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contents

review

Radiation therapy for melanoma brain metastases: a systematic review

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Background. Radiation therapy (RT) for melanoma brain metastases, delivered either as whole brain radiation therapy (WBRT) or as stereotactic radiosurgery (SRS), is an established component of treatment for this condition. However, evidence allowing comparison of the outcomes, advantages and disadvantages of the two RT modalities is scant, with very few randomised controlled trials having been conducted. This has led to considerable uncertainty and inconsistent guideline recommendations. The present systematic review identified 112 studies reporting outcomes for patients with melanoma brain metastases treated with RT. Three were randomised controlled trials but only one was of sufficient size to be considered informative. Most of the evidence was from non-randomised studies, either specific treatment series or disease cohorts. Criteria for determining treatment choice were reported in only 32 studies and the quality of these studies was variable. From the time of diagnosis of brain metastasis, the median survival after WBRT alone was 3.5 months (IQR 2.4–4.0 months) and for SRS alone it was 7.5 months (IQR 6.7–9.0 months). Overall patient survival increased over time (pre-1989 to 2015) but this was not apparent within specific treatment groups.
Conclusions. These survival estimates provide a baseline for determining the incremental benefits of recently introduced systemic treatments using targeted therapy or immunotherapy for melanoma brain metastases.

Key words: radiation therapy; stereotactic radiosurgery; melanoma; brain metastases

Introduction

Brain metastases are common in patients with advanced-stage melanoma, with a 20%–30% incidence in the first year after diagnosis of Stage IV disease, a 30%–40% incidence by 3-years, and an incidence of up to 73% in autopsy series.[1-3] For patients with untreated, symptomatic brain metastases, the reported average survival times range from several weeks to a few months.[4,5] Patients who have melanoma brain metastases have a worse prognosis than patients who have brain metastases from other solid tumours.[6]

The two main radiation therapy (RT) techniques used to treat melanoma brain metastases are whole brain radiotherapy (WBRT) and stereotactic radiosurgery (SRS). WBRT has largely fallen out of favour in recent times due to its apparently limited benefits while SRS has gained favour, especially as modern imaging has enabled earlier identification of smaller lesions before they become symptomatic.

Other treatment options for melanoma brain metastases include surgery and systemic therapy. Newer systemic therapies with immune checkpoint inhibitors and BRAF-targeted agents have shown considerable benefit in patients with metastatic melanoma[7-9] and evidence is accumulating that they can effectively treat brain metastases.[10,11] Combinations of surgery, RT and systemic therapy are now often used, sometimes sequentially, sometimes concurrently. Many contemporary clinical management guidelines suggest multidisciplinary advice tailored for individual patients, given the complexity of treatment options and sequencing.[12-15] Surgery can provide rapid symptomatic relief and may be the treatment of choice for single or few[2-3] lesions or larger symptomatic metastases in surgically-accessible sites. SRS can be an alternative to surgical resection as a local therapy in patients with smaller metastases, multiple lesions or surgically-inaccessible ones.[16-18] Although WBRT was a common treatment in the past, it is used much less frequently today[19,20], and is often reserved for patients whose brain metastases progress during systemic therapy and who are not suitable for further surgery or SRS.[21,22]

The evidence base for assessing the efficacy of RT to treat melanoma brain metastases has been weak because few well-designed randomised controlled trials have been conducted. Clinical practice guidelines have therefore been based largely on low-level evidence or consensus opinion and, as a result, recommendations vary considerably. Guidelines in the USA[23] suggest that some patients should receive systemic therapy as their sole initial treatment modality with no need for brain-directed local therapy unless there is intracranial progression, and advise that many patients will require a combined modality approach. European guidelines[24] recommend combination immunotherapy or targeted therapy as the preferred initial option and their consensus-based recommendations are to treat melanoma brain metastases with SRS, but with surgery when SRS is not possible, restricting WBRT to patients without systemic therapy or local therapy options. Australian guidelines[13] provide a practice point that concurs with European opinion about the use of systemic drug therapy and suggest that this be considered as first-line treatment in asymptomatic patients; the evidence-based recommendation, however, is for SRS to be considered in patients with single or few brain metastases, while WBRT may be used for palliation. Another practice point states that surgical resection of brain metastases is recommended for metastases >1 cm in diameter in non-eloquent areas or for symptomatic metastases.

The aim of this systematic review was to analyse the results of all published studies documenting the results of RT, without systemic immunotherapy or targeted therapy, as treatment for melanoma brain metastases. The review was prompted by the need to provide a benchmark for assessing the outcomes of upfront systemic therapy for patients with melanoma brain metastases.

Materials and methods

Terms covering melanoma, brain metastases and RT (WBRT or SRS) were used in the search strategy of Medline (1947 – 24 September 2020), Embase (1947 – 25 September 2020), the Cochrane Database of systematic reviews and the Cochrane Central Trials Registry (to 30 September 2020). Full details and results are provided in Supplementary Table 1. No language restrictions were used. Included studies were those reporting outcomes in patients with melanoma brain metastases treated with RT. Studies reporting patients with a mixture of cancer types including melanoma were excluded, as were studies of melanoma in which not all patients had brain metastases. Single case reports were also excluded, as were studies in which all patients received a combination of radiation and some form of contemporary systemic therapy (immune checkpoint inhibitors, BRAF –directed targeted therapies) without a radiation-only cohort.Non-contemporary systemic immunotherapies included interferon, interleukin, BCG vaccine, and non-contemporary systemic chemotherapies included temolozolmide, fotemustine, dacarbazine, razoxane, cisplatin and lomustine.

Complete search results were imported into Endnote, duplicates were removed and references were coded for inclusion/exclusion with reasons. Those included in the review had their data extracted by one author (GW). Reference lists of identified studies and review articles were examined to identify additional studies. Extracted data included article identifiers, design features, inclusion criteria, method of diagnosis, patient characteristics, treatment details, follow-up duration, deaths, adverse events, survival data and details of recurrences or new intracranial lesions. Quality assessment was performed using a specific tool for cohort studies[25] and the Cochrane collaboration risk of bias assessment for randomised controlled trials (RCTs).[26]

Descriptive statistics were generated using SPSS v25[27] with medians and interquartile ranges (IQR),

TABLE 1. Studies of radiation treatment in patients with melanoma brain metastases

Reference	Year	Country	Treated years	Total patients	Prospective data	Design	Surgery	WBRT	SRS		Non-contemp
									LA	GK	
Carella[50]	1980	US	1971–NS	60	×	Treatment cohort	✓	✓			✓
Katz[51]	1981	US	1971–1980	63	×	Treatment cohort	✓	✓			
Vlock[52]	1982	US	1970–1980	46	×	Treatment cohort	✓	✓			✓
Byrne[43]	1983	US	1978–1980	80	×	Treatment cohort	✓	✓			
Stridsklev[53]	1984	Norway	1973–1980	39	×	Treatment cohort		✓			✓
Choi (A)[54]	1985	US	1972–1977	194	×	Treatment cohort	✓	✓			✓
Choi (B)[55]	1985	US	1972–1977	59	×	Treatment cohort	✓	✓			
Ziegler[56]	1986	US	1972–1984	72	×	Treatment cohort	✓	✓			
Rate[44]	1988	US	1980–1987	77	×	Treatment cohort	✓	✓			✓
Hagen[57]	1990	US	1972–1987	35	×	Treatment cohort	✓	✓			
Stevens[38]	1992	Australia	1982–1990	129	×	Treatment cohort	✓	✓			
Somaza[58]	1993	US	1988–1992	23	×	Treatment cohort		✓		✓	✓
Willner[59]	1995	Germany	1985–1993	30	×	Disease cohort	✓	✓			✓
Isokangas[40]	1996	Finland	1980–1994	60	×	Treatment cohort	✓	✓			✓
Skibber[60]	1996	US	1979–1991	34	×	Treatment cohort	✓	✓			
Gieger[36]	1997	US	1992–1994	12	×	Treatment cohort	✓	✓	✓		
Gupta[61]	1997	UK	1991–1996	31	×	Treatment cohort	✓	✓			
Grob[62]	1998	France	1993–1996	35	×	Treatment cohort	✓			✓	✓
Sampson[63]	1998	US	past 20 years	670	×	Disease cohort	✓	✓			
Seung[64]	1998	US	1991–1995	55	×	Treatment cohort		✓		✓	
Lavine[65]	1999	US	1994–1997	45	×	Treatment cohort	✓	✓		✓	
Kontsadoulakis[66]	2000	US	1970–1992	136	×	Disease cohort	✓	✓			✓
Ellerhorst[67]	2001	US	1992–1995	87	×	Treatment cohort	✓	✓			
Buchsbaum[68]	2002	US	1994–1998	74	×	Disease cohort	✓	✓	✓	✓	
Gonzalez-Martinez[69]	2002	US	1996–NS	24	×	Treatment cohort		✓		✓	✓
Mingione[70]	2002	US	1989–1999	45	×	Treatment cohort	✓	✓		✓	✓
Noel[71]	2002	France	1994–2001	25	×	Treatment cohort			✓		
Yu[72]	2002	US	1994–1999	122	×	Treatment cohort		✓		✓	
Zacest[73]	2002	Australia	1979–1999	147	×	Treatment cohort	✓	✓			✓
Harrison[74]	2003	US	1990–1997	65	×	Treatment cohort	✓	✓			
Conill[75]	2004	Spain	1997–2002	26	×	Treatment cohort		✓			✓

Reference	Year	Country	Treated years	Total patients	Prospective data	Design	Surgery	WBRT	SRS		Non-contemp
									LA	GK	
Fife[37]	2004	Australia	1985–2000 (also 1952–1984)	686 (+ 451)	×	Disease cohort	✓	✓			
Meier[76]	2004	Switzerland	1966–2002	100	×	Disease cohort	✓	✓	✓	✓	✓
Morris[77]	2004	UK	1998–2003	102	×	Treatment cohort	✓	✓	?	?	✓
Radbill[78]	2004	US	1996–2001	51	×	Treatment cohort	✓	✓		✓	
Selek[79]	2004	US	1991–2001	103	×	Treatment cohort	✓	✓	✓		
Stone[80]	2004	US	1989–1999	83	×	Disease cohort	✓	✓		✓	
Koc[81]	2005	US	1999–2003	26	×	Treatment cohort				✓	✓
Panagiotou[82]	2005	Greece	1986–2001	64	×	Disease cohort	✓	✓			✓
Rhomberg[83]	2005	Austria	1982–2002	19	×	Treatment cohort		✓			✓
Christopoulou[84]	2006	UK	1998–2004	29	×	Treatment cohort	✓	✓		✓	
Gaudy-Marquesta[85]	2006	France	1997–2003	106	×	Treatment cohort				✓	✓
Conill[86]	2007	Spain	1997–2004	37	×	Treatment cohort		✓			✓
Mathieu[87]	2007	US	1987–2005	245	×	Treatment cohort	✓	✓		✓	
Samlowski[32]	2007	US	1999–2004	44	×	Treatment cohort	✓	✓	✓		✓
Raizer[5]	2008	US	1991–2001	355	×	Disease cohort	✓	✓	?	?	✓
Redmond[88]	2008	US	1998–2007	59	×	Treatment cohort	✓	✓		✓	
Carrubba[89]	2009	US	2002–2007	37	×	Disease cohort	✓	✓	?	?	
Ahmad[90]	2010	UK	2001–2009	65	×	Treatment cohort	✓	✓			
Rades[91]	2010	Germany	1989–2008	51	×	Treatment cohort		✓			
Schild[92]	2010	US	NS	7 (+ 53)	Y+N	Treatment cohort		✓			✓
Staudt[93]	2010	Germany	1986–2003	265	×	Disease cohort	✓	✓	✓		✓
Davies[34]	2011	US	1986–2004	330	×	Disease cohort	✓	✓	?	?	
Eigentler[94]	2011	Germany	1986–2007	672	×	Disease cohort	✓	✓	?	?	✓
Liew[95]	2011	US	1987–2008	333	×	Treatment cohort		✓		✓	
Skeie[96]	2011	Norway	1996–2006	77	×	Treatment cohort	✓	✓		✓	
Zakrzewski[97]	2011	US	2002–2008	89	×	Disease cohort	✓		?	?	✓
Bernard[98]	2012	US	2004–2010	54	×	Treatment cohort	✓		✓		
Hauswald[33]	2012	Germany	2000–2011	87	×	Treatment cohort	✓	✓	?	?	
Knisely[35]	2012	US	2002–2010	77	×	Treatment cohort		✓		✓	✓
Koay[99]	2012	US	2005–2011	296	×	Disease cohort	✓	✓	?	?	✓
Lo[100]	2012	US	2000–2007	28	×	Treatment cohort		✓		✓	✓
Salvati[101]	2012	Italy	1997–2007	84	×	Treatment cohort	✓	✓	?	?	

Reference	Year	Country	Treated years	Total patients	Prospective data	Design	Surgery	WBRT	SRS		Non-contemp
									LA	GK	
Mathew[102]	2013	US	2008–2011	58	×	Treatment cohort				✓	✓
Miller[103]	2013	Germany	2000–2010	34	×	Treatment cohort	✓			✓	✓
Partl[31]	2013	Austria	1988–2009	87	×	Treatment cohort	✓	✓	?	?	
Silk[41]	2013	US	2005–2012	70	×	Treatment cohort		✓	?	?	✓
Zukauskaite[104]	2013	Denmark	1995–2009	80	×	Treatment cohort	✓	✓	?	?	✓
Dyer[105]	2014	US	2000–2010	147	×	Treatment cohort		✓	✓		✓
Marcus[106]	2014	US	1998–2010	135	×	Treatment cohort	✓	✓	?	?	✓
Neal[107]	2014	US	2000–2009	129	×	Treatment cohort	✓	✓		✓	✓
Rades [108]	2014	Germany	2000–2013	54	×	Treatment cohort			✓	✓	
Vecchio[109]	2014	Italy	1994–2010	115	×	Disease cohort	✓	✓	?	?	✓
Christ[110]	2015	US	2005–2011	103	×	Treatment cohort	✓	✓	✓		✓
Frakes[111]	2015	US	2008–2012	28	×	Treatment cohort		✓	✓		
Hauswald[112]	2015	Germany	1990–2011	84	×	Treatment cohort	✓	✓	✓		✓
Ivanov[113]	2015	Russia	2009–2013	95	×	Treatment cohort	✓	✓		✓	
Ly[114]	2015	US	2009–2012	52	×	Treatment cohort	✓		✓		
Ostheimer[115]	2015	Germany	1992–2011	100	×	Treatment cohort	✓	✓	?	?	✓
Gallaher[116]	2016	US	since 2006	19	×	Treatment cohort		✓		✓	
Gupta[29]	2016	UK	NS	18	Yes	RCT		✓			✓
Patel[117]	2016	US	2007–2014 (abstract says 2005–2013)	87	×	Treatment cohort			✓		✓
Rades[118]	2016	Germany	2000–2015	23	×	Treatment cohort		✓			
Szyszka-Chare[39]	2016	Poland	1985–2012	110	×	Disease cohort	✓	✓	?	?	✓
Wolf[119]	2016	US	2012–2015	80	×	Treatment cohort	✓	✓		✓	✓
Acharya[120]	2017	US	2006–2016	72	×	Treatment cohort		✓		✓	✓
All[121]	2017	US	2008–2016	58	×	Treatment cohort			?	?	
Feng[122]	2017	US	2007–2014	87	×	Treatment cohort	✓	✓	✓	✓	✓
Kaidar-Person[123]	2017	US	2007–2015	58	×	Treatment cohort		✓	✓		✓
Minniti[124]	2017	Italy	2008–2015	120	×	Treatment cohort	✓		✓		✓
Patel[125]	2017	US	2009–2013	54	×	Treatment cohort	✓		✓		✓
Pessina[126]	2017	Italy	2011–2015	53	×	Treatment cohort	✓		?	?	
Sperduto[127]	2017	US	2006–2013	823/481	×	Disease cohort	✓	✓	?	?	
Xu[128]	2017	US	2010–2014	65	×	Treatment cohort	✓	✓		✓	✓
Diao(A) [129]	2018	US	2006–2015	72	×	Treatment cohort				✓	✓

Reference	Year	Country	Treated years	Total patients	Prospective data	Design	Surgery	WBRT	SRS		Non-contemp
									LA	GK	
Diao(B) [130]	2018	US	2006–2015	91	×	Treatment cohort				✓	✓
Fang[131]	2018	US	2005–2011	235	×	Disease cohort	✓	✓	?	?	✓
Gabani[132]	2018	US	2011–2013	1104	×	Treatment cohort		✓	?	?	✓
Kano[133]	2018	US	1988–2012	422	×	Treatment cohort	✓	✓		✓	✓
Kotecha[134]	2018	US	1987–2014	366	×	Disease cohort	✓	✓	✓	✓	✓
Ladwa[135]	2018	Australia	2009–2016	142	×	Disease cohort	✓	✓	?	?	
Matsunaga[136]	2018	Japan	1991–2015	177	×	Treatment cohort	✓	✓		✓	✓
Tio[137]	2018	Australia	2011–2014	355	×	Disease cohort	✓	✓	?	?	✓
Zubatkina[138]	2018	Russia	2009–2014	78	×	Treatment cohort	✓	✓		✓	
Hauswald[28]	2019	Germany	2013–2017	7	Yes	RCT		✓			
Hong[30]	2019	Australia	2009–2017	215	Yes	RCT	✓	✓	?	?	✓
Jardim[139]	2019	Australia	2015–2017	43	×	Treatment cohort	✓	✓		✓	✓
Mastorakos[140]	2019	US	2011–2015	198	×	Treatment cohort	✓	✓		✓	✓
Phillips[42]	2019	Canada	2000–2018	277	NS	Disease cohort		✓	?	?	
Tjong[141]	2019	Canada	2008–2017	97	×	Treatment cohort	✓	✓	?	?	
McHugh[142]	2020	New Zealand	2005–2017	110	×	Treatment cohort	✓	✓	?	?	✓
Pomeranz-Krumme[143]	2020	US	2010–2018	25	×	Treatment cohort	✓	✓	✓	✓	✓

GK = Gamma Knife methods; Non-contemp = non-contemporary systemic therapy; LA = linear accelerator; SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy stereotactic radiosurgery

as data were not normally distributed. Medians were tested for difference using the non-parametric median test, Fisher's exact (2-sided). When three or more studies reported the same outcome for the same treatment group, data were pooled and analysed.

Results

Search results

Search results and exclusions are shown in Figure 1. There were 142 publications between 1980–2020, 112 of which were unique studies or were the primary publication of a series of publications, and 30 were duplicates or non-primary publications (Table 1.) Seven studies were published only as abstracts. 57.1% (64/112) of the publications were from the USA, 30.4% from Europe, and 11.6% from other countries. Sample sizes ranged from 7–1304 patients (median 77). While our focus was on RT, most articles (96/112) included patients who had received a variety of other therapies for their brain metastases. Surgery was reported in 79 studies, WBRT in 95 studies, SRS in 84 studies and 64 reports included subsets of patients who received some form of systemic therapy as well as RT. Outcomes for the subsets of patients treated with both RT and any form of contemporary systemic therapy were not analysed. Amongst studies reporting the use of SRS, five reported using both linear accelerator and Gamma Knife methods, 32 used Gamma Knife only, 18 used linear accelerator only and 29 did not report which was used.

Only three studies were prospectively-conducted RCTs; the remainder were retrospective studies of patient cohorts of specific treatment/s (n = 85) or cohorts of patients treated for melanoma brain metastases (n = 24). A comparison of outcomes for different treatment regimens was reported in 97 studies, while 15 studies were non-comparative, reporting outcome data for a single treatment group.

Risk of bias assessment

Details of the risk of bias assessment for each study are provided in Supplementary Table 2 and summarised in Figure 2. Of the three RCTs, none reported how their randomisation sequence was generated but all reported complete outcome data and clinically-relevant outcomes. Two of the three trials[28,29] were closed early due to poor accrual and had sample sizes of 7 and 18, greatly limiting the reliability of their results. Baseline characteristics for the different treatment arms were reported and similar for the trial of 18 patients[29] but were not reported for the trial of 7 patients.[28] The largest trial[30] had 215 patients, and while not stratifying for previous treatments, randomisation was effective as previous treatments were well balanced between the two study arms, as were baseline characteristics. This trial provided the most reliable data for identifying the effects of adjuvant WBRT in conjunction with surgery and/or SRS for patients with 1–3 brain metastases.

For the 109 cohort studies, selection bias was a significant concern, as 77 studies (71%) did not provide information specifying how a treatment choice was made. Sixteen studies reported that there were no significant differences in patient characteristics such as age, number of brain metastases and tumour volume between treatment groups, while 11 studies reported significant differences between patients treated using different modalities. The remaining 82 studies did not report similarities or differences in patient characteristics, although in 21 studies the patient characteristics were provided. Fifty-six studies did not report if or how the diagnosis of melanoma brain metastases was verified; this may have resulted in misclassification of disease, although this is probably a relatively inconsequential source of bias.

Many studies reported analyses of one treatment without considering prior and subsequent forms of treatment (e.g. SRS preceded by surgical resection). Our analysis was based on grouping data based on all treatments received for brain metastases.

Treatment decisions

Five studies (651 patients) included only asymptomatic patients, nine studies (532 patients) included only symptomatic patients, 30 studies (5906 patients) had a mixture of asymptomatic and symptomatic patients and 57 studies (5452 patients) did not report this detail.

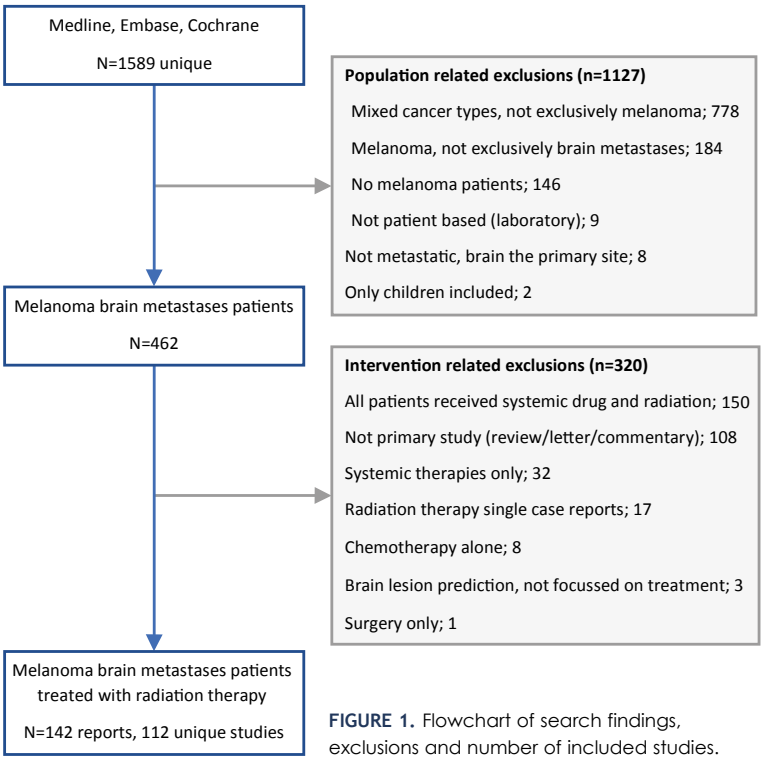

FIGURE 1. Flowchart of search findings, exclusions and number of included studies.

Seven of the 95 studies provided specific criteria for choosing WBRT in their patients; four stated that it was used for multiple brain metastases[31-34], three reported its use for progression of brain metastases[31,35,36], one its use for single, large metastases[36] and one its use for symptomatic metastases.[37]

Nineteen studies reported criteria for choosing SRS. Twelve stated that it was used for small metastases, often <30mm in diameter, nine required good performance status as measured by Karnofsky performance score (KPS), with four of these using a cut-off of KPS ≥ 70. Seven studies used SRS for a small number of brain metastases (usually 1–3). Five studies used SRS when metastases were inaccessible for surgery, four used it in for multiple metastases, but only one specified a number (≤ 9), and three stated that it was used for asymptomatic lesions. Other infrequently used criteria were; expected survival > 3 months, non-life threatening lesions, high risk for surgery, including proximity to the brain stem or optic nerve. A single study[32] reported criteria for using a combination of SRS and WBRT, stating that this was used for ≥ 5 lesions.

Fourteen studies provided criteria for surgery; a single metastasis (5 studies), few or <3 metastases

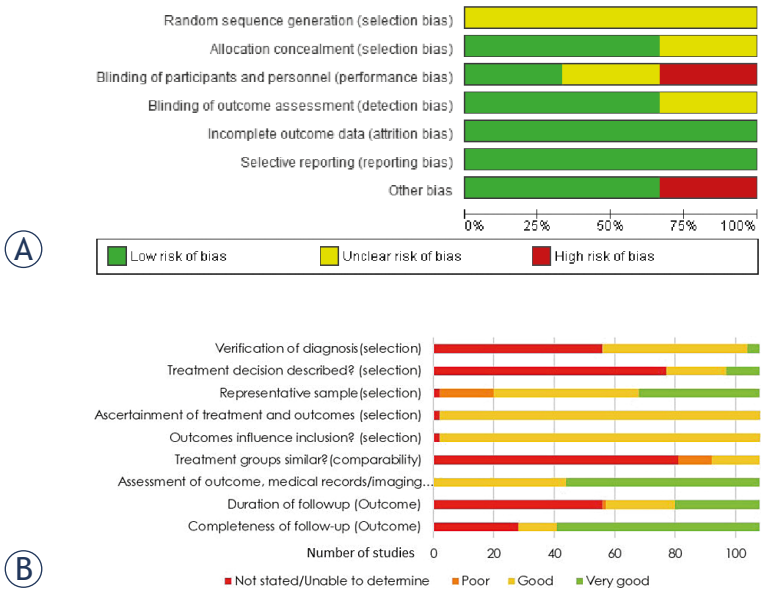

FIGURE 2. (A) Risk of bias assessments for randomised controlled trials evaluating radiation therapies in patients with melanoma brain metastases. **(B)** Quality assessment of cohort studies of patients with melanoma brain metastases treated with radiation therapy.

(2 studies), accessible metastases (8 studies), symptomatic metastases (4 studies), stable extracranial disease (4 studies), good KPS (1 study), life expectancy > 3 months (1 study), and 2–3 brain metastases if one was life-threatening (2 studies).

Treatment groupings

Many treatment groupings that included RT were reported but outcomes were not reported for all groups. Given the limited amount of data for clearly-defined treatment groups, we re-grouped data into two additional treatment options; (i) patients treated with WBRT and any of SRS, surgery or non-contemporary systemic therapy, and (ii) patients treated with SRS and any of WBRT, surgery, or non-contemporary systemic therapy.

Patient characteristics within treatment groups

Patient characteristics within different treatment groups are summarised in Table 2. For all treatment types, there was a predominance of males. Patients treated with WBRT alone were somewhat younger than those receiving SRS and patients undergoing WBRT were less likely to have a single brain metastasis. While the data were sparse, there was considerable overlap in patient characteristics across different treatment modalities, indicating that the choice of treatment was not consistently determined by age, presence of symptoms, number of metastases or control of primary disease.

Median survival

Ninety-six studies reported median survival for all patients or subsets of patients and there were 49 different treatment groupings.

Within-study comparisons were possible for six treatment groupings (Table 3.). Eleven studies reported median survival for patients treated with WBRT alone or with WBRT and surgery. The median survival in the WBRT alone group was 4.0 months (IQR 3.0–4.0 months), significantly less than for those treated with surgery and WBRT (11.0 months, IQR 8.8–11.8 months) (p = 0.002). Expressing these findings as a median difference between treatments, patients who had WBRT and surgery had a 5.4 month (IQR 4.6–8.0 months) longer survival compared with those treated with WBRT alone. In this group of 11 studies, three reported using surgery in patients with a single brain metastasis[37-39], and two studies reported features for treatment with WBRT, this being good performance status alone in one study[40] and multiple lesions, good performance and symptoms in the second study.[37] Significant differences in median survival were also apparent between WBRT alone and surgery alone (6.0 months longer for surgery) and median survival for patients treated with WBRT plus SRS was 3.4 months longer than with WBRT alone. In the five studies with groups treated with WBRT alone or surgery alone, three reported that surgery was used for a single or few brain metastases[34,37,38] and WBRT was used for patients with more than one brain metastasis (1 study)[38] and for patients with good performance status (1 study[37]). None of the four studies reporting patients treated with WBRT alone or WBRT+SRS described features leading to these treatment choices. There were no significant differences in median survival between WBRT alone and SRS alone, or between WBRT alone and WBRT with chemotherapy, or SRS alone compared to WBRT with SRS.

Summarised findings for median survival in all studies are detailed in Table 3 and for other groupings in Supplementary Table 4. The group treated with WBRT alone had the shortest survival; 3.5 months (IQR 2.4–4.0 months). For the group treated with surgery and WBRT, the median survival was 11.0 months (IQR 7.8–12.0 months). Adding chemotherapy to WBRT appeared to provide lit-

TABLE 2. Patient characteristics within treatment group for the 51 studies that reported baseline characteristics

Treatment group		Age in years	Proportion of patients			
			Males	Asymptomatic	With single brain metastasis	With controlled primary disease
WBRT alone	Median IQR N studies(pts)	53.0 49.0–55.8 10 (295)	63.8% 42.5–73.7 % 13 (496)	*i11.5%, i32.2%* NA 2 (85)	26.6% 18.5–48.6% 7 (339)	29.0% 13.9–47.8% 10 (329)
SRS alone	Median IQR N studies(pts)	60.2 56.25–62.25 9 (444)	66.7% 54.0–76.3% 12 (822)	66.8% 59.65–78.79% 4 (359)	54.8% 41.19–61.22 9 (706)	32.2% 26.16–36.32% 8 (669)
WBRT and any of SRS, surgery, chemotherapy, non-contemp	Median IQR N studies(pts)	53.0 47.00–58.75 5 (243)	63.4% 51.64–73.88% 6 (266)	- - 0	51.7% 40.72–72.91% 4 (223)	45.9% 30.18–68.06% 6 (262)
SRS and any of; surgery, WBRT, chemotherapy, non-contemp	Median IQR N studies(pts)	56.9 52.5 – 59.25 17 (1127)	59.1% 54.82 – 68.04% 20 (1838)	65.4% 51.28 – 66.38% 5 (953)	38.8% 30.11 – 51.74% 16 (1697)	24.0% 17.56 – 39.70% 16 (1660)

GK = Gamma Knife methods; non-contemp = non-contemporary systemic therapy; *i* = individual study data; IQR = interquartile range; NA = not applicable; SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy stereotactic radiosurgery

tle benefit, with six studies of 137 patients reporting a median survival of 4.3 months (IQR 2.8–6.0 months) (Supplementary Table 4). The compiled grouping of WBRT with any of SRS, surgery, or non-contemporary systemic therapy had a median survival of 7.2 months (IQR 4.6–9.4 months) across 47 studies with 2230 patients.

Median survival after SRS treatment alone was reported in eight studies, giving a median survival of 7.5 months (IQR 6.7–9.0 months). The median survival for patients treated with Gamma Knife SRS (5 studies, 208 patients) was 7.0 months (IQR 5.6–7.8 months) and for the three studies (980 patients) that did not report which SRS technology was used the median survival was 8.8 months. Eighteen studies reported using linear accelerator SRS but none reported the median survival for patients treated with SRS alone. Adding non-contemporary systemic therapy to SRS treatment did not improve the median survival (7.9 months, IQR 6.1–9.9 months) but the addition of surgery was associated with an increase in median survival of around 5 months (13.0 months, IQR 9.4, 13.5 months). Compiled grouping of SRS with any of WBRT, surgery, or non-contemporary systemic therapy gave a median survival of 8.0 months (IQR 6.2–10.9 months) over 42 studies involving 2702 patients.

In the only completed RCT in patients with melanoma brain metastases[30], median survival in the group treated with adjuvant WBRT after definitive local treatment of 1–3 metastases was 16.5 months (95% CI 13–24 months) compared to 13.0 months (95% CI 10–19 months) for those that did not receive adjuvant WBRT (p=0.86).

No studies reported median survival within treatment groups separately for asymptomatic and symptomatic patients. For the 5 studies that included only asymptomatic patients, there were no common treatment groups. In 4 studies of 89 symptomatic patients, the median survival for the treatment group WBRT with surgery was 9.2 months (IQR 5.4–12.8 months) and for WBRT with any other treatment; 5 months (IQR 2.5–10.0 months, 7 studies of 245 patients).

Median survival in different time periods

Median survival over time was explored by grouping the data into three time periods based on the first year of patient recruitment within each study (Table 4). Eighty-one studies reported median survival for their whole cohort irrespective of treatment, and these showed increasing survival over the years. There were significant differences in median survival between the pre-1989 group compared with the 1990–2002 (p = 0.017) and 2003–2015 groups (p = 0.002) and also between the groups first treated in 1990–2002 compared with 2003–2015 (p = 0.021).

Median survival within treatment groups over the three time periods showed a trend toward slightly increased survival in more recent years, but none of the differences was statistically significant.

One-year survival

Fifty-six studies reported 1-year survival for all patients or subsets of patients (Table 3). Pooled

TABLE 3. Pooled outcome results for studies of radiation treatments

Treatment	Groups	Median survival		1-year survival rate, %		1-year local control rate		6-mo new brain lesion rate		Serious adverse events
		Studies (Patients)	Months (IQR)	Studies (Patients)	% (IQR)	Studies (Patients)	% (IQR)	Studies (Patients)	% (IQR)	
WBRT *vs.* WBRT + Surgery										
Non-random, comparative	WBRT	11 (980)	4.0 (3.0, 4.0)	0	-	0	-	0	-	Not reported
	WBRT+Surg	11 (439)	11.0 (8.8,11.8)	0	-	0	-	0	-	
All studies	WBRT	26 (2185)	3.5 (2.4, 4.0)	7 (189)	9.0 (0.0, 22.5)	1 (74)	5.5 (0.0,	0	-	Post-op death;
	WBRT+Surg	16 (619)	11.0 (7.8, 12.0)	1 (19)	*i41.0*	0	12.0)	0	-	2% (1 study), Hemorrhage; 3/72 lesions (1 Study)
WBRT *vs.* Surgery										
Non-random, comparative	WBRT	5 (699)	3.9 (3.6, 5.0)	0	-	0	-	0	-	-
	Surgery	5 (234)	9.8 (7.6, 16.5)	0	-	0	-	0	-	Gr3 tox; 3/39 (1 study)
All studies	Surgery	9 (359	8.7 (6.2, 10.4)	1 (16)	*i36.0*	0	-	0	-	Post-op death; 2% (1 Study)
WBRT *vs.* SRS										
Non-random, comparative	WBRT	3 (931)	4.1 (3.2, 5.6)	0	-	0	-	0	-	-
	SRS	3 (980)	8.8 (7.2, 11.4)	0	-	0	-	0	-	Hemorrhage; 4/56
All studies	SRS	8 (1188)	7.5 (6.7, 9.0)	6 (330)	35.5 (20.8, 47.8) *i26.0*	4 (260)	76.0 (62.8, 88.5)	0	-	lesions (1 study) (SRS-GK)
SRS Type	SRS-GK	5 (208)	7.0 (5.6, 7.8)	1 (83)	-	0	-	0	-	
	SRS-LA	0	-	0	-	0	-	0	-	
	SRS-NS	3 (980)	8.8 (7.2, 11.4)	0		0	-	0	-	
WBRT *vs.* WBRT + Chemotherapy										
Non-random, comparative	WBRT	4 (148)	2.5 (1.0, 4.2)	0	-	0	-	0	-	Gr3 tox; 3/39 (1 study)
	WBRT +Chemo	4 (62)	5.5 (4.0, 6.0)	1 (7)	*i0.0*	0	-	0	-	
All studies	WBRT+Chemo	6 (137)	4.3 (2.8, 6.0)	2 (15)	*i0.0, i37.0*	0	-	0	-	Leukopenia; 2/8 (1 study), Toxicity; 9/14 (1 study)
SRS *vs.* WBRT+SRS										
Non-random, comparative	SRS	5 (881)	7.0 (6.0, 8.1)	1 (83)	*i26.0*	0	-	0	-	Swelling
	WBRT+SRS	5 (344)	6.5 (5.7, 6.5)	1 (39)	*i23.0*	0	-	0	-	requiring surgical decompression; 3/77 pts (1 study)
All studies	WBRT+SRS	12 (516)	7.0 (6.0, 8.0)	3 (58)	36.0 (29.5, 37.0)	0	-	0	-	-
WBRT *vs.* WBRT+SRS										
Non-random, comparative	WBRT	4 (337)	3.6 (2.7, 5.0)	1 (59)	*i10.0*	0	-	0	-	-
	WBRT+SRS	4 (197)	7.4 (6.5, 10.7)	1 (8)	*i38.0*	0	-	0	-	-
SRS+ Chemotherapy										
AI studies	SRS+Chemo	1 (23)	*i6.5*	0	-	0	-	0	-	-
	SRS+/- Chemo	7 (580)	*7.9 (6.1, 9.9)*	2 (358)	*i13.2, i27.9*	1 (106)	*i69.0*	1 (106)	*i12.0*	Hemorrhage; 1/106 pt (1 study), 4/56 lesions (1 study). Radiation necrosis; 1/106 pts (1 study)
SRS + Surgery										
All studies	SRS+Surg	4 (200)	13 (9.4, 13.5)	1 (60)	*i58.0*	1 (34)	*i52.0*	1 (34)	*i32.0*	Hemorrhage; 18% (1 study)
WBRT+ other treatments										
All studies	WBRT and any of surgery, SRS, non-contemp	47 (2230)	7.2 (4.6, 9.4)	19 (827)	21.4 (13.6, 37.0)	5 (208)	1.0 (0.0, 16.0)	8 (986)	46.5 (39.8, 55.5)	WBRT specific; Deaths;6/194 (1 study), headache; 12/26 (1 study), Toxicity > Gr3; 3/7 (1 study) LeukopeniaGr1-2; 2/9 (1 study) Hemorrhage; 1/20 (1 study)
SRS+ other treatments										
All studies	SRS and any of surgery, WBRT, non-contemp	42 (2702)	8.0 (6.2, 10.9)	35 (2644)	31.0 (25.0, 39.0)	16 (1043)	69.0 (60.0, 82.0)	10 (1261)	49.0 (42.0, 56.0)	SRS specific; Hemorrhage; 14% (4 studies, 441 patients) Radiation necrosis; 6.6% (4 studies, 241 patients Seizure-edema-death; 1/55 (1 study) Complications; 6/106 (1 study)

Chemo = chemotherapy; GK = Gamma Knife methods; Gr = grade; *i* = individual study data; IQR = interquartile range; LA = linear accelerator; non-contemp = non contemporary systemic therapy; SRS = stereotactic radiosurgery; Surg = surgery; WBRT = whole brain radiation therapy

TABLE 4. Median survival within treatment groups and grouped by the first year of patient recruitment

First year of recruitment	Pre–1989			1990–2002			2003–2015			Not reported		
	No. of studies	Median survival	IQR	No. of studies	Median survival	IQR	No. of studies	Median survival	IQR	No. of studies	Median survival	IQR
All patients	25	4.8	3.25–8.05	33	6.0	4.35–8.00	22	9.2	6.90–11.43	1	*i3.0*	NA
WBRT alone	17	3.6	2.49–4.0	7	2.5	2.3–4.0	6	4.2	2.75–4.80	3	4.3	3.40–6.40
SRS alone	2	*i6.4, i7.7*		4	7.3	5.78–7.88	2	*i10.0, i11.9*		0		
WBRT and any of surgery, SRS, non-contemporary systemic therapy	43	7.4	4.00–9.20	23	7.3	5.50–10.00	4	8.0	5.73–10.50	2	*i3.6, i4.3*	
SRS and any of surgery, WBRT, non-contemporary systemic therapy	17	8.3	5.90–9.65	29	7.9	5.85–10.04	18	9.0	6.90-13.00	0		

i = individual study data; IQR = interquartile range; NA = not applicable; SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy

data from 7 studies of 189 patients treated with WBRT alone gave a 1-year survival rate of 9.0% (IQR 0.0–22.5%) while for SRS alone the 1-year survival was 35.5% (IQR 20.8–47.8%, p = 0.041) in six studies of 330 patients. In the compiled grouping of WBRT with or without any other therapy, the 1-year survival was 21.4% (IQR 13.6%–37.0%). For the SRS grouping with or without any other therapy (WBRT, surgery, non-contemporary systemic therapy), the 1-year survival was 31.0% (IQR 25.0–39.0%) across 35 studies of 2644 patients. In the only completed RCT[30], 1-year survival in the group treated with adjuvant WBRT was 58.4% (95%CI 49.6%–68.9%) compared with 54% (95%CI 45.3%–64.3%, p = 0.89) for those treated without WBRT.

Local control

Most studies defined local control as a reduction in metastasis size or stability of metastasis size, as determined by follow-up imaging. Fifty-three studies reported local control data, 21 without a defined time frame and for almost all it was reported for the total patient group, not separately for different treatment groupings. The 1-year local control rate was highest for those treated with SRS; 76% (IQR 62.8%–88.5%). The 1-year local control rate after WBRT was 5.5% (IQR 0.0%–12.0%, 1 study, 74 patients). For the 550 patients in 11 studies that treated patients with any combination of WBRT, SRS, surgery and non-contemporary systemic therapy, the 1-year local control rate was 68.0% (IQR 66.072.0%). There was no difference in the 1-year local control rate between Gamma Knife SRS and linear accelerator-based SRS (69% *vs.* 72.0%).

New brain lesions

Thirty-six studies reported rates of new brain lesions developing during the follow-up period. For the 23 studies that reported new brain lesions at 6-months the median rate was 44% (IQR 32.0%–53.0%) and at 12-months 67% (IQR 62.3%–71.5%, 14 studies). Three studies (189 patients) reported a 6-month new brain lesion rate in patients treated with WBRT and other treatment, giving a median rate of 39% (IQR 34.0%–44.5%) and for SRS and other treatment a rate of 47% (IQR 34.5%–55.5%, 11 studies, 1306 patients). At 12 months, the RCT of patients with 1–3 brain metastases reported a new brain lesion rate of 42% in the adjuvant WBRT group and 50.5% in those who did not receive adjuvant WBRT (p = 0.22). For patients treated with SRS, the proportion who developed a new brain lesion by 12 months was 67% (IQR 57.0%–75.0%; 11 studies, 1278 patients).

Neurologic deaths

Neurologic death was reported in 40 studies but only 11 reported this for a defined treatment group. The definition of neurologic death was variable. Only one study provided a definition that combined an objective measurement with radiological and clinical neurologic changes.[41] Other studies used brain lesion progression and/or recurrence (18 studies), brain hemorrhage alone (4 studies), neurologic dysfunction alone (4 studies) or other features (3 studies) as criteria for designating a death as neurologic. The reported proportion of patients with a neurologic cause of death ranged from 0% to 90%. Three studies reported the propor-

tion of patients treated with WBRT with or without surgery who experienced neurologic death (0%, 24%, 83%). Two studies reported neurologic deaths for patients treated with WBRT alone (14%, 88%), two studies reported on SRS alone (41%, 90%), two studies reported neurologic deaths in those treated with SRS and WBRT (50%, 58%) and two studies reported neurologic death in patients treated with WBRT with or without systemic therapy (57%, 75%). Re-grouping the data into SRS with any other treatment (6 studies), gave a median neurologic death rate of 53% (IQR 44.8%–69.4%), while for WBRT and any other treatment (10 studies), the median was 50% (IQR 19.3%–62.3%).

Effect of number of brain metastases on survival

Seventy-six studies assessed whether the number of brain metastases present at diagnosis impacted survival, with 58 reporting significantly improved survival for patients with single lesions while 18 reported no impact. In the RCT[30], the number of brain metastases (1 *vs.* 2–3) did not influence overall survival. Only three studies reported these data within specific treatment groups.[42-44] Two studies[43,44] reported survival in patients treated with WBRT alone comparing those with one metastasis to those with ≥ 2 brain metastases; one study[44] reported better survival in the single metastasis group (16 weeks) compared to the multiple metastases group (12 weeks) while the other study[43] did not (9 weeks for a single metastases and 11 weeks for multiple metastases).

Adverse effects of radiation therapy

Adverse effects of RT were reported in 41 studies, but only 17 reported events within treatment groups and these were primarily studies that included systemic therapy. Radiation necrosis (with various radiological and/or pathological definitions) was reported in 13 studies, 11 of which focussed on SRS. The median rate was 8.1% (IQR 3.4%–22.2%). In the four studies using Gamma Knife SRS, the median radiation necrosis rate was 3.4% (IQR 0.47%–5.49%) and for the 4 studies using linear accelerator SRS it was 22.2% (IQR 15.59%–25.66%). Two studies reported this for WBRT, with rates of 1.9% and 3.6%. Eight studies reported intracranial haemorrhage in their patients, with seven studies focussed on SRS, giving a median rate of 14.7% (IQR 0.94–18.8%). Three studies using Gamma Knife SRS reported brain haemorrhage rates with a median rate of 18.8% (IQR 9.86–24.01%) and two studies used linear accelerator SRS, with brain haemorrhage rates of 15% and 16%. Other reported adverse effects included headaches, seizures, skin reactions, fatigue, nausea, alopecia and confusion but because data were sparse and pooled analysis was not possible.

Discussion

For unbiased comparisons of an intervention, prospective randomised controlled trials are required. Although RT has long been used in the management of patients with melanoma brain metastases, there have been only three randomised trials of RT for this condition, and only one of these[30] recruited sufficient patients for meaningful analysis. However, a large number of non-randomised studies (n = 109) have published outcomes for patients with melanoma brain metastases treated with various RT regimens. The number of patients in each study varied, but most (86%) had fewer than 200 patients and medians of 20–30 for different treatment groups. This low number of patients per treatment group reduces the precision of estimates of survival duration within each study but when pooled over many studies, greater precision can be achieved. These non-randomised studies were of variable quality with multiple study design features poorly reported, hindering our understanding of how patients were selected for the studies and how representative they were. Over the 40-year period encompassed by this review there was a consistent trend towards improvement in the median survival of patients with melanoma brain metastases. This is likely due to earlier diagnosis of small brain metastases using newer imaging technologies, as well as a general improvement in treatment. However, we were unable to demonstrate an improvement in median survival within treatment groups over time, possibly due to a paucity of data for individual treatment groups.

Within-study comparisons were possible for only six treatment groupings. These analyses demonstrated significantly longer median survival times for patients who were treated with surgery alone (+6 months), WBRT and surgery (+7 months) and WBRT and SRS (+4 months) compared to those treated with WBRT alone (4 months). The better survival after surgery or SRS than after WBRT is almost certainly due mainly to selection issues since patients with fewer lesions, better performance status and a lower burden of extracranial disease were more likely to receive surgery or SRS and these fea-

tures are associated with improved survival. The benefit of within-study comparisons is the presence of a "control" group in the same study, meaning that treatment decisions, management and outcome assessment were likely to be more consistent than comparisons with studies performed at different institutions and at different times.

Many treatment groups were not represented in the within-study comparisons and were therefore reviewed across studies to provide estimates of median and 1-year survival rates for major treatment groupings. Patients treated with WBRT alone had a median survival of only 3.5 months, while those treated with SRS had a median survival of 7.5 months. Data were somewhat limited but suggest that linear accelerator-based SRS resulted in similar local control rates as Gamma Knife-based SRS. This is a reassuring finding as there is no randomised comparison of different SRS techniques for brain metastases. The combination of surgical removal of the lesion/s and WBRT was associated with substantially improved median survival, apparently adding 7.5 months of life, with median survival 11.0 months. These across-study median survival estimates are reassuringly consistent with the within-study findings. These findings, however, conflict with those of the randomised controlled trial that showed no survival gain and no improvement in intracranial control or performance status with adjuvant WBRT after adequate local treatment of 1–3 brain metastases.[30] This may be because about one third of the patients in the RCT also received SRS, which may have enhanced survival and limits our ability to compare their outcomes with those of patients treated with WBRT and surgery but no SRS.

Median survival for patients treated with surgery and SRS also showed benefit (+5.5 months), with a median survival of 13 months. Again, this is likely attributable to selection of patients with fewer metastases for surgery and SRS. Importantly, the data confirmed a lack of any survival benefit from the addition of non-contemporary systemic chemotherapy or non-contemporary forms of immunotherapy.

Limitations

Risk of bias assessment for these studies showed that many of the non-randomised studies included patients who were treated without explanation of how treatment choices were made. In the 30% of studies that did report treatment selection criteria there was considerable variation, reflecting the diversity of clinical practice between and even within individual centres and over the 40-year study period. This selection bias limited our ability to apply results to specific patient groups as we could not be sure in many instances which types of patients received particular treatments. Also, important prognostic factors such as performance status and extent of extracranial disease were rarely reported within treatment groups. Compiling the rather limited patient characteristics data for the different treatment groups showed that there was considerable overlap in the types of patients receiving WBRT and SRS. There was a degree of consistency in offering surgery to patients with a single or few brain metastases, as almost half of the studies that reported criteria for surgery stated this. However, it was not possible to determine survival outcomes for patients who underwent surgery for a single brain metastasis followed by RT as this was not reported. Most studies that analysed the effect on survival of having a single versus multiple brain metastases, irrespective of other treatments, reported improved survival with a single metastasis. This suggests that patients who undergo surgery have a greater likelihood of increased survival at baseline. A valid comparison of different RT modalities should consider or control for factors that have a major impact on survival, an issue not possible to evaluate using the current evidence.

Further difficulties arose in relation to the multitude of different outcomes reported that could not be easily combined. For example, median, 6-month, 1 and 2-year survival rates were often reported but recurrence/regrowth at a treated site versus new lesions at new sites were often not clearly specified within treatment groups or time frames. Similarly, the definitions of neurologic death varied between studies. Only one study provided a robust, measurable definition of this while others relied on less precise features. Definitions of radiation necrosis were also variable, provided in only eight studies, each of which was different; three relied solely on various imaging features, one solely on clinical signs of bleeding and four on combined imaging features and clinical signs. Radiation necrosis and neurologic death are important endpoints being measured in current clinical trials and an assurance of similar definitions and measurements will greatly aid interpretation of these outcomes across studies. A possible solution to the diverse and variably-defined outcomes in studies would be for clinicians, researchers and patients to agree on a minimum required and consistently-defined outcome reporting set, as has been done for other diseases

such as rheumatoid arthritis, ulcerative colitis, and lung cancer.[45-47] Researchers have developed a process for selecting outcomes of interest to clinicians and patients, and deciding how these can be implemented in their respective settings.[48,49] A similar strategy for future studies of patients with melanoma brain metastases would be feasible.

Few studies reported whether the treatments resulted in relief of symptoms for symptomatic patients. Australian guidelines suggest that WBRT may be considered in a palliative setting for relief of symptoms, and there are many anecdotal reports of its value in this situation, but we found little reported evidence to support the effectiveness of this option.

Use of treatment groupings was a substantial limitation to interpretation, as many studies grouped together patients who received different treatment combinations. Ideally more uniform treatment groups should be used but this would require studies of much greater size to achieve adequate numbers within each group.

Conclusions

One randomised trial and many observational studies have reported survival outcomes for patients treated with RT for melanoma brain metastases. WBRT alone and SRS alone resulted in median survival times of about 4 and 8 months respectively. For patients who were selected to have surgery in addition to RT, there was a 5–7-month improvement in survival, however, this likely reflects the tendency to select patients with a better baseline prognosis relative to patients not offered surgery. While most studies included in this review were not optimal for determining the efficacy of an intervention, they provide the only evidence currently available. Given the improved efficacy of newer systemic therapies in the treatment of metastatic melanoma, RT alone today has a diminished role in the management of melanoma brain metastases, and large-scale trials or cohort studies of RT alone would be considered unethical. Therefore, this systematic review of the various forms of RT with or without surgery provides baseline estimates for measuring the incremental benefits of contemporary systemic therapies over RT with or without surgery in the treatment of patients with melanoma brain metastases.

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review

Electrochemotherapy for solid tumors: literature review and presentation of a novel endoscopic approach

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Background. Electrochemotherapy (ECT) is a minimally invasive and safe treatment gaining positive and long-lasting antitumoral results that are receiving the attention of the scientific community. It is a local treatment that combines the use of electroporation and the administration of cytotoxic drugs to induce cell death in the target tissue. ECT is largely used for the treatment of cutaneous and subcutaneous lesions, and good results have been reported for the treatment of deep visceral tumors. The latest literature review is provided. Moreover, in line with its development for the treatment of visceral tumors in this article, we describe a novel approach of ECT: endoscopic treatment of colorectal cancer. Endoscopic ECT application was combined with systemic chemotherapy in the treatment of obstructing rectal cancer without prospective surgery. A good response after ECT was described: concentric involvement of the rectum was reduced, and no stenosing lesions were detected.

Conclusions. Clinical studies have demonstrated that ECT is a very effective treatment for tumors of different histologic types and localizations. Endoscopic treatment for gastrointestinal cancer is an innovative application of ECT. The combination of systemic treatment and ECT was safe and highly effective in the treatment of colorectal cancer, especially when obstructive, giving the patient a significant gain in quality of life.

Key words: electrochemotherapy; colorectal cancer; endoscopy

Introduction

Electrochemotherapy (ECT) is a local ablative therapy based on electroporation, a physical approach for enhanced delivery of cytotoxic drugs into tumors. Electroporation is the application of short-intensity high-voltage electric pulses to tumors, where it creates transient pores in the cell membrane of tumor cells and consequently increases the permeability of the cell membrane to allow hydrophilic drugs to enter the cytoplasm and induce cell death.[1]

Electroporation can be reversible or irreversible. Irreversible electroporation is a nonthermal ablation procedure employing multiple short-term electrical pulses that irreversibly destroy cells in the application area; reversible electroporation, on the other hand, can induce apoptosis instead of necrotic cell death due to the action of cytotoxic drugs, which can result in a superior immune effect. The reversibility or irreversibility of electroporation mainly depends on two electric pulse characteristics: electric field strength and time pulse length. For reversible electroporation, pulses of 1000 V/cm intensity and 100 μsec duration are used. In irreversible electroporation, higher electric field strengths or time pulse lengths are commonly used.[2]

Several clinically approved drugs have been tested in preclinical studies, but bleomycin and cisplatin are currently the most suitable and most commonly used chemotherapeutic drugs in association with ECT. In ECT after drug administration, a short time interval is needed for anticancer drugs, either administered intravenously or intratumorally, to distribute in the tumors. Bleomycin causes multiple DNA breaks and cisplatin intra- and interstrand DNA bonds in tumor cells. The cytotoxic effect of antitumoral drugs in ECT is increased 1000-fold for bleomycin and 80-fold for cisplatin.[3]

For several years, ECT has received attention in the scientific community because it is a minimally invasive and safe treatment, gaining positive and long-lasting antitumoral results. Great experience has been collected in the literature in the treatment of cutaneous and subcutaneous lesions, and in recent years, the first experiences with ECT treatment in deep-seated tumors have been reported with promising results.[4]

This article aims to describe the technique and clinical applications of ECT as a review of the latest publications. The review focuses on the treatment results and indications of this interesting treatment option. Furthermore, a new technical advancement is reported in a case of an obstructing rectal cancer not indicated for radiotherapy, treated with systemic chemotherapy combined with ECT, gaining systemic and local response after 3 months of FOLFOXIRI and bevacizumab chemotherapy and a single ECT treatment conducted endoscopically.

ECT indications, contraindications

The indication for ECT should be made by clinicians after a multidisciplinary team discussion to better evaluate the localization, size, characteristics of the lesions, general conditions and expected survival of the patient to define the purpose of the treatment. The technique can be applied in a variety of malignant lesions; fields of applications of ECT may be classified into three groups: treatment of cutaneous and subcutaneous lesions, treatment of deep metastases located in liver, bone, renal cancer, soft tissues and treatment of primary tumors.[3] During patient selection, it is important to evaluate laboratory tests (number of platelets, coagulation, creatinine), cardiac, renal and lung function, body surface, and performance status (PS).

The choice of treatment modality is standardized for use in cutaneous and subcutaneous lesions.[6] Physicians have to accurately select, on the basis of current recommendations, the employed drug (bleomycin or cisplatin), delivery route (intratumoral or intravenous), type of anesthesia (local or general) and type of electrodes.

The ECT procedure may vary depending on the size and location of the lesions. A correlation between tumor size and effectiveness of ECT has been evaluated for cutaneous and subcutaneous tumors by Mali *et al.* on cutaneous tumors: ECT was less effective on tumors larger than 3 cm. In the case of large lesions, the multiple application and repositioning of electrodes needs to be considered.[7]

As a standardized procedure in the treatment of deep-seated tumors is lacking, it is difficult to design a general ECT protocol.

The time of response may vary depending on tumor characteristics and adopted techniques: for cutaneous tumors, the 2-month follow-up time is used as a reference for response, but for deep-seated tumors, it is difficult to establish a specific follow-up scheduled time after ECT treatment.

ECT is not indicated in pregnancy or lactation or in cases of allergy or hypersensitivity to bleomycin or cisplatin.

Literature review

The earliest *in vivo* studies on the efficacy of ECT were carried out in the early 1990s: ECT was proven to be very efficient in animal studies, and safety and dosage were assessed in phase I and II studies.[8,9] Several drugs commonly used in clinical settings were experimentally investigated, and the data supported the role of bleomycin, a hydrophilic molecule, as the drug of choice for ECT and demonstrated an increase in carboplatin and cisplatin cytotoxicity when used in ECT.[10]

Treatment of cutaneous and subcutaneous tumor nodules was one of the first effective applications of ECT.[5] The management of this tumor location often represents a challenge for clinicians, particularly when surgical excision is not possible or when lesions are radiotherapy-resistant.

Several trials confirmed the efficacy of ECT in the treatment of cutaneous and subcutaneous primary tumors or metastases from different malignancies (melanoma, basal cell carcinoma, squamous cell carcinoma, breast cancer, sarcomas): both bleomycin and cisplatin ECT were proven to be safe and well tolerated. Moreover, efficacy was excellent, reaching high objective response rates (ORs) and a high percentage of long-lasting responses. ECT was effective regardless of the histological type of tumor.[11]

The analysis of recent literature showed a wide variability according to the number, size and histological type of treated cutaneous and subcutaneous metastases.[12,13] Benevento *et al*. reported a 75.3% complete response (CR) and a 92.3% objective response rate (ORR) without intolerable side effects[14] in breast cancer patients. Campana *et al.* identified a subgroup of elderly breast cancer patients as the most responsive to ECT.[15] A recent review of the literature by a Swiss group confirmed ECT as a feasible, safe and easy-to-perform procedure; moreover, their analysis identified an OR rate for ECT across all the selected studies of 84.0%.[16] Borgognoni *et al*. found similar results in the treatment of cutaneous melanoma metastases and rare nonmelanoma skin cancer: the OR rate was 88.6% in melanoma and 91.7% in nonmelanoma tumors.[17] Caracò *et al*. reported a long-lasting response after ECT, with a mean duration of follow-up of 27.5 months for melanoma patients.[18] Other authors analyzed data on ECT treatment of cutaneous metastasis from head and neck (HN) cancer and noticed a significant correlation between response to ECT and tumor size: ECT seems more effective in small tumor nodules (< 3 cm).[19,20]

Because of the results obtained with ECT in cutaneous and lesions, many efforts have recently been made to translate the application of ECT into the treatment of non-superficial tumors. Preclinical *in vitro* studies were carried out on colorectal carcinoma cells, showing an increased cytotoxicity of bleomycin combined with electroporation.[21]

Bianchi *et al*. performed a prospective phase II trial to evaluate the safety and efficacy of ECT in the treatment of bone metastases.[22] Twenty-nine patients affected by painful bone metastases from various malignancies in different skeleton sites were treated: 27 patients obtained a partial response (PR) or stable disease (SD) after 3 months, 20 patients reported improvement of pain condition (>50%) and reduction of consumption of analgesic drugs, and no complications were observed during and after ECT treatment. These results demonstrate that ECT might be safe and feasible for the treatment of painful bone metastases.

Furthermore, in 2014, a pilot study was performed on 16 patients to evaluate the feasibility, safety and efficacy of intraoperative ECT in the treatment of colorectal liver metastasis: the technique was proven to be safe (no serious adverse events were observed) and effective (85% of patients with complete response and 15% of those with partial response).[23] A similar Italian study conducted in 2017 obtained comparable results.[24]

The long-term safety and effectiveness of ECT was recently confirmed in the treatment of colorectal liver metastases in the vicinity of major hepatic vessels and was thus unsuitable for surgery, radiofrequency or microwave ablation.[25,26]

Based on the encouraging clinical results obtained in the treatment of colorectal liver metastases, Djokic *et al*. conducted a prospective pilot study to evaluate the role of ECT in the treatment of hepatocellular carcinoma (HCC).[27] Ten patients resistant or not suitable for surgery or local ablative technique (TACE/RFA) were enrolled; they were treated with ECT during open surgery; bleomycin was the injected drug. Treatment was safe and well tolerated; at the first radiologic follow-up after 1 month, 88% of the lesions achieved a complete response (CR), and the others achieved a PR. The treatment was safe and effective even in tumors located in proximity to the hepatic or portal vessels and for tumors larger than 3 cm. ECT was shown to be a safe and effective treatment; it is minimally invasive with short hospitalization and good patient compliance; moreover, in selected cases, it may be considered a technique with curative intent; more studies need to be carried out to confirm these results. Recently, the first percutaneous ECT of HCC was performed.[28]

In 2018, Tarantino *et al*. published the results of the first study evaluating the safety and effectiveness of percutaneous ECT in the treatment of unresectable perihilar-cholangiocarcinoma (PHCCA).[29] Five patients were enrolled in the study: two patients had CR, and in one patient, a complete *restoration ad integrum* of liver parenchyma and high local efficacy without hepatic recurrence during follow-up were observed. These results confirmed the efficacy of ECT, even in combination with systemic chemotherapy. An Italian group of works recently obtained similar results.[30]

In the last 10 years, several studies have been conducted to evaluate the potential adjunctive role of ECT and IRE in patients affected by locally advanced pancreatic cancer, who currently have a poor prognosis, with a 5-year survival rate of approximately 12%.[31] Preclinical studies were performed to evaluate ECT's role: the results of an animal study conducted on 10 mice with orthotropic human pancreatic carcinoma suggested that ECT might allow increased drug delivery in tumor cells, increasing gemcitabine efficacy and potentially reducing local tumor recurrence.[32] IRE's role has been evaluated in clinical studies: results of a multicenter prospective trial published in 2014 conducted on 200 patients with pancreatic can-

cer demonstrated that the addition of irreversible electroporation (IRE) to systemic chemotherapy could prolong survival.[33] Irreversible laparotomic and laparoscopic electroporation in addition to systemic chemotherapy was performed in a total of 70 patients with locally advanced pancreatic cancer enrolled in a multicenter Asian study.[34] The results suggested that adding IRE to chemotherapy might provide a survival advantage. The necessity to evaluate the combination of IRE and multidrug systemic chemotherapy for the treatment of pancreatic cancer led to the creation of the American Hepato-Pancreato-Biliary Association (AHPBA) Pancreatic Registry. Holland *et al.* reported initial outcomes of the AHPBA registry on 152 patients. The median overall survival (OS) was 30.7 months, the median progression-free survival (PFS) was 22.8 months, and the median time to tumor progression (TTP) was 27.3 months. The combination of ECT and systemic chemotherapy for pancreatic cancer is a safe treatment with encouraging survival.[35]

The efficacy of ECT was evaluated in the treatment of refractory cases of vulvar cancer in a multicenter observational study carried out in five Italian centers.[36] Sixty-one patients were treated: a clinical response rate was obtained in 83.6% of cases, and the procedure was safe and well tolerated. Corrado *et al*. observed similar results in the palliative treatment of primary or recurrent vulvar cancer.[37] The overall response rate (ORR) was 80% after 1 month, 61.5% of patients were alive at the 6-month follow-up and 50% at 1 year. ECT may have a role in the management of vulvar cancer in palliative setting. ECT with bleomycin may be considered also for vulvar metastasis to reduce pain and bleeding.[38]

ECT may be used for the treatment of different histological types of cancer. Andresciani *et al.* described the application of ECT as a treatment option in local recurrences of renal cell carcinoma 2 years after radical nephrectomy: ECT was effective, without evidence of residual disease 6 months after the procedure.[1]

Endoscopic treatment for gastrointestinal cancer is a novel application of ECT. The first human phase I study in patients affected by advanced esophageal cancer was conducted in 2018: ECT treatment was well tolerated, and tumor regression was gained and endoscopically confirmed in all patients.[39]

A similar phase I clinical study was conducted by Hansen *et al.* to investigate the safety and efficacy of endoscopically delivered ECT in patients with colorectal tumors.[40] The results were encouraging: all the treated patients, elderly and with multiple comorbidities, were successfully treated and gained local complete or partial tumor response after one treatment; the procedure was well tolerated without relevant side effects.

Case report

A 61-year-old male patient affected by metastatic colorectal cancer was treated by ECT in January 2021. The study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained, also for the publication of the results. The patient had no known significant comorbidity; he was overweight and suffered from hypertension controlled with therapies. The diagnosis of cancer was obtained in November 2020. During the colonoscopy, a substenosing, ulcerated bleeding lesion was observed from 20 cm to 10 cm before the anal edge. The lesion was histologically proven to be poorly differentiated adenocarcinoma.

At the basal CT scan, multiple hepatic lesions and a single lung metastasis were described.

The low abdominal MRI, performed in December 2020 (Figure 1A), described a bulky pathological cancer mass extending from the distal part of the sigma to the rectum until 8 centimeters from the anal border. The rectum was concentrically involved by the tumor, and local nodes were increased in volume.

The radiotherapist rejected the possibility of combined chemoradiotherapy: local radiotherapy was not indicated because of the high risk of colorectal obstruction.

First-line systemic chemotherapy with fluorouracil, leucovorin, irinotecan and oxaliplatin (FOLFOXIRI) plus BEVACIZUMAB was started in December 2020.

Four cycles of chemotherapy were led between December 2020 and January 2021; therapy was well tolerated, and a dose reduction of 25% of IRINOTECAN was required for IV cycles of gastroenteric G3 toxicity therapy.

At the first abdominal MRI evaluation (Figure 1B), a good response was described. Concentrical involvement of the rectum was reduced, there were no stenosing lesions, and pathological local nodes were reduced in volume and number.

Local treatment with ECT was endoscopically conducted in the region of colorectal cancer by employing colonoscopy. One day before the treat-

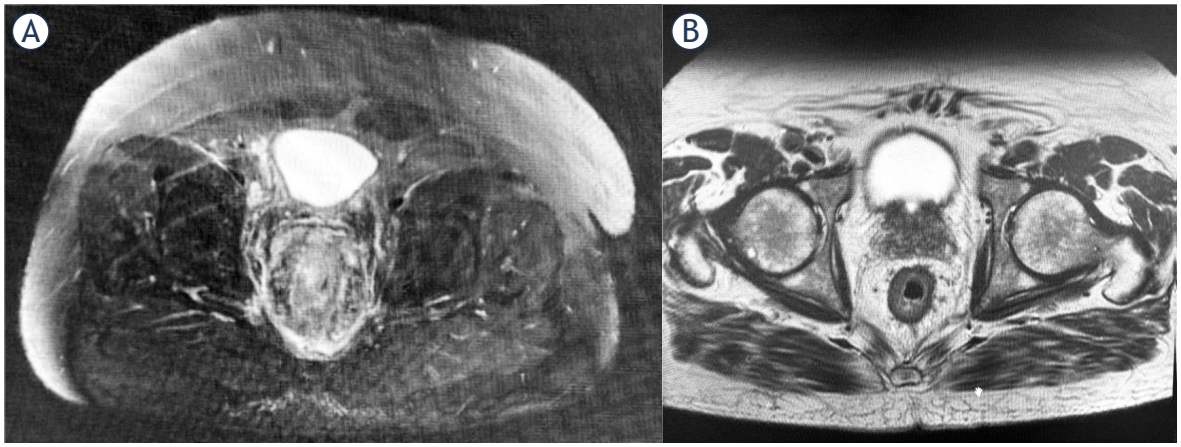

FIGURE 1. (A) NMR imaging before Electrochemotherapy (ECT) and **(B)** follow-up evaluation 4 weeks after the ECT session.

ment, physical examination, laboratory tests and cardiologic evaluation were carried out. Treatment was performed under general anesthesia (deep sedation with Propofol) with standard hemodynamic monitoring.

This procedure was conducted in the presence of a multidisciplinary team constituted by an oncologist, gastroenterologist, anesthetist and specialized nurse. The Cliniporator EPS-02 produced by IGEA was used. Software was used to optimize the placement of electrodes in the predefined area. Electric pulses were delivered by linear needle electrodes. The electrode used (Stinger E_L2_00_S4) has been developed by IGEA and is dedicated to laparoscopic/endoscopic approach, being equipped with a long connector cable (20 cm) and 4 expandable needle electrodes positioned at a fixed distance of 0.4 cm in a square geometry and parallel one to each other. Total needle electrode length is 4 cm, with an active part at the 2 cm extremity (Figure 2)

In accordance with European Standard Operating Procedures of Electrochemotherapy (ESOPE) guidelines, ECT was performed 8 minutes after the end of slow bleomycin intravenous infusion (bleomycin 15000 IU/m^2 of body surface area diluted in 100 cc of physiologic solution in 10 minutes).[5]

Rectosigmoidoscopy was performed, and then the electrode was endoscopically introduced to perform electroporation of the lesion (Figure 3). Five applications of the electric pulses were performed with repositioning of the electrode, in order to completely cover the lesion.

The duration of the procedure was 30 minutes. Antibiotic prophylaxis with cephalosporin was employed. The hospitalization time was 2 days.

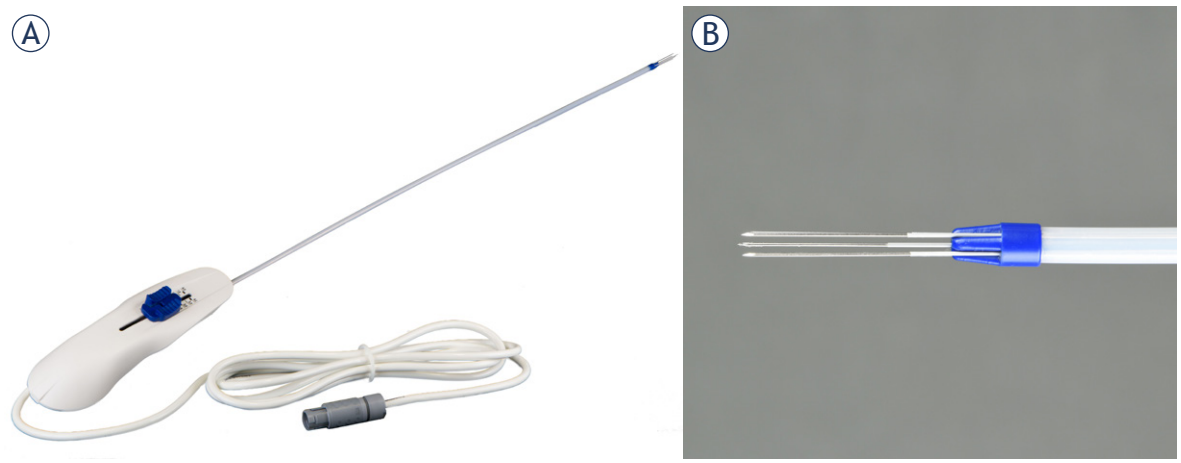

FIGURE 2. The new Stinger electrode. **(A)** The overall support with the connecting cable. **(B)** The needle expandable electrodes at the extremity.

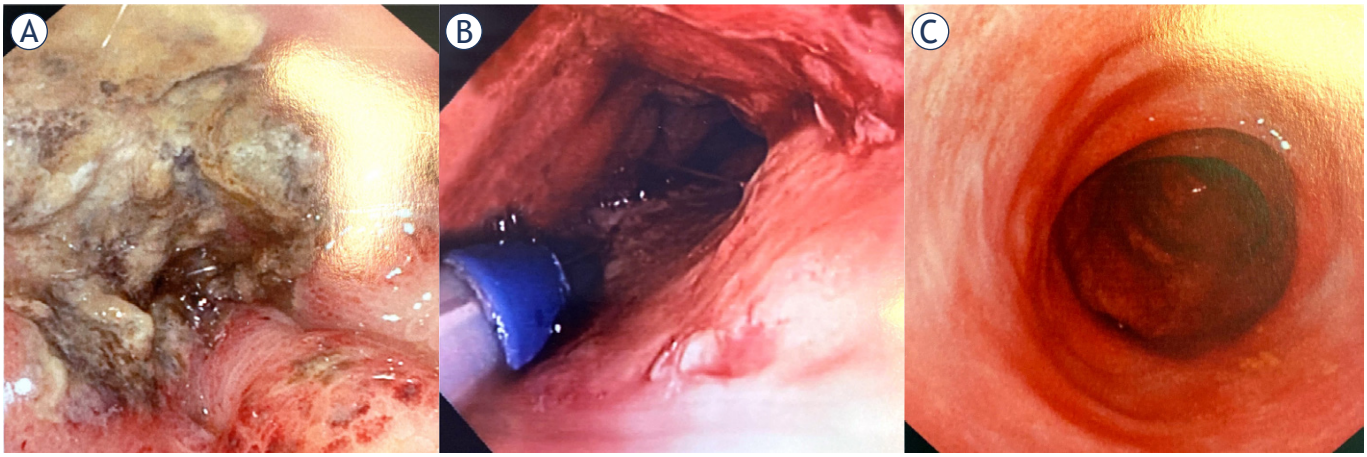

FIGURE 3. Electrochemotherapy (ECT) session. **(A)** before ECT, **(B)** electrode positioning, **(C)** at the 4-week follow-up.

The procedure was well tolerated, without side effects.

At the first colonoscopy performed 4 days after ECT treatment, tumor downstaging was confirmed, the endoscopic device could easily pass through the lesions, and all the colon tubes were studied. Moreover, at the total body CT performed 4 weeks after ECT treatment, lung and hepatic metastasis were reduced in volume (Figure 1B, 2C).

Systemic chemotherapy treatment was carried out after the procedure. After one month, no adverse events were reported; moreover, the patient reported a reduction in gastroenteric symptomatology and a subjective improvement in his well-being and quality of life.

Conclusions

ECT was the main topic of the present article. Literature data show the role of ECT as both curative and palliative treatment, improving quality of life of patients with different tumour types.

Moreover, the present article described our center's preliminary experience with ECT combined with standard systemic chemotherapy in the treatment of advanced colorectal adenocarcinoma not indicated for surgery or radiotherapy.

The combination of systemic treatment and ECT was highly effective and safe in the treatment of this tumor: a good local response was observed with a resolution of the local stenosis caused by cancer and an improvement in patient quality of life because of a reduction in gastroenteric symptoms.

To the best of our knowledge, at the time of writing this paper, no data concerning colorectal endoscopic ECT treatment are available. Preliminary results may be considered a proof of concept for future prospective studies that are needed to confirm these data.

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research article

Portal hypertension may influence the registration of hypointensity of small hepatocellular carcinoma in the hepatobiliary phase in gadoxetic acid MR

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Carla Caparroz and Alejandro Forner contributed equally and share the first authorship.

Background. The aim of the study was to analyze the association between the liver uptake of Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) in the hepatobiliary phase (HBP) in cirrhotic patients and the presence of clinically significant portal hypertension (CSPH), and how these features impact on hepatocellular carcinoma (HCC) detection in the HBP.

Patients and methods. Post-hoc analysis of a prospective cohort of 62 cirrhotic patients with newly US-detected nodule between 1–2 cm (study group). Twenty healthy subjects were used as control group. Qualitative and quantitative analysis of the liver contrast uptake in the HBP assessed by Relative Liver-Enhancement (RLE), Liver-Spleen (LSCR), Liver-Muscle (LMCR), and Liver-Kidney Contrast-Ratio (LKCR), Contrast Enhancement Index (CEI), and Hepatic Uptake (HUI), and biliary excretion, were registered. CSPH was confirmed invasively (HVPG > 10 mmHg) or by indirect parameters. The appearance of HCC at the HBP was analyzed.

Results. Nineteen patients (30.6%) did not have CSPH. In 41 patients (66.1%) the final diagnosis was HCC. All indices were significantly higher in the control group, indicating a more intense HBP liver signal intensity compared to patients with cirrhosis, even if the comparison was restricted to patients with no CSPH. CSPH was associated to a lower rate of HCC hypointensity in the HBP (51.9% *vs.* 85.7% without CSPH, p = 0.004).

Conclusions. Liver uptake of Gd-EOB-DTPA at the HBP is decreased in cirrhosis even if the liver function is minimally impaired and it falls down significantly in patients with CSPH compromising the recognition of hypointense lesions. This fact may represent a limitation for the detection of small HCC in patients with cirrhosis and CSPH.

Key words: liver cirrhosis; magnetic resonance imaging; hepatocellular carcinoma

Introduction

Portal hypertension (PH) is a clinical syndrome that often complicates cirrhosis. It is related to the increased hepatic resistance to portal blood flow through the liver because of the architectural disruption of the liver vascular anatomy caused by fibrosis and nodule formation.[1] An important step in the pathophysiology of PH is the dysfunction at the hepatic sinusoidal cells in response to different liver injuries in the early stages of the cirrhosis development.[1] PH is defined as clinically significant (CSPH) when the portal pressure increases above a critical threshold value of 10 mmHg.[2] Although under this value there are no complications related to PH (such as ascites or variceal bleeding), significant changes in the hepatic sinusoidal system are already present in cirrhotic patients with hepatic venous pressure gradient below 10 mmHg. Interestingly, development of CSPH is the well-established key event defining a clinically significant risk of hepatocellular carcinoma development.[3-5]

Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA; Primovist/Eovist®), also known as gadoxetic acid, has an early phase with distribution into the extracellular space similar to other gadolinium-based contrast agents, followed by the uptake into hepatocytes and excretion into bile during the hepatobiliary phase (HPB).[6-8] The uptake of Gd-EOB-DTPA by the hepatocytes during this delayed phase is impaired in chronic liver diseases and in recent years, there have been several attempts to evaluate the potential of magnetic resonance (MR) with Gd-EOB-DTPA as a reliable tool for liver dysfunction assessment.[9] Increased model for end-stage liver disease (MELD), bilirubin and indocyanine green clearance ratio at 15 minutes, and decreased cholesterol have been associated with suboptimal HBP[10-14], and the parenchymal enhancement among different HBP phases (at 5, 10, 15 and 20 minutes) is lower in Child-Pugh (CP) B and C compared to CP-A patients.[15,16] Also, MR with Gd-EOB-DTPA has been tested for preoperative identification of those patients with major contraindications for hepatectomy.[17-20]

A recent experimental study in animals evaluating the impact of PH in the pharmacokinetics of gadobenate dimeglumine (Gd-BOPTA) has shown that the clearance across sinusoidal membranes of contrast agents is modified by changes in portal flow rates and as a result, at a given perfused concentration, portal flow rates modified Gd-BOPTA hepatocyte concentrations.[21] However, the impact of these changes related to PH in the contrast uptake in the HBP and in the diagnostic accuracy of the MR with Gd-EOB-DTPA has not been extensively studied.

Accordingly, the aim of the present study is to evaluate the impact of CSPH and liver function impairment on the liver uptake of Gd-EOB-DTPA during the HBP, and consequently, how they may impact on hepatocellular carcinoma (HCC) detection in the HBP.

Patients and methods

Between July 2012 and October 2015, we prospectively included consecutive asymptomatic patients with Child-Pugh A-B cirrhosis with no previous history of hepatocellular carcinoma (HCC), in whom a new solitary well defined solid nodule between 1 and 2 cm was detected by screening ultrasonography (US). The Institutional Ethics Committee for Clinical Research approved the study and all patients provided written informed consent before enrollment. These patients were included in a prospective study conducted in our Unit.[22] Hepatic extracellular-contrast-enhanced MR (EC-MR) followed by Gd-EOB-DTPA MR were obtained in less than 1-month interval. The final HCC diagnosis was established by EC-MR according to the accepted non-invasive criteria[5], or by biopsy in lesions with atypical vascular profile. All these patients (n = 62) were considered the study group. We also included patients with healthy liver who were submitted to Gd-EOB-DTPA MR for the study of a solitary hepatocellular liver lesion (focal nodular hyperplasia, or hepatocellular adenoma) during the same period, and they were included as a control group for evaluating the liver uptake of Gd-EOB-DTPA during HBP (control group).

Diagnosis of CSPH in patients in the study group was established based on Baveno recommendations[2] including 1) hepatic venous pressure gradient (HVPG) greater than 10 mm Hg, or 2) by indirect clinical findings when: a) the hepatic elastography (Fibroscan®-Echosens, Paris, France) registered a value greater than 21 Kpa,[23] b) presence of venous shunts, and/or ascites at imaging techniques and/or 3) presence of gastroesophageal varices by upper endoscopy.

MR imaging

Two 1.5-T MR units were used: SIGNA HDxt, GE Healthcare and Magnetom AERA, Siemens

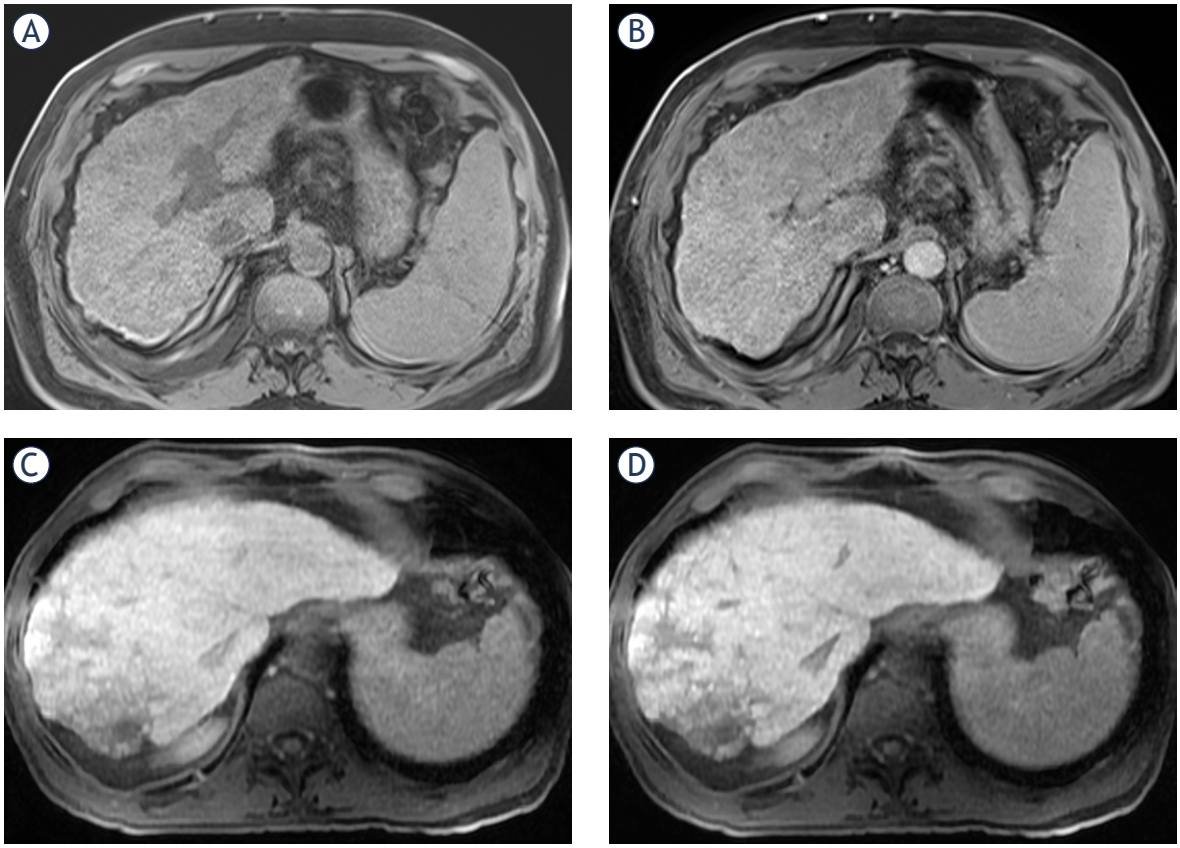

FIGURE 1. Non-adequate quality in the liver uptake of Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) in the 20 minutes hepatobiliary phase. **(A)** and **(B)**: Patient with liver cirrhosis Child-Pugh B and clinically significant portal hypertension. **(A)**: Baseline T1w-3D VIBE sequence. **(B)**: The liver showed poor or non-apparent contrast uptake compared to the liver before contrast injection. **(C)** and **(D)**: Patient with liver cirrhosis Child-Pugh A 5 points with no clinically significant portal hypertension. **(C)**: Baseline T1w-3D VIBE sequence. **(D)**: The liver parenchyma showed very heterogeneous uptake of the contrast media, especially in the periphery of the right hepatic lobe.

Medical Solutions. The sequence protocol for Gd-EOB-DTPA MR is detailed in supplemental material. Dynamic images were acquired after IV injection of gadoxetic acid 0,25 mmol/ml (Primovist; Bayer) at a dose of 0.1 ml/kg body weight at a rate of 1,5 ml/s followed by a 20 ml saline flush at the same rate. The arterial phase was acquired 7 s after the arrival of contrast medium in the aortic arch. Portal and transitional and HB phases were acquired 50 s, 90 s, 10 min and 20 min thereafter respectively. The 20 minutes HBP was not acquired in those patients without underlying chronic liver disease.

Evaluation of liver uptake of Gd-EOB-DTPA in the hepatobiliary phase

Qualitative analysis

All 10 and 20 minutes HBPs were reviewed by two independent radiologists (A.D. and J.R). The quality of the HBP was classified as 1) adequate, when the liver parenchyma showed signal intensity (SI), higher than the SI of the intrahepatic vessels, or 2) Non-adequate quality, when the SI in the liver parenchyma was non-superior to the SI in intrahepatic vessels (Figure 1).[24] Also, the biliary contrast excretion was evaluated qualitatively according to the extension (intrahepatic only and extrahepatic). Inadequate hepatobiliary contrast excretion was defined as the lack of contrast agent in the extrahepatic bile ducts in the hepatobiliary phase at 20 minutes

Quantitative analysis

Different MR-derived parameters focused to estimate the amount of Gd-EOB-DTPA liver uptake were calculated in the 10 and 20 minutes HBP. In all these indices, greater values mean more liver uptake of Gd-EOB-DTPA in the HBP. The quantita-

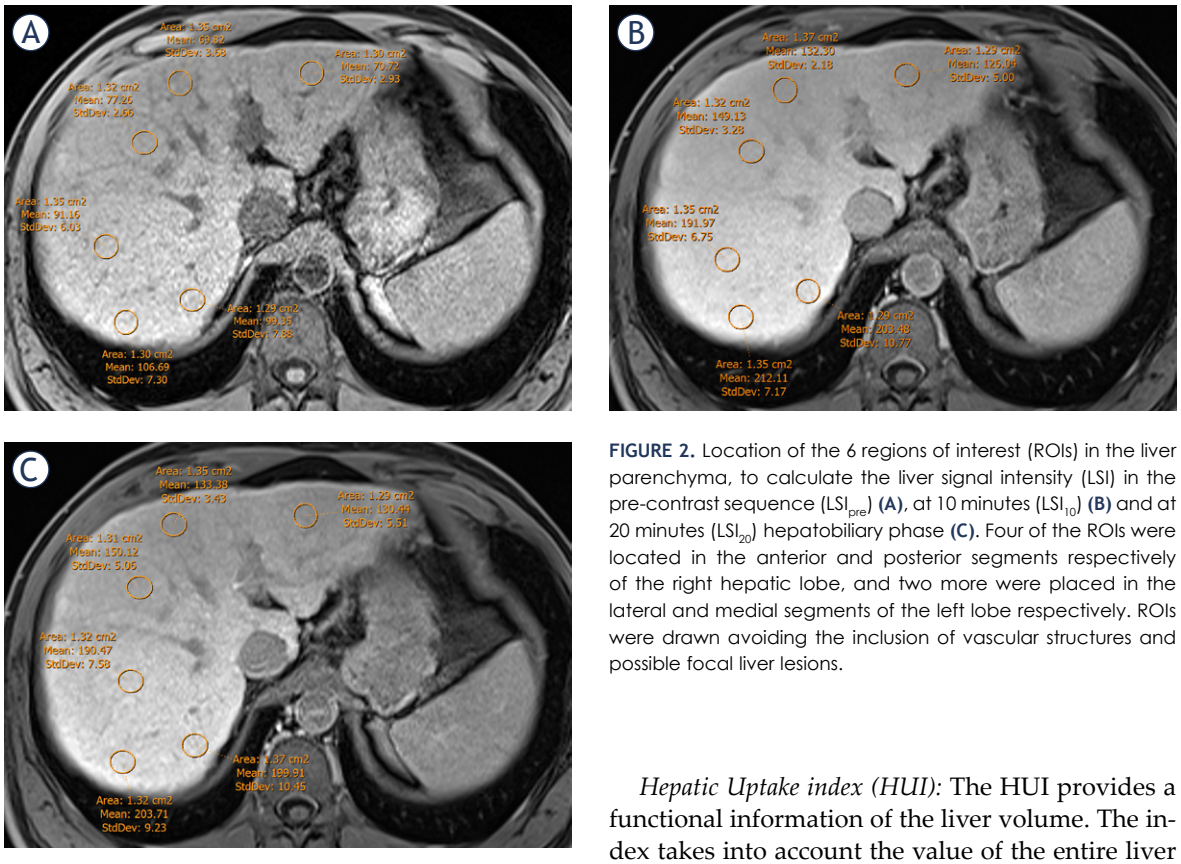

FIGURE 2. Location of the 6 regions of interest (ROIs) in the liver parenchyma, to calculate the liver signal intensity (LSI) in the pre-contrast sequence (LSI_{pre}) **(A)**, at 10 minutes (LSI_{10}) **(B)** and at 20 minutes (LSI_{20}) hepatobiliary phase **(C)**. Four of the ROIs were located in the anterior and posterior segments respectively of the right hepatic lobe, and two more were placed in the lateral and medial segments of the left lobe respectively. ROIs were drawn avoiding the inclusion of vascular structures and possible focal liver lesions.

tive assessments were done by C.C. (Figure 2). All formulas are summarized in Table 1 and described in detail in supplemental material.

Relative Liver Enhancement (RLE): RLE_{10} and RLE_{20} establish the relationship between the SI of the liver parenchyma in the 10 minutes (LSI_{10}) and the 20 minutes HBP (LSI_{20}), and the liver SI before contrast injection (LSI_{pre}).[25]

Liver to Spleen/muscle/Kidney Contrast Ratios: These indices determine the relationship between the SI of the liver and the SI of the spleen (LSCR), muscle (LMCR) and kidney (LKCR). To estimate the LSCR, an additional ROI was drawn on the spleen, over the same three images selected previously.[26] (Figure 3A) For LMCR, an additional ROI with an average area of 100 mm^2 was drawn on the right paraspinal muscle. (Figure 3B). Finally, for LKCR an additional ROI with an average area of 0,5 to 1 cm^2 was drawn on the upper pole of the right kidney (Figure 3C).

Contrast Enhancement Index (CEI): The CEI_{10} and CEI_{20} were calculated as a ratio between the liver-to-muscle SI ratio 10 and 20 minutes after contrast injection ($LMCR_{10}$ and $LMCR_{20}$) respectively, and the liver-to muscle SI ratio before contrast injection ($LMCR_{pre}$).[27]

Hepatic Uptake index (HUI): The HUI provides a functional information of the liver volume. The index takes into account the value of the entire liver volume (Vol_{Liver}), and the liver and spleen signal intensity and the formula is described in Table 1. Vol_{Liver} was calculated in the late venous T1WI sequence obtained 5 minutes after contrast injection. For this purpose, a free hand irregular-ROI was drawn delineating the liver contour in every one of the images (ALMA 3D Workstation®), defining a liver area by liver plane. The Vol_{Liver}, expressed in cm^3 was the sum of all the measured liver areas.

Analysis of the focal liver lesions

The imaging characteristics of the target lesion (TL) were independently registered in an electronic case report form by A.D. and J.R. They were blinded to final diagnosis, and imaging findings registered by each other. Any discrepancies during image analysis were solved by consensus discussion between the two investigators. Qualitative appearance of the lesion on delayed post-contrast sequences were registered as hypo, hyper or isointense lesions respect to the surrounding liver parenchyma.

Statistical analysis

Baseline characteristics of the patients were expressed as median and range or count and propor-

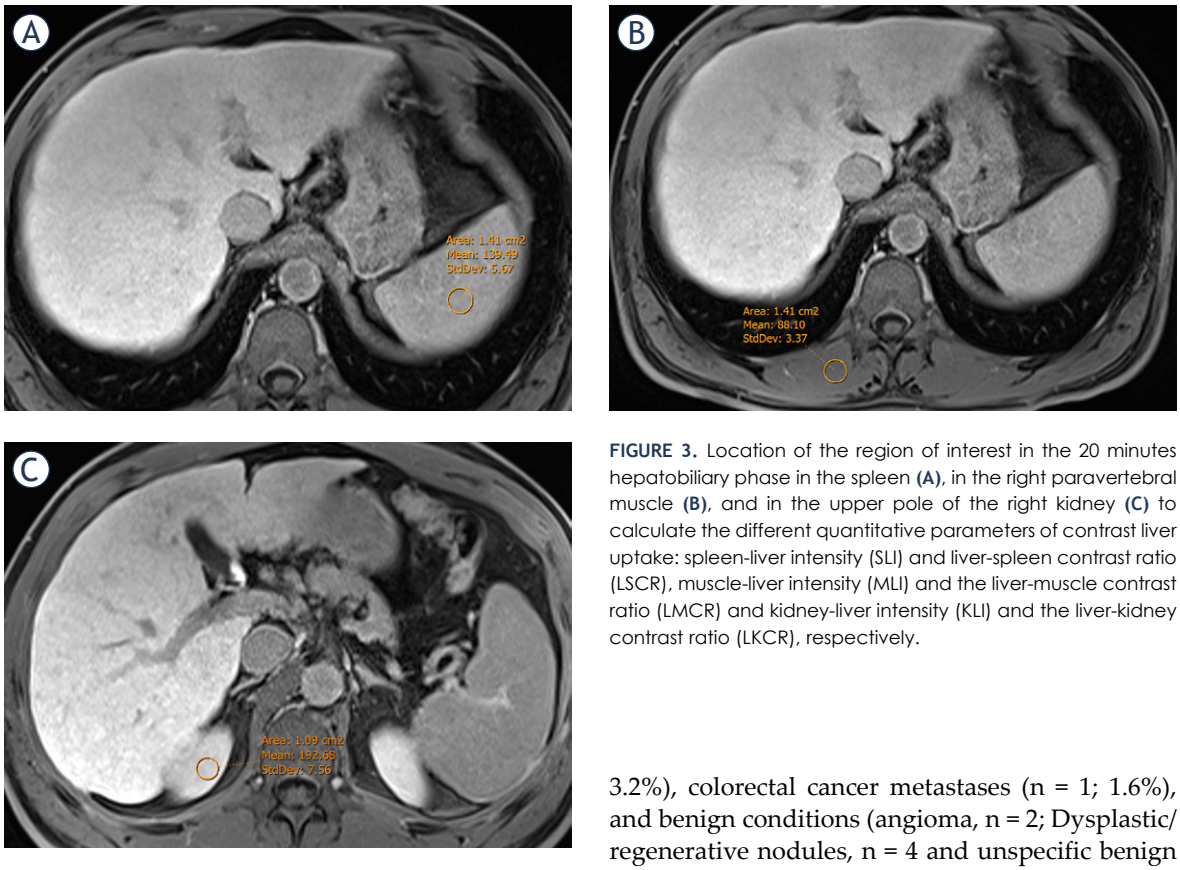

FIGURE 3. Location of the region of interest in the 20 minutes hepatobiliary phase in the spleen **(A)**, in the right paravertebral muscle **(B)**, and in the upper pole of the right kidney **(C)** to calculate the different quantitative parameters of contrast liver uptake: spleen-liver intensity (SLI) and liver-spleen contrast ratio (LSCR), muscle-liver intensity (MLI) and the liver-muscle contrast ratio (LMCR) and kidney-liver intensity (KLI) and the liver-kidney contrast ratio (LKCR), respectively.

tion. Comparisons were done by using the Student t test or the Mann-Whitney test for continuous variables and the chi-square test or Fisher-exact test for categorical variables. A *p* value of less than 0.05 was considered significant. Calculations were done with the SPSS package version 20 (SPSS, Inc., Chicago, IL)

Results

A total of 62 cirrhotic patients were included in the study group and their characteristics are summarized in supplemental Table 1. Fifty-three out of 62 (85.5%) were CP-A patients [A-5 points (n = 44) and A-6 points (n = 9)] and 9 (14.5%) were CP-B [B-7 points (n = 4), B-8 points (n = 4) and B-9 points (n = 1)]. Forty-three (69.4%) had CSPH: 11 confirmed by HVPG > 10 mm Hg (n = 11) and 32 by indirect signs. All patients with Child-Pugh score ≥ 6 (n = 18) had CSPH. The control group included 20 patients without chronic liver disease and normal liver function.

Final diagnosis of the 62 target lesions in the study group patients was: HCC (n = 41; 66.1%), intrahepatic cholangiocarcinoma (ICC) (n = 2; 3.2%), colorectal cancer metastases (n = 1; 1.6%), and benign conditions (angioma, n = 2; Dysplastic/regenerative nodules, n = 4 and unspecific benign lesions, n = 12).[22] The HBP at 20 minutes was not available in one patient with a final diagnosis of HCC. Thirty-one out of 41 HCC were diagnosed by non-invasive criteria (in 8 cases, pathology confirmation was also available) and by pathology in the remaining 10 cases.

Impact of liver function on the Gd-EOB-DTPA uptake in the HBP

Table 2 describes the different quantitative parameters evaluating the contrast liver uptake in the 10 and 20 minutes HBP considering the degree of liver function impairment according to Child-Pugh classification. All quantitative indices were significantly higher in CP-A patients compared to CP-B. We also focused our analysis in those CP-A patients comparing between 5 and 6 points: Except in LMCR, all indices were significantly higher in CP-A 5 points patients.

Impact of CSPH on the Gd-EOB-DTPA liver uptake in the HBP

We further evaluated the impact of CSPH on the liver enhancement in the HBPs (Table 3). All indices except RLE at 20 minutes and CEI were signifi-

TABLE 1. Formulas used for qualitative assessment of liver uptake of Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) in the hepatobiliary phase (HPB)

	Formula	Variables definition
Relative Liver Enhancement (RLE)	$RLE10 = \frac{(LSI10 - LSIPRE)}{LSIPRE}$ $RLE20 = \frac{(LSI20 - LSIPRE)}{LSIPRE}$	RLE_{10} and RLE_{20}: RLE at 10- and 20-min HBP LSI_{pre}, LSI_{10} and LSI_{20}: Liver signal intensity pre-contrast, at 10- and 20-min HBP, respectively
Liver to Spleen Contrast Ratio (LSCR)	$LSCRpre = \frac{LSIPRE}{SSIPRE}$ $LSCR10 = \frac{LSI10}{SSI10}$ $LSCR20 = \frac{LSI20}{SSI20}$	$LSCR_{pre}$, $LSCR_{10}$ and $LSCR_{20r}$: LSCR pre-contrast, at 10- and 20- min HBP, respectively LSI_{pre}, LSI_{10} and LSI_{20}: Liver signal intensity pre-contrast, at 10- and 20- min HBP, respectively SSI_{pre}, SSI_{10} and SSI_{20}: Spleen signal intensity pre-contrast, at 10- and 20- min HBP, respectively
Liver to muscle Contrast Ratio (LMCR)	$LMCRpre = \frac{LSIPRE}{MSIPRE}$ $LMCR10 = \frac{LSI10}{MSI10}$ $LMCR20 = \frac{LSI20}{MSI20}$	$LMCR_{pre}$, $LMCR_{10}$ and $LMCR_{20r}$: LMCR pre-contrast, at 10- and 20- min HBP, respectively LSI_{pre}, LSI_{10} and LSI_{20}: Liver signal intensity pre-contrast, at 10- and 20- min HBP, respectively MSI_{pre}, MSI_{10} and MSI_{20}: Muscle signal intensity pre-contrast, at 10- and 20- min HBP, respectively
Liver to Kidney Contrast Ratios (LKCR)	$LKCRpre = \frac{LSIPRE}{KSIPRE}$ $LKCR10 = \frac{LSI10}{KSI10}$ $LKCR20 = \frac{LSI20}{KSI20}$	$LKCR_{pre}$, $LKCR_{10}$ and $LKCR_{20r}$: LKCR pre-contrast, at 10- and 20- min HBP, respectively LSI_{pre}, LSI_{10} and LSI_{20}: Liver signal intensity pre-contrast, at 10- and 20- min HBP, respectively KSI_{pre}, KSI_{10} and KSI_{20}: Kidney signal intensity pre-contrast, at 10- and 20- min HBP, respectively
Contrast Enhancement Index (CEI)	$CEI10 = \frac{LMCR10}{LMCRPRE}$ $CEI20 = \frac{LMCR20}{LMCRPRE}$	- $LMCR_{pre}$, $LMCR_{10}$ and $LMCR_{20r}$: LMCR pre-contrast, at 10- and 20- min HBP, respectively - CEI_{10} and CEI_{20}: Contrast Enhancement Index at 10- and 20- min HBP, respectively
Hepatic Uptake index (HUI)	$HUI_{10}=VOL_{LIVER}\left(\frac{LSI10}{SSI10}\right)$-1 $HUI_{20}=VOL_{LIVER}\left(\frac{LSI20}{SSI20}\right)$-1	- HUI_{10} and HUI_{20}: Hepatic Uptake index at 10- and 20- min HBP, respectively LSI_{10} and LSI_{20}: Liver signal intensity at 10- and 20- min HBP, respectively SSI_{10} and SSI_{20}: Spleen signal intensity at 10- and 20- min HBP, respectively

cantly higher in absence of CSPH. To confirm the impact of CSPH irrespectively of liver function, we evaluated those patients with well-preserved liver function (CP-A 5 points) and in them, CSPH impacted in the liver Gd-EOB-DTPA uptake since LSCR, LMCR and LKCR were significantly higher in those patients without CSPH. Finally, we compared the liver contrast uptake in patients with very well-preserved liver function (all CP-A 5 points and those without CSPH) and patients with normal liver (control group B) and all scores were significantly higher in patients with healthy liver (Table 4).

Gd-EOB-DTPA liver uptake through the different HBP (10 and 20 minutes)

The quantitative assessment is exposed in supplemental Table 2. All parameters that quantify the liver contrast uptake were significantly higher at 20 minutes compared to at 10 minutes. These differences were maintained in CP-A and CP-A 5 points patients. The calculations were not done in Child-Pugh B due to low number of patients.

Impact of CSPH over the biliary excretion of Gd-EOB-DTPA in the HBP 20 minutes

In the 49 patients with adequate HBP at 20 minutes, the biliary excretion of the contrast media arrived to the extrahepatic bile duct (n = 20) and the intestinal lumen (n = 28). Contrarily, only in 6 out 12 cases with poor HBP quality, the biliary excretion was present in the extrahepatic bile duct ($p < 0.001$). In all patients without CSPH (n = 18), the biliary excretion arrived to extrahepatic bile duct. Contrarily, in 7 out of 43 patients with CSPH (16.3%), the biliary excretion was not identified in the extrahepatic biliary tree.

Impact of the quality of the HBP and the presence of CSPH in the registration of hypointense HCC lesions in HBP

In 49 out of 62 MR studies (79%), the HBP was categorized as adequate, in 12 (19.4%) non-adequate an in one patient (1.6%), the 20 minutes HBP was not available. Six out of 12 patients with non-ade-

TABLE 2. Quantitative assessment of liver uptake of Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) during the hepatobiliary phase (HPB) at 10 and 20 minutes considering the degree of liver function impairment according to Child-Pugh classification. Variables described as median and interquartile range

INDEX	Study group	Child-Pugh A	Child-Pugh B	P value	Child-Pugh A-5 points	Child-Pugh A-6 points + B	P value
N	62	53	9		44	18	
RLE_{10}	0.65 [0.49-0.75]	0.68 [0.51-0.81]	0.41 [0.30-0.52]	<0.001	0.68 [0.52-0.82]	0.50 [0.40-0.58]	0.005
RLE_{20}	0.63 [0.51-0.78]	0.67 [0.55-0.82]	0.41 [0.30-0.52]	<0.001	0.68 [0.56-0.83]	0.52 [0.40-0.61]	0.006
$LSCR_{10}$	1.32 [1.17-1.55]	1.35 [1.11-1.59]	1.20 [0.99-1.28]	0.016	1.43 [1.19-1.61]	1.20 [1,11-1.28]	0.001
$LSCR_{20}$	1.48 [1.23-1.71]	1.51 [1.30-1.80]	1.20 [1.01-1.43]	0.006	1.54 [1.32-1.81]	1.25 [1.17-1.53]	0.008
$LMCR_{10}$	2.14 [1.78-2.51]	2.16 [1.81-2.62]	1.78 [1.52-2.13]	0.030	2.26 [1.81-2.71]	2.08 [1.73-2.16]	NS
$LMCR_{20}$	2.25 [1.86-2.63]	2.29 [1.89-2.69]	1.79 [1.52-2.23]	0.030	2.34 [1.88-2.70]	2.05 [1.75-2.31]	NS
$LKCR_{10}$	1.02 [0.85-1.17]	1.04 [0.89-1.21]	0.83 [0.74-0.90]	0.005	1.06 [0.91-1.25]	0.87 [0.76-1.05]	0.008
$LKCR_{20}$	1.14 [0.90-1.13]	1.19 [0.96-1.33]	0.89 [0.80-0.96]	0.009	1.20 [0.96-1.38]	0.91 [0.81-1.22]	0.012
CEI_{10}	1.39 [1.27-1.57]	1.43 [1.34-1.1.58]	1.26 [1.22-1.32]	0.006	1.47 [1.34-1.58]	1.33 [1.25-1.38]	0.003
CEI_{20}	1.43 [1.30-1.61]	1.45 [1.35-1.63]	1.32 [1.19-1.37]	0.007	1.50 [1.39-1.66]	1.35 [1.19-1.42]	0.001
HUI_{10}	407.3 [223.7-640.9]	486.2 [235.3-845.4]	312.4 [-3.31-400]	0.022	522.4 [284.9-1036.4]	265.7 [130.5-383.7]	<0.001
HUI_{20}	660.5 [301.5-956.5]	697.8 [367.1-1028.9]	265 [72.5-620.4]	0.009	720.1 [450.5-1062.4]	327.2 [198.1-772.2]	0.004

CEI10 and CEI20 = contrast enhancement index at 10-20 min; HUI = hepatic uptake index; LKCR10 and LKCR20 = liver-kidney contrast ratio at 10-20 min; LMCR10 and LMCR20 = liver-muscle contrast ratio at 10-20 min; LSCR10 and LSCR20 = liver-spleen contrast ratio at 10-20 min; N = number; NS = non-significant; RLE10 and RLE20 = relative liver enhancement at 10-20

quate HBP at 20 minutes were CP-B patients and all of them had CSPH. Contrarily, in all 19 patients without CSPH, the HBP was classified as adequate. Table 5 describes the different quantitative parameters evaluating the Gd-EOB-DTPA liver uptake in the 20 minutes HBP considering the quality of the HBP according to the subjective assessment. All indices were significantly higher in those studies classified as having an adequate HBP compared to those categorized as non-adequate. We further evaluated the impact of the quality of the HBP in the registration of the signal intensity of HCC lesions related to the surrounding liver parenchyma. At the 20 minutes HBP, 26 out of 41 HCC nodules (63.4%) were hypointense, 8 (19.5%) isointense, 6 hyperintense (14,6%) and 1 HCC (2.4%) was heterogenous. In the 49 patients with an adequate Gd-EOB-DTPA liver uptake, 25 out of 32 HCC nodules (78.1%) were hypointense. Contrarily, in the 12 patients with non-adequate Gd-EOB-DTPA liver uptake, one of the 9 nodules diagnosed as HCC were hypointense ($p<0.001$). We further compared the appearance of HCC in the HBP according to the presence of CSPH. In the 19 patients without CSPH, 12 out of 14 HCCs (85.7%) were hypointense. On the other hand, only 14 out of 27 HCCs (51.9%) in the 43 patients with CSPH appeared as hypointense ($p = 0.044$).

Discussion

The results of our study show that the presence of CSPH in cirrhotic patients determines an impairment of the liver Gd-EOB-DTPA uptake reflected by significant lower values by almost all quantitative indices evaluated. In addition, the patients with healthy liver displayed more intense liver contrast uptake during the 10 minutes HBP, even when they were compared with cirrhotic patients with very well-preserved liver function defined as Child-Pugh A 5 points and absence of CSPH. Very interestingly, the rate of hypointense HCC in the HBP is significantly lower in those patients with CSPH, having an impact on HCC detection by MR with Gd-EOB-DTPA. Our findings are clinically relevant since cirrhotic patients who present CSPH and impaired liver function are those at high risk of HCC development in whom an early diagnosis and accurate tumor staging are critical. This fact represents a severe limitation for the detection of new HCC nodules in cirrhotic patients when dynamic sequences are skipped in the abbreviated MRI protocol for the HCC screening.[28-31]

There are several hypotheses that could justify the suboptimal HBP in the cirrhotic liver. The potential decrease in the number of hepatocytes due to the increase of fibrous tissue in the cirrhotic

TABLE 3. Quantitative assessment of liver uptake of Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) during hepatobiliary phase (HPB) at 10 and 20 minutes in the study group considering the presence of clinically significant portal hypertension (CSPH). On the left, including all study cohort and on the right, considering only cirrhotic patients with preserved liver function (Child-Pugh A 5 points). Variables described as median and interquartile range

INDEX	No CSPH (all CP A-5 points)	CSPH	P value	No CSPH (all CP A-5 points)	CP A-5 points and CSPH	P value
N	19	43		19	25	
RLE_{10}	0.73 [0.52-0.89]	0.57 [0.47-0.69]	0.027	0.73 [0.52-0.89]	0.65 [0.51-0.77]	NS
RLE_{20}	0.73 [0.56-0.85]	0.59 [0.47-0.74]	NS	0.73 [0.56-0.85]	0.66 [0.54-0.77]	NS
$LSCR_{10}$	1.54 [1.37-1.67]	1.23 [1.15-1.39]	< 0.001	1.54 [1.37-1.67]	1.29 [1,15-1.51]	0.014
$LSCR_{20}$	1.68 [1.53-1.84]	1.35 [1.20-1.57]	0.003	1.68 [1.53-1.84]	1.37 [1.26-1.74]	0.036
$LMCR_{10}$	2.41 [2.12-2.83]	2.06 [1.75-2.45]	0.006	2.41 [2.12-2.83]	1.88 [1.76-2.50]	0.034
$LMCR_{20}$	2.49 [2.24-2.96]	1.95 [1.77-2.39]	0.005	2.49 [2.24-2.96]	1.92 [1.78-2.6]	0.036
$LKCR_{10}$	1.15 [0.98-1.34]	0.93 [0.81-1.13]	0.001	1.15 [0.98-1.34]	1.01 [0.84-1.21]	0.032
$LKCR_{20}$	1.25 [1.16-1.43]	0.98 [0.88-1.28]	0.001	1.25 [1.16-1.43]	1.05 [0.88-1.03]	0.017
CEI_{10}	1.46 [1.34-1.59]	1.36 [1.26-1.52]	NS	1.46 [1.34-1.59]	1.47 [1.31-1.58]	NS
CEI_{20}	1.50 [1.38-1.71]	1.41 [1.24-1.54]	NS	1.50 [1.38-1.71]	1.48 [1.33-1.65]	NS
HUI_{10}	609.7 [501.4-970.3]	327.8 [191.7-511.8]	0.004	609.7 [501.4-970.3]	411.4 [223.4-1210.7]	NS
HUI_{20}	803.6 [678.7-1091.2]	450.5 [274.8-864.4]	0.033	803.6 [678.7-1091.2]	569.0 [341.8-987.1]	NS

CEI10 and CEI20 = contrast enhancement index at 10-20 min; HUI = hepatic uptake index; LKCR10 and LKCR20 = liver-kidney contrast ratio at 10-20 min; LMCR10 and LMCR20 = liver-muscle contrast ratio at 10-20 min; LSCR10 and LSCR20 = liver-spleen contrast ratio at 10-20 min; N = number; NS = non-significant; RLE10 and RLE20 = relative liver enhancement at 10-20 min

TABLE 4. Quantitative assessment of liver uptake of Gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) during hepatobiliary phase (HPB) at 10 minutes in patients with normal liver (control group) compared with all Child-Pugh (CP) A 5 points patients (left panel) and with patients Child-Pugh A 5 points patients without clinically significant portal hypertension (CSPH). Variables described as median and interquartile range

INDEX	Control group	Child-Pugh A-5 points	P value	Control group	No CSPH (all CP A-5 points)	P value
N	20	44		20	19	
RLE_{10}	1.06 [0.82-2.16]	0.68 [0.52-0.82]	< 0.001	1.06 [0.82-2.16]	0.73 [0.52-0.89]	< 0.001
$LSCR_{10}$	2,27 [2.06-2.88]	1.43 [1.19-1.61]	< 0.001	2,27 [2.06-2.88]	1.54 [1.37-1.67]	< 0.001
$LMCR_{10}$	3.11 [2.90-3.55]	2.26 [1.81-2.71]	< 0.001	3.11 [2.90-3.55]	2.41 [2.12-2.83]	< 0.001
$LKCR_{10}$	1.82 [1.51-1.99]	1.06 [0.91-1.25]	< 0.001	1.82 [1.51-1.99]	1.15 [0.98-1.34]	< 0.001
CEI_{10}	1.71 [1.55-1.85]	1.47 [1.34-1.58]	0.001	1.71 [1.55-1.85]	1.46 [1.34-1.59]	0.007
HUI_{10}	1449.6 [1259-1717.7]	522.4 [284.9-1036.4]	< 0.001	1449.6 [1259-1717.7]	609.7 [501.4-970.3]	< 0.001

CEI10 = contrast enhancement index at 10 min; HUI = hepatic uptake index; LKCR10 = liver-kidney contrast ratio at 10 min; LMCR10 = liver-muscle contrast ratio at 10 min; LSCR10 = liver-spleen contrast ratio at 10 min; N = number; RLE10 = relative liver enhancement at 10 min

liver may have a role[10,32] and also, the eventual alteration of the Gd-EOB-DTPA transport system in the hepatocellular membrane, with decrease of the expression of the organic anion transporting polypeptides (OATP1B1 and OATP1B3) and/or the increase of the multidrug resistance protein MRP2 expression.[6,32,33] Furthermore, structural and biochemical changes at the sinusoidal system may also contribute to a suboptimal HBP in the MR with Gd-EOB-DTPA, as shown in the sinusoidal obstruction syndrome (SOS), characterized by sinusoidal congestion and dilatation due to detachment of the cellular endothelium that obstructs the sinusoidal fenestrations in the centrilobular space, associated with hepatocellular necrosis and perisinusoidal fibrosis.[34,35]

To our knowledge, there are few studies evaluating the impact of PH in the Gd-EOB-DTPA MR.

TABLE 5. Quantitative parameters evaluating the liver uptake in the 20 minutes hepatobiliary phase (HPB) of Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) according to the quality of the HBP. In one patient the HBP at 20 minutes was not available (n = 61). Variables described as median and interquartile range

INDEX	Qualitative assessment adequate	Qualitative assessment Non-adequate	P value
N	49	12	
RLE_{20}	0.68 [0.57-0.83]	0.40 [0.31-0.51]	< 0.001
$LSCR_{20}$	1.53 [1.34-1.78]	1.19 [1.06-1.22]	< 0.001
$LMCR_{20}$	2.34 [1.88-2.78]	1.91 [1.50-2.11]	0.002
$LKCR_{20}$	1.20 [0.95-1.35]	0.89 [0.78-0.98]	0.001
CEI_{20}	1.23 [1.09-1.32]	1.00 [0.90-1.10]	0.001
HUI_{20}	744.1 [444.8-1024.1]	251.5 [65-4-331.0]	< 0.001

CEI10 and CEI20 = contrast enhancement index at 10-20 min; HUI = hepatic uptake index; LKCR10 and LKCR20 = liver-kidney contrast ratio at 10-20 min; LMCR10 and LMCR20 = liver-muscle contrast ratio at 10-20 min; LSCR10 and LSCR20 = liver-spleen contrast ratio at 10-20 min; N = number; RLE10 and RLE20 = relative liver enhancement at 10-20 min

Asenbaum *et al.*[36] included in a retrospective study 178 patients with chronic liver disease without superimposed HCC, 109 (61.2%) with CSPH. The authors demonstrated an inverse correlation between HVPG and RLE (r2 = 0.18, $p< 0.0001$), findings in line with our results. Regrettably, in this study the authors did not include a control group and thus, were not able to demonstrate the worse contrast uptake in the HBP in cirrhotics without CSPH compared with healthy liver patients. In addition, in our study the quantitative analysis of the contrast uptake was done not only by the measurement of RLE, but also by the determination of contrast enhancement index, hepatic uptake index, and liver to spleen, muscle and kidney contrast indices, thus confirming the impact of CSPH in the Gd-EOB-DTPA hepatocyte uptake. More recently, Hectors *et al.* conducted a prospective study with 35 patients with chronic liver disease who underwent HVPG measurements and dynamic Gd-EOB-DTPA MR. Twenty-one (60%) patients had PH, of whom 9 had CSPH, and the authors report a statistically significant decrease of liver contrast uptake in presence of CSPH.[37]

A very relevant finding of our study is the unexpected high rate of HCC nodules that were hyper or isointense (36.6%) compared to the surrounding liver parenchyma in the HBP, since previous studies have described that less than 15% of HCC nodules were not hypointense in the HBP.[8,28,38-40] Interestingly, this rate is significantly higher in those patients with CSPH (48.1%) compared to those without (14.3%). This finding is supported by the poorer contrast uptake of the non-tumoral liver parenchyma in those patients with CSPH. Consequently, the diagnostic capacity of Gd-EOB-DTPA is significantly impaired in those patients at higher risk of HCC and thus, in higher need of properly establishing if a hepatic nodule corresponds to a malignant focus or not. According to our results, portal pressure determines the target population for the optimal use of Gd-EOB-DTPA MR in patients with chronic liver disease, and those patients with no CSPH potentially candidates to resection in case an early HCC is diagnosed may benefit most from Gd-EOB-DTPA MR.

Our study has some limitations. First, it includes a small number of patients and the number of patients with no CSPH or with impaired liver function was relatively low. Finally, it could be argued that the determination of CSPH was done invasively in 11 out of 43 cases. However, we applied a very stringent, internationally validated non-invasive criteria based on available evidence, which minimizes a potential misclassification. Finally, we did not conduct T1 relaxation time measurements at HBP, which has been suggested as an accurate approach for evaluating liver function.[41,42]

In conclusion, our study shows that the liver uptake of Gd-EOB-DTPA at the HBP is impaired in cirrhosis compared to healthy livers regardless the degree of liver function impairment. Even in patients with compensated cirrhosis categorized as CP-A 5 points, the liver contrast uptake is impaired when CSPH is present. This limits the ability to register hypointensity in the HBP and thus, hampers the detection capacity of HCC when using MR with organ-specific contrast and the dynamic sequences are skipped of the MRI protocol for HCC screening.

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research article

Early isolated subarachnoid hemorrhage versus hemorrhagic infarction in cerebral venous thrombosis

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Background. Cerebral venous thrombosis (CVT) is a rare cerebral vascular disease, the presentation of which is highly variable clinically and radiologically. A recent study demonstrated that isolated subarachnoid hemorrhage (iSAH) in CVT is not as rare as thought previously and may have a good prognostic significance. Hemorrhagic venous infarction, however, is an indicator of an unfavorable outcome. We therefore hypothesized that patients who initially suffered iSAH would have a better clinical outcome than those who suffered hemorrhagic cerebral infarction.

Patients and methods. We selected patients hospitalized due to CVT, who presented either with isolated SAH or cerebral hemorrhagic infarction at admission or during the following 24 hours: 23 (10 men) aged 22–73 years. The data were extracted from hospital admission records, our computer data system, and the hospital radiological database.

Results. The iSAH group consisted of 8 (6 men) aged 49.3 ± 16.2 and the hemorrhagic infarction group included 15 (4 men) aged 47.9 ± 16.8. Despite having a significantly greater number of thrombosed venous sinuses/deep veins (Mann-Whitney Rank Sum Test, p = 0.002), the isolated SAH group had a significantly better outcome on its modified Rankin Score (mRs) than the hemorrhagic infarction group (Mann-Whitney Rank Sum Test, p = 0.026). Additional variables of significant impact were edema formation (p = 0.004) and sulcal obliteration (p = 0.014).

Conclusions. The patients who suffer iSAH initially had a significantly better outcome prognosis than the hemorrhagic infarction patients, despite the greater number of thrombosed sinuses/veins in the iSAH group. A possible explanation might include patent superficial cerebral communicating veins.

Key words; cerebral venous thrombosis; subarachnoid hemorrhage; hemorrhagic brain infarction; superficial communicating veins

Introduction

Cerebral venous thrombosis (CVT) is a rare cerebral vascular disease that represents a minor proportion of all strokes. A recent Dutch multicentric study revealed an incidence of 1.3 per 100,000 adults.[1] The presentation of CVT is highly variable, not only clinically but also radiologically.[2,3] Improvement in imaging techniques has enabled the identification of less obvious CVT cases; the incidence of CVT is increasing.[4] Symptoms and signs depend not only on the location but also on the rate of thrombus progression. Involvement of multiple venous sinuses/veins may cause a wide variety of symptoms, e.g., headache, seizures, focal deficits, and disturbed consciousness, which may even proceed to coma.

The wide spectrum of possible radiological presentations ranges from brain edema accompanying venous sinus or cortical vein thrombosis to venous infarction, which may be hemorrhagic and accompanied by SAH and hematocephalus. Isolated SAH (iSAH) may also appear without venous infarction.[5,6] Superficial CVT manifestations such as cortical or perimesencephalic SAH secondary to cerebral venous thrombosis are considered to be very rare.[7,8] A recent study, however, demonstrated that 33 CVT patients in a series of 332 presented with SAH and 22 of those with iSAH mostly accompanied by thrombosis in cortical veins, lateral sinus, and/or superior sagittal sinus. The outcome was favorable in all but one patient, who died of pulmonary embolism.[9] In contrast, a prospective and retrospective Pakistani and Middle East study revealed hemorrhagic infarction to be the most significant feature of a long-term unfavorable outcome.[10] Another study from Pakistan revealed hemorrhagic brain infarction to be usually associated with multiple venous sinus/vein occlusions. Superior sagittal, transverse, and sigmoid sinuses were most often occluded, as well as the internal jugular vein, straight sinus, cortical and deep cerebral veins.[11] Hemorrhagic infarction as a factor in an unfavorable outcome, therefore, seems to be associated with multiple venous sinus, superficial and deep vein occlusions. Nevertheless, unsolved dilemmas about hemodynamics and the therapeutic approach still exist.[12,13] Multiple cerebral sinuses/veins may be occluded in hemorrhagic infarction patients as also iSAH patients. An interesting recent hypothesis suggests that isolated SAH in CVT may be a consequence of blood leakage from fragile dilated bridging cortical veins, which have no valves or muscular layer.[7,14,15] Bridging cortical veins are abundant near the tentorial and dural venous sinuses.[16,17]

According to a previous experimental study, cortical SAH and perimesencephalic SAH in CVT patients indicates an increased blood flow in the superficial communicating veins from which the bridging veins originate.[18] We, therefore, propose that early iSAH, either cortical or perimesencephal, indicates persistent collateral venous flow and is a good prognostic sign in CVT patients.

To find out what features may influence the outcome in CVT patients, we decided to analyze retrospectively files of CVT patients hospitalized at our clinical ward for vascular neurology in the past 11 years. We hypothesized that patients with a better clinical outcome might have retained patent superficial communicating veins and consequently suffer iSAH rather than hemorrhagic infarct. They would therefore be spared from the mass effect of a hemorrhagic infarct. We consequently decided to examine by hand clinical and radiological details in files of our CVT patients who presented with iSAH or hemorrhagic infarction within the first 24 hours after admission; we sought to identify radiological features that might explain their clinical outcome.

Patients and methods

Patients and methods

Sixty-three CVT patients were admitted to the Department of Neurology, UMC Ljubljana, between January 1, 2008, and December 31, 2018.[19] The patients were diagnosed and treated in our hospital, and only those in whom CVT was proven clinically and radiologically were identified as such. Among those, we chose patients who presented either with iSAH or cerebral hemorrhagic infarction at admission or within the next 24 hours. Twenty-three patients from our inventory were enrolled in our retrospective observational study. Clinical and radiological data were analyzed.

The patients were 13 women and 10 men aged 22–73 years. The data were reviewed by an experienced neurologist working in the vascular neurology ward of the Department of Neurology, UMC Ljubljana. At admission, all the patients presented with either hemorrhagic venous infarction or iSAH due to CVT. Patients presenting with other intracranial and/or systemic pathology that can cause hemorrhagic lesions and/or isolated SAH (e.g., ruptured aneurysms, arteriovenous malformation, amyloidosis, PRES syndrome) were not included.[20,21] The data were extracted from hospital admission records, our computer data system, and the AGFA radiological database, all in accord with the Helsinki Declaration. Reports and scans were de-identified and coded before evaluation. The study was approved by the Slovenian National Medical Ethics Committee (163/02/09).

CVT was diagnosed by clinical examination, followed by brain CT, CT venography (CTV), brain MR and MR venography (MRV), whenever necessary, and laboratory findings, (e.g., d-dimer, C-reactive protein, coagulation screening, along with a complete blood count and biochemical profile). The following sinuses/deep veins were found to be obstructed in the iSAH group: the transverse sinus in 8 (bilaterally in 4 patients), the sigmoid sinus in 7 (bilaterally in 1), the superior sagittal sinus in 5, the jugular vein bulb in 5, the confluence of sinuses in 3, the straight sinus in 2, and the vein

of Galen in 1 patient. In the hemorrhagic infarction group, we found the superior sagittal sinus obstructed in 8 patients, the straight and sigmoid sinus in 6, the jugular vein bulb in 3, the internal cerebral vein in 2 (in 1 bilaterally), the basilar vein in 1 (bilaterally), the straight sinus in 1, the petrosal sinus in 1, and the vein of Galen in 1 patient. We also searched for environmental precipitating factors for CVT (e.g., trauma) as well as known intrinsic and acquired predisposing/precipitating factors (e.g., infections, contraceptive abuse, malignant disease, hematologic conditions, noninfective inflammatory disease, intracranial hypotension, acquired and genetic prothrombotic states).[21] All the patients received anticoagulant treatment immediately after the diagnostic procedures were completed. They were started on low molecular heparin in a therapeutic dose and switched to warfarin before discharge. Average discharge time was about 3 weeks from admission, and a control clinical examination was typically performed 3–4 but not more than 6 months after discharge.

Radiological analysis

The type/location of venous infarct, intracerebral hemorrhage, and/or subarachnoid hemorrhage was determined by CT and/or MR whenever needed to confirm the presence of a venous hemorrhagic infarct in the perfusion area of cerebral veins and/or blood in the subarachnoid space and/or thrombosed sinuses/veins. The thrombus location in cerebral veins and major cerebral venous sinuses was determined by CTV and/or MRV. An initial CT was typically used as an accurate and fast method to detect hemorrhagic lesions and CTV as a fast and reliable method to investigate the structure of deep cerebral sinuses/veins.[22]

Brain CT was performed on a CT 40-slice multi-detector row CT scan (SIEMENS SOMATOM Sensation Open 40). CT imaging was obtained with a 3-mm section thickness through the posterior fossa and basal brain structures and a 4.8-mm section thickness through the supratentorial hemispheres. CTV, as a fast thin-section volumetric helical CT examination, was performed with a time-optimized bolus of contrast medium to enhance the cerebral venous system. A 75–100 mL non-ionic contrast medium (iodine, 300 mg/mL) was administered at a rate of 3 mL/sec with a 45-second pre-scanning delay. Helical scanning was performed on the cranial region, from the first vertebral body to the calvaria vertex. Post-processing included two-dimensional (2D) and sometimes three-dimensional (3D) multiplanar images, slice thickness 3mm with 1 mm overlap.

MRI was performed on a 1.5T unit (Philips Achieva 1.5T MRI system) using a standardized protocol for brain examination, including the following sequences: axial T1, axial T2, axial fluid-attenuated inversion recovery (FLAIR), T2*, diffusion-weighted imaging (DWI) sequences, apparent diffusion coefficient (ADC) map and axial, sagittal and coronal T1 after the application of paramagnetic contrast agent. MR images were not used for statistics and were therefore not described in further detail.

Data analysis

A multiple linear regression was performed to test the effects upon clinical outcome of age, gender, predisposing/precipitating factors (e.g. genetic or acquired thrombophilia, steroid hormonal therapy, autoimmune disorders, malignancy, pregnancy), clinical signs/symptoms (e.g. headache, seizures, focal signs, nausea/vomiting, disturbed consciousness), and CT diagnostics (e.g herniation, brain edema, sulcal effacement, ventricular compression). The impact of any pattern and burden of venous sinus/deep vein thrombosis on venous stroke was tested. A Spearman rank correlation coefficient was determined.

The patients were divided into 2 groups. The first group consisted of patients who were diagnosed at admission as having isolated SAH associated with CVST, and the second group included patients who initially suffered from hemorrhagic venous brain infarction. The outcomes were clinically ranked according to the modified Rankin score of 0 to 6.[23] The clinical outcome results were revised by a neurologist experienced in cerebrovascular pathology.

After comparing the baseline and demographic data of the groups, the laboratory and radiological data of the 2 groups were compared using the Mann-Whitney Rank sum test and/or Spearman rank-order correlation, as appropriate. The statistical analyses were performed using the Sigma plot statistic package.

Results

With the multiple linear regression test, a positive correlation between CT diagnostic scores at admission and mRS outcome scores at discharge was observed (Spearman rank correlation coefficient, R

TABLE 1. The basic data and predisposing/precipitating factors in isolated subarachnoid hemorrhage (iSAH) and haemorrhagic infarction groups of patients

	iSAH group N = 8	Hem. inf. group N = 15
Age (mean ± SD)	49.3 ± 16.2	47.9 ± 16.8
Gender	6 M, 2 W	4 M, 11 W*
Genetic thrombophilia (%)	4 (50.0%)	2 (13.3%)
Acquired thrombophilia (%)	0 (0%)	4 (26.7%)
Autoimmune disorder (%)	4 (50.0%)	4 (26.7%)
Hypothyroid disorder (%)	1 (12.5%)	1 (6.7%)
Venous sinuses injury (%)	1 (12.5%)	0 (0%)
Malignancy (%)	1 (12.5%)	1 (6.7%)
Pregnancy (%)	0 (0%)	1 (6.7%)
Glucocorticoid/sex steroid therapy (%)	1 (12.5%)	6 (40.0%)

* statistically significant difference between the two at $p < 0.05$; Hem. inf. group = haemorrhagic infarction groups; M = men; N = number; W = women

= 0.850; $p < 0.001$). A positive correlation was also found between CT diagnostic scores at admission and mRS outcome scores at a 6-month control ($R = 0.911$; $p < 0.001$). There was no correlation between age, gender, predisposing/precipitating factors or clinical signs/symptoms at admission and the mRS outcome at discharge or at the 6-month control.

The iSAH group consisted of 8 patients (6 men) aged 49.3 ± 16.2 years. In 7 of them, we identified isolated cortical SAH and in 1, perimesencephal. The hemorrhagic infarction group was composed of 15 patients (4 men) aged 47.9 ± 16.8 years. We observed significant gender differences ($p = 0.032$), with men significantly predominating in the iSAH group and women in the hemorrhagic infarction group. Genetic thrombophilia and autoimmune disorders prevailed among the risk/provoking factors in the iSAH group. In the hemorrhagic infarction group, the results were more dispersed; however, glucocorticoid/sex steroid therapy was most frequently observed (Table 1).

TABLE 2. Clinical signs on admission in isolated subarachnoid hemorrhage (iSAH) and haemorrhagic infarction groups

	iSAH group N = 8	Hem. Inf group N = 15
Headache (%)	6 (75.0%)	9 (60.0%)
Seizure (%)	3 (37.5%)	8 (53.3%)
Focal signs (%)	2 (25.0%)	5 (33.3%)
Nausea/vomiting (%)	2 (25.0%)	3 (20.0%)
Confusion (%)	0 (0%)	2 (13.3%)
Disturbed consciousness(%)	0 (0%)	4 (26.7%)

Hem. inf. group = haemorrhagic infarction groups; N = number

The most frequently reported symptoms/signs on admission in both groups are presented in Table 2. Headache and seizures predominated in both groups.

There was a statistically significant difference between the groups (Mann-Whitney Rank Sum Test, $p = 0.026$; Table 2) in the mRS outcome score at the control examination but not at discharge. The iSAH group had a significantly better outcome than the hemorrhagic infarction group. Nevertheless, the number of thrombosed venous sinuses/deep veins in the iSAH group was significantly greater ($p = 0.002$). In this group, we also observed significantly more occlusions of a confluence of sinuses ($p = 0.015$), transverse sinuses ($p = 0.015$), sigmoid sinuses ($p = 0.023$), and jugular vein bulbs ($p = 0.013$) than in the hemorrhagic infarction group. In contrast, there was a larger number of sulcal obliteration ($p = 0.014$) and edema formation ($p = 0.004$) in the hemorrhagic infarction group. There was no statistically significant difference between the two groups of patients ($p = 0.128$) in the number of herniations. However, herniation was observed in all 3 patients with a fatal outcome in the hemorrhagic infarction group (mRS 6); minor subfalcine herniation was observed in another patient in this group (Table 3).

Discussion

In this retrospective observational study, we found that patients with CVT who suffered initially from iSAH had a better clinical outcome than patients suffering from hemorrhagic brain infarct. Isolated SAH patients had significantly more venous sinuses and deep veins obstructed than those in the hemorrhagic infarction group. Due to the CT venography that was routinely performed, however, the detection of cortical vein thrombosis was not accurate enough to perform statistics.[24] Nevertheless, we did observe a specific pattern of occluded sinuses in this group. The confluence of sinuses, transverse sinuses, sigmoid sinuses, and jugular vein bulbs were occluded significantly more often than in the hemorrhagic infarction group.

Despite more sinus/deep vein obstructions in the iSAH group, all the fatal cases occurred in the hemorrhagic infarction group as also significantly more edema formation/sulcal effacement. We observed gender differences, with men significantly predominating in the isolated SAH group

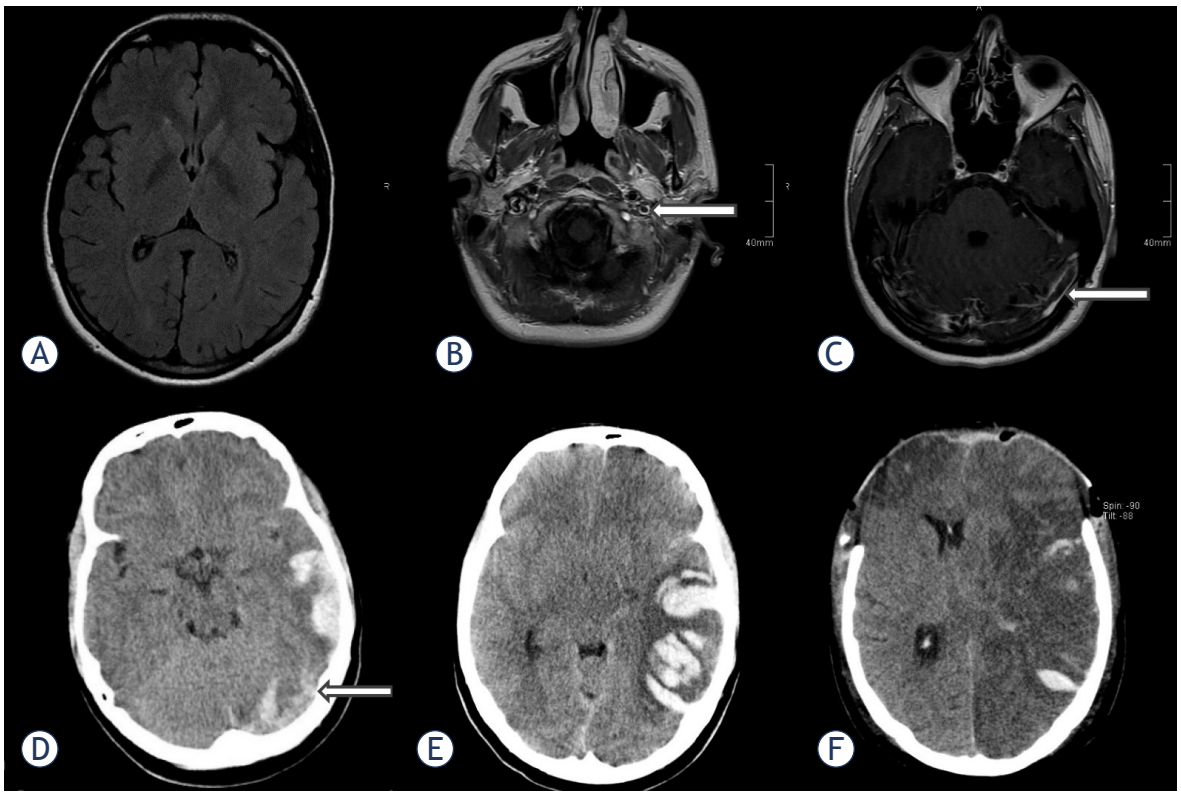

FIGURE 1. A 23-year old woman with headache followed by seizure and focal neurological deficit MRI on admission showed no focal lesions/oedema **(A)**; contrast material-enhanced (CE) T1 and T2 showed occlusion of the left sigmoid sinus **(B)** and left transverse **(C)**. Despite immediate anticoagulant treatment (fractioned heparin), the next day the patient became drowsy. CT revealed hemorrhagic infarction; in addition to the transverse sinus (arrow), the Labbe vein was suspected to be occluded due to the infarction territory **(D)**. Decompressive craniotomy failed to prevent progression to irreversible coma **(E,F)**.

and women in the hemorrhagic infarction group. Significant differences in predisposing/precipitating factors were not found. There were also no significant differences regarding clinical symptoms/signs between the groups.

Previous experience has indicated that hemorrhagic infarction is a major long-term factor of unfavorable outcome[10], especially when it presents as a space-occupying lesion.[25] In our patients who presented with hemorrhagic infarction accompanied by edema formation/sulcal obliteration, the clinical outcome at the control examination was significantly worse than in isolated SAH patients. There were also 3 lethal outcomes (mRS 6) among

TABLE 3. Comparison of thrombosed veins/sinuses (CVS), oedema formation, herniation, sulcal obliteration, modified Rankin Scores (mRs) at discharge and control examination in both groups of patients

	iSAH group N = 8	Hem. Inf group N = 15
Average No. of thrombosed CVS (median, 25%, 75% percentiles)	4 (25% 3.25, 75% 5.75)	2 (25% 1, 75% 3)*
Sulcal obliteration	0 (0.0%)	13 (86.7%)*
Subfalcine/uncal herniation	0 (0.0%)	4 (26.7%)
Oedema formation	2 (25.0%)	8 (53.3%)*
Average mRS at discharge (median, 25% , 75% percentiles)	1 (25% 0, 75% 1.75)	2 (25% 0, 75% 3)
Average mRS at control (median, 25% , 75% percentiles)	0 (25% 0, 75% 0)	1 (25% 0, 75% 3)*

*statistically significant difference between the two groups at $p < 0.05$; Hem. inf. group = haemorrhagic infarction groups; iSAH = isolated subarachnoid hemorrhage; N = number

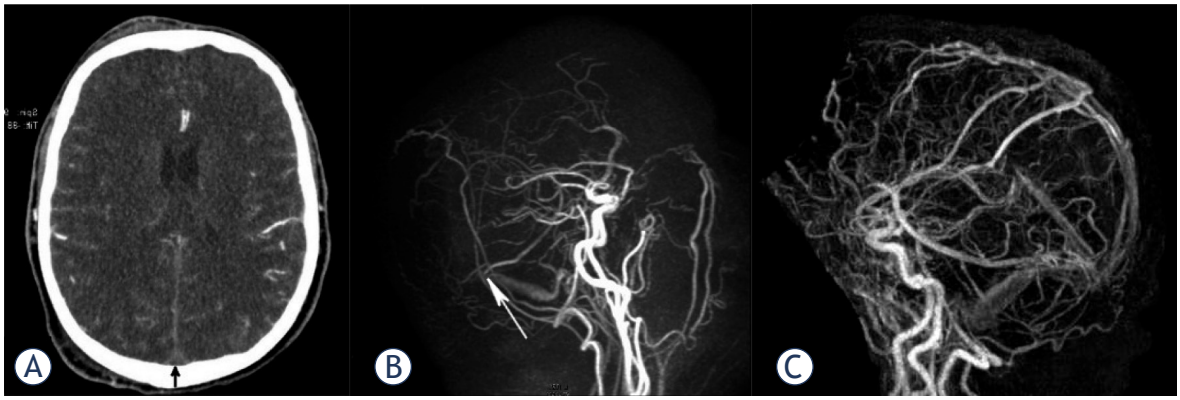

FIGURE 2. A 59-year old man was examined after 5 days of headaches and a seizure. CT revealed bilateral cortical subarachnoid hemorrhage (SAH) and moderate diffuse brain edema, but no hemorrhagic infarction was formed **(A)**. An extensive thrombosis of cerebral sinuses/veins including the superior sagittal sinus, transversal sinuses, left sigmoid sinus and jugular bulb was observed. The right transversal sinus was occluded to the point of Labbe vein inflow **(B)**, arrow showing confluence of vein to sinus). Fractured heparin and later warfarin were introduced; the patient scored 0 according modified Rankin Score (mRs) at control examination. Complete recanalization of the occluded sinuses occurred **(C)**.

hemorrhagic infarction patients. In each of those patients, we observed brain herniation within 24 hours of admission. Brain edema and sulcal obliteration were also present in each; brain edema of predominately the infratentorial region due to deep venous system obliteration was found in 1 of them. Edema formation, sulcal obliteration, and herniation are indicators of a space-occupying lesion and a worse clinical outcome.[25] Venous infarction formation may be due to venous reflux in the cerebral veins, which have no valves; or perhaps, similarly to superficial communicating veins, due to increased venous and capillary tissue pressure leading to diapedesis of erythrocytes, blood-brain barrier disruption, blood vessel damage and blood leak, all of which further lead to hemorrhagic infarction formation[26]. Given a rigid skull and meninges, the brain cannot distend, which leads to increased intracranial pressure, as also reduced cerebral perfusion pressure, cerebral blood flow, and oxygenation.[27] However, given the experimental study by Ungersböck K. *et al*.[18], which revealed the progression of thrombosis from the venous sinus to the bridging and cortical communicating veins completing an obstruction of venous collaterals, we presume this same progression in our patients and its eventuating a fatal outcome. The occlusion of the venous sinus alone seems to be not enough to cause cerebral infarction (Figure 1).[18] In addition, it seems that iSAH in CVT might be connected to a specific pattern of sinuses being occluded.

The patients with iSAH, either cortical or in the posterior fossa (e.g., perimesencephal), in our study had an excellent outcome in that they were practically free of functional disability at the control examination. None of them showed sulcal obliteration, although 3 of them experienced supratentorial edema. Isolated SAH patients were also found to have a good outcome in previous studies and reports.[9,14,15,28] In the present study, we focused on clinical and radiological features that may influence different presentations of CVT. The previous studies examined patients experiencing either iSAH [9,15] or hemorrhagic brain infarct.[10] We found no clinical studies focusing on the manner of iSAH and hemorrhagic venous brain infarct formation after CVT.

Venous blood flows along veins by the pressure gradient to the nearest venous sinus.[16,29] If there is no communication through which blood flows, then venous stasis, edema, blood leakage, and infarction develop.[26] The leakage and formation of iSAH presumably evolve from congested superficial communicating veins and/or overstretched thin-walled bridging veins.[7,27]

According to a previous experimental study, cortical SAH/perimesencephalic SAH in CVT patients indicate increased blood flow in the superficial communicating veins from which the bridging veins originate.[18] Persistent communication through the communicating/bridging venous system may reduce venous stasis and attenuate brain edema when the venous sinus is occluded. We suggest that iSAH is an indicator of that process. It seems that in patients who initially suffer iSAH, venous blood flow is partly transferred from the occluded veins/sinuses to superficial anastomotic veins, which carry venous blood towards venous

sinuses that are not occluded; blood flow is also partly transferred by thin superficial communicating veins leading blood towards meningeal veins and perhaps diploic veins (Figure 2).[7,30]

The findings in our patients are consistent with a recent neuroradiological study demonstrating that occlusion of the Labbe's vein significantly correlates with occlusion of the ipsilateral transversal sinus.[31]

Predisposing/precipitating factors in the iSAH and hemorrhagic infarction groups were not significantly different. Five women were receiving sex steroid therapy, 1 of which was in the isolated SAH group. One man in the hemorrhagic infarction group was receiving corticosteroid therapy. Hence, gender differences between the iSAH and hemorrhagic infarction groups cannot be explained by the effect of women-specific risk factors. Men predominated in our iSAH group, similar to a study from India.[7] In contrast, a French study of 22 of such patients included only 4 men.[9] In each of the case series, sampling was relatively small, possibly due to the rarity of the pathology; hence, bias is possible.

Anticoagulant therapy was introduced in all the patients as recommended.[32]

In patients experiencing large hemispheric lesions, a decompressive craniotomy was found to be effective.[33] Decompressive craniotomy relieves pressure on patent venous pathways, although it does not open the occluded ones. We propose that craniotomy should be attempted soon enough to prevent large edema/sulcal displacement, which is followed by compression and thrombosis of superficial communicating veins (e.g., Labbe's vein), as in line with recent updates/neuroradiologic studies.[31,33]

Among the limitations of the present study are the retrospective and observational methods, since such methods may involve bias due to differences in the approach of various clinicians/radiologists. The examinations were likewise not performed according to a standardized protocol. This is a limitation in the value of the results. The available clinical results and radiological images were, however, reviewed and interpreted by an experienced clinical neurologist and neuroradiologist. At the same time, an observational method might be a strength, since it originates from real life and real clinical problems, possibly providing insights on how to perform future clinical and neuroradiological evaluations. Another limitation is the small number of patients in the iSAH/hemorrhagic infarction groups, which can be explained by the rarity of the pathology and the strict inclusion criteria.

In short, this retrospective study has shown that patients with CVT who have suffered from cortical subarachnoid hemorrhage have an excellent clinical outcome, despite a higher number of occluded deep cerebral veins/sinuses. Further, a specific pattern of occluded venous sinuses was found, a clue to which might be patent communicant superficial venous pathways, e.g., vein of Labbe, vein of Trolard, and other less defined communicating cortical veins that drain to the nearest patent venous sinus. The pattern of sinuses that are occluded may have a role. Patients who suffered an early hemorrhagic venous infarction had a worse outcome – a mass effect leading to brain edema and superficial vein obliteration may be the explanation.

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research article

Safety and efficacy of drug-eluting microspheres chemoembolization under cone beam computed tomography control in patients with early and intermediate stage hepatocellular carcinoma

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Background. Drug-eluting microsphere transarterial chemoembolization (DEM-TACE) is the standard of care in patients with intermediate-stage hepatocellular carcinoma and ensures targeted and controlled cytotoxic and ischemic effects. Proper patient selection and optimized treatment techniques are associated with longer median survival. The aim of this single-institution retrospective study was to evaluate safety and efficacy of DEM-TACE under cone beam computed tomography (CBCT) control in patients with early and intermediate stage hepatocellular carcinoma.

Patients and methods. A total of 144 patients (mean age 67.9 ± 8.0 years, 127 males and 17 females) between February 2010 and December 2018 were studied. Microparticles of different dimensions according to two manufacturers (diameter of 70–150 μm, 100–300 μm or 300–500 μm and 40-μm, 75-μm or 100-μm) were used and loaded with 50–150 mg of doxorubicin. The objective tumour response according to the modified Response Evaluation Criteria in Solid Tumours (mRECIST), the time to progression, adverse events and overall survival were (OS) evaluated.

Results. In total, 452 procedures were performed (median, 3 per patient). Four (0.9% of all procedures) major complications were noted. Postembolization syndrome occurred after 35% of procedures. At the first imaging follow-up 2–3 months after first treatment, 91% of patients achieved an objective response. The median time to progression was 10.2 months (95% CI: 8.3-12.1 months). OS rates at 1, 2, 3, 4, and 5 years were 85%, 53%, 33%, 20% and 14%, respectively. The median survival time was 25.8 months (95% CI: 22.1–29.5 months).

Conclusions. DEM-TACE under CBCT control in patients with early and intermediate stage hepatocellular carcinoma is a safe and effective method of treatment with high objective tumour response and survival rates.

Key words: hepatocellular carcinoma; drug-eluting microspheres; doxorubicin; transarterial chemoembolization; cone beam computed tomography; safety; efficacy

Introduction

Hepatocellular carcinoma (HCC) accounts for most primary liver tumours and is the fourth most common cause of cancer-related death in the world.[1] The prognosis of the disease correlates strongly with liver function (defined by Child-Pugh's class, bilirubin, albumin, clinically relevant portal hyper-

tension, ascites), tumour status (defined by number and size of nodules, presence of vascular invasion, extrahepatic spread) and general tumour-related health status.[2] The Barcelona Clinic of Liver Cancer (BCLC) staging system divides patients into five groups to facilitate treatment selection and prognosis prediction.[2] According to the guidelines of the European Association for the Study of the Liver (EASL) and the European Society of Medical Oncology (ESMO), transarterial chemoembolization (TACE) is the standard treatment for HCC in patients with intermediate-stage disease, *i.e.*, BCLC stage B.[2,3] Furthermore, a clinical situation known as "treatment stage migration" has been introduced since not all patients with early-stage disease can be treated with surgery or ablation but may benefit from TACE.[4] The intention of treatment stage migration is to offer the next most suitable option within the same stage or the next prognostic stage to patients who do not respond to the recommended treatment or do not qualify for it, thus improving their outcomes.[4–6]

The principle of TACE is selective delivery of a high local concentration of a chemotherapeutic drug mixed with embolic material, which results in strong cytotoxic and ischemic effects.[2] Two techniques are currently recommended: conventional TACE (cTACE) and drug-eluting microsphere transarterial chemoembolization (DEM-TACE).[2,3] Conventional TACE (cTACE) uses a mixture of Lipiodol and chemotherapeutics, while DEM-TACE uses microparticles that can be loaded with a chemotherapeutic drug that is released in the target tissue slowly and in a controlled manner. The most frequently used drug in DEM-TACE is doxorubicin.[7]

Untreated patients with BCLC stage B disease have a median OS of 10 to 16 months after HCC diagnosis.[2,8] The median OS for patients treated with TACE is approximately 20 months after treatment initiation.[5,9] In well-selected patients, median OS can be prolonged to 30–50 months.[2,3] A difference in OS between patients treated with doxorubicin-loaded DEM-TACE and those treated with cTACE has not been observed.[10,11]

A further improvement in TACE was the introduction of cone beam CT (CBCT) control during the procedure. CBCT is mounted on a C-arm fluoroscopy unit in the Institute of Radiology suite, allowing enhanced visibility in soft-tissue and vascular procedures. CBCT enables more precise implementation of TACE from planning to microcatheter positioning, which ensures visualization of the tumour-feeding vessels and parenchymal staining during TACE, achieving a detection accuracy significantly superior to that of standard 2D angiography.[12,13]

The aim of this single-institution retrospective study was to evaluate the safety and effectiveness of doxorubicin drug-eluting microsphere chemoembolization under CBCT control in patients with early and intermediate stage HCC.

Patients and methods

Patient selection

The present retrospective study included 144 patients with early and intermediate stage HCC who underwent doxorubicin-loaded DEM-TACE at our interventional oncology centre between February 2010 and December 2018. The last date for follow-up evaluation was 31 January 2020. Clinical examination, laboratory tests and contrast-enhanced four-phase computed tomography (CT) or magnetic resonance (MR) imaging with hepatobiliary contrast media (Primovist; Bayer HealthCare, Germany) were performed for each patient at baseline. A decision in favour of treatment with doxorubicin-loaded DEM-TACE was reached by consensus at a multidisciplinary hepatopancreatobilliary tumour board (MTB) at our institution, consisting of abdominal and interventional radiologist, gastroenterologist, hepatic surgeon, nuclear medicine physician, oncologist, and pathologist. The inclusion and exclusion criteria for the study are presented in Table 1.

Written informed consent for the procedure was obtained from all patients before each treatment. The need for informed consent for publication was waived by the national ethics committee due to the retrospective, anonymized study design. The study was performed in accordance with the Helsinki Declaration ethical standards for biomedical studies on humans and was approved by the Republic of Slovenia National Medical Ethics Committee on the 18th of April 2017 (decision number 60/04/17). All data were collected from patient charts held at the Clinical Institute of Radiology, Clinical Department of Gastroenterology and Clinical Department of Abdominal Surgery at University Medical Centre Ljubljana.

Treatment

The first treatment cycle was defined by at least two doxorubicin-loaded DEM-TACE procedures at intervals of 4–6 weeks (*i.e.* TACE 1a and 1b).

Additional procedures prior to the first dynamic contrast enhanced CT or MR evaluation were performed if the multifocality of the disease did not allow complete targeting of the tumours. All procedures were carried out under CBCT control (using Allura Xper FD20; Philips Healthcare and Artis Zee floor with DynaCT; Siemens, Forchheim, Germany). Typically, a 2.4-French microcatheter (Progreat®, Terumo Europe N. V, Belgium) was advanced into either a subsegmental or a segmental tumour-feeding artery depending on the location of the targeted tumour. CBCT was performed with the administration of a nonionic contrast agent (Ultravist 370®, Bayer HealthCare, Germany; Visipaque 320, GE Healthtcare, Norway) through a power injector (Avanta®, Medrad, Bayer HealthCare, Germany). The injection rate for the initial lesion visualization with catheter placed in main hepatic artery was typically 1 mL/s with a total injected volume of contrast agent of 10 mL and a delay time of 8–10 seconds.[13] For each CBCT scan, the area of interest was positioned in the system isocenter, and, over approximately 10 seconds, 310–321 projection images were acquired with the motorized C-arm. X-ray parameters of 51–120 kV and 101–125 mA, covering approximately 180–200° clockwise arc at a rotation speed of 20° per second were used. Multiple two-dimensional projections were acquired and reconstructed by using Feldkamp algorithm to generate three-dimensional volumetric images. The matrix size was 512 x 512, and the field of view (FOV) 38 x 38 cm.

Selective or superselective chemoembolization was performed with microparticles with varying diameters from different manufacturers loaded with 50–150 mg of doxorubicin: DC Beads (DC Bead®, Boston Scientific, Marlborough, Massachusetts) with a diameter of 70–150 μm, 100–300 μm or 300–500 μm; Tandem (Tandem®, Boston Scientific, Marlborough, Massachusetts) 2 ml or 3 ml of 40-μm, 75-μm and 100-μm, LifePearl microspheres (LifePearl®, Terumo Europe N. V, Belgium). The same size of the microparticles were then used for the following procedures in each patient. Since 2013, we have been using small microparticles (*i.e.* 70–150 μm) in all patients.

In patients with multifocal tumours, the position of the microcatheter was changed within the same session to ensure superselective delivery to each lesion.[13] Prior to microparticle delivery, CBCT was repeated to confirm the catheter position in feeding artery and complete coverage of the targeted lesions.

TABLE 1. Inclusion and exclusion criteria (Child-Pugh; CP)

Inclusion criteria	early-stage HCC patients ineligible for resection, transplantation or ablation; intermediate-stage HCC patients with a CP score of A or B (up to 7 points); treatment with DEM-TACE under cone beam CT control.
Exclusion criteria	DEM-TACE prior to liver transplantation; inability of regular follow-up.

DEM-TACE = drug-eluting microsphere transarterial chemoembolization; HCC = hepatocellular carcinoma

Treatment complications

Procedure-related complications were classified as complications occurring during the procedure or complications detected up to 1 month after the procedure. The complications were classified as minor and major according to the CIRSE Quality-Improvement Guidelines for Hepatic Transarterial Chemoembolization.[14] Postembolization syndrome (PES) was defined as fever, pain, nausea, elevation of liver transaminases (*i.e.* doubling of baseline value of aspartate aminotransferase (AST)) and an increased white blood cell (WBC) count that occur 24–72 hours after the procedure and was not considered a complication in accordance with the CIRSE guidelines.

Treatment response

Tumour treatment responses were evaluated with dynamic contrast-enhanced CT or dynamic MR imaging with hepatobiliary contrast media 2–3 months after the last doxorubicin-loaded DEM-TACE procedure according to the modified Response Evaluation Criteria in Solid Tumours (mRECIST).[15] Patients with a complete response or a partial response were classified as having an objective response to treatment. In cases of an objective response to treatment, a radiological follow-up was performed every 3 months for the first 2 years, mostly with MRI of the liver. If no progression occurred after 2 years, follow-up imaging was then scheduled every 6 months. Doxorubicin-loaded DEM-TACE treatment was repeated when necessary in patients with residual or additional tumours observed on imaging, *i.e.* retreatment "on demand".[13] A decision for retreatment was again reached by MTB.

Statistical analysis

Categorical variables were expressed as frequencies and percentages. Continuous variables were

TABLE 2. Patient characteristics at baseline

Gender, number of patients (%)	
Male/Female	127 (88.2)/17 (11.8)
Age, years	
Mean ± SD	67.9 ± 8
Imaging characteristics	
Number of lesions per patient, median (range)	3 (1–10)
Bilobar, n. (%)	52 (36.1)
Unilobar, n. (%)	92 (63.9)
Right lobe, n. (%)	71 (49.3)
Left lobe, n. (%)	21 (14.6)
Signs of portal hypertension, n. (%)	
Yes/No	76 (52.8)/68 (47.2)
Ascites, n. (%)	
Yes/No	34 (23.6)/110 (76.4)
Cirrhosis, n. (%)	
Yes/No	120 (83.3)/24 (16.7)
Cirrhosis aetiology, n. (%)	
Alcohol	63 (52.5)
HBV	16 (13.3)
HCV	14 (11.6)
Primary biliary cirrhosis	2 (1.8)
Other	25 (20.8)
Child-Pugh score (avg. points ± SD)	5.7 ± 0.8
Child-Pugh class, n. (%)	
A/B	91 (75.8)/29 (24.2)
Barcelona Clinic of Liver Cancer stage, n. (%)	
A/B	50(34.7)/94 (65.3)
Laboratory characteristics, median (range)	
Albumin, [g/l]	39.5 (28–50)
Total bilirubin, [µmol/l]	18 (5–83)
AFP, [ng/ml]	14.4 (1.1–12809.8)
AST, [µkat/l]	0.82 (0.35–3.29)
GGT, [µkat/l]	1.77 (0.25–24.95)
Creatinine, [µmol/l]	78 (39.0–148.0)

AFP = alpha fetoprotein; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase); HBV = hepatitis B virus; HCV = hepatitis C virus; SD = standard deviation

expressed as the mean ± standard deviation or the median and range in case of skewed distributions. Survival rates and the time to progression (TTP) were calculated using the Kaplan–Meier method and compared using the log rank test. The limit of statistical significance was set at $p < 0.05$.

The follow-up time was determined as the number of months from the first doxorubicin-loaded DEM-TACE procedure until death or until 31 January 2020. The time to progression was calculated as the time until the date of imaging control showing progression or, in censored cases, until 31 January 2020, or patient death.

The analysis was performed using IBM® SPSS® Statistics 22 (International Business Machines Corp., Armonk, NY) for Windows.

Results

Patient characteristics

The baseline demographic, clinical, laboratory and imaging characteristics of 144 patients included in the analysis are summarized in Table 2. The mean patient age was 67.9 ± 8.0 years, and most patients were male (88.2%). The median number of lesions per patient was 3 (range, 1–10), and the median size of the largest lesion was 3.8 cm. Ninety-one patients were classified as Child-Pugh class A (75.8%), and the remaining 29 were classified as class B (24.2%). Twenty-four of the 144 (16.7%) patients were not cirrhotic. The most common aetiology of cirrhosis was alcohol abuse (52.5%, n = 63), followed by other (20.8%, n = 25), hepatitis B (13.3%, n = 16) and hepatitis C (11.6%, n = 14). Ninety-two (63.9%) patients had unilobar, predominantly right lobe, disease. Clinical signs of portal hypertension were observed in 76 (52.8%) patients, and ascites was observed in 34 (23.6%) patients. Sixty-eight of 101 (67.3%) patients with available AFP data had an elevated AFP level (> 7.5 ng/mL).

Procedure

Overall, 452 doxorubicin-loaded DEM-TACE procedures were performed in 144 patients. The median number of procedures per patient was 3 (range, 1–8). In 136 (94.4%) patients, at least two doxorubicin-loaded DEM-TACE procedures were performed. The remaining 8 (5.6%) patients had only one procedure at the time of their follow-up. Large microparticles (300–500 µm) were used in 16% of patients (n = 23), intermediate-size microparticles (100–300 µm) in 19.4% of patients (n = 28) and small microparticles (40–100 µm) in 64.6% of patients (n = 93) in the first doxorubicin-loaded DEM-TACE procedure. The maximum cumulative dose of doxorubicin per procedure was 150 mg (range 50–150 mg). Doxorubicin-loaded DEM-TACE was the primary treatment for 120 patients

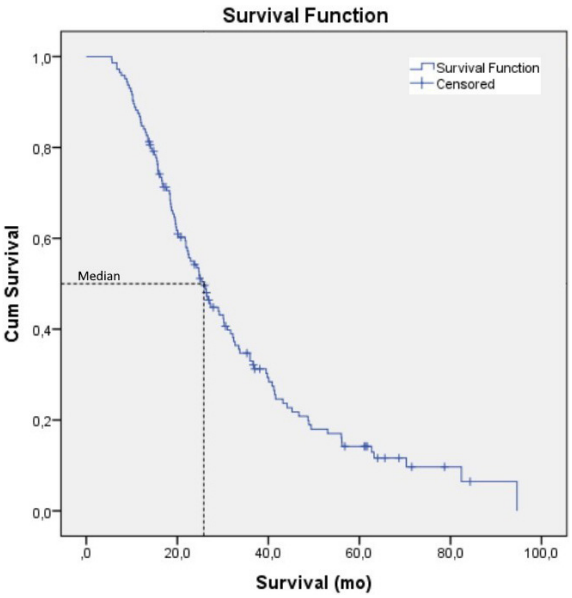

FIGURE 1. Kaplan-Meier curve of overall survival (entire study population).

(83.0%). Other treatments for HCC preceding the first doxorubicin-loaded DEM-TACE are presented in Table 3.

Procedure complications

PES occurred in 158 procedures (35.0%). All PES cases were managed medically and did not prolong hospitalization. In addition to PES, eleven (2.4% of all procedures) minor complications occurred and are presented in Table 4. Gastric erosions in symptomatic patients were detected by gastroscopy and treated medically. Intraprocedural small arterial branch rupture was immediately successfully treated with coil embolization.

Four (0.9% of all procedures) major complications were noted: ischemic cerebrovascular insult to the cerebellum, radial artery thrombosis following a transradial approach, variceal bleeding resulting from emesis after the procedure and infection of the necrotic tumour, which resolved after antibiotic treatment.

Overall survival

After a median follow-up of 23.8 months (range, 5.6–94.6), 115 patients had died. The one-, two-, three-, four- and five-year survival rates were 85%, 53%, 33%, 20% and 14%, respectively (Figure 1). The median OS was 25.8 months (95% CI: 22.1–29.5 months).

TABLE 3. Hepatocellular carcinoma (HCC) treatment prior to initial doxorubicin-loaded drug-eluting microsphere transarterial chemoembolization (DEM-TACE)

Treatment	Number of patients (%)
No previous treatment	120 (83.0)
cTACE	2 (1.4)
Surgical resection	13 (9.3)
Transplantation	1 (0.7)
Surgical resection and RFA	1 (0.7)
Surgical resection and cTACE	1 (0.7)
RFA	2 (1.4)
MWA	1 (0.7)
ECT	3 (2.1)

cTACE = conventional TACE; ECT = electrochemotherapy; MWA = microwave ablation; RFA = radiofrequency ablation

TABLE 4. Minor complications after drug-eluting microsphere transarterial chemoembolization (DEM-TACE) in the study population

Complications	Number of procedures (%)
Chest pain	4 (0.9)
Pain in the right shoulder	1 (0.2)
Hematoma at the puncture site	3 (0.7)
Gastric erosions	2 (0.4)
Intraprocedural small arterial branch rupture	1 (0.2)

Child-Pugh class, ascites and portal hypertension showed statistically significant differences with respect to OS on univariate analysis (Table 5) ($p = 0.008$; $p = 0.001$; $p = 0.016$).

No statistically significant difference was observed between unilobar and bilobar disease ($p = 0.609$).

Treatment response and time to progression

An objective response was achieved in 131 (91%) of 144 patients at the first dynamic contrast enhanced imaging follow-up. A complete response was achieved in 72 of 131 patients (55.0%) patients, and a partial response was achieved in 59 (45.0%) patients.

Progression was observed at the first imaging follow-up in 13 of 144 patients. Eight patients had target lesion progression, 3 patients had a new

TABLE 5. Overall survival after DEM-TACE in the study population Senči polja med vrsticami

Factor	Number of patients	Median survival	95% CI	*p* value
Child-Pugh A	91	26.6	17.7–35.5	0.008
Child-Pugh B	29	19.6	18.4–20.8	
Portal hypertension	76	20.2	14.7–25.7	0.001
No portal hypertension	68	32.4	24.0–40.8	
Ascites	34	19.6	15.3–23.9	0.016
No ascites	110	29	23.0–35.0	
Unilobar disease	92	24.9	21.0–28.8	0.609
Bilobar disease	52	26.3	21.5–31.1	

intrahepatic lesion, and 2 developed extrahepatic lesions. Further decisions were based on clinical evaluations, laboratory values and imaging data. Patients with target lesion progression were further treated with doxorubicin-loaded DEM-TACE (37.5%, n = 3) or systemic chemotherapy (25%, n = 2) or received best supportive care (37.5%, n = 3). Patients with new intrahepatic lesions were further treated with doxorubicin-loaded DEM-TACE (n = 1), systemic chemotherapy (n = 1), or both (n = 1). Both patients with extrahepatic lesions received systemic chemotherapy.

During the follow-up, progression was observed in 115 patients (79.9%). Of these, 27 patients (23.5%) had target lesion progression. The median time to progression was 10.2 months (95% CI: 8.3–12.1 months). Overall, further HCC treatment after progression was performed in 93 patients. Doxorubicin-loaded DEM-TACE was performed in 66 patients, MWA in 2 patients and both in 1 patient. Twenty-one patients were treated with systemic therapy alone, and 3 were treated with systemic therapy and doxorubicin-loaded DEM-TACE. The remaining 22 patients received best supportive care.

Discussion

The purpose of this retrospective study was to evaluate the safety and effectiveness of DEM-TACE in patients with early and intermediate stage HCC. TACE is the standard of care for HCC patients with intermediate-stage disease. Moreover, for patients who are ineligible for surgical resection or percutaneous ablation, TACE is also considered a first-line treatment option in the early stage.[16,17] However, intermediate HCC corresponds to a highly heterogeneous group of patients with significant variations in number and size of tumour, patient performance status and liver function, resulting in variable survival rates.[18] The median OS for patients treated with TACE is reported to range from approximately 19 months to 40–50 months in well-selected patients[2,3,9], which is consistent with our results. The median OS in our study was 25.8 months, with a range from 5.6 months to more than 7 years. A multicentric study by Han *et al*. in 2019 showed a median OS of 19.9 months with a range from 7 months to more than 4 years.[19] A study by Burrel *et al.* showed that the median OS can be prolonged up to 48.6 months with careful patient selection.[4] Several prognostic factors, such as Child Pugh class and tumour number, have been linked to higher survival rates and can be used to select ideal candidates for TACE.[19,20] The Child Pugh score is a valuable tool for assessing liver function, and our results support its correlation with survival. However, this score does not consider some events that may indicate end-stage liver disease (*e.g.*, renal failure, spontaneous bacterial peritonitis, hyponatremia, recurrent encephalopathy, and malnutrition) and may be replaced by other selection criteria in the future.[16] Another factor that may lead to better survival rates in some studies is treatment stage migration, as the survival of BCLC-A patients is expected to be better than that of BCLC-B patients.[6] Treatment stage migration also applies to BCLC stage B. Although most patients achieve an objective response after treatment, they can present with new tumour sites during their follow-up and thus qualify as having disease progression. Intrahepatic treatable progression can be treated with repeat TACE. On the other hand, the untreatable progression may necessitate initiation of systemic therapy. In this study, migration to systemic therapy was recorded in 24 patients.

Higher OS may be related to the high objective response rates achievable with TACE, which is supported by the fact that an objective response to treatment measured by mRECIST correlates with prolonged OS.[21,22] According to the European Association for the Study of the Liver mRECIST represents the gold standard for radiologically evaluating tumour response during HCC locoregional treatment.[2] Previously used EASL criteria express the change in the two dimensions of hyperenhancing tumour and therefore also reflect the extent of necrosis caused by the treatment. mRECIST criteria adopted a single long-axis measurement and are simplified objective measure of treatment response, especially with the use of su-

perselective approach where little or no viable tumour is expected. An objective response to TACE is achieved in approximately 50% of patients, with the lowest rate reported to be approximately 16% and the highest reported to be approximately 85.6%.[16,17,23] A prospective, randomized phase II study comparing doxorubicin-loaded DEM-TACE with cTACE showed higher objective response rates in the doxorubicin-loaded DEM-TACE group – 51.6% *vs*. 43.5%, respectively.[11] In a study by Suk *et al*. assessing CBCT after doxorubicin-loaded DEM-TACE, an objective response was achieved in 85.6% of patients (63.8% complete response, 21.8% partial response).[23] Our results exceed those of the above studies, showing a 91% rate of an objective response defined according to the mRECIST.

The benefits of CBCT for intra-arterial liver procedures are now well established and documented. The detection accuracy of CBCT for HCC lesions is equivalent to those of multidetector CT and MRI and superior to that of angiography. CBCT is the most accurate imaging technique to identify tumour-feeding arteries and can be used to rule out nontarget embolization of nontumour-feeding extrahepatic arteries. These advantages of CBCT can result in better treatment efficacy of DEM-TACE and other intra-arterial therapies.[13]

DEM-TACE has been demonstrated to result in fewer complications than cTACE.[24] In a systematic review of cTACE by Lencioni *et al*., 274 articles with a total of 34,137 patients were analyzed and adverse events were observed in 15,351 patients in a total of 217 selected studies.[9] The most common procedure-related complication was PES, with the incidence of 47.7%. Other studies describe an even higher incidence of PES, with an estimated rate of 48%.[9] According to the CIRSE guidelines, PES by itself is considered an expected outcome of the procedure rather than a complication.[14,25] Procedural complications, including intraprocedural small arterial branch rupture, hematoma at the puncture site, cerebellar stroke and radial artery thrombosis following a transradial approach, occurred in 1.1% of our cases. Eosophageal variceal bleeding was managed with endoscopic variceal banding. To our knowledge there is no known mechanism through which TACE could facilitate variceal bleeding. Since the bleeding occurred only once in otherwise large cohort of patients with high risk for variceal bleeding, we believe that this was probably a coincidental event. Other complications are all recognized although extremely rare following TACE procedures and all of them were one-time events.[26-28] Intraprocedural small arterial branch rupture was managed with endovascular coil embolization and this complication didn't prolong the patient's hospital stay. Ischemic stroke is an extremely rare, but recognized event.[27] A recent literature review describes twelve cases in patients undergoing DEB-TACE or cTACE with mechanisms including intrahepatic arteriovenous shunts, hepatopulmonary shunts and intracardiac shunts. The presence of arteriovenous shunting to hepatic or pulmonary veins is routinely checked for at intraprocedural angiography and is a well-known contraindication to performing TACE, while checking for intracardiac shunts is not routinely performed in these patients. The patient in our cohort had a cerebellar stroke, which occurred after the procedure being performed via the right internal mamarian artery. Due to the cerebellar location, we hypothesize this non-target embolization was the result of the reflux of the embolic agent into the vertebral artery. In our patient the symptoms of ischemic insult resolved spontaneously, and no specific treatment was applied. Thrombosis of the radial artery was a result of transradial approach in a patient with a severe stenosis of iliac arteries preventing transfemoral approach. Radial artery occlusion is not a TACE specific complication and it occurs in 1 – 10% of patients following transradial interventions with lower numbers of complications occurring in experienced centers.[26] Our patient was managed conservatively while hospitalized but was then lost to angiological follow-up.

Our study has some limitations. This was a retrospective study including only a limited number of patients and no control group. A standard treatment methodology and patient selection criteria for TACE are lacking due to the heterogeneity of the HCC patient population with BCLC stages A and B. Therefore, patient selection should be carefully considered to achieve the best outcomes. Subsequent studies should include a larger group of patients. Other medical centres should also be included since the results of this single-centre study may not be applicable to other centres and geographic regions. Furthermore, although four interventional radiologists performed DEM-TACE according to uniform protocols, minor variations, such as in catheterization selectivity, were inevitable. Finally, the treatment effect of DEM-TACE may also be significantly influenced by multimodality treatment; thus, the long-term outcomes might not be representative.

In conclusion, DEM-TACE under CBCT control in patients with early and intermediate stage HCC is a safe and effective method of care. The results

of this study are consistent with that in our study from 2016, confirming that proper patient selection, routine utilization of CBCT control for superselective TACE guidance, regular treatment response evaluation and liver function tests, together with retreatment "on demand" results in high objective tumour response and survival rates.

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research article

Cone-beam computed tomography guided nusinersen administrations in adult spinal muscular atrophy patients with challenging access: a single- center experience

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Background. The challenging anatomic predispositions in adult patients with spinal muscular atrophy (SMA) preclude the conventional lumbar punctures. Consequently, an introduction of alternative method for intrathecal delivery of nusinersen is required. Cone-beam CT (CBCT) allows volumetric display of the area of interest, pre-procedural planning and real time needle guidance which results in accurate anatomic navigation. The aim of the study was to evaluate technical success, safety, and feasibility of CBCT lumbar intrathecal delivery of nusinersen in the adult SMA patients with challenging anatomical access.

Patients and methods. Thirty-eight adult SMA patients were treated in our institution. Patients with challenging access were selected by multidisciplinary board for image guided administration of nusinersen either due to implantation of the posterior fusion instrumentation, severe scoliosis defined as Cobb's angle > 40° or body mass index over 35. Technical success, radiation exposure and occurrence of adverse events were assessed.

Results. Twenty patients were selected, and 108 CBCT-guided procedures were performed. Each patient underwent at least 4 administrations. Transforaminal approach was performed in 82% of patients. The technical success was 100%, with primary success of 93.5%. The median radiation effective dose of the administrations was 5 mSv, the mean value equalled 10 mSv. Only mild adverse events were reported in the study.

Conclusions. CBCT-guided lumbar intrathecal administrations of nusinersen in an adult SMA population with challenging access was feasible and safe image guided method.

Key words: nusinersen; cone-beam CT; lumbar puncture

Introduction

Antisense-oligonucleotide nusinersen (Spinraza, Biogen Netherlands, Netherlands) was the first approved intrathecal drug for treatment of 5q spinal muscular atrophy (SMA) experienced as safe and clinically beneficial for lifelong treatment of infants and children.[1,2] Growing evidence indicate safety and efficacy even in some subgroups of adult patients.[3-5]

Intrathecal administrations of nusinersen require lumbar puncture. In the natural progression of SMA, particularly in type 2 and 3, patients may develop severe scoliosis.[6] In the most severe, type 1 SMA the patients rarely survive to adulthood, where as in type 4 being the mildest, involvement of the spine is

rare.[6] Debilitating scoliosis which requires surgery may not develop up to the onset of puberty and during childhood a conventional lumbar puncture is usually feasible.[6] In older patients factors such as altered spine anatomy, obliterated interlaminar space and obesity are commonly present and preclude the conventional lumbar puncture.[6-8] Consequentially, the need for reproducible and safe image-guided method for intrathecal administrations in adult patients has been accentuated.[9]

Several studies described successful techniques for administration of nusinersen in patients with challenging access. In these patients, successful interlaminar (IL) or transforaminal (TF) lumbar accesses have been performed under CT-, fluoroscopic- or ultrasound-guidance.[7,10-14] However, the possibility of spine deformity progression and the need for repetitive injections require a method with satisfactory deep soft tissue resolution and following the "as low as reasonable achievable" radiation principle.[10,15,16] The cone-beam CT (CBCT) allows volumetric display of the area of interest, pre-procedural planning and real time needle guidance which results in accurate anatomic navigation.[17] So far, only three studies described the use of CBCT for intrathecal delivery of nusinersen in children and adults.[11,18,19] However, up to our knowledge there are no larger studies on CBCT guided intrathecal nusinersen delivery that would present data only on adult SMA patients.

The purpose of this prospective study was to present a single-center experience on implementation of lumbar spine CBCT-guided intrathecal nusinersen delivery in consecutive adult SMA patients with challenging access to investigate the technical success, feasibility, and safety.

Patients and methods

Patients

The treatment with nusinersen has been available to the adult Slovenian patients with SMA in the University Medical Centre Ljubljana since the beginning of 2019. Thirty-eight adult SMA patients have undergone the repetitive treatment with intrathecal administrations in our institution from April 2019 to May 2021. Patients with challenging access were selected for CBCT-guided intrathecal nusinersen delivery.

The study was approved by the National Ethics Committee. An informed written consent from the patients was obtained before the beginning of the study.

Inclusion criteria

The criteria for the group eligible for CBCT-guided lumbar punctures were determined by a multidisciplinary board team of neurologists and interventional radiologists. Patients with history of scoliosis corrective surgery with implantation of posterior fusion instrumentation, severe scoliosis defined as Cobb's angle > 40° or patients with body mass index (BMI) over 35 were included in CBCT-guided group.[8,20,21] The remaining patients underwent conventional lumbar puncture.

Procedure

All procedures were performed on Siemens Artis Q (Siemens Healthineers, Erlangen, Germany) CBCT system with C-arm and navigational overlay in outpatient setting in the interventional radiology suite by interventional radiologists with more than 10-year experience in image-guided procedures.

Upon arrival to the interventional suite, the patients with implanted posterior instrumentation were placed in half lateral position to expose the curvature of the spine for TF approach. For IL approach either prone or half-lateral position was used. Following optimal patient positioning, a single rotation of a low dose CBCT was performed. Using the navigational computer program, the entry and target point were defined by the performing radiologist considering the safest and most feasible approach. For patients with history of spine surgery and posterior fusion instrumentation a TF approach was used as it was the only feasible option.[20] In this approach the trajectory line was positioned via the inferior portion of the intervertebral foramen to evade the exiting neurovascular bundle. IL approach was selected for patients without spinal instrumentation and visible interlaminar space.[12]

Lumbar punctures were performed under sterile conditions, local anesthesia was used. For the procedures 20 G spinal needles were used, the length of needle based on the distance from the skin to the target point.

Integrated laser of the C-arm marked the entry position of the needle on the skin. The target point position was visualized under intermittent fluoroscopic guidance using two orthogonal views (Figures 1, 2, 3). Once the needle tip reached the target point, an aspiration of cerebrospinal fluid (CSF) was performed to confirm the intrathecal position. Afterwards, 5 ml of CSF was aspirated, and 5 ml nusinersen solution was intrathecally delivered according to the manufacturer's instructions.

After the procedure the patients were surveilled for 4-6 hours before being discharged.

Variables and data collection

Patient age, sex, BMI, type of SMA were recorded and spine anatomy was evaluated. For each procedure the type of the approach (TF or IL) with level of injection, total duration of the procedure from the arrival in the interventional suite to exiting, the technical success and peri-procedural adverse events (AE) were noted. Radiation exposure was calculated as effective dose (ED) as a product from dose-area product (DAP) and theoretical coefficient. The theoretical coefficient used in the equation was 0.0012 mSv/μGy*m^2. The data was obtained through patient databases and systematic questionnaire.

Technical success

The technical success was defined by extraction of a macroscopically clear, no-blood-contaminated cerebrospinal fluid (CSF) with successful intrathecal application of 12 mg nusinersen.[11] Primary success was determined when a macroscopically clear CSF was extracted at first attempt without further repositioning of the needle. Secondary success was defined for procedures that required additional attempts for secure access at the same or another level during the same procedure.

Adverse events

Peri-procedural AEs that occurred during the first 24 hours were recorded in accordance with the Society of Interventional Radiology guidelines.[22] AEs were noted during the peri-procedural surveillance period and reported by the patients upon their next regular clinical visit at the outpatient department. The patients assessed the overall discomfort and pain level during the CBCT-guided nusinersen intrathecal delivery using the Visual Analogue Scale (VAS).

Statistical analysis

Calculations were performed with statistical spreadsheet computer program SPSS Inc. (SPSS for Windows, Version 22.0. Chicago, SPSS Inc). The normality of variable distribution was obtained using the Shapiro-Wilk test. Statistical analyses were performed using t-test for independent variables and Mann-Whitney test.

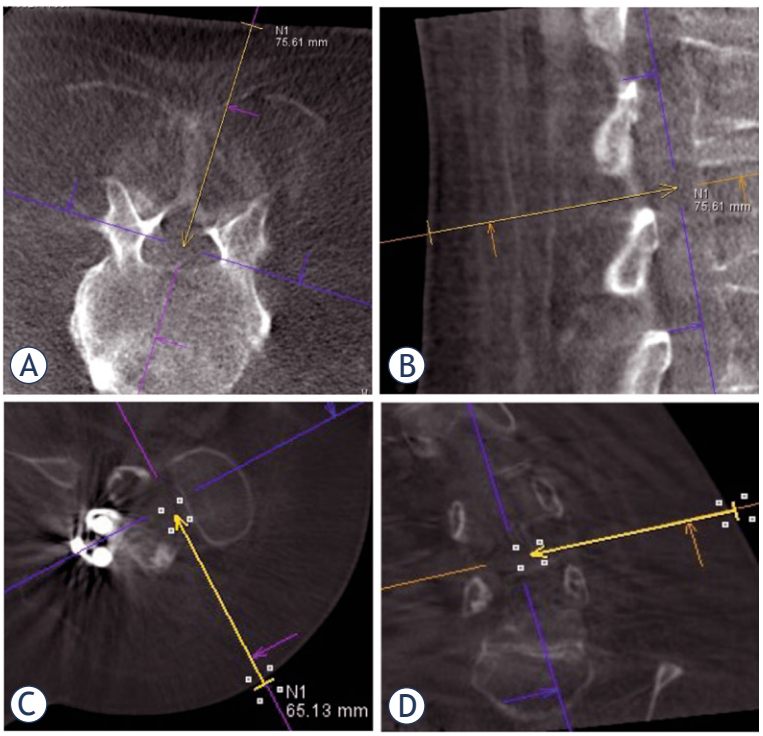

FIGURE 1. Cone-beam CT orthogonal reconstructions demonstrating the planned needle trajectory. **(A), (B).** Planning the interlaminar approach (yellow arrows); **(C), (D).** Planning the transforaminal approach (yellow arrows).

Results

Patients

20 patients (53%) were found eligible for CBCT-guided intrathecal nusinersen delivery. Seventeen patients had severe scoliosis, ten patients had posterior fusion instrumentation and two patients were obese. In 18 patients (47%) intrathecal administrations were possible by conventional lumbar puncture and were not included in our study. Patient characteristics are presented in Table 1. None of the patients withdrew from the CBCT-guided intrathecal nusinersen delivery.

Procedure

During the study 108 CBCT-guided procedures were performed. Each patient underwent at least 4 administrations. The patient with most administrations had 8 administrations. The predominant approach to the spine was TF with L2-L3 or L3-L4 being the most frequent levels of lumbar puncture. For IL approach L3-L4 level was most frequently chosen (Table 2). Total procedure time was approximately 1 hour (Table 2).

TABLE 1. Patient characteristics for cone-beam CT (CBCT)-guided intrathecal nusinersen delivery patients and classical lumbar puncture patients

	CBCT-guided	Classical lumbar	P-value
Male sex (%)	10 (50)	12 (67)	
Age at first administration, median (range) year	33.5 (20–62)	44.5 (19–69)	0.62
BMI, median (range) kg/m2	23.4 (14.2–41.8)	24.7 (14.3–33.6)	0.08
SMA type 2 (%)	13 (65)	0	
SMA type 3 (%)	7 (35)	15 (83)	
SMA type 4 (%)	0	3 (17)	
Posterior fusion instrumentation due to scoliosis (%)	10 (50)	0	
Severe scoliosis (%)	17 (85)	N/A	

BMI = body mass index; N/A = not available; SD = standard deviation; SMA = spinal muscle atrophy

TABLE 2. Procedure summary

	Interlaminar	Transforaminal	Total
L1-L2 (%)	0 (0)	4 (4)	4 (4)
L2-L3 (%)	7 (6)	48 (44)	55 (50)
L3-L4 (%)	10 (9)	35 (32)	45 (41)
L4-L5 (%)	2 (2)	3 (3)	4 (5)
Number of procedures (%)	19 (18)	89 (82)	108 (100)
Duration per procedure, mean ± SD min	63 ± 21	60 ± 26	62 ± 25

SD = standard deviation

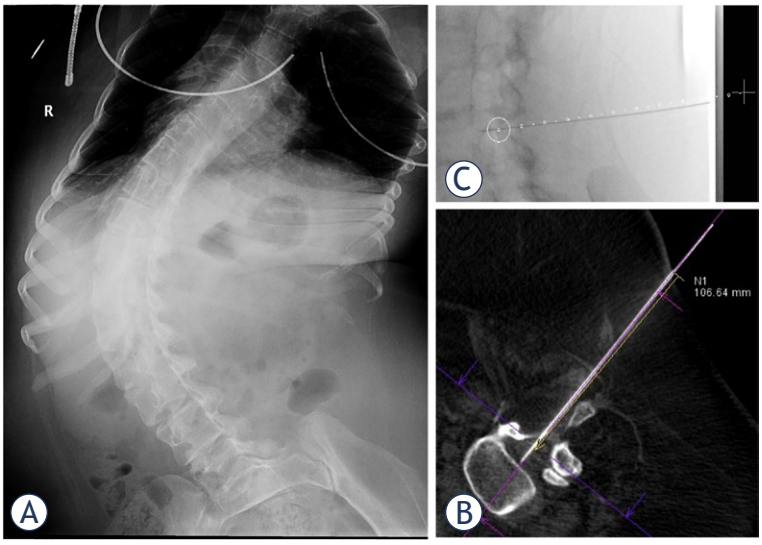

FIGURE 2. (A). A 22-year-old female with severe scoliotic deformity of the spine; **(B).** The nusinersen administration was performed with interlaminar approach under cone-beam CT -guidance; **(C).** Introduction of the needle following the planned trajectory (white dotted line) to the target point (white circle).

Technical success

All CBCT-guided procedures were technically successful. Primary success was achieved in 101 (94%) procedures. In others secondary success was achieved; 2 IL and 5 TF approach.

Effective dose

The median ED for all administrations was 5.5 mSv (interquartile range 2.7–13 mSv) and mean 10 mSv (standard deviation 11 mSv). There was no difference in median ED between patients with posterior fusion instrumentation in comparison to the patients without (5 mSv *vs.* 5.8 mSv). There was a statistically important difference in median ED for obese patients in comparison to other patients (12 mSv *vs.* 5 mSv, p = 0.004). ED for every application is presented in Figure 4.

Adverse events

Median value of patients' subjective assessment of pain level on the VAS scale for CBCT-guided procedures was 4. After the CBCT-guided procedures, twelve patients (60 %) at least once experienced headaches or low back pain (Table 3). Two patients (10 %) additionally experienced pain in the upper extremity due to positioning during the procedure. One patient (5%) reported radiating pain in the leg. AEs were labeled as mild, since no or nominal therapy was required.[22] There were no AEs in the remaining 5 patients (25%).

Discussion

We report our experience on implementation of lumbar spine CBCT-guided intrathecal nusinersen delivery in consecutive adult SMA patients with challenging access. Evidence gained support good technical success, safety, and feasibility of CBCT-guided intrathecal nusinersen delivery.

The patient selection in our study was multidisciplinary, prospective, and based on inclusion criteria which considered the anatomy of the adult SMA patients. Additionally, to criteria regarding scoliosis and history of corrective surgery, we also acknowledged the patients' general constitution. This problem was so far addressed in only one study in a patient with BMI of 28, in which the intrathecal administration was performed under CT-guidance.[10]

Only few studies have reported their experience in CBCT-guided intrathecal administrations

of nusinersen.[11,18,19] An early study utilized CBCT for needle positioning in three TF procedures in children.[18] The authors reported later switch to fluoroscopy guidance as the performing physicians gained confidence with the intrathecal deliveries.[18] A later study analyzed lumbar punctures with TF approach in seven adult patients.[19] The latest study by Weaver *et al.* presented the largest group of 28 patients for CBCT-guided intrathecal nusinersen delivery.[11] In these studies the CBCT-guided lumbar intrathecal delivery was performed in both children and adult patients, whereas our study focused only on adult patients.[11,18,19] In our study CBCT-guided procedures were predominantly performed by the TF approach, a similar experience previously described by Weaver *et al.*[11] IL approach was not possible either due to severe scoliosis or no visible interlaminar space on CT after the posterior fusion instrumentation. Therefore, our data is in line with previous study, which acknowledges that a growing population of SMA patients requires alternative to the IL approach.[18]

Technical success of the CBCT-guided administrations was achieved in all patients. Only in few procedures secondary success was noted. This is in accordance with the study by Weaver *et al.* which reported high primary success rate for TF approach performed by both CBCT and fluoroscopy.[11] Similarly, high technical success is reported in studies utilizing CT as image-guidance.[7,12,20,23] However, while we specifically determined the technical success according to successful approach to the intrathecal space, other studies defined technical successes ambiguously; namely a primary success in most of the studies was not defined.[7,12,20,23] Two CT-guided studies reported a high (95% and 96.2%) single puncture attempt, which was comparable to our primary technical success.[12,23]

In contrast to other CBCT studies, only mild peri-procedural AEs were reported in ours. Weaver *et al.* reported 4% occurrence of mild AEs such as radicular pain and headaches as well as 0.5% of severe AE such as meningitis.[11] Shokuhfar *et al.* recorded one case (10%) of bilateral radiculopathy.[19] Although no severe AEs were noted in our study, the aforementioned studies raise attention to the potential risks one must take into consideration before the procedure. In the study we also report AEs from the patients that underwent conventional lumbar punctures. In comparison to patients after the conventional lumbar puncture, patients after the CBCT-guided punctures reported lower intensity and duration of low back pain. This finding is contrary to the findings by Carrera-Garcia *et al.*[8] A possible explanation might be the high proportion of primary technical success which minimized the trauma to the spinal meninges and the peridural

TABLE 3. Adverse events for cone-beam CT (CBCT)-guided intrathecal nusinersen delivery patients and classical lumbar puncture patients

	CBCT- guided (n = 108)	Conventional lumbar (n = 112)	P-value
Headache occurence (%)	18 (17)	42 (37)	
Headaches VAS, median (range)	2 (0–10)	4.5 (0–10)	0.12
Headaches duration day, median (range)	0.05 (0–5)	2 (0–6)	0.05
Low back pain occurrence (%)	11 (10)	40 (36)	
Low back pain VAS, median (range)	0 (0–2)	2.75 (6)	< 0.01
Low back pain duration day, median (range)	0 (0–4)	2.45 (0–14)	< 0.01

VAS = visual analogue scale

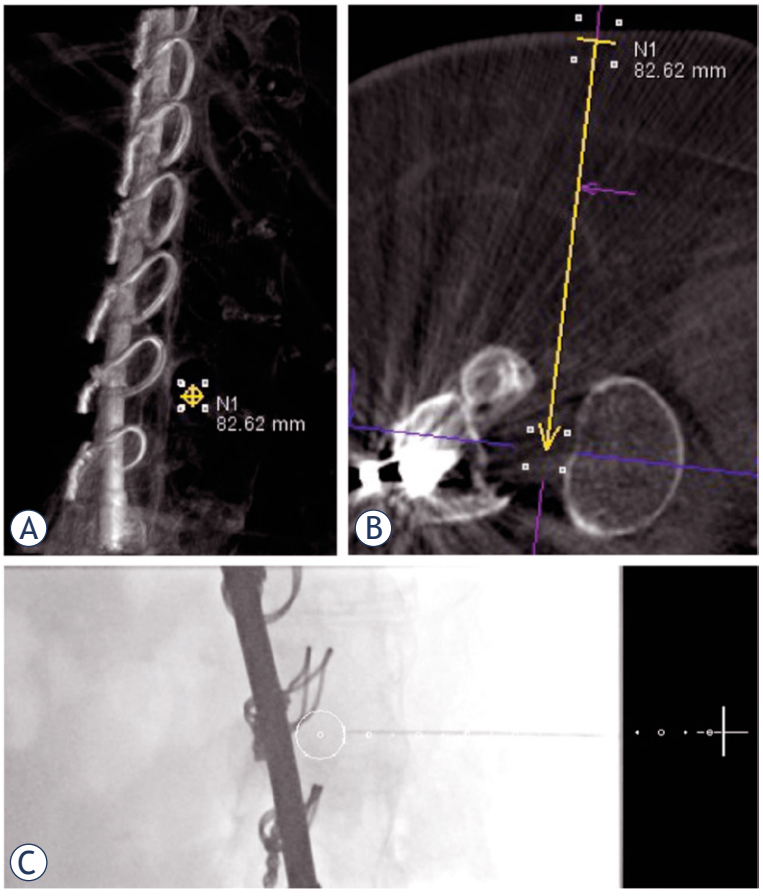

FIGURE 3. (A). A 42-year old female after corrective surgery for scoliosis; **(B).** Transforaminal approach planning before needle introduction (yellow arrow); **(C).** Introduction of the needle following the planned trajectory (white dotted line) to the target position (white circle).

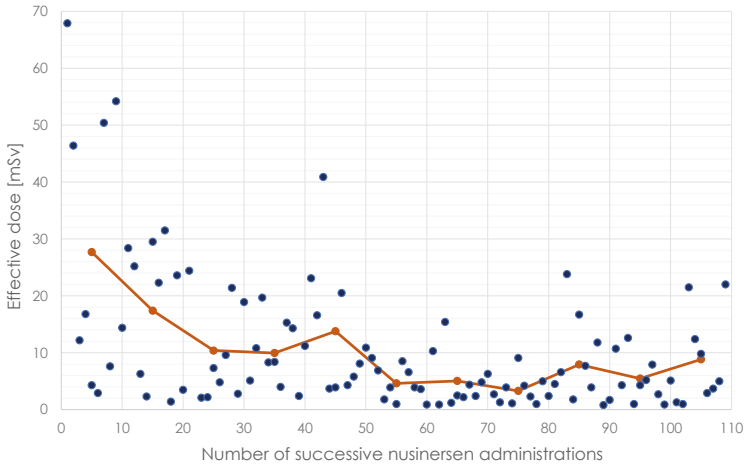

FIGURE 4. Scatter plot presenting effective dose for each cone-beam CT-guided procedure (blue dots) and calculated average trend line with orange dots for every ten procedures.

mSv = milli sievert

membrane which are abundant in nociceptors and other sensory receptors.[24]

Direct comparison with other CBCT-guided studies regarding radiation was not possible due to differences in reported units.[11,19] In comparison to our study, three CT-guided studies performed predominantly on adult patients reported lower ED of around 2.5 mSv, whereas Spiliopolus *et al.* reported higher average ED of 12.7 mSv.[3,7,23,25] Plausible rationale for these findings may be differences in population characteristics. Patients with posterior spinal instrumentation are expected to receive higher radiation exposure than the patients without fusion instrumentation.[25,26] Contrary to this observation our data did not reveal any ED differences regarding spine instrumentation. However, the EDs for the two obese patients proved to be higher in comparison to the other patients.

There was a decline in the average ED over the course of the study time, a finding that is in line with other studies.[12,26] The following factors need to be accounted for the initial high ED. The highest contributing factor to the initial high ED was patients' repositioning with CBCT reacquisition. The patients were repositioned as they either found the initial position uncomfortable or the target region was insufficiently depicted on acquired CBCT or the most comfortable patients' position did not allow optimal CBCT acquisition. Physicians' experience to adjust for specific anatomical considerations was also important in ED reduction. Thus, good cooperation between radiographers and performing physicians plays a key role in radiation exposure reduction. It is important to understand that each adult SMA patient is specific with differences in anatomical considerations, thus patient-tailored approach needs to be implemented.

There are few limitations that need to be noted. One might argue that this is only a single-center study. A multi-center comparison is not realizable since we are the only institution performing these procedures in our country. Furthermore, it would be difficult to standardize protocol in between organizations due to specifics of such a group. Here we report consecutive patients with standard protocol, which provides important insight in clinical work. Additionally, only partial comparison between other studies with CBCT-guidance was possible since there were differences in patient characteristics and methodology.[11,18,19]

Conclusions

This single-center prospective study supported the use of CBCT-guided lumbar intrathecal administrations of nusinersen in an adult SMA population with challenging access as feasible, technically successful, and safe image guided method.

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research article

Nanosecond electric pulses are equally effective in electrochemotherapy with cisplatin as microsecond pulses

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Background. Nanosecond electric pulses showed promising results in electrochemotherapy, but the underlying mechanisms of action are still unexplored. The aim of this work was to correlate cellular cisplatin amount with cell survival of cells electroporated with nanosecond or standardly used 8 × 100 µs pulses and to investigate the effects of electric pulses on cisplatin structure.

Materials and methods. Chinese hamster ovary CHO and mouse melanoma B16F1 cells were exposed to 1 × 200 ns pulse at 12.6 kV/cm or 25 × 400 ns pulses at 3.9 kV/cm, 10 Hz repetition rate or 8 × 100 µs pulses at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz repetition rate at three cisplatin concentrations. Cell survival was determined by the clonogenic assay, cellular platinum was measured by inductively coupled plasma mass spectrometry. Effects on the structure of cisplatin were investigated by nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry.

Results. Nanosecond pulses equivalent to 8 × 100 µs pulses were established *in vitro* based on membrane permeabilization and cell survival. Equivalent nanosecond pulses were equally efficient in decreasing the cell survival and accumulating cisplatin intracellularly as 8 × 100 µs pulses after electrochemotherapy. The number of intracellular cisplatin molecules strongly correlates with cell survival for B16F1 cells, but less for CHO cells, implying the possible involvement of other mechanisms in electrochemotherapy. The high-voltage electric pulses did not alter the structure of cisplatin.

Conclusions. Equivalent nanosecond pulses are equally effective in electrochemotherapy as standardly used 8 × 100 µs pulses.

Key words: electroporation; electrochemotherapy; nanosecond pulses; cisplatin

Introduction

Electrochemotherapy (ECT) is a local cancer treatment. The dominant mechanism of ECT is increased cellular uptake of impermeant or low permeant anticancer drugs with high intrinsic cytotoxicity - most commonly bleomycin and *cis*-diaminedichloroplatinum(II) (cisplatin) - due to transiently increased membrane permeability of cells/tumors after exposure to short high-voltage electric pulses.[1]

Over the past ten years, the number of ECT treatments performed for superficial tumors has increased dramatically and new indications have been added, such as treatment of skin metastases from visceral or hematological malignancies, vulvar cancer, deep-seated malignancies, and some noncancerous skin lesions.[2] ECT has become

broadly accepted mainly because of its simplicity (it is easy to master) and versatility (it allows treating a variety of cancers). Its efficacy, tolerability, and high patient satisfaction have been demonstrated in several studies, but also some side effects have been reported. According to the reports, the main side effects are unpleasant sensations, which can be painful, and muscle contractions triggered by applied high voltage electric pulses.[3,4] Most commonly, electric pulses are administrated as trains of eight monophasic pulses with a duration of 100 μs at 1 Hz or 5 kHz pulse repetition rate.

Nanosecond pulses have shown potential advantages over micro- and millisecond pulses in electroporation-based applications. The use of pulses with high electric field strength, but very short duration (i.e., in the nanosecond range) results in low energy transfer by the pulses to the treated volume, resulting in a low heating[5,6] and thereby minimizing the possibility of thermal damage to the tissue, which is very important for sparing delicate structures in and around the treated area.[7] In addition, nanosecond pulses limit electrochemical reactions at the electrode-electrolyte interface[8] which may affect the treated medium or cells/tissues.[9-11] Although a much higher electric field strength is required to achieve a comparable biological effect, excitation thresholds appear to be higher than the electroporation thresholds with nanosecond pulses[12-16], implying that shortening the pulse duration to nanosecond pulses could also reduce neuromuscular stimulation in electroporation-based applications.

Recently, nanosecond pulses have been explored in ECT and calcium electroporation and have shown promising results – either tumor regression *in vivo* or a decrease in cell survival *in vitro*.[8,17-21] We have previously reported that nanosecond pulses of an appropriately chosen amplitude in combination with cisplatin decreased cell survival in *in vitro* assays to the same extent as standard 8 × 100 μs pulses.[8] The aim of our present work was to investigate the underlying mechanisms of ECT with nanosecond pulses and cisplatin *in vitro* on Chinese hamster ovary CHO and mouse skin melanoma B16F1 cells. Two nanosecond pulse protocols (1 × 200 ns pulse at 12.6 kV/cm and 25 × 400 ns pulses at 3.9 kV/cm, 10 Hz repetition rate) were compared with 8 × 100 μs pulses at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz repetition rate standardly used in ECT. Accumulation of cisplatin and cell survival after *in vitro* ECT were measured and effects of high voltage electric pulses on the cisplatin molecular structure were investigated by nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HRMS).

Materials and methods

Cell culture of Chinese hamster ovary (CHO) cells and *in vitro* cell survival after ECT experiment protocols were described previously.[8] Mouse skin melanoma cell line B16F1 (European Collection of Authenticated Cell Cultures, cat. no. 92101203, Sigma Aldrich, Germany, mycoplasma free) was cultured in the same way as CHO cells except that Dulbecco's Modified Eagle Medium (DMEM, cat. no. D5671, Sigma-Aldrich, Missouri, United States) supplemented with 10% FBS (cat. no. F9665, Sigma-Aldrich), 2.0 mM L-glutamine, 1 U/ml penicillin/streptomycin and 50 μg/ml gentamycin was used instead of Nutrient Mixture F-12 Ham. Briefly, cisplatin (Cisplatin Kabi, 1 mg/mL, Fresenius Kabi, Germany or Cisplatin Accord, 1 mg/ml, Accord, UK) diluted in saline was added to cells suspended in complete growth medium DMEM just before electroporation so that the final concentration was 4×10^6 cell/ml and 0, 10, 30 or 50 μM cisplatin. The cell suspension was exposed to monophasic rectangular pulses (1 × 200 ns pulse at 12.6 kV/cm or 25 × 400 ns at 3.9 kV/cm, 10 Hz repetition rate or 8 × 100 μs at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz pulse repetition rate) or no pulses (non-electroporated controls). Cell survival was determined by the clonogenic assay.

For determination of cellular cisplatin, 125 μl of the treated cell suspension was diluted 40–100 times in complete growth medium Ham F-12 (CHO) or complete growth medium DMEM (B16F1) 25 min after electroporation (or addition of cisplatin/saline for non-electroporated controls) and centrifuged at 900 g for 5 min at 23°C in 15 ml centrifuge tubes. The supernatant was separated from the cell pellet and the pellet was washed with 2 ml saline and centrifuged again. After centrifugation, saline was discarded, and the cell pellet was kept at −20°C until digestion. For digestion, 0.1 ml H_2O_2 and 0.1 ml HNO_3 (both from Merck, Germany) were added to the cell pellets, and the tubes were closed and sealed with Teflon tape and left overnight at 80°C. After digestion, 1.8 ml of Milli-Q water (18.2 MΩ obtained from a Direct-Q 5 Ultrapure water system, Merck Millipore, Massachusetts, USA) was added and samples were measured by inductively coupled plasma mass spectrometry (7900 ICP-MS Agilent Technologies, Japan) with ^{193}Ir used as an inter-

nal standard during the measurement. The experiments were repeated 4–7 times. The number of cisplatin molecules per cell was calculated by first dividing the measured total mass of Pt in the cell pellet by the number of cells in the pellet, then subtracting the average mass of Pt per cell of non-electroporated cell pellets that were not incubated with cisplatin, and finally calculating the number of cisplatin molecules per cell from the difference of the mass of Pt per cell in samples (assuming 1 mol of Pt is equivalent to 1 mol of cisplatin).

Cell survival and amount of Pt data (after outliers, defined using the interquartile range method, were removed) were analyzed using the Kruskal–Wallis test and p-values were adjusted with the post-hoc Holm method test (α = 0.05) because the Shapiro-Wilk normality test failed (α = 0.05). The Spearman correlation coefficient was calculated to test the correlation between the number of cisplatin molecules per cell and cell survival. The data were processed and visualized using Microsoft Excel 2016 and R 3.6.1.[22]

Potential structural changes of cisplatin in the solution treated with high voltage electric pulses were investigated by NMR spectroscopy and HRMS. For practical reasons, both microsecond and nanosecond pulses were delivered to electroporation cuvettes with 2 mm gap with the laboratory prototype pulse generator based on an H-bridge digital amplifier for this set of experiments. For microsecond pulses, 8 × 100 μs at 1.1 kV/cm at 1 Hz pulse repetition rate were delivered (same pulse protocol as in cellular electrochemotherapy experiments). For nanosecond pulses, 25 × 400 ns at 2.2 kV/cm at 10 Hz repetition rate were delivered – the electric field strength for this pulse protocol was lower than in cellular electrochemotherapy experiments because of the technical limitations of the prototype pulse generator. 1 × 200 ns pulse was not applied because the pulse generator used is not capable of generating such short pulses. ^{1}H NMR spectra were obtained on NMR Bruker Ascend™ 600 MHz spectrometer at room temperature at 600 MHz. Chemical shifts, reported in ppm, are referenced to residual peaks of D_2O at 4.79 ppm. Spectra were recorded in D_2O (with and without NaCl) as well as in 90% H_2O/10% D_2O (with or without NaCl) using water suppression (WATERGATE) method. NMR data were processed with MestReNova 11.0.4. To approximately 1–2 mg of cisplatin (Sigma Aldrich) 1 mL of a) D_2O, b) D_2O containing 154 mM NaCl, c) 90% H_2O/10% D_2O or d) 90% H_2O/10% D_2O containing 154 mM NaCl was added. The obtained suspension was filtered through Minisart NML Cellulose Acetate Syringe Filter (28 mm, 0.2 μL). ^{1}H NMR spectra were recorded immediately after the filtration when not treated with any pulse protocol or directly after microsecond or nanosecond pulse application. HRMS spectra were recorded on Agilent 6224 Accurate Mass Time of Flight (TOF) Liquid Chromatography-Mass Spectrometry (LC-MS) instrument using water-acetonitrile solution (80:20, v/v) as the mobile phase. Fragmentor voltage was set to 150.0 V. To approximately 1–2 mg of cisplatin (Sigma Aldrich) 1 mL of distilled water or saline was added and obtained suspension was filtered through Minisart NML Cellulose Acetate Syringe Filter (28 mm, 0.2 μL). Filtered solutions underwent a) no pulses, b) microsecond pulses, or c) nanosecond pulses application as mentioned above, followed by immediate injection of such solutions into the LC-MS.

Results

CHO and B16F1 cells were electroporated in presence of 10, 30 and 50 μM cisplatin with: 1 × 200 ns pulse at 12.6 kV/cm; 25 × 400 ns pulses at 3.9 kV/cm, 10 Hz pulse repetition rate; or 8 × 100 μs pulses at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz pulse repetition rate. The electric field strengths for specific pulse parameters were selected based on survival-permeabilization curves (refer to Vižintin *et al.*[8] for graphs for CHO cells and to Figure S1 in the Supplementary material for graphs for B16F1 cells).

Cell survival results after ECT determined by the clonogenic assay are shown in Figure 1. Survival data of CHO cells were combined from the previous[8] (for non-electroporated cells and cells electroporated with 25 × 400 ns and 8 × 100 μs pulses) and the present study (additional non-electroporated cells and cells electroporated with 1 × 200 ns pulse). As intended, electroporation alone (i.e., in the absence of cisplatin) did not decrease cell survival in both cell lines compared with the non-electroporated control for any of the pulse protocols tested. For the non-electroporated cells treated with cisplatin, a statistically significant decrease in cell survival was observed only for CHO cells at the highest (50 μM) cisplatin concentration tested. On the other hand, electroporation in the presence of cisplatin decreased cell survival except for B16F1 cells treated with 1 × 200 ns pulse. For CHO cells, 1 × 200 ns, 25 × 400 ns, and 8 × 100 μs pulse protocols were all equally effective at decreasing cell survival at all the three tested cisplatin concentrations (Figure 1A). In B16F1 cells, 25 × 400 ns and 8

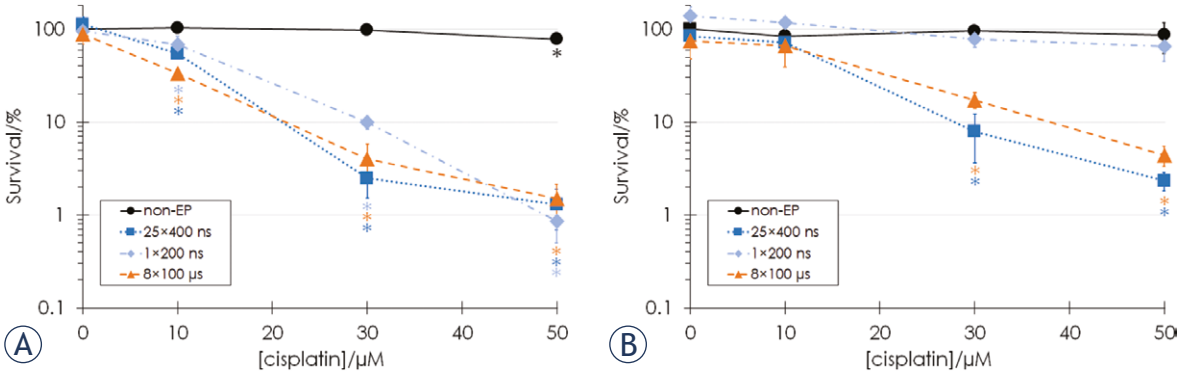

FIGURE 1. Cell survival of **(A)** CHO and **(B)** B16F1 cells at different cisplatin concentrations determined by the clonogenic assay for non-electroporated (non-EP) cells (black circles) and cells electroporated with 25 x 400 ns pulses at 3.9 kV/cm, 10 Hz repetition rate (dark blue squares), 1 × 200 ns pulse at 12.6 kV/cm (light blue diamonds) or 8 × 100 µs pulses at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz pulse repetition rate (orange triangles). Bars represent standard deviation, asterisks (*) show statistically significant differences ($p < 0.05$) to the survival of non-electroporated cells without cisplatin. Survival data were combined from the previous[8] (for non-electroporated cells and cells electroporated with 25 × 400 ns and 8 × 100 µs pulses) and the present study (for B16F1 cells, additional non-electroporated CHO cells and CHO cells electroporated with 1 × 200 ns pulse).

× 100 µs pulses were equally effective, whereas 1 × 200 ns pulse protocol was less effective (Figure 1B).

The amount of Pt in the cells was determined by measuring the total mass of Pt in the cell pellets by ICP-MS. Electroporation increased the cellular Pt amount. For both cell lines, there were no statistically significant differences in the measured Pt amount in cells electroporated with 25 × 400 ns or 8 × 100 µs pulses at the same cisplatin concentration. For CHO cells, the amount of Pt in cells electroporated with 1 × 200 ns pulse was statistically significantly lower compared to the amount of Pt in cells electroporated with 25 × 400 ns and 8 × 100 µs pulse incubated only at 50 µM cisplatin (Figure 2A). For B16F1 cells, lower cellular Pt was measured after application of 1 × 200 ns pulse compared to 25 × 400 ns and 8 × 100 µs pulses at all tested cisplatin concentrations (Figure 2B).

From the measured Pt content, the number of cisplatin molecules per cell was calculated and plotted against the cell survival data. The number of cisplatin molecules per cell and cell survival were more strongly correlated for B16F1 cells (Spearman's correlation coefficient: $\varrho = -0.85$, $p < 0.001$ for CHO and $\varrho = -0.92$, $p < 0.01$ for B16F1). In the case of CHO cells, at the same number of cisplatin molecules per cell, notably lower cell survival was measured for electroporated cells compared to non-electroporated cells (Figure 3A). For example, cell survival of 98% was achieved for non-electroporated cells with 9.4×10^6 cisplatin molecules per cell, whereas cell survival of 68.5%

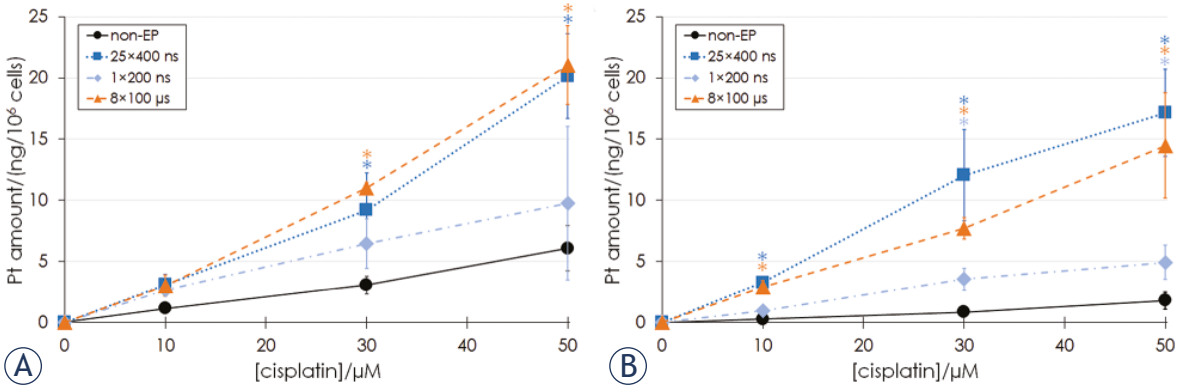

FIGURE 2. Pt amount in cell pellets of **(A)** CHO and **(B)** B16F1 cells after 25 min incubation at different extracellular cisplatin concentrations in non-electroporated (non-EP) cells (black circles) and cells electroporated with 25 x 400 ns pulses at 3.9 kV/cm, 10 Hz repetition rate (dark blue squares), 1 × 200 ns pulse at 12.6 kV/cm (light blue diamonds) or 8 × 100 µs pulses at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz pulse repetition rate (orange triangles). Bars represent standard deviation, asterisks (*) show statistically significant differences ($p < 0.05$) to the measured number of cisplatin molecules in non-electroporated cells at the same extracellular cisplatin concentration.

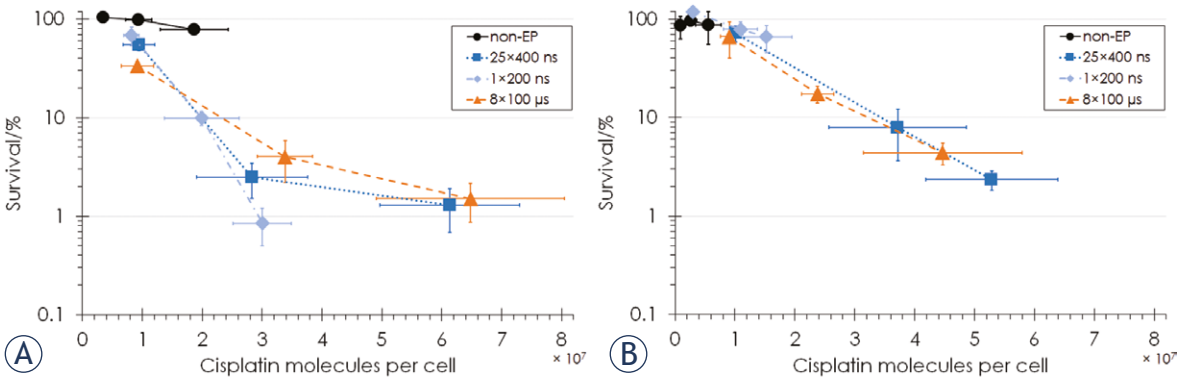

FIGURE 3. Cell survival as a function of the number of cisplatin molecules per cell for **(A)** CHO cells and **(B)** B16F1 cells in non-electroporated (non-EP) cells (black circles) and cells electroporated with 25 x 400 ns pulses at 3.9 kV/cm, 10 Hz repetition rate (dark blue squares), 1 × 200 ns pulse at 12.6 kV/cm (light blue diamonds) or 8 × 100 µs pulses at 1.1 (CHO) or 0.9 (B16F1) kV/cm, 1 Hz pulse repetition rate (orange triangles). Bars represent standard deviation. Survival data were combined from the previous[8] (for non-electroporated CHO cells and CHO cells electroporated with 25 × 400 ns and 8 × 100 µs pulses) and the present study (for B16F1 cells, additional non-electroporated CHO cells and CHO cells electroporated with 1 × 200 ns pulse).

was measured for cells electroporated with 1 × 200 ns pulse with 8.2 × 10^6 cisplatin molecules per cell, cell survival of 54.8% was measured for cells electroporated with 25 × 400 ns pulses with 9.5 × 10^6 cisplatin molecules per cell, and cell survival of 33.7% was measured for cells electroporated with 8 × 100 µs pulses with 9.2 × 10^6 cisplatin molecules per cell. From the data acquired, it could not be concluded if also in B16F1 cells a lower number of cisplatin molecules per cell causes a larger decrease in cell survival because the range of the number of cisplatin molecules in electroporated and non-electroporated cells did not overlap and thus survival could not be compared at approximately the same number of cisplatin molecules per cells (Figure 3B).

Cisplatin has been widely investigated for its biospeciation in aqueous solutions due to its diverse stepwise ligand displacement reactions.[23] Therefore, ^{1}H NMR spectroscopy was applied to investigate potential structural changes of cisplatin due to high voltage electric pulses. First, spectra of cisplatin in D_2O and D_2O with 154 mM NaCl (corresponding to physiological saline 0.9% NaCl) not exposed to electric pulses were recorded (Figure 4A–B). Weak broadened peaks for hydrogen atoms of amino ligands (NH_3) were found at approximately 4.08 ppm. Similarly, also representative peaks of cisplatin after treatment with 8 × 100 µs pulses at 1.1 kV/cm at 1 Hz pulse repetition rate or 25 × 400 ns pulses at 2.2 kV/cm at 10 Hz repetition rate remained at the same shift. The only major difference was observed in the spectrum of cisplatin recorded in D_2O with 154 mM NaCl after treatment with microsecond pulses (Figure 4B), where the broad peak for hydrogens of cisplatin disappeared. This can be attributed to the fast hydrogen-deuterium (H/D) exchange of deuterium from D_2O with hydrogen atoms of NH_3 ligands.[24] However, when spectra of cisplatin were recorded in 90% H_2O/10% D_2O solution containing 154 mM NaCl acquiring water suppression (to minimize the intensity of water signal to obtain a stronger signal of the NH_3 ligand) no such disappearance of the peak was observed (Figure 4D). Comparable spectra with peaks at 4.08 ppm were obtained also when no electric pulses or nanosecond pulses were applied. Similarly, the hydrogen peak of NH_3 was observed in the samples recorded in a 90% H_2O/10% D_2O solution without NaCl (Figure 4C). It is also important to note that no new peaks appeared in other regions of the NMR spectra.

High-resolution mass spectrometry (HRMS), which can also provide abundant information on molecular structure, was also performed to investigate possible newly formed cisplatin species. In some reports, authors detected hydrolysis products corresponding to mono-, di- and trimeric species, by mass spectrometry.[25-28] Therefore, HRMS was used in our structural investigation of cisplatin in water and saline (0.9% NaCl) exposed to micro- and nanosecond pulses.

First, cisplatin in H_2O was investigated and on the full-scan positive-ion mass spectrum (mass range of *m*/*z* 100–1100) presented in Figure S2 in Supplementary Material. It can be observed that the most abundant peaks occur in the mass range of *m*/*z* 280–330, where the following fragments were observed: [Pt(NH_3)$_2$(N_2)

Cl]$^+$ (*m/z* 292.9909), [M+NH_4]$^+$ (*M – indicates molecular formula for cisplatin, i.e. [Pt(NH3)2Cl2]*) (*m/z* 317.9872) (both Figure S3), [M+H]$^+$ (*m/z* 300.9601) (Figure S4), [Pt(NH_3)$_2$(CH_3CN)Cl]$^+$ (*m/z* 306.0101) (Figure S5) and [M+Na]$^+$ (*m/z* 322.9425) (Figure S6). Additionally, three lower abundant clusters can be found in the mass range of *m/z* 540–590. Two of them were identified as [Pt(NH_3)$_2$$Cl_2$·Pt($NH_3$)Cl]$^+$ (*m/z* 547.9121) and [Pt(NH_3)$_2$$Cl_2$·Pt($NH_3$)$_2$Cl]$^+$ (*m/z* 564.9378) (Figure S7). Additionally, one cluster at *m/z* 610–630 with the main ion fragment at *m/z* 617.9408 belongs to [2M+NH_4]$^+$ (Figure S8). Similar fragments have been observed when the samples were treated with micro- and nanosecond pulses (Figure S9–10 and Figure S11–S12). The species observed are in agreement with those reported in the literature.[26] Figure S19 represents the spectrum of water from the electroporation cuvette without the application of electric pulses. No differences were observed between the solutions treated with either nanosecond or microsecond pulses or untreated control.

HRMS experiments have been further performed in saline, where more extensive fragmentation was observed throughout the mass range of *m/z* 100–1100 (Figure S13). However, these peaks are comparable to the ones in the spectrum of saline from electroporation cuvette without the application of electric pulses (Figure S20). Similarly to spectra without NaCl, peaks of [Pt(NH_3)$_2$(N_2)Cl]$^+$ fragment and sodium [M+Na]$^+$ adduct were identified on zoom-scan spectrum (Figure S14). Again, spectra recorded in saline that was not treated with electric pulses are comparable with the spectra where cisplatin in saline solutions were treated with micro- and nanosecond pulses (Figures S15–16 and Figures S17–18, respectively).

Overall, NMR, as well as HRMS investigations, point to cisplatin remaining structurally comparable after the exposure to high voltage electric pulses similar to those used in *in vitro* ECT experiments with respect to its aqueous solutions without electric pulses.

Discussion

ECT has been shown to be a safe and effective cancer treatment, requiring much lower doses of the chemotherapeutic agent than conventional chemotherapy. However, pain and muscle contractions were reported as a drawback. Nanosecond pulses and high-frequency biphasic pulses of a few microsecond duration (H-FIRE)[29-31] were suggested

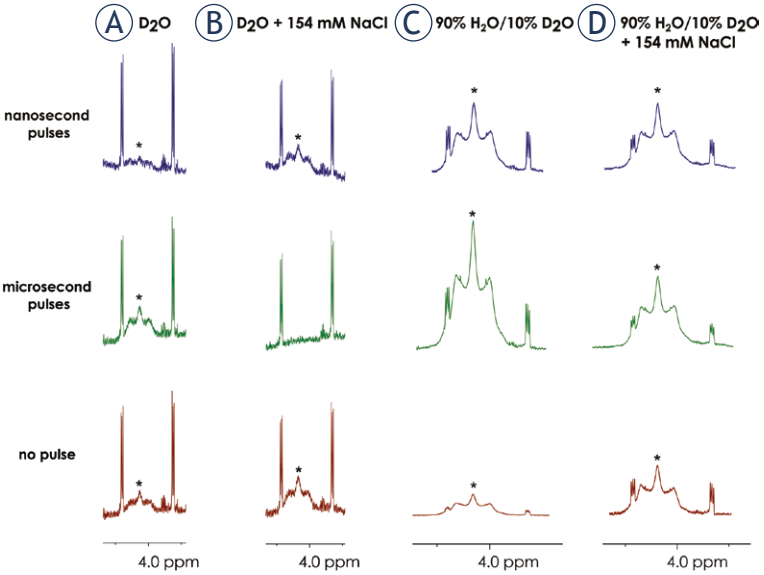

FIGURE 4. ^{1}H NMR spectra of cisplatin, showing the signals for hydrogens of NH_3 ligands labeled with asterisks (*). Spectra were recorded in a) D_2O, b) D_2O containing 154 mM NaCl, c) 90% H_2O/10% D_2O and d) 90% H_2O/10% D_2O containing 154 mM NaCl treated with 25 × 400 ns pulses (blue), 8 × 100 µs pulses (green) or no pulses (red).

to limit neuromuscular stimulation and contractions.[15,16] Additionally, with nanosecond pulses, the possibility of thermal damage to the tissue is minimized[5,6] due to low energy being transferred to the treated area and electrochemical reactions are reduced.[8] ECT with nanosecond pulses has shown promising results[8,17-19], but the underlying mechanisms of the observed decrease in cell survival and tumor regression remain to be explained.

In this study, we measured cell survival and cisplatin accumulation after *in vitro* ECT with 8 × 100 µs pulses, which are standardly used in ECT procedures, and equivalent nanosecond pulses, i.e. pulse protocols that have an equivalent biological effect on cell survival and cell membrane permeabilization. The electric field strength was chosen for each pulse protocol at a value that resulted in the highest permeabilization (determined as the percentage YO-PRO1 fluorescing cells) of the cell membrane without a decrease in cell survival (measured by the metabolic MTS assay). In the case of 8 × 100 µs pulses, 1.1 kV/cm was selected for CHO cells, but the survival for B16F1 cells was around 55% at this electric field strength, thus a lower (i.e. 0.9 kV/cm) electric field strength was used for electroporating B16F1 cells with this pulse protocol. For 25 × 400 ns pulses, the same electric field strength (3.9 kV/cm) was determined to be optimal for both cell lines. For 1 × 200 ns pulse, we used the highest experimentally achievable electric field

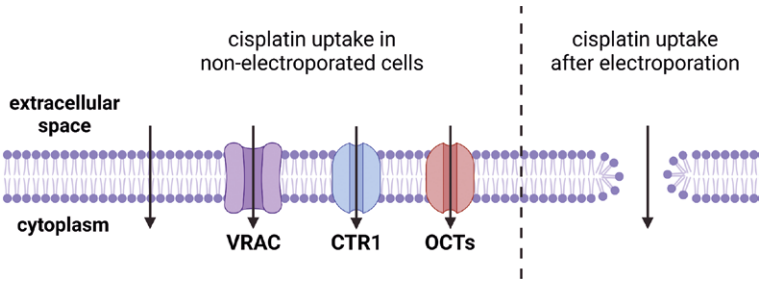

FIGURE 5. The mechanism of cisplatin uptake into cells is not completely elucidated. In non-electroporated cells, cisplatin enters partially through passive diffusion and facilitated diffusion through ion channels including LRRC8 volume-regulated anion channels (VRAC) and membrane transporters like copper transporter 1 (CTR1) and organic cation transporters (OCTs). In electroporated cells, more cisplatin can enter through the permeabilized cell membrane (pore is a symbolic presentation of increased membrane permeability even though the mechanisms behind electroporation are more complex – refer to[34]).

strength (i.e. 12.6 kV/cm), which did not decrease the cell survival in either cell line. Electroporating both cell lines with 8 × 100 μs or 25 × 400 ns pulses at the selected electric field strengths resulted in > 95% permeabilization (optimal for ECT), while for the 1 × 200 ns pulse at 12.6 kV/cm the permeabilization was 85% for CHO and only 42% for B16F1 cells (suboptimal for ECT). However, 1 × 200 ns pulse protocol was also included in the study based on results of cell survival of CHO cells after ECT determined by the metabolic MTS assay that showed that this pulse protocol was as effective in decreasing cell survival in ECT with cisplatin as the 25 × 400 ns protocol at all cisplatin concentrations.[8]

The aim was to test whether the combination of permeabilizing electric pulses (that alone do not cause a decrease in cell survival) and cisplatin results in increased cellular cisplatin accumulation (compared to non-electroporated cells) and whether the amount of cellular cisplatin is correlated to cell survival due to the increase of intracellular accumulation of the chemotherapeutic agent being one of the main mechanisms of action of ECT. To exert its cytotoxic effect, cisplatin must enter the cell. The exact mechanisms of cisplatin uptake have not been fully elucidated. Cisplatin is only slightly permeant; thus, it only partially enters the cell through passive diffusion across the cell membrane. Recent studies pointed out active transport mechanisms such as facilitated diffusion involved in cisplatin uptake - and LRRC8 volume-regulated anion channels (VRAC), copper transporter 1 (CTR1), and organic cation transporters (OCTs) were shown to be involved in cisplatin uptake.[32,33] Electroporation makes the cell membrane non-selectively permeable, allowing a larger quantity of cisplatin to enter the cell (Figure 5).

As expected, the measured amount of Pt was higher in electroporated cells when compared to non-electroporated cells incubated at the same cisplatin concentration, although the differences were not always statistically significant (Figure 2). These results indicate that the application of electric pulses indeed increases the intracellular accumulation of cisplatin. Overall, the amount of Pt in B16F1 was lower than in CHO cells exposed to the same cisplatin concentration, with or without electroporation, which also correlates with the higher cell survival of B16F1 cells (Figure 1). A comparison of cell survival of CHO and B16F1 cells with a similar number of cisplatin molecules per cell (Figure 3) reveals that a higher number of cisplatin molecules is needed to decrease the cell survival of B16F1 cells compared to CHO.

There were no statistically significant differences in the cell survival and amount of cellular Pt obtained in cells electroporated with 25 × 400 ns and 8 × 100 μs pulses at the same cisplatin concentration when comparing within the same cell line. Thus, it can be assumed that by using equivalent nanosecond pulses, it is possible to achieve the same decrease in cell survival and same cisplatin accumulation in cells and the as with the standard 8 × 100 μs pulses; in other words, equivalent nanosecond pulses are equally effective in ECT as 8 × 100 μs pulses.

The 1 × 200 ns pulse in combination with cisplatin did not decrease cell survival in B16F1 cells. This could be explained by the fact that 1 × 200 ns pulse permeabilizes less than half of the cell population of B16F1 and is also consistent with the measured Pt amount which was not significantly higher as in non-electroporated cells (Figure 2B). Application of 1 × 200 ns pulse alone (i.e., in the absence of cisplatin) seemed to even slightly promote cell growth (although the cell survival was not statistically significantly higher compared to the non-electroporated control). More interestingly, however, is that application of 1 × 200 ns pulse to CHO cells resulted in a lower amount of Pt in cells electroporated with 1 × 200 ns pulse as with 25 × 400 ns or 8 × 100 μs pulses, but the same decrease in cell survival was achieved with the 1 × 200 ns pulse as with 25 × 400 ns or 8 × 100 μs pulses. The lower amount of cisplatin in CHO cells electroporated with 1 × 200 ns could be explained *per se* by the fact that this pulse protocol achieved suboptimal cell membrane permeabilization compared to the 25 × 400 ns and 8 × 100 μs pulse protocols.

Nevertheless, a comparable decrease in cell survival was achieved, suggesting that increased accumulation of cisplatin into cells may not be the only cause of cell death in ECT. Figure 2A indicates that in electroporated CHO cells, a lower number of cisplatin molecules per cell is required to decrease cell survival to the same extent as in non-electroporated cells. Similar results have been reported previously in the literature[35,36], but not discussed. There may be a synergistic effect of cisplatin and electroporation, i.e., the observed decrease in cell survival in ECT is not the sum of the decrease in cell survival caused by electric pulses and cisplatin alone, but electroporation appears to make cells more susceptible to cisplatin.

The results of survival and number of internalized cisplatin molecules for B16F1 cells, however, do not show a similar synergistic effect of cisplatin and electroporation. Contrary to CHO cells, the number of cisplatin molecules per cell seems to linearly correlate with the logarithm of cell survival for B16F1 cells (Figure 3). Nonetheless, as mentioned above, lower cellular cisplatin was consistently measured for the B16F1 cell line and there is only one experimental point from the electroporated cells (cells electroporated with 1 × 200 ns pulse at 10 μM cisplatin) that falls in the range of the number of molecules of the non-electroporated cells. A similar number of internalized cisplatin molecules was measured for non-electroporated cells at 30 μM cisplatin and for cells electroporated with 1 × 200 ns pulse at 10 μM cisplatin, but the cell survival was even slightly higher for the latter. As discussed above, however, the 1 × 200 ns pulse protocol did not effectively permeabilize B16F1 cells. More data (from non-electroporated cells incubated at higher cisplatin concentrations) would thus be needed to determine if also in the case of B16F1 cells a lower number of internalized cisplatin molecules is needed to decrease cell survival in electroporated cells.

To test whether electric pulses could affect cisplatin by modifying the structure of the molecule as proposed in theoretical studies[37], we used NMR spectroscopy and HRMS spectrometry and found that the structure of cisplatin remains comparable after the application of electric pulses to either its saline or water solution (representing a simplified extra- and intracellular environment, respectively). Thus, high voltage electric pulses did not affect the structure of the studied complex under the conditions used in our experiments. Therefore, the reason for the observed increased susceptibility of the electroporated CHO cells to cisplatin is probably a consequence of the effect of electroporation on the cells. The cytotoxicity of cisplatin is thought to be mediated primarily by the formation of DNA adducts and the resulting impairment of transcriptional and/or DNA replication mechanisms. It was shown that electroporation increases the amount of cisplatin bound to the DNA, which could increase cisplatin cytotoxicity in electroporated cells.[35,38] However, additional mechanisms play an important role in exerting the toxic effects of cisplatin, including generation of ROS, mitochondrial dysfunction, increase in intracellular Ca^{2+} concentration, and activation of signal transduction pathways.[39] Electric pulses can also lead to generation of intracellular reactive oxygen species (ROS)[40,41], damage mitochondria[42,43], and disrupt calcium homeostasis through the entry of Ca^{2+} from the extracellular space or intracellular stores.[44,45] It has been shown that an increase in ROS enhances the efficacy of cisplatin and vice versa.[46,47] Moreover, an increase in intracellular Ca^{2+} concentration enhances cisplatin-mediated ROS production and increases cisplatin cytotoxicity.[48-50] This type of potentiation of cisplatin cytotoxicity may be responsible for the enhanced cisplatin cytotoxicity in electroporated cells, but it yet needs to be elucidated. Michel *et al*.[51] observed an increased immunoreactivity with SOD-2 (an enzyme that clears mitochondrial ROS) in cells subjected to ECT with cisplatin. To the best of our knowledge, this is the only report that measured ROS after ECT with cisplatin.

Our study also has limitations. Two different pulse generators and electrode geometries (i.e., electroporation cuvettes with 2 or 4 mm gap) were used in the cell experiments because of the technical limitations of the pulse generators used. Also in cell experiments, we did not directly measure the amount of cisplatin in cell pellets, but Pt was measured instead and assumed that cisplatin most likely accounts for the majority of the measured amount of Pt in cells incubated with cisplatin. This assumption is supported by the fact that the amount of Pt in non-electroporated cells that were not incubated with cisplatin was 2–3 orders of magnitude lower than in samples incubated with cisplatin or even below the detection limit. We also do not know whether the measured Pt was located inside the cells or was e.g. bound to the surface of the cell membrane. However, the formation of reactive hydrolyzed cisplatin products that would bind immediately and irreversibly to cell membrane phospholipids is not expected because the electroporation medium used has a high concentration of chloride ions so cisplatin should be stable in it and the measured Pt most probably

comes from intracellular cisplatin.[52] Additionally, in experiments investigating the effects of electric pulses on cisplatin structure, the conditions before the measurements by NMR spectroscopy and HRMS spectrometry could not be fully matched with the conditions in the cell experiments due to several reasons. First, it was namely not possible to record spectra of cisplatin in growth media due to many species present in the growth medium which interfere with cisplatin signals; thus, pulses were delivered to cisplatin dissolved in water or saline for NMR spectroscopy and HRMS spectrometry. Second, because of the limitations of the pulse generator used for NMR spectroscopy and HRMS spectrometry experiments, 25 × 400 ns pulses were delivered at lower amplitudes than in the cell experiments. Third, because of the difference in conductivity, electric pulses delivered to H_2O and D_2O had a notably different shape than pulses delivered to saline or cells in growth medium; due to the low conductivity of the load, they resembled an exponentially decaying rather than a rectangular pulse shape.

In conclusion, we have shown that by using equivalent nanosecond pulses in ECT, the same decrease in cell survival is achieved and the same amount of cisplatin accumulates in the cells as with the standard 8 × 100 μs pulses, i.e., that in ECT, equivalent nanosecond pulses are equally efficient as 8 × 100 μs pulses. By investigating the underlying mechanisms in nanosecond pulse ECT, we discovered that electroporated CHO cells are more susceptible to cisplatin than non-electroporated cells (regardless of the pulse protocol). The electric pulses used for electroporation do not appear to alter the structure of the cisplatin molecule, so the observed increased susceptibility is likely a consequence of the effect of electroporation on the cells. The use of nanosecond pulses in ECT is promising as it was demonstrated to be effective with the potential to mitigate muscle contractions. Because extensive preclinical data and solid evidence of mechanisms of action have been the basis for introducing ECT into clinical practice, further studies of nanosecond pulse ECT *in vivo* are necessary to enable translation into clinical trials.

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research article

Impact of *AKT1* polymorphism on DNA damage, *BTG2* expression, and risk of colorectal cancer development

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Background. AKT, also called protein kinase B, is a serine-threonine kinase that functions as a mediator of PI3K-Akt-mTOR signaling pathway and plays an important role in an array of cellular processes. Many single nucleotide polymorphisms (SNP) in *AKT* gene have been observed to be associated with various types of cancers. In the current research the association of a functional SNP rs1130233 in *AKT*, depicting G to A transition, was studied with AKT activation, DNA damage, an early response B-cell translocation gene 2 (Btg2) expression and risk of colorectal cancer (CRC) development.

Patients and methods. A total 197 population-based controls and 200 CRC patients were genotyped for SNP rs1130233. AKT expression, activation and *BTG2* expression were determined in GG, AG and AA genotype carriers. DNA damage was determined through comet assay.

Results. The heterozygous AG genotype (55.67%) was more prevalent in the local population compared to homozygous wild type GG (37.78%) and homozygous AA genotypes (6.55%). Moreover, AG and AA alleles were observed to be significant contributors (P = 0.01, OR = 1.80, CI = 1.18 to 2.74, and P = 0.001, OR = 5.00, CI = 1.90 to 13.18, respectively) in increasing the risk of CRC. The immunoblot analysis revealed that G to A transition decreased the expression and activation of AKT. Moreover, AG and AA genotypes of *AKT1* rs1130233 showed a significant increase in DNA damage and Btg2 expression.

Conclusions. The data concludes that G to A substitution is a risk factor for CRC development involving a decrease in AKT expression and activation and increase in DNA damage.

Key words: *AKT1;* BTG2; colorectal cancer; DNA damage; rs1130233

Introduction

Colorectal cancer is a multifactorial disease, and its life time risk in the general population increases ~5% with age.[1] This may be caused by carcinogenic compounds ingestion through foods and importantly individual differences in the metabolism of carcinogens as caused by both genetic and environmental risk factors which play essential roles in the development of colorectal cancer. Many genes sequence variations lead to the pathogenesis of inherited and sporadic forms of colorectal cancer.[2,3]

AKT, also known as Protein Kinase B (PKB), is a serine/threonine protein kinase which was originally discovered as an oncogene transduced by the acute transforming retrovirus (PKB-8/AKT8), isolated from mouse leukemia.[4,5] AKT is the downstream target of PI3K signaling that triggers a number of biological processes including cell survival, cell growth, glucose metabolism, angiogenesis, cell cycle entry cell motility, and also stimulate malignant transformation of cells and tumor progression. PI3K/AKT/mTOR pathway is one of the central nodes in many physiological abnormali-

ties including cancer.[6-10] Mammalian *AKT* gene has three isoforms; *AKT1*, *AKT2*, and *AKT3*. All these isoforms show broad tissue distribution and a broad range of functions. AKT1 also known as AKT kinase, is ubiquitously expressed isoform.[8,11]

Constitutive activation of AKT is mainly attributed to the aberrant activation of upstream signaling such as mutation or hyperactivation of receptor tyrosine kinases (Src, Ras and PTEN proteins) and increased synthesis of growth factors, as has been observed in several types of cancers.[12-14] Genetic variations in *AKT* gene (e.g. rs1130233, rs2498801, rs2494752) is linked with various types of cancers including liver, lungs and bladder cancers.[15,16] These polymorphic forms of *AKT* differ widely in their role as oncogene and exert their actions by regulating a diverse array of genes including NF-kB, Btg2 etc.

Human B-cell translocation gene 2 (*BTG2*), an ortholog of mouse *TIS21*, is a tumor suppressor gene that belongs to an antiproliferative gene family. BTG2 is implicated in a variety of physiological processes including cell differentiation, development, cell cycle arrest at G1/S and G2/M phases, cells death, DNA damage repair and antioxidant defenses. The downregulation of BTG2 thus has various physiological effects including cancer development.[17-20]

Genetic variability in *AKT* can affect an array of cellular processes including genes regulation, cancer development or regression etc. Previous studies have shown a strong correlation between *AKT* gene polymorphism and the prevalence of different types of cancers. The effect of genetic variability of this feedback loop hasn't been worked out on colorectal cancer development. It was hypothesized that sequence variations in AKT may affect colorectal cancer development *via* BTG2 regulation and DNA damage. The study was designed to identify a risk factor for the prevalence of colorectal cancer development and the possible underlying mechanism of tumorigenesis. The data from CRC patients and control individuals revealed that *AKT* rs1130233 single nucleotide polymorphism increases the risk of CRC development through increased DNA damage and downregulation of a tumor suppressor BTG2.

Patients and methods

Patients

This case-control study involved a total of 397 individuals including both colorectal cancer patients (CRC; n = 200) and population-based controls (n = 197). CRC patients (n = 200) from both sex, having age ≤ 60 and with documentary evidence of pathologically confirmed adenocarcinoma of colorectal cancer were included in this study. Subjects with mixed ethnic background, comorbidity, and patients who developed CRC at the age of above 60 years at the diagnosis were excluded. Determination of tumor stages and types were done by experienced pathologist at Institute of radiation and nuclear medicine (IRNUM), Peshawar. All patients and their guardians were informed about the nature of the study and important information of patients such as age, sex, ethnicity, medical records, pathology reports, drug history, family history, tumor size, tumor location and lymph node status etc. were obtained on a pre-designed proforma. Colorectal cancer risk factors such as taking red meat, vegetables, fibers, fruits and cooking choices and smoking history were also obtained. For control blood samples were collected from healthy individuals (n = 197) who had no sign of present or previous malignancy and no indication of CRC or nor any family history of cancer and had no blood relation with patients. Selection of control group of healthy donors was done on the basis of sex, age, smoking history and habits, residential, occupational and food intake. Informed consent of all the enrolled subjects was obtained on a questionnaire. The ethical approval was obtained from the institutional ethical board at Department of Biotechnology, University of Peshawar, Pakistan. Blood samples were collected both from colorectal cancer patients and controls at IRNUM Peshawar in 5 mL EDTA tubes and were stored at -20°C till further analysis.

DNA extraction and genotyping

DNA was extracted using DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific, USA) and was quantified using UV-visible spectrophotometer (752 PC, China). Akt single nucleotide polymorphism was determined using polymerase chain reaction (PCR, Multigene Optimax, Labnet International, USA). PCR was performed in a 20 μL reaction mixture using allele specific primers. The sequences of primers and amplification condition are given in Table 1. The AA (379 bp), AG (245 and 379 bp) and GG (245 bp) genotypes were visualized with ethidium bromide and identified on agarose gel (2%) using UV transilluminator (Wealtec, USA).

TABLE 1. Primers sequences and amplification conditions for genes

Genes	Direction	Primer sequence	Amplification condition
BTG2	Forward	5′-CCTGGGCAGAGAGTGAAAAG-3′	95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 58°C for 45 s, 72°C for 45 s and 72°C for 10 min
	Reverse	5′-CCTTCCATCCTAACCCCAAT-3′	
GAPDH	Forward	5′-CCATGGAGAAGGCTGGGG-3′	
	Reverse	5′-CAAAGTTGTCATGGATGACC-3′	
AKT1	Forward	F1-5′-ATAGGGAGTCATGGAGGGTTTG-3′	95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 45 s, 72°C for 45 s and 72°C for 10 min
	Reverse	R1-5′-CTTTACCAAATCCTGGTCACTGAA-3′	
	Forward	F2-5′-AAAAAATTGATTGATGGGAGGAAG-3′	
	Reverse	R2-5′-TAATCCCTGGCCTGCTCAG-3′	

Isolation of lymphocytes

Lymphocytes were isolated from fresh blood as described previously.[21] Briefly, blood containing EDTA was mixed with phosphate buffer saline (PBS; Ca^{+2} and Mg^{+2} free) and layered over 2 mL ficoll / lymphocytes separation medium (LSM™1077; Catalog Number: HiSep LSMTM 1077-LS001) in a 15 mL falcon tube. The mixture was centrifuged (2000 RPM for 30 min) that led to the formation of four distinct layers; the upper plasma layer, the second buffy coat layer containing lymphocyte and monocyte, the third ficoll layer (LSM) and the bottom layer of RBCs and cell debris. The buffy coat was isolated, mixed with 1 Ml PBS and centrifuged at 1500 RPM for 10 min. The pellet containing isolated lymphocytes washed with PBS, gently suspended in 1 ml PBS and used in subsequent experiments.

Comet assay for DNA damage

DNA damage in lymphocytes was assessed using comet assay, (also called single cell gel electrophoresis assay), as described previously.[22] Briefly, cells were fixed in ethanol for 20 min, then hydrated in distilled water for 30 min followed by staining. The slides were washed with cold distilled water and mounted with the cover glass. For scoring of DNA comets, 100 stained nuclei were selected randomly from each group under the fluorescent microscope at 200x magnification and images were recorded. Total comet score was calculated as described previously.[21]

Immunoblot analysis

The isolated lymphocytes were lysed in a buffer containing Tris (50 mM, pH 7.4, Nacl (150 mM), EDTA (1.0 mM), phenylmethylsulphonyl fluoride (1.0 mM), aprotinin (1.0 µg/ml), leupeptin (1.0 µg/ml), NaF (1.0 mM, 1.0 mM) sodium orthovanadate, sodium deoxycholate (0.25 %) and Nonidet P-40 (1.0%). The extracted proteins were quantified and electrophoresed on SDS-PAGE, transferred onto a nitrocellulose membrane using immunoblotting kit. Membrane was incubated with anti-Akt, anti-pAkt and anti-tubulin and proteins were detected using immunoblotting detection kit Ab Signal™ (AbClon, Seoul, Republic of Korea). Antibodies for AKT and pAKT were purchased from Cell Signaling Technology while α-tubulin from Santa Cruz Biotechnology. α-Tubulin was used as a loading control.

RNA extraction and polymerase chain reaction (PCR)

Total RNA was extracted from purified PBMCs using Trizol reagent. RNA was reverse transcribed to cDNA using reverse transcription kit (Invitrogen). *BTG2* expression was determined using conventional PCR followed by agarose gel electrophoresis. *GAPDH* was used as an endogenous control. The primer sequences and amplification conditions for *BTG2* and *GAPDH* are given in Table 1.

Statistical analysis

Data was analysed using Minitab® 17 and was presented as Mean ± SD. Odds ratio (OR), 95% confidence interval (CI) were used to find out the association between *AKT1* single nucleotide polymorphism and CRC risk. $P \leq 0.05$ was considered as statically significant.

Results

Association of Akt1 rs1130233 with risk of colorectal cancer development

Frequency of selected demographic and risk factors in CRC cases and controls

A total of 200 CRC patients and 197 age, and sex matched CRC free healthy subjects were enrolled

TABLE 2. Demographic and clinical information of control subjects and colorectal cancer patients

Characteristics	Cases n = 200(%)	Control n = 197(%)
Age*		
40 ≤	131 (65.5)	120 (60.91)
40 >	69 (34.5)	77 (39.09)
Sex*		
Male	119 (59.50)	112 (57.50)
Female	81 (40.50)	85 (42.50)
Food consumption*		
Mainly vegetables	106 (53.00)	97 (49.24)
Mixed Food	94 (47)	100 (50.76)
Smoking*		
Ever	36 (18.00%)	29 (14.72%)
Never	164 (82.00%)	168 (85.28%)
Cancer family history*		
Yes	27 (13.50)	17 (8.63)
No	173 (86.50)	180 (91.37)
Cancer Stages		
I	1 (0.50)	
II	33 (16.50)	
III	112 (56.00)	
IV	54 (27.00)	

*Non-significant (P > 0.05) difference between cases and control

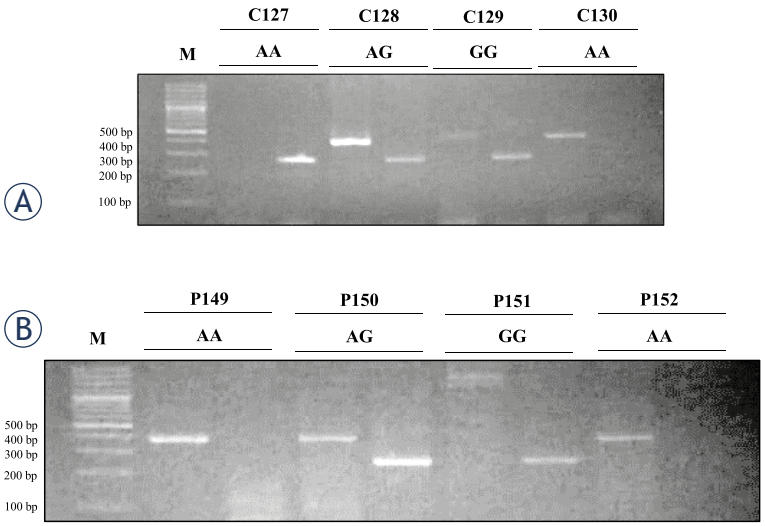

FIGURE 1. Akt rs1130233 single nucleotide polymorphism in **(A)** Control and **(B)** colorectal cancer patients. Representative images have been shown. AA genotype (379 bp band); AG genotype (245 and 379 bp bands); GG genotype (245 bp band). The number above the lanes indicate subjects identity.

C = Control, P = CRC Patient, M = DNA Marker

in this study. The data about the demographic information is given in Table 2. Among 200 clinically diagnosed CRC cases, there were 81 (59.50%) female and 119 (40.5%) male patients which shows that in female population of Khyber Pakhtunkhwa CRC frequency is relatively less than males as indicated by the higher incidence of CRC among males. There were 85 (42.50%) women and 112 (57.50%) men among the control group. The age and sex related differences were non-significant between the CRC and control groups (P > 0.05). The smoking status indicated that most of the subjects, including both patients and control, were non-smokers and non-significantly different in case and control cohorts (P > 0.05). Food intake especially vegetables consumption plays an important role in maintaining proper health, however, with regard to vegetable consumption the difference between control and patients was non-significant (P = 0.249). The family history data indicate that the prevalence of CRC was not linked with family history of any type of cancer as 173 CRC patients did not have family history of any type of cancer. All CRC patients were divided into four groups based on Tumor Node Metastasis (TNM) staging criteria; where patients with stage I: 1 (0.50%); stag II: 33 (16.50%); stage III: 112 (56.00%), and stage IV: 54 (27.00%).

Frequencies of Akt1 rs1130233 polymorphism and alleles distribution in colorectal cancer patients and control

Overall 397 subjects including 200 colorectal cancer patients (cases) and 197 healthy individuals (control) were enrolled in this study and genotyping of *AKT1* rs1130233 was performed to evaluate the status of rs1130233 polymorphism in case and control groups. Representative images of wild type GG, heterozygous mutatnt AG and homozygous mutatnt AA alleles for both control and CRC patients are given in Figure 1. The data has been presentated in Table 3. Among 200 colorectal cancer patients, 60 (30.0%) had wild type GG genotype, 120 (60.0%) had heterozygous mutatnt AG genotype while remaining 20 (10.0%) had homozygous mutant AA genotype. In control population, 90 subjects (45.69%) had GG genotype, 101 (51.27%) had AG and 6 (3.04%) had AA genotypes. The presence of AG and AA alleles were assoiated with

TABLE 3. Gene and allele frequencies of *AKT1* rs1130233 polymorphism and its association with colorectal cancer

Type of polymorphism	Genotype	Cases n = 200 (%)	Control n = 197 (%)	P value	OR (95% CI)
Genotype Frequency	GG	60 (30.00)	90 (45.69)		Reference
	AG	120 (60.00)	101 (51.27)	0.01	1.80 (1.18–2.74)
	AA	20 (10.00)	6 (3.04)	0.001	5.00 (1.90–13.18)
Dominant Model	GG	60 (30.00)	90 (45.69)	0.001	1.96 (1.30–2.96)
	AG+AA	140 (70.00)	107 (54.31)		
Recessive Model	GG+AG	180 (90.00)	191 (96.96)	0.01	0.28 (0.11–0.72)
	AA	20 (10.00)	6 (3.04)		
Allele Frequency	G	0.6000	0.7132		
	A	0.4000	0.2868		

colorectal cancer risk (OR = 1.80, CI = 1.18–2.74, P = 0.01 for AG and OR = 5.00, CI = 1.90–3.18, P = 0.001 for AA). The association between *AKT1* rs1130233 polymorphism and colorectal cancer was also assessed using dominent and recessive models. For dominant model (GG vs AG+AA), homozygous wild type (GG) was present in 60 patients (30.0%) and 90 controls (45.69%) while heterozygous and homozygous mutatant alleles (AG+AA) were collectively present in 140 patients (70.00%) and 107 controls (54.31%). An increased risk for colorectal cancer (OR = 1.96, CI = 1.30–2.96, P = 0.001) was observed for dominant model. For recessive model, (AA *vs* GG+AG), homozygous polymorphism was observed in 20 patients (10.00%) and 6 controls (3.04%) while homozygous wild type and heterozygous polymorphism (GG+AG) was collectively observed in 180 patients (90.00%) and 191 controls (96.96%). An increased risk for colorectal cancer was also observed for recessive model (OR = 0.28, CI = 0.11–0.72, P = 0.01). Similarly, G and A allele frequencies were 0.60 and 0.40 respectively for cases and 0.7132 and 0.2868 respecively for control and hence follows Hardy Weinberg equilibrium. Overall genotype frequency of GG, AG and AA for both cases and control was 150 (37.78%), 221 (55.67%) and 26 (6.55%) respectively indicating that heterozygous AG genotype is more prevalent than GG and AA.

Frequencies of *AKT1* rs1130233 polymorphism and alleles in colon cancer cases and control

The colorectal cancer patients were sub grouped into colon and rectum cancer pateints and their association *AKT1* rs1130233 polymorphism was determined (Table 4). Among overall 200 colorectal cancer pateints, 102 (51%) were colon cancer patients while 98 (49%) were rectum cancer patients. Among colon cancer patients, GG, AG and AA genoptes frequency were 29.41, 59.80 and 10.79% respectively. The AG and AA genotypes were assoiated with higher risk for development of colon cancer (OR = 1.81, CI = 1.08–3.05; P = 0.02 for AG and OR = 5.50, CI = 1.87–16.15; P = 0.001 for AA). An increased risk for colon cancer was observed for dominant (OR = 2.02, CI = 1.21–3.36; P = 0.006) and recessive (OR = 3.85, CI = 1.38–10.73; P = 0.01) models.

The association between *AKT1* rs1130233 polymorphism and rectum cancer was also evalutaed (Table 5). Among 98 rectum cancer patients, GG, AG and AA genotypes frequencies were 30.61, 60.20, and 9.19% respectively. Both AG (OR = 1.75, CI = 1.04–2.96; P = 0.04) and AA (OR = 4.50, CI = 1.48–13.69; P = 0.008) were assoiated with higher risk for development of recctum cancer. An increased risk for rectum cancer (OR = 1.91, CI = 1.14–3.18; P = 0.01) was observed for dominant and recessive models (OR = 3.22, CI = 1.11-9.32; P = 0.03).

Association of *AKT1* rs1130233 polymorphism with tumor location

On the basis of tumor location, the colorectal cancer patients were separted as colon and rectum cancer pateints and the association of *AKT1* polymorphism was assessed. Among overall 200 colorectal cancer pateints, 102 pateints (51%) had colon cancer while 98 patients (49%) were rectum cancer patients. Among 102 colon cancer patients, 32 pateints (31.37%) had GG, 67 patients (65.69%) had AG and 3 patients (2.94%) possessed AA genotypes. Among 98 rectum cancer patients, 36

TABLE 4. Frequencies of *AKT1* rs1130233 polymorphism and alleles in colon cancer cases and control

Type of polymorphism	Genotype	Colon n = 102 (%)	Control n = 197 (%)	P value	OR (95% CI)
Genotype frequency	GG	30 (29.41)	90 (45.69)		Reference
	AG	61 (59.80)	101 (51.27)	0.02	1.81 (1.08–3.05)
	AA	11 (10.79)	6 (3.04)	0.001	5.50 (1.87–16.15)
Dominant model	GG	30 (29.41)	90 (45.69)	0.006	2.02 (1.21–3.36)
	AG+AA	72 (70.59)	107 (54.31)		
Recessive model	GG+AG	91 (89.21)	191 (96.96)	0.01	3.85 (1.38–10.73)
	AA	11 (10.79)	6 (3.04)		

pateints (36.74%) had GG genotype, 55 patients (56.12%) had AG genotype while remaining 7 patients (7.14%) had AA genotype. The AG and AA polymorphism had equaleffect on the prevalce of both colon and rectum cancer as both of them showed non-significant difference (P > 0.05) for AG and AA transition.

Effect of rs1130233 on AKT protein expression and phosphorylation

To find out whether rs1130233 G to A transition can have effect on AKT expression, lymphocytes were isolated from various subjects of different genotypes (GG = 21, AG = 25 and AA = 06) and their AKT and pAKT proteins levels were determined using immunoblotting. The data indicated that G to A transition decreased AKT expression in both healthy in various individuals independent of their age, sex and health status (Figure 2A). The densitometry analysis revealed GG genotype carriers had significantly (P < 0.05) higher level of AKT followed by heterozygous AG carriers while the AKT expression was lowest in AA genotypes (Figure 2B). pAKT represents the active kinase, therefore, the phosphorylation status of AKT was also determined in GG, AG and AA carriers. pAKT level also showed a decreased intensity in GG>AG>AA order (Figure 2C). The data shows that substitution of G by A have a significant impact on AKT expression and activation and hence could have an effect on colorectal cancer development in different ways.

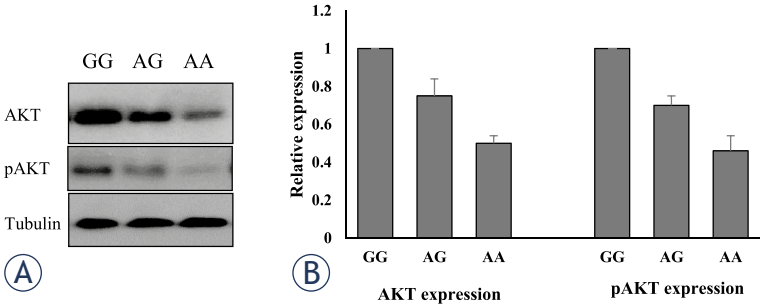

FIGURE 2. Association of *AKT* rs1130233 single nucleotide polymorphism with AKT expression and phosphorylation. **(A)** Representative images of immunoblotting showing expression of AKT and pAKT in lymphocytes of GG, AG and AA carriers. **(B)** Mean densitometry profile of AKT and pAkt expression of different subjects of various genotypes. P < 0.05 GG vs AG, and P < 0.01 GG vs AA.

Association of *AKT1* rs1130233 with DNA damage

To find out the association of rs1130233 with genome integrity, DNA damage was assessed by comet assay. Because cancer patients have multiple genes mutation leading to DNA damages, therefore comet assay was performed only in control individuals carrying GG (n = 13), AG (n = 15) or AA (n = 4) alleles. Because age and life style can have an impact on DNA damage, therefore comet assay was performed in individuals of similar age groups, non-smoking subjects and subjects with similar dietary habits. Moreover, the frequency of AA genotype carriers in control individuals was very less, therefore, the combined total comet score was calculated for AG and AA genotype carriers (AG+AA) (Figure 3). The total comet score for individuals carrying AA genotype was 190 ± 30.5, AG genotype (110 ± 20.54) and GG 63 ± 15.70. The total comet score of AA genotypes was significantly (P < 0.05) greater than AG and GG genotypes. Also, AG carriers had significantly higher (P < 0.05) comet score than GG genotype indicating a greater DNA damage. The data thus indicates that GG allele of *AKT1* contributes to genome stability.

TABLE 5. Frequencies of *AKT1*rs1130233 polymorphism and alleles in rectum cancer cases and control

Type of polymorphism	Genotype	Rectum n = 98 (%)	Control n = 197 (%)	P value	OR (95% CI)
Genotype frequency	GG	30 (30.61)	90 (45.69)		Reference
	AG	59 (60.20)	101 (51.27)	0.04	1.75 (1.04–2.96)
	AA	9 (9.19)	6 (3.04)	0.008	4.50 (1.48–13.69)
Dominant model	GG	30 (30.61)	90 (45.69)	0.01	1.91 (1.14–3.18)
	AG+AA	68 (69.39)	107 (54.31)		
Recessive model	GG+AG	89 (90.81)	191 (96.96)	0.03	3.22 (1.11–9.32)
	AA	9 (9.19)	6 (3.04)		

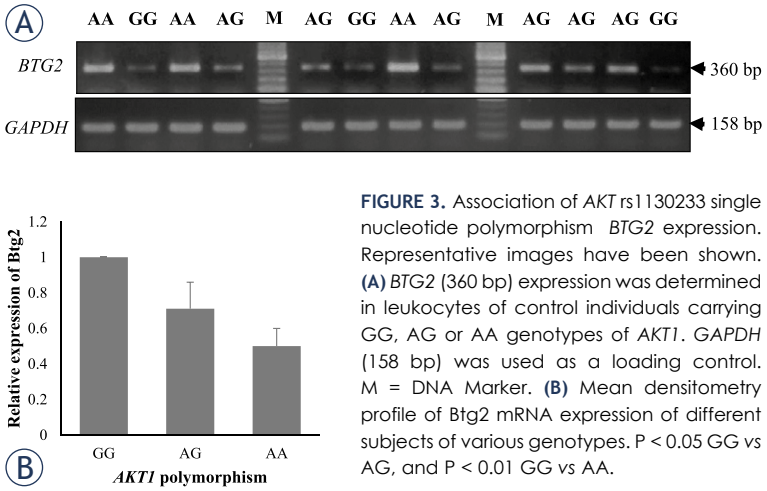

FIGURE 3. Association of *AKT* rs1130233 single nucleotide polymorphism *BTG2* expression. Representative images have been shown. **(A)** *BTG2* (360 bp) expression was determined in leukocytes of control individuals carrying GG, AG or AA genotypes of *AKT1*. *GAPDH* (158 bp) was used as a loading control. M = DNA Marker. **(B)** Mean densitometry profile of Btg2 mRNA expression of different subjects of various genotypes. P < 0.05 GG *vs* AG, and P < 0.01 GG *vs* AA.

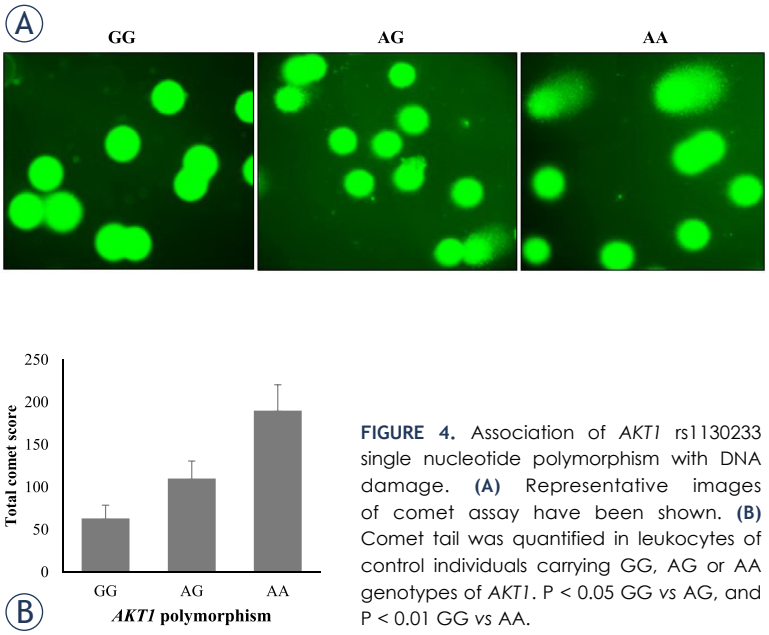

FIGURE 4. Association of *AKT1* rs1130233 single nucleotide polymorphism with DNA damage. **(A)** Representative images of comet assay have been shown. **(B)** Comet tail was quantified in leukocytes of control individuals carrying GG, AG or AA genotypes of *AKT1*. P < 0.05 GG *vs* AG, and P < 0.01 GG *vs* AA.

Association of *AKT1* rs1130233 with *BTG2* expression

Previously we have reported that AKT downregulates *BTG2* expression in various types of cells.[20] Moreover, BTG2 has been shown to be involved in DNA damage repair, we therefore determined the expression of BTG2 in GG (n = 20), AG (n = 24) and AA (n = 6) carriers of *AKT1* rs1130233 single nucleotide polymorphism. BTG2 expression was determined in lymphocytes of control individuals of similar age groups and life style. The *BTG2* expression profile and its densitometric analysis is given in Figure 4. The data shows that an inverse association between AKT activation and *BTG2* expression in genotype dependent manner. *BTG2* expression was significantly (P < 0.05) higher in AA and AG carriers compared to GG individuals indicating that various genotypes of *AKT* differentially regulate Btg2 gene expression and hence will impart distinct effect of various cellular processes.

Discussion

CRC is a multifactorial disease. Exposure to environmental toxins, life style and internal factors including genetic variations are important factors responsible for CRC development.[23] It has been demonstrated that lifestyle factors, including diet has a significant association with risk of CRC. Dietary pattern contributes to risk of CRC and mortality among CRC survivors. Higher intake of red and processed meat is associated with increased risk of CRC, while higher intake of vegetables, whole grains, dairy products, and fish show inverse associations with CRC risk.[24] In the current research project, vegetable consumption was however, not significantly associated with CRC risk, as nearly all patients were from low economic background

who most of the time rely on vegetable sources for their daily diet. Moreover, the smoking behavior in the current population is in general less and hence smoking was also a non-significant contributor to CRC risk in the current model, as most of the patients were non-smokers.

The genetic factor involving genes sequence variations have been linked with an increased risk for various types of cancers. AKT has a key role in controlling various cellular functions like cell growth, proliferation, DNA damage repair and cell survival etc.[25] Various research based evidences suggest that AKT is activated in various types of cancers.[16] Furthermore, genetic variations in *AKT* are reported to affect the AKT functioning and hence can have a crucial role in tumorigenesis.[26] So, we investigated the association between *AKT1* rs1130233 polymorphism and colorectal cancer risk in Pashtun population of Khyber Pakhtunkhwa Pakistan.

The presence of allele (AG/AA) of *AKT1* rs1130233 polymorphism was significantly associated with risk of colorectal cancer. The AA genotype was found to be more profound risk factor compared to AG. The association was also assessed using dominant and recessive genetic models and mutant polymorphic forms were observed to be the risk factors for colorectal cancer. The allele frequencies of *AKT1* rs1130233 differ widely in different ethnic groups. For example, the A allele frequency of 0.300 in Caucasians, 0.051 in Africans and 0.575 in East Asians (consisting of Japanese and Chinese)[27] and 0.3438 in Pashtun population of Pakistan**,** suggesting the population specific susceptibility to cancer. When patients were divided on the basis of age, sex, cancer history and food style, no significant differences in genotype frequencies were observed. The association of mutant alleles (AG and AA) with CRC was independent of patients' age, sex, and life style. A sub group analysis also showed an increased risk both for colon and rectum cancers.

AKT1 rs1130233 polymorphism has been observed to be associated with bladder cancer in Iranian population[16] and head and neck squamous cell carcinoma in Northeast Chinese population.[28] *AKT1* rs1130233 A/A genotype has also been observed to have a significant impact on drug response. Giovannetti *et al*. report that *AKT1* rs1130233 A/A genotype was associated with shorter time-to-progression (P = 0.04) and overall survival (P = 0.007) among non–small cell lung cancer patients treated with gefitinib.[29] Similarly, the *AKT1* rs1130233 has been found to play an important role in modulating the acute effects of delta-9- tetrahydrocannabinol-induced medial temporal function during fear processing, with these being associated with the A allele presence.[30] Furthermore, *AKT1* rs1130233 G/A+A/A genotypes have been observed to favor apoptosis, resulting in the higher risk of muscle atrophy and cachexia and weight loss in human cachexia cancer. The underlying mechanism involves the increased production of inflammatory cytokines in patients who suffer from tumor induced inflammation.[27]

Various genetic variations of *AKT*s, such as single nucleotide polymorphisms (SNPs), have also been well recognized to modulate gene function. The G to A substitution significantly decreased AKT1 expression and phosphorylation. The *AKT1* rs1130233 polymorphism is located in exon 8 and the G→A variation is located at the boundary of exon 8 and intron 7.[31] Because of this unique localization the G to A transition interferes the posttranscriptional modification of *AKT1* gene leading to decrease in its expression that in turn causes low AKT1 protein synthesis and activation.[32]

AKT1 rs1130233 (AG and AA genotypes) was observed to be linked with an increase in DNA damage. There are however, conflicting reports about the role of AKT in DNA damage. The deregulation of the PI3K-AKT/ mTORC1/ p70S6K pathway has been observed to have profound effects on genome stability *via* suppression of MRE11 expression leading to escalation of Ras-induced DNA damage.[33] Gol *et al.* has shown that both AKT1 and AKT2 isoforms are involved in radiation induced-DNA double strand break repair through homologous recombination in colon cancer cells.[34] Because AG and AA genotypes are characterized by a decrease in AKT activation (phosphorylation), which in turn leads to an increase in genome instability and hence provides a possible link between AG and AA genotypes and associated DNA damage.

AKT1 is shown to exert its effects through various mediators, such as protein kinases and phosphatases, survival factors, regulators of protein synthesis etc.[35] Previously we have reported that AKT increases cells survival and proliferation of cancer cells through downregulation of *BTG2* expression.[20] The current study shows a strong correlation between Btg2 upregulation and a decreased in AKT expression and activation as depicted in AG and AA carriers. Btg2 gene has also been shown to be upregulated in response to DNA damage and hence acts as a marker of DNA damage and repair pathway.[36]

In the current research a decreased in AKT expression and activation is linked with an increase in DNA damage indicating an important mechanism for *BTG2* upregulation. However, more work is required to underpin this signaling mechanism. The AA genotype of *AKT* rs1130233 is present at a low frequency, therefore a large set of population is required to further confirm the impact of AA allele in CRC. AKT is widely employed in a number of different types of cancers and it is important to determine the association of *AKT* rs1130233 polymorphism with other types of cancers also. Moreover, how G to A transition in AKT rs1130233 effects posttranscriptional modification of *AKT* needs to be addressed.

Conclusions

The present study concludes the possibly important role of Akt1 in the development of colorectal cancer. The study determined that *AKT1* rs1130233 polymorphism is a risk factor for the development of colon and rectum cancers and is significantly associated with DNA damage.

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research article

Real-life long-term outcomes of upfront surgery in patients with resectable stage I-IIIA non-small cell lung cancer

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Background. Treatment of early-stage non-small cell lung cancer (NSCLC) is rapidly evolving. When introducing novelties, real-life data on effectiveness of currently used treatment strategies are needed. The present study evaluated outcomes of stage I–IIIA NSCLC patients treated with upfront radical surgery in everyday clinical practice, between 2010–2017.

Patients and methods. Data of 539 consecutive patients were retrieved from a prospective hospital-based registry. All diagnostic, treatment and follow-up procedures were performed at the same thoracic oncology centre according to the valid guidelines. The primary outcome was overall survival (OS) analysed by clinical(c) and pathological(p) TNM (tumour, node, metastases) stage. The impact of clinicopathological characteristics on OS was evaluated using univariable (UVA) and multivariable regression analysis (MVA).

Results. With a median follow-up of 53.9 months, median OS and 5-year OS rate in the overall population were 90.4 months and 64.4%. Five-year OS rates by pTNM stage I, II and IIIA were 70.2%, 60.21%, and 49.9%, respectively. Both cTNM and pTNM stages were associated with OS; but only pTNM retained its independent prognostic value ($p = 0.003$) in MVA. Agreement between cTNM and pTNM was 69.0%. Next to pTNM, age ($p = 0.001$) and gender ($p = 0.004$) retained their independent prognostic value for OS.

Conclusions. The study showed favourable outcomes of resectable stage I–IIIA NSCLC treated with upfront surgery in real-life. Relatively low agreement between cTNM and pTNM stages and independent prognostic value of only pTNM, observed in real-life data, suggest that surgery remains the most accurate provider of the anatomical stage of disease and important upfront therapy.

Key words: resectable NSCLC; upfront surgery; real-life data; overall survival; prognostic factors

Introduction

Lung cancer is a major public health issue worldwide, with an estimated 2.2 million new cases and 1.8 million deaths in 2020 making it the second most common cancer and the leading cause of cancer death worldwide.[1] After decades of poor control of lung cancer, the mortality rates began to decrease in the last two decades.[1] This trend coincides with a slow, but steady increase in lung cancer survival rates, that was up to now mostly noticeable in localized (stage I and II) non-small cell lung cancer

(NSCLC). Currently the 5-year net survival of localized lung cancer is around 60%.[2,3]

Localized lung cancer accounts for around 25% of newly diagnosed lung cancers, with a vast majority of them having NSCLC histology.[3] Surgery with curative intent remains fundamental treatment for stage I–II and for selected stage IIIA NSCLC patients.[4] With the introduction of novel, less invasive surgical techniques, such as video-assisted thoracoscopic surgery and improved perioperative care, the outcomes of patients with resectable NSCLC improved substantially.[3,4] Platinum-based adjuvant chemotherapy, which is nowadays considered as a standard adjuvant treatment of early-stage NSCLC, further improved cure rates.[5] With the incorporation of novel targeted therapies and immunotherapy with immune checkpoint inhibitors (ICIs) additional increase in overall survival is expected. Targeted therapy with osimertinib, which led to significant reduction in distant recurrence or death in a prospective phase 3 trial has already been incorporated into treatment recommendations for epidermal growth factor receptor (EGFR) positive patients.[5] Based on the positive results of some recently published adjuvant trials, it is expected that ICIs will soon become a part of standard adjuvant therapy for early-stage NSCLC as well. There is growing evidence that neoadjuvant treatment with ICI leads to major or even complete pathologic responses in a substantial percentage of patients without compromising surgery for resectable NSCLC[6], thus making neoadjuvant immunotherapy an appealing approach in the future.

It is expected that the percentage of patients diagnosed with resectable NSCLC will increase in the next years. Several international clinical trials, including the European NELSON study confirmed the efficacy of low-dose CT screening in decreasing lung cancer mortality in the high-risk population of heavy smokers.[7,8] With the introduction of screening programs, we expect not only an increase of patients diagnosed with localized NSCLC but it might also become necessary to redefine treatment paradigms for early-stage NSCLC.

There is no doubt that major changes in the detection and treatment of early-stage NSCLC are expected shortly. To better predict and evaluate the effectiveness of those novel strategies in everyday clinical practice and to develop individualized risk-adjusted treatment strategies for individual patients, more data on clinicopathological characteristics and outcomes of early-stage NSCLC patients treated in a real-life before the introduction of those novelties, are needed. The International Association for the Study of Lung Cancer (IASLC) recommendations for TNM classification scheme, based on a database of nearly 90.000 patients[9] as well as some IASCL validation studies performed on the Caucasian population[10] provide valuable data on survival of patients treated in routine clinical practice. Next to the IASLC data, there is almost complete lack of information on the outcomes of the cohorts of resectable stage I–IIIA NSCLC patients, treated in a real-life scenario in the last decade. Most of the real-life observational trials reported recently present data for specific subpopulations of resectable NSCLC, such as patients treated with adjuvant chemotherapy[11] or patients with stage IIIA or N2 disease.[12-14] Our study aimed to evaluate overall survival of consecutive resectable TNM stage I–IIIA NSCLC patients treated with upfront radical surgery in a real-life practice, using prospectively collected hospital-based registry data. We also assessed the impact of clinicopathological characteristics, particularly TNM stage, on survival.

Patients and methods

Data source and study population

Data were retrieved from the hospital-based lung cancer registry, which prospectively collects demographics, clinicopathological, treatment, and survival data for all lung cancer patients diagnosed and treated at the centre. In hospital follow-up data are supplemented with the death certificates provided by the National Health Institute on a regular basis. All data was collected in an anonymised fashion. For the purpose of this study, survival status was updated and the data were retrieved in January 2020.

We retrieved the data of consecutive patients with resectable cTNM stage I–III NSCLC, treated with upfront radical surgical resection at a single thoracic oncology centre in Slovenia, between January 2010 and December 2017. All patients had pathologically confirmed NSCLC. Diagnostic and treatment procedures were performed as recommended by the international guidelines valid at the time.[15,16] Lymph nodes showing (18) F-fluorodeoxyglucose (FDG) uptake on preoperative PET-CT scans, or their short axis > 1 cm on CT scans were marked as clinically positive. In patients with clinically positive mediastinal lymph nodes endobronchial ultrasound-guided lymph node biopsy (EBUS TBNB) was performed, whenever

feasible. For all patients, including those with cN2 disease, the institutional multidisciplinary tumour board concluded that they have resectable NSCLC and were referred to upfront surgery.

All patients underwent radical surgical resection (R0) with lobectomy, bilobectomy, or pneumonectomy with complete lymph node dissection as a standard surgical procedur.[16,17] Adjuvant chemotherapy and/or postoperative radiotherapy were performed according to the international guidelines valid at that time.[15,16] Patients with neoadjuvant treatment were not included in the study population.

Clinical stage was defined as the last stage determined before surgical resection. All resected tissue including lymph nodes was examined by board certified pathologists. Clinical and pathological stages were assigned based on the 7th edition TNM classification for NSCLC[17], valid at the time. Testing for EGFR mutations and anaplastic lymphoma kinase (ALK) rearrangements has been introduced gradually as recommended by the international societies.[18] Testing was performed on formalin fixed, paraffin embedded tumour tissue specimens or different cytological specimens. For EGFR testing allele-specific PCR method with commercial kits, either Cobas EGFR mutation test (Roche, USA) or Therascreen EGFR PCR Kit (Qiagen, UK). ALK immunohistochemical detection was based on ALK CDx assay (Ventana, Roche, USA). Patients were followed-up with physical examination and chest CT scan, first biannually and after two years annually.

The hospital-based registry data collection and all subsequent analyses for academic purposes were approved by the Slovenian National Committee for Medical Ethics (approval number 135/07/09 and 40/04/12). All patients consented for data collection and subsequent analyses.

Outcome measures and statistical analyses

The primary endpoint was overall survival (OS), defined as the time in months from the date of surgery until either the date of death from any cause or the date the patient was last known to be alive (censored data). Patient and treatment characteristics were analysed using descriptive statistics. The agreement between clinical and pathological TNM staging variables was calculated as simple percent agreement to ease the interpretation of the results. Survival curves were estimated using the Kaplan-Meier estimator. The independent prognostic value of each included characteristic was tested in a Cox proportional hazards regression model. All variables with $p \leq 0.250$ in univariable regression analysis (UVA) were considered for and included in the multivariable regression analysis (MVA), except EGFR and ALK status due to being applicable only to a subset of patients. A p-value below 0.05 was considered statistically significant. All reported p-values are two-tailed. All statistical analyses were carried out using IBM SPSS Statistics software (version 21).

Results

We identified 539 consecutive stage I–IIIA NSCLC patients treated with upfront radical surgery. Demographic, clinicopathological, and treatment characteristics of the study population are presented in Table 1. The median age was 64 years (range, 39–83), males accounted for 58.4% of patients. Most patients were current or former smokers, with only 12.7% of never smokers included in the study. Adenocarcinoma appeared most frequently (63.3%), followed by squamous-cell carcinoma (36.2%) and other rare types of NSCLC (0.6%). EGFR mutations and ALK rearrangements were detected in 12.3% and 5.3 % of tested patients, with low completeness of ALK testing due to the introduction of testing to routine clinical practice from 2014 onward. Lobectomy was performed in a vast majority of patients, bilobectomy or pneumonectomy was required in only 5.8% and 9.1% of patients, respectively. Adjuvant platinum doublet chemotherapy was delivered in 146 (27.1%) of patients, the vast majority of whom had pathologically confirmed lymph node involvement. Postoperative radiotherapy was used in 36 (6.7%) patients; all of them had pathological N2 disease.

PET-CT was performed in 94.8% of patients (511/539). EBUS TBNB was gradually introduced in the routine clinical practice during the study period and was applied in 112 patients, with cN1 and cN2 disease according to CT and/or PET-CT scan. Lymph node involvement was confirmed in 65.5% of the samples obtained from the patients with cN2 disease. Mediastinoscopy was performed in five patients with cN2 and negative EBUS TNBN of mediastinal nodes; all lymph node samples obtained by mediastinoscopy were negative. Most patients were diagnosed with clinical stage I (57.3%) or stage II (26.9%). Clinical stage IIIA was determined in 15.8% of patients. All patients had either a single zone cN2 involvement or cT3/T4 disease without

TABLE 1. Demographic, clinicopathological and treatment characteristics of study population

Characteristic	N (%)
No. of patients	**539**
Age in years: median (range)	64 (39–83)
< 65 years	271 (50.3)
≥ 65 years	268 (49.7)
Gender	
Male	315 (58.4)
Female	224 (41.6)
Smoking status (n = 537; completeness = 99.6 %)	
Current	257 (47.8)
Former	212 (39.5)
Never	68 (12.7)
Histology	
Adenocarcinoma	341 (63.3)
Squamous-cell carcinoma	195 (36.2)
NSCLC other rare types	3 (0.6)
EGFR[a] status in non-squamous NSCLC (n = 334; completeness = 99.7%)	
Positive	41 (12.3)
Negative	292 (87.7)
ALK[b] status in non-squamous NSCLC (n = 334; completeness = 39.2%)	
Positive	7 (5.3)
Negative	124 (94.7)
Clinical TNM stage[c]	
I	309 (57.3)
II	145 (26.9)
IIIA	85 (15.8)
Clinical T stage	
T1	242 (44.9)
T2	193 (35.8)
T3	96 (17.8)
T4	8 (1.5)
Clinical N stage	
N0	393 (72.9)
N1	102 (18.9)
N2	44 (8.2)
Pathological TNM stage[c] (n = 532; completeness = 98.7%)	
I	296 (55.6)
II	150 (28.2)
III	86 (16.2)
Pathological T stage (n = 537; completeness = 99.6%)	
T1	223 (41.5)
T2	248 (46.2)
T3	58 (10.8)
T4	8 (1.5)
Pathological N stage (n = 534; completeness = 99.1%	
N0	386 (72.3)
N1	81 (15.2)
N2	67 (12.5)
Surgery type	
Lobectomy	459 (85.2)
Bilobectomy	31 (5.8)
Pneumonectomy	49 (9.1)
Adjuvant treatment	146 (27.1)
Platinum-based chemotherapy	36 (6.7)
Postoperative radiotherapy	

[a]EGFR: epidermal growth factor receptor; [b]ALK: anaplastic lymphoma kinase; [c]stage defined by American Joint Committee on Cancer staging

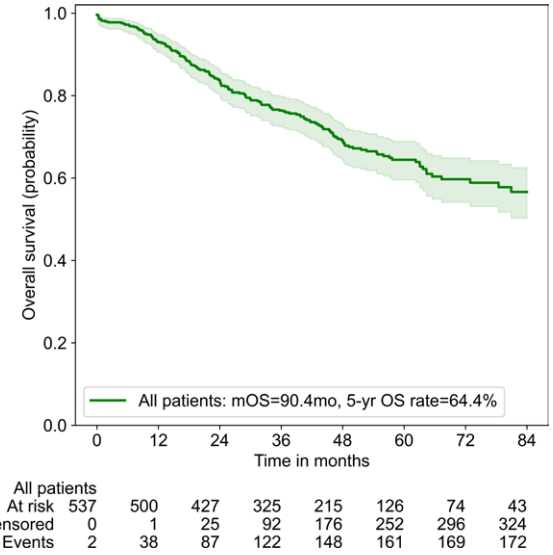

FIGURE 1. Overall survival of patients with completely resected stage I–III A non-small cell lung cancer.

tumour invasion to the adjacent vessels or organs. Postoperative pathological examination and staging also revealed high rate of pathological stage I (55.6%) or stage II (28.2%), with low percentage of stage IIIA disease (16.2%). However, the agreement between clinical and pathological staging was relatively low.

Table 2 shows the comparison between clinical (cTNM) and pathological (pTNM) staging according to TNM staging categories. The agreement between cTNM and pTNM stages was the highest for stage I (81%) and much lower for stage II (55%) and stage IIIA (49%). Of note, cTNM stage IIIA turned out to be pTNM stage II or stage I in 36% and 14% of patients, respectively. When analysing T and N descriptors separately, the accuracy of cT-descriptor decreased with increasing stage while for cN-descriptor the lowest accuracy rate was observed for cN1 stage. The overall agreement between clinical and pathological stage were quite similar for all three descriptors, TNM stage, T stage and N stage, i.e., 69.0%, 72.3% and 71.9%, respectively.

The median follow-up time was 53.9 (50.9–56.9) months. At the end of follow-up, 177/539 patients (32.8%) died. The median OS (mOS) for the whole cohort of patients was 90.4 months (95% CI calculation unreliable due to few events after mOS), with an estimated 5-year OS rate of 64.4% (Figure 1). The overall survival of patients grouped by cTNM, pTNM, cN and pN stage is depicted in Figure 2. The mOS has not been reached in the majority of the subgroups. The estimated 5-year OS rates for patients with cTNM stage I, stage II, and stage IIIA were 70.6%, 56.9%, and 55.3%; while the estimated 5-year OS rates for patients with pTNM stage I, stage II, and stage IIIA were 70.2%, 60.2%, and

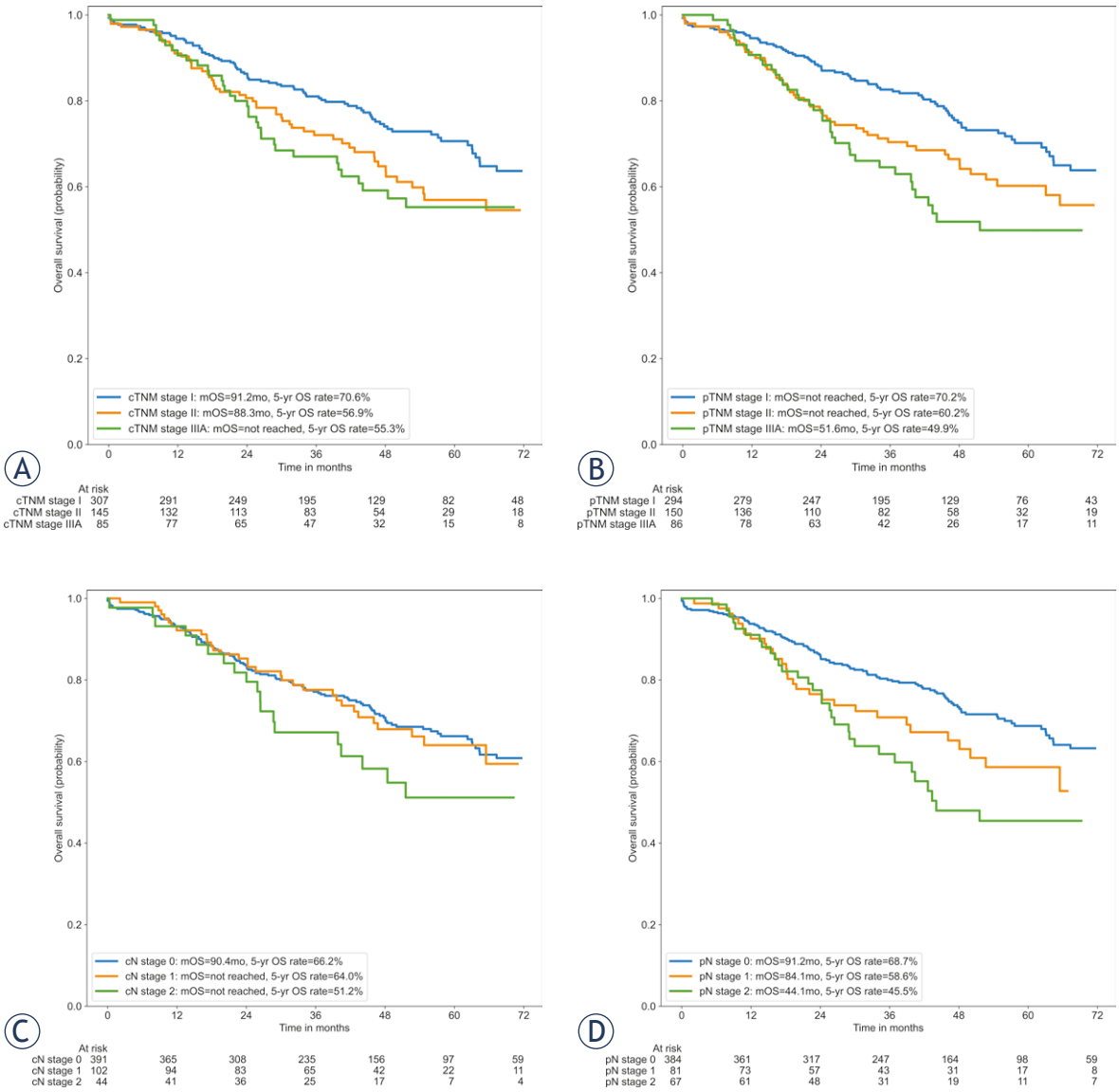

FIGURE 2. Overall survival by clinical TNM stage **(A)**, pathological TNM stage **(B)**, clinical N stage **(C)** and pathological N stage **(D)**.

49.9%, respectively, (Figures 2A and 2B). When observing the N status alone, Figures 2C and 2D show that pN stage provides a much clearer separation of survival curves than cN stage – as also demonstrated by UVA below.

In UVA the factors significantly associated with shorter overall survival were age ≥ 65 years and male gender. Furthermore, with respect to the anatomical stages, all stage categories, except cN (p = 0.313), were significantly associated with OS in the UVA (Table 3). However, in MVA that included either cTNM or pTNM stage as a determinator of the anatomical extent of disease, pTNM retained its significant and independent impact on OS (p = 0.003), next to age and gender, while cTNM stage lost its independent prognostic value (p = 0.092) (Table 4). Of note, TNM stage (clinical or pathological) was always included in the model for multivariate analyses, while the other factors were included in the stepwise procedure (thus only the significant factors are reported in Table 4).

Discussion

This observational cohort study presents real-life data on long-term survival and the impact of clinicopathological characteristics on overall survival

of resectable stage I–IIIA NSCLC patients, treated with upfront radical surgery at a single thoracic oncology centre in the period 2010–2017. The median OS time of 90.4 months and estimated 5-year survival rate of 64.4% observed in our real-life cohort of 539 consecutive patients are encouraging. Our data exceed the median OS of 63 months observed in a German cohort of patients with radically resected stage I–IIIB NSCLC, treated at a single academic centre in a very similar period (from 2009 to 2014), which also included patients with a higher stage IIIB disease.[10] When comparing by pTNM stage I, II and IIIA, the estimated 5-year survival rates of 70.2%, 60.2% and 49.9%, respectively, observed in our study, correspond very well to the 5-year survival rates in the German study.[10] Our findings also slightly exceed the 5-year survival rates of 83%–71%, 57%–49% and 36% for pTNM stage IA–B, II A–B and IIIA, published by IASLC.[9] Furthermore, our findings are also in line with 5-year survival rates between 37%–47%, observed in real-life cohorts of patients with resectable stage IIIA–N2 NSCLC, treated with upfront surgery in a similar period.[12-14] Thus, our observation supports the idea that selected patients with stage IIIA NSCLC might have a favourable outcome when treated by upfront radical surgery followed by adjuvant chemotherapy and/or irradiation.

As expected, the observed survival rates decreased with increasing stage of all staging variables (T, N, and TNM). But of note, while significant differences in survival were observed according to both clinical and pathological T and both clinical and pathological TNM stage, clinical N stage (as opposed to pathological N stage) did not prove a significant prognostic factor already in the UVA. Furthermore, in the multivariate analyses in which only TNM stage as a comprehensive denominator of T and N stages was included, only pTNM stage retained its significant and independent impact on overall survival, while cTNM stage failed to do so (likely due to its N stage part). This clearly points towards a much stronger prognostic value of pathological compared to clinical staging variables in resectable NSCLC. Also, in many previous studies evaluating prognostic impact of clinical and pathological TNM or N stage on OS the information on pathological stage improved prognostic value of the model.[9,14,17] There is evidence suggesting quite a high rate of disagreement between clinical and pathological staging in operable NSCLC patients treated in everyday practice. Even in studies performed after introduction of PET-CT and EBUS TBNB in routine clinical practice, relatively high rate of disagreement between clinical and pathological N and TNM staging was observed. In the Dutch observational study performed in patients with pathological stage IIIA disease, the agreement between clinical and pathological T and N stage was 57.1% and 28.5%, respectively.[19] The agreement rates observed in our study were relatively high for all three descriptors T, N and TNM stage (72.3%, 71.9% and 69.0%, respectively), but still not optimal. However, EBUS TBNB have only been introduced in our everyday clinical practice during the study period. With the incoming era of neoadjuvant systemic therapy, the accurate non-surgical staging of not only mediastinal lymph nodes but also hilar lymph nodes were becoming important. In our study the lowest agreement between clinical and pathological N status was observed particularly for cN1 stage (33%). Very interesting and clinically important observation is that almost half (48%) of cN1 patients were down staged to pN0, while upgrading to pN2 was found in a smaller, 19% proportion of patients. With recent dilemmas whether more invasive mediastinal lymph node

TABLE 2. Comparison between clinical (c) and pathological (p) TNM staging

2A. Comparison between clinical and pathological TNM stage (n = 532; completeness = 98.7%)

	c Stage I (N = 303) N (%)	c Stage II (N = 144) N (%)	c Stage IIIA (N = 85) N (%)
p Stage I	**246 (81%)**	38 (26%)	12 (14%)
p Stage II	40 (13%)	**79 (55%)**	31 (36%)
p Stage IIIA	17 (6%)	27 (19%)	**42 (49%)**

Overall agreement: 367 out of 532 cases (69.0%)

2B. Comparison between clinical and pathological T stage (n = 537; completeness = 99.6%)

	cT1 (N = 240) N (%)	cT2 (N = 193) N (%)	cT3 (N = 96) N (%)	cT4 (N = 8) N (%)
pT1	**187 (78%)**	24 (13%)	10 (10%)	2 (25%)
pT2	46 (19%)	**158 (82%)**	41 (43%)	3 (37%)
pT3	5 (2%)	9 (4%)	**42 (44%)**	2 (25%)
pT4	2 (1%)	2 (1%)	3 (3%)	**1 (13%)**

Overall agreement between: 388 out of 537 cases (72.3%)

2C. Comparison between clinical and pathological N stage (n = 534; completeness = 99.1%)

	cN0 (N = 388) N (%)	cN1 (N = 102) N (%)	cN2 (N = 44) N (%)
pN0	**324 (84%)**	49 (48%)	13 (30%)
pN1	42 (11%)	**34 (33%)**	5 (11%)
pN2	22 (6%)	19 (19%)	**26 (59%)**

Overall agreement: 384 out of 534 cases (71.9%)

TABLE 3. Univariate analyses of overall survival

Factor	p-value	HR (95% CI)
Age		
< 65		1
≥ 65	0.002	1.59 (1.18 – 2.15)
Gender		
Male		1
Female	0.001	0.59 (0.43 – 0.81)
Smoking status		
never		1
current or former	0.115	1.50 (0.91 – 2.47)
Histology		
adenocarcinoma or NOS		1
squamous cell carcinoma	0.111	1.28 (0.95 – 1.73)
EGFR status[a] (positive vs negative)		
negative		1
positive	0.111	0.56 (0.27 – 1.14)
Clinical TNM stage	0.027*	
I		1
II	0.034	1.44 (1.03 – 2.02)
IIIA	0.025	1.57 (1.06 – 2.34)
Clinical T stage	0.001*	
T1		1
T2	0.882	0.97 (0.69 – 1.38)
T3 or T4	0.001	1.86 (1.29 – 2.68)
Clinical N stage	0.317*	
N0		1
N1	0.958	0.99 (0.67 – 1.46)
N2	0.137	1.44 (0.89 – 2.34)
Pathological TNM stage	0.003*	
I		1
II	0.030	1.46 (1.04 – 2.06)
IIIA	0.001	1.90 (1.29 – 2.79)
Pathological T stage	0.007*	
T1		1
T2	0.019	1.49 (1.07 – 2.07)
T3 or T4	0.004	1.92 (1.23 – 2.98)
Pathological N stage	0.002*	
N0		1
N1	0.054	1.48 (0.99 – 2.20)
N2	0.001	1.93 (1.29 – 2.87)

[a]only in non-squamous NSCLC; *for the whole variable

staging might change the treatment paradigm and outcomes of NSCLC patients with cN1 disease our data become even more appealing.

Notably, the survival rates observed in our current study far exceed those observed in a retrospective analysis of NSCLC patients treated at our centre in 2006.[20] The latter revealed much shorter median overall survival rates for all clinical TNM stages I, II and IIIA NSCLC with the largest differences observed in stages II–IIIA. In that analysis all consecutive patients were included, regardless of whether they received treatment with curative intent or not, which is definitively one of the reasons for worse survival rates. But still, improvement in overall survival achieved over the last years is obvious. This can be attributed to major advances in diagnostic procedures, surgical techniques, postoperative care and adjuvant therapies for early NSCLC that we witnessed in the last decade and their rapid transfer into everyday clinical practice at our institution.[21]

The clinicopathological characteristics of our cohort of patients mirror the typical population of NSCLC patients in our country and region at the beginning of this century, with prevailing smokers and squamous-cell histology.[21] Next to pTNM stage, age and gender retained their significant and independent prognostic value for OS in MVA; while smoking status and histology failed to show prognostic value already in the UVA. Our results are in concordance with the observations made on a large series of patients with NSCLC confirming older age and male gender as independent prognostic factors for worse survival.[22,23] Male gender was confirmed as an independent prognostic factor for worse survival in published trials, however this has been seen particularly in patients with advanced NSCLC and adenocarcinomas.[23] In our study male gender turned out to be an independent predictor of worse survival in early-stage NSCLC and irrespective of histology, thus suggesting other probable causes of poor survival in male NSCLC patients which need to be further investigated.

Our study also provides valuable data on the frequency of EGFR mutations and their prognostic value in early-stage NSCLC. The findings are in line with the results of recently published large individual study[24] which failed to confirm prognostic impact of EGFR status on survival of patients with resectable NSCLC. There are still uncertainties about the percentage of EGFR mutated tumours in early-stage NSCLC. In our study, EGFR testing performed on a large series of 334 patients with resectable non-squamous cell NSCLC, revealed a 12.3% positivity rate which is quite comparable to the 13.8% positivity rate observed in advanced NSCLC in the countries and the centres which participated in the INSIGHT registry trial.[25] Similarly, ALK positivity rate of 5.3% observed in our series of resectable NSCLC corresponds very well with the positivity rates observed in advanced NSCLC.[26]

The results of our study should be considered in the context of its strengths and limitations. The study provides a wealth of information on clinicopathological characteristics and survival outcomes of a large cohort of resectable NSCLC patients, treated with upfront surgery in real-life practice. Additionally, all data were collected prospectively by the hospital-based lung cancer registry. Looking at potential limitations, results from a single centre study might not be generalisable to the overall population in the country or region. However, at our centre more than a half of the country's newly

diagnosed resectable NSCLC are treated, thus representing the entire population quite well. It is also encouraging that the activities on establishing a nationwide register of lung cancer patients collecting detailed data on clinicopathological characteristics and individual treatments at the Cancer Registry of Slovenia are ongoing. Since our hospital-based registry does not capture data on the cause of death, we do not present data on cancer specific survival but on overall survival, which might be influenced by comorbidities and other conditions often present in fairly old population of patients with resectable NSCLC. The hospital registry also does not collect precise data on modality of preoperative staging (imaging *versus* invasive procedures) to determine clinical N stage in each individual patient. Therefore, the data on mediastinal staging by EBUS TNBN and mediastinoscopy were collected retrospectively and might be subject to bias.

Our study with a lengthy follow-up, showed a favourable outcome for patients with resectable stage I–IIIA NSCLC treated with upfront surgery in a real-life setting. Particularly encouraging are the survival rates observed in patients with stage IIIA disease indicating that selected patients with N2 disease are candidates for upfront surgery. Relatively low agreement between cTNM and pTNM stages and the independent prognostic value of pTNM but not cTNM stage observed in our study, suggest that we should aim to further improve preoperative staging. Until then we should always weight our decisions about upfront treatment of resectable NSCLC very carefully for each individual patient. Currently, surgery remains the most reliable provider of information on anatomical TNM stage as one of the strongest prognostic factors and enables us to make an informed decision on adjuvant systemic treatment in each individual patient.

Finally, it is inspiring to notice a substantial improvement in overall survival rates of early-stage NSCLC patients treated over the last decades at the same large thoracic oncology centre. With the aim of further improving our results, we are planning an additional study which will strive to evaluate preoperative staging of nodal involvement more profoundly, thus providing for better multimodality treatment selection for each individual patient.

TABLE 4. Multivariate analyses of overall survival (separate for clinical and for pathological stage)

Cox regression model with clinical stage	p-value	HR (95% CI)
Age		
< 65		1
≥ 65	0.003	1.58 (1.17 – 2.14)
Gender		
Male		1
Female	0.006	0.63 (0.46 – 0.88)
Clinical TNM stage	0.092*	
I		1
II	0.078	1.36 (0.97 – 1.91)
IIIA	0.068	1.46 (0.97 – 2.18)
Cox regression model with pathological stage	**p-value**	**HR (95% CI)**
Age		
< 65		1
≥ 65	0.001	1.68 (1.24 – 2.28)
Gender		
Male		1
Female	0.004	0.62 (0.45 – 0.86)
Pathological TNM stage	0.003*	
I		1
II	0.076	1.37 (0.97 – 1.93)
IIIA	0.001	1.95 (1.32 – 2.88)

*for the whole variable

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The raw data underlying this article are available in the article. Due to data privacy, and hospital registry-related restrictions, the clinicopathological data cannot be made public, i.e., accessible to anyone for any purpose without a review process and without putting an agreement in place.

Data availability statement and author contribution statement

Marko Bitenc: conceptualization, writing – original draft, formal analysis, writing – review & editing. **Tanja Cufer**: conceptualization, formal analysis, writing – original draft, writing – review & editing, supervision. **Izidor Kern**: investigation, writing—original draft, formal analysis. **Martina Miklavcic**: data curation, investigation, writing – original draft, writing – review & editing. **Sabrina Petrovic**: data curation, investigation, writing – original draft. **Vida Groznik**: software, data curation. **Aleksander Sadikov**: software, formal analy-

sis, visualization, writing – review & editing, supervision.

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research article

Identification of women with high grade histopathology results after conisation by artificial neural networks

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Background. The aim of the study was to evaluate if artificial neural networks can predict high-grade histopathology results after conisation from risk factors and their combinations in patients undergoing conisation because of pathological changes on uterine cervix.

Patients and methods. We analysed 1475 patients who had conisation surgery at the University Clinic for Gynaecology and Obstetrics of University Clinical Centre Maribor from 1993–2005. The database in different datasets was arranged to deal with unbalance data and enhance classification performance. Weka open-source software was used for analysis with artificial neural networks. Last Papanicolaou smear (PAP) and risk factors for development of cervical dysplasia and carcinoma were used as input and high-grade dysplasia Yes/No as output result. 10-fold cross validation was used for defining training and holdout set for analysis.

Results. Baseline classification and multiple runs of artificial neural network on various risk factors settings were performed. We achieved 84.19% correct classifications, area under the curve 0.87, kappa 0.64, F-measure 0.884 and Matthews correlation coefficient (MCC) 0.640 in model, where baseline prediction was 69.79%.

Conclusions. With artificial neural networks we were able to identify more patients who developed high-grade squamous intraepithelial lesion on final histopathology result of conisation as with baseline prediction. But, characteristics of 1475 patients who had conisation in years 1993–2005 at the University Clinical Centre Maribor did not allow reliable prediction with artificial neural networks for every-day clinical practice.

Key words: uterine cervical dysplasia; uterine cervical cancer; conisation; artificial neural networks

Introduction

Cervical cancer is a preventable disease. Effective measures are organised cervical cancer screening programme in combination with vaccination against human papilloma virus (HPV) and treatment of precancerous lesions.[1] There are many risk factors, which can facilitate development of cervical dysplasia and cancer. Among them are early onset of sexual activity, multiple sex partners, parity, marital status, socioeconomic status, factors that influence persistent infection (genetics, sex hormones, immunological impairment as in human immunodeficiency virus (HIV) infection, sexually transmitted diseases (HPV, HIV, Herpes simplex virus [HSV], Chlamydia), factors related

to HPV (genotype, numbers of viral copies), long term use of hormonal contraception, smoking and obesity.[2-13]

HPV is very important risk factor necessary for development of cervical dysplasia and cancer. [14-15] After initiation of sexual activity, almost all women acquire infection with HPV. This infection can only be transitory, clears spontaneously and does not progress to dysplasia.[16] Patients aged 30–35 years are tested positive in 13.5% compared to 5.4% patients older than 35 years.[17]

In computer science artificial neural networks (ANN) are part of artificial intelligence and represent deep machine learning. ANN are nonlinear computational models. They are able to perform tasks, similar to human brain. Just by analysing examples (training set) can perform classification, decision-making, prediction, visualisation, recognition and other. The name neural networks came from similarities with structure and behaviour of that of human brain.[18] There are many types of different ANN. They are very important tool in processing large amount of data, image processing, image recognition, computer vision and natural language processing. Because of their ability to learn and make prediction make them very useful tool in medicine.[19,20] They are used in every day clinical practice in cancer diagnostics where they help radiologists to recognise pathological features, help to predict malignant tumour response to treatment, help in triage and others.[21-25]

This study has been designed to evaluate if neural networks can help us to identify patients with higher risk for high grade squamous intraepithelial lesion (HSIL) and cervical cancer based only on the evaluation of their risk factors for cervical dysplasia and result of the last Papanicolaou smear (PAP). If neural networks are successful in predicting high risk patients, we could use them to identify and take special measures in situation when such patients became non-responders in organised cervical cancer screening programme. With such special attention, we could prevent them from acquiring cervical cancer.

Patients and methods

Our study has been approved by Medical Ethics Committee of the Republic of Slovenia on 10. 11. 2015, No.: 0120-553/2015-2 KME63/11/15. Data from patients who had conisation in the years 1993–2005 were collected in database: age at the time of surgery, age at first intercourse, number of sexual partners, number of pregnancies (births, spontaneous and legal abortions), socio-economic status, marital status, type of contraception, smoking habits, menstrual pain, vaginal discharge, coagulopathy, colposcopic findings, result of last PAP smear, histopathology of cervical biopsy prior conisation, indication for conisation, additional smears (HPV 16, 18, 31 and 33 and possible other pathogens), vaginal therapy before conisation, type of conisation, data regarding complications after conisation if present, final histopathology and data if margins of the cone were free of disease. Records from database were anonymised and we used only data of suspected risk factors for HSIL regarding age at the time of surgery, age at menarche, age at first intercourse, number of sexual partners, number of deliveries, spontaneous and legal abortions, type of contraception, marital status, socioeconomic status, smoking habits, last PAP smear result and final histopathology of the cone. All patients with incomplete data were removed from analysis.

The sample is relatively small and is not representative of the real-life situation because more patients have dysplasia or carcinoma and only smaller portion of patients have low risk squamous intraepithelial lesion (LSIL) or no dysplasia at all. In Slovenia, healthy women without dysplasia represent majority of women who attend organised Cervical cancer screening programme ZORA. In year 2019 in Slovenia, we diagnosed 105 new cases of cervical carcinoma and 1056 cases of HSIL. In the same period, we analysed 220301 PAP smears from 206323 women.[26] First line treatment for dysplastic changes on uterine cervix is conisation or large loop excision of transformation zone (LLETZ) in majority of cases.[27] In 2019, we performed 2017 conisation procedures. 1334 (66%) patients had conisation because of HSIL (cervical intraepithelial neoplasm [CIN]), 400 (20%) patients because of low-grade squamous intraepithelial lesions (LSIL) and 283 (14%) had no dysplasia.[26] In Slovenia number of conisations is decreasing in favour of LLETZ.[28]

We constructed two basic settings of our database. In *Raw* setting we used previously mentioned risk factors with age in years and last PAP result. For better classification performance we constructed another classification (*Class*) setting in which we grouped patients by *Age at the time of surgery* in 15 age groups with 5 years interval and divided *Last PAP smear result* in two groups (high risk PAP smear Yes: PAP III–V and No: PAP I–II). We divided *Final histopathology result of conisation* in two groups (HSIL: CIN 2, 3, CIS [carcinoma *in situ*], Ca

[carcinoma] and NO-HSIL: CIN 1, 1–2 and non-dysplastic changes).

In our database are complete data of 1475 patients, 26 (1.8%) without dysplasia on final histological result of conisation, 160 (10.8%) with L-SIL and 1289 (87.4%) with HSIL. Last PAP smear was high risk in 16 patients (61.5%) without dysplasia, 127 patients (79.4%) with LSIL and in 1169 patients (90.7%) with HSIL.

Mean age of patients without HSIL was 38.6 years (13–83 years, standard deviation 10.47) and 34.9 (13–81, standard deviation 8.98) in the group of patients with HSIL. Mean age at menarche was 13.7 (10–19, standard deviation 1.84) in group of patients without HSIL and 13.5 (9–20, standard deviation 1.16) in HSIL patients. Mean age at first intercourse was 17.6 (13–25, standard deviation 1.59) in patients without HSIL and 17.4 (12–25, standard deviation 1.66) in patients with HSIL. HSIL and NO-HSIL group of patients were statistically different regarding age ($p < 0.01$), age at 1st intercourse ($p < 0.035$), number of sex partners ($p < 0.004$) and high risk PAP smear ($p < 0.01$).

In our group of patients without HSIL 57% tested HPV 16 negative and 27% positive (16% not tested) and in the group of patients with HSIL 54% tested negative and 33% positive (14% not tested). In the NO-HSIL group 65% tested HPV 18 negative, 21% positive (15% not tested) and in HSIL group 60% tested negative and 27% positive (13% not tested).

Because many patients did not have HPV testing, we decided to remove such patients from analysis. When we analysed removed patients because of no HPV testing (HPV 16, 18, 31 or 33), we discovered that numerous patients with HSIL would be missed (Table 1).

Chi-square test ($\chi = 1.631$, $p = 0.202$) found no statistically difference of HPV 16, 18 status and presence of HSIL in our group of patients. In this time period we didn't routinely tested presence of HPV infection. Because of a chance that we detected transitory infection with HPV testing and that over 400 patients with HSIL would be excluded from analysis because they were not tested against HPV, we decided to exclude HPV from further analysis. HPV 16 and 18 statuses in our patients are presented in Table 2.

TABLE 1. Final histology of the cone in patients without human papilloma virus (HPV) testing

	Frequency	Percent
NO dysplasia	9	1.8
CIN 1	26	5.3
CIN 1–2	27	5.4
CIN 2	90	18.1
CIN 2–3	55	11.1
CIN 3	223	45.0
CIS	55	11.1
invasive ca	11	2.2
Total	**496**	**100.0**

CIN = cervical intraepithelial neoplasm

Human neuron or nerve cell is a cell, which can be electrically or chemically excited. It has body – soma and dendrites – which lead signal to neuron and single axon which lead signal from neuron and interconnects with other neural cells. Information is transferred via electrical or chemical mechanism.[29]

In ANN we have different neurones. There are two main types. Input neurone called perceptron receives information. Output neurone produces final output. All neurones are arranged in layers. First layer is input layer with perceptrons, last layer is layer with output neurones. In between there can be one or many hidden layers. Every neuron interconnect with all neurones from previous and

TABLE 2. Number and percentage of patients according to human papilloma virus (HPV) 16 and 18 statuses in high grade squamous intraepithelial lesion (HSIL) and NO-HSIL group

	HPV 16				HPV 18			
	HSIL group		NO-HSIL group		HSIL group		NO-HSIL group	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
not performed	177	14	29	16	172	13	27	15
negative	693	54	106	57	775	60	120	65
positive	419	32	51	27	342	27	39	20
Total	**1289**	**100**	**186**	**100**	**1289**	**100**	**186**	**100**

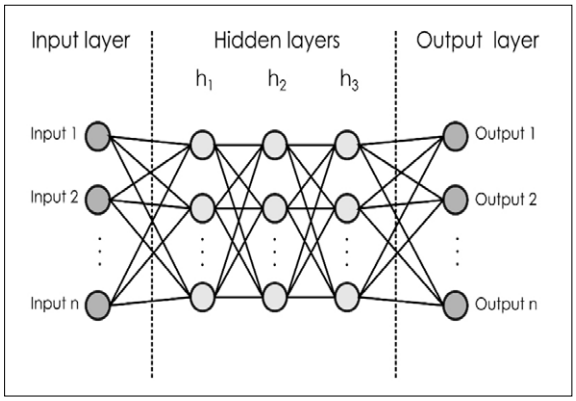

FIGURE 1. Schematic of simple neural network with input, output and three hidden layers.

next layer.[30] Diagram of simple ANN is presented in Figure 1.

As in neural cell, artificial neurones in neural networks receive information and became excited. When excitation level (weight) is reached, they promote signal to other neurones. Before weight is reached no output signal is produced. There are many different mathematical functions for neuron activation. Activation function of output neurons can be different from that of previous layers. Output of the last neuron is numerical value which can range from 0–1. Threshold for classified positive/negative is by default 0.5, meaning that cases with values > 0.5 are classified as positive and cases with value ≤ 0.5 as negative. Threshold value can be changed according to the performance of the algorithm and our goals.[18]

Dataset must be split in two parts-training and holdout set. Training set is used to build model, test relations between input variables and determining weights of the neurones. Algorithms are then tested on holdout set in which are instances unknown to neural network. Training set must be larger than holdout.[18]

In every classification process, we have actual positive and negative cases, which can be classified correctly as positives or negatives or classified incorrectly. The best way to visualize the situation is to use confusion matrix.

Effectiveness of ANN or any other classification system or algorithm can be measured. In our study we used p*recision* (positive predicted value; PPV), r*ecall* (*sensitivity*, t*rue positive rate;* TPR), receiver operator characteristic curve (ROC curve), area under the ROC curve (AUC).[31] *F*-measure and Matthews correlation coefficient (MCC) are another measure for efficiency. F-measure is combined measure of precision and recall:

$$F = \frac{2 * \text{Precission} * \text{Recall}}{\text{Precision} + \text{Recall}}.$$

It ranges between 0 (worst) and 1 (best). MCC ranges between −1 and +1. −1 meaning perfect misclassification, 0 means as expected in random guessing and +1 perfect classification.[32] Precision-recall curve (PRC curve) is another measure of classification efficiency. Precision (PPV) is plotted on y-axis and Recall (TPR) on x-axis. It is more informative than ROC Curve in imbalanced data settings because it analyses fraction of true positives among all positive predictions.[33]

Quality of data is of vital importance – sufficient numbers of instances (collection of attributes in database) and qualitative attributes (features that measure or describe different aspect of instances). Before running classification algorithm, it is necessary to run simulation of baseline classification. We can then compare results derived from our model with baseline results and decide how good (or bad) our model is in classification and prediction.

Dealing with unbalanced data

When we have imbalanced datasets where one of the variables represents only a small proportion of the sample, baseline prediction for majority class is very high. For example – if majority class represents 88% of instances as in our case, baseline prediction is high – 88%. If prediction algorithm predicts with 92% accuracy this is not statistically significant. There are some methods, how to deal with unbalanced data:

- Under-sampling: randomised reduction of majority class to match minority class
- Over-sampling: n-fold replication of minority class to match majority class
- SMOTE: synthetic minority over-sampling technique creates new synthetic instances, which have similar characteristics as original ones in minority class.[34,35]

Experiment with WEKA

Weka (1999–2020 The University of Waikato, Hamilton, New Zealand) is open-source application for data mining with many other possibilities beside ANN as are Bayesian networks, Logistic regression, Classification trees, K-nearest neighbours and others.[36] It enables us to test classification algorithm on whole dataset, we can split dataset by percentage, test whole dataset against separate training dataset from different dataset which we import in Weka and n-fold cross validation. When

we manually or randomly split dataset in training and holdout part, there is always a chance that we collect all important instances in one of the sets, especially if one kind of instances represent small proportion of all instances. N-fold cross validation is powerful option which can minimise the chance of such situation. It divides entire database into n parts. Each n-1 part is used as training and each n part as holdout set. All combinations of n and n-1 parts are then tested against each other and algorithm at the end presents the best result of tested combinations. In our experiment, we used 10-fold cross validation.[37]

Preparation of datasets for analysis

We prepared eight data sets:

- Raw set: we used as variable original risk factors and as output HSIL_Y/N.
- Class set: same as raw set except age groups instead of age and PAP_HR_Y/N instead of last PAP.
- Raw and class with under-sampling, over-sampling and SMOTE method for equalising imbalanced dataset.

Original dataset consisted of 186 No-HSIL and 1289 HSIL patients. To prepare over-sampling dataset we duplicated HSIL negative patients to get 558 No-HSIL and original 1289 HSIL patients. For under-sampling, we randomly selected and deleted HSIL patients to get 272 HSIL and original 186 No-HSIL patients. With SMOTE algorithm, we created data set with original 1289 HSIL patients and 744 No-HSIL patients.

Baseline prediction was calculated for each set and results for multi-layer perceptron with 10-fold cross validation was recorded. Results are presented in Table 4.

Results

In first part of analysis, we analysed original database with artificial neural network, multi-layer perceptron (MLP). We achieved 81.42% correct predictions which is worse than baseline – ZeroR prediction 87.39% (kappa = 0.08 showing no level of agreement between predicted and actual status, AUC 0.594, MCC 0.086, F-Measure 0.806, precision 0.799 and recall 0.814). When we corrected minority class with over-sampling method ZeroR prediction was 69,79%, achieved 79,21% (kappa = 0.523 showing weak level of agreement between predicted and actual status, AUC 0.837, MCC 0.525,

TABLE 3. Confusion matrix for classification with all possible outcomes

	Predicted pos (PP)	Predicted neg (PN)
Actual pos (P)	True positives (TP)	False negatives (FN)
Actual neg (N)	False positives (FP)	True negatives (TN)

Neg = negatives; Pos = positives

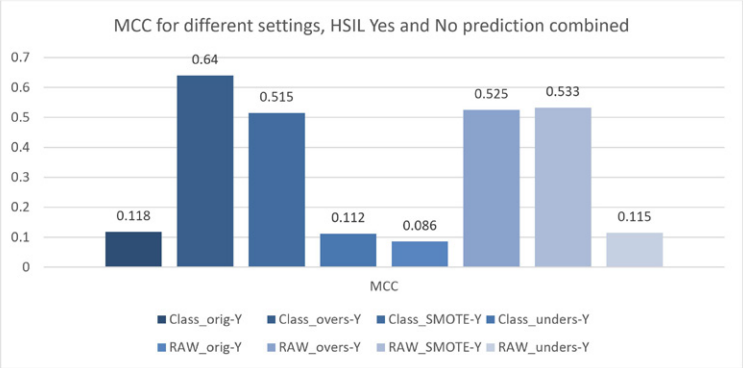

FIGURE 2. Matthews correlation coefficient (MCC) for categorisation squamous intraepithelial lesion (HSIL)-combined for YES and NO prediction for different equalisation methods (no correction of minority class, under-sampling, over-sampling and synthetic minority over-sampling technique [SMOTE]) for both RAW and Class settings. Best performance of multi-layer perceptron (MLP) is on dataset with data organised in classes and over-sampling method for minority class – MCC = 0.64. Lowest performance is with original dataset without correction for minority class – MCC = 0.086.

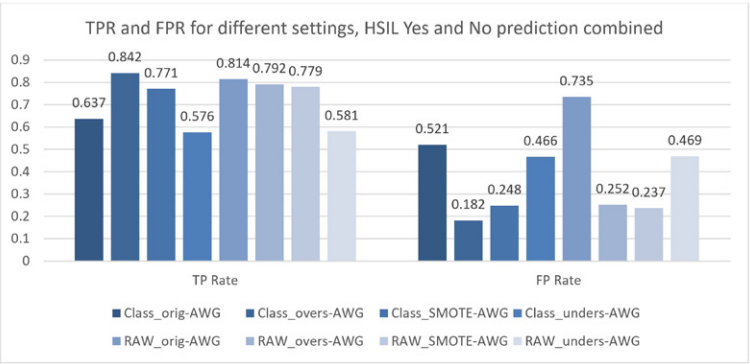

FIGURE 3. True positive and False positive rate for different settings for prediction Yes and No combined and for different equalisation methods (no correction of minority class, under-sampling, over-sampling and synthetic minority over-sampling technique [SMOTE]) for both RAW and Class settings. Best performance model from Figure 2 has 0.842 true positive rate and 0.182 false positive rate. Lowest performance model from Figure 2 has high 0.814 true positive rate which is almost as high as best performance model but also high false positive rate 0.735.

Raw = original settings; Class = class setting; FPR = false positive rate; HSIL = high grade squamous intraepithelial lesion; overs = oversampling; TPR = true positive rate; unders = undersampling; SMOTE = synthetic minority over-sampling technique

TABLE 4. Results of multi-layer perceptron (MLP) classifications for different settings with baseline prediction – ZeroR, percentage of correct classification and Kappa statistic for all analysis. Results are for prediction high grade squamous intraepithelial lesion (HSIL)-Yes (Y), prediction NO-HSIL (N) and weighted average for whole model (YES and NO combined) – Weighted average (AVG). In bold-type letters are results, where prediction by MLP is better than baseline prediction ZeroR

	TP Rate	FP Rate	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area	Class	% Correct	Kappa	ZeroR %
Class_orig–Y	0.751	0.634	0.739	0.751	0.745	0.118	0.567	0.735	Yes	82.10	0.0965	87.39
Class_orig–N	0.366	0.249	0.308	0.366	0.373	0.118	0.567	0.377	No			
Class_orig–AVG	0.637	0.521	0.633	0.637	0.635	0.118	0.567	0.629	Weighted Avg			
Class_overs–Y	**0.860**	**0.201**	**0.908**	**0.860**	**0.884**	**0.640**	**0.870**	**0.920**	**Yes**	**84.19**	**0.6376**	**69.79**
Class_overs–N	**0.799**	**0.140**	**0.712**	**0.799**	**0.753**	**0.640**	**0.870**	**0.703**	**No**			
Class_overs–AVG	**0.842**	**0.182**	**0.849**	**0.842**	**0.844**	**0.640**	**0.870**	**0.855**	**Weighted Avg**			
Class_SMOTE–Y	**0.797**	**0.274**	**0.834**	**0.797**	**0.815**	**0.515**	**0.802**	**0.850**	**Yes**	**77.08**	**0.5141**	**63.40**
Class_SMOTE–N	**0.726**	**0.203**	**0.673**	**0.726**	**0.699**	**0.515**	**0.802**	**0.669**	**No**			
Class_SMOTE–AVG	**0.771**	**0.248**	**0.775**	**0.771**	**0.772**	**0.515**	**0.802**	**0.784**	**Weighted Avg**			
Class_unders–Y	0.669	0.559	0.636	0.669	0.652	0.112	0.542	0.608	Yes	57.64	0.1113	59.39
Class_unders–N	0.441	0.331	0.477	0.441	0.458	0.112	0.542	0.448	No			
Class_unders–AVG	0.576	0.466	0.572	0.576	0.573	0.112	0.542	0.543	Weighted Avg			
RAW_orig–Y	0.907	0.828	0.884	0.907	0.895	0.086	0.594	0.905	Yes	81.42	0.0856	87.39
RAW_orig–N	0.172	0.093	0.211	0.172	0.189	0.086	0.594	0.174	No			
RAW_orig–AVG	0.814	0.735	0.799	0.814	0.806	0.086	0.594	0.813	Weighted Avg			
RAW_overs–Y	**0.825**	**0.285**	**0.870**	**0.825**	**0.847**	**0.525**	**0.837**	**0.905**	**Yes**	**79.21**	**0.523**	**69.79**
RAW_overs–N	**0.715**	**0.175**	**0.639**	**0.715**	**0.675**	**0.525**	**0.837**	**0.661**	**No**			
RAW_overs–AVG	**0.792**	**0.252**	**0.800**	**0.792**	**0.795**	**0.525**	**0.837**	**0.831**	**Weighted Avg**			
RAW_SMOTE–Y	**0.800**	**0.258**	**0.843**	**0.800**	**0.821**	**0.533**	**0.814**	**0.867**	**Yes**	**77.87**	**0.5318**	**63.4**
RAW_SMOTE–N	**0.742**	**0.200**	**0.681**	**0.742**	**0.710**	**0.533**	**0.814**	**0.691**	**No**			
RAW_SMOTE–AVG	**0.779**	**0.237**	**0.784**	**0.779**	**0.780**	**0.533**	**0.814**	**0.802**	**Weighted Avg**			
RAW_unders–Y	0.688	0.575	0.636	0.688	0.661	0.115	0.551	0.614	Yes	58.08	**0.1144**	**59.39**
RAW_unders–N	0.425	0.313	0.482	0.425	0.451	0.115	0.551	0.466	No			
RAW_unders–AVG	0.581	0.469	0.573	0.581	0.576	0.115	0.551	0.554	Weighted Avg			

Raw = original settings; Class= class setting; overs = oversampling; SMOTE = synthetic minority over-sampling technique; unders = undersampling

F-Measure 0.795, precision 0.800 and recall 0.792). SMOTE performed inferior than over-sampling with baseline ZeroR 63.40% and achieved 77.87% (kappa = 0.53 showing weak level of agreement between predicted and actual status, AUC 0.814, MCC 0.533, F-Measure 0.780, precision 0.784 and recall 0.779). Under-sampling method performed worse than analysis on original dataset with ZeroR prediction 59.39%, achieved 58.08% (kappa = 0.11 showing no level of agreement between predicted and actual status, AUC 0.551, MCC 0.115, F-Measure 0.576, Precision 0.573 and Recall 0.581).

In second part of analysis, we grouped data in classes as described previously. Analysis with MLP on original data achieved 82.10% correct prediction which is less than baseline 87.39% ZeroR prediction (kappa = 0.09 showing no agreement between predicted and actual status, AUC 0.567, MCC 0.118, F-Measure 0.635, precision 0.633 and recall 0.637). Performance of MLP was better with over-sampling method, where baseline ZeroR prediction was 69.79% and MLP achieved 84.19% correct predictions (kappa = 0.64 showing moderate level of agreement between predicted and actual

status, AUC 0.870, MCC 0.640, F-Measure 0.844, precision 0.849 and recall 0.842). With SMOTE method baseline ZeroR prediction was 63,40% and achieved prediction 77,08% (kappa = 0.51 showing weak level of agreement between predicted and actual status, AUC 0.802, MCC 0.515, F-Measure 0.772, precision 0.775 and recall 0.771). Undersampling method performed worse than analysis on original data with ZeroR prediction 59.39% and 57,64% correct predictions (kappa = 0.11 showing no agreement between predicted and actual status, AUC 0.542, MCC 0.112, F-Measure 0.573, precision 0.572 and recall 0.576).

All results are presented in Table 4. MCC for all models is graphically presented in Figure 2 for prediction HSIL-Yes and NO combined. True positive rate and false positive rate for all models are graphically presented in Figure 3. ROC curve for worst performance model is represented on Figure 4 and for best performance model on Figure 5.

Discussion

In medicine, we mostly deal with imbalanced classes. In such data sets baseline prediction is high for majority class. In most cases, we have situation in which we must precisely and accurately classify patients from minority class.[38] Misclassification of patient with severe disease as negative means that we potentially endanger their health and because of delayed diagnosis, disease can progress to life-threatening situation or death. Such situation endangers only patient involved. In case that we classify patients, for example, who have very contagious disease, misclassification as negative means that such false negative patients will spread the disease and endanger other healthy people. Misclassification of healthy patients as positive results in further diagnostic tests and eventually leads to correct diagnosis. Unnecessary procedures result in greater stress for patient, higher expenses and bigger load for health system. Good classification algorithms therefore must have very high sensitivity and specificity.

Cervical cancer is preventable disease.[1] Artificial intelligence (AI) and deep learning methods are used for optimisation of screening, diagnostic and treatment procedures and are also present in the field of cervical cancer. Cervical cytology is of vital importance in screening programmes. Mango *et* Laurie[39] published article of computer assisted cervical cancer screening using neural networks in 1993. They used robotic arm for loading and un-

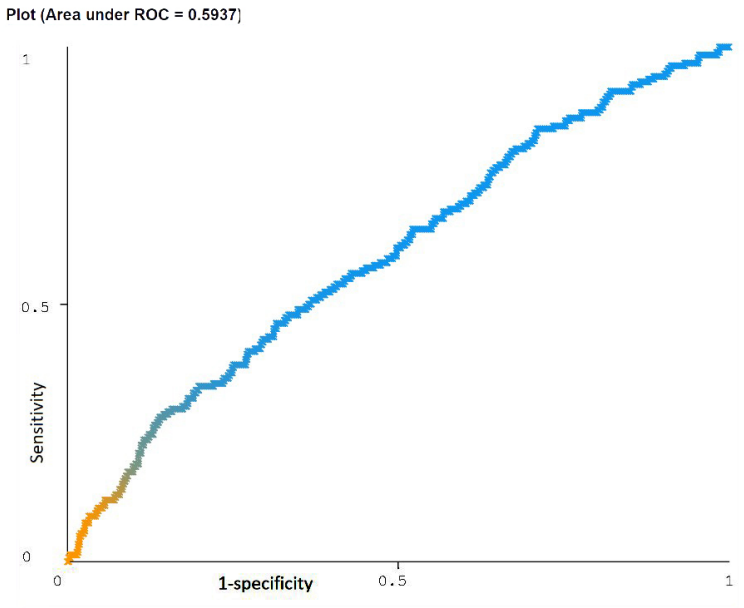

FIGURE 4. Receiver operator characteristic (ROC) curve for multi-layer perceptron (MLP) performance on dataset without grouping in classes and no correction for minority class where X axis represent 1- specificity (false positive rate) and Y axis represents sensitivity (true positive rate). Area under the ROC curve (AUC) = 0.594. AUC for categorisation with random guessing is 0.5. This Figure represents model with lowest performance of MLP from our study.

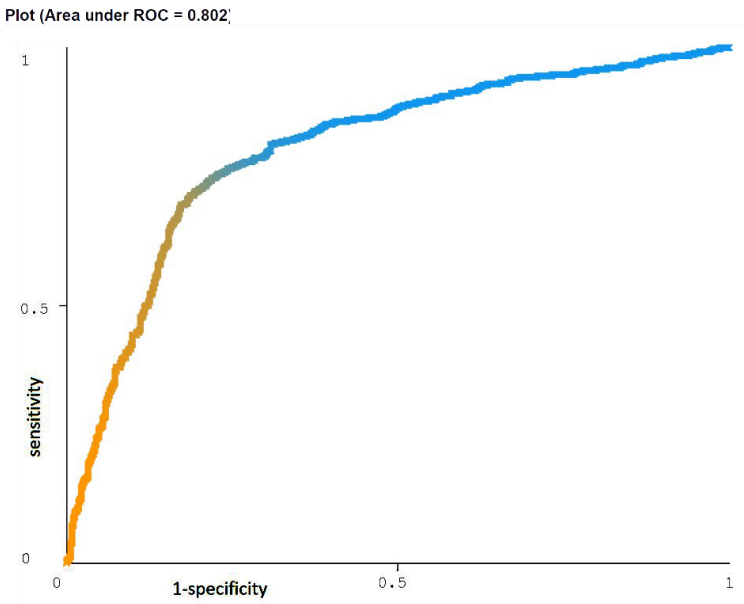

FIGURE 5. Receiver operator characteristic (ROC) curve for multi-layer perceptron (MLP) performance on dataset with patients grouping in classes and synthetic minority over-sampling technique (SMOTE) correction for minority class where X axis represent 1- specificity (false positive rate) and Y axis represents sensitivity (true positive rate). Area under the ROC curve (AUC) = 0.802 which is well above classification with random guessing where AUC is 0.5. This Figure represents best performance model of MLP from our study.

loading slides of PAP smears from storage container, automated microscope and automated high-definition camera for imaging the slide. Multiple pictures from each slide were recorded. In the review station cytologists examined pictures. They used ANN to recognise different cells from images. After training neural network on sample pictures overall ANN sensitivity for all cytologic findings was 96% compared to 81% of that of cytologists.[39]

Sompawong *et al.* used ANN on images of liquid-based cytology (LBC) PAP smears to detect and analyse features of nucleus of the cervical cell and to screen normal and abnormal morphological features. In his study they achieved 57.8% mean average precision and 91.7% accuracy, sensitivity and specificity. This could help technicians and cytologists in their work.[40]

Holmström *et al.* tested the use of ANN to analyse PAP smears to detect pathological changes in rural Kenya where cervical cancer represent significant health burden with high mortality rate. PAP smears were digitalised with portable scanner, uploaded to cloud and analysed in regional medical centre. Sensitivity of ANN was 95.7% and specificity 84.7% compared to 100% sensitivity and 78.4% specificity of human examinator. AUC for ANN was 0.94. NPV was very high 99–100% particularly for HSIL. They concluded, that such model can be very helpful in cervical cancer screening in areas with low resources of health care professionals.[41]

Bao *et al.* [42] and Turic *et al.* [43] published study of AI assisted cytology in cancer screening programme in China. They digitalised LBC images of cervical smears and analysed them with AI. PAP smears were also analysed by cytologists. Agreement between AI and manual reading was 94.7 with kappa 0.92 which is almost perfect agreement and AI assisted cytology was more sensitive for detection CIN2+ lesions than manual reading by 5,8% with slight reduction in specificity.

Colposcopy is very important diagnostic procedure. Clinical experience is important for accurate colposcopic result.[44] With the use of AI - deep convolutional networks it is possible to analyse colposcopic images with higher accuracy than subjective assessment by human. In his study Chandran and colleagues published 92,4% sensitivity, 96.2% specificity and kappa 0.88 which showed strong association between predicted and actual status of colposcopic changes.[45] It is important, that women referred for colposcopy are correctly selected to prevent overload in colposcopic clinics. Such overload with improper patients can result in miss diagnostics, unnecessary procedures and can be a threat for subsequent pregnancies.[46] Karakitsos *et al.*[47] used learning vector quantizer neural network to identify patients who need referral for colposcopy. They analysed PAP smear using LBC and several markers of HR-HPV infection. All women had colposcopic directed biopsy performed by experienced colposcopist and histologic result was golden standard to determine if colposcopy was necessary or not. They did not only identified more patients in need for immediate colposcopy with the use of AI but also reduced number of patients with clinical insignificant lesions compared to other methods. Combined sensitivity for training and testing set was 85.16% with specificity 98.01%, PPV 85.71%, NPV 97.92% and overall accuracy of 96.42%. ANN are very good in recognising pathological morphological features on images and all parameters are very good in all studies.[47] Pouliakis *et al.* obtained similar results with study of classification and regression trees (CART) for the triage of women for referral to colposcopy and risk estimation for CIN. They used LBC and several markers of HR-HPV infection. This study is important because they used missing data, which can be a problem and most studies exclude them from analysis. CART has 83.28% sensitivity, 94.26% specificity, 79.04 PPV, 95.06 NPV and 100%valid cases while other methods have only 67.75%-96.25% valid cases depending on the method used. CART performed superiorly compared to cytology alone when used ASCUS+ threshold level ($p < 0.0001$).[48]

In our study we used MLP, which is back propagation artificial neural network on our dataset of patients, which had conisation surgery in University Gynaecologic clinic Maribor in years 1993–2005. As input layer, we used known risk factors for development of cervical dysplasia and carcinoma, High-risk dysplasia CIN2+ Yes/No as output layer. Risk factors are important and increase risk for development of disease but not all patients with risk factors develop disease.[49] All patients with incomplete data were removed from analysis as are in majority of studies. Original dataset was imbalanced and patients without HSIL represented minority class. To our knowledge this is first study with such settings.

MLP performed worse on original dataset in comparison with baseline prediction. Such outcome can be expected in dataset where data are imbalanced.[36] There are several methods to equalise imbalanced data. We can reduce the majority class by randomly selecting and removing instances from majority class with under-sampling method.[34] When we balanced dataset with under-sampling

method, prediction did not improve and stayed below baseline. Reason for this may be in removing instances with important variables from training and/or testing set. We prepared dataset with under-sampling method few more times but with all settings, we could not achieve better performance. MLP correctly classified 57.64% cases which is inferior compared to baseline zeroR 59.39% and also kappa statistic 0.1113 showed no agreement between real and predicted status.

SMOTE and over-sampling methods improved performance of MLP.[35] With over-sampling method we multiplicate instances from minority class to match that of majority class. In this case is always a chance, that we can find equal instances in training and testing set.[34] SMOTE method uses k-nearest neighbour algorithm to create new synthetic instances which are all unique.[35] In best performance model where baseline prediction ZeroR was 69,79% MLP correctly classified 84,19% cases and kappa statistic 0.64 showed moderate agreement between real and predicted status.

In real clinical practice, many patients have multiple risk factors but never develop disease or, many with only a few became ill. It is possible that patients do not tell the truth about risk factors because they are too intimate, they are ashamed or they do not remember. Collection of all risk factors from patients participating in screening or other programme in nationwide database is also questionable because of ethical considerations.[50] With our experiment we proved, that with the use of ANN we can predict more patients who will develop HSIL based only on the analysis of their risk factors for developing HSIL and result of last PAP smear than with baseline prediction. But performance and classification accuracy of ANN is not high enough for every day clinical practice.

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research article

Advancements in the radiooncological treatment of high-risk prostate cancer: a quarter century of achievements

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Background. The aim of the study was to evaluate the development of treatment of primary high-risk prostate cancer in regards to biochemical no evidence of disease (bNED), acute and late gastrointestinal (GI) and genitourinary (GU) side effects.

Patients and methods. Primary high-risk prostate cancer patients treated between 1994 and 2016 were included. Applied doses ranged from 60 to 80 Gy, with a dose of 1.8 or 2 Gy per fraction. Techniques were either 3D conformal or intensity modulated radiotherapy and volumetric intensity modulated arc therapy.

Results. 142 patients were treated with doses up to 70 Gy (median dose 66 Gy; 66 Gy group), 282 with doses between 70 and 76 Gy (median dose 74 Gy; 74 Gy group), and 141 with doses >76 Gy (median dose 78 Gy; 78 Gy group). The median follow-up was 48 months. The bNED rates were 50% after 5 years and 44% after 9 years in the 66 Gy group; 65% and 54%, respectively, in the 74 Gy group; and 83% and 66%, respectively, in the 78 Gy group ($p = 0.03$ *vs.* 74 Gy and $p < 0.0001$ *vs.* 66 Gy). We found a higher rate of acute GI side effects in the 78 Gy group compared to the other groups, but not in maximum acute GU side effects and late maximum GI and GU effects.

Conclusions. High-risk prostate cancer patients treated with doses of 78 Gy had significantly better bNED rates. Compared to the historical 66 Gy group, 50% more patients achieved bNED after a follow-up of 9 years.

Key words: biochemical control; gastrointestinal toxicity; genitourinary toxicity; dose escalation

Introduction

Prostate cancer is the most common cancer in men in the US and central Europe, accounting for 20–25% of all cases.[1-3] One in five of these cases is diagnosed with high-risk prostate cancer.[4] However, prostate cancer is only responsible for cancer mortality rates of 6–10%[3,5,6] and death from other reasons is much more likely after being diagnosed with prostate cancer[7] in study conditions.

In the last 25 years, many improvements have been introduced in the field of prostate cancer. In regards to diagnostics and staging, comprehensive PSA screening[1,2], use of ultrasound-guided biopsy[8], and computed tomography (CT)[9], magnetic resonance imagining (MRI)[10], and prostate-specific membrane antigen (PSMA) positron emission tomography (PET)/CT[11] have found their way into clinical routine, especially for high-risk prostate cancer.

In addition, external beam radiotherapy (EBRT) has taken a leap forward within the last three decades. Starting with 3D conventional radiotherapy[12] and the use of lead blocks, and ending with volumetric intensity modulated arc therapy (VMAT)[13], new techniques allow dose escalation to 72 Gy with tolerable side effects[14], and even to ≥74 Gy[15-17], while also providing similar results as radical prostatectomy (RPE)[7,14] in localized prostate cancer. This dose escalation has significantly increased the curability

of prostate cancer and is, therefore, in our opinion, the most important advancement in the field of prostate cancer radiotherapy over the last quarter century.

Although a final conclusion has not yet been reached about the optimal duration[18-20], evidence-based androgen deprivation therapy (ADT)[2,19-22], especially in high-risk prostate cancer, has improved the outcome after radiotherapy.

With similar oncological results between RPE and EBRT, the focus of patient decision-making shifts more and more to side effects. Therefore, the goal of our study was not only to evaluate the development of high-risk prostate cancer treatment over the last 25 years and the resulting biochemical no evidence of disease (bNED), but also to compare the gastrointestinal (GI) and genitourinary (GU) side effects of radiotherapy.

Patients and methods

The study protocol was approved by the ethical review board of our medical university according to local laws and regulations (EK Nr: 1291/2020).

All patients included in this study were treated at our Department of Radiation Oncology between 1994 and 2016. The inclusion criteria were high-risk prostate cancer as defined by the NCCN classification[1] (PSA > 20 ng/ml, Gleason Score 8-10, or T stage ≥ T3). The required staging was localized cancer without evidence of locoregional or distant metastases. The lymph nodes of all patients were staged using CT. Bone scintigraphy and ADT were performed at the discretion of the treating urologist but were recommended for 3 years according to Bolla *et al*.[23] Patients were primarily treated locally with EBRT.

The definition of the clinical target volume was determined using CT and, from 1997 onwards, MRI for planning. The total prescribed dose ranged from 60 Gy to 80 Gy, with a dose of 1.8 to 2 Gy per fraction. Pelvic lymph nodes were irradiated with a dose of 1.8 or 2 Gy per fraction up to 45–50.4 Gy. Treatment groups were based on the median dose; 58% of patients in the 66 Gy group received 66 Gy, with a maximum of < 70 Gy, 63% in the 74 Gy group received 74 Gy, with doses between 70 and 76 Gy and 90% in the 78 Gy group received 78 Gy, with doses > 76 Gy. The dose was prescribed to 95% of the planning target volume (PTV) according to ICRU report 62.[24] Clinical target volumes (CTV) were defined as the prostate and the seminal vesicles. If pelvic lymph nodes were treated, the CTV also included the iliac vessels up to the aortic bifurcation. The safety margin around the clinical target volume was 5 mm in all directions with gold marker fiducials, 7 mm in all directions without fiducials for the 78 Gy group, and 10 mm in the 74 Gy group for the first 66 Gy and 5 mm dorsally for the last 8 Gy. For 66 Gy, the safety margin varied between 10 and 20 mm. Due to the broad time period of our study, safety margins varied over time. All patients received a rectal balloon[25] as internal immobilization. The irradiation was performed in supine position via either a 3D conformal 4-field box up until January 2013 or intensity-modulated radiation therapy (IMRT) or the VMAT technique from then on.

Follow-up was scheduled for 3 and 12 months after treatment, and then yearly thereafter. We defined bNED failure using the Phoenix criteria (nadir + 2 ng/ml).[26] Recent PSA values and late GI and GU side effects according to RTOG grading[27] were compiled by the physician during each follow-up. Survival data were retrieved from the population census (Statistik Austria).

Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, San Diego, USA) and R version 3.6.1 (2019-07-05) with RStudio 1.2.1335 (packages: survival version 2.44-1.1, survminer version 0.4.6). A p-value < 0.05 was considered significant. The bNED and survival rates were estimated using the Kaplan-Meier method. The resulting curves were compared using the log-rank test. Multivariable Cox regression models were created including the initial PSA value (log2 transformed); Gleason score ≤ 6/Histograding 1, 7/Histograding 2, and 8-10/Histograding 3; applied dose in Gy; T stage 1a-c and 2a/X (reference), 2b/c and 3, or 4 according to the NCCN guidelines[1]; and pelvic irradiation. Side effects were analysed using the Mann-Whitney U test.

Results

Patient characteristics are provided in Table 1. As our observation period covers decades, irradiation techniques changed. Therefore, almost all patients in the 78 Gy group were treated using IMRT or VMAT. With the implementation of IMRT, we also introduced routine irradiation of the pelvic lymph nodes for high-risk prostate cancer patients. Thus, almost all patients in the 78 Gy group were also irradiated in the region of the pelvic lymph nodes. Exceptions were made for, for example, patients with earlier intestinal surgery.

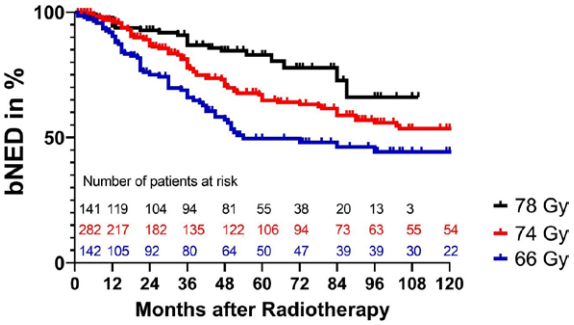

FIGURE 1. Biochemical no evidence of disease (bNED) rates in the 66 Gy, 74 Gy, and 78 Gy groups. The difference between the groups is highly significant ($p < 0.0001$).

Observed bNED rates for the 66 Gy group were 50% after 5 years and 44% after 9 years. For the 74 Gy group, these values were 65% and 54%, respectively, and for the 78 Gy group, 83% and 66%, respectively. A significant difference was found when comparing all groups at once ($p < 0.0001$; Figure 1).

Regarding survival, we detected 7 disease-specific deaths and 40 other causes of death in the 66 Gy group, 11 and 44, respectively, in the 74 Gy group, and 0 and 7, respectively, in the 78 Gy group, respectively.

Disease-specific survival rates after 5 years were 95% in the 66 Gy group, 97% in the 74 Gy group, and 100% in the 78 Gy group ($p = 0.11$). The overall survival rates after 5 years were 74%, 82%, and 96% ($p = 0.0002$), respectively.

The results of the multivariable analysis are displayed in Table 2. The log2-transformed PSA value has to be interpreted as a twice as high initial PSA value leading to a 19% increased risk of bNED failure when comparing two patients.

Maximum acute GI and GU side effects are provided in Table 3. Significantly more acute GI side effects occurred in the 78 Gy group compared to the 74 Gy and 66 Gy groups ($p < 0.001$ and $p = 0.02$, respectively). No significant differences were observed for acute GU side effects ($p = 0.19$ for 78 *vs.* 66 Gy, and $p = 0.88$ 78 *vs.* 74 Gy).

Table 4 provides the maximum late GI and GU side effects. No significant differences were found (GI side effects: $p = 0.40$ for 78 *vs.* 66 Gy, and $p = 0.74$ for 78 *vs.* 66 Gy; GU side effects: $p = 0.13$ and 0.37, respectively).

The onset of RTOG grade 2 or higher is shown in Figure 2 for late GI side effects and Figure 3 for late GU side effects. No significant difference was found for late GI side effects ($p = 0.96$). For late GU side effects, we detected a significant difference ($p = 0.006$).

TABLE 1. Patient characteristics

Median Dose	78 Gy	N = 141	74 Gy	N = 282	66 Gy	N = 142
Dose distribution in Gy						
Min	76		70.4		60	
Max	80		75		70	
N with median dose	127	90%	178	63%	83	58%
T category						
T1	43	30%	48	17%	21	15%
T2	61	43%	108	38%	53	37%
T3	36	26%	121	43%	60	42%
T4	1	1%	5	2%	8	6%
Gleason score						
≤6 or histological grading 1	20	14%	94	31%	40	11%
7 or histological grading 2	29	21%	66	20%	52	4%
8–10 or histol. grading 3	92	65%	118	42%	42	30%
X	0	0%	4	1%	8	6%
iPSA in ng/ml						
Median	15.7		20.6		21	
Technique						
3D-conformal	11	8%	281	100%	142	100%
IMRT or VMAT	130	92%	1	0%	0	0%
Inclusion of LN	133	94%	105	37%	15	11%
ADT	126	89%	259	92%	113	80%
Mean in months	21		16		23	
Follow-up in months						
Min	3		2		3	
Max	116		240		240	
Median	48		47		59	
Age in years						
Min	49		51		53	
Max	84		86		93	
Median	75		73		71	
Gold marker fiducials	53%		1%		0%	

ADT = androgen deprivation therapy; iPSA = initial prostate specific antigen, IMRT = intensity modulated radiotherapy, T = Tumour extension; VMAT = volumetric intensity modulated arc therapy; LN = lymph nodes; X = no Gleason score or histological grading available

We also performed a subgroup analysis and compared the onset of late GU toxicity in patients with irradiated lymph nodes. No significant differences were found when comparing all dose groups at once and 78 Gy with 74 Gy ($p = 0.15$ and 0.17, respectively).

One case of RTOG grade 4 acute GU toxicity was observed in a patient treated with 74 Gy without irradiation of the pelvic lymph nodes. That patient

TABLE 2. Multivariate analysis of potential predictors of biochemical no evidence of disease (bNED)

Variable	HR	95% CI	p-value
iPSA (log2 transformed)	1.193	1.058–1.345	0.004
Gleason ≤ 6 or Histograding 1	reference		
Gleason 7 or Histograding 2	1.254	0.797–1.890	0.280
Gleason 8-10 or Histograding 3	1.687	1.132–2.515	0.010
Pelvic irradiation	0.783	0.540–1.135	0.196
T stage ≤ 2a	reference		
T stage 2b/c	1.466	0.950-2.262	0.084
T stage 3/4	1.517	1.054-2.181	0.025
Dose (Gy)	0.928	0.890-0.969	< 0.001

CI =confidence interval; HR = hazard ratio; iPSA = initial PSA; T stage low = T1a-c and 2a/X; intermediate = 2b/c; high = 3 or 4

TABLE 3. Maximum acute side effects

GI acute	0	1	2	3	GU acute	0	1	2	3
78 Gy	11%	50%	39%	1%	**78 Gy**	13%	54%	32%	1%
74 Gy	35%	35%	29%	1%	**74 Gy**	19%	45%	34%	1%
66 Gy	38%	22%	40%	0%	**66 Gy**	25%	44%	30%	1%

GI = gastrointestinal; GU = genitourinary

TABLE 4. Maximum late side effects

GI late	0	1	2	3	GU late	0	1	2	3
78 Gy	62%	21%	13%	4%	**78 Gy**	49%	23%	23%	5%
74 Gy	63%	22%	13%	1%	**74 Gy**	53%	21%	22%	3%
66 Gy	66%	22%	12%	0%	**66 Gy**	54%	29%	15%	2%

GI = gastrointestinal; GU = genitourinary

developed overflow incontinence and required surgery. No other grade 4 side effects were observed.

Discussion

The goal of our study was to evaluate the development of high-risk prostate cancer treatment over more than two decades in our department. As surgery and radiotherapy are comparable treatment alternatives, side effects are an important factor in choosing a therapy based on informed decision-making.[1,2,7]

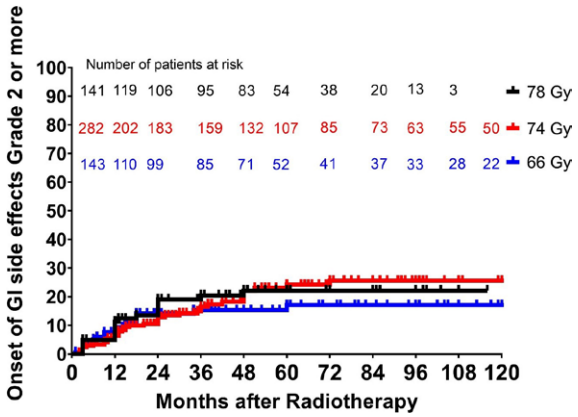

FIGURE 2. Onset of RTOG grade ≥2 gastrointestinal (GI) side effects after treatment over a follow-up period of 120 months.

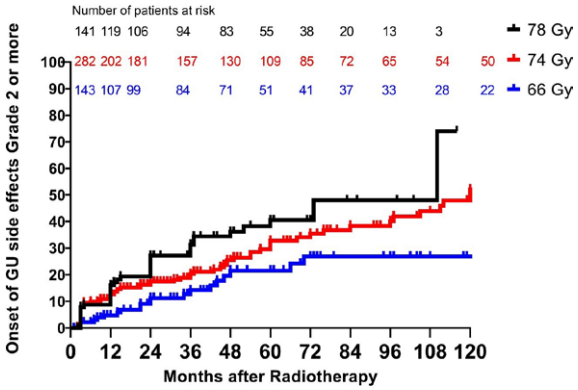

FIGURE 3. Onset of RTOG grade ≥ 2 genitourinary (GU) side effects after treatment over a follow-up period of 120 months.

Starting in the late 1990s, several important studies regarding dose escalation were initiated. Dearnaley *et al.*[16] showed a 10-year bNED rate of 55% in patients treated with 74 Gy compared to 43% after treatment with 64 Gy. Even higher rates were reported in the M.D. Anderson trial[15] and by Peeters *et al.*[17], who escalated the dose from 70 Gy or 68 Gy to 78 Gy. Peeters *et al*. reported a 5-year bNED rate of approximately 70%, and the M.D. Anderson trial reported 75% after 10 years in high-risk patients.

Concerning biochemical control, we are able to reproduce the increased bNED rates by escalating the dose as in the above studies.[15-17] Our bNED rates of 54% and 66% after 9 years for 74 Gy and 78 Gy, respectively, are comparable to the 55% bNED for 74 Gy after 10 years[16] and 70% after 5 years[17] and to the 75% after 10 years for 78 Gy.[15] Notably, our mean ADT duration was higher in the 78 Gy group than in the 74 Gy group, possibly shifting

the bNED rates additionally in favour of the 78 Gy group.[19] Regarding our 78 Gy group, the bNED rate of 83% after 5 years is similar to the 78% described by Ozyigit *et al*.[28] However, the mean ADT duration was 21 months in the 78 Gy group, which is lower than the suggested 24 to 36 months of ADT[2] after Bolla *et al*.[20] showed inferior survival after only 6 months of ADT compared to 36 months. Evidence indicates that 18 months leads to no worse outcomes than 36 months[18], possibly reducing the recommended duration of ADT in the future. Regarding pelvic lymph node irradiation, we were able to detect a tendency of increased bNED rates in our multivariable analysis but no significance, leaving this question unanswered.

Regarding follow-up and survival, as our department has a large catchment area, it is difficult to gather reliable data concerning disease-specific and overall survival, as patients often die in another hospital not associated with our digital infrastructure. Therefore, with a median follow-up of 48 to 59 months, we decided to report only 5-year disease-specific and overall survival rates. However, the similar follow-up does not harm the comparability between groups.

That said, our data suggest great success of high-risk prostate cancer treatment, as 78 Gy provides a 50% increase in bNED rates after 9 years compared to 66 Gy. With absolute bNED rates in the 78 Gy group of 83% and 66% after 5 and 9 years, respectively, and a median age of 75 years in that treatment group, life-long curation of high-risk prostate cancer can be achieved in many cases.

A direct comparison of side effects between our groups is hampered by the fact that our 78 Gy group was almost completely treated using VMAT with reduced margins. Therefore, as IMRT leads to lower GI toxicity[29], caution in making comparisons is advised. However, almost all patients in this group received pelvic lymph node irradiation, which increases toxicity[30], though only by a small amount. Over time, we were able to detect significantly more late GU side effects with increased dose while seeing no difference in late GI side effects. This is possibly due to smaller safety margins, especially when gold markers were implanted, as well as broader use of the IMRT and VMAT technique. Maximum late GI and GU side effects were not significantly different when comparing the 78 Gy group to the other groups. However, when defining the onset of late GU side effects ≥ grade 2 as an event, we detected a significant difference. As the subgroup analysis including only patients with irradiated lymph nodes did not show a significant difference, the cause for this is more likely in the dose escalation to the prostatic urethra and the bottom of the bladder.

A limitation of this study is its retrospective nature. In addition, due to the broad time period of the study, not only doses, but also irradiation technique and irradiated volume, varied over the 25-year observation period. Furthermore, our groups varied in regards to the percentage of patients with lymph node irradiation.

A strength of our study is that it is monocentric with systematic recording of GI/GU side effects. Thus, it provides consistent acquisition of side effects. This is especially important because of the large difference in reported toxicity by patients and physicians.[31] Moreover, we include a large collective of only one risk group for which we are able to present the development of daily routine without any bias due to study conditions.

Over the last quarter century, long-term bNED rates of patients treated with EBRT have increased by 50%. If such success could be achieved by a new drug, it would be all over the news. Sadly, our discipline fails to market this great success accordingly compared to developments in the areas of surgery and systemic treatments, especially with new, promising developments in high-risk prostate cancer treatment, such as simultaneously integrated boosts, as displayed in the FLAME-trial.[32]

Conclusions

Great progress has been made in the treatment of high-risk prostate cancer. Doses of 78 Gy result in significantly higher biochemical control rates and acceptable side effects. Therefore, dose escalation in EBRT for high-risk prostate cancer patients is an appropriate standard of care.

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research article

Real-world outcomes, treatment patterns and T790M testing rates in non-small cell lung cancer patients treated with first-line first- or second-generation epidermal growth factor receptor tyrosine kinase inhibitors from the Slovenian cohort of the REFLECT study

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Background. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are effective treatments for EGFR mutation-positive (EGFRm) non-small cell lung cancer (NSCLC). However, routine clinical practice is different between countries/institutions.

Patients and methods. The REFLECT study (NCT04031898) is a retrospective medical chart review that explored real-life treatment and outcomes of EGFRm NSCLC patients receiving first-line (1L) first-/second-generation (1G/2G) EGFR TKIs in 8 countries. This study included adult patients with documented advanced/metastatic EGFRm NSCLC with 1L 1G/2G EGFR TKIs initiated between Jan 2015 – Jun 2018. We reviewed data on clinical characteristics, treatments, EGFR/T790M testing patterns, and survival outcomes. Here, we report data from 120 medical charts in 3 study sites from Slovenia.

Results. The Slovenian cohort (median age 70 years, 74% females) received 37% erlotinib, 32% afatinib, 31% gefitinib. At the time of data collection, 94 (78%) discontinuations of 1L TKI, and 89 (74%) progression events on 1L treatment were reported. Among patients progressing on 1L, 73 (82%) were tested for T790M mutation yielding 50 (68%) positive results, and 62 (85%) received 2L treatment. 82% of patients received osimertinib. Attrition rate between 1L and 2L was 10%. The median (95% CI) real-world progression free survival on 1L EGFR TKIs was 15.6 (12.6, 19.2) months; median overall survival (95% CI) was 28.9 (25.0, 34.3) months.

Conclusions. This real-world study provides valuable information about 1G/2G EGFR TKIs treatment outcomes and attrition rates in Slovenian EGFRm NSCLC patients. The reduced attrition rate and improved survival outcomes emphasize the importance of 1L treatment decision.

Key words: real-world study; non-small cell lung cancer; epidermal growth factor receptor; T790M testing, attrition

Introduction

Lung cancer remains a major public health challenge worldwide, due to its diagnosis in advanced stages and high rate of mortality.[1,2] The discovery of sensitizing mutations to epidermal growth factor receptor (EGFR) has changed the treatment paradigm for lung cancer and has allowed for improved outcomes in patients with tumours harboring such actionable mutations.[3,4] Tyrosine kinase inhibitors (TKIs) targeting EGFR have proven efficacy for the treatment of EGFR-mutated (EGFRm) non-small cell lung cancer (NSCLC) and are the treatment of choice when this sensitizing mutation is found.[1] Several generations of EGFR TKIs have been developed and have become gradually available – from the first-generation erlotinib and gefitinib, to second-generation afatinib and dacomitinib, and third-generation osimertinib.[5]

Initial treatment recommendations for metastatic EGFRm NSCLC relied on first- and second-generation (1G/2G) EGFR TKIs, but despite promising initial responses to these therapies, the disease inevitably develops resistance and the progression requires treatment change.[6] In approximately half of cases, the resistance is mediated by the EGFR secondary mutation T790M[1,7], which is targeted by osimertinib in exon 20.[8] Based on AURA3 study results, the standard of care is now testing for the T790M mutation in all patients whose disease has progressed on 1G/2G EGFR TKIs and treatment with osimertinib when the T790M resistance mutation is identified.[1,7,8] Based on the FLAURA study results, which showed significant survival benefit with osimertinib versus comparator EGFR TKIs, osimertinib received approval by the European Medicines Agency in 2018 and became the preferred first-line (1L) treatment option in advanced or metastatic EGFRm NSCLC.[1,9]

The implementation of testing and treatment recommendations in clinical practice is not always a simple process. Access to new methods of molecular testing and novel therapies may be affected by lengthy local approvals and reimbursement processes, particularly in Central Eastern Europe (CEE).[10,11] Among countries in this region, Slovenia benefits from having a long tradition in cancer care and one of the oldest population-based cancer registries in Europe.[12,13] The advantage of having implemented a national cancer registry consists in the objective evaluation of the burden of disease and trends over time and is in direct conjunction with adequate setting and resource allocation at institutional level.[12,14]

In Slovenia, the molecular testing of EGFRm is reflex and it was partially covered by pharmaceutical companies until July 2020, when it became fully reimbursed by the public health system.[15] However, the reimbursement of innovative anti-cancer therapies is still not optimal, and it exceeds 2 years.[16] For example, the newly approved osimertinib as 1L therapy was reimbursed only in October 2020.

In addition to patient and tumour characteristics, the treatment decisions in real-world (RW) practice are driven by clinical and cost-effectiveness, safety, and availability of treatments.[17] As shown by the recent RW experience with 1L 1G/2G EGFR TKIs, the efficacy and safety of these agents proven in registration trials usually translate in real-life practice; yet, the testing rates of the resistance mutation T790M are not optimal.[18-26] To what extent the same findings apply in the Slovenian population is unknown. For this reason, Slovenia participated in this multinational medical chart review with the overarching goals of understanding the outcomes of EGFRm NSCLC patients initiated on 1L 1G/2G EGFR TKIs, treatment and T790M testing patterns, and attrition rates in various locations from Europe and Israel.[27] Here we present the results of the Slovenian patients included in this study.

Patients and methods

Study design and participants

The retrospective medical chart review "Real-world treatment patterns, clinical outcomes, and EGFR / T790M testing practices in EGFR-mutated advanced non–small cell lung cancer patients receiving First-Line EGFR TKI Therapy" (REFLECT, ClinicalTrials.gov: NCT04031898) was conducted in 7 European countries and Israel. Overall, medical chart review and data collection were carried out in 49 clinical centres from May to December 2019, and 3 comprehensive cancer care centres in Slovenia participated in this study. In Slovenia, data abstraction was conducted from October to December 2019.

The study design has been reported elsewhere.[27] Briefly, eligible patients for this study were ≥ 18 years of age with a confirmed diagnosis of locally advanced or metastatic EGFRm NSCLC who initiated 1L therapy with a 1G/2G EGFR TKI (afatinib, gefitinib or erlotinib) between January 1, 2015 and June 30, 2018. At the time of medical chart review, patients could have been alive or deceased, provided that the date of last follow-up or death was known. Patients were identified in the chrono-

logical order of initiating 1L EGFR TKIs within the study period of interest (i.e., starting with January 1, 2015) and enrolled consecutively in the electronic data collection form until the site's quota was reached. Patients enrolled in a clinical trial for experimental treatments related to EGFRm NSCLC and patients receiving systemic treatment for their locally advanced or metastatic NSCLC prior to the 1L EGFR TKIs were excluded.

In each participating country, the Institutional Review Boards (IRBs) or Ethics Committees (ECs) approved the protocol and study conduct. This medical chart review did not require informed, written consent from patients who were alive at the time of data collection unless the local IRBs/ECs required otherwise. In Slovenia, the Agency for Medicinal Products and Medical Devices (JAZMP) and the National Medical Ethics Committee (KME) approved the study, and an informed consent waiver was granted.

Outcomes and definitions

The primary outcome included progression events during treatment with 1L EGFR TKIs and time to progression, defined as time from initiation of 1L 1G/2G EGFR TKI therapy until the earliest sign of progression or death prior to start of a new therapy line or start of a new therapy line. Progression was defined as radiological progression according to any imaging method, start of new therapy line, death, or other record indicative of progression, such as documented evaluation of the clinician. To differentiate this primary outcome from the progression free survival (PFS) reported in randomized clinical trials, we use the term "real-world PFS" (rwPFS).

The secondary outcomes of this study included attrition rates and T790M testing rates among patients progressing on 1L 1G/2G EGFR TKIs, types of treatments received in subsequent lines, incidence of central nervous system (CNS) metastases and leptomeningeal disease (LMD) and time to their development, overall survival (OS) from the start of 1L EGFR TKI therapy, and OS from first diagnosis of CNS metastases and/or LMD to the date of death from any cause, with patients last known to be alive censored at the date of last available follow-up.

Data collection

Patient- and disease-specific data were obtained from the patient's medical records and registered by participating investigators in an electronic case report form. Each patient's case was allocated an anonymized, encrypted identifier. Data were collected from the time of initial NSCLC diagnosis until death or the last available follow-up at the time of the patient's inclusion in the study.

TABLE 1. Clinical characteristics at the time of initial NSCLC diagnosis

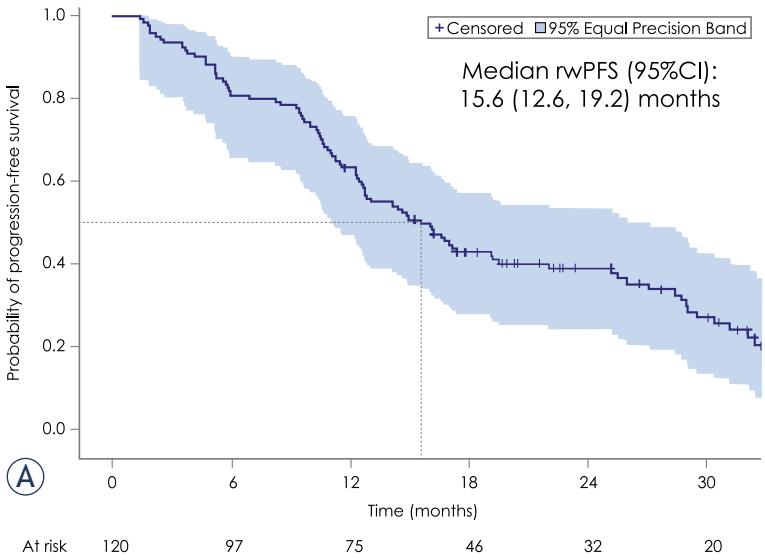
Characteristic	N = 120 n (%)
Smoking history	
Current smoker	7 (6)
Former smoker	33 (28)
Never smoker	76 (63)
Unknown	4 (3)
ECOG performance status	
0	28 (23)
1	62 (52)
2	22 (18)
3	6 (5)
4	1 (1)
Unknown	1 (1)
Stage at initial diagnosis	
Early stage (I-II)	13 (11)
Limited regional (IIIA)	4 (3)
Locally advanced (IIIB)	0
Metastatic (IV)	103 (86)
Site of distant metastases	
Adrenal	12 (10)
Bone	54 (45)
Brain	33 (28)
Liver	19 (16)
Lung	60 (50)
Lymph nodes	60 (50)
Peritoneal	2 (2)
Pleura	38 (32)
Skin/soft tissue	3 (3)
Other*	10 (8)

* Other sites of distant metastases included: bone marrow, eye, kidney, spleen, and pericardium.

ECOG=Eastern Cooperative Oncology Group

Statistical analysis

Sample size was based on the feasibility information received from each country, taking into ac-



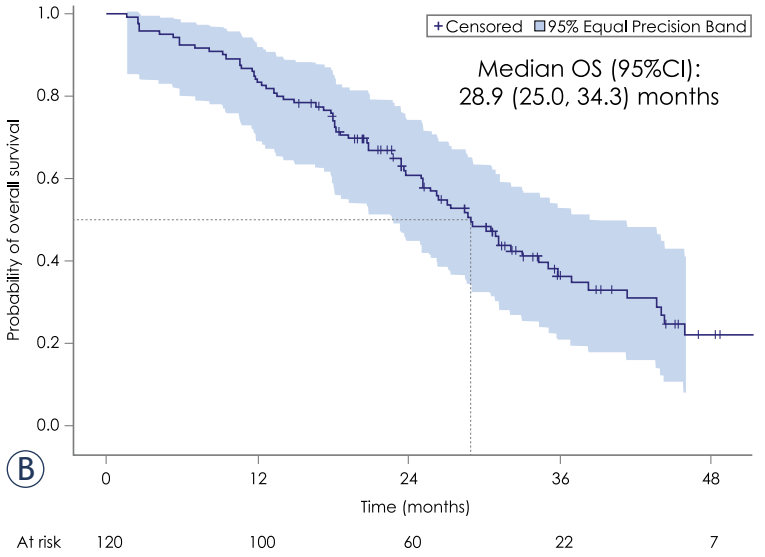


FIGURE 1. (A) Kaplan-Meier curves for median real-world progression free survival on first-line (1L) epidermal growth factor (EGFR) tyrosine kinase inhibitors (TKIs) therapy. **(B)** Kaplan-Meier curves for median overall survival from start of 1L EGFR TKI therapy. Censored patients are indicated with a cross.

CI = confidence interval; OS = overall survival; rwPFS = real-world progression-free survival

count the volume of potentially eligible patients treated with 1L EGFR TKIs in the period of interest for the study. It was anticipated that each participating physician would contribute with 5–30 case records to the study and each country would collect data from 50–180 medical records.

This study had no formal statistical hypothesis; descriptive statistics were used to assess the demographic and clinical characteristics, treatment patterns, and attrition rate. Kaplan-Meier estimators were used to describe median PFS and OS with 95% confidence interval (95% CIs). All analyses were performed in the full analysis set. The stratified OS analysis required > 20 number of events and a level of maturity of > 50%. The study was not powered for group comparisons.

Results

In total, 120 medical charts were included in this medical chart review from 3 study sites in Slovenia. The sites participating in the REFLECT study were also the only centres where lung cancer is being treated in Slovenia: 1 national cancer centre and 2 university hospitals.

Demographic, clinical and EGFR mutation characteristics at baseline

The median age (range) of patients was 70 (33–93) years, the majority were female (74%) and had never smoked (63%). At the initial diagnosis of NSCLC, adenocarcinoma was the predominant histological subtype (99%), and the majority of patients (86%) had metastatic stage. Most patients (75%) had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1. The most frequent sites of metastases at the time of initial diagnosis were lung and lymph nodes (50% each), bone (45%), pleura (32%), and brain (28%) (Table 1). The median (range) follow-up time was 24.3 (1.6–57.7) months.

EGFR mutation status was determined from tissue biopsy (75%) or cytology specimens (25%). The specimen was extracted from the primary tumour in most cases (73%). In 2% of patients, the biopsy site was unknown. The most frequent EGFR mutation was exon 19 deletion (58%) followed by exon 21 L858R point mutation (28%); uncommon mutations (15%) included G719X, L861Q, S768I, T790M, and exon 20 insertions.

First-line EGFR TKI therapy, progression and survival

The 1L EGFR TKI therapies initiated during the period of interest for the study had a balanced distribution: 37% of patients received erlotinib, 32% gefitinib and 31% afatinib. At the time of data collection, 94 patients (78%) discontinued 1L EGFR TKIs due to progression events or toxicities. Toxicities occurred in 9 cases (8%), with 5 of them (4%) not

starting any further treatment line. A number of 26 patients (22%) continued 1L treatment.

In total, 89 progression events per protocol were reported: 47 radiological progression events (39%), 22 clinical progression events (18%), 16 deaths (13%) and 4 cases (3%) with start of a new therapy line without documented progression.

Median (95% CI) rwPFS was 15.6 (12.6, 19.2) months (Figure 1A). Estimated probabilities for rwPFS (95% CI) at 12, 24 and 36 months were 63% (54%, 71%), 39% (30%, 48%) and 18%% (10%, 28%), respectively. Median (95% CI) OS from start of 1L EGFR TKI was 28.9 (25.0, 34.3) months (Figure 1B). Estimated probabilities for OS (95% CI) at 12, 24 and 36 months were 83% (75%, 89%), 61% (51%, 69%) and 36% (27%, 46%), respectively.

T790M mutation testing and osimertinib treatment

Of the 89 patients with progression events on 1L EGFR TKI therapy, 73 (82%) were tested for the T790M mutation at any time. Of the 73 patients tested for T790M mutation, the mutation was identified in 50 patients (68%) and the test was negative for 23 patients (32%). Of the 73 patients with disease progression on 1L EGFR TKIs who were tested for the T790M mutation, 62 (85%) received second-line (2L) treatment. In these patients, the 2L included osimertinib (84%), chemotherapy (15%) or targeted therapy (1%).

Among the rest of the 16 patients with progression on 1L EGFR TKI therapy who were not tested for T790M mutation, 4 patients (25%) received 2L treatment, with either chemotherapy or osimertinib (50% each).

Testing for the T790M mutation was performed by using liquid biopsy in most cases (77%), followed by tissue biopsy (14%) or cytology specimen (9%). Most tests (97%) were based on Cobas® EGFR mutation test (Roche). The mean time (standard deviation) between the initiation of 1L EGFR TKIs and T790M testing was 14.4 (9.0) months.

Second and subsequent therapy lines

Of the 89 patients with disease progression on 1L EGFR TKIs, 66 (74%) initiated 2L treatment. In the Slovenian cohort of patients, 16 (13%) patients who discontinued 1L died before receiving 2L treatment, while 12 (10%) patients alive of the time of 1L discontinuation did not receive any further line. The 2L treatments included osimertinib (82%), chemotherapy (17%) and other targeted therapy (1%). At the time of data collection 18 patients (28%) were still receiving 2L treatment (Figure 2).

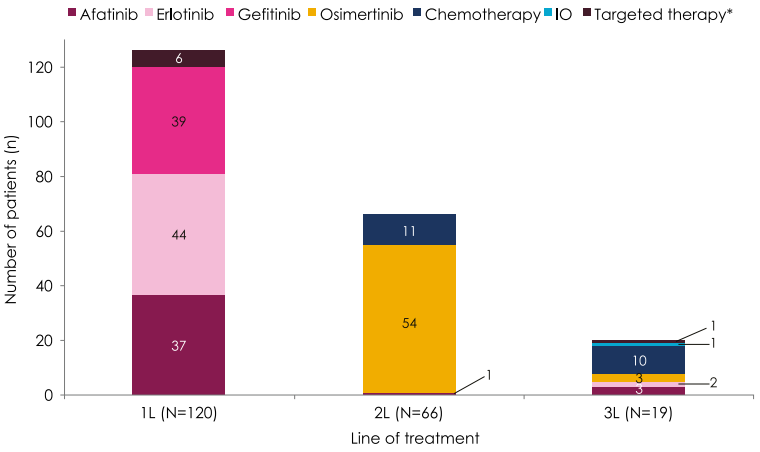


FIGURE 2. Treatment patterns patients in with locally advanced or metastatic epidermal growth factor receptor mutated (EGFRm) non-small cell lung cancer (NSCLC) treated with first-line (1L) first-/second-generation (1G/2G) EGFR tyrosine kinase inhibitors (TKIs). Note that multiple treatments could have been administered at each line of treatment.

* Targeted therapy besides afatinib, erlotinib, gefitinib and osimertinib (1L: not specified; 3L: crizotinib); 2L = second-line; 3L =third-line; IO = immuno-oncology

Of the 48 patients discontinuing 2L, 19 (40%) received third-line (3L) treatment, which consisted of chemotherapy (53%), targeted therapy (26%), osimertinib (16%), or immuno-oncological therapy (5%) (Figure 2). At the end of data collection, 1 patient (5%) was still on 3L treatment. Attrition rates on 1L, 2L, and 3L treatment are shown in Figure 3.

Of the 18 patients discontinuing 3L, 5 (28%) received fourth-line (4L) treatment, which consisted of targeted therapy (60%) or osimertinib (40%). All patients discontinued 4L, with one case of death being registered, while the remaining 4 patients received the fifth-line of treatment (5L), which consisted of targeted therapy (50%), chemotherapy (25%), and osimertinib (25%) (Figure 2). All patients discontinued 5L treatment.

Central nervous system metastases

The medical charts of 46 patients (38%) recorded the presence of central nervous system (CNS) metastases: in 33 cases (28%) these were present at the start of 1L EGFR TKIs, and in 13 cases (11%) the CNS metastases developed after the start of 1L treatment. In all cases (100%), an imaging examination (computed tomography or magnetic resonance imaging scan) was used for the diagnosis of the CNS metastases, and in 2 cases (4%) tissue biopsy was also performed. Patients with CNS metastases

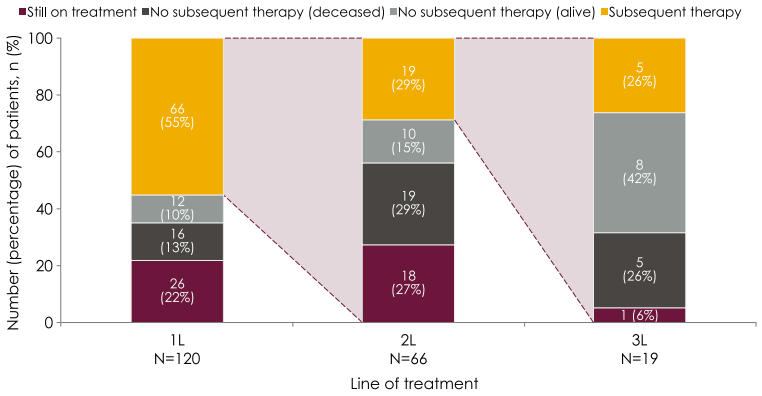


FIGURE 3. Attrition rates at first-line (1L), second-line (2L) and third-line (3L) in patients with locally advanced or metastatic epidermal growth factor receptor mutated (EGFRm) non-small cell lung cancer (NSCLC).

had a median age (range) of 67.5 (33.0–87.0) years and most (70%) were female. Treatments applied for CNS metastases included whole brain radiation therapy (63%), targeted therapy (63%), stereotactic radiosurgery (11%) and surgical resection (9%); in 4% of cases no treatment was provided.

The median (range) time from the initiation of 1L EGFR TKIs to CNS metastases diagnosed during 1L or later lines treatment was 19.8 (7.7, 34.6) months. The median (95% CI) OS in patients with CNS metastases at the start of 1L EGFR TKIs was 24.3 (18.4, 41.5) months, with 24 events reported. In the group of patients with CNS metastases developed during treatment, the number of events was too small to allow reporting of OS.

Leptomeningeal disease

Leptomeningeal disease (LMD) was reported in 4 patients: for 1 patient before and for 3 after the start of 1L EGFR TKI therapy. In all patients the diagnosis relied on imaging examinations only. The median (range) time from the initiation of 1L EGFR TKIs to LMD diagnosed during treatment was 19.6 (4.5, 28.7) months. The number of events was too small to allow reporting of OS.

Discussion

This is the first comprehensive analysis of the outcomes, treatment patterns, and testing rates in metastatic EGFRm NSCLC patients who received 1L 1G/2G EGFR TKI therapy in Slovenia over 3.5 years, from 2015 to 2018. This is a nationally representative dataset for our clinical practice because all 3 large-volume centers from Slovenia that ensure an integrated oncology care of lung cancer patients, with national coverage, participated in the REFLECT study.

Considering the real-life setting, the unselected population of patients with EGFRm NSCLC and the relatively equal distribution of 1G/2G EGFR TKIs (37% erlotinib, 32% afatinib, 31% gefitinib), our findings indicate positive treatment outcomes with 1L EGFR TKIs with a median rwPFS of 15.6 months. In the overall cohort from the REFLECT study (n=896), the median rwPFS was 13.0 (95% CI 12.3, 14.1) months and more patients received afatinib (45%).[27] In clinical trials of 1G/2G EGFR TKI therapy, the acquired resistance developed after a median of 9.2–14.7 months of targeted treatment.[6] Other European RW studies that partially overlap with the limits of the data collection set for the REFLECT study, but with a different distribution of the 1G/2G EGFR TKI therapies have shown PFS ranging from 7.6 to 11.0 months.[18-20,22,26] The enhanced rwPFS outcomes observed in the Slovenian cohort may be the result of more standardized and homogenous cancer care across centers, including established pathways for EGFR and T790M mutation testing, as well as effective control policies. Furthermore, in many cases the treatment may have continued beyond radiological progression, a common approach in patients with genetic actionable alterations.[1] Another observational study specifically exploring the continuation of EGFR TKIs beyond radiological progression showed that patients continued treatment without clinical deterioration for a median of 5.1 months and had a median PFS of 15.3 months.[28]

The median OS from the start of the 1L 1G/2G EGFR TKI therapy was 28.9 months in the Slovenian cohort and 26.2 (95% CI 23.6, 28.4) months in the overall REFLECT study cohort.[27] In general, the median OS reported in RW studies with 1L 1G/2G EGFR TKIs varies greatly, due to timelines set for the analysis, factors related to the healthcare system and access to EGFR TKIs, patient characteristics and data quality. Our findings are in line with those of other reports and are relevant for the period under study, when third-generation EGFR TKI osimertinib was not yet approved as 1L treatment.[18,20,22,24,26] Following osimertinib 1L approval and subsequent market entries, more data on the effectiveness of osimertinib in various geographies are awaited.

Upon progression on 1L 1G/2G EGFR TKIs, Slovenian national guidelines for the treatment of NSCLC, in accordance with European guidelines, recommend testing for resistance mutation

T790M and, in patients with positive test results initiation of osimertinib.[1,29] In this cohort, 82% of patients were tested for the presence of T790M upon progression on 1L; the resistance mutation was identified in two-thirds (68%) of these patients, thereby providing an opportunity for treatment that is effective against disease with T790M mutation. Expressed at the level of the overall Slovenian cohort (42%), this positive rate of T790M is in line with other RW data from European cohorts.[21,23,30,31] Additionally, in most patients (n=66) receiving 2L treatment in our cohort, post-progression treatment consisted of osimertinib (82%), preponderantly in patients with the T790M mutation. These results support a unified approach to T790M testing and subsequent treatment at the national level, consistent with guidelines recommendations.[1,29] In current local practice, when a clinical progression is suspected (even before radiologic progression), an active search with minimally invasive liquid biopsy for the presence of resistance T790M mutation is begun. This approach allows for early initiation of 2L systemic therapy with the goal of improving patient outcomes.

Over the course of the lung cancer disease, many patients develop CNS metastases, which confer a poor prognosis and present additional treatment challenges.[32] CNS metastases are often identified in patients with adenocarcinoma and molecular alterations, and their incidence is significantly correlated with the presence of EGFR-activating mutations.[1,33,34] In this cohort, 38% of patients had CNS metastases, most of them present at the time of diagnosis of the metastatic stage of lung cancer (28%). In a local retrospective analysis exploring the cumulative incidence of brain metastases in 629 patients with adenocarcinomas tested for EGFRm, those with the EGFR activating mutation had a longer time to CNS progression (25.9 vs. 11.9 months, p=0.002).[35] In this REFLECT study cohort, the time to CNS progression was 19.8 months, with a median OS of 24.3 months in patients with CNS metastases at the start of 1L 1G/2G EGFR TKI therapy. The difference may be due to advances in radiological techniques used to identify CNS metastases, as well as practice changes. The dynamic landscape of technology, improved local control and reduced morbidity are reflected in the current management of CNS metastases as stereotactic radiosurgery has become the foremost treatment modality in patients with "limited" intracranial disease.[36]

REFLECT was primarily a study of attrition rates between treatment lines. In this cohort, of the 78 patients who started 1L 1G/2G EGFR TKIs and were alive at the time of treatment discontinuation, 12 (15%) did not receive 2L treatment. The trend of not receiving further treatment was sustained in subsequent lines, although the number of patients alive at the time of treatment discontinuation progressively decreased. The rate of patients not receiving 2L treatment after the 1L EGFR TKIs was initially reported in clinical trials and it was approximately 35%, whereas in RW studies the rate varies more widely (10–62%).[25,37,38] Although the REFLECT study did not explore the reasons why patients did not receive further treatment lines, data reported in the literature suggest various causes, including lack of genetic testing, low T790M mutation rate, poor performance status and even patient's preference not to receive the next line of treatment, which would be chemotherapy in many cases.[37] In our cohort we noticed that 18% patients progressing on 1L EGFR TKI were not tested for presence of T790M mutation. The rationale behind the lack of T790M testing at progression was not investigated, but such finding might be explained by rapid deterioration of clinical status followed by death on 1L EGFR TKI, presence of exon 20 insertion, which is associated with limited efficacy of common EGFR TKIs and unfavorable prognosis or poor performance status at the time of disease progression rendering patient ineligible for any further systemic therapy.[37,39,40] Hence, the true T790M positivity rate and proportion of patients eligible for targeted 2L may be different in real-life. Beyond possible differences in healthcare setting and availability of effective treatment options, exploring locally in more depth the reasons behind attrition rates is crucial to further improve patient outcomes.

The real-life character of this study confers both strengths and limitations. With a minimal set of inclusion and exclusion criteria, and a representative dataset for Slovenia, this study allowed for building RW evidence on 1L 1G/2G EGFR TKI therapy at the national level based on a 3.5-year data review (2015–2018). The fact that data collection relied entirely on information existing in patients' records, which sometimes have insufficient or missing data, is a key limitation in such designs. Nevertheless, Slovenia benefited from the participation of all 3 of the country's institutions in which lung cancer is treated. As a result, data availability was very good, with minimal cases of unknown information in patients' histories. In general, secondary data collection may be subject to selection bias, including of sites and patients. To reduce site selection bi-

as and potential patients' spreading between sites, all 3 Slovenian comprehensive cancer centers were included in the study. To reduce patient selection bias, the ethics review package submitted has requested an informed consent waiver, which was granted by the National Ethics Committee. Thus, all medical records of eligible patients were considered, irrespective of the vital status at the time of data collection and patients were enrolled consecutively in the electronic data collection form in the chronological order of starting the 1L 1G/2G EGFR TKI therapy. In contrast to clinical trials design, disease progression was not confirmed through a standardized, objective method, and the study definition reflects the RW situation (start of a new line of therapy or any other records indicative of progression, besides radiological tests). Finally, the study was not powered to compare the individual 1G/2G EGFR TKIs, and therefore outcomes could not be further characterized by molecule.

Conclusions

This real-world study, performed in a representative dataset for Slovenian clinical practice, provides insights into the effectiveness of 1G/2G EGFR TKIs and T790M testing patterns in EGFRm NSCLC patients receiving routine care. The survival outcomes and reduced attrition rate reported in this real-life setting from our country are encouraging. Newer 1L treatment options require follow-up studies to reflect the dynamic changes in clinical practice.

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research article

Trends in treatment of childhood cancer and subsequent primary neoplasm risk

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Background. The aim of the study was to investigate long-term risk and spectrum of subsequent neoplasm (SN) in childhood cancer survivors and to identify how trends in therapy influenced cumulative incidence of SN.

Patients and methods. The population-based cohort comprises 3271 childhood cancer patients diagnosed in Slovenia aged ≤ 18 years between 1st January 1961 and 31st December 2013 with a follow-up through 31st December 2018. Main outcome measures are standardised incidence ratios (SIRs), absolute excess risks (AERs), and cumulative incidence of SN.

Results. After median follow-up time of 21.5 years for 5-year survivors, 230 patients experienced 273 SN, including 183 subsequent malignant neoplasm (SMN), 34 meningiomas and 56 nonmelanoma skin cancers. 10.5% patients received radiotherapy only, 31% chemotherapy only, 26.9% a combination of chemotherapy and radiotherapy and 16.1% surgery only. The overall SIR was almost 3 times more than expected (SIR 2.9), with survivors still at 2-fold increased risk after attained age 50 years. The observed cumulative incidence of SMN at 30-year after diagnosis was significantly lower for those diagnosed in 1960s, compared with the 1970s and the 1980s (P heterogeneity < 0.001). Despite reduced use of radiotherapy over time, the difference in cumulative incidence for the first 15 years after diagnosis was not significant for patients treated before or after 1995 (p = 0.11).

Conclusions. Risks of developing a SMN in this study are similar to other European population-based cohorts. The intensity of treatment peaked later and use of radiotherapy declined slower compared to high income countries, making continuous surveillance even more important in the future.

Key words: population-based study, childhood cancer survivors, subsequent neoplasm

Introduction

Currently, ~ 80% of children with cancer are long-term survivors with possible late sequelae.[1,2] Treatment of childhood cancer depends on surgery, radiotherapy, and chemotherapy despite their potential toxicity. Late effects of cancer treatment are important causes of morbidity and mortality in survivors of childhood cancer.[3] The burden of therapy was reduced, through clinical trials, in childhood cancers with good or excellent survival.[4] However, for many children with cancer relapse of primary disease is still the leading cause of death.[4] Death due to subsequent neoplasm (SN) is the most common non-relapse related event.[5]

Large population-based studies in childhood cancer survivors have been conducted in the Nordic countries and Britain with long and almost

complete follow-up.[6,7] Cancer registries generally have limited or no information on treatment variables. Multicentre studies conducted in Netherlands and US collect data through questionnaires or hospital registries, with up to one third of patients lost to follow up.[8-14] However, detailed treatment data extracted from hospital registries provided important information about the risk factors for SN.[8-14]

Population based analysis of SN after treatment of childhood cancer in Slovenia was first published in 2004.[15] The aims of present analysis were to assess long-term risk and spectrum of SN in Slovenia; identify how trends in therapy influenced cumulative incidence of SN.

Patients and methods

Cohort ascertainment and subsequent neoplasm ascertainment

The study cohort comprises patients in Slovenia aged ≤ 18 years with childhood cancer diagnosis between 1st January 1961 and 31st December 2013 and a follow-up through 31st December 2018.

The cohort was ascertained through the population-based Cancer Registry of Slovenia (CRS). The registry combines data from University Children Hospital Ljubljana and Institute of Oncology Ljubljana, representing all institutions where childhood cancer patients are treated and subjected to follow-up.[16] Data coverage is estimated to be close to complete. CRS is linked to the Central population registry for information on vital status and causes of death.

Childhood cancers were coded according to International Classification of Diseases for Oncology (ICD, 3rd version).[17] For every patient basic treatment information (use of surgery, chemotherapy, and radiation) and outcome (recurrence of primary cancer, subsequent neoplasms, cause of death) were reported.

Subsequent neoplasms (SNs) for the entire cohort were defined as a neoplasm on new location, which is not a direct spread or metastasis of the primary neoplasm, or neoplasm on the same location with a different histological type (18.). SNs were validated through pathology reports or in some cases with other means through a clinical diagnosis (e.g., meningioma). SN were classified as subsequent malignant neoplasm (SMN), having ICD-O behaviour code of 3, meningioma, non-melanoma skin cancer (NMSC).

As registration of neoplasms with ICD-O behaviour code 2 is close to complete in CRS, these were included in SMN (*in situ* cervical carcinoma, *in situ* carcinoma of bladder, ductal *in situ* carcinoma of breast and *in situ* melanoma). As registration of meningioma and NMSC is incomplete for general population, they were reported for our cohort but excluded from further statistical analysis.

Statistical analysis

Time at risk for SN was set at diagnosis of childhood cancer (at latest 31st December 2013) and ended at the earliest occurrence of loss of follow up, death or study exit date (31st December 2018). During this period 3350 children were diagnosed with cancer, 79 patients were excluded from analysis after reviewing diagnosis (histology missing, benign tumours or Langerhans cell histiocytosis).

Standardized incidence ratio (SIR) was calculated as the observed divided by expected number of SMN. The expected number of SMNs were calculated by multiplying the number of person-years at risk in the cohort within specific sex, five-year age strata and single calendar year interval by corresponding neoplasm incidence rates in Slovenian population extracted from CRS. Absolute excess risk (AER) was calculated as observed minus expected number of SMN divided by person-years at risk and multiplied by 1000, unless otherwise specified. AER is the number of extra SMN observed beyond that expected per 1000 persons per year. Meningioma and NMSC were excluded from SIR and AER calculations since their ascertainment is not complete in CRS.

SIRs and AERs were stratified by sex, age at diagnosis of primary cancer, attained age (age of the subjects at the study exit date, death or lost of follow up), primary neoplasm type, treatment period of childhood cancer, years from diagnosis of childhood cancer and childhood cancer therapy. A multivariable Poisson regression model was used to calculate relative risk (RR) and relative excess risk (RER) and analyse the potential simultaneous effect of this explanatory factors on the SIR and AER. Relative risk represents ratio of SIRs adjusted for explanatory factors and RER as ratio of AERs adjusted for explanatory factors (19.). Results relating to overall SIRs and AERs were only reported in text whenever there were at least 3 observed SMNs. For SIR, AER, RR and RER 95% confidence intervals were estimated (95% CI).

The cumulative incidence for the first occurrence of SN, SMN, NMSC and meningioma was computed as a function of time from childhood cancer diagnosis with death due to any other cause

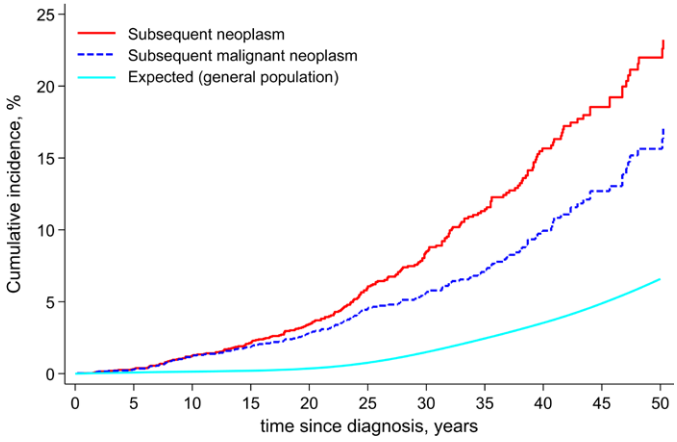

FIGURE 1. Cumulative incidence of all subsequent neoplasms and subsequent malignant neoplasms.

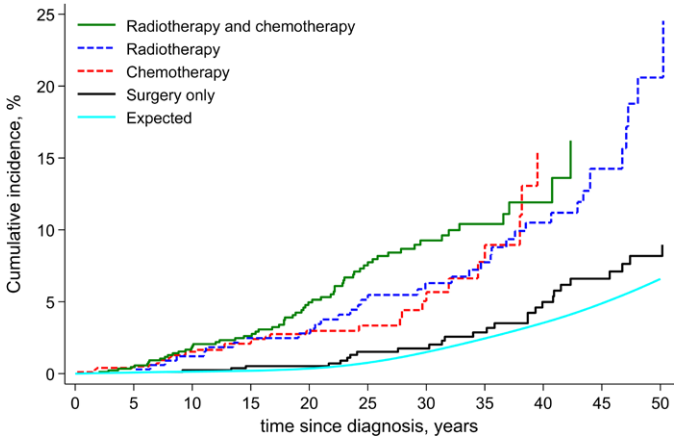

FIGURE 2. Cumulative incidence of subsequent malignant neoplasm by treatment modality of childhood cancer.

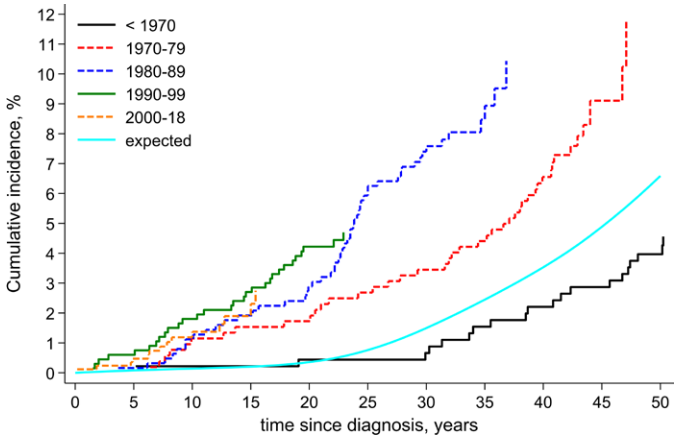

FIGURE 3. Cumulative incidence of subsequent malignant neoplasm by decade of diagnosis of childhood cancer.

prior to developing SN considered as a competing event. Expected cumulative incidence for SMNs was calculated using the Ederer II method.[20]

Five-year relative survival following an SN was estimated using the Stata command strs.[21]All statistical analysis were conducted using Stata statistical software, version 17.0. All tests were 2-sided, with p value < 0.05 considered statistically significant.

Results

Cohort characteristics

In this retrospective cohort study 3,271 childhood cancer patients accrued a total of 46,464 person-years of follow-up, with median follow-up time of 21.5 years (range, 5.25–57.8 years) for 5-year survivors. The most common types of childhood cancer were leukaemia (26.6%), CNS tumours (19.1%), Hodgkin's lymphoma (9.6%) and non-Hodgkin's lymphoma (8.5%) (Table 1).

In total, 230 patients experienced 273 SN, including 183 SMN, 34 meningiomas and 56 NMSC. Of all individuals with an SN, 192 had one, 33 two and 5 three SNs. At the study exit date 53% (n = 1744) of patients were alive (Table 2). A total of 10.5% patients received radiotherapy only, 31% chemotherapy only, 26.9% a combination of chemotherapy and radiotherapy and 16.1% surgery only. The proportion of patients treated with radiotherapy was highest for those diagnosed from 1970 to 1989 (> 50%) and decreased over time (29.7% > 2000). Simultaneously, the number of patients treated with chemotherapy increased from 49.1% in 1970s to 75.2% after year 2000. In the cohort 16% (n = 527) patients had no therapy, of whom 75% were diagnosed before 1970 and majority died of childhood cancer. After 1980 there is approximately 4% of children with cancer undergoing observation only (e.g., low grade glioma, low risk neuroblastoma) (Table 3).

The overall risk of developing an SNs and SMNs

The estimated cumulative incidence of developing an SMN in the cohort was 2.8% at 20 years and increased to 5.7% at 30 years after childhood cancer diagnosis. The cumulative incidence of SNs and SMNs increased with attained age without plateauing (Figure 1).

Cumulative incidence of developing an SMN at 40 years after childhood cancer diagnosis was significantly lower for patients having surgery only (P

TABLE 1. Characteristics of all individuals in study and number of subsequent neoplasms

		Number (%)	Any subsequent malignant neoplasm	Non-melanoma skin cancer	Benign meningioma
All survivors		3271 (100%)	183 (100%)	56 (100%)	34 (100%)
Gender	Male	1830 (55.9%)	77 (42.1%)	30 (54%)	14 (41%)
	Female	1441 (44.1%)	106 (57.9%)	26 (46%)	20 (59%)
Childhood cancer type	Leukaemia	870 (26.6%)	23 (12.6%)	11 (20%)	14 (41%)
	Hodgkin's lymphoma	315 (9.6%)	51 (27.9%)	17 (30%)	2 (6%)
	Non-Hodgkin's lymphoma	277 (8.5%)	16 (8.7%)	4 (7%)	2 (6%)
	Central nervous system tumour	625 (19.1%)	25 (13.7%)	12 (21%)	15 (44%)
	Neuroblastoma	124 (3.8%)	6 (3.3%)	1 (2%)	0 (0%)
	Retinoblastoma	60 (1.8%)	0 (0%)	0 (0%)	0 (0%)
	Wilms' tumour	143 (4.4%)	9 (4.9%)	1 (2%)	1 (3%)
	Bone tumour	199 (6.1%)	13 (7.1%)	0 (0%)	0 (0%)
	Soft-tissue sarcoma	224 (6.8%)	14 (7.7%)	4 (7%)	0 (0%)
	Germ cell	168 (5.1%)	8 (4.4%)	3 (5%)	0 (0%)
	Liver	27 (0.8%)	1 (0.5%)	1 (2%)	0 (0%)
	Thyroid	86 (2.6%)	8 (4.4%)	1 (2%)	0 (0%)
	Nasopharyngeal carcinoma	13 (0.4%)	4 (2.2%)	1 (2%)	0 (0%)
	Melanoma	75 (2.3%)	2 (1.1%)	0 (0%)	0 (0%)
	Carcinoma	59 (1.8%)	3 (1.6%)	0 (0%)	0 (0%)
	Other	6 (0.2%)	0 (%)	0 (0%)	0 (0%)
Age at childhood cancer diagnosis (years)	Mean	9.4 (6.0)	11.2 (5.7)	11.3(6.0)	7.0 (4.1)
	0–4	1065 (32.6%)	39 (21.3%)	13 (23%)	12 (35%)
	5–9	656 (20.1%)	34 (18.6%)	9 (16%)	15 (44%)
	10–14	690 (21.1%)	49 (26.8%)	13 (23%)	5 (15%)
	15–19	860 (26.3%)	61 (33.3%)	21 (38%)	2 (6%)
Decade of diagnosis of childhood cancer	< 1970	528 (16.1%)	22 (12.0%)	4 (7%)	3 (9%)
	1970–79	560 (17.1%)	50 (27.3%)	18 (32%)	11 (32%)
	1980–89	651 (19.9%)	63 (34.4%)	22 (39%)	16 (47%)
	1990–2000	679 (20.8%)	32 (17.5%)	9 (16%)	3 (9%)
	2000–2018	853 (26.1%)	16 (8.7%)	3 (5%)	1 (3%)
Attained age (years)	0–19	1643 (50.2%)	31 (16.9%)	4 (7%)	2 (6%)
	20–29	550 (16.8%)	33 (18.0%)	4 (7%)	7 (21%)
	30–39	494 (15.1%)	59 (32.2%)	20 (36%)	19 (56%)
	40–49	353 (10.8%)	33 (18.0%)	19 (34%)	5 (15%)
	50–59	151 (4.6%)	19 (10.4%)	7 (12%)	0 (0%)
	60+	80 (2.4%)	8 (4.4%)	2 (4%)	1 (3%)
Treatment of childhood cancer	No therapy	527 (16.1%)	4 (2.2%)	9 (16%)	2 (6%)
	Surgery only	506 (15.5%)	24 (13.1%)	3 (5%)	0 (0%)
	Chemotherapy	1014 (31.0%)	40 (21.9%)	17 (30%)	2 (6%)
	Radiotherapy	345 (10.5%)	44 (24.0%)	27 (48%)	11 (32%)
	Radiotherapy and chemotherapy	879 (26.9%)	71 (38.8%)	9 (16%)	19 (56%)

TABLE 2. Vital status by decade of childhood cancer diagnosis

Decade of diagnosis	All survivors	
	Dead	Alive
< 1970	447	81
1970–1979	394	166
1980–1989	309	342
1990–2000	222	457
2000–2013	155	698
Total	1527	1744

heterogeneity < 0.001) (Figure 2). The observed cumulative incidence of SMN at 30 years after childhood cancer diagnosis was significantly lower for those diagnosed in 1960s (P heterogeneity < 0.001) (Figure 3). Despite reduced use of radiotherapy after 1995 difference in cumulative incidence of SMN for the first 15 years after diagnosis was not significant (Pepe Mori's test for difference, p = 0.11).

The risk of developing any SMN was almost 3-fold (SIR 2.9; 95% CI: 2.5–3.3) in the cohort compared with the general population, corresponding to an absolute excess risk of 2.6 per 1000 person-years (95% CI: 2.1–3.2.). Males appeared to be at higher risk than females in terms of the SIR (P heterogeneity < 0.001). With increasing attained age, the SIR gradually decreased, and AER increased (Table 4), with survivors still at 2-fold increased risk after age 50 years (SIR = 2.0; 95% CI: 1.3–3.1). The risk of an SMN was highest among patients with nasopharyngeal carcinoma (SIR 7.5; 95% CI: 2.8–20.0), neuroblastoma (SIR 5.1; 95% CI: 2.3–11.3) and Hodgkin's lymphoma (SIR 5.0; 95% CI: 3.8–6.6) (Table 4).

Elevated SIRs and AERs were evident for all childhood cancers, except for retinoblastomas, melanomas, and carcinomas. Not a single retinoblastoma patient in cohort developed SN.

Five-year overall survival was estimated for children with different solid tumours through decades to enable interpretation of results. Survival for patients with retinoblastoma was 50%, 56%, 88% and 100% for those diagnosed in 1960s, 1970s, 1980s and after 2000, respectively. Patients with central nervous system (CNS) tumours, sarcomas and Wilms tumours diagnosed in 1970s and 1990s experienced increase of five-year overall survival from 44% to 65%, 46% to 62% and 58% to 76%, respectively.

Risk of specific subsequent primary neoplasms

The most frequent SMNs were those of the thyroid (n = 37), genitourinary (n = 36; 15 cervical carcinoma *in situ*) and breast (n = 26) carcinoma. The majority of breast (n = 13) and thyroid (n = 19) carcinoma occurred in Hodgkin's lymphoma. Most genitourinary cancers occurred among bone and soft tissue sarcoma survivors (n = 12). Seventy percent of SN occurred in patients with CNS tumours, leukaemia, and lymphoma (Table 5).

The greatest risk for SMN was observed for thyroid, (SIR 21.6; 95% CI: 15.2–29.7), CNS (SIR 13.4; 95% CI: 7.9–21.2), soft tissue sarcoma (SIR 9.5; 95% CI: 3.1–22.2) and head and neck carcinoma (SIR 6.4; 95% CI: 2.9–12.1). SMNs of the thyroid (AER 76), breast (AER 41) and CNS (AER 36) contributed together almost 60% to the total AER. The distribution of observed excess SMN changed with attained age. In patients up to 40 years of age thyroid (AER 71), breast (AER 35), CNS tumours (AER 30) and leukaemia (AER 17) represent the majority of SMNs. After 40 years of age thyroid (AER 109), genitourinary (AER 87), breast (AER 84), CNS (AER 78) and respiratory (AER 75) tumours were responsible for 80% of the total AER (Table 6).

Because follow up commenced at the time of childhood cancer diagnosis all subsequent leukae-

TABLE 3. Treatment modality by decade of childhood cancer diagnosis

Treatment	< 1970	1970–79	1980–89	1990–99	2000–2013
No therapy	399 (75.6%)	38 (6.8%)	27 (4.2%)	30 (4.4%)	33 (3.9%)
Surgery only	41 (7.8%)	102 (18.2%)	85 (13.1%)	116 (17.1%)	162 (19.0%)
Chemotherapy only	30 (5.7%)	135 (24.1%)	174 (26.7%)	270 (39.8%)	405 (47.5%)
Radiotherapy only	49 (9.3%)	145 (25.9%)	88 (13.5%)	46 (6.8%)	17 (2.0%)
Radiotherapy and chemotherapy	9 (1.7%)	140 (25.0%)	277 (42.6%)	217 (32.0%)	236 (27.7%)
Total	528 (100%)	560 (100%)	651 (100%)	679 (100%)	853 (100%)

TABLE 4. Standardized incidence ratios (SIR), absolute excess risks (AER), relative risk (RR) and relative excess risk (RER) for any subequent malignant neoplasm (SMN)

Factor	Level	any SMN			AER (95%CI)	RER (95%CI)
		O	SIR (95%CI)	RR (95%CI)		
Overall	All combined	183	2.9 (2.5,3.3)	–	2.6 (2.1,3.2)	--
Sex	Male	77	4.0 (3.2,5.0)	1.0 (ref.)	2.3 (1.7,3.1)	1.0 (ref.)
	Female	106	2.4 (2.0,2.9)	0.7 (0.5-1.0)	2.9 (2.1,4.0)	1.4 (0.9-2.1)
	$P_{heterogeneity*}$		<0.001	0.03	0.30	0.16
Age at diagnosis of childhood cancer (years)	0–4	39	3.9 (2.8,5.3)	1.0 (ref.)	2.0 (1.3,3.1)	1.0 (ref.)
	5–9	34	3.3 (2.3,4.6)	0.9 (0.6-1.6)	2.4 (1.5,4.0)	0.8 (0.4-1.6)
	10–14	49	3.1 (2.3,4.1)	0.9 (0.5-1.5)	3.2 (2.1,4.9)	0.7 (0.4-1.5)
	15–19	61	2.3 (1.8,2.9)	0.8 (0.5-1.3)	2.7 (1.7,4.3)	0.6 (0.3-1.2)
	P_{trend*}		0.01	0.3	0.23	0.13
Decade of diagnosis of childhood cancer	< 1970	22	1.4 (1.0,2.2)	1.0 (ref.)	1.2 (0.3,4.8)	1.0 (ref.)
	1970–1979	50	3.4 (2.6,4.5)	1.7 (1.0-3.0)	4.1 (2.8,6.1)	3.4 (1.0-11.9)
	1980–1989	63	4.0 (3.1,5.1)	1.7 (0.9-3.0)	3.8 (2.7,5.3)	3.5 (1.0-12.5)
	1990–2000	32	2.7 (1.9,3.8)	1.1 (0.5-2.1)	1.8 (1.0,3.1)	2.6 (0.7-9.7)
	2000–2018	16	2.7 (1.7,4.4)	0.9 (0.4-2.0)	1.2 (0.5,2.5)	2.5 (0.6-10.4)
	P_{trend*}		0.07	0.3	0.02	0.61
Era diagnosis	< 1995	151	2.9 (2.5,3.4)	1.0 (ref.)	3.1 (2.4,3.9)	1.0 (ref.)
	> = 1995	32	2.8 (2.0,4.0)	0.7 (0.5-1.1)	1.4 (0.8,2.5)	1.0 (0.6-1.8)
	$P_{heterogeneity*}$		0.88	0.15	0.01	0.9
Attained Age (yrs)	< 20	31	10.6 (7.4,15.0)	1.0 (ref.)	1.5 (1.0,2.2)	1.0 (ref.)
	20–29	33	2.2 (1.6,3.1)	0.2 (0.1-0.4)	1.4 (0.7,2.5)	1.0 (0.5-2.0)
	30–39	59	3.5 (2.7,4.5)	0.3 (0.2-0.5)	5.1 (3.6,7.3)	3.4 (1.9-6.1)
	40–49	33	2.7 (1.9,3.8)	0.2 (0.1-0.4)	5.2 (3.0,9.0)	3.4 (1.5-7.4)
	50–59	19	2.0 (1.3,3.1)	0.2 (0.1-0.4)	6.6 (2.7,16.3)	7.5 (2.8-20.4)
	60+	8	1.3 (0.6,2.6)	0.1 (0.1-0.4)	3.7 (0.2,90.4)	10.8 (1.6-74.0)
	P_{trend*}		<0.001	<0.001	<0.001	<0.001
Time since diagnosis of childhood cancer (years)	0–9	38	6.0 (4.3,8.2)	1.0 (ref.)	1.6 (1.1,2.3)	1.0 (ref.)
	10–19	37	2.6 (1.9,3.6)	0.4 (0.3-0.7)	1.7 (1.0,2.9)	1.1 (0.6-2.0)
	20–29	51	3.1 (2.4,4.1)	0.4 (0.2-0.6)	4.3 (2.9,6.5)	2.5 (1.4-4.4)
	20–39	36	2.6 (1.9,3.6)	0.3 (0.2-0.6)	5.7 (3.4,9.6)	3.4 (1.7-6.9)
	40+	21	1.7 (1.1,2.5)	0.2 (0.1-0.4)	5.3 (1.8,15.5)	5.2 (2.1-12.4)
	P_{trend*}		<0.001	<0.001	<0.001	<0.001
Type of childhood cancer	Leukaemia	23	2.7 (1.8,4.0)	1.0 (ref.)	1.6 (0.8,3.0)	1.0 (ref.)
	Hodgkin's lymphoma	51	5.0 (3.8,6.6)	2.5 (1.4-4.2)	6.5 (4.6,9.1)	2.8 (1.4-5.7)
	non-Hodgkin's lymphoma	16	4.3 (2.7,7.1)	1.7 (0.9-3.3)	3.3 (1.7,6.2)	1.3 (0.5-3.6)
	Central nervous system tumour	25	2.8 (1.9,4.2)	1.2 (0.7-2.2)	2.1 (1.1,3.8)	1.1 (0.5-2.4)
	Neuroblastoma	6	5.1 (2.3,11.3)	1.8 (0.7-4.5)	3.2 (1.2,8.6)	1.8 (0.6-5.5)
	Retinoblastoma	0	0	-	0	-
	Wilms Tumour	9	3.8 (2.0,7.3)	1.4 (0.6-3.1)	2.6 (1.1,6.3)	1.0 (0.3-3.3)
	Bone sarcoma	13	2.7 (1.6,4.6)	1.6 (0.8-3.3)	3.3 (1.4,7.8)	1.8 (0.6-5.0)
	Soft-tissue sarcoma	14	2.6 (1.5,4.4)	1.2 (0.6-2.3)	2.3 (1.0,5.4)	1.1 (0.4-2.9)
	Germ-cell	8	1.6 (0.8,3.1)	0.9 (0.4-2.1)	1.0 (0.1,6.7)	0.6 (0.1-3.0)
	Liver	1	7.9 (1.1,56.1)	2.2 (0.3-16.3)	3.2 (0.3,30.4)	2.1 (0.2-19.2)
	Thyroid	8	2.0 (1.0,4.0)	1.2 (0.5-2.7)	2.3 (0.6,8.9)	0.9 (0.2-4.0)
	Nasopharyngeal carcinoma	4	7.5 (2.8,20.0)	4.2 (1.4-12.8)	12.6 (4.1,39.1)	6.9 (1.9-24.6)
	Melanoma	2	0.4 (0.1,1.8)	0.3 (0.1-1.4)	0.1	0
	Carcinoma	3	1.1 (0.4,3.4)	0.8 (0.2-2.6)	0.2	0
	$P_{heterogeneity*}$		<0.001	<0.001	<0.001	<0.001
Treatment of childhood cancer	No therapy treatment of childhood	4	0.4 (0.2,1.1)	0.3 (0.1-0.8)	0	-
	Surgery only	24	1.7 (1.1,2.5)	1.0 (ref.)	1.0 (0.4,2.7)	1.0 (ref.)
	Chemotherapy	40	3.3 (2.4,4.4)	1.8 (1.1-3.1)	2.2 (1.4,3.4)	4.6 (1.0-20.9)
	Radiotherapy	44	4.4 (3.3,5.9)	2.6 (1.6-4.3)	5.7 (3.9,8.4)	7.3 (1.6-33.5)
	Radio and chemotherapy	71	4.3 (3.4,5.4)	2.4 (1.5-3.9)	3.8 (2.8,5.2)	7.0 (1.6-30.8)
	$P_{heterogeneity*}$		<0.001	<0.001	<0.001	<0.001

* = observed

TABLE 5. Number and type of subsequent neoplasms (SN) by childhood cancer type

Childhood cancer type / SN	ALL AML	HL	NHL	CNS	Neuroblastoma	Retinoblastoma	Wilms	Bone sarcoma	Soft tissue sarcoma	Germ cell	Liver	Thyroid	Nasopharyngeal carcinoma	Melanoma	Carcinoma	Total
Meningioma	14	2	2	15	0	0	1	0	0	0	0	0	0	0	0	34
NMSC	11	17	4	12	1	0	1	0	4	3	1	1	1	0	0	56
Breast (C50 D05)	1	14	0	0	0	0	1	1	2	2	0	1	1	1	2	26
CNS (C70-C72)	6	0	0	11	1	0	0	0	0	0	0	0	0	0	0	18
Digestive (C15-C26)	1	3	4	0	1	0	0	2	1	0	0	1	0	0	0	13
Genitourinary (C51-C68, D09, D06)	3	3	2	4	0	0	2	5	7	3	1	5	0	1	0	36
Leukaemia (C90-C93)	3	2	1	1	0	0	0	1	1	0	0	0	0	0	0	9
Lymphoma (C81-C85)	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Melanoma (C43, D03)	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	4
Bone (C40-C41)	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
Head&Neck (C00-C14)	2	1	1	1	0	0	0	2	0	0	0	0	1	0	1	9
Other	1	3	1	1	0	0	2	0	0	0	0	1	0	0	0	9
Respiratory (C30-C39)	0	4	3	0	1	0	0	0	2	1	0	0	1	0	0	12
Soft-tissue (C49)	0	2	0	0	2	0	0	0	0	1	0	0	0	0	0	5
Thyroid (C73)	2	19	3	6	1	0	3	1	1	0	0	0	1	0	0	37
Total	48	70	22	52	7	0	11	13	18	11	2	9	5	2	3	273

ALL/AML = acute lymphoblastic/myelolastic leukaemia; CNS = central nervous system; HL = Hodgkin's lymphoma; NHL = non-Hodgkin's lymphoma; NMSC = non-melanoma skin cancer

mias (SL) were reported. There were 9 cases of subsequent leukaemia. Compared to general population, childhood cancer survivors had a 6-fold overall increased risk of leukaemia and an 8-fold (95% CI: 3.5–15.8) increased risk before age 40 (Table 6). Six patients developed SL within first 5 years of childhood cancer diagnosis. Only two out of nine patients survived the disease.

Mortality and survival following SMN

Fifty-nine patients out of 183 with SMN died within study period (1961 – 2018); 52 due to SMN and 7 of other causes. Five-year relative survival for patients with a SMN was 69 % (95% CI: 61–76). Most deaths were attributed to CNS tumours, SL, gastrointestinal, respiratory, head and neck carcinomas. Six patients developed lethal SMNs outside the radiotherapy field or without radiotherapy, two of them were with a known cancer predisposition syndrome.

Discussion

Main findings

Our study reports almost 3-fold increase in SMN among survivors of childhood cancer compared with general population. The SIRs reported by attained age are similar to other population-based studies, but somewhat lower than in non-population-based studies, particularly for those after age 40 years.[22]

For the first time we provided treatment data for our cohort. Intensive radiotherapy and chemotherapy started in 1970s, with highest proportion of patients having radiotherapy in 1980s. Low intensity treatment in 1960s consequently resulted in only sporadic survival. Childhood cancer patients diagnosed in 1980s had the most intensive cancer treatment (56% radiotherapy and 70% chemotherapy). In high income countries radiotherapy for childhood cancer was already declining from 75% before 1980 to 43% after 1980 and chemotherapy was given to more than 80% of patients after 1980.[11,13,23] The maximum proportion of patients treated with radiotherapy and chemotherapy at any time was lower in our cohort and became comparable only recently, with approximately 30% of children with cancer having radiotherapy and 75% chemotherapy.[11,13] The previous study on our cohort reported 48 SNs compared to 273 in current study, emphasizing need for continuous follow up despite lower risk.[15] This is even more important since use of radiotherapy declined later. Namely,

TABLE 6. Standardized incidence ratios and absolute excess risks for specific subsequent malignant neoplasm overall and by attained age (0-39, 40+ years). Absolute excess risks are per 100,000 person-years

SMN (ICD10)	All ages				0-39 years				40+ years			
	Obs	Exp	SIR (95%CI)	AER (95%CI)	Obs	Exp	SIR (95%CI)	AER (95%CI)	Obs	Exp	SIR (95%CI)	AER (95%CI)
All sites	183	63.2	2.9 (2.5,3.3)	257 (213,307)	123	34.9	3.5 (2.9,4.2)	216 (173,266)	60	28.2	2.1 (1.6,2.7)	545 (372,770)
Head & Neck (C00-C14)	9	1.4	6.4 (2.9,12.1)	16 (7,33)	5	0.3	17.8 (5.8,41.5)	12 (4,28)	4	1.1	3.5 (1.0,9.0)	49 (10,147)
Digestive organs (C15-C26)	13	5.5	2.4 (1.3,4.1)	16 (7,32)	7	1.0	6.8 (2.8,14.1)	15 (5,32)	6	4.4	1.4 (0.5,2.9)	27 (2,112)
Respiratory organs (C30-C39)	12	2.9	4.2 (2.2,7.4)	20 (9,37)	5	0.2	23.3 (7.6,54.5)	12 (4,28)	7	2.6	2.7 (1.1,5.5)	75 (22,185)
Bone (C40-C41)	2	0.4	5.1 (0.6,18.3)	3 (0,14)	2	0.3	5.8 (0.7,21.0)	4 (0,16)	0	0.0	0.0 (.,73.9)	0
Melanoma of skin (C43, D03)	4	4.2	1.0 (0.3,2.4)	0	3	2.2	1.4 (0.3,4.0)	2 (0,13)	1	2.0	0.5 (0.0,2.8)	0
Soft tissue (C49)	5	0.5	9.5 (3.1,22.2)	10 (3,23)	4	0.4	11.2 (3.1,28.7)	9 (2,24)	1	0.2	5.9 (0.1,32.9)	14 (0,90)
Breast (C50, D05)	26	6.6	3.9 (2.6,5.7)	41 (25,65)	16	1.6	10.3 (5.9,16.7)	35 (20,59)	10	5.1	2.0 (0.9,3.6)	84 (27,198)
Genitourinary (C51-C68, D09, D06)	36	32.2	1.1 (0.8,1.5)	8 (2,21)	22	23.3	0.9 (0.6,1.4)	0	14	8.9	1.6 (0.9,2.6)	87 (28,201)
Central nervous system (C70-C72)	18	1.3	13.4 (7.9,21.2)	36 (21,57)	13	0.9	14.3 (7.6,24.4)	30 (15,52)	5	0.4	11.6 (3.8,27.0)	78 (24,190)
Thyroid gland (C73)	37	1.7	21.6 (15.2,29.7)	76 (53,105)	30	1.1	27.3 (18.5,39.0)	71 (47,102)	7	0.6	11.3 (4.6,23.4)	109 (42,233)
Lymphoma (C81-C85)	3	2.8	1.1 (0.2,3.2)	0 (0,9)	3	2.0	1.5 (0.3,4.4)	3 (0,14)	0	0.8	0.0 (.,4.6)	0
Leukemia (C90-C93)	9	1.5	6.0 (2.8,11.4)	16 (7,32)	8	1.0	8.0 (3.5,15.8)	17 (7,35)	1	0.5	2.0 (0.1,11.2)	9 (0,80)

AER = absolute excess risks; Exp = expected; Obs = observed; SIR - Standardized incidence ratios; SMN - subequent malignant neoplasm

prophylactic cranial radiotherapy (CRT) in patients with acute lymphoblastic leukaemia (ALL) was gradually omitted in Slovenia after 1995 and for majority of patients after year 2002. Systematic review of randomized trials addressing prophylactic CRT in ALL patients conducted between the 1970s and 1990s showed that radiotherapy can generally be replaced by intrathecal therapy.[24] There is substantial variation in percentage of irradiated patients between different childhood ALL treatment groups, however children from high income countries included in randomized trials had prophylactic CRT omitted a decade earlier then our patients.[25] How different trends in treatment will correlate with cumulative incidence of SN in our cohort needs longer observation time.

Risk of SMNs in retinoblastoma survivors

In our cohort, no SNs were observed among retinoblastoma patients, which is likely related to the fact that less than 20% had external beam radiotherapy. In countries using external beam radiotherapy, five-year overall survival of retinoblastoma patients diagnosed in 1966–1970 and 1996–2000 increased from 86% to 96%.[26] In Slovenia only half of patients with retinoblastoma survived the disease in the 1960s and 1970s. With the use of chemotherapy and modern local therapies, survival increased to 88% in the 1980s and is 100% nowadays.[27] The risk for SMN in nonhereditary retinoblastoma patients treated with surgery only, is comparable to general population and only hereditary retinoblastoma patients treated with radiotherapy have higher risk for SMN.[28] In our study only four long term survivors with probable hereditary retinoblastoma had radiotherapy.

Risk of subsequent sarcomas

In our study the risk of subsequent soft tissue (SIR 9.5, 95% CI: 3.1–22.2 *vs*. 15.7 95% CI: 14–17.6) and bone sarcomas (SIR 5.1, 95% CI: 0.6–18.3 *vs*. 21.65, 95% CI: 18.97–24.6) was significantly lower than in PanCareSurFup cohort, that comprises data from 12 European countries.[29,30] The risk of subsequent soft tissue (SIR 12.1, 95% CI: 9.1–16) and bone sarcoma (SIR 10.1, 95% CI: 7.2–14) is more comparable to Nordic population-based cohort study then British, where highest overall SIR for any specific subsequent neoplasm was observed for subsequent bone neoplasms (SIR, 30.5; 95% CI, 24.9–37.3).[6,7] Again, the greatest risk for subsequent primary sarcomas was observed in survivors of hereditary retinoblastoma treated with radiotherapy, but there are only few of such patients in our cohort. [29,30] Similar

trends in survival are seen for other childhood cancers contributing to subsequent sarcomas, namely patients with CNS tumours, sarcomas, and Wilms tumours.[7,29,30] Survival of children diagnosed with CNS tumours, sarcomas, and Wilms tumours in 1970s and 1990s increased from 44% to 65%, 46% to 62% and 58% to 76%, respectively. As radiotherapy and chemotherapy are known risk factors for subsequent sarcomas, we might never see such an increase as in British and PanCareSurFup studies, since less patients were exposed to high-dose, high-volume radiotherapy and chemotherapy at any time. [31,32] As the most intensive treatment in our cohort was implemented later, we might expect increased risk with continued follow up.

Risk of subsequent leukaemia (SL)

In our cohort risk of SL is somewhat higher (SIR 6.0, 95% CI 2.8–11.4) compared to PanCareSurFup cohort (SIR 3.7, 95% CI 3.1–4.5).[33] The risk of SL is estimated for five-year survivors in published studies, making comparison difficult.[6,33,34] By studying five-year survivors two thirds of SL in our cohort would be lost, with majority of patients dead due to high mortality of SL. Determining risk of SL before patients became 5-year survivors may have implications for other studies despite low numbers in our cohort.

Mortality and causes of death following SMNs

Recurrence of primary cancer is still the leading cause of death in childhood cancer patients up to 15 years after diagnosis, afterwards death due to SMN takes the lead.[5,35] Ten percent of patients that died of SMN had either no radiotherapy or SMN outside radiotherapy field. One third had known genetic cancer predisposition syndrome. Even these small numbers could stress the importance of surveillance for patients after radiotherapy or with known genetic predisposition syndromes.[22]

Clinical implications

The fact that the risks of developing an SMN in this study are similar to other European population-based cohorts is important knowledge as it shows that follow-up guidelines for potential surveillance of SMNs developed for European survivors are relevant to the Slovenian childhood cancer survivor population. Follow up provided by a dedicated physician applying current guidelines, as in Slovenia, is probably the best care possible for long-term survivors.

Study limitations

Strength of our study is almost complete follow up in population-based setting with little heterogeneity in data collection and patient's management. Potential limitations are the relatively small number of SPNs and unavailable detailed treatment information not allowing for investigations into the risks by specific cumulative radiotherapy and chemotherapy doses.

Conclusions

Within this population-based study with nearly complete follow we observed almost 3-fold increased risk for SMN among childhood cancer survivors. What is new, are treatment data for our cohort, showing that most intensive treatment with radiotherapy and chemotherapy was implemented later in practice and radiotherapy also declined slower compared to high income countries. The evidence assembled in this study stresses the importance of continuous surveillance according to European guidelines and further studies to assess whether risk of SMNs in childhood cancers survivors in Slovenia will be different in the future.

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research article

Abbreviated ^{13}C-mixed triglyceride breath test for detection of pancreatic exocrine insufficiency performs equally as standard 5-hour test in patients after gastrectomy performed for gastric cancer

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Background. ^{13}C-mixed triglyceride breath test (^{13}C-MTGT) is a non-invasive test for the detection of moderate and severe pancreatic exocrine insufficiency (PEI), but it requires prolonged breath sampling. The aim of this study was to determine the diagnostic power of abbreviated ^{13}C-MTGT in detecting PEI in patients after subtotal and total gastrectomy performed due to gastric cancer.

Subjects and methods. This cross-sectional observational study included 3 groups of subjects; healthy controls, patients with subtotal and patients with total gastrectomy. Demographic and clinical data of patients were collected. Stool samples to determine faecal elastase (Fe-1) and chymotrypsin were collected and measured by ELISA. All subjects performed 5-hour ^{13}C-MTGT breath test. The concentration and relative content of ^{13}C in exhaled air was measured by isotope ratio mass spectrometer (IRMS). PEI was confirmed as values of ^{13}C-exhalation < 26.8% after 5 hours.

Results. Overall, 65 participants were included into analysis, 22 having PEI (n = 11 after subtotal and n = 11 after total gastrectomy, both performed for gastric cancer). ^{13}C-MTGT breath test showed difference in percent of exhaled ^{13}C between PEI and non-PEI patients already after 60 minutes (p = 0.034). Receiver operating characteristic (ROC) curve analysis showed that cut-off value of 13.74% after 150 minutes is showing equivalent diagnostic power to the longer test with sensitivity and specificity both above 90% for the exclusion of PEI in patients after subtotal and/or total gastrectomy.

Conclusions. In this study abbreviated ^{13}C-MTGT test could be shortened from 5 to 2.5 hours without decrease in its diagnostic accuracy for detection of PEI in patients with subtotal or total gastrectomy performed for gastric cancer. This allows significant time savings in the diagnostics of PEI in this subgroup of patients.

Key words: abbreviated ^{13}C-mixed triglyceride breath test; pancreatic exocrine insufficiency; gastrectomy; faecal elastase; gastric cancer

Introduction

Pancreatic exocrine insufficiency (PEI) is a malabsorption syndrome caused by deficiency or inactivation of pancreatic enzymes and/or bicarbonate in the gastrointestinal tract. This leads to maldigestion, malabsorption, and malnutrition with consequent higher morbidity, higher long-term mortal-

ity, and reduced quality of life.[1] We distinguish primary PEI, in which the mechanism is tied to the pancreas itself (various diseases of the pancreatic parenchyma or pancreatic duct) and secondary PEI, which is often unrecognized because the mechanisms of PEI are extrapancreatic.[1,2]

Secondary PEI also includes PEI in patients with altered anatomy due to gastric surgery (subtotal and total gastrectomy). Subtotal and total gastrectomy are common surgical procedures, very often performed in patients with gastric tumours, mostly with resectable adenocarcinoma.[3] Thus, besides chronic pancreatitis, diabetes mellitus (DM), coeliac disease, cystic fibrosis, inflammatory bowel disease and pancreatic cancer, the surgical procedures are the most common causes of PEI. In these diseases, there is a presence of decreased exocrine secretion.[2,4]

The pathophysiology of PEI post-gastrectomy is attributed to several factors. Firstly, the loss of the gastric reservoir leads to an absence of the initial mechanical digestion of food and faster transit of osmotically active food particles into the small intestine. The less digested food particles are less potent stimulators of cholecystokinin (CCK), resulting in a decrease in endogenous stimulation to release digestive enzymes. Secondly, loss of duodenal transit of food with reconstructive techniques bypassing the duodenum, such as Billroth-II (B2) and Roux-en Y (RY) reconstructions, leads to less CCK being released in response to the detection of chyme in the duodenum and upper jejunum. Thirdly, the release of pancreatic enzymes is not coordinated with the intestinal transit of food and inadequate mixing occurs (post-cibal asynchrony), leading to ineffective digestion. Finally, truncal vagotomy has been shown to reduce secretin-stimulated pancreatic trypsin and lipase secretion by 50-60%. This is attributed to the interruption of the cephalic phase of pancreatic digestion, during which sensory inputs are transmitted to the exocrine pancreas through the vagus nerve.[5]

PEI has great impact on the quality of life, morbidity and mortality of these patients also in patients after gastrectomy, especially if the condition remains unrecognised.[6]

Diagnostic tests for the direct pancreatic function are gold standard as they are most sensitive for the detection of PEI but are invasive. The alternative are non-invasive tests such as faecal elastase-I (Fe-1), however with low sensitivity and specificity falls in diagnosing mild to moderate PEI.[7,8] The specificity of this test seems to be even reduced after total and subtotal gastrectomy.[8-10] Therefore, diagnostics of PEI patients after gastrectomy may be difficult, as faecal elastase (Fe-1), the standard of PEI detection, may be of normal range values. The sensitivity of these tests is low due to its extrapancreatic mechanism of PEI in these patients.[11] On the other hand, the ^{13}C mixed triglyceride breath test (^{13}C-MTGT) is a non-invasive assay that indirectly evaluates pancreatic lipase activity and pancreatic exocrine function. The disadvantage of this breath test is the long time required for the test (5–6 hours). This is time consuming for patients and for medical staff, so there is a great need for a test that will be of shorter duration and therefore more patient-friendly.[1,2,11-14] Limited number of studies have tested patients with suspected PEI who underwent a long 6-hour or modified, shortened ^{13}C-MTGT breath test and showed some good results for shortening the test from 6 to 4 hours.[15-17] As mentioned, the long time period of breath sampling and immobilization is a drawback and the period less than 6 hours led to decreased sensitivity of ^{13}C-MTGT. Even though with shorter times (1-5 hours), sensitivity and specificity ranged from 73% to 85% and 83% to 100%, respectively.[16] However, none of these studies were performed in a subgroup of patients after subtotal and total gastrectomy with test whether even shorter duration of test can be performed. We hypothesised that in this specific subgroup of post-gastrectomy patients the ^{13}C-MTGT breath test could be significantly shortened due to the changed anatomy after resection of the stomach. Since this has not been explored before we performed this prospective observational study that specifically focused on patients with resectable gastric cancer.

Therefore, the purpose of our study is to determine the diagnostic value of abbreviated ^{13}C mixed triglyceride respiratory test (^{13}C-MTGT) for the evaluation of PEI in patients after subtotal and total gastrectomy performed for gastric cancer. The goal was to determine and confirm the equivalence of the sensitivity of the shortened and standard ^{13}C-MTGT breath test in detecting PEI, while determining the optimal required cut-off time of the abbreviated ^{13}C-MTGT breath test with preserved sensitivity and specificity of PEI determination.

Subjects and methods

Participants

The study was designed as a cross-sectional, observational study from a single centre, University Medical Centre of Ljubljana. The subjects were

divided into three groups: healthy controls, subjects with subtotal gastrectomy and subjects with total gastrectomy. The group of healthy controls served as a base population for better estimation of diagnostic accuracy for abbreviated ^{13}C-MTGT breath test. All subjects were adults, 18 years of age or older. Before voluntary participation all participants needed to give the written informed consent. The study design and execution were approved by National Ethics Committee of Republic of Slovenia for Medical Ethics (registration number 140/02/10).

Exclusion criteria for patients in both gastrectomy groups, as well as in the group of healthy individuals, were conditions often associated with PEI (type 1 and 2 diabetes, celiac disease, acute pancreatitis, chronic pancreatitis, surgical conditions such as pancreatectomy, pancreatic head tumours, etc.). Since we studied the impact of changed anatomy after gastric surgery on the performance of ^{13}C-MTGT breath test we excluded patients with primary metastatic gastric cancer. The other exclusion criteria were other metastatic diseases, liver disease in which bile secretion is impaired, inability to participate in research due to psychiatric illness, pregnancy, lactation and allergy to butter or chocolate (these patients are unable to performed the test). Healthy controls were without any clinical signs and symptoms of gastric diseases, normal pancreatic elastase and without concomitant diseases.

Upon inclusion, demographic and clinical data on patients (gender, age, associated diseases, regular therapy, eating habits, smoking, coffee drinking, physical activity, weight, height, calculated body mass index [BMI]) were collected. Gastrointestinal symptoms and the degree of expression (diarrhoea, steatorrhea, abdominal pain, weight loss, flatulence, anorexia, increased appetite) were also recorded.

Blood analyses

Moreover, peripheral blood for haemogram, amylase, lipase, CRP, hepatogram (AST, ALT, total and direct bilirubin, AF and GGT, prothrombin time, INR), electrolytes, urea, creatinine, calcium, lipidogram was drawn from all subjects in the morning after a 12-hour fast. Subjects submitted the first morning urine for amylase, lipase, glucose and stool to determine faecal elastase and faecal chymotrypsin. Analysing pancreatic faecal elastase (FE-1) and chymotrypsin levels subjects needed to pass the stool samples. The concentrations were measured by Enzyme-Linked ImmunoSorbent Assay method (ELISA) and detected photometrically.

Performance of ^{13}C-mixed triglyceride breath test (^{13}C-MTGT)

All subjects underwent our standardized ^{13}C-MTGT breath test that took in total 5 hours (= 300 min). This is in line to already published procedure and is standard of care in our institution.[12] The first exhalation is done as a baseline, before eating test meal and then at 30-minute intervals. After the first exhalation the subject ate a test meal consisting of two slices of white bread, each weighing 100 g, with one piece of bread accompanied by a piece of butter weighing 20 g. With another piece of bread, subjects consumed 30 g of chocolate spread (Nutella, Ferrero Rocher, Germany). The chocolate cream was mixed with 250 mg of a substrate of mixed triglycerides (1,3-distearyl-2-octanyl glycerol) labelled with the isotope ^{13}C (Euriso-top, Saarbrücken, Germany). After eating the bread, the subjects drank 200 mL of water. They were requested to sit throughout the whole examination. The subjects blew exhaled air into test tubes at intervals of 30 minutes. Based on the difference in concentration between ^{13}C and ^{12}C, the relative isotope ratio (IRMS) mass spectrometer was used to determine the relative ^{13}C content of exhaled CO_2. The concentration of ^{13}C in exhaled air and measurement of the ratio of ^{13}C to ^{12}C in exhaled CO_2 were analysed. The measured isotope ratio in the samples were expressed as the relative difference (δ per mL in %) and subtracted from the baseline. The values of ^{13}C exhalation were compared with the standard parameter of ^{13}C of healthy volunteers. PEI was confirmed according to Keller *et al*. if patients had a ratio of ^{13}C below 26.8%.[17]

Statistical analysis

Statistical analyses were performed using the software package SPSS 21.0 (IBM Inc., Chicago, USA). Normally distributed variables were expressed as arithmetic mean and standard deviation, and One-Way ANOVA test was used for comparisons between variables. In case of abnormally distributed variables the differences between continuous variables were analysed by the nonparametric Mann-Whitney test. Differences between categorical variables and calculation of the positive/negative predictive values (PPV, NPV) were performed by using the Pearson's chi-square test. The optimal time of the ^{13}C MTGT breath test was tested with a receiver operating characteristic (ROC) analysis with area under curve (AUC), sensitivity and specificity. Respective cut-off values were performed for each

TABLE 1. Basic characteristics of subjects enrolled in the study

	All subjects N = 65	Healthy controls N = 20	Subtotal resection N = 23	Total resection N = 22	p-value
Sex M/F	38 (58.5%)/27 (41.5%)	7/13	14/9	17/5	0.020
Age [years]	59.3 ± 16.9	43.4 ± 13.4	62.7 ± 13.8	70.2 ± 11.4	< 0.001
Age group					< 0.001
18−40 y	8 (12.3%)	7 (35.0%)	1 (4.3%)	0	
41−65 y	35 (53.8%)	13 (65.0%)	15 (65.3%)	7 (31.8%)	
> 65 let	22 (33.8%)	0	7 (30.4%)	15 (62.2%)	
Weight [kg]	67.9 ± 15.3	66.6 ± 16.0	70.0 ± 13.7	67.0 ± 16.8	0.738
Height [m]	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	0.576
BMI	23.2 ± 4.2	22.3 ± 4.3	24.1 ± 4.0	23.0 ± 4.2	0.333
PEI (< 26.8)#	22 (33.8%)	0	11 (47.8%)	11 (50.0%)	0.001
Pancreatic elastase (mcg/g)	349.8 ± 182.1	440.2 ± 126.3	312.2 ± 188.8	307.0 ± 195.2	0.026
Normal	50 (76.9%)	20 (100%)	16 (69.6%)	14 (63.6%)	
Mild decrease	7 (10.8%)	0	2 (8.7%)	5 (22.7%)	
Moderate decrease	2 (3.1%)	0	2 (8.7%)	0	
Severe decrease	6 (9.2%)	0	3 (13.0%)	3 (13.6%)	
Chymotrypsin (U/g)	225.3 ± 160.8	301.7 ± 187.4	181.9 ± 130.3	201.3 ± 144.8	0.033
Normal	52 (80.0%)	20 (100%)	14 (60.9%)	18 (81.8%)	
PEI	13 (20.0%)	0	9 (39.1%)	4 (18.2%)	
Smoking					0.445
No	56 (86.2%)	19 (95.0%)	18 (78.3%)	19 (86.4%)	
< 15 cigarettes /day	4 (6.2%)	0	3 (13.0%)	1 (4.5%)	
> 15 cigarettes /day	5 (7.7%)	1 (5.0%)	2 (8.7%)	2 (9.1%)	
Coffee					0.136
No	23 (35.4%)	4 (20.0%)	7 (30.4%)	12 (54.5%)	
1−2 cups /day	29 (44.6%)	10 (50.0%)	12 (52.2%)	7 (31.8%)	
> 2 cups /day	12 (18.5%)	6 (30.0%)	3 (13.0%)	3 (13.6%)	
Alcohol					0.108
No	47 (72.3%)	12 (60.0%)	15 (65.2%)	17 (77.3%)	
< 2 units/day	16 (24.6)	8 (40.0%)	5 (21.7%)	3 (13.6%)	
> 2 units/day	2 (3.1%)	0	0	2 (9.1%)	
Cholesterol (mmol/l)	4.5 ± 0.8	4.5 ± 0.7	4.4 ± 1.0	4.5 ± 0.7	0.736
HDL_cholesterol	1.5 ± 0.5	1.5 ± 0.4	1.4 ± 0.4	1.5 ± 0.6	0.637
LDL_cholesterol	2.5 ± 0.7	2.5 ± 0.6	2.5 ± 0.8	2.5 ± 0.7	0.921
Triglycerides	1.2 ± 0.8	0.9 ± 0.2	1.2 ± 0.9	1.5 ± 0.9	0.033

According to the ^{13}C-mixed triglyceride breath test; BMI = body mass index; F = female; M = male; PEI = pancreatic exocrine insufficiency

timepoint of ^{13}C measurements, and their diagnostic accuracy for PEI was calculated. Calculation of statistical power assumed a sample of 20 patients in each group with 80% in order to confirm or refuse the hypothesis when assuming an error α below 0.05. Thus, statistical significance for all tests was determined as p-value below 0.05.

Results

Overall, 65 participants were included into analysis and were then divided into 3 groups: (i) healthy controls (n = 20), (ii) group of patients with subtotal resection (n = 23) and (iii) group of patients with total gastrectomy (n = 22). Baseline characteristics

TABLE 2. Differences between patient with positive and negative pancreatic exocrine insufficiency (PEI) determined by ^{13}C-mixed triglyceride breath*

	PEI		p-value
	Positive < 26.8 (n = 22)	Negative > 26.8 (n = 43)	
Sex			
Male/Female	16/6	22/21	0.095
Age [years]	63.5 ± 12.8	57.2 ± 18.5	0.157
Weight [kg]	72.8 ± 15.8	65.5 ± 14.7	0.069
Height [m]	1.7 ± 0.1	1.7 ± 0.1	0.447
BMI	24.4 ± 4.0	22.5 ± 4.1	0.082
Smoking			0.181
No	17 (77.3%)	39 (90.7%)	
< 15/day	3 (13.6%)	(2.3%)	
15/day	2 (9.1%)	3 (7.0%)	
Coffee			0.107
No	9 (40.9%)	14 (32.6%)	
1–2/day	12 (54.5%)	17 (39.5%)	
> 2/day	1 (4.5%)	11 (25.6%)	
Alcohol			0.527
No	14 (63.6%)	33 (76.7%)	
< 2 units/day	7 (31.8%)	9 (20.9%)	
> 2 units/day	1 (4.5%)	1 (2.3%)	
Cholesterol	4.5 ± 0.8	4.4 ± 0.8	0.523
HDL_cholesterol	1.3 ± 0.4	1.5 ± 0.5	0.091
LDL_cholesterol	2.6 ± 0.8	2.5 ± 0.6	0.630
Triglycerides	1.6 ± 1.0	1.0 ± 0.6	0.011

* Determined by ^{13}C-mixed triglyceride breath test @300 min: PEI group 19.6 ± 9.5; non-PEI group 40.9 ± 10.4 (< 0.001); BMI = body mass index

TABLE 3. Percent of exhaled ^{13}C in patients with pancreatic exocrine insufficiency (PEI) and without PEI at respective timepoint of ^{13}C measurement

	PEI		p-value*
	Positive < 26.8 (n = 22)	Negative > 26.8 (n = 43)	
30 min	0.29 ± 0.71	0.47 ± 0.62	0.233
60 min	0.97 ± 1.87	2.23 ± 2.00	0.034
90 min	2.05 ± 2.96	5.23 ± 3.24	< 0.001
120 min	3.53 ± 3.94	8.85 ± 4.05	< 0.001
150 min	8.39 ± 6.36	20.88 ± 5.96	< 0.001
180 min	10.12 ± 7.03	25.07 ± 6.63	< 0.001
210 min	12.21 ± 7.37	29.33 ± 7.29	< 0.001
240 min	14.50 ± 7.66	33.52 ± 8.06	< 0.001
270 min	17.10 ± 8.25	37.33 ± 9.09	< 0.001
300 min	17.72 ± 9.44	40.76 ± 10.43	< 0.001

*Determined by Mann-Whitney test

of subject at enrolment are presented in Table 1. According to the baseline the groups of patients with gastrectomy differed in age and in gender ratio when compared to the healthy controls.

Moreover, PEI were identified in patients after subtotal and total gastrectomy with ^{13}C-MTGT breath test, FE-1, and faecal chymotrypsin, but not with 100% coverage. ^{13}C-MTGT breath test after 300 minutes with a score < 26.8% was taken as a reference to determine PEI[17,18], and at the end determined 22/45 (48,9%) patients with PEI.

Approximately half of the patients in group of patients after subtotal and total gastrectomy had PEI confirmed. Meanwhile, no significant differences in patients' characteristics or habits were determined between patients with PEI and patients without PEI (Table 2).

After performing ^{13}C-MTGT breath test there has been an observation of difference in percent of exhaled ^{13}C between the patients without PEI and patients with PEI soon after 2 measurements at 60 minutes (Table 3). In later timepoints, after 3rd measurement at 90 minutes the differences were increasing ($p < 0.001$) confirming that the abbreviated of ^{13}C-MTGT breath test to exclude PEI can be reliably used in patients after gastrectomy.

The required test time was not shorter in patients with total gastrectomy than in those after subtotal gastrectomy (Table 4) as no differences were observed in any of the timepoints.

The optimal duration of the abbreviated ^{13}C-MTGT breath test was determined by cut-off values and ROC analysis showing that shortening the test to 150 minutes with the cut-off value of 13.74% is showing high sensitivity and specificity, both above 90% and high PPV and NPV for the exclusion of PEI in patients after subtotal and/or total gastrectomy. The reliability of the abbreviated ^{13}C-MTGT breath test showed an equivalence of sensitivity in comparison to the standard, 5-hour ^{13}C-MTGT breath test (Table 5, Figure 1) in this subgroup of patients.

Discussion

PEI in patients after subtotal and total gastrectomy should be detected as early as possible and with a highly sensitive test as early treatment of PEI improves outcome for these patients. Diagnostic of measuring fecal elastase in stool is most commonly performed, but it is important to supplement or even substitute it with more sensitive tests such as the ^{13}C-MTGT breath test as it is crucial to treat PEI

TABLE 4. Percent of exhaled ^{13}C according to diagnosis of in respective timepoint of C13 measurement

	Healthy controls (n = 20)	Diagnosis		p-value*	p-value**
		Subtotal resection (n = 23)	Total resection (n = 22)		
30 min	0.54 ± 0.74	0.32 ± 0.71	0.38 ± 0.51	0.700	0.936
60 min	2.28 ± 2.32	1.27 ± 1.88	1.92 ± 1.86	0.193	0.534
90 min	5.12 ± 3.76	3.15 ± 3.12	4.32 ± 3.45	0.202	0.495
120 min	8.63 ± 4.76	5.669 ± 4.54	7.04 ± 4.63	0.162	0.593
150 min	20.60 ± 7.09	13.96 ± 9.00	15.87 ± 8.11	0.056	0.714
180 min	24.91 ± 7.86	16.94 ± 10.74	18.77 ± 9.00	0.031	0.790
210 min	29.31 ± 8.61	20.45 ± 12.33	21.51 ± 9.55	0.023	0.937
240 min	33.67 ± 9.26	24.18 ± 13.81	24.14 ± 10.11	0.014	1.000
270 min	37.71 ± 9.82	28.04 ± 15.26	26.47 ± 10.63	0.010	1.000
300 min	41.14 ± 10.40	32.01 ± 17.21	28.51 ± 11.00	0.008	0.658

* Mann-Whitney test; ** Tukey Post-hoc analysis between subtotal and total resection groups

early in these patients. However, the execution of this test takes significant time, generally 5–6 hours. The test is feasible as soon as patients are able to eat, and all of our patients have passed the test within 6 months of gastrectomy.

All patients underwent a C13 breath test less than 6 months after gastrectomy. Previously it has been shown that the time required for a breath test in patients with fast food passage in the upper gastrointestinal tract may be shorter[16,17], so it is still necessary to determine the most optimal time required for breath test in patients with gastrectomy where changed anatomy impacts test meal transition time even more. The aim of the current study was to find the optimal duration and cut-off value for the ^{13}C-MTGT breath test in secondary PEI at respective timepoints in two groups of patients, namely with subtotal and with total gastrectomy performed for gastric cancer. Because the time after gastrectomy in which patients underwent 13C-MTGT breath test after gastrectomy was too short, laboratory-detectable malnutrition had not yet occurred. If the 13C-MTGT breath test would be performed after a longer period of time, we would expect reduced laboratory nutritional markers at the same time as the pathological 13C-MTGT breath test, which in principle would not affect the 13C-MTGT breath test itself.

Other conditions that could simultaneously lead to PEI and consequently a change in the 13C-MTGT breath test could be ruled out by additional investigations (DM, etc).

Our analysis showed that the diagnostic sensitivity and specificity of the abbreviated ^{13}C-MTGT breath test for detection of PEI was equivalent to the sensitivity of the longer 5-hour ^{13}C-MTGT breath

TABLE 5. Cut-off values for prediction of non-pancreatic exocrine insufficiency (non-PEI) within respective timepoints in all subjects

	All patients							
	cut-off	AUC	95% CI	p-value	Sensitivity	Specificity	PPV	NPV
30 min	0.25	0.591	0.444–0.737	0.233	53.5%	68.2%	42.9%	76.7%
60 min	1.16	0.662	0.522–0.801	0.034	67.4%	54.5%	46.2%	74.4%
90 min	3.79	0.776	0.654–0.898	< 0.001	67.4%	81.8%	56.3%	87.9%
120 min	4.71	0.845	0.738–0.952	< 0.001	88.4%	72.7%	76.2%	86.4%
150 min	13.74	0.929	0.853–1.000	< 0.001	93.0%	90.9%	87.0%	95.2%
180 min	16.19	0.938	0.869–1.000	< 0.001	93.0%	90.9%	87.0%	95.2%
210 min	18.64	0.948	0.888–1.000	< 0.001	95.3%	90.9%	90.9%	95.3%
240 min	20.85	0.962	0.902–1.000	< 0.001	97.7%	90.9%	95.2%	95.5%
270 min	25.71	0.962	0.891–1.000	< 0.001	97.7%	95.5%	95.5%	97.7%
300 min	26.95	0.962	0.889–1.000	< 0.001	100%	95.5%	100%	97.7%

AUC = area under curve; NPV = negative predictive values; PPV = positive predictive values

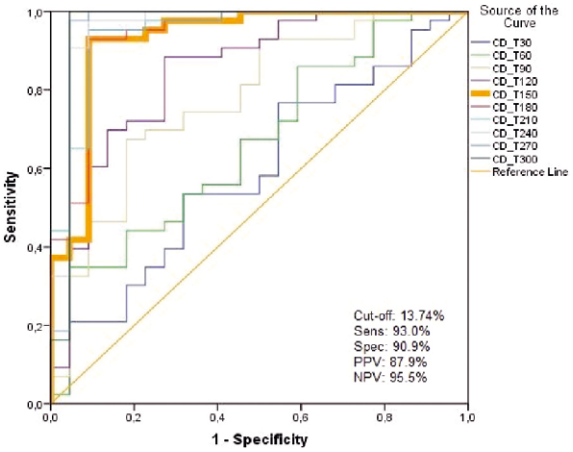

FIGURE 1. Receiver operating characteristic (ROC) curves for respective time points of breathing test for all subjects.

NPV = negative predictive values; PPV = positive predictive values; Sens = sensitivity; Spec = specificity

test in patients after subtotal and total gastrectomy. Two and half hours have been determined as optimal to detect patients with PEI with the cut-off value of exhaled ^{13}C at 13.74% after 2.5 hours (Table 5). Taking all that, there was also no difference in the required duration of the ^{13}C-MTGT breath test when comparing patients after total gastrectomy and the duration of the test in patients after subtotal gastrectomy, even though the transit time in the upper gastrointestinal tract depends on the type of gastrectomy and affects the time required for a breath test. Since the number of patients was small, no significant differences occurred, but with a larger number, we would expect a shorter duration time of the ^{13}C-MTGT breath test required when used in patients after total gastrectomy compared to subtotal gastrectomy patients. Our study was the first in this regard to perform the sub-analysis of patients with subtotal and total gastrectomy, and at the same time confirming shortening of the test.[16,17] Keller *et al*. in 2011 indicated that shortening the test to less than 6 hours, decreases the sensitivity, however, even with considerable shorter sampling, the sensitivity and specificity ranged from 73% to 85% and 83% to 100%, respectively, and reached even higher sensitivity and specificity rates in mild to moderate PEI (100% and 92%, respectively).[17] They also showed that abbreviated version of the test was promising. Abbreviated test as such makes the examination more acceptable and comfortable in time, both for patients and medical staff. Our study contributes to the innovation in the diagnostics and treatment of patients with PEI after gastrectomy and improves their quality of life, as well as facilitates the diagnostic process of these patients. This is important as in patients after gastrectomy, fecal elastase in the faeces may be preserved and the sensitivity of this test is expected to be low because the mechanism of PEI is extrapancreatic.[18,19] Therefore, there is a presence of the risk that patients may be deprived of appropriate treatment with pancreatic enzyme replacement therapy (PERT).[4] Because PEI has a strong impact on quality of life, additional tests such as ^{13}C-MTGT breath test in addition to Fe-1 or ^{13}C-MTGT breath test on its own detects more patients. Meanwhile the ^{13}C-MTGT breath test is a non-invasive test that indirectly assesses pancreatic lipase activity and pancreatic exocrine, detects levels of undigested or digested products following gastric resection, so it is appropriate for patients after gastrectomy.[11-14,18,20] Other trials have also tested patients with suspected PEI who underwent a modified shortened ^{13}C-MTGT breath test, but some did not include patients after gastrectomy.[15-17] On the other hand, they demonstrated high sensitivity for severe PEI ranging 90% to 100% and specificity ranging 80% to 90%.[4,17,21]

Our data are though in concordance with these results. The current study showed that our abbreviated version of the ^{13}C-MTGT after 2.5 hours shows valuable diagnostic power. Both sensitivity and specificity exceeded 90% which represent a strong performance and importantly, after 2.5 hours might detect almost all patients with moderate or severe PEI. Similar performance was observed by Keller *et al*. in two of their previous research with similar cut-off value but rather after 4 hours.[13,14] The findings of all studies suggested that for clinical purposes the testing period may be shortened. Keller *et al*. performed their study in 181 patients and revealed that cumulative ^{13}C-exhalation with ^{13}C-MTGT breath test over 4 hours had 88% sensitivity and 94% specificity for detection of PEI when compared to the standard 6-hour test.[17] This reliability has been previously confirmed.[16] Thus, the evaluation of pancreatic exocrine function using abbreviated test was in concordance with several studies showing that the abbreviated test might be of diagnostic value and used in clinical practice.[16,17] However, due to different optimal timepoint more studies need to be evaluated confirming the exact time point for determine PEI as previous studies showed only minor abbreviation when compared to our data showing the abbreviation of more than 3 hours.

Nevertheless, our findings are significant for bringing innovation into clinical practice and the study design encompassed two groups of patients

that might develop PEI, our analysis had limitations. The sample size that was used is relatively small. Out of 65 subjects, only 22 had PEI. Secondly, the patients were not split by the surgical procedure. They had undergone the Roux-en-Y method or the Billroth I (BI) and the types might be associated with differences in fat digestive and absorptive function as BI reconstruction was proven to be superior to that after Roux-en-Y reconstruction.[13] Furthermore, the basic characteristics of healthy controls did not match in age with patients' group. Here it must be highlighted that the controls were used only as a baseline group stimulating statistical power of PPV and NPV in subjects. The testing time was not compared to other treatment modalities and possible diet was not evaluated to impact the testing results. Finally, in our study gastric emptying was not performed so its influence on the duration of the test or on the rates of abbreviated ^{13}C-exhalation was not covered, despite that gastric emptying parameter was proven similar in patients and controls, and correction for these did not improve accuracy of ^{13}C-MTGT.[13] One of the limitations was also not regarding the possible concomitant adjuvant/ neoadjuvant chemotherapy.

The importance of our study is that it demonstrated the possibility of shortening the ^{13}C-MTGT breath test for patients after total and subtotal gastrectomy, which may make the test less time consuming and therefore more patient-friendly and medical stuff-friendly and suitable for wider clinical use in these two groups of patients for the assessment of PEI.

Conclusions

The negative side of the breath test for detection of PEI is the long 5-hour procedure, which is burdensome for patients and medical personnel. Because of this there is a great clinical need for the test to be shortened. In the study we confirmed that this can be performed in a subgroup of patients with resected stomach due to gastric cancer. The abbreviated ^{13}C-MTGT breath test to 2.5 hours performed equally as the standard 5-hour test in this subgroup of patients. The results of study support the use of abbreviated test in patients after gastrectomy.

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study protocol

Treatment of skin tumors with intratumoral interleukin 12 gene electrotransfer in the head and neck region: a first-in-human clinical trial protocol

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Background. Immune therapies are currently under intensive investigation providing in many cases excellent responses in different tumors. Other possible approach for immunotherapy is a targeted intratumoral delivery of interleukin 12 (IL-12), a cytokine with anti-tumor effectiveness. Due to its immunomodulatory action, it can be used as an imunostimulating component to in situ vaccinating effect of local ablative therapies. We have developed a phIL12 plasmid devoid of antibiotic resistance marker with a transgene for human IL-12 p70 protein. The plasmid can be delivered intratumorally by gene electrotransfer (GET).

Patients and methods. Here we present a first-in-human clinical trial protocol for phIL12 GET (ISRCTN15479959, ClinicalTrials NCT05077033). The study is aimed at evaluating the safety and tolerability of phIL12 GET in treatment of basal cell carcinomas in patients with operable tumors in the head and neck region. The study is designed as an exploratory, dose escalating study with the aim to determine the safety and tolerability of the treatment and to identify the dose of plasmid phIL12 that is safe and elicits its biological activity.

Conclusions. The results of this trail protocol will therefore provide the basis for the use of phIL12 GET as an adjuvant treatment to local ablative therapies, to potentially increase their local and elicit a systemic response.

Key words: gene therapy; interleukin 12; gene electrotransfer; basal cell carcinoma; head and neck region

Introduction

Immune therapies are currently under intensive investigation. Immune checkpoint inhibitors became standard of care for variety of tumor types, providing in many cases excellent responses in patients with advanced or progressive disease, unsuitable for treatment with local therapies. However, there are still patients that are non-responders and the reasons for that are still not well understood.[1]

The other category of immune stimulators are cytokines. These have been extensively investigat-

ed, predominantly in combined treatment schemes. The IL-12 is a soluble cytokine with anti-tumor effectiveness. It stimulates adoptive and natural immunity in the organism, predominantly through production of interferon gamma (IFN-γ).[2] Besides immunostimulatory effectiveness it also has anti-angiogenic mode of action.[2] Treatment with recombinant IL-12 has confirmed its anti-tumor effectiveness, but due to the high drug concentrations required to induce effect also severe toxicity were observed, which eventually lead to abrogation of IL-12 use.[3]

Gene therapy provides a new technical approach for more localized delivery of the therapeutic proteins to tissues, therefore reducing their eventual systemic toxicity. In most of the clinical trials, genes are delivered by viral vectors; however, non-viral gene delivery systems like gene electrotransfer (GET) also provide a safe approach for naked plasmid DNA delivery to tumors.[4,5] GET is based on electroporation that uses electric pulses to destabilize the cell membrane for delivery of large and non-permeant molecules into the cells.[6] The transport of plasmids through the cell membrane and into the nucleus for transcription is not well understood.[7] However, there are several clinical studies providing evidence of feasibility, safety, and effectiveness of this non-viral gene delivery.[4,5,8,9]

Studies on murine tumor models have shown that GET with plasmid DNA encoding interleukin 12 (IL-12 GET) is especially successful in the treatment of skin tumors and their metastases.[10] Furthermore, the safety and efficacy data of such treatment on skin melanoma metastases were already published.[4] In a human clinical study with IL-12 GET of melanoma metastases, local and also systemic effectiveness on the distant non-treated tumors were demonstrated. The study included 24 patients with skin metastatic melanoma.[4] The plasmid encoding human IL-12 under the control of the CMV promoter and with resistance to kanamycin, as the selection gene, was used. Gene therapy was performed three times on each tumor, resulting in promoted local clinical response of treated tumors and in systemic anti-tumor effect on distant non-treated nodules in 53% of patients. This treatment approach is currently being investigated in the treatment of melanoma, Merkel cell carcinoma, breast cancer[8,9,11,12], and also in combination with immune checkpoint inhibitors.[13,14]

However, despite these encouraging results, IL-12 plasmid used in the described studies contain antibiotic resistance gene serving as a selection marker for the production of plasmid DNA. As the presence of antibiotic-resistance genes raises safety concerns, European Union regulatory requirements endorse the use of plasmids without the selective genes. Therefore, we developed the phIL12 plasmid and its clinical grade production process in our previous studies.[15,16] In the proposed first-in-human study (EudraCT: 2021-000852-21, ISRCTN15479959, ClinicalTrials NCT05077033) we intend to study the safety and tolerability of phIL12 GET in treatment of basal cell carcinomas in patients with operable tumors in the head and neck region. The study is designed as an exploratory, dose escalating study with the aim to determine the safety and tolerability of the treatment and to identify the dose of plasmid phIL12 that is safe and elicits its biological activity.

Trial rational

Basal cell carcinoma accounts for 80% of all non-melanoma skin cancers and is the most common malignant tumor that occurs on the skin. It grows slowly and rarely metastasizes. However, basal cell carcinoma comprises heterogeneous histologic variants, from highly biologically benevolent, well-limited and superficially growing tumors to aggressive, infiltratively growing or deeply invasive lesions. Due to complex anatomical conditions in some parts of the head (nose, orbital area, ears) combined with a possibly more aggressive form of growth, an inadequate first treatment may present a serious therapeutic problem. The basic therapeutic options in basal cell carcinoma are surgery, radiotherapy and electrochemotherapy, that result in comparable local control when early lesions are treated.[17] Since the baseline tumor assessment could be inadequate and the disease underestimated, relapses at the treatment site are not uncommon. Repeated treatment, whether surgical or radiotherapeutic, is associated with reduced efficacy and/or functional or cosmetic impairment in the treated area. Therefore, from clinical perspective, searching for new therapeutic alternatives is of high importance. Recently, immunotherapy became important for the treatment of locally advanced or metastatic basal cell carcinoma, with first approved immune checkpoint inhibitor cemiplimab, which has set the stage for investigation of other immunotherapies.[18]

Basal cell carcinoma has the highest mutational burden compared to other skin cancers resulting in the activation of the local immune response that

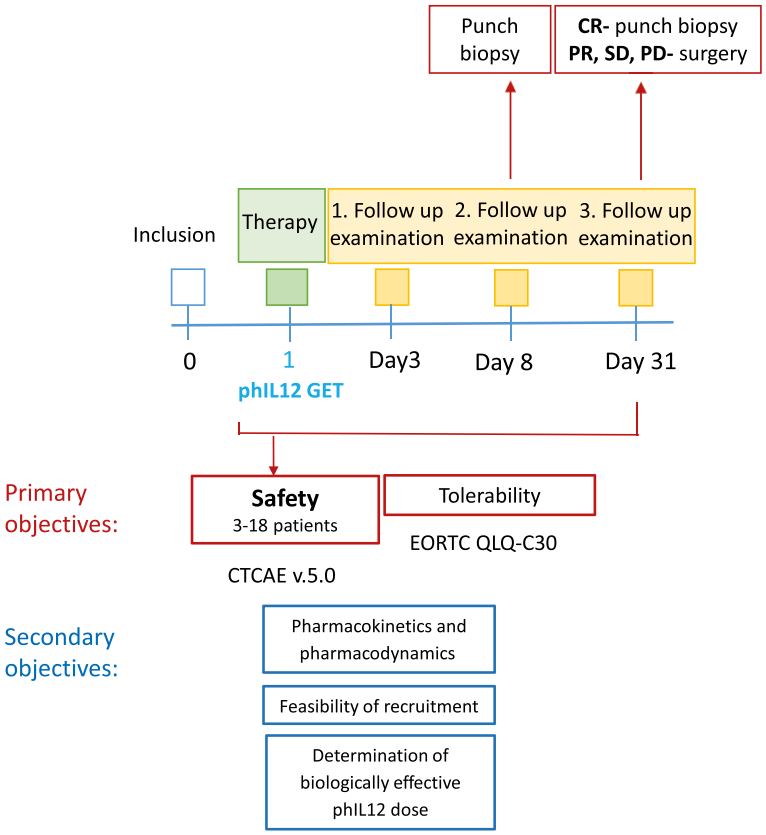

FIGURE 1. Clinical trial design.

CTCAE v.5 = Common Terminology Criteria for Adverse Events version 5.0; CR = complete response; EORTC QLQ-C30 = European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30; PR = partial response; PD = progressive disease; SD = stable disease

prevents rapid tumor growth and metastasis.[19] Indeed, clinical studies confirmed that electrochemotherapy is more effective in basal cell carcinoma than in other histological tumor types.[20,21] This can be explained by a higher mutational load and an increased presence of tumor antigens or neoantigens that are triggered after electrochemotherapy and are required for effective stimulation of immune system and elimination of tumor cells.[22]

In accordance with these findings we have designed the proposed clinical study. It is based on GET of plasmid DNA encoding IL 12 into the tumor, aiming to subsequently stimulate local immune response that would result in tumor eradication. If this therapeutic approach with IL-12 will prove to be safe and effective, it will be the first "proof of principle" of its kind in clinic and a valuable addition to the existing therapeutic armamentarium. However, in the case that our study will confirm the safety of the proposed therapy, but will not yield expected therapeutic response, the tumors will be treated with standard treatment, i.e. surgery. The prolonged interval to surgery should not affect prognosis of treatment outcome, due to slow course of basal cell carcinoma growth.[23]

Trial design

This is a clinical, interventional, open label, single arm, Phase I trial for intratumoral phIL12 GET. It is intended to treat basal cell carcinomas in patients with operable tumors in the head and neck region. The aim of the study is to evaluate the safety and tolerability of phIL12 GET. The study is designed as an exploratory, dose escalating study with the aim to determine the dose that produces IL-12 expression in the tumors with best biological activity, infiltration of the immune cells and no toxicity. Study hypothesis: Intratumoral phIL12 GET is safe and tolerable in treatment of skin tumors (Figure 1).

The aim will be achieved with i) controlled application of the treatment, ii) evaluation of trial and obtained clinical results, iii) presentation of the results by providing written and audio-visual material, participation at and organization of expert meetings and scientific meetings.

In the study 3–6 patients per IL-12 dose level will be included; 3 doses, for a total estimated number of 9 patients (depending on the course of the study, from 3 and up to 18 patients will be included). The enrollment of the patients will be staggered: the waiting period will be 30 days after the treatment of previous patient, based on expected duration of acute and subacute toxicity. Consecutive cohorts of 3 to 6 patients will be treated with increasing doses of phIL12 at three dose levels (0.5 mg/ml, 1 mg/ml and 2 mg/ml) according to an adapted 3 + 3 design as described below. There will be no intra-patient dose escalation. The clinical study will be conducted in accordance with this protocol and GCP guidelines and in accordance with the regulations.

The study is conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Institute of Oncology Ljubljana (protocol code ERIDEK-0086/2020, date of approval 25 November 2020). The study was also approved by the National Ethics Committee of the Republic of Slovenia (0120-524/2020-12) and the Agency for Medicinal Products and Medical Devices of the Republic of Slovenia. The study was registered in the ISRCTN (ISRCTN15479959) and Clinical Trials (NCT05077033) database.

TABLE 1. Primary objectives

Primary objective	Definition of objectives	Timepoint of objectives evaluation
Assessment of the safety of intratumoral phIL12 GET	Assessment of adverse events in accordance with the CTCAE v5 criteria	From the beginning of therapy until the follow-up examination on day 30 after the treatment (day 1, 3, 8 and 31)
Assessment of the tolerability of intratumoral phIL12 GET	Assessment of patient reported outcome by the quality of life questionnaire EORTC QLQ-C30	A follow-up examination on day 0, 8 and 31

CTCAE = Common Terminology Criteria for Adverse Events; GET = gene electrotransfer

TABLE 2. Secondary objectives

Secondary objective	Definition of objectives	Timepoint of objectives evaluation
Pharmacokinetics and biodistribution.	Determination of serum levels of IL-12 cytokine.	A follow-up examination according to clinical trial protocol (day 0, 3, 8 and 31).
Pharmacodynamics	Determination of tumor IL-12 and IFN-γ levels in tumor biopsies. Determination of plasmid DNA in tumor biopsies.	A follow-up examination according to clinical trial protocol (day 8 and 31).
Feasibility of recruitment	Evaluation of the appropriateness and execution of the treatment and follow up procedures.	During recruitment, execution of the treatment and follow up.
Determination of recommended dose for confirmatory studies	Measurement of pharmacodynamics data and selection of the phIL12 dose that produces IL-12 expression in the tumors with best biological activity, infiltration of the immune cells and no toxicity.	Based on all measurements during follow up.

Objectives

Primary and secondary objective of the study are presented in Table 1 and Table 2, respectively.

Inclusion and exclusion criteria

In the study, only patients with confirmed basal cell skin carcinoma of the head and neck will be included. Their inclusion eligibility will be assessed in accordance with the inclusion and exclusion criteria (Table 3).

Trial procedures

Inclusion of patients

Patients with basal cell skin carcinoma of the head and neck, discussed at the multidisciplinary oncology advisory team meeting where all patients with confirm malignancies in the head and neck are presented and discussed, will be reviewed and informed of their eligibility to participate in the SmartGeneH&N clinical study. They will be presented with all information (in written and oral form) regarding the study and other possible treatment options. Prior to inclusion, they will sign an informed consent form. The patients included in the study will be the one that meet all the inclusion criteria and will not have any exclusion criteria (Table 3).

The enrollment of the patients will be staggered. The waiting period between each individual will be 30 days after completion of therapy, based on expected duration of acute and subacute toxicity.

Consecutive cohorts of 3 to 6 patients will be treated with increasing doses of phIL12 at three dose levels (0.5 mg/mL, 1 mg/mL and 2 mg/mL) according to the adapted 3 + 3 design (see below). There will be no intra-patient dose escalation. Patients will be assigned to a treatment cohort and will receive phIL12 at a single dose-level.

If no dose limiting toxicity (DLTs) are observed during the therapy, until day 30 after the treatment (day 31), in the first 3 patients treated at a dose level, 3 additional patients will be enrolled and treated at the next higher dose level. Doses will be escalated until ≥ 2 of 3 patients (67–100%) in a dose cohort have at least 1 DLT. If exactly 1 of the first 3 patients treated at a dose level experiences at least one DLT, 3 additional patients will be enrolled and treated at that dose level. If none of the additional 3 patients (i.e., 1 of 6 [16.7%] total patients in this dose cohort) experiences at least 1 DLT, dose escalation may proceed. If any patient of the additional

TABLE 3. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Histologically or cytologically confirmed, previously untreated cutaneous basal cell carcinoma located in the head and neck region	Other malignancy at the time of inclusion
Solitary tumors, with largest diameter up to 3 cm, in the region where curative (R0) surgery is feasible	Lesions not suitable for treatment with GET (invasion into the bone, infiltration of large vessels)
Age 18-years or older	A life-threatening infection and/or severe heart failure and/or liver failure and/or other life-threatening systemic diseases
Life expectancy > 3 months	Significantly reduced lung function, which requires the determination of DLCO. Patients should not be treated if DLCO is abnormal
Physical performance in accordance with the Karnofsky scale ≥ 70 or < 2 in accordance with World Health Organization (WHO) scale	Treatment with immunosuppressive drugs, steroids and other drugs that would affect poor wound healing
The patient must be capable of understanding the treatment procedure and possible adverse events, which may arise during treatment	Age under 18-years
The patient must be capable of signing the informed consent to participate in the clinical study (voluntary and conscientious consent after education)	Major disruptions in the coagulation system (who does not respond to the standard therapy – replacement of vitamin K or freshly frozen plasma)
Prior to inclusion in the trial, the patient must be presented at a multidisciplinary advisory team meeting	A chronic decline in the kidney function (creatinine > 150 μmol/L)
	Epilepsy
	Pregnancy and breast-feeding
	The patient's incapability of comprehending the purpose or course of the trial, or not agreeing to be included in the trial
	Patients unwilling or unable to comply with the protocol requirements and scheduled visits

DLCO = Diffusing Capacity of the Lungs for carbon monoxide; GET = gene electrotransfer

3 patients (i.e., ≥ 2 of 6 [33.3%] total patients in this cohort) experiences at least 1 DLT, dose escalation will be stopped.

A DLT is defined as any grade 4 clinical or biological event related to the study treatment and occurring during the first 30 days after the treatment with phIL12 GET.

Randomization

This is a one arm, open label clinical study without randomization.

Treatment

The application of the general or local anesthesia or sedation will be selected by the anesthesiologist. The phIL12 will be injected intratumorally, and 5 minutes thereafter the electric pulses will be applied to the tumor for the transfection of tumor cells. Drug dosage and regime was determined based on non-clinical data. A single dose of phIL12 will be administered by intratumoral injection. In the study we will use three doses of phIL12: 0.5 mg/ml, 1 mg/ml, 2 mg/ml, which were determined based on non-clinical data. The lowest dose, 0.5 mg/ml, is the dose that, according to the guidelines of the European Medicines Agency (EMA), elicits a pharmacological effect in the mouse model in preclinical testing. The next two doses are also determined in accordance with the guidelines with 2 mg/ml being the maximum feasible dose (MFD). Preclinical testing of all three doses has shown satisfactory results in mice that received a single pmIL12 GET therapy (mouse orthologue of phIL12).[15] An electrical pulse generator CLINIPORATOR™ (IGEA, s.p.A.) holding authorization for the use in the clinical environment with designation CE, will be used. It generates electric pulses appropriate for phIL12 GET. The electrodes of the same manufacturer with parallel row needle array will be used. The application of electric pulses will be performed as described in the updated Standard Operating Procedures where a detailed information on electrodes and their usage in different clinical aspects is defined.[24] At the end of the treatment, we will take care of the lesions with standard wound-dressing techniques and move the patient into the post-anesthetic care for further monitoring and pain management. When patient will recover from anesthesia, treatment toxicity, possible side effects (Common Terminology Criteria for Adverse Events version 5.0 [CTCAE v.5]) and post-procedure pain (visual analog scale [VAS] scale) will be assessed.

TABLE 4. Trial procedures

Procedures	Inclusion	Therapy	Follow-up examinations		
	Day 0	Day 1	Day 3	Day 8	Day 31
Informed consent	X				
Concurrent treatments[1]	X				
Clinical examination	X		X	X	X
Complete blood count, biochemistry, serum cytokines	X[2]		X	X	X
Coagulation profile	X[2]				
Digital imaging of the tumor and tumor measurement	X[3]	X	X	X	X
Immune profile determination[4]	X		X	X	X
Saliva sample and a skin swab from the location of therapy	X	X	X	X	X
EORTC QLQ-C30	X			X	X
ECOG	X				
Examination prior to anesthesia[5]	X				
phIL12 GET		X			
Pain assessment in accordance with the VAS scale		X	X	X	X
CTCAE v.5		X	X	X	X
Punch biopsy				X	X[6]
Excision of tumor lesion					X[7]

[1] A detailed description of concurrent treatments (name of the medicinal products, dosage and treatment protocol, beginning of the treatment and reason of the treatment).
[2] Complete blood count, biochemistry and coagulation profile must be carried out after the inclusion examination, no more than 7 calendar days prior to therapy.
[3] Temporary measurement of the size of the tumor and initial imaging must be carried out no more than 7 days prior to therapy.
[4] Peripheral blood mononuclear cells will be examined with flow cytometry to determine content of different subgroups.
[5] Prior to therapy, patients will be examined and assessed by anesthesiologists in accordance with the ASA scale.
[6] Punch biopsy will be performed in case of complete response.
[7] Tumor lesion will be excised if tumor will NOT completely respond to the treatment.

CTCAE = Common Terminology Criteria for Adverse Events; ECOG = Eastern Cooperative Oncology Group; EORTC QLQ-C30 = European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30; GET = gene electrotransfer; VAS scale = visual analog scale scale

Post-treatment analgesia will be prescribed by the principal investigator in accordance with standard practice. The principal investigator will decide on the need for post-treatment hospitalization regarding the patient's condition following phIL12 GET therapy and anesthesia in accordance with standard practice following invasive procedures. In this case, hospitalization is not considered as a negative side effect.

Course of the study

All of the trail procedures are listed in Table 4.

Visit 1 (day -6 to 0): Screening and inclusion of the patient into the study

Patients that will meet all inclusion/exclusion criteria, will be acquainted with the course of the study and will sign the informed consent form.

An overview of medical history and treatment (names and dosages of medicinal products, start date and reason for treatment, and therapy duration) will be done. The investigator will collect the patient's medical history documents with a special emphasis on oncological diseases, previous treatments and response to therapies thus far. A clinical overview will include the measurement of weight, height, blood pressure, pulse. A complete blood count, biochemistry and coagulation profile will be determined at visit 1. Digital imaging of tumor will be performed. The tumor location and its size will be determined. Peripheral blood mononuclear cells will be examined with flow cytometry to determine the content of different subgroups. A saliva sample and skin swabs from the location of the planned application of plasmid DNA will be collected. The quality of life questionnaire (European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30, EORTC QLQ-C30) will be completed. EORTC QLQ-C30 is a validated questionnaire for the assessment of quality of life in oncology patients with different cancer types in four areas (physical condition, social/fam-

ily condition, psychological condition, functional condition).[25] As part of the questionnaire, patients answer on a four-point scale: not at all/a little/quite a bit/very much or with scores from 1–4. Patient performance status will be evaluated in accordance with Eastern Cooperative Oncology Group (ECOG) criteria. Patients given a score of 0, 1 or 2 are eligible to participate in the clinical study.

In all patients, the eligibility status for anesthesia will be evaluated with the standard pre-anesthetic procedure (ASA scale). Patients not eligible for general anesthesia or sedation, will not be included in the clinical study.

Visit 2 (day 1): Therapy with phIL12 GET

No more than 7 calendar days may pass from the visit 1 to the visit 2. On visit 2, a follow-up examination will include: confirmation of the eligibility of the patient to continue treatment in the clinical study, the measurement and digital imaging of the tumor, therapy with phIL12 GET, pain assessment in accordance with Visual Analog Scale (VAS) criteria following treatment, collection of saliva sample and skin swabs from the location of plasmid DNA application, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria.

Visit 3 (day 3): Follow-up examination 2 days after the treatment

On visit 3, a follow-up examination will include: pain assessment in accordance with VAS criteria following treatment, a complete blood count and biochemistry, a blood draw for determining the patient's immune profile (PBMC and subgroups of lymphocytes T), collection of saliva sample and skin swabs from the location of the application of plasmid DNA, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria, the measurement and digital imaging of the tumor.

Visit 4 (day 8 after the treatment): Follow-up examination 7–10 days after the treatment

Visit 4 may vary ± 3 day from the planned visit. On visit 4, a follow-up examination of the patient will include: a clinical examination, pain assessment in accordance with VAS criteria following treatment, a complete blood count and biochemistry, a blood draw for determining the patient's immune profile (PBMC and subgroups of lymphocytes T), collection of saliva sample and skin swabs from the location of the plasmid DNA application, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria, measurement and digital imaging of the tumor, punch biopsy to determine the efficiency of transfection.

Visit 5 (day 31): Follow-up examination 30 days after treatment

Visit 5 may vary ± 3 day from the planned visit. On visit 5, a follow-up examination of the patient will include: a clinical examination, pain assessment in accordance with VAS criteria following treatment, a complete blood count and biochemistry, a blood draw for determining the patient's immune profile (PBMC and subgroups of lymphocytes T), collection of saliva sample and skin swabs from the location of the plasmid DNA application, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria, the measurement and digital imaging of the tumor, quality of life questionnaires (EORTC QLQ-C30), preliminary evaluation of treatment effectiveness according to the RECIST v1.1 criteria, punch biopsy for assessment of viability of the tumor. Tumors that will not respond to the treatment completely will be excised on day 30 after the treatment. Patients will be instructed to immediately report new symptoms to the doctor.

Follow-up after the therapy

The endpoint of clinical trial is 30 days after the treatment (visit 5). Nevertheless, the patients will be followed up in accordance with national Recommendation for diagnosis, treatment and follow-up of patients with basal cell carcinoma and Guidance on follow up on patients administered with gene therapy medicinal products.[26,27] The clinical follow up will be performed 3, 6 and 12 months after the treatment. Since the plasmid DNA is associated with low risk of delayed adverse reaction the yearly follow up will be arranged in form of the questionnaire forwarded to the patient (as defined in Guidance on follow up on patients administered with gene therapy medicinal products).

Endpoints evaluation criteria

Safety of intratumoral phIL12 GET

At each visit, the investigators will note all adverse events (Adverse Event - AE and Serious Adverse Event - SAE) in the appropriate Clinical Report Form (CRF), from the inclusion of the patient into the clinical study until the end of patient follow-up. The investigator will evaluate and record:

- Symptoms or a diagnosis associated with AE;
- The date of the beginning and end of AE;
- The severity of symptoms;
- A causal link to the disease or therapy in a clinical trial;
- A description of measures regarding elimination of AE.

Recording adverse reactions of phIL12 GET will be in the scope of the CTCAE v.5 criteria (NIH, 2017).

The investigator will evaluate the cause-effect link between the adverse event of phIL12 GET in accordance with the following criteria:

- Not linked to the study;
- Probably not linked to the study;
- Possibly linked to the study;
- Probably linked to the study;
- Surely linked to the study.

The severity of adverse reactions will be evaluated based on the level:

- Grade 1 – mild;
- Grade 2 – moderate;
- Grade 3 - a difficult complication requiring medical care and treatment;
- Grade 4 - a life-threatening condition requiring immediate medical attention;
- Grade 5 – death.

The investigator will follow-up on patients during the presence of the adverse reactions. According to the investigator assessment of the patient condition, the latter could be withdrawn from the clinical study. The reason for withdrawal of treatment within the clinical study will be recorded on the CRF form for monitoring adverse reactions and on the CRF form upon study completion.

All adverse reactions (AR) that might have a link to AE and the clinical study will investigator report to the sponsor. If a serious adverse reaction or a suspicion on it (SAR and SUSAR) is recorded during the study, within 24 hours the principal investigator will in writing inform the study coordinator. The latter will further inform the National Centre for Pharmacovigilance of the Agency for Medicinal Products and Medical Devices of the Republic of Slovenia and the Ethics Committee of the Institute of Oncology Ljubljana. The principal investigator will report to the study coordinator within 24 hours on all changes of the condition regarding a serious adverse reaction. In accordance with the regulation, the sponsor shall also submit regular periodic and annual safety reports. In case of death of a patient, the principal investigator will report on this to the sponsor regardless of whether the death was associated with progressive disease, therapy or the investigational product or with an unrelated.

Assessment of the tolerability of intratumoral phIL12 GET

The patients will fill out the quality of life questionnaire prior to treatment and in determined intervals following the treatment (Table 4). We will monitor the hospitalization duration and medicinal products required for pain management during hospitalization and at each control examination.

We will use the following questionnaires:

- Quality of life questionnaires EORTC QLQ-C30;
- Pain assessment in accordance with VAS criteria.

Preliminary evaluation of treatment effectiveness according to the RECIST v1.1 Criteria

Preliminary objective tumor response will be assessed in accordance with RECIST v1.1. criteria. The responses to be measured are:

- Complete Response (CR): Disappearance of target lesion;
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesion, taking as reference the baseline sum diameters;
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesion, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (the appearance of one or more new lesions is also considered progression);
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Pharmacokinetics, pharmacodynamics of the treatment

Levels of plasmid phIL12 and IL-12 and IFN-γ protein levels will be evaluated in tumor biopsies. Serum levels of IL-12 cytokine will be monitored during each visit. As a part of the clinical study, the swabs will be taken prior to treatment, after treatment and at control checkups from the location of

plasmid DNA application. Furthermore, the saliva samples will be collected at each visit. Saliva samples and skin swabs will be used to determine the shedding of plasmid DNA. From the samples, the presence of plasmid DNA will be evaluated with the quantitative PCR method in real time. Changes in blood parameters will be followed at each visit determining complete blood count, biochemistry and immune subgroups profile.

Sample size and statistical analysis

The study is exploratory; therefore, no formal sample size calculation was performed. The design (3 + 3 design) and the corresponding sample size are usual for Phase I trials in oncology. All statistical analyses will be descriptive.

Discussion

The current trial is designed to evaluate the safety and tolerability of the intratumoral phIL12 plasmid DNA gene electrotransfer. The drug dosage that will be established as safe and capable to elicits local immune response will be used for design of next clinical trials.

The gene electrotransfer utilizing IL-12 plasmid DNA was tested extensively in preclinical models and was also used in clinical trials before. To our knowledge, one Phase 1 clinical study (NCT00323206)[4] and four Phase 2 clinical studies have been conducted in USA in patients with malignant melanoma, cutaneous lymphoma, squamous cell carcinoma of the head and neck, and Merkel cell cancer (NCT01502293, NCT01579318, NCT02345330, NCT01440816).[9,28] Further, four new studies (NCT03132675, NCT04526730, NCT03567720, NCT03823131) are active, also in mucosal head and neck squamous cell carcinoma patients, to evaluate a combination of GET of plasmid DNA encoding IL-12 in combination with pembrolizumab or nivolumab.

The above-mentioned Phase 2 clinical studies were performed with tavokinogene telseplasmid (TAVO™), a plasmid encoding IL-12 produced by OncoSec Medical Incorporated (USA). It is a plasmid that encodes genes for the p35 and p40 subunits of the heterodimeric human IL-12 protein separated by an internal ribosome entry site. Constitutive expression of the subunits is driven by a single cytomegalovirus promoter. Once the plasmid is introduced into a mammalian cell, a functional IL-12 p70 protein is expressed and secreted into the tumor microenvironment. Intratumoral GET of accessible lesions is performed using an *in situ* EP device.[8] The TAVO™ plasmid contains a kanamycin resistance gene as the selection gene for the production process, a feature that is discouraged by EMA.

Plasmid phIL12, like TAVO™, is a plasmid DNA, encoding for p35 and p40 subunits of the heterodimeric human IL-12 protein. However, the plasmid backbone of phIL12 is different, since we aimed for the plasmid devoid of antibiotic resistance marker and for the treatment-inducible, tumor-specific promoter, whereas the transgene product is the same in both plasmids, a functional IL-12 p70 protein. In phIL12 plasmid, operator-repressor titration ORT® technology, which is based on providing the titration of repressor of essential gene for propagation of bacteria in plasmid, was used. Multiple operators present on the plasmid titrate the repressor leading to expression of essential gene. In our case, the essential gene in bacteria was *dapD*, which was under transcriptional control of lac operator/promotor (lacO/P). Genome encoded *Lacl* repressor prevents bacterial growth, unless transformed with plasmid containing the lac operator (lacO) that titrates the Lacl repressor from the operator.[29] In addition, since the use of phIL12 GET is foreseen as an adjuvant treatment to local ablative therapies, to increase their local and elicit a systemic response, the IL12 coding sequence was placed under the transcriptional control of an inducible p21 (or cyclin dependent kinase inhibitor 1A (CDKN1A)) promoter that can be activated by the hypoxic tumor microenvironment and/or by the genotoxic stress induced by local ablative therapies, such as tumor irradiation of electrochemotherapy.[16]

Based on preclinical data for TAVO™ that are adequately comparable to phIL12 and since the expression product and a mode of action are the same (i.e. protein IL-12), we decided to use the same doses as in the above clinical studies as a basis for selecting a starting dose in non-clinical study and in this first-in-human phase I study.

The treatment protocol with TAVO™ was designed as a repetitive protocol at days 1, 5 and 8, with possible repetitive cycles at 6- or 12-weeks intervals. However, our intent is to use phIL12 as adjuvant immunotherapeutic to the established local ablative therapies. As already reported, ablation of tumors induces local immune response with immunogenic cell death.[30,31] Therefore, studies combining local ablative therapies like radiotherapy or electrochemotherapy can be boosted from local to

locoregional or even systemic response by immune checkpoint inhibitors. The recent study combining electrochemotherapy with pembrolizumab, demonstrated that this approach is feasible. The patients treated with pembrolizumab and ECT experienced lower disease progression rates and longer survival than those who received pembrolizumab alone.[32] Similar approach could be by immunostimulation using phIL12 intratumoral GET. Further, different studies combining tumor irradiation and immune checkpoint inhibitors demonstrated the same.[33–36] Therefore, in the future clinical trials phIL12 is envisioned as an immunostimulating component to *in situ* vaccinating effect of local ablative therapies such as electrochemotherapy or radiotherapy. We believe that in the frame of such treatment combination, a single treatment with IL-12 GET would be sufficient to elicit pronounced antitumor effect.

Conclusions

The designed first-in-human clinical trial is aimed at evaluation of safety and tolerability of phIL12 GET, thus setting the stage for the use of phIL12 GET as an adjuvant treatment to local ablative therapies, to increase their local and elicit a systemic response.

Author contributions

GS, AG, MC, PS, MB, TJ and BM contributed to conception and design of the study. MB, TJ and GS wrote the first draft of the manuscript. AG, MC, PS and BM wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Sevalno zdravljenje možganskih metastaz melanoma. Sistematični pregled

Thompson JF, Williams GJ, Hong AM

Izhodišča. Sevalno zdravljenje možganskih metastaz melanoma, ki ga izvajamo v obliki obsevalnega zdravljenja celotnih možganov ali stereotaktične radiokirurgije, je uveljavljena oblika zdravljenja te bolezni. Dokazov, ki bi omogočili primerjavo rezultatov, prednosti in slabosti obeh načinov sevalnega zdravljenja pa je malo. Do sedaj je bilo tudi zelo malo randomiziranih kontroliranih raziskav. To je povzročilo precejšnjo negotovost in nedosledna priporočila v smernicah. V pričujočem sistematičnem pregledu smo obravnavali 112 raziskav, ki so poročale o rezultatih zdravljenja bolnikov z metastazami melanoma v možganih in ki so jih sevalno zdravili. Tri so bile randomizirane kontrolirane raziskave, vendar je bila le ena dovolj obsežna, da jo je bila dovolj povedna. Večina izsledkov je izvirala iz nerandomiziranih raziskav, kjer so izvajali posebna zdravljenja ali pa so obravnavali kohorte bolnikov z določeno obliko bolezni. O merilih za izbiro zdravljenja je poročalo le 32 raziskav in kakovost teh raziskav je bila različna. Srednje preživetje, merjeno od časa diagnoze možganskih metastaz, je bilo ob obsevanju celotnih možganov kot edinim zdravljenju samo 3,5 meseca (interkvartilni razpon [IQR] 2,4–4,0 meseca), če pa so bolnike zdravili samo s stereotaktično radiokirurgijo je bilo srednje preživetje 7,5 meseca (IQR 6,7–9,0 meseca). Skupno preživetje bolnikov se je s časom povečevalo (od obdobja pred letom 1989 do leta 2015), vendar to ni bilo razvidno v različnih skupinah zdravljenja.

Zaključki. Sistematični pregled preživetja bolnikov z možganskimi metastazami melanoma lahko omogoči primerjavo z rezultati učinkovitosti zdravljenja nedavno uvedenih sistemskih zdravljenj, kot so tarčno zdravljenje ali imunoterapija.

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Elektrokemoterapija solidnih tumorjev. Pregled literature in predstavitev novega endoskopskega pristopa

Schipilliti FM, Onorato M, Arrivi G, Panebianco M, Lerinò D, Milano A, Roberto M, Capalbo C, Mazzuca F

Izhodišča. Elektrokemoterapija (ECT) je minimalno invazivno in varno zdravljenje, s katerim dosegamo dobre in dolgotrajne protitumorske učinke, ki so zbudili pozornost med znanstvenimi raziskavami. To je lokalno zdravljenje, ki združuje uporabo elektroporacije in citotoksičnih zdravil za povzročitev celične smrti v ciljnem tkivu. ECT večinoma uporabljamo za zdravljenje kožnih in podkožnih sprememb, o dobrih rezultatih pa so poročali tudi pri zdravljenju globokih visceralnih tumorjev. Sodoben pregled literature to potrjuje. Eno od najnovejših zdravljenj visceralnih tumorjev je elektrokemoterapevtsko endoskopsko zdravljenje kolorektalnega raka. Predstavljamo primer endoskopske uporabe EKT obstruktivnega raka danke, ker operacija ne bi omogočila dolgotrajnejšega učinka. Ugotovili smo dober odziv na EKT. Koncentrično razraščanje tumorja danke se je zmanjšalo, stenoze pa nismo zaznali.

Zaključki. Klinične raziskave so pokazale, da je EKT zelo učinkovito zdravljenje tumorjev različnih histoloških tipov in lokalizacij. Endoskopsko zdravljenje raka prebavil je inovativna uporaba ECT. Kombinacija sistemskega zdravljenja in ECT je bila varna in zelo učinkovita pri zdravljenju raka debelega črevesa in danke, zlasti obstruktivnega, kar je bolniku omogočilo znatno izboljšanje kakovosti življenja.

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Portalna hipertenzija lahko vpliva na zaznavo hipointenzivnosti kontrastnega sredstva pri majhnih lezijah hepatocelularnega raka v hepatobiliarni fazi pri MR in uporabi gadoksetične kisline

Caparroz C, Forner A, Rimola J, Darnell A, García-Criado A, Ayuso JR, Reig M, Bruix J, Ayuso C

Izhodišča. Namen raziskave je bil analizirati povezavo med privzemom gadoksetične kisline v jetrih (Gd-EOB-DTPA) v hepatobiliarni fazi in prisotnostjo klinično pomembne portalne hipertenzije pri bolnikih s cirozo ter oceniti. kako ta pojav vpliva na zaznavo hepatocelularnega raka.

Bolniki in metode. Opravili smo naknadno analizo prospektivne skupine 62 bolnikov z jetrno cirozo, ki smo jim ultrazvočno na novo odkrili nodul velikosti med 1–2 cm (študijska skupina). Kontrolna skupina je predstavljalo 20 zdravih oseb. Kvalitativno in kvantitativno smo analizirali privzem kontrasta v jetrih v hepatobiliarni fazi, ga ocenili z relativnim privzemom kontrasta oz. razmerjem med kontrastnim obarvanjem jeter in vranice, jeter in mišičja ter jeter in ledvic. Opazovali smo tudi indeks privzema kontrasta, indeks jetrnega privzema in biliarno izločanje kontrasta. Klinično pomembna portalna hipertenzija smo potrdili invazivno (hepatični venski tlačni gradient >10 mm Hg) ali z indirektnimi parametri. Analizirali smo zaznavo hepatocelularnega raka v hepatobiliarni fazi.

Rezultati. 19 bolnikov (30,6 %) ni imelo klinično pomembne portalne hipertenzije. Pri 41 bolniki (66,1 %) je bila končna diagnoza hepatocelularni rak. Vsi indeksi so bili bistveno višji v kontrolni skupini, kar kaže, da je imela kontrolna skupina intenzivnejši jetrni signal v hepatobiliarni fazi kot skupina s cirozo. To smo zaznali, tudi če smo naredili primerjavo, kjer pri preiskovancih nismo zaznali klinično pomembno portalno hipertenzijo. Klinično pomembna portalna hipertenzija je bila povezana z nižjo stopnjo hipointenzivnosti privzema kontrasta pri hepatocelularnem raku v hepatobiliarni fazi preiskave (51,9 % proti 85,7 % brez klinično pomembne portalne hipertenzije, p = 0,004).

Zaključki. Privzem gadoksetične kisline v jetrih v hepatobialiarni fazi se zmanjša, kadar ima bolnik cirozo, tudi če je delovanje jeter minimalno okvarjeno ter znatno pade pri bolnikih z klinično pomembno portalno hipertenzijo. Ta pojav ogroža prepoznavanje hipointenzivnih lezij in lahko predstavlja omejitev pri odkrivanju majhnega hepatocelularnega raka, kadar imajo bolniki cirozo in klinično pomembno portalno hipertenzijo.

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Primerjava zgodnje izolirane subarahnoidalne krvavitve in hemoragičnega infarkta pri možganski venski trombozi

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Izhodišča. Možganska venska tromboza je redko žilno obolenje. Radiološko in klinično se zelo različno pojavlja. Novejše raziskave so pokazale, da izolirana subarahnoidna krvavitev ob možganski venski trombozi ni tako redka in je povezana z dobro napovedjo poteka bolezni. Nasprotno pa je hemoragični možganski infarkt napovedni dejavnik, ki napoveduje slabši potek bolezni in je večinoma povezan z okluzijo več ven ali/in sinusov. Postavili smo hipotezo, da bodo imeli bolniki v raziskavi z izolirano subarahnoidno krvavitvijo boljši klinični izid bolezni v primerjavi s tistimi, ki so doživeli hemoragični možganski infarkt.

Bolniki in metode. Izbrali smo bolnike hospitalizirane zaradi možganske venske tromboze, ki so imeli izolirano subarahnoidno krvavitev ali hemoragični infarkt ob sprejemu oziroma v času 24 ur od sprejema. Kriterijem je ustrezalo 23 bolnikov (10 moških), starih 22-73 let. Podatke smo pridobili iz bolniškega arhiva ter računalniške in radiološke podatkovne baze.

Rezultati. V skupini z izolirano subarahnoidno krvavitvijo je bilo 8 bolnikov (6 moških) starih 49,3 ± 16,2 let in v skupini s hemoragičnim možganskim infarktom 15 bolnikov (4 moških) starih 47,9 ± 16,8 let. Skupina z izolirano subarahnoidno krvavitvijo je imela po 3 mesecih bistveno boljši izid zdravljenja ocenjen z modificirano Rankinivo lestvico kot skupina s hemoragičnim infarktom (Mann-Whitney Rank Sum Test, p = 0,026) kljub značilno večjemu številu tromboziranih venskih sinusov in/ali globokih ven (Mann-Whitney Rank Sum Test, p = 0,002). Dodatne spremenljivki z značilnim vplivom na potek bolezni sta bili tvorba možganskega edema (p = 0,004) in obliteracija sulkusov (p = 0,014).

Zaključki. Bolniki, ki so utrpeli izolirano subarahnoidno krvavitev, so imeli boljši izid bolezni kljub značilno večjemu številu tromboziranih sinusov in/ali ven. Možen razlog bi lahko bile prehodne povrhnje komunikantne vene.

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Varnost in učinkovitost transarterijske kemoembolizacije z delci pod nadzorom računalniške tomografije s stožčastim snopom pri bolnikih z jetrnoceličnim rakom v zgodnjem in srednjem stadiju bolezni

Koršič S, Levasič N, Dežman R, Lešnik Zupan LA, Trotovšek B, Janša R, Šmid A, Popovič P

Izhodišča. Transarterijska kemombolizacija z mikrodelci, ki nase vežejo kemoterapevtik (*angl. drug-eluting microspheres transarterial chemoembolization,* DEM-TACE), izboljša preživetje bolnikov z jetrnoceličnim rakom v zgodnjem in srednjem stadiju bolezni ter zagotavlja tarčne nadzorovane citotoksične in ishemične učinke zdravljenja. Ustrezen izbor bolnikov in izboljšana tehnika zdravljenja so povezane z daljšim srednjim preživetjem. Namen pričujoče raziskave je bil preveriti varnost in učinkovitost DEM-TACE pod nadzorom računalniške tomografije s stožčastim snopom (*angl. cone-beam computed tomography,* CBCT) pri bolnikih z jetrnoceličnim rakom v zgodnjem in srednjem stadiju bolezni.

Bolniki in metode. Raziskava je bila retrospektivna analiza 144-ih bolnikov (srednja starost 67,9 ± 8,0 let, 127 moških in 17 žensk), ki smo jih zdravili z DEM-TACE z doksorubicinom med februarjem 2010 in decembrom 2018. Uporabili smo mikrodelce različnih dimenzij dveh proizvajalcev (premera 70–150 µm, 100–300 µm ali 300–500 µm in 40-µm, 75-µm ali 100-µm). Mikrodelci so vsebovali 50–150 mg doksorubicina. Beležili smo objektivni odgovor na zdravljenje po kriterijih mRECIST, čas do napredovanja bolezni, neželene učinke zdravljenja in celokupno preživetje.

Rezultati. Naredili smo 452 posegov DEM-TACE. Zabeležili smo štiri večje zaplete (0,9 % od vseh posegov). Postembolizacijski sindrom se je pojavil pri 35 % posegov. Ob prvi slikovni kontroli 2–3 mesece po zdravljenju smo videli objektivni odgovor na zdravljenje pri 91 % bolnikov. Srednji čas do napredovanja bolezni je bil 10,2 mesecev (95 % interval zaupanja [IZ]: 8,3–12,1 mesecev). 1- 2-, 3-, 4- in 5-letno celokupno preživetje je bilo 85 %, 53 %, 33 %, 20 % in 14 %; srednje preživetje pa 25,8 mesecev (95 % IZ: 22,1–29,5 mesecev).

Zaključki. DEM-TACE z doksorubicinom pod nadzorom CBCT je varna in učinkovita metoda zdravljenja bolnikov, ki imajo jetrnocelični rak v zgodnjem in srednjem stadiju bolezni. Dosegli smo dober objektivni odgovor na zdravljenje in primerljivo preživetje glede na objavljene raziskave.

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Aplikacije nusinersena pod nadzorom računalniške tomografije s stožčastim snopom pri odraslih bolniki s spinalno mišično atrofijo in z zapletenimi anatomskimi predispozicijami. Izkušnje posamičnega terciarnega centra

Salapura V, Snoj Ž, Lea Leonardis L, Koritnik B, Kostadinova V

Izhodišča. Zaradi zapletenih anatomskih predispozicij ni moč vedno izvesti običajno lumbalno punkcijo pri odraslih bolnikih s spinalno mišično atrofijo. Potrebno je vpeljati novo metodo, ki bi omogočala varno aplikacijo zdravila nusinersen. Metoda CT slikanja s stožčastim snopom nam omogoča dober prostorski prikaz predela, kamor želimo aplicirati zdravilo, načrtovanje pred posegom in nadzor nad položajem igle med uvajanjem. Tako smo v raziskavi ugotavljali tehnično uspešnost, varnost in izvedljivost lumbalnih intratekalnih aplikacij zdravila nusinersen s pomočjo CT slikanja s stožčastim snopom pri bolnikih s spinalno mišično atrofijo in zapletenimi anatomskimi predispozicijami.

Bolniki in metode. Na Inštitutu za radiologijo, UKC Ljubljana smo zdravili 38 bolnikov s spinalno mišično atrofijo. Multidisciplinarni tim je izbral za slikovno vodeno aplikacijo nusinersena tiste bolnike, ki so imeli zapletene anatomske predispozicije. Vključili smo bolnike z operativnim zdravljenjem hrbtenice zaradi skolioze, bolnike s hudo skoliozo, ki smo jo definirali s Cobbovim kotom > 40°, ter bolnike z indeksom telesne mase nad 35. Opravili smo analizo tehnične uspešnosti, izpostavljenosti sevanju in stranskih učinkov.

Rezultati. V raziskavo smo vključili 20 bolnikov in opravili 108 aplikacij zdravila s pomočjo CT slikanja s stožčastim snopom. Vsakemu bolniku smo zdravilo aplicirali vsaj štirikrat. V 82 % smo izvedli transforaminalni pristop. Tehnična uspešnost metode je bila 100 %. V prvem poskusu smo uspešno aplicirali zdravilo v 93,5 % primerov. Srednja vrednost sevalne efektivne doze pri vseh aplikacijah zdravila je bila 5 mSv. V raziskavi smo zabeležili zgolj blage stranske učinke zaradi obravnave bolnikov.

Zaključki. Aplikacija nusinersena pod nadzorom CT slikanja s stožčastim snopom je bila varna in izvedljiva metoda pri bolnikih s spinalno mišično atrofijo in zapletenimi anatomskimi predispozicijami.

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Nanosekundni električni pulzi so enako učinkoviti v elektrokemoterapiji s cisplatinom kot mikrosekundni pulzi

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Izhodišča. Uporaba nanosekundnih električnih pulzov v elektrokemoterapiji je pokazala spodbudne rezultate, vendar osnovni mehanizmi delovanja še vedno niso raziskani. Namen raziskave je bil povezati količino celičnega cisplatina s preživetjem celic, elektroporiranih z nanosekundnimi ali standardnimi 8 × 100 µs pulzi ter preučiti učinke električnih pulzov na strukturo cisplatina.

Materiali in metode. Ovarijske celice kitajskega hrčka CHO in celice mišjega melanoma B16F1 smo izpostavili 1 × 200 ns pulzu pri 12,6 kV/cm ali 25 × 400 ns pulzom pri 3,9 kV/cm s ponavljalno frekvenco 10 Hz ali 8 × 100 µs pulzom pri 1,1 kV/cm (CHO) ali 0,9 kV/cm (B16F1) s ponavljalno frekvenco 1 Hz in trem različnim koncentracijam cisplatina. Celično preživetje smo določili s testom klonogenosti, količino platine v celičnih peletih pa s spektroskopijo z induktivno sklopljeno plazmo. Učinke na strukturo cisplatina smo spremljali z jedrsko magnetno resonanco in masno spektrometrijo visoke ločljivosti.

Rezultati. Parametri nanosekundnih pulzov, ekvivalentni 8 × 100 µs pulzom, so bili določeni *in vitro* na podlagi permeabilizacije celične membrane in preživetja celic. Ekvivalentni nanosekundi pulzi so bili pri elektrokemoterapiji enako učinkoviti pri zmanjševanju preživetja celic in kopičenju cisplatina v celicah kot 8 × 100 µs pulzi. Število znotrajceličnih molekul cisplatina je močno korelirano s celičnim preživetjem za celice B16F1, manj pa za celice CHO, kar nakazuje na možno vpletenost drugih mehanizmov v elektrokemoterapiji. Visokonapetostni električni pulzi niso vplivali na strukturo cisplatina.

Zaključki. Ekvivalentni nanosekundni pulzi so enako učinkoviti pri elektrokemoterapiji kot standardno uporabljeni 8 × 100 µs pulzi.

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Vpliv polimorfizma AKT1 na poškodbe DNK, ekspresijo BTG2 in tveganje za razvoj kolorektalnega raka

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Izhodišča. AKT, imenovana tudi protein kinaza B, je serin-treonin kinaza, ki deluje kot mediator signalne poti PI3K-Akt-mTOR in ima pomembno vlogo v nizu celičnih procesov. Ugotovili so, da so številni polimorfizmi enega nukleotida (SNP) v genu AKT povezani z različnimi vrstami raka. Namen pričujoče raziskave je bil proučiti povezavo med funkcionalnim SNP rs1130233 v AKT, ki prikazuje prehod G v A, in tveganjem za razvoj kolorektalnega raka. Zato smo ugotavljali aktivacijo AKT, poškodbo DNK in izražanje gena 2 (Btg2) za translokacijo B-celic v zgodnjem odzivu.

Bolniki in metode. V raziskavo smo vključili 200 bolnikov s kolorektalnim rakom in 197 preiskovancev iz populacije kot kontrolno skupino. Naredili smo genotipizacijo za SNP rs1130233. Izražanje AKT ter aktivacijo in izražanje BTG2 smo določili pri nosilcih genotipa GG, AG in AA. Poškodbe DNK smo določili s kometnim testom.

Rezultati. Heterozigotni genotip AG (55,67 %) je bil v lokalni populaciji bolj razširjen v primerjavi s homozigotnim nemutiranim tipom GG (37,78 %) in homozigotnim mutiranim genotipom AA (6,55 %). Ob tem smo opazili, da alela AG in AA pomembno prispevata (P = 0,01; razmerje obetov [OR] = 1,80; interval zaupanja [CI] = 1,18–2,74 ter P = 0,001; OR = 5,00; CI = 1,90–13,18) k povečanju tveganja za kolorektalnega raka. Analiza *imunoblot* je pokazala, da je prehod G v A zmanjšal izražanje in aktivacijo AKT. Poleg tega sta genotipa AG in AA AKT1 rs1130233 pokazala znatno povečanje poškodbe DNA in izražanja Btg2.

Zaključki. Raziskava je pokazala, da je zamenjava G z A lahko dejavnik tveganja za razvoj kolorektalnega raka, ki vključuje zmanjšanje izražanja in aktivacije AKT ter povečanje poškodbe DNK.

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Rezultati zdravljenja pri bolnikih z začetno operacijo in resektabilnim nedrobnoceličnim rakom pljuč stadija I-IIIA v vsakodnevni klinični praksi

Bitenc M, Čufer T, Kern I, Miklavčič M, Petrovič S, Groznik V, Sadikov A

Izhodišča. Zdravljenje nedrobnoceličnega pljučnega raka v zgodnjem stadiju se hitro razvija. Pri uvajanju novosti so potrebni dejanski podatki o učinkovitosti obravnave v dosedanji vsakodnevni klinični praksi. Proučili smo preživetja bolnikov z nedrobnoceličnim pljučnim rakom stadijev I–IIIA, ki smo jih zdravili v obdobju 2010–2017 z začetno radikalno kirurgijo.

Bolniki in metode. Podatke o 539 zaporednih bolnikih smo pridobili iz prospektivnega bolnišničnega registra. Vse diagnostične postopke, zdravljenje in sledenje bolnikov smo opravili v istem centru in v skladu s takrat veljavnimi smernicami. Primarni cilj raziskave je bila analiza celokupnega preživetja glede na klinični (k) in patološki (p) stadij TNM (tumor, bezgavke, metastaze). Z metodama univariatne in multivariatne regresijske analize pa smo analizirali tudi vpliv drugih klinično patoloških značilnosti.

Rezultati. Po srednji opazovalni dobi 53,9 mesecev je bilo srednje in 5-letno celokupno preživetje celotne kohorte 90,4 mesecev in 64,4 %. 5-letno celokupno preživetje bolnikov s stadiji pTNM I, II in IIIA je bilo 70,2 %, 60,21 % in 49,9 %. S celokupnim preživetjem sta bila značilno povezana stadija kTNM in pTNM, vendar pa je samo pTNM v multivariatni analizi ohranil neodvisno napovedno vrednost (p = 0,003). Skladnost med kTNM in pTNM je bila 69,0 %. Poleg stadija pTNM sta starost (p = 0,001) in spol (p = 0,004) ohranila neodvisno napovedno vrednost za celokupno preživetje.

Zaključki. Raziskava je pokazala dobra celokupna preživetja bolnikov z resektabilnim nedrobnoceličnim pljučnim rakom, ki smo jih zdravili z začetno operacijo v vsakodnevni klinični praksi. Nepopolno ujemanje med stadijema kTNM in pTNM in neodvisna napovedno vrednost samo stadija pTNM za celokupno preživetje pa kažeta, da začetna kirurgija še vedno zagotavlja najzanesljivejšo določitev anatomskega stadija in ostaja pomembno zdravljenje zgodnjega nedrobnoceličnega pljučnega raka.

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Prepoznavanje žensk s histopatološkimi izvidi visoke stopnje po konizaciji s pomočjo umetnih nevronskih mrež

Mlinarič M, Križmarič M, Takač I, Repše Fokter A

Izhodišča. Namen raziskave je bil oceniti, ali lahko z uporabo umetnih nevronskih mrež napovemo histopatološki izvid visoke stopnje pri bolnicah, ki so imele konizacijo zaradi sprememb na maternicnem vratu.

Materiali in metode. Analizirali smo 1475 bolnic, pri katerih smo naredili konizacijo na Univerzitetni kliniki za ginekologijo in porodništvo Univerzitetnega kliničnega centra Maribor v letih 1993–2005. Zaradi neuravnoteženega števila bolnic z in brez sprememb ter z namenom izboljšanja klasifikacije smo bazo podatkov uredili v različne sklope. Za analizo z umetnimi nevronskimi mrežami smo uporabili odprtokodni program Weka. Za vhodne podatke pa smo uporabili zadnji bris po Papanikolaouju in dejavnike tveganja za razvoj patoloških sprememb na maternicnem vratu ter visokorizično displazijo DA/NE kot izhodni rezultat. 10-kratno navzkrižno preverjanje smo uporabili za definiranje učnega in testnega seta za analizo.

Rezultati. Z nevronskimi mrežami smo izvedli simulacijo bazalne klasifikacije in več testiranj glede na različne dejavnike tveganja. Nevronske mreže so v modelu, v katerem je bila bazalna uspešnost 69,79 %, pravilno razvrstile 84,19 % primerov; področje pod krivuljo je bilo 0,87; kappa vrednost 0,64: F-mera 0,884 in Matthewsov korelacijski koeficient (MCC) 0,640.

Zaključki. Z umetnimi nevronskimi mrežami smo uspeli prepoznati več bolnic, ki bodo imele visoko rizičen izvid po konizaciji kot pa z bazalno napovedjo. Kljub temu pa na podlagi značilnosti 1475 bolnic, ki so imele konizacijo, nevronske mreže niso dosegle zanesljivosti, ki bi bila primerna za vsakodnevno klinično delo.

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Napredek v radioonkološkem zdravljenju raka prostate z visokim tveganjem. Četrt stoletja dosežkov

Moll M, Herrmann H, Zaharie A, Goldner G

Izhodišča. Namen raziskave je bil ovrednotiti napredek pri zdravljenju bolnikov, ki so zboleli zaradi primarnega raka prostate z visokim tveganjem. Analizirali smo biokemično odsotnost bolezni ter akutne in pozne gastrointestinalne in genitourinarne stranske učinke.

Bolniki in metode. Vključili smo bolnike s primarnim rakom prostate z visokim tveganjem, ki smo jih zdravili v letih 1904–2016. Prejete obsevalne doze so znašale od 60 do 80 Gy, doza na frakcijo pa 1,8 ali 2 Gy. Uporabili smo konformno tehniko 3D ali intenzitetno modulirano radioterapijo ali pa volumetrično intenzitetno modulirano ločno terapijo.

Rezultati. Bolnike smo razdelili v 3 skupine: 142 bolnikov smo obsevali z dozami do 70 Gy (srednja doza 66 Gy; skupina 66 Gy), 282 z dozami med 70 in 76 Gy (srednja doza 74 Gy; skupina 74 Gy) in 141 z dozami > 76 Gy (srednja doza 78 Gy; skupina 78 Gy). Srednji čas spremljanja je bil 48 mesecev. V skupini 66 Gy je bil delež biokemične odsotnosti bolezni po petih letih 50 % in po devetih letih 44 %; v skupini 74 Gy 65 % in 54 %; ter v skupini 78 Gy 83 % in 66 % ($p = 0,03$ proti skupini 74 Gy in $p < 0,0001$ proti skupini 66 Gy). V skupini 78 Gy smo ugotovili višji delež akutnih gastrointestinalnih stranskih učinkov kot v drugih skupinah, ne pa tudi razlik v maksimalnih akutnih genitourinarnih stranskih učinkih in tudi ne v kasnih maksimalnih gastrointestinalnih in genitourinarnih stranskih učinkih.

Zaključki. Bolniki z rakom prostate z visokim tveganjem, ki so bili zdravljeni z dozami 78 Gy, so imeli statistično pomembno boljše deleže biokemične odsotnosti bolezni. V primerjavi s historično skupino 66 Gy je po devetih letih sledenja 50 % več bolnikov doseglo biokemično odsotnost bolezni.

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Preživetje, načini zdravljenja in deleži testiranja T790M pri bolnikih z nedrobnoceličnim rakom pljuč, ki smo jih primarno zdravili z zaviralci tirozinske kinaze receptorja epidermalnega rastnega dejavnika prve ali druge generacije v vsakdanji klinični praksi. Podatki za bolnike iz slovenske kohorte v klinični raziskavi REFLECT

Turnšek N, Devjak R, Edelbaher N, Osrajnik I, Unk M, Vidovič D, Jerič T, Janžič U

Izhodišča. Zaviralci tirozinske kinaze (*angl. tyrosine kinase inhibitors*; TKI) receptorja epidermalnega rastnega dejavnika (*angl. epidermal growth factor receptor*; EGFR) so učinkoviti v zdravljenju napredovalega nedrobnoceličnega raka pljuč s pozitivnimi mutacijami *EGFR* (EGFRm). Ker se vsakdanje zdravljenje bolnikov razlikuje med posameznimi centri, smo želeli analizirati obravnavo bolnikov v Sloveniji.

Bolniki in metode. Retrospektivna raziskava REFLECT (NCT04031898) je analizirala podatke bolnikov z napredovalim EGFRm nedrobnoceličnega raka pljuč, ki so pričeli rutinsko zdravljenje med leti 2015 in 2018 in so v 1. redu zdravljenja prejeli 1. ali 2.generacijo EGFR TKI. Pridobili smo podatke o kliničnih značilnostih, načinu zdravljenja, vzorcih testiranja *EGFR/T790M* in izhodih zdravljenja. Raziskava je potekala v 8 državah, predstavljamo pa slovenske podatke 120 bolnikov, zdravljenih v 3 onkoloških centrih v državi.

Rezultati. Med slovenskimi bolniki je bila srednja starost 70 let, žensk je bilo 74 %, v 1. redu zdravljenja so prejeli: erlotinib (37 %), afatinib (32 %) in gefitinib (31 %). V času analize je 94 (78 %) bolnikov prenehalo zdravljenje s 1. redom EGFR TKI, 89 (74%) zaradi napredovanja bolezni. Med bolniki z napredovanjem bolezni je bilo 73 (82 %) testiranih za mutacijo *T790M*, pri 50 (68 %) smo to mutacijo odkrili in 62 (85 %) bolnikov je prejelo 2. red zdravljenja, od tega 82% z osimertinibom. Stopnja osipa med 1. in 2. redom zdravljenja je znašala 10 %. Srednje preživetje brez napredovanja bolezni (95 % interval zaupanja) s 1. redom zdravljenja je znašalo 15,6 (12,6–19,2) mesecev, srednje celokupno preživetje pa 28,9 (25,0–34,3) mesecev.

Zaključki. Raziskava vsakdanje klinične prakse ponuja koristne informacije o učinkovitosti zdravljenja z EGFR TKI pri bolnikih z napredovalim EGFRm nedrobnoceličnega raka pljuč. Stopnja osipa med zdravljenjem 1. in 2. reda ter odlični rezultati preživetja kažejo na pomembnost pričetka zdravljenja z najbolj učinkovitim EGFR TKI.

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Spremembe pri zdravljenju raka v otroštvu in tveganje za sekundarne maligne neoplazme

Mazić Česen M, Reulen CR, Jazbec J, Zadravec Zaletel L

Izhodišča. Namen raziskave je oceniti tveganje in opredeliti sekundarne neoplazme po zdravljenju raka v otroštvu v povezavi s spremembami v zdravljenju.

Bolniki in metode. V populacijsko raziskavo smo vključili 3271 bolnikov, ki smo jih zdravili zaradi raka do dopolnjnega 18. leta starosti v obdobju med letoma 1961 in 2013 in sledili do vključno leta 2018. Uporabili smo statistične metode: standardizirano incidenčno stopnjo, absolutno presežno tveganje in kumulativno incidenco.

Rezultati. V povprečnem času sledenja 23,2 let smo pri 230 bolnikih zabeležili 273 primerov sekundarnih neoplazem; od tega 183 malignih neoplazem, 34 meningiomov in 56 primerov nemelanomskega raka kože. 10,5 % bolnikov smo zdravili z obsevanjem, 31 % s kemoterapijo, 26,9 % s kombinacijo kemoterapije in obsevanja in 16,1 % izključno z operacijo. Tveganje za sekundarno maligno neoplazmo je bilo skoraj 3-krat večje kot v splošni populaciji (standardizirana incidenčna stopnja 2,9) in je ostalo 2-krat večje tudi po dopolnjenem 50. letu starosti. Kumulativna incidenca za sekundarne maligne neoplazme 30 let po diagnozi otroškega raka je bila pomembno nižja za bolnike, ki smo jih zdravili med leti 1960–1970 v primerjavi z bolniki, ki smo jih zdravili med leti 1970–1990 ($p < 0.001$). Kljub pomembnemu zmanjšanju deleža obsevanih bolnikov po letu 1995, kumulativna incidenca za sekundarne maligne neoplazme prvih 15 let po diagnozi ni pomembno nižja po letu 1995 ($p = 0.11$).

Zaključki. Tveganje za sekundarne maligne neoplazme pri bolnikih, ki smo jih zdravili zaradi raka v otroštvu, je v pričujoči raziskavi primerljivo s tveganjem v drugih populacijskih raziskavah. Razlika v intenzivnosti onkološkega zdravljenja, ki je doseglo vrhunec in upad kasneje kot v drugih evropskih državah, pomeni, da se tveganje za sekundarne neoplazme v prihodnosti lahko poveča.

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doi: 10.2478/raon-2022-0034

Skrajšani dihalni test z mešanimi trigliceridi C^{13} je pri odkrivanju eksokrine insuficience trebušne slinavke enako natančen kot standardni 5-urni test pri bolnikih po gastrektomiji zaradi raka želodca

Siuka D, Kumer K, Štabuc B, Štubljar D, Drobne D, Janša R

Izhodišča. Dihalni test z mešanimi trigliceridi C^{13} (C^{13}-MTGT) je neinvazivni test za odkrivanje zmerne in hude eksokrine insuficience trebušne slinavke, potrebno pa je dolgotrajno vzorčenje izdihanega zraka. Namen pričujoče raziskave je bil ugotoviti diagnostično moč skrajšanega C^{13}-MTGT pri odkrivanju eksokrine insuficience trebušne slinavke pri bolnikih po subtotalni in totalni gastrektomiji, ki je bila narejena zaradi raka želodca.

Preiskovanci in metode. V presečno opazovalno raziskavo smo vključili 3 skupine preiskovancev: zdrave kontrolne osebe ter bolnike po subtotalni in bolnike po totalni gastrektomiji zaradi raka želodca. Zbrali smo demografske in klinične podatke bolnikov. Vzorce blata za določanje fekalne elastaze (Fe-1) in kimotripsina smo izmerili s testom ELISA. Vsi preiskovanci so opravili 5-urni dihalni test C^{13}-MTGT. Koncentracijo in relativno vsebnost C^{13} v izdihanem zraku smo izmerili z masnim spektrometrom za razmerje izotopov. Eksokrino insuficienco trebušne slinavke smo potrdili kot vrednosti C^{13}-izdiha < 26,8 % po 5 urah.

Rezultati. V analizi smo obravnavali 65 udeležencev, 22 jih je imelo eksokrino insuficienco trebušne slinavke (11 po subtotalni gastrektomiji in 11 po totalni gastrektomiji). Dihalni test C^{13}-MTGT je pokazal razliko v odstotkih izdihanega C^{13} med bolniki z ali brez eksokrine insuficience trebušne slinavke že po 60 minutah (p = 0,034). Analiza krivulje karakteristike delovanja sprejemnika (ROC) je pokazala, da mejna vrednost 13,74 % po 150 minutah kaže enakovredno diagnostično moč daljšemu testu z občutljivostjo in specifičnostjo nad 90 % za izključitev eksokrine insuficience trebušne slinavke pri bolnikih po subtotalni ali totalni gastrektomiji.

Zaključki. Raziskava je pokazala, da skrajšanje testa C^{13}-MTGT s 5 na 2,5 ure ne zmanjša njegove diagnostične natančnosti za odkrivanje eksokrine insuficience trebušne slinavke pri bolnikih po subtotalni ali totalni gastrektomiji, opravljeni zaradi raka želodca, kar lahko omogoča znatne prihranke časa pri diagnostiki te podskupine bolnikov.

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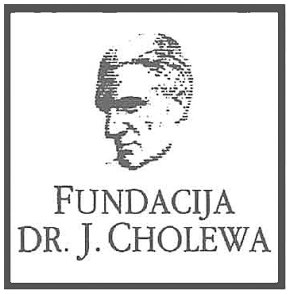
Zdravljenje kožnih tumorjev glave in vratu z intratumorskim genskim elektroprenosom gena za interlevkin 12. Protokol prvega kliničnega preskušanja pri ljudeh

Grošelj A, Bošnjak M, Jesenko T, Čemažar M, Markelc B, Strojan P, Serša G

Izhodišča. Imunske terapije so trenutno predmet mnogih raziskav, saj pogosto zagotavljajo odlične odgovore na zdravljenje pri različnih tumorjih. Možen način imunoterapije je ciljna intratumorska dostava interlevkina 12 (IL-12), citokina z znano protitumorsko učinkovitostjo. Takšen način zaradi svojega imunomodulatornega delovanja omogoči stimulacijo imunskega odziva, ki lahko okrepi učinek vakcinacije *in situ* pri lokalnih ablativnih terapijah. Razvili smo plazmid phIL12, ki je brez gena za odpornost proti antibiotikom in nosi zapis za humani protein IL-12. Plazmid lahko dostavimo v tumor z uporabo genskega elektroprenosa (GET).

Bolniki in metode. Predstavljamo protokol prvega kliničnega preskušanja pri ljudeh za phIL12 GET (*ISRCTN15479959, ClinicalTrials NCT05077033*). Raziskava je namenjena oceni varnosti in tolerančnosti phIL12 GET pri zdravljenju bazalnoceličnega raka pri bolnikih z operabilnimi tumorji v predelu glave in vratu. Raziskavo smo zasnovali kot I. klinično fazo, pri kateri smo povečevali odmerek plazmida phIL12 z namenom ugotoviti varnost in tolerančnost zdravljenja. Želeli smo določiti odmerek plazmida phIL12, ki je varen in izzove biološko aktivnost.

Zaključki. Rezultati pričujočega kliničnega protokola bodo zagotovili osnovo, ki bo omogočila uporabo phIL12 GET kot dopolnilnega zdravljenja k lokalnim ablativnim terapijam. Namen takšne terapije je povečati lokalni odgovor na zdravljenje in tudi izzvati sistemski odziv.



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Tantum Verde 1,5 mg/ml oralno pršilo, raztopina
Tantum Verde 3 mg/ml oralno pršilo, raztopina

Sestava: 1,5 mg/ml: 1 ml raztopine vsebuje 1,5 mg benzidaminijevega klorida, kar ustreza 1,34 mg benzidamina. V enem razpršku je 0,17 ml raztopine. En razpršek vsebuje 0,255 mg benzidaminijevega klorida, kar ustreza 0,2278 mg benzidamina. **Sestava 3 mg/ml:** 1 ml raztopine vsebuje 3 mg benzidaminijevega klorida, kar ustreza 2,68 mg benzidamina. V enem razpršku je 0,17 ml raztopine. En razpršek vsebuje 0,51 mg benzidaminijevega klorida, kar ustreza 0,4556 mg benzidamina. **Terapevtske indikacije:** Samozdravljenje: Lajšanje bolečine in oteklin pri vnetju v ustni votlini in žrelu, ki so lahko posledica okužb in stanj po operaciji. Po nasvetu in navodilu zdravnika: Lajšanje bolečine in oteklin v ustni votlini in žrelu, ki so posledica radiomukozitisa. **Odmerjanje in način uporabe:** Uporaba: 2- do 6-krat na dan (vsake 1,5 do 3 ure). Odmerjanje 1,5 mg/ml: Odrasli: 4 do 8 razprškov 2- do 6-krat na dan. Pediatrična populacija: Mladostniki, stari od 12 do 18 let: 4-8 razprškov 2- do 6-krat na dan. Otroci od 6 do 12 let: 4 razprški 2- do 6-krat na dan. Otroci, mlajši od 6 let: 1 razpršek na 4 kg telesne mase; do največ 4 razprške 2- do 6-krat na dan. Odmerjanje 3 mg/ml: Odrasli: 2 do 4 razprški 2- do 6-krat na dan. Pediatrična populacija: Mladostniki, stari od 12 do 18 let: 2 do 4 razprški 2- do 6-krat na dan. Otroci od 6 do 12 let: 2 razprška 2- do 6-krat na dan. Otroci, mlajši od 6 let: 1 razpršek na 8 kg telesne mase; do največ 2 razprška 2- do 6-krat na dan. Starejši bolniki, bolniki z jetrno okvaro in bolniki z ledvično okvaro: niso potrebni posebni previdnostni ukrepi. Trajanje zdravljenja ne sme biti daljše od 7 dni. Način uporabe: Za orofaringealno uporabo. Zdravilo se razprši v usta in žrelo. **Kontraindikacije:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Pri nekaterih bolnikih lahko resne bolezni povzročijo ustne/žrelne ulceracije. Če se simptomi v treh dneh ne izboljšajo, se mora bolnik posvetovati z zdravnikom ali zobozdravnikom, kot je primerno. Uporaba benzidamina ni priporočljiva za bolnike s preobčutljivostjo na salicilno kislino ali druga nesteroidna protivnetna zdravila. Pri bolnikih, ki imajo ali so imeli bronhialno astmo, lahko pride do bronhospazma. Pri takih bolnikih je potrebna previdnost. To zdravilo vsebuje 13,6 mg alkohola (etanola) v enem razpršku (0,17 ml), kar ustreza manj kot 0,34 ml piva oziroma 0,14 ml vina. Majhna količina alkohola v zdravilu ne bo imela nobenih opaznih učinkov. To zdravilo vsebuje metilparahidroksibenzoat (E218). Lahko povzroči alergijske reakcije (lahko zapoznele). To zdravilo vsebuje manj kot 1 mmol (23 mg) natrija v enem razpršku (0,17 ml), kar v bistvu pomeni 'brez natrija'. Zdravilo vsebuje aromo poprove mete z benzilalkoholom, cinamilalkoholom, citralom, citronelolom, geraniolom, izoevgenolom, linalolom, evgenolom in D-limonen, ki lahko povzročijo alergijske reakcije. Zdravilo z jakostjo 3 mg/ml vsebuje makrogolglicerol hidroksistearat 40. Lahko povzroči želodčne težave in drisko. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Študij medsebojnega delovanja niso izvedli. **Nosečnost in dojenje:** O uporabi benzidamina pri nosečnicah in doječih ženskah ni zadostnih podatkov. Uporaba zdravila med nosečnostjo in dojenjem ni priporočljiva. **Vpliv na sposobnost vožnje in upravljanja strojev:** Zdravilo v priporočenem odmerku nima vpliva na sposobnost vožnje in upravljanja strojev. **Neželeni učinki:** Neznana pogostnost (ni mogoče oceniti iz razpoložljivih podatkov): anafilaktične reakcije, preobčutljivostne reakcije, odrevenelost, laringospazem, suha usta, navzea in bruhanje, oralna hipestezija, angioedem, fotosenzitivnost, pekoč občutek v ustih. Neposredno po uporabi se lahko pojavi občutek odrevenelosti v ustih in v žrelu. Ta učinek se pojavi zaradi načina delovanja zdravila in po kratkem času izgine. **Način in režim izdaje zdravila:** BRp-Izdaja zdravila je brez recepta v lekarnah in specializiranih prodajalnah. **Imetnik dovoljenja za promet:** Aziende Chimiche Riunite Angelini Francesco – A.C.R.A.F. S.p.A., Viale Amelia 70, 00181 Rim, Italija **Datum zadnje revizije besedila:** 05. 04. 2022

Pred svetovanjem ali izdajo preberite celoten Povzetek glavnih značilnosti zdravila.

Samo za strokovno javnost.

Datum priprave informacije: april 2022

ANGELINI

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Zdravilo LUMYKRAS® še ni krito iz obveznega zdravstvenega zavarovanja.
Literatura: 1. Mullard A, et al. Nat Rev Drug Discov 2021;20:496. 2. Povzetek glavnih značilnosti zdravila LUMYKRAS®, Amgen. 3. Skoulidis F, et al. N Engl J Med 2021;384:2371–81.

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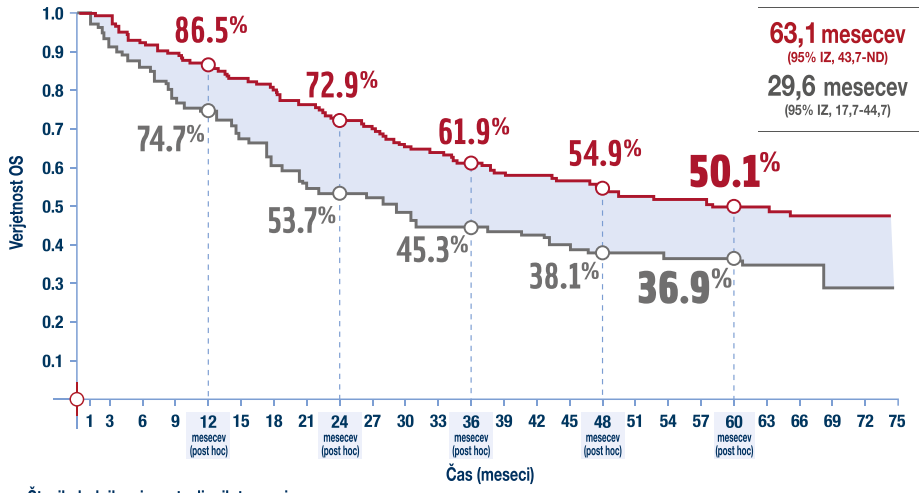
▾Za to zdravilo se izvaja dodatno spremljanje varnosti. Poročati je potrebno o vseh domnevnih neželenih učinkih zdravila.
SESTAVA ZDRAVILA: Ena filmsko obložena tableta vsebuje 120 mg sotorasiba. **TERAPEVTSKE INDIKACIJE:** Kot monoterapija za zdravljenje odraslih z napredovalim nedrobnoceličnim rakom pljuč (NDRP) z mutacijo *KRAS G12C*, pri katerih je bolezen napredovala po vsaj eni predhodni liniji sistemskega zdravljenja. **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje mora uvesti zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje raka. Pred uvedbo zdravljenja je treba z validiranim testom potrditi prisotnost mutacije *KRAS G12C*. Odmerjanje: Priporočeni odmerek je 960 mg sotorasiba (osem 120mg tablet) enkrat dnevno, vsak dan ob istem času. Zdravljenje se priporoča do napredovanja bolezni ali pojava nesprejemljive toksičnosti. *Izpuščeni odmerki ali bruhanje:* Če je od načrtovanega odmerka preteklo manj kot 6 ur, mora bolnik vzeti odmerek kot običajno. Če je od načrtovanega odmerka preteklo več kot 6 ur, bolnik ne sme vzeti odmerka. Zdravljenje mora nadaljevati naslednji dan, kot je prepisano. Če bolnik po zaužitju zdravila LUMYKRAS bruha, tisti dan ne sme vzeti dodatnega odmerka in mora zdravljenje nadaljevati naslednji dan, kot je predpisano. *Prilagajanje odmerka:* Odmerjanje je treba prilagajati glede na toksičnost zdravila LUMYKRAS. Za stopnje zmanjšanja odmerka in prilagoditve odmerka zaradi neželenih učinkov med zdravljenjem glejte celoten Povzetek glavnih značilnosti zdravila. Če se pojavijo toksični učinki, je odmerek dovoljeno zmanjšati največ dvakrat. Če bolniki ne prenašajo najmanjšega odmerka, tj. 240 mg enkrat dnevno, je treba zdravljenje z zdravilom LUMYKRAS prekiniti. Sočasna uporaba zdravila LUMYKRAS z zdravili za zmanjševanje kisline: Sočasna uporaba zaviralcev protonske črpalke (PPI) ali antagonistov receptorjev H2 z zdravilom LUMYKRAS ni priporočena. Če je potrebno zdravljenje z zdravilom za zmanjševanje kisline, se lahko uporabi nesistemski antacid, zdravilo LUMYKRAS pa je treba vzeti bodisi 4 ure pred uporabo nesistemskega antacida bodisi 10 ur po njem. Posebne populacije: Pri bolnikih, starih 75 let in več, prilagajanje odmerka ni potrebno. Prilagajanje odmerka pri bolnikih z blago okvaro jeter ni priporočeno. Uporaba sotorasiba pri bolnikih z zmerno in hudo okvaro jeter ni priporočena. Prilagajanje odmerka pri bolnikih z blago okvaro ledvic (CrCl ≥ 60 ml/min) ni priporočeno. Zdravila LUMYKRAS pri bolnikih z zmerno ali hudo okvaro ledvic (CrCl < 60 ml/min) niso preučevali. Pri zdravljenju bolnikov z zmerno ali hudo okvaro in končno odpovedjo ledvic je zato potrebna previdnost. Zdravilo LUMYKRAS ni namenjeno za uporabo pri pediatrični populaciji za zdravljenje nedrobnoceličnega raka pljuč. Način uporabe: Zdravilo LUMYKRAS je namenjeno za peroralno uporabo. Tablete je treba pogoltniti cele. Ni podatkov, ki bi podpirali jemanje zdravila LUMYKRAS tako, da se tablete žveči, zdrobi ali razpolovi, vendar je tablete mogoče raztopiti v vodi. Tablete se lahko jemljejo s hrano ali brez nje. Za uporabo pri bolnikih, ki težko pogoltnejo zdravila v trdni obliki glejte celoten Povzetek glavnih značilnosti zdravila. **KONTRAINDIKACIJE:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Hepatotoksičnost: Sotorasib lahko povzroči hepatotoksičnost, ki lahko privede do z zdravilom povzročene poškodbe jeter in hepatitisa. Sotorasib je bil povezan s prehodnimi zvišanji ravni transaminaz (ALT in AST) v serumu. Zvišane ravni so se izboljšale ali izzvenele po prilagoditvi odmerka ali trajni prekinitvi zdravljenja in v kliničnih študijah niso privedle do primerov odpovedi jeter ali smrtnih primerov. Pri bolnikih je treba spremljati delovanje jeter (ALT, AST in celokupni bilirubin), in sicer pred začetkom zdravljenja z zdravilom LUMYKRAS, nato v prvih 3 mesecih zdravljenja vsake 3 tedne, nato pa enkrat mesečno ali kot je klinično indicirano, preiskave pa je treba opravljati pogosteje pri bolnikih, pri katerih se pojavijo zvišanja ravni transaminaz in/ali bilirubina. Glede na stopnjo resnosti nenormalnih izvidov laboratorijskih preiskav je treba zdravljenje z zdravilom LUMYKRAS prekiniti do izboljšanja na stopnjo ≤ 1 ali na izhodiščno stopnjo ter v skladu s priporočili bodisi prilagoditi odmerek bodisi trajno prekiniti zdravljenje. Intersticijska pljučna bolezen (ILD)/pnevmonitis: Bolnike spremljajte za pojav novih ali poslabšanje obstoječih pljučnih simptomov, ki bi kazali na ILD/pnevmonitis (npr. dispneja, kašelj, vročina). Nemudoma prekinite zdravljenje z zdravilom LUMYKRAS pri bolnikih z domnevno ILD/pnevmonitisom in trajno prekinite zdravljenje z zdravilom LUMYKRAS, če ne odkrijete nobenih drugih morebitnih vzrokov za ILD/pnevmonitis. Intoleranca za laktozo: Zdravilo LUMYKRAS vsebuje laktozo. Bolniki z redko dedno intoleranco za galaktozo, odsotnostjo encima laktaze ali malabsorpcijo glukoze/galaktoze ne smejo jemati tega zdravila. Natrij: To zdravilo vsebuje manj kot 1 mmol (23 mg) natrija na tableto, kar v bistvu pomeni „brez natrija". **INTERAKCIJE:** Vpliv drugih zdravil na sotorasib: Sočasna uporaba sotorasiba s PPI (omeprazolom) ali antagonistom receptorjev H2 (famotidinom) je privedla do zmanjšanja koncentracij sotorasiba. Sočasna uporaba PPI in antagonistov receptorjev H2 z zdravilom LUMYKRAS ni priporočena, saj ni znano, kakšen bi bil vpliv na učinkovitost sotorasiba. Če je potrebno zdravljenje z zdravilom za zmanjševanje kisline, je zdravilo LUMYKRAS treba vzeti 4 ure pred uporabo nesistemskega antacida ali 10 ur po njem. Sočasna uporaba večkratnih odmerkov itrakonazola (močnega zaviralca CYP3A4 in Pgp) ni povečala izpostavljenosti sotorasibu v klinično pomembnem obsegu. Prilagoditev odmerka zdravila LUMYKRAS pri sočasni uporabi z zaviralci CYP3A4 ni priporočena. Sočasna uporaba močnih induktorjev CYP3A4 (npr. rifampicina, karbamazepina, enzalutamida, mitotana, fenitoina in šentjanževke) z zdravilom LUMYKRAS ni priporočena, saj lahko ta zdravila zmanjšajo izpostavljenost sotorasibu. Vpliv sotorasiba na druga zdravila: Sotorasib je zmeren induktor CYP3A4. Sočasna uporaba sotorasiba s substrati CYP3A4 je privedla do zmanjšanja njihovih plazemskih koncentracij, kar bi lahko zmanjšalo učinkovitost teh substratov. Izogibajte se sočasni uporabi zdravila LUMYKRAS s substrati CYP3A4 z ozkimi terapevtskimi indeksi, ki med drugim vključujejo alfentanil, ciklosporin, dihidroergotamin, ergotamin, fentanil, hormonske kontraceptive, pimozid, kinidin, sirolimus in takrolimus. Če se sočasni uporabi ni mogoče izogniti, prilagodite odmerek substrata CYP3A4 v skladu s trenutnim Povzetkom glavnih značilnosti zdravila. Podatki *in vitro* so pokazali, da bi sotorasib morda lahko inducival CYP2B6, CYP2C8, CYP2C9 in CYP2C19; klinični pomen teh ugotovitev ni znan. Pri sočasni uporabi sotorasiba z drugimi zdravili, ki se presnavljajo s pomočjo teh encimov, se priporoča ustrezno spremljanje. Podatki *in vitro* so pokazali, da bi sotorasib morda lahko zaviral CYP2D6; klinični pomen teh ugotovitev ni znan. Pri sočasni uporabi zdravila LUMYKRAS s substrati CYP2D6 (npr. flekainidom, propafenonom, metoprololom), se priporoča ustrezno spremljanje. Podatki *in vitro* so pokazali, da bi sotorasib morda lahko zaviral BCRP; klinični pomen teh ugotovitev ni znan. Pri sočasni uporabi zdravila LUMYKRAS s substrati BCRP (npr. metotreksatom, mitoksantronom, topotekanom in lapatinibom), se priporoča ustrezno spremljanje. Sočasna uporaba zdravila LUMYKRAS s substrati Pgp z ozkimi terapevtskimi indeksi ni priporočena. Če se sočasni uporabi ni mogoče izogniti, prilagodite odmerek substrata Pgp v skladu s trenutnim Povzetkom glavnih značilnosti zdravila. **PLODNOST, NOSEČNOST IN DOJENJE:** Ženskam v rodni dobi je treba svetovati, naj se med zdravljenjem z zdravilom LUMYKRAS izogibajo zanositvi. Bolnice v rodni dobi, ki prejemajo zdravilo LUMYKRAS, morajo med zdravljenjem in še najmanj 7 dni po zadnjem odmerku zdravila LUMYKRAS uporabljati visokoučinkovite metode kontracepcije. Zdravilo LUMYKRAS lahko zmanjša učinkovitost hormonskih kontraceptivov, zato morajo ženske, ki uporabljajo hormonske kontraceptive, uporabljati tudi dodatno pregradno metodo. Zdravila LUMYKRAS ne uporabljajte pri nosečnicah in pri ženskah v rodni dobi, ki ne uporabljajo učinkovite kontracepcije. Bolnice je treba seznaniti z morebitnimi tveganji za plod, če se zdravilo LUMYKRAS uporablja med nosečnostjo ali če bolnica zanosi med jemanjem zdravila LUMYKRAS. **NEŽELENI UČINKI:** Zelo pogosti: anemija, glavobol, kašelj, dispneja, driska, navzea, bruhanje, zaprtje, bolečine v trebuhu, artralgija, bolečine v hrbtu, utrujenost, piruksija, zvišane ravni AST, zvišane ravni ALT. Pogosti: z zdravilom povzročena poškodba jeter, zvišane ravni alkalne fosfataze v krvi, zvišane ravni bilirubina v krvi, zvišane ravni gama glutamiltransferaze. Občasni: ILD/pnevmonitis. **REŽIM IZDAJE:** Rp/Spec. **IMETNIK DOVOLJENJA ZA PROMET:** Amgen Europe B.V., Minervum 7061, 4817 ZK Breda, Nizozemska. **Dodatne informacije:** Amgen zdravila d.o.o., Ameriška ulica 2, 1000 Ljubljana. **DATUM ZADNJE REVIZIJE BESEDILA:** Januar 2022. **DATUM PRIPRAVE INFORMACIJE:** Maj 2022.

SI-510-0522-00002

IMFINZI™
durvalumab
50 mg/ml koncentrat za raztopino za infundiranje

AKTIVIRA IMUNSKI SISTEM.
PREPOZNA. **REAGIRA.**

5-LETNO CELOKUPNO PREŽIVETJE BOLNIKOV Z LOKALNO NAPREDOVALIM, NEOPERABILNIM NSCLC S PDL-1 ≥ 1%



Število bolnikov izpostavljenih tveganju

Zdravilo IMFINZI	212	208	193	186	178	171	165	156	146	141	132	129	124	118	117	114	109	105	103	98	74	52	29	14	1	0
Placebo	91	81	75	67	64	58	52	47	45	44	41	38	38	37	36	33	31	31	30	29	24	14	8	5	2	0

Po petih letih je bilo živih še 50% bolnikov zdravljenih z zdravilom Imfinzi po zaključeni sočasni kemoradioterapiji na osnovi platine.

NSCLC... nedrobnocelični rak pljuč (non small cell lung cancer)

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

Imfinzi 50 mg/ml koncentrat za raztopino za infundiranje

SESTAVA: 1 ml koncentrata za raztopino za infundiranje vsebuje 50 mg durvalumaba. Ena viala z 2,4 ml koncentrata vsebuje 120 mg durvalumaba. Ena viala z 10 ml koncentrata vsebuje 500 mg durvalumaba.**INDIKACIJE:** Zdravilo Imfinzi je kot samostojno zdravljenje indicirano za zdravljenje lokalno napredovalega, neoperabilnega nedrobnoceličnega raka pljuč (NSCLC – "non small cell lung cancer") pri odraslih, ki imajo tumorje z ≥ 1 % izraženostjo PD-L1 na tumorskih celicah in pri katerih bolezen ni napredovala po kemoradioterapiji na osnovi platine. Zdravilo Imfinzi je v kombinaciji z etopozidom in bodisi karboplatinom bodisi cisplatinom indicirano za prvo linijo zdravljenja odraslih z razsejanim drobnoceličnim rakom pljuč (ES SCLC – "extensive stage small cell lung cancer"). **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje mora uvesti in nadzorovati zdravnik, ki ima izkušnje na področju zdravljenja raka. Bolnike z lokalno napredovalim nedrobnoceličnim rakom pljuč je treba za zdravljenje izbrati na podlagi izraženosti PD-L1, ugotovljene z validirano testno metodo. **Odmerjanje:** Priporočeni odmerek zdravila Imfinzi pri samostojnem zdravljenju je 10 mg/kg na 2 tedna ali 1500 mg na 4 tedne do napredovanja bolezni, nesprejemljivih toksičnih učinkov ali največ 12 mesecev . Bolniki s telesno maso 30 kg ali manj morajo prejemati odmerke na podlagi telesne mase, in sicer 10 mg/kg na 2 tedna ali 20 mg/kg na 4 tedne kot samostojno zdravljenje, dokler se telesna masa ne poveča na več kot 30 kg. V kombinaciji s kemoterapijo je priporočeni odmerek zdravila Imfinzi 1500 mg v kombinaciji s kemoterapijo na 3 tedne (21 dni) 4 cikluse in nato 1500 mg na 4 tedne kot samostojno zdravljenje. Zdravilo Imfinzi se uporablja v intravenski infuziji, ki traja 1 uro. Povečevanje ali zmanjševanje odmerka ni priporočljivo. Glede na individualno varnost in prenašanje je lahko potrebna odložitev odmerka ali prenehanje uporabe zdravila. V primeru domnevnih imunsko pogojenih neželenih učinkov je treba opraviti ustrezno ovrednotenje za potrditev etiologije ozioma izključitev druge etiologije. Glede na resnost neželenega učinka je treba prenehati uporabljati zdravilo IMFINZI in pričeti z dajanjem kortikosteroidov. Če se stanje ne izboljša ali se poslabša, pride v poštev povečanje odmerka kortikosteroidov in/ali dodatna uporaba sistemskih imunosupresivov. Po izboljšanju na ≤ 1. stopnjo je treba začeti s postopnim zmanjševanjem kortikosteroida in ga zmanjševati v obdobju vsaj 1 meseca. Po odložitvi uporabe je mogoče zdravilo Imfinzi znova začeti uporabljati v času 12 tednov, če se neželeni učinki izboljšajo na ≤ 1. stopnjo in je odmerek kortikosteroida zmanjšan na ≤ 10 mg prednizona ali ekvivalenta na dan. Zdravilo IMFINZI je treba dokončno ukiniti, če se imunsko pogojeni neželeni učinki 3. stopnje (močno izraženi) ponavljajo in pri katerih koli imunsko pogojenih neželenih učinkih 4. stopnje (življenjsko nevarni), razen pri endokrinopatijah, ki jih nadziramo z nadomestnimi hormoni. Pri ne imunsko pogojenih neželenih učinkih, v primeru 2. ali 3. stopnje izraženosti, odložite uporabo zdravila IMFINZI do izboljšanja neželenih učinkov na ≤ 1. stopnjo ali na izhodiščno raven. Z uporabo zdravila Imfinzi je treba prenehati v primeru neželenih učinkov 4. stopnje (razen v primeru laboratorijskih nepravilnosti 4. stopnje, pri katerih naj odločitev o prekinitvi uporabe zdravila temelji na spremljajočih kliničnih znakih oziroma simptomih in na klinični presoji zdravnika). Zdravilo Imfinzi je namenjeno za intravensko uporabo. Dati ga je treba kot raztopino za intravensko infundiranje v obdobju 1 ure. **KONTRAINDIKACIJE:** Preobčutljivost na učinkovino (učinkovine) ali katero koli pomožno snov. **OPOZORILA IN PREVIDNOSTNI UKREPI:** Za izboljšanje sledljivosti bioloških zdravil je treba jasno zabeležiti lastniško ime in številko serije uporabljenega zdravila. Imunsko pogojeni pnevmonitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojeni pnevmonitis ali intersticijska bolezen pljuč, opredeljeno kot potreba po uporabi sistemskih kortikosteroidov in brez jasne druge etiologije. Pri bolnikih, zdravljenih z radioterapijo pljuč, je pogost radiacijski pnevmonitis in klinična slika pnevmonitisa in radiacijskega pnevmonitisa je zelo podobna. V študiji PACIFIC sta se pri bolnikih, ki so opravili zdravljenje z najmanj 2 cikloma sočasne kemoradioterapije od 1 do 42 dni pred začetkom preizkušanja, pnevmonitis ali radiacijski pnevmonitis pojavila pri 161 (33,9 %) bolnikih v skupini z zdravilom Imfinzi in pri 58 (24,8 %) bolnikih v skupini s placebom, vključno s 3. stopnjo (3,4 % in 3,0 %) in 5. stopnjo (1,1 % in 1,7 %). Bolnike je treba spremljati glede znakov in simptomov pnevmonitisa ali radiacijskega pnevmonitisa. Imunsko pogojeni hepatitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavil imunsko pogojeni hepatitis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojeni kolitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojeni kolitis ali driska, opredeljeno kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojene endokrinopatije: Imunsko pogojeni hipotiroidizem, hipertiroidizem in tiroiditis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, so se pojavili imunsko pogojeni hipotiroidizem, hipertiroidizem in tiroiditis; hipertiroidizmu lahko sledi hipotiroidizem. Bolnike je treba spremljati glede nenormalnih izvidov delovanje ščitnice pred zdravljenjem in redno med zdravljenjem ter kot je potrebno glede na klinično oceno. Imunsko pogojeni hipotiroidizem, hipertiroidizem in tiroiditis je treba obravnavati in ukrepati, kot je priporočeno v povzetku glavnih značilnosti zdravila. Imunsko pogojena adrenalna insuficienca: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavila imunsko pogojena adrenalna insuficienca. Bolnike je treba spremljati glede kliničnih znakov in simptomov adrenalne insuficience. Imunsko pogojena sladkorna bolezen tipa 1: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavila imunsko pogojena sladkorna bolezen tipa 1, ki se lahko najprej kaže kot diabetična ketoacidoza, ki je lahko smrtno nevarna, če je dovolj zgodaj ne odkrijemo. Bolnike je treba spremljati glede kliničnih znakov in simptomov sladkorne bolezni tipa 1. Imunsko pogojeni hipofizitis/hipopituitarizem: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojena hipofizitis ali hipopituitarizem. Bolnike je treba spremljati glede kliničnih znakov in simptomov hipofizitisa ali hipopituitarizma. Imunsko pogojeni nefritis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavil imunsko pogojeni nefritis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojeni izpuščaj: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavil imunsko pogojeni izpuščaj ali dermatitis (vključno s pemfigoidom), opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Pri bolnikih, ki so bili zdravljeni z zaviralci PD-1, so poročali o pojavljanju Stevens-Johnsonovega sindroma ali toksične epidermalne nekrolize. Drugi imunsko pogojeni neželeni učinki: Glede na mehanizem delovanja zdravila Imfinzi se lahko pojavijo še drugi potencialno imunsko pogojeni učinki. Naslednji imunsko pogojeni neželeni učinki so bili opaženi pri bolnikih, ki so prejemali samostojno zdravljenje z zdravilom Imfinzi: miastenija gravis, miokarditis, miozitis, polimiozitis, meningitis, encefalitis, Guillain Barréjev sindrom, imunska trombocitopenija, neinfektivni cistitis in pankreatitis. Bolnike je treba spremljati glede znakov in simptomov in ukrepati, kot je priporočeno za druge imunsko pogojene neželene učinke. Z infundiranjem povezane reakcije: Bolnike je treba spremljati glede znakov in simptomov z infundiranjem povezanih reakcij. Pri bolnikih, ki so prejemali zdravilo Imfinzi, so bile opisane hude z infundiranjem povezane reakcije. Bolniki, ki niso bili vključeni v klinična preizkušanja: V klinična preskušanja niso bili vključeni bolniki z naslednjimi značilnostmi: izhodiščno oceno zmogljivosti ECOG ≥ 2; aktivno ali predhodno dokumentirano avtoimunsko boleznijo v 2 letih pred začetkom študije; anamnezo imunske pomanjkljivosti; anamnezo hudih imunsko pogojenih neželenih učinkov; boleznimi, ki so zahtevale sistemsko imunosupresijo, razen fiziološkega odmerka sistemskih kortikosteroidov (≤ 10 mg na dan prednizona ali ekvivalenta); neobvladanimi sočasnimi boleznimi; aktivno tuberkulozo ali okužbo s hepatitisom B ali C ali HIV; bolniki, ki so prejeli živo oslabljeno cepivo v 30 dneh pred začetkom zdravljenja z zdravilom Imfinzi ali v 30 dneh po začetku. Dokler takšnih podatkov ni, je treba durvalumab v teh skupinah bolnikov uporabljati previdno ter po skrbnem individualnem pretehtanju možnih koristi in tveganj za posameznega bolnika. Varnost sočasnega profilaktičnega kranialnega obsevanja obsevanj z zdravilom Imfinzi pri bolnikih z ES SCLC ni znana. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI:** Razen fizioloških odmerkov sistemskih kortikosteroidov (≤ 10 mg na dan prednizona ali ekvivalenta) pred uvedbo durvalumaba ni priporočljivo uporabljati sistemskih kortikosteroidov ali imunosupresivov, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost durvalumaba. Vendar pa je mogoče kortikosteroide ali druge imunosupresivne uporabiti po začetku zdravljenja z durvalumabom za zdravljenje imunsko pogojenih neželenih učinkov. Z durvalumabom niso izvedli formalnih farmakokinetičnih (PK) študij medsebojnega delovanja zdravil. Primarni poti odstranjevanja durvalumaba sta katabolizem beljakovin preko retikuloendoteljiskega sistema ozioroma tarčno posredovano odstranjevanje, zato ni pričakovati presnovnih medsebojnih delovanj med zdravili. Farmakokinetična medsebojna delovanja med durvalumabom in kemoterapijo so ocenjevali v študiji CASPIAN: izkazalo se je, da sočasno zdravljenje z durvalumabom ne vpliva na farmakokinetiko etopozida, karboplatina ali cisplatina. Poleg tega populacijska farmakokinetična analiza kaže, da sočasno zdravljenje s kemoterapijo ne vpliva pomembno na farmakokinetiko durvalumaba. **PLODNOST, NOSEČNOST IN DOJENJE:** Ženske v rodni dobi morajo med zdravljenjem z durvalumabom in vsaj še 3 mesece po zadnjem odmerku durvalumaba uporabljati učinkovito kontracepcijo. Podatkov o uporabi durvalumaba pri nosečnicah ni. Glede na mehanizem delovanja durvalumab lahko vpliva na vzdrževanje nosečnosti; v alogenskem modelu nosečnosti pri miših je bilo ugotovljeno, da moteno signaliziranje PD-L1 poveča izgubo plodov. Pri nosečnici uporabljeni durvalumab lahko škoduje plodu in ga ni priporočljivo uporabljati med nosečnostjo in pri ženskah v rodni dobi, ki ne uporabljajo učinkovite kontracepcije med zdravljenjem in vsaj še 3 mesece po zadnjem odmerku. Ni znano, ali se durvalumab pri človeku izloča v materinem mleku. Pri človeku protitelesa lahko prehajajo v materino mleko, a možnost absorpcije in škode za novorojenčka ni znana. Toda možnega tveganja za dojenega otroka ni mogoče izključiti. Odločiti se je treba, ali naj ženska prekine z dojenjem ali naj prekine zdravljenje z durvalumabom oziroma sploh ne začne zdravljenja z njim, pri čemer je treba upoštevati koristi dojenja za otroka in koristi zdravljenja za žensko. Podatkov o možnih vplivih durvalumaba na plodnost pri človeku ali živalih ni. **NEŽELENI UČINKI:** Ugotovitve o varnosti zdravila Imfinzi pri samostojnem zdravljenju temeljijo na kumulativnih podatkih 3006 bolnikov z več vrstami tumorjev. Zdravilo Imfinzi so uporabljali v odmerku 10 mg/kg na 2 tedna ali v odmerku 20 mg/kg na 4 tedne. Najpogostejši neželeni učinki (> 10 %) so bili kašelj/produktiven kašelj (21,5 %), driska (16,3 %), izpuščaj (16,0 %), zvišana telesna temperatura (13,8 %), okužbe zgornjih dihal (13,5 %), bolečine v trebuhu (12,7 %), srbenje (10,8 %) in hipotiroidizem (10,1 %). Ugotovitve o varnosti zdravila Imfinzi v kombinaciji s kemoterapijo temeljijo na podatkih 265 bolnikov z SCLC. Zdravilo Imfinzi so uporabljali v odmerku 1500 mg na 3 tedne v kombinaciji s kemoterapijo in nato pri samostojnem zdravljenju na 4 tedne. Najpogostejši neželeni učinki (> 20 %) so bili nevtropenija (48,7 %), anemija (38,5 %), navzea (33,6 %), utrujenost (32,1 %), alopecija (31,3 %), trombocitopenija (21,1 %) in levkopenija (20,0 %). Zdravilo Imfinzi pri samostojnem zdravljenju: Zelo pogosti neželeni učinki: okužbe zgornjih dihal, hipotiroidizem, kašelj/produktiven kašelj, driska, bolečine v trebuhu, izpuščaj, srbenje, zvišana telesna temperatura. Pogosti neželeni učinki: pljučnica, zobne okužbe in okužbe ustnih mehkih tkiv, oralna kandidoza, gripa, hipertiroidizem, pnevmonitis, disfonija, zvišanje vrednosti aspartat aminotransferaze ali zvišanje vrednosti alanin aminotransferaze, nočno znojenje, dermatitis, mialgija, zvišanje vrednosti kreatinina v krvi, disurija, periferni edemi, z infundiranjem povezane reakcije. Občasni neželeni učinki: tiroiditis, adrenalna insuficienca, intersticijska bolezen pljuč, kolitis, hepatitis, dermatitis, miozitis, nefritis, pankreatitis. Redki neželeni učinki: sladkorna bolezen tipa 1, hipofizitis / hipopituitarizem, diabetes insipidus, miastenija gravis, meningitis, miokarditis, pemfigois, polimiozitis, imunska trombocitopenija. Zdravilo Imfinzi v kombinaciji s kemoterapijo: Zelo pogosti neželeni učinki: nevtropenija, anemija, trombocitopenija, levkopenija, zmanjšan apetit, kašelj/produktiven kašelj, navzea, zaprtost, bruhanje, izpadanje las, utrujenost. Pogosti neželeni učinki: okužbe zgornjih dihal, pljučnica, zobne okužbe in okužbe ustnih mehkih tkiv, febrilna nevtropenija, pancitopenija, hipotiroidizem, hipertiroidizem, tiroiditis, adrenalna insuficienca, pnevmonitis, driska, bolečine v trebuhu, stomatitis, zvišanje vrednosti aspartat aminotransferaze ali zvišanje vrednosti alanin aminotransferaze, hepatitis, izpuščaj, srbenje, dermatitis, mialgija, zvišanje vrednosti kreatinina v krvi, disurija, zvišana telesna temperatura. Občasni neželeni učinki: gripa, sladkorna bolezen tipa 1, disfonija, intersticijska bolezen pljuč. **VRSTA IN VSEBINA OVOJNINE:** 2,4 ml koncentrata v stekleni viali iz stekla tipa 1 z elastomernim zamaškom in sivo snemno aluminijsko zaporo; viala vsebuje 120 mg durvalumaba. Pakiranje vsebuje 1 vialo. 10 ml koncentrata v stekleni viali iz stekla tipa 1 z elastomernim zamaškom in belo snemno aluminijsko zaporo; viala vsebuje 500 mg durvalumaba. Pakiranje vsebuje 1 vialo. **NAČIN IZDAJANJA ZDRAVILA:** H - Predpisovanje in izdaja zdravila je le na recept. **DATUM REVIZIJA BESEDILA:** februar 2022 (SI-2065) **IMETNIK DOVOLJENJA ZA PROMET:** AstraZeneca AB, S-151 85, Sodertalje, Švedska

Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila.
Dodatne informacije so na voljo pri družbi AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, Ljubljana, telefon +386 1 51 35 600.

Reference: 1.Spigel DR, Faivre-Finn C, Gray JE, et al. Five-year survival outcomes from the PACIFIC trial: durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. J Clin Oncol. Forthcoming 2021.

AstraZeneca

SI-2123

Prvo v Evropski uniji odobreno bispecifično protitelo za zdravljenje folikularnega limfoma, ki je usmerjeno proti CD20xCD3.

Lunsumio®▼

mosunetuzumab

Zdravilo Lunsumio je kot monoterapija indicirano za zdravljenje odraslih bolnikov s ponovljenim ali neodzivnim (refraktarnim) folikularnim limfomom (FL), ki so prejeli vsaj dve predhodni sistemski zdravljenji.[1]

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti, kar označuje navzdol obrnjen črn trikotnik. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

Vir: 1 Povzetek glavnih značilnosti zdravila Lunsumio. Dostopano julij 2022 na https://www.ema.europa.eu/en/documents/product-information/lunsumio-epar-product-information_sl.pdf

Skrajšan povzetek glavnih značilnosti zdravila Lunsumio

Ime zdravila: Lunsumio 1 mg/30 mg koncentrat za raztopino za infundiranje
Kakovostna in količinska sestava: Lunsumio 1 mg koncentrat za raztopino za infundiranje: Ena viala vsebuje 1 mg mosunetuzumaba v 1 ml, v koncentraciji 1 mg/ml. Lunsumio 30 mg koncentrat za raztopino za infundiranje: Ena viala vsebuje 30 mg mosunetuzumaba v 30 ml, v koncentraciji 1 mg/ml. Mosunetuzumab je humanizirano protitelo (imunoglobulin) polne dolžine, izotipa G1 (IgG1), usmerjeno proti CD20/CD3, pridobljeno iz celic jajčnika kitajskega hrčka s tehnologijo rekombinantne DNA. **Terapevtske indikacije:** Zdravilo Lunsumio je kot monoterapija indicirano za zdravljenje odraslih bolnikov s ponovljenim ali neodzivnim (refraktarnim) folikularnim limfomom (FL), ki so prejeli vsaj dve predhodni sistemski zdravljenji. **Odmerjanje in način uporabe:** Zdravilo Lunsumio se sme dajati le pod nadzorom zdravnika, usposobljenega za uporabo zdravil za zdravljenje raka, in v okolju, ki omogoča ustrezno zdravstveno obravnavo in obvladanje hudih reakcij, kot je sindrom sproščanja citokinov (cytokine release syndrome - CRS). Odmerjanje: *Profilaksa in premedikacija*: Bolniki, ki prejmejo zdravilo Lunsumio, morajo biti dobro hidrirani. Priporočena premedikacija in priporočeni odmerki za vsak 21-dnevni cikel so podrobno prikazani v Povzetku glavnih značilnosti zdravila. Trajanje zdravljenja: Zdravilo Lunsumio je treba dajati 8 ciklov, razen če se pri bolniku pojavi nesprejemljiva toksičnost ali bolezen napreduje. Bolniki, ki dosežejo popolni odziv, po 8 ciklih ne potrebujejo nadaljnjega zdravljenja. Bolniki, ki po 8 ciklih zdravljenja z zdravilom Lunsumio dosežejo delni odziv ali imajo stabilno bolezen, morajo dobiti dodatnih 9 ciklov zdravljenja, razen če se pojavijo nesprejemljiva toksičnost ali bolezen napreduje. Prilagoditev odmerka: Zdravljenje bolnikov, pri katerih se pojavijo učinki 3. ali 4. stopnje, je treba začasno prekiniti, dokler simptomi ne minejo. Način uporabe: Zdravilo Lunsumio je namenjeno le za intravensko uporabo. Zdravilo Lunsumio je treba razredčiti z upoštevanjem aseptičnega postopka. Dati ga je treba v intravenski infuziji po namenski infuzijski liniji. Za dajanje zdravila Lunsumio ne uporabljajte linijskega filtra, uporabite pa lahko filtre v kapalni komori. Zdravila Lunsumio se ne sme dati kot hiter intravenski odmerek ali bolus. **Kontraindikacije:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Sindrom CRS: Pri nekaterih bolnikih, ki so prejemali zdravilo Lunsumio, se je pojavil CRS, vključno z življenje ogrožajočimi reakcijami. Z infundiranjem povezane reakcije se lahko kažejo z enako klinično sliko kot CRS. Premedikacijo morajo bolniki prejeti vsaj od začetka zdravljenja do konca 2. cikla zdravljenja. Bolnike je treba nadzorovati glede znakov ali simptomov CRS. Naročiti jim je treba, naj nemudoma poiščejo zdravniško pomoč, če se kadar koli pojavijo znaki ali simptomi CRS. V primeru pojava CRS morajo zdravniki uvesti podporno zdravljenje. Resne okužbe: Pri nekaterih bolnikih, ki so prejemali zdravilo Lunsumio, so se pojavile resne okužbe. Po infundiranju zdravila Lunsumio so pri nekaterih bolnikih opažali febrilno nevtropenijo. Bolniki z aktivnimi okužbami ne smejo prejeti zdravila Lunsumio. Zdravilo Lunsumio je treba previdno uporabljati pri bolnikih z anamnezo ponavljajočih se ali kroničnih okužb, s pridruženimi boleznimi, ki lahko povečajo nagnjenost k okužbam, ali z intenzivnim predhodnim imunosupresivnim zdravljenjem. Bolniki morajo dobiti ustrezna profilaktična zdravila. Bolnike je treba nadzorovati glede znakov in simptomov okužbe pred in po dajanju zdravila Lunsumio ter jih ustrezno zdraviti. Zagon tumorja: Pri nekaterih bolnikih, zdravljenih z zdravilom Lunsumio, so poročali o zagonu tumorja. Specifičnih dejavnikov tveganja za pojav zagona tumorja niso ugotovili. Vendar pa obstaja tveganje za prizadetost in umrljivost bolnika zaradi učinka mase tumorja ob zagonu tumorja pri bolnikih z obsežnimi tumorji, ki se nahajajo v neposredni bližini dihalnih poti in/ali vitalnih organov. Pri bolnikih, ki prejemajo zdravilo Lunsumio, je treba nadzorovati in ocenjevati kritična anatomska mesta glede pojava zagona tumorja. Sindrom razpada tumorja: Pri bolnikih, ki so prejemali zdravilo Lunsumio, so poročali o sindromu razpada tumorja. Bolniki morajo dobiti ustrezno profilaktično zdravljenje. Bolnike je treba nadzorovati glede znakov in simptomov sidroma razpada tumorja; to še zlasti velja za bolnike z velikim tumorskim bremenom ali hitro rastočimi tumorji ter bolnike z zmanjšanim delovanjem ledvic. Spremljati je treba biokemijske izvide bolnikov in v primeru odstopanj takoj ukrepati. Imunizacija: Sočasno z zdravilom Lunsumio se ne sme dajati živih in/ali živih oslabljenih cepiv. Kartica za bolnika: Zdravnik, ki zdravilo predpiše, se mora z bolnikom pogovoriti o tveganjih zdravljenja z zdravilom Lunsumio. Bolnik mora dobiti kartico za bolnika in izrecno navodilo, naj jo ima vedno pri sebi. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Prehodnega klinično pomembnega učinka na substrate CYP450 z ozkim terapevtskim indeksom ni mogoče izključiti, saj uvedba zdravila Lunsumio povzroči prehoden porast citokinov, to pa lahko povzroči zavrtje encimov CYP450. Pri uvedbi zdravila Lunsumio bolnikom, ki se zdravijo s substrati CYP450 z ozkim terapevtskim indeksom, je treba razmisliti o spremljanju zdravljenja. Odmerek sočasno uporabljanega zdravila je treba ustrezno prilagoditi. **Neželeni učinki:** Najpogostejši neželeni učinki so bili sindrom sproščanja citokinov, nevtropenija, zvišana telesna temperatura, hipofosfatemija in glavobol. Najpogostejši resni neželeni učinki so bili CRS, zvišana telesna temperatura in pljučnica. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Javna agencija Republike Slovenije za zdravila in medicinske pripomočke, Sektor za farmakovigilanco, Nacionalni center za farmakovigilanco, Slovenčeva ulica 22, SI-1000 Ljubljana, Tel: +386 (0)8 2000 500, Faks: +386 (0)8 2000 510, e-pošta: h-farmakovigilanca@jazmp.si, spletna stran: www.jazmp.si. Za zagotavljanje sledljivosti zdravila je pomembno, da pri izpolnjevanju obrazca o domnevnih neželenih učinkih zdravila navedete številko serije biološkega zdravila. **Režim izdaje zdravila:** H. **Imetnik dovoljenja za promet:** Roche Registration GmbH, Emil-Barell-Strasse 1, 79639 Grenzach-Wyhlen, Nemčija. **Verzija: 1.0/22**

Dodatne informacije so na voljo pri:
Roche farmacevtska družba d.o.o., Stegne 13g, 1000 Ljubljana
Samo za strokovno javnost.

Zdravilo še ni krito iz obveznega zdravstvenega zavarovanja.
Datum priprave informacije: avgust 2022
M-SI-00000555(v1.0)

Zdravilo Lonsurf je indicirano v monoterapiji za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (KRR), ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF – Vascular Endothelial Growth Factor) in zaviralci receptorjev za epidermalni rastni dejavnik (EGFR – Epidermal Growth Factor Receptor).[1]

Zdravilo Lonsurf je indicirano v monoterapiji za zdravljenje odraslih bolnikov z metastatskim rakom želodca vključno z adenokarcinomom gastro-ezofagealnega prehoda, ki so bili predhodno že zdravljeni z najmanj dvema sistemskima režimoma zdravljenja za napredovalo bolezen.[1]

VEČ ČASA

za več trenutkov, ki štejejo

Podaljša celokupno preživetje
v 3. liniji zdravljenja bolnikov z mCRC in mGC[2,3]

Literatura: 1. Povzetek glavnih značilnosti zdravila Lonsurf, december 2020. 2. Mayer R et al. N Engl J Med. 2015;372:1909-19. 3. Shitara K et al. Lancet Oncol. 2018;19:1437-1448.
Družba Servier ima licenco družbe Taiho za zdravilo Lonsurf®. Pri globalnem razvoju zdravila sodelujeta obe družbi in ga tržita na svojih določenih področjih.

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Skrajšan povzetek glavnih značilnosti zdravila: Lonsurf 15 mg/6,14 mg filmsko obložene tablete in Lonsurf 20 mg/8,19 mg filmsko obložene tablete

SESTAVA*: Lonsurf 15 mg/6,14 mg: Ena filmsko obložena tableta vsebuje 15 mg trifluridina in 6,14 mg tipiracila (v obliki klorida). **Lonsurf 20 mg/8,19 mg**: Ena filmsko obložena tableta vsebuje 20 mg trifluridina in 8,19 mg tipiracila (v obliki klorida). **TERAPEVTSKE INDIKACIJE*:** Kolorektalni rak ‡ v monoterapiji za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom, ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF ‡ Vascular Endothelial Growth Factor) in zaviralci receptorjev za epidermalni rastni dejavnik (EGFR ‡ Epidermal Growth Factor Receptor). Rak želodca ‡ v monoterapiji za zdravljenje odraslih bolnikov z metastatskim rakom želodca vključno z adenokarcinomom gastro-ezofagealnega prehoda, ki so bili predhodno že zdravljeni z najmanj dvema sistemskima režimoma zdravljenja za napredovalo bolezen. **ODMERJANJE IN NAČIN UPORABE*:** Priporočeni začetni odmerek zdravila Lonsurf pri odraslih je 35 mg/m^2/odmerek peroralno dvakrat dnevno na 1. do 5. dan in 8. do 12. dan vsakega 28-dnevnega cikla zdravljenja, najpozneje 1 uro po zaključku jutranjega in večernega obroka (20 mg/m^2/odmerek dvakrat dnevno pri bolnikih s hudo ledvično okvaro). Odmerek, izračunan glede na telesno površino, ne sme preseči 80 mg/odmerek. Možne prilagoditve odmerka glede na varnost in prenašanje zdravila: dovoljena so zmanjšanja odmerka na najmanjši odmerek 20 mg/m^2 dvakrat dnevno (oz. 15 mg/m^2 dvakrat dnevno pri bolnikih s hudo ledvično okvaro). Potem ko je bil odmerek zmanjšan, povečanje ni dovoljeno. **KONTRAINDIKACIJE*:** Preobčutljivost na učinkovini ali katero koli pomožno snov. **OPOZORILA IN PREVIDNOSTNI UKREPI*:** Supresija kostnega mozga: Pred uvedbo zdravljenja in po potrebi za spremljanje toksičnosti zdravila, najmanj pred vsakim ciklom zdravljenja, je treba pregledati celotno krvno sliko. Zdravljenja ne smete začeti, če je absolutno število nevtrofilcev < 1,5 x 10^9/l, če je število trombocitov < 75 x 10^9/l ali če se je pri bolniku zaradi predhodnih zdravljenj pojavila klinično pomembna nehematološka toksičnost 3. ali 4. stopnje, ki še traja. Bolnike je treba skrbno spremljati zaradi morebitnih okužb, uvesti je treba ustrezne ukrepe, kot je klinično indicirano. Toksičnost za prebavila: Potrebna je uporaba antiemetikov, antidiaroikov ter drugih ukrepov, kot je klinično indicirano. Če je potrebno, prilagodite odmerke. Ledvična okvara: Uporaba zdravila ni priporočljiva pri bolnikih s končno stopnjo ledvične okvare. Bolnike z ledvično okvaro je potrebno med zdravljenjem skrbno spremljati; bolnike z zmerno ali hudo ledvično okvaro je treba zaradi hematološke toksičnosti bolj pogosto spremljati. Jetrna okvara: Uporaba zdravila Lonsurf pri bolnikih z obstoječo zmerno ali hudo jetrno okvaro ni priporočljiva. Proteinurija: Pred začetkom zdravljenja in med njim je priporočljivo spremljanje proteinurije z urinskimi testnimi lističi. Pomožne snovi: Zdravilo vsebuje laktozo. **INTERAKCIJE*:** Previdnost: Zdravila, ki medsebojno delujejo z nukleozidnimi prenašalci CNT1, ENT1 in ENT2, zaviralci OCT2 ali MATE1, substrati humane timidin-kinaze (npr. zidovudin), hormonski kontraceptivi. **PLODNOST*. NOSEČNOST IN DOJENJE*:** Ni priporočljivo. **KONTRACEPCIJA*:** Ženske in moški morajo uporabljati zelo učinkovite metode kontracepcije med zdravljenjem in do 6 mesecev po zaključku zdravljenja. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA STROJEV*:** Med zdravljenjem se lahko pojavijo utrujenost, omotica ali splošno slabo počutje. **NEŽELENI UČINKI*:** Zelo pogosti: nevtropenija, levkopenija, anemija, trombocitopenija, zmanjšan apetit, diareja, navzea, bruhanje, utrujenost. Pogosti: okužba spodnjih dihal, febrilna nevtropenija, limfopenija, hipoalbuminemija, disgevzija, periferna nevropatija, dispneja, bolečina v trebuhu, zaprtje, stomatitis, bolezni ustne votline, hiperbilirubinemija, sindrom palmarne plantarne eritrodisestezije, izpuščaj, alopecija, pruritus, suha koža, proteinurija, pireksija, edem, vnetje sluznice, splošno slabo počutje, zvišanje jetrnih encimov, zvišanje alkalne fosfataze v krvi, zmanjšanje telesne mase. Občasni: septični šok, infekcijski enteritis, pljučnica, okužba žolčevoda, gripa, okužba sečil, gingivitis, herpes zoster, tinea pedis, okužba s kandido, bakterijska okužba, okužba, nevtropenična sepsa, okužba zgornjih dihal, konjunktivitis, bolečina zaradi raka, pancitopenija, granulocitopenija, monocitopenija, eritropenija, levkocitoza, monocitoza, dehidracija, hiperglikemija, hiperkaliemija, hipokaliemija, hipofosfatemija, hipernatriemija, hiponatriemija, hipokalciemija, protin, anksioznost, nespečnost, nevrotoksičnost, disestezija, hiperestezija, hipoestezija, sinkopa, parestezija, pekoč občutek, letargija, omotica, glavobol, zmanjšana ostrina vida, zamegljen vid, diplopija, kataraka, suho oko, vrtoglavica, neugodje v ušesu, angina pektoris, aritmija, palpitacije, embolija, hipertenzija, hipotenzija, vročinski oblivi, pljučna embolija, plevralni izliv, izcedek iz nosu, disfonija, orofaringealna bolečina, epistaksa, kašelj, hemoragični enterokolitis, krvavitev v prebavilih, akutni pankreatitis, ascites, ileus, subileus, kolitis, gastritis, refluksni gastritis, ezofagitis, moteno praznjenje želodca, abdominalna distenzija, analno vnetje, razjede v ustih, dispepsija, gastroezofagealna refluksna bolezen, proktalgija, bukalni polip, krvavitev dlesni, glositis, parodontalna bolezen, bolezen zob, siljenje na bruhanje, flatulenca, slab zadah, hepatotoksičnost, razširitev žolčnih vodov, luščenje kože, urtikarija, preobčutljivostne reakcije na svetlobo, eritem, akne, hiperhidroza, žulj, bolezni nohtov, otekanje sklepov, artralgija, bolečina v kosteh, mialgija, mišično-skeletna bolečina, mišična oslabelost, mišični krči, bolečina v okončinah, ledvična odpoved, neinfektivni cistitis, motnje mikcije, hematurija, levkociturija, motnje menstruacije, poslabšanje splošnega zdravstvenega stanja, bolečina, občutek spremembe telesne temperature, kseroza, nelagodje, zvišanje kreatinina v krvi, podaljšanje intervala QT na elektrokardiogramu, povečanje mednarodnega umerjenega razmerja (INR), podaljšanje aktiviranega parcialnega tromboplastinskega časa (aPTČ), zvišanje sečnine v krvi, zvišanje laktatne dehidrogenaze v krvi, znižanje celokupnih proteinov, zvišanje C-reaktivnega proteina, zmanjšan hematokrit. Post-marketinške izkušnje: intersticijska bolezen pljuč. **PREVELIKO ODMERJANJE*:** Neželeni učinki, o katerih so poročali v povezavi s prevelikim odmerjanjem, so bili v skladu z uveljavljenim varnostnim profilom. Glavni pričakovani zaplet prevelikega odmerjanja je supresija kostnega mozga. **FARMAKODINAMIČNE LASTNOSTI*:** *Farmakoterapevtska skupina:* zdravila z delovanjem na novotvorbe, antimetaboliti, oznaka ATC: L01BC59. Zdravilo Lonsurf sestavljata antineoplastični timidinski nukleozidni analog, trifluridin, in zaviralec timidin-fosforilaze (TPaze), tipiracilijev klorid. Po privzemu v rakave celice timidin-kinaza fosforilira trifluridin. Ta se v celicah nato presnovi v substrat deoksiribonukleinske kisline (DNA), ki se vgradi neposredno v DNA ter tako prepreči celično proliferacijo. TPaza hitro razgradi trifluridin in njegova presnova po peroralni uporabi je hitra zaradi učinka prvega prehoda, zato je v zdravilo vključen zaviralec TPaze, tipiracilijev klorid. **PAKIRANJE*:** 20 filmsko obloženih tablet. **NAČIN PREDPISOVANJA IN IZDAJE ZDRAVILA:** Rp/Spec. **Imetnik dovoljenja za promet:** Les Laboratoires Servier, 50, rue Carnot, 92284 Suresnes cedex, Francija. Številka dovoljenja za promet z zdravilom: EU/1/16/1096/001 (Lonsurf 15 mg/6,14 mg), EU/1/16/1096/004 (Lonsurf 20 mg/8,19 mg). **Datum zadnje revizije besedila: december 2020. *Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila. Celoten povzetek glavnih značilnosti zdravila in podrobnejše informacije so na voljo pri: Servier Pharma d.o.o., Podmilščakova ulica 24, 1000 Ljubljana, tel: 01 563 48 11, www.servier.si.**

LON AD1 C1 2021-22. Samo za strokovno javnost.
Datum priprave informacije: september 2021.

SEDAJ ODOBRENO PO VSAJ ENI PREDHODNI TERAPIJI NA PODLAGI ANTI-HER2[1]

ENHERTU®
trastuzumab derukstekan

NEPRIMERLJIVO PREŽIVETJE*
POSTAVLJA NOVE STANDARDE ZDRAVLJENJA HER2+ METASTATSKEGA RAKA DOJK[2]

Zdravilo ENHERTU je v raziskavi DESTINY-Breast03 dokazalo neprimerljivo podaljšanje PFS v primerjavi s trenutnim standardom zdravljenja (T-DM1).[1,2]

72 % manjše

tveganje za napredovanje bolezni ob zdravljenju z zdravilom ENHERTU v primerjavi s **T-DM1** (ključni opazovani dogodek raziskave: PFS glede na BICR; HR: 0,28; 95 % IZ: 0,22, 0,37; p<0,000001)[1,2]

mPFS > 2 LETI

po oceni raziskovalca je mediani PFS znašal **25,1 mesecev** pri bolnikih, ki so prejemali ENHERTU, v primerjavi s **7,2 mesecev** pri bolnikih zdravljenih s T-DM1 (sekundarni opazovani dogodek; HR: 0,26; 95 % IZ: 0,20, 0,35)[2]

Pri zdravilu ENHERTU so poročali o primerih intersticijske pljučne bolezni (ILD) in pnevmonitisa. Za diagnozo je ključno prepoznavanje simptomov. Bolnike je treba spremljati in pričeti z zdravljenjem ob prvih znakih ILD.[1,2]

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

ENHERTU 100 mg prašek za koncentrat za raztopino za infundiranje

SESTAVA: Ena viala praška za koncentrat za raztopino za infundiranje vsebuje 100 mg trastuzumab derukstekana. Po rekonstituciji ena viala s 5 ml raztopine vsebuje 20 mg/ml trastuzumab derukstekana. Trastuzumab derukstekan je konjugat protitelesa in zdravila, ki vsebuje humanizirano monoklonsko protitelo IgG1 proti HER2 z istim zaporedjem aminokislin, kot ga ima trastuzumab. Proizvajajo ga sesalske celice (ovarij kitajskega hrčka) in je prek razcepljivega veznika na tetrapeptidni bazi kovalentno vezan na DXd, ki je derivat eksatekana in zaviralec topoizomeraze I. Na vsako molekulo protitelesa je vezanih približno 8 molekul derukstekana. *Pomožne snovi*: L-histidin, L-histidinijev klorid monohidrat, saharoza, polisorbat 80. **TERAPEVTSKE INDIKACIJE:** Zdravilo Enhertu kot monoterapija je indicirano za zdravljenje odraslih bolnikov z neresektabilnim ali metastatskim HER2-pozitivnim rakom dojk, ki so pred tem že prejeli eno ali več shem zdravljenja na podlagi anti-HER2. **ODMERJANJE IN NAČIN UPORABE:** Zdravilo Enhertu mora predpisati zdravnik in njegovo dajanje nadzorovati zdravstveni delavec, ki sta izkušena v uporabi zdravil proti raku. Za preprečitev napak, povezanih z zdravili, je pomembno, da preverite nalepke na vialah in se prepričate, da je zdravilo, ki se pripravlja in daje, res zdravilo Enhertu (trastuzumab derukstekan), in ne trastuzumab ali trastuzumab emtanzin. Zdravilo Enhertu se ne sme zamenjati s trastuzumabom ali trastuzumab emtanzinom. Bolniki, ki se zdravijo s trastuzumab derukstekanom, morajo imeti dokumentiran HER2pozitiven status tumorja, ki je opredeljen kot ocena 3 + na podlagi imunohistokemije (IHC) ali razmerje ≥ 2,0 na podlagi *in situ* hibridizacije (ISH) ali fluorescenčne *in situ* hibridizacije (FISH), ocenjeno z *in vitro* diagnostičnim (IVD) medicinskim pripomočkom z oznako CE. Če IVD z oznako CE ni na voljo, je treba status HER2 oceniti z drugim potrjenim testom. Odmerjanje: Priporočeni odmerek zdravila Enhertu je 5,4 mg/kg, ki se daje z intravensko infuzijo enkrat vsake 3 tedne (21dnevni cikel) do napredovanja bolezni ali nesprejemljive toksičnosti. Začetni odmerek je treba dati z 90-minutno intravensko infuzijo. Če bolnik prejšnjo infuzijo dobro prenaša, se lahko naslednji odmerki zdravila Enhertu dajejo kot 30-minutne infuzije. Za profilakso ali obvladovanje se bolniku smejo dati antiemetiki, skladno z lokalno klinično prakso in prenašanjem pri bolniku. Hitrost infundiranja zdravila Enhertu je treba zmanjšati ali infundiranje prekiniti, če se pri bolniku razvijejo simptomi, povezani z infuzijo. V primeru hudih reakcij na infuzijo je treba zdravilo Enhertu trajno ukiniti. Prilagajanje odmerka: Obvladovanje neželenih učinkov lahko zajema začasno prekinitev uporabe, zmanjšanje odmerka ali ukinitev zdravljenja z zdravilom Enhertu, skladno s smernicami, podanimi v povzetku glavnih značilnosti zdravila (preglednici 1 in 2). Po zmanjšanju odmerka zdravila Enhertu se odmerek ne sme več ponovno povečati. Načrt zmanjševanja odmerka: Začetni odmerek je 5,4 mg/kg, prvo zmanjšanje odmerka (4,4 mg/kg), drugo zmanjšanje odmerka (3,2 mg/kg), pri potrebi po nadaljnjem zmanjšanju odmerka ukinite zdravljenje. *Prosimo, glejte celoten povzetek glavnih značilnosti zdravila Enhertu za prilagajanje odmerka zaradi neželenih učinkov: intersticijska pljučna bolezen (ILD)/pnevmonitis* (asimptomatska ILD/asimptomatski pnevmonitis (stopnja 1), simptomatska ILD/simptomatski pnevmonitis (stopnja 2 ali višja)), *nevtropenija* (stopnja 3 (manj kot 1,00,5 × 10^9/l), stopnja 4 (manj kot 0,5 × 10^9/l)), *febrilna nevtropenija* (absolutno število nevtrofilcev manj kot 1,0 × 10^9/l in telesna temperatura, višja od 38,3 °C, ali telesna temperatura 38 °C ali višja, ki vztraja več kot eno uro), *zmanjšan iztisni delež levega prekata* (LVEF) (LVEF več kot 45 % in absolutno zmanjšanje glede na izhodiščno vrednost za 10 % do 20 %; LVEF 40 % do 45 %; LVEF manj kot 40 % ali absolutno zmanjšanje glede na izhodiščno vrednost za več kot 20 %; simptomatično kongestivno srčno popuščanje). Zakasnjen ali izpuščen odmerek: Če se načrtovani odmerek zakasni ali izpusti, ga je treba dati takoj, ko je mogoče, brez čakanja na naslednji načrtovani cikel. Časovni načrt dajanja je treba prilagoditi, da se ohrani 3-tedenski razmik med odmerki. Infuzijo je treba dati s hitrostjo in odmerkom, ki ga je bolnik prenašal pri zadnji infuziji. Posebne populacije: *Starejši:* Pri bolnikih, starih 65 let ali starejših, prilagajanje odmerka zdravila Enhertu ni potrebno. Podatki pri bolnikih, starih ≥ 75 let, so omejeni. *Okvara ledvic:* Prilagajanje odmerka pri bolnikih z blago (očistek kreatinina [CLcr] ≥ 60 in < 90 ml/min) ali zmerno (CLcr ≥ 30 in < 60 ml/min) okvaro ledvic ni potrebno. Morebitne potrebe po prilagajanju odmerka pri bolnikih s hudo okvaro ledvic ni mogoče opredeliti zaradi pomanjkanja podatkov. Pri bolnikih z zmerno okvaro ledvic so opazili višjo incidenco ILD stopnje 1 in 2/pnevmonitisa, ki sta vodila do zvečanja števila prekinitev zdravljenja. Bolnike z zmerno ali hudo okvaro ledvic je treba natančno spremljati glede neželenih učinkov, vključno z ILD/pnevmonitisom. *Okvara jeter:* Pri bolnikih, ki imajo celokupni bilirubin ≤ 1,5kratnik zgornje meje normalnih vrednosti (ZMN), ne glede na vrednost aspartat transaminaze (AST), odmerka ni treba prilagajati. Morebitne potrebe po prilagajanju odmerka pri bolnikih, ki imajo celokupni bilirubin > 1,5kratnik ZMN, ne glede na vrednost AST, ni mogoče opredeliti zaradi pomanjkanja podatkov. Zato je treba te bolnike natančno spremljati. Način uporabe: Zdravilo Enhertu je za intravensko uporabo. Zdravstveni delavec ga mora rekonstituirati in razredčiti. Treba ga je dati z intravenskim infundiranjem. Zdravilo Enhertu se ne sme dati kot hitro intravensko injekcijo ali bolus. **KONTRAINDIKACIJE:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Intersticijska pljučna bolezen/pnevmonitis: Pri zdravilu Enhertu so poročali o primerih intersticijske pljučne bolezni (ILD) in/ali pnevmonitisa. Nekateri primeri so bili smrtni. Bolnikom je treba naročiti, naj takoj poročajo o kašlju, dispneji, zvišani telesni temperaturi in/ali katerih koli novih dihalnih simptomih ali poslabšanju obstoječih. Bolnike je treba spremljati glede znakov in simptomov ILD/pnevmonitisa. Dokaze za ILD/pnevmonitis je treba takoj proučiti. Bolnike s sumom na ILD/pnevmonitis je treba oceniti z radiografskimi posnetki, najbolje z računalniško tomografijo (CT). Treba je razmisliti o posvetu s pulmologom. Nevtropenija: V kliničnih študijah z zdravilom Enhertu so poročali o primerih nevtropenije, vključno s primeri febrilne nevtropenije. Pred uvedbo zdravila Enhertu in pred vsakim odmerkom ter vsakič, ko je klinično indicirano, je treba preveriti celotno krvno sliko. Morda bo treba začasno prekiniti dajanje zdravila Enhertu ali zmanjšati odmerek, odvisno od tega, kako huda je nevtropenija. Zmanjšanje iztisnega deleža levega prekata: Pri zdravljenjih antiHER2 so poročali o zmanjšanem iztisnem deležu levega prekata (LVEF). Pred uvedbo zdravljenja z zdravilom Enhertu in v rednih intervalih med njim (v skladu s kliničnimi indikacijami) je treba izvesti standardne preiskave delovanja srca (ehokardiografija ali slikanje MUGA) za oceno LVEF. Zmanjšanje LVEF je treba obvladovati s prekinitvami zdravljenja. Zdravljenje z zdravilom Enhertu je treba trajno ukiniti, če se potrdi LVEF manj kot 40 % ali absolutno zmanjšanje glede na izhodiščno vrednost za več kot 20 %. Zdravilo Enhertu je treba trajno ukiniti pri bolnikih s simptomatskim kongestivnim srčnim popuščanjem. Embrio-fetalna toksičnost: Zdravilo Enhertu lahko ima škodljiv vpliv na plod, če se da nosečnici. Pri ženskah v rodni dobi je treba pred uvedbo zdravljenja z zdravilom Enhertu preveriti status nosečnosti. Bolnice je treba seznaniti z možnimi tveganji za plod. Ženskam v rodni dobi je treba svetovati, da uporabljajo učinkovito kontracepcijo med zdravljenjem in še vsaj 7 mesecev po zadnjem odmerku zdravila Enhertu. Moškim bolnikom s partnerkami v rodni dobi je treba svetovati, da uporabljajo učinkovito kontracepcijo med zdravljenjem z zdravilom Enhertu in še vsaj 4 mesece po zadnjem odmerku zdravila Enhertu. Bolniki z zmerno ali hudo okvaro jeter: Zdravilo Enhertu je treba pri bolnikih z zmerno in hudo okvaro jeter dajati previdno. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Pri sočasnem dajanju trastuzumab derukstekana z zdravili, ki so zaviralci CYP3A ali OATP1B ali prenašalcev Pgp, odmerka ni treba prilagajati. **PLODNOST, NOSEČNOST IN DOJENJE:** Nosečnost: Dajanje zdravila Enhertu nosečnicam se ne priporoča. Bolnice je treba seznaniti z možnimi tveganji za plod, preden zanosijo. Ženske, ki zanosijo, se morajo takoj obrniti na zdravnika. Če ženska zanosi med zdravljenjem z zdravilom Enhertu ali v obdobju 7 mesecev po zadnjem odmerku zdravila Enhertu, se priporoča natančno spremljanje. Dojenje: Ni znano, ali se trastuzumab derukstekan izloča v materino mleko. Humani IgG se izloča v materino mleko in potencial za absorpcijo in resne neželene učinke na dojenčka ni znan. Zato ženske ne smejo dojiti med zdravljenjem z zdravilom Enhertu in še 7 mesecev po zadnjem odmerku. Odločiti se je treba med prenehanjem dojenja in prenehanjem zdravljenja z zdravilom Enhertu, pri čemer je treba pretehtati prednosti dojenja za otroka in prednosti zdravljenja za mater. Plodnost: Namenskih študij plodnosti s trastuzumab derukstekanom niso izvedli. Ni znano, ali so trastuzumab derukstekan ali njegovi presnovki prisotni v semenski tekočini. Pred začetkom zdravljenja je treba moškim bolnikom svetovati, da se posvetujejo o možnosti shranjevanja semena. Moški bolniki v celotnem obdobju zdravljenja in še najmanj 4 mesece po zadnjem odmerku zdravila Enhertu ne smejo zamrzniti ali darovati semena. **NEŽELENI UČINKI:** Združeno varnostno populacijo so ocenili pri bolnikih, ki so v kliničnih študijah dobili vsaj en odmerek 5,4 mg/kg zdravila Enhertu (n = 573) zaradi različnih vrst tumorjev. Mediani čas trajanja zdravljenja v tej združeni populaciji je bil 11,3 meseca (razpon: 0,7-37,9 meseca). *Zelo pogosti:* okužba zgornjih dihal, nevtropenija, anemija, levkopenija, trombocitopenija, limfopenija, hipokaliemija, zmanjšan apetit, glavobol, omotica, intersticijska pljučna bolezen, dispneja, kašelj, epistaksa, navzea, bruhanje, driska, bolečina v trebuhu, zaprtje, stomatitis, dispepsija, zvišane transaminaze, alopecija, mišično-skeletna bolečina, utrujenost, pireksija, zmanjšan iztisni delež, izguba telesne mase. *Pogosti:* pljučnica, febrilna nevtropenija, dehidracija, disgevzija, zameglјen vid, izpuščaj, hiperpigmentacija kože, pruritus, periferni edem, zvišana alkalna fosfataza v krvi, zvišan bilirubin v krvi, zvišan kreatinin v krvi, reakcije povezane z infuzijo. **IMETNIK DOVOLJENJA ZA PROMET Z ZDRAVILOM:** Daiichi Sankyo Europe GmbH, Zielstattstrasse 48, 81379 München, Nemčija **DATUM ZADNJE REVIZIJE BESEDILA:** 11.07.2022 (SI-2310) **REŽIM PREDPISOVANJA IN IZDAJE:** H **Prosimo, da pred predpisovanjem preberete celoten povzetek glavnih značilnosti zdravila.** Dodatne informacije so na voljo pri podjetju AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, 1000 Ljubljana, telefon: 01/51 35 600.

* zmanjšanje tveganja za napredovanje bolezni ali smrti (PFS)

PFS - preživetje brez napredovanja bolezni, **mPFS** - mediano preživetje brez napredovanja bolezni, **T-DM1** - trastuzumab emtazin, **BICR** - ocena slepega neodvisnega pregleda (blinded independent central review), **IZ** - interval zaupanja, **HR** - razmerje ogroženosti

Literatura: 1. Povzetek glavnih značilnosti zdravila ENHERTU, dostopano 8.8.2022 **2.** J.Cortes et al; Trastuzumab Deruxtecan versus Trastuzumab Emtasine for Breast Cancer; NEJM 2022;386(12):1143-1154

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Datum priprave materiala: avgust 2022. Samo za strokovno javnost. SI-2322

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Referenca: 1. Keytruda EU SmPC

Ime zdravila: KEYTRUDA 25 mg/ml koncentrat za raztopino za infundiranje vsebuje pembrolizumab. Terapevtske indikacije: Zdravilo KEYTRUDA je kot samostojno zdravljenje indicirano za zdravljenje: odraslih in mladostnikov, starih 12 let ali več, z napredovalim (neoperabilnim ali metastatskim) melanomom; za adjuvantno zdravljenje odraslih in mladostnikov, starih 12 let ali več, z melanomom v stadiju IIB, IIC ali III, in sicer po popolni kirurški odstranitvi; metastatskega nedrobnoceličnega pljučnega raka (NSCLC) v prvi liniji zdravljenja pri odraslih, ki imajo tumorje z ≥ 50 % izraženostjo PD-L1 (TPS) in brez pozitivnih tumorskih mutacij EGFR ali ALK; lokalno napredovalega ali metastatskega NSCLC pri odraslih, ki imajo tumorje z ≥ 1 % izraženostjo PD-L1 (TPS) in so bili predhodno zdravljeni z vsaj eno shemo kemoterapije, bolniki s pozitivnimi tumorskimi mutacijami EGFR ali ALK so pred prejemom zdravila KEYTRUDA morali prejeti tudi tarčno zdravljenje; odraslih in pediatričnih bolnikov, starih 3 leta ali več, s ponovljenim ali neodzivnim klasičnim Hodgkinovim limfomom (cHL), pri katerih avtologna presaditev matičnih celic (ASCT) ni bila uspešna, ali po najmanj dveh predhodnih zdravljenjih kadar ASCT ne pride v poštev kot možnost zdravljenja; lokalno napredovalega ali metastatskega urotelijskega raka pri odraslih, predhodno zdravljenih s kemoterapijo, ki je vključevala platino; lokalno napredovalega ali metastatskega urotelijskega raka pri odraslih, ki niso primerni za zdravljenje s kemoterapijo, ki vsebuje cisplatin in imajo tumorje z izraženostjo PD-L1 ≥ 10, ocenjeno s kombinirano pozitivno oceno (CPS); ponovljenega ali metastatskega ploščatoceličnega raka glave in vratu (HNSCC) pri odraslih, ki imajo tumorje z ≥ 50 % izraženostjo PD-L1 (TPS), in pri katerih je bolezen napredovala med zdravljenjem ali po zdravljenju s kemoterapijo, ki je vključevala platino; za adjuvantno zdravljenje odraslih z rakom ledvičnih celic s povišanim tveganjem za ponovitev bolezni po nefrektomiji, ali po nefrektomiji in kirurški odstranitvi metastatskih lezij, za zdravljenje odraslih z MSI-H (microsatellite instability- high) ali dMMR (mismatch repair deficient) kolorektalnim rakom v naslednjih terapevtskih okoliščinah: prva linija zdravljenja metastatskega kolorektalnega raka; zdravljenje neoperabilnega ali metastatskega kolorektalnega raka po predhodnem kombiniranem zdravljenju, ki je temeljilo na fluoropirimidinu; in za zdravljenje MSI-H ali dMMR tumorjev pri odraslih z: napredovalim ali ponovljenim rakom endometrija, pri katerih je bolezen napredovala med ali po predhodnem zdravljenju, ki je vključevalo platino, v katerih koli terapevtskih okoliščinah, in ki niso kandidati za kurativno operacijo ali obsevanje; neoperabilnim ali metastatskim rakom želodca, tankega črevesa ali žolčnika in žolčnih vodov, pri katerih je bolezen napredovala med ali po vsaj enem predhodnem zdravljenju. Zdravilo KEYTRUDA je kot samostojno zdravljenje ali v kombinaciji s kemoterapijo s platino in 5-fluorouracilom (5-FU) indicirano za prvo linijo zdravljenja metastatskega ali neoperabilnega ponovljenega ploščatoceličnega raka glave in vratu pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1. Zdravilo KEYTRUDA je v kombinaciji s pemetreksedom in kemoterapijo na osnovi platine indicirano za prvo linijo zdravljenja metastatskega neploščatoceličnega NSCLC pri odraslih, pri katerih tumorji nimajo pozitivnih mutacij EGFR ali ALK; v kombinaciji s karboplatinom in bodisi paklitakselom bodisi nab-paklitakselom je indicirano za prvo linijo zdravljenja metastatskega ploščatoceličnega NSCLC pri odraslih; v kombinaciji z aksitinibom ali v kombinaciji z lenvatinibom je indicirano za prvo linijo zdravljenja napredovalega raka ledvičnih celic (RCC) pri odraslih; v kombinaciji s kemoterapijo s platino in fluoropirimidinom je indicirano za prvo linijo zdravljenja lokalno napredovalega neoperabilnega ali metastatskega raka požiralnika ali HER-2 negativnega adenokarcinoma gastroezofagealnega prehoda pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 10; v kombinaciji s kemoterapijo za neoadjuvantno zdravljenje, in v nadaljevanju kot samostojno adjuvantno zdravljenje po kirurškem posegu, je indicirano za zdravljenje odraslih z lokalno napredovalim trojno negativnim rakom dojk ali trojno negativnim rakom dojk v zgodnjem stadiju z visokim tveganjem za ponovitev bolezni; v kombinaciji s kemoterapijo je indicirano za zdravljenje lokalno ponovljenega neoperabilnega ali metastatskega trojno negativnega raka dojk pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 10 in predhodno niso prejeli kemoterapije za metastatsko bolezen; v kombinaciji z lenvatinibom je indicirano za zdravljenje napredovalega ali ponovljenega raka endometrija (EC) pri odraslih z napredovalo boleznijo med ali po predhodnem zdravljenju s kemoterapijo, ki je vključevala platino, v katerih koli terapevtskih okoliščinah, in ki niso kandidati za kurativno operacijo ali obsevanje; v kombinaciji s kemoterapijo, z bevacizumabom ali brez njega, je indicirano za zdravljenje persistentnega, ponovljenega ali metastatskega raka materničnega vratu pri odraslih bolnicah, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1. **Odmerjanje in način uporabe:** Testiranje PD-L1: Če je navedeno v indikaciji, je treba izbiro bolnika za zdravljenje z zdravilom KEYTRUDA na podlagi izraženosti PD-L1 tumorja potrditi z validirano preiskavo. Testiranje MSI/MMR: Če je navedeno v indikaciji, je treba izbiro bolnika za zdravljenje z zdravilom KEYTRUDA na podlagi MSI-H/dMMR statusa tumorja potrditi z validirano preiskavo. Odmerjanje: Priporočeni odmerek zdravila KEYTRUDA pri odraslih je bodisi 200 mg na 3 tedne ali 400 mg na 6 tednov, apliciran z intravensko infuzijo v 30 minutah. Priporočeni odmerek zdravila KEYTRUDA za samostojno zdravljenje pri pediatričnih bolnikih s cHL, starih 3 leta ali več, ali bolnikih z melanomom, starih 12 let ali več, je 2 mg/kg telesne mase (do največ 200 mg) na 3 tedne, apliciran z intravensko infuzijo v 30 minutah. Za uporabo v kombinaciji glejte povzetke glavnih značilnosti zdravil sočasno uporabljenih zdravil. Če se uporablja kot del kombiniranega zdravljenja skupaj z intravensko kemoterapijo, je treba zdravilo KEYTRUDA aplicirati prvo. Bolnike je treba zdraviti do napredovanja bolezni ali nesprejemljivih toksičnih učinkov (in do maksimalnega trajanja zdravljenja, če je le to določeno za indikacijo). Pri adjuvantnem zdravljenju melanoma ali RCC je treba zdravilo uporabljati do ponovitve bolezni, pojava nesprejemljivih toksičnih učinkov oziroma mora zdravljenje trajati do enega leta. Za neoadjuvantno in adjuvantno zdravljenje TNBC morajo bolniki neoadjuvantno prejeti zdravilo KEYTRUDA v kombinaciji s kemoterapijo, in sicer 8 odmerkov po 200 mg na 3 tedne ali 4 odmerke po 400 mg na 6 tednov, ali do napredovanja bolezni, ki izključuje definitivni kirurški poseg, ali do pojava nesprejemljivih toksičnih učinkov, čemur sledi adjuvantno zdravljenje z zdravilom KEYTRUDA kot samostojnim zdravljenjem, in sicer 9 odmerkov po 200 mg na 3 tedne ali 5 odmerkov po 400 mg na 6 tednov ali do ponovitve bolezni ali pojava nesprejemljivih toksičnih učinkov. Bolniki, pri katerih pride do napredovanja bolezni, ki izključuje definitivni kirurški poseg, ali do nesprejemljivih toksičnih učinkov povezanih z zdravilom KEYTRUDA kot neoadjuvantnim zdravljenjem v kombinaciji s kemoterapijo, ne smejo prejeti zdravila KEYTRUDA kot samostojnega zdravljenja za adjuvantno zdravljenje. Če je aksitinib uporabljen v kombinaciji s pembrolizumabom, se lahko razmisli o povečanju odmerka aksitiniba nad začetnih 5 mg v presledkih šest tednov ali več. V primeru uporabe v kombinaciji z lenvatinibom je treba zdravljenje z enim ali obema zdraviloma prekiniti, kot je primerno. Uporabo lenvatiniba je treba zadržati, odmerek zmanjšati ali prenehati z uporabo, v skladu z navodili v povzetku glavnih značilnosti zdravila za lenvatinib, in sicer za kombinacijo s pembrolizumabom. Pri bolnikih starih ≥ 65 let, bolnikih z blago do zmerno okvaro ledvic, bolnikih z blago okvaro jeter prilagoditev odmerka ni potrebna. Odložitev odmerka ali ukinitev zdravljenja: Zmanjšanje odmerka zdravila KEYTRUDA ni priporočljivo. Za obvladovanje neželenih učinkov je treba uporabo zdravila KEYTRUDA zadržati ali ukiniti, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. **Kontraindikacije:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **Povzetek posebnih opozoril, previdnostnih ukrepov, interakcij in neželenih učinkov:** Imunsko pogojeni neželeni učinki (pnevmonitis, kolitis, hepatitis, nefritis, endokrinopatije, neželeni učinki na kožo in drugi): Pri bolnikih, ki so prejemali pembrolizumab, so se pojavili imunsko pogojeni neželeni učinki, vključno s hudimi in smrtnimi primeri. Večina imunsko pogojenih neželenih učinkov, ki so se pojavili med zdravljenjem s pembrolizumabom, je bila reverzibilnih in so jih obvladali s prekinitvami uporabe pembrolizumaba, uporabo kortikosteroidov in/ali podporno oskrbo. Pojavijo se lahko tudi po zadnjem odmerku pembrolizumaba in hkrati prizadanejo več organskih sistemov. V primeru suma na imunsko pogojene neželene učinke je treba poskrbeti za ustrezno oceno za potrditev etiologije oziroma izključitev drugih vzrokov. Glede na izrazitost neželenega učinka je treba zadržati uporabo pembrolizumaba in uporabiti kortikosteroide – za natančna navodila, prosimo, glejte Povzetek glavnih značilnosti zdravila Keytruda. Zdravljenje s pembrolizumabom lahko poveča tveganje za zavrnitev pri prejemnikih presadkov čvrstih organov. Pri bolnikih, ki so prejemali pembrolizumab, so poročali o hudih z infuzijo povezanih reakcijah, vključno s preobčutljivostjo in anafilaksijo. Pembrolizumab se iz obtoka odstrani s katabolizmom, zato presnovnih medsebojnih delovanj zdravil ni pričakovati. Uporabi sistemskih kortikosteroidov ali imunosupresivov pred uvedbo pembrolizumaba se je treba izogibati, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost pembrolizumaba. Vendar pa je kortikosteroide ali druge imunosupresive mogoče uporabiti za zdravljenje imunsko pogojenih neželenih učinkov. Kortikosteroide je mogoče uporabiti tudi kot premedikacijo, če je pembrolizumab uporabljen v kombinaciji s kemoterapijo, kot antiemetično profilakso in/ali za ublažitev neželenih učinkov, povezanih s kemoterapijo. Ženske v rodni dobi morajo med zdravljenjem s pembrolizumabom in vsaj še 4 mesece po zadnjem odmerku pembrolizumaba uporabljati učinkovito kontracepcijo, med nosečnostjo in dojenjem se ga ne sme uporabljati. Varnost pembrolizumaba pri samostojnem zdravljenju so v kliničnih študijah ocenili pri 7.631 bolnikih, ki so imeli različne vrste raka, s štirimi odmerki (2 mg/kg telesne mase na 3 tedne, 200 mg na 3 tedne in 10 mg/kg telesne mase na 2 ali 3 tedne). V tej populaciji bolnikov je mediani čas opazovanja znašal 8,5 meseca (v razponu od 1 dneva do 39 mesecev), najpogostejši neželeni učinki zdravljenja s pembrolizumabom pa so bili utrujenost (31 %), diareja (22 %) in navzea (20 %). Večina poročanih neželenih učinkov pri samostojnem zdravljenju je bila po izrazitosti 1. ali 2. stopnje. Najresnejši neželeni učinki so bili imunsko pogojeni neželeni učinki in hude z infuzijo povezane reakcije. Pojavnost imunsko pogojenih neželenih učinkov pri uporabi pembrolizumaba samega za adjuvantno zdravljenje (n = 1.480) je znašala 36,1 % za vse stopnje in 8,9 % od 3. do 5. stopnje, pri metastatski bolezni (n = 5.375) pa 24,2 % za vse stopnje in 6,4 % od 3. do 5. stopnje. Pri adjuvantnem zdravljenju niso zaznali nobenih novih imunsko pogojenih neželenih učinkov. Varnost pembrolizumaba pri kombiniranem zdravljenju s kemoterapijo so ocenili pri 3.123 bolnikih z različnimi vrstami raka, ki so v kliničnih študijah prejemali pembrolizumab v odmerkih 200 mg, 2 mg/kg telesne mase ali 10 mg/kg telesne mase na vsake 3 tedne. V tej populaciji bolnikov so bili najpogostejši neželeni učinki naslednji: anemija (55 %), navzea (54 %), utrujenost (38 %), nevtropenija (36 %), zaprtost (35 %), alopecija (35 %), diareja (34 %), bruhanje (28 %) in zmanjšanje apetita (27 %). Pojavnost neželenih učinkov 3. do 5. stopnje je pri bolnikih z NSCLC pri kombiniranem zdravljenju s pembrolizumabom znašala 67 % in pri zdravljenju samo s kemoterapijo 66 %, pri bolnikih s HNSCC pri kombiniranem zdravljenju s pembrolizumabom 85 % in pri zdravljenju s kemoterapijo v kombinaciji s cetuksimabom 84 %, pri bolnikih z rakom požiralnika pri kombiniranem zdravljenju s pembrolizumabom 86 % in pri zdravljenju samo s kemoterapijo 83 %, pri bolnikih s TNBC pri kombiniranem zdravljenju s pembrolizumabom 80 % in pri zdravljenju samo s kemoterapijo 77 % in pri bolnicah z rakom materničnega vratu pri kombiniranem zdravljenju s pembrolizumabom 82 % in pri zdravljenju samo s kemoterapijo 75 %. Varnost pembrolizumaba v kombinaciji z aksitinibom ali lenvatinibom pri napredovalem RCC in v kombinaciji z lenvatinibom pri napredovalem EC so ocenili pri skupno 1.456 bolnikih z napredovalim RCC ali napredovalim EC, ki so v kliničnih študijah prejemali 200 mg pembrolizumaba na 3 tedne skupaj s 5 mg aksitiniba dvakrat na dan ali z 20 mg lenvatiniba enkrat na dan, kot je bilo ustrezno. V teh populacijah bolnikov so bili najpogostejši neželeni učinki diareja (58 %), hipertenzija (54 %), hipotiroidizem (46 %), utrujenost (41 %), zmanjšan apetit (40 %), navzea (40 %), artralgija (30 %), bruhanje (28 %), zmanjšanje telesne mase (28 %), disfonija (28 %), bolečine v trebuhu (28 %), proteinurija (27 %), sindrom palmarno-plantarne eritrodizestezije (26 %), izpuščaj (26 %), stomatitis (25 %), zaprtost (25 %), mišično-skeletna bolečina (23 %), glavobol (23 %) in kašelj (21 %). Neželenih učinkov od 3. do 5. stopnje je bilo pri bolnikih z RCC med uporabo pembrolizumaba v kombinaciji z aksitinibom ali lenvatinibom 80 % in med uporabo sunitiniba samega 71 %. Pri bolnicah z EC je bilo neželenih učinkov od 3. do 5. stopnje med uporabo pembrolizumaba v kombinaciji z lenvatinibom 89 % in med uporabo kemoterapije same 73 %. Za celoten seznam neželenih učinkov, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. Za dodatne informacije o varnosti v primeru uporabe pembrolizumaba v kombinaciji glejte povzetke glavnih značilnosti zdravila za posamezne komponente kombiniranega zdravljenja. **Način in režim izdaje zdravila:** H – Predpisovanje in izdaja zdravila je le na recept, zdravilo se uporablja samo v bolnišnicah. **Imetnik dovoljenja za promet z zdravilom:** Merck Sharp & Dohme B.V. , Waarderweg 39, 2031 BN Haarlem, Nizozemska.

MSD
Merck Sharp & Dohme inovativna zdravila d.o.o.,
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Samo za strokovno javnost.
H - Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila Keytruda, ki je na voljo pri naših strokovnih sodelavcih ali na lokalnem sedežu družbe.

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Dent RAG, Cole P. In vitro maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95. doi: 10.1038/bjc.1981.71

Chapman S, Nakielny R. *A guide to radiological procedures*. London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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LORVIQUA®
LORLATINIB

Pri bolnikih z metastazami v CŽS ali brez njih

SOOČITE

ALK+ mNSCLC Z ZDRAVILOM LORVIQUA

Zdravilo **LORVIQUA** v monoterapiji je indicirano za zdravljenje odraslih bolnikov z napredovalim nedrobnoceličnim rakom pljuč (NSCLC), ki je ALK pozitiven, in se predhodno niso zdravili z zaviralcem ALK.[1]

Zdravilo **LORVIQUA** v monoterapiji je indicirano za zdravljenje odraslih bolnikov z napredovalim NSCLC, ki je ALK-pozitiven, pri katerih je bolezen napredovala po:

- zdravljenju z alektinibom ali ceritinibom kot prvim ALK zaviralcem tirozin kinaze (TKI); ali
- zdravljenju s krizotinibom in vsaj še 1 drugim ALK TKI.[1]

BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

Lorviqua 25 mg, 100 mg filmsko obložene tablete
▼Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o kateremkoli domnevnem neželenem učinku zdravila. Glejte poglavje 4.8 povzetka glavnih značilnosti zdravila, kako poročati o neželenih učinkih. **Sestava in oblika zdravila:** Ena filmsko obložena tableta vsebuje 25 mg ali 100 mg lorlatiniba in 1,58 mg oz. 4,20 mg laktoze monohidrata. **Indikacije:** Zdravljenje odraslih bolnikov z napredovalim nedrobnoceličnim rakom pljuč (NSCLC – *Non-Small Cell Lung Cancer*), ki je ALK (anaplastična limfomska kinaza) pozitiven in se predhodno niso zdravili z zaviralcem ALK, ter pri bolnikih, pri katerih je bolezen napredovala po: zdravljenju z alektinibom ali ceritinibom kot prvim ALK zaviralcem tirozin kinaze (TKI – *Tyrosine Kinase Inhibitor*) ali zdravljenju s krizotinibom in vsaj še 1 drugim ALK TKI. **Odmerjanje in način uporabe:** Zdravljenje mora uvesti in nadzorovati zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje rakavih bolezni. Odkrivanje ALK-pozitivnega NSCLC je potrebno pri izbiri bolnikov, saj so to edini bolniki, pri katerih so dokazali korist. Priporočeni odmerek je 100 mg peroralno enkrat na dan. Zdravljenje je treba nadaljevati do napredovanja bolezni ali nesprejemljive toksičnosti. Če bolnik izpusti odmerek, ga mora vzeti takoj, ko se spomni, razen če do naslednjega odmerka manjka manj kot 4 ure. Bolniki ne smejo vzeti 2 odmerkov hkrati, da bi nadomestili izpuščeni odmerek. Prilagajanje odmerkov: Ravni zmanjšanja odmerka: *prvo zmanjšanje odmerka*: 75 mg peroralno enkrat na dan; *drugo zmanjšanje odmerka*: 50 mg peroralno enkrat na dan. Zdravljenje je treba trajno prekiniti, če bolnik ne prenaša odmerka 50 mg peroralno enkrat na dan. Za prilagajanje odmerkov zaradi neželenih učinkov glejte preglednico 1 v SmPC-ju. Posebne populacije: *Starejši bolniki (≥ 65 let):* Zaradi omejenih podatkov priporočil o odmerjanju ni mogoče dati. *Okvara ledvic*: Prilagajanje odmerkov pri bolnikih z normalnim delovanjem in blago ali zmerno okvaro [absolutna ocena hitrosti glomerulne filtracije (eGFR – estimated Glomerular Filtration Rate): ≥ 30 ml/min] ni potrebno. Pri bolnikih s hudo okvaro ledvic (absolutna vrednost eGFR < 30 ml/min) je priporočljiv zmanjšan odmerek lorlatiniba, npr. začetni odmerek 75 mg peroralno enkrat na dan. Podatkov pri bolnikih na ledvični dializi ni na voljo. *Okvara jeter*: Pri bolnikih z blago okvaro ni potrebno prilagajanje odmerkov. Podatkov o uporabi pri zmerni ali hudi okvari ni, zato uporaba ni priporočljiva. *Pediatrična populacija*: Varnost in učinkovitost pri otrocih in mladostnikih, starih < 18 let, nista bili dokazani. Način uporabe: Peroralna uporaba, vsak dan ob približno istem času, s hrano ali brez nje. Tablete je treba pogoltniti cele. **Kontraindikacije:** Preobčutljivost na učinkovino ali katerokoli pomožno snov. Uporaba močnih induktorjev CYP3A4/5. **Posebna opozorila in previdnostni ukrepi:** Hiperlipidemija: Uporaba je povezana z zvečanji vrednosti holesterola in trigliceridov v serumu – morda bo treba uvesti ali povečati odmerek zdravil za zniževanje ravni lipidov. Učinki na osrednje živčevje: Opazili so učinke na osrednje živčevje, vključno s psihotičnimi učinki in spremembami v kognitivni funkciji, razpoloženju, duševnem stanju ali govoru – morda bo treba prilagoditi odmerek ali prekiniti zdravljenje. Atrioventrikularni blok: Pri bolnikih, ki so prejemali lorlatinib, so poročali o podaljšanju intervala PR in AV-bloku. Potrebno je spremljanje EKG in morda bo treba prilagoditi odmerek. Zmanjšanje iztisnega deleža levega prekata: Pri bolnikih, ki so prejemali lorlatinib in pri katerih so opravili izhodiščno in še vsaj eno nadaljnjo oceno iztisnega deleža levega prekata (LVEF – *Left Ventricular Ejection Fraction*), so poročali o zmanjšanju LVEF. Če imajo bolniki dejavnike tveganja za srce ali stanja, ki vplivajo na LVEF, ali se jim med zdravljenjem pojavijo pomembni srčni znaki/simptomi, je treba razmisliti o spremljanju srca, vključno z oceno LVEF. Zvečanje vrednosti lipaze in amilaze: Pri bolnikih, ki so prejemali lorlatinib, se je pojavilo zvečanje vrednosti lipaze in/ali amilaze. Zaradi sočasne hipertrigliceridemije in/ali morebitnega intrinzičnega mehanizma je treba upoštevati tveganje za pankreatitis. Bolnike je treba spremljati glede zvečanja vrednosti lipaze in amilaze. Intersticijska bolezen pljuč (ILD – *Interstitial Lung Disease*)/pnevmonitis: Pri uporabi lorlatiniba so se pojavili hudi ali življenjsko ogrožajoči pljučni neželeni učinki, skladni z ILD/pnevmonitisom. Vse bolnike, pri katerih pride do poslabšanja respiratornih simptomov, ki kažejo na ILD/pnevmonitis, je treba takoj pregledati glede ILD/pnevmonitisa. Hipertenzija: Pri bolnikih, ki so prejemali loratinib, so poročali o hipertenziji. Pred uvedbo lorlatiniba mora biti krvni tlak pod nadzorom. Med zdravljenjem je treba krvni tlak preveriti po 2 tednih in nato najmanj enkrat na mesec ter glede na stopnjo resnosti zdravljenje prekiniti in nato nadaljevati z zmanjšanim odmerkom ali trajno prekiniti. Hiperglikemija: Pri bolnikih, ki so prejemali loratinib, se je pojavila hiperglikemija. Pred uvedbo je treba oceniti koncentracijo glukoze v serumu na tešče in jo nato redno spremljati v skladu z nacionalnimi smernicami ter glede na stopnjo resnosti zdravljenje prekiniti in nato nadaljevati z zmanjšanim odmerkom ali trajno prekiniti. Laktoza: Vsebuje laktozo. Bolniki z redko dedno intoleranco za galaktozo, odsotnostjo encima laktaze ali malabsorpcijo glukoze/galaktoze ne smejo jemati tega zdravila. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Učinek zdravil na lorlatinib: *Induktorji CYP3A4/5:* Sočasna uporaba močnih induktorjev CYP3A4/5 (npr. rifampicin, karbamazepin, enzalutamid, mitotan, fenitoin in šentjanževka) je kontraindicirana. *Zaviralci CYP3A4/5:* Sočasni uporabi močnih zaviralcev CYP3A4/5 (npr. boceprevir, kobicistat, itrakonazol, ketokonazol, posakonazol, troleandomicin, vorikonazol, ritonavir, paritaprevir v kombinaciji z ritonavirom in ombitasvirom in/ali dasabuvirom ter ritonavir v kombinaciji z elvitegravirom, indinavirom, lopinavirom ali tipranavirom in grenivka ali grenivkin sok), se je treba izogibati, saj lahko pride do zvečanja koncentracij lorlatiniba v plazmi (če je sočasna uporaba nujna, je priporočljivo zmanjšati odmerek lorlatiniba). Učinek lorlatiniba na druga zdravila: *Substrati CYP3A4/5:* Izogibati se je treba sočasnemu dajanju lorlatiniba in substratov CYP3A4/5 z ozkimi terapevtskimi indeksi (npr. alfentanil, ciklosporin, dihidroergotamin, ergotamin, fentanil, hormonski kontraceptivi, pimozid, kinidin, sirolimus in takrolimus), saj lahko lorlatinib zmanjša koncentracije teh zdravil. *Substrati P-glikoproteina:* Substrate P-gp, ki imajo ozke terapevtske indekse (npr. digoksin, dabigatraneteksilat), je treba v kombinaciji z lorlatinibom uporabljati previdno, saj obstaja verjetnost, da se koncentracija teh substratov v plazmi zmanjša. *Študije in vitro s prenašalci zdravil, ki niso P-gp:* Lorlatinib je treba v kombinaciji s substrati BCRP, OATP1B1, OATP1B3, OCT1, MATE1 in OAT3 uporabljati previdno, saj klinično pomembnih sprememb v plazemski izpostavljenosti teh substratov ni mogoče izključiti. **Plodnost, nosečnost in dojenje:** Ženskam v rodni dobi je treba svetovati, naj se med zdravljenjem z lorlatinibom izogibajo zanositvi in naj med zdravljenjem uporabljajo visoko učinkovito nehormonsko metodo kontracepcije, saj lahko lorlatinib povzroči, da hormonski kontraceptivi postanejo neučinkoviti. Učinkovito kontracepcijo je treba uporabljati še vsaj 35 dni po zaključku zdravljenja. Med zdravljenjem in še vsaj 14 tednov po zadnjem odmerku morajo bolniki, ki imajo partnerice v rodni dobi, uporabljati učinkovito kontracepcijo. Nosečnost: Študije na živalih so pokazale embriofetalno toksičnost, zato uporaba med nosečnostjo ali pri ženskah v rodni dobi, ki ne uporabljajo kontracepcije, ni priporočljiva. Dojenje: Med zdravljenjem in še 7 dni po zadnjem odmerku je treba prenehati z dojenjem. Plodnost: Zdravljenje lahko ogrozi plodnost pri moških. **Vpliv na sposobnost vožnje in upravljanja strojev:** Ima zmeren vpliv na sposobnost vožnje in upravljanja strojev. Potrebna je previdnost, saj se pri bolnikih lahko pojavijo učinki na osrednje živčevje. **Neželeni učinki:** Zelo pogosti: anemija, hiperholesterolemija, hipertrigliceridemija, učinki na razpoloženje, učinki na kognitivne funkcije, periferna nevropatija, glavobol, motnja vida, hipertenzija, diareja, navzea, zaprtje, izpuščaj, artralgija, mialgija, edem, utrujenost, zvečanje telesne mase, zvečanje vrednosti lipaze, zvečanje vrednosti amilaze. **Način in režim izdaje:** Rp/Spec - Predpisovanje in izdaja zdravila je le na recept zdravnika specialista ustreznega področja medicine ali od njega pooblaščenega zdravnika. **Imetnik dovoljenja za promet:** Pfizer Europe MA EEIG, Boulevard de la Plaine 17, 1050 Bruxelles, Belgija. **Datum zadnje revizije besedila:** 04.04.2022

Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.

Literatura: 1. Povzetek glavnih značilnosti zdravila Lorviqua, 4.4.2022.

ALK = anaplastična limfomska kinaza, **CŽS** = centralni živčni sistem, **mNSCLC** = (Metastatic Non-Small Cell Lung Cancer) metastatski nedrobnocelični rak pljuč, **NSCLC** = (Non-Small Cell Lung Cancer) nedrobnocelični rak pljuč, **TKI** = (Tyrosine Kinase Inhibitor) zaviralec tirozin kinaze.

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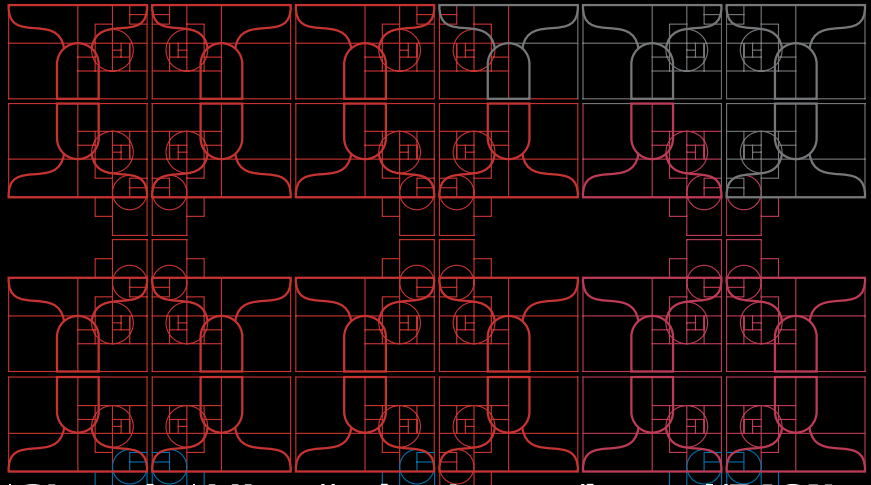

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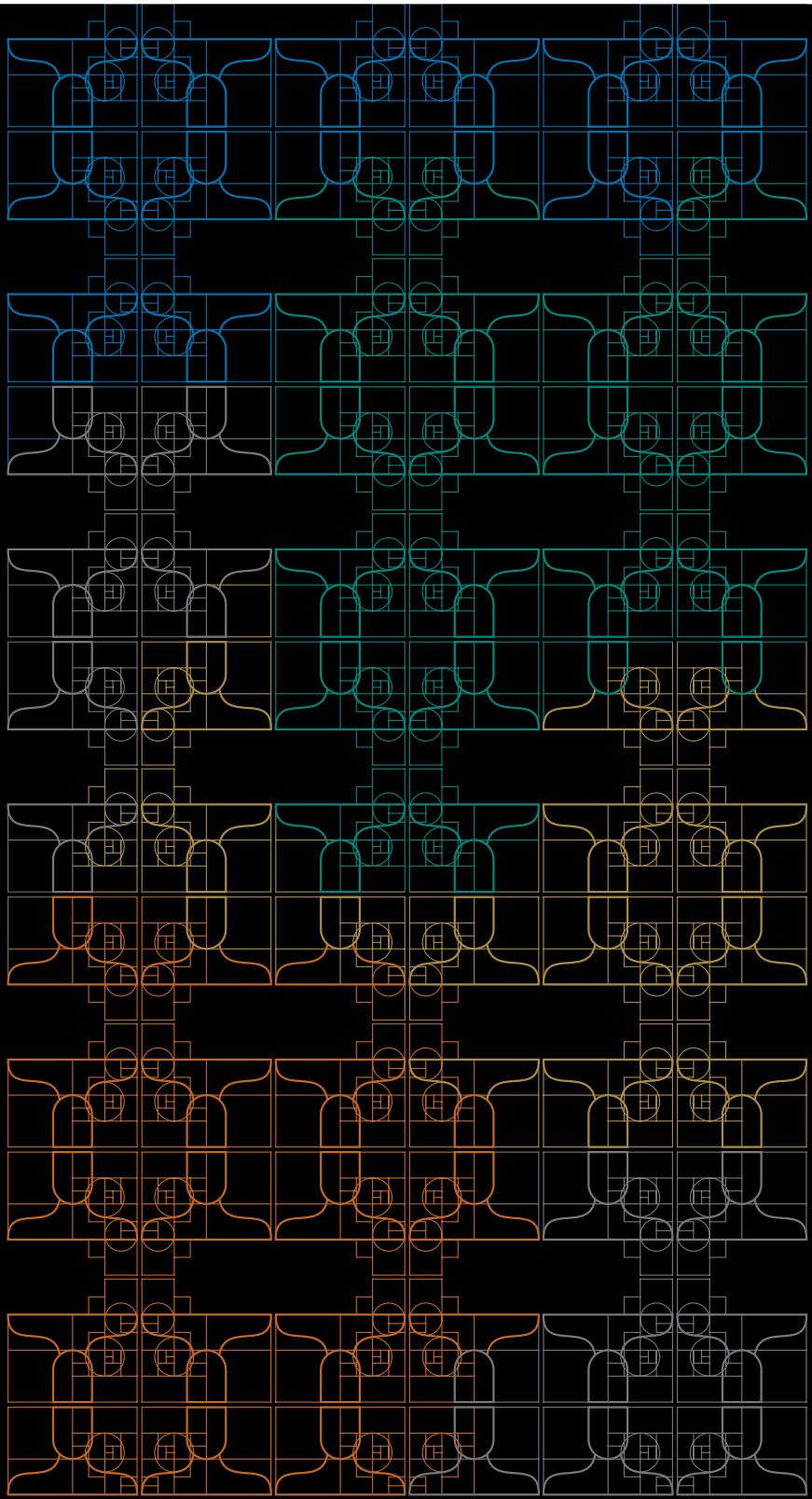

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