

PRECLINICAL MOUSE MODELS IN ADOPTIVE CELL THERAPIES OF CANCER

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Abstract: Engineered T cell-based therapies are an advanced approach for cancer immunotherapy using genetically modified T cells. To date, CD19 and BCMA targeting Chimeric Antigen Receptor (CAR) T cells have been approved for the treatment of certain hematologic malignancies. The success of CAR-T cells is offset by limited efficacy, particularly in solid tumors, and safety risks. Preclinical *in vivo* research, which is highly dependent on reliable mouse models, has been a cornerstone of the success story of adoptive cell therapies and continues to provide invaluable information for the development of the next generation of cellular immunotherapies. In this review we describe four of the most common preclinical mouse models: xenograft models, syngeneic models, immunocompetent transgenic models and humanized mouse models. All of these have advantages and disadvantages and no mouse model can fully recapitulate the human situation because of inherent differences and treatment complexity. Reports suggest that using a combination of mouse models in preclinical *in vivo* research prior to translating the treatment to humans in clinical trials can help incrementally improve the quality, safety, and efficacy of the treatment and provide more comprehensive information than a single model.

Key words: mouse model; xenograft; syngeneic; transgenic; humanized; CAR-T; adoptive cell therapy

Introduction

Adoptive cell therapy (ACT) is a next-generation approach to treating cancer based on immune cells engineered to specifically recognize and effectively eliminate cancer cells. T-cell therapy using chimeric antigen receptors (CARs) has emerged as a leading approach in ACT (1). CARs are designed receptor molecules that merge specificity of monoclonal antibodies with the signalling capacity and effector functions of the T cell receptor (TCR) in T cells (2). The initial CAR designs, referred to as first-generation CARs, include an extracellular antigen-binding domain, usually in the form of a single-chain variable fragment (scFv) derived

from an antibody, linked to intracellular signalling domains, most often derived from the components of the TCR complex, such as the CD3 zeta chain (3, 4). This molecule is capable of recognizing antigens independently of HLA (human leukocyte antigens) presentation but does not support long-term T cell persistence and effector responses due to its limited signalling capacity (5). The second-generation CAR design incorporates additional co-stimulatory domains such as CD28 and 4-1BB (TNFRSF9) that enhance expansion, effector functions and persistence of CAR-T cells (2). The second-generation CAR-T design was the basis for successful clinical trials in relapsed or refractory paediatric and adult blood malignancies (6-10) that have led the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to approve CD19-targeting CAR-T cells in 2017

and 2018, respectively and BCMA-targeting CAR-T cells in 2021 (11). However, adoptive cancer immunotherapy is associated with safety risks such as cytokine release syndrome (CRS) and neurologic toxicities (12), which have led to life-threatening complications (13). In addition, the efficacy of cellular immunotherapy in solid tumor as well as hematologic malignancies is limited, due in part to the immunosuppressive tumor microenvironments, intrinsic T-cell dysfunction (14) and lack of unique surface antigens (15). Various approaches have been pursued to increase the efficacy of adoptive immunotherapy in cancer, and preclinical mouse models are currently irreplaceable to validate their efficacy and safety. Excellent reviews present the use of preclinical models for adoptive cell therapies (16-18). Here, we focus on how preclinical mouse models have supported recent advances in the development of next-generation cellular immunotherapies.

Human xenograft models

A xenograft is a cell, tissue, or organ transplant from a donor that is of a different species than the recipient. In the use of mouse models for the study of human diseases, the most common type of xenograft is the transfer of human tissue, including cells and biopsies, to a mouse recipient. The recipient must be immunocompromised to avoid rejecting foreign human cells. Cells are applied either non-original place (ectopic model) or in the organ from which the tissue is derived (orthotopic model) (reviewed in (19)). In this way, researchers can translate *in vitro* findings into preclinical *in vivo* stage to evaluate the efficacy and safety of cellular immunotherapy in a given disease.

Patient-derived cancer xenografts (PDX) are a simulation of a specific patient's tumor in an animal model. The various types of immunocompromised mice used in tumor xenograft models lack part or most of the immune system so they cannot reflect the immune system of humans. Their immune response to tumors is simplified and does not capture the full complexity of the response. In the past, different types of immunocompromised mice were developed to mimic the human immune system to varying degrees.

The development of immunocompromised mice dates back to athymic mice reported as early as 1966 (20), non-obese diabetic severe combined

immune-deficient mice (NOD SCID) and improved NOD SCID mice with Interleukin 2 Receptor Subunit Gamma (IL2R γ) deficiency (16, 21, 22). Since then, the use of NSG (NOD SCID IL2R γ -) (reviewed in (23)) in CAR-T has been reported by several authors including (24). The NSG mouse was developed by backcrossing the *Il2rg*^{-/-} mouse resulting from a complete null mutation onto NOD/ShiLtSz-Prkdc SCID mouse (25). The NOG mouse was developed by backcrossing the *Il2rg*^{-/-} mouse resulting from a truncated intracellular signaling domain onto NOD/ShiLtSz-Prkdc SCID mouse (26). In NOG mice, the *Il2rg* mutant gene is expressed and produces a protein that binds cytokines but does not signal. Conversely, *Il2rg* gene expression does not occur in NSG mice (27, 28). The use of the xenograft models gives us opportunity to assess the effect of the human CAR-T cells on human tumors but there are no interactions with other immune cells or healthy tissues. Nevertheless, xenograft models are often used as the initial preclinical mouse model in proof-of-concept studies in the development of next-generation cellular immunotherapies. They also allow functional validation of engineered human T cells including tumor targeting, anti-tumor activity, secretion of cytokines, tonic signaling, and intrinsic dysfunction such as T cell exhaustion. Here are selected recent examples that represent only a snapshot of numerous studies in this highly active and explored field of research.

As described in the introduction, the development of second-generation CAR-T cells that contain costimulatory domains in addition to CD3 zeta has been critical for clinical efficacy. Currently, so-called third-generation CAR-T cells, which integrate multiple costimulatory domains into the same CAR molecule, are being tested for efficacy and safety (29). Xenograft models have been useful in the development of second and third generation CAR-T cells (30). Route of administration is important in solid tumors (31). Xenograft models revealed the main differences between the two domains, CD28 and 4-1BB (TNFRSF9). Exhaustion was ameliorated by 4-1BB (TNFRSF9) and exacerbated by CD28 (32). CD28 promoted faster tumor regression, while 4-1BB (TNFRSF9) promoted multiple cytokine secretion (33).

CAR-T cells, which can be remotely controlled by the addition of small-molecule were tested

in immunodeficient NSG mice. The authors demonstrated that the use of a split receptor, in which antigen recognition and intracellular signaling domains assemble into a functional unit only after the addition of a heterodimerizing small molecule, allows remote control of the activity of the engineered CAR-T cells. Such regulation provides additional control of the T cell activity, with the rationale of improving safety (34).

Another landmark study at the interface between cellular immunotherapy and synthetic biology is the development of designer T cells equipped with tailored therapeutic response programs through the use of synthetic Notch receptors (synNotch). Using NSG mice with subcutaneously implanted CD19 negative or CD19 positive target cells, authors demonstrate that their system functions as designed *in vivo*. Specifically, they demonstrated *in vivo* expression of cytokines and bi-specific tumor-targeting antibodies by the SynNotch T Cells (35).

Distinct approach that allows additional control over the injected CAR-T cells to increase safety, but also to allow multiple antigen targeting to mitigate potential antigen escape in CAR-T cell therapy, is the prototype universal immune receptor called SpyCatcher. This universal immune receptor enables covalent binding of targeting ligands to the T cell surface using SpyCatcher-SpyTag chemistry. The SpyCatcher immune receptor redirects primary human T cells by addition of SpyTag-labeled targeting ligands *in vivo* in a solid tumor xenograft model. (36).

As mentioned in the introduction, intrinsic dysfunction of T cells is one of the important limiting factors in cellular immunotherapies. One such example is T cell exhaustion, which leads to defects in T cell functionalities. In an attempt to counteract exhaustion, CAR-T cells were engineered to overexpress the transcription factor c-Jun. In this study, human xenograft models in immunocompromised NOD-SCID-*Il2rg*^{-/-} (NSG) mice were used as models to demonstrate enhanced expansion, improved function, limited terminal differentiation and enhanced anti-tumor activity (37).

Current clinically used CAR-T cells use lentiviral or retroviral vectors to introduce CARs into primary T cells. While this is effective, it poses certain problems related to random integration. With the advent of genome editing approaches most notably CRISPR/Cas systems, the CARs

(38) or TCRs (39, 40) were introduced into the endogenous TCR genomic locus. These protocols utilize CRISPR/Cas9 and the homology-directed repair (HDR) pathway with either viral (38) or non-viral (39, 40, 41) donor template delivery. Both landmark approaches were validated in NOD/SCID/ *IL2gr*-null (NSG) xenograft models. In these studies, xenograft models were sufficient to provide evidence of the concept of these novel platforms, specific targeting and tumor control, as well as improved functionalities of human T cells engineered with designed immune receptors.

Tasian reports that CAR-T's orchestration of off-target toxicities may only be found in early clinical trials (41). Mouse xenografts are useful in screening for basic CAR-T efficacy and for answering specific human biology questions. Additional studies in immune competent hosts are required to evaluate CAR safety. The hostile tumor microenvironment (TME) includes Tregs and MDSCs, but it's largely ignored in preclinical immunocompromised models (42). Tregs may partially explain worse CAR-T clinical trial results in solid tumors, and their inclusion in xenograft models may provide more accurate results.

In xenograft models it is difficult to distinguish between xenogeneic rejection, graft versus host disease (GVHD), allogenic response of human CAR-T cells to the tumor and actual CAR-T therapeutic efficacy. Therefore, appropriate and rigorous controls in experimental design are very important to obtain reliable results and draw correct conclusions. One such control that aids in differentiating the above-mentioned effects from the on-target responses of designed cellular immunotherapies are T cells engineered with a non-targeting immune receptor, such as CAR directed against target that is not expressed on tumor cells. In the absence of the host immune system, it is not possible to test the TME, tumor metastatic potential, or host response to CAR-T.

Taken together, xenograft models provide key insights into the function of human CAR-T cells against human tumors *in vivo*, which has been a basis for clinical success. This allowed the study of basic properties of human CAR-T cells such as anti-tumor activity, secretion of cytokines, expansion and persistence *in vivo*. However, in the absence of an interacting immune system, these models do not allow for comprehensive evaluation of immune-mediated mechanisms as well as on-target off-tumor toxicities (Figure 1).

To gain deeper insight into mechanism of action, interactions with the endogenous immune system and the role of endogenous immune response, xenograft models need to be complemented with syngeneic mouse models.

Syngeneic mouse models

Syngeneic models refer to genetically identical or sufficiently identical and immunologically compatible individuals to allow transplantation. The main feature of syngeneic mouse models is their immunocompetence, including full mouse immunity and comprehensive stroma. Important factor in their comprehensive use is their relative simplicity compared with other immunocompetent models.

The field of engineered cellular immunotherapies is moving towards increasing the efficacy and improving safety. Often, designed T cells rely upon recruiting endogenous immune response or counteracting immunosuppressive TME. Elucidation of the effects and mechanisms cannot be performed in a comprehensive manner in immunocompromised mice because the interacting immune system is lacking. Here, we list selected reports of upgraded CAR-T cells whose functions have been studied in various syngeneic mouse models.

In preclinical studies of adoptive cell therapy in a syngeneic model, the CAR-T, tumors, and target antigens are all mouse derived. Thus, the model allows observation of the CAR-T cells in the context of a functional immune system (16). This model can reveal on-target off-tumor toxicity (43). Its major drawback is that mouse biology does not fully recapitulate human biology. Mouse syngeneic models have largely been unable to mimic CRS. Mouse CAR-T have shorter persistence and are more susceptible to activation-induced cell death compared to human. Syngeneic models do not provide much insight into the mechanisms of human CAR-T cells (44).

Initial syngeneic models of CAR-T demonstrated the superior efficiency of CAR-T over monoclonal antibodies. Preconditioning of the patient by irradiation or chemoablation was crucial for the efficacy of CAR-T therapy (45, 46). In this way, B-cell aplasia was shown as toxicity – mice were clear of lymphoma, but B cells were also absent. Chedale (47) showed that first generation CAR-T were efficient in removing lymphoma, but not

persistent. Second generation CAR-T cells induced B-cell aplasia and chronic toxicity accompanied by CD11b+Gr1+ myeloid derived suppressor cells; elevated TNF α and IFN γ point toward CRS–BALB/c (48), but not in C3H/HeJ or C57BL/6J – side effects vary between strains.

Better and more accurate preclinical models are needed for CAR-T in solid tumors. There the combination with checkpoint blockade regimens was shown to be successful (49). Syngeneic model also demonstrated potential toxicities and strain-specific effects (50). Chinnasamy (51) emphasized that mouse strains must be carefully selected or tested on multiple strains to ensure that toxicity is accurately modeled.

Widely varying results in studies using the same tumor associated antigen (TAA) but different mouse strains as seen in anti-CD19, anti-NKG2D and anti-VEGF studies caution against using a single mouse strain to determine safety before moving to clinical trials (16).

One approach to generate CAR-T cells with improved functionalities is to overexpress an additional accessory molecule along with the immune receptor. Constitutive expression of IL-12 in CAR-T cells was shown to augment CAR-T cell functions in a syngeneic model. Additional modification of infused hCD19-targeted CAR-T cells to secrete IL-12 allowed for efficient eradication of systemic EL4 (hCD19) tumors, as well as induction of B-cell aplasias, in the absence of prior cyclophosphamide conditioning. This outcome was dependent on both CD4 and CD8 T-cell subsets and required continued *in vivo* autocrine stimulation of IL-12 as well as modified T cell-IFN γ secretion, which in turn resulted in resistance to Treg-mediated suppression (52).

In addition to IL-12, IL-18 emerged as a promising candidate (53-56). In one of these studies (54) syngeneic model of pancreatic and lung tumors revealed that release of IL-18 modulated the immune cell landscape in the tumor. Increased numbers of CD206- M1 macrophages and NKG2D+ NK cells were observed, while Tregs, suppressive CD103+ DCs, and M2 macrophages decreased. These observations were possible because an intact interaction immune system was present.

CAR-T cells were also engineered to secrete a combination of IL-7+CCL19 with the rationale that these factors contribute to the maintenance of T-cell zones in lymphoid organs. Upgraded CAR-T cells eradicated established solid tumors

and prolonged survival compared to conventional CAR-T cells. The syngeneic model showed increased infiltration of dendritic cells and T cells into tumor tissues. Depletion of recipient T cells reduced the therapeutic effects of upgraded CAR-T cell treatment, demonstrating that endogenous immune responses were induced (57).

In another study, CAR-T cells were engineered to secrete single-chain variable fragments (scFv) that block PD-1 (PDCD1). Clinically relevant syngeneic model with PD-L1 (CD274) positive tumor targets made it possible to demonstrate that secreted scFv acted on both CAR-T cells and bystander tumor-specific T cells to improve anti-tumor activity (58).

CAR-T cells constitutively expressing the immune-stimulatory molecule CD40 ligand (CD40L) demonstrated improved anti-tumor activity. In relevant syngeneic models, the authors investigated the underlying mechanisms and found that CD40L+ CAR-T cells were able to counteract tumor antigen escape variants via CD40/CD40L-mediated cytotoxicity and induction of an endogenous immune response. After adoptive cell transfer, upgraded CAR-T cells licensed antigen-presenting cells and recruited endogenous tumor-recognizing T cells (59).

Another study demonstrating the importance of syngeneic mouse models explored how depletion of immunosuppressive M2 tumor-associated macrophages (TAMs) may improve the efficacy of CAR-T cells. The authors found that a folate receptor β (FR β) positive subset of TAMs exhibited an immunosuppressive M2-like profile. When CAR-T cells were engineered to eliminate these FR β + TAMs, an enrichment of proinflammatory monocytes, a recruitment of endogenous tumor-specific CD8+ T cells, delayed tumor growth, and prolonged survival were observed (60).

Syngeneic models are useful for evaluating immunotherapies, *e.g.* in combination studies, particularly using checkpoint inhibitors. Syngeneic model panels can be extensively characterized (*e.g.* RNA sequencing of cell lines and tumors, immunophenotyping, biomarker identification), and these data can be combined with *in vivo* efficacy benchmarking profiling results from common checkpoint inhibitors (anti-PD-1, PD-L1, CTLA-4).

Syngeneic models are therefore an indispensable for evaluating the safety and efficacy of cellular immunotherapy, as they allow the study of

adoptively transferred cells in the context of an interacting immune system. This allows for a rigorous assessment of immune-mediated mechanisms involved in successful therapy as well as toxicities. Potential drawbacks of syngeneic models include difficulties in generating cellular products and limited persistence and efficacy after infusion (Figure 1).

Immunocompetent transgenic mice

Transgenic animals have been around for several decades (61). Immunocompetent transgenic mice tolerant to human tumor associated antigens (TAAs) have been described in hematologic and solid tumors and for evaluating the safety and efficacy of antitumor immunotherapies (62-67). Although most anti CD19 CAR-Ts are studied in syngeneic or xenograft models, immunocompetent transgenic mouse models can also be used to better determine CAR-T safety. In transgenic mice, human TAA (murine TAA knockout and human TAA knock in) are expressed in mice to highlight the on-target off-tumor effect in healthy tissues. The mice are bred to have TAA expression similar to humans (68). Mice have their own T cells and an intact immune system (like in syngeneic models), but allow the use of human TAA-specific CAR-Ts (like xenografts) (16). A study in C57BL/6J with mouse CD19 knockout and human CD19 knockng restricted to B cells showed no toxicities other than B-cell aplasia (52). CARs targeting different antigens (CD19, CEA, HER2) have been tested in the clinic. The positive effect of preconditioning on adoptively transferred cells was confirmed in transgenic and not xenograft models (16, 69, 70). Engrafted tumors do not recapitulate many of the properties of naturally occurring tumors. A transgenic model in which tumors develop spontaneously can better mimic clinical progression and predict off-tumor toxicities (16). Transgenic CEA mice were developed by Zimmerman and colleagues (71) and have been frequently used to test CEA-targeting- CAR-T-cell therapy. In this model with high CEA expression, the toxicity associated with CAR-T cells appears to be limited to high-affinity CAR-T cells (65, 67). One of transgenic mouse models with CEA levels equivalent to those in humans (72) has also shown severe side effects associated with CAR-T-cell therapy (73, 74).

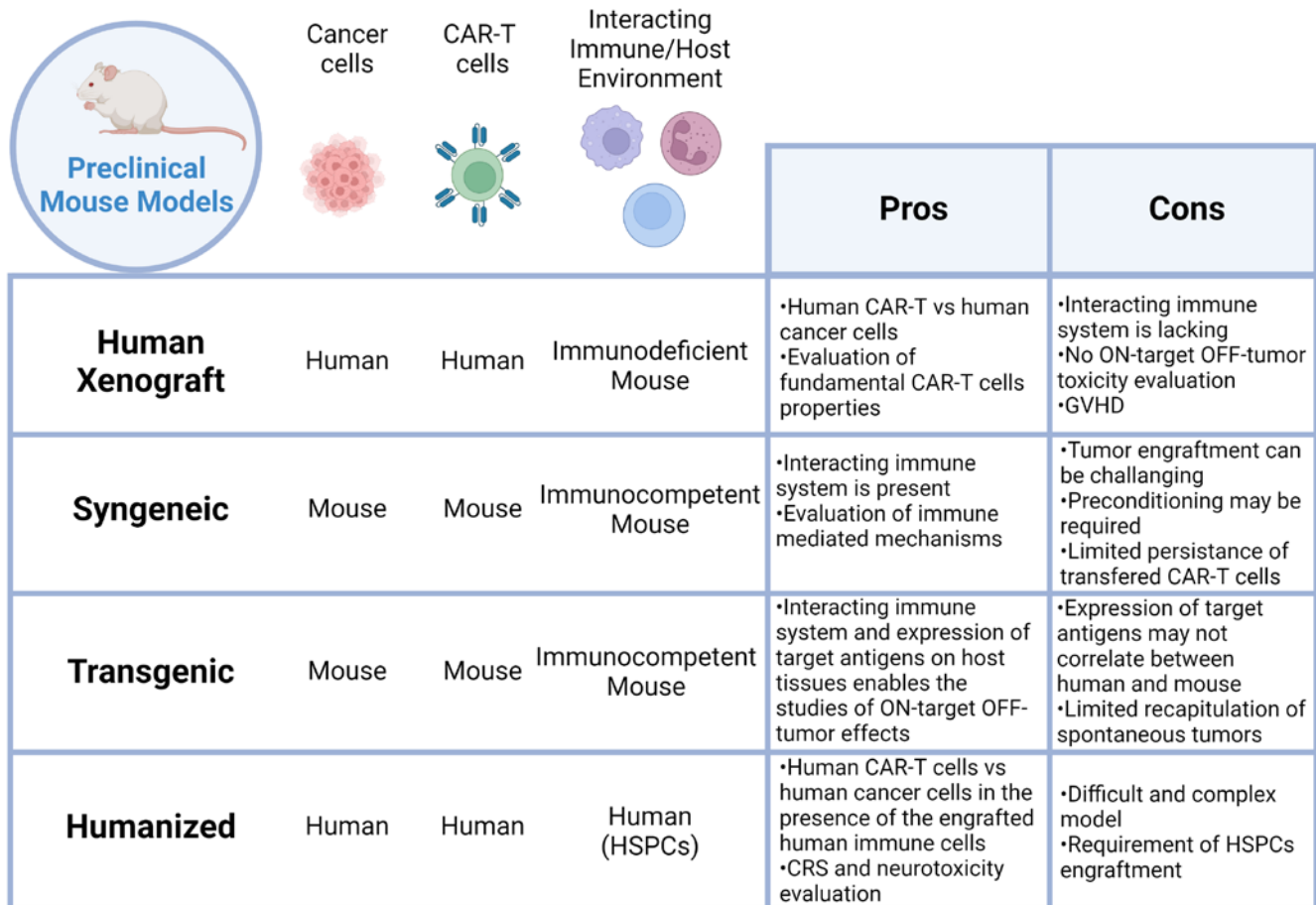


Figure 1: Schematic representation of preclinical mouse models for adoptive cell therapies

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Humanized models

Currently, NOD/SCID/*Il2rg*^{-/-} (NSG) or BALB/c/*RAG2*^{-/-}/*Il2rg*^{-/-} (BRG) mice are the standard recipients in the generation of humanized mice because they are deficient in mouse T cells, B cells, and NK cells (75-77). Transfer of human CD34⁺ hematopoietic stem and progenitor cells (HSPCs) into newborn NSG or BRG mice results in long-term engraftment of CD34⁺ cells and reconstitution of multilineage human immune cells (77-79). The comprehensive transfer protocol involves transfer of human CD34⁺ cells into NSG recipient mice and implantation of human fetal thymus and liver tissue under the kidney capsule of these mice (77, 80, 81). The resulting humanized mice are called bone marrow-liver-thymus (BLT) mice. They support the long-term engraftment and systemic reconstitution of a nearly complete human immune system, including multilineage human adaptive and innate immune cells consisting

of T cells, B cells, NK cells, dendritic cells, and macrophages (77, 80, 81). Importantly, human immune cells developed in BLT mice, particularly T cells, are functional and have shown productive responses to skin xenografts and various viral/bacterial infections (77, 80-82). The next generation of humanized mouse models including NSG-SGM3 (or NSGS), NSGW41, NOG-EXL and MISTRG, support human myelopoiesis at varying degrees and through different strategies (28).

Humanized mouse models may bridge the gap between the syngeneic and xenograft models, because they are tolerant to human cells and exhibit aspects of a functional human immune system (16).

Until recently, CRS, which typically develops within the first few days after infusion of CD19 CAR-T cells, and neurotoxicity, the two major toxicities associated with clinically used CD19 CAR-T cells in humans, could not be reproduced in preclinical mouse models. This changed with

the development of a novel xenograft model in humanized mice that faithfully recapitulated both major toxicities (83). This model was developed by engrafting human cord blood hematopoietic stem and progenitor cells (HSPCs) into sub lethally irradiated newborn triple transgenic NSG (SGM3) mice. These mice express human stem cell factor, granulocyte-macrophage colony-stimulating factor (CSF2), and IL-3 to support the engraftment. Successful reconstitution of hematopoiesis was demonstrated, which included human B cells, monocytes, and T cells as well as cells from other lineages. Interestingly, the timing of HSC injection shortly after birth was important for successful human T lymphopoiesis. Circulating T cells exhibited a physiological CD4/CD8 ratio and differentiated into all major T cell differentiation subsets. T cells from these mice were then used to generate second-generation CAR-T cells targeting either CD19 or CD44v6. These CAR-T cells were injected into adult SGM3 mice that had previously been engrafted with ALL-CM leukemia cells. CAR-T cells cleared leukemia, which was associated with CRS characterized by weight loss, fever, and elevated systemic levels of human inflammatory cytokines including IL-6, resembling CRS in humans receiving CD19-targeting CAR-T cells. The authors used this model to show that monocytes are the major sources of IL-1 and IL-6 during CRS and that the syndrome could be prevented by depleting monocytes or by blocking the IL-6 receptor with tocilizumab (IL-6 receptor-blocking antibody). This model also recapitulated the lethal neurotoxicity, characterized by inflammation of the meninges. Interestingly, authors demonstrated that anakinra (IL-1 receptor antagonist) but not tocilizumab ameliorated neurotoxicity.

In the follow-up study by the same group, this model was further developed to investigate the efficacy and safety of CAR-T cells derived from preselected T cell subsets. When preselected naive/stem memory T cells were used to generate the CAR-T cellular product, superior anti-tumor activity, expansion and functional phenotype were observed compared to unselected bulk T cells. Surprisingly, this was accompanied by limited incidence of severe CRS and neurotoxicity. Overall, this model revealed improved efficacy and safety when preselected T cells are used to generate CAR-T cell products (84).

Therefore, in contrast to all other *in vivo* models using mice, humanized mouse models were able to

reveal and investigate critical toxicities associated with CAR-T cell therapy (Figure 1).

In addition to humanized mouse models for T cell-based therapies, models have also been developed that allow adoptive transfer of engineered B cells. In this example, B cells were first isolated from the spleens of humanized donor mice, then genetically engineered and infused into “autologous” humanized recipient mice. Because the recipient mice were humanized with the same source of CD34+ cells as the humanized donor mice, such approach rendered engineered human B cells tolerant to the host (85).

Conclusions

Cellular immunotherapy with CAR-T cells is a paradigm shifting approach for the treatment of certain blood cancers. However, limited efficacy and safety risks are the major barriers to advance the field and plethora of academic research groups, biotech and pharmaceutical companies are developing innovative next-generation cellular immunotherapies. After a novel approach has been validated *in vitro*, the next steps are experiments in preclinical mouse models. Typically, the initial experiments are conducted in human xenograft models, which are well suited for evaluating of novel genetic constructs and designs, immune receptor signaling, anti-tumor activity and intrinsic properties of human CAR-T cells, such as expansion and persistence, as well as certain dysfunctions, including T cell exhaustion. When more in-depth information about the immune mechanisms is required, which is the case when improved cellular immunotherapies aim to recruit endogenous immune responses and/or counteract an immunosuppressive tumor microenvironment, xenograft models are inadequate because they lack an interacting immune response. To answer such questions, syngeneic models are needed and have already provided valuable information for clinical translation. Since syngeneic models are associated with certain challenges including difficulties in the manufacturing of mouse cellular products, *in vivo* expansion, persistence, and efficacy, studies that systematically develop optimized protocols and procedures are very important (86).

In human clinical trials using CAR-T cells, certain toxicities including CRS and neurotoxicity

were observed that were not predicted by any of the preclinical models available at the time. Recently, a humanized mouse model was successfully developed to specifically address this challenge and replicate the pathologies observed in humans in preclinical mouse models as well (83). This example highlights the importance of continued development of preclinical mouse models as the field of cellular immunotherapy rapidly grows.

Preclinical mouse models continue to be a cornerstone in the development of the next generation of cellular immunotherapies. In this review recent advances and use of the four most common preclinical mouse models are presented. In addition, we presented the selected recent studies in which these models have been used to demonstrate innovative CAR-T cell approaches. The use of multiple models may provide a better understanding of a particular CAR-T therapy than a single model and we anticipate that increasingly sophisticated models will be developed, aided in part by recent advances in genome editing technologies, to comprehensively address the complexities of immunology and cellular immunotherapy. However, we must be aware of the limitations of preclinical mouse models, including the inherent differences between human and mouse biology and immunology. Careful design of properly controlled experiments is essential to generate reliable, high-quality data and draw the right conclusions required for clinical translation of cellular immunotherapies.

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PREDKLINIČNI MIŠJI MODELI PRI ADOPTIVNIH CELIČNIH TERAPIJAH RAKA

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Izvleček: Napredne terapije na osnovi biotehnološko spremenjenih limfocitov T predstavljajo moderen pristop k imunoterapiji raka z uporabo genetsko spremenjenih limfocitov T. Do danes sta bili za zdravljenje hematoloških malignosti odobreni terapiji s himernimi antigenskimi receptorji usmerjenimi proti antigenoma CD19 in BCMA. Uspeh zdravljenja s celicami CAR-T pa ovirajo omejena učinkovitost, še posebej pri solidnih tumorjih in varnostna tveganja. Predklinične raziskave *in vivo*, ki so močno odvisne od zanesljivih mišjih modelov, so bile kritični dejavnik zgodbe o uspehu adoptivnih celičnih terapij in še vedno zagotavljajo neprecenljive podatke za razvoj naslednje generacije celičnih imunoterapij. V preglednem članku povzemamo štiri najpogostejše predklinične mišje modele: ksenografte, singenetske modele, imunokompetentne transgenske modele in humanizirane mišje modele. Vsi opisani modeli imajo svoje prednosti in slabosti in noben mišji model ne more do popolnosti preslikati situacije v človeškem pacientu zaradi medvrstnih razlik ter izjemne zapletenosti zdravljenja. Podatki iz literature kažejo na to, da lahko uporaba kombinacije mišjih modelov v predkliničnih in *in vivo* raziskavah pred translacijo zdravljenja na ljudi v kliničnih poskusih pripomore k postopnemu izboljšanju kakovosti, varnosti in učinkovitosti zdravljenja in zagotovi bolj celosten nabor podatkov kot en sam model.

Ključne besede: mišji model; ksenograft; singenetski; transgenski; humanizirani; CAR-T; adoptivna celična terapija