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Mouse Models in Bone Marrow Transplantation and Adoptive Cellular Therapy

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Abstract

Mouse models of transplantation have been indispensable to the development of bone marrow transplantation (BMT). Their role in the generation of basic science knowledge is invaluable and is subject to discussion below. However, this article focuses on the direct role and relevance of mouse models towards the clinical development and advances in BMT and adoptive T-cell therapy for human diseases. The authors aim to present a thoughtful perspective on the pros and cons of mouse models while noting that despite imperfections these models are obligatory for the development of science-based medicine.

Ever since we learned to apply science-based approaches to medicine and used in vivo model systems, the mouse has become a scientific powerhouse. One doesn't have to be true cognoscenti to appreciate the value of mice towards enhancing our understanding of most basic science knowledge. The application of these advances to the betterment of humanity and specifically towards clinical medicine is also apparent to every practicing physician. From the advances in the understanding of molecular biology, cloning, and recombinant technology human genetic and immune diseases, to the development of life-saving drugs, including penicillin,[1] vaccinations,[2,3] blood transfusions,[4] surgical sutures and techniques[5]—all marvels of modern medicine—mice have served as invaluable models. Nonetheless, the reasons for why so much medical research is done with mice, what makes them suitable for research, the relevancy of the information gained from them for human diseases, all need to be revisited periodically, just as any scientific method ought to be. It is obvious that humans and mice are different: mice are smaller, have a shorter life span, in most cases are genetically in-bred, live closer to the ground, and possess limitations that are germane to any scientific model system. However, humans and mice share >95% of genomes and also suffer from many of the same diseases, for many of the same genetic reasons.[6–9] Experimental findings in mice often correlate to human biology and have as such served as a research stand-in for a human patient.

This review will focus on the relevance of murine studies to hematopoietic stem cell transplantation or bone marrow transplantation (BMT) and adoptive cellular therapy against viruses and tumors. We will provide a perspective on the relevance and the advances that were made "directly" from mouse models towards the clinical development of BMT and

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cellular therapy in humans. To this end, we will not attempt to enumerate the “basic” discoveries (of which there are too many), but instead try to demonstrate how murine studies can be relevant and have *directly* led to translation of most aspects of BMT as are currently practiced today. However, we will also discuss how excessive reliance on imperfect animal models can needlessly impede successful clinical progress.

BENEFITS OF MOUSE MODELS

Mouse models have been of value from the inception to the development of BMT as a therapeutic modality, the use and development of hematopoietic stem cells (HSCs), the selection of donors, the understanding of complications like graft-versus-host disease (GVHD), the curative potency of graft-versus-tumor/leukemia (GVT/GVL), currently used immunosuppressive strategies, the utilization of growth factors, and the development of supportive care. In making a case for the utility, relevance, and value of murine models, our intent is neither to suggest that these are “infallible” nor “sufficient” models, nor to indicate that they somehow supplant the need for carefully designed human clinical trials. On the contrary, the intent is to demonstrate that they are “necessary,” “critical,” and have been absolutely essential for the development of clinical BMT.

Bone Marrow Transplantation

Murine models have directly led to many aspects of the current day practice of BMT. As listed above and in more detail elsewhere, there are several models that are employed in studying the different aspects of BMT.[10,11] While any single model system is likely to be better suited for any one specific feature/outcome of BMT than others, collectively over the years they have facilitated not only generation of new knowledge, but also better understanding and clinical development of both autologous and allogeneic BMT. Seminal studies by Medawar and colleagues in mice led to the scientific basis for the concept of allograft tolerance and transplantation.[12] Even earlier, at the turn of last century, observations by Loeb on the inability to transfer tumors across different strains of mice led to the notion of tumor rejection by alloreactivity.[13–16] Initially from Medawar's observations on the role of histocompatibility in allograft rejection, and later, more specifically, to the groundbreaking immunological research in mice by Gorer and Snell, we owe the identification of the major histocompatibility complex (MHC) genes, the H-2 system.[17–24] This directly led to the discovery and use of large animal and eventual human leukocyte antigen (HLA) typing in the selection of donors in human transplantation.[25–31] The first demonstration that HSCs exist and that they can be engrafted and give rise to multiple lineages following transfer into secondary hosts came from the pioneering murine studies by Till, McCulloch, and colleagues.[32,33] A series of critical shielding experiments in mice by Jacobson and later Lorenz et al and subsequently in other larger animals with radiation followed by transplantation of marrow grafts provided the basis not only for understanding concepts of allograft transplantation but also led directly to the use of radiation in modern day preparative regimens.[34–39] The notion that HSCs are required for recovery, ie, the fundamental step of BMT, was a direct consequence of observations by Barnes and Louitit and others in mice.[40] Experiments by Barnes and colleagues in the treatment of murine leukemia by supralethal irradiation and marrow grafting directly led to the first human clinical marrow transplantation by Mathe and colleagues in Europe and to the incomparable body of work by Don Thomas and colleagues in the United States. Thus, “direct” translation of observations from murine models led to the birth of the field of clinical BMT.[38,40–43] Even the clinical use of the intravenous route as the preferred method of administration of BMT and peripheral blood stem cell transplantation is directly based on murine studies.[44]

Graft-Versus-Host Disease

GVHD is the most significant toxicity of clinical allogeneic BMT and cell therapy. This entity was first noted, predicted, and understood in mice.[10] GVHD reaction was first recognized when irradiated mice were infused with allogeneic marrow and spleen cells.[45,46] Although mice recovered from radiation injury and marrow aplasia, they subsequently died with "secondary disease," a 'runting' syndrome that causes diarrhea, weight loss, skin changes, and liver abnormalities. This phenomenon was subsequently recognized as GVHD. Billingham postulated three requirements for the development of GVHD based on murine models,[46,47] and these postulates still hold true for both experimental and clinical GVHD. Over the subsequent decade the introduction of rudimentary tissue typing paved the way for the classical murine studies of Korngold and Sprent, which confirmed the T-cell dependence of GVHD.[48–50] This observation has directly led to the scientific and clinical development of targeting donor T cells for GVHD.[51]

Methotrexate is one of the first and still is a key component of most current immune-prophylaxis strategies. Its efficacy in reducing GVHD after allogeneic BMT was first shown in mice in 1958 by Uphoff and was then successfully translated into humans.[52] Cyclosporine, which is extracted from a species of fungus, was discovered in 1972 and found to be a potent immune-suppressor of GVHD in mice.[53–55] The development of cyclosporine and also FK506,[56,57] the calcineurin inhibitors, which form the foundation for current prophylaxis strategies, was thus directly translated from murine studies. The development of rapamycin for GVHD prevention was a result of direct translation of studies in murine models of GVHD by Blazar and colleagues in 1993.[58] Another currently used medication, mycophenolate mofetil, was also first shown to be efficacious in murine models of GVHD by van Leeuwen et al before its subsequent clinical translation.[59] Other model systems, including canine and nonhuman primate models, played important roles in the development of strategies for clinical prophylaxis and treatment of GVHD.

The presence of well-characterized inbred strains, availability of knock-out and transgenic animals, easy availability of reagents, and the relative low cost have made mouse models the most utilized systems for investigating the complex biological mechanisms of GVHD. They have and continue to facilitate a deeper, better, and more refined understanding of basic biology and also provide direction for development of potential translational avenues.[60] In light of space constraints, only a few examples will be provided of how the recent advances in our basic understanding of the biology of GVHD from mouse models have continued to lead to the translation of novel avenues for the prevention and treatment of human GVHD. The notion that cytokines are key amplifiers of GVHD and that their blockade could be useful was first observed in mice.[60–64] Although blockade of a single cytokine has not been as efficacious in clinical GVHD, recent clinical evidence suggests it might be a viable strategy in a selected few patients.[64] The discovery and role of regulatory T cells in GVHD was made by a series of seminal observations in mice.[65–67] These observations are now being translated into humans.[68] The identification of a role for co-stimulation and check-point blockade and the development of antibodies to target these check-points in mice has led to the recent, direct, and successful translation of these strategies in GVHD and also in immunotherapy against cancers.[60,69] The understanding and application of clinically altering gut microbiota was made in mice by van Bekkum and colleagues, and further refined by current studies in murine models.[70,71] Studies from mouse models on mixed chimerism and memory T-cell subsets are being directly translated into human clinical trials.[72–75] Other recent clear examples of murine studies that have led to "direct" translation and development of drug-based approaches for mitigating or preventing GVHD include the use of post-BMT cyclophosphamide, proteasome inhibition, and histone deacetylase inhibitors.[76–81] Many of these approaches have shown value in not only small but also large phase II trials and are now being tested in randomized multicenter clinical trials under

Clinical Trials Network consortium for their efficacy. The above examples are limited and do not include many other interventions that have been informed by murine studies.

Graft-Versus-Tumor/Leukemia

It is now widely appreciated that the potency and curative potential of allogeneic cell therapy comes from the immunological mechanisms commonly referred to as GVT/GVL effects, a term first used in murine models.[82] Mouse models have played, yet again, a fundamental role in the conception and in translation of this idea. The concept of leukemia/tumor destruction by the immune system was originally envisioned by Ehrlich and later refined by Burnet.[83,84] Although clinical observation of such a process was made by Coley,[85] who noted regression of tumors in patients that developed infections, the first tangible and seminal demonstration of superior leukemia elimination by adoptive transfer of allogeneic cells than syngeneic cells was made[40] in murine models. This was attributed to an immunologic reaction by the donor cells against the non-self–host type leukemia. Barnes noted that extrapolation of this observation from mouse to man for treatment of leukemia might be possible under certain contexts. The observation of the tight link between GVHD and GVL, their temporal association, and that lymphohematopoietic tissue is the primary target of GVH reaction was made from mouse models.[86] Thus, mouse and other animal models have been pivotal in making the early and fundamental observations on the presence and potency of GVL. Many of these observations have been confirmed in human patients. Recent murine studies have showed that cognate interactions between T cells and tumors and T-cell cytotoxic (CTL) pathways are critical for GVL,[87,88] elucidating the pathways of CTL that are critical for tumor elimination. Observations on the role of donor NK cells in murine models of GVT/GVHD have led to better understanding and potential clinical implications for these observations in large scale human studies.[89–94] The murine models used to study GVL do have several caveats, including dependence on the type of HSCT protocol, the timing and dose of leukemic cell infusion, homogeneity from inbreeding, lack of concomitant immuno-suppression, limitations of tumor cell line biology (despite exhibiting the classic hallmarks), and immune repertoire variations due to development in the context of absence of sculpting by the process of immunoediting.[11,95] Murine models clearly help in understanding the biological principles in a reductionist manner, and inform us about what is more probable for clinical translation despite not reflecting the entire complexity of the clinical situation. However, it is important to note, as has been observed by every clinician, that there is a spectrum of responses to currently established practices on and outcomes from BMT (GVHD, GVT) even between human patients, where results from subsets of patients also cannot be extrapolated to other contexts.

Stem Cell Mobilization

Although the half-lives of human and murine HSCs vary somewhat, on a weight-adjusted basis, the total number of HSCs stem cells and progenitors is comparable in the marrow. The daily production rate of red blood cells, platelets, and granulocytes in the mouse is also comparable on body weight basis to humans.[96] Thus, increased basic understanding of HSCs and their niche in mice has led to many insights into human HSCs, which will not be addressed here. But from the clinical translational perspective, the 1962 discovery that HSCs can be detected in peripheral blood of mice led to the subsequent concept and development of harvesting PBSCs in humans.[97] The development of dimethyl sulfoxide (DMSO)-based strategies for preservation of lymphocytes was also first developed in mice.[98,99] Pioneering murine-based studies by Stanley and Metcalf and colleagues in the 1970s and 1980s led to the identification and development of growth factors granulocyte colony-stimulating factor (GCSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF).[100–102] These results have directly been translated into HSC mobilization from PBMCs and to the use of growth factors for promoting engraftment after clinical BMT.[103,104]

Cord Blood

The development of human cord blood transplantation has been yet another major development in the field of BMT. As noted by Broxmeyer and Boyse et al in their seminal paper in 1989, the use of human umbilical cord blood for therapeutic reconstitution was proposed and developed as result of direct observation of successful hematopoietic reconstitution of lethally irradiated inbred mice with syngeneic neonatal blood by Gengozian et al in 1961.[87,105]

Low Intensity

The development of non-myeloablative BMT has been a significant advance in the history of BMT therapy and has allowed the expansion of allogeneic BMT as a therapy for hematologic and nonhematologic diseases, especially in the older population. The concept of mixed chimerism played a significant role in the eventual development of this approach. The finding by Ildstad and Sachs in 1984 that mice reconstituted with a mixture of donor and recipient strain bone marrow after myeloablative conditioning became specifically tolerant of skin allografts from the bone marrow donor laid the groundwork for further experimental studies.[106] Work by Sachs and Sykes et al demonstrated development of mixed chimerism with non-myeloablative regimens in murine models.[107] Seminal studies in murine models by Slavin and colleagues and by Storb and colleagues in canine models led directly to the development of non-myeloablative condition for allogeneic BMT.[108,109]

Supportive Care and Ethical Concerns

The ability to give blood products has not just facilitated clinical BMT but revolutionized medical and surgical care. Much of this ability is a result of the direct translation of the role of citrate for longer preservation of blood in murine models.[110] Finally, murine models serve to help prevent unethical medical practices. Their utilization with other animal model systems is in line with the Declaration of Helsinki and Nuremberg Code that outline the requirement of animal models for ethical concerns associated with human experimentation. Large experimental models in this context can be useful and have the advantages of being outbred with longer life spans, but they are too expensive, lack tools for mechanistic dissection, and the prohibitive processes involved in working with them often do not allow for replication and statistical robustness. Mouse models do not have these limitations and, more importantly, can be designed to better mimic certain clinical scenarios (like the use of aged mice, multiple strains, spontaneous tumor models, etc) that could provide for a more realistic assessment.

Antiviral and Anti-tumor–Directed T-Cell Therapy

Development of adoptive T-cell therapy for post-transplant viral/ lymphoproliferative diseases and T cells that express chimeric antigen receptors (CARs) targeting specific antigens has been an exciting development. As will be described below, these developments occurred largely without the utilization of or despite the lack of a relevant mouse model system. However, it is well known that many, if not all of the necessary key developmental principles and technology are direct off-shoots of seminal observations in mice. For example, the discovery of T cells by Miller,[111] CD8/4 T cells by Cantor and Boyse,[112,113] Th1/2 subsets by Mosmann and Coffman[114], T-cell receptor by Kappler et al,[115,116] McIntyre and Allison,[117] and Davis et al,[118] and dendritic cells by Steinman and Cohn[119] were made in mice. The ability of T cells to eliminate viral infections, the very first CTL studies of viral immunity, and the demonstration of MHC restriction by Doherty and Zinkernagel were in the murine lymphocytic choriomeningitis virus (LCMV) model.[120,121] The first example of “gene transfer” was made by Szybalski and Szybalska in 1962 using hypoxanthine-guanine phosphoribosyltransferase (HPRT)-deficient cells in murine

models.[122] The discovery of reverse transcriptase by Baltimore and many other key advances in the development of molecular biology have resulted from murine studies.[123] Thus, despite the lack of appropriate murine models that fully mirror human post-transplant viral infections and lymphoproliferative diseases, the development of adoptive T-cell therapy could not have been conceived or investigated in humans without the insights provided from murine studies. Thus, although a bit indirect, there nonetheless are murine "toe-prints" on each and every aspect of adoptive T-cell therapy strategies.

LIMITATIONS OF MURINE MODELS FOR ADOPTIVE CELLULAR THERAPY

The crucial contribution of "standalone" mouse models notwithstanding, cellular therapies, including stem cell transplantation, are complex biological treatments that can only be developed for human use by combining systematic and controlled preclinical (murine and human) and clinical studies; these studies may need to include iterative cycles of preclinical and small-scale clinical studies in which the approach can be validated in humans and the preclinical models modified to better reflect clinical realities. In other words, for almost every cell therapy project, the linkage between preclinical and clinical data is dynamic and evolving, and while datasets from a single murine model may contribute to the decision on whether or not to proceed to clinical study, it is unwise to allow these models to become the critical arbiter. Nowhere is the need for caution better illustrated than in the interpretation of murine models in which T-cell therapies are used to modulate allogeneic HSCT. In HSCT, T-cell therapies are used to prevent and/or treat post-transplant complications such as infections, disease relapse, or GVHD.[124] The history of HSCT clearly demonstrates the general axiom outlined above, that the absence of positive results from murine models should not preclude clinical development,[125] and that the clinical development of complex biological therapies may need to be parallel, or even sequential, to the development of animal models. When a specific cellular therapy is contemplated, therefore, it is crucial not just to determine whether an appropriate model exists, but also to be aware of its advantages and limitations and to draw only those conclusions that are adequately substantiated by this model. Table 1 gives an overview of several mouse models used in HSCT research and points out their advantages and limitations.

While syngeneic mouse models allow mechanistic studies of biological processes in a living organism, including genetic gain or loss-of-function studies, they have the inherent limitations of species-specificity and that human cells or tissues cannot be tested directly. Xenogeneic models, in contrast, allow the direct study of human cells and tissues in a living animal but have major limitations regarding their consistency, the influence of the murine microenvironment (eg, tumor stroma of murine origin), and the limited function of human immune system cells due to suboptimal cytokine and accessory cell support. A major advance in the use of mice to model cellular therapy after HSCT was the development of humanized mice that are mouse–human chimeric animals: severely immuno-compromised mice (such as NOD-SCID-γc-/- [NSG]) are engrafted with human HSCs and/or tissue(s) and may be genetically engineered to express human genes such as human MHC genes and/or cytokines/growth factors (recently reviewed[126,127]). This approach now allows functional study of the human immune system in specific disease settings. For example, human species-specific viruses such as the human immunodeficiency virus (HIV) or the human herpes virus Epstein-Barr virus (EBV) and their interaction with the human immune system can now be studied in mice, as can cancer stem cell populations, while regenerative medicine applications such as tissue repair can be examined in greater detail.

Adoptive T-Cell Therapy for Post-transplant Viral Infections and Lymphoproliferative Disorders

Despite the availability of potent pharmacologic anti-viral treatments, viral infections remain a major cause of morbidity and mortality after allogeneic HSCT. Most frequent are infections with herpes viruses such as cytomegalovirus (CMV) and EBV, or with adenoviruses, mainly occurring during the period of impaired T-cell function post-transplant. In immunocompromised patients, CMV reactivation or infection can cause organ diseases such as colitis, hepatitis, and pneumonitis, as well as retinitis in HIV patients; EBV can cause malignant transformation of B cells leading to lymphoproliferative diseases (LPD), while adenovirus may cause lethal damage to lung, liver, gut, and other organs. These diseases may occur or recur despite the administration of anti-viral agents, which are also expensive and frequently toxic. An alternative is the prophylactic or therapeutic adoptive transfer of antigen-specific CTLs targeting one or several viruses simultaneously. In many clinical studies conducted over the past 20 years, this approach has proven to be generally safe and effective, producing long-lasting viral control without inducing GVHD.[128] Moreover, virus-specific CTL therapy can rescue patients who have previously failed anti-viral drug treatment or developed drug resistance.[128] Virus-associated malignant post-transplant complications such as the EBV-associated post-transplant lymphoproliferative disorders (EBVLPD) are also very effectively prevented or treated with CTLs without discernible side effects.[129,130] More recently, EBV-CTLs have been explored for the treatment of other EBV-associated malignancies such as Hodgkin lymphomas or nasopharyngeal carcinomas. Safety and efficacy of EBV-CTLs were also demonstrated when using only partially HLA-matched banked allogeneic CTL lines in a phase II study for the treatment of EBV-LPD after solid organ transplant or HSCT in 33 patients that had failed conventional therapy. The response rates (complete or partial) were encouraging, at 64% at 5 weeks and 52% at 6 months.[131] No adverse effects were seen after partially matched EBV-CTL infusion. The possibility of using partially HLA-matched banked virus-specific CTLs as an “off-the-shelf” treatment option for multiple common viruses of the immunocompromised host (including CMV, EBV and adenoviruses) is currently being investigated in a multicenter phase II study. In this analysis, the safety and efficacy of most closely HLA-matched multivirus-specific CTL lines is being examined in HSCT patients with EBV, CMV, or adenovirus infections that are persistent despite standard therapy (ClinicalTrials.gov identifier NCT00711035).

The results outlined above showing the safe and apparently effective development of adoptive T-cell therapy for the prevention and treatment of post-transplant infection provide an excellent example of successful clinical translation of basic immunologic findings without the intermediate of *directly relevant* preclinical xenograft or humanized mouse models. Reliable mouse models to directly test the function of the human immune system in hosts infected with human herpes viruses only became available about 20 years after the first successful clinical translation of this approach.[132,133] This delay came about because herpes viruses are highly species-specific, having evolved in parallel with each species. Consequently the genomes of primate- and rodent-specific herpes viruses are very distinct.[134] Moreover, the fully humanized immunodeficient mice that support the development and maintenance of a functional human immune system have had a lengthy period of development.[126,127]

Because of the lack of directly relevant murine models, investigators relied instead on human in vitro studies and on general support from studies of unrelated murine viruses causing murine disease. These immunologic studies from the 1980s revealed how T cells play a crucial role in the control of herpes virus infections and that individuals with compromised T-cell functions (whether as a result of primary immune deficiency, HSCT, or

HIV infection) were at high risk of developing disseminated infection and multi-organ disease. Studies assessing the immune response to murine herpes viruses had suggested that virus-specific $CD8^+$ CTLs were a major contributor to effective virus control and that these cell types could be adoptively transferred to syngeneic mice to confer immunity to murine viruses.[135–139] Subsequently, $CD4^+$ T cells were shown to be important for sustained viral control and memory formation.[140] In vitro studies in human healthy donors and patients after allogeneic HSCT showed that HLA-matched CMV- or EBV-specific CTL lines specifically killed CMV- or EBV-infected target cells.[141,142] Moreover, in a prospective single-center cohort study of 20 patients undergoing allogeneic BMT from a CMV-seropositive HLA-identical sibling donor, patients with detectable CMV-specific T-cell responses in vitro were protected against CMV pneumonia (10/10), whereas 6/10 patients without T-cell responses died from this complication.[141] Investigators were sufficiently encouraged by these strong preclinical/associative clinical data to study the adoptive transfer of CMV- or of EBV-specific T cells to HSCT patients despite the lack of a directly relevant animal model.

In the first clinical study of CMV-specific T-cell transfer in three patients[143] and a subsequent phase I/II trial investigators showed that infusion of CMV-specific $CD8^+$ CTL clones was safe and prevented CMV reactivation in 14/14 patients without inducing GVHD.[144] However, sustained responses were only observed in patients that simultaneously reconstituted the $CD4^+$ T-cell compartment and the durability of this protection was not well established. To treat EBV-LPD after HSCT, investigators infused lymphocytes obtained from the stem cell donor. Although this adoptive transfer of unmanipulated donor lymphocytes containing T cells produced complete remissions of the lymphoproliferation, the presence of alloreactive T cells within the infused population induced severe GVHD in several patients, associated with significant morbidity and mortality.[145,146] Subsequently, therefore, investigators infused stem cell donor-derived T cells that were cultured ex vivo and shown to be EBV-specific. These cells were safe and effective as prophylaxis or therapy for EBV-LPD, resulting in sustained viral and disease control.[129] Because the T cells were genetically marked (using the neo gene), the fate of the infused T cells could be determined and demonstrated long-term persistence beyond 10 years.[130]

Thus, clinical translation of virus-specific T-cell therapies was possible primarily based on human in vitro preclinical and associative clinical studies. The supporting murine data—though conceptually crucial—addressed only the general principle of whether (murine) T cells could control (murine) viral disease. Although it might have been easier and faster to implement the clinical studies if investigators had animal models that were clinically relevant, clearly it would have been wholly inappropriate to have waited for these to appear and deny treatment in the interim. Nonetheless, the recent description of humanized mouse models to study human EBV infection in the context of the human immune system recapitulate with great accuracy the hallmarks of EBV infection and immune control in vivo and have matched clinical findings.[132,133] These models will allow us to dissect the in vivo molecular pathogenesis of EBV-associated malignancies such as diffuse large B-cell lymphoma[147] or hemophagocytic lymphocytosis[148] and to serve as preclinical treatment models. However, even these humanized models will never fully answer the question of risk for T-cell–mediated allo-reactivity or cross-reactivity with healthy adult human tissue, and the importance of linking iterative preclinical and clinical studies will remain paramount.

Tumor-Directed T-Cell Therapy Using Transgenic T-Cell Receptors

The environment following HSCT is ideally suited to the adoptive transfer of T cells. The lymphodepletion associated with the conditioning regimen favors production of homeostatic cytokines such as interleukin (IL)-7 and IL-15 that favor T-cell growth and expansion, while the absence of endogenous T cells reduces competition for these agents and for residual

antigen presenting cells. As described in the preceding section, generation of virus-specific CTLs to prevent and treat viral disease as well as virus-associated malignancies is now well established and the stage is set for widespread implementation.[149] Relatively few tumors, however, express “strong” antigens such as those associated with oncogenic viruses. Instead, most tumor-associated antigens (TAAs) are weakly immunogenic self-antigens such as cancer testis antigens (NY-ESO-1, MAGE, PRAME, SSX2), Wilms tumor 1 protein, or survivin, an inhibitor of apoptosis protein.[150] Cytotoxic T cells with high-affinity T-cell receptors (TCRs) directed against these weak self TAAs are often deleted or tolerized in the host, making it difficult to generate high-affinity tumor-directed T cells that will efficiently recognize and kill tumor target cells. To overcome this problem, we and others have redirected antigen-specificity against tumor cells by transfer of engineered artificial TCRs using either CARs targeting tumor-associated cell surface antigens or $\alpha\beta$-TCRs targeting intracellular TAAs that are processed and presented as peptides in the context of MHC molecules.[151,152] It seems likely that HSCT will represent a useful means of obtaining the lymphodepleted state that may be optimal for the expansion and anti-tumor activity of the transferred engineered T cells. While mouse models have proved useful in predicting the safety and efficacy of many of these approaches, they have also revealed undoubted inadequacies.

One of the most widely used clinical approaches is to adoptively transfer T cells that express CARs targeting the CD19 antigen, which is expressed on a high proportion of B-cell malignancies. In both human and mouse CD19 CAR studies, several critical factors influenced the persistence, expansion and anti-tumor activity of the CAR T cells, the most important of which appeared to be the choice of the co-stimulatory endodomains in the CAR construct, disease model and chemotherapy used for the lymphodepleting conditioning regimen pre-infusion. Thus, direct comparisons in lymphoblastic leukemia xenograft models compared the expansion and anti-tumor activity of T cells expressing a CD19 CAR with or without the CD28 co-stimulatory endo-domain.[153,154] While the addition of the CD28 endodomain was beneficial, substitution of an alternative co-stimulatory endodomain derived from CD137 (4-1BB) was clearly superior in terms of persistence, expansion and anti-tumor activity when the CAR T cells were infused in lymphodepleted hosts,[154] a superiority that appears to have been confirmed in clinical human studies and could not have been so clearly predicted from preclinical human in vitro analyses alone.[155,156] However, the murine data did not foreshadow the severe adverse effects from tumor lysis and cytokine storms observed in several of the human patients.[155–159]

Less successful have been mouse models that attempt to predict the outcome of clinical trials using adoptive T-cell therapy employing transgenic $\alpha\beta$-TCRs. Several of these studies reported lethal GVHD when lymphodepleted mice received synge-neic T-cells expressing TCR genes encoding receptors for a range of TAAs. This effect was attributed to cross-pairing of the transgenic TCR with endogenous (“native”) TCR chains, leading to the generation of mixed TCR dimers with self specificity.[160,161] This lethal GVHD can be prevented if endogenous TCR production is knocked down using zinc finger nucleases that target sequences in native $\alpha\beta$-TCR chains.[162] But despite this severe GVHD that frequently occurs in these murine models, to date no such gain of cross-reactivity has been observed in more than 100 human subjects receiving TCR gene-modified T cells for cancer, even after a lymphodepleting regimen and IL-2 infusions[163] and many of these patients with otherwise refractory metastatic melanoma and synovial cell sarcoma have had complete tumor responses from the therapy.[164,165] However, these and other studies using CARs to target TAAs have had toxicities due to expression of the *targeted* epitope by normal tissues or by cross-reactivity with related epitopes on normal tissue; some of these adverse event have produced lethal neurotoxicity or cardiotoxicity.[166] In no case has the murine model successfully predicted this genuine adverse event, and by directing our focus towards an as

yet non-problematic event could be argued to have provided a false sense of security and a misappropriation of effort and resources.

Hence, safety and toxicity assessments for CAR or TCR-redirected T cells must be performed through a careful clinical dose-escalation approach and cannot yet be predicted from mouse models. Fortunately, we have now additional tools to effectively improve safety of clinical gene therapy products by the incorporation of suicide genes such as the herpes simplex thymidine kinase[167] or the inducible caspase 9 (iC9)[168] systems. Incorporation of the iC9 in particular allows for the rapid elimination of transgenic T cells in patients within 30 minutes of administration of the activating drug, apparently without side effects.[168]

CONCLUSIONS

Many articles in this issue of *Seminars in Hematology*, including the current one, have illustrated how remarkably well laboratory mice can mirror human biology despite the huge differences in body size, life span, genetic diversity, metabolism, pharmacokinetics, and immune system composition.[169–172] Murine models can be excellent tools to understand BMT in humans, allowing us to learn from both their successes as well as failures. They illuminate the biological principles, facilitate better understanding of the processes, and inform the next generation of studies. Their contribution to the field has been almost immeasurable as has been pointed out here. Nonetheless, mice are not mini-patients and mouse models do not fully mirror all aspects of human diseases. Investigators must acknowledge the nuances and differences, and discuss the advantages and limitations of the models used for their research. Mouse studies do not replace clinical trials; they were never intended to nor expected to. They, however, always did, do, and will point to what is probable and what could be attempted in human studies. It is reasonable to ask why murine studies fail to translate. Yet other reasons can be the poorly conducted human translational clinical trials, both from lack of complete understanding and also from poor design. There are many examples of human phase I/II trials that have failed to show demonstrable efficacy in randomized phase III trials. The reasons for this are many, complex, and beyond the scope of this review. Failure of clinical trials leads to insights, better ideas, and refinement of the next generation of clinical studies. Likewise for studies with murine models—simply because an intervention that worked well in murine models has not translated into humans should not be viewed as an outright failure of mice as the model, but should lead to refinement and development of better model systems that mirror more closely the human complexity. Not doing so would be antiethical to scientific enterprise and inquiry. The biological principles revealed by mouse models are profound and it is also important to understand the limitations of their clinical implications. Nonetheless it is critical to recognize that while mouse models may be imperfect, they have been, and are, invaluable to the advancement of modern medicine. Stated otherwise, they are imperative for the development of science-based medicine.

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Table 1

Mouse Models for Cellular Therapies

	Advantage	Disadvantage
Syngeneic	– Immunocompetent host – Absence of major histocompatibility (MHC) barriers, no GVHD – Diverse genetically engineered models/strains available to study molecular mechanisms – Spontaneous tumor models available, including metastasis models – Detailed study of interactions between immune system, tumor and microenvironment possible – High reproducibility	– Human cells cannot be tested directly – Inherent differences between mouse and human species: immune system, tumor and microenvironment
Xenogeneic	– Direct testing of human immune cells against human tumor cells – Use of cell lines or primary patient tumor samples	– Immunocompromised host – Cytokine support for human cells suboptimal – Tumor stroma of mouse origin – Development of xenogenic GVHD – Variable reproducibility, depends on passaging conditions of the tumors
Humanized	– Mice reconstituted with a functional human immune system and/or tissue(s) – Allows to study oncogenic viruses with human cell tropism (eg, Epstein-Barr virus–associated malignancies) – Some transgenic strains available (HLA-A*0201, several cytokines, growth factors) – No or minimal xenograft GVHD due to tolerance induction	– Immunocompromised host – Need 10– 12 weeks to establish human immune system – Cytokine support for human cells suboptimal – Tumor stroma of mouse origin (except for the hematopoietic components) – Expensive