Molecular Therapy
Commentary

Predicting Dangerous Rides in CAR T Cells: Bridging the Gap between Mice and Humans

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Anti-CD19 chimeric antigen receptor (CAR) T cells (CART19) immunotherapy has proved to be extremely successful in B cell leukemia and lymphoma,[1] and based on the beneficial results of several clinical trials, two CART19 products are now commercially available in the US.[2,3] However, together with the striking antitumor effects, significant toxicities are associated with CAR T treatment—in particular, cytokine release syndrome (CRS) and neurotoxicity. CAR T toxicities are quite unique and specific to this potent treatment. Therefore, highly trained physicians are required to properly manage these patients. For this reason, the US Food and Drug Administration (FDA) approval for both Novartis and Kite/Gilead was contingent on a specific CAR T risk evaluation and mitigation strategy (REMS) management plan. Nevertheless, since the first pediatric patient treated with CART19 at the Children's Hospital of Philadelphia, we have significantly improved our expertise on the management of CRS and neurotoxicity. However, we still do not have comprehensive knowledge on its pathogenesis.[4–6] Of note, preclinical murine models of CAR T therapy developed before human clinical use failed to predict the observed clinical toxicities with CART19. There is, therefore, an urgent need for preclinical models able to predict the toxicity of future CAR T products and to give deeper insights into the pathogenesis of CART19 toxicities. In this issue of *Molecular Therapy*, Pennell et al.[7] describe the development of a new mouse model aimed to recapitulate key toxicities of chimeric antigen receptor immunotherapy, including CRS and neurotoxicity.

The authors modified a previously developed humanized murine model where all mouse B cells also expressed human CD19 (hCD19).[8] In the original homozygous model, hCD19 expression in mouse B cells was higher than in human B cells and murine CD19 expression and the absolute number of B cells were significantly reduced. Therefore, to overcome these issues, the authors generated a hemizygous version of the original model that resulted in an increased total B cell number and nearly physiologic levels of hCD19 expression.

This new animal model was then used to assess the toxicity of anti-CD19 CAR T cells. The CAR19 construct used in this study features a 4-1BB costimulatory domain and, although not FDA-approved, is being tested in human clinical trials (ClinicalTrials.gov: NCT02028455). Murine T cells retrovirally transduced with the human CAR19 were generated and showed both *in vitro* and *in vivo* activation in the presence of hCD19+ murine B cells. Importantly, *in vivo*, CART19 cells significantly expanded in the spleen, leading to almost complete loss of B cells in the spleen and significant reduction in the peripheral blood. CART19 expansion and the induction of B cell aplasia are key characteristics of CART19 function in the clinic and reflect the strong T cell activation triggered by the engagement of the CAR construct (K.T. Mueller et al., 2016, Blood, abstract).[9]

After demonstrating that, in this model, CART19 can efficiently recognize normal B cells, the authors began testing the antitumor efficacy. They engrafted the hCD19-humanized mice with a murine B cell lymphoma cell line (TBL12) that was engineered to express hCD19. Interestingly, in this model, CART19 were able to induce potent anti-lymphoma activity only when lymphodepletion was induced by cyclophosphamide, similar to observations in human adoptive cell therapy trials.[10–13] Importantly, the authors noted that a subset of mice was succumbing early after hCART19 infusion and speculated that they were experiencing CRS and/or neurotoxicity. To test the possible occurrence of on target and/or off tumor toxicity, non-tumor-bearing mice were injected with CART19 after lymphodepletion. Interestingly, in these mice, CART19 caused acute toxicity (weight loss, clinical signs) and deaths that correlated with higher CART19 and lymphodepletion doses. This is another important parallel of this preclinical murine model with human trials, where lymphodepletion and higher T cell doses correlate with severity of CRS.[14–16] In this context, the authors demonstrated the presence of intestinal-epithelial barrier damage, a finding that is not a classic sign of clinically evident CRS but may potentially be undiagnosed.

Neurotoxicity is a major adverse effect of CAR T treatment[6,17] that has been observed in both leukemia and lymphoma patients treated with CART19. The spectrum of neurotoxicity can span from mild confusion and agitation to aphasia, seizures, cerebral edema, coma, and, in rare cases, also death. In the present study, the authors observed behavioral abnormalities in mice treated with high CART19 doses, suggestive of neurotoxicity. Infiltration of T cells in the brain was detected, and fewer microglial cells were observed in the brain of mice experiencing CRS, potentially indicating cytotoxicity. A key unresolved issue is whether this finding is relevant to the neurotoxicity experienced in the clinic. A recent report from the Seattle group[17] suggests that severe

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neurotoxicity is associated with increased blood-brain barrier permeability, high concentrations of cytokines in the cerebrospinal fluid, and endothelial damage.

Diffuse activation of the immune system and, in particular, macrophages has been described as the main pathogenetic mechanism of CRS.[18] High levels of interleukin 6 (IL-6) and other innate immunity cytokines and markers of macrophage activation, such as ferritin, are found at very high levels in CRS.[19–21] In their study, Pennel and colleagues[7] found that, in the colon and spleens of mice treated with high doses of CART19, there was activation of pathways of both the innate and adaptive immune system, including upregulation of IL-6 signatures. However, neither increased levels of cytokines nor macrophage activation markers were detected in the serum. An interesting observation is the clear role of interferon γ (IFN-γ) signaling in the development of CRS. T cell-secreted IFN-γ could be the trigger of innate immune system activation and of the subsequent release of IL-6. This is in line with the finding of increased serum IFN-γ in CRS patients and, the preclinical beneficial effect of the IL-2-inducible T cell kinase (ITK) inhibitor ibrutinib that is thought to be mediated by reduction of IFN-γ release.[22]

As mentioned, the management of CRS has significantly improved over the years due, in part, to the development of risk-adapted scoring systems and treatment algorithms.[4] A key player in the management of CRS is tocilizumab. It is indeed thanks to the use of this anti-IL-6 receptor antibody, which is routinely used for juvenile arthritis, that the first pediatric patient treated with CART19 was rescued from a very severe form of CRS.[19] The use of tocilizumab was guided by the timely cytokine measurements performed in our patients that revealed extremely elevated levels of IL-6 together with several other cytokines. Remarkably, also in the animal model developed by Pennel et al.,[7] the use of an anti-IL-6 antibody reverses some of the consequences of CRS, such as weight loss.

In summary, the authors of this paper developed an animal model able to recapitulate some aspects of CAR T-mediated toxicity. Other CRS models have been described with both CART19[22] or HER2-CART,[23] but these models either were developed using immunodeficient mice or used first-generation CARs. Other immunocompetent murine models have been developed for CAR T treatment,[24–26] but, no toxicity was observed in those systems. This is the first animal model able to reproduce both CRS and neurotoxicity after CART19. The reasons for the occurrence of CRS and neurotoxicity in this model, but not in others, are not completely clear. Several factors may interplay, including the use of intense lymphodepletion and the special interaction between hCD19 and human CAR19, a system that has been optimized to produce the greatest T cell activation. It is also surprising that a fully human CAR construct works so well in murine T cells and does not cause immune rejection of adoptively transferred T cells before the induction of antitumor effects and CRS.

Despite the similarities of this model with human CART19 toxicities, several differences remain: the lack of IL-6, ferritin and C reactive protein elevation in the serum, and the lack of evident macrophage activation syndrome. These differences might be related to species-to-species variability but also the different function of murine versus human T cells and, not least, the lack of tumor cells in this model.

Another aspect of this work is that it allows the potential pre-clinical evaluation of the toxicity of human CAR constructs. It would be very beneficial to extend this system to future CAR-antigen models and use it to predict human clinical toxicities. However, it might be difficult to recapitulate these results with target antigens whose expression pattern is less known as compared to CD19. This model would be difficult to use in novel CAR-antigen systems where the exact distribution of expression of the antigen is not completely known and would be part of the evaluation for on target off tumor effects. An optimization of this model could be the insertion of the human target gene X in the murine gene X in one allele at embryonic level so that the progeny will express both the human and murine target in all naturally expressing tissues. However, even in this case, murine toxicities may not overlap human ones as target expression can vary among different species.

Overall, this report represents a milestone in the development of preclinical models able to predict human CAR T toxicity and efficacy and could be used to assess therapeutic approaches for CRS neurotoxicity and potentially acquire further insight on the mechanisms of CRS and/or neurotoxicity pathogenesis.

CONFLICTS OF INTEREST

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