Blood’s 70th anniversary: CARs on the Blood highway

As part of a year-long celebration in honor of Blood’s 70th anniversary, we are publishing a series of editorials written by past Editors-in-Chief of the journal. The authors reflect on their experience at Blood in light of the journal’s publication history. Each of these special pieces will highlight and discuss the impact of one or more original research articles that had a significant influence on the field or that mark a pioneering scientific development in hematology that appeared in the journal during the author’s term as Editor-in-Chief.

When invited to contribute an essay on the most important advance in hematology published in Blood during my 2008 to 2012 tenure as Editor-in-Chief, I seminal paper sprung to mind, namely, the first demonstration of clear target-specific activity of chimeric antigen receptor (CAR) T cells in a human clinical trial, contributed by James Kochenderfer, Steven Rosenberg, and coworkers at the National Cancer Institute (NCI).1 The paper consisted of only a single case report about the administration of CD19-CAR T cells to a patient with advanced follicular lymphoma, demonstrating profound persistent B-cell aplasia and marked tumor regression. The findings were so provocative that the editors and reviewers were uniformly enthusiastic, and the paper was published in July 2010. This Blood paper perhaps does not get sufficient credit in the timeline of CAR development, with more attention focused on a subsequent 2011 case report from Porter, June, and coworkers reporting a complete clinical remission and B-cell ablation in a patient with refractory chronic lymphocytic leukemia ( CLL) following CD19 CAR.2 Regardless, it is likely that any reader of Blood is well aware of the flurry of subsequent clinical research activity demonstrating the incredible potency of CAR in the treatment of lymphoid malignancies (reviewed in Maus et al3), culminating in the recent designation of “Cancer Immunotherapy” as the 2013 breakthrough of the year by Science magazine,4 and substantial investment in the technology by both biotechnology startups and pharmaceutical giants such as Novartis.

Zelig Eshhar and coworkers suggested that T-cell recognition and signaling could be rendered more independent, beginning the CAR story in 1989, when they demonstrated that replacement of T-cell receptor α and β (TCRα and TCRβ) extracellular domains with the immunoglobulin variable heavy and variable light chains to create what they termed “T-bodies” resulted in T-cell activation specific to the introduced immunoglobulin chains.5 They refined this concept several years later with single-chain constructs consisting of the variable region of a given antibody as the recognition moiety joined to the structural and signaling components of the CD3 component of the TCR.6 These CARs were shown to target and kill tumor cells in vitro as well as in several natural and artificial in vivo tumor models (reviewed in Maus et al).3

Looking back at the >20-year journey between the initial experiments supporting this groundbreaking concept and the recent accumulation of evidence for its potent clinical efficacy suggests that scientists’ creativity and vision can outpace more prosaic but critical factors necessary for clinical success. In the case of CAR T cells, the missing links were vectors for safe and effective transgene delivery and techniques to maintain functional T cells during ex vivo manipulations and following in vivo delivery. During those 20 years, Blood published many papers contributing to progress in these areas. One of the first areas of intense clinical development for CAR T cells was redirection of T cells to combat HIV infection, with the HIV-envelope (HIV-env)-binding portion of CD4 fused to CD3ζ shown to kill HIV-infected cells in vitro, and target HIV-env–expressing tumor cells in mice.7,8 This concept was rapidly moved into initial CAR clinical trials, with 2 Blood papers reporting low-level-long-term persistence of engineered CD4-CD3ζ T cells, but no biological or clinical evidence for efficacy.9,10 However, further progress was based on the critical discoveries by Michel Sadelain and coworkers that signal 1 TCR signaling was insufficient for a potent, sustained activation of engineered cells and that costimulation was required for sustained T-cell proliferation and activity via inclusion of a CD28 domain within CAR constructs.11-13 Discoveries regarding the functional heterogeneity of various T-cell subsets and development of culture conditions to facilitate survival and transduction of the desired T-cell target population were also important.14,15 A parallel area of clinical development with major implications for later successful clinical use of CAR T cells was treatment of active Epstein-Barr virus lymphoproliferative disease posttransplantation with genetically tagged virus-specific T cells, reported in Blood and elsewhere in the late 1990s.15,16

Development and optimization of efficient, stable, and safe methodologies for transfer of transgenes into T cells were also critical steps in CAR clinical development, and Blood has historically served as home for important papers describing gene therapy advances. Although electroporation of CAR transgene DNA was the delivery method initially used by Eshhar and eventually applied by Till and coworkers in a clinical trial targeting CD20 in lymphoma, efficiency of stable transfer was very low, requiring in vitro drug selection prior to infusion, likely resulting in impaired in vivo function and rapid disappearance of CAR T cells.17 Vector backbones derived from murine gammaretroviruses were employed in a number of clinical trials targeting both T cells and hematopoietic stem and progenitor cells (HSPCs) in the 1990s and early 2000s, with incremental improvements in vector design and transduction conditions culminating in clear clinical improvement for patients with several inherited immunodeficiencies (reviewed in Rivière et al18). However, the subsequent occurrence of leukemias in the HSPC trials and in relevant animal models, linked to insertional activation of proto-oncogenes via strong vector enhancers and gammaretrovirus integration patterns,19-21 temporarily stalled clinical development of clinical trials utilizing integrating vectors, including CAR T-cell studies. Integration profiling and long-term follow-up of patients enrolled in T-cell gene therapy trials did not reveal clonal expansions or concerning integration patterns with mature T-cell targets in contrast to HSPC targets.21,22 Blood published an important direct comparative experiment showing a much lower risk of insertional
leukemogenesis following transduction of mature murine T cells as compared with HSPCs.\textsuperscript{23} Due to continued concern regarding the safety of gammaretroviral vectors, many investigators, including most developing CAR T-cell therapies, redirected efforts toward development of safety-modified HIV-based lentiviral vectors. Multiple papers, including several in Blood, suggested a safer integration profile and a lower chance of proto-oncogene activation associated with the use of lentiviral as compared with gammaretroviral vectors, even when targeting HSPCs.\textsuperscript{24–26}

In 2003, a landmark paper from Brentjens, Sadelain, and coworkers reported that CD19-CAR transduced human T cells could eradicate a disseminated human B-cell tumor in immunodeficient mice, demonstrating the potency of these engineered T cells and the utility of CD19 as a target.\textsuperscript{27} The performance of extensive safety and efficacy studies in animal models, as well as the availability of data from initial human gene therapy clinical trials for both immunodeficiencies and cancer, reassured regulatory bodies, ethics committees, patients, and investors; facilitated initiation of the CAR T-cell trials reported in the 2010 and 2011 CD19-CAR case reports\textsuperscript{1,2;}; and contributed to the explosion of interest in CAR T cells that immediately followed. It is difficult to keep up with the pace of presentations and publications about CAR T cells in hematologic malignancies over the past 4 years. More extensive results from both the NCI (in Blood, Kochenderfer et al\textsuperscript{28}) and the University of Pennsylvania\textsuperscript{29} CD19-CAR T-cell trials, as well as results from a concurrent study from Memorial Sloan Kettering Cancer Center (in Blood\textsuperscript{30}) were published, demonstrating significant, but not always complete or lasting, disease eradication in CLL and low-grade lymphomas.\textsuperscript{28} Even more striking responses to CAR T cells in patients with CD19-positive acute lymphoblastic leukemia (ALL) were reported by Sadelain and coworkers\textsuperscript{31} followed by further encouraging results in both adult\textsuperscript{32} and pediatric\textsuperscript{33} ALL patients, with overall response rates of about 80%. CD19-CAR T cells have been used for disease eradication following allogeneic transplantation\textsuperscript{34,35} with little evidence these cells can cause graft-versus-host disease, and are in development for a range of other hematologic malignancies including large-cell lymphoma\textsuperscript{36} and multiple myeloma.\textsuperscript{37} Much remains to be learned regarding choice of vector backbone, costimulatory moiety, ex vivo culture and transduction conditions, and the need for patient conditioning preinfusion. Engraftment and long-term persistence of CAR T cells at a level detectable by CD4\textsuperscript{+} T lymphocytes was reported by Sadelain and coworkers,\textsuperscript{31} followed by further encouraging results in both adult\textsuperscript{32} and pediatric\textsuperscript{33} ALL patients, with overall response rates of about 80%. CD19-CAR T cells have been used for disease eradication following allogeneic transplantation\textsuperscript{34,35} with little evidence these cells can cause graft-versus-host disease, and are in development for a range of other hematologic malignancies including large-cell lymphoma\textsuperscript{36} and multiple myeloma.\textsuperscript{37} Much remains to be learned regarding choice of vector backbone, costimulatory moiety, ex vivo culture and transduction conditions, and the need for patient conditioning preinfusion. Engraftment and long-term persistence of CAR T cells at a level detectable by flow cytometry appears to be crucial. The pathophysiology and most effective treatment of cytokine release syndrome, landing many patients treated with CAR T cells in intensive care units, is being elucidated.\textsuperscript{38,39}

B-cell malignancies are particularly suited to CAR T-cell therapies because the collateral permanent loss of normal B cells resulting from long-term persistence of CD19-CAR T cells can be satisfactorily coped with via immunoglobulin infusions. However, extension of the approach to myeloid malignancies or to most solid tumors is hampered by the lack of tumor surface molecules that can be targeted specifically and safely, without permanently destroying myelopoiesis or damaging critical normal tissues. Inclusion of suicide genes in CAR T cells, potentially allowing controlled removal of CAR T cells once tumor ablation has occurred, is being explored, but at present, no available suicide gene systems are 100% efficient in ablation of transduced T cells, a requirement for targeting antigens such as CD33 in acute myeloid leukemia. The ability to remove CAR T cells from the patient might also permit use of an “off-the-shelf,” universal donor CAR T-cell product. Finally, natural killer (NK) cells or NKT cells have also been explored as vehicles for redirected CAR killing of tumors, with potentially much shorter in vivo half-lives, and less risk of allogeneic graft-versus-host effects.\textsuperscript{40,41}

The CAR story continues to develop, and I am certain Blood will continue to feature articles highlighting central advances in the preclinical and clinical development of these complex, exciting, and promising cellular therapies. I am proud that during my tenure as Editor-in-Chief from 2008 to 2012, and as Associate Editor covering gene therapy and stem cells from 1998 to 2007, Blood published so many landmark papers contributing to the successful development of CAR T cells as vehicles to combat human disease.

Cynthia E. Dunbar
Editor-in-Chief, Blood, 2008-2012

References

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