Is It Safer CARs That We Need, or Safer Rules of the Road?

To the editor:

The recent deaths with chimeric antigen receptor–modified T cells ("T-bodies," "designer T cells") have been a wake-up call for all of us to the potential for toxicity of these therapies.[1,2] This follows on a previous report of hepatotoxicity from anti-G250 designer T cells that did not result in deaths.[3] In the April 2010 issue of *Molecular Therapy*, Dr. Heslop wrote an excellent Commentary on the application of these cells and potential means to improve their safety.[4] Some additional thoughts may be warranted that go beyond the issues of target safety. In particular, two points could be made: (i) components useful for expanding tumor-infiltrating lymphocytes (TILs) and first-generation designer T cells (i.e., lymphodepletion and engraftment) may be unwarranted for newer, second-generation agents, and (ii) initial patient exposures for these new agents are most safely initiated at lower levels for untested antigens.

The major focus of preclinical research over the past decade has been to improve designer T-cell "quality" by supplementing with signals that co-opt components of the interaction between antigen-presenting cells and T cells.[5] Like TILs, first-generation designer T cells with signal 1 provided antitumor cytotoxicity for a limited time but ultimately succumbed to activation-induced cell death (AICD) or passed to a resting, anergic state. In contrast, the advanced second-generation agents in these studies added one or more costimulatory signals to obtain signal 1+2 that conferred a new potential to respond to antigen with proliferation and sustained cytotoxicity, with escape from AICD and resistance to regulatory T-cell suppression. Consequently, even a few T cells trafficking into tumor had the potential to respond *selectively* to antigen with *local* intratumoral expansion until tumor elimination. The application of lymphodepletion[6] before T-cell infusion vastly increases T-cell "quantity," superimposing a *nonselective* and *systemic* expansion ("engraftment") on these agents that were designed not to need it.

From the details presented, it is likely that the CD19 death was not due to T-cell toxicity but rather to a recognized complication of the conditioning regimen,[1] a reminder that conditioning, integral to engraftment strategies, is not a benign intervention. In contrast, the Her2 death appears to have been the result of on-target toxicity against normal tissues (lung, bowel, heart) previously known to express antigen.[2] This is reminiscent of the G250 study with toxicity from limited doses of designer T cells by infusion,[3] but in the Her2 case not reversible by steroids due to the vast numbers of Her2 self-reactive T-cells in the engraftment setting and the vigor of second-generation design.

The most instructive clinical parallel is that of donor lymphocyte infusion (DLI) in allo–bone marrow transplantation settings. Patients are engrafted with T cell–depleted marrow to create a chimeric state of donor and host tolerance, whereupon small numbers ($<10^9$) of allo-donor T cells are subsequently infused. With a fully competent alloimmune reaction, these exposures of allo–T cells can be safely managed with a balance of graft-versus-host reaction and antitumor benefit.[7,8] In this, we know that size of dose matters: too high a DLI dose kills. Translated to second-generation designer T cells, infusions under a graded dose-escalation plan should allow recognition of on-target autoimmune toxicities before grade V (death) in the same way that DLIs are "tuned,"[7] where high doses (as in engraftment) may be lethal.

In line with phase I goals, we seek means to safely increment patient exposures while advancing therapeutic aims. In contrast to the natural targets for TILs that were extensively vetted for safety via infusions before the first engraftment was ever tried, the targets of designer T cells are artificially selected and may be unsafe—especially for second-generation agents with their powerful engines for self-perpetuation under incorporated costimulation—thus warranting a cautious exposure. Accordingly, safety testing could be pursued via simple infusions, employing lower starting doses (perhaps 10^8 or 10^9 cells) in line with DLI dosing protocols.[7] The G250 on-target toxicities were recognized early and safely in exactly such a dose-escalation infusion protocol; the system worked: no one died.[3] Engraftment, by contrast, leads to much higher exposures that can be hard to project, with on-target toxicities that can be hard to control, as in the Her2 death.[2]

In terms of efficacy, there is as yet no proof that any of the second-generation designer T cells, with their incorporated costimulation signals, even *need* engraftment, a procedure devised in response to deficiencies of signal 1–only T cells (e.g., TILs). The first studies with second-generation signal 1+2 designer T cells under infusion protocols have just gotten under way, and it is too early to infer either sufficiency or deficiency of any existing second-generation reagents to eliminate tumors without systemic engraftment. Accordingly, it is plausible by this conception that a DLI-type infusion dose escalation could still be productive for Her2 targeting with this advanced agent, with a margin for antitumor benefit and safety, although the engraftment death may have the regrettable result of impeding this path.

In the end, I believe that these new agents merit new thinking, taking a step back from engrafting to permit them to reveal the potential they were designed for. T cells engaged by antigen-presenting cell costimulation eliminate infections with very few starting cognate effectors, and when we have successfully adopted those features into our engineering, I believe that we will likewise be able to eliminate cancers as efficiently, without engraftment. The proposed infusion escalations can be performed intrapatient, so that a personalized, optimally "tuned" dose can be delivered in the manner of DLI. But where infusions with these more advanced reagents are proven therapeutically inadequate at full doses (e.g., 10^{11} cells with cytokine support) and safe, then engraftment, with its higher cost and hazard, is a justifiable next step in the risk escalation.[9]

Finally, if a designer T cell is fully escalated under simple infusions, in which suicide genes are generally not needed, then a suicide gene suggested for engraftment[4] is also not needed if proceeding to this step, because safety of the target will have been established.

doi:10.1038/mt.2010.162

Richard P Junghans[1]
[1]Division of Surgical Research, Boston University School of Medicine, Roger Williams Medical Center, Providence, Rhode Island, USA

Correspondence: *R P Junghans (rpj@bu.edu)*

REFERENCES

1. Brentjens, R, Yeh, R, Bernal, Y, Riviere, I and Sadelain M (2010). Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. *Mol Ther* **18**: 666–668.
2. Morgan, RA, Yang, JC, Kitano, M, Dudley, ME, Laurencot, CM and Rosenberg, SA (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* **18**: 843–851.
3. Lamers, CHJ, Sleijfer, S, Vulto, AG, Kruit, WHJ, Kliffen, M, Debets, R *et al.* (2006). Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* **24**: e20–e22.
4. Heslop, HE (2010). Safer CARs. *Mol Ther* **18**: 661–662.
5. Eshhar, Z (2008). The T-body approach: redirecting T cells with antibody specificity. *Handb Exp Pharmacol* **181**: 329–342.
6. Dudley, ME, Wunderlich, JR, Yang, JC, Sherry, RM, Topalian, SL, Restifo, NP *et al.* (2005). Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* **23**: 2346–2357.
7. Mackinnon, S, Papadopoulos, EB, Carabasi, MH, Reich, L, Collins, NH, Boulad, F *et al.* (1995). Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood* **86**: 1261–1268.
8. Sykes, M and Spitzer, TR (2002). Non-myeloblative induction of mixed hematopoietic chimerism: application to transplantation tolerance and hematologic malignancies in experimental and clinical studies. *Cancer Treat Res* **110**: 79-99.
9. Junghans, RP (2010). Strategy escalation: an emerging paradigm for safe clinical development of T cell gene therapies. *J Transl Med* **8**:55.