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Engineered Stem Cells Provide Cancer-Killing iNKT Cells

Mallory Paynich Murray[1] and Mitchell Kronenberg[1,*]
[1]La Jolla Institute for Immunology, La Jolla, CA 92037, USA
*Correspondence: mitch@lji.org
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Invariant natural killer T (iNKT) cells have been tested for their potential use in cancer immune therapy, but their low frequency has limited their use. In this issue, Zhu and colleagues propose to overcome this by engineering hematopoietic stem cells (HSCs) to provide a continual source of iNKT cells. (Zhu et al., 2019).

Aside from T cell biologists, there is often confusion about the identity of iNKT cells. First, they are not natural killer (NK) cells. Unlike NK cells, iNKT cells are bona fide T cells that differentiate in the thymus, where they rearrange and express T cell receptor (TCR) genes (Crosby and Kronenberg, 2018). However, they express a TCR α chain, which is identical in nearly all of this cell population. Thus, they are called ''invariant'' NKT cells. Most T cells recognize peptide fragments presented by polymorphic genes encoded in the HLA locus, but iNKT cells recognize self and microbial lipids presented by CD1d, which is not polymorphic. Because CD1d is not polymorphic, iNKT cells will not cause graft-versus-host disease (GVHD), and there is evidence from this publication and others that they might even help to prevent GVHD (Karadimitris and Chaidos, 2012). iNKT cells have other properties that are imparted by their TCR during thymic education and that might make them useful for cancer immune therapy; these properties include the ability to carry out rapid effector functions, such as IFNγ release and cytotoxic activity. iNKT cells also home to tissues where they can persist. Furthermore, activated iNKT cells express CD40L, allowing them to stimulate dendritic cells (DCs) and other innate immune cells, therefore providing an adjuvant effect (Figure 1) (Crosby and Kronenberg, 2018). This adjuvant effect and the iNKT cells' reported ability to kill tumor-associated macrophages (Song et al., 2009) are particularly important because they suggest that iNKT cells could act against tumors, even against cancers that do not express CD1d (Figure 1, solid as opposed to dotted line).

Interest in stimulating iNKT cells to fight cancer was excited by the initial finding that a glycolipid antigen known as α-galactosyl ceramide (αGalCer), which prevented liver metastases in mice, had anti-metastatic activity because it is a potent stimulant for iNKT cells residing in the liver (Cui et al., 1997). The use of iNKT cells in tumor immune therapy in patients has been investigated in pre-clinical studies and clinical trials for nearly two decades, with benefit to some patients (Wolf et al., 2018). Two general kinds of strategies have been used to activate iNKT cells in the fight against cancers. One path has been the use of medicinal chemistry and various lipid antigens related to αGalCer, in some cases formulated in liposomes or after incubation with dendritic cells. The second path has been cell-based; examples include *ex vivo* expanded iNKT cells, or more recently, the use of iNKT cells as a platform for chimeric antigen receptors (CARs) for neuroblastoma or B cell malignancies. One problem is that the frequency of iNKT cells in peripheral blood is 0.01% or less in most individuals. This issue could be mitigated because iNKT cells are relatively easy to expand in culture to a level that might be sufficient for personalized immune therapy. The long-term persistence and function of the expanded cells might not be sufficient, however, although a recent publication reported that the survival of the iNKT cells expressing CARs was enhanced by engineering them to express IL-15 (Xu et al., 2019).

In the paper by Zhu and colleagues, the strategy was to engineer HSCs to express the rearranged TCR genes encoding the α and β chains of an iNKT cell TCR and to test these stem cells in a bone-marrow-liver-thymus (BLT) humanized mouse model. The HSCs could give rise to all blood cell types, but because of allelic exclusion, in which a productively rearranged TCR gene allele prevents the rearrangement and expression of any others, the percentage of T lymphocytes that were iNKT cells was greatly enhanced, up to 60% of the total. The percentage of iNKT cells in the T cell pool could be adjusted, however, by titrating the amount of the lentivirus vector used for the transduction. The authors demonstrated that the iNKT cells derived from the engineered HSCs (HSC-iNKT cells) have the expected properties of human iNKT cells in terms of their distribution, phenotype, and ability to secrete cytokines. Toxicity and tumor formation were not observed in BLT mice with increased iNKT cells as a result of HSC engineering. Although these mice did succumb to GVHD due to untransduced HSCs in the inoculum, which gave rise to human T cells reactive to mouse tissues, they were protected compared to control BLT recipients of HSCs that did not express the iNKT cell TCR. Importantly, the HSC-iNKT cells persisted over five months and in secondary recipients, as well.

iNKT cells can stimulate the anti-tumor response in multiple ways, including direct killing of $CD1d^+$ tumor targets, stimulation of NK cells to become more active killers, activation of DCs similar to adjuvants to boost CD8 T cell and other responses, or by killing tumor-associated macrophages (Figure 1)

(Song et al., 2009, Wolf et al., 2018). The investigators used *in vitro* models to show that HSC-iNKT cells could carry out all of these responses, although some of the *in vitro* model systems did not closely represent the *in vivo* situation or they required HSC-iNKT cell stimulation with αGalCer. This stimulation might not be feasible when the iNKT cell frequency is very high because of adverse events that have been reported with this or similar glycolipids (Tefit et al., 2014). It is impressive, however, that *in vivo* the HSC-iNKT cells could stimulate a protective immune response to a myeloma cell line and a melanoma cell line that expressed CD1d. The myeloma results are particularly striking because the CD1d-dependent anti-tumor response did not require the addition of αGalCer, and it was therefore presumably based on the recognition of autologous glycolipid antigens that can stimulate iNKT cells.

Zhu and colleagues demonstrate that engineering HSCs to generate iNKT cells can effectively increase a persistent population of iNKT cells, but is an increase in iNKT cells beneficial in cancer? In many types of cancers, including neuroblastoma, colorectal cancer, and head and neck squamous cell carcinoma, iNKT cells within the tumor or in circulation correlated with increased patient survival (Wolf et al., 2018). It remains uncertain whether the efficacy of iNKT cell therapies will depend on CD1d expression by the tumor, but this study and others demonstrate that iNKT cells mediate important adjuvant effects that may also occur in patients. iNKT cells are part of a larger family of innate-like lymphocytes, including NK cells and γδ T cells, which are T lymphocytes with a different type of antigen receptor. All of these lymphocyte types rapidly respond to stimuli and are easily amenable to expressing CARs and other forms of engineering, for example, "kill switches" to remove cells should they prove harmful. Clinical trials are currently underway using each of these innate-like lymphocytes, and stem cells are now being used to generate large numbers of NK cells for immune therapy (Wang et al., 2019). Clearly, HSC-derived innate-like lymphocytes provide advantages compared to traditional adoptive T cell therapies, and future studies will continue to shed light on which of these cell types prove to be the most effective.

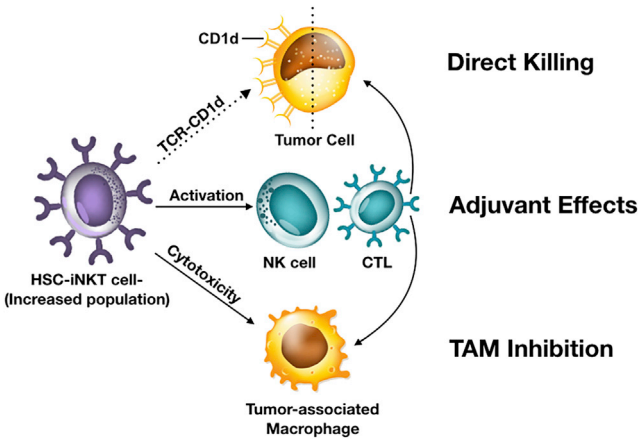

Figure 1. Mechanisms by which iNKT Cells Target Tumors
By genetically engineering HSCs with the iNKT cell TCR, total iNKT cells were sustained long-term in humanized mice. iNKT cells can directly lyse tumor cells by recognizing CD1d (Direct Killing, dotted line). iNKT cells also activate other cells that lyse tumor cells and/or tumor-associated macrophages (TAMs) in a CD1d-independent manner (Adjuvant Effects). iNKT cells can also directly kill TAMs through CD1d-dependent or independent mechanisms (TAM Inhibition).

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