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A milestone method to make natural killer T cells

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A differentiation protocol to produce off-the-shelf natural killer T cells may enable clinical application.

Autologous chimeric antigen receptor (CAR) cell therapies are manufactured individually for each patient in a costly, time-consuming process from cells that may be suboptimal, and there is a growing demand for off-the-shelf, allogeneic products that can expand accessibility to a wider range of patients. In *Nature Biotechnology*, Li et al.[1] describe a method to generate allogeneic CAR natural killer T cells (alloCAR-NKTs) that lends itself to clinical application. Building on previous work, they differentiate cord-blood hematopoietic stem and progenitor cells into alloCAR-NKTs using a culture system that avoids mouse feeder cells and serum. The alloCAR-NKTs can target various cancers (including multiple myeloma) and exhibit translatable therapeutic properties, such as tumor homing, expansion in vivo and robust antitumor functionality (Fig. 1). This work offers a major technological advance towards the clinical-scale manufacturing of alloCAR-NKT therapeutics.

NKTs have long been recognized for their potent antitumor properties on the basis of studies in mouse tumor models and correlative findings in patients with cancer. This rare cell type has properties of both NK cells and T cells. They are innate-like T cells that are evolved to react to self-derived and microbial glycolipids presented by a monomorphic HLA class I-like molecule, CD1d[2]. Type 1 (or invariant) NKTs — the subject of the study by Li et al. — are further defined by expression of the invariant T cell receptor α-chain TRAV10–TRAJ18 (formerly Vα24–Jα18), and by reactivity to the synthetic glycolipid α-galactosylceramide. They have been shown to effectively localize to tumor tissues and to colocalize with tumor-associated macrophages, and can kill these tumor-supportive cells in a CD1d-dependent manner[3]. NKTs also mediate activation of NK cells and help dendritic cells to induce tumor-specific T cell responses[4,5]. These intrinsic antitumor properties are absent in polyclonal T cell products, which makes NKTs an attractive alternative for adoptive cell therapy applications (including CAR-redirected cancer immunotherapy).

Our group and collaborators recently tested autologous NKTs that coexpress a GD2-specific CAR and the cytokine IL-15 in a first-in-human study[6]. Interim results showed objective responses in children with relapsed or refractory neuroblastoma, without dose-limiting toxicities[6]. However, a major limitation of autologous CAR-redirected immunotherapy is that immune cells derived from patients are often adversely affected by the disease and/or prior genotoxic therapies. Patient-specific products also require long manufacturing times and are expensive.

Allogeneic immunotherapies that use conventional T cells are limited by the risk of inducing life-threatening graft-versus-host disease. A range of strategies has been used to prevent this, including gene editing. These interventions introduce their own safety risks,

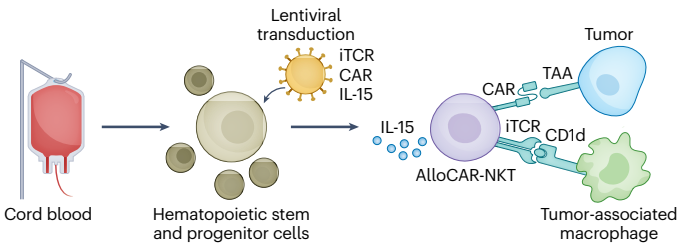

Fig. 1 | AlloCAR-NKT cells derived from hematopoietic stem and progenitor cells target tumor cells and tumor-supportive myeloid cells. Hematopoietic stem and progenitor cells from cord-blood donors are transduced with the NKT cell invariant T cell receptor (iTCR) and a tumor-specific CAR of choice, followed by large-scale ex vivo expansion and differentiation into mature CAR-NKTs for off-the-shelf cancer immunotherapy. AlloCAR-NKTs target tumor cells directly in a CAR-dependent manner and indirectly by eliminating tumor-supportive myeloid cells (such as tumor-associated macrophages) in a CD1d-dependent manner. TAA, tumor-associated antigen.

such as off-target genome editing, and can increase manufacturing time and complexity. Moreover, polyclonal T cells are not inherently tumor-reactive and when used as CAR effectors, their antitumor activity depends entirely on the specificity of the CAR — which can be circumvented by tumor escape variants. By contrast, innate or innate-like lymphocyte subsets (such as NK, γδ T and NKT cells) have inherent antitumor properties and do not mediate graft-versus-host disease. Moreover, NKTs protect against graft-versus-host disease, and their recovery is associated with increased disease-free survival in patients with leukemia who are undergoing allogeneic stem cell transplantation[7].

AlloCAR-NKTs can be obtained from various sources, such as peripheral blood mononuclear cells. Our group is currently testing alloCAR-NKTs derived from peripheral blood mononuclear cells in a phase 1 clinical trial in patients with B cell malignancies. Interim results demonstrate that the therapy is well-tolerated and that objective responses were reached in 4 of 9 evaluated patients[8]. However, because human peripheral blood mononuclear cells contain a very low frequency of NKTs (averaging about 0.1% of T cells), alternative sources are being investigated for cell therapy applications. Efforts in this direction have already demonstrated that large numbers of functional NKTs can be generated from induced pluripotent stem cells[9] and from hematopoietic stem and progenitor cells[1,10], which paves the way for the next generation of therapies based on alloCAR-NKTs.

In their study, Li et al. introduce an improved differentiation protocol that is compatible with clinical application. In previous work, the authors relied on artificial thymic organoids and mouse feeder cells to support cell growth[10], both of which preclude clinical manufacturing. The new protocol generates alloCAR-NKTs from cord-blood

hematopoietic stem and progenitor cells in a five-stage, six-week process that can be completed entirely without feeder cells. As part of this process, hematopoietic stem and progenitor cells are transduced with lentivectors that deliver the invariant NKT T cell receptor, a CAR of interest and, optionally, additional genes (including immune enhancement molecules, reporters or safety controls that can influence in vivo functionality), which showcases the remarkable versatility of this technology. The authors demonstrate a robust 10^6-fold expansion of hematopoietic stem and progenitor cells from diverse cord-blood donors, which generated mature alloCAR-NKTs of high yield and purity.

In a specific application, Li et al. use their protocol to generate alloCAR-NKTs that coexpress a B cell maturation antigen-specific CAR that is used for CAR T cell immunotherapy of multiple myeloma and the cytokine IL15, which is known to enhance antitumor potency and cell proliferation. Focusing on multiple myeloma, they comprehensively characterize these cells through phenotypic, functional and transcriptomic studies and show that IL-15-expressing alloCAR-NKTs exhibit potent antitumor activity, expansion and persistence, and effective tumor homing in vivo, while maintaining the innate advantages of NKTs (including a low risk for graft-versus-host disease and cytokine release syndrome).

The alloCAR-NKTs possess additional properties that appear promising for off-the-shelf immunotherapy. Compared with NKTs derived from peripheral blood mononuclear cells, alloCAR-NKTs from hematopoietic stem and progenitor cells skew toward NK-cell-like differentiation. This may be advantageous for targeting CD1d-negative tumors by engaging cytotoxicity receptors specific to NK cells. Moreover, the authors describe an intrinsic 'hypoimmunogenic' phenotype that enables alloCAR-NKTs to resist rejection by recipient T and NK cells. This phenotype is sustained at the level of transcription, epigenetics and signaling pathway modulation, and remains stable through in vivo antitumor responses. Finally, the authors show that their alloCAR-NKTs retain the ability of NKTs derived from peripheral blood mononuclear cells to target macrophages, as they selectively deplete immunosuppressive tumor-associated macrophages and myeloid-derived suppressor cells in the tumor microenvironment, which provides a substantial advantage over T cell therapies.

Despite the considerable technological advance presented in this work, several limitations remain. First, the multistage, six-week ex vivo manufacturing process is much longer and more complex than the two-week numeric expansion protocol for alloCAR-NKTs derived from peripheral blood mononuclear cells, which may prove challenging in clinical settings. Second, NKT cells derived from hematopoietic stem and progenitor cells have a similar, but not identical, phenotype to those made from peripheral blood mononuclear cells. Specifically, the former group lacks the $CD4^+$ subset (a major functional population of human NKTs) and the clinical consequences of missing this subset are unknown. It is worth mentioning that most CAR-NKTs isolated from patients in the neuroblastoma clinical trial were $CD4^+$, which is the dominant NKT subset in children[6]. Third, it is unclear how the low levels of HLA expression in these alloCAR-NKTs will translate clinically. The proposed 'Goldilocks' scenario in which HLA class I expression is below the level required for recognition by T cells but above the threshold for NK cell activation remains hypothetical. More broadly, protection of allogeneic cell therapy products from rejection is one of the most pressing challenges in the field to ensure that the cells persist long enough to mediate durable tumor regression.

In conclusion, the technology of Li et al. to engineer hematopoietic stem and progenitor cells into alloCAR-NKTs offers a new, versatile, and scalable platform for generating NKT products for off-the-shelf cancer immunotherapy. The study represents a major milestone on the way to eventual clinical testing of these products, and the initiation of such a clinical study in patients with cancer is eagerly anticipated.

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Competing interests

The author declares no competing interests.