Off-the-Shelf CAR-NK Cells for Cancer Immunotherapy

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CAR-T therapy has shown great success treating blood cancers, but drawbacks include high manufacturing costs and potentially fatal toxicities such as cytokine release syndrome. In this issue of *Cell Stem Cell*, Li et al. (2018) describe how engineered iPSC-derived NK cells armed with NK-tailored CAR constructs (CAR-iPSC-NK cells) provide better options for anti-cancer immunotherapy.

Immunotherapy is rapidly evolving into the "fifth pillar" of cancer treatment, increasingly used alongside more established cancer treatments such as surgery, chemotherapy, radiation, and targeted therapy. One promising branch of cancer immunotherapy is chimeric antigen receptor (CAR)-engineered immune cells. CARs are artificial receptors composed of an extracellular antibody-derived antigen binding domain, followed by a hinge and transmembrane region and intracellular costimulatory and main signaling domains, usually derived from T cell receptor signaling moieties (Sadelain et al., 2017). Antigen binding triggers an intracellular signaling cascade and subsequent activation of the immune cell against the antigen-expressing target cell. CAR therapy was pioneered using autologous human T cells (CAR-T) and has seen great success treating patients with hematological malignancies (June and Sadelain, 2018). However, CAR-T clinical trials for the treatment of solid tumors have been somewhat disappointing, in part because the highly immunosuppressive tumor microenvironment presents an obstacle that is not encountered in blood cancers (Lim and June, 2017). Furthermore, autologous T cells are costly and labor-intensive to manufacture and can be logistically challenging to deliver to patients due to the personalized nature of the treatment. It also introduces variability that makes the prediction of efficacy and safety for a given patient extremely difficult. There is a great need for an "off-the-shelf" product: allogenic immune cells that can be manufactured on a large scale and readily distributed to treat a broad range of cancer patients.

Natural killer (NK) cells mediate short-lived rapid responses against malignant cells and offer an alternative to T cells in CAR therapies, and CAR-NK clinical trials are already underway. NK cells have the potential to be an allogenic therapeutic, as they do not require strict HLA matching or carry the risk of graft-versus-host disease. CAR-NK cells do not present the same safety concerns as CAR-T cells, such as cytokine release syndrome observed in many CAR-T clinical trials (Rezvani et al., 2017). However, primary NK cells are difficult to isolate, purify, and transduce, resulting in a heterogeneous mixture of cells that often expand poorly (Zeng et al., 2017). The NK cell line NK92 has been used in the clinic, as it can expand easily and indefinitely, but it requires irradiation prior to infusion into patients due to chromosomal abnormalities and the risk of malignant transformation (Rezvani and Rouce, 2015). Induced pluripotent stem cells (iPSCs) offer another renewable source of NK cells that can be standardized as an off-the-shelf therapy.

In this issue of *Cell Stem Cell*, Li et al. (2018) demonstrate the feasibility of iPSC-derived CAR-NK cells as a treatment for mesothelin-overexpressing ovarian tumors. CAR-NK studies overwhelmingly use CAR constructs that were designed for T cells, containing T cell costimulatory domains such as CD28, which is not found in NK cells (Hermanson and Kaufman, 2015). In contrast, Li and colleagues expressed CAR constructs specifically tailored to enhance NK cell activation in their iPSC-derived NK cells and showed that these NK-tailored CAR constructs perform better than CAR constructs designed for T cells subsequently expressed in NK cells. First, they screened a panel of CAR constructs designed for NK cell activation in NK92 cells. It was found that they were more cytotoxic against target cells and expressed more proinflammatory cytokines than NK92 cells with third generation CARs typically expressed in T cells. In their chosen construct, they showed that the transmembrane NKG2D and intracellular 2B4 domains are necessary for the optimal NK signaling and antigen-induced NK cell-mediated cytotoxicity.

The authors then expressed the optimal NK-specific CAR construct in human iPSCs and demonstrated similar phenotypes between iPSC-derived and peripheral blood NK cells. CAR-iPSC-NK cells released CD107a, expressed interferon-γ, and mediated cytotoxicity against the appropriate target cells. After verifying *in vitro* activity against mesothelin-expressing target cells, Li and coworkers tested the CAR-iPSC-NK cells in a mouse xenograft model of ovarian cancer. Mice were injected with NK cells after tumor cell inoculation, and IL-2 and IL-15 were administered for 3 weeks to aid in NK cell expansion. iPSC-NK cells expressing the NK-specific CAR were more effective at lowering the tumor burden and lengthening survival than iPSC-NK cells expressing a traditional T cell CAR construct, further highlighting the benefits of adapting the CAR construct to the immune cell type in which it is expressed.

Li and colleagues also compared *in vivo* antitumor efficacy between iPSC-NK cells with the NK CAR construct and primary T cells with a T cell CAR construct. Although reduction of tumor burden was similar between the two groups, the NK

group had significantly longer survival due to lessened toxicity. Mice receiving CAR-T treatment lost significant body weight and sustained pathogenic damage to multiple organs including the liver and lungs, leading to early deaths unrelated to tumor burden. These mice had sustained elevated levels of interferon-γ and IL-6, correlating to cytokine release syndrome observed in the clinic. The CAR-NK-treated mice did not display weight loss, organ pathology, or elevated cytokine levels, indicating that it may be safer than traditional CAR-T therapy.

CAR-iPSC-NK cells may present an attractive alternative as a safer, renewable, off-the-shelf CAR therapy, but clinical efficacy and safety remain to be seen. Since antitumor efficacy was similar with CAR-NK and CAR-T with their respective optimal CAR constructs *in vivo*, CAR-iPSC-NK therapy will likely encounter many of the same obstacles as current CAR-T therapies in treating solid tumors, such as heterogeneous tumor populations, on-target off-tumor effects, and the immunosuppressive tumor microenvironment. The authors reported that CAR-iPSC-NK cells stopped proliferating after exogenous cytokine administration was stopped—systemic cytokine administration can translate to high costs and potentially unwanted side effects with clinical use. Although CAR-iPSC-NK cells showed antitumor effects after just a single injection in mice, patients may need multiple doses due to the shorter lifespan of NK cells. This may initially seem like a drawback, but may ultimately prove to be advantageous, as iPSCs are an unlimited NK source. Therefore, the therapy can be treated more like a drug with controlled and repeated doses, perhaps making it easier to control dangerous side effects with more frequent, short-lived infusions of cells. Further ablation of HLA-I and II expression on CAR-iPSC-NK cells can also be considered to avoid host-versus-graft depletion.

The authors illustrated that iPSC-derived NK cells were shown to have a diminished risk of cytokine release syndrome, and unlike T cells, NK cells are not strongly associated with graft-versus-host disease. However, CAR-iPSC-NK cells are not without their own set of safety concerns. Their safety profile may be equivalent to NK92 in that they have the potential for malignant transformation. Additionally, patient immune responses to allogenic iPSCs remain to be seen; few clinical studies of iPSC-derived cells have been completed (Trounson and DeWitt, 2016). Overall, the authors present a compelling study that takes a major step in exploring alternative off-the-shelf CAR therapies with CAR constructs specific to the immune cell type being utilized. Ultimately, CAR-iPSC-NK cells may have greater efficacy if armored with cytokines or checkpoint blockade inhibitors as seen in CAR-T studies (Li et al., 2017), and clinical studies will reveal safety concerns down the road.

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