



Editorial Overview

Natural killer cells for cancer immunotherapy: a new CAR is catching up



Immunotherapy has revolutionised cancer treatment. Since the approval of ipilimumab (an anti-cytotoxic T lymphocyte antigen [CTLA]-4 monoclonal antibody) in 2011 for the treatment of patients with malignant melanoma, immune checkpoint inhibitors such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 have demonstrated their power in the clinic to treat previously untreatable terminal tumours. These success stories finally led to James P Allison and Tasuku Honjo winning the Nobel Prize in Physiology or Medicine in 2018. The US Food and Drug Administration (FDA) approved the first cell-based immunotherapy, chimeric antigen receptor (CAR)-T cell therapy, for the treatment of refractory B-cell acute lymphoblastic leukaemia in 2017, and the second one for the treatment of B-cell non-Hodgkin lymphoma in 2018.

Undoubtedly both immunotherapy approaches have proved their own importance in fighting cancer, with a caveat: both require sufficient functional, primary T cells. This requirement can be challenging when treating so-called “cold tumours” that do not contain T cells to be unleashed, or when a patient’s T cells are almost entirely wiped out from a first-line therapy. It can be time-consuming to engineer donated T cells and expand them to obtain enough CAR-T cells, and currently available CAR-T treatments are expensive. Moreover, CAR-T therapy still has some way to go as a therapy for solid tumours. All these challenges highlight the need to seek other immunotherapy options, and recent developments have shown natural killer (NK) cell therapy as one of the most promising.

NK cells are a type of lymphoid cell essential for the innate immune system. They recognise “non-self” cells without the need for antibodies and major histocompatibility complex (MHC), executing a rapid immune reaction. The broad cytotoxicity and rapid killing make NK cells ideal for the use in cancer immunotherapy. Indeed, long before the era of CAR-T, researchers had attempted to harness NK cells to fight cancers. These attempts can be tracked back to clinical studies in the late 1980s, but technical, logistical and financial challenges excluded the application of blood NK cells as an exciting and promising cancer therapy at the time. Over the past decade, the field has witnessed numerous important developments. Pre-clinical and clinical studies have demonstrated the safety and efficacy of allogeneic NK cells against various hematological malignancies and solid tumours and several clinical trials are currently ongoing.

The huge success of CAR-T cells generated enthusiasm to genetically modify NK cells with CARs to sharpen their tumour-killing capacity. CAR-NK cells have several advantages over CAR-T cells. First, unlike CAR-T cells, CAR-NK cells retain an intrinsic capacity to recognise and target tumour cells through their native receptors, making the escaping of tumour cells through downregulation of the CAR target antigen less likely. Second, CAR-NK cells do not undergo clonal expansion or immune rejection within days to weeks, and thus they do not present

the same safety concerns, such as cytokine release syndrome, observed in many CAR-T clinical trials. Lastly, NK cells do not require strict HLA matching and lack the potential to cause graft-versus-host disease, an important risk imposed by CAR-T cell immunotherapy, which make it possible for CAR-NK cells to be an off-the-shelf allogeneic therapeutic.

Primary NK cells are difficult to isolate, purify, and transduce, often producing a heterogeneous cell population that expands poorly. However, the NK cell line NK-92 can expand easily and indefinitely in vitro and has been used in the clinic, which makes it a great renewable resource to generate CAR-NK-92 cells. Due to the usual concerns regarding immortal cell lines, such as chromosomal abnormalities and the risk of malignant transformation, NK-92 requires irradiation before infusion into patients, which can suppress proliferation of NK-92 cells while maintaining their full cytotoxic activity. Induced pluripotent stem cells (iPSCs) can offer another renewable and potentially better resources of NK cells. A recent pre-clinical study published in *Cell Stem Cell* on June 28, 2018, from Dan Kaufman’s group at University of California, San Diego (USA) explored this possibility. The researchers expressed an optimised, NK-specific CAR construct in human iPSCs and differentiated these genetically modified iPSCs into functional NK cells. They were able to show that these NK-CAR-iPSC-NK cells significantly inhibited tumour growth in an ovarian cancer xenograft mouse model. More importantly, the authors compared in vivo antitumour efficacy between iPSC-NK cells with CAR-T cells and found that although both approaches achieved similar tumour-killing efficacy, NK-CAR-iPSC-NK cell-treated mice exhibited significantly longer survival and did not suffer from weight loss, organ pathology, or increased cytokine levels compared with CAR-T treated mice, indicating that CAR-NK therapy might be a safer option than current CAR-T therapy. This difference would probably make it feasible to treat patients with multiple doses of CAR-NK cells, which might lead to better clinical outcomes than with a single dose, which is used for CAR-T cell therapy owing to limited availability of cells and high cost.

While CAR-T cells have produced exciting clinical results, studies using CAR-NK cells have been largely pre-clinical until very recently. Right now, there are more than a dozen clinical trials registered on clinicaltrials.gov to test CAR-NK cell therapy in both haematological and solid tumours, including glioblastoma, prostate cancer, and ovarian cancer. China launched several clinical trials targeting multiple tumours in 2016, and results from one phase 1 clinical trial (published in the *American Journal of Cancer Research* on June 1, 2018) demonstrated the safety of CD33-CAR-NK-92 cells in patients with relapsed and refractory acute myeloid leukaemia. A European trial testing HER2-specific CAR-NK-92 cells in glioblastoma patients was launched last year, with results expected in the next 2 years. Although both the safety and efficacy of CAR-NK therapy in patients with cancer need to be

further tested in more clinical trials, it is exciting that we are now witnessing a new CAR, with NK cells behind the wheel, that is rapidly catching up with CAR-T cells. It seems unlikely that CAR-NK would replace CAR-T, but it could be an addition to the armamentarium of cell-based immunotherapy. Although it is early to imagine a combinational treatment with CAR-NK and CAR-T cells, recent studies published in the *Journal of Clinical Investigation* (Sept 10, 2018) and *Cell* (Nov 29, 2018) have indicated that NK cells may play an important role in PD-1/PD-L1 blockade immunotherapy and that unleashing both NK cells and T cells simultaneously enhances anti-tumour activity.

Standing at the beginning of 2019, we are enthusiastic. We expect exciting news from CAR-NK therapy clinical trials. We look forward to embracing CAR-NK cells to join our continuing war against cancer in the coming years.

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