Her2-specific CAR-NK cells display enhanced cytotoxicity against breast cancer

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Recent advancements in immunotherapies targeting specific cancer antigens have positioned immunotherapy as a compelling alternative to traditional cancer treatments. Human epidermal growth factor receptor 2 (Her2) is a cancer antigen notably overexpressed in several malignancies, including breast, stomach, and ovarian cancers. Her2 overexpression is associated with aggressive tumor behavior and poor prognosis. Current therapies targeting Her2, such as the monoclonal antibody trastuzumab and chimeric antigen receptor (CAR)-T cells, have shown therapeutic potential but are often accompanied by significant side effects, including cardiotoxicity and graft-versus-host disease. To address these limitations, we engineered natural killer (NK) cells with a Her2-specific CAR, leveraging both the target specificity of CAR technology and the inherent cytotoxicity of NK cells to develop an off-the-shelf therapeutic product. Our aim was to evaluate the efficacy and specificity of these CAR-NK cells against tumor cell lines.

Here, expanded NK cells derived from peripheral blood were engineered to express a Her2-specific CAR. Using CRISPR/Cas9 and adeno-associated virus serotype 6 (AAV6) vectors, we performed precise knock-in of the CAR construct into the AAVS1 safe harbor site. Successful CAR integration was confirmed through nanopore sequencing at the genomic level and flow cytometry at the protein level. The cytotoxic activity of CAR-NK cells was assessed in vitro against Her2-expressing cancer cell lines utilizing live imaging via the Incucyte system.

We successfully generated Her2-specific CAR-NK cells via CRISPR/Cas9 editing and confirmed precise CAR integration by MinION sequencing. Flow cytometry revealed that over 40% of edited NK cells expressed the CAR on their surface, with CAR expression persisting for at least 30 days. In cytotoxicity assays, CAR-NK cells exhibited enhanced cytotoxicity against Her2-positive tumor cells compared to unmodified NK cells, while no significant difference in cytotoxicity was observed against Her2-negative tumor cells.

These findings highlight the successful engineering and functionality of Her2-specific CAR-NK cells, supporting their potential as a therapeutic strategy for Her2-positive cancers. Ongoing in vivo studies are aimed at further evaluating the clinical potential and safety of this approach. Additionally, integration of this technology into large-scale GMP-compliant manufacturing protocols is currently underway.