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Blood 142 (2023) 3632–3633

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

801.GENE THERAPIES

Development of Novel Lipid Nanoparticles and Virus-like Particles for In Vivo Engineering of Immune Cells for Targeted Cancer Therapy

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Recent progress in genetic and cellular engineering has revolutionized medicine and cancer care, offering new avenues for effective treatments and potential cures. However, the success of these therapies depends on safe and efficient delivery of therapeutic molecules to target cells. Despite challenges, viral delivery methods remain the gold standard because the lack of alternative non-viral approaches matching their efficiency and safety. Here, we developed and tested two different targeted engineered non-viral therapeutic vehicles with the goal to perform in vivo immune cell engineering to specifically engineer T cells, natural killer (NK) cells or macrophages to express chimeric antigen receptors (CARs) to mediate improved anti-tumor activity. These studies developed cell-targeted mRNA-lipid nanoparticles (LNPs) and cell-targeted mRNA-viral-like particles (VLPs). The LNPs are targeted using cell-type specific antibodies conjugated to the LNP, whereas the VLPs are targeted to specific immune cells by engineered expression of designed ankyrin repeat proteins (DARPins). Using the ab-LNPs approach, human T cell line (Jurkat) were transduced at 80% with an anti-CD3 vector, the NK cell line (NK92) at 75% with an anti-NKp46 vector, and macrophages at 85% with an anti-CD14 vector. In contrast, there was little non-specific expression in each cell type using naked (non-targeted) LNPs, ensuring specificity. We were also able to specifically engineer and target the VLPs using several times lower concentrations (MOI=1-5) than what is typically used for intact viral transduction. Here, Jurkat cells were transfected at 15% with the anti-CD3 vector, NK92 cells at 20% with the anti-NKp46 vector and macrophages at 60% with the anti-CD14 vector. Testing ab-LNPs in human PBMCs, 1mg of anti-CD3-LNPs transduced the 90% of T cells, the anti-NKp46-LNPs transduced the 20% of NK cells, and the anti-CD14-LNPs transduced the 65% of monocytes, with all of them showing little or non-expression in all other cell lines. Using targeted-VLPs to engineer PBMCs, anti-CD3 vector transduced T cells at 20%, anti-NKp46 transduced NK cells at 20%, and anti-CD14 transduced monocytes at 50%, with low levels of non-specific expression in other cell types. Testing the capacity of our new guided engineered therapeutic vehicles to increase the potential of specific immune cells to kill tumor cells, we co-cultured the different immune cells (T cells, NK cell and macrophages) transduced with our therapeutic vectors (either targeted-VLPs or ab-LNPs) to express an anti-mesothelin (meso) CAR construct. These engineered cells were tested against meso-expressing A1847 ovarian cancer cells. All the immune cells increase their capacity to kill ovarian cancer cells by 2 to 7 times in a standard cytotoxicity assay (Figure 1A and 1B). To assess the in vivo transduction capability of our novel therapeutic delivery vehicles, we are using both immunocompetent B6 mice (for LNPs) and humanized immunodeficient NSG mice (for human VLPs). Our initial studies using targeted-VLPs for in vivo cell engineering demonstrated impressive efficiencies ranging from 15% (for NK cells) to 65% (for T cells). We are currently doing similar in vivo studies to test the efficiency of ab-LNPs. Next, we will translate these promising results to an in vivo ovarian cancer mice model to engineer endogenous immune cells to directly express CARs, as new strategy to overcome the current problems of cell and gene therapies, starting by develop an effective and safety therapy for treatment of diverse refractory malignancies.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-184312

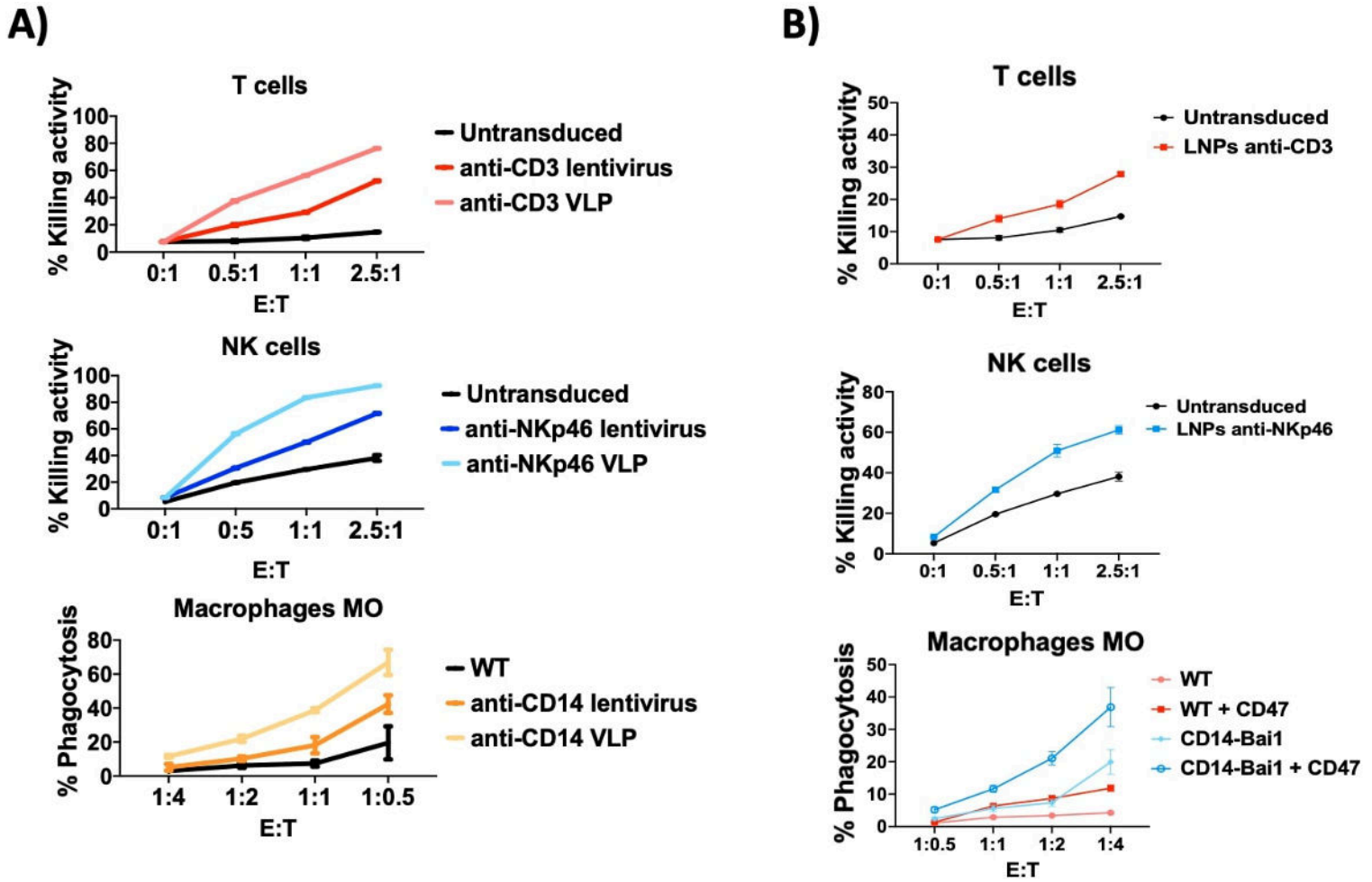

FIGURE 1: Lentivirus, VLP and LNP-mediated engineering of specific immune cells to mediate effective anti-tumor activity. A) For functional studies, lineage specific lentivirus or VLPs were used to transduce primary T cells, NK cells or Macrophages with lineage-specific anti-meso-CARs leading to improved killing of A1847 ovarian cancer cells. Each immune cell population was targeted with a lineage-specific DARPin, as indicated **B)** 1 mg of ab-LNPs were used to transduce primary T cells, NK cells or Macrophages with lineage-specific anti-meso-CARs leading to improved killing of A1847 ovarian cancer cells. Here, the ab-LNPs were conjugated to lineage-specific abs, as indicated. Studies with engineered macrophages were done with and without addition of anti-CD47 ab.

Figure 1