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In vivo HSC engineering generates lineage-restricted, multiplexed CAR-M, NK, and T cells that mediate synergistic anti-tumor activity in pre-clinical models

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Abstract:

Background

The clinical success of cancer immunotherapy, including chimeric antigen receptor (CAR) cell therapy, has revolutionized treatment paradigms and patient outcomes. While hematological tumors have benefited most from these approaches, such transformative success has yet to be achieved for refractory solid tumors. Limited access, complex manufacturing, and high cost of therapy exacerbate the burdens faced by these patients. Methods To address the unmet need of advanced solid tumor patients, we developed a helper-dependent adenovirus (HDAd) platform of virus-like particles (VLP) harboring a 35-kb cargo capacity. The VLP preferentially targets CD46 which is highly expressed on primitive hematopoietic stem cells (HSCs). Mobilization of HSCs into the bloodstream enables in situ transduction followed by stable transgene integration, ultimately aiming to generate a continuous supply of HSCderived engineered immune effectors from a single VLP dose. Results With the goal of programming a lineage-specific and potent multi-cellular anti-tumor immune response, we identified and validated genetic regulatory elements that drive functional CAR expression in human. We next built a vector comprising concatenated myeloid- and lymphoid-restricted HER2 CARs to generate a multiplexed CAR-M, NK, and T cell therapy. The lineage-restricted, multiplexed HER2 CAR VLPs were administered to HSC mobilized, immune competent mice expressing human CD46. Peripheral blood monitoring at Weeks 6 to 12 post-VLP dose revealed HER2 CAR-expressing myeloid, NK, and T cells; total immune cell blood counts were equivalent between VLP-HER2 CAR-treated animals and VLP-GFP or mock controls, demonstrating successful generation of HSC-derived engineered immune cells concomitant with normal hematopoiesis. Importantly, CAR expression in B cells and HSCs was below the limit of detection in vivo, demonstrating lineage-selective promoter specificity. At the study

endpoint, bone marrow from CAR- or GFP-expressing mice was transplanted to EO771/huHER2 orthotopic tumor-bearing mice. The multiplexed CAR-M, NK, and T cell therapy mediated superior tumor control compared to single-lineage CAR alone or GFP control. In the tumor microenvironment, HER2 CAR-M displayed a pro-inflammatory phenotype coupled to a significant increase in CAR-T and NK cell abundance and activation.

Conclusions

These data establish robust proof of concept for the generation and anti-tumor potency of HSC-derived, lineage-restricted CAR-M, NK, and T cell therapy generated via a single dose of VLPs in vivo. HSCs comprise a promising self-renewing reservoir of CAR-M, NK, and T cells mediating synergistic anti-tumor activity. This platform has the potential to overcome myriad therapeutic challenges in advanced solid tumors and provide an innovative off-the-shelf therapy for expanded patient access.