

**NOVEL IMMUNE RECONSTITUTION MODEL HIGHLIGHTS THE IMPORTANCE OF STEALTH STRATEGIES THAT POTENTIATE EFFECTOR CELL FUNCTION AND PROMOTE FUNCTIONAL PERSISTENCE OF NEXT-GENERATION ADOPTIVE CELL THERAPIES**

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**Background** Cyclophosphamide and Fludarabine (Cy/Flu)-based lymphodepletion conditioning (LDC) regimens are recognized as critical steps toward creating greater access to homeostatic cytokines and immune system modulation favorable for adoptively transferred, cell-based cancer therapy. However, protracted LDC has been associated with poor immune reconstitution and increased susceptibility to opportunistic infections. Therefore, the next-generation of cancer cell therapies should prioritize reducing the requirement for LDC regimens. Using a novel immune reconstitution *in vitro* assay, we provide promising evidence for unique stealth strategies designed to potentiate effector cell function without the need for Cy/Flu-based LDC; including, (1) engineering an alloimmune defense receptor (ADR)<sup>1</sup> which targets 4-1BB expressed on alloreactive immune cells and provides a CD3 $\zeta$  signaling boost to potentiate ADR-edited iPSC-derived NK (iNK) and T (iT) cells; (2) knockout of two costimulatory ligands to prevent immunological synapse formation; and (3) a combinatorial therapeutic strategy including CD38-null iNK and iT cells with the addition of anti-CD38 monoclonal antibody (mAb) to deplete alloreactive effector cells.

**Methods** By adjusting the dosage and temporal administration of Cy/Flu *in vitro*, we established a novel lymphodepletion model with a range of phosphoramidate mustard (the active metabolite of cyclophosphamide) and fludarabine-treated PBMCs to mimic the kinetics alloreactive immune depletion and reconstitution post-Cy/Flu LDC. Using high-resolution and mixed lineage- and source-detection flow cytometry panels, different sources and compartments of input cells were analyzed for persistence and activity.

**Results** Using our reconstitution model, preliminary data show that ADR potentiates iNK cells and extends functional persistence by selective targeting of 4-1BB-positive control or Cy/Flu-treated alloreactive immune cells. Double knockout of costimulatory ligands or supplementing PBMC and iNK cell co-cultures with anti-CD38 mAb protected costimulatory ligands-null and CD38-null iNK cells, respectively, and extended functional persistence by stagnating proliferation of alloreactive immune cells. Furthermore, we highlight translational data depicting the addition of anti-CD38 mAb to an LDC regimen further delays immune reconstitution in patients and may provide a prolonged window for therapeutic efficacy.

**Conclusions** Collectively, the data demonstrate that unique stealth strategies that potentiate effector cell function and promote functional persistence have the potential to eliminate the need for Cy/Flu mediated LDC. Notably, arming iNK cells with ADR extends functional persistence in the presence of an intact endogenous immune compartment. As exhibited by the immune reconstitution model, we show the first evidence of promising stealth strategies for allogeneic cell therapies that reduce or overcome the requirement of LDC while maintaining robust cytotoxic tumor responses.

**REFERENCE**

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