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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Engineered Natural Killer Cells Expressing Chimeric Ilt Receptors (CIR) Effectively Target HLA-G Positive AML Tumor Cells

Mylinh Duong, PhD[1], *Jihyun Park, PhD*[1], *Raphael Ognar, MSc, MBA*[1], *Simon Wain-Hobson, DPhil*[1], *Henri Bayle, PhD*[1]

[1] NKILT Therapeutics, Houston, TX

Background
Human leukocyte antigen-G (HLA-G), the immune checkpoint of pregnancy, directs immunosuppression by binding to Ig-like transcript (ILT) receptors (ILT2 in lymphoid cells and ILT4 in myeloid cells) via immunoreceptor tyrosine-based inhibitory motif (ITIM) signaling domains. HLA-G is expressed in more than 50% of solid tumors and leukemias as a mechanism to evade immune attack. As a result, HLA-G is an excellent tumor-specific antigen since its expression in normal tissues is low except for cytotrophoblasts in the placenta where it protects the fetus from the mother's immune system. We present here a novel approach to target HLA-G-expressing Acute Myeloid Leukemia (AML) cells by utilizing the natural receptors of ILT2 and ILT4 coupled to positive intracellular signaling domains. Such chimeric ILT receptors, or CIRs drive activation of Natural Killer (NK) cells. Further, we have identified unique intracellular activation modules that function to robustly enhance cytotoxicity and expansion of NK cells upon engagement with tumor cells.
Methods
CD56 $^{+}$ NK cells were isolated from PBMCs, cultured with IL-15 and activated with a feeder-free cocktail of an immobilized cytokine and activating ligand. Activated NK cells were transduced with γ-retroviruses directing expression of CIR proteins (CIR.4-1BB.CD3ζ or CIR.X.Y where X and Y are candidate activation and coactivation moieties), soluble IL-15 and a ΔCD19 marker or CAR control constructs directed to HLA-G or CD33. After 8 days of expansion, CIR- or CAR-NK cells were cocultured with GFP *ffluc*-expressing AML target cell lines with or without transgenic HLA-G (G1, G2 and G5) isoforms. Cytotoxicity of CIR-NK cells was determined by reduction of GFP fluorescence in Incucyte or Celligo instruments or luciferase activity and supernatants were analyzed for IFN-γ and TNF-α release by ELISA. Transduced NK cells were labeled with nuclear-RFP for measurement of expansion in coculture. In comparative experiments, T cells from PBMCs were transduced to produce CIR-T cells by standard methods.
Results
CIR (ILT2 or ILT4.4-1BB.ζ) constructs were efficiently and stably expressed in T and NK cells (>80% ΔCD19 $^{+}$ 8- and 14-days post activation). Mock-transduced NK cells displayed innate killing activity against HLA-G negative AML cells (THP1 and KG1) that was not augmented by CIR constructs (mock = 6.16E6 $\pm$ 0.77E6 vs. CIR = 6.43E6 $\pm$ 0.37E6 in coculture against KG1 cells). In cocultures against THP1 and KG1 target cells expressing exogenous G1, G2 or G5 isoforms, CIR-NK cells or CIR-T cells displayed enhanced target cell killing in 2 and 7-day assays relative to mock-transduced effector cells (mock = 1.47E6 $\pm$ 0.49E6 vs. CIR = 0.57E6 $\pm$ 0.6 in coculture against KG1-HLA-G1). Further, while mock NK cells transduced with RFP alone could kill one-third of AML cells expressing endogenous HLA-G1 and G5 (Molm13 and Kasumi1), CIR-NK cells eliminated more than 90% of these AMLs and their activivation resulted in 4-fold higher IFN-γ secretion. Notably, robust cytotoxicity of CIR-NK cells was maintained even at 28 days post activation (mock = 36.95E6 $\pm$ 21.85E6 vs. CIR = 7.38E6 $\pm$ 1.92 in coculture against Kasumi1). To further enhance the functionality of the CIR constructs, a screen of novel costimulatory molecules to replace 4-1BB and/or CD3ζ resulted in several potent candidates ultilizing MyD88 signaling that greatly improved the cytotoxicity (> 80% tumor elimination), proliferation (> 2-fold expansion) and proinflammatory cytokine production (IFN-γ and TNF-α) of NK cells in cocultures. A subset is displayed in Figure 1.
Conclusions
The use of natural receptors ILT2 and ILT4 to target HLA-G takes advantage of the physiological pairing of the ILT receptors with the multiple immunosuppressive HLA-G isoforms thereby circumventing the possibility of antigen escape often observed in CAR-directed therapy. We demonstrated that engineered CIR-NK cells can convert HLA-G expression in AML from a potent inhibitor of leukocyte activity into a target for anti-tumor control.

Disclosures Duong: *NKILT Therapeutics:* Consultancy, Current equity holder in private company, Current holder of *stock options* in a privately-held company, Patents & Royalties: Patents; *Bellicum Pharmaceuticals:* Current equity holder in publicly-traded company, Ended employment in the past 24 months, Patents & Royalties: Patents. **Park:** *NKILT Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Patents & Royalties: Patents; *Kiromic:* Divested equity in a private or publicly-traded company in the past 24 months, Ended employment in the past 24 months. **Ognar:** *NKILT Therapeutics:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Patents; *Alira Health, LLC:* Ended employment in the past 24 months; *American Cancer Society Board of Manhattan:* Membership on an entity's Board of Directors or advisory committees; *360MedLink, Inc.:* Other: Non-executive Board member. Ended 2021. **Wain-Hobson:** *Invectys Inc.:* Current equity holder in private company; *NKILT Therapeutics:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Patents. **Bayle:** *Bellicum Pharmaceuticals:* Consultancy, Current equity holder in publicly-traded company, Ended employment in the past 24 months, Patents & Royalties: Patents; *NKILT Therapeutics:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Patents.

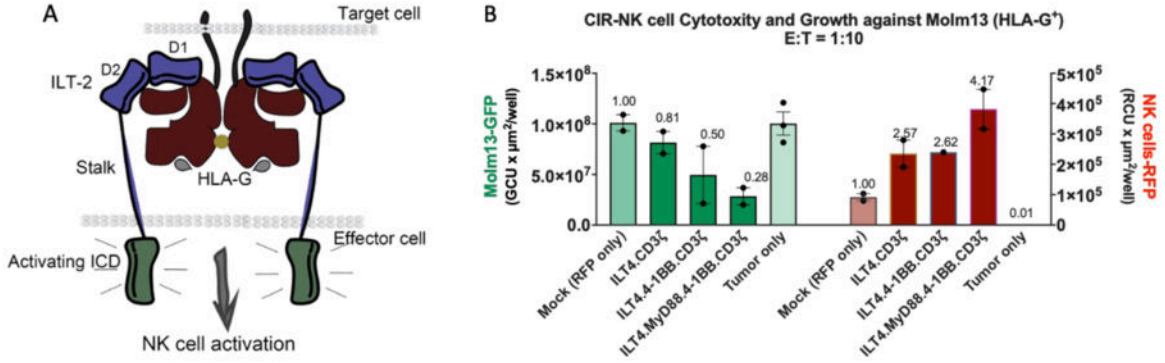

Figure 1. Anti-tumor efficacy of CIR-NK cells in vitro. (A) Schematic depiction of a CIR comprising the extracellular D1 and D2 domains of ILT2, a stalk and transmembrane domain derived from CD8-α and a replacement of the ITIM domains of ILT2 with engineered signaling domains driving NK cell activation. (B) Elimination of Molm13 AML tumor cells (left) and expansion of CIR-NK cells (right) in 7-day coculture at 1:10 E:T. Fold change relative to NK cells lacking CIR expression is displayed above each column.

Figure 1

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