INTRODUCTION

Natural Killer (NK) cells are highly responsive cytotoxic immune cells of the innate immune system with well characterized anti-tumor properties. Their ability to directly kill malignant cells and elicit an adaptive immune response makes them a promising candidate for a precision guided immunotherapy in oncology.

Bicycle® peptides are small (ca.1.5kDa), chemically synthetic, structurally constrained peptides discovered via phage display and optimized using structure-driven design and medicinal chemistry approaches. We have applied the Bicycle® platform technology to discover and evaluate a new class of fully synthetic molecules termed NK tumor immune cell agonists (NK-TICA®). The NK-TICA® molecule consists of chemically coupled Bicycle® peptides that bind specifically to the key activating receptor, NKp46, and to tumor antigens, that results in highly potent, antigen-dependent receptor activation and NK cell function.

We previously demonstrated potent, selective binding of our Bicycle® peptides to receptor-expressing cells and the capability of the bifunctional molecule to induce NK cell function in vitro (Rezvaya et al., 2022). With Bicycle’s novel NK-TICA® compound, we demonstrate the engagement of NK cells, the specific activation and function of NK cells, and enhanced tumor cytotoxicity in a tumor target- and dose-dependent manner.

RESULTS

Figure 3: NK cells specifically kill tumor (HT1080 (A) in the presence of NK-TICA® bearing EphA2 binding Bicycle® without EphA2 binding or NKp46 binding, no enhanced tumor killing was seen, compared to EphA2/Nkp46 binding NK-TICA®, EC50 2 pM. No change in viability of NK cells was seen at 24h with the addition of NK-TICA® (Figure 3B). Luminiscence for no NK-TICA® is shown at 3x10^4/mL, whereas for viability it is shown at 2.5x10^5/mL.

Figure 4: NK-TICA® enhanced NK cell cytokine production and activation. NK cells were co-cultured with HT1080-Suc and NK-TICAs: EphA2/Nkp46 binding NK-TICA®, or EphA2/Nkp46 non-binding NK-TICA®. Cytokine released measured at 48h (IFN-γ, 4A, EC50 = 10μM) and 48h (IL-12, 4B, EC50 = 4μM) into supernatants was measured by multiplex assay (Magellan Discovery) or ELISA. NK cell surface expression of CD25 was measured at 24h post co-culture with NK-TICA® and tumor cells (C).

CONCLUSIONS

- Building on success with CD137 Bicycle® TICAs, the Bicycle platform has now been successfully applied to build prototype NK cell engagers.
- NK-TICA® molecules promote the NK cell engagement to tumor cells by immune synapse formation.
- NK-TICA® molecules drive NK cell-mediated tumor cell killing and cytokine production in vitro.
- NK cells were capable of enhanced killing of successive rounds of EphA2+ve tumor cell addition.
- We hypothesize that utilization of Bicycle® NK-TICA® as a multifunctional immune cell engager will promote the modulation and anti-tumor activity of peripheral and intra-tumoral NK cells to solid tumors.

REFERENCES

2. Lani et al. PEGS-Boston (2017)
5. Rezvaya et al. AACR-JITC (2022)
6. Images created with BioRender.com (2022, 2023)