Generation of a *Bicycle* NK-TICA™, a novel NK cell engaging molecule designed to induce targeted tumor cytotoxicity

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INTRODUCTION

Natural killer (NK) cells are immune cells that can detect and eliminate tumor cells and bridge innate to adaptive immune responses. Tumor specific activation of NK cells is thus an area of active investigation in immune oncology, but to date has relied on complex biologic modalities (e.g., antibodies, fusion proteins, or cell therapies), each of which has inherent disadvantages in this application. Thus, alternative approaches are warranted.

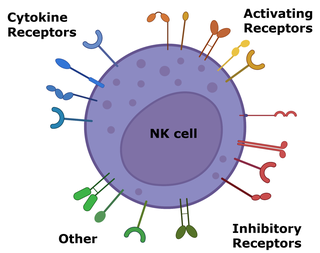


Figure 1: Surface receptors expressed on human NK cells (based on Chiossone *et al*., 2018). NK cells emanate from the bone marrow, patrol the body, last for several days, and can kill by direct contact-dependent cytotoxicity or signaling through death receptors. These innate cells use receptors to read the surface of cells for signs of stress, transformation, viral infection, or decoration with antibodies.

Bicycles are small (~1.5 kDa), chemically synthetic, structurally constrained peptides discovered via phage display and optimized using structure-driven design and medicinal chemistry approaches. We have now applied this technology to identify *Bicycles* that bind specifically to the key activating receptor, NKp46. When chemically coupled to tumor antigen binding *Bicycles,* this results in highly potent, antigen-dependent NK cell activation. We term this new class of fully synthetic molecules NK-TICAs and we will describe their discovery and evaluation in this presentation.

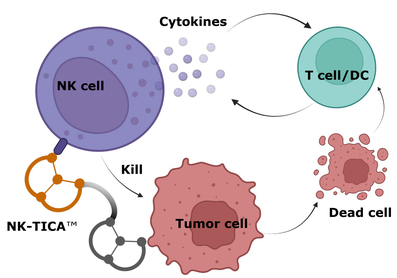
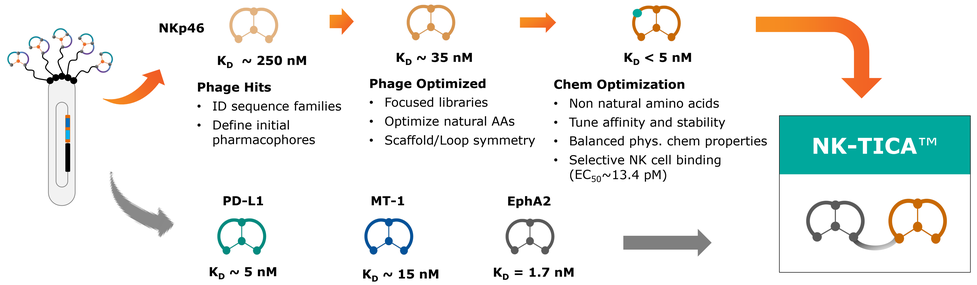


Figure 2: Recent work suggests a role of NK cell activation *in situ* to catalyze the development of anti-tumor immunity via release of tumor antigens (kill) and activation of DCs/T cells (cytokines) (Wang *et al.*, 2021).

We have developed a novel, fully synthetic tumor binding and NKp46 binding NK-TICA™ molecule that is capable of inducing NK cell activation in the presence of tumor. As an immunotherapeutic agent, Bicycle's NK-TICA™ molecules are positioned to engage NK cells in a tumor antigen dependent manner to kill and drive adaptive immunity in solid tumors.

GENERATION OF COMPONENT PARTS TO CONSTRUCT NK-TICAs

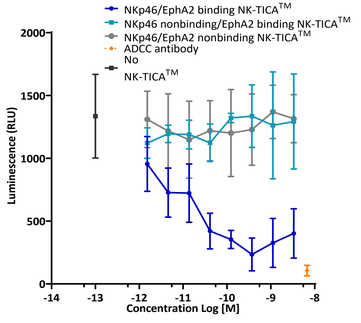
Using our unique phage display screening platform, we identified high affinity, selective binders to NKp46. For proof-of-concept studies, NKp46 binding *Bicycles* were conjugated with an EphA2-binding *Bicycle®.* The EphA2 and PD-L1 binding *Bicycle®* is specific and potent with ~1.7 nM and ~5 nM, respectively, evaluated by SPR (Upadhyaya *et al*., 2021). The MT-1 *Bicycle®* is specific and potent with ~15 nM evaluated by SPR (Lani *et al*., 2017). The resulting bifunctional NK-TICAs were then tested in primary human cell-based functional models.



RESULTS

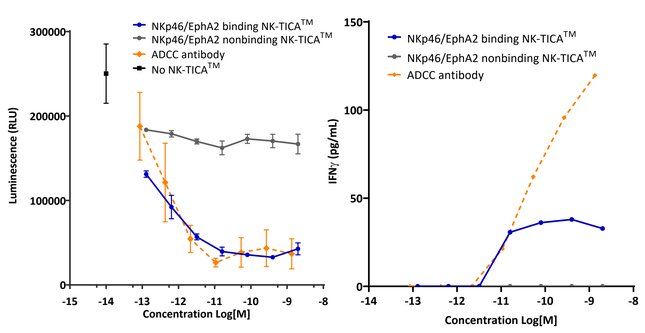
We have developed a novel modular compound with high affinity and selectivity to NK cell receptors with specific tumor targeting potential. We demonstrate potent, selective binding of our *Bicycles* to receptor-expressing cells and the capability of the bifunctional molecule to induce primary human NK cell function *in vitro*.

NK-TICAs enhanced NK killing that is dependent upon tumor antigen binding



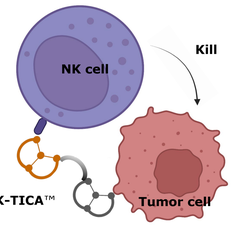
NK cells specifically kill tumor in the presence of NK-TICA™ bearing EphA2 binding *Bicycle®*. Without EphA2 binding, NKp46-binding/EphA2 nonbinding (BCY15666) and NKp46/EphA2 non-binding (BCY15667) did not induce tumor killing compared to NKp46/EphA2-binding (BCY15664, EC_{50}16pM). ADCC-capable anti-EGFR antibody was used as positive control. Luminescence for no NK-TICA™ is shown at 10-^{13}M.

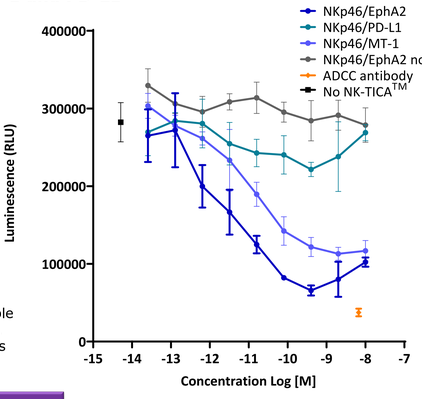
NK-TICAs afford both enhanced killing as well as cytokine production by NK cells



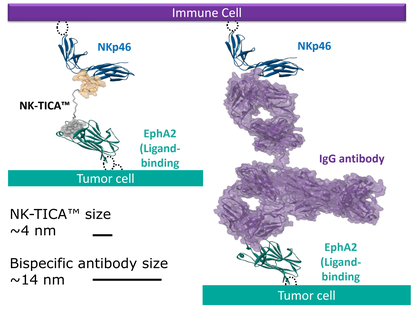
NK cells were co-cultured with HT1080-luc and NK-TICAs: NKp46/EphA2 binding NK-TICA™(BCY17226), or NKp46/EphA2 nonbinding NK-TICA™(BCY15667). Cytokine released (IFNγ) into supernatants (4hr) was measured by ELISA (RnD systems)(right). HT1080-luc cell death was measured at 24hr (BCY17226, EC_{50}6pM)(left). ADCC-capable anti-EGFR antibody was used as positive control.

NK cells can be directed to kill tumor cells by NKp46 NK-TICAs employing multiple different tumor antigens: EphA2, MT-1 and PD-L1





NK cells co-cultured with HT1080-luc cells in the presence of NKp46 NK-TICAs of varying tumor binding arms: EphA2 (BCY17226), MT-1 (BCY18604), or PD-L1 (BCY18603). ADCC-capable anti-EGFR antibody was used as positive control. Luminescence values for no NK-TICA™ addition is arbitrarily shown as 5 x 10-^{15} M.



Modeling of NK-TICAs in complex with NKp46 and EphA2 in comparison to a bispecific antibody

Graphical model demonstrating the approximate size of a NK-TICA™ and relative spacing when bound simultaneously to tumor target EphA2 and NKp46. Shown for comparison is the size and relative bound spacing for a bispecific antibody (IgG) binding to EphA2 and NKp46 at the same epitopes (PDB: 6rw2, 6iap, 1hzh, Gauthier *et al*. 2019).

CONCLUSIONS

- Building on success with CD137 *Bicycle®* TICAs, the Bicycle® platform has now been successfully applied to build prototype NK cell engagers
- NK-TICAs drive NK cell-mediated tumor cell killing and cytokine production *in vitro* and as such have the potential to catalyze the development of durable anti-tumor immunity in tumor types not well served by current therapies

REFERENCES

- Upadhyaya *et al*. J Immunother. 9:e001762 (2021)
- Lani *et al*. PEGS-Boston (2017)
- Gauthier *et al*. Cell. 177:1701 (2019)
- Wang *et al*. Oncogene. 40:717–730 (2021)
- Images created with BioRender.com (2022)
- PDB#6rw2,6iap, 1hzh

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