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

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ABSTRACT# 15699

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.

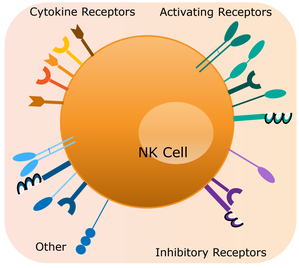


Figure 1: Surface receptors expressed on human NK cells (based on Chiossone *et al.*, 2018). NK cells emanate from the bone marrow, patrol the body, last for several days, and can kill by direct contact-dependent cytotoxicity or signaling through death receptors. These innate cells use receptors to read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

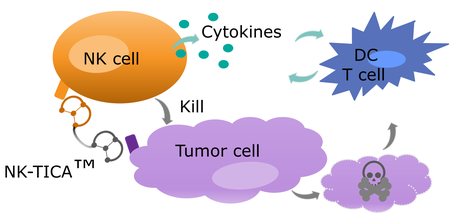
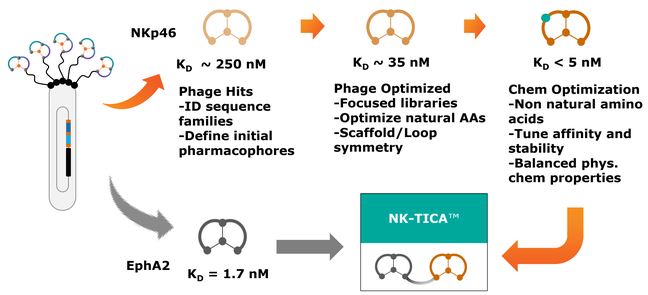


Figure 2 Recent work suggests a role of NK cell activation *in situ* to catalyze the development of anti-tumor immunity via release of tumor antigens (kill) and activation of DCs/T cells (cytokines) (Wang *et al.*, 2021).

We have developed a novel, fully synthetic EphA2 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

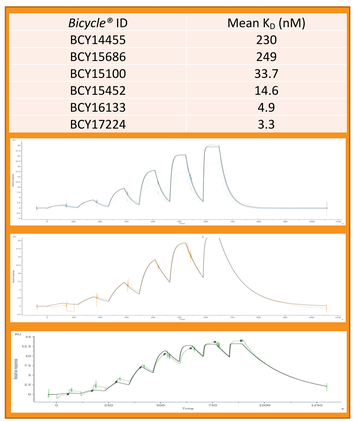
Using our unique phage display screening platform, we have identified high affinity, selective binders to NKp46.



By conjugating the *Bicycle*® NK cell-engaging binders to a model tumor antigen EphA2-binding *Bicycle*®, we have developed a bifunctional NK-TICA™ molecule. As has been shown in previous reports, the EphA2 binding *Bicycle*® is specific and potent with ~1.7nM evaluated by SPR (Upadhyaya *et al*., 2021).

SPR sensorgrams of NKp46 binding *Bicycle*® monomers

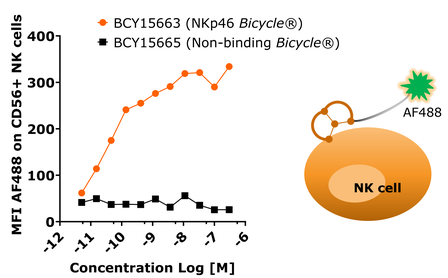
Bicycle® ID	Mean K_D (nM)
BCY14455	230
BCY15686	249
BCY15100	33.7
BCY15452	14.6
BCY16133	4.9
BCY17224	3.3



RESULTS

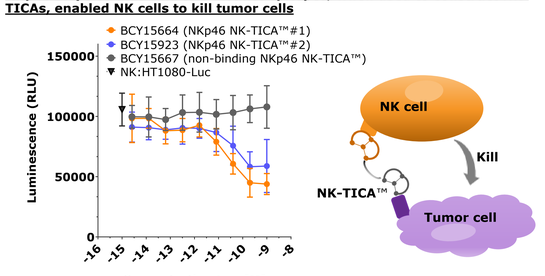
We have developed a novel modular compound with high affinity and selectivity to NK cell receptors with specific tumor targeting potential. We demonstrate potent, selective binding of our *Bicycles* to receptor-expressing cells and the capability of the bifunctional molecule to induce NK cell function *in vitro*. With Bicycle's novel NK-TICA™ compound, we demonstrate engagement of NK cells, specific activation and function of NK cells, and enhanced EphA2-expressing tumor cytotoxicity, in a dose dependent manner *in vitro*.

NKp46 NK-TICAs selectively bind primary NK cells



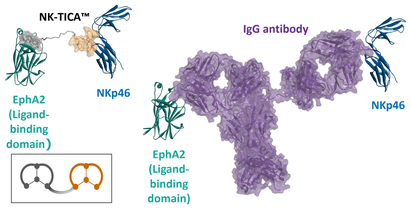
Binding of NKp46 *Bicycle*® was measured by flow cytometry. The fluorescently labeled (AF488-tagged) NKp46 *Bicycle*® bound only to NK cells in purified human PBMC (EC_{50}13.4pM). A non-binding control NK-TICA™ demonstrated no binding above background in either NK or T lymphocyte populations.

NKp46 *Bicycles* that bind two distinct epitopes, when used to construct NK-TICAs, enabled NK cells to kill tumor cells



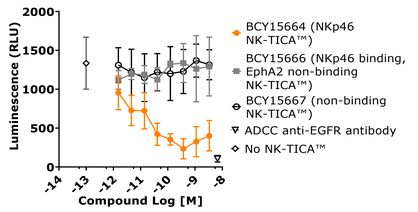
Primary human NK cells co-cultured with EphA2$^+$ve HT1080-luc cells EphA2-binding NK-TICAs: BCY15664 (NKp46 epitope 1, EC_{50}21pM) or BCY15923 (NKp46 epitope 2, EC_{50}44pM). BCY15667 does not bind NKp46 or EphA2. ADCC-capable anti-EGFR antibody was used as positive control. Luminescence values for no NK-TICA™ addition is arbitrarily shown as 10^{-15}M.

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



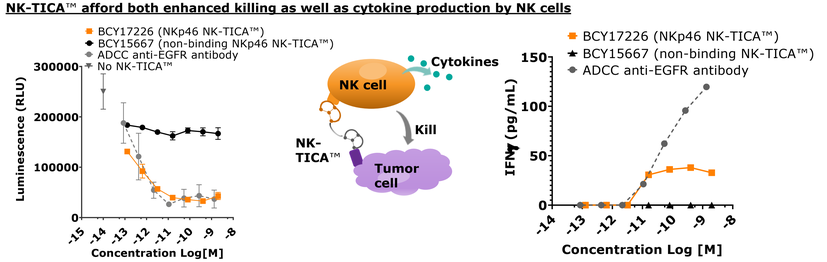
Graphical model demonstrating the approximate size of a NK-TICA™ and relative spacing when bound simultaneously to tumor target EphA2 and NKp46. Shown for comparison is the size and relative bound spacing for a bispecific antibody (IgG) binding to EphA2 and NKp46 at the same epitopes. NK-TICA™ size and geometries may permit much closer approach of the tumor and NK cell targets which may influence the receptor clustering and resulting signaling (PDB: 6rw2, 6iap, 1hzh, Gauthier *et al.*2019).

NK-TICA™ enhanced NK killing is dependent upon tumor antigen binding



NK cells specifically kill tumor in the presence of NK-TICA™ bearing EphA2 binding *Bicycle*®. Without EphA2 binding, both BCY15666 (NKp46 binding) and BCY15667 (NKp46 non-binding) do not induce tumor killing compared to 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 for reference.

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



NK cells were co-cultured with HT1080-luc and treated with NK-TICAs BCY17226:NKp46 NK-TICA™, or BCY15667: non-binding NK-TICA™. 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.

CONCLUSION/SUMMARY

- 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 catalyse the development of durable anti-tumor immunity in tumor types not well served by current therapies

REFERENCES

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- Chiossone *et al*. Nat. Rev. Immunol. 18:672 (2018)
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- Wang *et al*. Oncogene. 40:717–730 (2021)
- PDB#6rw2,6iap, 1hzh

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