# Bicycle®

# NKp46 engaging Bicycle NK-TICA™ drives tumor targeted cytotoxicity

Abstract Number

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## ABSTRACT

Natural killer (NK) cells are immune cells that can detect and eliminate tumor cells and bridge innate to adaptive immune responses. Bicycles are small (~2kDa), chemically synthetic, structurally constrained peptides discovered *via* phage display and optimized using medicinal chemistry approaches. We identified Bicycles that bind specifically to a key activating receptor, NKp46, combined them with tumor targeting Bicycles to generate NK-TICA™ and evaluated these molecules *in vitro*.

## INTRODUCTION

We developed a novel, fully synthetic tumor binding and NKp46 binding NK-TICA™ (natural killer-tumor-targeted immune cell agonist) 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.

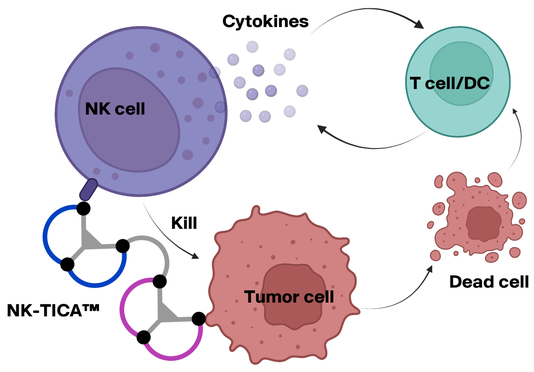


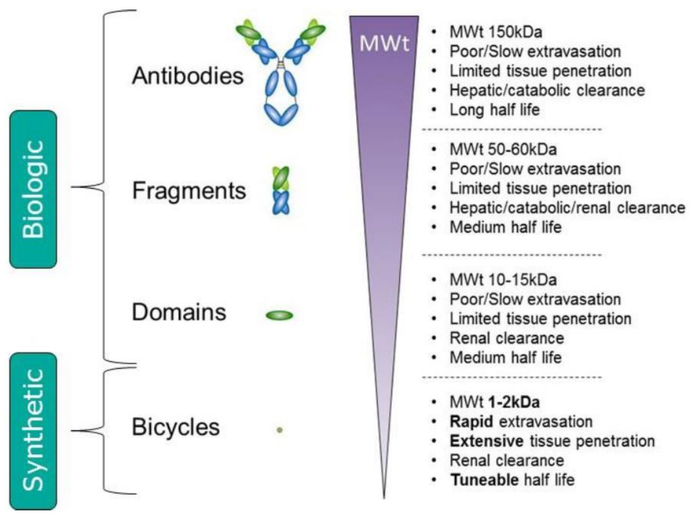

Figure 1: Program hypothesis: Catalysis of adaptive immunity by NK cells has potential to enable tumor rejection and enhance the action of established therapeutics such as targeted toxins and immune checkpoint inhibitors.

## ABOUT *BICYCLES*

*Bicycles* are a class of small (~2kDa) peptide-based therapeutic modality (Fig 2 A.). Peptides are selected through a phage display screening process (Fig 2 B. [1] Winter et al). The unique attributes of *Bicycles* include:

- High selectivity to target and tunable affinity
- Large binding footprint to biologically relevant 3D structures
- Readily conjugated to toxin payload, fluorochrome, other *Bicycles*, radionuclides, biotin/affinity tags etc.
- Compatible with multiple routes of administration, including IV, SQ, and inhalation
- Fully synthetic and scalable manufacturing

### A. *Bicycles* relative to other therapeutic compounds




### B. Phage display screening process and multi-valent *Bicycles*

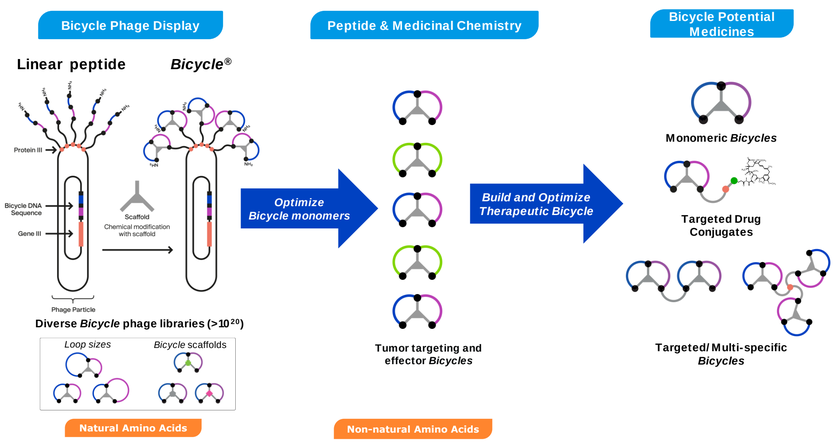



Figure 2 A. *Bicycles* relative to other therapeutic compounds, B. Phage display screening process and multi-valent *Bicycles*.

## METHODS

### NKp46 *Bicycles*: discovery and optimization by phage display and chemistry

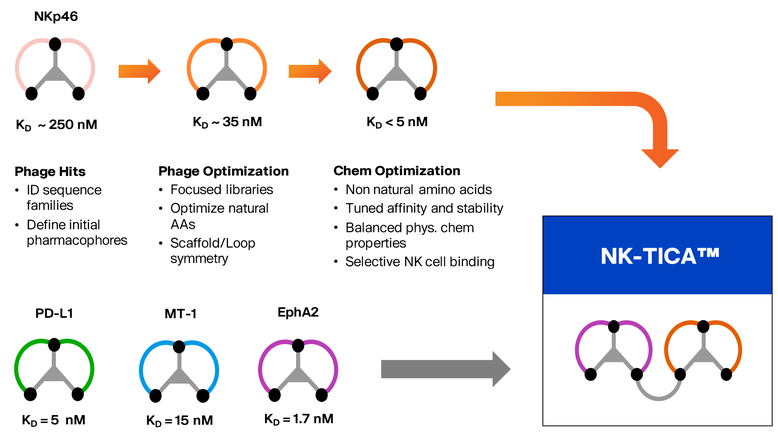



Figure 3: Phage display screening identified high affinity, selective binders to NKp46.
For proof-of-concept studies, NKp46 binding *Bicycles* were conjugated with an EphA2, PD-L1 and MT-1 binding NK-TICA™ *Bicycle*®.

## RESULTS

### NK-TICA™ selectively binds NKp46 by SPR and on primary NK cells

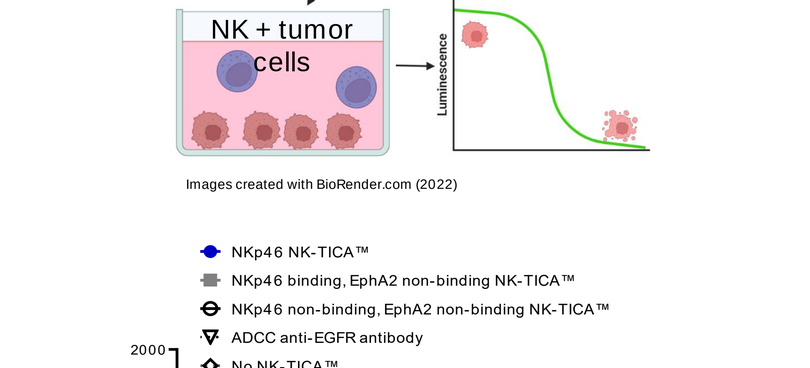
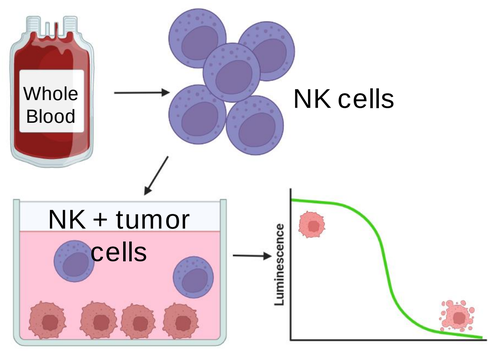



Figure 4: Binding of NKp46 Bicycle® monomers was measured by SPR (Figure 4A) and by flow cytometry (Figure 4B).
The AF647-fluorescently labelled NKp46 Bicycle® bound only to PBMC purified NK cells.
Non-binding NK-TICA™ control (D-amino acids ablating affinity to the target) demonstrated no binding above background in either CD56+ NK (Figure 4B) or T lymphocyte populations (data not shown).

### NK-TICA™ enhances NK killing across multiple EphA2+ cell lines




Images created with BioRender.com (2022)

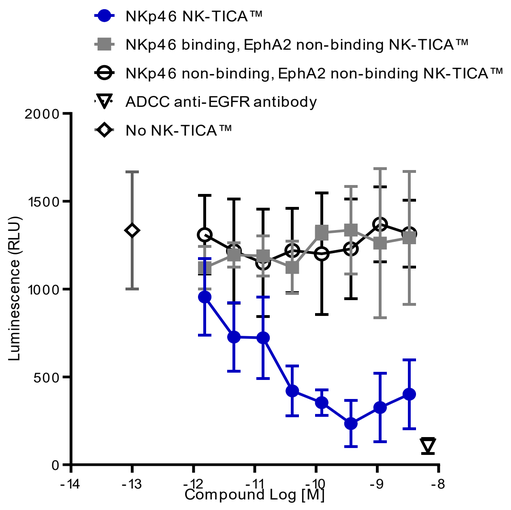



Figure 5: NK cells specifically kill EphA2+ cell line HT1080 in the presence of EphA2 binding Bicycle® NK-TICA™ ($EC_{50}$~16pM).
Without EphA2 binding, the *NK-TICAs* NKp46 non-binder/EphA2 non-binder and NKp46 binder/EphA2 non-binder did not enhance tumor killing compared to no NK-TICA™ control. ADCC-capable anti-EGFR antibody was used as positive control.

### NK-TICA™ enhances NK cytokine production in the presence of EphA2+ cell lines

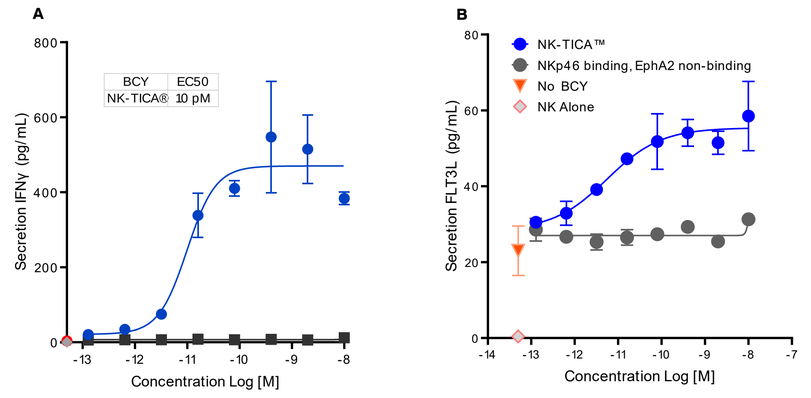



Figure 6: NK cells were co-cultured with HT1080-luc and NKp46/EphA2 binding or NKp46 binding/EphA2 non-binding NK-TICA®.
Cytokines in supernatants at 4hr (IFNγ, Figure 6A and TNFα, not shown) and 48hr (FLT3L, Figure 6B) were measured by MesoScale Discovery™ multiplex assay.

### NK-TICA™ drive NKp46 tumor killing with multiple tumor antigens: EphA2, MT-1 and PD-L1

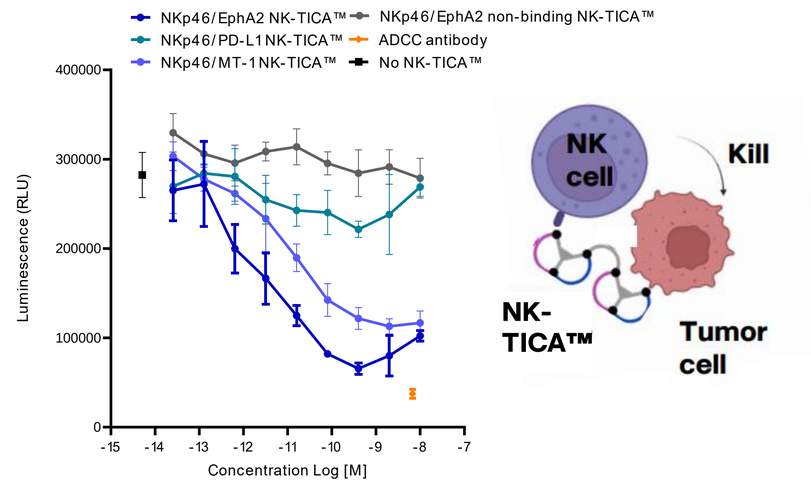



Figure 7: NK cells were co-cultured with EphA2, MT-1 or PD-L1 expressing cells in the presence of NKp46 NK-TICA™ with the varying tumor binding arms.
ADCC-capable anti-EGFR antibody was used as positive control.

### Modeling of NK-TICA™ in complex with NKp46 and EphA2 in comparison to a bispecific antibody

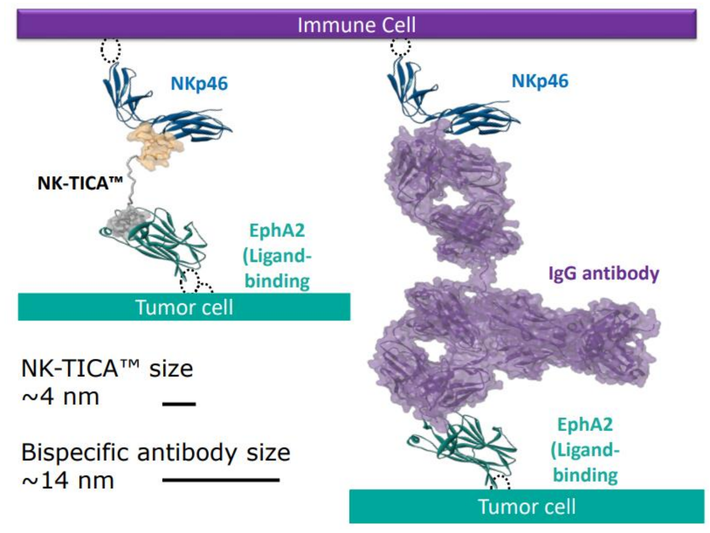



Figure 8: Graphical model demonstrating the approximate size of an NK-TICA™ and relative spacing when bound simultaneously to tumor target EphA2 and NKp46.
Shown for comparison is the size and relative bound spacing for a bispecific antibody (IgG) binding EphA2 and NKp46 at the same epitopes. (PDB: 6rw2, 6iap, 1hzh, Gauthier et al. 2019).

## CONCLUSION

- Building on progress made with CD137 Bicycle® TICAs, the Bicycle platform has now been successfully applied to build prototype NK cell engagers.
- NK-TICA™ 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

1. Winter et al. Nature Chem Bio (2009)
2. Upadhyaya et al. J. Immunother. (2021)
2. Lani et al. PEGS-Boston (2017 )
3. Gauthier et al. Cell (2019)
4. Wang et al. Oncogene (2021)

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