# Modulation of the natural killer cell immune response to tumor with a synthetic tumor-immune cell agonist, NK-TICA®

**Fay J. Dufort, Tucker R. Ezell, Alexandra Rezvaya, Kathleen Q.W. Ho, Christopher J. Leitheiser, Philip E Brandish, Kevin McDonnell, Nicholas Keen**

▶1340

## 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.

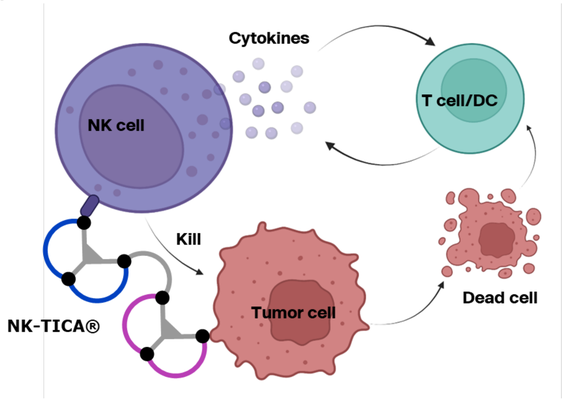
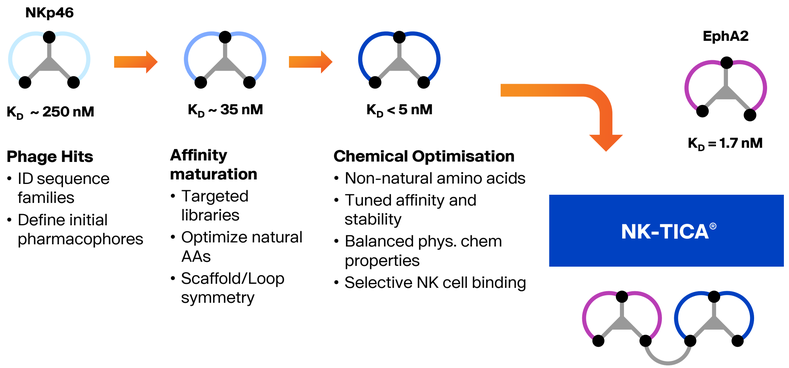


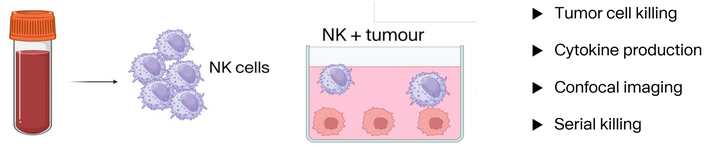

Figure 1: Surface receptors expressed on human NK cells (based on Chiossone *et al.*, 2018). They patrol the body, last for several days, and can kill by direct contact-dependent cytotoxicity or signaling through death receptors. These innate cells use receptors to read the surface of cells for signs of stress, transformation, viral infection, or decoration with antibodies. 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) (Gauthier *et al.*, 2019, Wang *et al.*, 2021).

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.

### NKp46 *Bicycles* are discovered using phage display and optimized using medicinal chemistry




## METHODS




## RESULTS

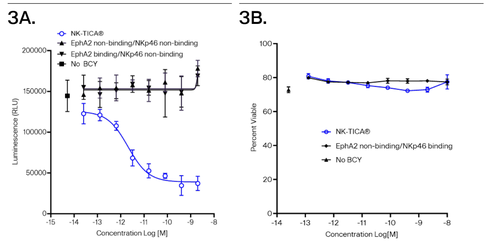



Figure 3: **NK cells specifically kill tumor (HT1080 (A) in the presence of NK-TICA® bearing EphA2 binding Bicycle®.** Without EphA2 binding or NKp46 binding, no enhanced tumor killing was seen, compared to EphA2/NKp46 binding NK-TICA®, $EC_{50}$ 2 pM. No change in viability of NK cells was seen at 24hr with the addition of NK-TICA® (Figure 3B). Luminescence for no NK-TICA® is shown at $5x10^{-15}$M, whereas for viability it is shown at $2.5x10^{-14}$M.

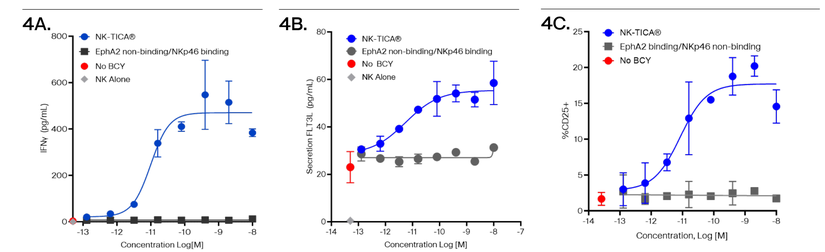
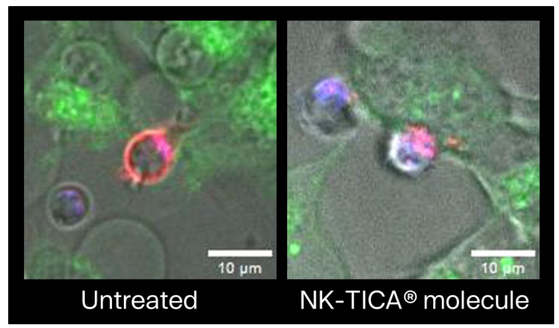



Figure 4: **NK-TICA® enhanced NK cell cytokine production and activation.** NK cells were co-cultured with HT1080-luc and NK-TICAs: EphA2/NKp46 binding NK-TICA®, or EphA2/NKp46 non-binding NK-TICA® Cytokine released measured at 4hr (IFNγ, 4A, $EC_{50}$ = 10 pM) and 48hr (FLT3L, 4B, $EC_{50}$ = 6 pM) into supernatants was measured by multiplex assay (MesoScale Discovery) or ELISA. NK cell surface expression of CD25 was measured at 24hr post co-culture with NK-TICA® and tumor cells (C).

Figure 5: **NKp46 clusters to synapse in presence of NK-TICA®.** NK cells stained with anti-NKp46-Alexa Fluor® 647 antibody (red) were plated with EphA2+ve fibrosarcoma cell line (HT1080). In the absence of NK-TICA® (left), NKp46 (red) is distributed on the NK cell surface (left). In the presence of NK-TICA® (right), NKp46 (red) clusters to the NK-tumor interface. Tumor cells: CellBrite®™ Steady 488 membrane dye (Biotium®, green). NK cells: Lysoview® 594 (blue) and anti-NKp46-AF647 antibody (red)



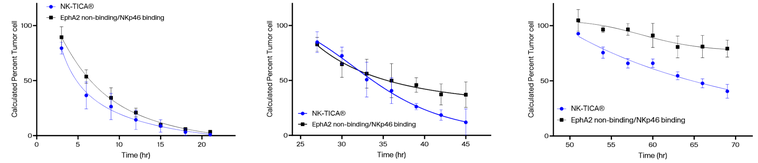




Figure 6**: NK cells demonstrated enhanced tumor cell killing for 3 rounds (A-C).** Monitored by an IncuCyte S3®, NK cells were placed in coculture with GFP-labeled tumor cells (HT1080-GFP) and NK-TICA® (2 nM). NK cells were treated only once with NK-TICA® and were able to enhance killing of 3 rounds of HT1080 tumor cells. (GFP tumor cells were added to the wells for monitoring capability to kill multiple rounds of target cells)

## 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

1. Upadhyaya *et al.* J Immunother. 9:e001762 (2021)
2. Lani *et al.* PEGS-Boston (2017)
3. Gauthier *et al.* Cell. 177:1701 (2019)
4. Wang *et al.* Oncogene. 40:717–730 (2021)
5. Rezvaya *et al.* AACR-JITC (2022)
6. Images created with BioRender.com (2022, 2023)

**Bicycle Therapeutics, Inc.**
35 Cambridgepark Drive, Suite 350
Cambridge, MA 02140
USA
T. +1 617-945-8155

**BicycleTx Limited**
Portway Building
Granta Park, Cambridge
CB21 6GS, UK
T. +44 (0)1223 261503

Company number 11036101.
Registered in England.

**bicycletherapeutics.com**