Modulation of the natural killer (NK) cell immune response to tumor with novel synthetic tumor -immune cell agonist, NK-TICA™

Fay J. Dufort[1], Tucker Ezell[1], Alexandra Rezvaya[1], Kathleen Ho[1], Christopher J. Leitheiser[1], Liuhong Chen[2], Peter Brown[2], Philip E Brandish[1], Michael Skynner[2], Kevin McDonnell[1], Nicholas Keen[1]

Abstract # 1806

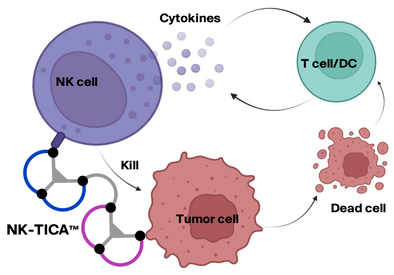
ABSTRACT

The tumor specific activation of natural killer (NK) cells with *Bicycles* is an area of active investigation in immune oncology. *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 applied the Bicycle platform technology to discover and evaluate a new class of fully synthetic molecules termed NK tumor immune cell agonists (NK-TICA™).

INTRODUCTION

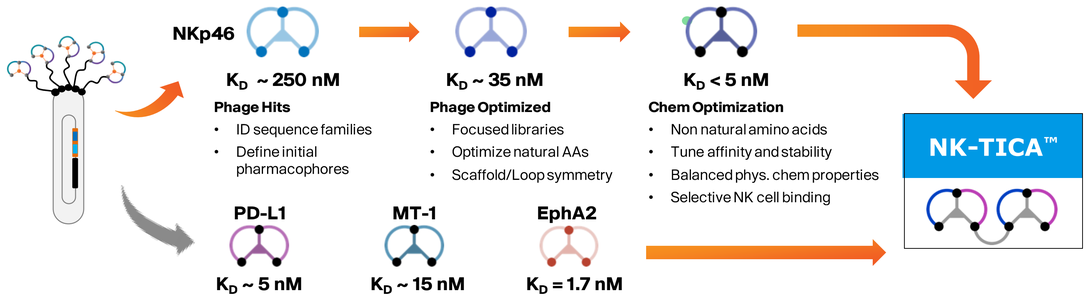
Natural killer (NK) cells are immune cells that can detect and eliminate tumor cells and bridge innate to adaptive immune responses. NK cells are highly responsive immune cells that can detect and eliminate tumor cells and bridge innate to adaptive immune responses. *Bicycles* are small (~1.5 kDa), chemically synthetic, structurally constrained peptides discovered via phage display and optimized using structure-driven design and medicinal chemistry approaches. We have 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™ consists of chemically coupled *Bicycles* 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 activation.

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 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 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. In conclusion, *NK-TICA*s 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. We hypothesize that utilization of Bicycle NK-TICA™ as a multifunctional immune cell engager will promote the modulation of NK cells, as well as the infiltration and anti-tumor activity of NK cells in solid tumors.

Generation of component parts to construct the *NK-TICA*s



Using our unique phage display screening platform, we identified high affinity, selective binders to NKp46. For *in vitro* 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-TICA*s were then tested in primary human cell-based functional models.

RESULTS

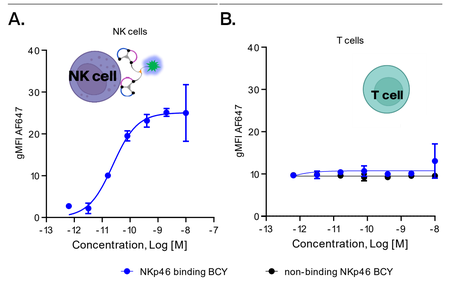


Figure 1. NKp46 *Bicycles* selectively bind primary NK cells. Binding of NKp46 *Bicycles* was measured by flow cytometry. The fluorescently labelled (AF647-tagged) NKp46 Bicycle bound only to NK cells in purified PBMC. A non-binding control NK-TICA™ demonstrated no binding above background in both CD56+ NK (A) and CD3+ T cell (shown here, B) and other immune cell populations.

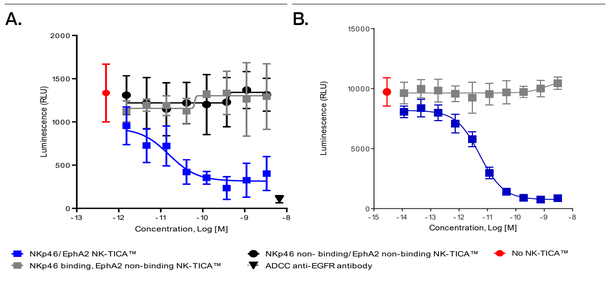
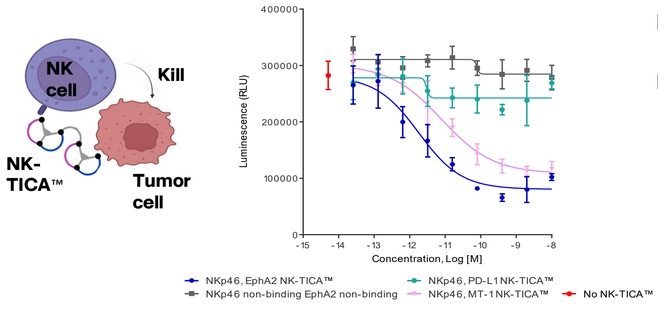


Figure 2. NK-TICA™ enhances specific NK killing of tumor cells. *NK-TICA*s bearing EphA2 binding Bicycle® enhanced NK killing across multiple cell lines and is dependent upon specific binding to tumor antigen bearing cells (A), HT1080 ~60000 EphA2-PE/cell and Figure 2B A431 ~43000 EphA2-PE/cell)). Without EphA2 or NKp46 binding, NKp46 binding /EphA2 non-binding or NKp46 non-binding/EphA2 non-binding TICAs did not induce tumor killing compared to NKp46 /EphA2 binding NK-TICA™, EC_{50} 2 pM (A) 5 pM (B). ADCC-capable anti-EGFR antibody was used as positive control. Luminescence for no NK-TICA™ is shown at $5e^{-14}$ M (A) or $3e^{-15}$ M (B).

Figure 3. 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-TICA*s of varying tumor binding arms: EphA2, MT-1, or PD-L1. Luminescence values for no NK-TICA™ addition is arbitrarily shown as 5×10^{-15} M.



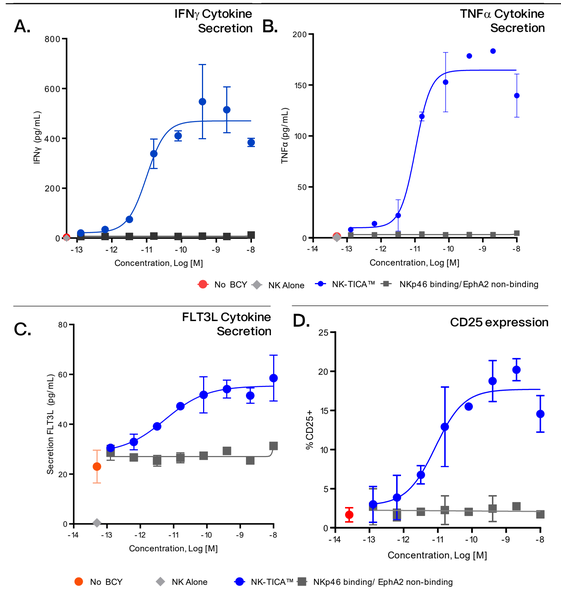
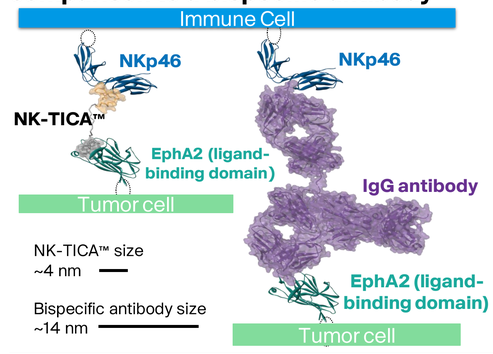


Figure 4. NK-TICA™ enhanced NK cell cytokine production and activation. NK cells were co-cultured with HT1080-luc and *NK-TICA*s: NKp46/EphA2 binding NK-TICA™, or NKp46 binding/EphA2 non-binding NK-TICA™. Cytokine secretion into supernatant medium measured at 4 hours (EC_{50} ~10 pM: IFNγ (A) and TNFα (B) and 48 hours (FLT3L, C) using a Mesosclae Discovery multiplex assay. NK cell surface expression of CD25 was measured at 24 hr post co-culture with NK-TICA™ and tumor cells (D).

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



Graphical model illustrating 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).

CONCLUSIONS

- Building on early success with CD137 Bicycle® TICAs, the Bicycle platform has now been successfully applied to build prototype NK cell engagers
- *NK-TICA*s 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. 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. Images created with BioRender.com (2022)

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

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

Company number 11036101.
Registered in England.

bicycletherapeutics.com