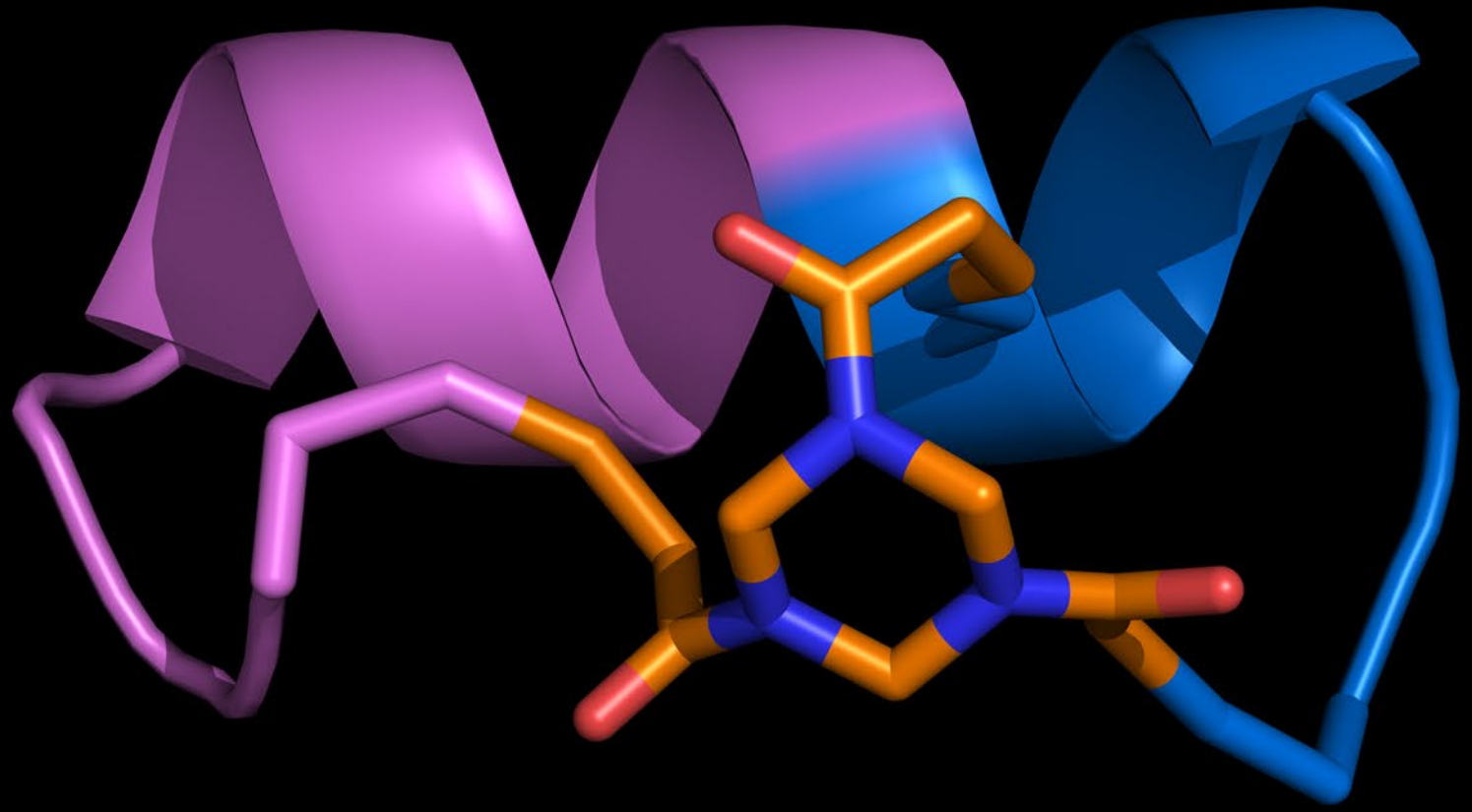
Bicycles: Bispecific, Precision-guided NK Cell Activators for the Treatment of Solid Tumors



Innate Killer Summit
March 29, 2023
Fay Dufort, PhD

Bicycle®

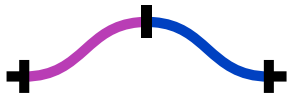
Forward-looking statement

This presentation may contain forward-looking statements made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. These statements may be identified by words such as “aims,” “anticipates,” “believes,” “could,” “estimates,” “expects,” “forecasts”, “goal,” “intends,” “may” “plans,” “possible,” “potential,” “seeks,” “will,” and variations of these words or similar expressions that are intended to identify forward-looking statements. All statements other than statements of historical facts contained in this presentation are forward-looking statements, including statements regarding: our future financial or business performance, conditions, plans, prospects, trends or strategies and other financial and business matters; our current and prospective product candidates, planned clinical trials and preclinical activities, current and prospective collaborations and the timing and success of our development of our anticipated product candidates.

Forward-looking statements are neither historical facts nor assurances of future performance. Instead, they are based on our current beliefs, expectations and assumptions regarding the future of our business, future plans and strategies, our development plans, our preclinical results, our plans to initiate clinical trials and the designs of the planned trials and other future conditions, and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in or implied by such forward-looking statements. These risks and uncertainties include, but are not limited to, the risk that any one or more of our product candidates will not be successfully developed or commercialized, the risk of cessation or delay of any ongoing or planned clinical trials, the risk that we may not realize the intended benefits of our technology, including that we may not identify and develop additional product candidates for our pipeline the risk that prior results will not be replicated or will not continue in ongoing or future studies or trials. For a discussion of these and other risks and uncertainties, and other important factors, any of which could cause our actual results to differ from those contained in the forward-looking statements, see the section entitled “Risk Factors” in our Annual Report on Form 10-K, filed with the Securities and Exchange Commission on February 28, 2023, as well as in other filings we may make with the SEC in the future, as well as discussions of potential risks, uncertainties and other important factors in our subsequent filings with the Securities and Exchange Commission. New risks and uncertainties may emerge from time to time, and it is not possible to predict all risks and uncertainties. Except as required by applicable law, we do not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

This presentation does not constitute an offer to sell or a solicitation of an offer to buy securities, nor shall there be any sale of any securities in any state or jurisdiction in which such offer, solicitation or sale would be unlawful prior to registration or qualification under the securities laws of any such state or jurisdiction.

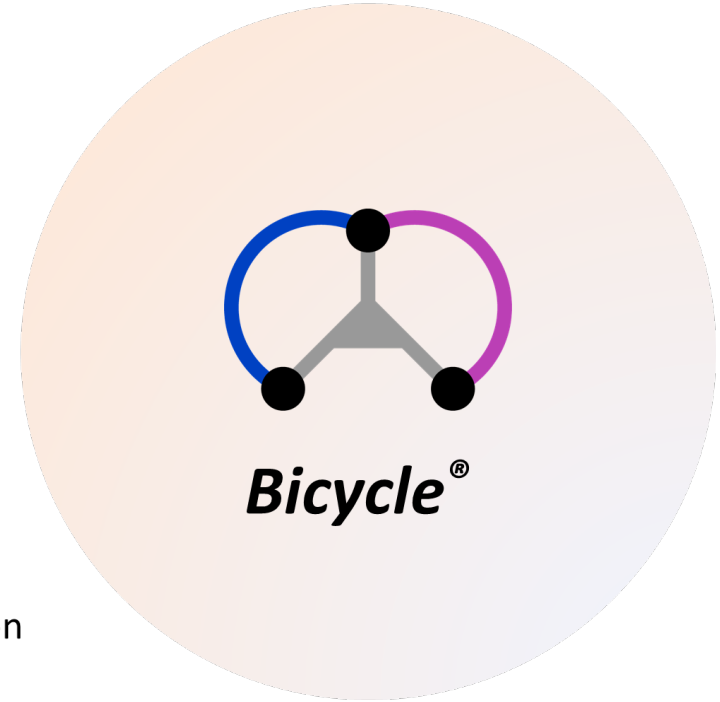
Bicycles are short peptides chemically constrained with a central scaffold

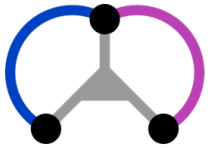


Short linear peptide

+

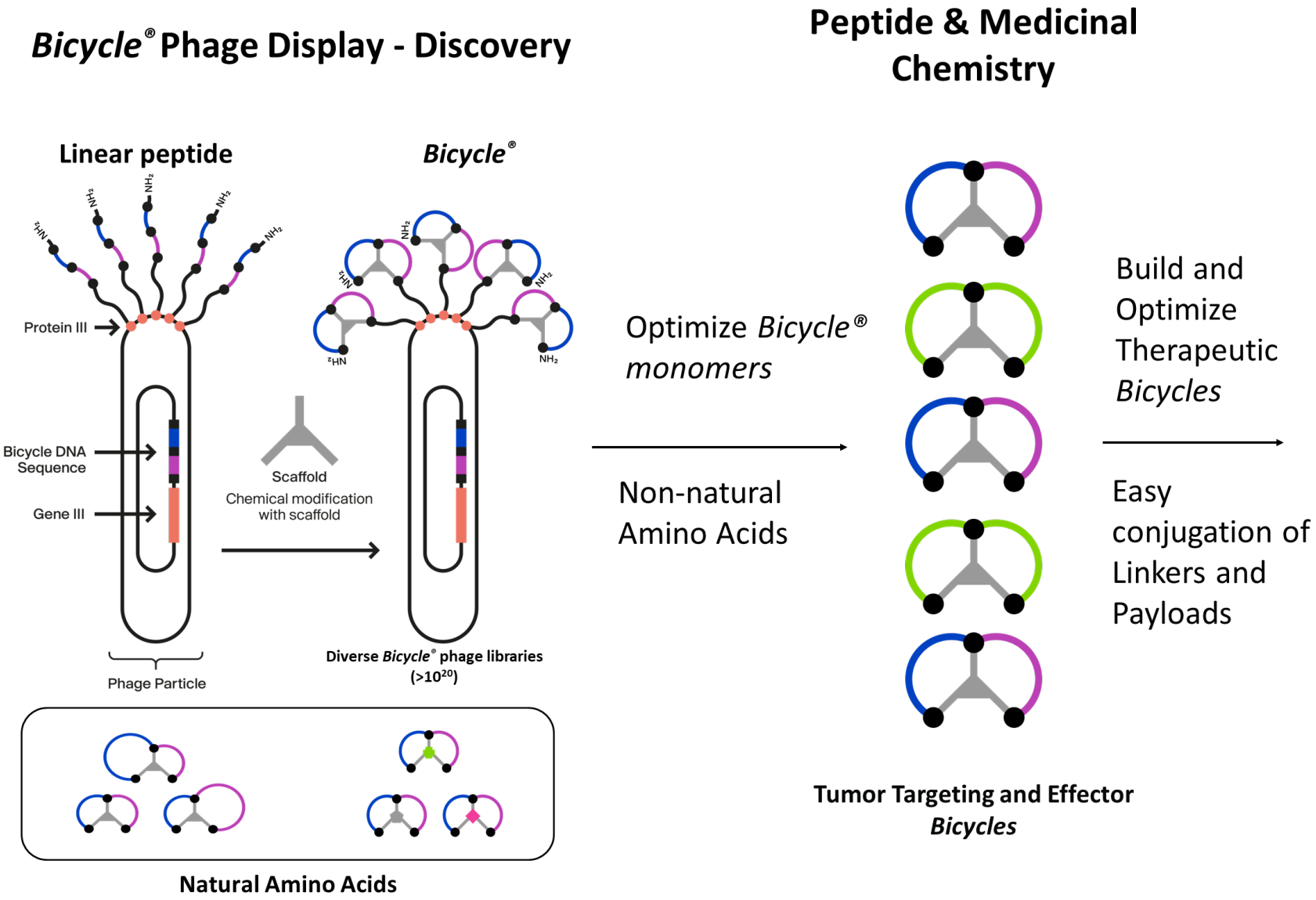
Scaffold
Chemical modification with scaffold



	 Bicycle®
Small size	**Yes 1.5 to 2 kDa**
Specificity	**High**
Chemical synthesis (NCEs)	**Yes**
Rapid tissue penetration	**Yes**
Complex protein targets druggable	**Yes**
Route of elimination	**Renal**

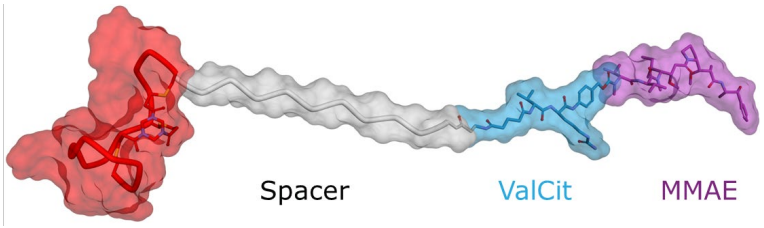
Based on the work of Sir Greg Winter to define the minimal mAb pharmacophore
- 2018 Nobel Prize in Chemistry
- Founded Bicycle Therapeutics

How *Bicycles* are discovered and why they work



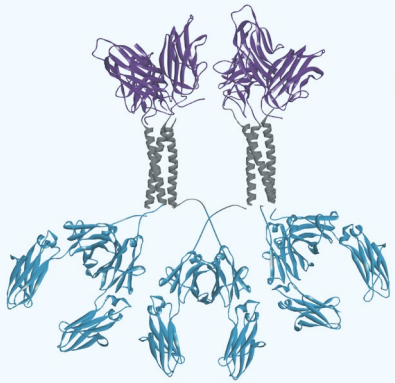
- Cyclization on the surface of the phage means we screen for the constrained 3D structure, not the sequence
- Switching to chemical synthesis after screening introduces non-natural amino acids & leverages enormous proprietary data sets

Bicycle Therapeutics – creating versatile new precision-guided medicines with potential to fill major gaps in cancer therapy

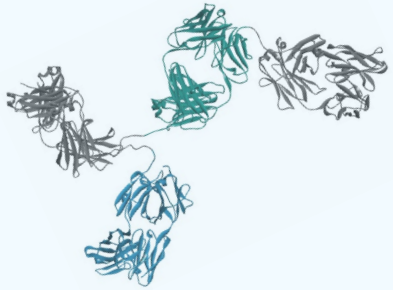


- **Bicycle Toxin Conjugates (BTCs)**
  - Precision delivery of MMAE: BT8009 & BT5528
  - Fast tissue distribution and clearance
  - Emerging clinical data

Properties of biologics not well matched to IO agonists



Medi6383
(OX40)
MW ~300 kDa

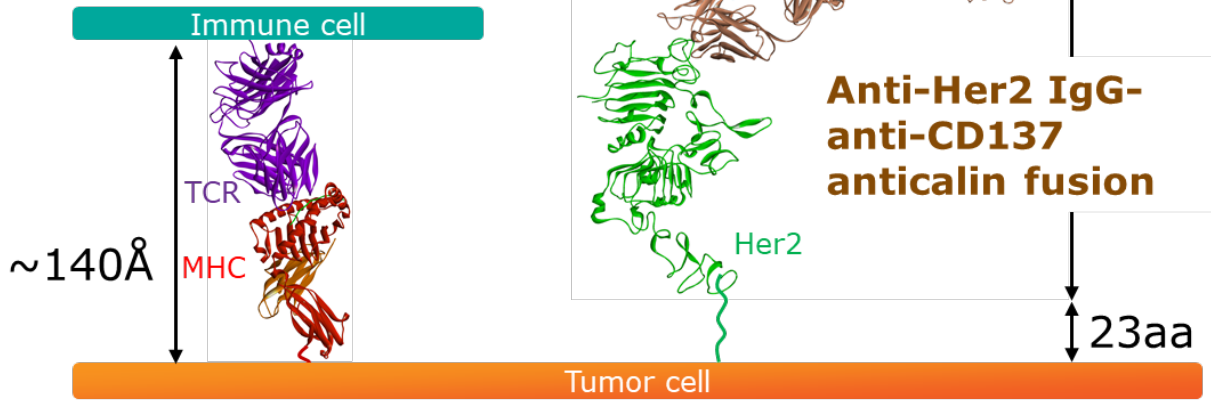


RO6958688
(CEA/CD3)
MW ~200 kDa

CD137L-HERA
MW ~170 kDa

Blincyto
(CD19/CD3)
MW ~54 kDa

- High MW = low tissue penetration
- Inflexible architecture = difficult “med chem”
- Complex manufacturing = $$$
- Non–tunable PK = not ideal for immune activation (side effects / tox)
- Prone to immunogenicity



The properties that make *Bicycles* ideal for toxin delivery also make them ideal for immune agonism, but for different reasons

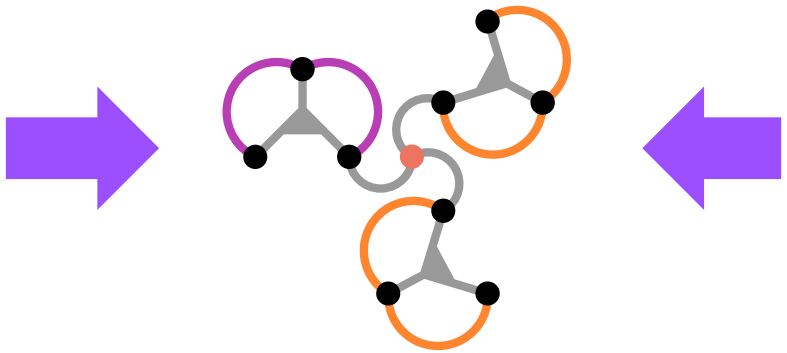
- In the body, activating signals (agonists) are local, rapid, and then stop
  - Cytokines, neurotransmitters, stress hormones
- Sustained (pathologic) signaling can lead to desensitization and dysregulation

Bicycles match the biology

precision-guided (local)
distribute quickly (rapid)
cleared rapidly (stop)

Localize action to the tumor

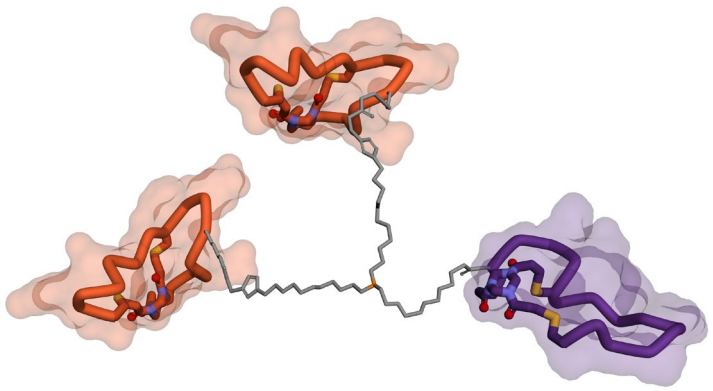
We choose targets that are present in solid tumors not well-served by current therapies



Activation of immune cells

We choose targets where knowledge of human biology says that activation is likely to help, and where other drug technologies, like antibodies, aren't working

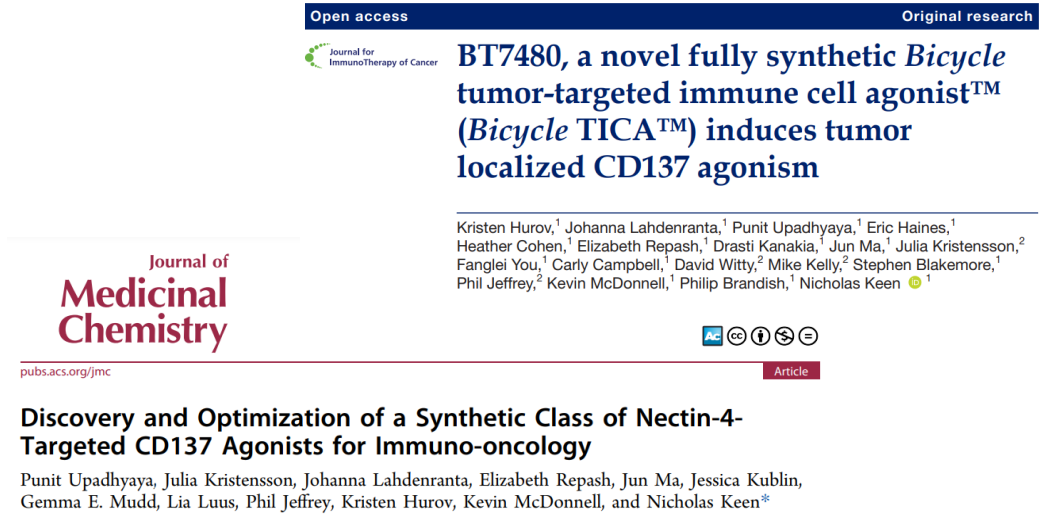
Bicycle Therapeutics – creating versatile new precision-guided medicines with potential to fill major gaps in cancer therapy



- **Bicycle Tumor-targeted Immune Cell Agonist™ (TICAs)**
  - Rapid, local and controlled immune agonism
  - Pathfinder molecule for CD137; BT7480 in phase 1
  - Pathfinder molecule for NKp46: preclinical

BT7480 – first chemically synthetic, conditionally active targeted CD137 activator

- Activity of the CD137 agonist arm is dependent on ligation of the Nectin-4 arm, leading to tumor specificity
- Causes complete regressions and anti-tumor activity with only intermittent dosing in syngeneic mouse models
- Causes an early increase in chemotactic cytokine production that precedes an increase in CD8+ T cell infiltration into the tumor
- Is well-tolerated in preclinical safety species
- Entered phase 1 clinical testing in November 2021


Open access Original research

Journal for ImmunoTherapy of Cancer

BT7480, a novel fully synthetic *Bicycle* tumor-targeted immune cell agonist™ (*Bicycle* TICA™) induces tumor localized CD137 agonism

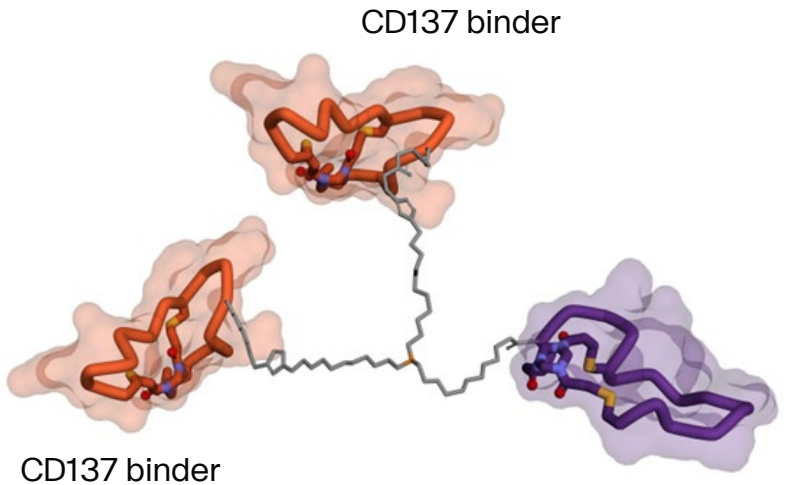
Kristen Hurov,[1] Johanna Lahdenranta,[1] Punit Upadhyaya,[1] Eric Haines,[1] Heather Cohen,[1] Elizabeth Repash,[1] Drasti Kanakia,[1] Jun Ma,[1] Julia Kristensson,[2] Fanglei You,[1] Carly Campbell,[1] David Witty,[2] Mike Kelly,[2] Stephen Blakemore,[1] Phil Jeffrey,[2] Kevin McDonnell,[1] Philip Brandish,[1] Nicholas Keen [1]

Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

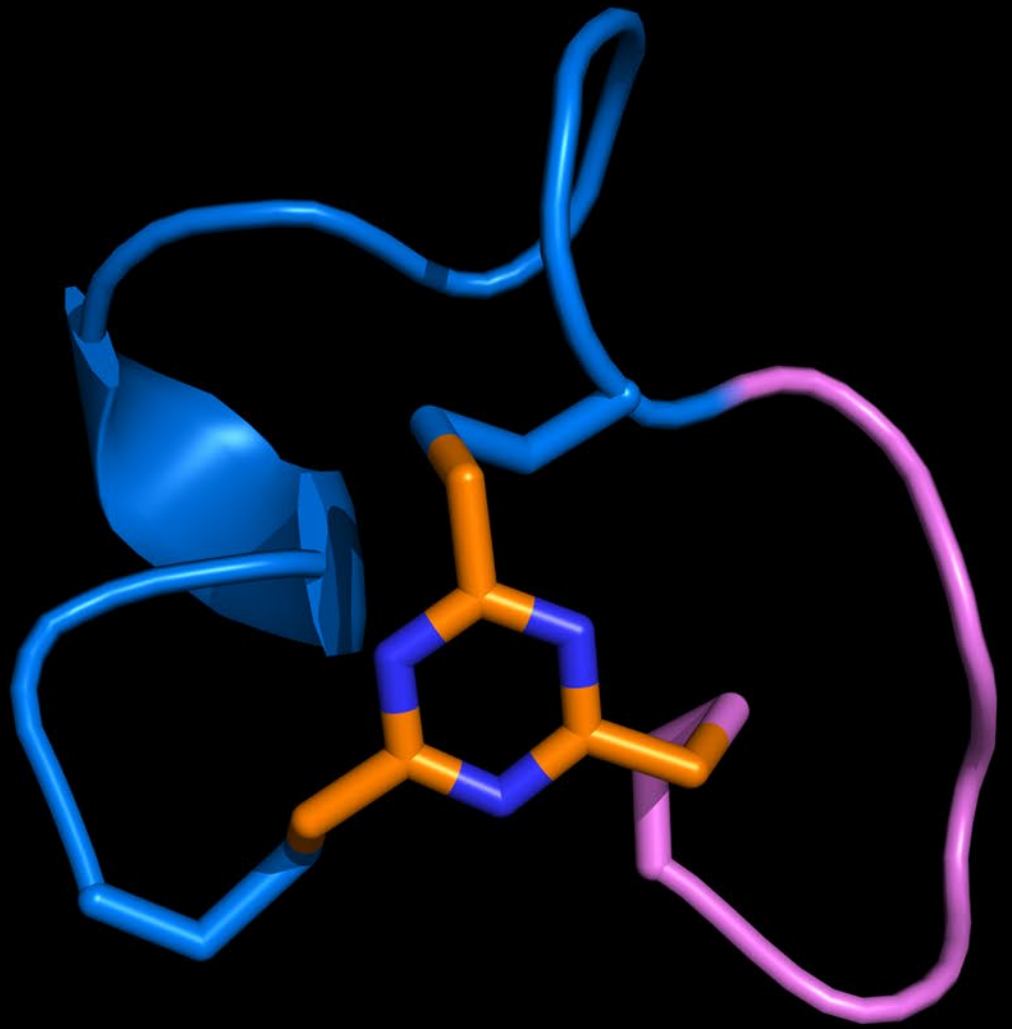
Discovery and Optimization of a Synthetic Class of Nectin-4-Targeted CD137 Agonists for Immuno-oncology

Punit Upadhyaya, Julia Kristensson, Johanna Lahdenranta, Elizabeth Repash, Jun Ma, Jessica Kublin, Gemma E. Mudd, Lia Luus, Phil Jeffrey, Kristen Hurov, Kevin McDonnell, and Nicholas Keen*



BT7480

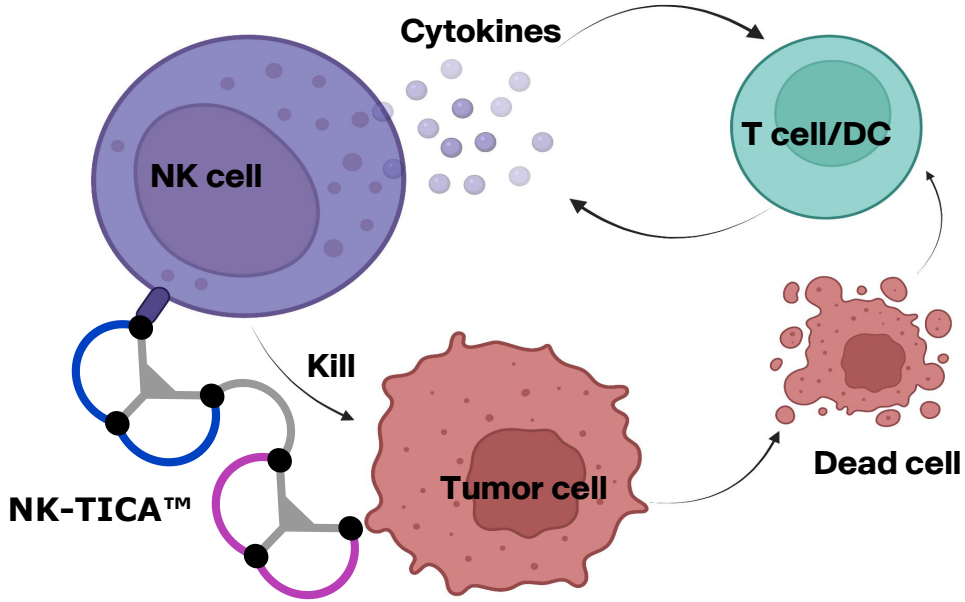
Bicycle® precision-guided NK cell activation



Bicycle®

Natural killer (NK) cells have emerged as important early drivers of the adaptive anti-tumor immune response

- Traditional understanding – NK cells kill tumor cells through direct cytotoxic mechanisms
- New science has revealed a role for NK cells in orchestration of adaptive immunity catalysis
- NK cell therapy emerging as an important new approach to cancer

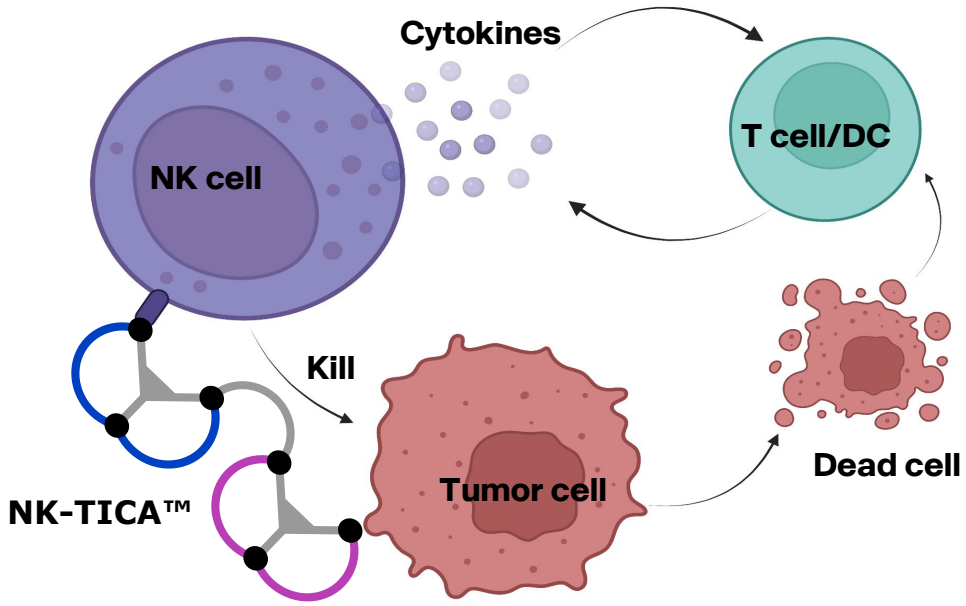


Chiossone et al., (2018) Nat. Rev. Immunol. 18, 672
Huntington et al., (2020) Nat. Rev. Cancer 20, 437
Bald et al., (2020) Nat. Immunol. 21, 835

Images created with BioRender.com (2022)

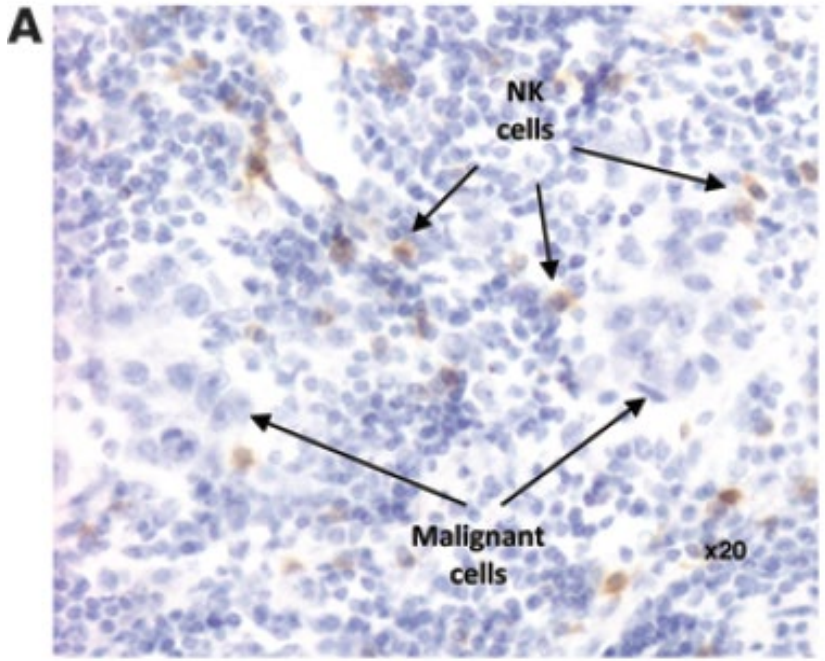
Program hypothesis

Catalysis of adaptive immunity by NK cells has potential to enable tumor rejection and enhance the action of established therapeutics (toxins and PD-1 blockers)



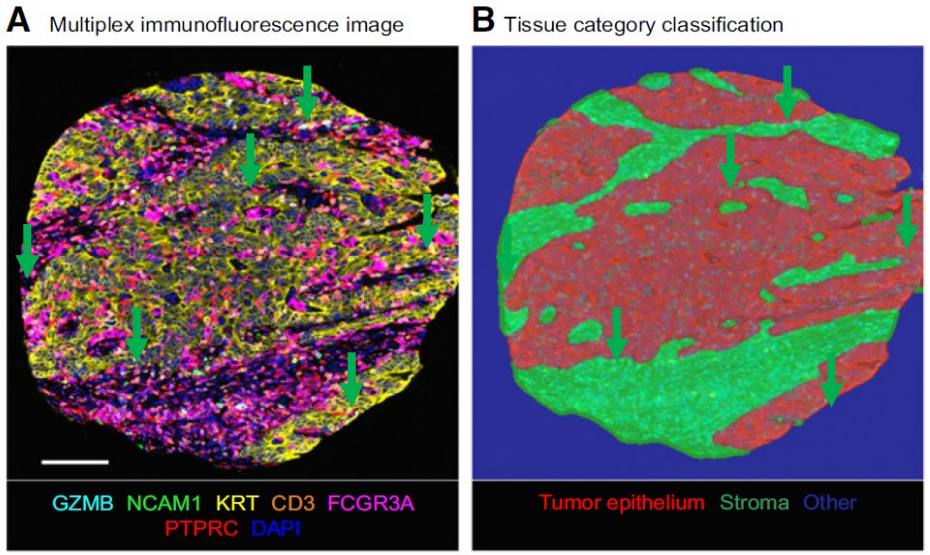
NK targeted *Bicycles* for solid tumor NK cell engagers

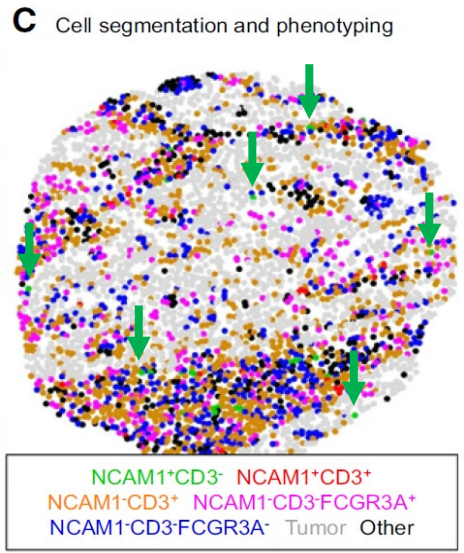
NK cells are present in appreciable numbers in the tumor, proximal to tumor cells



Mamessier (2011) J. Clin Invest – Enumeration & phenotyping of NK cells – tumor infiltrating cells had higher NKp46 expression than NK cells in normal tissue

Rigorous characterization of NK and NKT cell infiltrates in CRC and association with survival

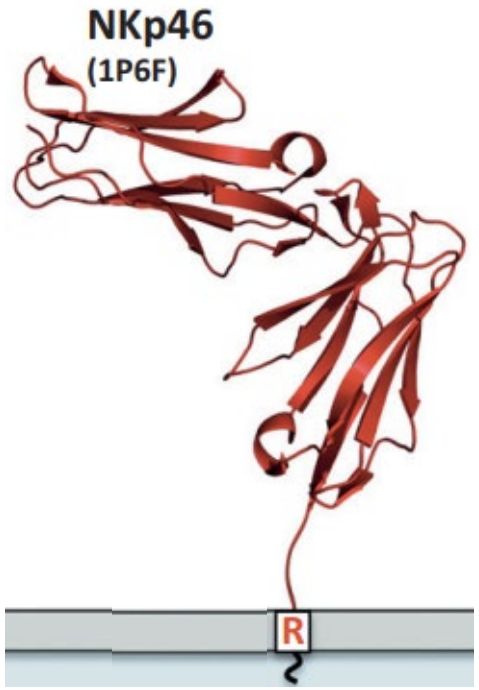




Vayrynen (2022) Cancer Immunol. Res- Example of multiplexed immunofluorescence sample from a set of 907 CRC cases.
Green arrows highlight NK cells in tumor nests.

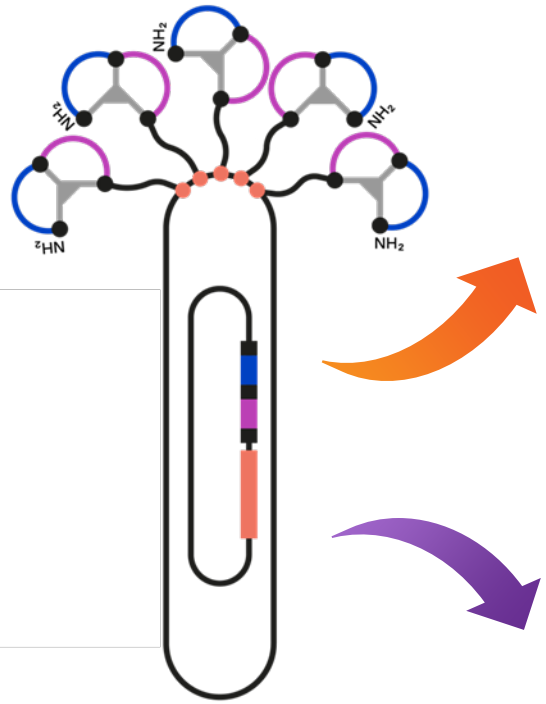
NKp46 targeted *Bicycle®* for solid tumor NK cell engagers

- Activating receptor specifically and constitutively expressed on NK cells. Not on T cells or myeloid cells. (Pessino *et al*, 1998)
- Well characterized with respect to structure and signaling (Foster *et al*, 2003; Barrow *et al*, 2019)
- NKp46, unlike NKG2D, is highly specific to NK cells
- Preclinical data for bispecific NKCE with NKp46 achieved by Innate Pharma with three antigens (Gauthier *et al*, 2019)
- NKp46 +ve NK cells are present in appreciable numbers in the tumor, proximal to tumor cells

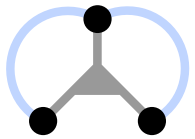


Koch *et al*. 2013 Trends in Immunology

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



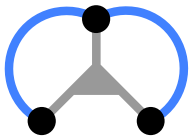
NKp46



K_D ~ 250 nM

Phage Hits

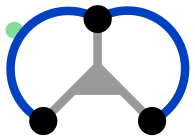
- ID sequence families
- Define initial pharmacophores



K_D ~ 35 nM

Phage Optimization

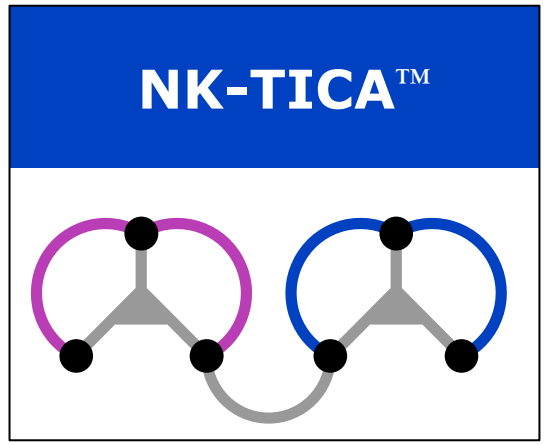
- Focused libraries
- Optimize natural AAs
- Scaffold/Loop symmetry



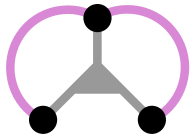
K_D < 5 nM

Chem Optimization

- Non natural amino acids
- Tuned affinity and stability
- Balanced phys. chem properties
- Selective NK cell binding

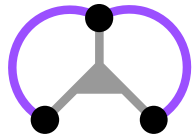


PD-L1



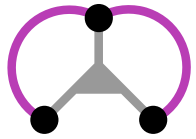
K_D = 5 nM

MT-1



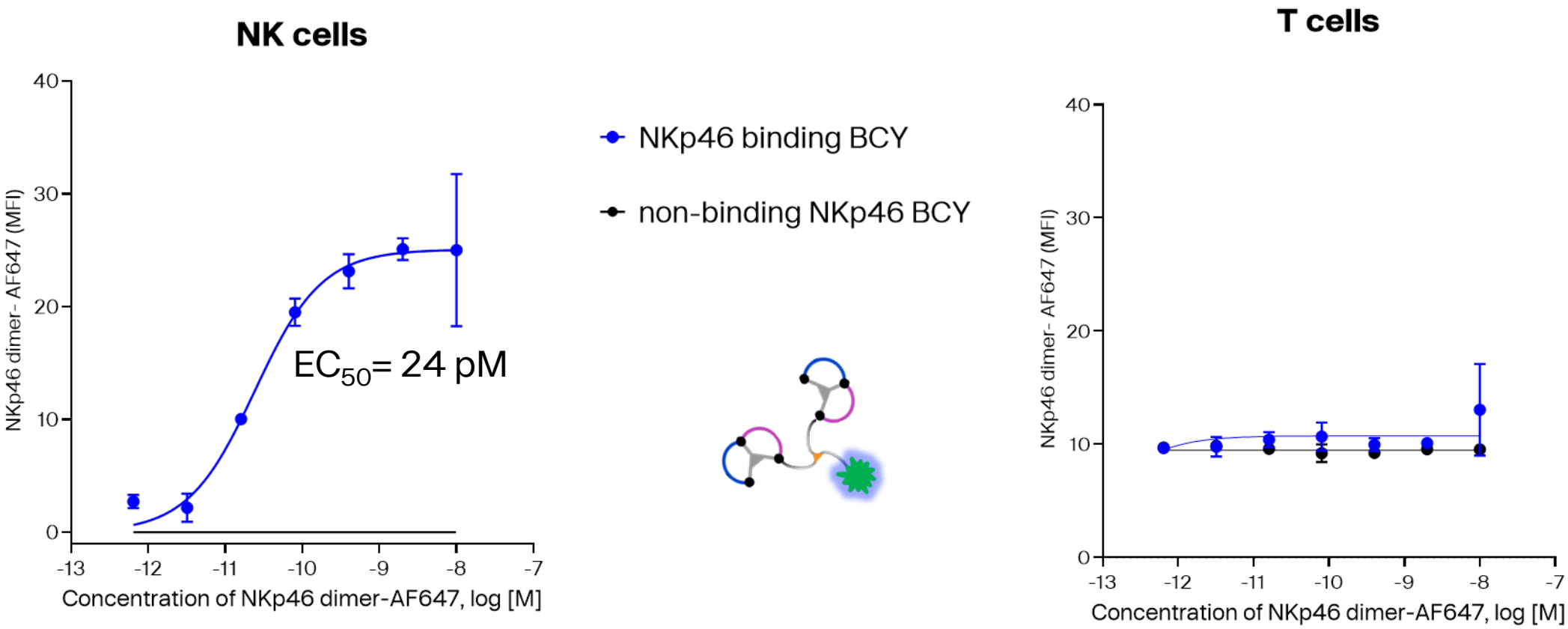
K_D = 15 nM

EphA2



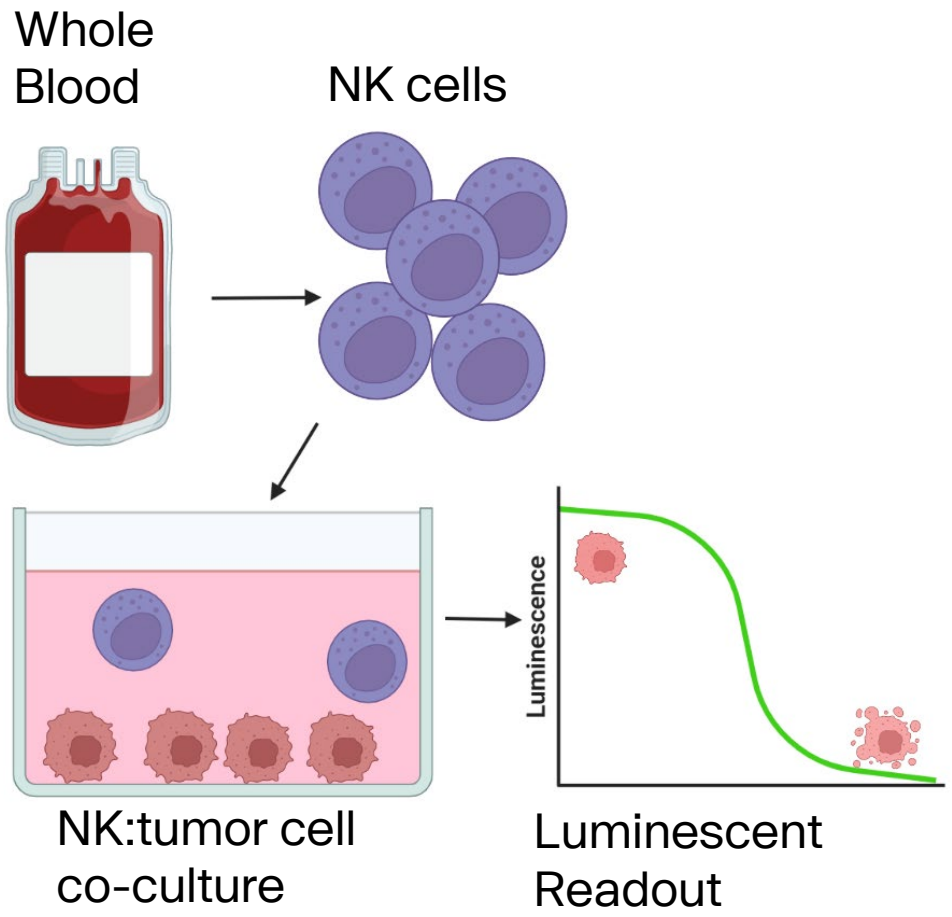
K_D = 1.7 nM

NKp46 *Bicycles* selectively bind to NK cells not T cells



- The fluorescently labeled (AF647-tagged) NKp46 *Bicycle*® bound only to NK cells in purified human PBMC (EC_{50} 24 pM).
- A non-binding control (all D isomer-analog) demonstrated no binding above background in either NK or T lymphocyte populations.

Evaluating NK-TICA™ in NK cell killing assay



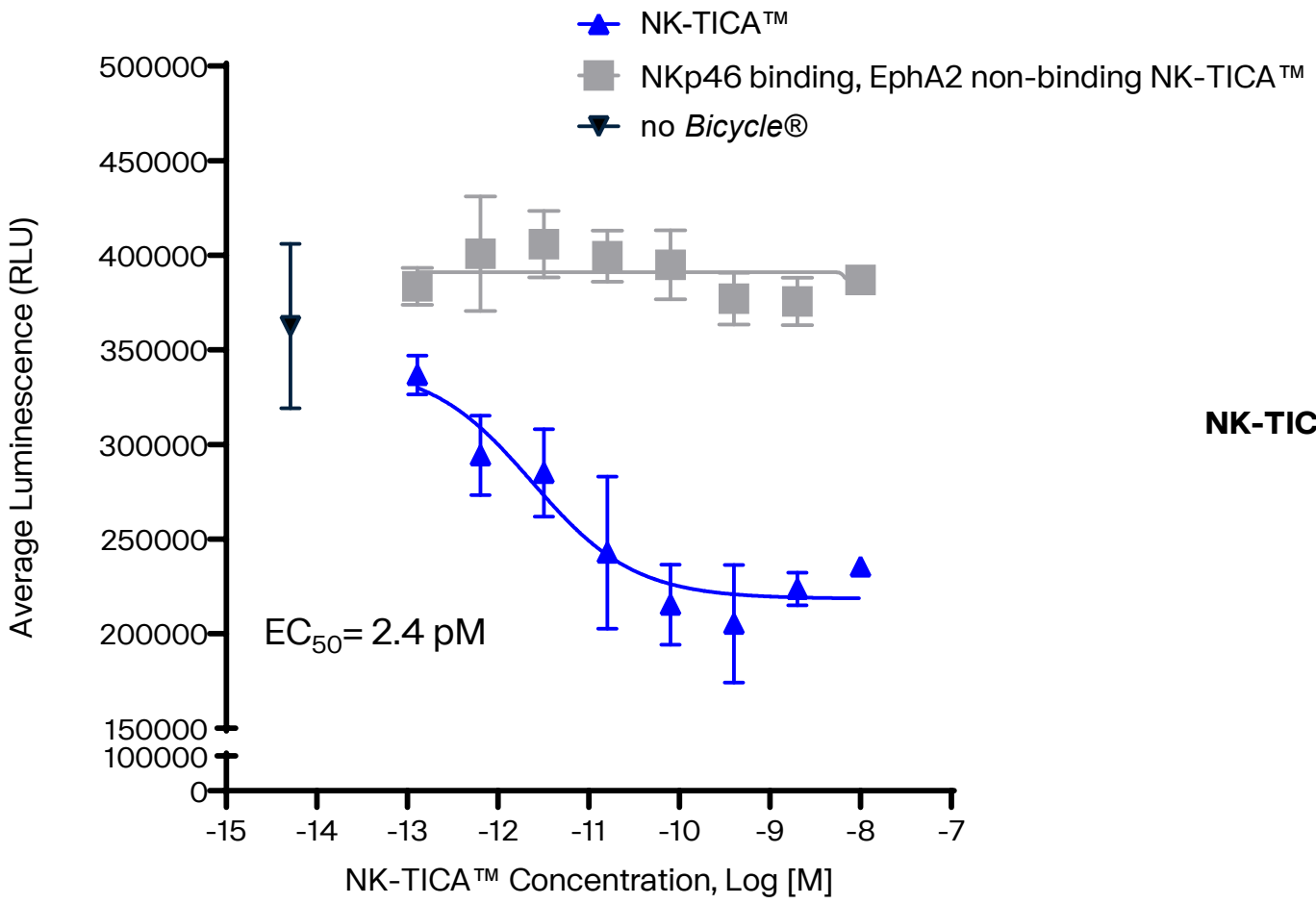
NK cells are isolated from whole blood through negative selection

NK cells are co-cultured with tumor cell lines expressing luciferase in presence of NK-TICA™

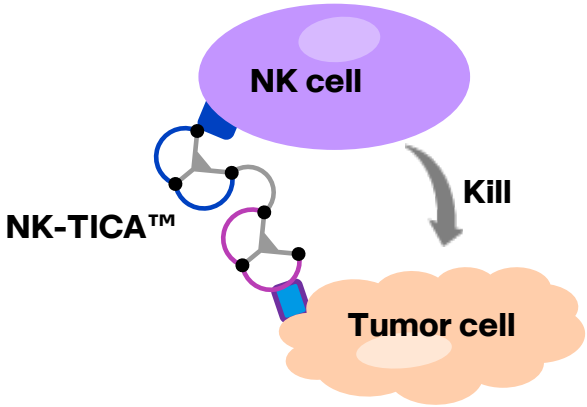
NK cells kill those tumor cells, measured by drop in luminescence

Images created with BioRender.com (2022)

Enhanced NK tumor killing with NK-TICA™ is dependent upon tumor antigen binding

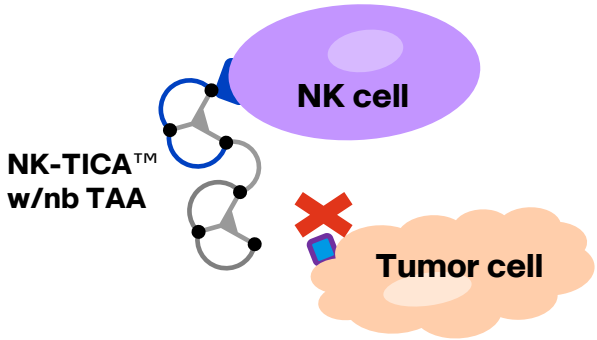


- NK cells specifically kill tumor in the presence of NK-TICA bearing EphA2 binding *Bicycle*®, not with non-binding *Bicycles*



– NK-TICA EC_{50} 2.4pM

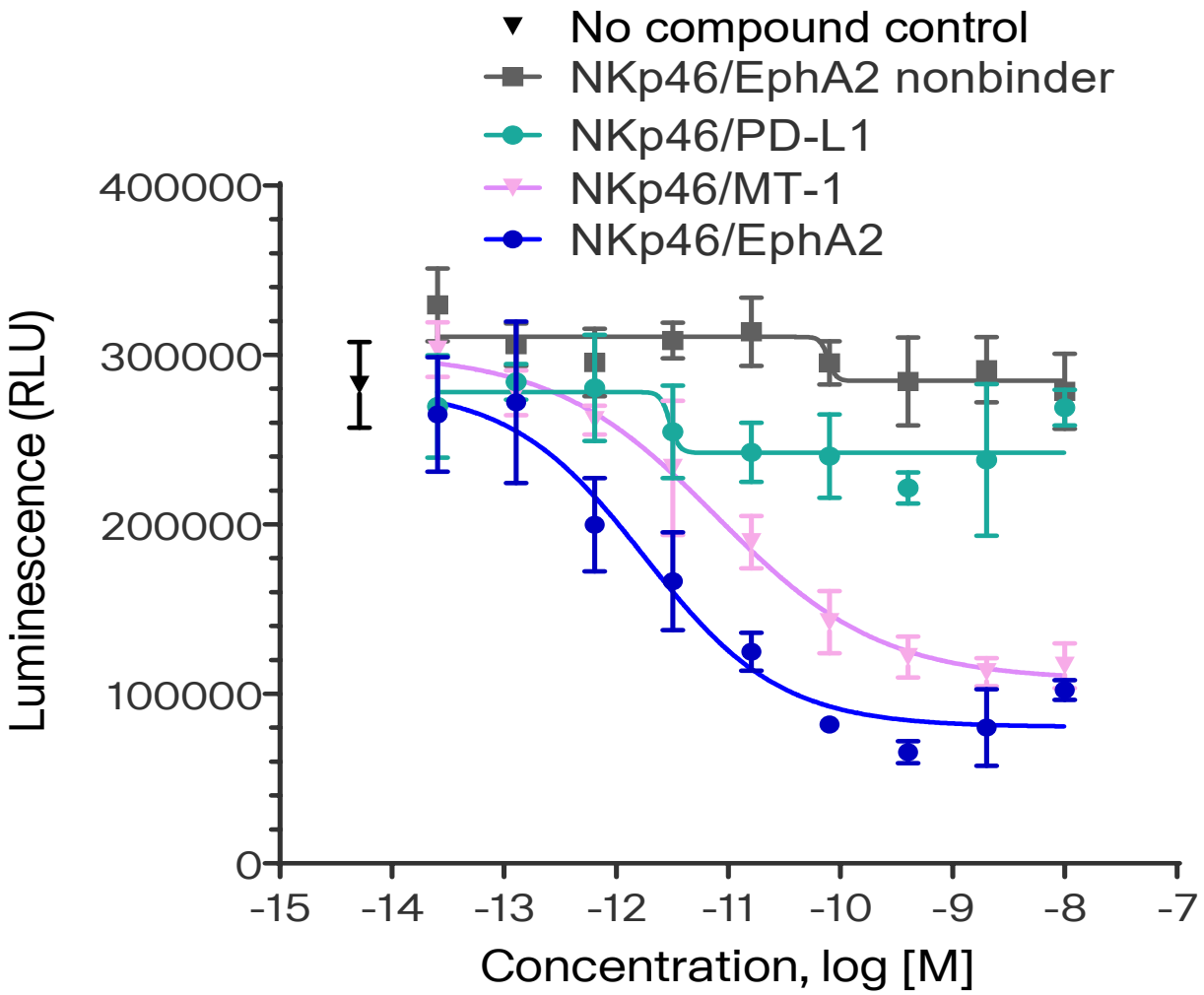
– NKp46 non-binding, EphA2 non-binding *Bicycle*®, do not enhance tumor killing



NKp46 *Bicycles* coupled to multiple tumor antigen targets drive potent tumor cell killing

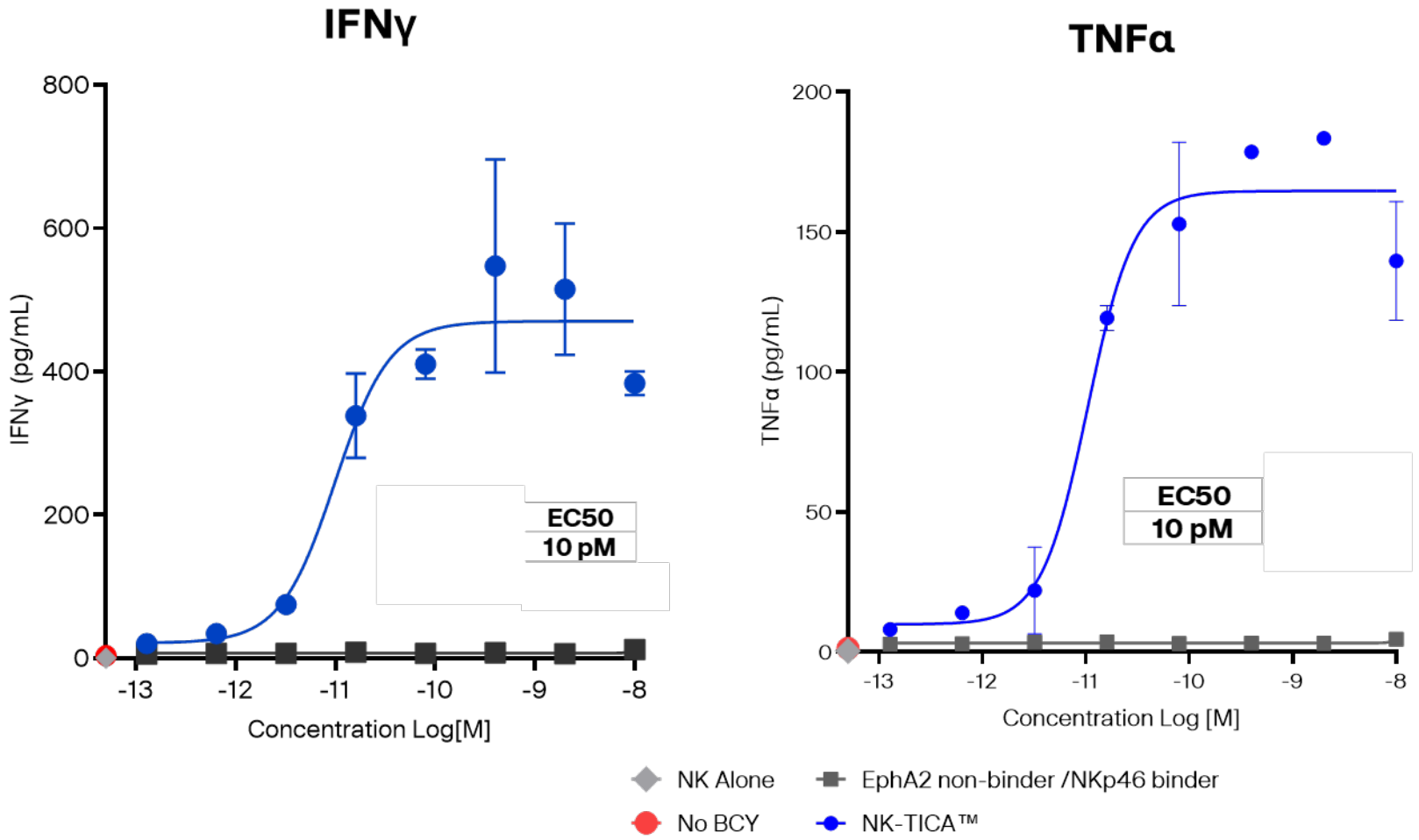
- HT1080-luc cells in co-culture with primary human NK cells
- HT1080 cells express EphA2, MT-1, and PD-L1

Potential to create *NK-TICAs* to address multiple solid tumor indications



Dufort et al., AACR 2022

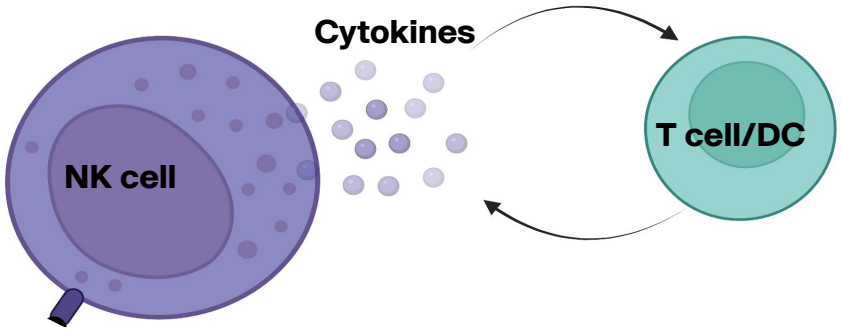
At pM concentrations, NK-TICA™ potently induce secretion of pro-inflammatory cytokines from NK cells *in vitro*

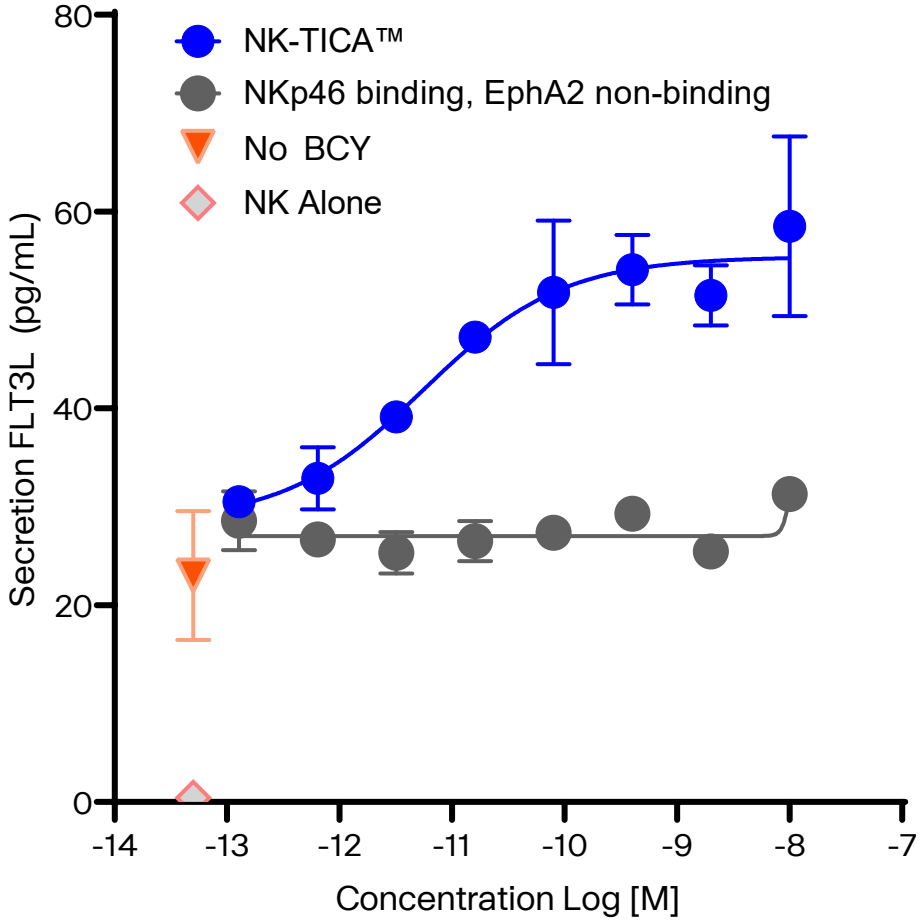


- NK cells secrete IFNγ and TNFα in the presence of NK-TICA™
- Cytokine secretion was completely dependent on binding to tumor antigen – “switch-like” behavior

NK-TICA™ enhances NK cell secretion of FLT3L

- NK cells support the recruitment and maturation of DCs
- FMS-related tyrosine kinase 3 ligand (FLT3L) is mainly produced by NK cells in the tumor microenvironment and is essential for the *in situ* development and proliferation of conventional DCs (cDCs)

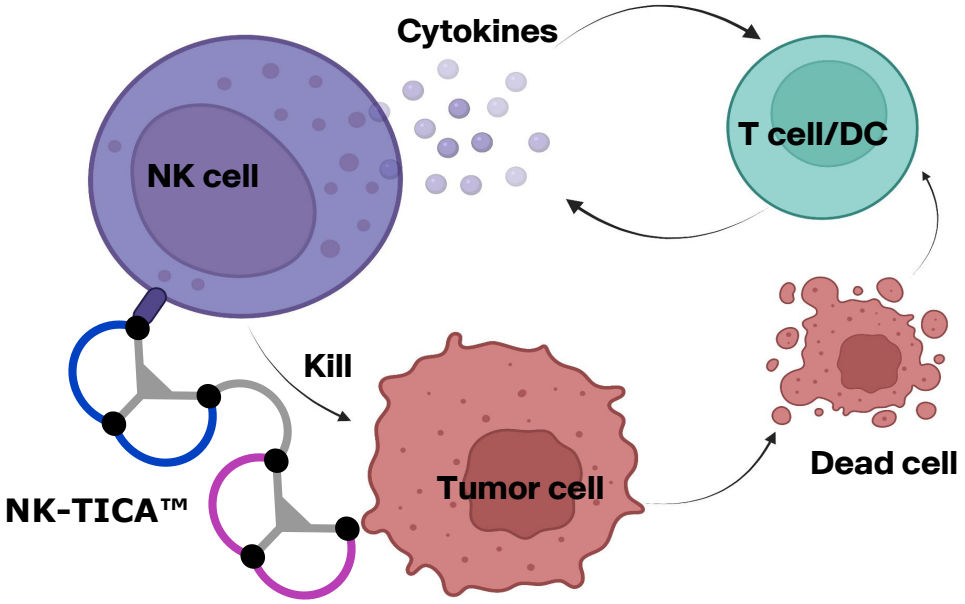




- Wculek *et al.* Nat Rev Immunol. 2020, Allen F *et al.* Oncoimmun 2018, Bottcher *et al.* Cell, 2018 , Holmes *et al.* PNAS 2014
- Zhou, Y. *et al.* Mol Cancer 2023, Salmon H *et al.* Immunity 2016 Barry *et al.* Nat Med 2018,

Bicycle®

We have created a series of chemically synthetic, conditionally active, targeted NKp46 activators



Potential for activity as a monotherapy and as an adaptor molecule to combine with universal NK cell therapy

- Building on success with CD137 Bicycle® TICAs, the Bicycle platform has now been successfully applied to build prototype NK cell engagers
- NKp46 *Bicycles* in a Bispecific TICA™ enhances cytokine production and tumor cell killing which has the potential to drive adaptive anti-tumor immunity
- 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

Thank you

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