Antitumor immunity induced by antibody-based natural killer cell engager therapeutics armed with not-alpha IL-2 variant

Olivier Demaria[1], Laurent Gauthier[1], Marie Vetizou[1], Audrey Blanchard Alvarez[1], Guillaume Habif[1], Roger Le Grand[2], Cécile Bonnafous[1], Agnès Represa[1], Sabrina Carpentier[1], Benjamin Rossi[1], Ariane Morel[1], Stéphanie Cornen[1], Ivan Perrot[1], Yannis Morel[1] and Eric Vivier[1,3,4].

[1]Innate Pharma, Marseille, France. [2]Université Paris-Saclay, Inserm, CEA, Center for Immunology of Viral, Auto-immune, Hematological and Bacterial Diseases (IMVA-HB/IDMIT), Fontenay-aux-Roses & Le Kremlin-Bicêtre, France. [3]Aix Marseille University, CNRS, INSERM, CIML, Marseille, France. [4]Assistance Publique des Hôpitaux de Marseille, Hôpital de la Timone, Marseille-Immunopôle, Marseille, France Contact: olivier.demaria@innate-pharma.fr

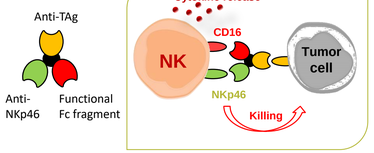
2SITO21 WASHINGTON, D.C.

Poster #851

Introduction

Harnessing NK cells is emerging as a promising therapeutic approach to improving the efficacy of cancer treatment and overcoming resistance to current immunotherapies targeting T cells. Synthetic biology offers unprecedented opportunities to manipulate biological functions of innate immune cells and boost their capacity to directly kill tumor cells and to indirectly stimulate T cell responses. We previously reported the generation of trispecific antibody-based natural killer cell engager therapeutics (ANKET), which co-engage NKp46 and CD16 on NK cells and a tumor antigen (TAg) on cancer cells, inducing NK cell activation and better tumor control as compared to approved therapeutic antibodies targeting the same tumor antigen[1].

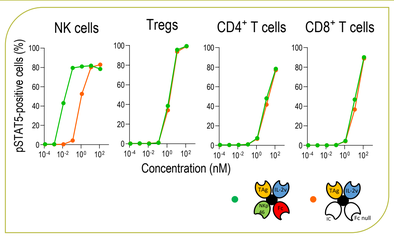
Trispecific ANKET:



Here, we describe the characterisation of tetraspecific ANKET, which incorporates a variant of interleukin-2 (IL-2v), deficient in binding to the IL-2Rα subunit, in addition to NKp46, CD16 and TAg interacting elements. IL-2v activity is redirected by tetraspecific ANKET toward NK cells through the binding in cis of NKp46 and CD16, and strongly promotes antitumor efficacy. Tetraspecific ANKET constitute a new technological platform for harnessing the functions of NK cells and inducing strong preclinical antitumor efficacy, supporting their development as next-generation cancer immunotherapies.

Tetraspecific ANKET:

Tetraspecific ANKET redirects IL-2v activity toward NK cells

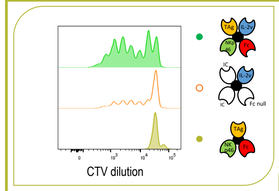


a Tetraspecific ANKET redirects IL-2R activation on NK cells. PBMCs from healthy donors were activated with the indicated molecules. pSTAT5 was analysed by flow cytometry.

Reference

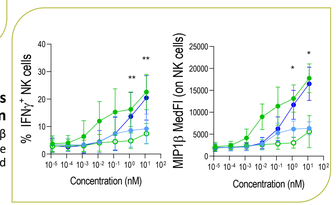
(1) Gauthier et al., Multifunctional Natural Killer Cell Engagers Targeting NKp46 Trigger Protective Tumor Immunity. **Cell, 2019**

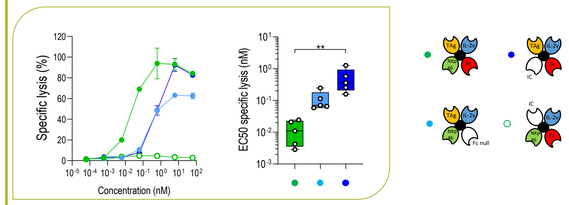
Induction of human NK cell activation by tetraspecific ANKET



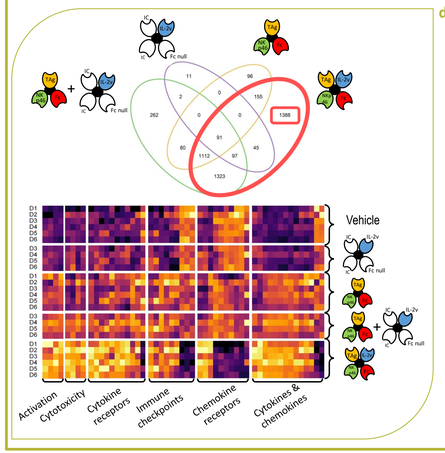
a Tetraspecific ANKET induces NK cell proliferation. proliferation of purified NK cells activated with 3.7nM of the indicated molecules in the absence of target tumor cells.

b Tetraspecific ANKET promotes cytokine production only upon target engagement. IFN-γ and MIP1-β production by purified NK cells in the presence of Raji tumor cells activated by the indicated molecules.



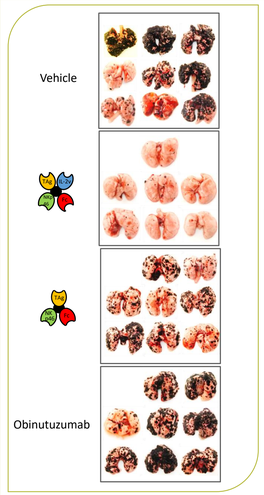


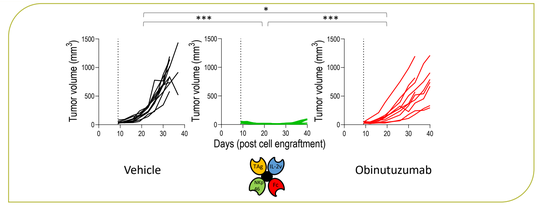
c Tetraspecific ANKET promotes cytotoxicity only upon target engagement. NK cell cytotoxicity towards Raji tumor cells induced by the indicated molecules.



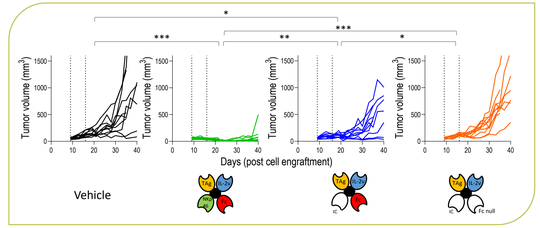
d Tetraspecific ANKET leads to the activation of a unique transcriptional program in NK cells associated with enhanced effector responses. Purified human NK cells were activated with the indicated molecules in the presence of the murine huCD20-B16F10 target cells. RNA sequencing was performed and reads were aligned with the human genome. Upper chart; Venn diagram representing the number of genes upregulated in NK cells following activation with the indicated molecules. Lower chart; Heatmap representing the expression of a selection of differentially regulated genes.

Induction of antitumor immunity by tetraspecific ANKET



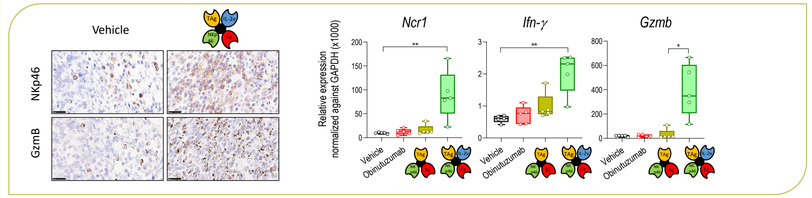


b Tetraspecific ANKET anti-tumor efficacy: solid tumor model. Raji B-cell lymphoma cells were engrafted s.c in CB17 SCID mice. Mice were treated i.v with a single dose of CD20-targeted tetraspecific ANKET (70µg) or obinutuzumab (1500µg), nine days after tumor engraftment (dotted line).

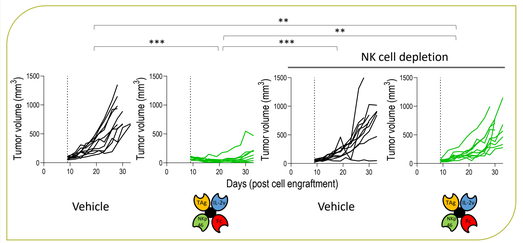


a Tetraspecific ANKET anti-tumor efficacy: disseminated tumor model. HuCD20-B16F10 were injected i.v in C57BL6 mice. Mice were treated i.v at day +1 with CD20-targeted ANKET (70µg) or obinutuzumab (600µg). Lungs were analysed at day 14.

c NKp46 targeting is mandatory to tetraspecific ANKET antitumor efficacy. Raji cells were engrafted s.c in CB17 SCID mice. Mice were treated with the indicated molecules targeting CD20 at the dose of 25 µg/mouse injected day 9 and 16 after tumor engraftment (dotted lines).

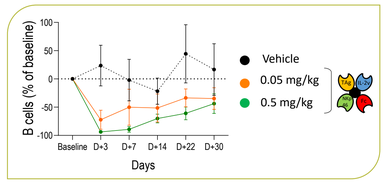


d Tetraspecific ANKET promotes accumulation of activated NK cells in the TME. Left, immunostaining for human NKp46 and GzmB on sections of s.c Raji tumors engrafted in RAG1ko huNKp46Tg mice, 3 days after treatment with CD20-targeted tetraspecific ANKET. Right, levels of *Ncr1*, *Ifn-γ* and *GzmB* transcripts, evaluated by qPCR in Raji tumors engrafted in CB17 SCID mice.

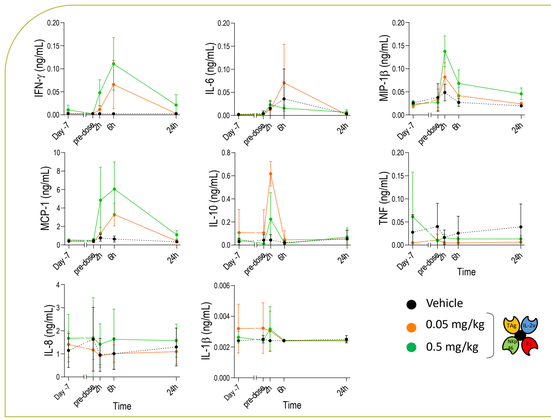


e NK cells drive tetraspecific ANKET antitumor activity. Raji cells were engrafted s.c in CB17 SCID mice. Mice were treated with a single i.v injection of tetraspecific ANKET (25µg, green) with or without NK cell depletion induced by anti-Asialo-GM1.

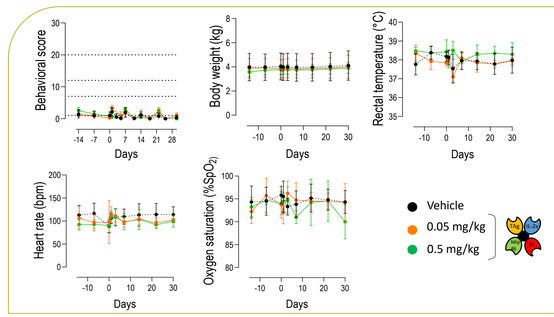
Efficacy and safety of tetraspecific ANKET treatment in Non-Human Primates (NHP)



a Single dose treatment with CD20-targeted tetraspecific ANKET induces profound depletion of circulating B cells in NHPs. Counts of circulating B cells, expressed as a % of the baseline counts in NHPs receiving a single injection of vehicle (n=6, black), or CD20-targeted tetraspecific ANKET at a dose of 0.05 mg/kg (n=4, orange) or 0.5 mg/kg (n=4, green).



b CD20-targeted tetraspecific ANKET induces minimal circulating cytokine release in NHPs. Cytokine concentrations in the plasma of NHPs receiving a single injection of vehicle (n=6, black), or CD20-targeted tetraspecific ANKET at a dose of 0.05 mg/kg (n=4, orange) or 0.5 mg/kg (n=4, green).



c No sign of toxicity induces by CD20-targeted tetraspecific ANKET in NHPs. NHPs receiving a single injection of vehicle (n=6, black) or CD20-targeted tetraspecific ANKET at a dose of 0.05 mg/kg (n=4, orange) or 0.5 mg/kg (n=4, green) were followed over time. Monitoring data are shown for behavior score, body weight, rectal temperature, heart rate and pulsed oximetry (SpO2).

Conclusion

We report here the design of new antibody-based natural killer cell engager therapeutics (ANKET):

- **Tetraspecific ANKET: a single tetraspecific molecule engaging the NK cell activating receptors NKp46 and CD16, the β chain of the interleukin-2 receptor (IL-2R) and a tumor-associated antigen (TAg). The IL-2R-interacting element is a variant of IL-2 (IL-2v) that cannot bind the IL-2Rα subunit.**
- **Tetraspecific ANKET redirects IL-2v activity on NK cells through the binding of NKp46 and CD16, and promotes IL-2R signaling in NK cells with approximately 2-log greater potency than non-targeted IL-2v, enhancing the proliferation of these cells.**
- **TAg binding by the ANKET, connecting the NK cell to the tumor, is needed to trigger the cytotoxic activity of NK cells and the secretion of cytokines and chemokines.**
- **Tetraspecific ANKET induces a cooperative crosstalk between the ITAM and JAK/STAT signaling pathways resulting in a specific transcriptomic effector program associated with enhanced NK cell effector responses, that could not be reproduced by treatment with the combination of soluble cytokine and trispecific ANKET .**
- **In mouse models of both disseminated and solid tumors, tetraspecific ANKET induces NK cell proliferation, accumulation at the tumor bed, and an antitumor efficacy greater than that of approved therapeutic antibodies.**
- **The treatment of non-human primates with CD20-targeted tetraspecific ANKET induces profound depletion of CD20+ circulating B cells with minimal systemic cytokine release and no sign of toxicity.**

ANKET™ Antibody-based NK cell Engager Therapeutics

innate pharma