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

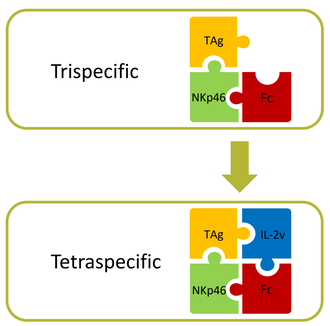
Olivier Demaria[1], Laurent Gauthier[1], Marie Vetizou[1], Audrey Blanchard Alvarez[1], Guillaume Habif[1], Roger Le Grand[2], Cécile Bonnafous[1], Agnès Represa[1], Sabrina Carpentier[1], Benjamin Rossi[1], Ariane Morel[1], Stéphanie Cornen[1], Ivan Perrot[1], Yannis Morel[1] and Eric Vivier[1,3,4].

[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

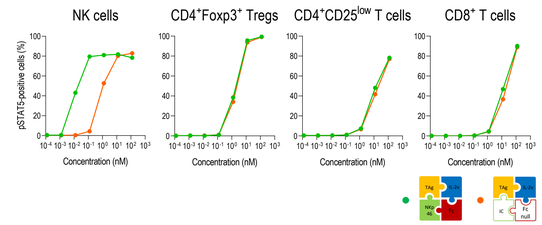
Abstract

In cancer, primary and secondary resistances to T-cell centered immunotherapies are prompting the manipulation of innate immunity to improve current treatments. Synthetic biology offers unprecedented opportunities to harness 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 Natural Killer cell engagers (ANKET3), which co-engage NKp46 and CD16 on NK cells and a tumor antigen (TAg) on cancer cells, improving NK cell activation and tumor control as compared to approved therapeutic antibodies targeting the same tumor antigen (1). Here, we described the generation of tetraspecific ANKET or ANKET4, by incorporating into trifunctional NKCEs a variant of interleukin-2 (IL-2v) deficient in binding to the α-subunit of its receptor. In vitro, ANKET4 promoted specifically IL-2R signaling in NK cells as compared to other lymphocytes, and induced primary human NK cell proliferation, and, only upon tumor target engagement, cytolytic activity and secretion of cytokines and chemokines. In mouse tumor models, ANKET4 induced NK cell proliferation and accumulation at the tumor bed and showed superior anti-tumor efficacy to ANKET3. In non-human primates, CD20-directed ANKET4 was well tolerated and led to CD20+ B cell depletion with minimal systemic cytokine release. Tetraspecific ANKET thus constitutes a new technological platform combining the induction of NK cell proliferation and effector functions in absence of toxicity, supporting their clinical development as next generation cancer immunotherapies.

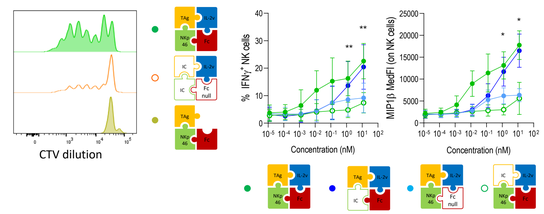
From ANKET3 to ANKET4



Induction of human NK cell activation by tetraspecific ANKET

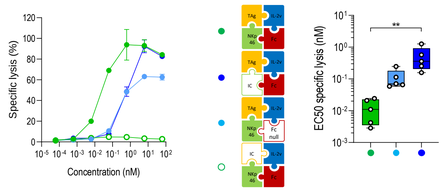


a Tetraspecific ANKET redirected IL-2R activation on NK cells. PBMC were activated for 20 min with the indicated molecules. pSTAT5 was analysed by flow cytometry on various immune cell populations.



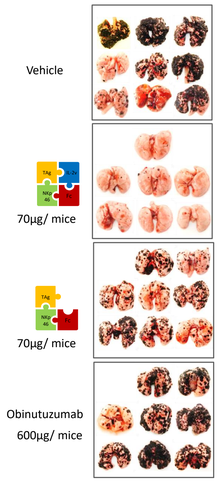
b Tetraspecific ANKET induced NK cell proliferation. Representative proliferation pattern of purified NK cells activated for 5 days with the indicated molecules in the absence of the target tumor cells.

c Tetraspecific ANKET induced cytokine production 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 for 4 h. The data shown are the mean ± SD for three donors.

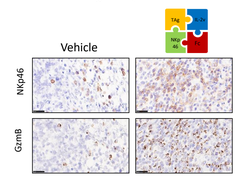


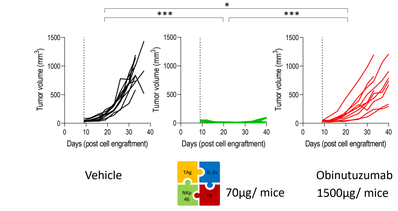
d Tetraspecific ANKET induced cytokine production upon target engagement. NK cell cytotoxicity towards Raji tumor cells induced by the indicated molecules. Left, representative donor; Right; boxplot depicting the EC50 obtained for five donors.

Induction of tumor immunity by ANKET

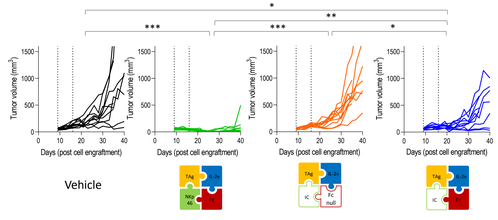


a Tetraspecific ANKET anti-tumor efficacy: disseminated tumor model. HuCD20-B16F10 were ijected i.v in C57BL6 mice. Mice were treated at day +1 with the indicated molecules and lungs analysed at day 14.

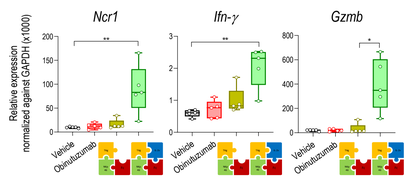




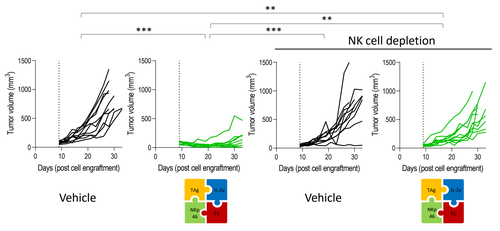
b Tetraspecific ANKET anti-tumor efficacy: solid tumor model. Raji B-cell lymphoma cells were subcutaneously engrafted in CB17 SCID mice. Efficacy of the indicated molecules injected i.v once, nine days after tumor engraftment.



c NKp46 targeting was mandatory to tetraspecific ANKET antitumor efficacy. Raji B-cell lymphoma cells were subcutaneously engrafted in CB17 SCID mice. Efficacy of the indicated molecules injected i.v once at the dose of 25 µg/mice, nine days after tumor engraftment.

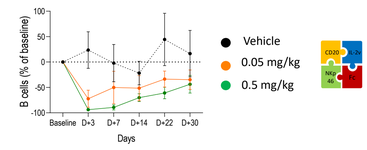


d Tetraspecific ANKET promoted accumulation of activated NK cells in the TME. Right, Immunostaining for human NKp46 and GzmB on sections of Raji tumors grown subcutaneously in RAG1ko huNKp46Tg mice, 3 days after treatment with ANKET4. Left, levels of Ncr1, Ifn-γ and GzmB transcripts, evaluated by qPCR in Raji tumors harvested from CB17 SCID mice three days after treatment with the indicated molecules.

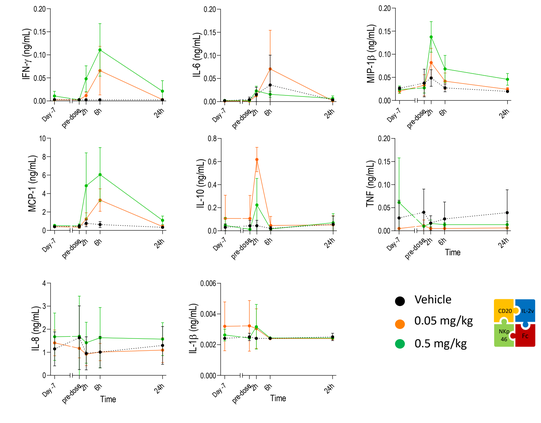


e NK cells drove tetraspecific ANKET antitumor activity. Raji B-cell lymphoma cells were subcutaneously engrafted in CB17 SCID mice. Efficacy of a single i.v injection of 25 µg of IL2v/aNKp46/Fc/aCD20 (green) in the context of NK cell depletion with anti-Asialo-GM1.

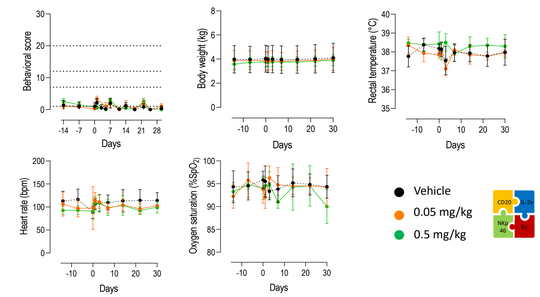
Efficacy and safety of ANKET treatment in non-human primates



a CD20 targeting tetraspecific ANKET induced profound circulating B cell depletion in Non Human Primates. 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 targeting-ANKET4 at a dose of 0.05 mg/kg (n=4, orange) or 0.5 mg/kg (n=4, green).



b CD20 targeting tetraspecific ANKET induced minimal circulating cytokine release in NHPs. Cytokine concentrations in the plasma of NHPs receiving a single injection of vehicle (n=6, black), or CD20 targeting-ANKET4 at a dose of 0.05 mg/kg (n=4, orange) or 0.5 mg/kg (n=4, green).



c No sign of toxicity induced by CD20 targeting tetraspecific ANKET in NHPs. NHPs receiving a single injection of vehicle (n=6, black) or CD20 targeting ANKET4 at a dose of 0.05 mg/kg (n=4, orange) or 0.5 mg/kg (n=4, green) were followed over time. Representative 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):

- **ANKET4: 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 and is redirected toward NK cells through the binding in cis of NKp46 and CD16.**
- **ANKET4 promotes IL-2R signaling in NK cells with approximately 2-log greater potency than non-targeted IL-2, leading to the preferential proliferation of these cells.**
- **Only the binding of the ANKET to the TAA, connecting the NK cell to the tumor, was able to trigger the cytotoxic activity of NK cells and the secretion of cytokines and chemokines.**
- **In mouse models of both invasive and solid tumors, ANKET4 induced NK cell proliferation and accumulation at the tumor bed, with an antitumor efficacy greater than that of approved therapeutic antibodies targeting the same tumor antigen.**
- **The treatment of non-human primates with CD20-directed ANKET4 promoted the depletion of CD20+ circulating B cells with minimal systemic cytokine release and no sign of toxicity.**
- **Tetraspecific ANKET, thus, 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.**

ANKET™ Antibody-based NK cell Engager Therapeutics

References

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