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

Olivier Demaria1, Laurent Gautheir1, Marie Vetru2, Audrey Blanchard Alvarez1, Guillaume Habib2, Roger Le Grand3, Cécile Bonnaud4, Agnès Represa4, Sabrina Carpentier5, Benjamin Ross1, Ariane Morel1, Stéphanie Cornen1, Ivan Perrot1, Yannis Morel1 and Eric Vivier1,4,6

1Innate Pharma, Marseille, France. 2Université Paris-Saclay, Inserm, CEA, Center for Immunology of Vital, Auto-Immune, Hematological and Bacterial Diseases (INRA-IBID/CHIM), Fontenay-aux-Roses & Le Kremlin-Bicêtre, France. 3Aix Marseille University, CNRS, INSERM, CISM, Marseille, France. 4Assistance Publique des Hôpitaux de Marseille, Hôpital de la Timone, Marseille-Innopolis, Marseille, France. Contact: olivier.demaria@innate-pharma.fr

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 engagers therapeutics (ANKET), which co-engage Nkp46 and CD16 in 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.

Trispecific ANKET

Here, we describe the characterisation of trispecific ANKET, which incorporates a variant of interleukin-2 (IL-2c), deficient in binding to the IL-2R beta subunit, in addition to Nkp46 and CD16, and Tag, interacting elements. IL-2c activity is redirected by trispecific ANKET toward NK cells through the binding in cis of Nkp46 and CD16, and strongly promotes antitumor efficacy. ANKET technology constitutes a new technological platform for harnessing the features of NK cells and inducing strong preclinical antitumor efficacy, supporting their development as next-generation cancer immunotherapeutics.

Tetraspecific ANKET

Tetraspecific ANKET redirects IL-2 activity toward NK cells

Induction of human NK cell activation by tetraspecific ANKET

Tetraspecific ANKET promotes cytokine production only upon target engagement, by synergy with the IL-2c subunit, and IL-2c was found to be the key mechanism of IL-2 activity in packed NK cells activated by the indicated mechanism.

Induction of antitumor immunity by tetraspecific ANKET

Tetraspecific ANKET promotes cytotoxicity only upon target engagement, to cell cytotoxicity towards NK cells induced by the indicated mechanisms.

Tetraspecific ANKET leads to the activation of a unique transmembrane signaling program in NK cells associated with enhanced effector responses. Purified human NK cells were cultured in the presence of the indicated cytokines in cis, in the absence of the indicated cytokines in trans. Data are expressed as the percentage of NK cells expressing granzyme B or CD107a, calculated on day 24h.

Tetraspecific ANKET promotes cytokine and cytotoxicity accumulation in induced NK cells in the TME, likely immunoactivating for human NKp46 and stimulate secretion of IL-2c at a higher rate.

Tetraspecific ANKET induces minimal circulating cytokine release in NHPs. Cytokine concentrations in the plasma of NHPs injected with a single injection of vehicle (saline), or Obinutuzumab-targeted tetraspecific ANKET at a dose of 0.5 mg/kg (10x), or Obinutuzumab-targeted ANKET at a dose of 0.5 mg/kg (5x), or Obinutuzumab-targeted ANKET at a dose of 0.5 mg/kg (2.5x).

Efficacy of tetraspecific ANKET in NHPs

No sign of toxicity induces by Obinutuzumab-targeted tetraspecific ANKET in NHPs, terms scoring a single injection of vehicle (saline), Obinutuzumab-targeted tetraspecific ANKET at a dose of 0.5 mg/kg (5x), or Obinutuzumab-targeted ANKET at a dose of 0.5 mg/kg (2.5x).

Conclusion

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

- Tetraspecific ANKET: a single tetraspecific molecule engaging the NK cell activating receptors Nkp46 and CD16, the beta 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-2c) that cannot bind the IL-2R beta subunit.
- Tetraspecific ANKET redirects IL-2 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-2c, 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 TNF and the JAK/STAT signaling pathways resulting in a specific trispecific receptor program associated with enhanced NK cell effector responses, that could not be reproduced by treatment with the combination of soluble cytokines 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 Obinutuzumab-targeted tetraspecific ANKET induces profound depletion of circulating B cells with minimal systemic cytokine release and no sign of toxicity.