Multifunctional natural killer cell engagers targeting NKp46 trigger protective tumor immunity

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Background

Over the last decades, various new therapies have been developed to promote anti-tumor immunity. Despite interesting clinical results in neoadjuvant malignancies, the development of novel NK cell engagers targeting NKp46, an activating receptor on NK cells and a tumor antigen on cancer cells, has been challenging. Tetravalent NKp46/NKp30 NK cell engagers (NKCEs) could trigger NK cell activation and anti-tumor activity. Tetravalent NKCEs were more potent in vitro than those less immunocompetent targeting the same tumor antigens. They have similar in vivo pharmacokinetics to Fc (anti-NKp46, anti-CD19/EGFR) antibodies, no off-target effects and efficiently controlled tumor growth in mouse models of solid and invasive tumors. Tetravalent NKCEs thus constitute a new generation of molecules for fighting cancer.

A

Comparison of the cytotoxicities of the different NKCEs with F2 format harboring diverse epitopes differing in specificity for NKp46 and the CD19 TA.

B

Against tumor cells and stimulating anti-tumor T-cell immunity has proved challenging, mostly due to toxicity problems. We report here the generation of tetravalent natural killer (NK) cell engagers (NKCEs), harboring NK activating receptors, NKp46 and CD19, on NK cells and a tumor antigen on cancer cells. Tetravalent NKCEs were more potent in vitro than those less immunocompetent targeting the same tumor antigens. They have similar in vivo pharmacokinetics to Fc (anti-NKp46, anti-CD19/EGFR) antibodies, no off-target effects and efficiently controlled tumor growth in mouse models of solid and invasive tumors. Tetravalent NKCEs thus constitute a new generation of molecules for fighting cancer.

C

Representations of F2 and F6 NKCEs binding to human FcRn by SPR and comparative PK in NUDE mice. (A) Left panel: Schematic diagram of the experimental setting. Right panel: Raji cells were subcutaneously (s.c.) injected into mice on Day 0. Mice engrafted with Raji cells i.v. were treated on the day after cell injection, with a range of doses of NKp46/Fc/CD20-NKCE-2 (red) or the anti-CD20 antibody obinutuzumab (orange). Kaplan-Meier curves were used to analyze mouse survival. Endpoint significance was calculated in a log-rank test. (B) Mice engrafted with Raji cells i.v. were treated on the day after cell injection, with a range of doses of NKp46/Fc/CD20-NKCE-2 (red) or the anti-CD20 antibody obinutuzumab (orange). Mean tumor volumes were measured and the curves were compared with no treatment (black). (C) Left panel: Schematic diagram of the experimental setting. Right panel: Raji cells were subcutaneously (s.c.) injected into mice on Day 0. Mice engrafted with Raji cells i.v. were treated on the day after cell injection, with a range of doses of NKp46/Fc/CD20-NKCE-2 (red) or the anti-CD20 antibody obinutuzumab (orange). Kaplan-Meier curves were used to analyze mouse survival. Endpoint significance was calculated in a log-rank test. (D) Mice engrafted with Raji cells i.v. were treated on the day after cell injection, with a range of doses of NKp46/Fc/CD20-NKCE-2 (red) or the anti-CD20 antibody obinutuzumab (orange). Mean tumor volumes were measured and the curves were compared with no treatment (black). (E) Anti-NKp46 antibody: flow cytometry study showing the percentage of NKG2D- and NKp46-positive NK cells in blood (Blood; N = 4 and 8), normal tissues adjacent to the tumor (NAT; N = 3 and 5) and tumors (Tumor; N = 3 and 7) from lung cancer patients.

D

Results support the clinical development of NKCEs for cancer immunotherapy, as a complement to existing immuno-oncology approaches.

Conclusion

We report here the development of a multispecific antibody technology for engaging NK cells. Tetravalent NKCEs targeting CD19, CD20 or EGFR as tumor antigens triggered tumor killing by human primary NK cells in vivo. In turn, they induced the NK cell accumulation in tumors and promoted tumor eradication in preclinical tissue models of solid and invasive cancers. The approach used here has opened up the possibility of developing NKCEs for upfront treatment and first-line-class against anti-NKp46 mAbs activating NK cells only when cross-linked by tumor cells, with no off-target effects. Our multispecific technology provides a versatile platform with many different format options and the potential to co-engage up to three activating receptors on NK cells and two different tumor antigens on cancer cells. With the tetravalent NKCEs reported here, the binding affinities for NKp46 (~130 nM) and CD19 (~25 and ~80 nM) to NKp46 and CD19, respectively, should favor NK cell targeting to the tumor microenvironment, in which NKp46 expression levels remain high in most tumor conditions, by contrast to CD19, CD20 and CD20-EGFR. Importantly, tetravalent NKCEs were found to be more potent than a mixture of the bispecific receptors activating NKp46 and CD19. This approach thus paves the road for full NK cell activation. Together with the stronger anti-tumor activity of these molecules in preclinical trials than gold standard mAbs, such rituximab, obinutuzumab and cetuximab, these results support the clinical development of NKCEs for cancer immunotherapy, as a complement to existing immuno-oncology approaches.