Combined blockade of PD-L1 and NKG2A checkpoints enhances anti-tumor CD8+ T cell response

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**Background**

- Inhibitory CD94-NKG2A receptors are expressed on subsets of cytotoxic NK cells, γδ and CD8+ T cells.
- NKG2A interacts with a non-classical MHC class I molecule, HLA-E (Qa-1) in mice), that is frequently upregulated on cancer cells of several solid tumors, providing a negative regulatory signal to tumor-infiltrating lymphocytes (TILs).
- Subcutaneous (s.c.) injection of A20 cells into BALB/c mice results in induction of Qa-1b expression and upregulation of PD-L1 expression on tumor cells.
- NKG2A blockade with mAbs enhances:
  - NK cell responses toward tumor cells in vitro and in humanized mice.
  - PD-1 checkpoint blockers in a syngeneic mouse tumor model and improves survival.
- Here, we further studied the effects of in vivo and ex vivo targeting NKG2A and PD-1 pathways with the emphasis on anti-tumor CD8+ T cell responses.

**Mechanism of action**

**NKG2A/Qa-1 and PD-1/PD-L1 pathways control A20 tumor growth in a CD8+ T cell-dependent manner**

**A)** Individual A20, A20 Qa-1+ KO and A20 PD-L1 KO tumor growths after s.c. engraftment (5x10⁶ cells) in BALB/c mice (n=10). TF: Tumor Free, CR: Complete Regression. **B)** Day 14 after tumor cell engraftment. Bars are medians. One-way ANOVA followed by Dunn’s test, P<0,001 (**), P=0,0005 (***) (n=11-12).

**Combined NKG2A and PD-L1 blockade increases complete response rate in a CD8+ T and NK cell-dependent manner**

**A)** Isotype control Anti-NKG2A Anti-PD-L1 Anti-NKG2A + anti-PD-L1

Mice (n=10-11) were treated with anti-NKG2A or anti-PD-L1 mAbs alone or combined. A. Individual tumor volumes. One representative experiment out 3 is shown. B & C. Individual tumor volumes of mice treated with combined anti-NKG2A or anti-PD-L1 mAbs with (dashed lines) or without (full lines) lymphocyte depletion: CD8+ cell (B) and NK cell depletion (C).

**Conclusions**

- Qa-1+ and PD-L1 are involved in the immune-escape of A20 tumor growth.
- Combined blockade of NKG2A and PD-L1 increases complete response rate in A20 tumor-bearing mice in a CD8+ T and NK cell-dependent manner.
- CD8+ TILs expressing high levels of PD-1 co-express high levels of NKG2A.
- Ex vivo combined NKG2A and PD-L1 blockade enhances anti-tumor efficacy of tumor infiltrating NKG2A/PD-1/CD8+ T cells.