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

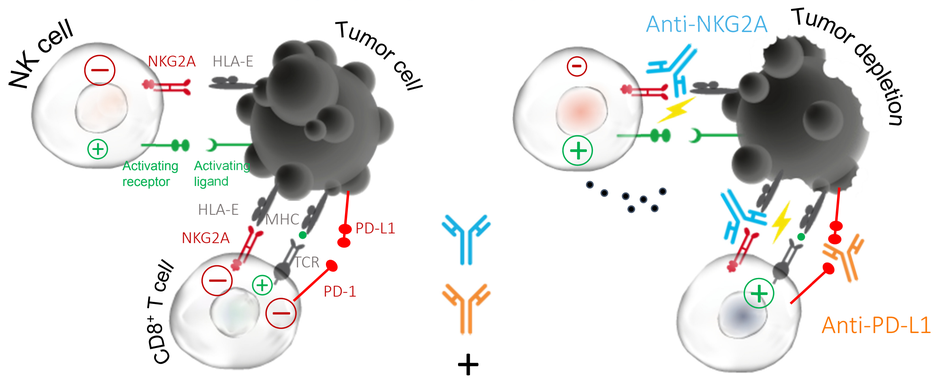
Poster # A130

Caroline Denis, Vedran Brezar, Thomas Arnoux, Julie Lopez, Clarisse Caillet, Fabien Chanuc, Nicolas Fuseri, Nicolai Wagtmann, Pascale André, Caroline Soulas - Innate Pharma, 117 Avenue de Luminy, 13009 Marseille, France

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-1b 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



NK and T cell inhibition by NKG2A & PD-1

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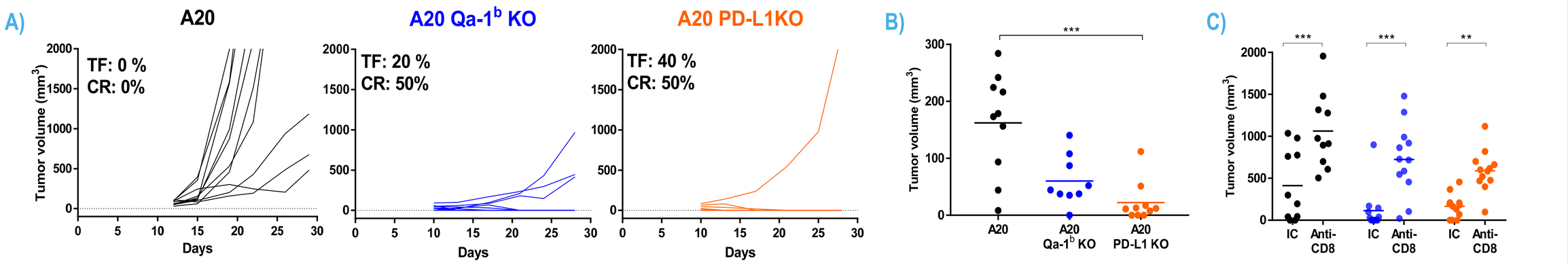
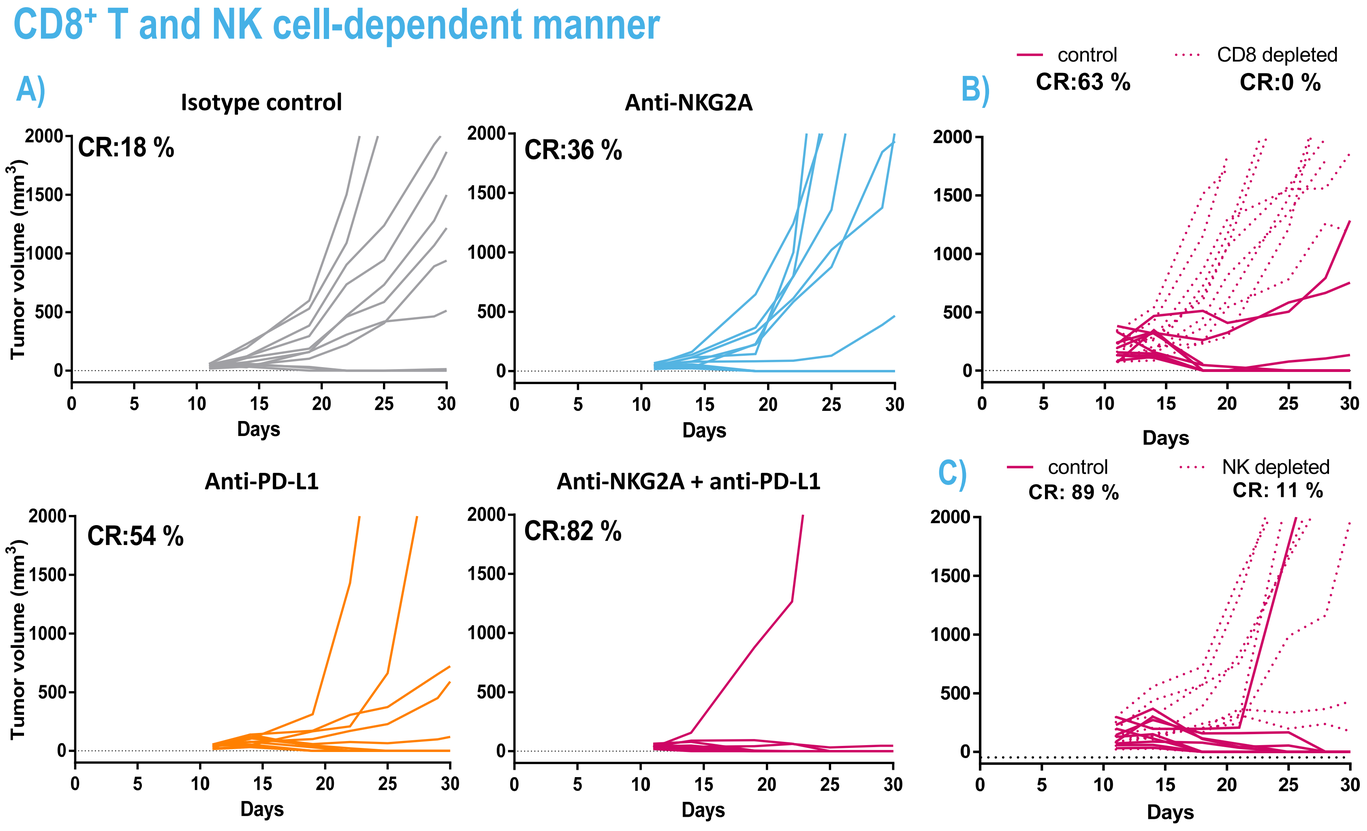
Activation by NKG2A & PD-L1 blockade

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



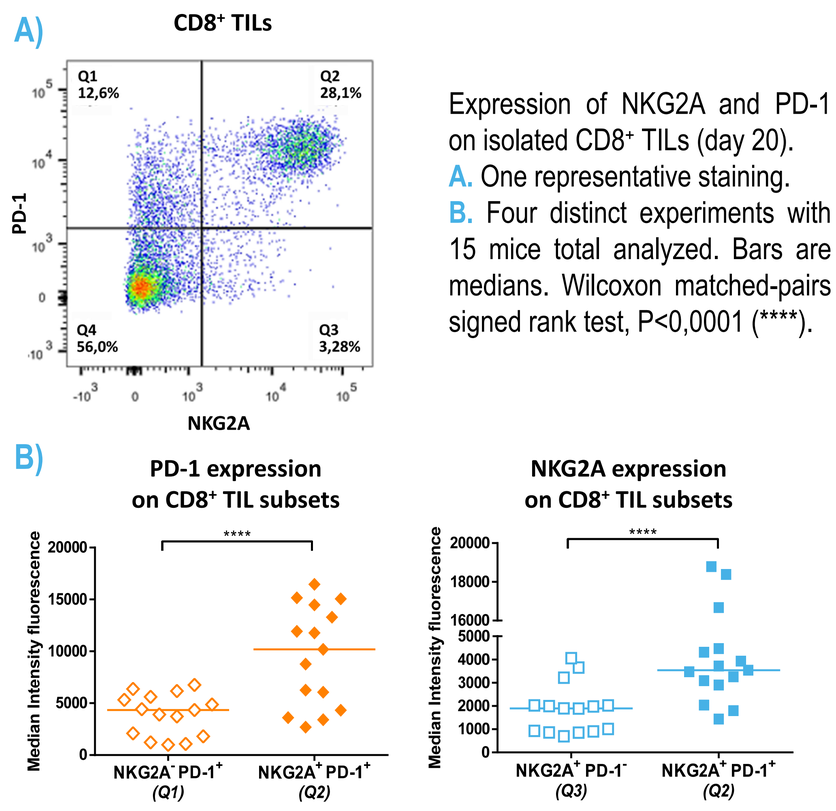
A) Individual A20, A20 Qa-1b KO and A20 PD-L1 KO tumor growths after s.c. engraftment (5x10^6 cells) in BALB/c mice (n=10). TF: Tumor Free, CR: Complete Regression. B) Day 14 after tumor cell engraftment. Bars are medians. One-way ANOVA followed by Dunn's test, P<0,001 (***). C) Day 18 after CD8+ cell depletion. Two-way ANOVA followed by Sidak's test, P<0,01 (**), P<0,0001 (***); n=11-12.

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



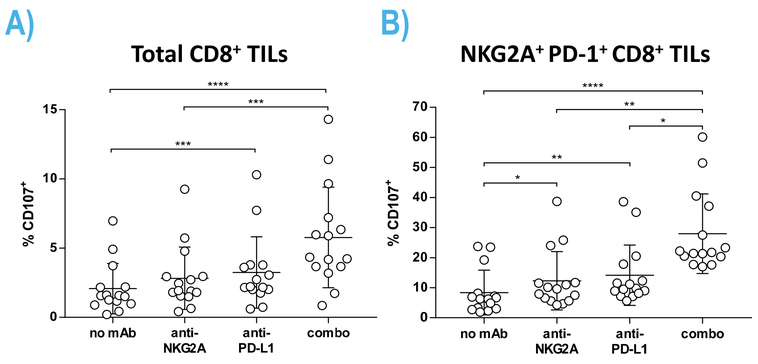
Mice (n=10-11) were treated with anti-NKG2A or anti-PD-L1 mAbs alone or combined. A. Individual tumor volumes. One representative experiment out 3 is shown. B & C. Individual tumor volumes of mice treated with combined anti-NKG2A or anti-PD-L1 mAbs with (dashed lines) or without (full lines) lymphocyte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

CD8+ TILs expressing high levels of PD-1 co-express high levels of NKG2A



Expression of NKG2A and PD-1 on isolated CD8+ TILs (day 20).
A. One representative staining.
B. Four distinct experiments with 15 mice total analyzed. Bars are medians. Wilcoxon matched-pairs signed rank test, P<0,0001 (****).

Ex vivo combined NKG2A and PD-L1 blockade enhances anti-tumor efficacy of tumor infiltrating NKG2A+PD-1+CD8+ T cells



CD107 mobilization on total CD8+ TILs (A) and NKG2A+PD-1+CD8+ TILs (B) in response to A20 tumor cells and in presence of the indicated mAbs. Four distinct *ex vivo* experiments, n=15 mice. One-way ANOVA followed by Dunn's test, P=0,043 (*), P=0,0014 (**), P=0,0005 (***), P <0,0001(****).

Conclusions

- Qa-1b 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-expressed high levels of NKG2A.
- *Ex vivo*, NKG2A blockade potentiates PD-L1 blockers by directly enhancing tumor-infiltrating CD8+ T cell-mediated killing of A20 tumors.

➢ **These data indicate that blocking both NKG2A/HLA-E and PD-1/PD-L1 pathways could enhance anti-tumor efficacy of CD8+ T cells.**

➢ **These data support the rationale for ongoing clinical trials with anti-NKG2A (monalizumab) and anti-PD-L1 (durvalumab, NCT02671435) therapeutic mAbs combination.**