NK and T cells, and IFN-γ are required for the anti-tumor efficacy of combination-treatment with NKG2A and PD-1/PD-L1 checkpoint inhibitors in preclinical models

Poster# 196

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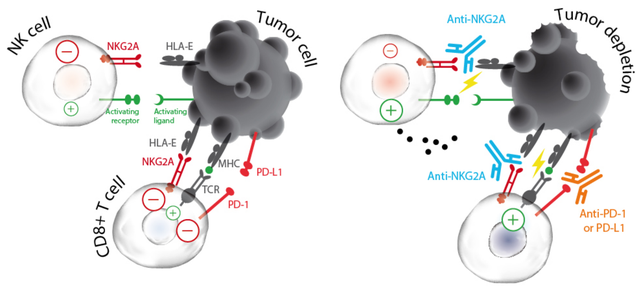
Background

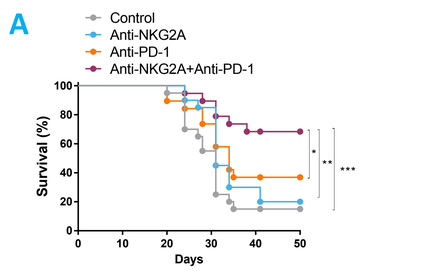
Monalizumab (IPH2201) is a first-in-class humanized IgG4 targeting NKG2A, which is expressed as a heterodimer with CD94 on subsets of NK cells, γδ T cells and tumor infiltrating CD8⁺ T cells. This inhibitory receptor binds to HLA-E in humans and to Qa-1b in mice. HLA-E is frequently up-regulated on cancer cells, protecting from killing by NKG2A⁺ cells. Monalizumab blocks binding of CD94-NKG2A to HLA-E, reducing inhibitory signaling and thereby enhancing NK and T cell responses.

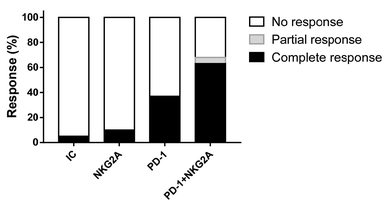
PD1/PD-L1 inhibitors are successfully being used to treat patients with a wide variety of cancers. Combined blockade of NKG2A/HLA-E and PD1/PD-L1 may be a promising strategy to better fight cancer by activating both the adaptive and innate immune systems.

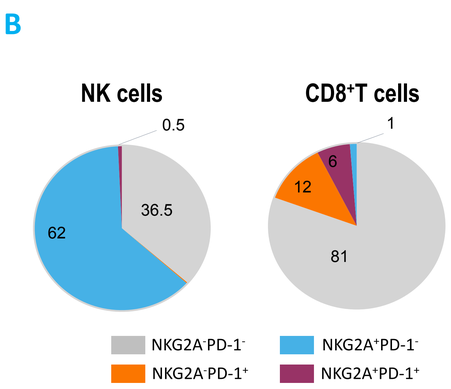
Mechanism of action



Combined NKG2A and PD-1 blockade increases complete response rate and survival

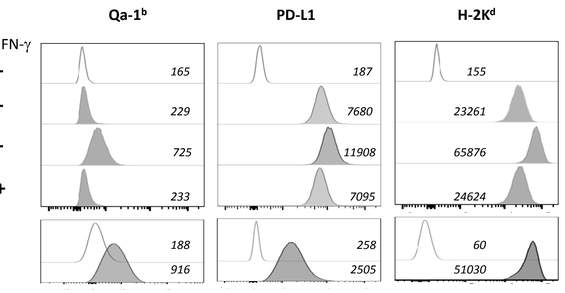






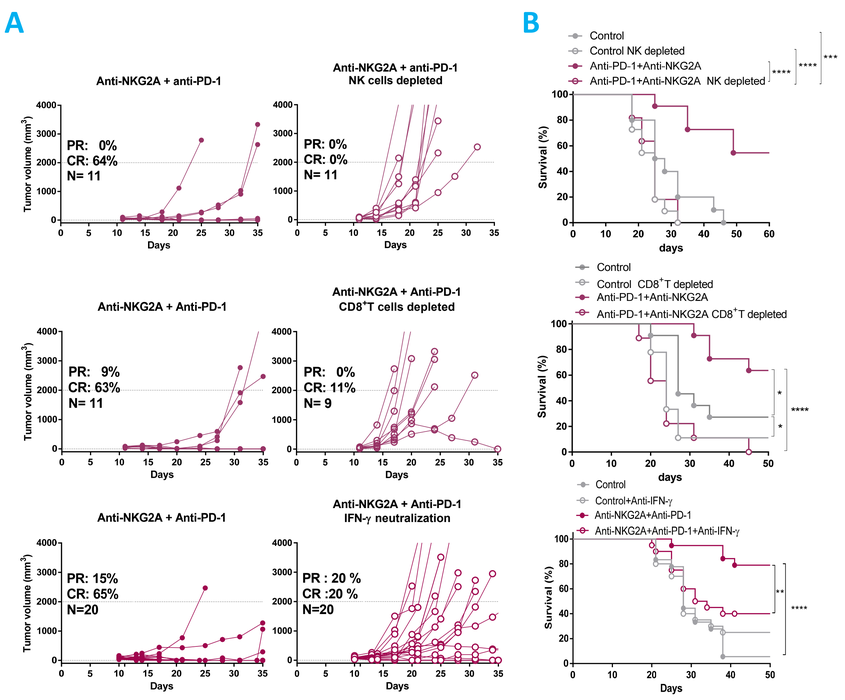
Balb/C mice were randomized when A20 tumor volumes ≈ 70 mm³ (n=19-20 mice/group) and treated 3 times (every 3-4 days) with IC, anti-NKG2A (200 µg, iv), anti-PD-1 (200 µg, ip) or anti-NKG2A and anti-PD-1 mAb. **A: upper panel:** Kaplan-Meier survival. Log Rank test, P<0.05 (*), P<0.005 (**), P<0.0005 (***). **Lower panel:** Response rate evaluated in each group by measurement of tumor volume.
B: Distribution of NKG2A and PD-1 receptors on tumors infiltrating NK and CD8⁺ T cells of untreated mice. Analysis performed by flow cytometry on day 22 after A20 tumor cell s.c engraftment (n=6 mice).

Qa-1 expression is induced in vitro by IFN-γ and in vivo after tumor cell engraftment in mice



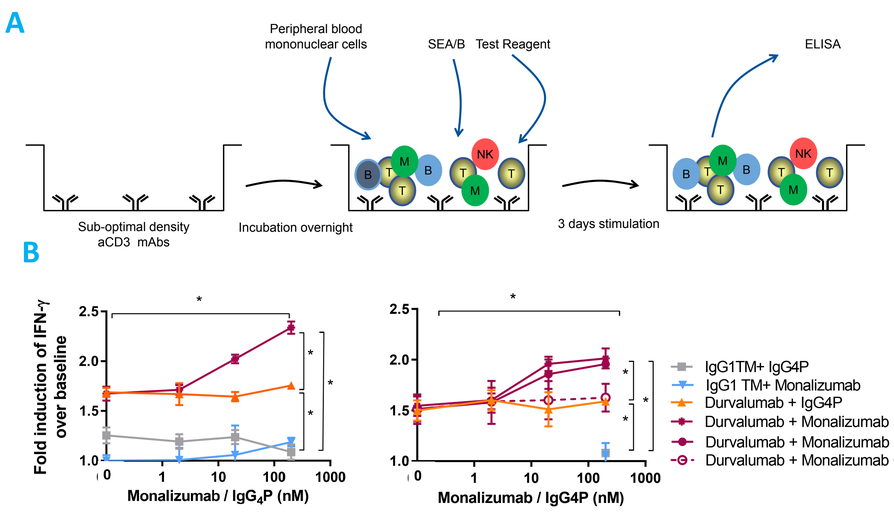
Expressions of Qa-1ᵇ, PD-L1 and H-2Kᵈ (dark histograms) and isotype control (white histograms) on A20 mouse B lymphoma cells. **A:** *In vitro* after overnight stimulation with IFN-γ,125 UI/mL, which induces increased expressions of Qa-1ᵇ (fold increase: $2^{+/-}0.5$), PD-L1 (fold increase:$1.7^{+/-}0.5$) and H-2Kᵈ (fold increase: $2.5^{+/-}0.5$) and neutralizing anti-mouse IFN-γ mAb. **B:** *Ex vivo* day 19 post tumor cell engraftment. In italic, Medians of Fluorescence Intensity (MFI). Data are representative of four independent experiments.

Anti-tumor efficacy of anti-NKG2A/anti-PD-1 combination is mediated by NK cells and CD8⁺ T cells and IFN-γ



Mice were randomized when tumor volumes ≈ 50 mm³ and treated 3 times (every 3-4 days) with IC, anti-NKG2A, anti-PD-1 or combination of both mAbs. NK or CD8⁺T cells were depleted by injection of anti-asialo-GM1 or anti-CD8 mAbs (3 times, once a week) and IFN-γ neutralized by injection of anti-IFN-γ mAb (6 times, every 2 days from day 10). **A:** Individual tumor volumes. **B:** Kaplan-Meier survival. Log Rank test, P<0.05 (**)P<0.005 (***), P<0.0005 (****).

Combination of durvalumab and monalizumab increases secretion of IFN-γ in SEB assay and is dependent on IFN-γ pathway



SEB (staphylococcal enterotoxin b) assay to analyze the effect of the combination of durvalumab and monalizumab on human cells.
A: Schematic depicting the assay set-up: PBMC from fresh blood are incubated in the presence of anti-CD3 antibodies and SEB for 72 hours before secretion of cytokines was analyzed by ELISA.
B: Induction of IFN-γ by durvalumab, and monalizumab alone or in combination over baseline. Addition of an antibody blocking IFN-γ receptor I (IFN-γR1) resulted in abrogation of IFN-γ secretion over baseline. Data are representative of four independent experiments, performed in triplicate.

Conclusions

Together, these data indicate that blocking NKG2A in conjunction with PD-1/PD-L1 checkpoint blockers provides increased anti-tumor efficacy by a mechanism that depends on IFN-γ. These data strengthen the rationale for assessing this combination in clinical trials.

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