NKG2A immune checkpoint blockade enhances the anti-tumor efficacy of PD-1/PD-L1 inhibitors in a preclinical model

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#2342

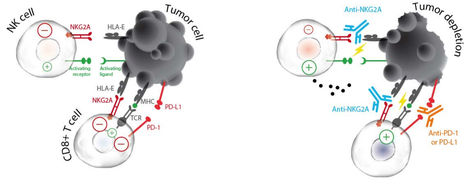
Introduction

Monalizumab (IPH2201) is a novel, first-in-class humanized IgG4 targeting the immune checkpoint receptor NKG2A (Natural Killer Group 2A). NKG2A is expressed as a heterodimer with CD94 on the surface of subsets of cytotoxic lymphocytes: NK (Natural Killer) cells, γδ T cells and tumor infiltrating CD8⁺ T lymphocytes. CD94-NKG2A is an inhibitory receptor specific for HLA-E (Human Leukocyte Antigen-E) in humans and orthologous Qa-1^b in mice. Upon ligand binding, CD94-NKG2A triggers inhibitory signaling that reduces NK and CD8⁺ T cell responses. HLA-E is frequently up-regulated on cancer cells of many solid tumors or hematological malignancies, protecting from killing by NKG2A⁺ immune cells. By blocking the binding of CD94-NKG2A to HLA-E, monalizumab leads to enhancement of NK and cytotoxic T cell responses.

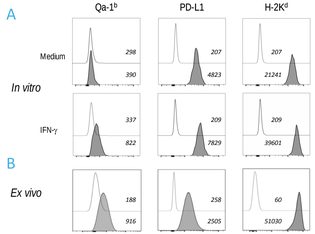
Blocking the PD-1 pathway has proven efficient as anti-tumor therapy. Nevertheless many patients remain refractory to these therapeutics. Combination treatment with PD-1 blockers and mAb to a second checkpoint receptor, CTLA-4, have proven effective only for some patients, suggesting a need for combining with other checkpoint blockers.

Here, we tested the combination of NKG2A and PD-1 blockade in an *in vivo* model where A20 solid tumors were established in Balb/c mice.

1. Mechanism of Action

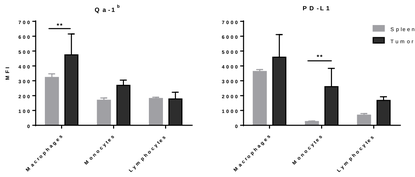


2. Qa-1^b is induced on A20 cells *in vitro* by IFN-γ and *in vivo* after engraftment in mice



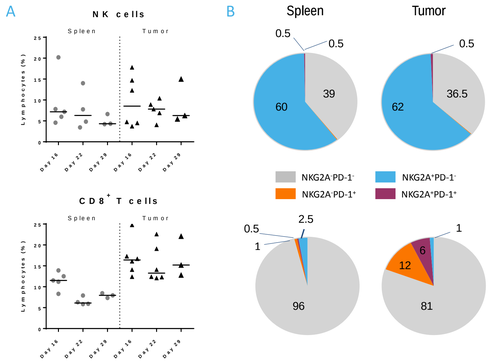
Expression of Qa-1^b, PD-L1 and H-2K^d (dark histograms) measured by flow cytometry A: after o/n stimulation with IFN-γ and B: day 19 post tumor cell engraftment.
Mean Fluorescence Intensity (MFI) is indicated in each histogram.

3. Qa-1^b and PD-L1 are increased on A20 tumor infiltrating macrophages and monocytes



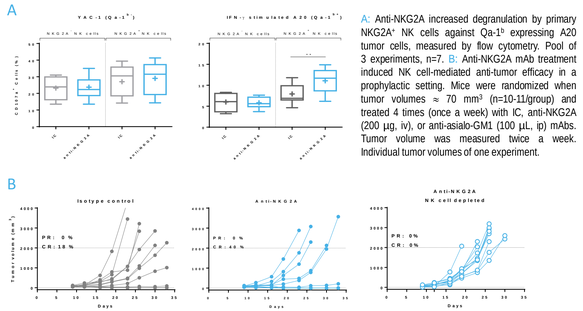
Mice (n=4) were euthanized on day 22 (tumor vol between 200 and 2000 mm^3). Qa-1^b and PD-L1 expression was measured by flow cytometry. Means +/- SD of MFI. Two way ANOVA followed by Sidak's test. P<0.05 (**).

4. NKG2A and PD-1 expression on A20 tumor infiltrating NK and CD8⁺ T cells



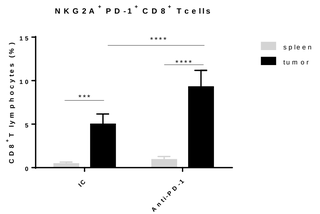
A: Mice were euthanized at the indicated time points following tumor cells engraftment. NK and CD8⁺T cells analyzed by flow cytometry (n=3-6 mice/time point). Each symbol represents an individual mouse, black horizontal line represents mean value.
B: Distributions of NKG2A and PD-1 receptors were analyzed on day 22 (means of 6 mice).

5. Anti-NKG2A *in vitro* and *in vivo* efficacy



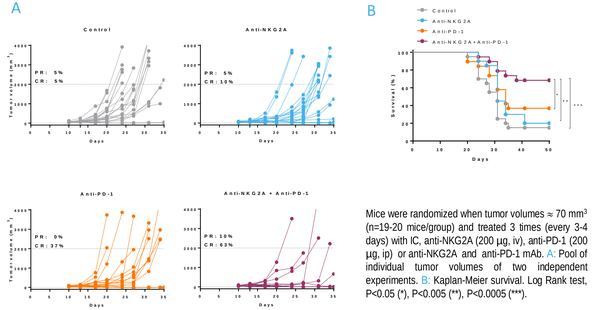
A: Anti-NKG2A increased degranulation by primary NKG2A⁺ NK cells against Qa-1^b expressing A20 tumor cells, measured by flow cytometry. Pool of 3 experiments, n=7. B: Anti-NKG2A mAb treatment induced NK cell-mediated anti-tumor efficacy in a prophylactic setting. Mice were randomized when tumor volumes ≈ 70 mm^3 (n=10-11/group) and treated 4 times (once a week) with IC, anti-NKG2A (200 µg, iv), or anti-asialo-GM1 (100 µL, ip) mAbs. Tumor volume was measured twice a week. Individual tumor volumes of one experiment.

6. Increased frequency of NKG2A⁺ PD-1⁺ CD8⁺ T cells in tumors of anti-PD-1 resistant mice



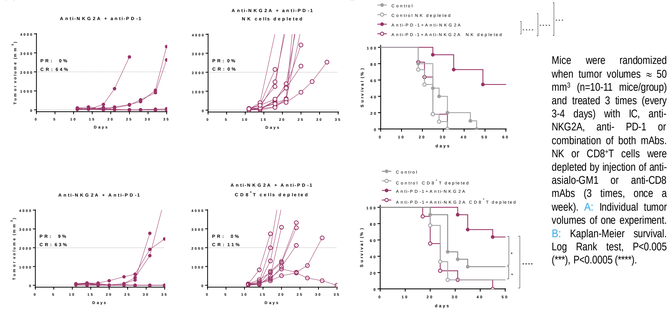
Mice treated with indicated mAbs (200 µg, ip, 3 times every 3-4 days) after tumor engraftment were sacrificed on days 21 and 28. CD8⁺T cells were characterized by flow cytometry. Means +/- SD of % NKG2A⁺ PD-1⁺ CD8⁺ among CD8⁺T cells. P<0.005 (***), P<0.0005 (****). N=3-6. % NKG2A⁺ NK cells among NK cells were not modified by anti-PD-1 treatment (data not shown).

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



Mice were randomized when tumor volumes ≈ 70 mm^3 (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: Pool of individual tumor volumes of two independent experiments. B: Kaplan-Meier survival. Log Rank test, P<0.05 (*), P<0.005 (**), P<0.0005 (***).

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



Mice were randomized when tumor volumes ≈ 50 mm^3 (n=10-11 mice/group) 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). A: Individual tumor volumes of one experiment. B: Kaplan-Meier survival. Log Rank test, P<0.005 (***), P<0.0005 (****).

Conclusion

- NKG2A is expressed on tumor infiltrating NK cells.
- NKG2A is induced on a subset of CD8⁺ T cells that also expressed PD-1 and is further increased in PD-1 resistant mice.
- NKG2A blockade delays A20 tumor growth.
- Combination of PD-1 and NKG2A blockade results in significant anti-tumor responses, characterized by an increased frequency of complete tumor cell regression.
- These data support the rationale for the clinical trial testing the combination with monalizumab and durvalumab (NCT02671435).

References

- Sagiv-Barfi I et al. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. *PNAS*, 2015.
- Zhu X et al. Progression of large lymphoma is significantly impeded with a combination of gemcitabine chemotherapy and dendritic cells intra-tumor vaccination. *Plos one*, 2015.