Discovery and preclinical characterization of dual antagonist antibodies targeting both LILRB1 and LILRB2 that enhance innate and adaptive anti-cancer immune responses

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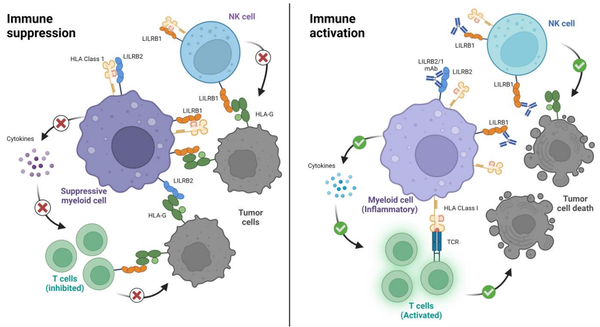
Background: One cause for the failure of checkpoint inhibitors is the immunosuppressive nature of the tumor microenvironment. LILRB1 (ILT2) and LILRB2 (ILT4) are ITIM-containing inhibitory receptors that recognize classical HLA Class 1 (e.g., HLA-A, HLA-B, etc.) and nonclassical ligands (e.g., HLA-G). LILRB1 is expressed on myeloid cells and subsets of B, NK, and T cells, while LILRB2 expression is mostly restricted to myeloid cells. Interaction of LILRB1 and LILRB2 receptors with HLA ligands promotes an inhibitory milieu that prevents T cells from attacking cancer cells. The distinct pattern of expression and function of these lymphoid and myeloid checkpoints suggests complementary targeting approaches for cancer immunotherapy. Dual blockade of LILRB1 and LILRB2 receptors by a single antibody that restores both innate and adaptive immune responses is a promising strategy to enhance efficacy of checkpoint inhibitors.

Methods: Dual LILRB1 and LILRB2 targeting antibodies were cloned from B-cells derived from rabbits immunized with human LILRB2 recombinant protein, and subsequently humanized. Clones were evaluated for binding to human LILRB1 and LILRB2 proteins. Dual targeting antibodies (one parental clone and several humanized variants) were evaluated in a panel of functional and phenotypic assays. The parental chimera antibody was further tested for efficacy in a humanized NSG-SGM3 mouse tumor model.

Results: Dual antibodies were selected based on binding to recombinant human LILRB1 and LILRB2 protein, as well as blocking of HLA-G binding. These antibodies demonstrated binding to cells expressing LILRB1 and LILRB2, with no appreciable binding to other family members. Lead antibodies demonstrated activity in functional cell-based assays modeling LILRB1- or LILRB2-mediated immunosuppression. Dual antibodies also enhanced IFN-γ production by LPS-stimulated human PBMC. Selected clones restored T cell function from M2c macrophage-mediated suppression in coculture with CD8⁺ T cells, and enhanced the tumoricidal activity of NK cells. Importantly, the lead antibody demonstrated in vivo efficacy with significant tumor growth inhibition and tumor regression in an SK-MEL-5 tumor model in humanized NSG-SGM3 mice.

Conclusions: We have identified dual antagonist antibodies targeting both LILRB1 and LILRB2 that restore both innate and adaptive immune responses. Additionally, dual antibodies restored CD8⁺ T cell activation from macrophage-mediated suppression and enhanced NK cell cytotoxic activity. These data provide a strong rationale for further development of dual antibodies as an anti-cancer immunotherapy.

LILRB1 and LILRB2 dual antagonism drives anti-tumor activity



RESULTS

Anti-LILRB2/1 antibodies demonstrate high affinity binding to recombinant LILRB1 and LILRB2 proteins

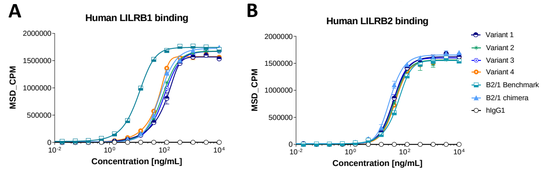


Figure 2. Anti-LILRB2/1 antibody binding to A) LILRB1 and B) LILRB2 by ELISA

Anti-LILRB2/1 antibodies bind to LILRB1 and LILRB2 on cell lines and myeloid cells

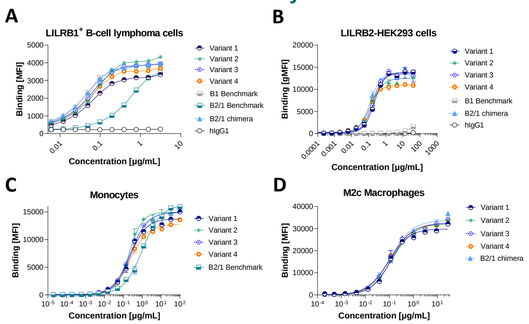


Figure 3. Anti-LILRB2/1 antibodies bind to A) 721.221 cells expressing human LILRB1, B) HEK293T cells expressing human LILRB2, C) human primary monocytes and D) human M2c macrophages, measured via flow cytometry.

Anti-LILRB2/1 antibodies block binding of human LILRB1 and LILRB2 to HLA-G on 721.221 B-cell lymphoma cells

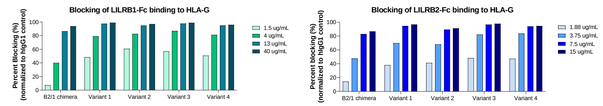


Figure 4. Anti-LILRB2/1 antibodies block the interaction of A) human LILRB1-Fc and B) human LILRB2-Fc with HLA-G expressed on B-cell lymphoma 721.221 cells.

Anti-LILRB2/1 antibodies relieve CD8⁺ T cells from M2c macrophage-mediated immune suppression

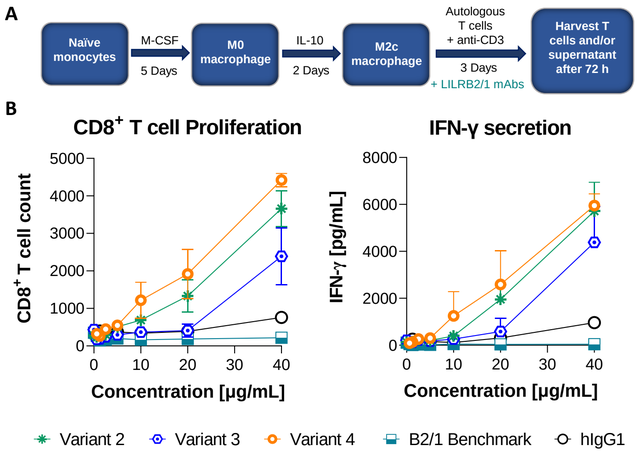


Figure 5. Treatment with anti-LILRB2/1 antibodies rescues T cells from M2c macrophage-mediated immune suppression.

Anti-LILRB2/1 antibodies enhance IFN-γ secretion in human PBMCs stimulated with LPS

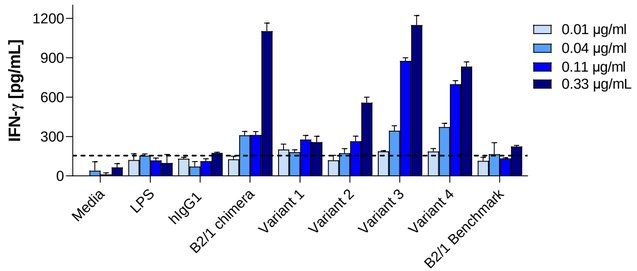


Figure 6. Human PBMCs are treated with anti-LILRB2/1 antibodies, stimulated with LPS, incubated for 24 h, then assayed for IFN-γ secretion.

Anti-LILRB2/1 antibodies enhance HLA-G mediated NK cell cytotoxicity

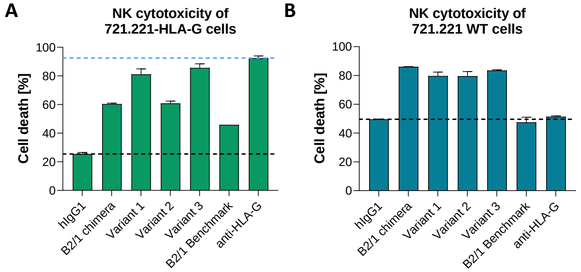


Figure 7. Treatment with anti-LILRB2/1 antibodies enhance NK cell mediated cytotoxicity of A) HLA-G expressing and B) Wild type 721.221 B-cell lymphoma cells.

PK profile and in vivo anti-tumor activity of humanized variant and parental anti-LILRB2/1 chimera antibody

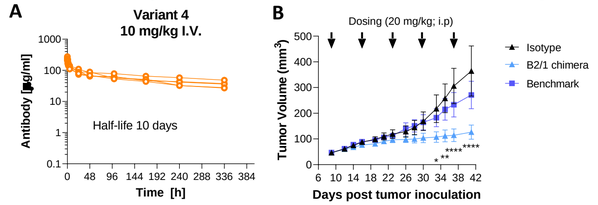


Figure 8. Pharmacokinetic profile and in vivo efficacy. A) Variant 4 has a half-life of ~10 days in humanized FcRn mice following intravenous (i.v.) single dose (10 mg/kg). B) Parental B2/1 chimera demonstrates anti-tumor activity in SK-MEL-5 tumor model in humanized NSG-SGM3 mice. Humanized NSG-SGM3 mice were injected i.p. with 20 mg/kg antibody every 7 days starting on day 9 post SK-MEL-5 subcutaneous tumor inoculation (N= 9/group). *p< 0.05, **p<0.01 and ****p<0.0001.

Summary

- Developed humanized antibodies that bind to human LILRB1 and LILRB2, and block binding to HLA-G
- Antibodies enhance IFN-γ secretion in PBMC stimulated with LPS
- Antibodies modulate the immunosuppressive function of TAMs and enhance adaptive anti-tumor responses in M2c/T cell coculture assays
- Antibodies enhance NK cell mediated cytotoxicity of wild-type and HLA-G expressing lymphoma B-cells
- Parental chimera antibody demonstrates robust anti-tumor activity

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