Discovery and preclinical characterization of anti-LILRB2 antibodies that rescue T cells from macrophage-mediated immune suppression

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Abstract #276

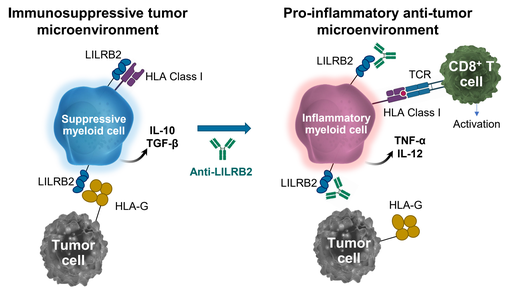
Background: The inhibitory receptor leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2, ILT4), is expressed on immunosuppressive myeloid cells, and has emerged as a key immune checkpoint in the tumor microenvironment (TME). Interaction of LILRB2 with the HLA class I ligands (e.g., HLA-G, HLA-A, etc.) mediates immune suppression by myeloid cells and promotes tumor immune evasion. Targeting this pathway in the TME may enhance efficacy of T cell checkpoint inhibitors. Antibodies targeting LILRB2 are currently being evaluated in clinical trials for the treatment of cancer.

Methods: Anti-LILRB2 antibodies were cloned from B cells derived from rabbits immunized with human LILRB2 recombinant protein. Cells were cultured at clonal density, and IgG antibodies in supernatants were evaluated for binding to human and cynomolgus LILRB2. Variable-regions from positive hits were sequenced, cloned, and expressed as recombinant rabbit-human chimeras. Anti-LILRB2 chimeric antibodies were evaluated in a panel of functional and phenotypic assays using primary human macrophages and T cells, and then prioritized for evaluation in a humanized NSG-SGM3 tumor model.

Results: Twenty-seven rabbit anti-LILRB2 clones were selected and expressed as rabbit-human IgG4 chimeras based on binding to recombinant human LILRB2 protein and blocking of HLA-G binding to LILRB2. A subset of chimeric clones demonstrated binding to stably expressing LILRB2 cells, and lack of binding to other LILRB or LILRA family members by enzyme-linked immunosorbent assay and by flow cytometry of transiently transfected HEK cells. Lead clones were identified based on their ability to block interaction of LILRB2 to HLA-G expressed on tumor cells, and activity in functional cell-based assays modeling LILRB2-mediated immune suppression. These clones enhanced LPS-induced IFN-γ production by PBMCs and increased the release of TNF-α by CD40L-activated macrophages. Selected clones also relieved M2c-macrophage-mediated immune suppression in a M2c/CD8⁺ T cell coculture assay by restoring T-cell proliferation and secretion of pro-inflammatory cytokines. Importantly, lead chimeric LILRB2 clones 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 identified novel anti-LILRB2 antibodies that restore innate and adaptive immune responses by modulating immunosuppressive macrophages. These data provide a strong rationale for further development of these antibodies as an anti-cancer immunotherapy.

1 Anti-LILRB2 antibodies can reprogram immuno-suppressive functions of myeloid cells



RESULTS

2 Screening cascade for the discovery of anti-LILRB2 antibodies

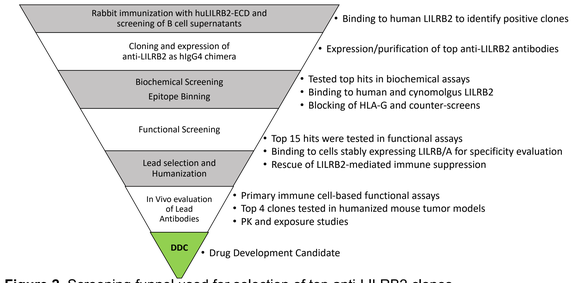


Figure 2. Screening funnel used for selection of top anti-LILRB2 clones

3 Anti-LILRB2 clones block HLA-G binding to LILRB2-Fc

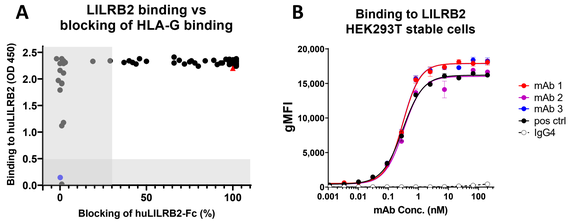


Figure 3. Top antibody clones were selected based on binding to A) soluble huLILRB2-Fc by ELISA, blocking of HLA-G binding to LILRB2, and B) binding to HEK293 cells stably expressing human LILRB2. In Figure 3A, the red triangle and blue circle represent the benchmark and isotype control, respectively.

4 Anti-LILRB2 antibodies do not cross-react with other LILRA/B family members

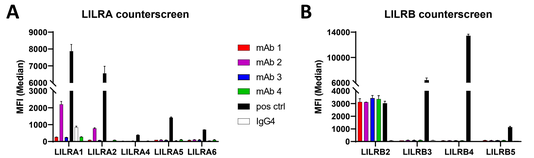


Figure 4. Top anti-LILRB2 clones (10 μg/mL) bind specifically to HEK293T cells expressing LILRB2 and not to cells expressing other A) LILRA or B) LILRB family members, as measured by flow cytometry. Cell surface expression of LILRA/LILRB was confirmed by monoclonal antibody specific to each protein. Anti-LILRB2 antibodies did not bind to LILRB1 by ELISA or HEK293T cells expressing LILRB1 by flow cytometry.

5 Anti-LILRB2 antibodies enhance IFN-γ production by PBMCs and TNF-α secretion by macrophages

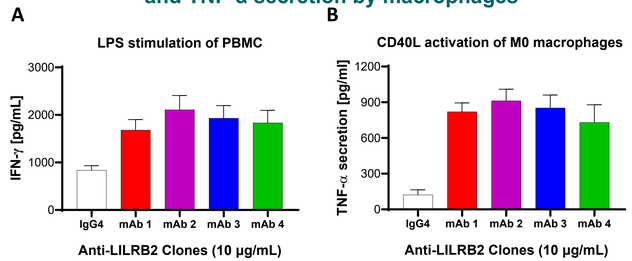


Figure 5. Anti-LILRB2 antibodies enhance LPS-mediated IFN-γ secretion by PBMCs, and TNF-α secretion by M0 macrophages in response to CD40L stimulation. A) Human PBMCs were stimulated with 1 μg/mL LPS in the presence of anti-LILRB2 antibodies for 24 h. IFN-γ in supernatant was measured by ELISA. B) Human M0 macrophages were cocultured with HEK293 cells expressing CD40L in the presence of anti-LILRB2 antibodies for 24 h. TNF-α secretion was measured in supernatant by ELISA.

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

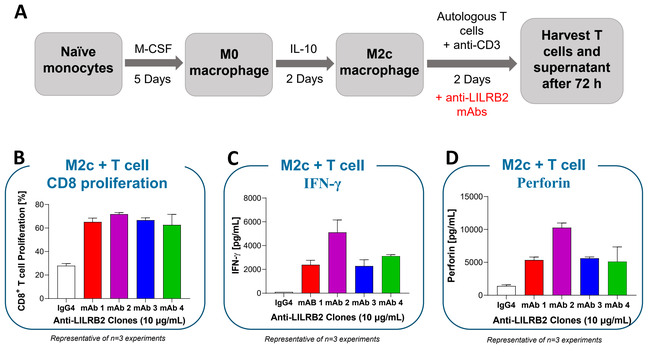


Figure 6. Anti-LILRB2 antibodies restore human CD8⁺ T cell proliferation, as well as IFN-γ secretion and perforin release. A) Schematic of M2c/T cell coculture assay. Anti-LILRB2 or isotype control antibodies treated M2c macrophages were cocultured with CD8⁺ T cells in presence of anti-CD3 for 72 h. B) Treatment with anti-LILRB2 antibodies restores human CD8⁺ T cell proliferation, C) enhances IFN-γ secretion and D) increases perforin release by T cells.

7 SK-MEL-5 tumor model in humanized NSG-SGM-3 mice

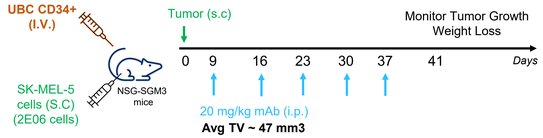
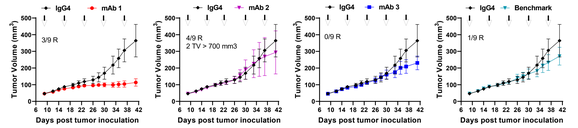


Figure 7. Humanized NSG-SGM3 mice were injected with 2 x 10^6 SK-MEL 5 cells. Tumor growth proceeded for 9 days prior to initiating antibody treatment.

8 In vivo efficacy of anti-LILRB2 antibodies in SK-MEL-5 tumor model in humanized NSG-SGM-3 mice



	Tumor Growth Inhibition (%)					Regression (%)
mAb	d30	d33	d35	d37	d41	d41
mAb 1	57	69	74	78	79	33
mAb 2	-18	-5	3	14	2	44
mAb 3	6	25	27	37	42	0
Benchmark	3	16	17	24	26	11

Figure 8. Anti-LILRB2 antibodies demonstrate anti-tumor activity in SK-MEL-5 tumor model in humanized NSG-SGM3 mice. NSG-SGM3 humanized mice were injected i.p. with 20 mg/kg antibody at Days 9, 16, 23, 30 and 37. N = 9 mice per group. Average tumor volumes were measured at ~47 mm^3 at start of dosing.

Summary

- Identified anti-LILRB2 lead antibodies that:
  - Enhance IFN-γ production by PBMCs and TNF-α secretion by macrophages
  - Relieve CD8⁺ T cells from M2c macrophage-mediated immunosuppression
  - Demonstrate anti-tumor activity in SK-MEL-5 tumor model in humanized NSG-SGM3 mice with up to 79% TGI and 42% tumor regression
- These results support further development of lead anti-LILRB2 antibodies for cancer immunotherapy

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