Preclinical characterization of OR502, an anti-LILRB2 antibody that rescues innate and adaptive immune responses from LILRB2 mediated immune suppression

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Abstract #498

Background: The inhibitory receptor leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2, ILT4) is mostly expressed on immunosuppressive myeloid cells and expression correlates with poor survival in multiple cancers and contributes to anti-PD1 resistance. 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 in the tumor microenvironment (TME). Blocking this interaction 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. The clones were humanized after selection based on activity in a panel of functional and phenotypic assays using primary human macrophages and T cells. The humanized variants were screened for their ability to rescue T cell activity (proliferation and IFN-γ) from M2c macrophage-mediated suppression and enhance LPS-induced IFN-γ production by PBMCs. The top variants were also evaluated for cytokine release in whole blood. The pharmacokinetic profiles of lead LILRB2 antibodies were determined in humanized FcRn mice.

Results: We have identified a panel of humanized anti-LILRB2 antibodies that specifically bind to human LILRB2-expressing cell lines and human myeloid cells without detectable binding to other LILRA or LILRB family members. These antibodies block LILRB2 interaction with HLA-G expressed on tumor cells. The lead antibody, OR502, enhanced LPS-induced IFN-γ production by PBMCs, and relieved M2c macrophage-mediated suppression of proliferation and IFN-γ secretion by anti-CD3-activated human CD8+ T cells in coculture assays. Furthermore, OR502 restored the ability of exhausted T cells to secrete IFN-γ in the presence of M2c macrophages and significantly enhanced the activity of anti-PD-1 in combination studies. OR502-treatment did not trigger inflammatory cytokine release or activation of neutrophils in human whole blood. The pharmacokinetics of OR502 in humanized FcRn mice demonstrated a half-life of 6-10 days.

Conclusions: We have identified a novel humanized anti-LILRB2 antibody, OR502, that restores innate and adaptive immune responses by modulating immunosuppressive myeloid cells. These data provide a strong rationale for further development of OR502 for cancer treatment.

1 LILRB2 promotes immunosuppression and blockade drives anti-tumor activity

Immunosuppressive TME — Anti-LILRB2 promotes tumor killing

Cold Tumor — Hot Tumor

RESULTS

2 OR502 demonstrates specific binding to LILRB2 and blocks LILRB2-Fc binding to HLA-G

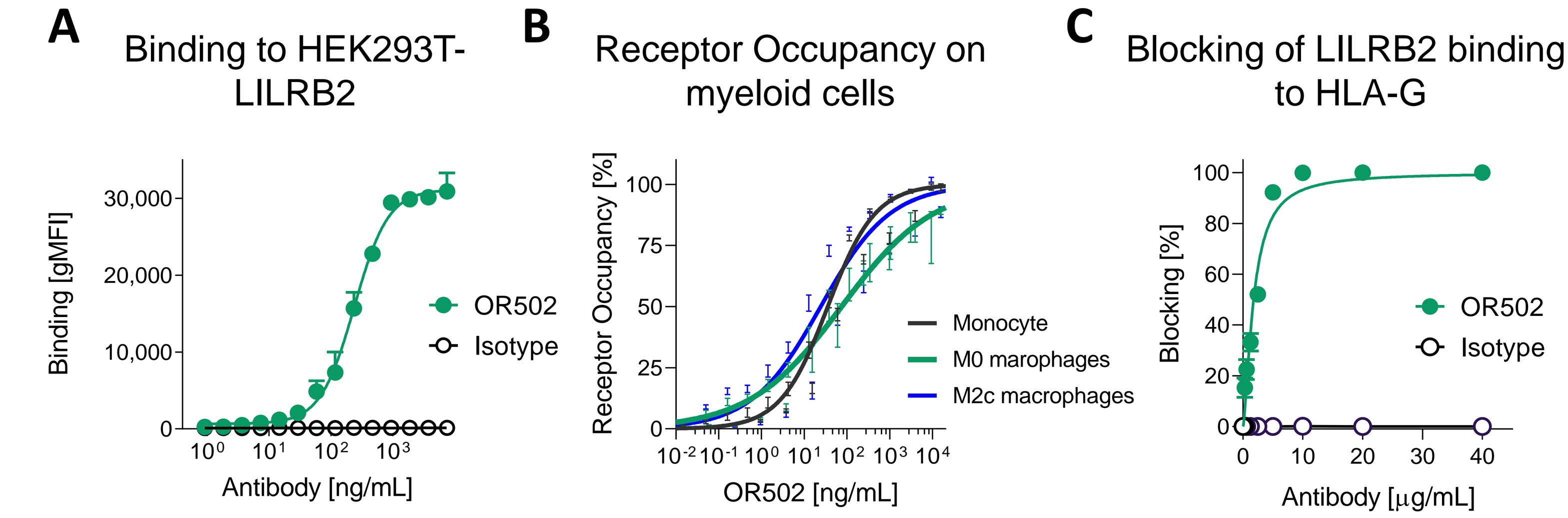

Figure 2. A) OR502 binding to LILRB2 expressing HEK293T cells. B) Binding of OR502 to human monocytes and monocyte-derived macrophages. C) OR502 blocks the interaction of LILRB2-Fc with HLA-G expressed on B-cell lymphoma 721.221 cells

3 OR502 does not bind to other LILRA/B family members

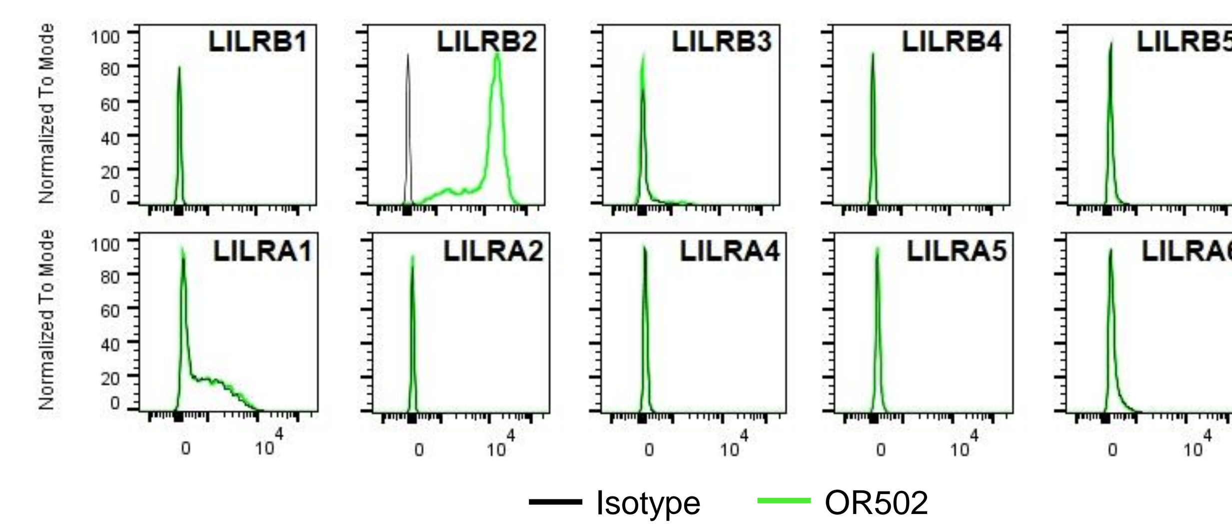

Figure 3. OR502 (1 μg/mL) binds specifically to HEK293T cells expressing human LILRB2 and not to cells expressing either LILRA or LILRB family members, as measured by flow cytometry. Cell surface expression of LILRA/LILRB was confirmed by monoclonal antibody specific to each protein.

4 OR502 boosts LPS-induced IFN-γ secretion by PBMC

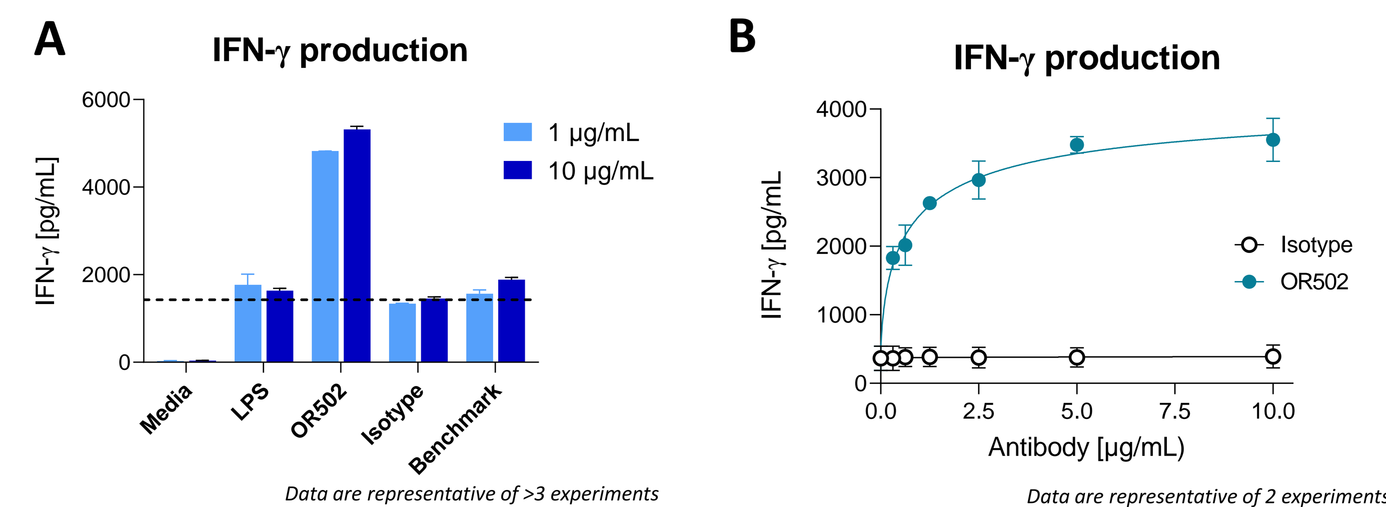

Figure 4. A) OR502 enhances LPS-mediated IFN-γ secretion by PBMCs and outperforms benchmark mAb. B) OR502 enhances IFN-γ release by LPS-stimulated PBMC in a dose dependent manner.

5 OR502 relieves CD8⁺ T cells from M2c macrophage-mediated immune suppression

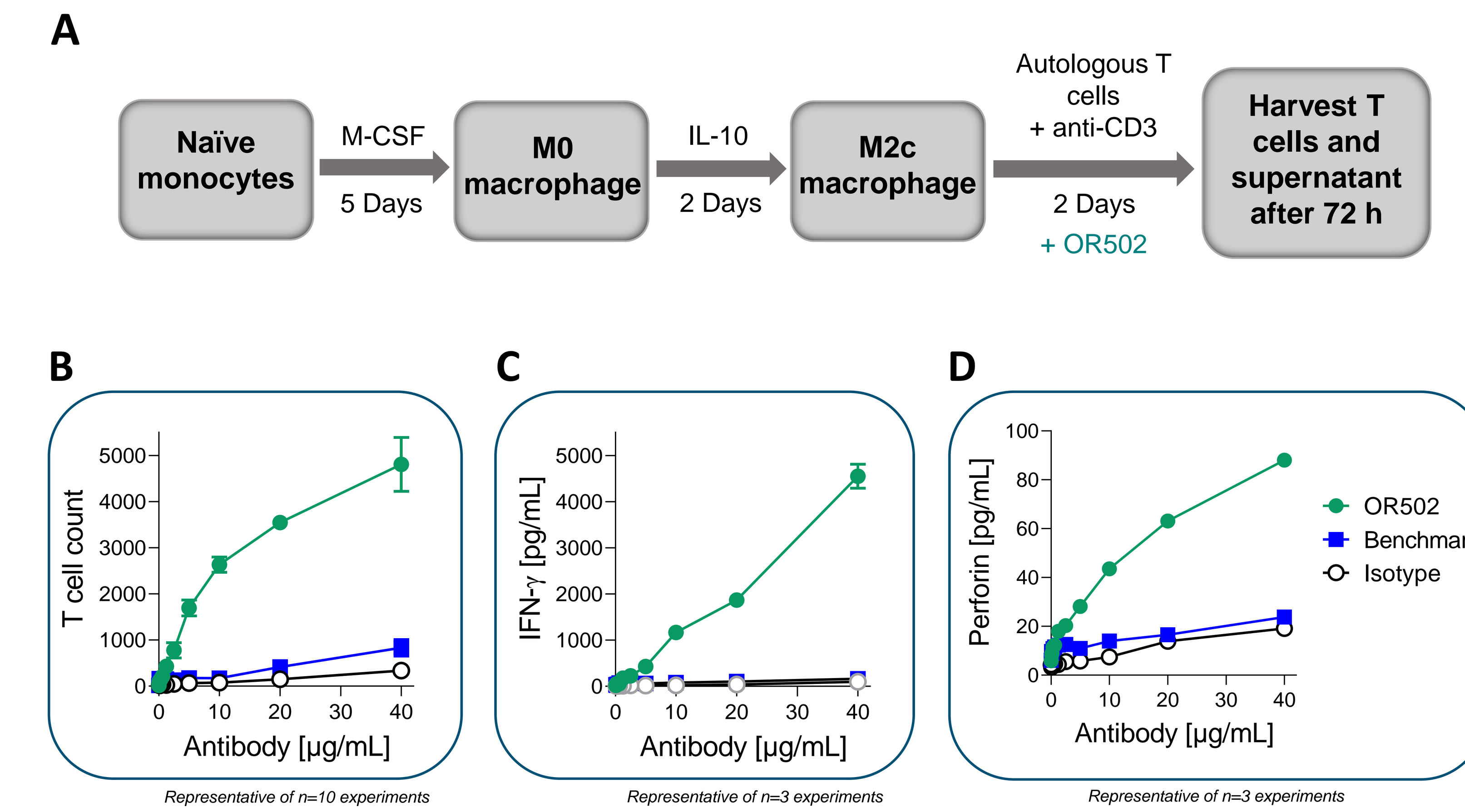

Figure 5. OR502 treated M2c macrophages enhance human CD8⁺ T cell proliferation, IFN-γ secretion and perforin release. A) Timeline and assay design of M2c macrophage/T cell coculture assay. M2c macrophages were incubated with anti-CD3, then cocultured with CD8⁺ T cells and anti-LILRB2 antibodies or isotype control for 72 h. After 72 h coculture, A) treatment with OR502 restores human CD8⁺ T cell proliferation, B) enhances IFN-γ secretion and C) increases perforin release by T cells.

6 Combination with OR502 is required for anti-PD-1 efficacy in M2c/exhausted T cell coculture assay

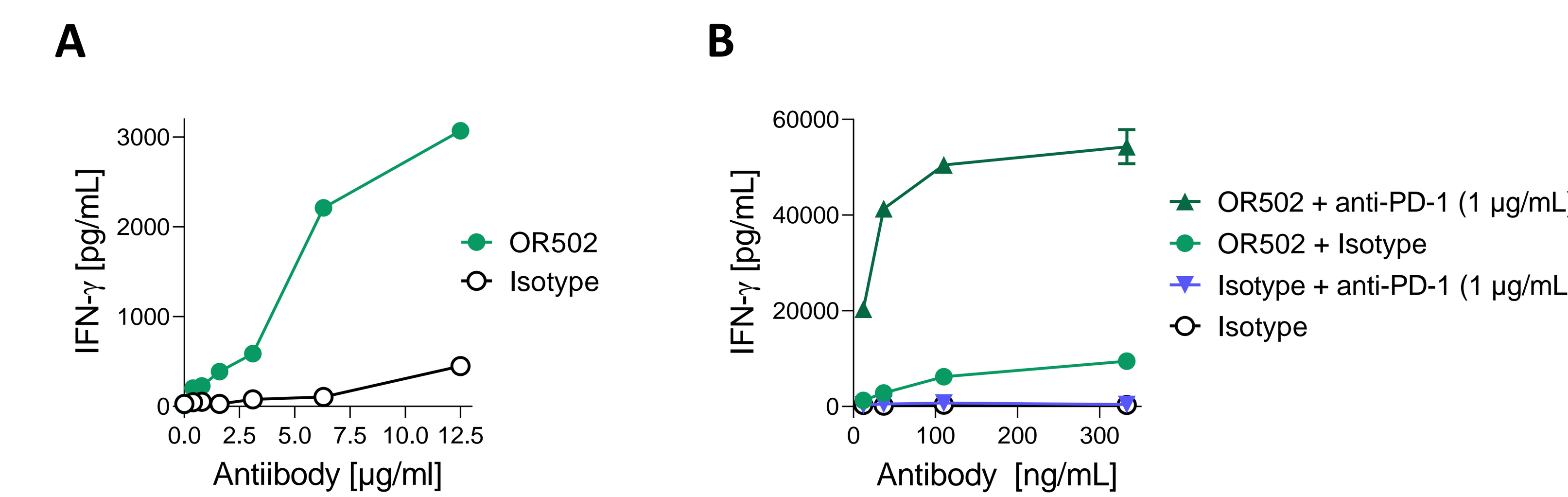

Figure 6. OR502 rescues exhausted T cells from M2c macrophage-mediated immune suppression. A) OR502-treated M2c macrophages enhance IFN-γ secretion by exhausted T cells. B) OR502 treated macrophages amplify the anti-PD-1 (1 μg/mL) induced IFN-γ production by exhausted T cells.

7 OR502 pharmacokinetic profile and in vivo anti-tumor activity of parental antibody

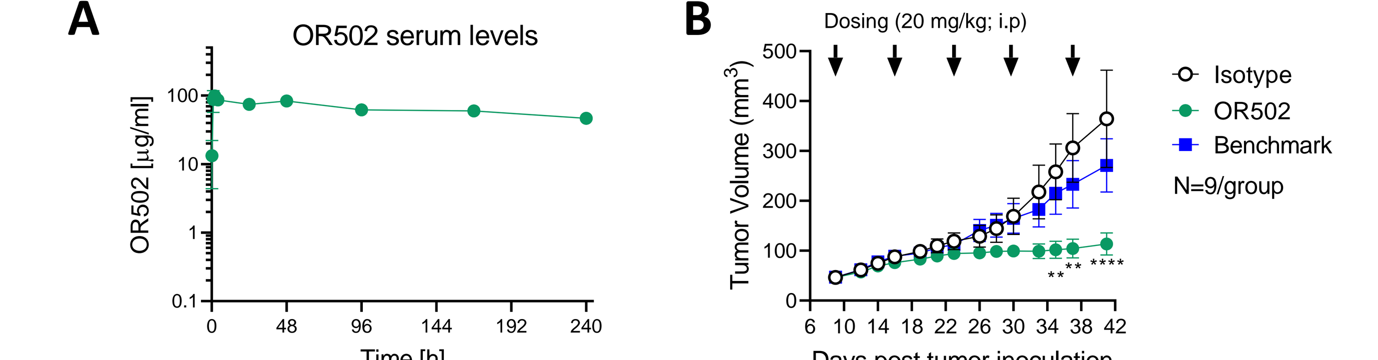

Figure 7. PK profile and in vivo efficacy. A) OR502 has a half-life of ~10 days in humanized FcRn mice following intraperitoneal (i.p.) single dose (10 mg/kg). B) OR502 parent antibody demonstrates anti-tumor activity in SK-MEL-5 tumor model in humanized NSG-SGM3 mice. NSG-SGM3 humanized mice were injected i.p. with 20 mg/kg antibody every 7 days starting on day 9 post SK-MEL-5 subcutaneous tumor inoculation (N= 9/group).

8 OR502 has minimal cytokine release in whole blood and does not induce neutrophil activation or depletion

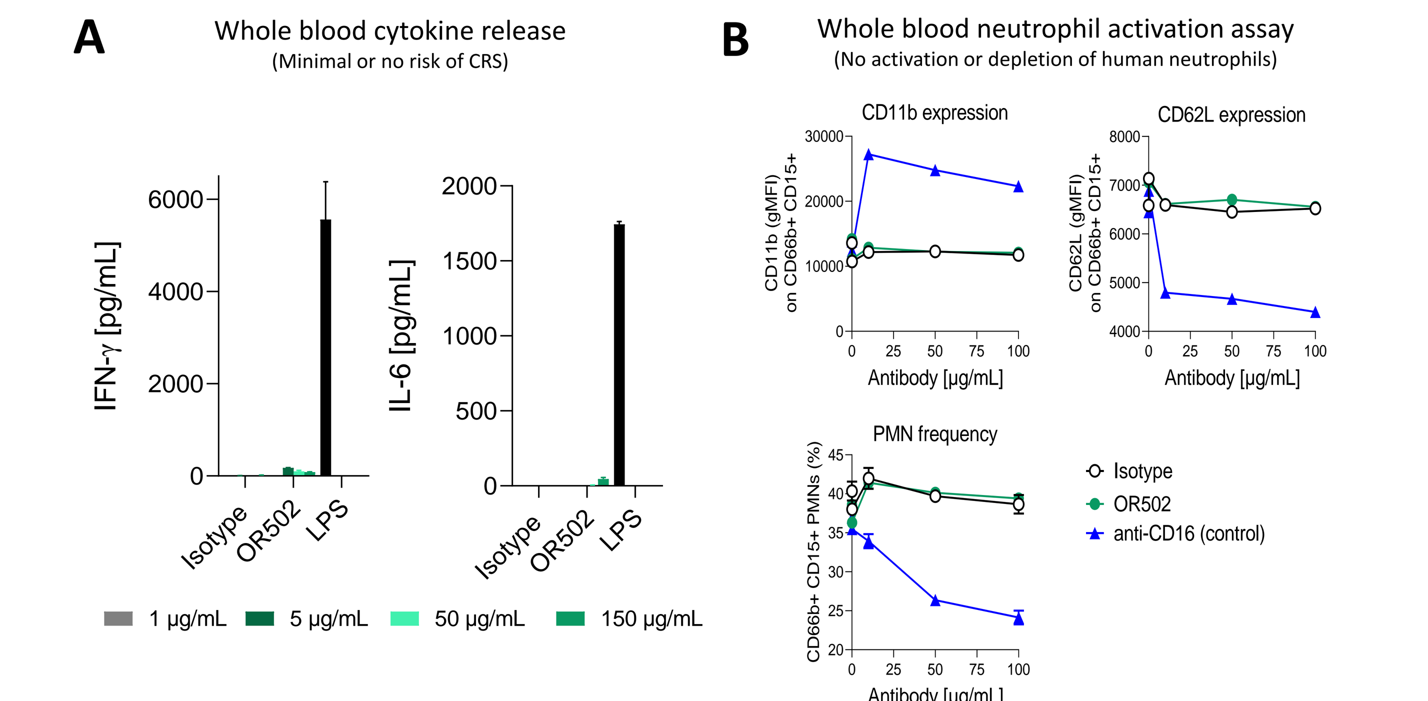

Figure 8. OR502 whole blood data predict acceptable safety profile. A) Whole blood ex-vivo cytokine release assay. Cytokine levels (IL-6, IFN-γ, IL-1β, and TNF-α) observed with OR502 treatment were suggestive of minimal risk of cytokine release syndrome (CRS). B) OR502 does not activate (no increase in CD11b or decrease in CD62L) nor deplete human neutrophils (%CD66b⁺ CD15⁺) in whole blood, compared to anti-CD16 positive control (data representative of 4 donors).

Summary

- OR502 blocks the binding of LILRB2-Fc with HLA-G and other HLA-class I molecules
- LILRB2 blockade by OR502 modulates the immunosuppressive function of TAMs and enhances adaptive anti-tumor responses in vitro in M2c/CD8⁺ T cell coculture assays
- OR502 enhances cytokine secretion following PBMC activation with proinflammatory stimuli
- OR502 parental antibody demonstrates robust anti-tumor activity
- In vitro, OR502 treatment in combination with anti-PD-1 amplifies its efficacy on exhausted T cells

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