Safety and Activity of LRRC15-IFNα, a Conditionally Active Biologic Targeting IFN-α Specifically to LRRC15$^+$ CAFs

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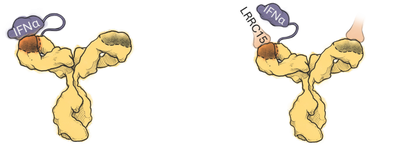
Introduction

IFN-α drives anti-tumor activity through both innate and adaptive immune responses, including dendritic cell maturation, repolarization of suppressive myeloid cells, and CD8+ T cell activation. Additionally, IFN-α can reprogram immunosuppressive cancer-associated fibroblasts (CAFs) by countering TGF-β activity. However, IFN-α therapy has been limited by significant dose-limiting toxicities. We have generated a conditionally active LRRC15-IFNα that targets IFN-α activity to the TME while remaining inactive systemically. LRRC15 is a TGF-β-induced cell surface protein selectively expressed by an immunosuppressive CAF population present in the majority of solid tumor types. Our approach uses a dual-binding antibody (DBA) mechanism that exploits the ability of an antibody to bind competitively to two distinct antigens. Once localized to the surface of an LRRC15+ CAF, LRRC15-IFNα exerts anti-tumor activity both by direct cis-signaling of IFN-α on CAFs and by trans-signaling to adjacent immune cells. Here we present preclinical data supporting the conditional activity of LRRC15-IFNα and the broader potential of the DBA platform.

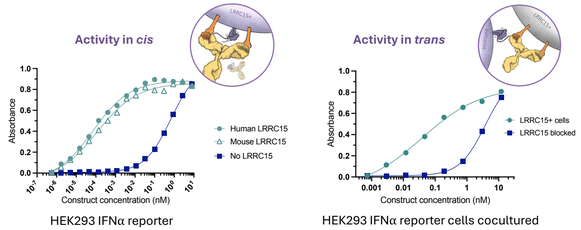
LRRC15 binding-dependent IFN-α activation using dual-binding antibody-based regulation

In the absence of LRRC15 the IFN-α is **OFF**

In the presence of LRRC15 the IFN-α is **ON**



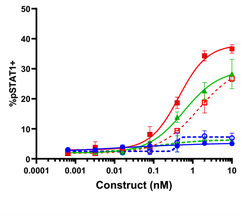
LRRC15-IFNα signals in cis and trans following binding to LRRC15-expressing cells



HEK293 IFNα reporter cells

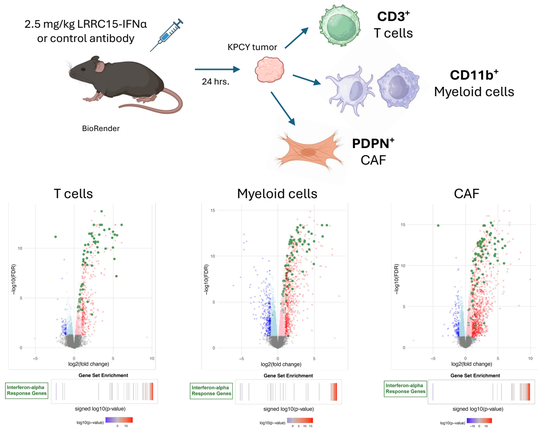
HEK293 IFNα reporter cells cocultured with LRRC15+ cell line

Figure 1: LRRC15-IFNα demonstrates LRRC15-dependent signaling on primary human fibroblasts



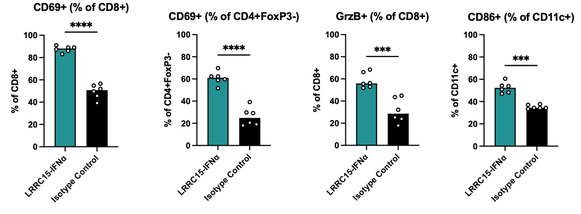
Normal human lung fibroblasts (NHLF) were cultured with 10 ng/mL TGF-β for 48 hours to induce LRRC15 expression and then treated with either blocking anti-LRRC15 or an isotype control. NHLF were treated with titrating concentrations of LRRC15-IFNα or non-conditional control constructs for 20 min. Frequency of pSTAT1$^+$ cells was assessed by flow cytometry.

Figure 2: LRRC15-IFNα induces an IFN-α gene signature in tumor CAF and immune cell populations



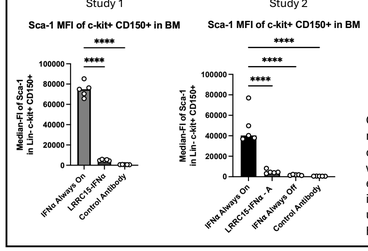
C57BL/6 mice bearing KPCY 6419c5 tumors were treated with 2.5 mg/kg LRRC15-IFNα or a control antibody. Tumors were harvested after 24 hrs. Cell populations were isolated by cell sorting and analyzed for differential gene expression by bulk RNA-seq. Genes associated with an IFN-α signature (volcano plots, green dots) were upregulated in all three populations.

Figure 3: Modulation of intratumor immune cell activation 24 hrs following LRRC15-IFNα treatment



C57BL/6 mice bearing KPCY 6419c5 tumors were treated with 2.5 mg/kg LRRC15-IFNα or a control antibody. Tumors were harvested after 24 hrs., and immune cell populations were characterized by flow cytometry. *P* values were determined using Welch's t test. *** p<0.001, **** p<0.0001

Figure 4: LRRC15-IFNα drives minimal Sca-1 induction on bone marrow HSCs

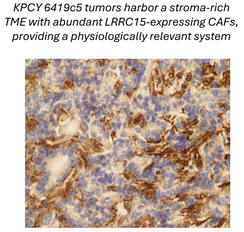


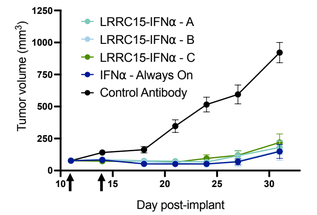
Absence of Sca-1 induction on bone marrow HSCs demonstrates the limited systemic activity of LRRC15-IFNα

C57BL/6 mice were treated with 2.5 mg/kg LRRC15-IFNα or control constructs. After 24 hrs., bone marrow was collected and HSCs were examined for IFN-α-mediated Sca-1 induction. *P* values were determined using one-way ANOVA with Tukey post hoc test. **** p<0.0001

Figure 5: LRRC15-IFNα demonstrates robust anti-tumor activity in the LRRC15$^+$ stroma-rich 2838c3 tumor model

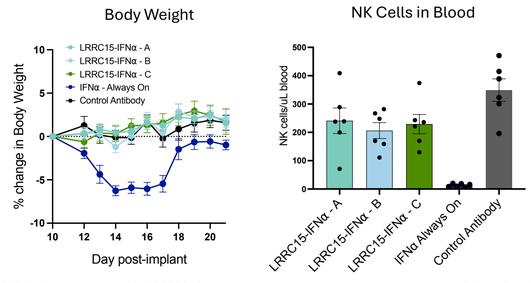
KPCY 6419c5 tumors harbor a stroma-rich TME with abundant LRRC15-expressing CAFs, providing a physiologically relevant system





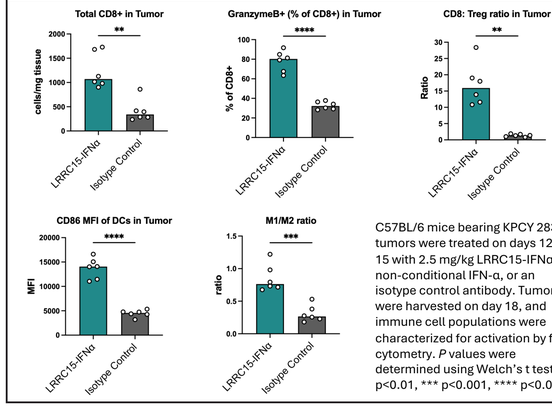
C57BL/6 mice bearing KPCY 2838c3 tumors were treated on days 11 and 14 with a 2.5 mg/kg LRRC15-IFNα variant, non-conditional IFN-α in a matched format, or an isotype control antibody. Tumor volume was measured twice weekly.

Figure 6: LRRC15-IFNα treatment avoids IFN-α-mediated toxicity



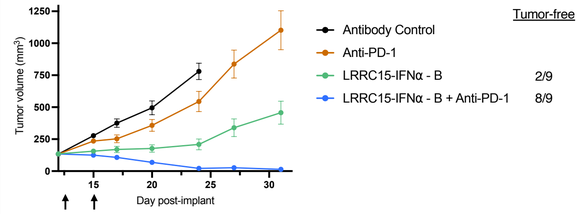
C57BL/6 mice bearing KPCY 2838c3 tumors were treated on days 11 and 14 with a 2.5 mg/kg LRRC15-IFNα variant, non-conditional IFN-α in a matched format, or an isotype control antibody. Body weight was measured daily, and lymphopenia was assessed in peripheral blood on day 17.

Figure 7: LRRC15-IFNα promotes intratumoral innate and adaptive immune activation



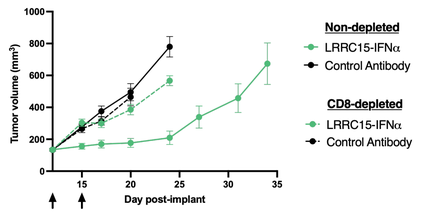
C57BL/6 mice bearing KPCY 2838c3 tumors were treated on days 12 and 15 with 2.5 mg/kg LRRC15-IFNα, non-conditional IFN-α, or an isotype control antibody. Tumors were harvested on day 18, and immune cell populations were characterized for activation by flow cytometry. *P* values were determined using Welch's t test. ** p<0.01, *** p<0.001, **** p<0.0001

Figure 8: LRRC15-IFNα drives tumor regression as monotherapy and in combination with anti-PD-1



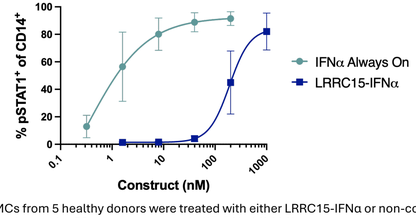
C57BL/6 mice bearing KPCY 2838c3 tumors were treated on days 12 and 15 with LRRC15-IFNα (2.5 mg/kg), LRRC15-IFNα + anti-PD-1 (2.5 + 10 mg/kg), anti-PD-1 (10 mg/kg), or an isotype control antibody (2.5 mg/kg). Tumor volume was measured twice weekly.

Figure 9: LRRC15-IFNα antitumor activity requires CD8$^+$ T cells



C57BL/6 mice bearing KPCY 2838c3 tumors were treated on days 12 and 15 with 2.5 mg/kg LRRC15-IFNα or a control antibody. CD8 depletion: anti-CD8 (clone 2.43, BioXcell #BE0061) was administered intraperitoneally two times per week, beginning 1 week before the first administration of therapy. CD8+ cell depletion was confirmed by flow cytometry

Figure 10: LRRC15-IFNα shows low immunostimulatory activity in human whole blood



Total human PBMCs from 5 healthy donors were treated with either LRRC15-IFNα or non-conditional IFN-α in a matched format for 20 min. Frequency of pSTAT1$^+$ of CD14$^+$ cells was assessed by flow cytometry. Data represent mean ± SD of 5 independent donors.

Summary

- LRRC15-IFNα specifically targets IFN-α activity to LRRC15$^+$ CAFs, with >100-fold preferential activity in the presence of LRRC15
- Targeted IFN-α delivery reprograms the immunosuppressive CAF-rich TME, and drives intratumoral CD8$^+$ T cell and innate immune activation
- LRRC15-IFNα demonstrates robust antitumor activity as monotherapy and in combination with anti-PD-1, without clinical signs of systemic IFN-α toxicity
- These results validate the DBA platform and support clinical development of LRRC15-IFNα