BONUM

# Identification of a Human IFNα2b Variant Active in Mice and its Use in A New Class of Targeted and Conditional IFN-α Therapeutics

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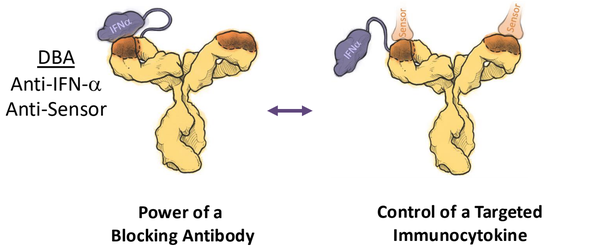
- IFN-α plays a central role in cancer immunology but has not been broadly aplied as an immunotherapy.
- Our novel dual-binding antibody (DBA)-based platform allows for the generation of conditionally-active immunocytokines. This platform enables IFN-α to be targeted to specific cell populations while remaining inactive on the majority of IFNAR$^+$ cells.
- This focused activity of a conditional IFN-α DBA therapeutic allows the separation of the potential toxic or immunosuppressive effects of IFN-α from the desired immune supportive response.
- To test the activity of a conditional IFN-α DBA therapeutic in mice, we developed a mouse cross-reactive human IFNα2b, referred to as the CKRL variant, using only four amino acid substitutions.
- Importantly, the CKRL variant provides mouse cross-reactivity while preserving the critical IFNα2b epitope recognized by the DBA

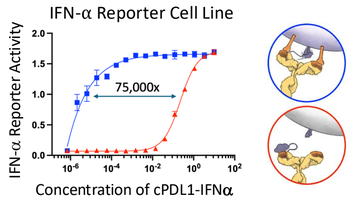
### Conditional IFN-α Targeting using Dual-Binding Antibodies

Dual Binding Antibody (DBA): Recognizes two distinct antigens
Bonum's regulated therapeutics utilize standard antibody and linker components
DBA-cytokine regulation domains are portable to multiple formats




**Power of a Blocking Antibody** | **Control of a Targeted Immunocytokine**

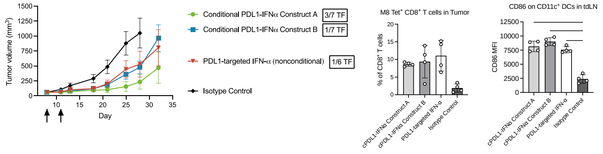
### Conditional IFN-α Activity in Cell-based Assays





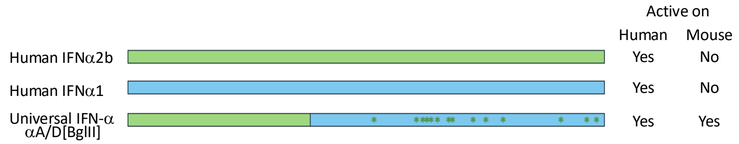

A conditional PDL1-IFN signals in the presence of PDL1 but not in the absence of PDL1 or when PDL1 is blocked

### PDL1-IFNα Activity in Vivo




huPD-1/huPD-L1 KI mice bearing huPD-L1-expressing MC38 tumors were dosed I.V. on days 8 and 11 with conditional PDL1-IFNα, non-conditional PD-L1-targeted IFN-α, or an isotype control at 5 mg/kg. Tumor volume was measured twice weekly (A). Tissues were harvested on day 13 from a subset of mice to assess frequency of intratumoral M8 tet$^+$ T cells (B) and expression of CD86 on DCs within the tumor-draining lymph node (C). *P* values were determined using one-way ANOVA with Tukey post hoc test. **** p<0.0001

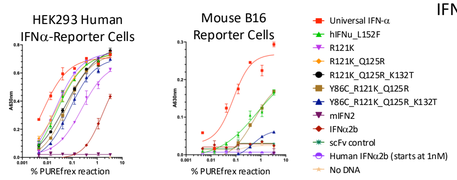
## Universal IFN-α is a hybrid of IFNα2b and IFNα1



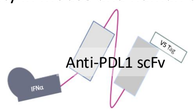

Universal IFN-α is a hybrid of human IFNα2b and IFNα1 that is commonly used in applications requiring activity across species. The hybrid was first generated using a shared BglII restriction site. Fourteen amino acids differ between IFNα2b and IFNα1

*Rehberg et al., J.Biol.Chem. 1982*

## Round 1: IFN-α variants tested by in vitro transcription/translation

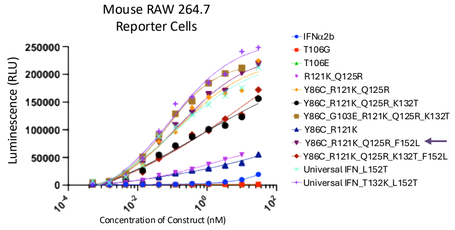



IFN-α variants were generated as IFNα-scFv fusions to identify mutations affecting activity in mouse and human cells




IFNa-scFv protein was generated with an anti-PDL1 antibody by in vitro transcription/translation using the PURExfrex® system (CosmoBIO USA). Protein mixture was serially diluted and protein was captured via an anti-V5 tag coated to the wells of the plate. Proteins were assayed for PD-L1 binding to assess expression (data not shown) or the ability to stimulate an IFN-α response in receptor reporter cell lines

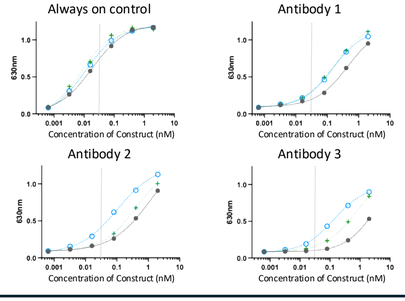
## Round 2: Mammalian expressed IFN-α variants




IFN-α activity in mouse cells was used to select the CKRL variant

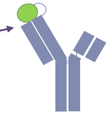
Constructs were made with the IFN-α variants in a monovalent format with an anti-PDL1 antibody. The IFN-α activity was tested in the mouse RAW 264.7 IFN-reporter cell assay (InvivoGen)

## Testing antibody blocking of IFN-α variants by expression as immunocytokines




Constructs were made with the three IFN-α variants in a format that is monovalent for IFN-α using anti-IFNα monospecific or DBAs without cell targeting, then tested in the human HEK293 IFN-reporter cell assay

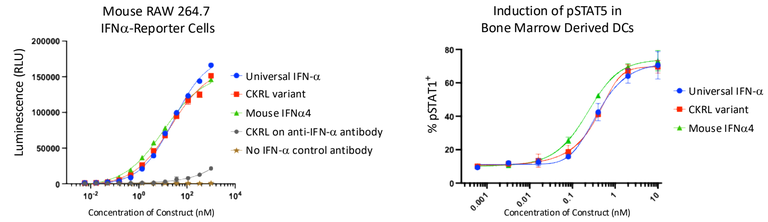
Fused IFN-α bound to anti-IFN-α antibody to test for blocking



## In vitro and in vivo activity of CKRL vs universal IFN-α and mouse IFNα4


IFN-α constructs were generated in a monovalent format with non-binding control antibodies to compare the activity of universal IFN-α, CKRL, and mouse IFNα4 in vitro and in vivo

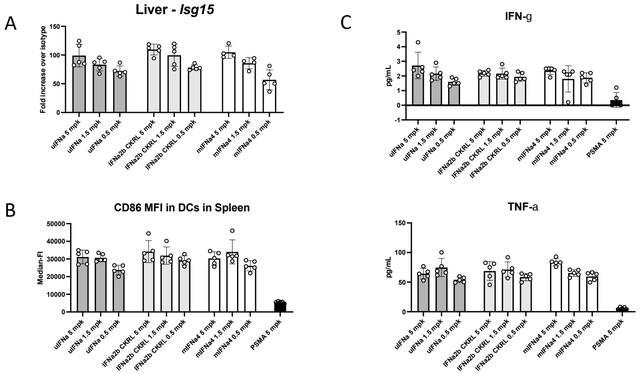
### In vitro




IFN-α constructs were tested for the ability to stimulate IFN-α in a mouse reporter cell and in primary mouse BMDCs. BMDCs were derived from C57BL/6 bone marrow cells by mCSF treatment. pSTAT1 was assessed by flow cytometry.

### In vivo

The in vivo activity of IFN-α CKRL is comparable to Universal IFN-α and mouse IFNα4




C57BL/6 mice received a single IV dose of either uIFN-α, IFN-α CKRL, or mouse IFNα4. Tissues were collected 24 hours later to assess *Isg15* induction in the liver (A), DC activation in the spleen (B), and cytokine levels in the serum (C).

## Conclusions

- We have developed a novel human IFN-α variant, CKRL, that displays robust mouse cross-reactivity while only differing from the human IFNα2b sequence by 4 amino acids.
- The CKRL IFN-α variant shows similar activity as human IFNα2b in our conditional DBA-containing therapeutics
- CKRL shows activity comparable to mouse IFNα4 both in vitro and in vivo
- The CKRL variant maintains the critical human IFNα2b epitopes required for the binding of our DBAs, allowing for testing in mouse models without the need for surrogate anti-mouse IFNα DBAs