Targeting IL1RAP To Eradicate Leukemia Stem Cells (LSC) In Acute Myeloid Leukemia (AML)

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Summary

- Target – IL1RAP
- MOA – eliminate stem cells that drive AML and are responsible for remission
- Indication - AML
- Current status
  - POC established in AML animal models
  - Humanized, cyno/human cross-reactive CD3 (wholly owned by COH)
  - Dozens of IL1RAP mAbs to optimize therapeutic activity (also wholly owned by COH)
  - Clinical candidate identification and lead optimization underway
  - Highly validated disease models established.
- Major Risk
  - Novel target, Therapeutic Benefit, Cytokine Release Syndrome
- COH IP status – Provisional patent applications filed.

Target Product Profile

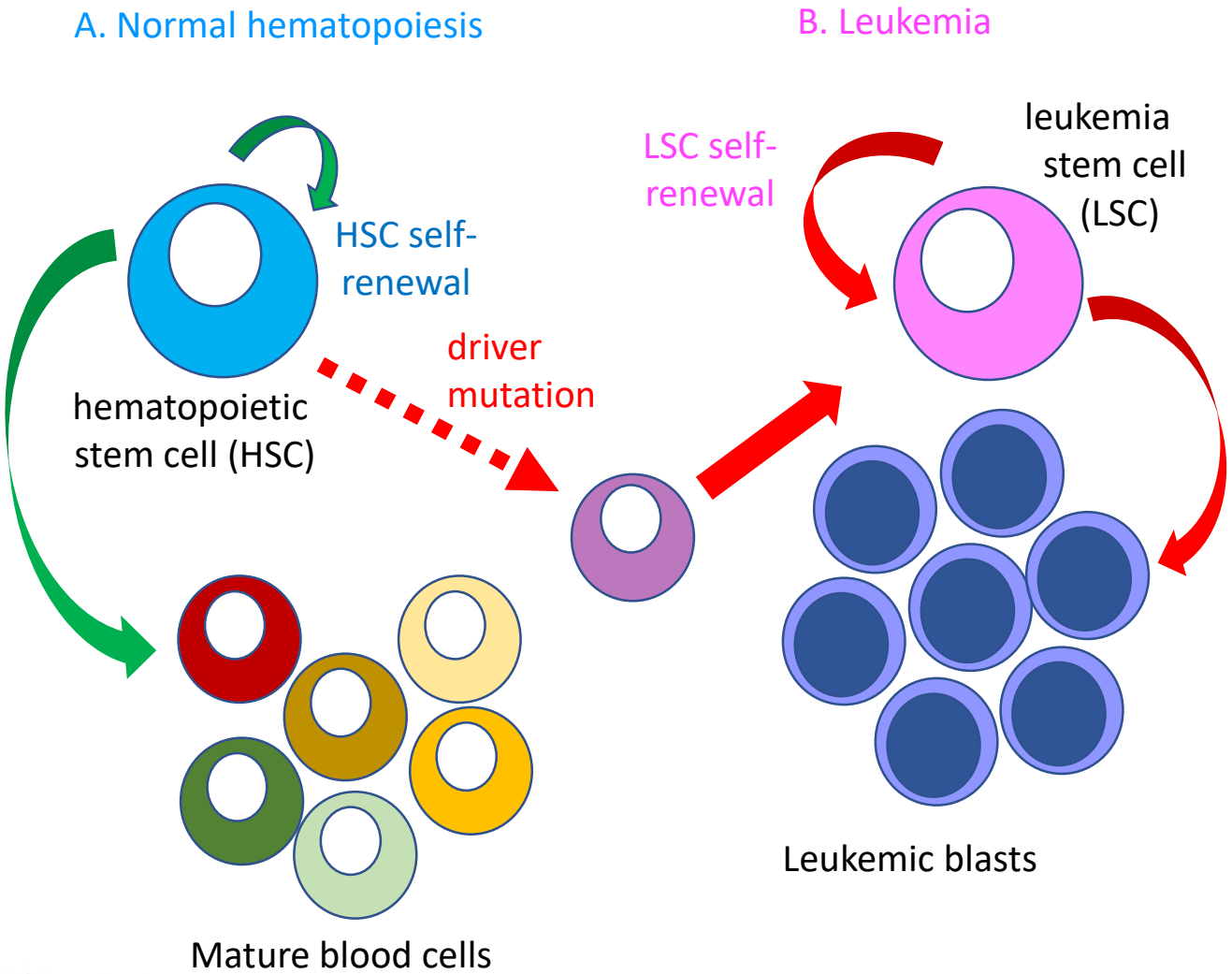
Attribute	Desired Criteria
Product	Bispecific Antibody addressing both IL1RAP and CD3
Indication and patent population	Patients with AML
Selectivity	Preferential expression of Leukemic stem cells that overexpress antigen, minimal engagement in healthy tissues
MOA	T-cell engager with optimized geometry to enhance efficacy
Safety	Comparable safety to first line treatment / other T cell engagers
Desired Dose & schedule	Weekly (up to 8 weeks), 0.5 to 100 ug/m^2
Pharma properties	i.v.
PK ADME properties	Uptake over 2 days, blood half-life 14 to 21 days, eliminated through metabolize/proteolysis

Acute Myeloid Leukemia (AML) is an incurable disease

- AML is the most common acute leukemias. Approximately 20,240 new cases are predicted in 2021 and 11,400 will die of the disease (SEER.cancer.gov)
- The 5-year relative survival rate of AML patients in the US was a dismal 29.5% (2011– 2017; SEER.cancer.gov)
- The mutational landscape of AML has been well-described and continues to evolve. Precision Medicine is used for risk stratification and treatment decisions; also, to develop new molecularly targeted therapies (MTT).
- Despite new approved MTTs, relapse occurs as therapies are not curative.
- Allogeneic (allo-) hematopoietic stem cell transplantation (HCT) can cure AML but not all patients are fit for HCT and some who qualify cannot identify suitable donors. Furthermore, allo-HCT can have debilitating side effects such as graft vs host disease.
- The success of allo-HCT supports the thesis that immune-based therapies can achieve durable remissions or cures.

There is clear evidence of unmet medical treatments for this patient population

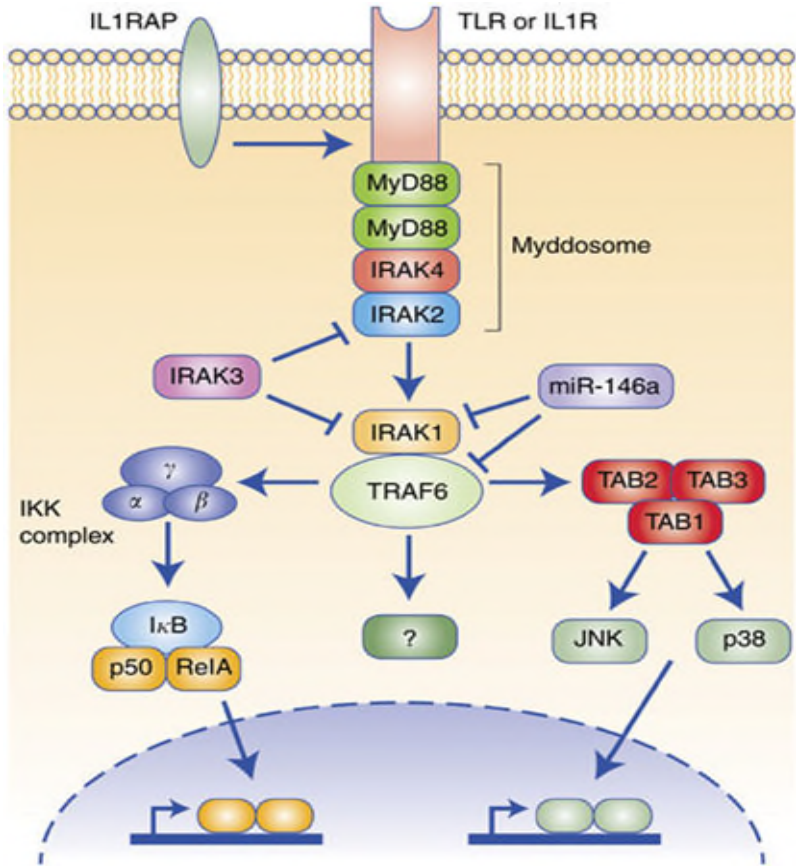
Targeting Leukemia Stem Cells (LSCs) to Cure AML



- AML is organized as a cellular hierarchy initiated and maintained by a pool of self-renewing LSCs.
- While LSCs are enriched in the CD34+CD38- subpopulation of AML blasts, they are defined only by the ability to engraft rather than by specific immunophenotypic features.
- A higher engraftment capacity is noted in quiescent LSC.
- Unfortunately, quiescent LSCs are resistant to the treatments approved and available for AML as many require cells to divide or cycle.
- Therefore, it is accepted in the field that LSC persistence causes disease refractoriness or relapse in AML patients
- Immune therapy has the potential to target both LSCs and blasts if an appropriate target can be identified.

IL1RAP is a good target for immune therapy

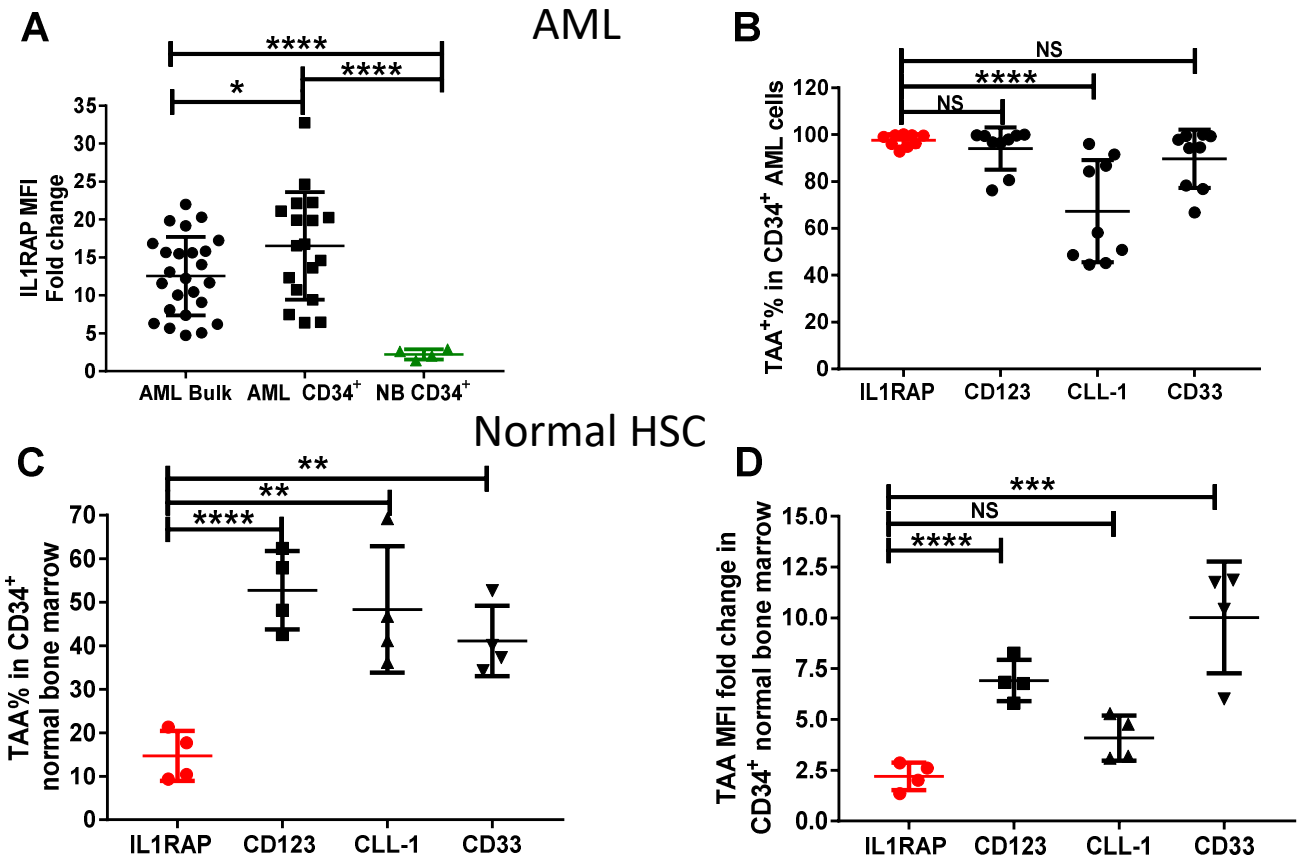
- Interleukin-1 receptor accessory protein (IL1RAP, IL1R3) is a coreceptor involved in several signaling pathways including interleukin-1 (IL-1), IL-33, IL-36G, and stem cell factor (SCF).
- Signaling by IL-1 requires the formation of a cell surface receptor complex involving IL-1, interleukin-1 receptor type 1 (IL1R1), and IL1RAP. Lack of IL1RAP completely abrogates cellular responses to IL-1.
- IL1RAP is present on the cell surface of candidate chronic myeloid leukemia LSCs, AML blasts and CD34+ cells which include LSCs, and myelodysplastic syndrome, but not on normal HSC.
- Studies with IL1RAP$^{-/-}$ knockout mice indicate that IL1RAP is dispensable for normal HSC function.



Bin Zhang, et al. Blood. 2016
Barreyro L, et al. Blood. 2012
G W Rhyasen, et al. British Journal of Cancer. 2015
Helena Ågerstam et al. Blood. 2016

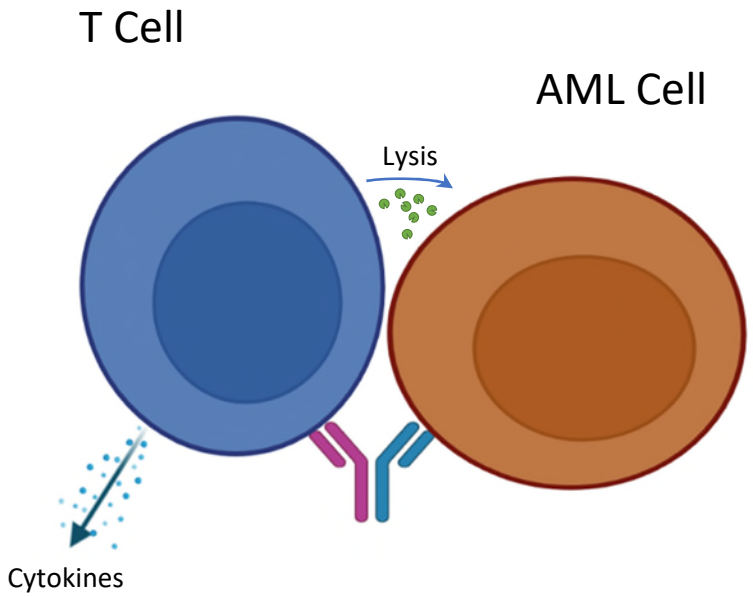
Expression of IL1RAP is specific for AML

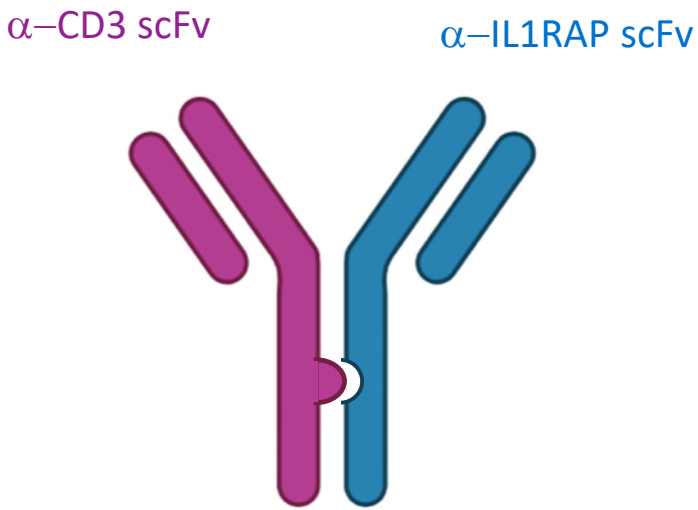
- IL1RAP is highly upregulated in AML, especially in the CD34$^+$ enriched fraction which includes LSCs. A mean of 97.5% CD34$^+$ cells are IL1RAP+ in clinical samples (n=30). This level is comparable to CD123 and CD33 presently in clinical trial, and much higher than CLL1, another target against AML in clinical trial.
- In normal bone marrow, 14.7% CD34$^+$ cells are IL1RAP+ much lower compared to 52.8% for CD123, 41.1% for CD33 and 48.4% for CLL-1. These antigens are being investigated for immune therapy of AML, and IL1RAP is expressed at significantly lower levels in normal HSCs than these. Thus, IL1RAP targeting is more selective.
- In CD34$^+$ AML, IL1RAP expression is higher in cells in G_0 phase of the cell cycle than those in G_1 phase. This suggests IL1RAP will target quiescent AML blasts and LSCs.



IL1RAP expression. A. IL1RAP is highly unregulated in both bulk and CD34+ enriched AML cells but not normal bone (NB) marrow CD34+ cells. B. IL1RAP+ cells in CD34+ enriched AML cells is as high as %CD123+ and %CD33+ both targeted by immune therapies. C and D. The % of IL1RAP+ cells in CD34+ enriched normal bone marrow is significantly lower than %CD123, %CLL-1, and %CD33, thus should be more specific and less toxic. (TAA = tumor associated antigen, MFI = mean fluorescence intensity)

Proof of Concept - IL1RAP Bispecific



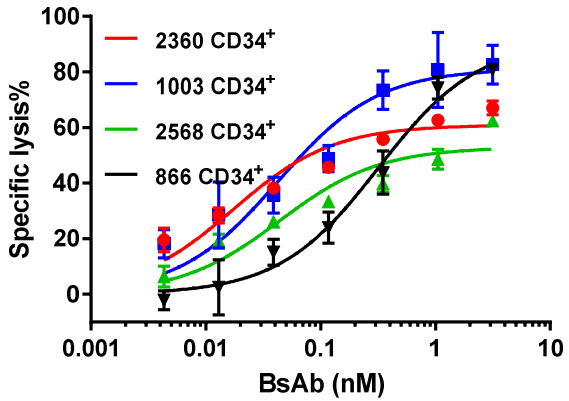


Bispecific antibodies (BsAb) can bridge a tumor cell to a T cell, leading to an immunological synapse, T cell directed cytotoxicity and expanding the immune response.

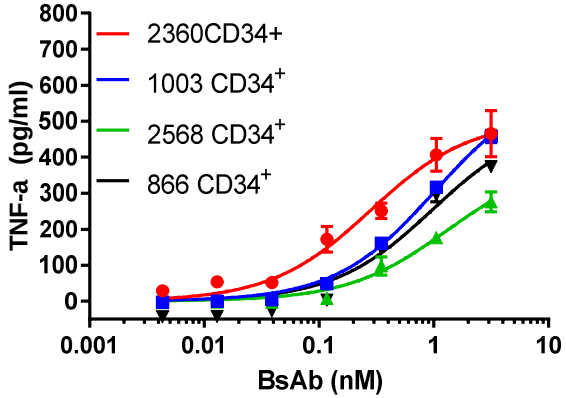
Design: Knobs-into-holes design of bispecific using scFvs of Il1RAP and CD3.

Proof of concept: IL1RAP BsAb kills AML patient cells *in vitro* and *in vivo*, activates immune response

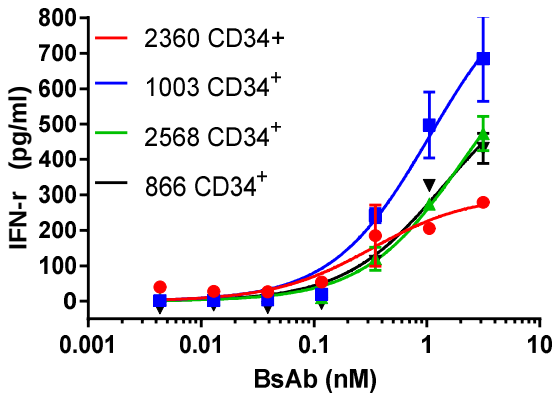
Cell Lysis



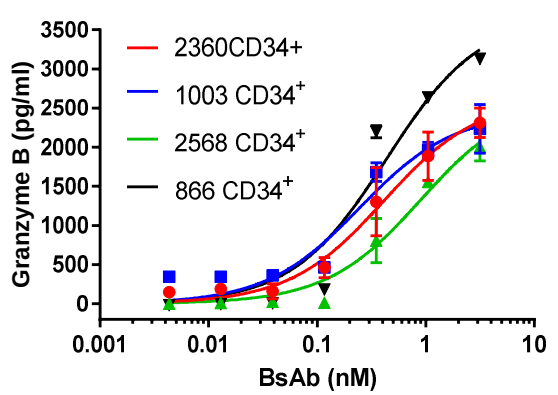
TNF-α



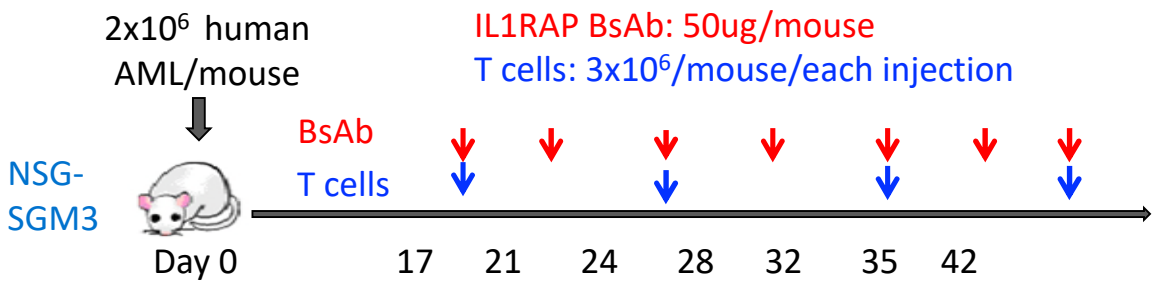
IFN-γ



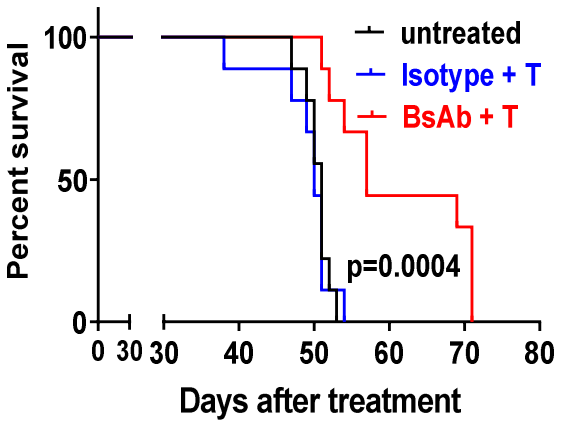
Granzyme B



Animal Experiment



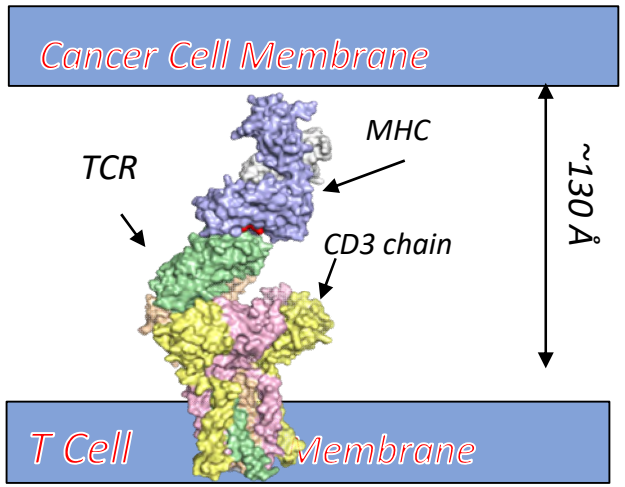
In Vivo Efficacy



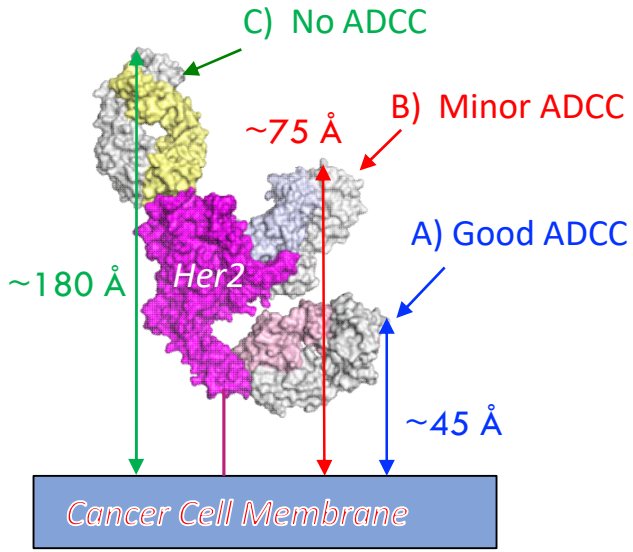
City of Hope

Development of a Clinical Product – Geometry as a critical parameter

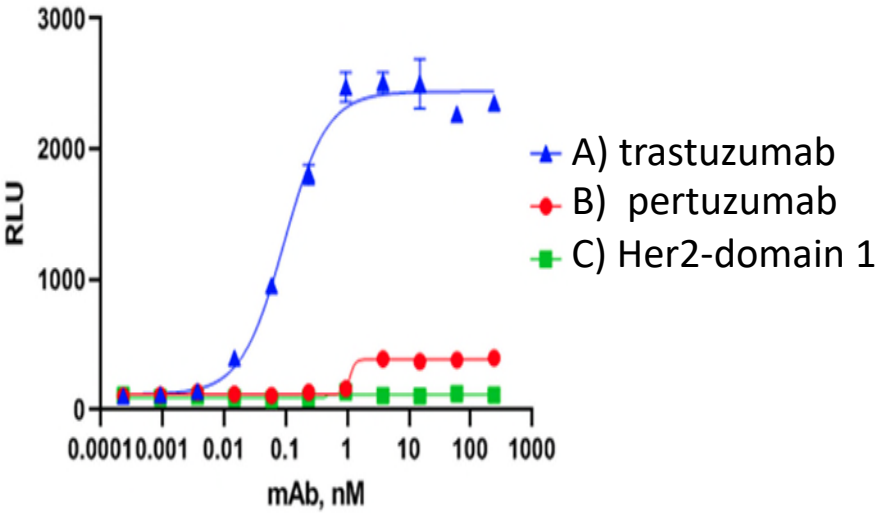
TCR/CD3 Complex
Involved in T cell activation



Geometry is critical to
achieve effective ADCC activity



T Cell Activation Assay



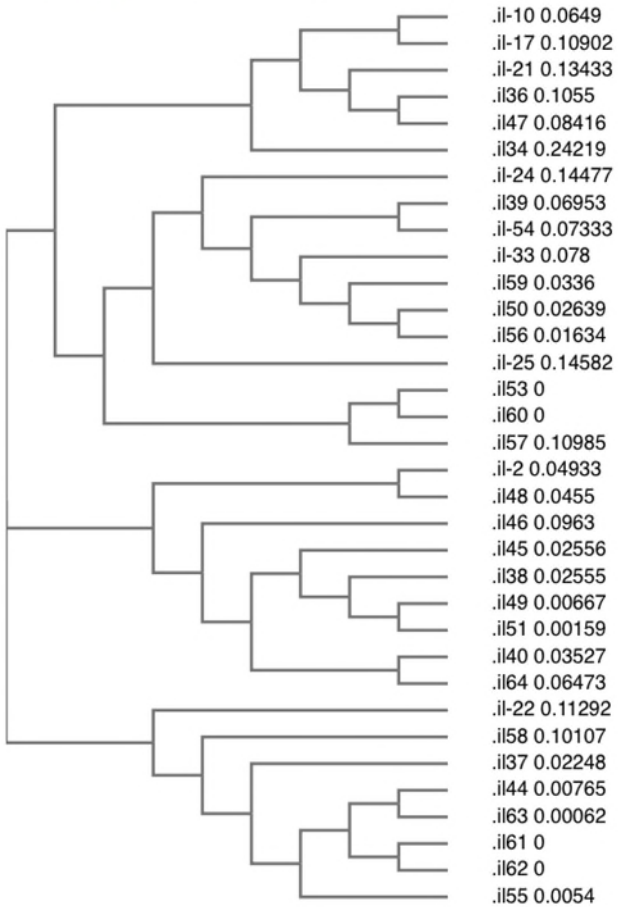
Biology indicates distance of ~130 Å between membranes is optimal. The design of a bispecific engager should match this.

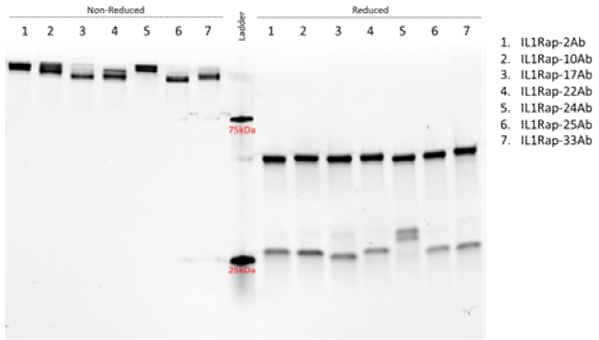
Development of a Clinical Product - Practically

- Generate library of IL1RAP mAbs to generate best in class T cell engager
  - Used next gen screening to identify 34 unique mAbs
  - Produced individual members within a cluster
  - Characterized
  - Generating clinical candidates
- Obtained COH wholly-owned CD3 that is cyno-human cross reactive
  - Humanized
  - Thermally stable
  - Affinity tuned (improve biodistribution)

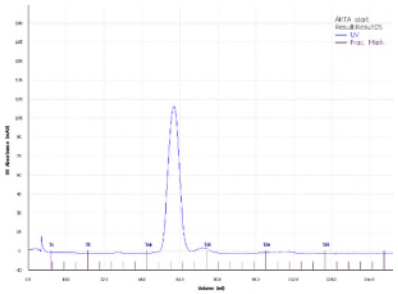
Development of a Clinical Product, Practically.
Next Gen Screening allows us to identify best in class

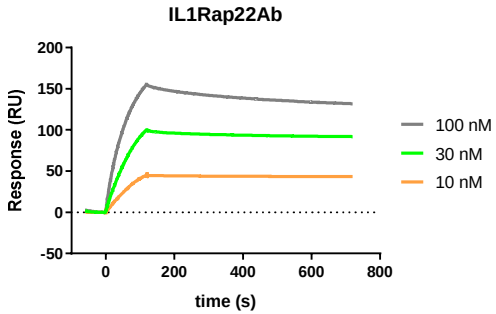
Cladogram of Hits



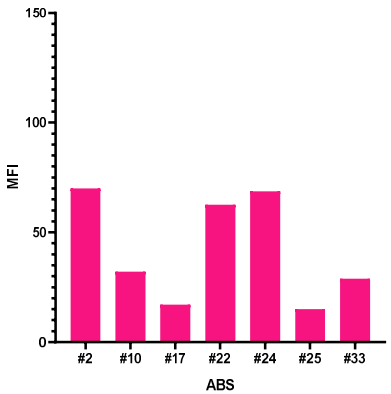


Size Exclusion
IL1Rap-22 Ab

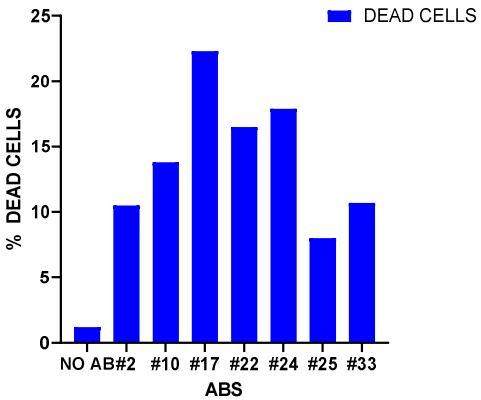




Specific binding



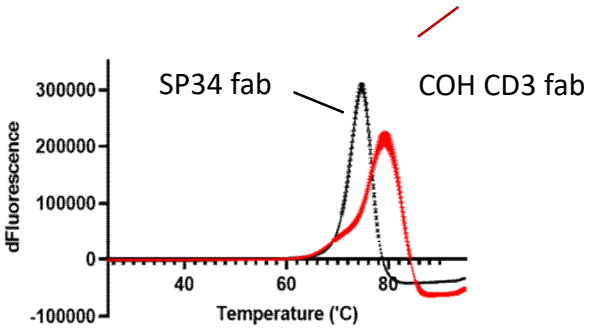
MV411 killing by ADCC



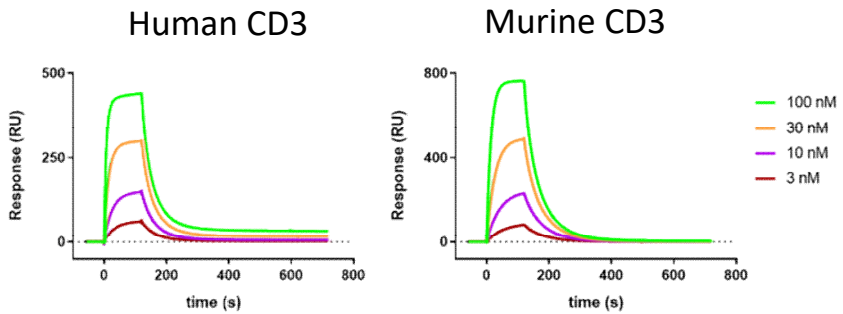
City of Hope.

Development of a Clinical Product, Practically
Generation of humanized, cross-reactive anti-CD3

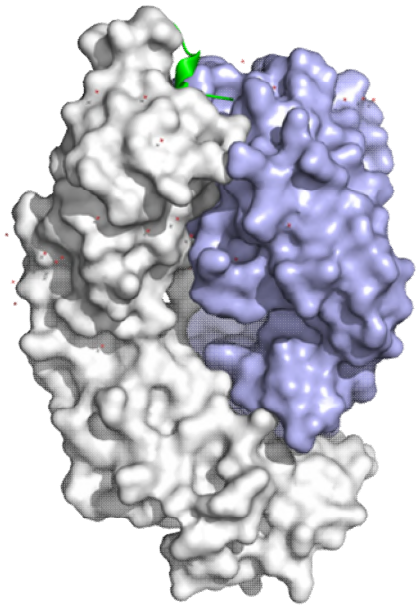
Improved stability compared to clinical α-CD3 Fab



Cross-reactive CD3 Fab with similar affinities



Structure of CD3 bound to humanized Fab

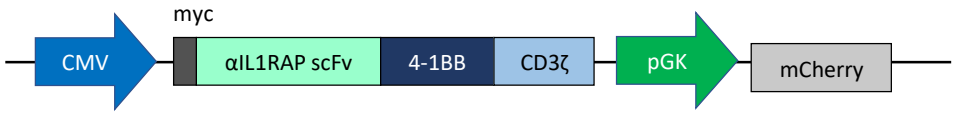


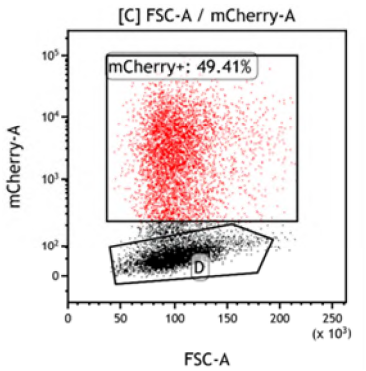
Next Steps

- Generation of multiple CD3 T cell engager (in progress)
- Selection of family by ADCC assays
- Humanize IL1RAP
- Finish IND enabling studies.

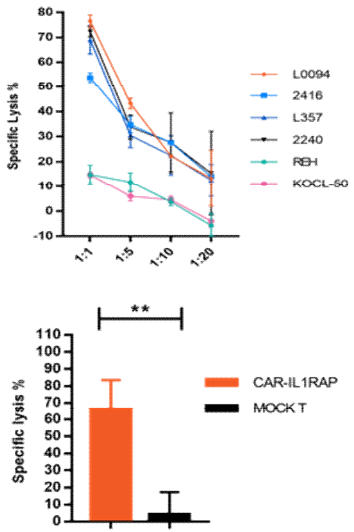
IL1RAP CAR T cells - POC

IL1RAP-redirected CAR T cell Design

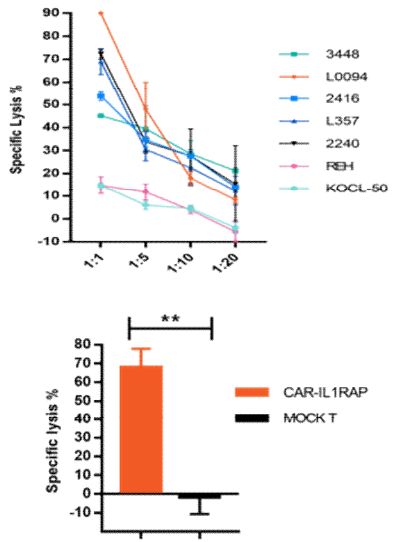




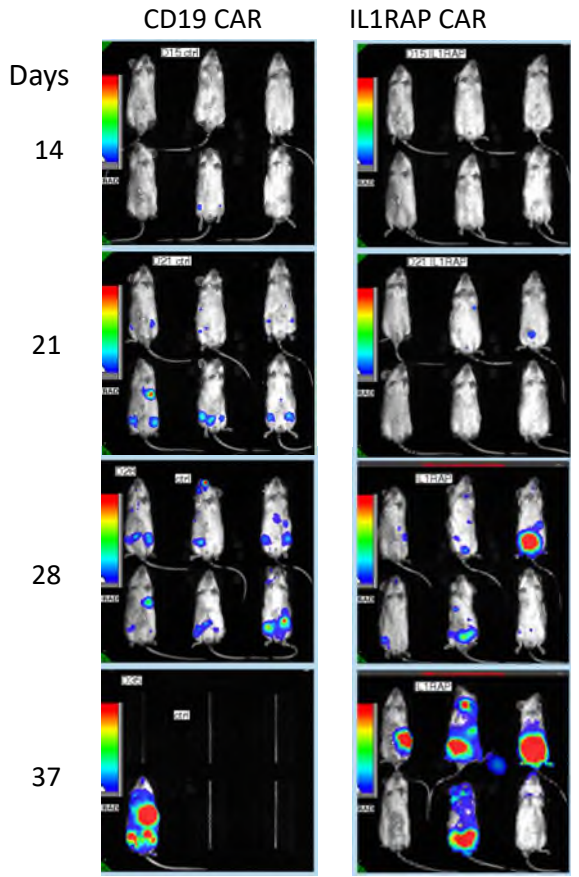
AML blasts

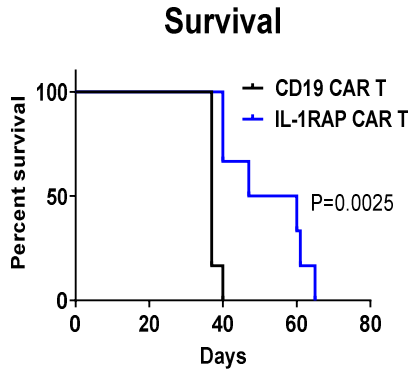


AML CD34+ cells



NSGS mice engrafted with MV4-11 human AML cell line.





CAR T cells - Next Steps

- Selection of humanized Fabs identified above underway
  - In-house Jurkat nFat luminescent assay to identify candidate
  - CAR construct using humanized Fabs to avoid tonic signaling and immunogenicity
- Compatible with FabRack technology opening possibility for polyvalent approach {antigen escape}, safety and additional technologies being developed in Williams/Brown lab.

Summary

- Thorough search for IL1RAP mAbs enabling the production of best-in- class T cell engagers
- COH-owned, cross-reactive, humanized CD3
- Compatible as stand-alone CAR T cells or combined with FabRack for next, next-gen CAR T cell therapy

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