4th Annual TPD Targeted Protein Degradation

KYMERA EST. 2016

Considerations for E3 Ligase Pairing and Screening of Immune-Inflammation Targets

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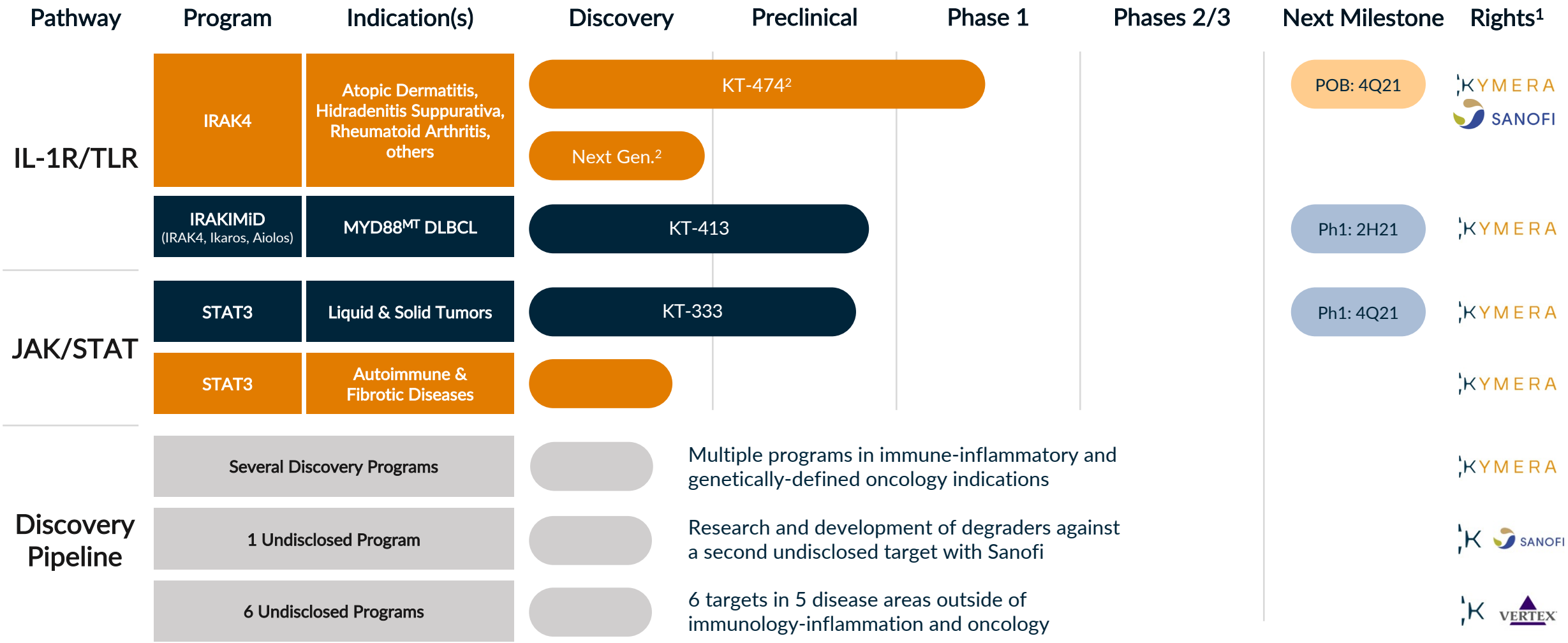
Disclosure Statement

Veronica Campbell is an employee of Kymera Therapeutics

Outline

- IRAK4 Immunology-Inflammation (I/I) Program
- Considerations for E3 pairing and introduction to Kymera Pegasus Platform
- Development of multiplex flow cytometry assay to monitor IRAK4 and characterize degrader activity across circulating primary immune cells
- Screening efforts identified IRAK4 selective degraders with broad degradation profile and potent activity across blood immune subsets
- Demonstration of IRAK4 target degradation in diseased blood and in HV Phase I study (KT-474)

Kymera's Pipeline of Novel Protein Degraders



1. Option to participate equally in the development and commercialization of Sanofi-partnered programs in the US.
2. Sanofi collaboration to develop IRAK4 degrader candidates, including KT-474 (SAR444656), outside of oncology and immuno-oncology fields.

● = Oncology ● = Immunology-Inflammation

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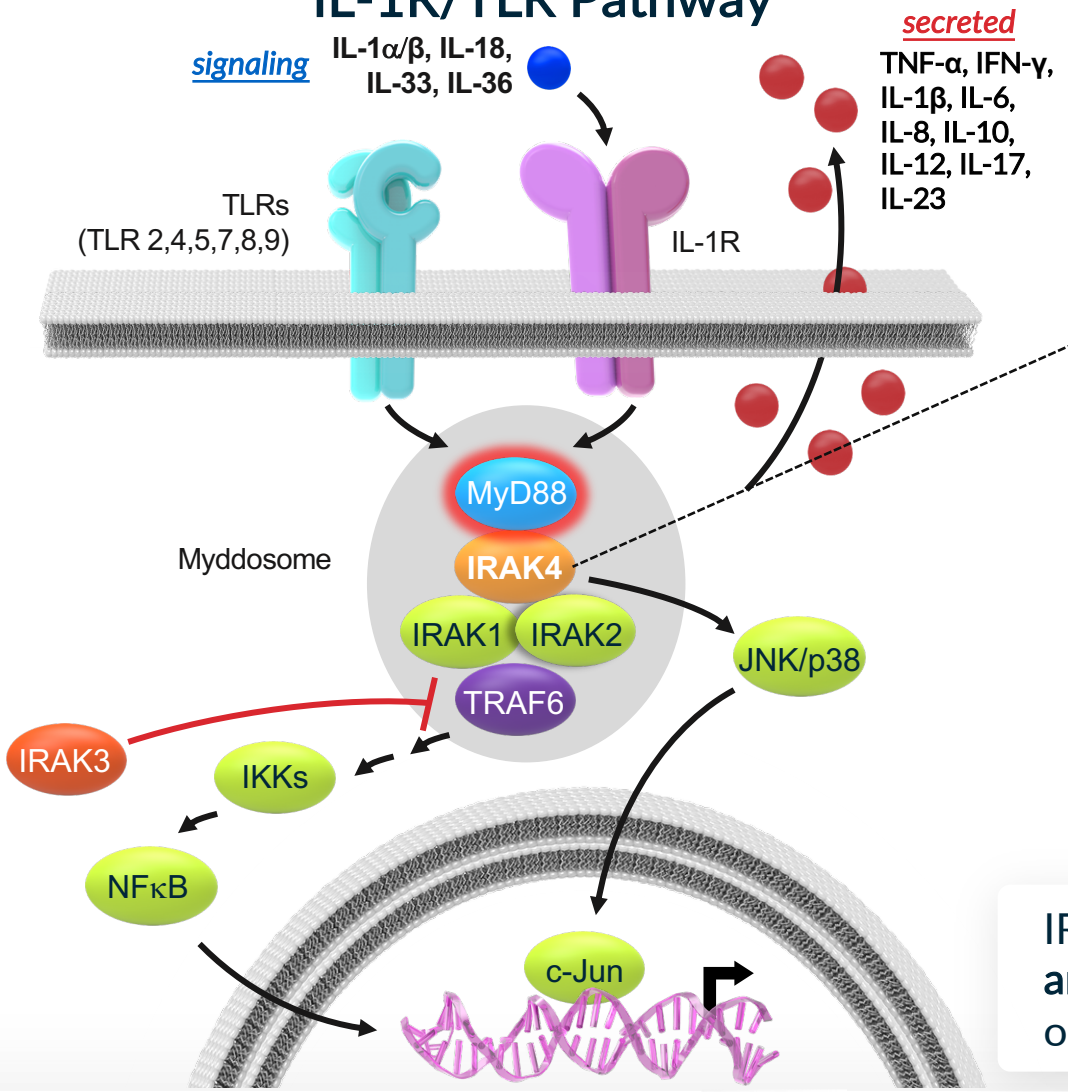
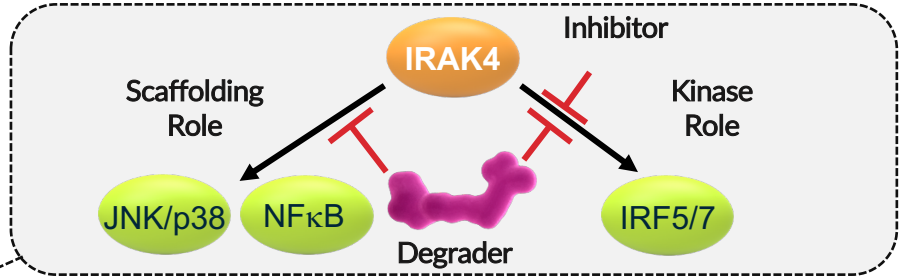
IRAK4 Targeting: Degrader Advantage, Clinical Validation, and Human Genetics De-risking

IL-1R/TLR Pathway



Degrader Advantage



Clinical Pathway Validation

IL-1α/IL-1β : Rheumatoid Arthritis, CAPS, Hidradenitis Suppurativa

IL-1α: Atopic Dermatitis

IL-1β: Gout; CANTOS Outcomes Data in Atherosclerosis and Lung Cancer

IL-18: Macrophage Activation Syndrome

IL-36: Generalized Pustular Psoriasis

IRAK4 SMI: Rheumatoid Arthritis

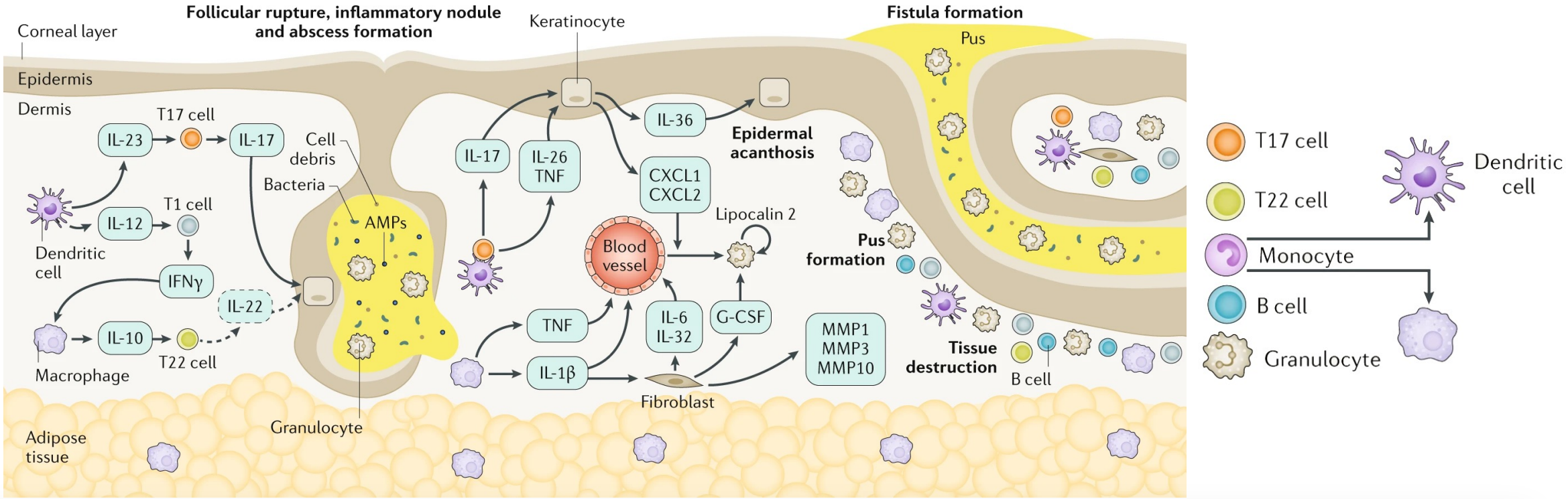
Human Genetics

Humans with **IRAK4 Null Mutation are healthy**

IRAK4 degrader has potential to achieve a **broad, well-tolerated anti-inflammatory effect,** providing multiple development opportunities in autoimmune inflammatory diseases

Targeting Autoimmune Disease May Require Targeting Multiple Cell Inflammatory and Resident Cell Types

HS Pathogenesis as an Example of Chronic Skin Disease



- Ensure characterization of target, E3 ligase and degraders in all pharmacology relevant cell types
- IRAK4 is expressed in innate and adaptive immune cells and structural skin cell types

Source: Sabat et al., Nature Reviews, 2020

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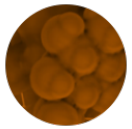
Considerations for E3 Ligase Pairing of I/I Targets

- What is the desired degradation profile for chronic inflammatory disease target?
  - Is your target ubiquitously expressed?
  - What are the pharmacology relevant cell types?
  - How safe is your target?
- What are the desired properties for E3 Ligands?

Pegasus: E3 Ligase Whole-Body Atlas

Different Expression Profiles of E3's Provide Opportunity for Broad, Tissue Selective or Restrictive Degradation



E3 Ligase Whole-Body Atlas

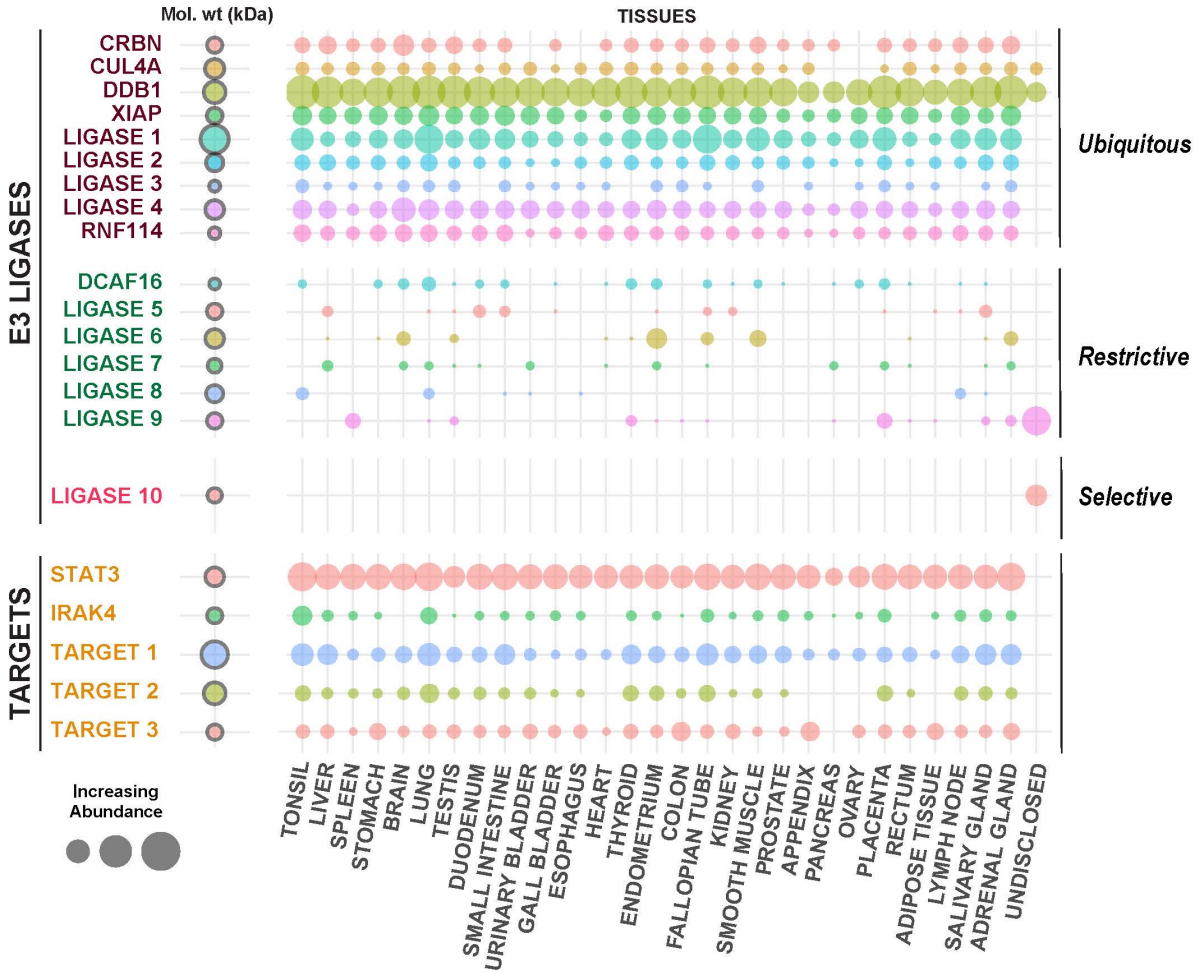
E3 Ligase Binders Toolbox

Ternary Complex Modeling

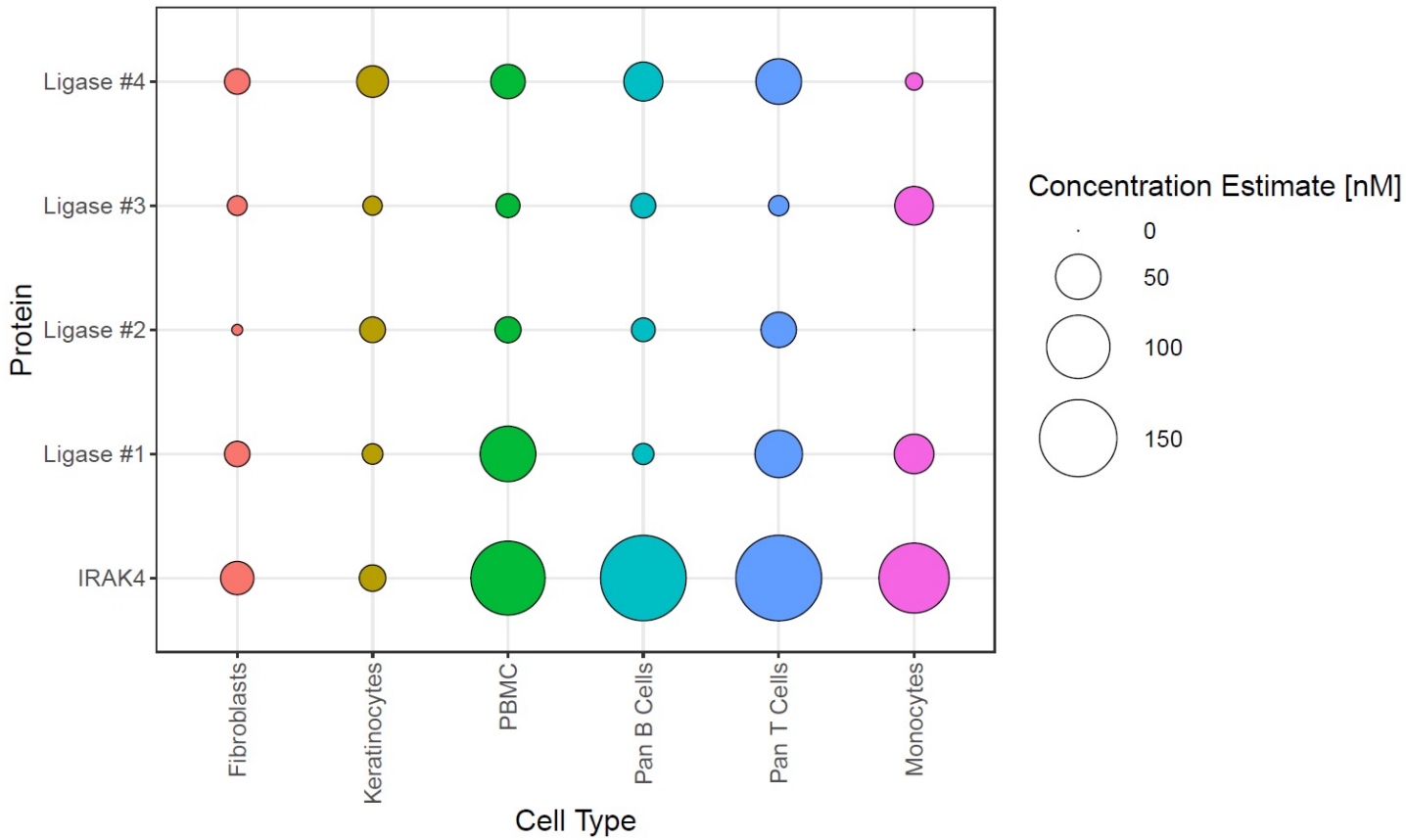
Quantitative System Pharmacology Model

Proprietary Chemistry

- Focused on determining the expression profiles of ~600 unique E3 ligases
- Patterns mapped in both disease and healthy contexts
- Ability to match a target protein with appropriate E3 ligase based on expression and biology
- Vision to develop tissue-selective or tissue-restrictive degraders to enable novel therapeutic opportunities



Target and E3 Ligase Expression across Primary Immune & Tissue Cell Types



- Select expression that matches target and E3 across all relevant cell types
- For IRAK4 I/I program, broad E3 expression and degradation profiling was desired

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Several Methods to Determine Degrader Potency and Degradation Profile

Method	Advantages	Considerations
Flow	**Interrogate multiple cell subsets in parallel, single cell resolution and no isolation required, small sample volume**, mod-throughput	Reagent-availability, semi-quantitative
Targeted mass spectrometry	Mod-high sensitivity, specificity, quantitative	Requires pre-isolation of cell subsets, large sample volume
Multiplex Immunoassay (i.e. ELISA)	Mod-high sensitivity and dynamic range, high-throughput, small volume	Reagent-availability, requires pre-isolation of cell subsets
Western Blotting	Visual, can see target degraded	Reagent-availability, low-throughput, requires pre-isolation of cell subsets, large sample volume

Flow Cytometry Workstreams

Internal Flow Capabilities

4 laser Attune + Autosampler

- Clog resistant – great for sticky cell types/aggregates
- Autosampler takes 45 min for a 96 well plate (newer versions take about 22 min)

Preclinical to Clinic

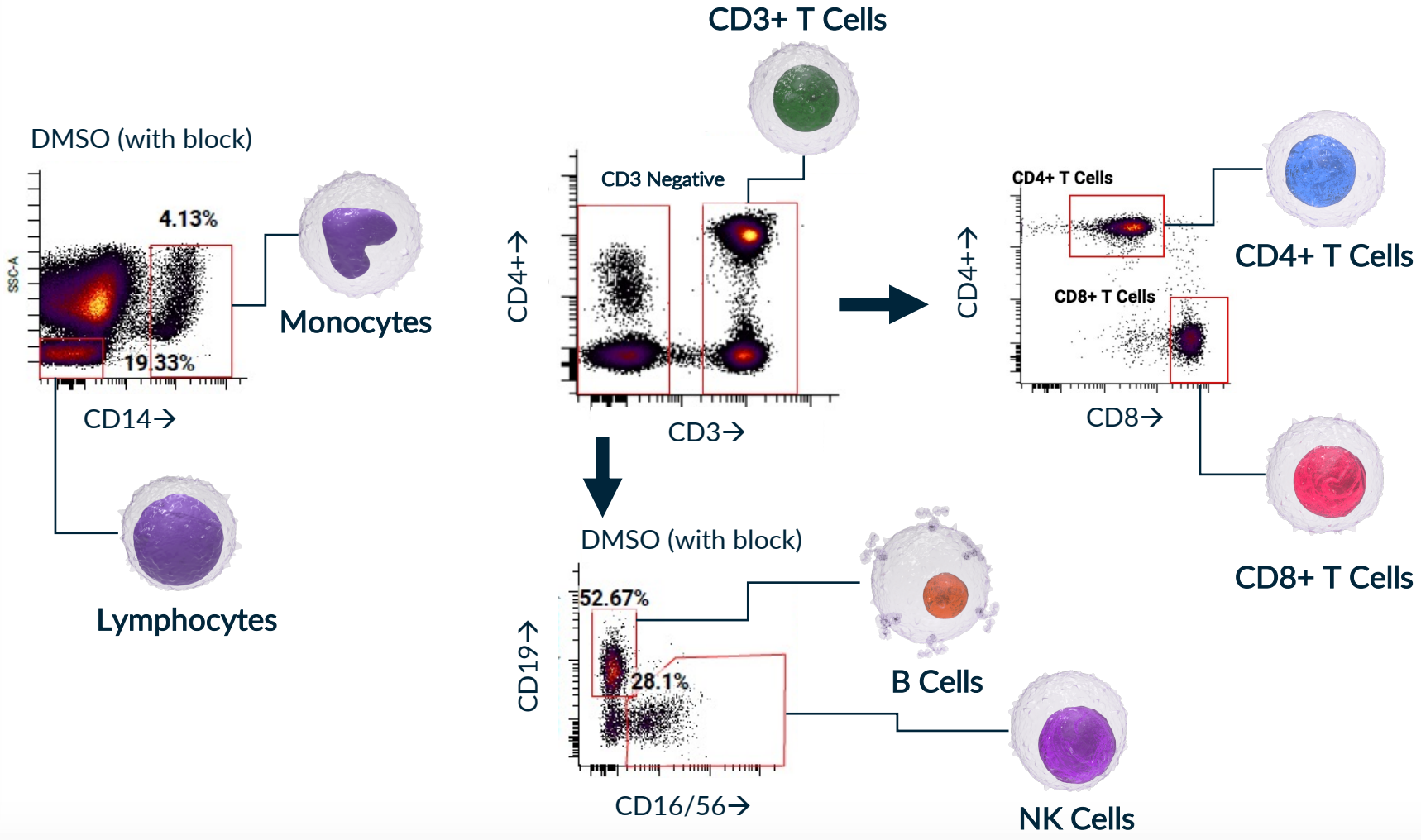
- Start building immunophenotyping panel
- Test and Validate several primary antibodies
- Confirm results using orthogonal methods

- Transfer discovery methods to CRO
- Complete method development, stability, precision and accuracy studies to implement in the clinic
- Fit-for-purpose assays

Human Primary Cell based Screening Assay using IRAK4 Multiplex Flow

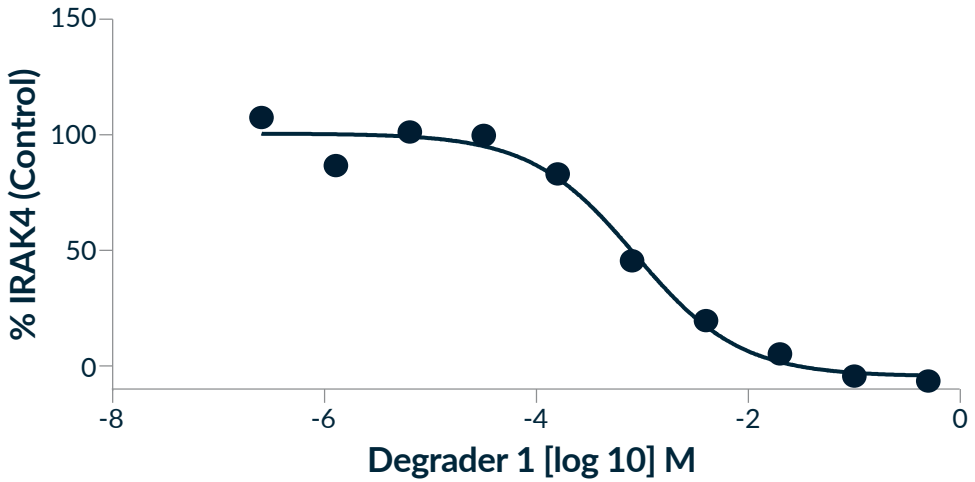
- Multiplex flow assay developed to monitor IRAK4 in peripheral immune subsets
- Immunophenotyping panel identifies Lymphocytes (T cells (CD4+/CD8+, B cells, NK cells) and monocytes
- Each subset gated to determine IRAK4 levels

Example of Immunophenotyping

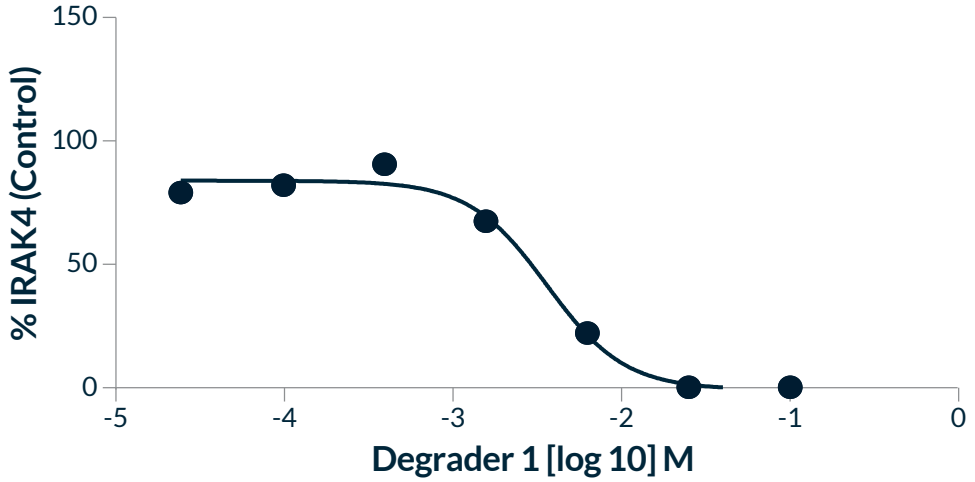


Confirmation of Degrader Potency across Multiple Methods

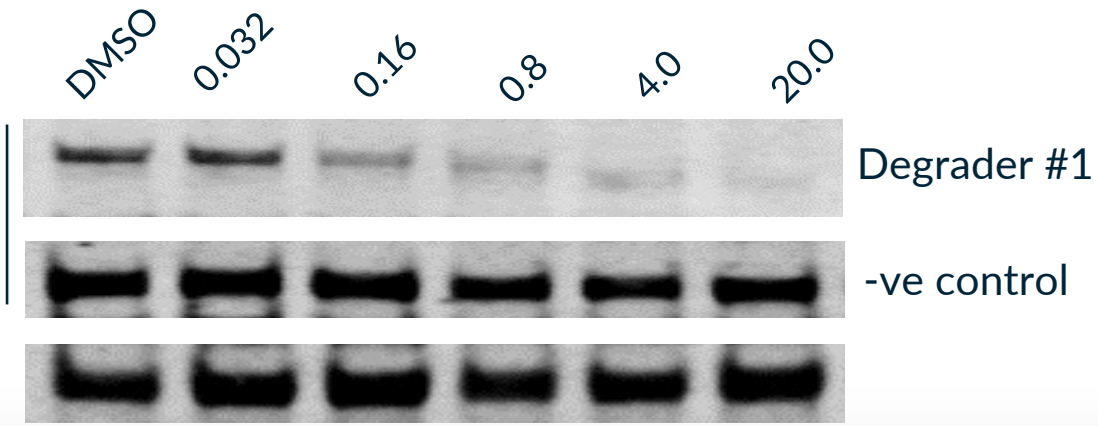
Multiplex Flow



Targeted MS



Western



- Calculated DC50 across different methods 0.5-1.1nM range

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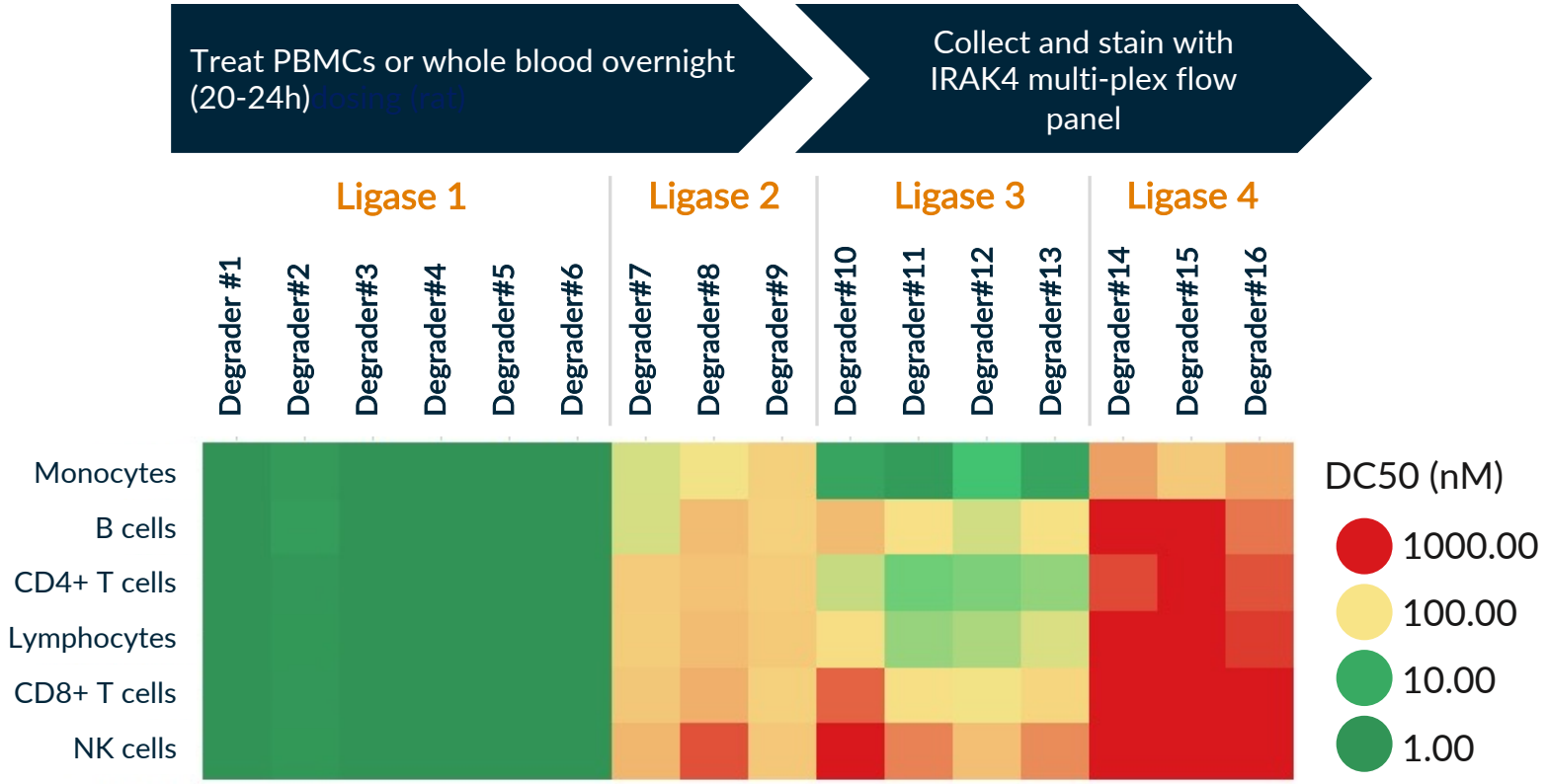
Differential Degradation Profile across PBMC subsets

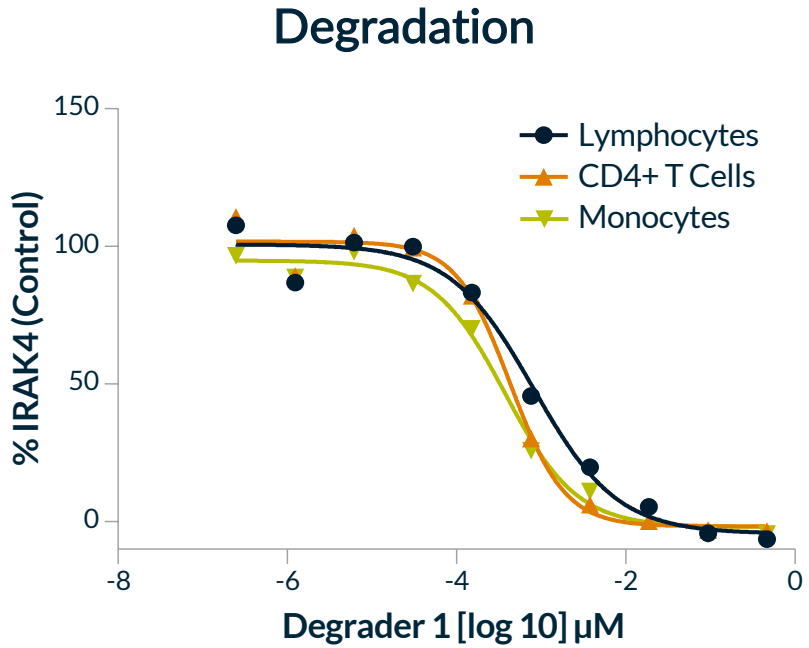
Key Questions:

1. Can we achieve degradation in primary human cells?
2. Can we achieve equal potency in key cell types?

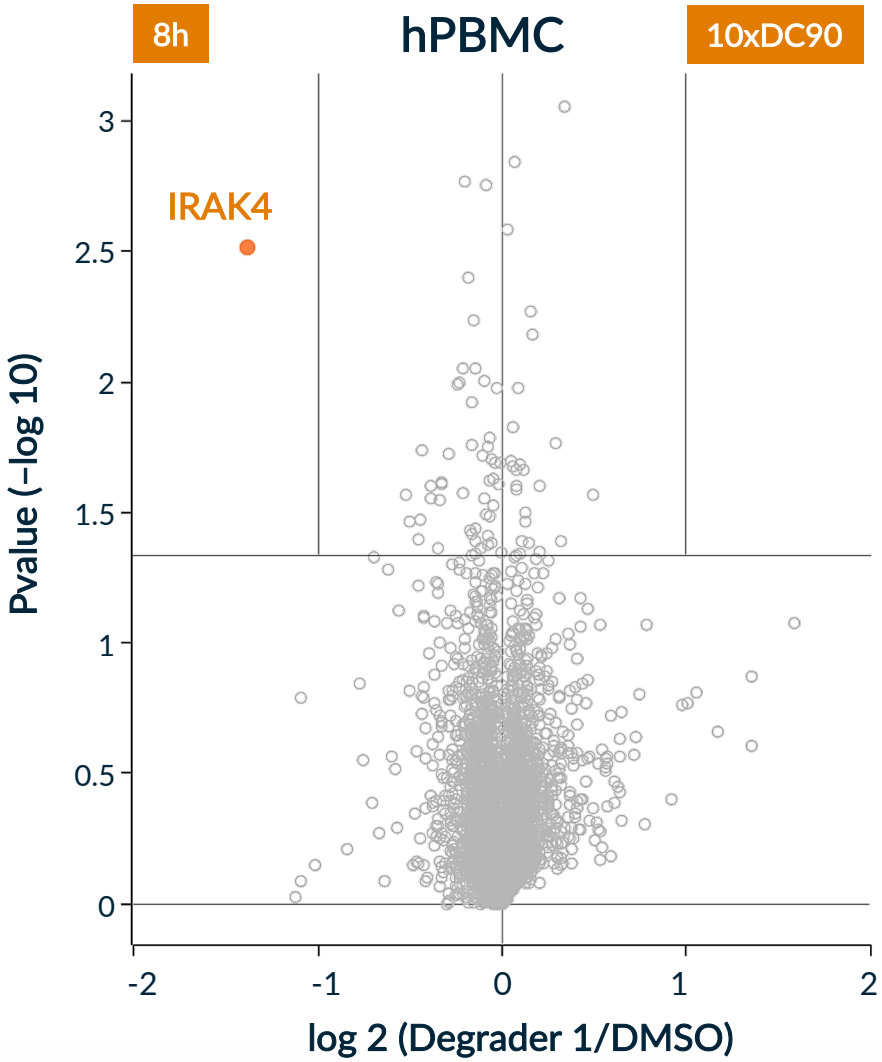


- Flow screening assay detects different IRAK4 degradation profiles in peripheral immune subsets (normal) based on E3 ligase pairing
- Ligase 1 provides desired potency and degradation profile

Identification of Potent and Selective IRAK4 Degraders



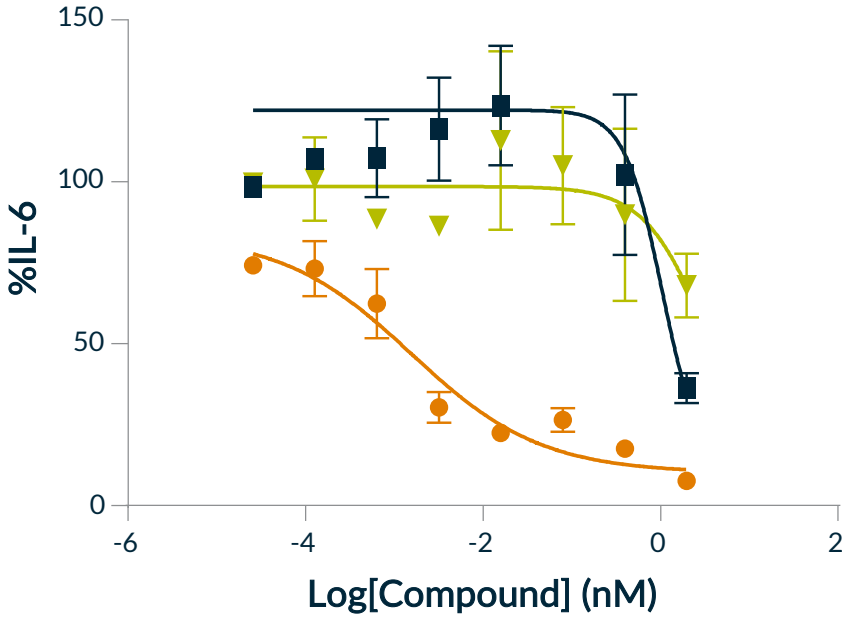
Cell Type	IRAK4 DC_{50} (nM)
Lymphocytes	1.1 ± 0.53
CD4+ T cells	1.0
Monocytes	0.86 ± 0.68



Degrader 1= tool degrader

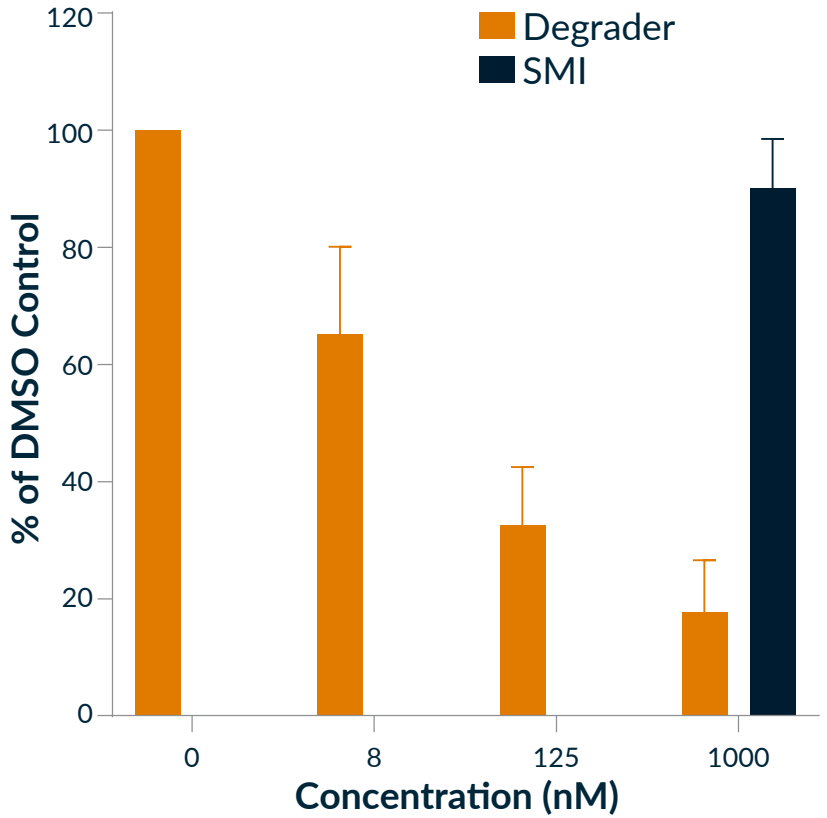
IRAK4 Broad Degradation Potently Inhibits IL-6 and IL-17 Production in vitro

LPS + IL-1B → IL-6 (PBMC)



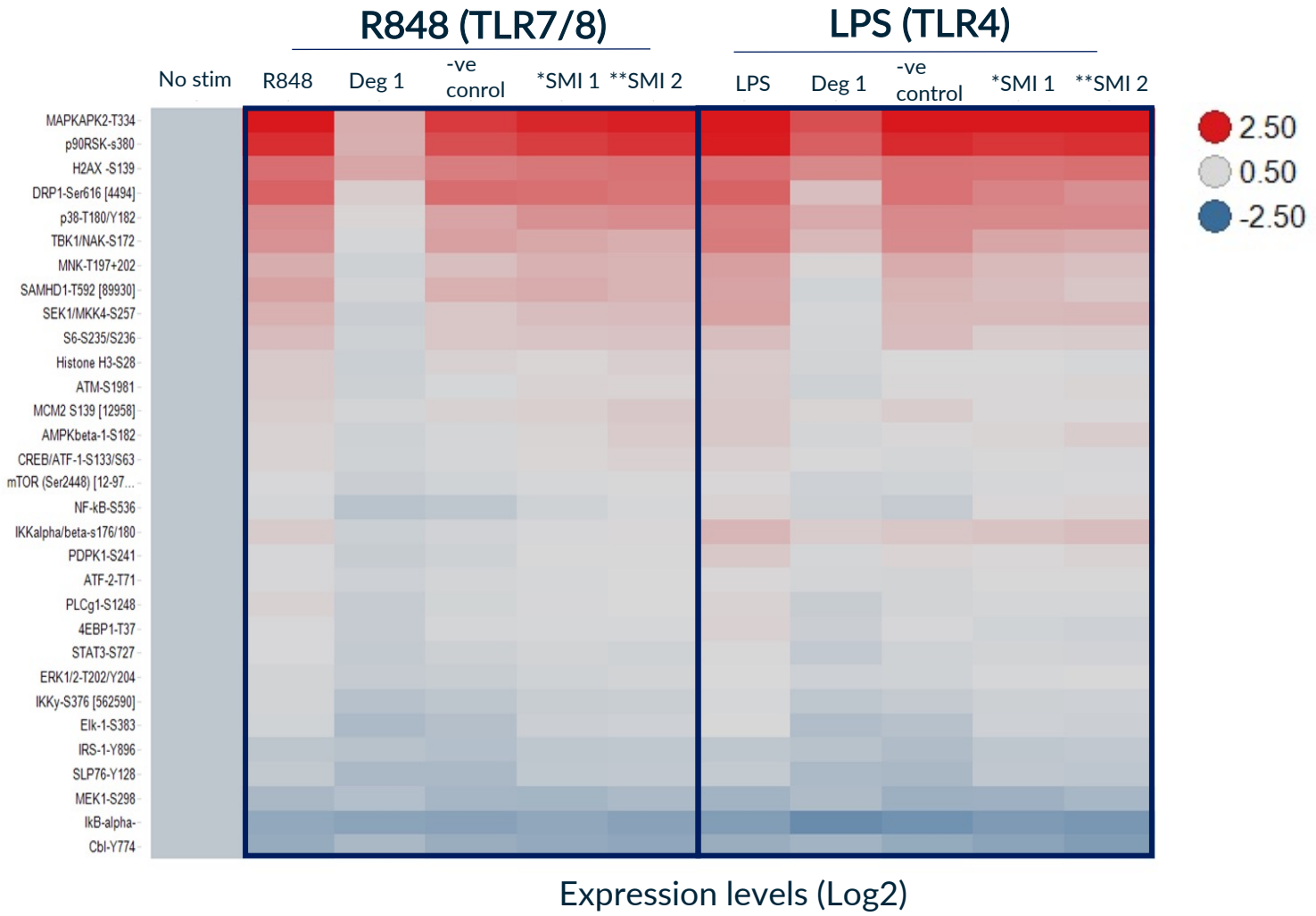
Legend	Compound	IL-6 IC_{50} (nM)
●	IRAK4 Degrader	0.8
■	Negative control	450
▼	IRAK4 SMI *(PF-06550833)*	N/A

IL-17 Release by CD4+ Th17 Cells



IRAK4 Degradation Blocks TLR Activation in Monocytes

- PBMCs pre-treated with compounds
- Multi-plex phospho-flow panel to evaluate signaling events in monocytes
- Degradation blocks activation downstream of TLR more than kinase inhibition alone



*SMI 1 = Bayer IRAK4 Ki
**SMI 2 = PF-06550833

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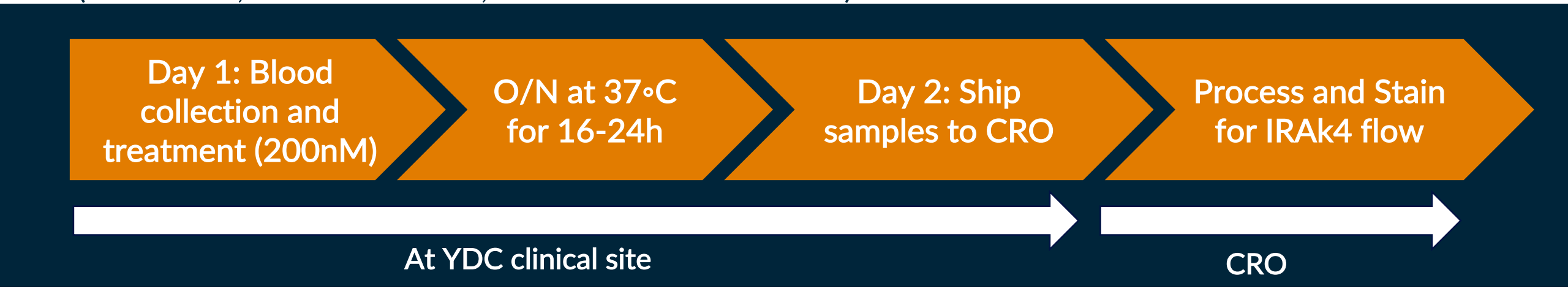
Overview Non-Interventional Trial to Assess Biomarkers in HS Patient Blood and Tissues

- Purpose of ex vivo treatment with KT-474: To assess performance of biomarker assays and demonstrate activity in circulating immune cells from diseased subjects (Hidradenitis Supurrativa)
- Blood drawn from consented HS patients in collaboration with Dr. Alavi at York Dermatology Center

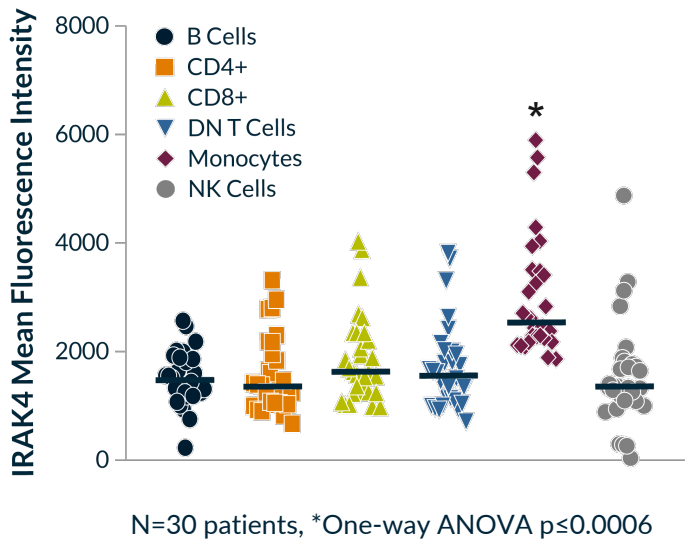
(N=10 mild, N=10 moderate, N=10 severe iHS4 score)



- Samples were stained with multi-plex immune panel and IRAK4 +/- Blocking control
- Assessed baseline levels of IRAK4 expression and ability of KT-474 to degrade IRAK4 in blood immune subsets

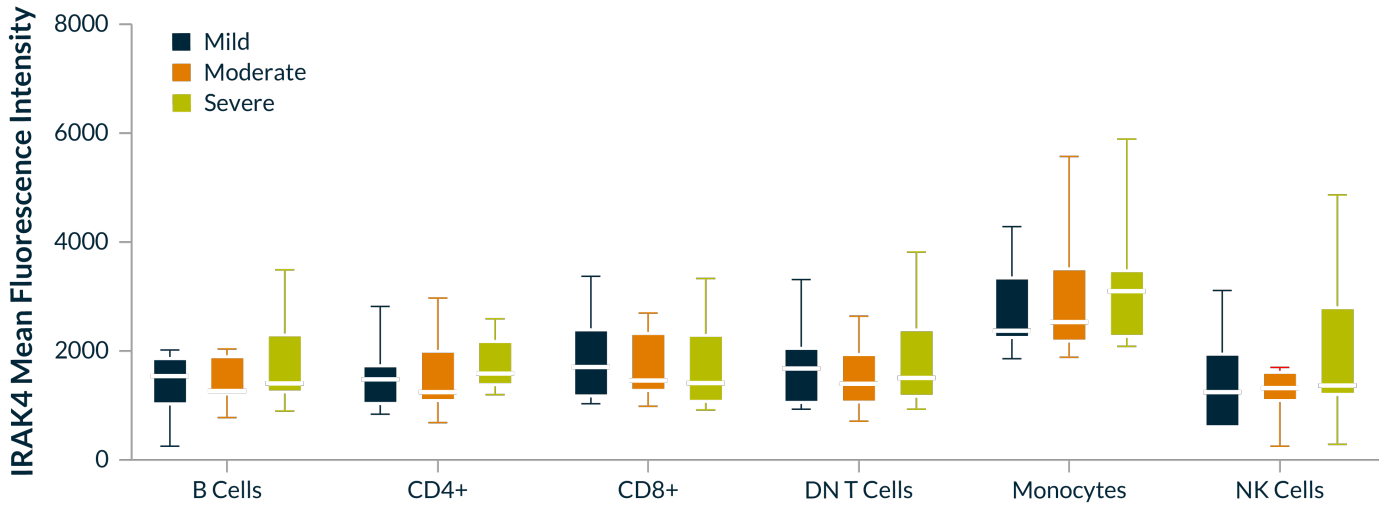
IRAK4 Expression Levels in HS Blood Immune Cells

IRAK4 Expression in Blood Immune Cells



N=30 patients, *One-way ANOVA p≤0.0006

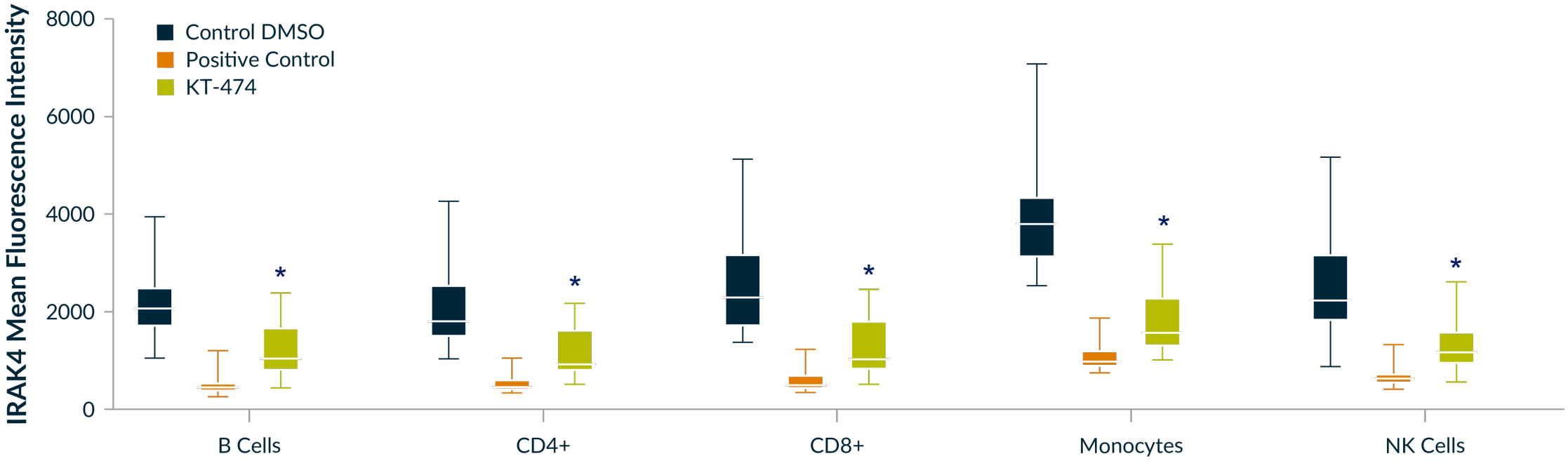
IRAK4 Expression in Blood Immune Cells by Disease Severity (IHS4)



- IRAK4 levels detected in circulating cells from HS patients
- Monocytes express IRAK4 at significantly higher levels compared to other immune subsets

- IRAK4 levels remain the same in patients across disease severity (same results obtained with HS-PGA and Hurley (Max) staging)

IRAK4 Degrader Downregulates IRAK4 Expression across All PBMC Subsets



N=30 patients, One-way ANOVA* KT-474 vs DMSO Control
Positive Control: cells treated with IRAK4 blocking antibody prior to IRAK4 staining

KEY TAKEAWAYS

Treatment with an IRAK4 degrader (KT-474) led to reduction of IRAK4 to a similar level approaching the lower limits of detection as determined by an anti-IRAK4 blocking antibody (Positive Control) across all PBMC subsets in HS patient blood, irrespective of baseline IRAK4 expression intensity

KT-474 Phase 1 Trial Design

Double-blind, Placebo-controlled, Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD) trial

Three-part Phase 1 Design

1 SAD Portion
Healthy Volunteers

- **7** cohorts (up to 56 adult healthy subjects)
- **8** per cohort (6:2 randomization)
- **Single** dosing (starting dose 25 mg)

2 MAD Portion
Healthy Volunteers

- **5** cohorts (up to 60 adult healthy subjects)
- **12** per cohort (9:3 randomization)
- **14x** daily doses (starting dose 25 mg)

3 MAD Portion
Patient Cohort

- **1** cohort (up to 20 AD and HS patients)
- Open-label
- **14x** daily doses

Endpoints

Primary
- Safety & tolerability

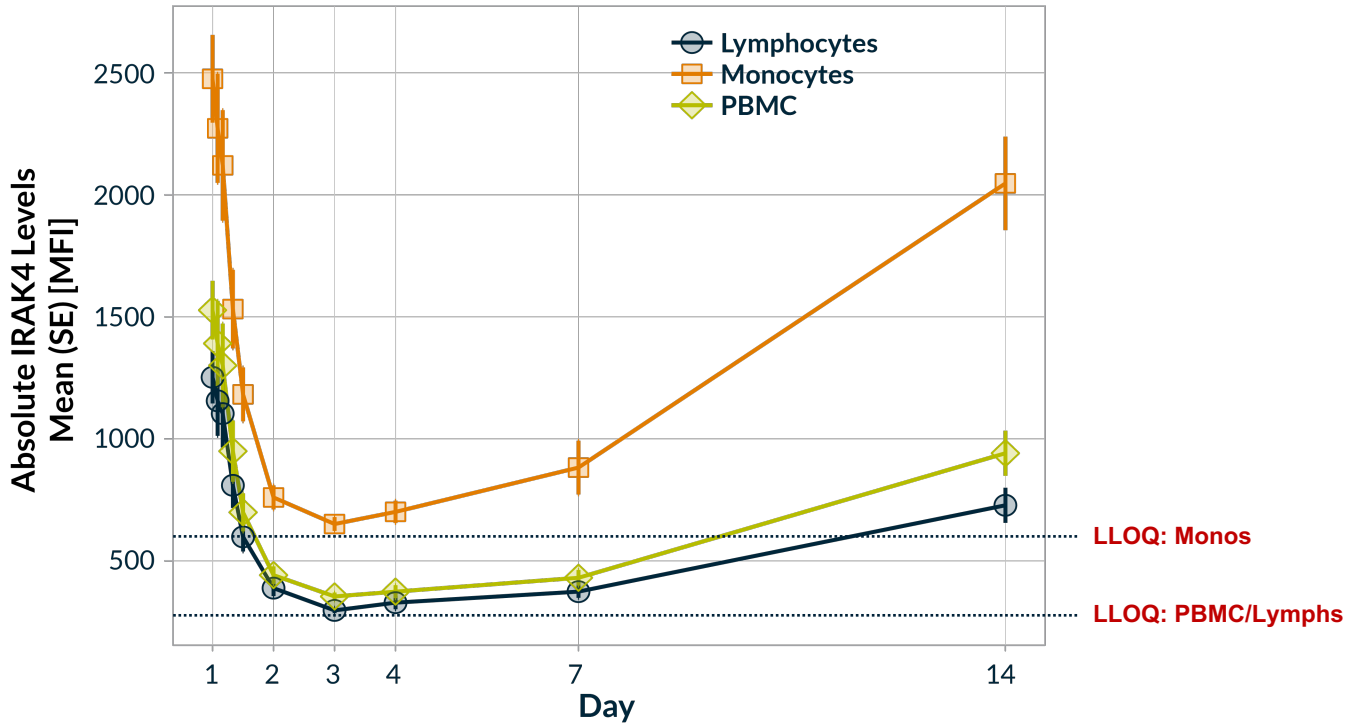
Secondary/ Exploratory
SAD & MAD
- Pharmacokinetic measures (half-life, bioavailability)
- IRAK4 knockdown in PBMC

Exploratory
SAD & MAD
- *Ex vivo* response of whole blood to TLR agonists (SAD & MAD) and IL-1β (MAD only)

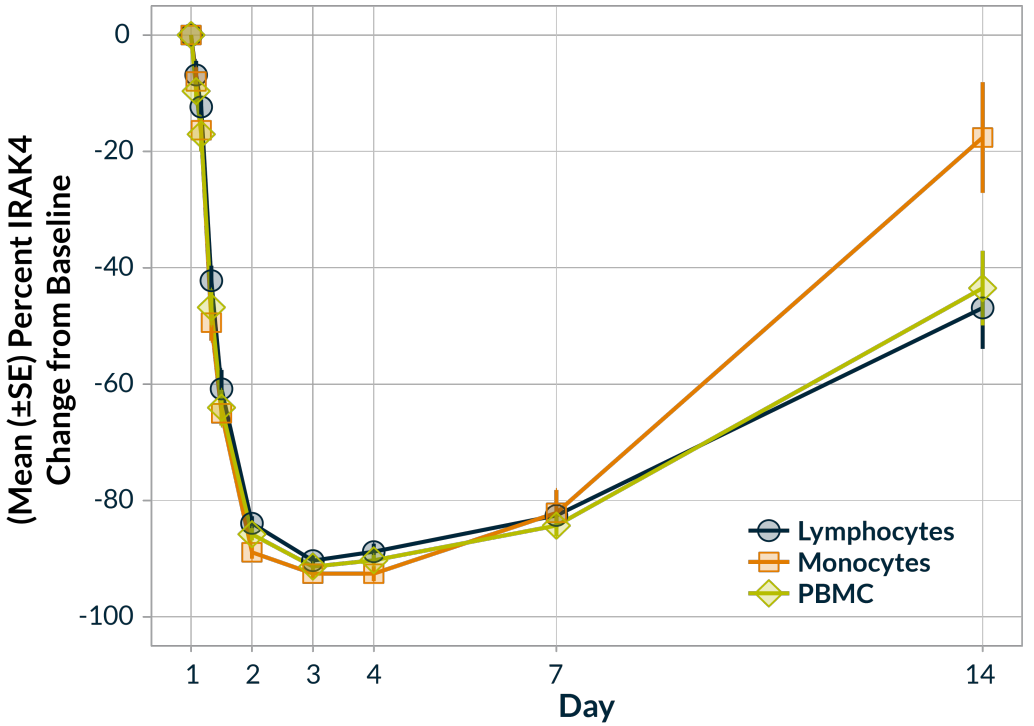
Exploratory
MAD Only
- IRAK4 knockdown in skin biopsies
- Proinflammatory cytokine and chemokine levels in skin biopsies (Patients only)
- Plasma C-reactive protein (HV and Patients) and cytokine levels (Patients only)

Robust IRAK4 Degradation Observed in Lymphocytes and Monocytes: Flow Cytometry Results at SAD 7

Absolute IRAK4 Levels



Mean % Reduction of IRAK4



Summary

- Incorporation of human primary cell screening assays early on, is critical to ensure E3 ligase provides desired degradation profile of POI
- Multi-plex flow Cytometry can be successfully integrated into TPD workstreams from preclinical through clinical development, as demonstrated with Kymera's IRAK4 I/I program
- Determined potency, degradation profile and functional impact across multiple primary cell types from normal and diseased samples
- Achieved desired broad degradation profile and functional impact on multiple immune cell types, that was differentiated from SMI
- Demonstrated IRAK4 degradation in HS blood immune subsets
- Implement into the clinic to monitor IRAK4 levels
- KT-474 degrades in multiple immune subsets during SAD portion of study consistent with preclinical findings

THANK YOU

KYMERA
EST. 2016

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