INTERIM RESULTS FROM NON-INTERVENTIONAL STUDY

To Evaluate Cutaneous & Circulating Biomarkers for a Novel IRAK4-Targeted Therapeutic

IN PATIENTS WITH HIDRADENITIS SUPPURATIVA

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LEARNING OBJECTIVES

CHARACTERIZE
IRAK4 expression in the skin and blood of patients with HS

HIGHLIGHT
the *ex vivo* pharmacodynamic activity of an IRAK4 degrader on peripheral blood mononuclear cells (PBMC) from patients with HS

TAKE AWAY MESSAGES

1. IRAK4 expression in the skin detected by immunofluorescence (IF) and mass spectrometry (MS) is higher in lesional (L) and peri-lesional (PL) skin compared to non-lesional (NL) skin
2. IRAK4 is detected by flow cytometry across all PBMC subsets, with highest expression in monocytes
3. An IRAK4 degrader, KT-474, reduces IRAK4 to a level approaching the lower limits of detection across all PBMC subsets irrespective of baseline expression intensity, whereas an IRAK4 kinase inhibitor increases IRAK4 levels in T and NK cells

KYMERA

Plain Language Summary

Why this study was needed

- Novel therapeutics targeting IRAK4 based on protein degradation are being developed for the treatment of HS. It is therefore important to characterize IRAK4 expression in the skin and blood of patients with HS
- Important to demonstrate how IRAK4 degraders differentiate from kinase inhibitors in their effect on IRAK4 protein levels to subsequently impact IL-1R/TLR biology.

What it showed

- IRAK4 expression is higher in L and PL HS skin compared to unaffected skin
- IRAK4 is expressed across all PBMC subsets in the blood, with highest expression in monocytes
- Ex vivo treatment with an IRAK4 degrader substantially lowers IRAK4 levels across all PBMC subsets in the blood, whereas an IRAK4 kinase inhibitor increases IRAK4 levels in T and NK cells

Why it's important

- This study provides the first information about IRAK4 expression in the skin and blood of HS patients and the ability of an IRAK4 degrader to lower IRAK4 levels in the blood of patients with HS.

Study Design and Baseline Demographics

Design

Number of Sites	Single center (York Dermatology Clinic and Research Center, Ontario, Canada) PI: Dr. Afsaneh Alavi, MD, MSch, FRCPC
Number of Patients	40 (30 HS and 10 AD)
Inclusion Criteria	1. Age 18 or older 2. Active Hidradenitis Suppurativa (HS) or Atopic Dermatitis (AD), diagnosed by PI 3. Mild, moderate, and severe HS patients (by IHS4 score), and moderate to severe AD (by EASI score)
Exclusion Criteria	1) Patients currently on a biologic or other immunosuppressive treatment for HS or AD 2) Use of biologic treatment for HS or AD within 3 months or 5 half-lives, whichever is longer 3) Use of non-biologic immunosuppressive treatment (eg. Cyclosporin) in the last 4 weeks.
Data Collection at Study Entry	Medical history, disease severity in HS (Hurley, PGA, IHS4, HASI) and AD (EASI), prior treatments, comorbidities, duration of disease
Sample Collection	Whole blood, plasma, skin (lesional, peri-lesional, non-lesional)

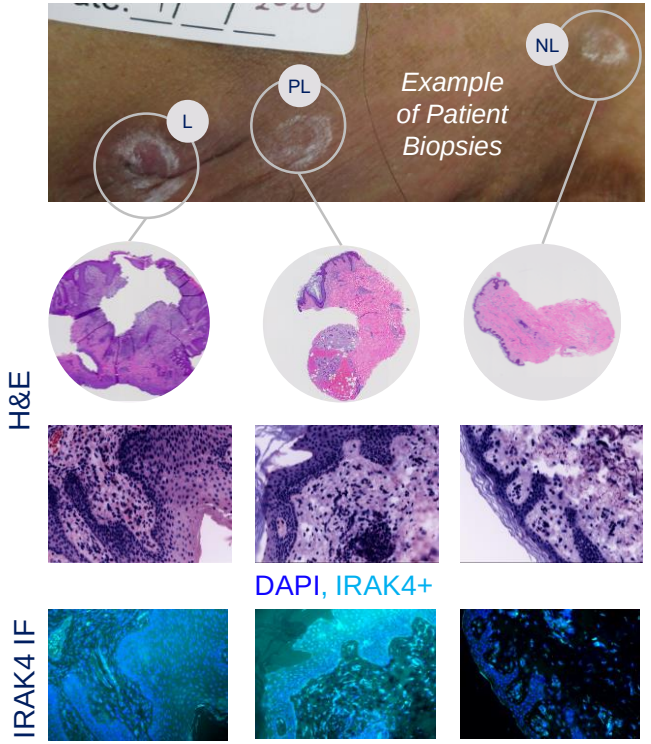
Baseline Demographics & Biomarkers

Study Duration	• FPI: 28May2020 • HS accrual completed; enrollment of AD patients ongoing
Patients Enrolled to Date	• 30 HS: 9 mild, 10 moderate, 11 severe • 2 AD
Demographics	• Age 19-56 yrs • 9 male, 23 Female • Duration of disease: 1-38 years • Race: 97% were non-Hispanic or Latino
Biomarker Endpoints	• Flow cytometry for IRAK4 in ex vivo treated whole blood • Targeted MS of IRAK4 in skin biopsies • IRAK4 immunofluorescence in skin biopsies • Cytokines from ex vivo treated whole blood • Plasma cytokines and acute phase reactants • Cytokines in skin biopsies

IRAK4 Expression is Highest in Lesional (L) & Peri-Lesional (PL) Skin

IRAK4 Immunofluorescence (IF)

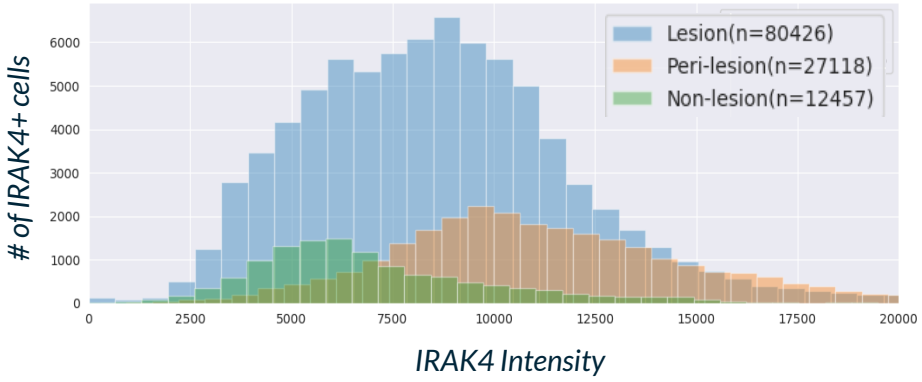
N=10 | **IHS4 severity:** 4 mild, 3 moderate, 3 severe



IF Analysis

- L, PL, NL IRAK4 positive cells counted and binned into intensity ranges as depicted by the horizontal bars below
- Cell counts per intensity bin were summed from the 3 biopsy locations

Cell count by intensity per biopsy location



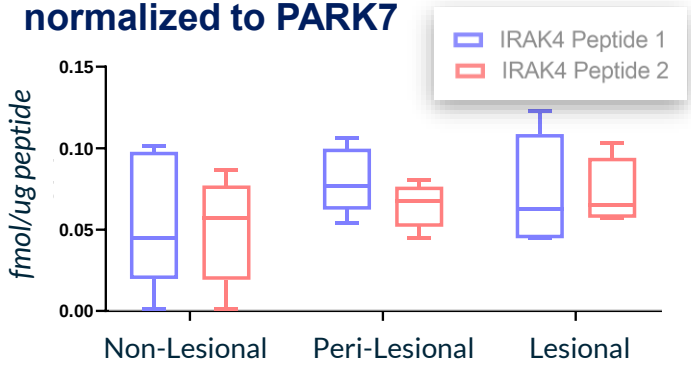
IRAK4 Mass Spectrometry (MS)

N=5 | **IHS4 severity:** 0 mild, 2 mod, 3 severe

MS Analysis

- Two peptides were chosen providing strong concordance in absolute quantification
- Plot represents the range of fmol/ug peptide across the 3 biopsy locations

IRAK4 absolute quantification normalized to PARK7



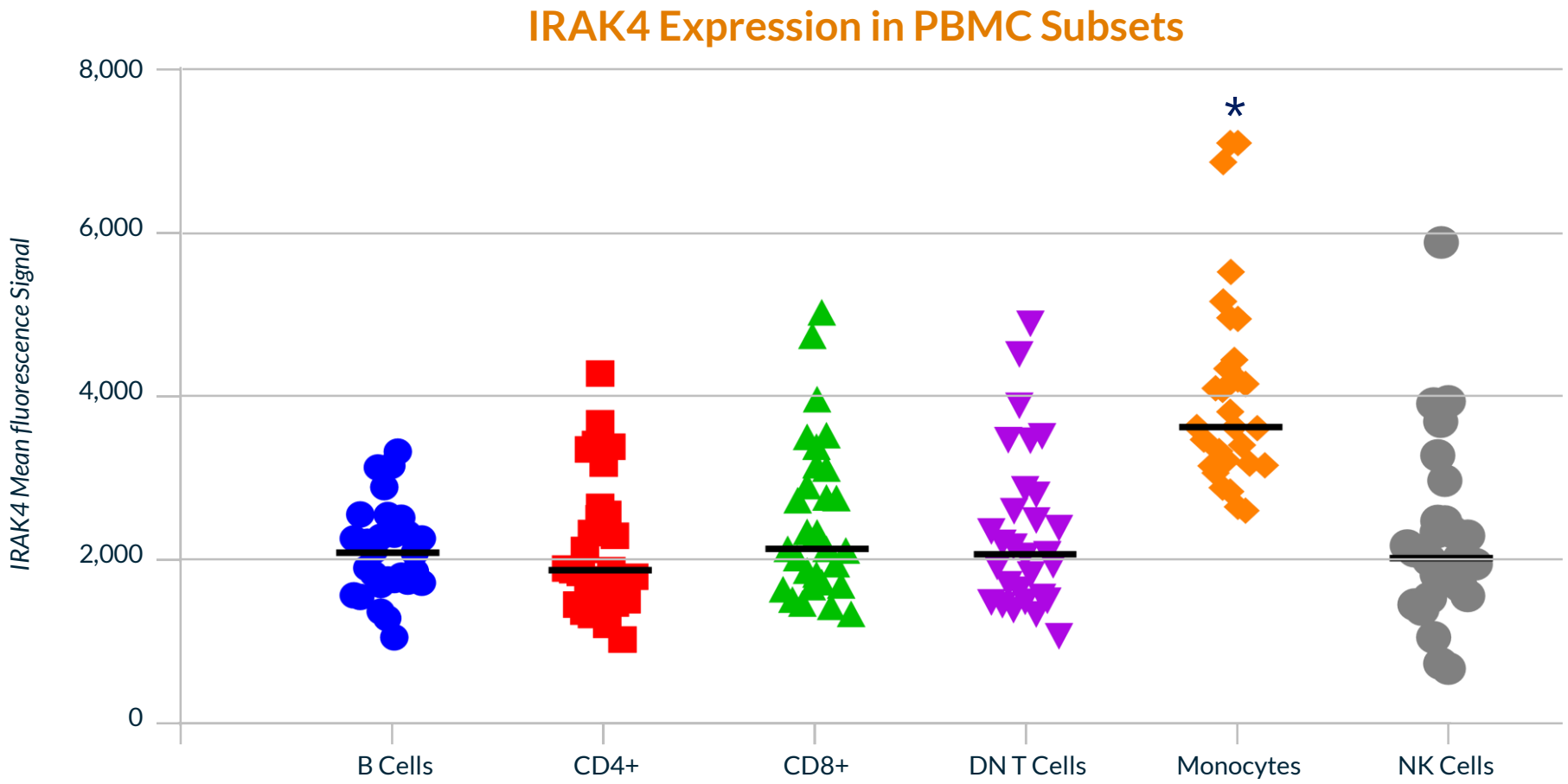
CONCLUSIONS

L and PL biopsies have more IRAK4+ cells and higher intensity IRAK4 staining than NL as measured by IF. MS with trend towards higher level of IRAK4 in L and PL compared to NL.

IRAK4 Expression in Peripheral Blood Mononuclear Cells

is Highest in Monocytes

- IRAK4 levels are significantly higher in monocytes compared to other PBMC subsets



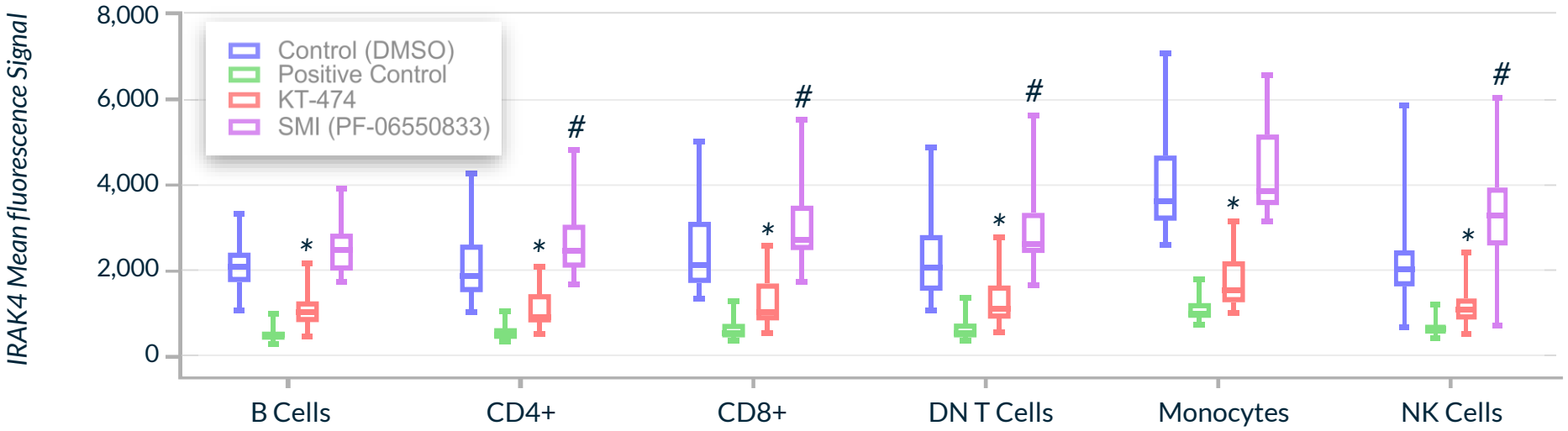
*N=30 (IHS4 severity: 9 mild, 10 moderate, 11 severe); *One-way ANOVA p≤0.0006*

IRAK4 Degrader Downregulates IRAK4 Expression Across All PBMC Subsets

Ex Vivo Blood Treatment

- Patient blood was treated with DMSO control or 200nM of KT-474 IRAK4 degrader or 200nM of IRAK4 kinase inhibitor (PF-06550833)
- Blood was incubated overnight at 37◦C (16-24 hours)
- Blood was shipped and processed for IRAK4 and lineage specific cell surface staining by flow

IRAK4 Levels Following Treatment with IRAK4 Degrader or Kinase Inhibitor



N=30 patients, One-way ANOVA KT-474 vs DMSO Control p≤0.0001, #SMI (PF-06550833) vs DMSO Control p≤0.02*
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
- Treatment with an IRAK4 kinase inhibitor led to an increase in IRAK4 levels of up to 2.6-fold in T and NK cells

Conclusions

1. **IRAK4 expression in HS patients can be quantified in the skin**
using immunofluorescence and mass spectrometry and in peripheral blood mononuclear cells (PBMC) by flow cytometry

2. **IRAK4 levels are higher in L and PL skin compared to NL skin**
supporting the relevance of the IRAK4 signaling pathway in HS

3. **IRAK4 expression in PBMC is highest in monocytes**
a cell type central to the pathogenesis of HS

4. **Ex vivo incubation of HS blood with the IRAK4 degrader KT-474 reduces IRAK4**
to a level approaching the lower limits of detection across all PBMC subsets, irrespective of baseline expression intensity, whereas an IRAK4 kinase inhibitor increases IRAK4 levels in T and NK cells

5. **Findings supports clinical development of KT-474 in HS** and other IL-1R/TLR-driven inflammatory diseases, with plans to **initiate Phase 1 in H1 2021**