SELECTED OPPORTUNITIES IN IMMUNOLOGY

USE OF IRAP INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISEASES

(BIO21469)

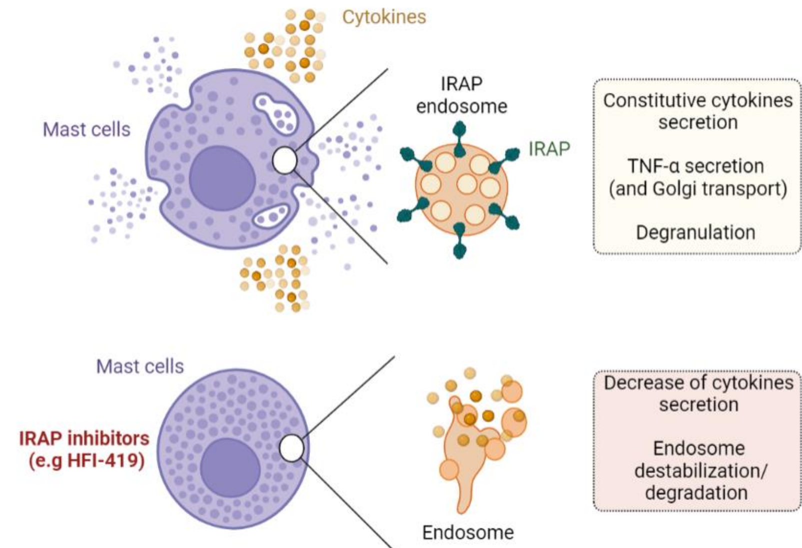
USE OF IRAP INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISEASES (BIO21469)

Product factsheet

Target, Product & Proof-of-Concept

- **Target:** IRAP (IRAP$^+$ endosomes) in mast cells and macrophages
- **Product:** IRAP inhibition strategies including IRAP inhibition by destabilization/degradation (as chemical compound used, HFI-419)
- **Application:** Primary or secondary Inflammatory diseases
  - (*In-vivo* Proof-of-Concept demonstrated in arthritis and kidney injury cisplatin-induced model)

- **Rational / POC:**
  - IRAP endosomes are dispensable for secretory granule exocytosis
  - Constitutive secretion of cytokines relies on IRAP endosomes in mast cells
  - IRAP endosomes are required for TNF-α secretion in vivo
  - IRAP is required for Golgi export of TNF-α transport vesicles
  - IRAP inhibition by HFI-419 destabilizes IRAP endosomes



IRAP is a therapeutic target in inflammatory diseases.

- **Patent and publication:**

USE OF IRAP INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISEASES - EP21306677

Mast cell-mediated inflammation relies on insulin-regulated aminopeptidase controlling cytokine export from the Golgi. bioRxiv. 2022.

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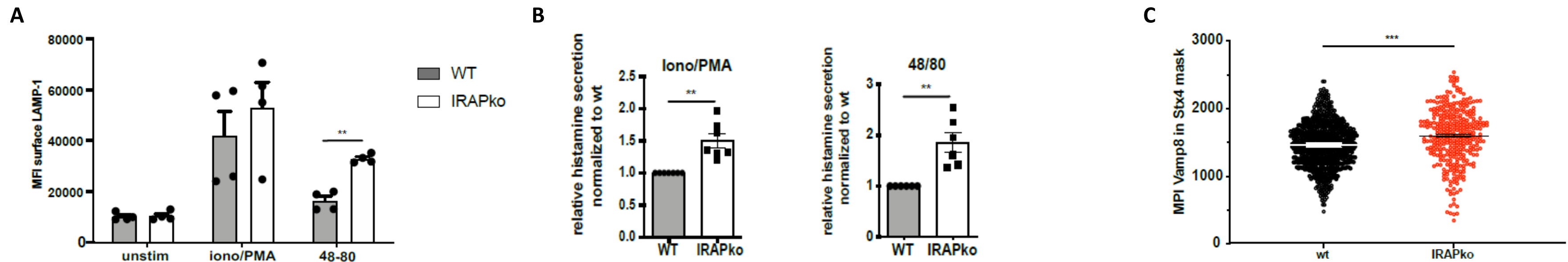
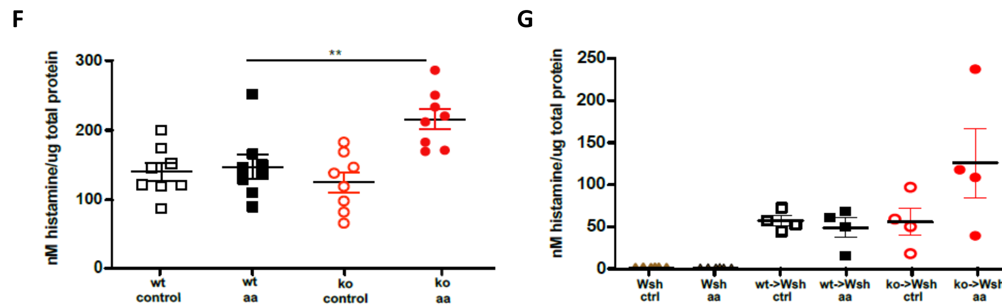
Rational : IRAP is dispensable for degranulation in mast cells

IRAP as a target to contain mast-cells degranulation



- **(A)** Degranulation analysis by flow cytometry via LAMP-1 surface staining of live peritoneal mast cells stimulated for 30min with ionomycin/PMA or 48/80 or left unstimulated.
- **(B)** Histamine secretion by peritoneal mast cells stimulated for 30min with ionomycin/PMA or 48/80.
- **(C)** Imaging flow cytometry quantification of VAMP8 mean pixel intensity (MPI) in the Stx4 mask of ionomycin/PMA-stimulated BMMC.



- **(F, G)** IRAP^{wt} and ^{ko} mice or **(G)** mast-cell deficient W^{sh} mice reconstituted with IRAP^{wt} and ^{ko} BMMC were challenged on one ear with 30mg/ml arachidonic acid while the control ear was left untreated. Histamine concentration was quantified in ear tissue homogenates and normalized to total protein concentration.

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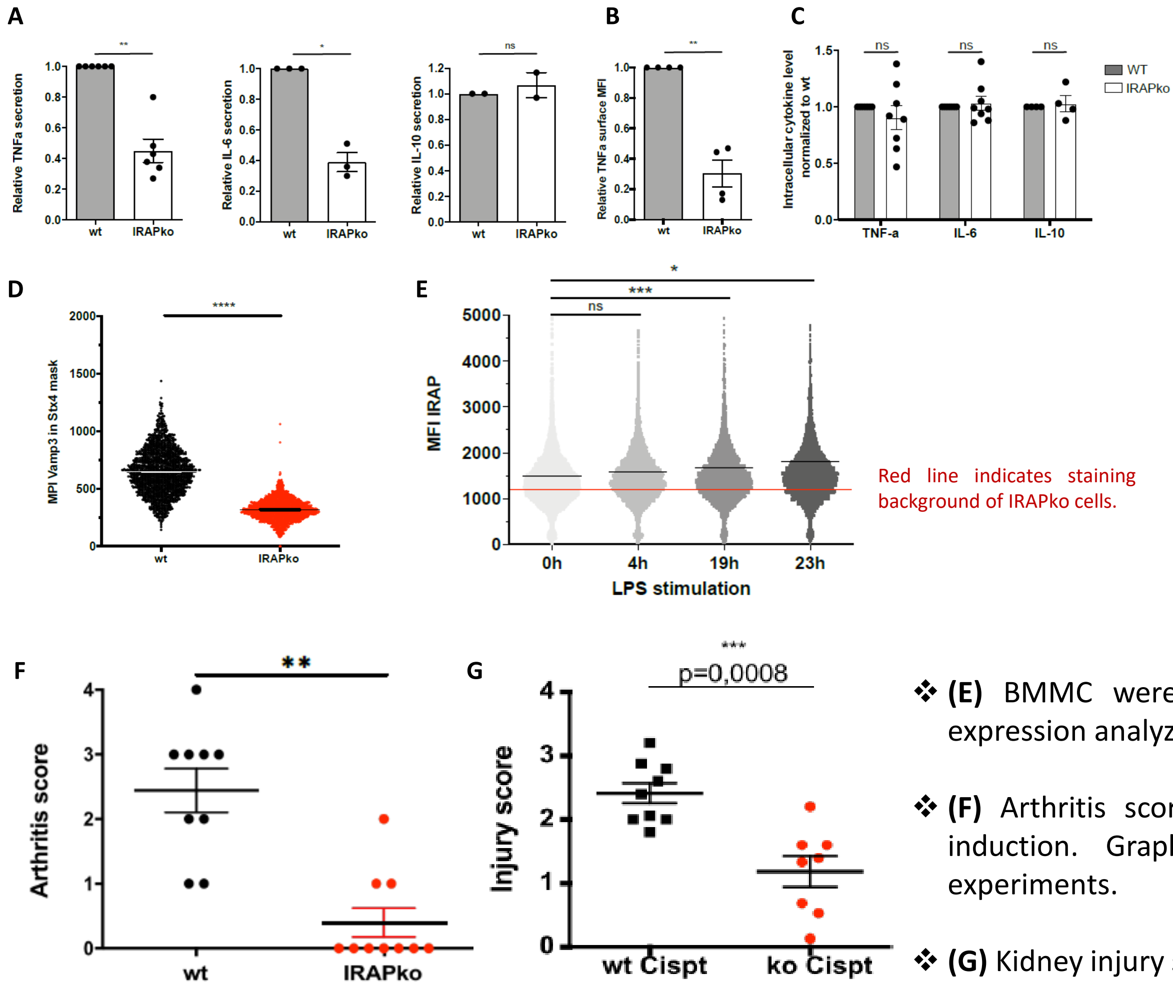
USE OF IRAP INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISEASES (BIO21469)

Rational : IRAP endosomes are required for inflammatory cytokine secretion in mast cells and inflammatory diseases.

IRAP as a target to contain cytokines related to inflammatory diseases



❖ **(A)** Peritoneal mast cells were stimulated with iono/PMA for 18h and secreted cytokines were quantified by ELISA in the culture supernatant.

❖ **(B)** Peritoneal mast cells were stimulated for 4h with iono/PMA and TAPI-I, and plasma membrane-bound TNF-α on live cells was detected via FACS.

❖ **(C)** Intracellular cytokine expression was determined by FACS of fixed and permeabilized peritoneal mast cells after 4h of treatment with iono/PMA and brefeldin A.

❖ **(D)** Imaging flow cytometry quantification of VAMP3 mean pixel intensity (MPI) in the Stx4 mask of iono/PMA stimulated mast cells.

❖ **(E)** BMMC were exposed to LPS for indicated times and IRAP expression analyzed by FACS in permeabilized cells.

❖ **(F)** Arthritis score of $IRAP^{wt}$ and $IRAP^{ko}$ mice 8 days after CAIA induction. Graphs show pooled data from two independent experiments.

❖ **(G)** Kidney injury score of cisplatin-treated $IRAP^{wt}$ and $IRAP^{ko}$ mice.

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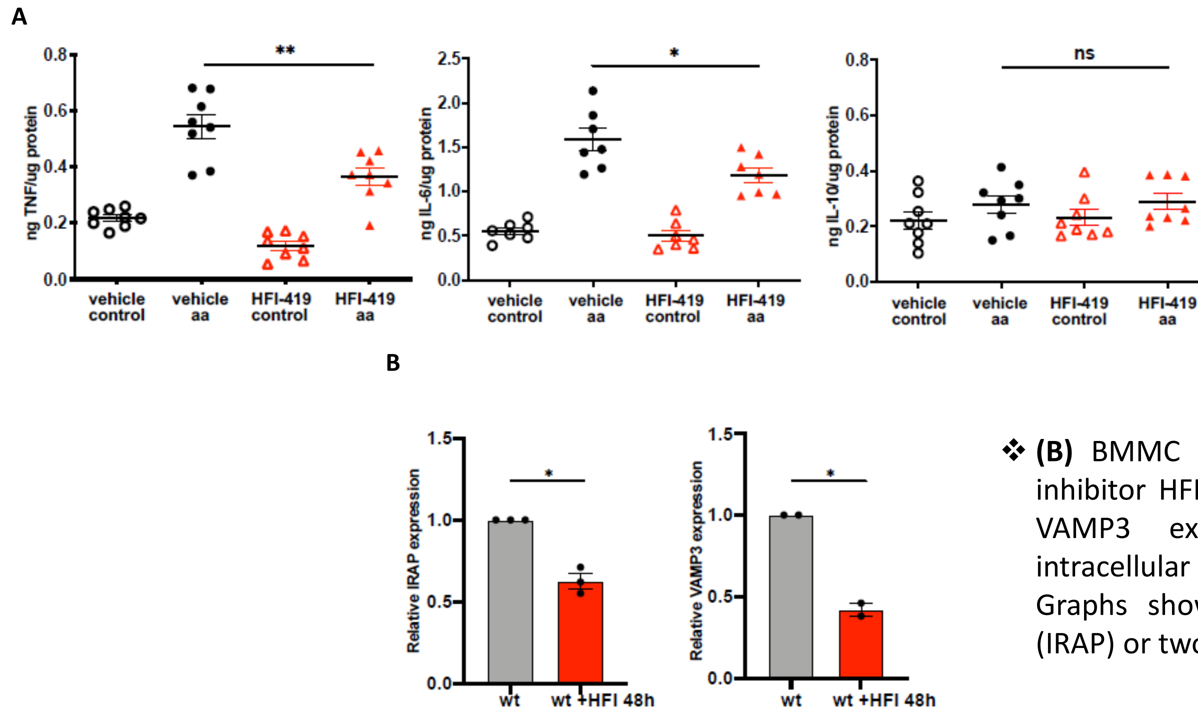
USE OF IRAP INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISEASES (BIO21469)

Proof-of-Concept : IRAP inhibitor HFI-419 blocks cytokine secretion via destabilization of IRAP and VAMP3$^+$ endosomes

Inhibition of IRAP as a therapeutic strategy in inflammatory diseases



- **(A)** IRAP wt mice were injected i.v. with 6µg HFI-419 or vehicle 24h and 15min prior to ear challenge. Cytokine concentrations were quantified in ear tissue homogenates and normalized to total protein concentration. Graph shows one representative out two experiments.

- **(B)** BMMC were exposed to the IRAP inhibitor HFI-419 for 24h, and IRAP and VAMP3 expression determined via intracellular staining in flow cytometry. Graphs show means ± SEM of three (IRAP) or two (VAMP3) experiments.

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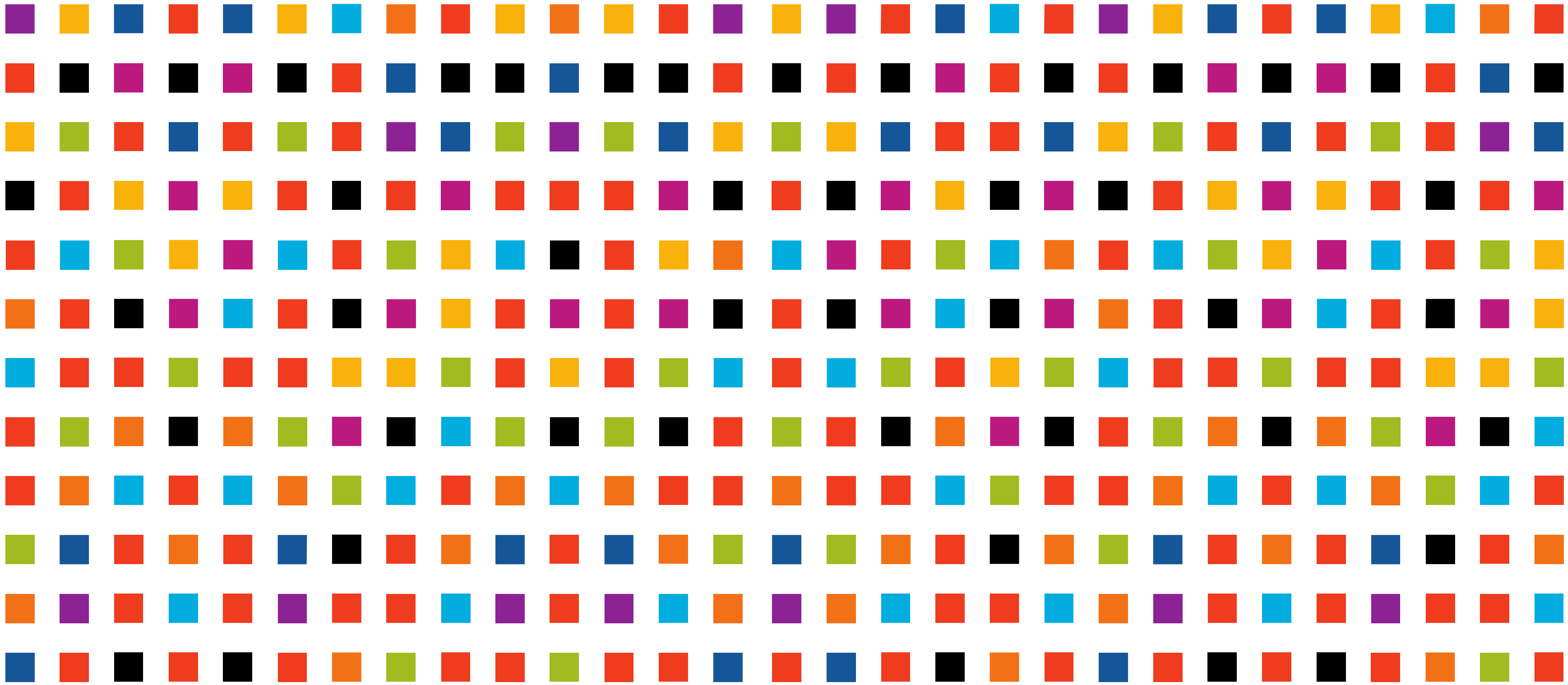

NATHAN.POMORSKI@INSERM-TRANSFERT.FR

Inserm Transfert - Paris Biopark
7 Rue Watt - 75013 Paris
Tel: +33 1 55 03 01 00 / Fax: +33 55 03 01 60
www.inserm-transfert.fr