

Incontro online Aggiornamento attività e rendicontazione

Aggiornamento attività WP 2
Capitalizzazione dello studio di
fattibilità di un diagnostico per le
malattie pediatriche rare

Raffaella Franca, PhD
DSM-UNITS

Spletno srečanje Posodabljanje dejavnosti in poročanje

*Posodobitev dejavnosti delovnega
paketa 2*

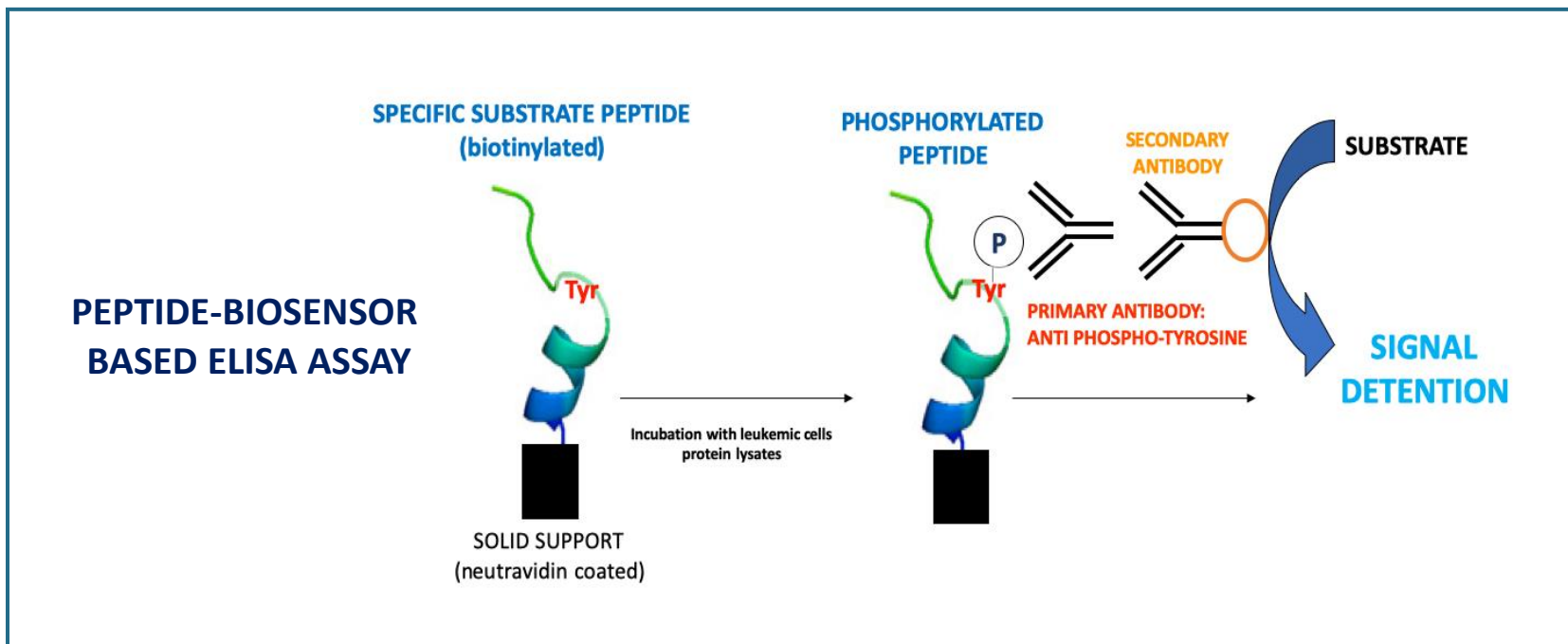
*Kapitalizacija študije izvedljivosti za
diagnostiko redkih pediatričnih
bolezni*

*Raffaella Franca, PhD
DSM-UNITS*



Aim of the project (WP2):

to set the basis for the development of a «**POINT-OF-CARE DEVICE**» for measuring the aberrant JAK kinase enzymatic activity in patients who are affected by JAK-related diseases.



JAK2 Peptide biosensors

PEPTIDES	SEQUENCE	MOLECULAR WEIGHT	REFERENCE
P _{JAK2-L}	GGDNDPDEYITLDEDGGK(biotin)GG	2250.28 Da	Lipchik et al. 2015
P _{JAK2-S}	LAKAVDGYVKPQI--Ttds-Lys(biotin)	2057.52 Da	Sanz et al. 2011
P _{PHOSPHO-JAK2}	GGDNDPDEY(P)ITLDEDGGK(biotin)GG	2330.25 Da	Lipchik et al. 2015

Lipchik et al, J Am Chem Soc. 2015 Feb 25;137(7):2484-94

Sanz et al., PLOS ONE, 2011. 6(4): p. e18522.

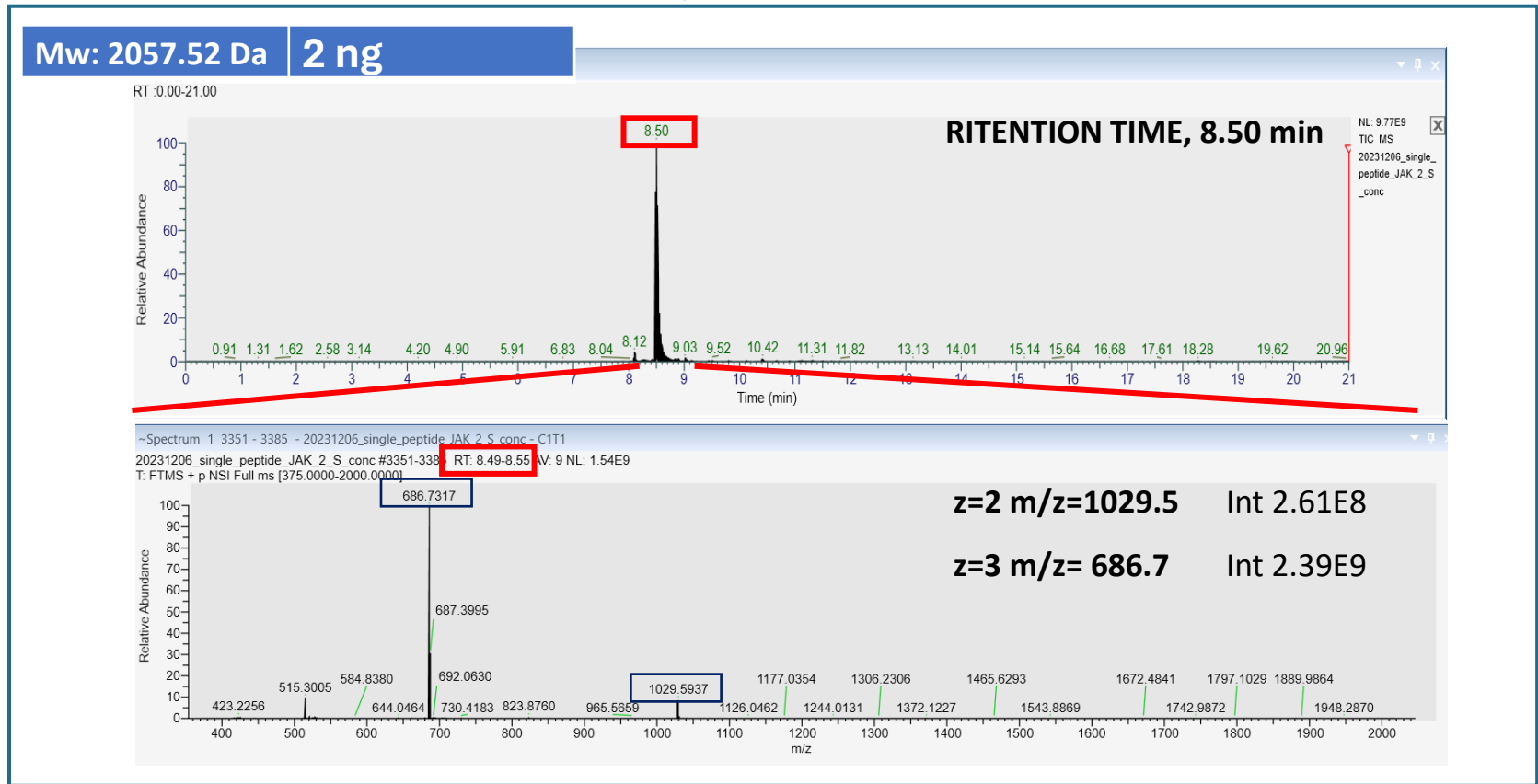
Peptide integrity BY MASS SPECTROMETRY

Thermo Scientific™ Vanquish™ Neo UHPLC
Orbitrap Exploris™ 240 Mass Spectrometer

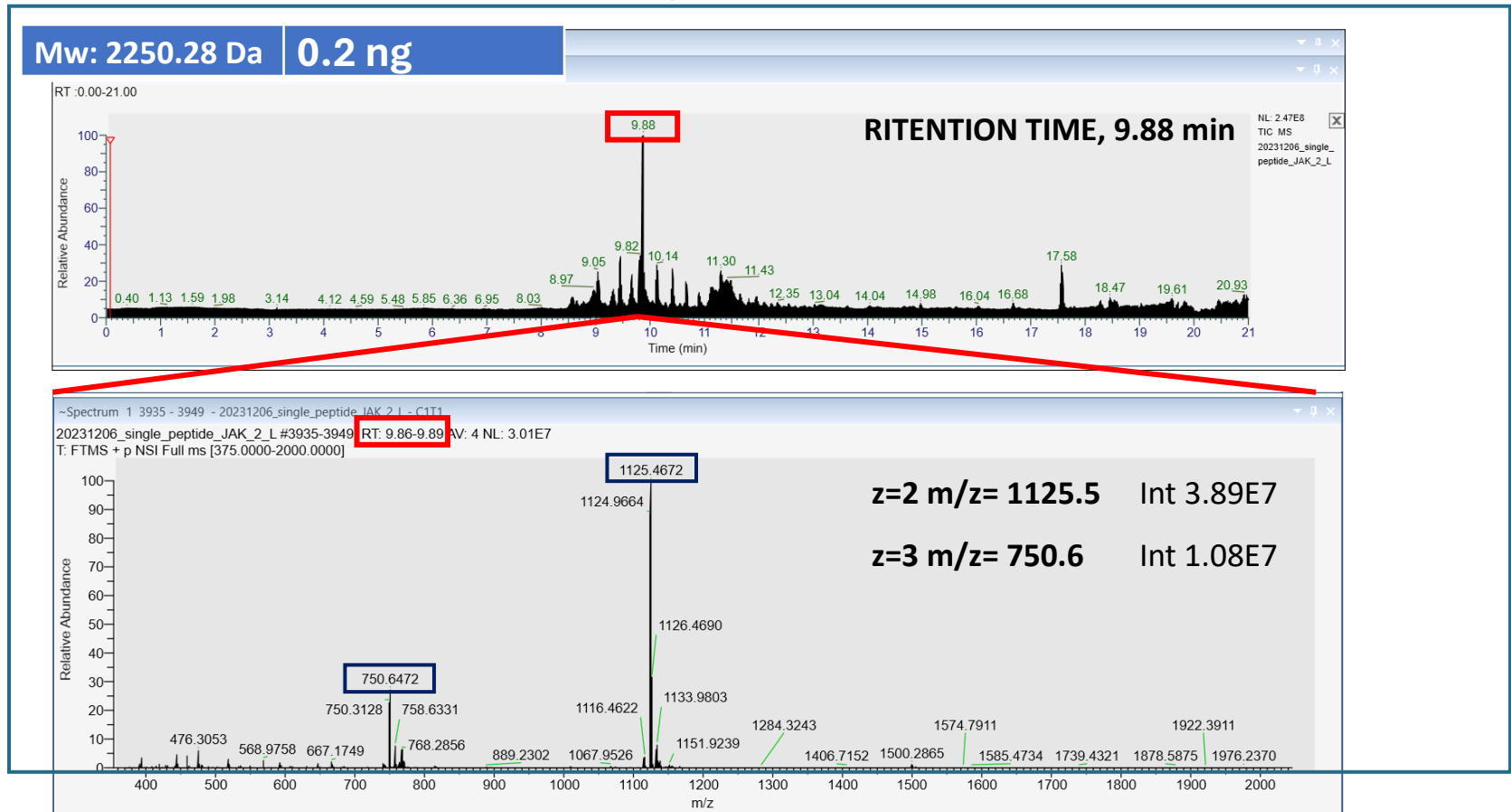
Available at DSM-UNITS



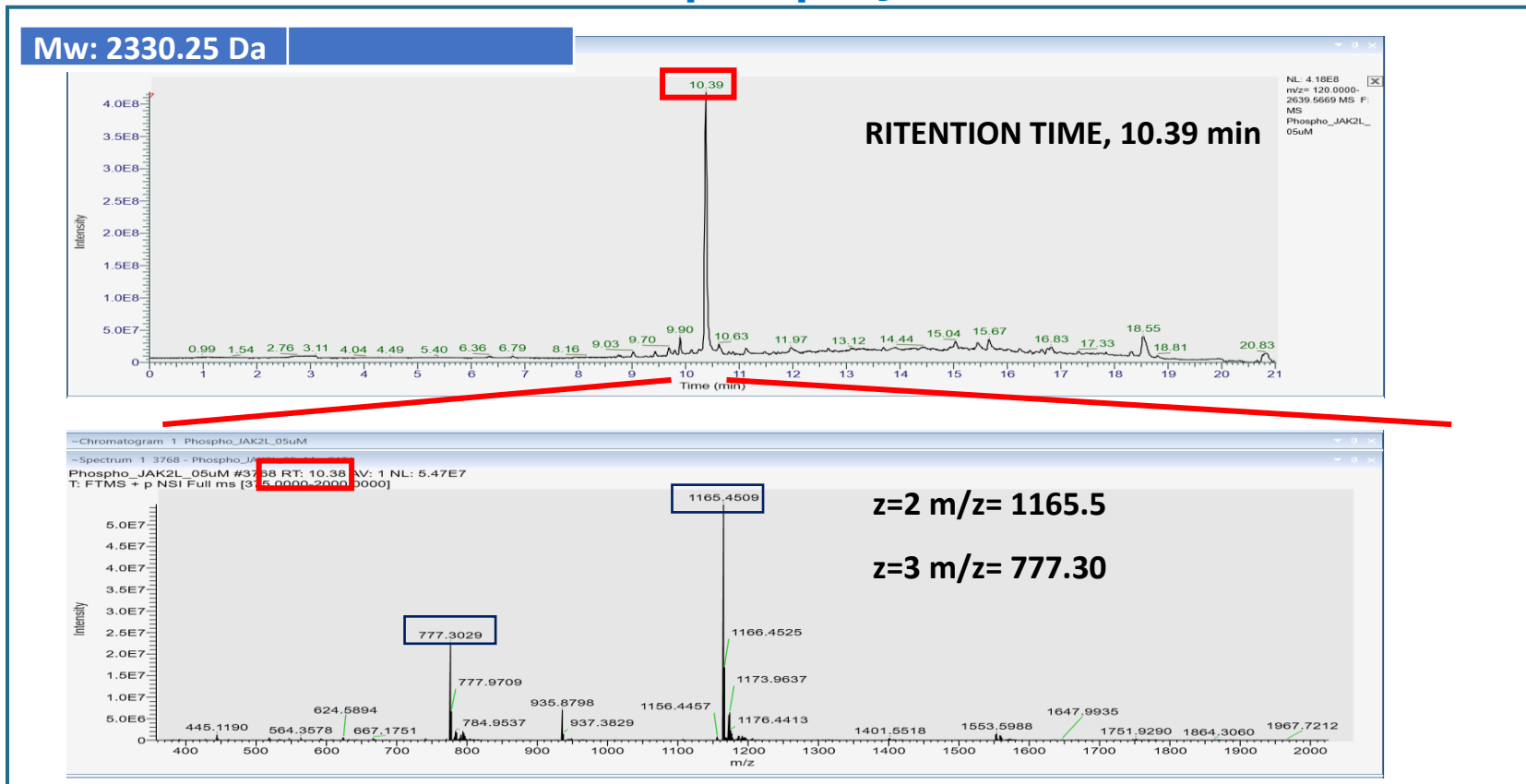
Peptide integrity : P_{JAK2-s}



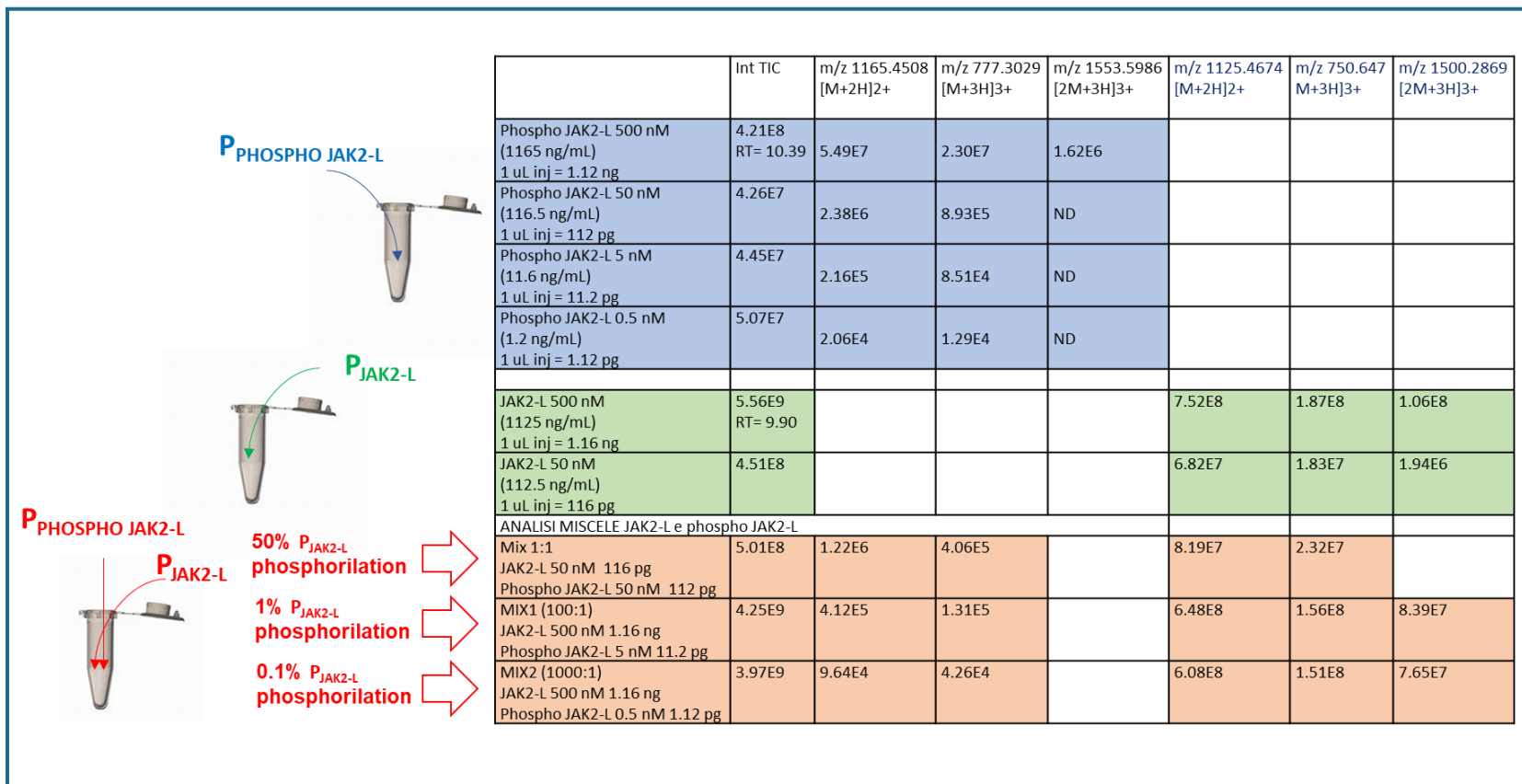
Peptide integrity : P_{JAK2-L}



Peptide integrity : P_{phospho}JAK2-L



Peptide mix



JAK2 Peptide biosensors

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Peptide structural integrity of the peptides is preserved

PEPTIDE-BIOSENSOR BASED ELISA ASSAY

1

In vitro studies on immortalized cell lines

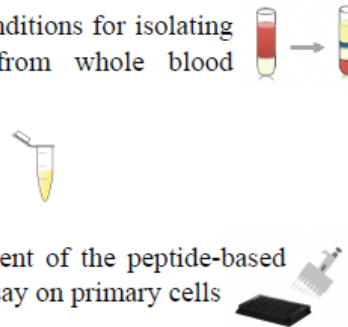
- Cell lines characterization
- Protein expression of **JAK1**, **JAK2**, **JAK3** and their relative **phosphoproteins**
- Immunoprecipitation of JAK2
- Development of the peptide-based ELISA assay



2

Optimization of the peptide- based assay on PBMCs from healthy donors

- Set up conditions for isolating PBMCs from whole blood samples
- Cell lysis
- Development of the peptide-based ELISA assay on primary cells



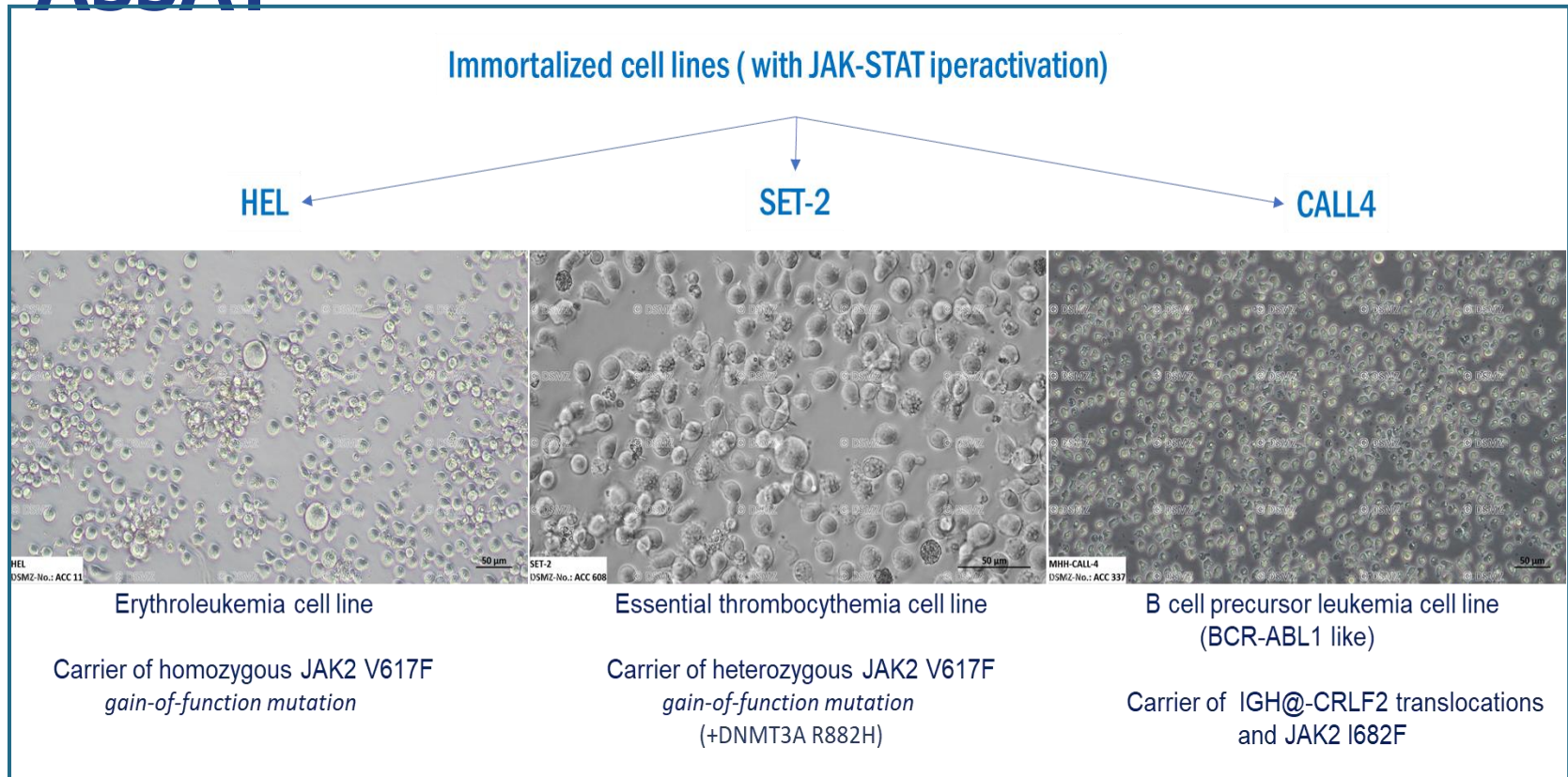
3

Application of the assay on primary cells from selected pediatric patients with JAKs mutations

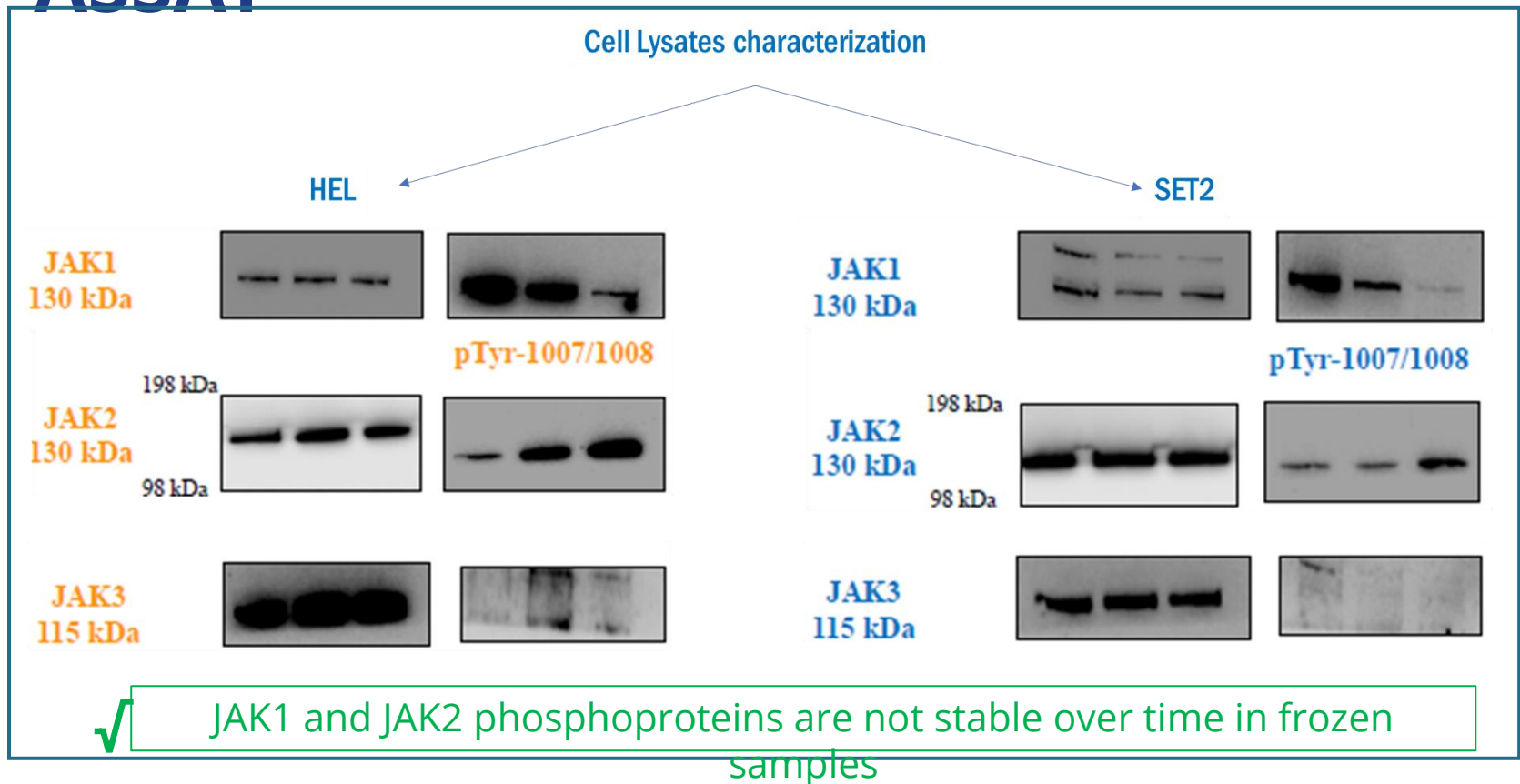
FINAL AIM



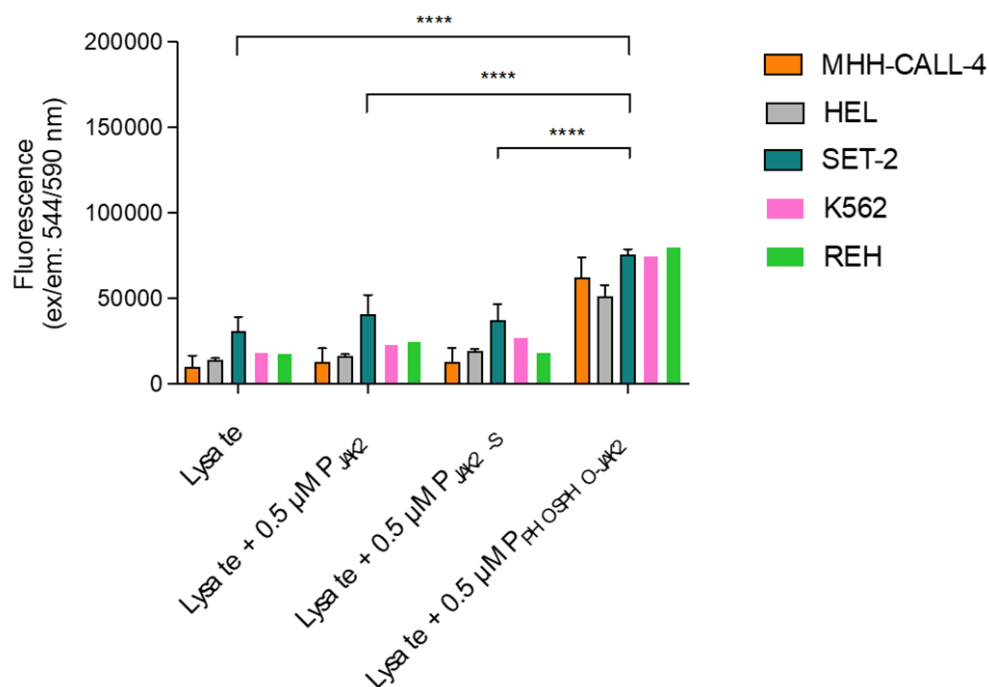
PEPTIDE-BIOSENSOR BASED ELISA ASSAY



PEPTIDE-BIOSENSOR BASED ELISA ASSAY

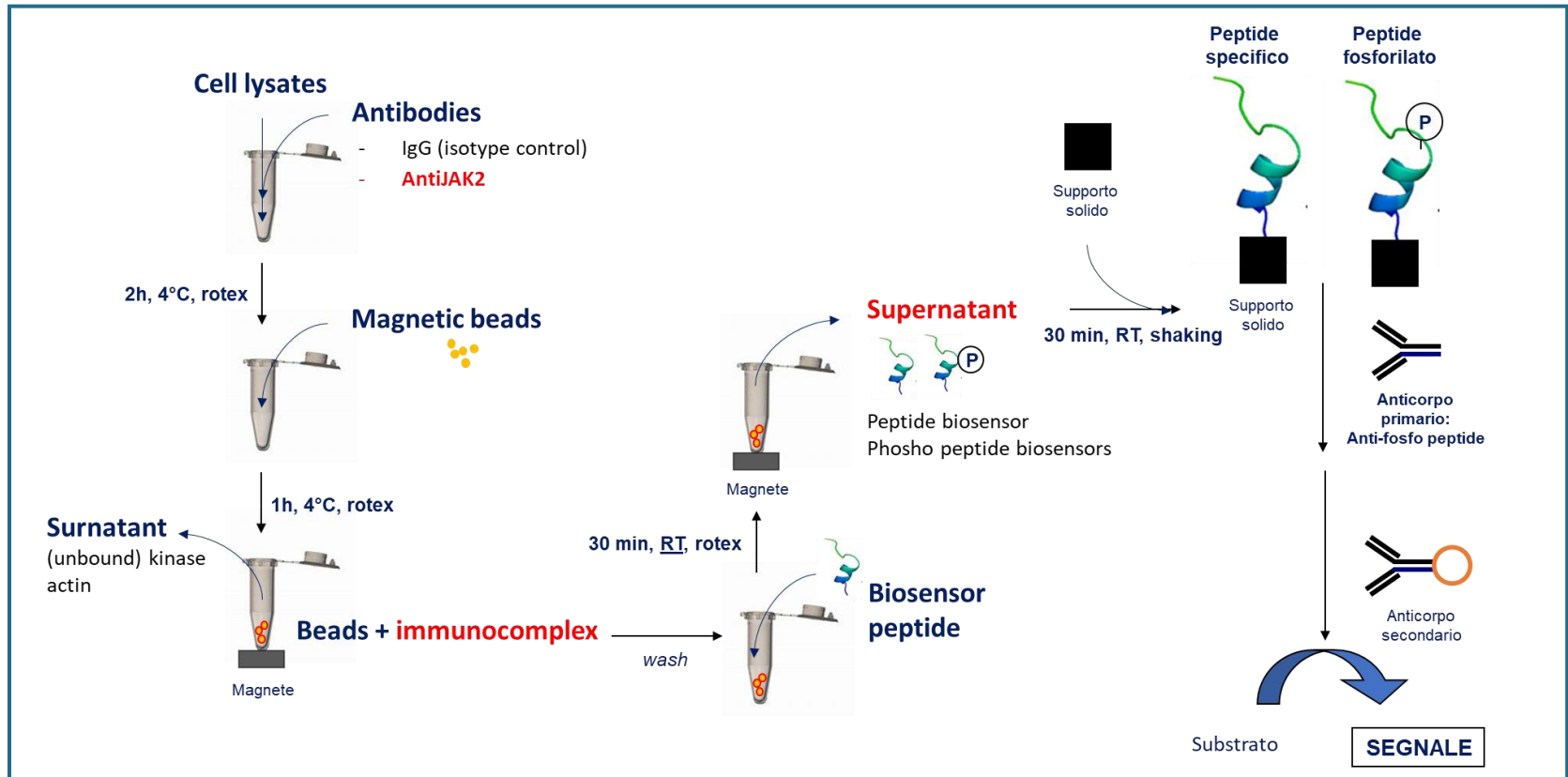


PEPTIDE-BIOSENSOR BASED ELISA ASSAY WHOLE LYSATES OF IMMORTALIZED CELL LINES

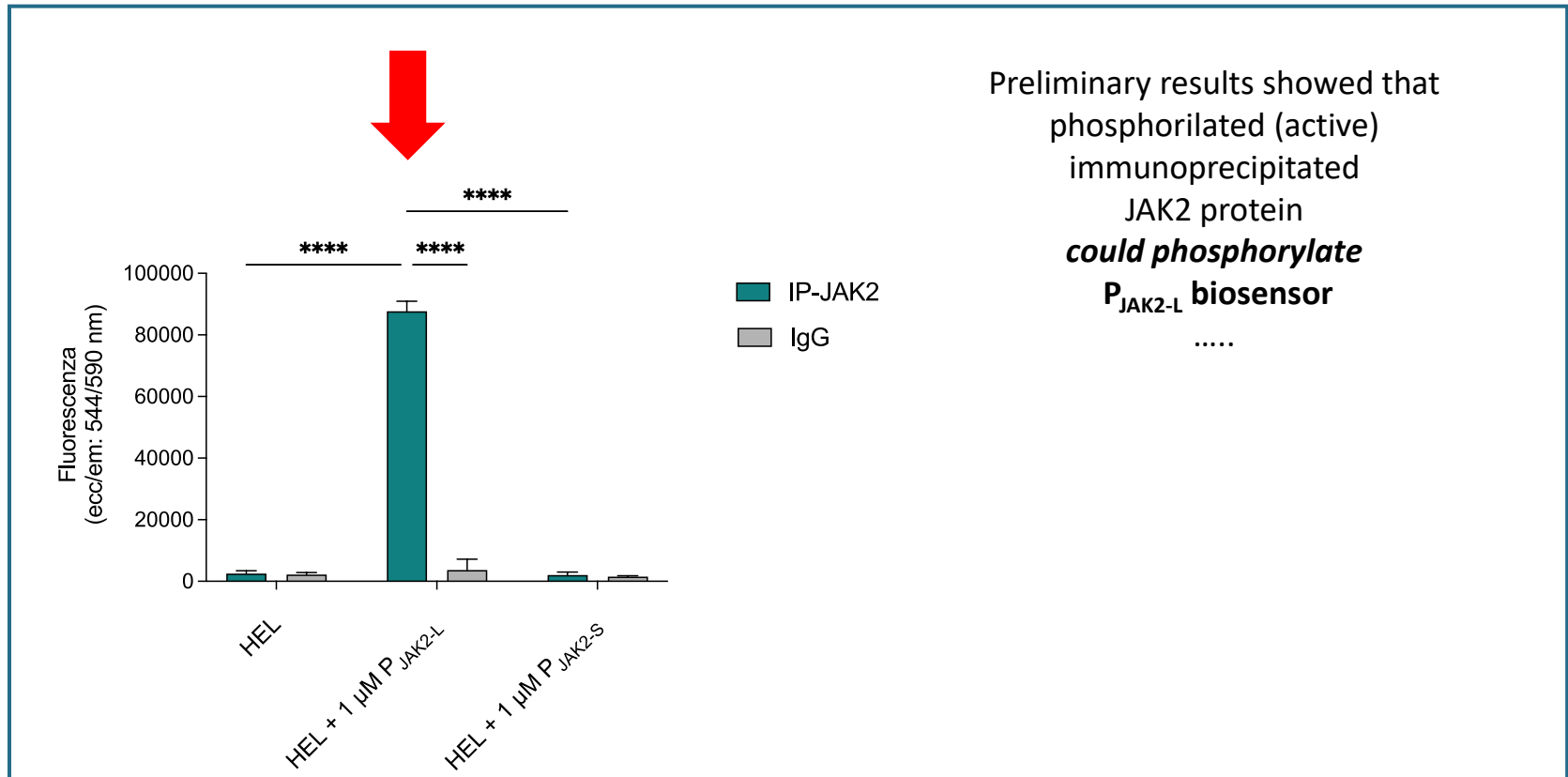


Peptide phosphorylation levels do not exceed lysate background

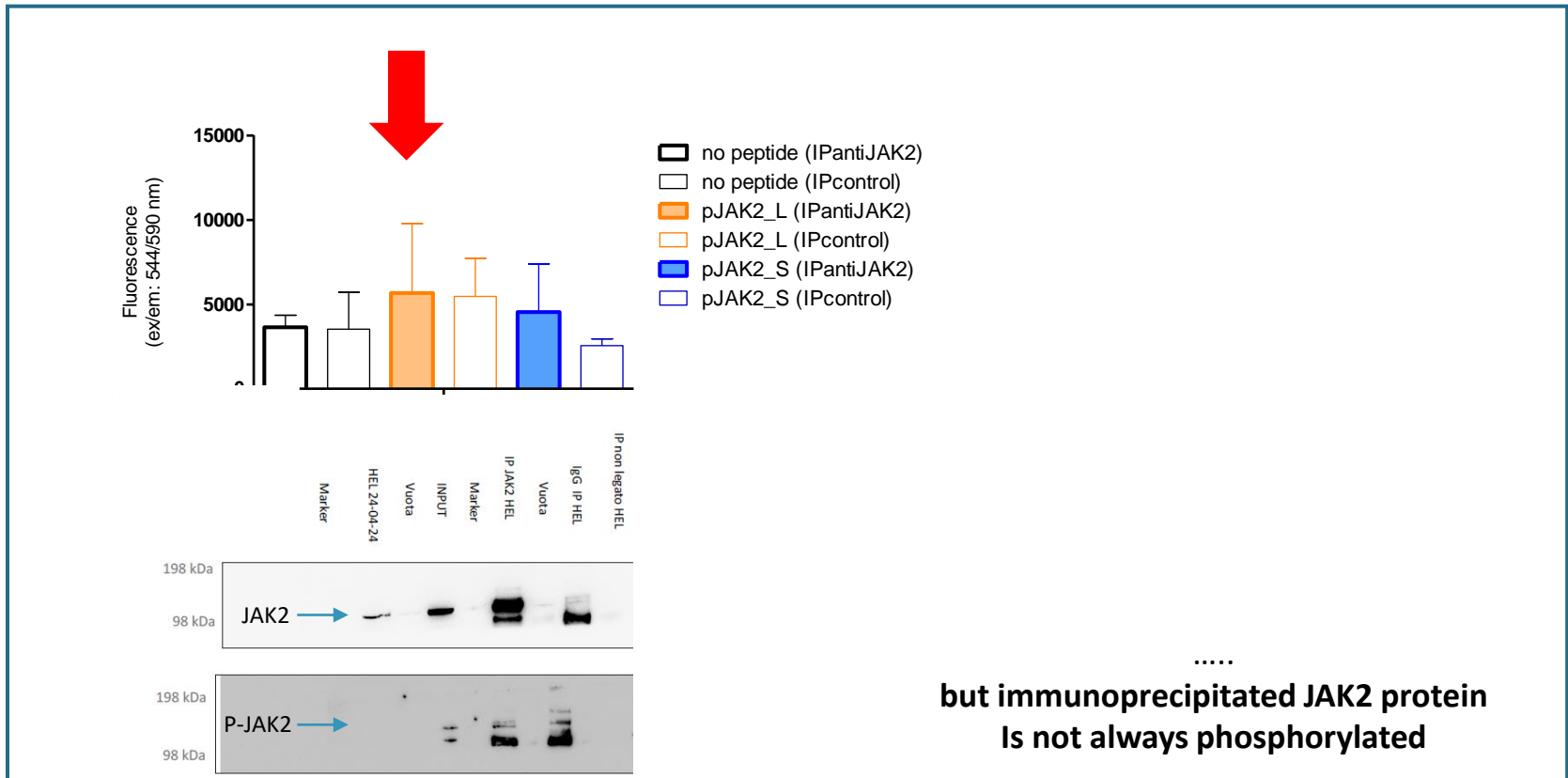
PEPTIDE-BIOSENSOR BASED ELISA ASSAY: JAK2 IMMUNOPRECIPITATION



PEPTIDE-BIOSENSOR BASED ELISA ASSAY: JAK2 IMMUNOPRECIPITATION



PEPTIDE-BIOSENSOR BASED ELISA ASSAY: IMMUNOPRECIPITATION





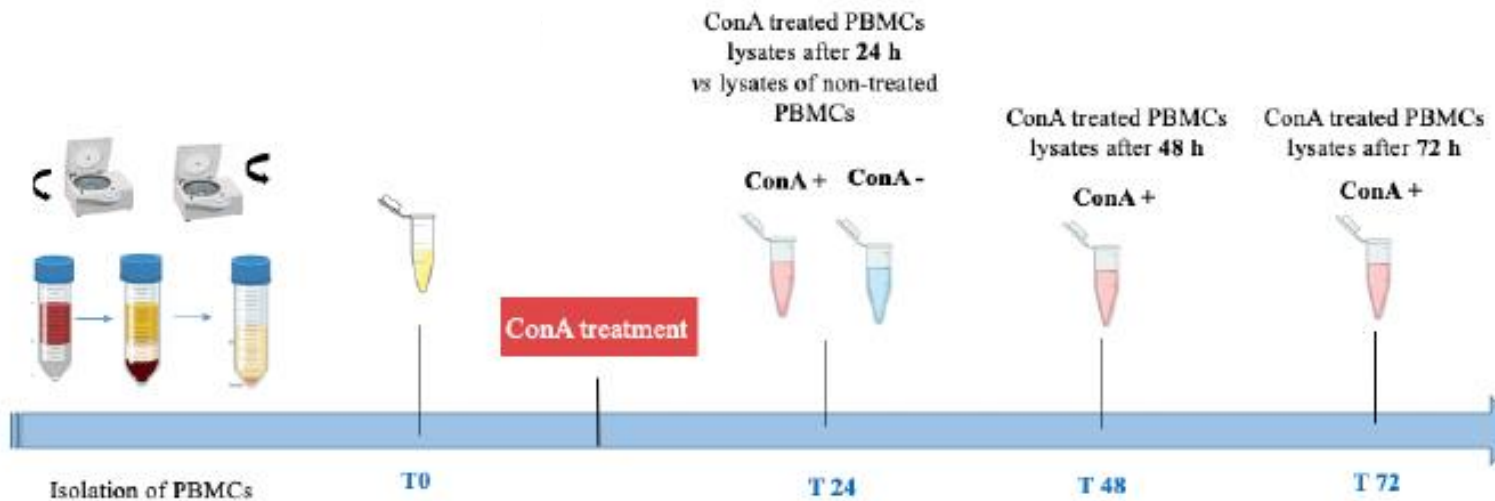
PEPTIDE-BIOSENSOR BASED ELISA ASSAY: JAK2 IMMUNOPRECIPITATION

NEXT STEPS

1. **PHOSPHO-JAK2 enrichment (IP with an anti-phosphoJAK2)**
2. **Alternative detection for the enzymatic reaction**
3. **Targeting sequence?**

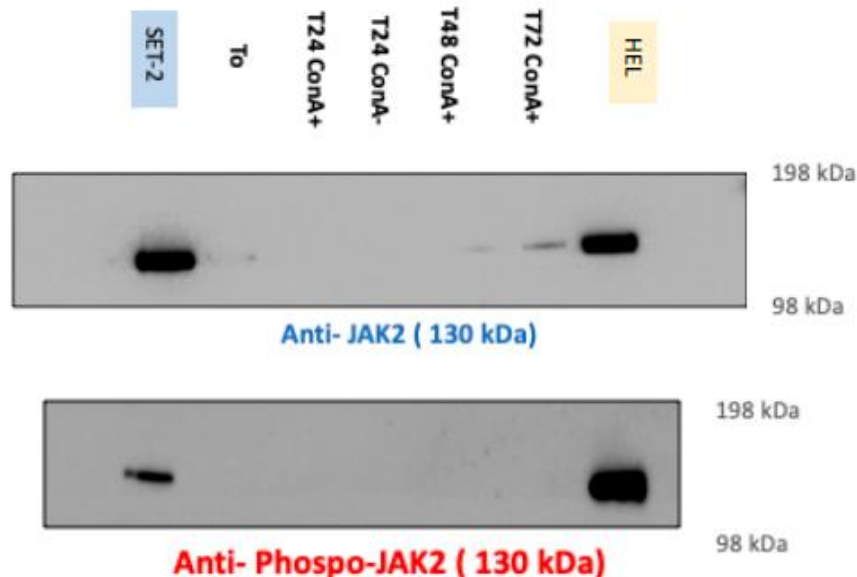
HEALTHY DONORS

PBMC isolation and cell culturing with Concanavalin-A



HEALTHY DONORS

PBMC isolation and cell culturing with Concanavalin-A



Conc-A is affecting JAK2 expression
after 72h exposure

PATIENTS: SAMPLE COLLECTION

Prof. Tadej Avčin

Prof. Andrea Taddio

PBMCs

PROJECT: “CONCERTO - CROSSBORDER NETWORK FOR CHILDREN RHEUMATOLOGY”

BIOLOGICAL SAMPLE COLLECTION

RATIONALE: Collection of biological samples (6-8 ml peripheral blood in heparin) of patients with **JAK kinase activating mutations** or with activation in the interferon pathway for isolation of peripheral blood mononuclear cells (PBMC)

- PBMC lysates will be analyzed with the novel peptide biosensor-based ELISA assay.
- Aim of the project is to provide the **proof-of-concept** that peptide biosensor (so far under development in in vitro studies on immortalized cell lines) is suitable for the detection of aberrant **JAK/STAT pathway** in pediatric patients affected by rare immune-mediated inflammatory disorders.
- In the future, this assay might have multiple applications and might be used as a **diagnostic test**, as an assay for **monitoring** patient response to therapy and/or as a tool for **screening a panel of JAK inhibitors** (JAKi) looking for the most appropriate one in an effort to **personalize patient therapy**.

SPECIFIC SUBSTRATE PEPTIDE (Bioylated)

Tyr

SOLID SUPPORT (Neutravidine coated)

Incubation with immunoprecipitated JAK2 protein

PRIMARY ANTIBODY Anti-phosphotyrosine

SECONDARY ANTIBODY

SIGNAL DETECTION

SUBSTRATE



Grazie per l'attenzione! / Hvala za pozornost!

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