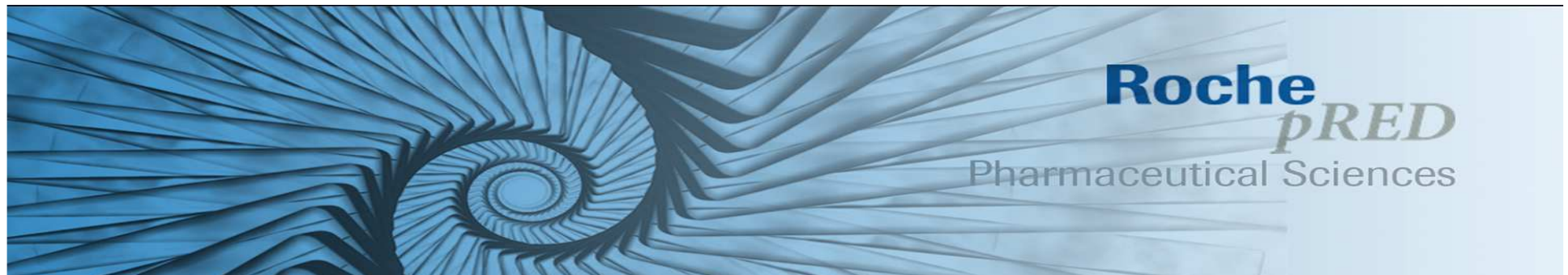
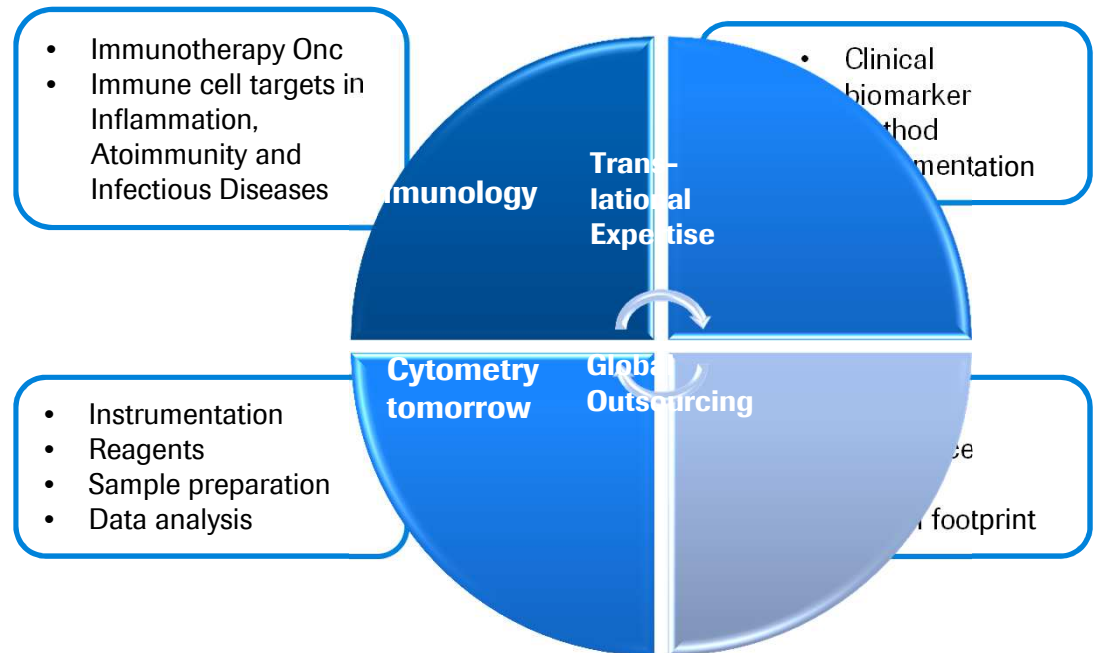

Cellular biomarker method validation and application in phase 1 clinical trials

*Bernhard Reis, Roche Pharmaceutical Research and Early Development,
Pharmaceutical Sciences, Roche Innovation Center Basel*

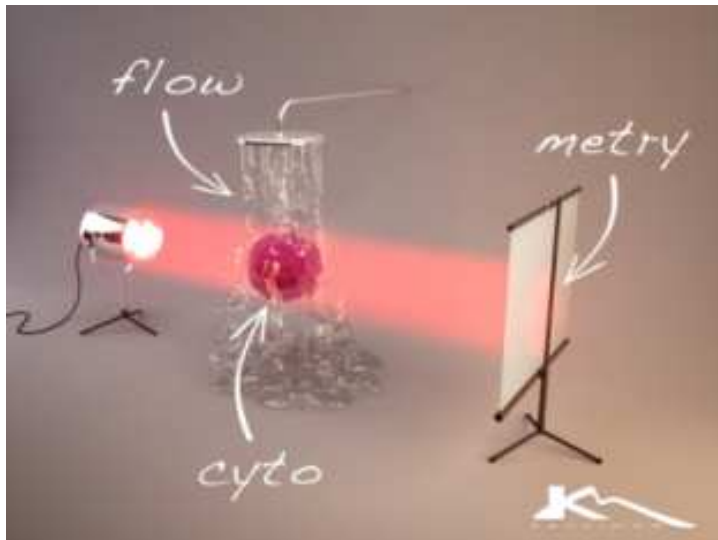


Cell Based Protein Biomarkers Group

- **Growing immunotherapy portfolio** in cancer, autoimmunity, inflammation and infectious diseases
- **Apply state of the art technology** for characterizing and quantifying immune cell subsets
- **Deliver decision enabling information** during clinical development



How we apply Flow Cytometry in early clinical development



We use flow to address in the clinic...

- Confirm MoA *increase confidence in molecule or target*
- Dose finding *decrease attrition rate*
- Combination partner hypothesis generation *increase efficacy*
- Explore patient stratification *PHC goals*

Biomarker method validation

INTRODUCTION



- Created by the NIH-FDA Biomarker Working Group
  - A glossary of terminology and uses of biomarkers and endpoints in basic biomedical research, medical product development, and clinical care
-
- Bioanalytical Method Validation Guidance for Industry, FDA May 2018

Terminology

- **Analytical validation**

- validation of the test's, tool's, or instrument's technical performance, but not validation of the item's usefulness.

- **Clinical validation**

- Establishing that the test, tool, or instrument acceptably identifies, measures, or predicts the concept of interest.

- **Biomarker qualification**

- A conclusion, based on a formal regulatory process, that within the stated context of use, a medical product development tool can be relied upon to have a specific interpretation and application in medical product development and regulatory review.

Bioanalytical Method Validation FDA Guidance for Industry



Accuracy

Precision

Sensitivity

Selectivity

Parallelism

Range

Reproducibility

Stability

The approach used for drug assays should be the **starting point for validation of biomarker assays**, although the FDA realizes that some characteristics may not apply or that different considerations may need to be addressed.

Fit-For-Purpose method validation Concept

- **Appropriate for the intended purpose of the study**
- **Relative to the stage of drug development**
- Pivotal studies submitted in an NDA, BLA, or ANDA that require regulatory decision making for approval, safety or labeling, such as BE or pharmacokinetic studies, should include bioanalytical methods that are fully validated
- **Exploratory methods that would not be used to support regulatory decision making (e.g., candidate selection) may not require such stringent validation**
- When biomarker data will be used to support a regulatory decision making, such as the pivotal determination of safety and/or effectiveness or to support dosing instructions in product labeling, the assay should be fully validated.

Biomarker categories modified based on FDA BEST*



- Diagnostic Biomarker
- Patient Stratification/ Disease
  - Predictive Biomarker
  - Monitoring Biomarker
  - Prognostic Biomarker
  - Susceptibility/ Risk Biomarker
- Target Engagement/ Pharmacodynamic biomarker
- Safety Biomarker

*<http://www.ncbi.nlm.nih.gov/books/NBK326791/>

Guiding principles of fit for purpose validation

- Does the method measure the intended analyte?
- How is the biomarker data going to be used?
- What is the variability associated with these measurements?
- What is the range in measurements that provide reliable data?
- How do sample collection, handling, and storage affect the reliability of the data from the biomarker method?

Technology specific – Flow Cytometry

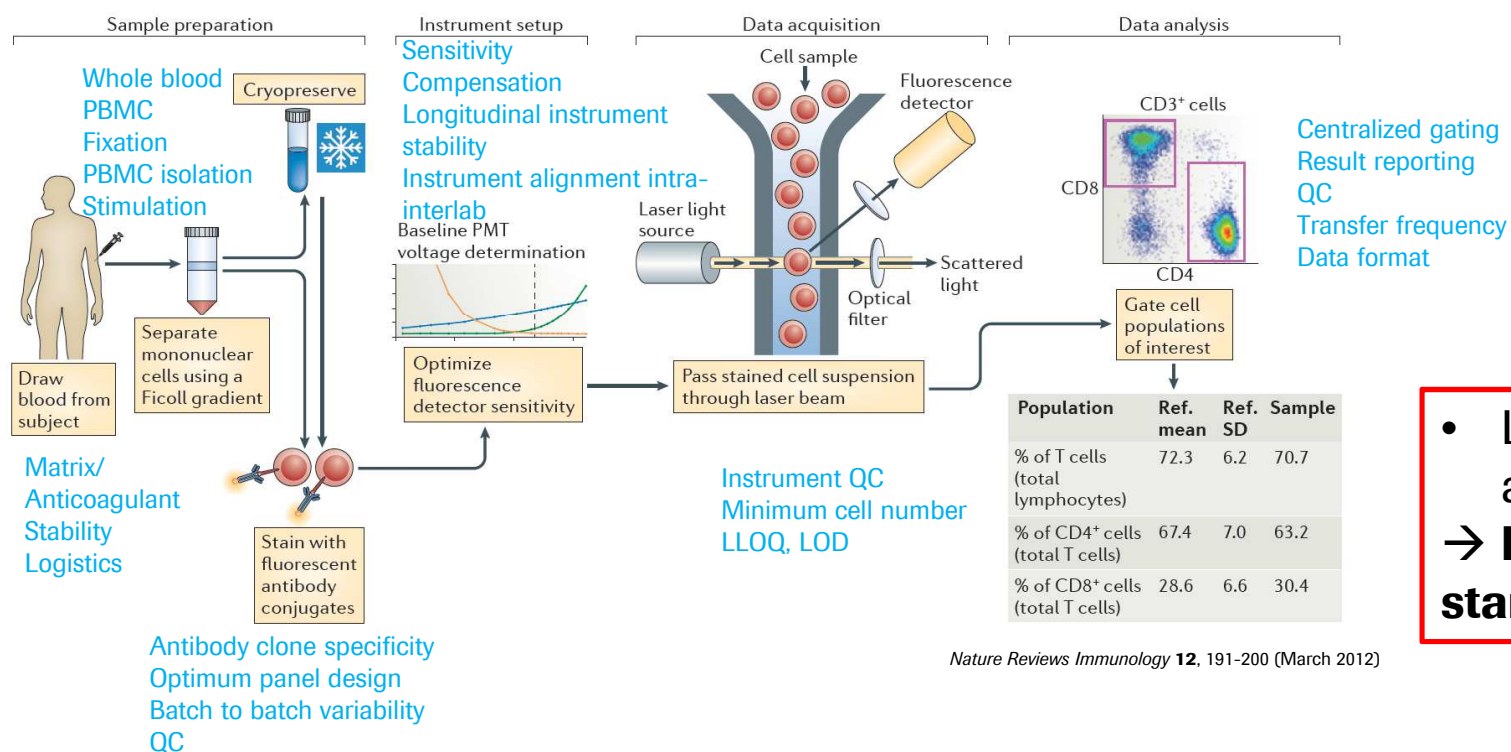
METHOD STANDARDIZATION AND VALIDATION

Flow cytometry – Complex workflows



Measurement of **individual cells** and their properties in a **flowing stream**

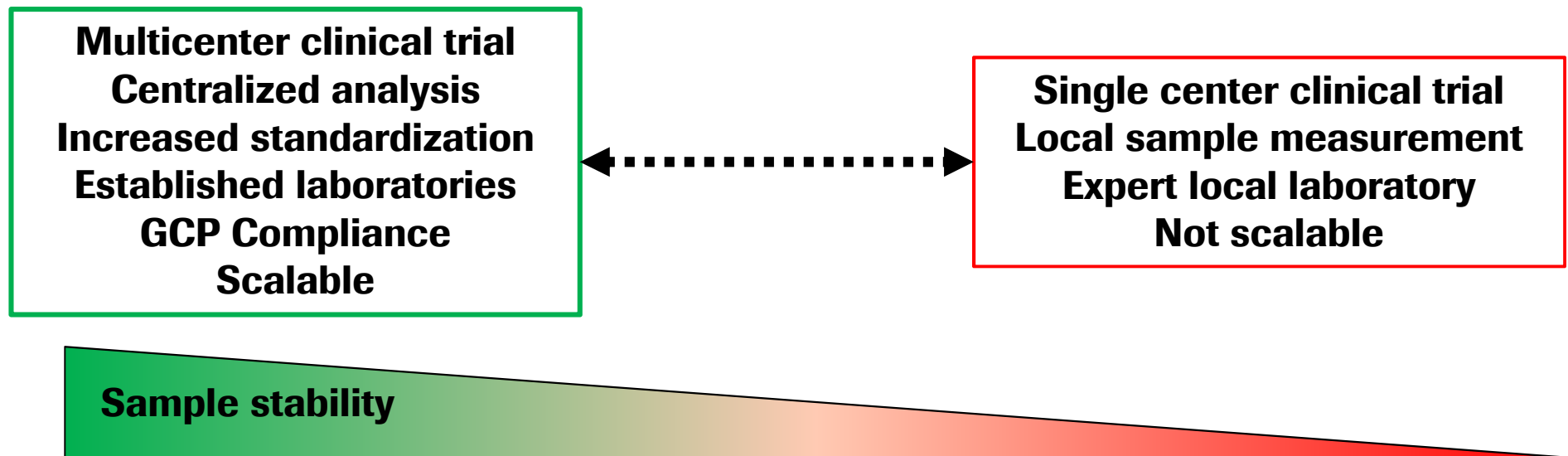
Flow Cytometry Workflow for blood samples



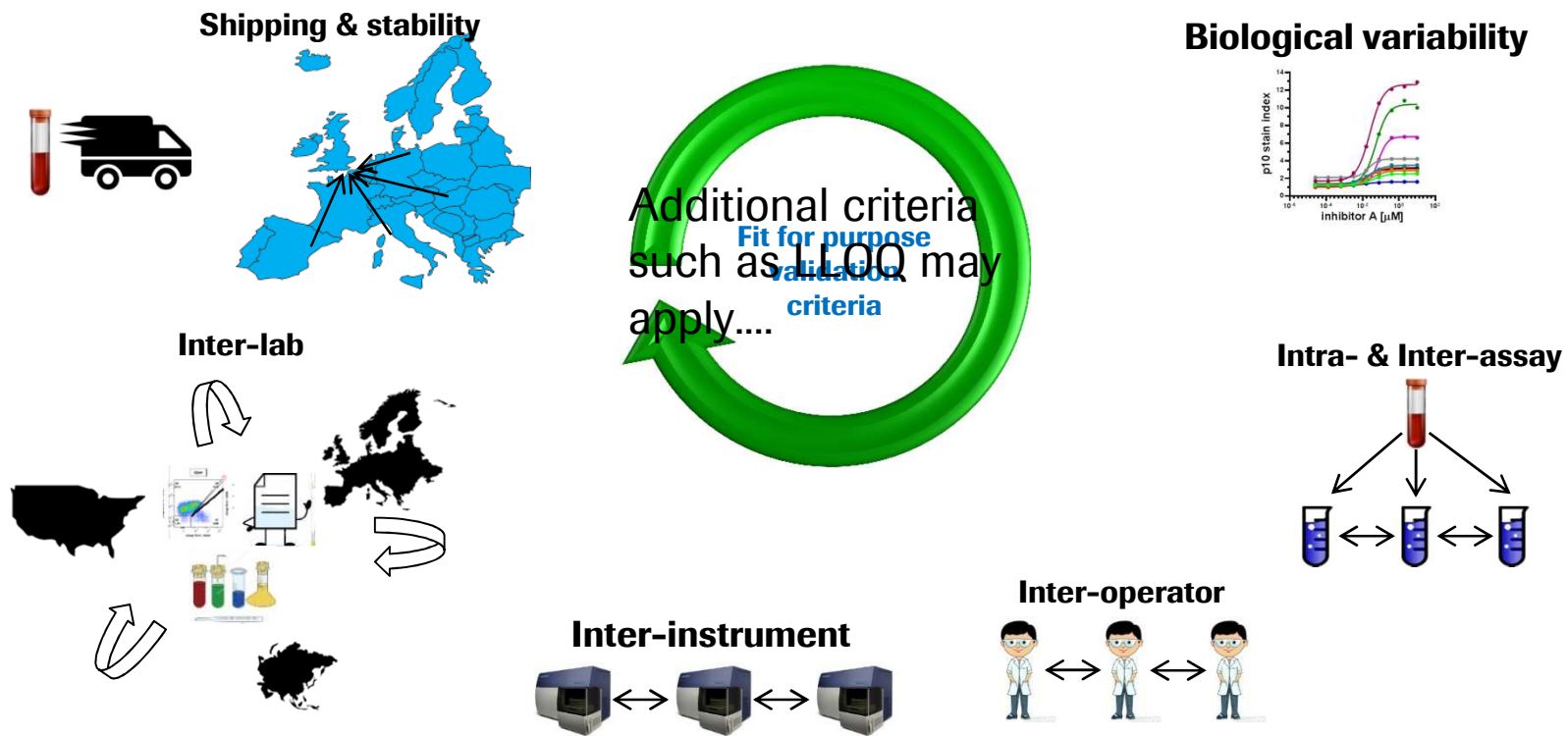
- Lack of automation
→ **Difficult to standardize**

Nature Reviews Immunology **12**, 191-200 (March 2012)

Sample stability is a major challenge for analysis of intact living cells



Flow cytometry method validation criteria in global clinical trials





A pharmacodynamic biomarker to support dose finding example

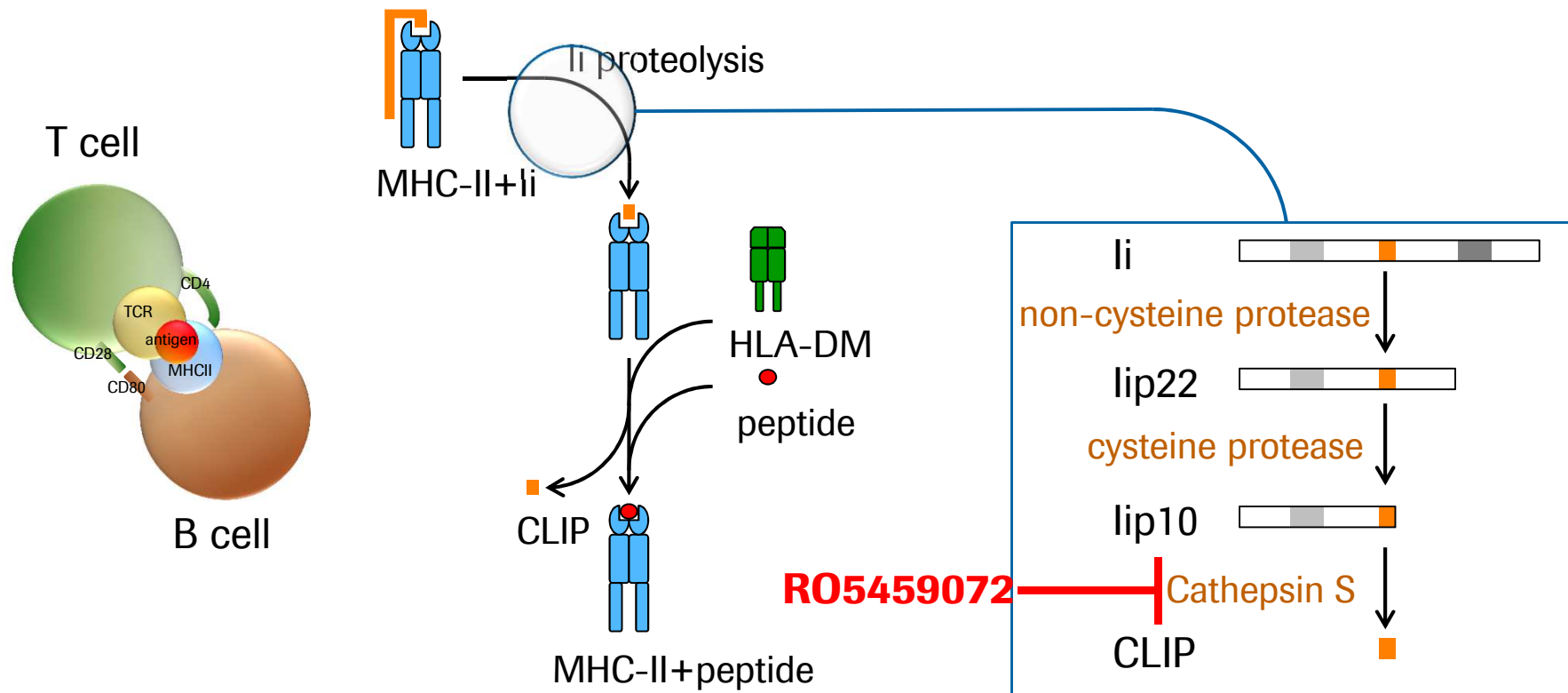
PROJECT APPLICATIONS

Pharmacodynamic Monitoring of RO5459072, a Small Molecule Inhibitor of Cathepsin S

 Michel Theron¹,  Darren Bentley²,  Sandra Nagel²,  Marianne Manchester¹,  Michael Gerg³, 
Thomas Schindler⁴,  Ana Silva¹,  Barbara Ecabert¹,  Priscila Teixeira¹,  Camille Perret¹ and  Bernhard
Reis^{1*}

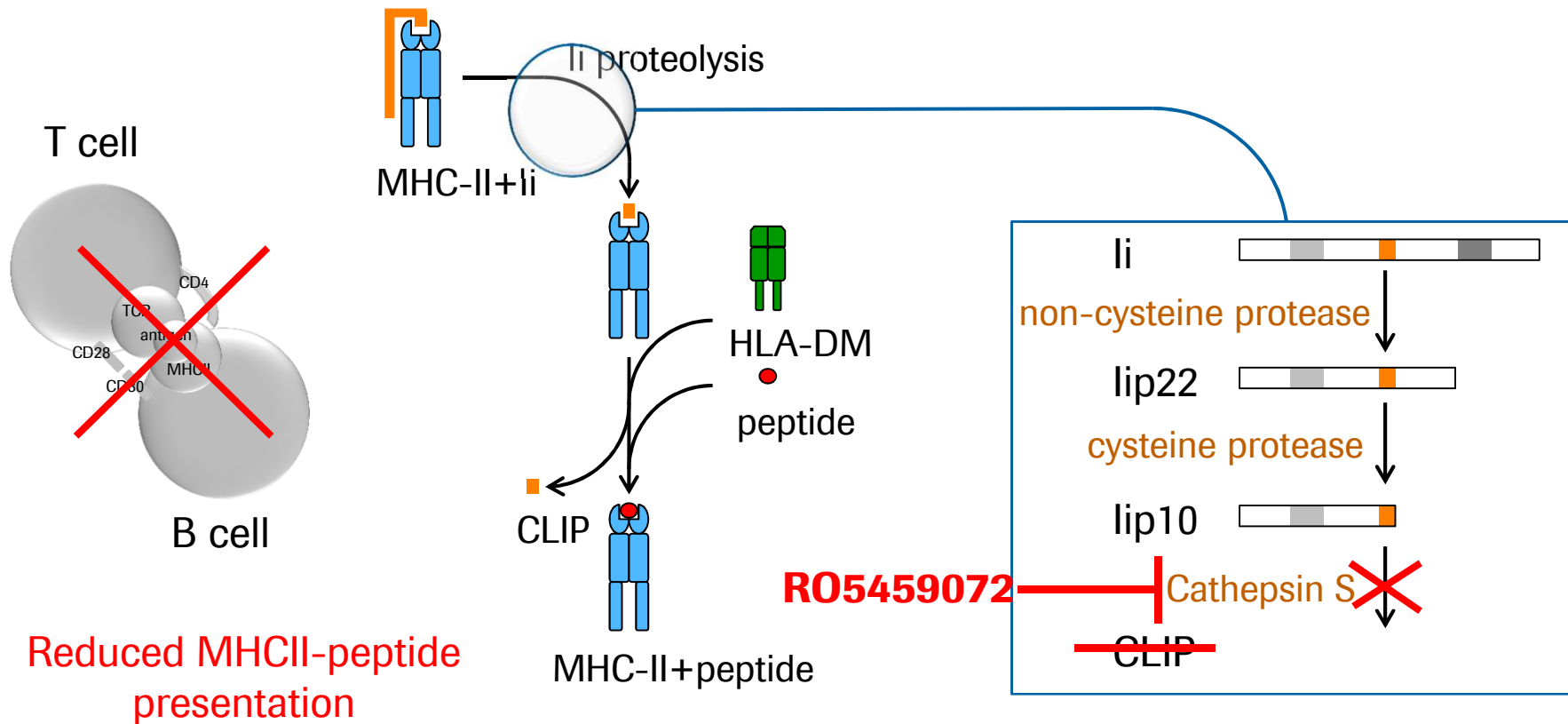
CD4+ T-cell and B-cell TCR-antigen-MHC II interaction

Proteolytic cascade MHC II maturation



CD4+ T-cell and B-cell TCR-antigen-MHC II interaction

Proteolytic cascade MHC II maturation



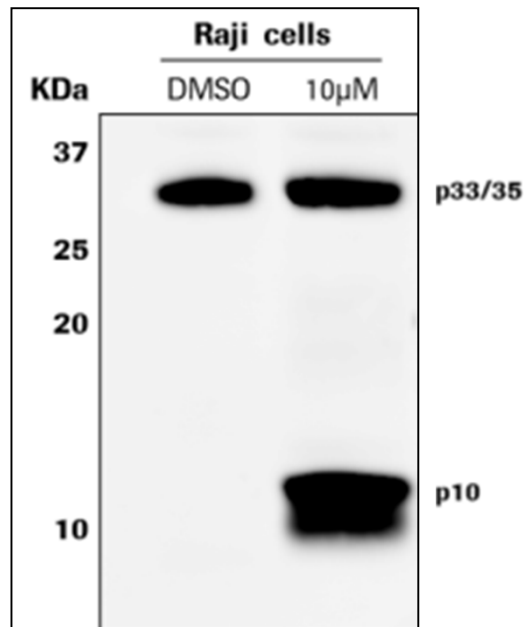
Cathepsin S small molecule antagonist requirement for a Pharmacodynamic biomarker

- Is the drug reaching its cellular target and does it have the desired effect?
- What is the optimal dosing?
- Feasibility in a healthy volunteer clinical trial setting
  - Whole blood, not tissue
  - Cellular marker in B cells only, a small variable subset in whole blood

⇒ **PD biomarker to provide proof of mechanism and identify optimal dose**

⇒ **Assuming prolonged $\geq 95\%$ target inhibition is required for efficacy**

li p10 as pharmacodynamic biomarker



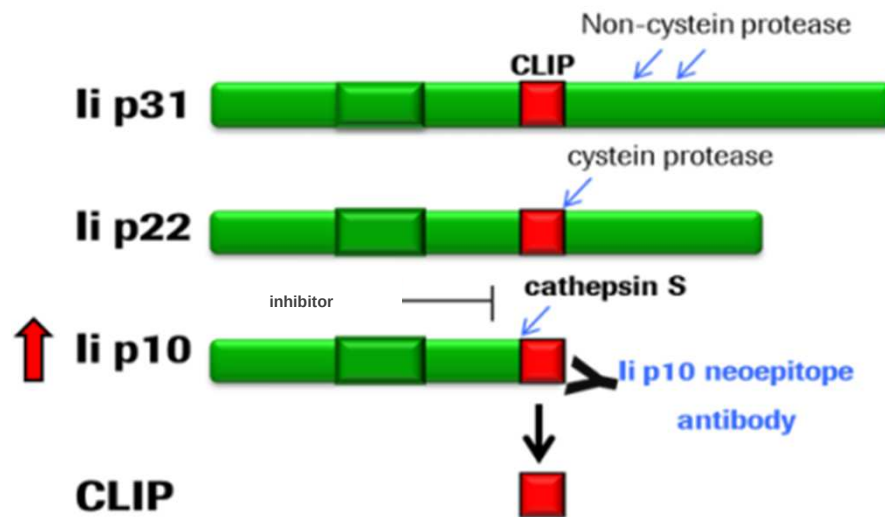
Western blot not an option
Other technologies considered were
Mass spectrometry and ELISA

Dismissed due to precision,
sensitivity, specificity reasons

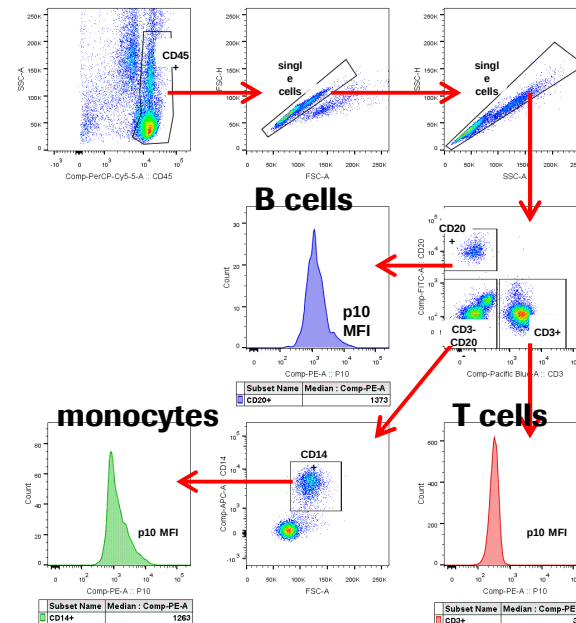
Inhibition of cathepsin S in Raji cells leads to
accumulation of li p10

Development of a Neoepitope specific antibody in a multiplexed combined surface-intracellular flow cytometry format

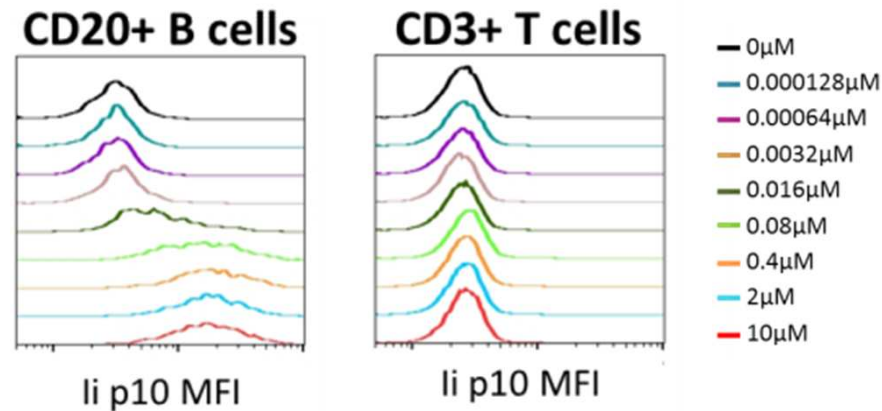
1. Neoepitope-specific antibody



2. Combine with flow cytometry for additional multiplexed detection of lymphocyte markers



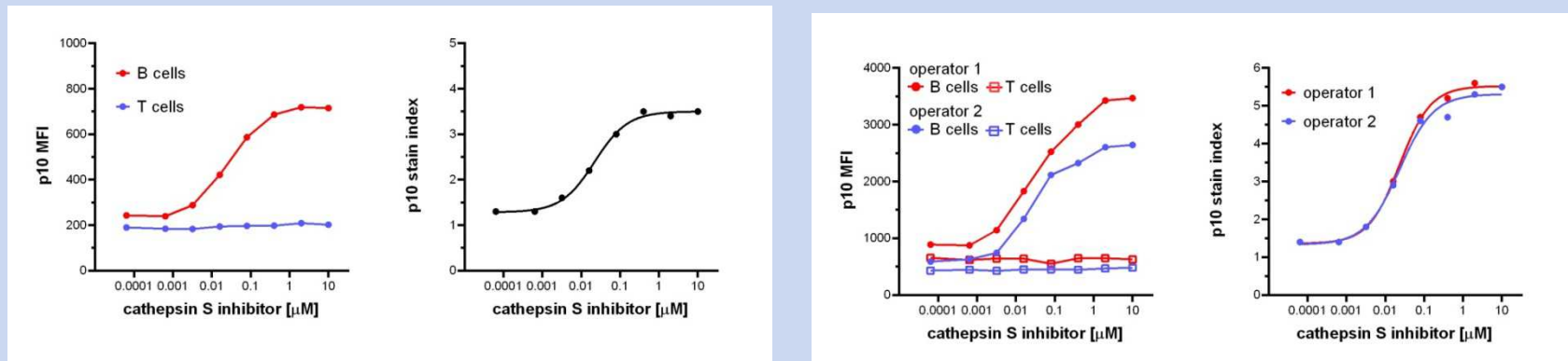
Detection of cell type-specific li p10 accumulation by flow cytometry using a neoepitope antibody



Dose-dependent li p10 accumulation was detected in B cells upon incubation with a cathepsin S inhibitor. T cells are shown as controls for specificity and background signal and do not exhibit p10 accumulation.

Demonstrate specificity, increase robustness

T cells as an internal negative control

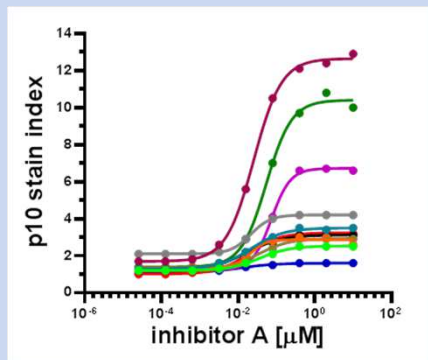


⇒ The p10 stain index improves data comparability by reducing operator introduced and daily performance variability

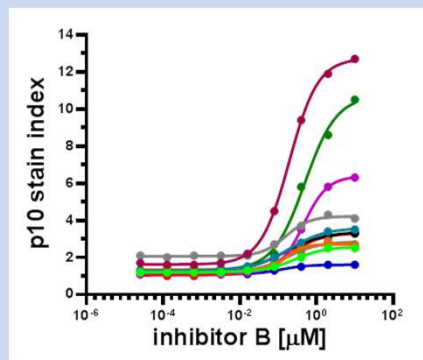
Variability in healthy volunteers

Heterogeneity in dynamic range, constant potency

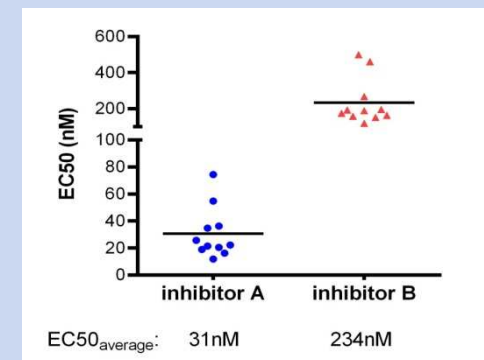
Heterogeneity in maximum p10 accumulation within B cells observed in response to *in vitro* CatS inhibition



Heterogeneity in maximum p10 accumulation tested with a less potent inhibitor

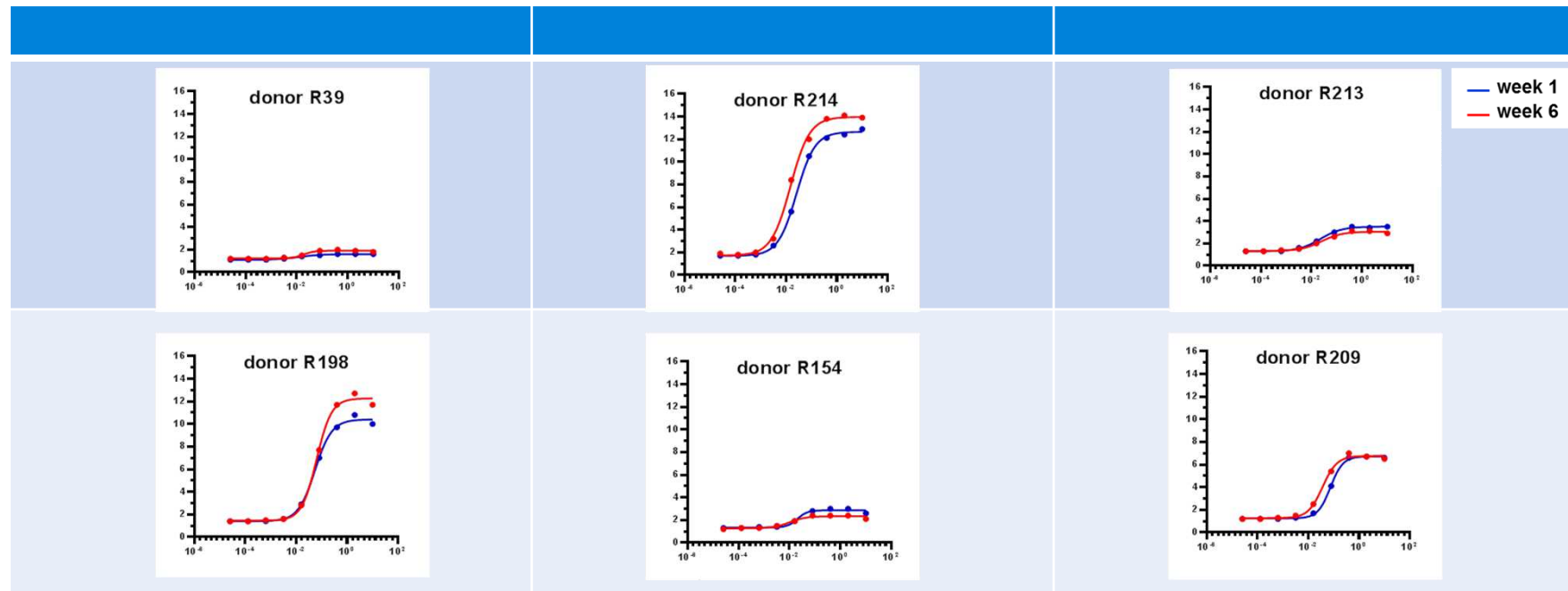


Inhibitors potency consistent across healthy volunteers, with values in agreement with *in vitro* potency on CatS activity



⇒ Inter-individual heterogeneity in maximum response but not inhibitor potency indicates a biological rather than pharmacological cause

Reproducible detection of donor and compound effect variability on li p10 accumulation

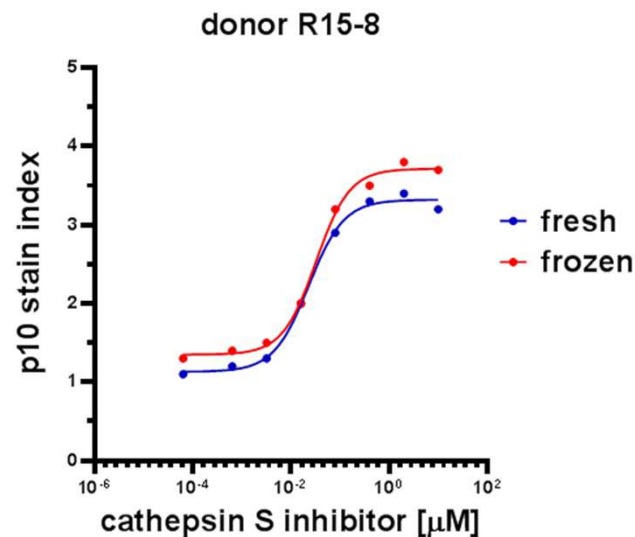


⇒ Acceptable longitudinal heterogeneity for use as a PD marker for intended length of treatment in clinical trial

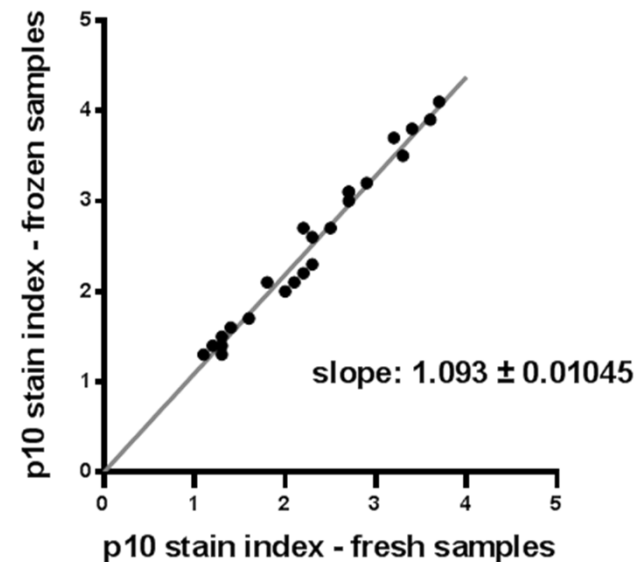
Validation parameter data not shown

- Precision
  - Intraassay
  - Interassay
  - Interinstrument
  - Interoperator
- Stability
- Quality controls
- Datapoint exclusion criteria

Stability of li p10 biomarker in samples in cryogenic storage for 2 weeks



Samples flash-frozen immediately after fixation yielded comparable data on dose-dependent li p10 accumulation in B cells after 2 weeks at -80°C



The cryogenic stability of the li p10 biomarker for assessment with the flow cytometry-based neoepitope assay allows for batch sample processing.

Single ascending dose study in healthy volunteers

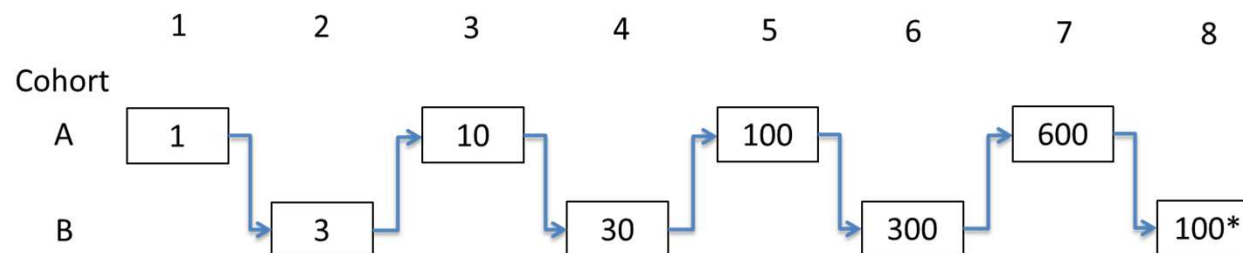
EXPERIENCE IN ACTUAL CLINICAL TRIAL

Objective: to investigate the safety and tolerability of RO5459072 in healthy volunteers



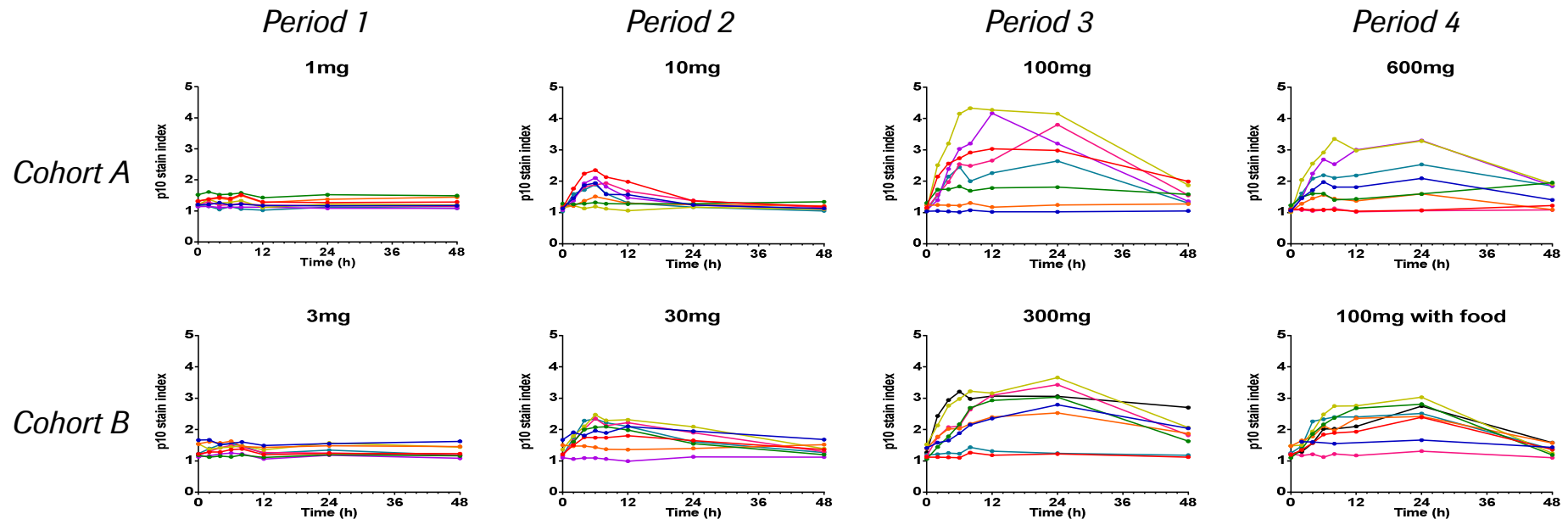
PK of RO5459072 and PD measure of CatS activity (p10) as key secondary endpoints

Clinical study design taking into account donor PD heterogeneity



Minimize effect of inter-individual maximum response
2 cohorts leapfrog design with wash out period
Placebo controlled (6+2)

Dose-dependent p10 accumulation in B cells in response to inhibitor *in vivo*

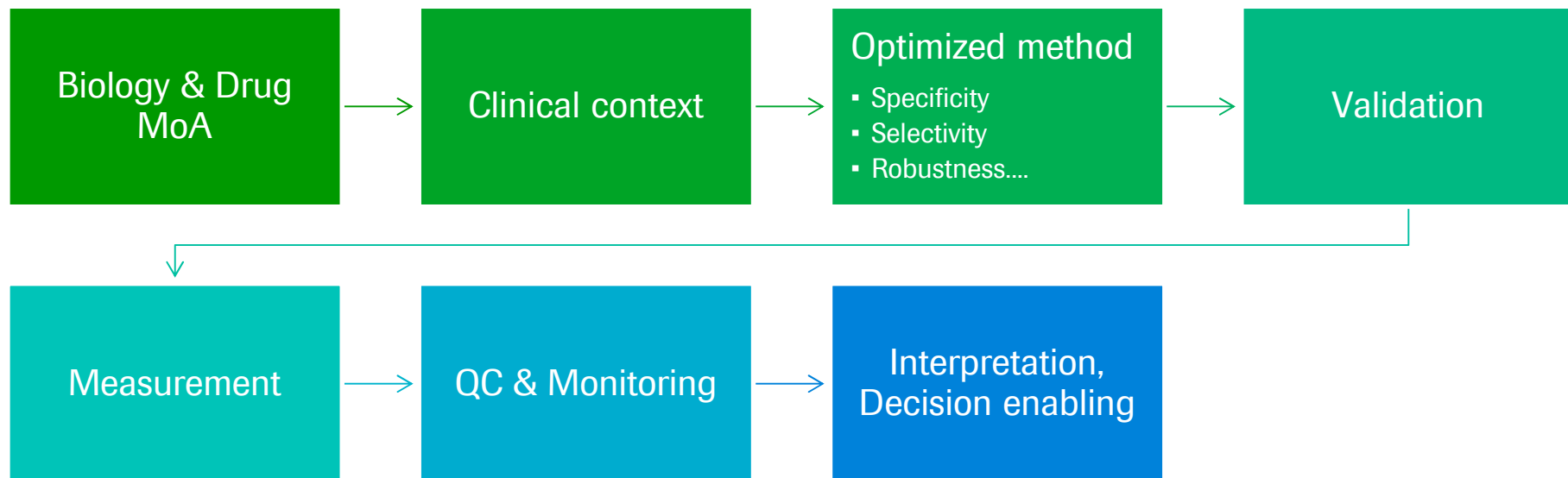


Peaked at 6-12h after administration, returning to near-basal levels at 48h
Detectable in all volunteers, with doses as low as 10mg
Maximum accumulation observed with doses 100-300mg

Multiple Ascending Dose Target engagement demonstrated Go for Phase 2

- Dose-dependent increase confirmed in MAD
  - Inter-individual heterogeneity in maximum response
  - Required PK/PD modelling for data interpretation
- ⇒ **100 mg bid achieves ~95% enzyme inhibition throughout dosing interval**
- ⇒ **Currently tested in various autoimmune diseases Phase 2**

Essential steps from concept to decision enabling biomarker data generation



Acknowledgements

P10 neoepitope antibody development

M. Gerg
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S. Nagel

Method development and sample measurement oversight

M. Theron
B. Ecabert
A. Silva
P. Teixeira

....and many more



*Doing now what patients need
next*