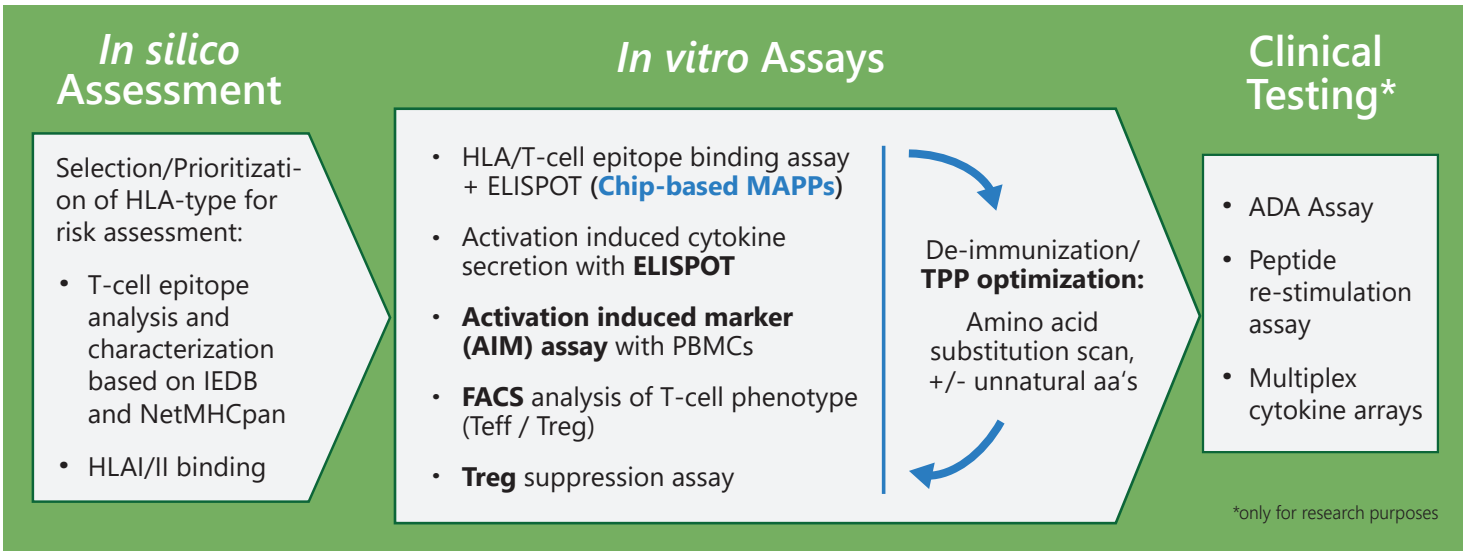
PEPperPRINT
A NEW DIVERSITY

Immunogenicity Risk Assessment Tools for Therapeutic Protein Products

Immunogenicity Risk Assessment (IRA) is used in therapeutic protein product (TPP) development to select high benefit drug candidates for the clinics. The measurement of anti-drug antibodies (ADA) is a standard method in clinical immunogenicity to evaluate TPP. ADA production is T-cell dependent since B-cell maturation is driven by CD4+ T-cells that have been activated by human leukocyte (HLA) class II bound epitopes. Therefore, the IRA considers various T-cell assays and HLA analyses.

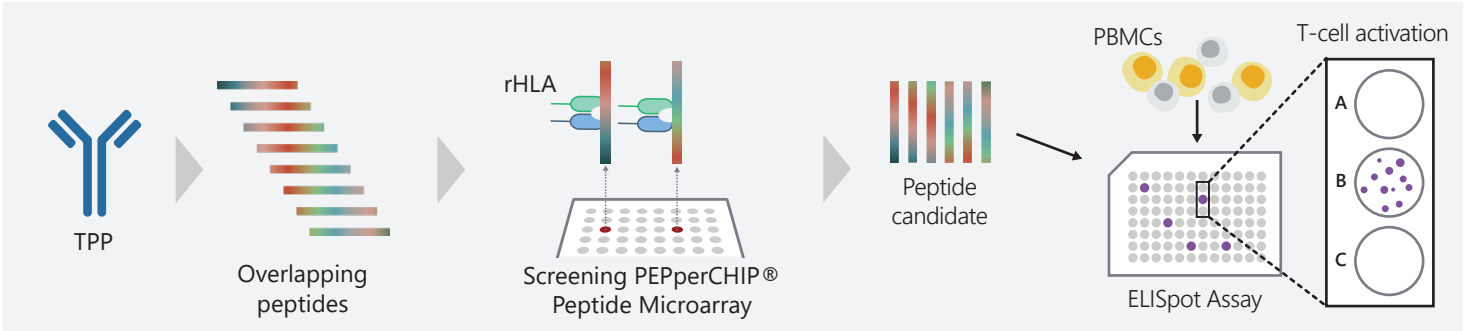


Immunogenicity screening starts with *in silico* assessment. FDA guidance recommends drug developers to profile potential immunogenicity with *in vitro* and *ex vivo* analyses, such as HLA binding assays, T-cell assays, and MHC associated peptide proteomics (MAPPs). PEPperPRINT develops a novel chip-based MAPP assay to screen TPPs without the need for patient material. Potential drug candidates are optimized by amino acid substitution scans. In that way, T-cell dependent immunogenicity risk can be decreased.

PEPperPRINT's IRA Tools in a Nutshell

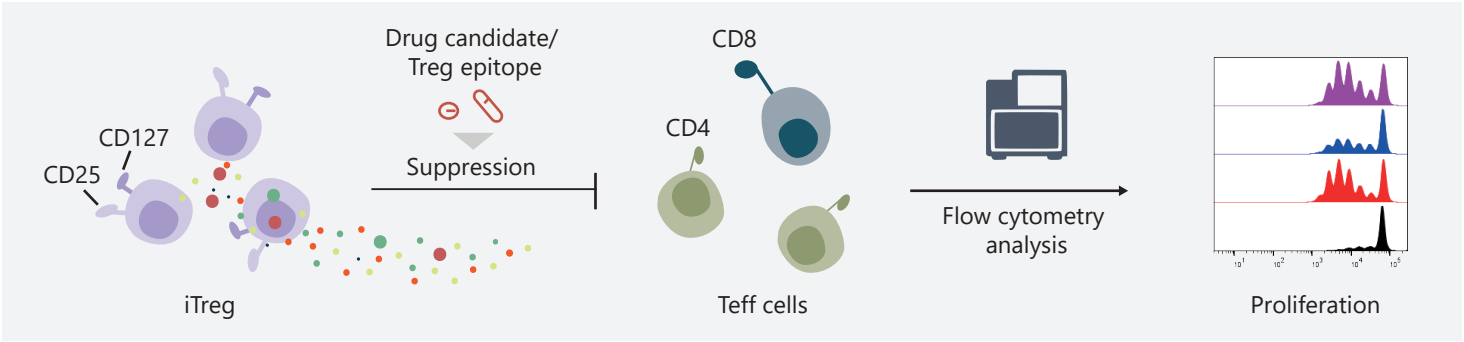
PEPperPRINT offers a collection of innovative IRA tools and assays that can be employed to detect anti-drug T-cell responses and develop a risk assessment profile as outlined below.

In vitro Assay: Chip-Based MAPP Assays



PEPperCHIP® Peptide Microarrays allow screening of thousands of peptides against recombinant HLA (rHLA) molecules, enabling the ranking of biological candidates by immunogenic potential. Identified peptide candidates are synthesized and tested in ELISpot/FluoroSpot assays with HLA-matched PBMCs.

In vitro Assay: Treg Suppression Assays

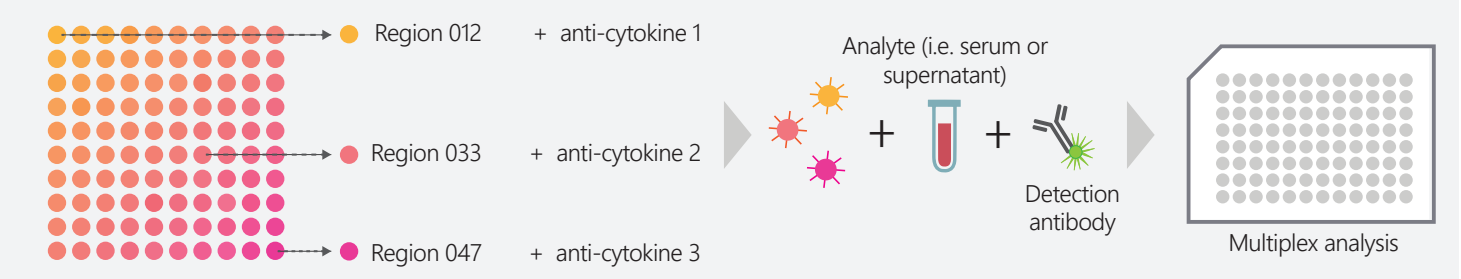


The TPP amino acid sequences can contain effector T-cell (Teff) and regulatory T-cell (Treg) epitopes. Teff epitopes lead to Teff cell activation and subsequent B-cell maturation resulting in unwanted ADA development. By contrast, Treg epitopes activate Treg cells to inhibit Teff cells and ADA formation. The presence of Treg epitopes is analyzed by co-incubation of Treg cells with Teff cells and immunogenic peptides. In our setup, PBMCs are cultured for 6 days in the presence of candidate Treg epitope at varying concentrations. Teff cells are stained for analysis by flow cytometry, and proliferation is measured by CellTrace.

In vitro Assay: Activation Induced Marker (AIM) Assays

AIM assays are widely-used tools for measuring ex vivo T-cell responses as a result of antigen-specific stimulation, without the knowledge of exact antigenic peptides and HLA restriction. PBMCs are stimulated with candidate peptide pools. Afterwards, cells are stained with our validated antibodies for early/late T-cell activation markers, and flow cytometry data is acquired. Customized phenotypic and characteristic T-cell response analyses are possible.

In vitro Assay: Multiplex Cytokine Arrays



Multiplex cytokine assays are used to measure cytokine secretion upon T-cell activation. While ELISPOT and flow cytometry are very robust methods for focused (IL-2, IL-4, IFN-γ) immunoassays, large multiplexed cytokine arrays (customized for a product's mechanism of action and potential process-related impurities) contribute to modelling of immunomodulatory pharmacology and safety endpoints (cytokine release syndromes) of a TPP.