NCIRA Best Practices recommendations for MAPPs assays; development and application

MAPPs Assays For Non-Clinical Immunogenicity Risk Assessment: Best Practices Recommended By The European Immunogenicity Platform

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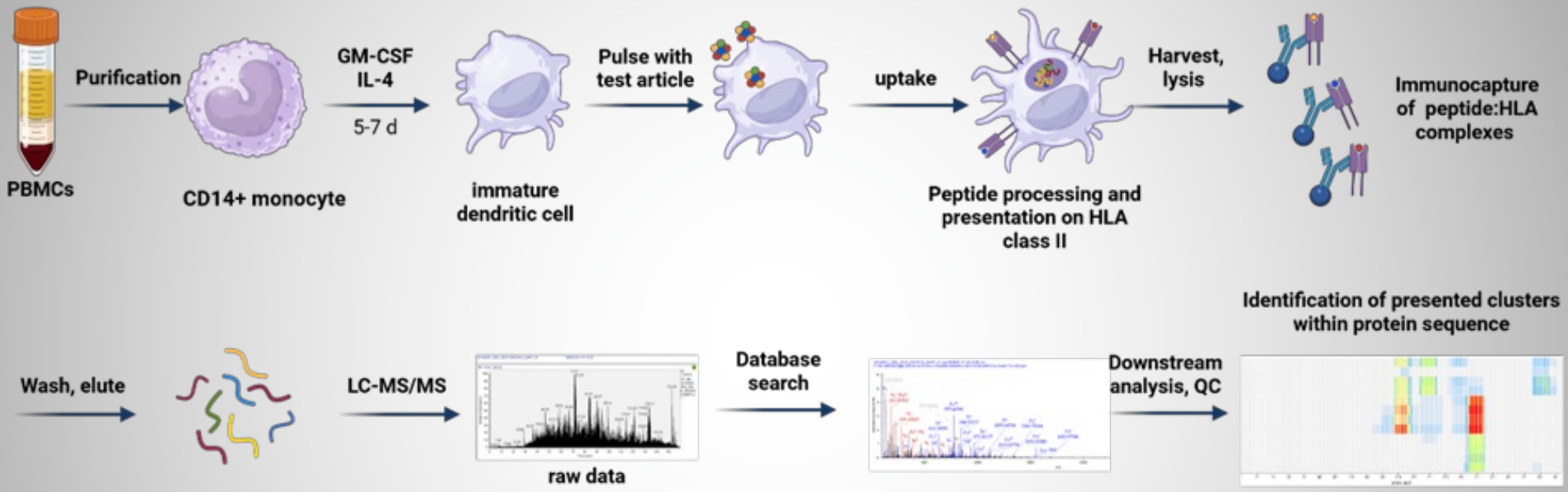
Diversity of protocols



Protocol

- Source, type and number of APCs
- Assay media
- Differentiation, pulsing and activation conditions
- Immunocapture steps
- LC-MS equipment
- Data analysis

Test article

- Whole proteins: to take into account antigen uptake and processing
- Quality of protein

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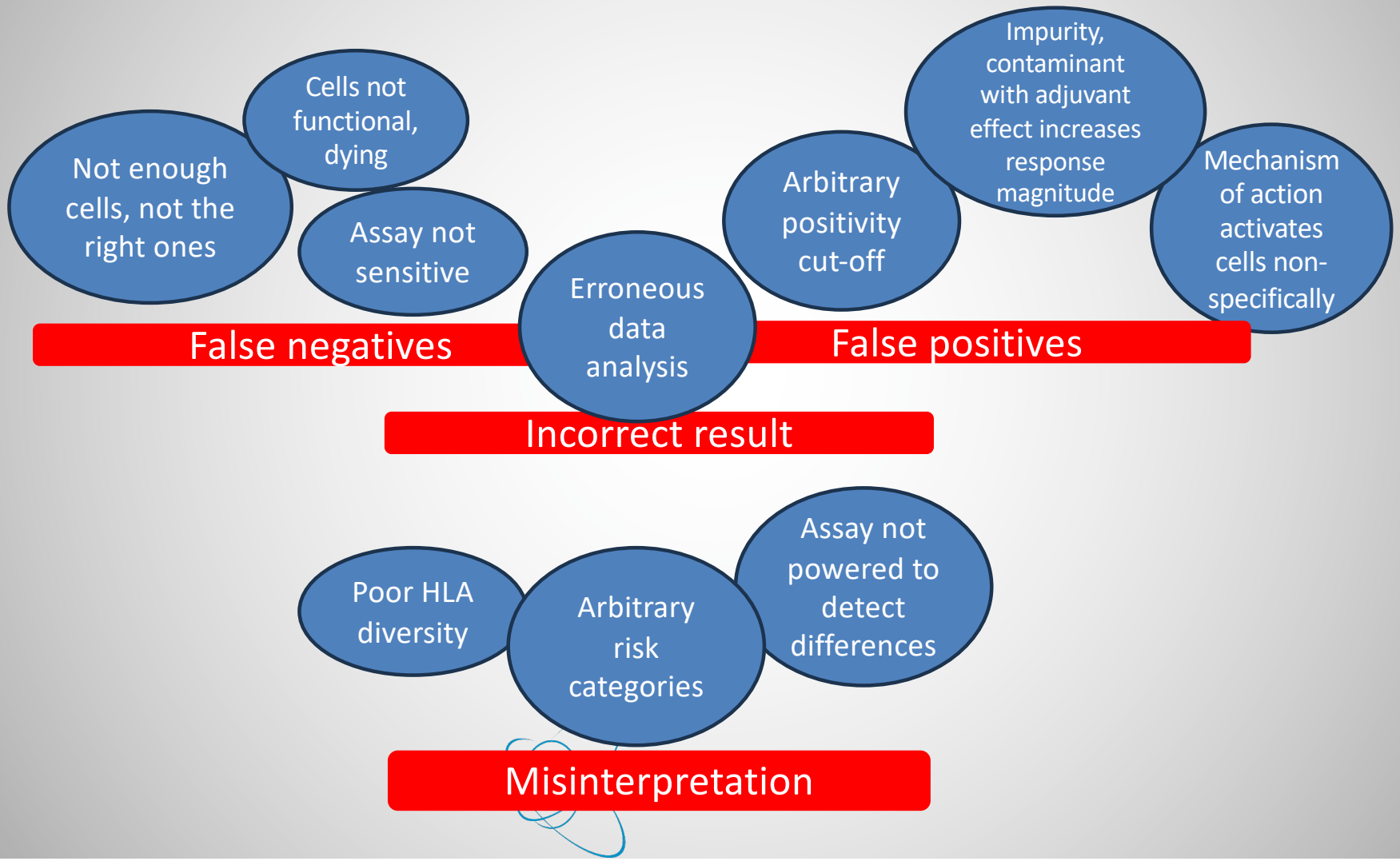
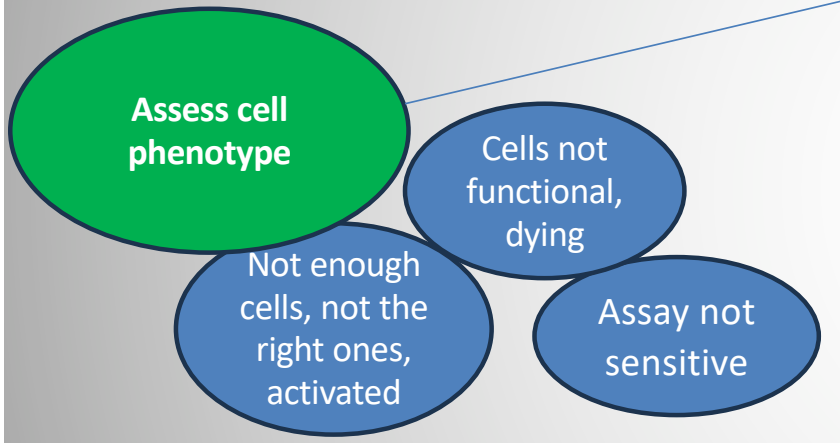
Potential points of failure



False negative remediation



Assess cell phenotype

- Source and nature of APCs used (fresh/cryo)
- CD14 differentiated into moDC
- Number of moDCs
- Media
- Cell viability
- DC activation state - expression levels of CD11c, CD83, CD86, CD40, HLA-DR on APCs (flow cytometry)

False negatives

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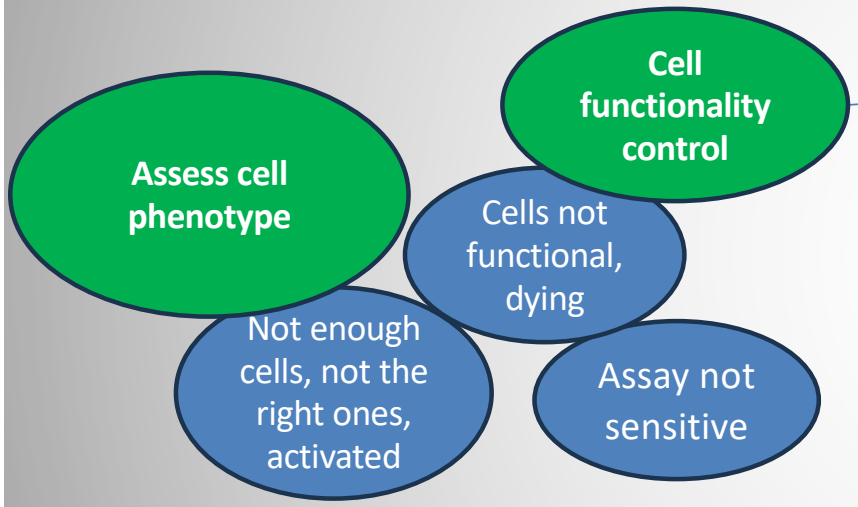
False negative remediation



False negatives

Cell functionality control

- Endogenous protein: verifies processing and presentation occurs at expected level.
- e.g. HLA-DRA, GAPDH, Cathepsin, Annexin or the human proteome

- Exogenous protein with known presentation pattern: verifies that cells are capable of internalization. e.g. Ovalbumin, Betv 1a, infliximab, rituximab, bovine protein if using FBS in culture media, human albumin if using human serum or serum-free medium

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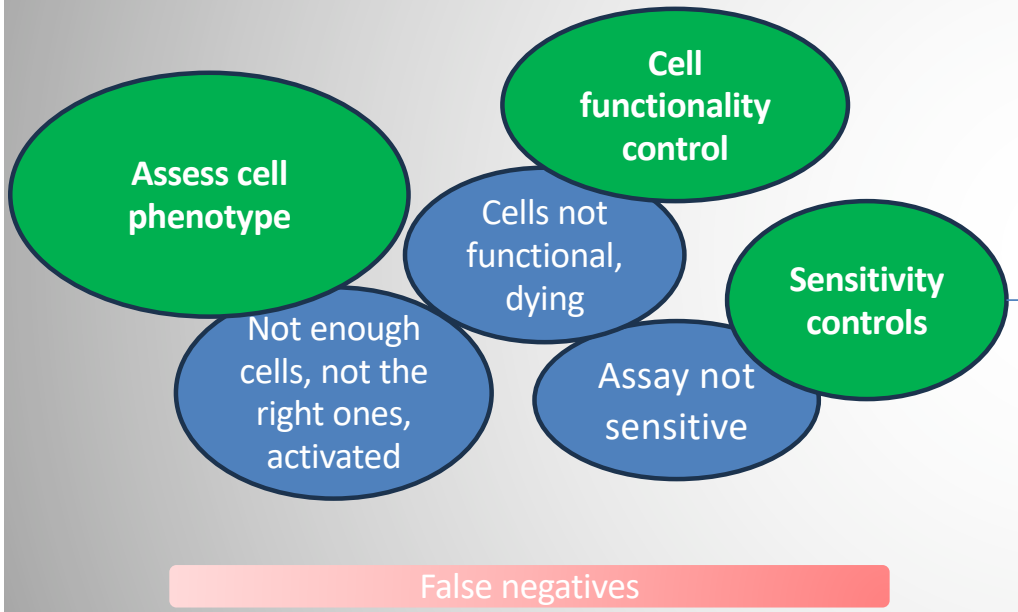
False negative remediation



Sensitivity controls

1. Antigen presentation: verifies the capacity of the assay to detect low number as well as high numbers of presented peptides. e.g. adalimumab, rituximab as high sensitivity and ustekinumab or trastuzumab as low sensitivity controls
2. LC-MS instrumentation performance control: low-level peptide identification standards (e.g., HeLa digests). Verifies broad and reproducibility elution profile across hydrophilic and hydrophobic peptides.
3. Western blot: assess HLA solubilization and pull-down efficacy
4. Histogram of size distribution: verifies that peptide lengths are those expected for HLA class II
5. Include common PTM in the search database to identify modified peptides

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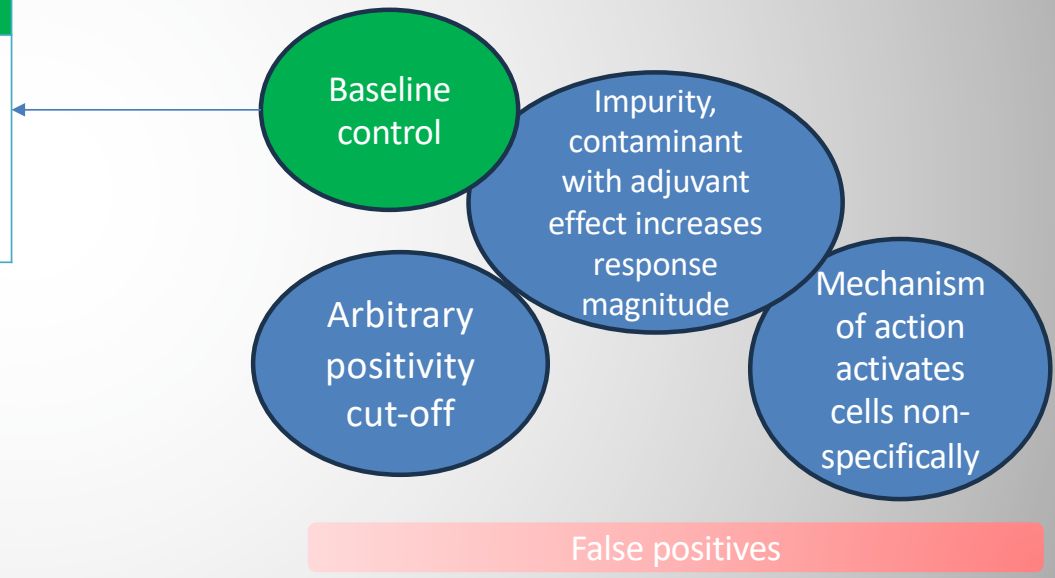
False positive remediation

Baseline control

Untreated samples for each donor

Evaluate whether drug-derived peptides detected in treated samples could share partial identity with other proteins and therefore be false positives



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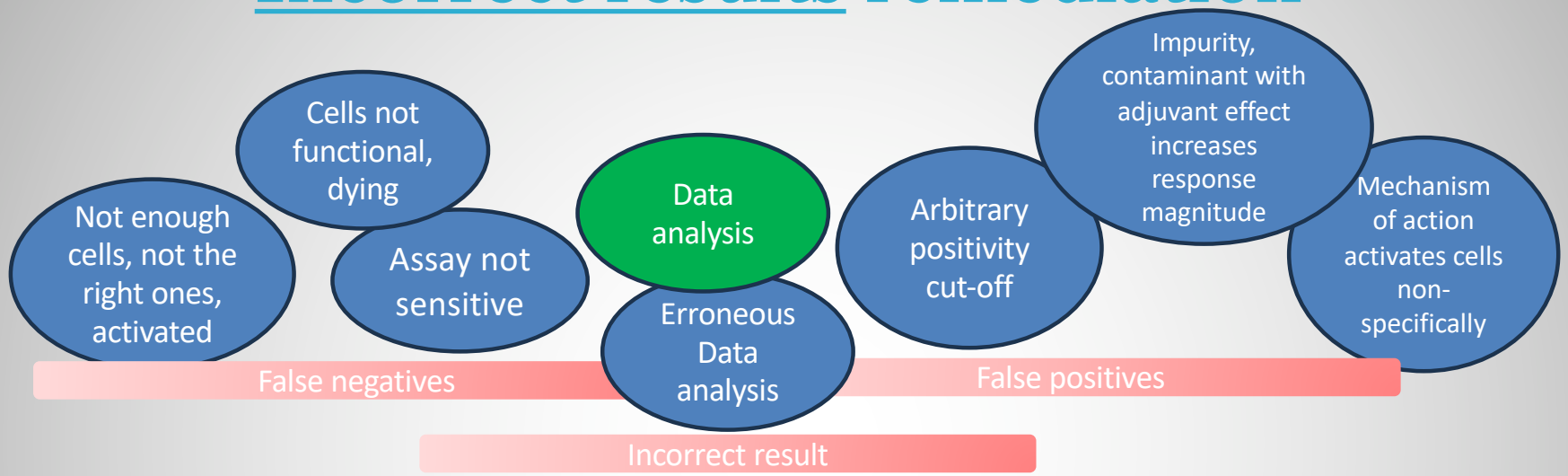
Incorrect results remediation



Data Analysis	
LC-MS analysis	• MS data collected in DDA acquisition mode • DIA may be more accurate but requires a pre-study • FDR (typically 1%) • Immunopeptidomics analysis (not proteomics) • Number of total and unique peptide clusters • Regions with multiple peptides that are presented across multiple donors are generally higher risk

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Concluding remarks

- The HLA class II MAPPs assay has become a major tool to mitigate unwanted immunogenicity of protein therapeutics at the design phase.
- A technical limitation of the MAPPs assay remains its relatively low throughput, typically 6-8 molecules and controls tested in 12-24 donors per month.
- Automation and increased computing power may also help with throughput.
- A critical step of the MAPPs assay has been to generate moDCs from monocytes, which requires a steady supply of large quantities of fresh blood and a rather long differentiation protocol.
- To date, there has been no cellular substitute for moDCs, for example, in the form of stable, immortalized cell lines with sufficient HLA coverage that could be rapidly generated.
- Other alternatives have been proposed, such as multiplexing the MAPPs assay by combining several test articles in one sample.
- Use of HLA class I MAPPs for mRNA and CGT (where CTL epitopes are more important).
- Potential for the use of patient DCs for MAPPs.

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