

NCIRA Best Practices recommendations for DC maturation assay development and application

Dendritic cell maturation assay for non-clinical immunogenicity risk assessment: best practices recommended by the European Immunogenicity Platform

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DC maturation process

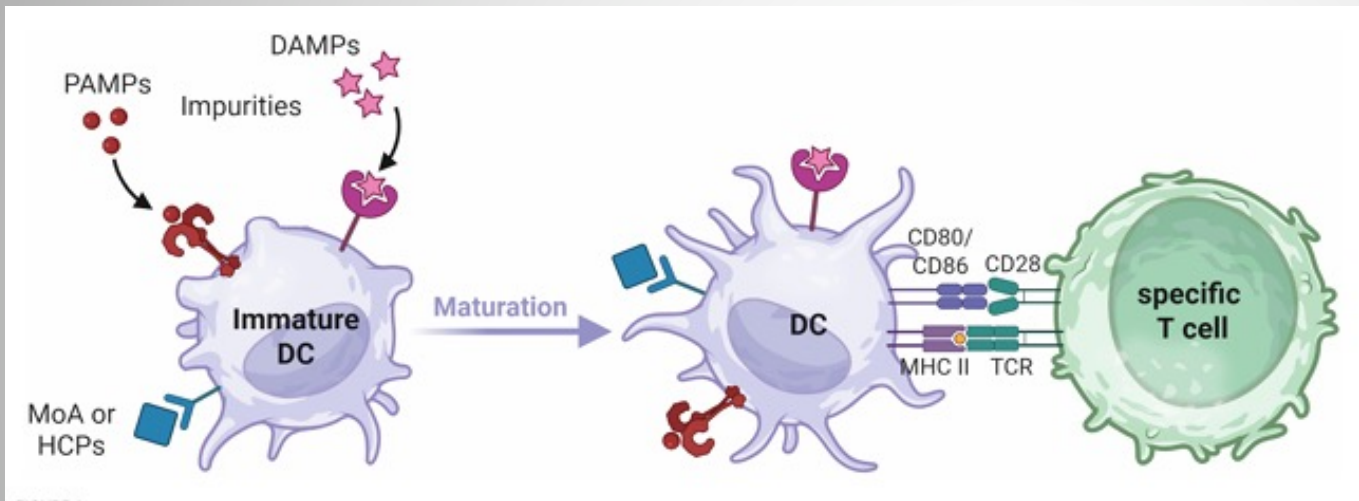


FIGURE 1

DC maturation process. Upon encountering PAMPs, DAMPs, MOA related stimuli, or impurities that trigger corresponding pathways, immature DCs undergo maturation. This process leads to morphological changes, increased processing and presentation of internalized antigens via HLA class II molecules and the upregulation of costimulatory molecules on the DC surface. T cell activation requires recognition of cognate antigen-derived peptides on HLA class II molecules (Signal 1) as well as interaction with costimulatory molecules on mature DCs (Signal 2). Created in BioRender. Karle, A. (2025) <https://BioRender.com/fmrjm0v>.

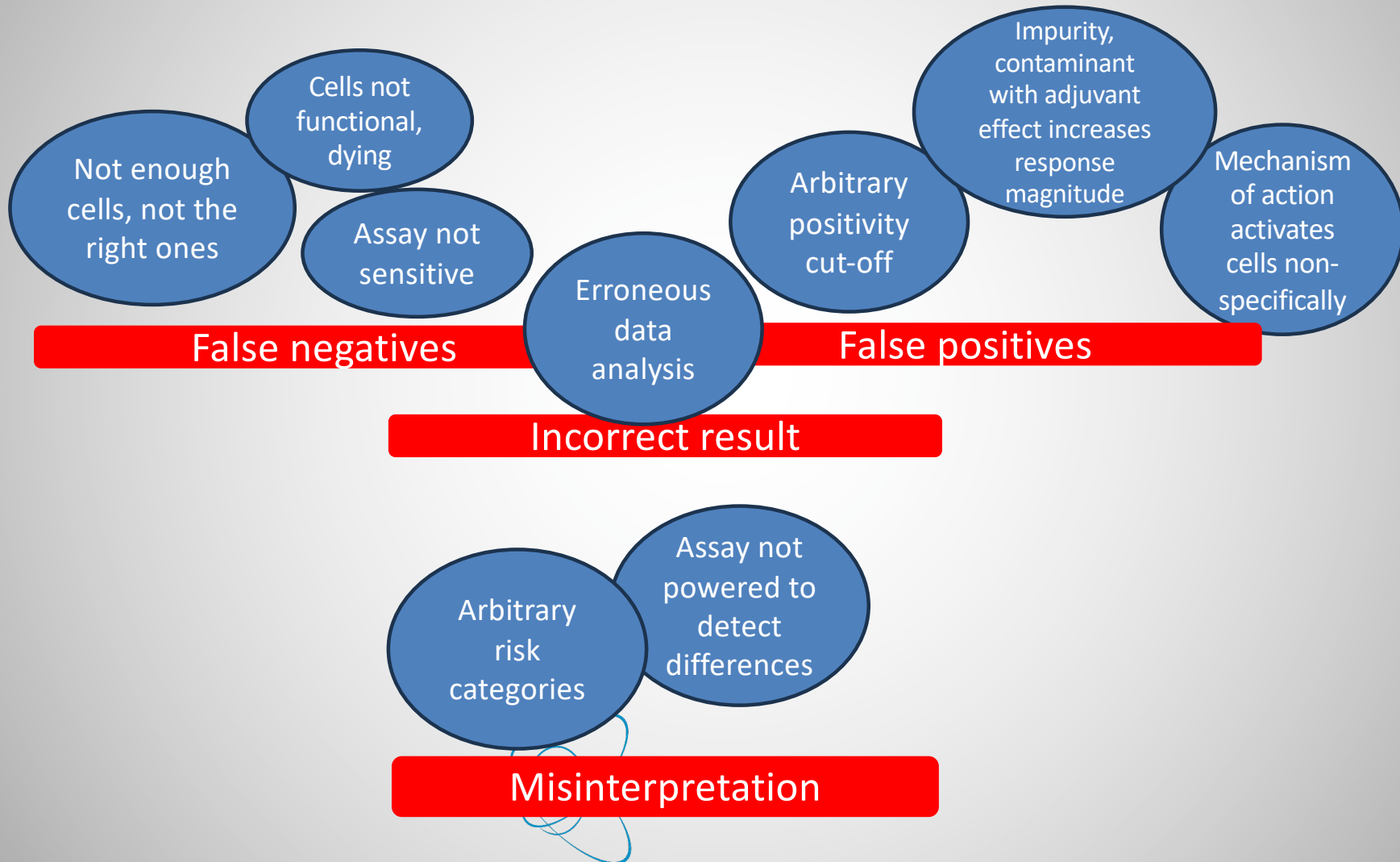
DCs

- Link innate & adaptive immune response
- Provide signal 1, 2 and 3 for T cell stimulation & differentiation
- Assay designed to evaluate a therapeutic's potential to trigger innate immune activation

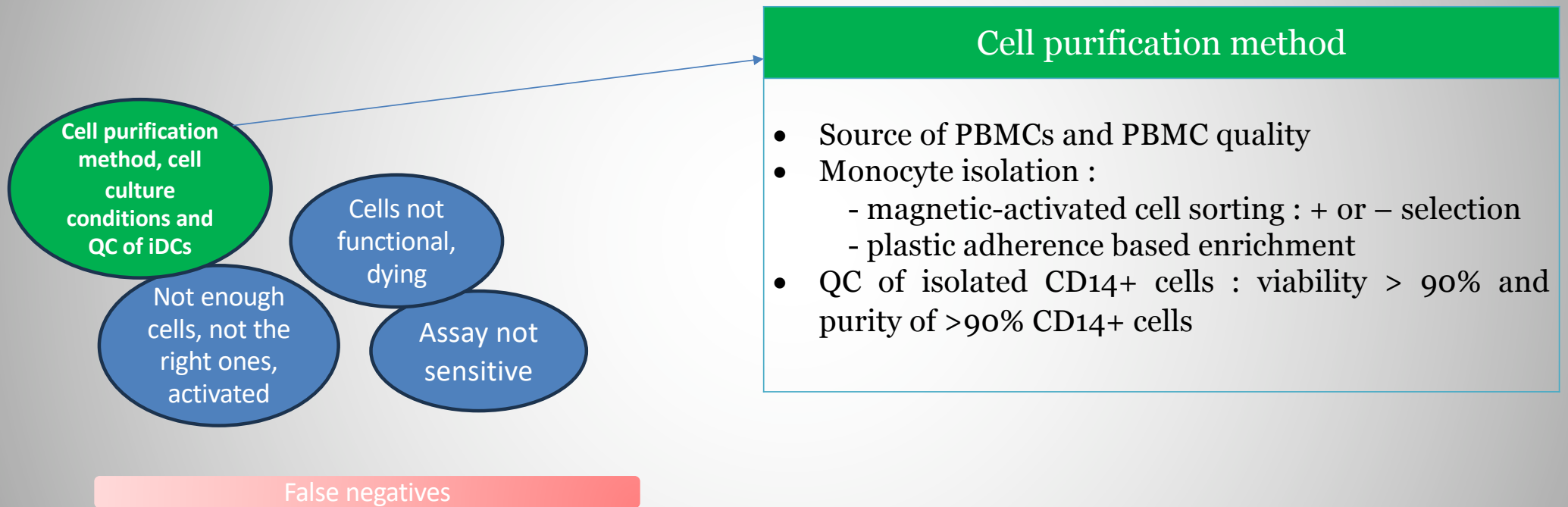
Purpose/objective

- assesses the ability of a test article to induce the maturation of immature moDC
- comparison and ranking of different test candidates in terms of their ability to induce DC maturation
- assessing the mechanistic impact of aggregated species within therapeutic products
- mechanistic studies

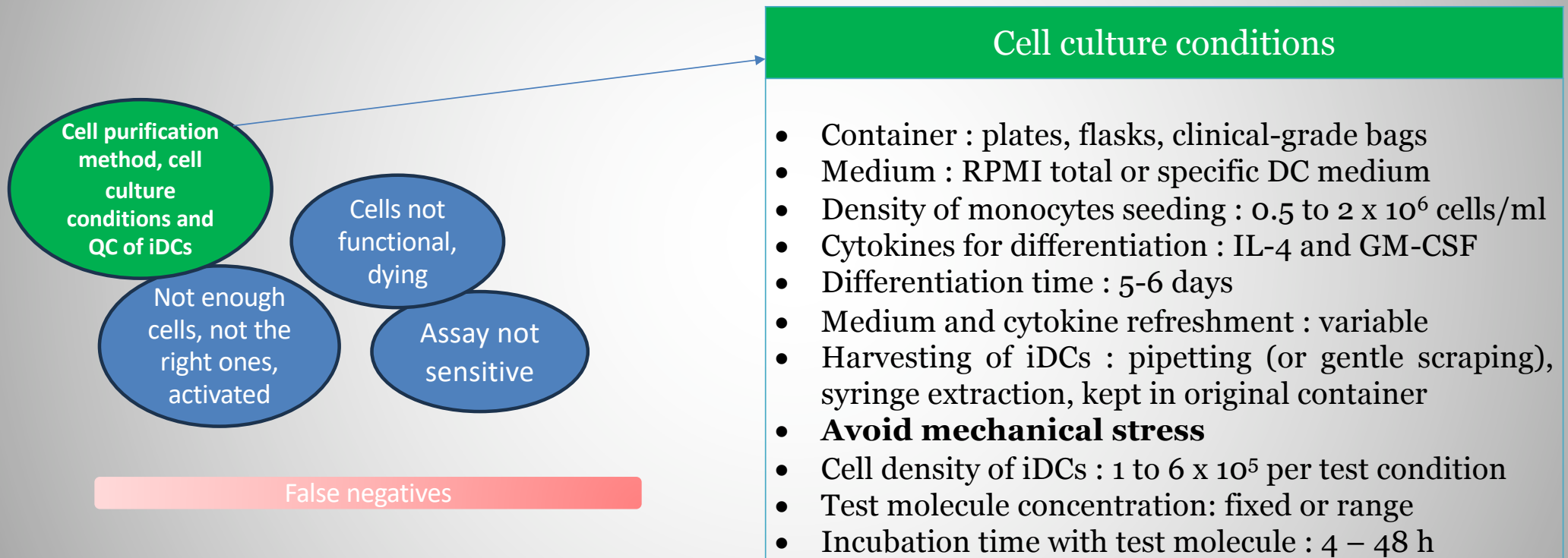
Potential points of failure



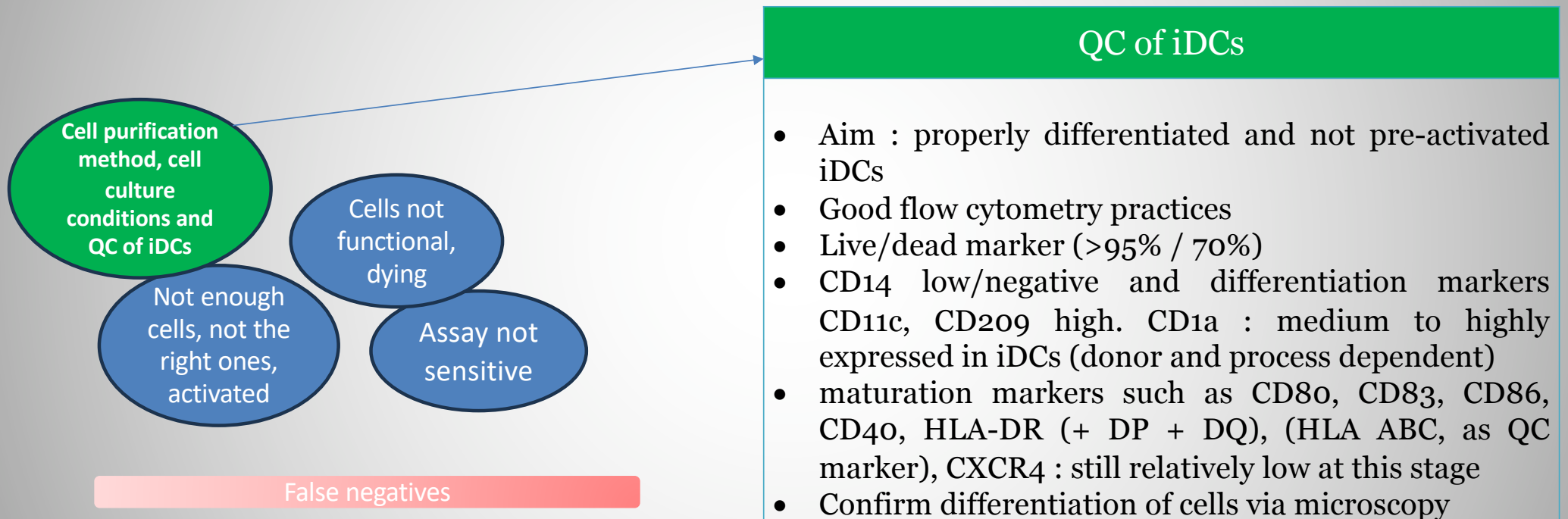
False negatives remediation -1



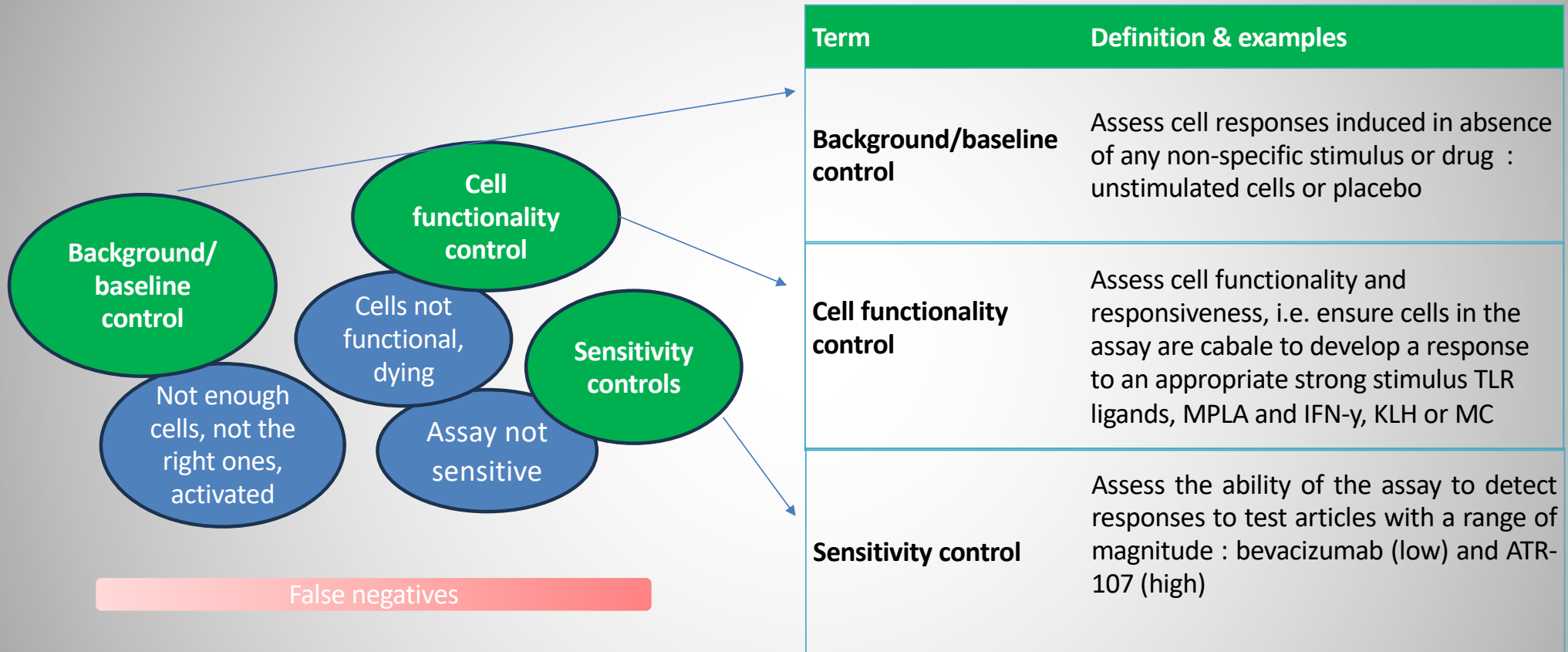
False negatives remediation -1



False negatives remediation -1

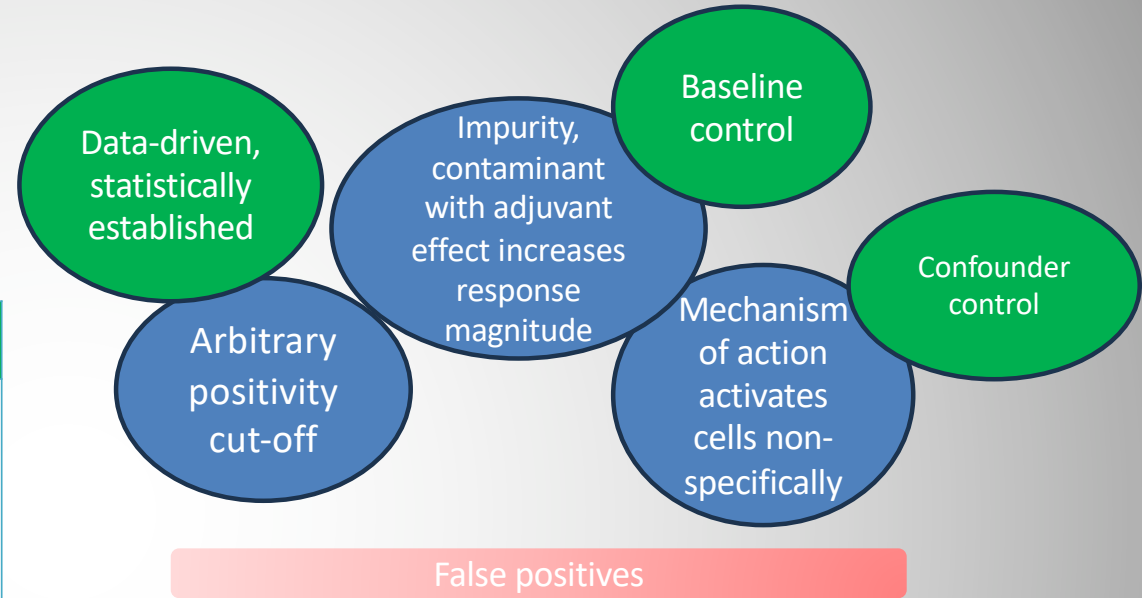


False negative remediation - 2



False positives remediation

Term	Definition
Baseline response control	Assess cell responses induced in absence of any non-specific stimulus or drug : unstimulated cells or placebo
Confounder control	To differentiate undetermined activation mechanisms from biological engagement : target on DCs



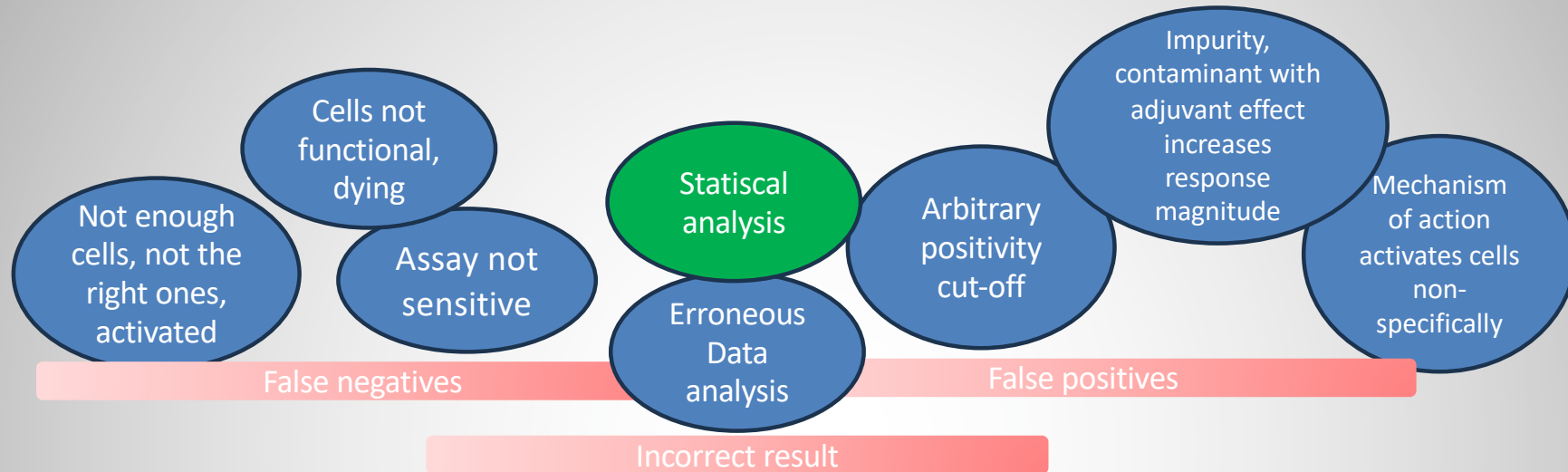
Quality of controls!

QC and assessment of mature DCs

Category	Markers
Viability	Live/dead viability dye
Phenotypic characterization	CD11c, CD1a and/or CD2019 and (optional) CD14
Maturation markers	CD80, CD83, CD86, CD40, HLA-DR (DP, DQ), HLA ABC, CXCR4
Minimal QC panel	L/D dye and CD80/CD83/CD86 and HLA-DR
Exclusion criteria	<ol style="list-style-type: none">1) Viability < 80%2) No maturation with functionality control3) Strong activation in baseline control
DC maturation read-out	<ol style="list-style-type: none">1) Flow cytometry : CD80, CD83, CD86, CD40, HLA-DR (DP, DQ), HLA ABC, CXCR42) Cytokine secretion in SN : IL-1β, IL-6, IL-8, IL-12p40, TNF-α3) PCR : mRNA expression4) Cell signaling studies

Remediation

Incorrect results



Interpretation of results	
Parameter	Details
Positivity	Specific values depend on FACS reagents and settings Positivity always in context of the sensitivity controls (low – medium – high) used Predefined markers and ratios Fold changes to overcome inter-donor variations
Replicates	Depending on stage of assay development
Number of donors	5 to 10
Statistical analysis	Fold change, 2-way ANOVA, equivalence testing

Concluding remarks

