NCIRA Best Practices recommendations for T cell assays development and application

T cell assays for non-clinical immunogenicity risk assessment: best practices recommended by the European Immunogenicity Platform.

Tourdot, S., Karle, A. C., Rosenbaum, M., Ackaert, C., Le Vu, P., Gutknecht, M., Ahmadi, M., Turksma, A. W., & Hickling, T. P.

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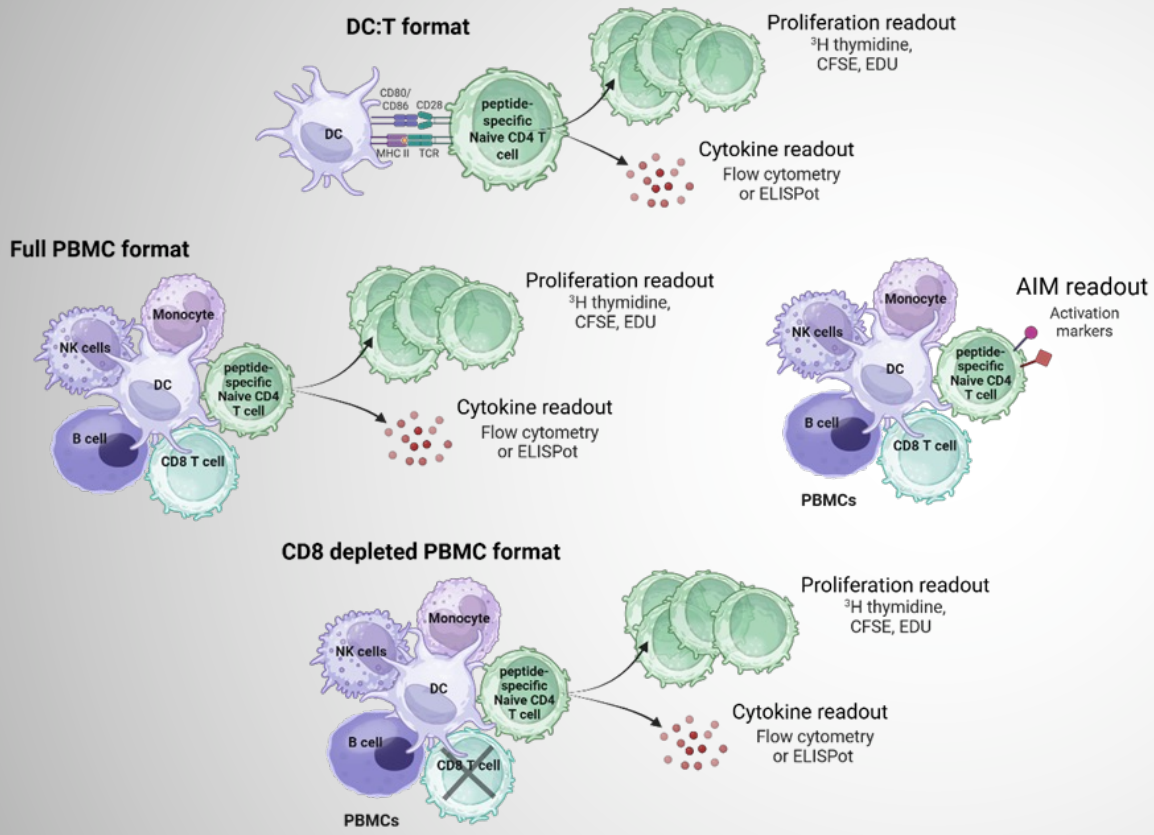
Diversity of formats and protocols



Protocol

- Test article in contact / not in contact with all cells
- Restimulation
- Timing of measurement
- Readout

Test article

- Whole protein & long peptides: antigen uptake and processing is involved
- Short peptides that represent segments of a protein/long peptide: does not inform processing

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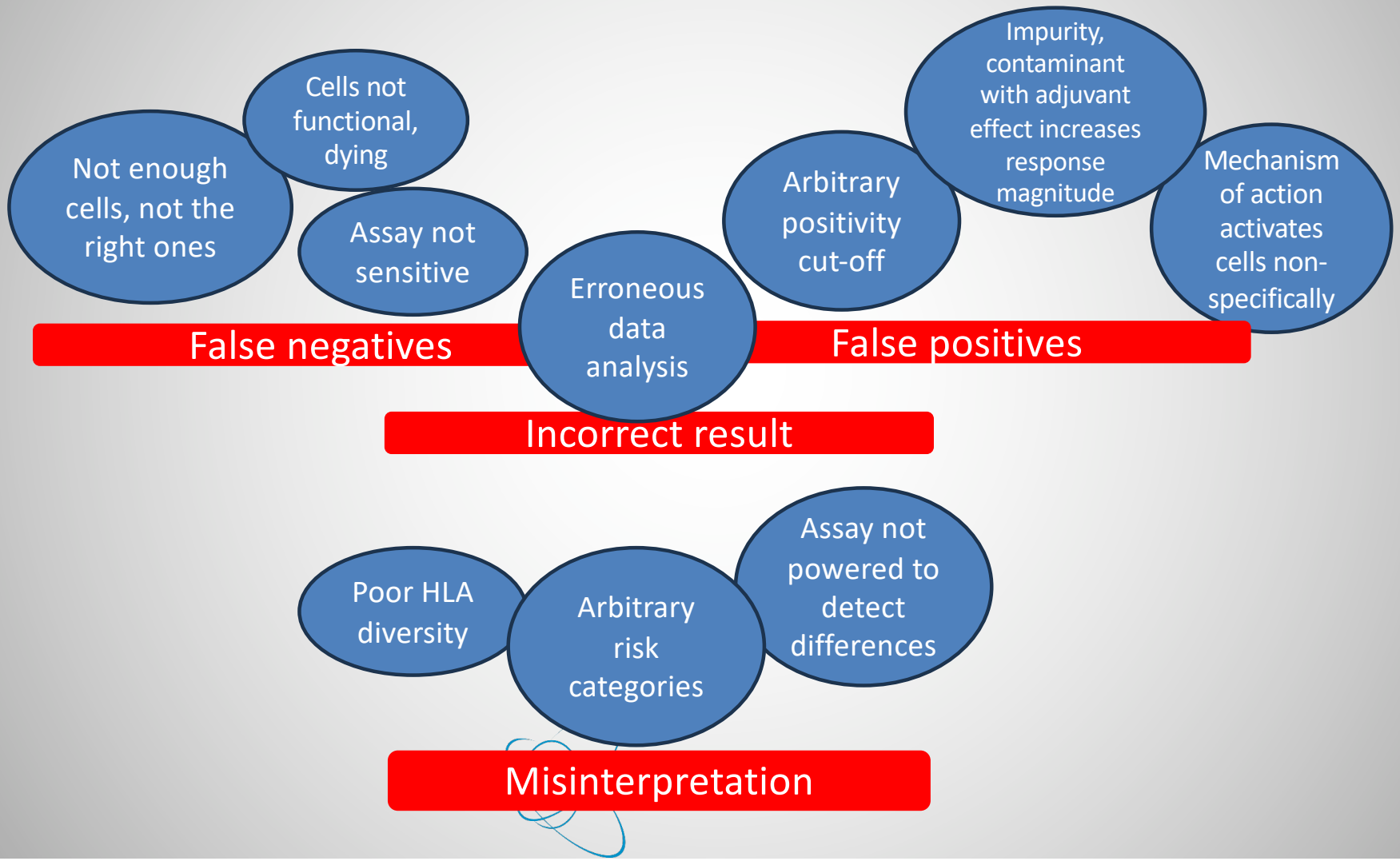
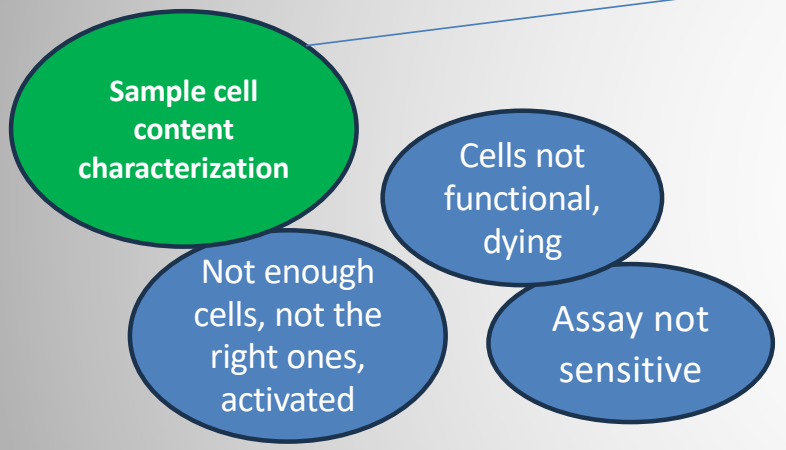
Potential points of failure



False negatives remediation -1



False negatives

Assess cell composition and phenotypes by flow cytometry

- Ratio between B and T cells
- Frequency of APCs
- Content of naïve vs memory CD4+/CD8+ T cells (CD45RA, CD45RO)
- Content of regulatory T cell (Tregs) (CD25, CD127, or intracellular staining for FoxP3)
- Activation state:
- Expression levels of CD69, CD40L on T cells
- Expression levels of CD83, CD86, CD40, HLA-DR on APCs

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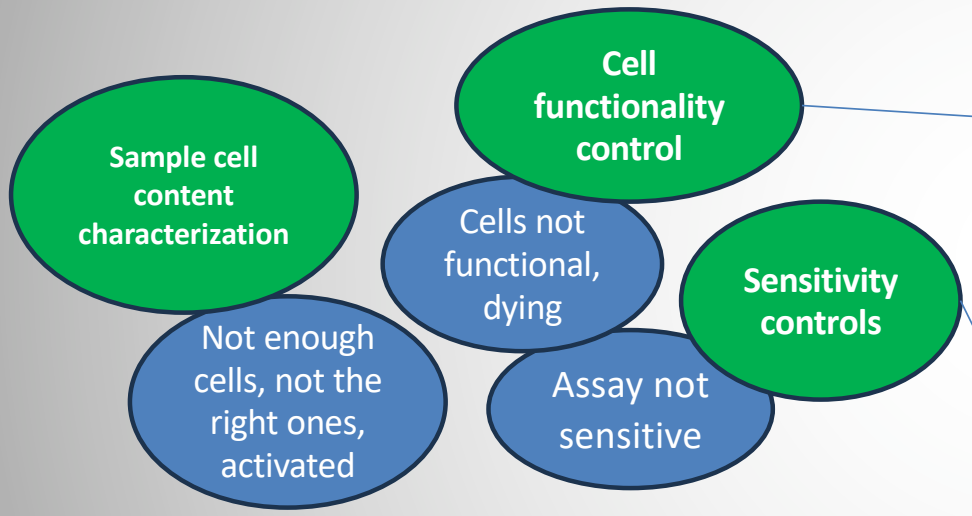
False negative remediation - 2



Term	Definition
Cell functionality control	Assess cell functionality and responsiveness, i.e., ensure cells in the assays are capable to develop a response to an appropriate strong stimulus
Sensitivity control	Assess the ability of the assay to detect responses to test articles with a range of magnitude.

False negatives

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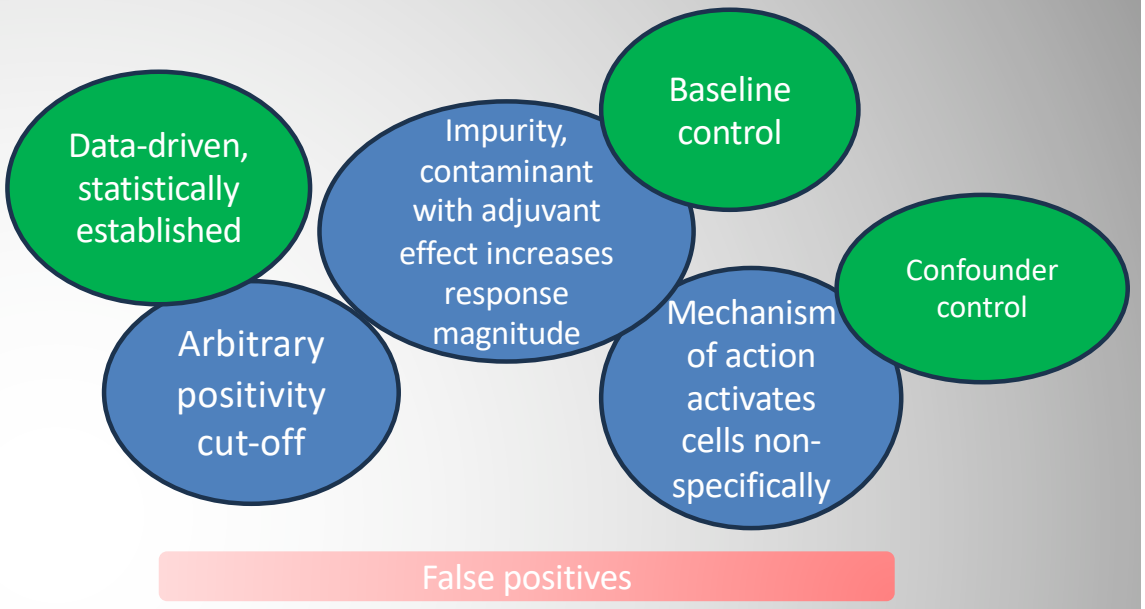
Cell functionality and Sensitivity Controls – T cell assays

Category	Details	Examples
Cell functionality control	Assess cell functionality and responsiveness, i.e., ensure T cells in the assay are capable to develop a response to an appropriate strong specific or non-specific stimulus	Strong antigen-specific naive responses: Keyhole limpet hemocyanin (KLH) Strong non-specific responses: anti-CD3 and anti-CD28 antibodies; Phytohemagglutinin (PHA) Of note, staphylococcal enterotoxin B (SEB) should be used with caution as aerosols could contaminate neighboring wells
Sensitivity controls	Assess the ability of the assay to detect responses to test articles with a range of donor response frequencies Biologically relevant sensitivity controls should: • Where possible, be of the closest format and length as test articles i.e., protein, short peptides, longer peptides • For short peptides, individual peptides are preferred as peptide pools tend to give larger responses. Pairs of control peptides can be used to cover required HLA breadth • Induce a naïve or recall response based on the type of donor material used: healthy donors (naïve) or treated-patient samples (recall) • Exhibit the anticipated donor response frequencies or represent a range between lower and higher responses • Be commonly used, chosen based on own experience or the scientific literature	<u>Short synthetic peptides:</u> Single peptide with promiscuous HLA binding - *Naïve responses:* therapeutic-derived peptides validated ex vivo (CD4+ T cell epitopes from e.g., rituximab, infliximab and natalizumab); PADRE [91] in assays with rounds of restimulation - *Recall responses:* Peptides from viruses most donors will have been exposed to (infection or vaccination, e.g., HLA II-restricted CEFT, i.e. peptides derived from human Cytomegalovirus (CMV), Epstein-Barr Virus (and Influenza Virus (Flu) and Tetanus Toxoid (TT); HLA I-restricted CEFT peptides) <u>Whole proteins:</u> Naïve responses: Therapeutics commonly used in in vitro assays such as ATR-107 (high T cell response frequencies), bococizumab (high T cell response frequencies), bevacizumab (low T cell response frequencies) and natalizumab (intermediate T cell response frequencies) Recall responses: same as for peptide controls but whole protein format e.g., tuberculin purified protein derivative (PPD), tetanus toxoid (TT), Influenza virus hemagglutinin (Flu HA) <u>Generic peptides:</u> reference product, lixisenatide (high T cell response frequencies), salmon calcitonin (high T cell response frequencies)

False positives remediation

Term	Definition
Baseline response control	Assess cell responses induced in absence of any non-specific stimulus or drug
Confounder control	Evaluate cell responses triggered by drug components that are not the focus but could interfere with the T cell response assessment



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Baseline response and Confounder Controls – T cell assays		
Category	Details	Examples
Baseline response control	Assess cell responses induced in absence of stimulus	Reconstitution medium, e.g. DMSO for synthetic peptides Relevant buffer/formulation Medium alone
Confounder control	Concern complex modalities Evaluate cell responses triggered by drug components that are not the focus of but could interfere with the T cell response assessment	Cytokines for cytokine fusion proteins Vector alone, cargo alone for products comprising a vector and a cargo (e.g., mRNA-LNPs)

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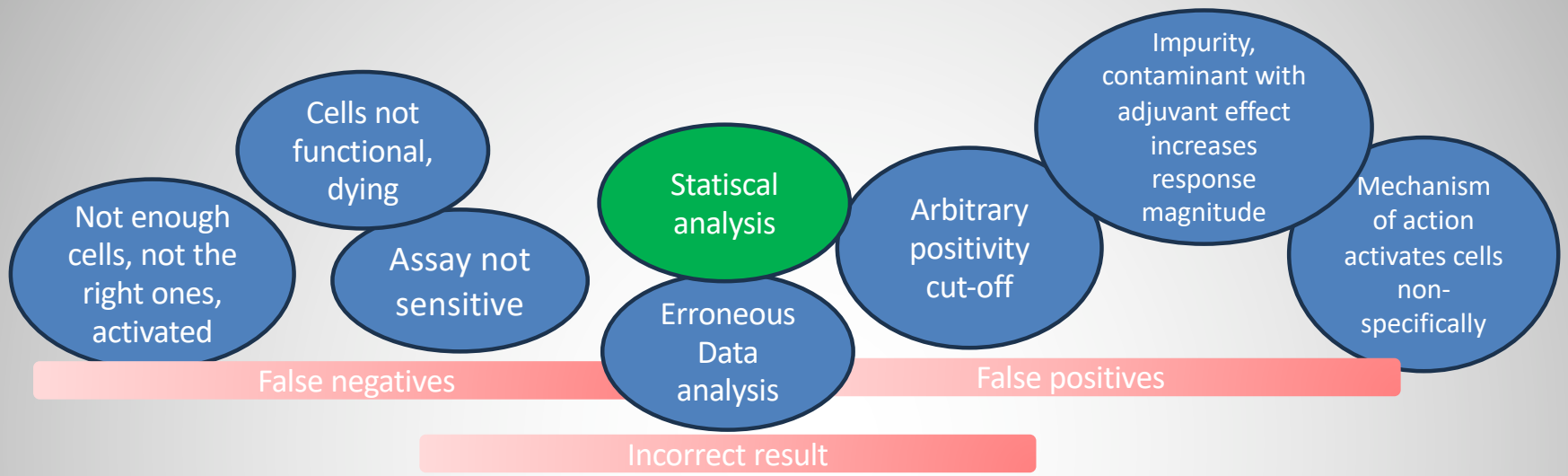
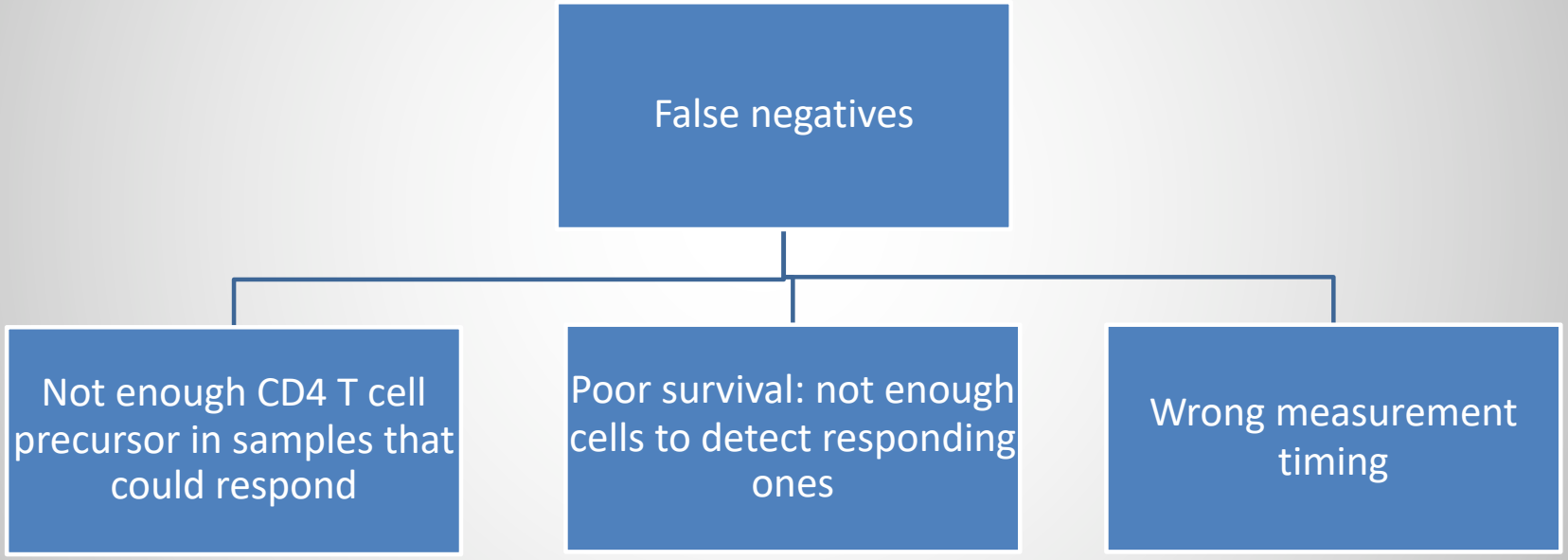
Remediation

Incorrect results



Data Analysis	
Parameter	Details
Donor response frequency to a test article	Can be used to rank-order molecules and estimate an immunogenicity potential relative to the sensitivity controls Donor response frequency differences between compounds should be interrogated statistically and the degree of confidence should be specified
Donor response magnitude to a test article	Can indicate donor-specific factors that influence the response and might not be detected with the baseline control. Statistical analysis should include relative responsiveness of the donor across all tests to identify hyper- or hypo-responsiveness.

False negatives: additional considerations



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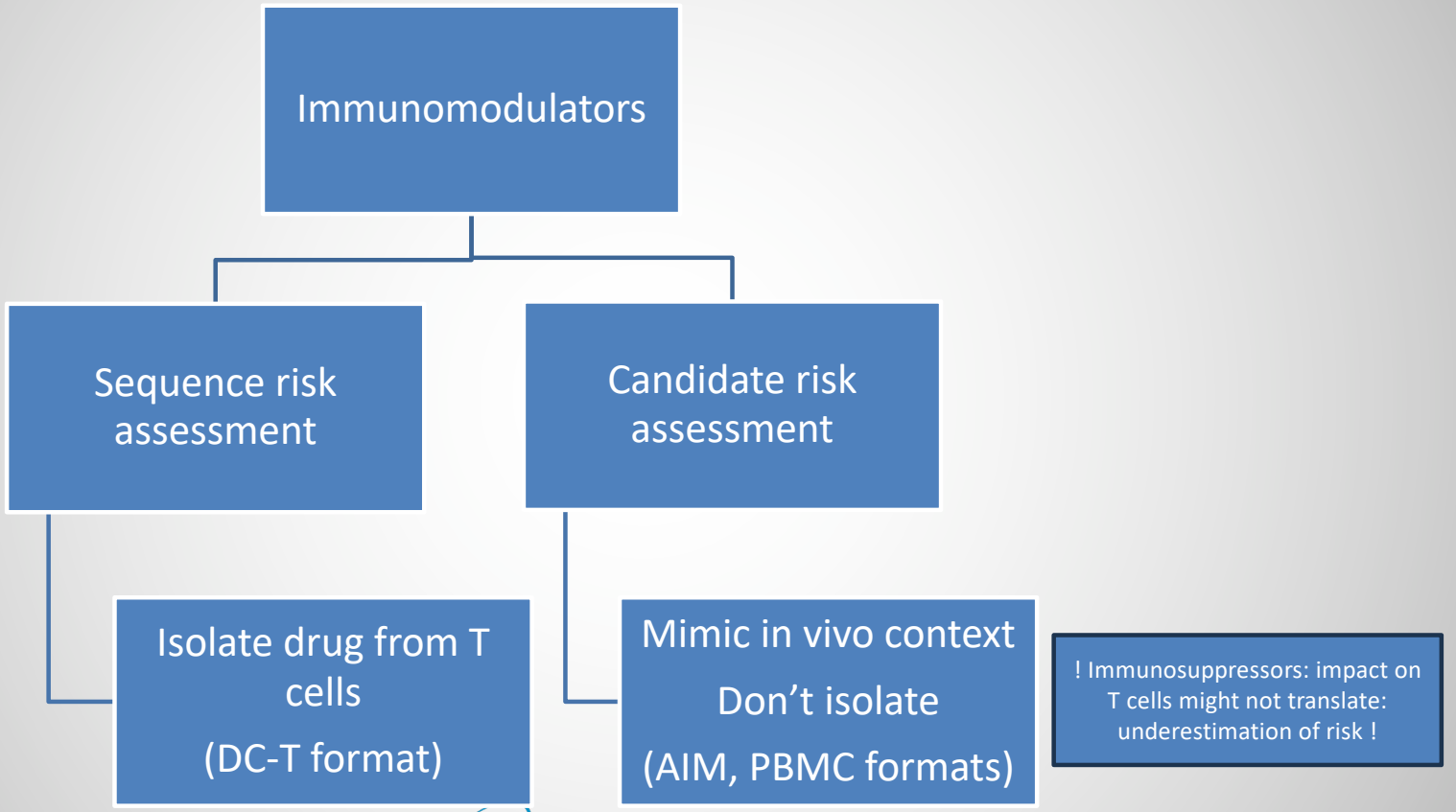
Additional considerations – Mechanism of Action



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