Go with the flow?

Ligand binding versus flow cytometry methods for the analysis of anti-drug antibodies in support of CAR-T cell trials

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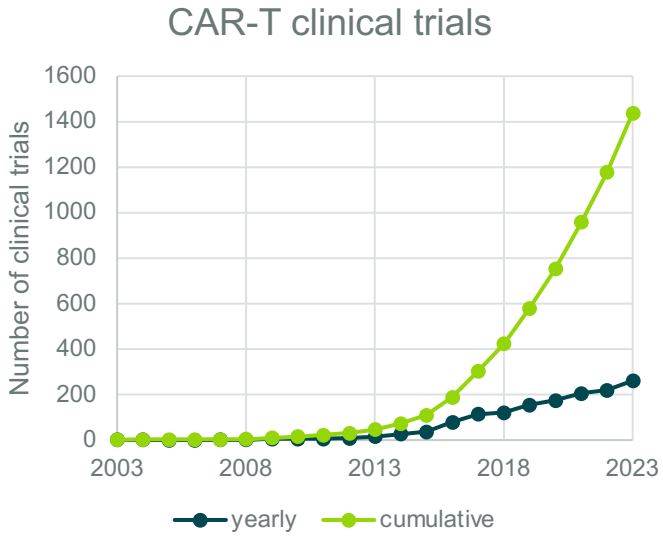
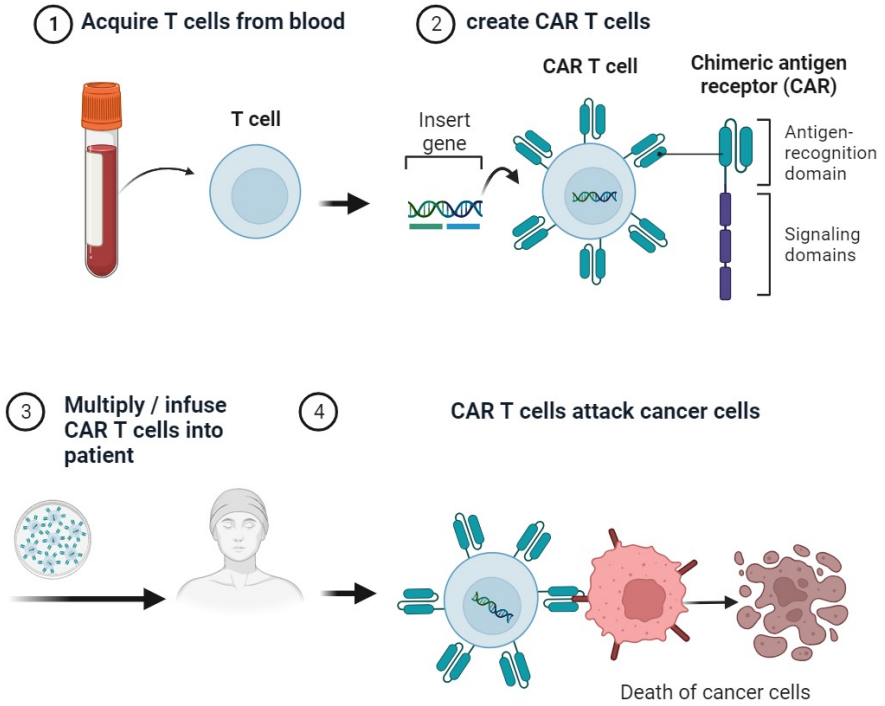
Outline

What method should be used for measuring anti-drug antibodies (ADA) against CAR-T?



- Background CAR-T
- Methodology and case studies
  - ➞Ligand binding assays (LBA)
  - ➞Flow cytometry assays
- Pros/cons
- Suggested workflow

Chimeric Antigen Receptor T-cells (CAR-T) can trigger ADA



ADA assays with immobilized drug

For CAR-T, surrogate:

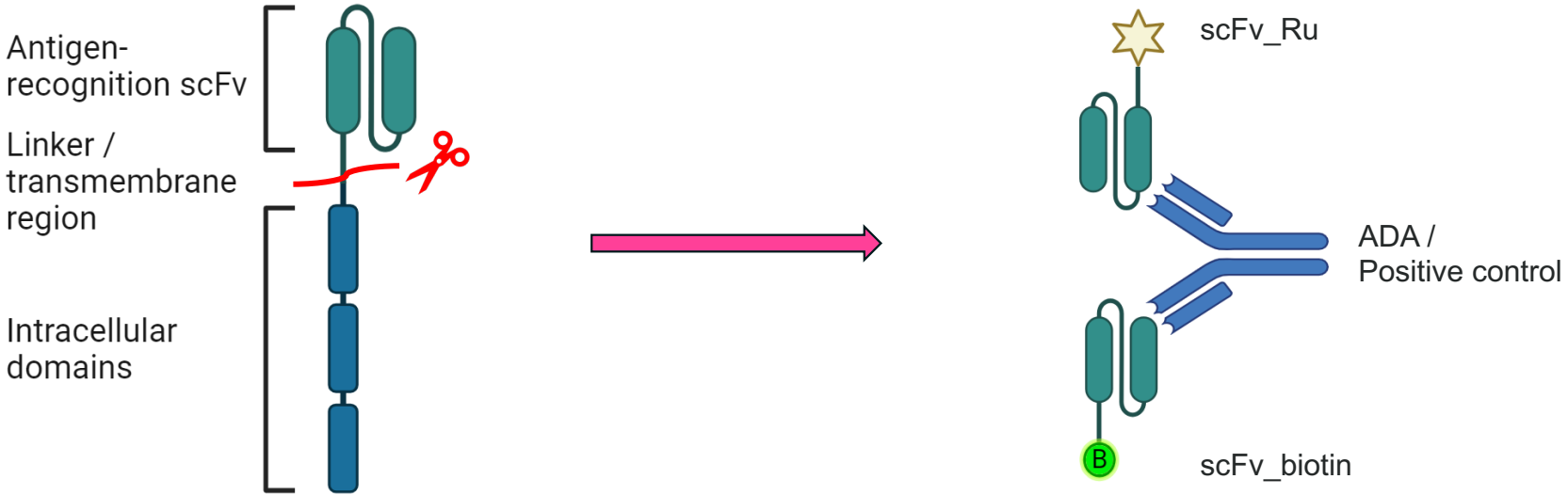
- CAR protein (ligand binding)
- CAR cell line (flow cytometry)

Methodology: ligand binding using purified CAR domain

Acquire truncated protein

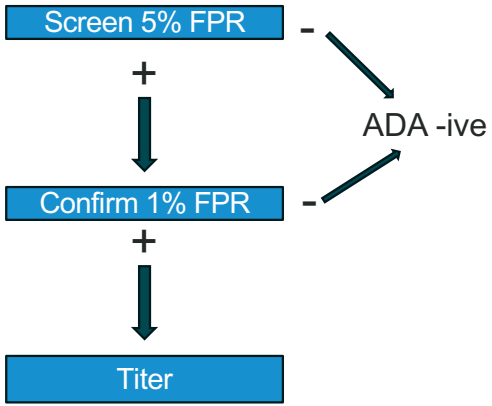
Label protein

Bridging immunoassay

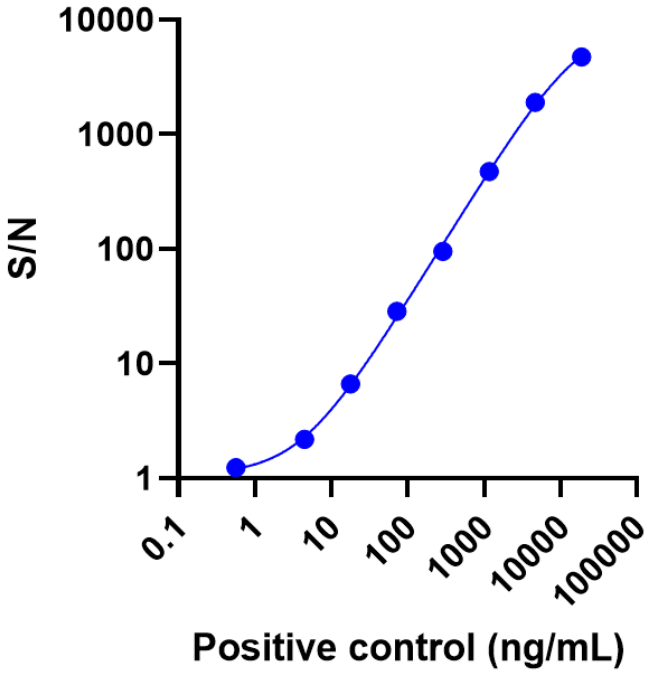


LBA: tiered analysis and high sensitivity

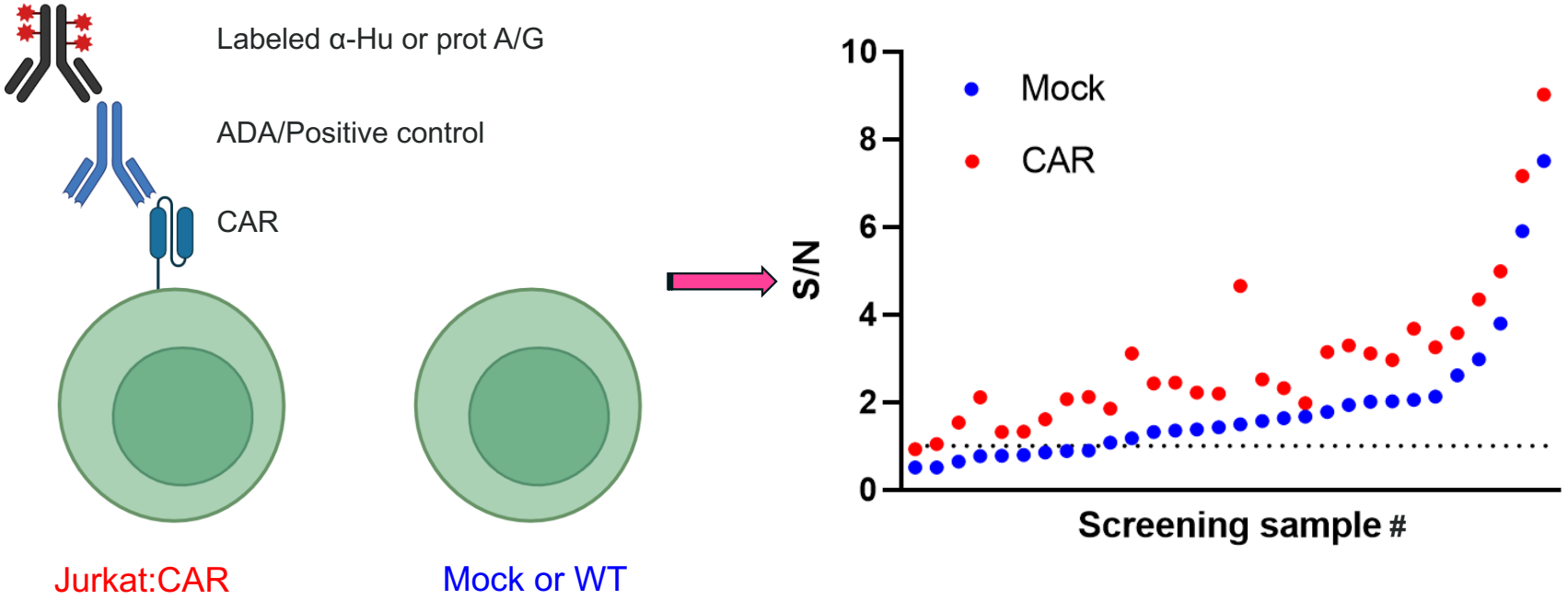
Tiered analysis



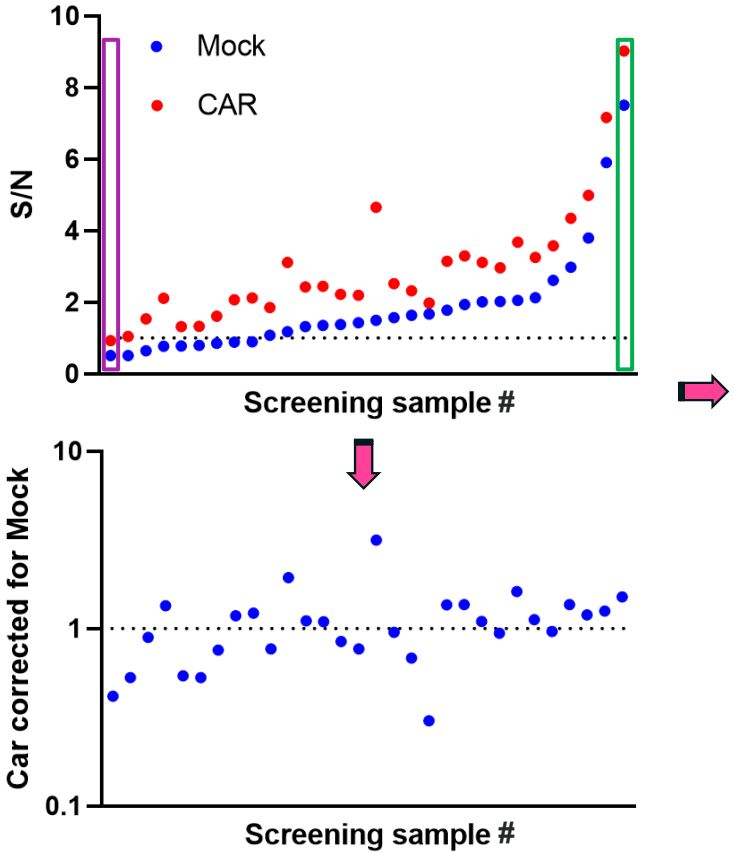
Sensitivity

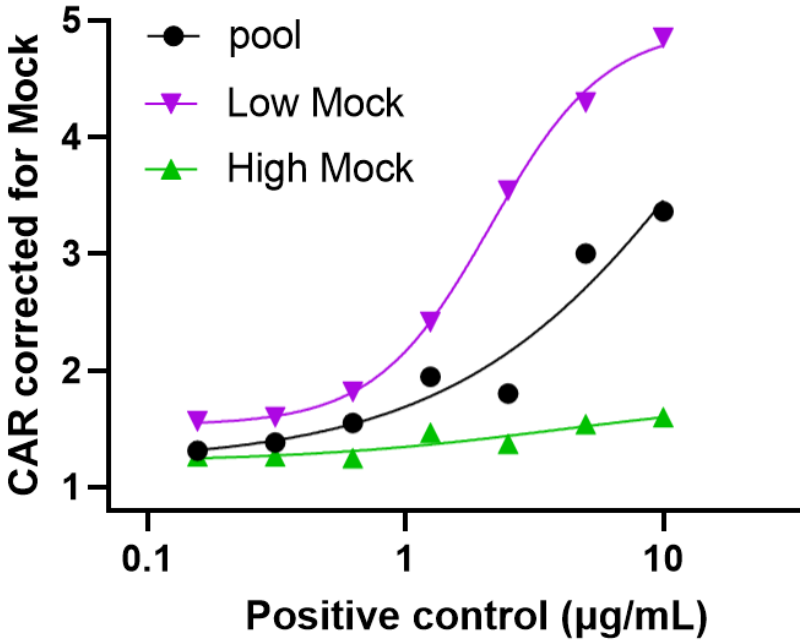


Methodology: flow cytometry using CAR expressing cell line

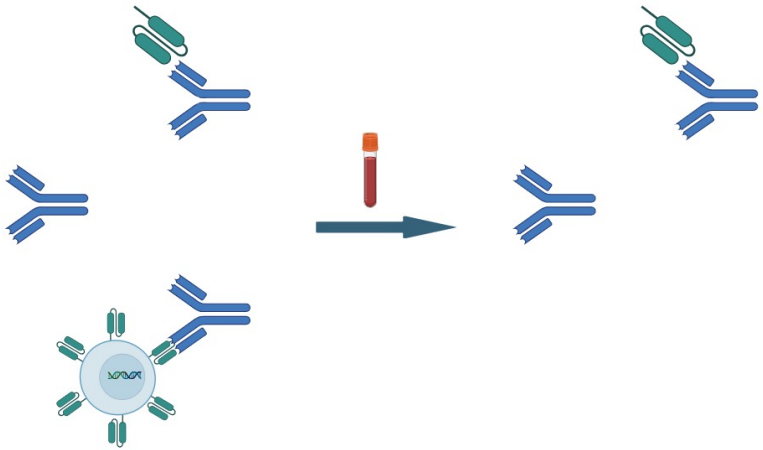


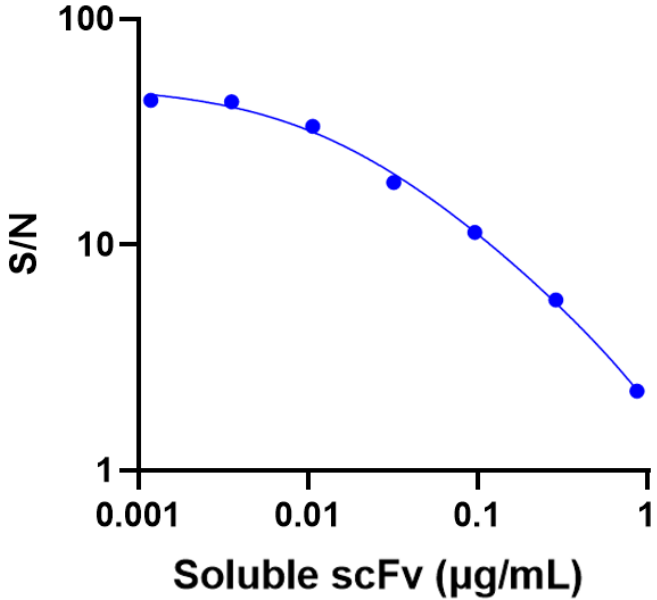
Flow cytometry: screening and sensitivity





Assay drug tolerance does not capture the full picture





Drug-ADA complexes *in vivo* | scFv interference *in vitro*

- Applies to LBA and flow
- DT optimization not performed

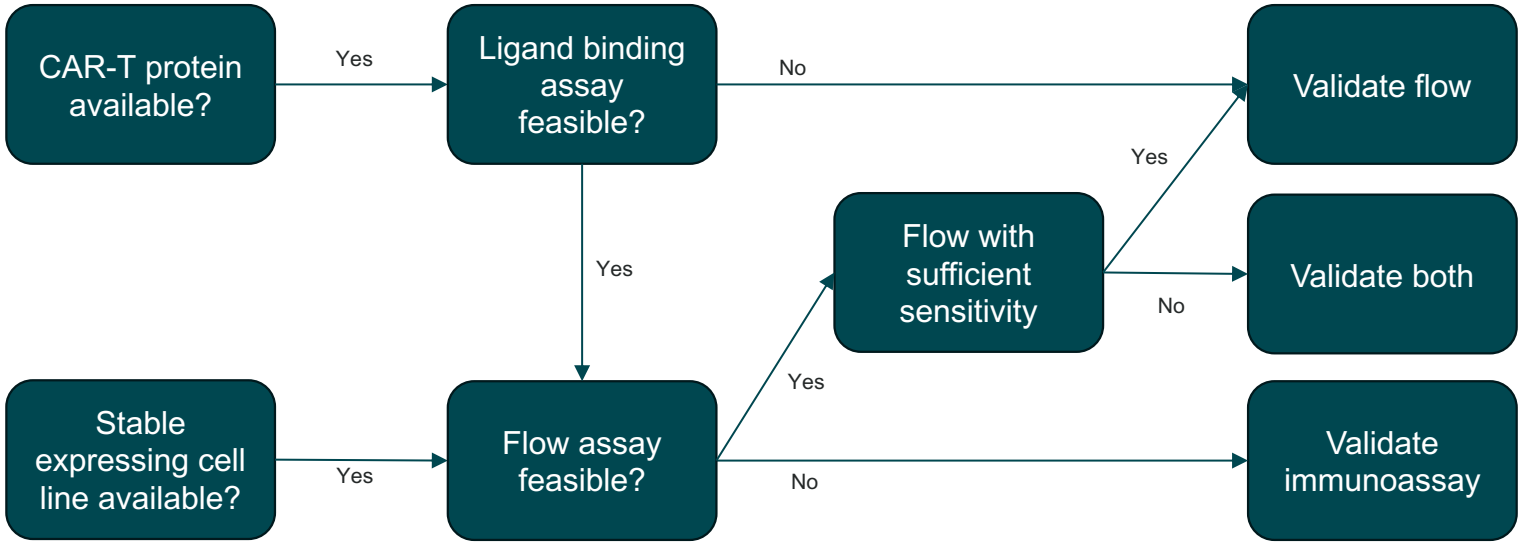
Go with the flow?

± Availability of protein or stable expressing cell line

Immunoassay: sensitive/robust	Flow: complete protein expressed on cells
+ Robust	- Technically challenging
+ Limited matrix effects	- Matrix effects
+ Sensitive	- Less sensitive
- Truncated (surrogate) protein	+ Complete protein
- Conformational epitopes?	+ Conformational epitopes

Suggested workflow

Develop CAR-T protein / cell line / (human) PC antibody well in advance
Assess feasibility in parallel



Thank you!

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