

# Neutralising Assay Methodologies

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*EMA workshop on immunogenicity assessment of biotechnology  
derived therapeutic proteins*

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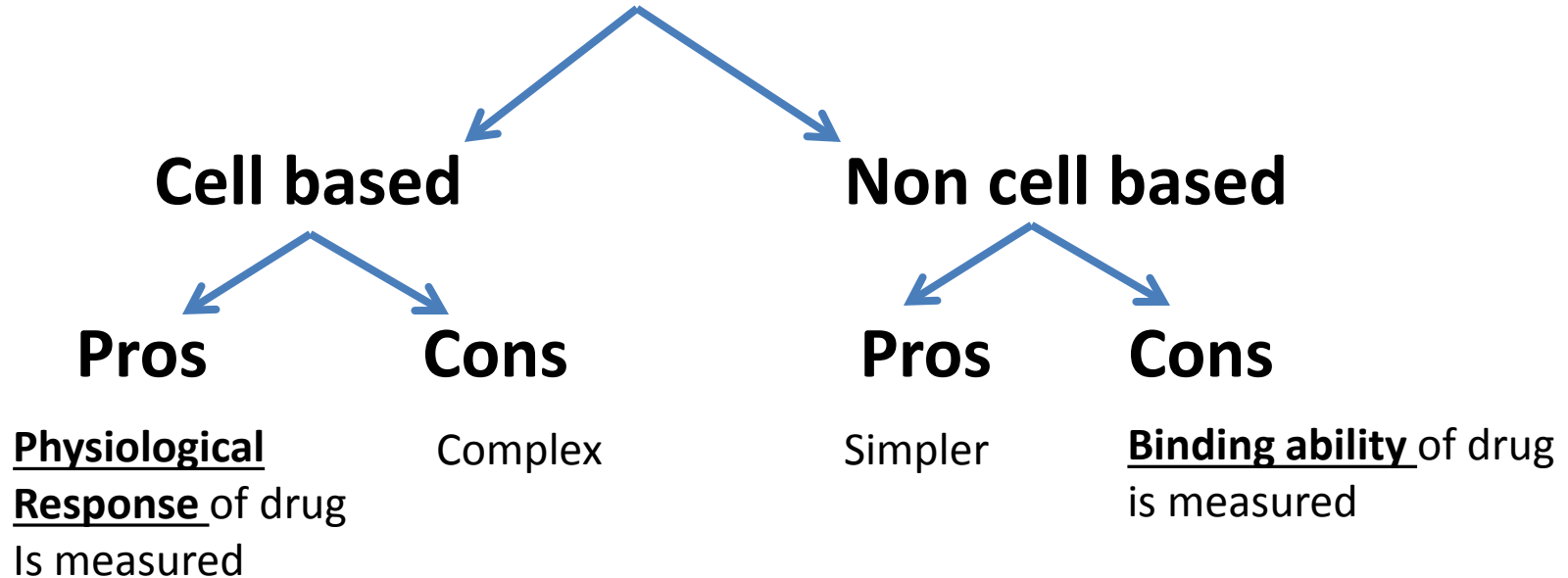
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# Key Points About Neutralising Antibodies

<b>1. Definition</b>	<ul style="list-style-type: none"><li>• These types of anti-drug antibodies (ADAs) interfere with the <b>biological activity</b> of the drug</li></ul>
<b>2. Impact of NAb to patient</b>	<ul style="list-style-type: none"><li>• Could range from none to serious impact depending upon the magnitude of NAb produced resulting in reduced exposure and/or efficacy</li></ul>
<b>3. NAb testing strategy</b>	<ul style="list-style-type: none"><li>• 2<sup>nd</sup> tier of immunogenicity assays</li><li>• NAb test is usually preceded by a binding ADA test</li></ul>
<b>4. NAb methodologies</b>	<ul style="list-style-type: none"><li>• Functional <i>in vitro</i> serum-based assays capable of assessing change in the biological activity of the drug</li><li>• Cell-based</li><li>• Non cell-based (Ligand binding)</li><li>• Qualitative determination</li></ul>
<b>5. Characterization of NAb</b>	<ul style="list-style-type: none"><li>• Titer (serial dilution of sample/fixed drug conc)</li><li>• Quasi-quantitative determination</li></ul>

# NAb Assay Methodologies

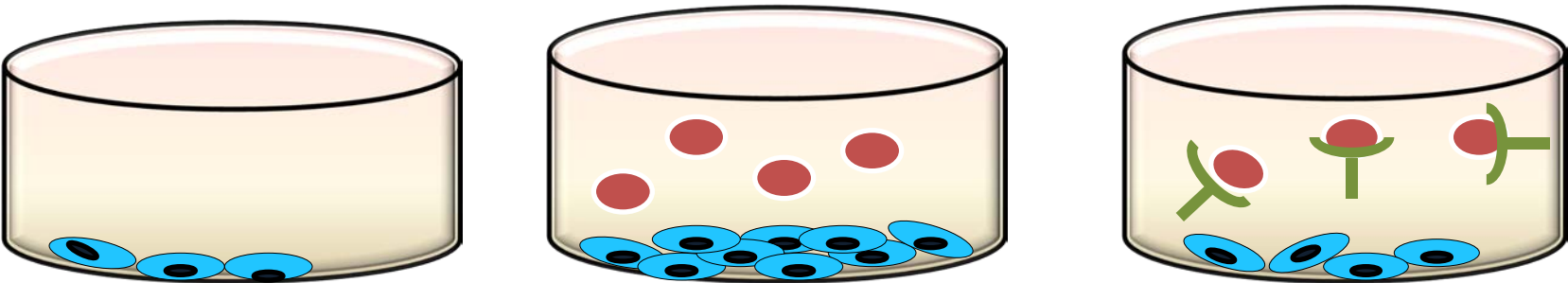
***Goal : To detect clinically relevant NABs***



**Assay characteristics : Sensitivity, specificity, drug product tolerance, reproducibility**

**The decision to use a cell-based or non cell based NAb assay is inconsistent across industry**

# Direct Cell-Based Assay Measuring Proliferation



Cells only

Cells + Drug

Cells + Drug + Anti-Drug Ab

Assay Response

Baseline

Proliferation

Inhibition of Proliferation

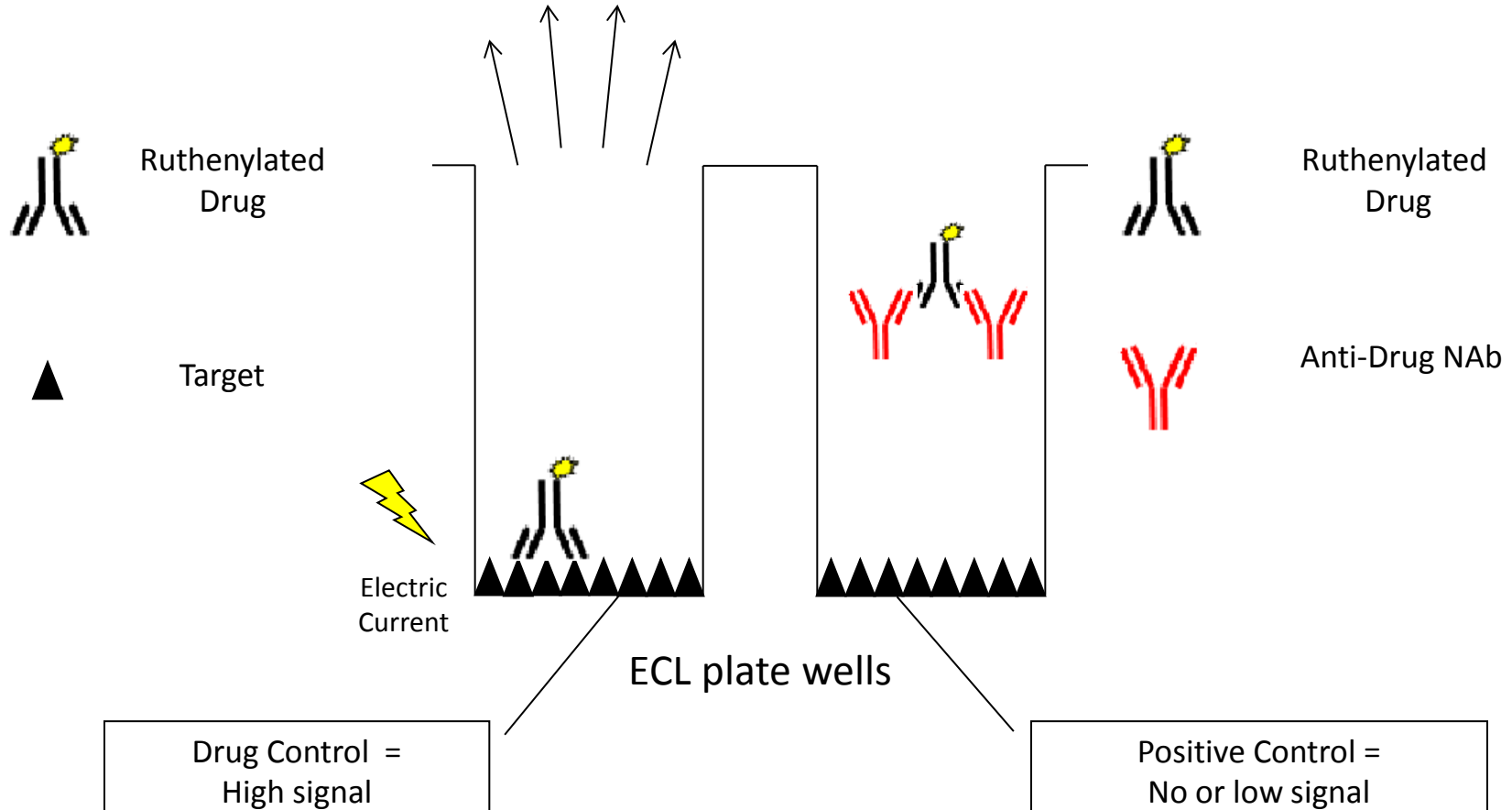
Cells only

Drug Control

NAb Positive Control



# Direct Competitive Ligand Binding Assay



**Instead of cells, the assay uses purified preparation of the target protein that the drug binds to**

# Assessment of Specificity of NAb assays

NAb Assay Format	Matrix interference Assay Format	MIA Type	Examples
<p><b><u>Direct Cell Based</u></b> <i>(uses a factor-dependent cell line)</i></p>	<p>Effect of sample on cell response by another stimulus</p>	<p>ALTERNATIVE STIMULUS</p>	<p>Cytokines, Growth Factors, agonistic MAb</p>
<p><b><u>Indirect Cell Based</u></b></p>	<p>Effect of sample on cell response in absence of added drug and/or ligand</p>	<p>SAMPLE INDUCED RESPONSE</p>	<p>MAbs, soluble receptors</p>
<p><b><u>Direct or Indirect Cell based or Non cell based</u></b></p>	<p>Sample pretreatment with protein G/L followed by test in NAb assay</p>	<p>IMMUNO DEPLETION</p>	<p>All types of biological therapeutics</p>

# Need for a consistent NAb assay selection strategy across Industry

Regulatory Agency	Year	Type of Biological Therapeutic/Area	Guidance for NAb assay Format	Additional text
EMA	2007	Biotechnology-derived therapeutic proteins	Functional bioassay	Adaptation of potency assay
EMA	2012	Monoclonal Antibodies	Competitive ligand binding assays	Cell based assays may offer an advantage
FDA (draft)	2009	Therapeutic proteins (Assay Development)	Cell based bioassay	Should reflect the product's mechanism of action
FDA	2014	Therapeutic proteins (Immunogenicity assessment)	Appropriate immunogenicity assays should be implemented	Impact of NAb on life saving drugs

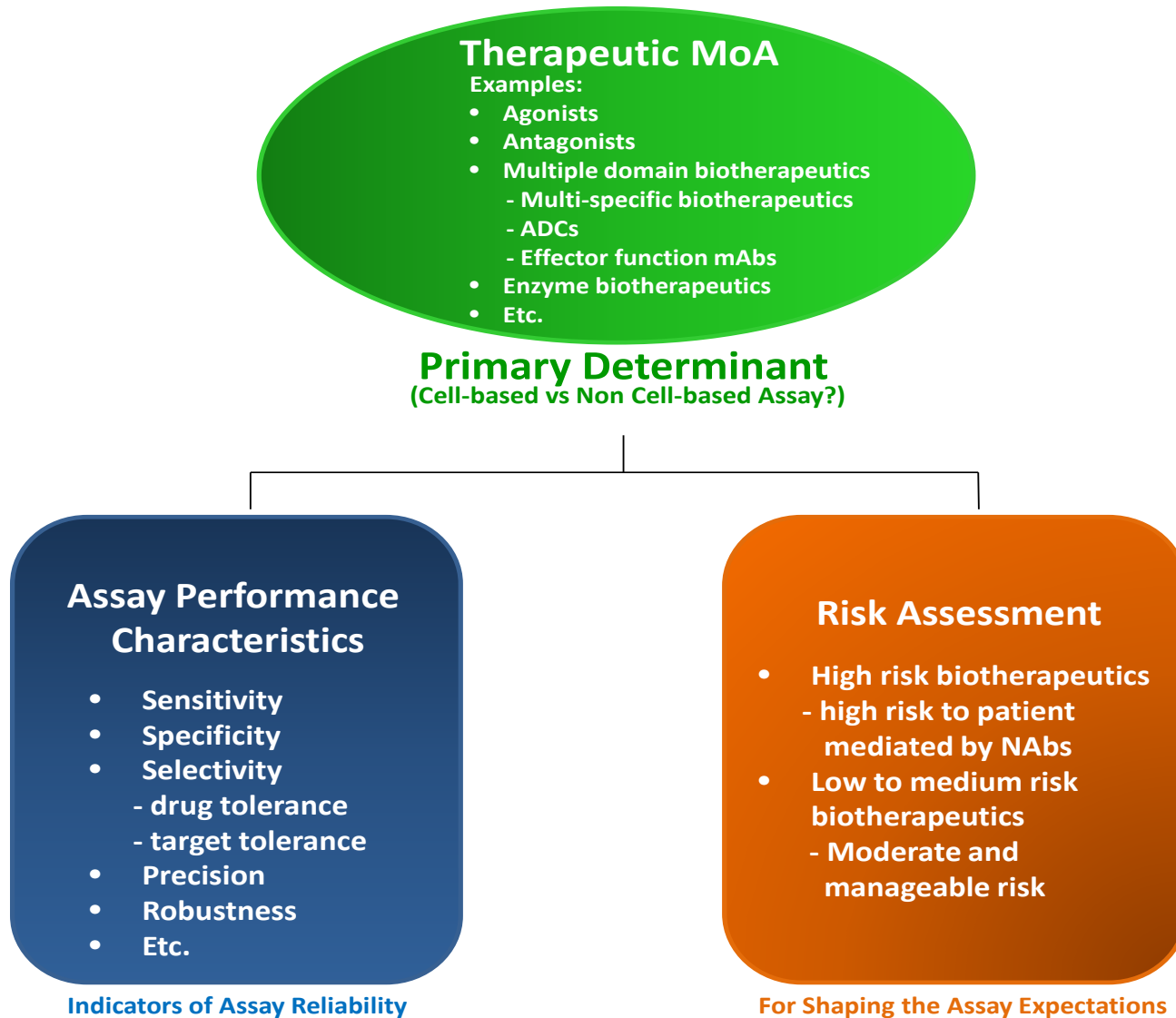
# AAPS LBABFG/Immunogenicity Assay Working Group Committee

Name	Company
Shalini Gupta, Chair	Amgen
Renuka Pillutla	Bristol Myers Squibb
Yuanxin Xu	Genzyme, Sanofi
Xu-Rong Jiang	MedImmune, Astra Zeneca
Shan Chung	Genentech
Manoj Rajadhyaksha	Regeneron
Joleen White	EMD Serono
Jim McNally	EMD Serono
Bonnie Wu (Lead Author)	Janssen, J&J
Joao Pedras-Vasconcelos	FDA

# White Paper (in draft)

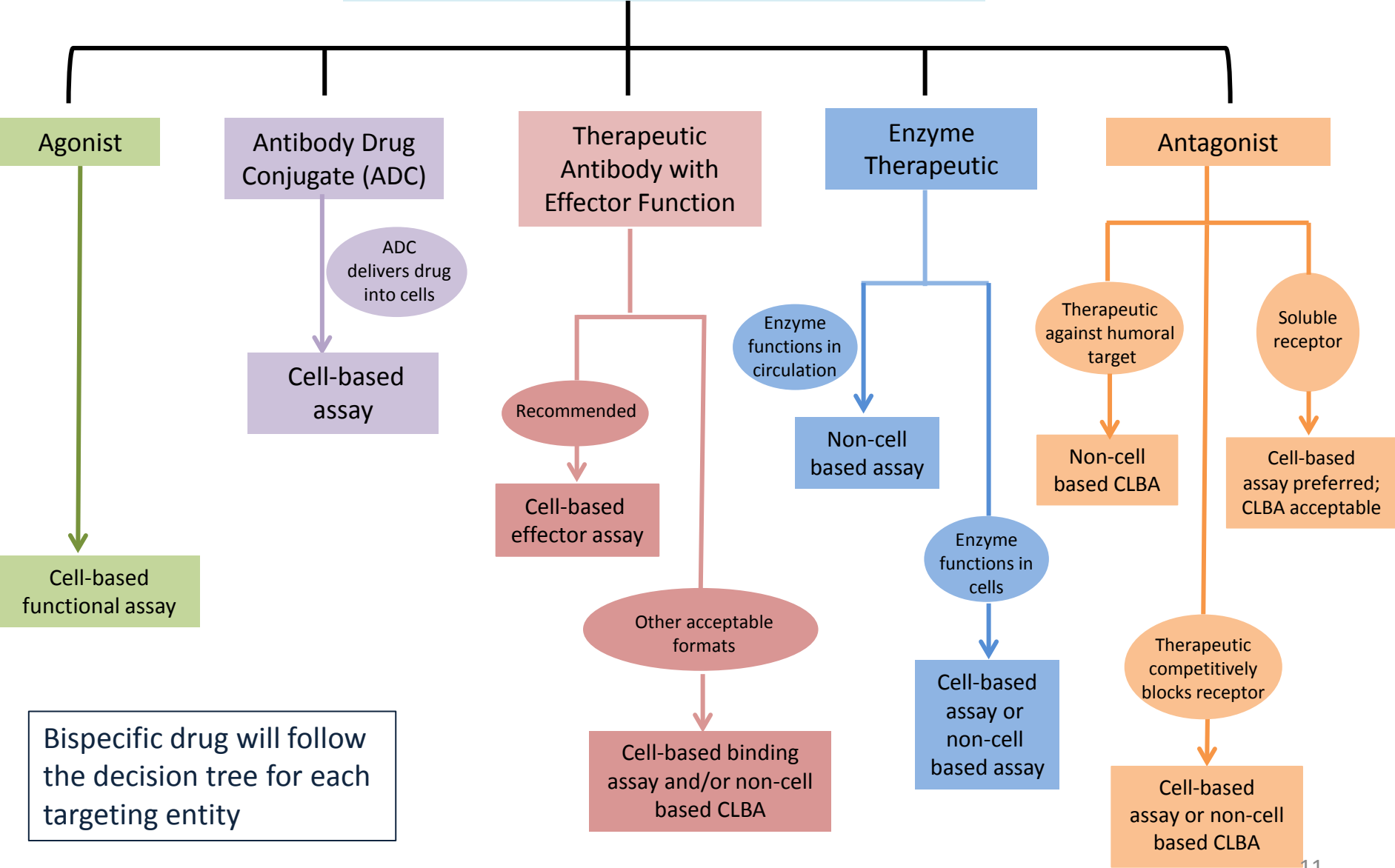
- **Title**
  - **Strategies to Determine Assay Format for the Assessment of Neutralizing Antibody Responses to Biotherapeutics**
- **In-Scope**
  - **Provide guiding principles for decision making strategy to use a cell-based or non cell-based NAb assay**
- **Out of Scope**
  - **Recommendation to include/exclude NAb testing in immunogenicity strategy**
- **Timing**
  - **Paper submission to Journal targeted by Q1 2016**

# Considerations for selection of NAb assay format



**A reliable NAb assay should be able to detect clinical relevant NAb.**

# Biologic Therapeutics Categorized by MoA



Bispecific drug will follow the decision tree for each targeting entity

# Bioanalytical Perspective

## On Trigger-based NAb testing

- Regulatory input is important for this approach
- The “trigger” needs to be well-defined
- Bioanalytical efforts are still needed to prepare if a clinical “trigger” is observed
  - NAb assay format selection
  - Availability of a NAb positive control
  - Level of assay development, qualification and/or validation needs thought
- NAb+/- results originating from a NAb test enable easier study interpretation

# Conclusions

- **NAb assays are qualitative assays**
- **A well-designed NAb assay should be able to detect clinically relevant NABs**
- **Efforts are ongoing in industry to compile recommendations for consistent use of cell-based and non cell-based NAb assays**
- **Bioanalytical approaches for “Trigger”-based NAb assay availability need discussion**