Biomedical Research
Preclinical Safety

In vitro assessments of Immunogenicity

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CASSS CMC Strategy Forum Europe: Immunogenicity Scientific Session
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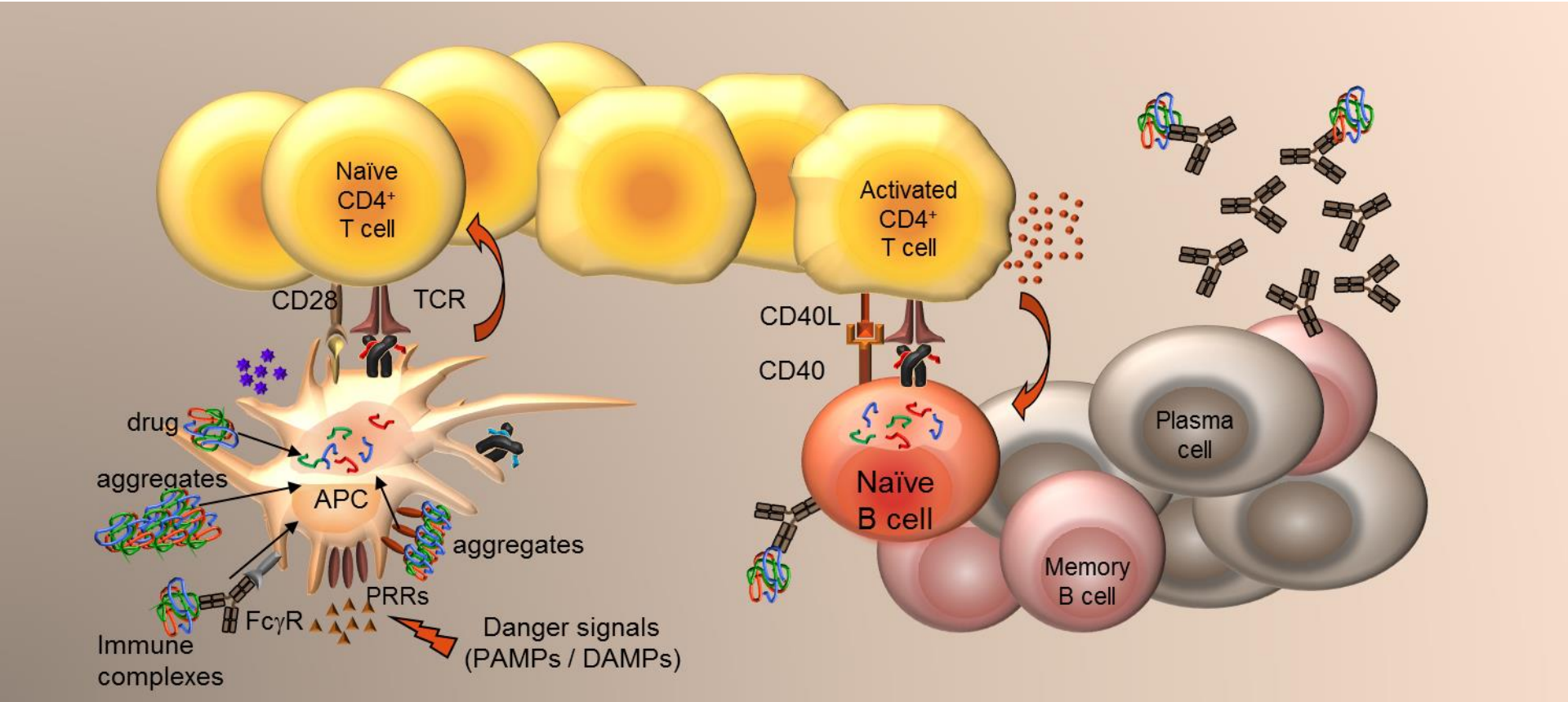
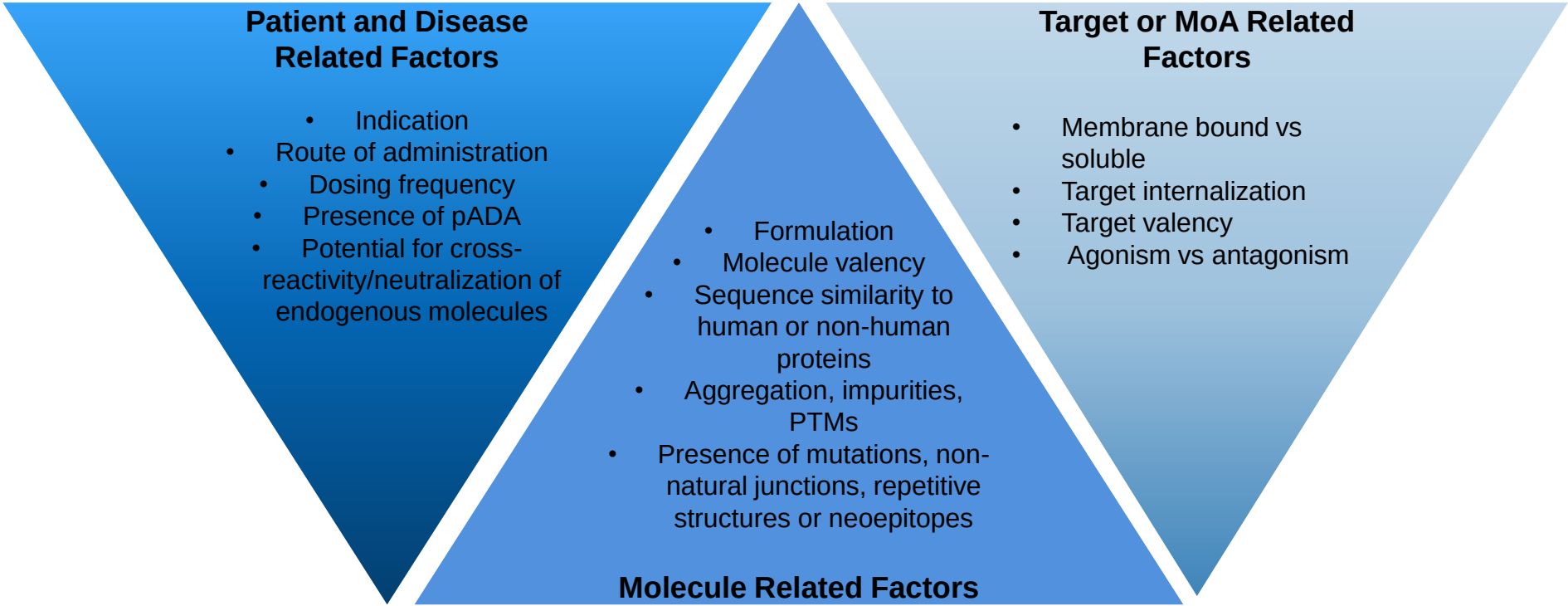
How do we define immunogenicity?

- **Immunogenicity** is the ability of a particular substance (i.e. antigen) to induce a **humoral-** or **cellular-** immune response
- However, in the biotherapeutics field:
  - Immunogenicity is used to describe the formation of **anti-drug antibodies (ADAs)** against a protein-based therapeutic
- The development of immunogenicity is usually triggered by sequence and/or structural differences between a ‘foreign’ biotherapeutic and the body’s natural protein
- As most biotherapeutics contain unique epitopes – immune system recognition results in an immune response to the therapeutic with varying incidence and magnitude

The immune response to therapeutic proteins requires a complex interplay of different immune cell subsets



Multiple factors need to be taken into consideration to define IG risk



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Immunogenicity Risk Classifications

IG Risk = Probability x Consequences

Classification	Probability	Efficacy Consequences	Safety Consequences
Tier 1 (low risk) Probability – Low Safety – Low	No mechanistic flag for the generation of ADA in human e.g. mAb against soluble targets	Possible	None to minimal potential to elicit clinical consequences
Tier 2a (low-intermediate risk) Probability – High Safety – Low	Mechanistic flag for the generation of high ADA levels in human e.g. mAb against receptor target internalized by APCs	Possible	None to minimal potential to elicit severe clinical consequences
Tier 2b (intermediate-high risk) Probability – Low Safety – High	No mechanistic flag for the generation of ADA levels in human	Possible	Potential to elicit adverse clinical consequences e.g. thromboembolism, anaphylaxis, immune activation e.g. targeting receptors on platelets, mast cells, cytokine-producing cells that could be cross-linked/activated by ADA
Tier 3 (high risk) Probability – High Safety – High	Mechanistic flag for the generation of high ADA levels in human e.g. receptor target internalized by APCs; modified human protein	Possible	Potential to elicit severe adverse clinical consequences e.g. neutralization of endogenous counterpart with non-redundant function

➢ **IG Risk classification guides the selection of the in vitro IG assays used for each project**

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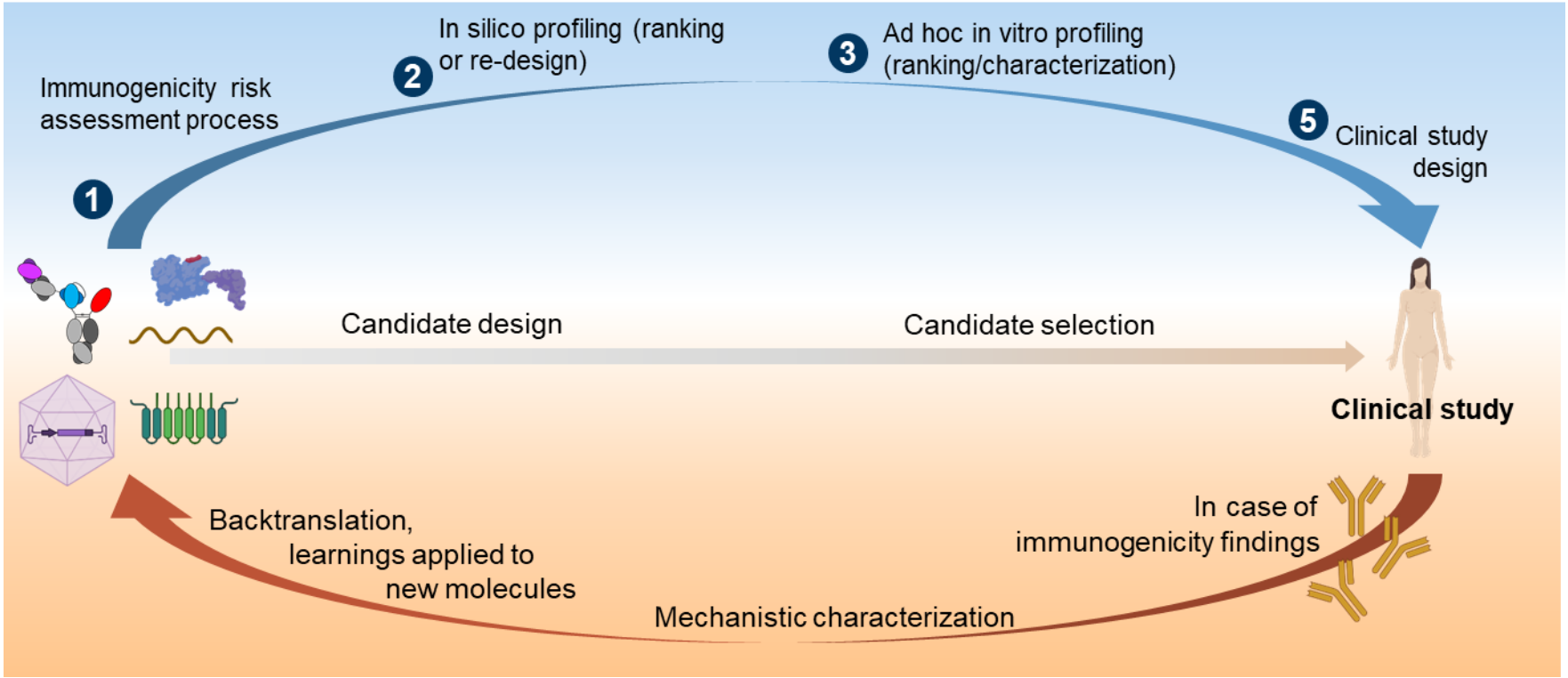
Immunogenicity strategy for biotherapeutics



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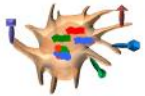
In vitro immunogenicity potential assessment platforms at Novartis

DC assay
Evaluation of DC maturation potential

CD14+ isolation from healthy donor PBMCs

Immature DCs loaded with drug

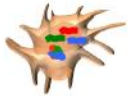


Flow cytometry

DC uptake assay
Evaluation of drug uptake kinetics

CD14+ isolation from healthy donor PBMCs

Immature DCs loaded with labeled drug



IncuCyte

MAPPs assay
Identification of potential T epitopes

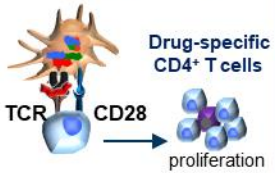
CD14+ isolation from healthy donor PBMCs

Immature DCs loaded with drug

Peptide isolation and nano LC-MS/MS

T cell assay
T cell proliferation and activation

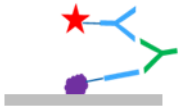
healthy donor PBMCs



Flow cytometry

SeroPro assay
Serological profiling of drug candidates

Patient- or healthy donor sera



Screening for pre-existing or induced drug-specific antibodies

ELISA

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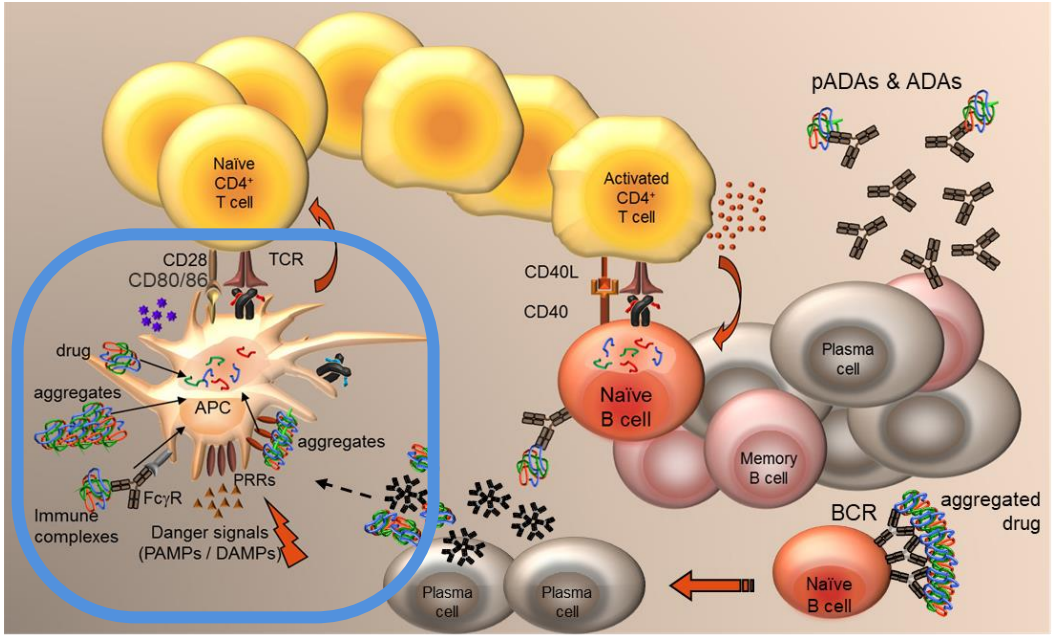
Assays focussing on Antigen Presenting Cells

Professional Antigen Presenting Cells (APCs)

- Sentinels of the immune system
- Specialized in antigen uptake, processing and presentation
- Expression of HLA class II
- These cells are used to address questions around antigen presentation, maturation signals and antigen uptake



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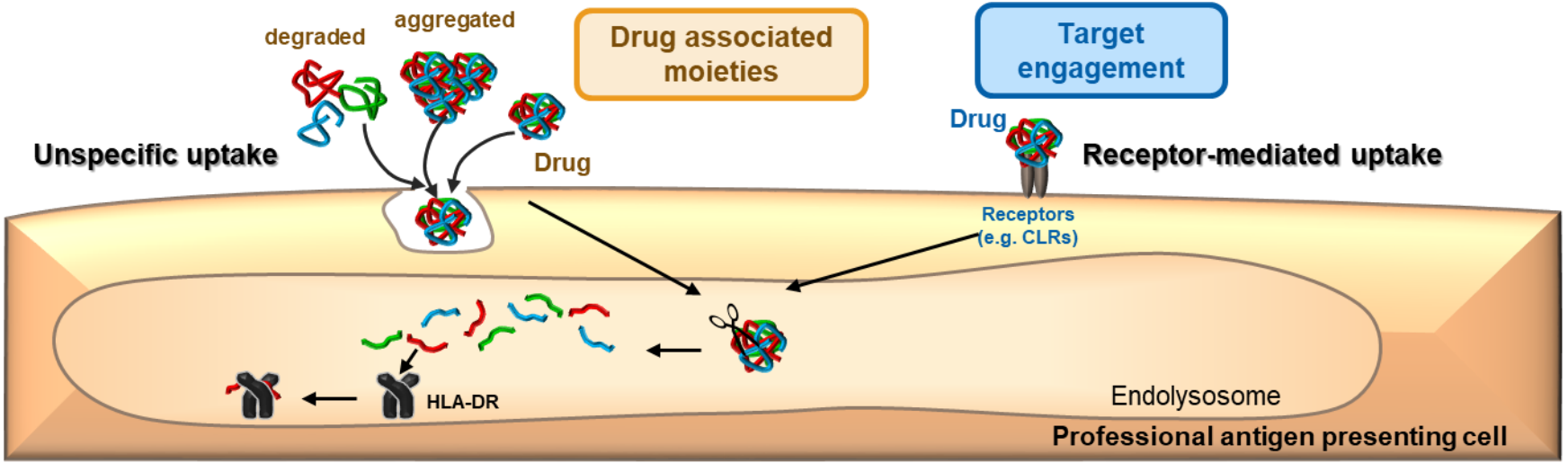
DC uptake assay

Detection of:

- Enhanced antigen uptake due to drug associated moieties or formats or target engagement as a risk factor for increased antigen presentation



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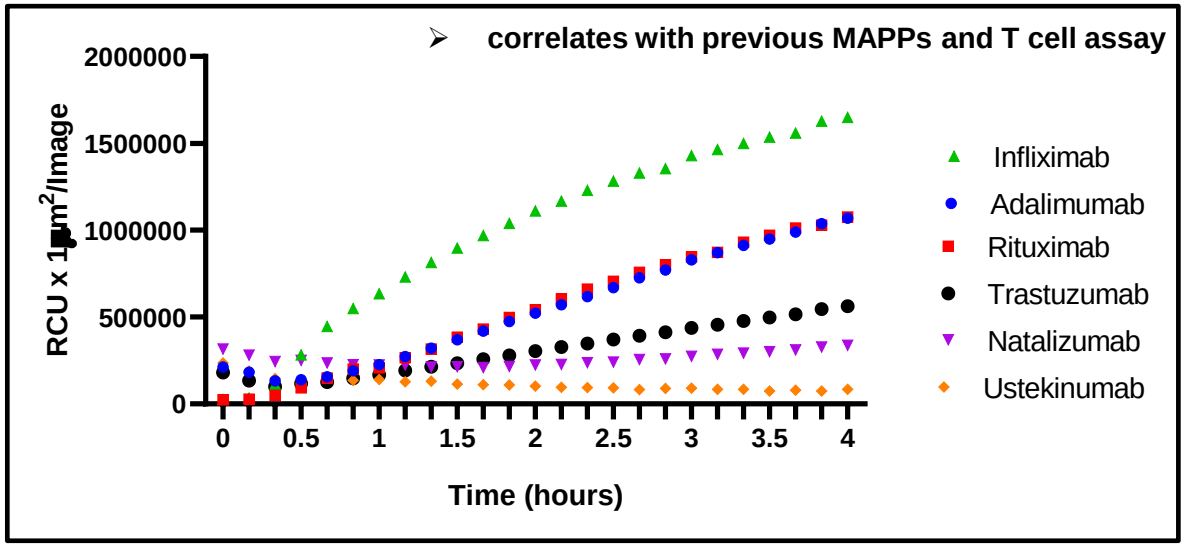
DC uptake results based on real-time live cell imaging analysis



Uptake Rank (high-low)	Estimate of Clinical Incidence*
Infliximab	High
=Adalimumab	Mid/High
=Rituximab	Mid
Trastuzumab	Low
Natalizumab	Mid
Ustekinumab	Low

Exploratory assay to determine the uptake rate of fluorophore labeled biotherapeutics

DC uptake was monitored over 4 hours every 10 min.
Results are based on the average of **10 human donors**.

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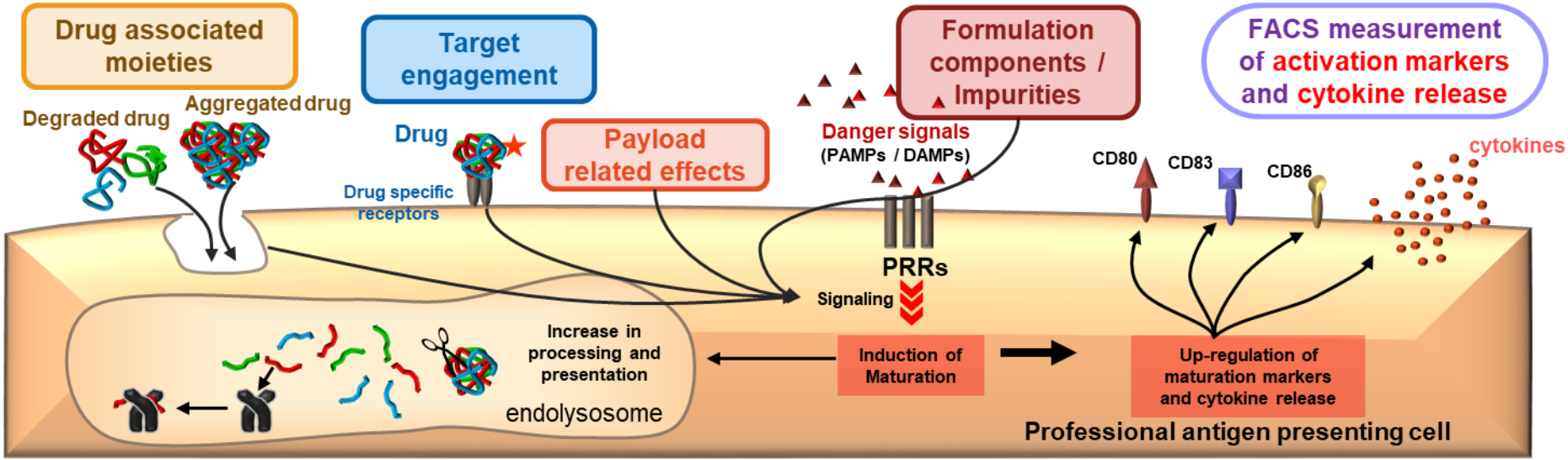
DC maturation assay

Detection of:

- drug associated & formulation mediated risk factors
- stimulatory effects mediated by target engagement on DCs or candidate payload effects



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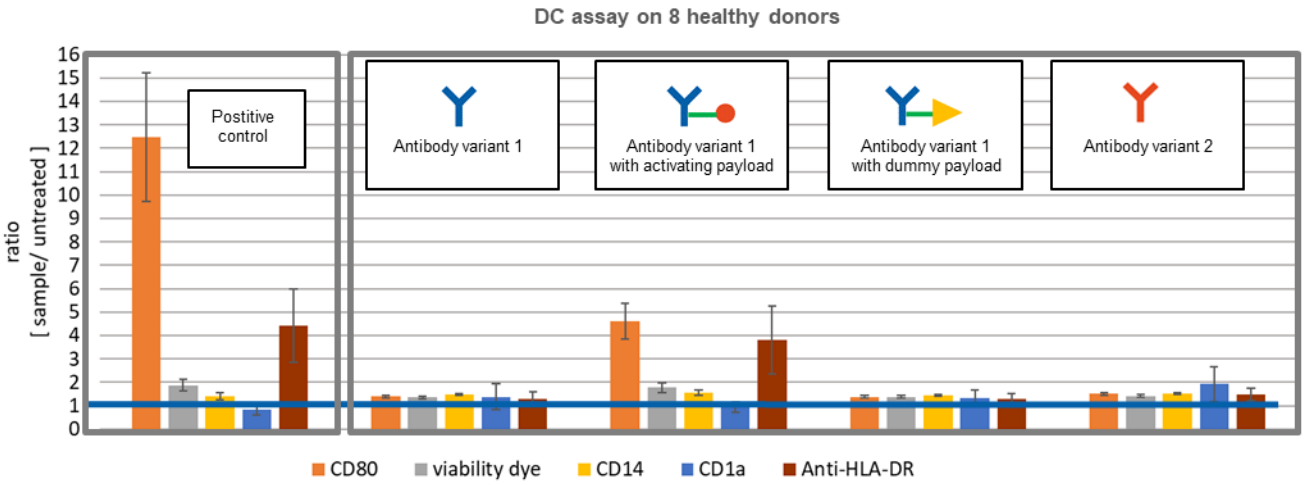
Immune stimulating antibody conjugate drives DC maturation as mode of action

Marker		Expected change upon activation with LPS *(S. enterica)*
CD80		increase ↑↑↑
Cell death		increase ↑
CD14		time dependent ↑↓
CD1a		decrease ↓
HLA-DR		increase ↑↑↑

***** CD14 up- or down- regulation depends on the type of stimulus used for activation and the length of activation



➢ payload effect: Stimulator induces maturation as expected

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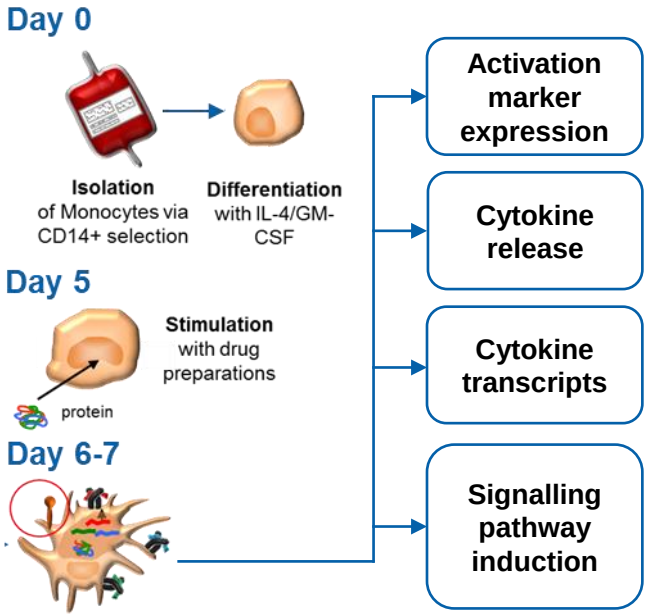
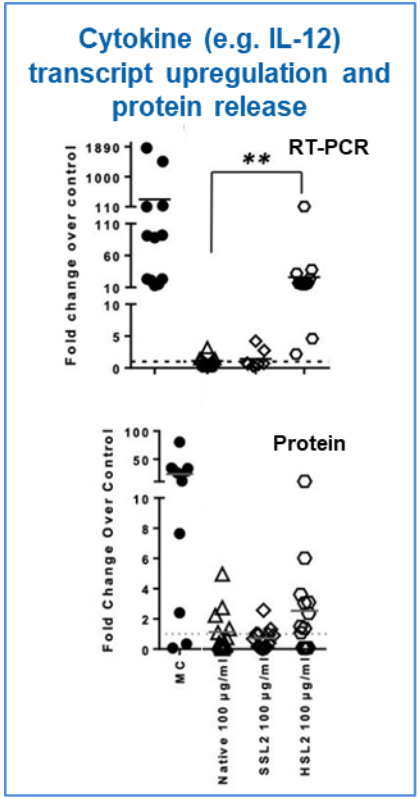
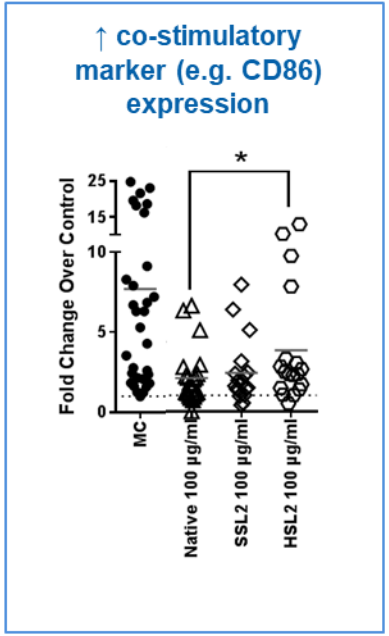
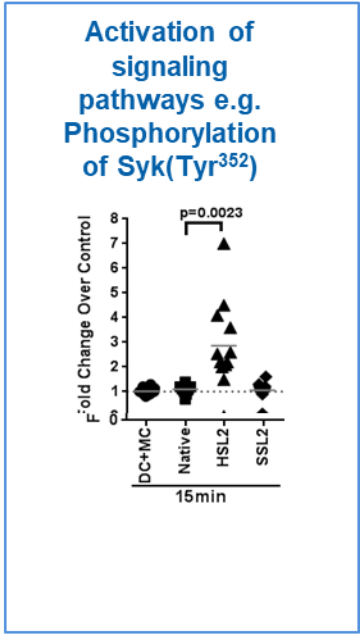
Aggregated infliximab induced DC maturation measured by orthogonal readouts









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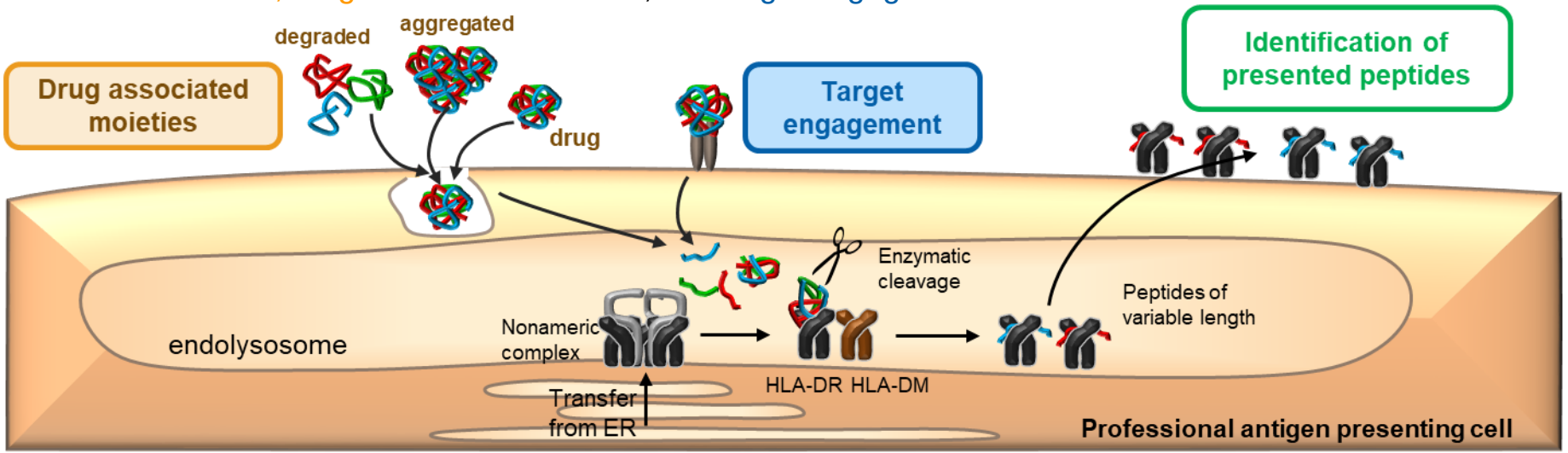
Morgan et al. 2019; *Front Immunol* 10:601

MAPPs assay (MHC-associated Peptide Proteomics)

Detection of:

- naturally processed HLA-DR associated peptides for protein design, ranking, mechanistic studies
- Effect of PTMs, drug associated moieties, and target engagement



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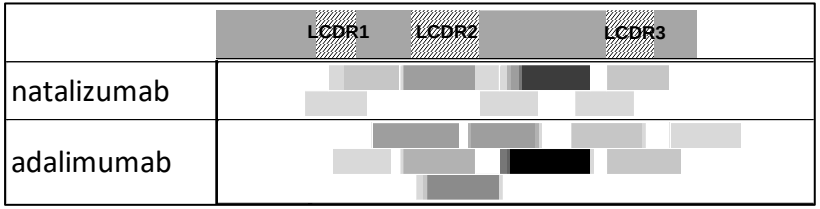
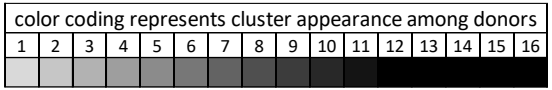
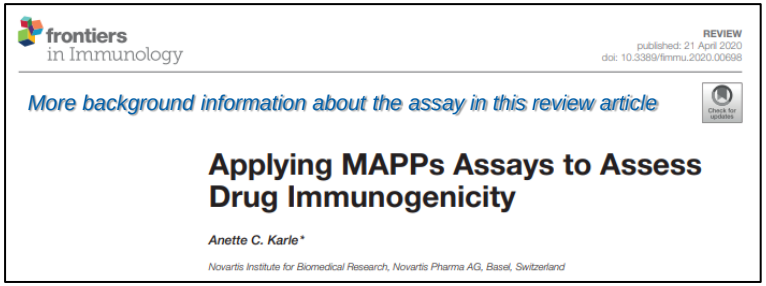
MAPPs assay output examples

1. Example of a presented cluster heatmap for two mAbs;

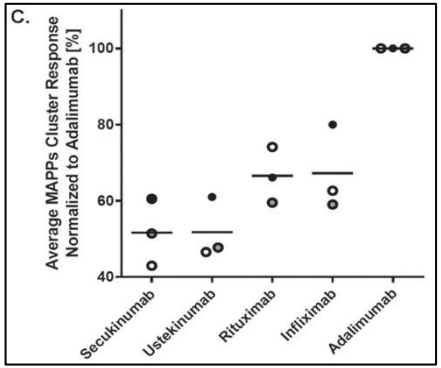




The grayscale representation indicates how many donors showed peptides in the respective area.

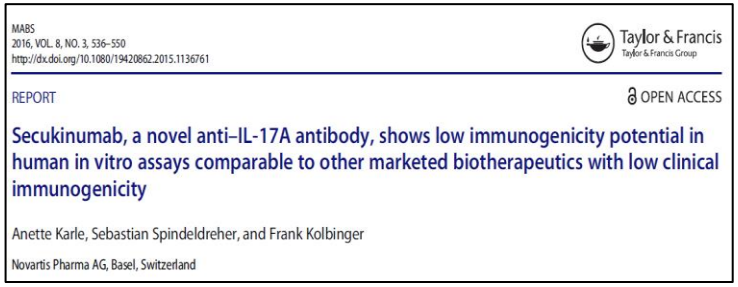


2. Example of normalized cluster numbers across mAbs



The number of clusters does not directly translate into immunogenicity in human but higher numbers of clusters increase the immunogenicity potential of a biotherapeutic.

The impact of the “humanness” of the sequences plays an additional role, since the level of immune tolerance towards the presented peptides can differ.



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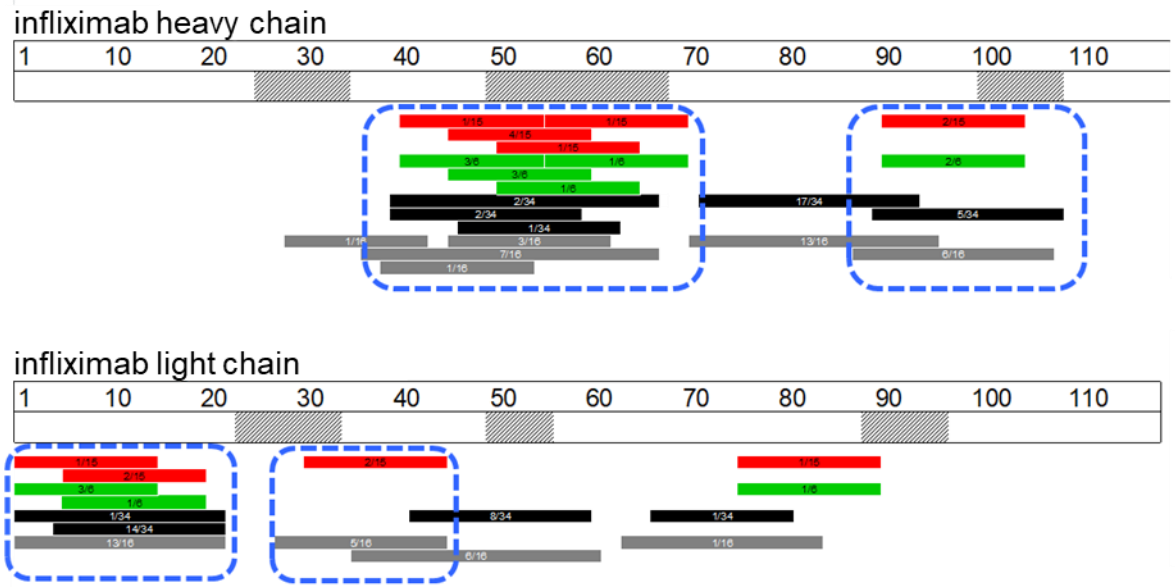
T cell epitopes of infliximab



T cell epitopes from healthy donors; N=15

T cell epitopes from ADA+ patients; N=6

MAPPs on native material with healthy donors (n=34)

MAPPs on aggregated material with healthy donors (n=16)

MAPPs assay performed by lab A. Karle at Novartis. T cell assay performed by lab Bernard Maillère at CEA Paris.

Different donor sets were used, therefore no perfect match expected due to different HLA distributions

- MAPPs clusters nicely align with T cell epitopes from healthy donors and ADA-positive patients.
- Infliximab stressed under exaggerated temperature conditions (55°C; 24h) shows increased antigen presentation in several sequence areas.

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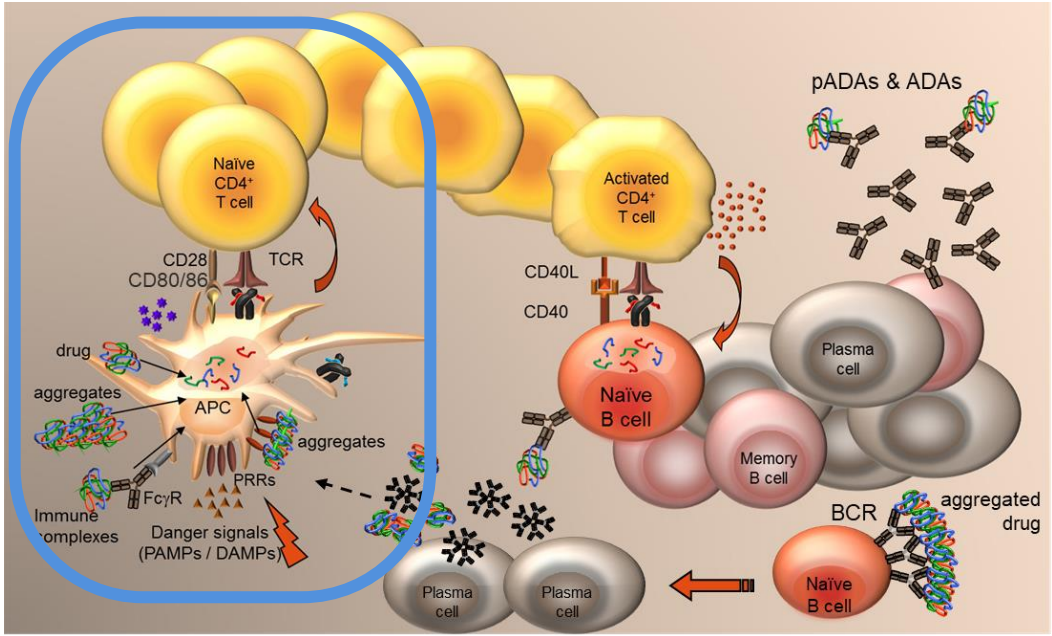
Assays focussing on APC / T cell interaction

CD4+ T helper cells

- Link between APCs and ADA producing B cells
- Essential for B cell activation and Ig class switch
- These cells are used to address questions around T cell recognition.



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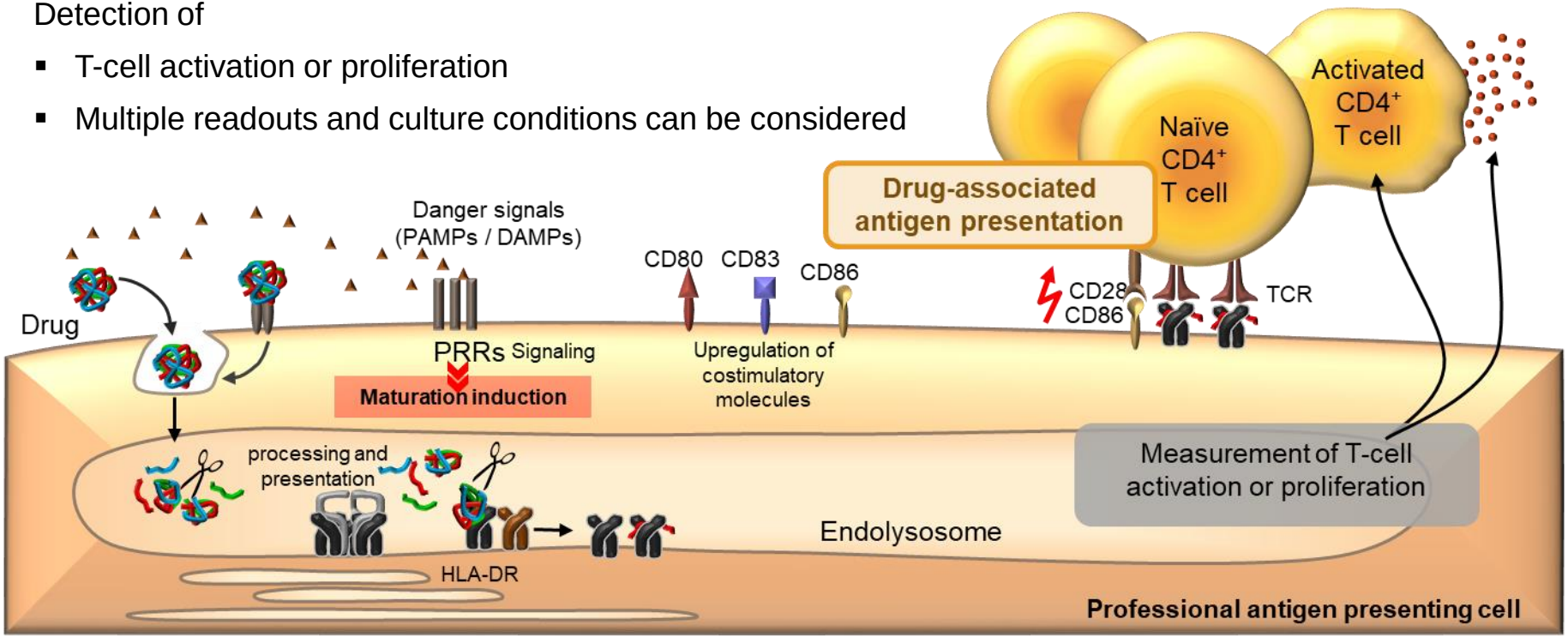
T Cell Assays

Detection of

- T-cell activation or proliferation
- Multiple readouts and culture conditions can be considered



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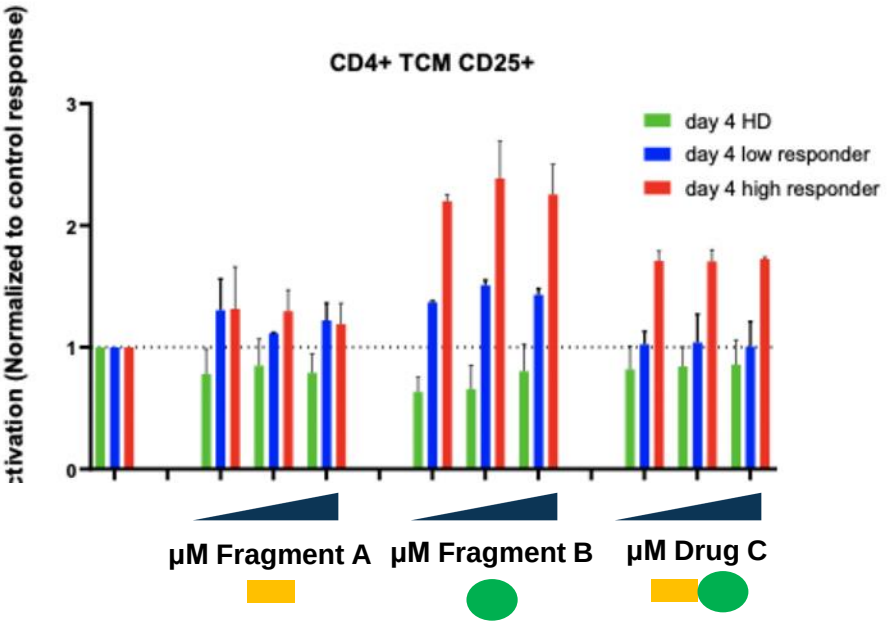
Memory T cell assay output

- Patients with high ADA responses to Drug C show clear T cell memory responses to Drug C or its component parts 10 months after treatment.



- Fragment A, Fragment B and Drug C induced slight total $CD4^+$ and memory T cell activation (T_{CM}) in high ADA responders.
- Healthy volunteers (HD) did not show any T cell responses to any molecule.

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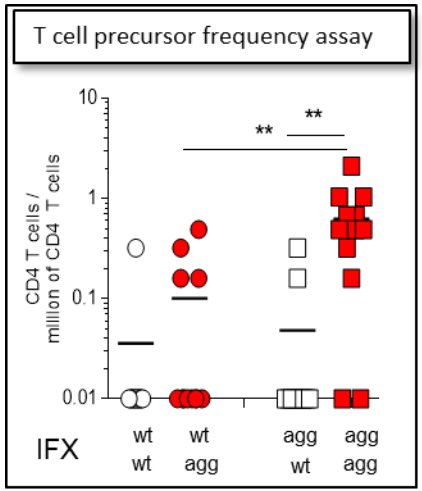
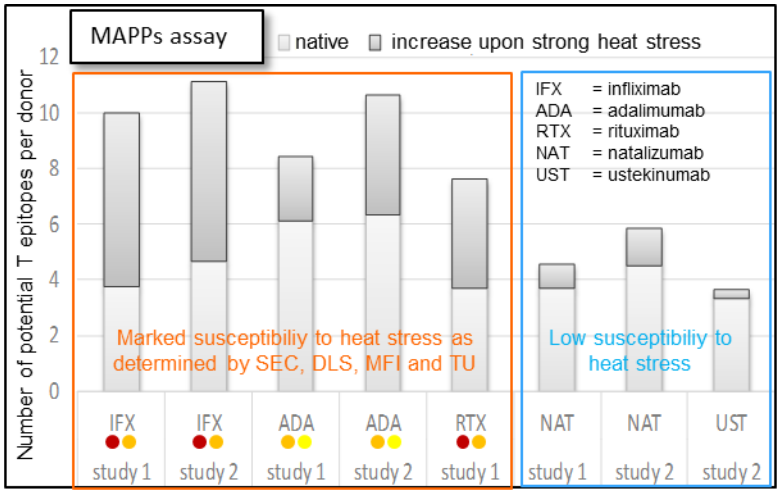
Aggregated infliximab increases peptide presentation and induces T cells





- Effect in subvisible paticle range (MFI)
- Effect in polydispersity (DLS)
- Degradation products (SEC)

MAPPs assay performed by lab A. Karle at Novartis. T cell assay performed by lab Bernard Maillère at CEA Paris.

Different donor sets were used.

- MAPPs assay: mAbs with relatively higher immunogenicity either showed elevated baseline peptide presentation and/or strong increase in presentation upon stress, while mAbs with rather low clinical immunogenicity show low baseline presentation and minor increase upon stress.
- T cell assay: some heat stressed mAbs induce large increase in pre-existing T cells.

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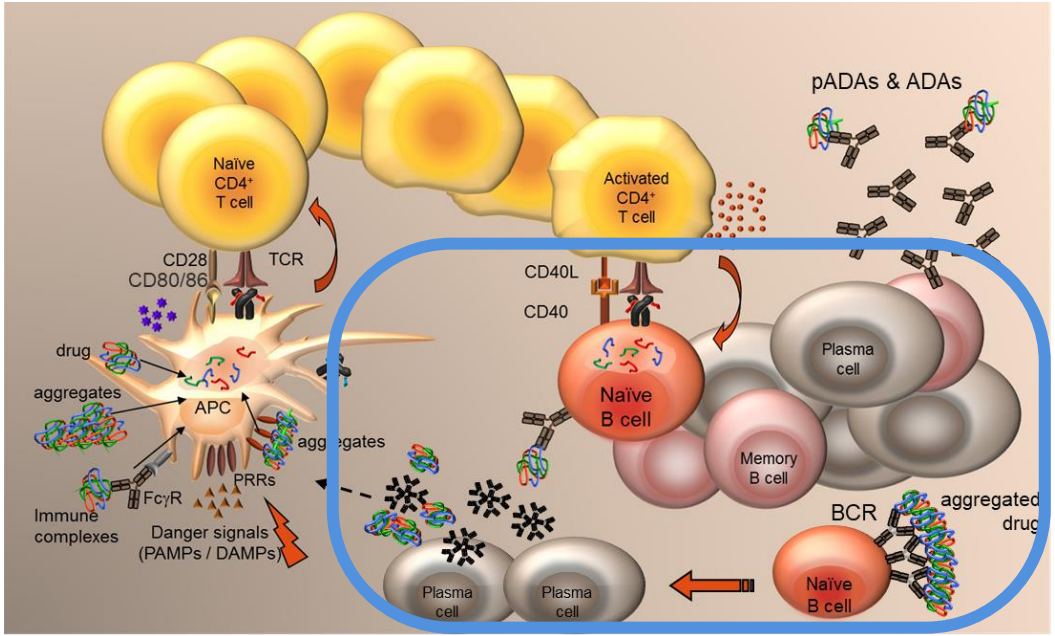
Assays focussing on B Cells

B Cells and Plasma Cells

- Drivers of the humoral response
- Production of ADAs (drug-induced and pre-existing)
- Class switch from IgM to IgG ADA production is only possible if activated by drug specific T cells



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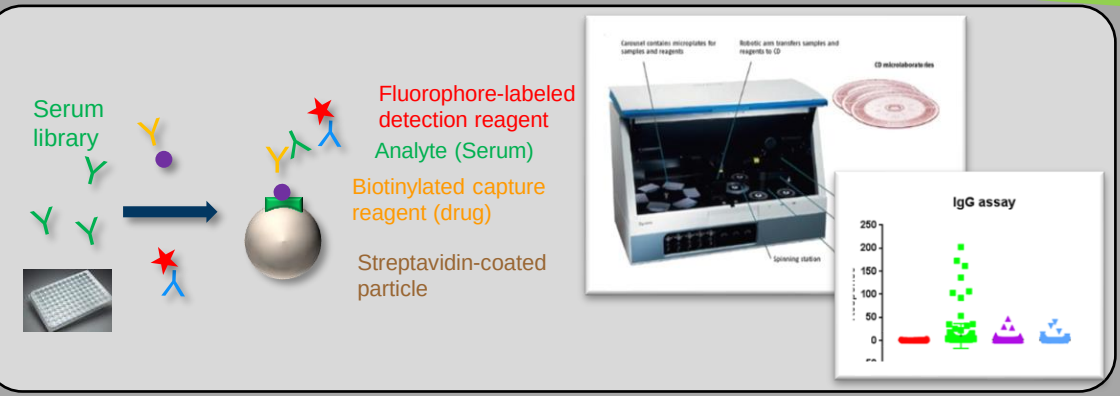
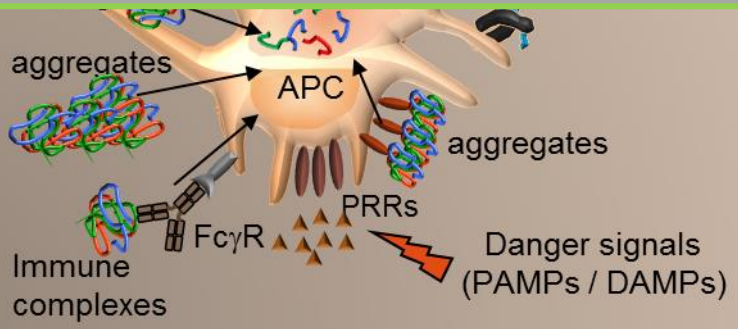
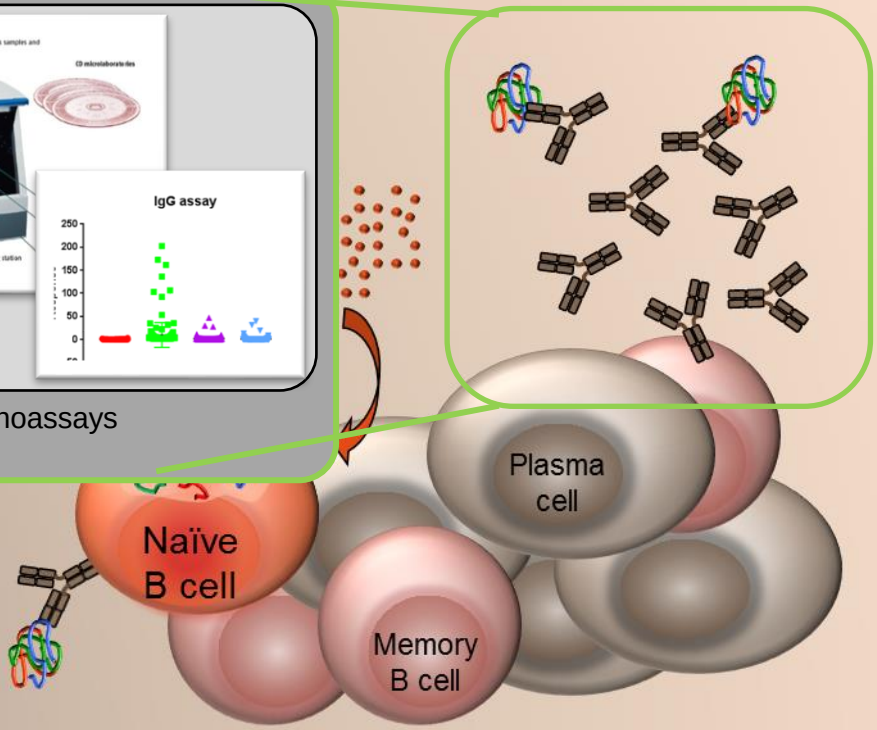
Serological profiling assay

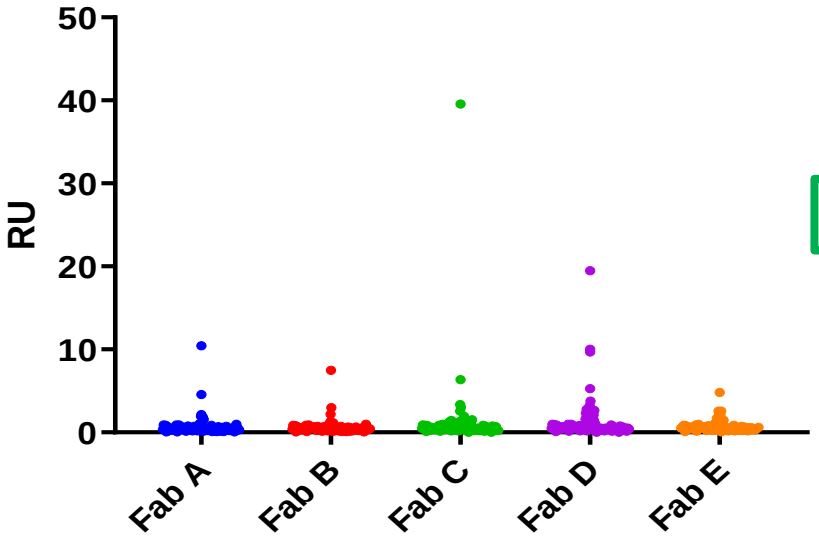


→ Serological response analysis by ELISA or Gyrolab Immunoassays
→ Testing of serum library against different biotherapeutics





SeroPro – pre-existing antibody (pADA) assay on Fabs– anti-human IgG Fc response



	Fab A	Fab B	Fab C	Fab D	Fab E
# of samples higher than threshold:	**6/94**	**6/94**	**7/94**	**15/94**	**5/94**
% of samples higher than threshold:	**6.38**	**6.38**	**7.45**	**16**	**5.32**
Blank value substracted (PBS, Rexxip H):	**0.03**	**0.04**	**0.02**	**0.02**	**0.04**
mixADA-Threshold:	**1.43**	**1.13**	**2.62**	**1.82**	**1.83**

- Fab D has the highest frequency of donors with a positive anti-drug response
- Deprioitised from development

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n = 2 technical replicates; Displayed average responses of 2 runs (94 donors/run)

Pro's/Con's of commonly applied assays for immunogenicity assessment

	MAPPs assay	DC assays	T cell assays	B cell assays
Pros	- Allows ranking of different drug candidates - Reveals only relevant binders - Mimicry of processing and presentation of in vivo situation - Effect of PTMs and aggregation can be assessed - Only moderate purity necessary - Good correlation with clinical immunogenicity	- Allows ranking of different drug candidates - Can be used to assess drug intrinsic and formulation related danger signals - Effect of PTMs and different formulations can be assessed - Orthogonal readouts allow the interrogation of mechanisms of DC activation - Medium throughput available dependent on readout of activation/uptake	- Allows ranking of different drug candidates - Effect of PTMs and different formulations can be assessed - As compared to the other tools, it is the closest to the in vivo situation - Can be used with patient samples to allow for back-translation/correlation to the clinical situation - Good correlation with clinical immunogenicity	- Allows ranking of different drug candidates - Can be used with patient samples to allow for back-translation/correlation to the clinical situation - Medium/high throughput assay available
Cons	- Does not predict T cell activation - Time-consuming (about 2 months per project) - Assay sensitivity may not be sufficient to detect the impact of CQAs	- Does not predict T cell activation - Very high purity of protein necessary - Correlation to clinical IG not yet well understood - Assay sensitivity may not be sufficient to detect the impact of CQAs	- Potential interference of drug function - Time-consuming (3 months / project) - High purity of protein necessary - Assay sensitivity may not be sufficient to detect the impact of CQAs	- Impact of pre-existing antibodies on the clinical response is not well understood - Impact of CQAs cannot be addressed

Summary

- At Novartis, the selection of in vitro immunogenicity potential assays for each project are selected based on the initial IG risk-assessment reflecting the relevant target/molecule related biology
- Different cell-based assays can be applied to evaluate and mechanistically investigate the immunogenicity potential of biotherapeutics, with good correlation to the clinical situation
- The impact of certain CQAs (e.g. aggregation, glycosylation) on the immunogenic responses can be assessed in these in vitro assays although sensitivity and translatability is still under evaluation

- **Support candidate design and selection**
- **Understand immunogenicity** mechanisms / risk factors
- **Root cause analysis** of adverse events in internal clinical trials

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Thank you

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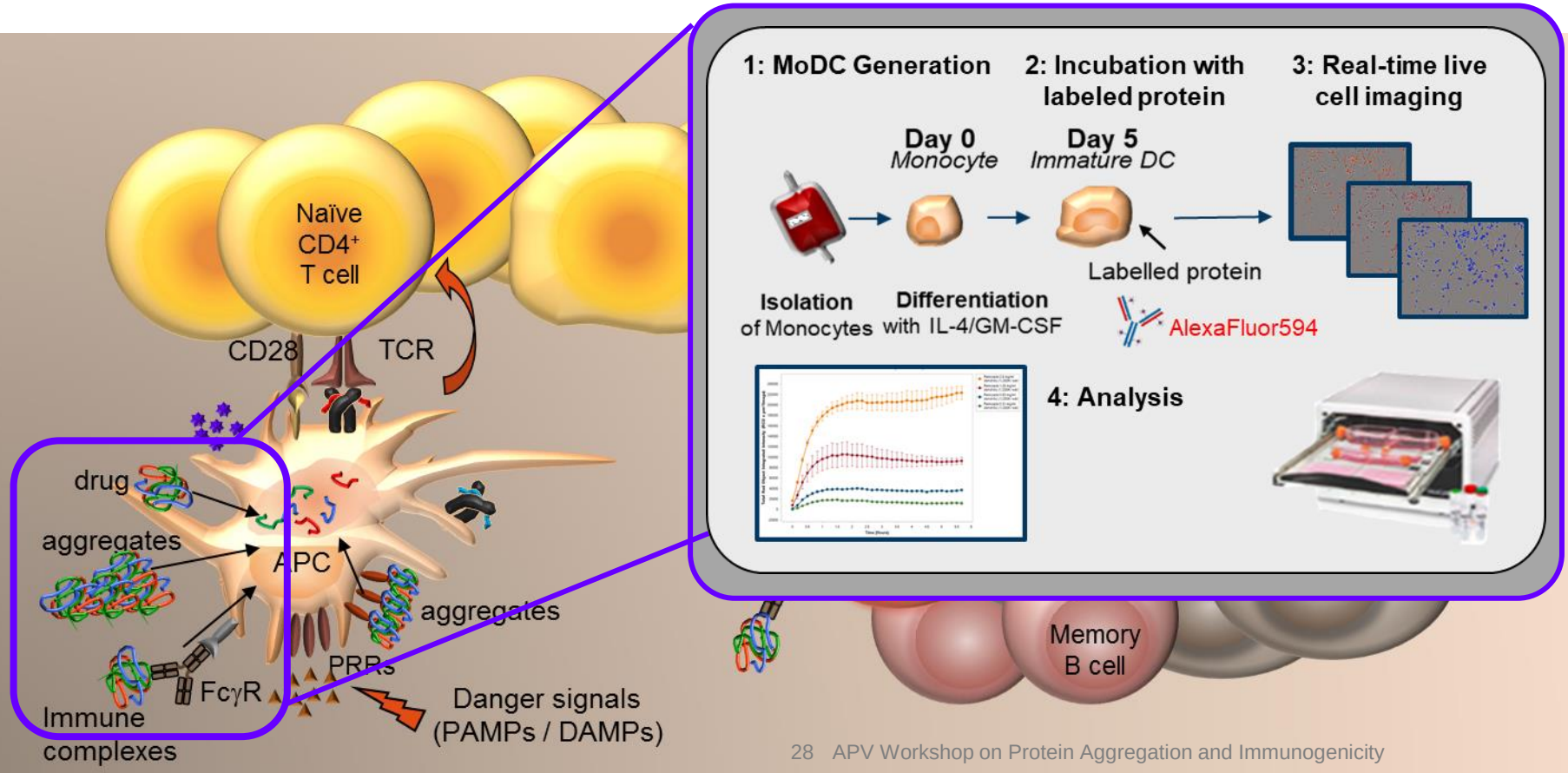
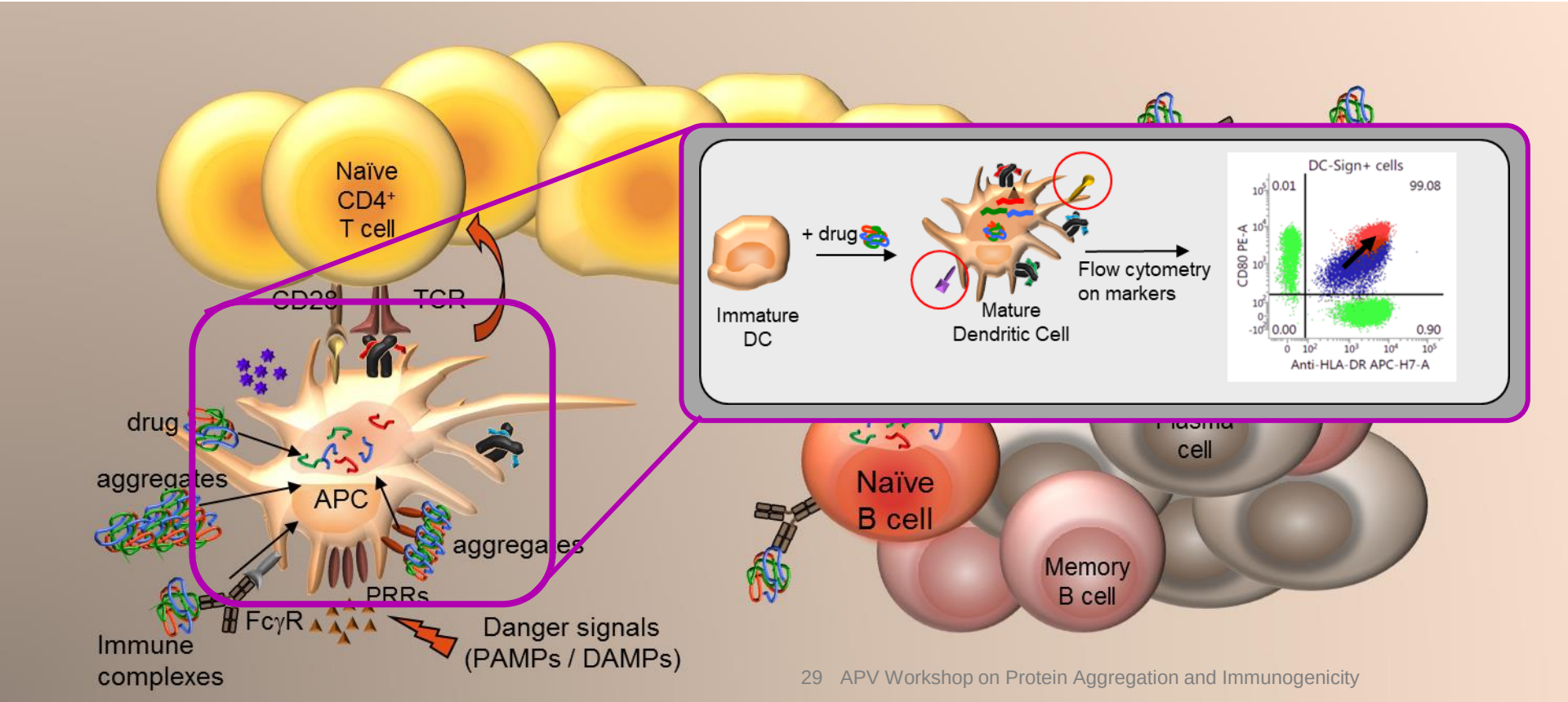
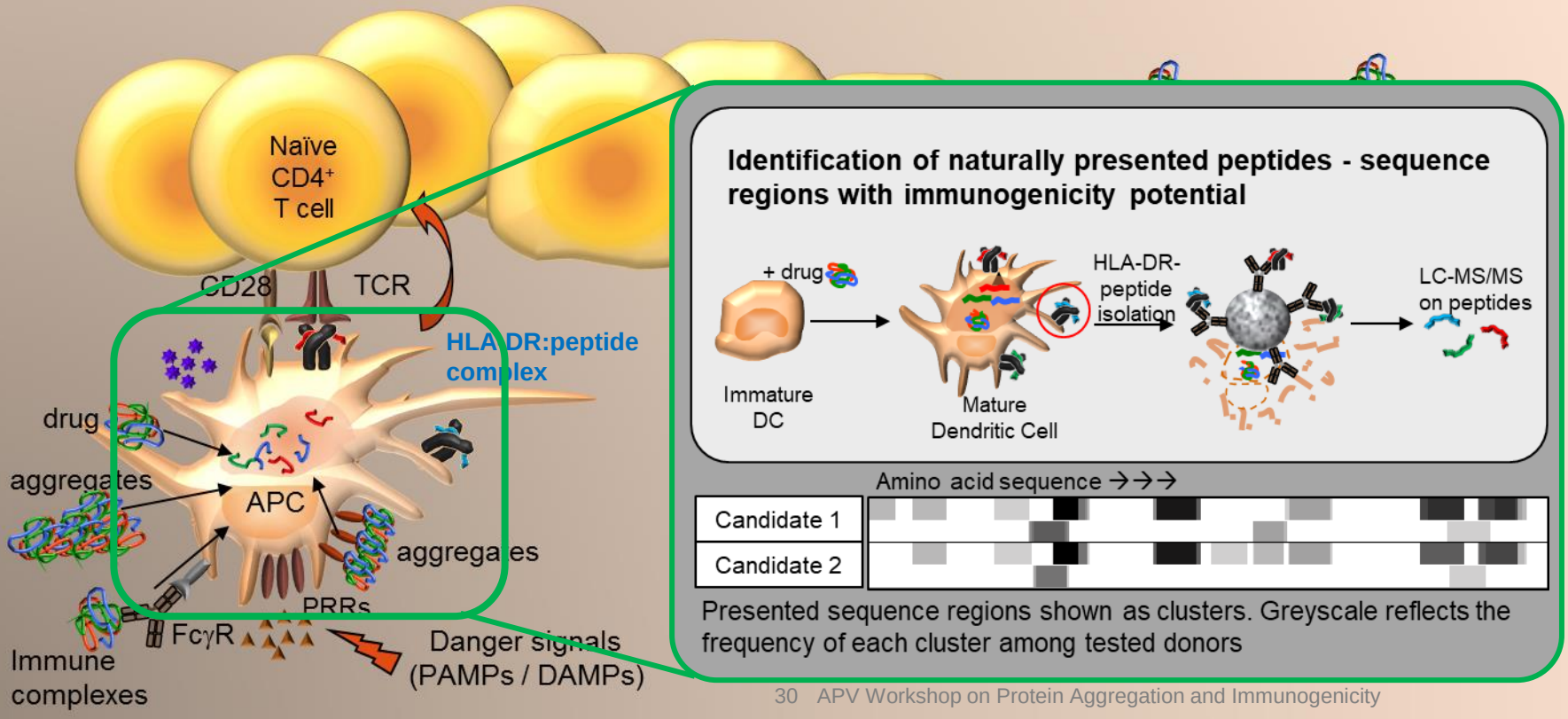
DC uptake assay



DC maturation assay



MHC Associated Peptide Proteomics (MAPPs)



Multiple different formats exist in the literature and can be applied for IG assessment

T Cell Assays (PBMC format)