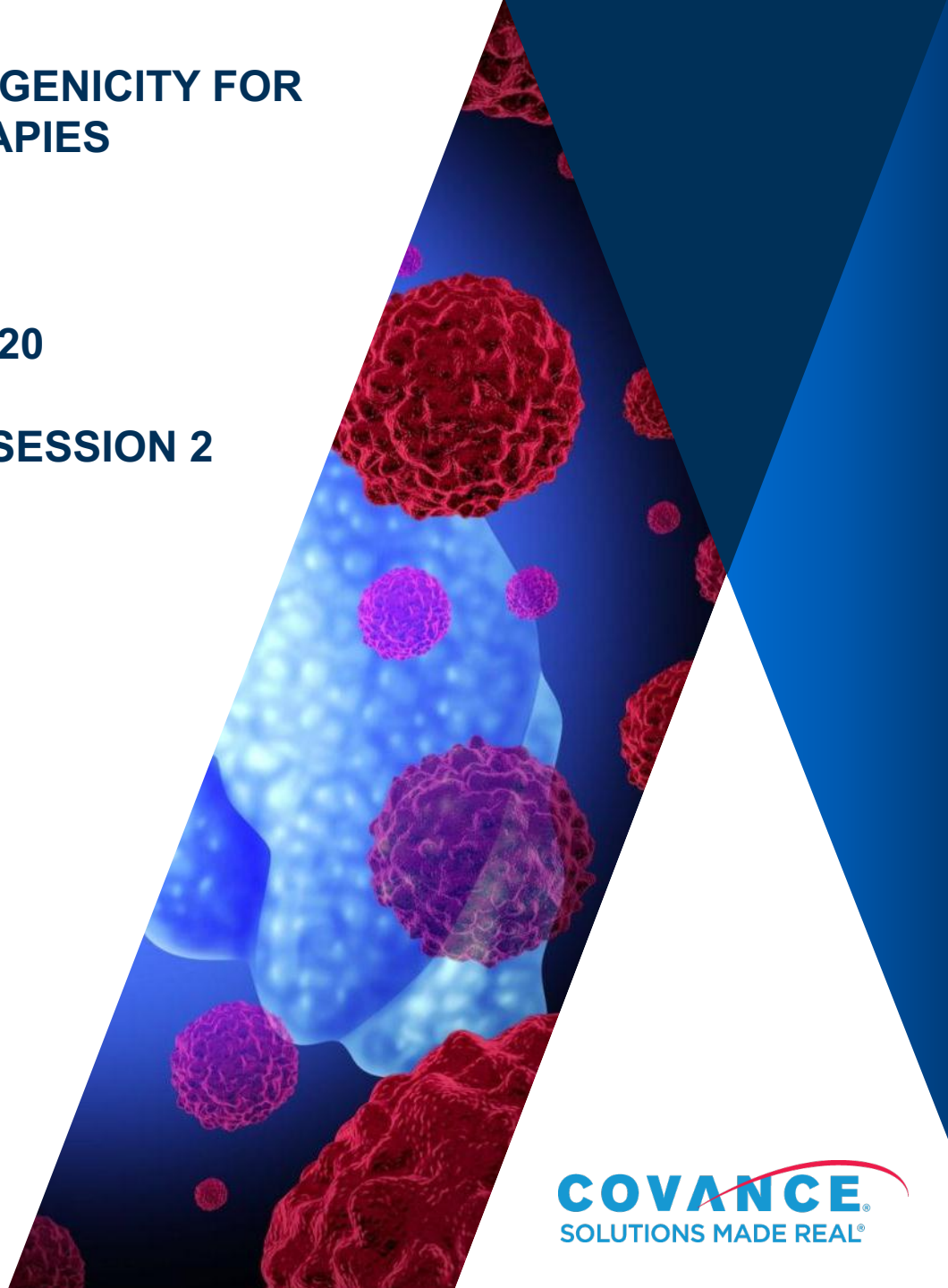
ASSESSING CELLULAR IMMUNOGENICITY FOR CELL AND GENE THERAPIES

JAMES MUNDAY

16TH SEPTEMBER 2020

EBF C&GT TRAINING DAY – SESSION 2



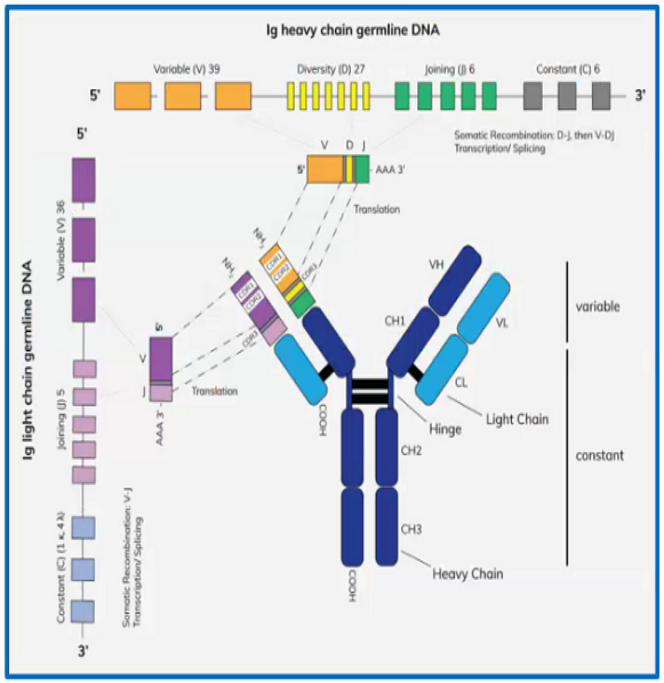
COVANCE
SOLUTIONS MADE REAL®

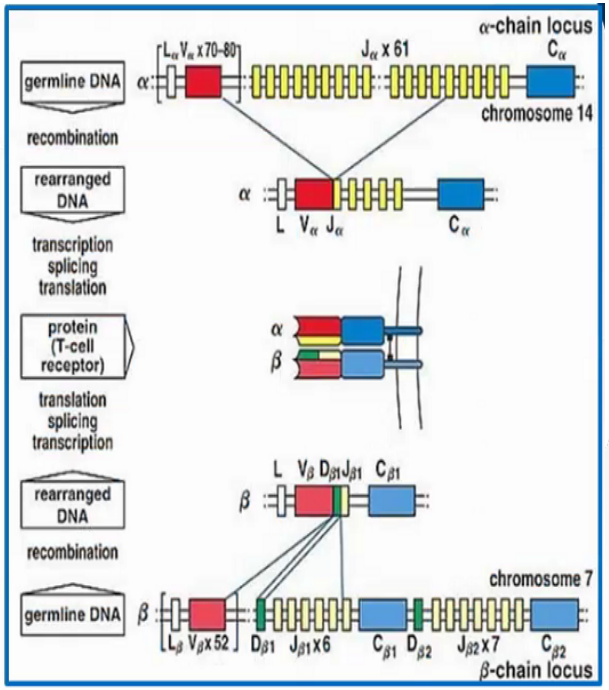
Meeting Agenda

- B-cells and T-cells are key components of the Adaptive Immune response
- Current regulatory guidance for Immunogenicity assessment for Cell and Gene therapy
- Methods/Technologies available to assess cellular Immunogenicity
  - ELISPOT
  - Flow Cytometry
- Statistics – Compare and contrast to ADA
- Summary

COVANCE
SOLUTIONS MADE REAL®

B-Cells AND T-Cells are key components of the adaptive Immune response





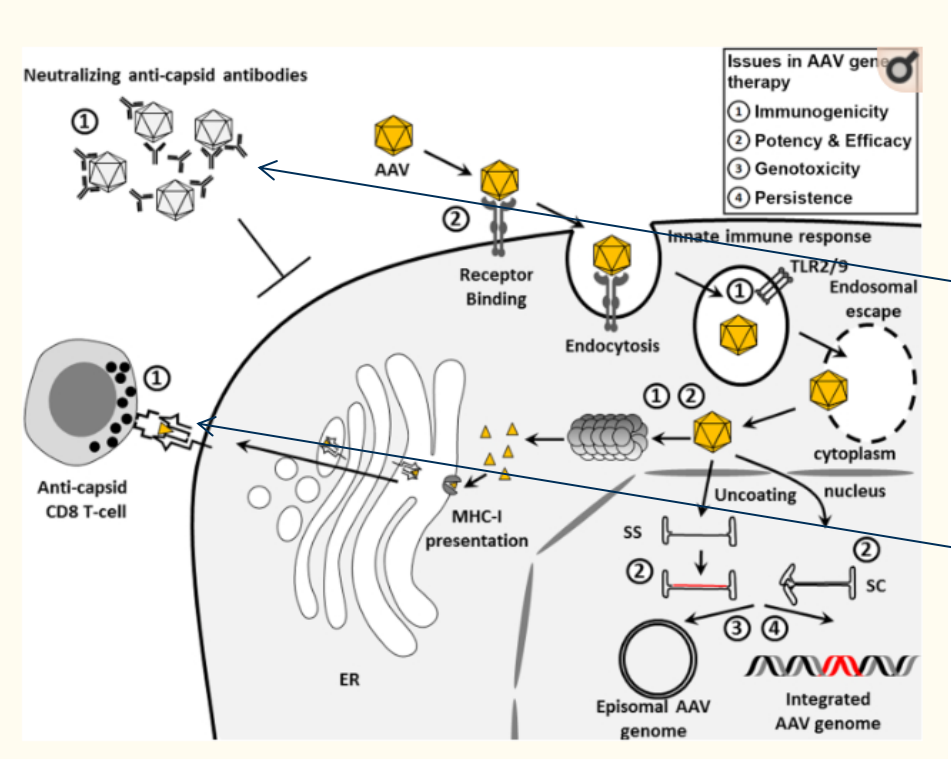
B-cells secrete antibodies that bind tightly to an antigen (generally a small “piece of protein”)

T-cells recognise an antigen in combination with a “self” MHC molecule, and secrete immunoactive cytokines in response

Cell therapies can stimulate a B-cell and a T-cell response

COVANCE
SOLUTIONS MADE REAL®

Cellular Immunogenicity



B-Cell mediated response

T-Cell mediated response

Image from: Emerging Issues in AAV-Mediated In Vivo Gene Therapy. Pasqualina Colella, Giuseppe Ronzitti, and Federico Mingozzi. Molecular Therapy: Methods & Clinical Development Vol. 8 March 2018

Current Immunogenicity regulatory guidance does not focus on T-cell response

COVANCE
SOLUTIONS MADE REAL®

Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC

No mention of T-cells in whole text

Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products

Guidance for Industry

Page 2

Features of some CGT products that may contribute to their risks include the potential for prolonged biological activity after a single administration, a high potential for immunogenicity,

Page 19

If immunogenicity is a concern (e.g., with viral capsids or allogeneic cellular products), then each subject's immune response to the product should be evaluated. This evaluation may include monitoring for evidence of **both cellular** and humoral immune responses.

COVANCE
SOLUTIONS MADE REAL®

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 May 2017
EMEA/CHMP/BMWP/14327/2006 Rev 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

Page 5

4. Factors that may influence the development of an immune response against a therapeutic protein
4.1. Patient- and disease-related factors • Genetic factors modulating the immune response
Genetic factors may influence immune responses to a therapeutic protein and lead to inter-patient variability. Genetic variation at *the level of MHC molecules and T-cell receptor will m*odify the immune recognition whereas genetic variation at the level of the modulating factors, such as cytokines and cytokine receptors, may influence the evolution and the intensity of the response.

Page 10

Both humoral and **cellular immune** responses should be considered. Cellular responses may be relevant when pharmacodynamic or adverse effects are (suspected to be) mediated by immune cells, e.g. when delayed type hypersensitivity occurs or when a cytotoxic T-cell response is suspected.

COVANCE
SOLUTIONS MADE REAL®

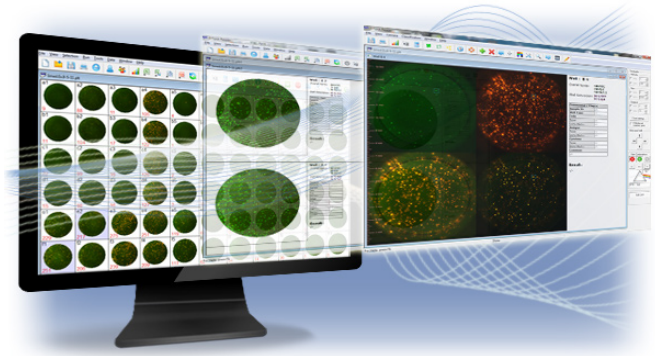
Methods/Technologies available to assess cellular Immunogenicity for T and B-Cell activities

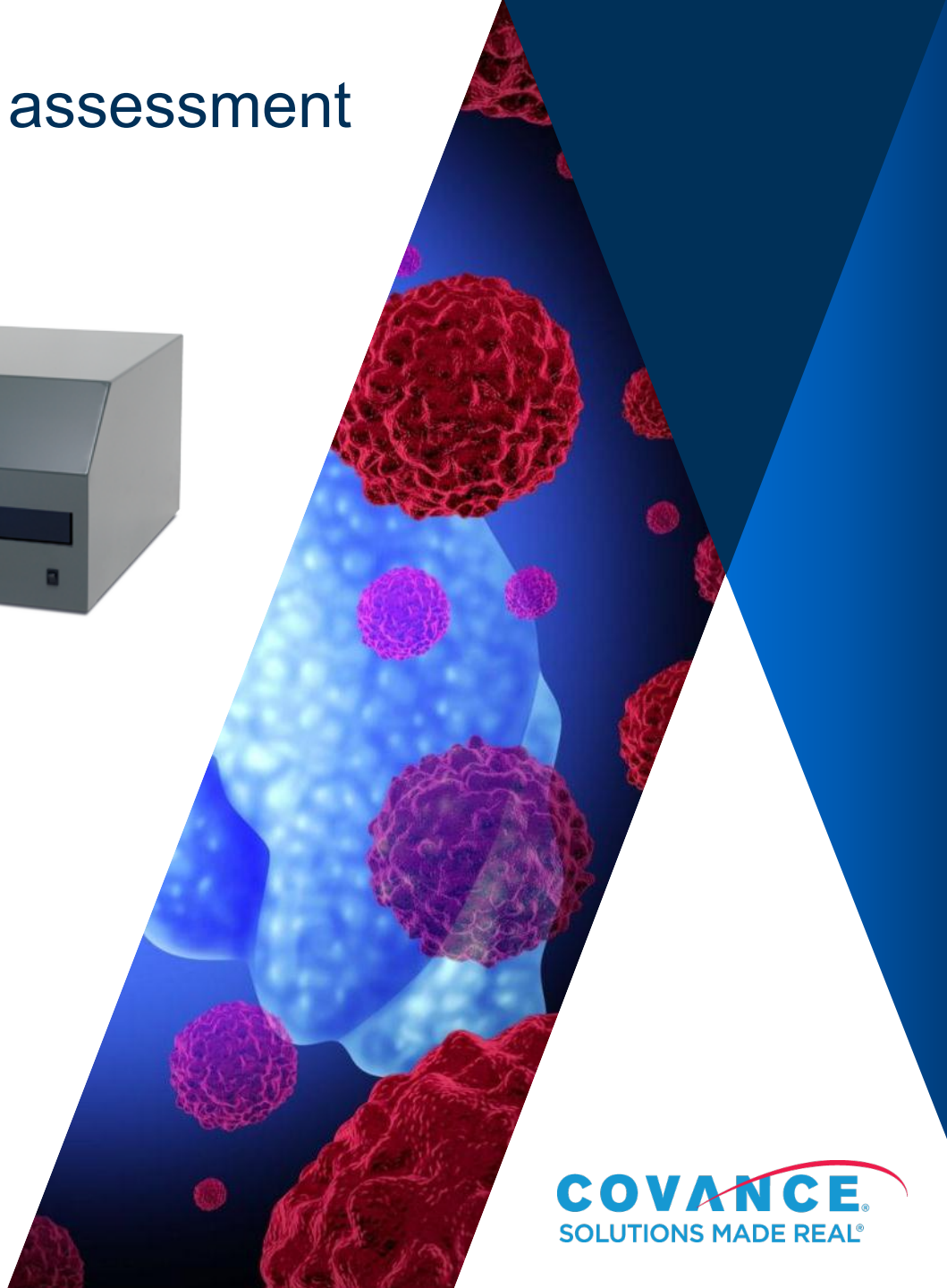
1. ELISPOT

2. Flow Cytometry

COVANCE
SOLUTIONS MADE REAL®

Cellular Immunogenicity assessment by ELISPOT





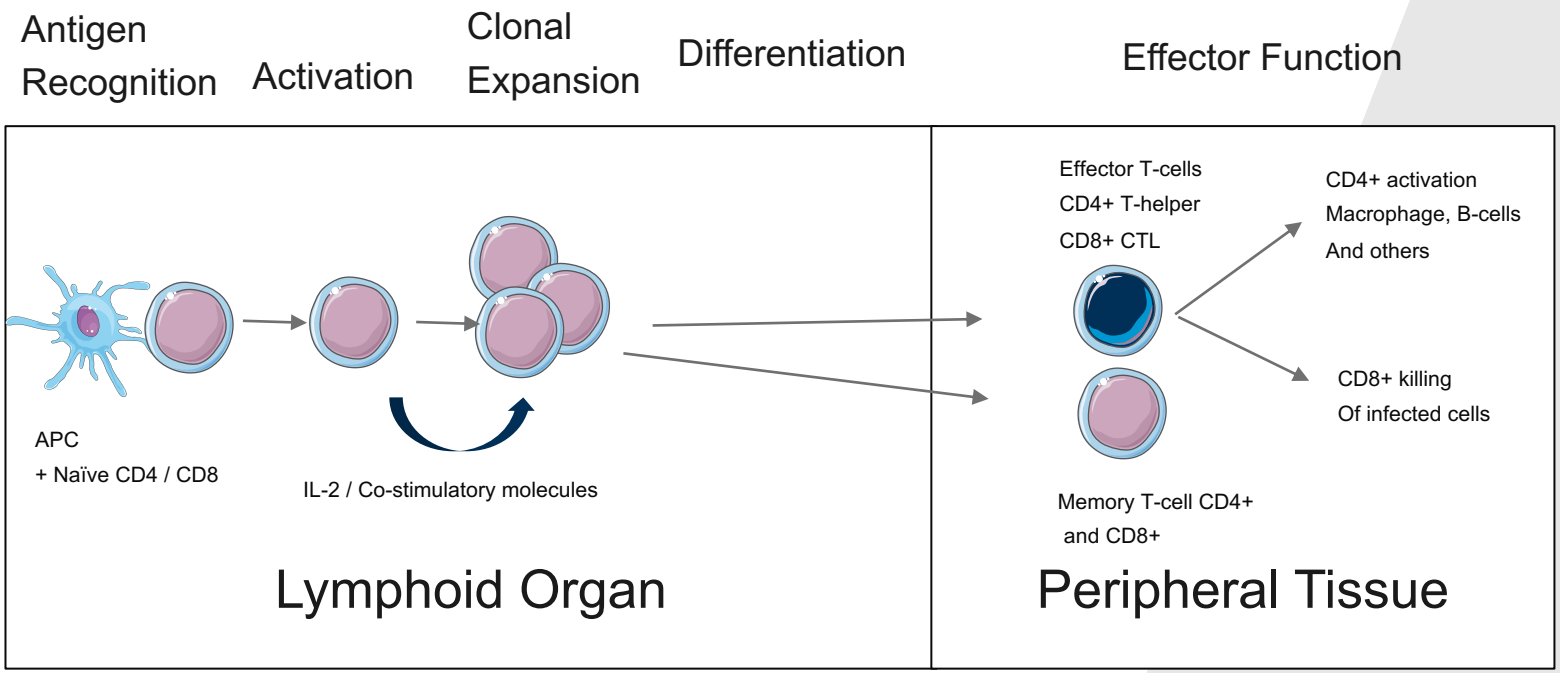
COVANCE
SOLUTIONS MADE REAL®

ELISPOT ASSAY

- Enzyme-Linked ImmunoSpot (ELISpot) is highly sensitive cell based Immunoassay designed to measure the frequency of cytokine, protein and antibody secreting cells at a single cell level.
- Invented by Cecil Czerkinsky in 1983 by his research group in Gothenburg, Sweden. The assay was originally developed for the detection of antigen-specific antibody secreting B cells.
- Technique can detect cellular product secretion down to 1 event in 100,000
- ELISpot ~200x more sensitive compared to supernatant ELISA analysis.
- ~20x more sensitive compared to Intracellular Cytokine Staining (ICS)

COVANCE
SOLUTIONS MADE REAL®

Cellular immune response that ELISPOT assay can measure



ELISPOT readouts

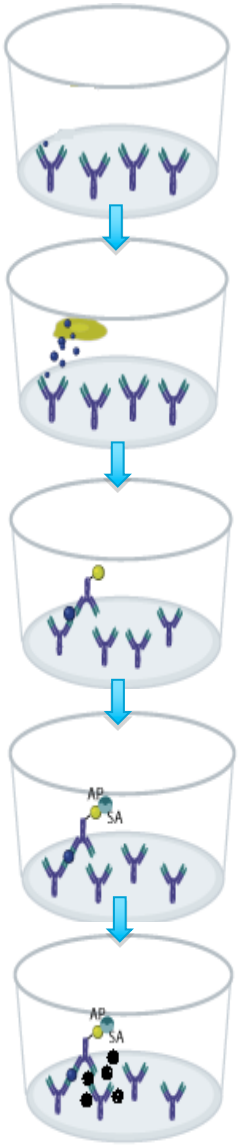
COVANCE
SOLUTIONS MADE REAL®

Application of ELISPOT Technique – Context of Use

- **Vaccine Immunogenicity testing –** T cell ELISpot (IFN-γ commonly used) assessment of T cell function following inoculation of vaccine material. Ex-vivo assessment of cell mediated immune response to vaccine.
- **Gene Therapy – Cellular Immunogenicity testing**
- **CAR-T efficacy testing** - Follow activation status of cells
- **Recall antigen testing** –Screening for prior immune interaction with bacteria, yeast or viral antigens in clinical trials. KLH & TT – standard antigens can be used
- **Epitope mapping/discovery –** Identification and characterization of novel epitopes from a protein recognized by the immune system.
- **Adjuvant discovery/selection/screening** – In-vitro or ex-vivo plate screening of adjuvant to improve antigen presentation and drug delivery.

COVANCE
SOLUTIONS MADE REAL®

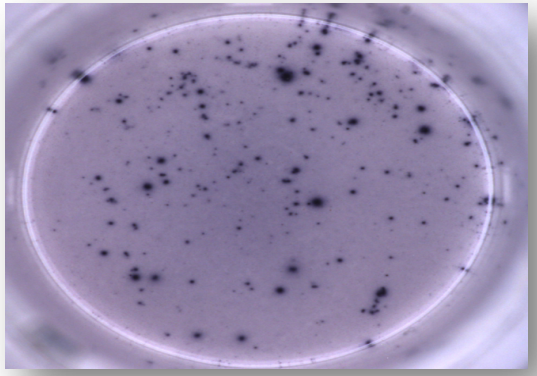
ELISpot – T cell assay principal



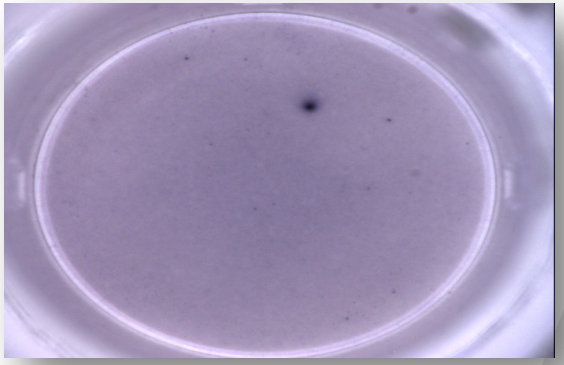
- Plates membrane is coated with anti-cytokine capture antibody.
- PBMC's/Splenocytes are isolated or thawed and added to the plate in addition to stimulus (viral, peptide subunit or large molecule). The plates are incubated at 37° C in 5% CO2 for approximately 15-20 hours.
- Well contents removed and washed. The remaining cytokine is labelled using a biotinylated detection antibody
- Well contents washed and bound antibody labelled using an enzyme
- Chromogenic substrate is then added to the well to develop the spots

ELISPOT Example data

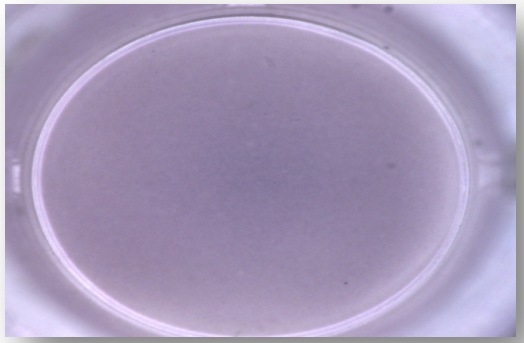
Positive stimulated sample



Negative un-stimulated sample



Assay background



COVANCE
SOLUTIONS MADE REAL®

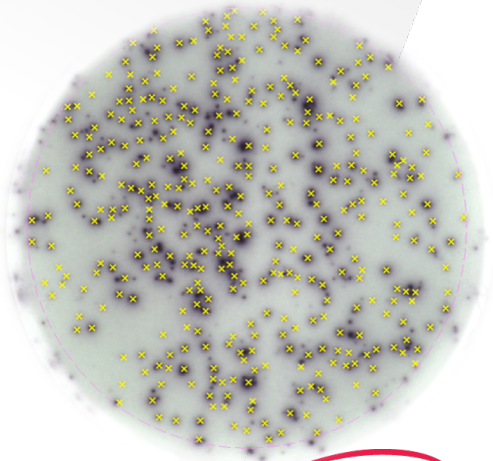
ELISpot Data Analysis – Count Settings

Spot sizes and intensity vary across T cell responses and stimuli (Mitogenic, Antigenic & negative control)

Antigen specific T cell response spots to CEF pool or pp65 CMV are large and dark in appearance. Comparatively, negative control sample spots are usually more uniform and regular (for optimal sample)

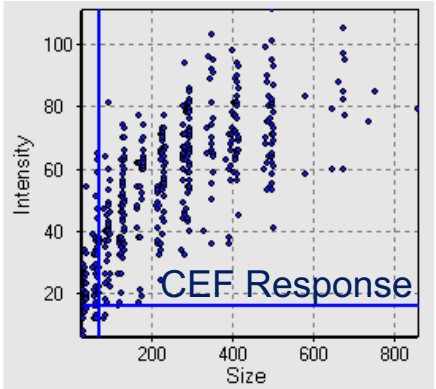
Spot count setting gating starter parameter are critical to determining accurate cellular response determination.

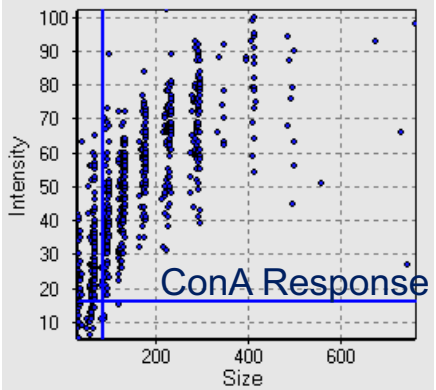
Count setting validation is a key aspect which requires optimisation & fixing during the assay validation stage.

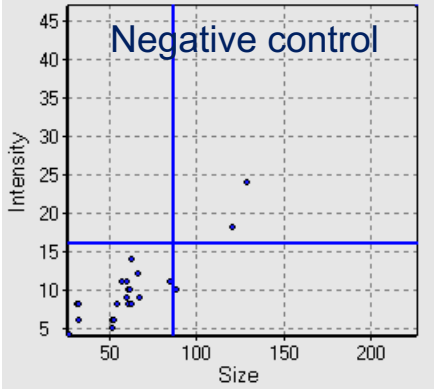


COVANCE
SOLUTIONS MADE REAL®

ELISpot Data Analysis – Gating Strategy







For some AAV gene therapy dosed at immune privileged sites immunogenicity it not always possible to determine a positive response

Count setting are even more critical for these types of therapies due to the unknown responses. Extra stringency and diligence is required when analysing the data.

Instruments can also determine not just spot number but also size and intensity, these parameters can also be adopted to determine sample positivity.

Variety of statistical approaches are applied. No standard approach defined

COVANCE
SOLUTIONS MADE REAL®

Future Directions for ELISpot

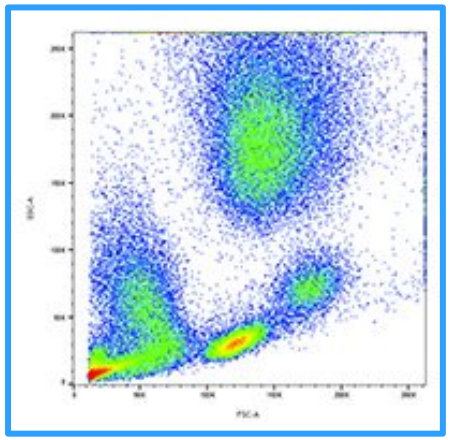
WRIB recommendations for ELISPOT:

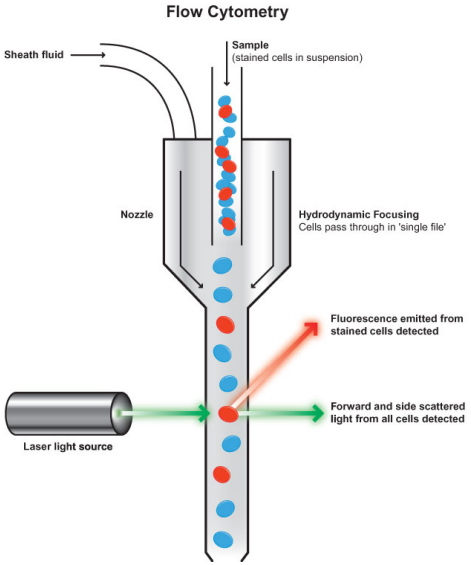
- ELISpot may be used to monitor for cellular immunity, if used it should be developed using a risk-based approach factoring in the route of administration

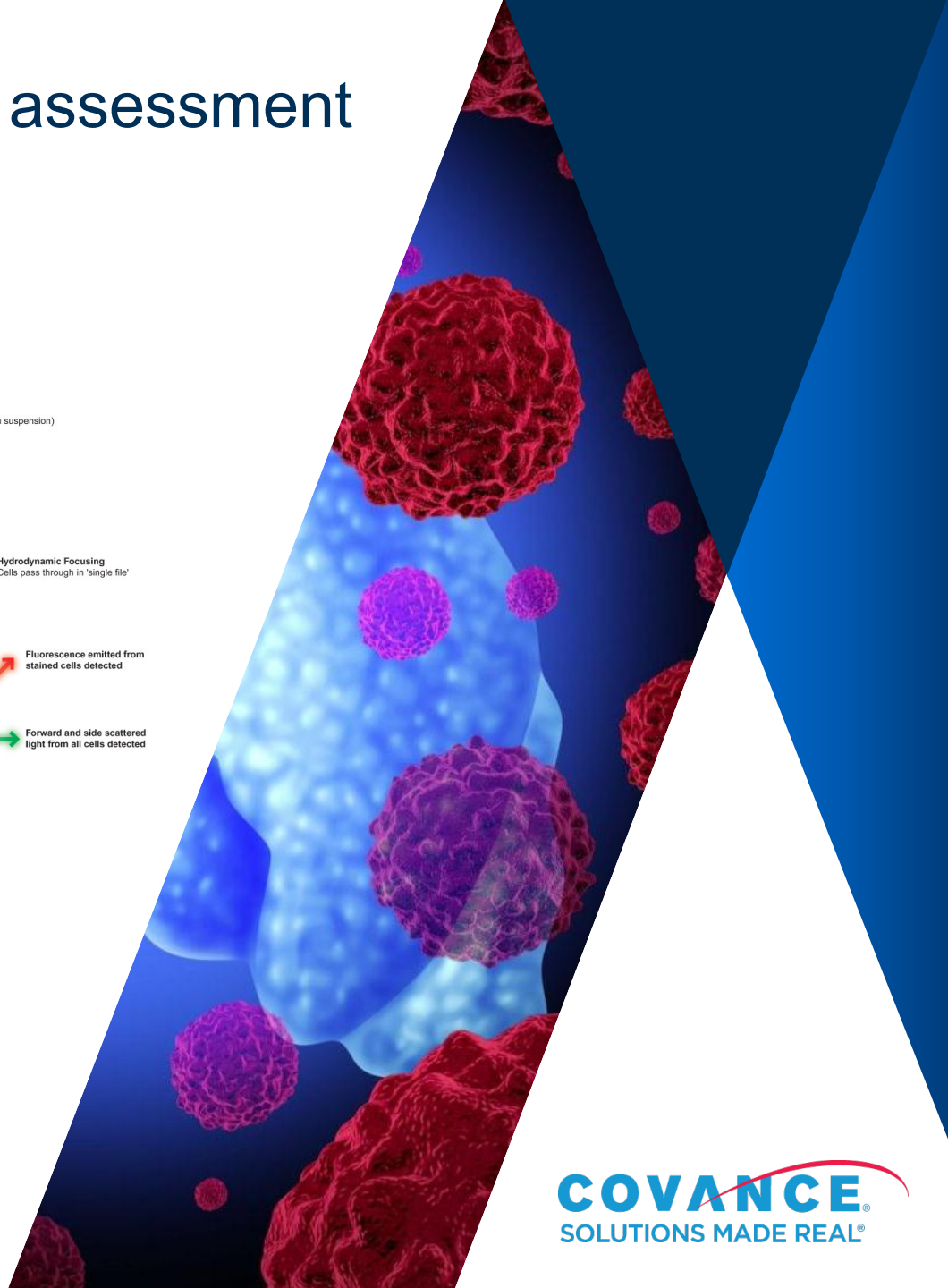
- ELISpot results can be normalized (intra- and inter-subject), special accommodation should be made for particular sites taking into account known variabilities in the assay. Not every site may be required to conduct every analysis. Multiple baselines can be used

- ELISpot harmonization consortium White Paper which outlines assay expectations and performance criteria may be helpful to develop ELISpot assays

Cellular Immunogenicity assessment by flow cytometry







COVANCE
SOLUTIONS MADE REAL®

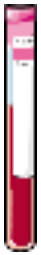
Applications of flow cytometry for assessing immunogenicity

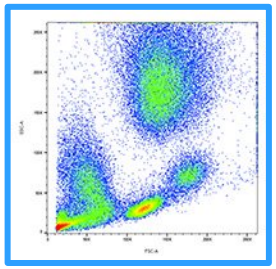
- Cytokine Analysis
- Immunogenic epitope analysis – Tetramer binding
- Immunophenotype analysis
- Receptor occupancy / ADA / NAb analysis

COVANCE
SOLUTIONS MADE REAL®

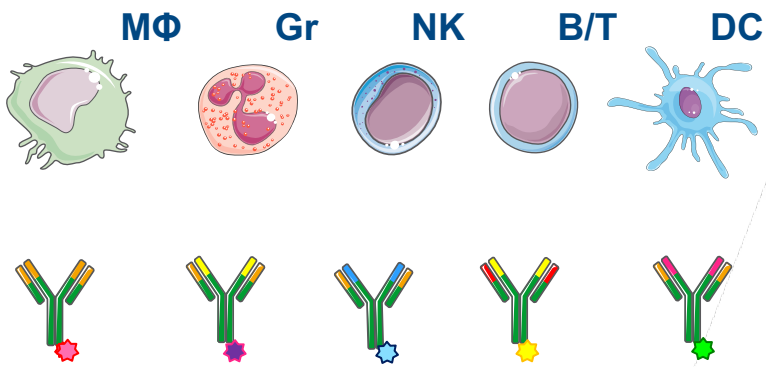
Flow Cytometry + Fluorochrome Conjugated MAbs to monitor Immune Cells subsets

Blood/Spleen: complex tissues composed by different immune cells directly/indirectly targeted by different therapeutics





Discrimination based on size and “complexity”



Additional layer of specificity with fluorochrome conjugated Mab

Cytoplasmic/Membrane proteins

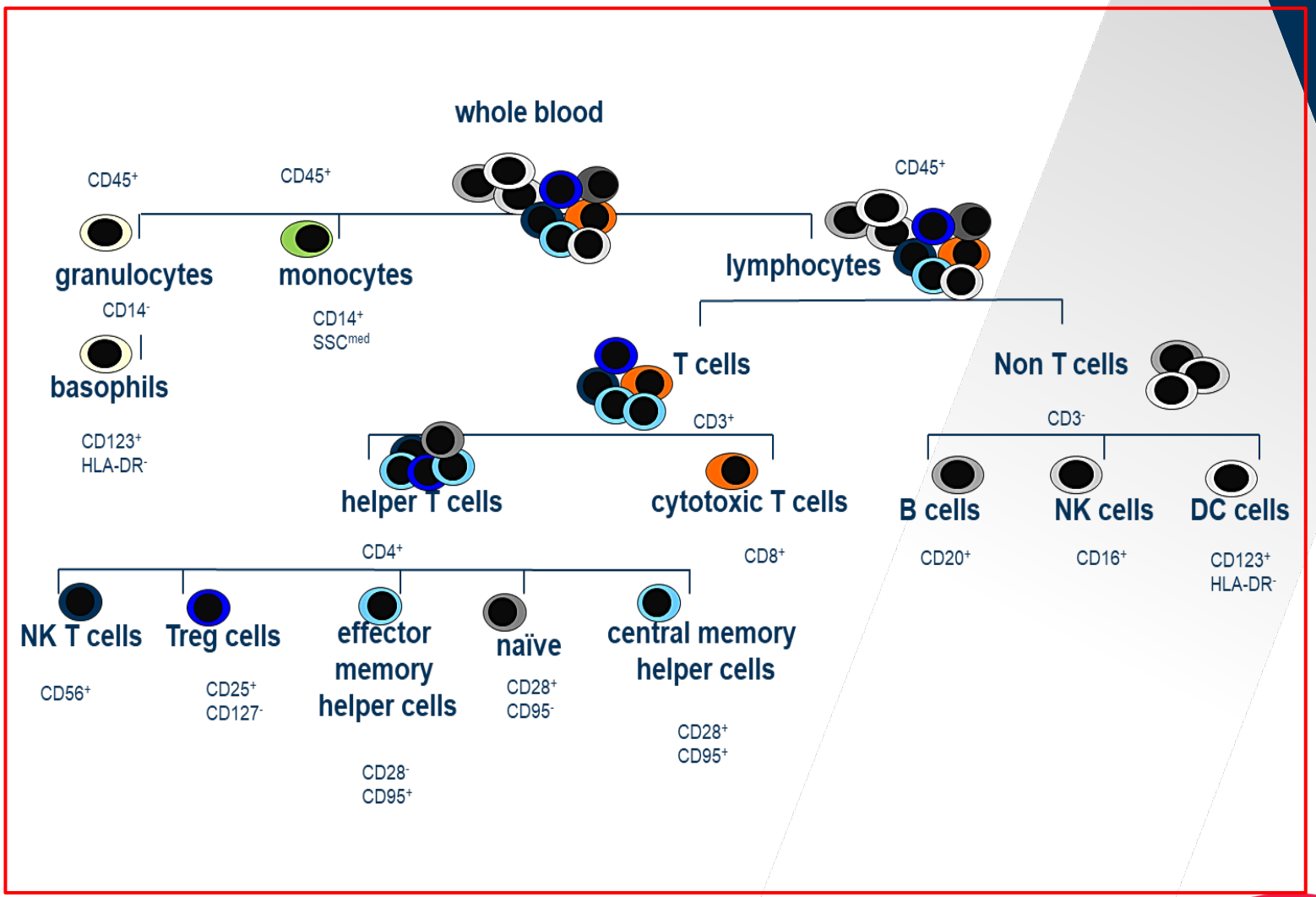
Phenotypical Characterisation (Resting/Activation)

Concentration (Cells/mL)

Frequency (%)

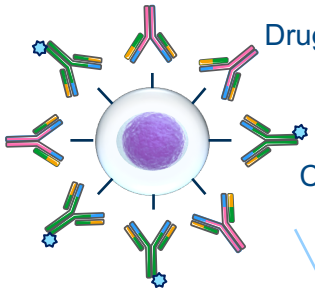
COVANCE
SOLUTIONS MADE REAL®

Phenotypical Characterisation of Immune Cells in a Broad Range of Animal Species

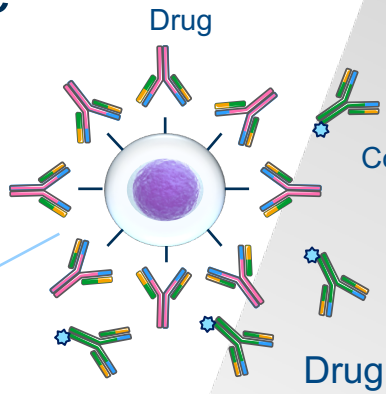


COVANCE
SOLUTIONS MADE REAL®

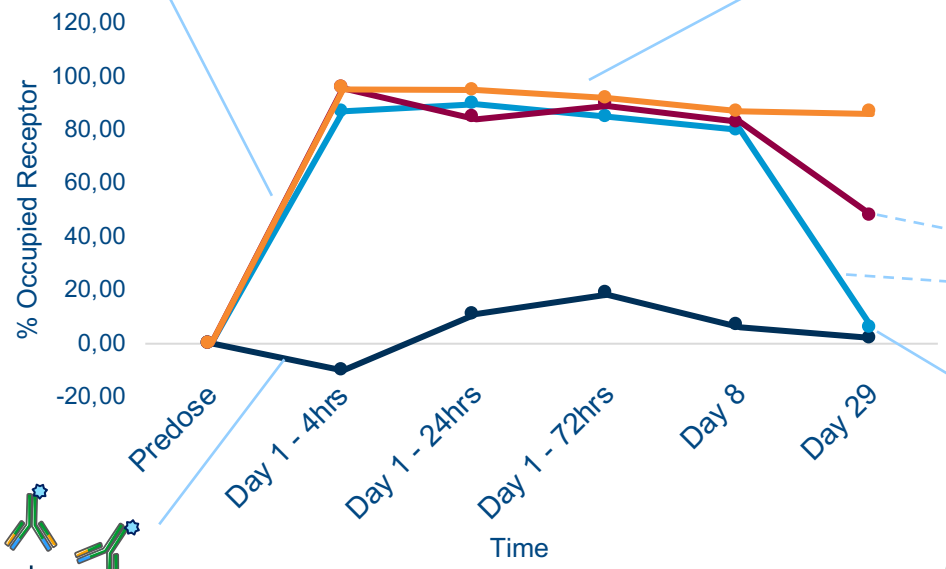
Receptor Occupancy (RO) by Flow – Can be used to Characterise & Quantify the binding profile of therapeutics to targets on the Cell Surface



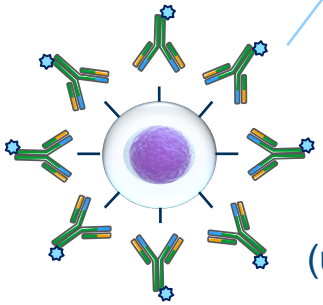
Some Free/Some Bound



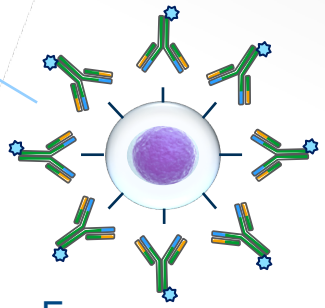
Drug Saturation
(All Receptors Bound)



Anti-Drug Ab (ADA)



Receptors Free
(unoccupied by drug)



Receptors Free
(unoccupied by drug)

COVANCE
SOLUTIONS MADE REAL®

Example of ADA in Study X and How RO Was Used to Interpret Findings

- **INCIDENCE OF ADA IN STUDY X:**

Anti-Drug Antibodies

	Anti-drug Antibody Analysis (Positive/Total)					
	Control (1)		Low (2)		High (3)	
Sampling time-point	M	F	M	F	M	F
Week 5	0/6	0/6	3/5	5/6	0/6	0/6
Week 14	0/6	0/6	6/6	5/5	0/6	1/6
Week 27	0/6	0/6	4/6	5/5	0/6	1/6
Week 31	2/6	0/6	3/6	6/6	0/5	0/6

No ADA detected in majority of high dose animals – May indicate drug tolerance constraint for ADA assay

ADA detected in majority of animals at low dose

Data: Covance

COVANCE
SOLUTIONS MADE REAL®

RO for treatment groups

Group/Sex	An No.	%Free	Group/Sex	An No.	%Free	Group/Sex	An No.	%Free
				Males				
1M	16	53.2	2M	145	3.3	3M	274	2.7
1M	17	96.1	2M	146	3.0	3M	275	2.4
1M	18	91.0	2M	147	2.8	3M	276	2.4
1M	19	100.0	2M	148	2.6	3M	277	3.3
1M	20	93.3	2M	149	3.0	3M	278	3.3
1M	21	100.1	2M	150	3.4	3M	279	2.6
1M	21	84.9	2M	151	52.3	3M	280	3.3
1M	23	82.1	2M	152	3.2	3M	281	2.8
1M	24	83.5	2M	153	21.4	3M	282	2.9
1M	25	69.4	2M	154	2.8	3M	283	2.5
1M	26	86.8	2M	155	2.7	3M	284	3.0
1M	27	87.4	2M	156	3.1	3M	285	2.8
1M	28	69.1	2M	157	4.2	3M	286	2.9
1M	29	77.2	2M	158	3.0	3M	287	2.9
1M	30	80.5	2M	159	3.0			
				Females				
1F	409	75.2	2F	538	50.3	3F	667	3.2
1F	410	80.3	2F	539	89.0	3F	668	3.3
1F	411	86.6	2F	540	103.5	3F	669	3
1F	412	82.3	2F	541	3.1	3F	670	3.1
1F	413	91.5	2F	542	2.6	3F	671	2.8
1F	414	68.2	2F	543	3.0	3F	672	2.5
1F	415	72.9	2F	544	67.8	3F	673	2.5
1F	416	94.7	2F	545	2.7	3F	674	2.9
1F	417	82.3	2F	546	2.8	3F	675	3
1F	418	71.3	2F	547	2.5	3F	676	2.8
1F	419	76.7	2F	548	2.8	3F	677	3
1F	420	73.6	2F	549	2.4	3F	678	3.4
1F	421	63.4	2F	550	2.9	3F	679	2.9
1F	422	68.3	2F	551	57.4	3F	680	2.9
1F	423	61.8	2F	552	91.3	3F	681	2.7

Data: Covance

13 of **15 males** and
9 of **15 females** show free occupancy <10%

90% receptor occupancy. Therefore, in the majority of animals ADA is not affecting pharmacological activity

COVANCE
SOLUTIONS MADE REAL®

Summary

- B-cells and T-cell responses are important to evaluate for cellular immunogenicity understanding
- Regulatory guidance for Cell and Gene therapies still evolving
- ELISPOT and Flow Cytometry technologies are key components for understanding cellular immunogenicity
- Immunogenicity assessment relies on multi-parameter assessment to understand all cellular components of the immune response

COVANCE
SOLUTIONS MADE REAL®

Acknowledgements

- EBF
- All of Team I&I
- Specific acknowledgments:
- Chris Cooper

COVANCE
SOLUTIONS MADE REAL®