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WP3
Evaluation of different T cell assay formats

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on behalf of WP3 partners

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ABIRISK objectives

- Investigation of the clinical relevance of biopharmaceutical-associated immunogenicity …
- Evaluation of the predictive value of existing tools and newly developed *ex vivo* methods, along with investigations into the immunological mechanisms that form the basis of the development of anti-drug antibodies….
- Provide data-driven feed-back to regulators and healthcare professionals.

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Work package 3.1: Evaluation of different T cell assay approaches

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In vitro T cell assays provided by selected European based CROs

- Antitope EpiScreen™ – sponsored by Merck
- Lonza EpiBase™ – *sponsored by Novartis*
- Platine Immuno'line™ – *sponsored by CEA*
- ProImmune REVEAL® – *sponsored by Sanofi*

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Considered that...

- This study was not done to identify the best CRO but to understand how robust the T cell data is
- Data in this presentation is blinded but if you know the assays provided by the CROs you will be able to identify the data
- CROs received identical batches of test items with SOP how to handle them (freeze thaw cycles, etc.)
- Not all CROs were blinded but were asked to apply their standard assay format and not to optimize

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Test articles

MAb	Type	Target	Adm. route	Indications	ADA incidence[1-6]
Infliximab	Chimeric Ab (IgG1)	TNF-α	i.v.	Crohn's, RA, Cutaneous systemic sclerosis, Ankylosing spondylitis	7-61%
Rituximab	Chimeric Ab (IgG1)	CD20	i.v.	Non-Hodgkin's lymphoma, SLE, Vasculitis, Primary Sjögren's syndrome, Severe pemphigus, RA	0-50%
Adalimumab	Human Ab (IgG1)	TNF-α	s.c.	RA, Crohn's, PsO, PsA	2.6-50%
Natalizumab	Chimeric Ab (IgG4)	VLA-4 Integrin	i.v.	MS, Crohn's	9%
Rebif®	Cytokine	IFNAR	s.c.	MS	12-28%
Betaferon®	Cytokine	IFNAR	s.c.	MS	16.5–47%

[1]Delluc S *et al*. FASEB J, 2011; [2]Baker M *et al*. Self/Nonself, 2010; [3]Sauerborn M. Handbook of Therapeutic antibodies 2nd edition, 2014; [4]Bertolotto *et al*. J Neurol, 2004; [5]Zisapel M *et al*. J Rheumatol, 2015; [6]Hsu L *et al*. Expert Rev Clin Immunol, 2013

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Evaluation / Validation?

- Assay validation
  - Robustness and consistency of data
  - Ranking of test items relative to antigenicity risk or immunogenicity potential
- Biological validation
  - Do *in vitro* assays with heathy donor cells reflect in vivo T cell responses in treated patients?
- Clinical validation
  - Predicting clinical incidence / outcome

- Is clinical validation possible at all?
- T cell data consistency?

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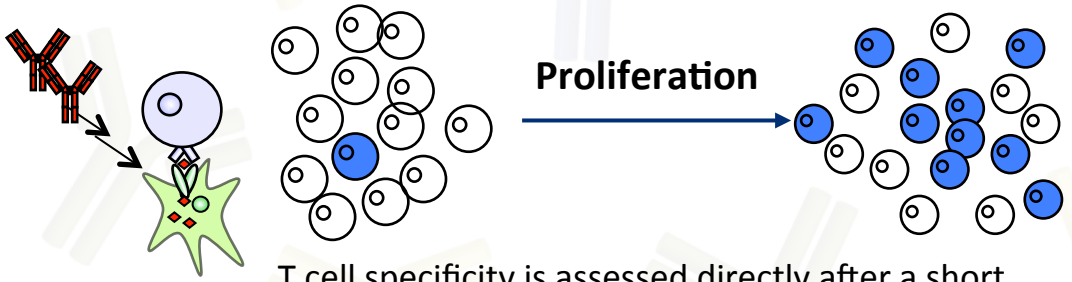
T cell assay formats

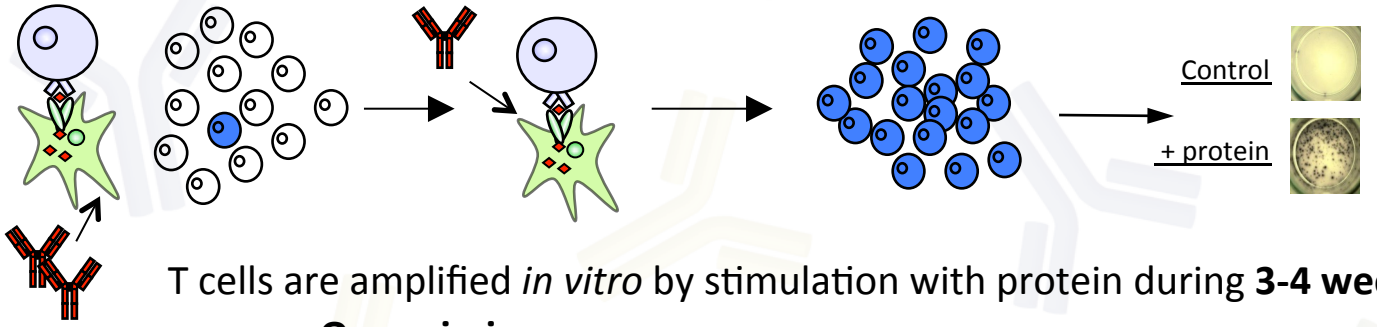
- **Short-term T cell assays** → **Providers 1, 3, 4**



Measure of cell proliferation : **CFSE$^+$, ^{3}H or EdU$^+$**
Measure of cytokine secretion: **IL-2 ELISPOT**

T cell specificity is assessed directly after a short culture step **7-10 days**

- **Long-term T cell assays** → **Provider 2**



Measure of the frequency of IFNγ secreting cells: **IFNγ ELISPOT**

T cells are amplified *in vitro* by stimulation with protein during **3-4 weeks**

- **One priming**
- **2 restimulation rounds (precursor amplification)**

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Comparison of different T cell assay approaches

Assay parameter	Provider 1	Provider 2	Provider 3	Provider 4
Tested antigens	Infliximab, adalimumab, rituximab, natalizumab, Betaferon® and Rebif®			
No of donors	50	16	50	50
Cells	Ag-loaded DC (maturation stim not specified) + CFSE-labelled CD8-depelted PBMC	Ag-loaded DC (matured with LPS) + CD4 T cells	Ag-loaded DC (matured with TNFα + IL-1β) + CD4 T cells	Ag-loaded DC (matured TNFα) + CD4 T cells
Readout	CFSE FACS	IFN-γ ELISPOT	EdU FACS	Thymidine incorporation and IL-2 ELISPOT
Data evaluation	Positive if % stimulation ≥ 0.5% and 2 SEM above background	Positive when spot count ≥ 2x background and minimal difference of 25 spots	Positive if SI ≥ 2 and significant vs control ($p<0.05$)	Positive if SI ≥2 and significant vs control ($p<0.05$)
Ranking	Ranking based on donor frequency and magnitude	Ranking based on precursor & donor frequency	Ranking based on donor frequency and magnitude	Ranking based on donor frequency

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Results provider 1

Protein ID	Percentage Antigenicity	Strength of Response (mean % stimulation)	Response Index (RI)
PPD (Study I / II)	98.08 / 96.00	26.46 / 17.34	25.946 / 16.643
KLH (Study I / II)	78.85 / 100	6.30 / 18.86	4.964 / 18.858
Adalimumab	3.85	1.42	0.055
Infliximab	7.69	1.11	0.085
Natalizumab	1.92	0.90	0.017
Rituximab	3.85	0.72	0.028
Rebif	4	0.75	0.030
Betaferon	0	0.00	0.00

- "Overall, taking into account the low numbers of responding donors, the low levels of % stimulation and lack of significant responses, the data suggests that **these test proteins are unlikely to be strongly antigenic**. **<u>However</u>**, external factors such as length and/or concentration of exposure, repeated exposure events, and mode(s) of action may affect responses elicited in vivo."

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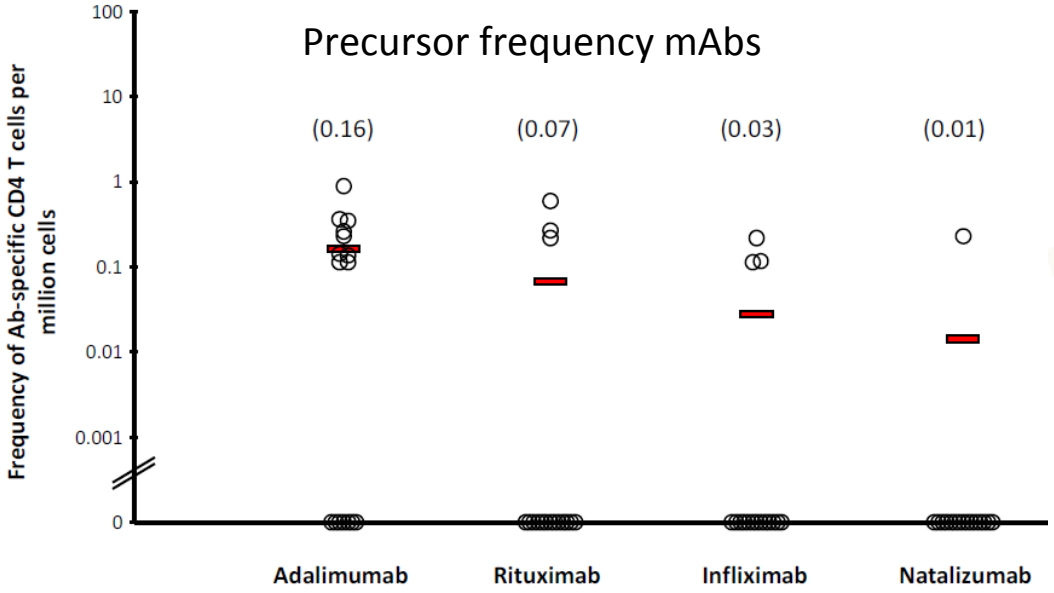
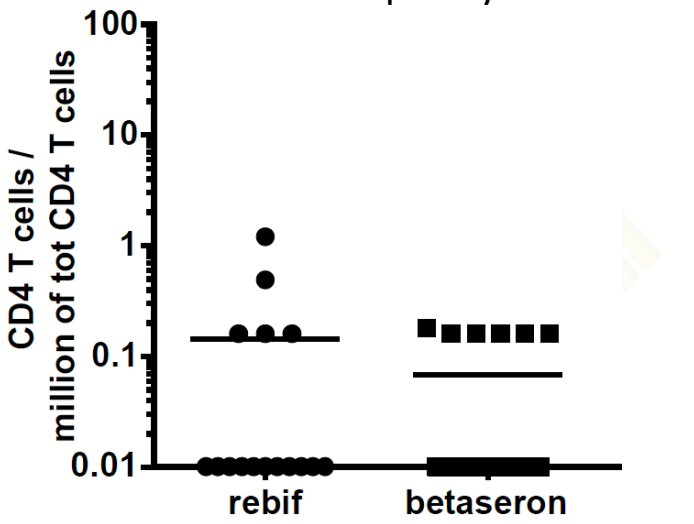
Results provider 2





- "**In contrast to the three antibodies Rituximab, Infliximab and Natalizumab which are less immunogenic, the antibody Adalimumab appears to be moderately immunogenic**. Rituximab is not significantly different from the Adalimumab but is also similar to the antibodies Infliximab and Natalizumab."
- "On the basis of these data, **both forms of IFN-β would have been considered as molecules with moderate risk of immunogenicity**"

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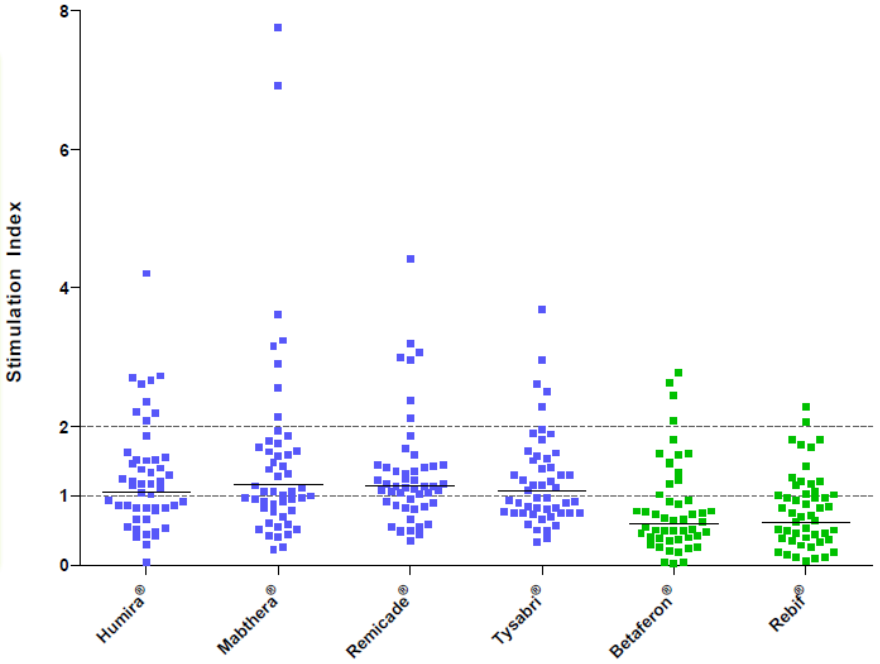
Results provider 3

Product	Number of responding donors	Frequency of responding donors (%)	Mean SI of responding donors
KLH	50	100	15.16
Adalimumab	9	18	2.59
Rituximab	8	16	3.65
Infliximab	7	14	2.95
Natalizumab	5	10	2.77
Betaferon	4	8	2.47
Rebif	2	4	2.17



- **"Rituximab, adalimumab and infliximab are higher risk compared to natalizumab."**
- **"Direct comparison of the IFNβ products suggests that Betaferon® is at higher risk than Rebif®."**

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Results provider 4

	Rebif	Betaferon	Infliximab	Rituximab	Adalimumab	Natalizumab	KLH (Novartis)	A33	KLH
Proliferation %	2	2	12	12	4	2	40	10	32
ELISpot %	4	2	8	8	4	4	46	14	38
Proliferation and ELISpot %	2	2	8	6	4	2	38	10	32
Correlation %	100	100	67	50	100	100	95	100	100

- **All test items < 10% cut-off, suggesting that they all fall into the 'low risk' category** for potential clinical immunogenicity (based on historic data with this assay)
- **However**, β-IFNs and anti-α-TNFs may have affected the outcome due to direct effects on DC viability and/or maturation

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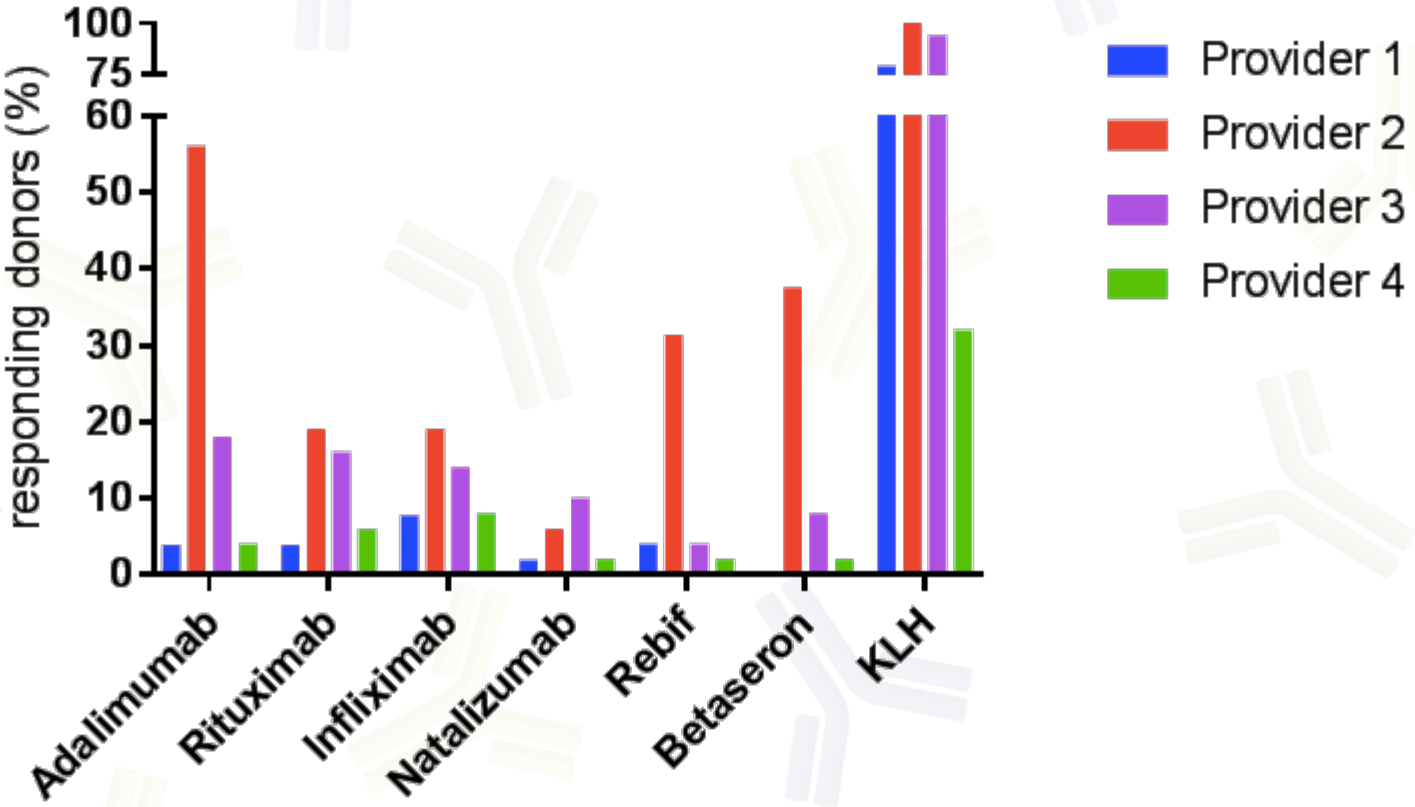
Comparison of responses across all assays



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Comparison of ranking

Ranking on this slide does not necessarily reflect statistically significant differences!

	Infliximab	Rituximab	Adalimumab	Natalizumab	Betaferon®	Rebif®
Provider 1	1	3	2	4	2	1
Provider 2	3	2	1	4	1	1
Provider 3	3	1	1	4	1	2
Provider 4	1	2	3	4	1	1

Colour coding indicates ranking, from high to low

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Overall conclusions

- All compounds tested have demonstrated immunogenicity in clinical *in vivo* studies, but only one assay could show strong *in vitro* immunogenicity
  - Assays not sensitive / accurate enough to differentiate
- No good correlation in terms of ranking between different assays
- Understanding MoA of the compounds is essential
  - βIFNs not suitable to use in DC:T cell assays due to their interaction with DCs
  - Anti-αTNFs possibly interfere with the DC maturation when TNFα is used for maturation

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General conclusions

- The comparison and “indirect early validation” of selected predictive immunogenicity tools was one of the initial key goals of ABIRISK
- This project has been logistically carried out according to planned strategy
- This data demonstrates a lack of correlation between the different assays used in this project (NB, no optimizations were allowed)
- However, low and high responses could be differentiated consistently and matched clinical experience, although some assays failed to predict a high risk at all.
- There is a need for globally accepted reference standards and quality controls to ensure comparable performance of such assays; not just strong antigens such as KLH.

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