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Immune response against particles in protein therapeutics

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Assessing the contribution of aggregation to the immunogenicity of protein therapeutics

- Protein aggregates can impact efficacy and safety
  - Highly undesirable; decreased biological activity of the protein, and the potential to trigger unintended immune responses
  - Enhanced immune responses to protein aggregates have been reported in animal and clinical studies
  - Aggregates displaying non-native protein conformations may be seen by the immune system as neoantigens, which could trigger antibody formation
- *In vitro* T cell assays can be used to dissect characteristics of protein aggregates that contribute to immunogenicity as well as provide an understanding of the immunological mechanism

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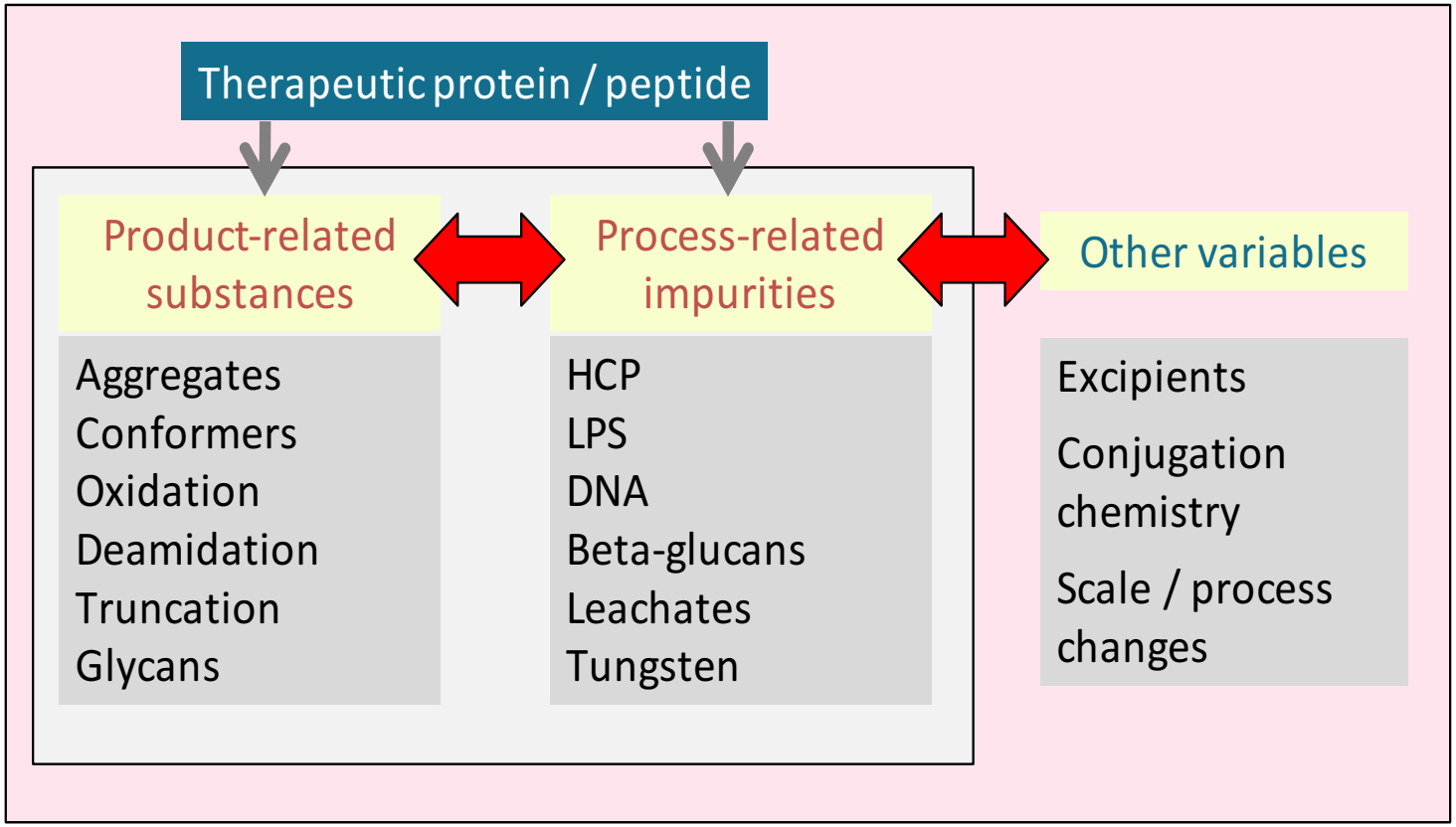
CMC variables that potentially influence immunogenicity



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Biotherapeutic aggregates may be generated at all stages of drug manufacture and delivery

Steps During Manufacturing/ Delivery

- Fermentation
- Purification
- Formulation
- Storage
- Shipping
- Administration

Potential Stress Conditions

- Heat
- Freeze-thaw
- Cross-linking
- Protein concentration
- Formulation – pH, salt….
- Extractables/leachables
- Chemical
- Mechanical
- Surface effects
- Nano-particles

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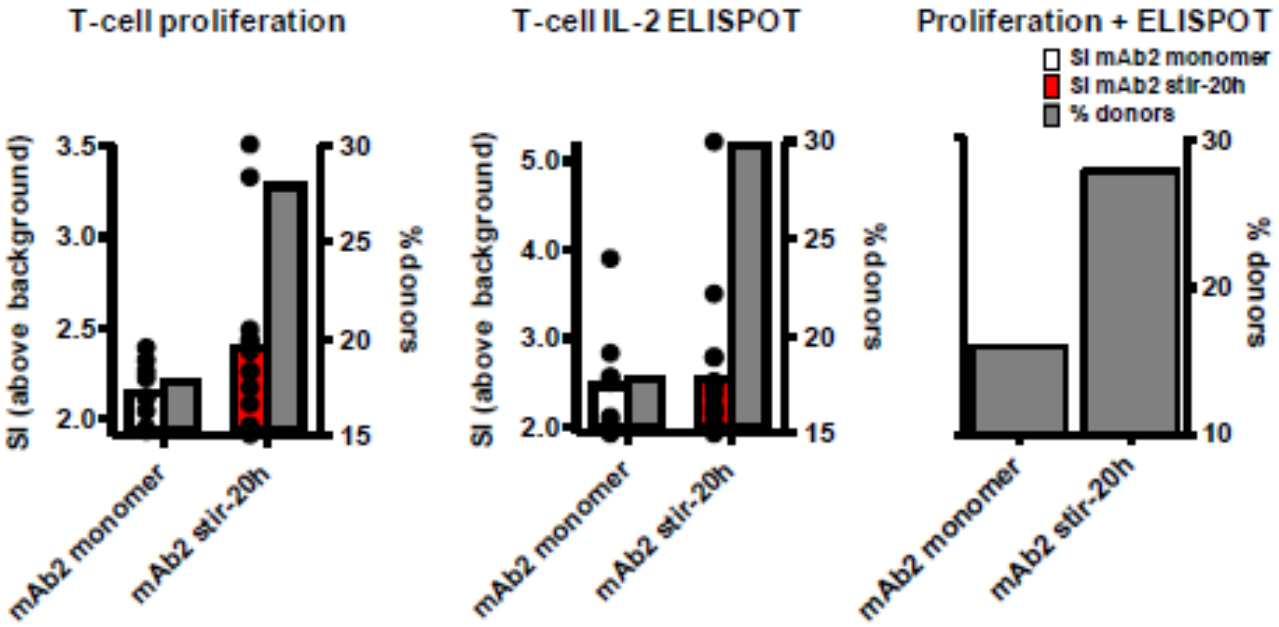
T cell proliferation in response to stress-induced aggregates



Stir stress >50,000 micron size particles/ml

Joubert et al. J. Bio Chem (2012)

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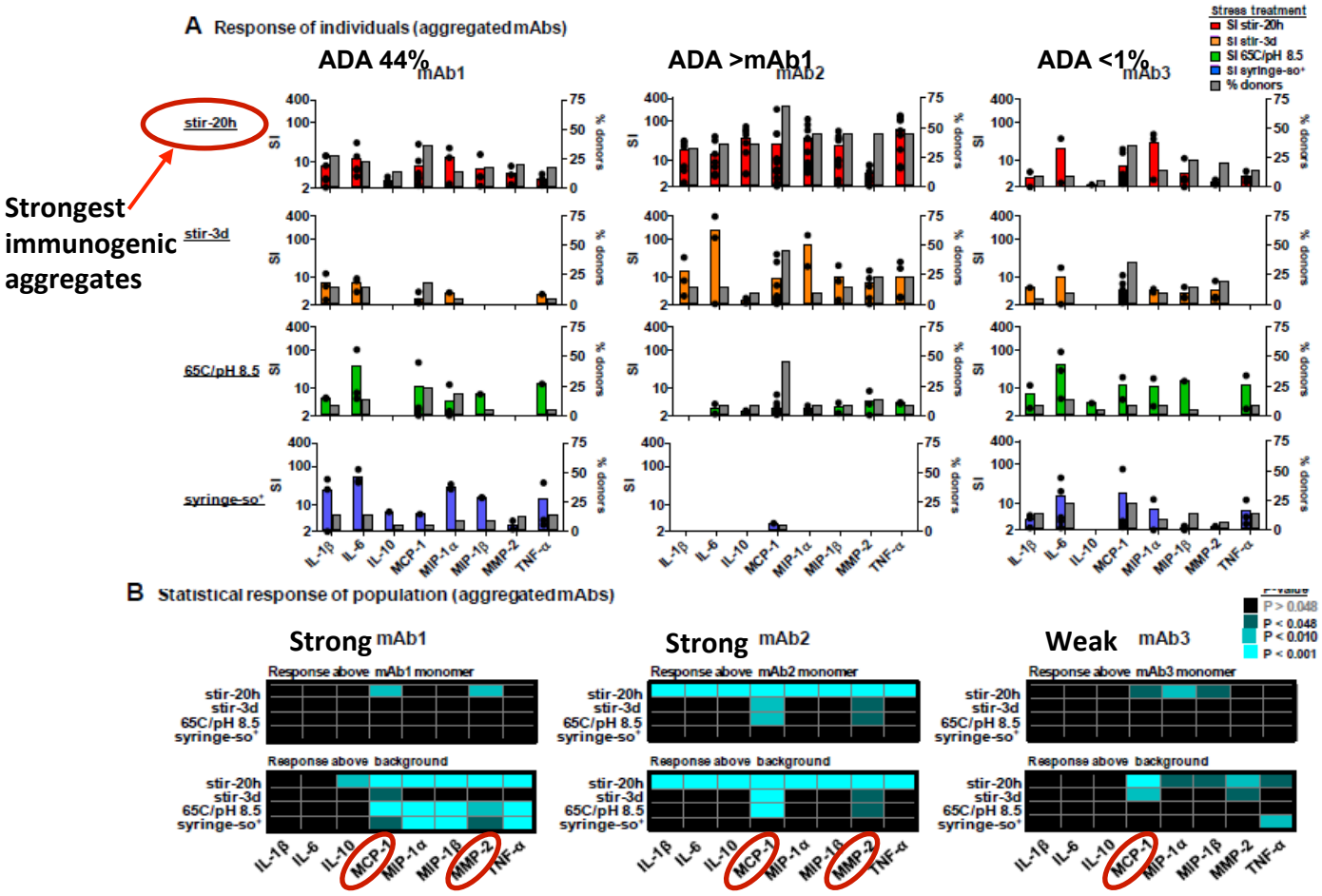
Cytokine release from stress-aggregated antibodies



Joubert et al. J. Bio Chem (2012)

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Immunogenicity of low levels of stress-induced aggregates

Determine effects of stress-induced aggregates of 2 clinically relevant therapeutics

Determine effects of stress-induced aggregation at levels that have relevance in manufactured products

- Rituximab (Rituxan) – clinical immunogenicity of 10% in RA (immune competent) patients
- Trastuzumab (Herceptin) – no significant clinical immunogenicity reported

Stage I – Antibody stress and analytics

- Four stress conditions induce aggregates of rituximab and trastuzumab
- Characterisation of stress-induced aggregates
- Containing aggregates at clinically relevant levels

Stage II - Assessment of PBMC proliferation and cytokine response

- T cell proliferation
- Cytokines

Stage III – Assessment of DC phenotype and cytokine response

- Monocyte derived DC used as model
- DC phenotype
- DC cytokines
- DC aggregate uptake

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Comparative analysis of rituximab and trastuzumab stress induced aggregate particles

Antibody	Stress condition	HP-SEC		DLS		Light obscuration (particles/ml)		
		Recovery, relative to unstressed (%)	Monomer content (%)	Z-average diameter (nm)	PDI	>1 um	>10 um	>25 um
Rituximab	Unstressed	100	99.1	10.2 ± 0.3	0.09 ± 0.02	287	58	0
	Stir (200rpm/ 30min)	103.1	99.1	1540 ± 134	1.00 ± 0.00	2,125	10	0
	Heat (70°C/10min)	98.7	99.3	19.7 ± 0.4	0.38 ± 0.14	1,314	29	19
	Freeze/thaw 10 cycles	100.5	98.9	10.1 ± 0.1	0.18 ± 0.01	520	19	0
Trastuzumab	Unstressed	100	97.5	10.3 ± 0.1	0.14 ± 0.02	904	0	10
	Stir (200rpm/ 30min)	96.7	97.7	2203 ± 857	0.77 ± 0.24	33,073	67	0
	Heat (70°C/10min)	96.9	97.6	28.7 ± 6.2	0.11 ± 0.01	1,021	10	0
	Freeze/thaw 10 cycles	95.6	96.8	53.3 ± 30.1	0.14 ± 0.03	10,404	67	0

- Overall stress-induced aggregates comprise <3% of total protein
- ~90% less >1um size particles in ritiuximab samples under stir stress conditions
- More representative of clinical products
- As observed previously mechanical stress induced highest levels of micron sized particles

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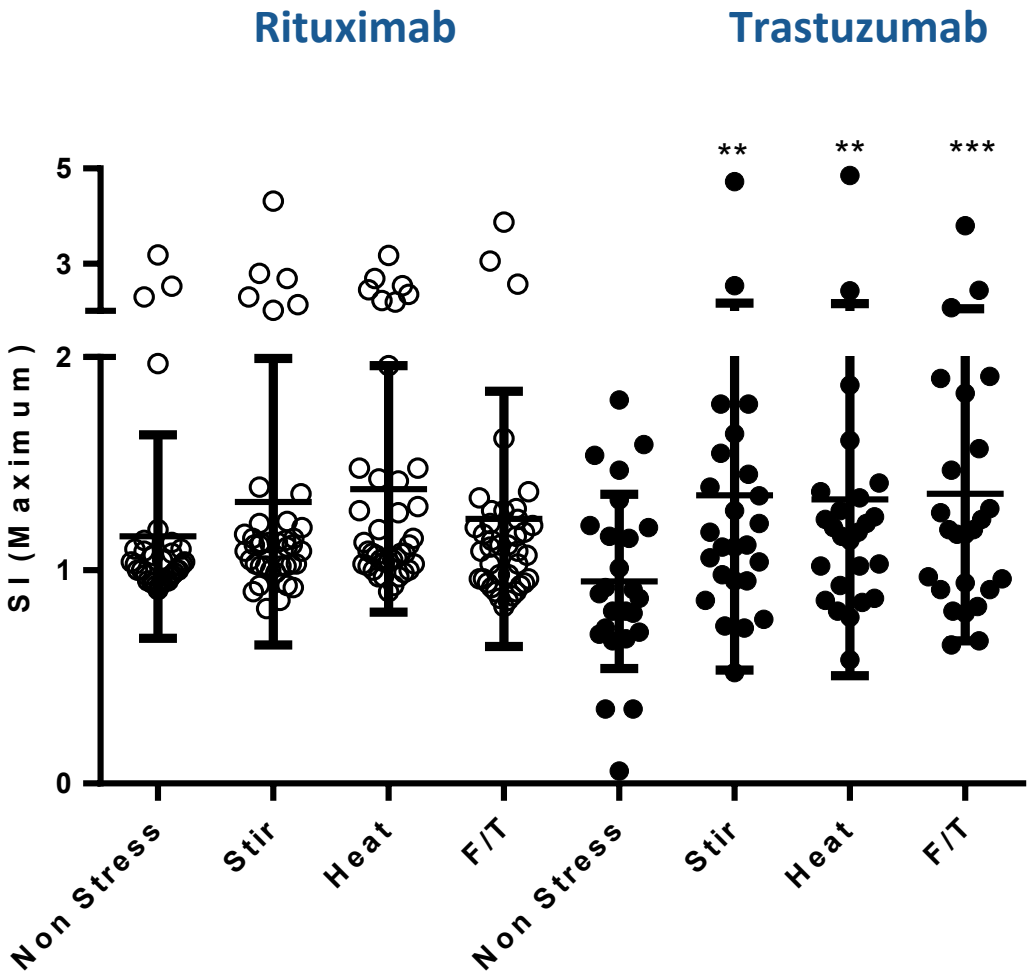
Effects of stress-induced aggregate particles on proliferation of PBMC



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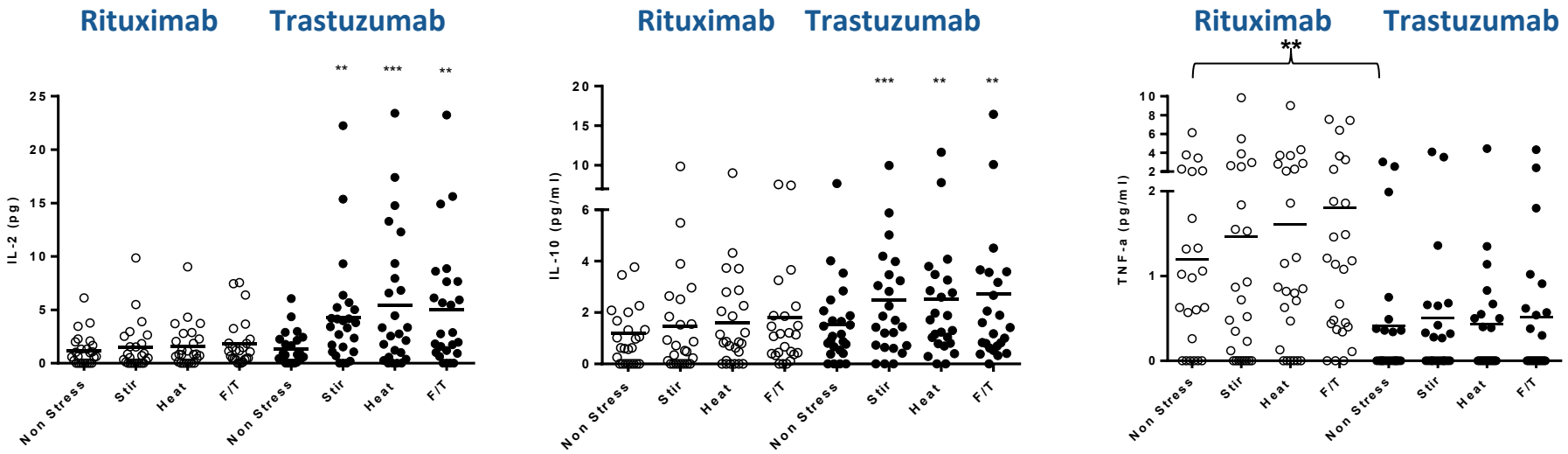
Effect of stress-induced aggregate particles on cytokine production by PBMC



- Increased IL-2 and IL-10 production associated with increased proliferation to trastuzumab
- Monomeric rituximab increased TNF-α compared to trastuzumab
- Variable levels of other proinflammatory cytokines

Paired Students t test p values for change in cytokine levels compared to monomeric antibody

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Effects of stress-induced aggregate particles on DC

Marker	Rituxamab			Trastuzumab		
	Stir	Heat	Freeze/Thaw	Stir	Heat	Freeze/Thaw
CD80	ns	ns	ns	ns	ns	ns
CD86	ns	ns	ns	**0.035**	**0.039**	ns
HLA-DR	ns	ns	ns	**0.047**	ns	ns
CD40	ns	ns	ns	ns	ns	ns
CD83	ns	ns	ns	ns	ns	ns
CD209	ns	ns	ns	ns	ns	ns

- MFI of activation markers CD86 and HLA-DR was significantly increased in the presence of stir stressed trastuzumab
- No significant changes with rituximab

Cytokine	Rituxamab			Trastuzumab		
	Stir	Heat	Freeze/Thaw	Stir	Heat	Freeze/Thaw
IL-1B	ns	ns	ns	ns	ns	ns
IL-12	ns	ns	ns	ns	ns	ns
IL-10	ns	ns	ns	0.042	0.045	ns
IL-8	ns	ns	ns	ns	ns	0.02 (reduced)
IL-6	ns	ns	ns	ns	ns	ns

- As observed with PBMC, significantly increased levels of IL-10 in presence of aggregated trastuzumab
- Reported in other studies (Joubert 2012 *et al*)

Paired Students t test p values for change in cytokine levels compared to monomeric antibody

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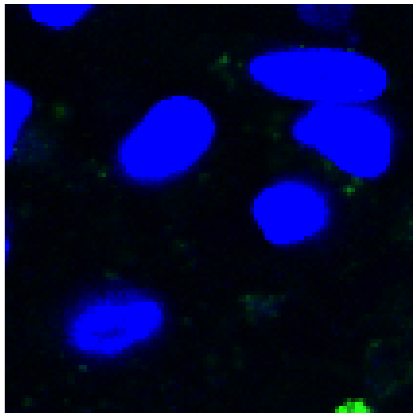
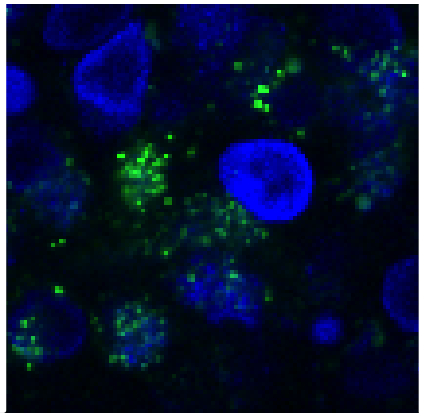
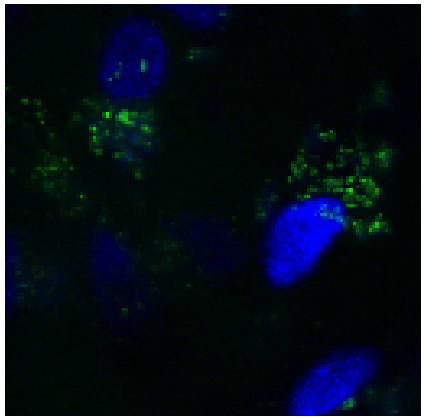
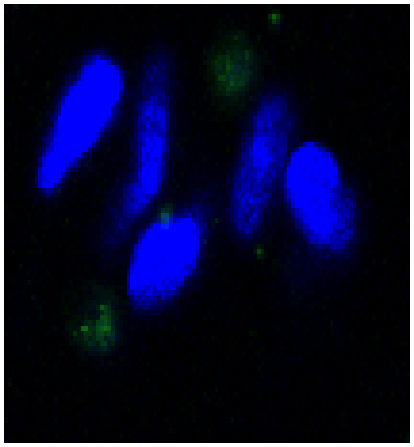
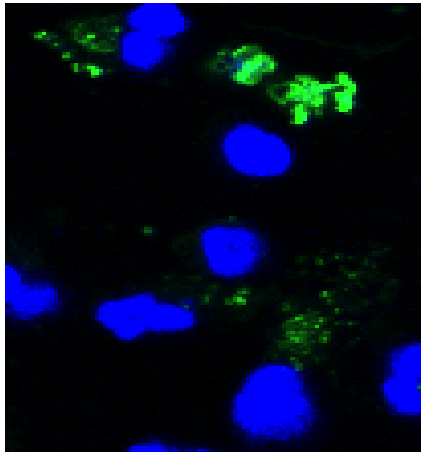
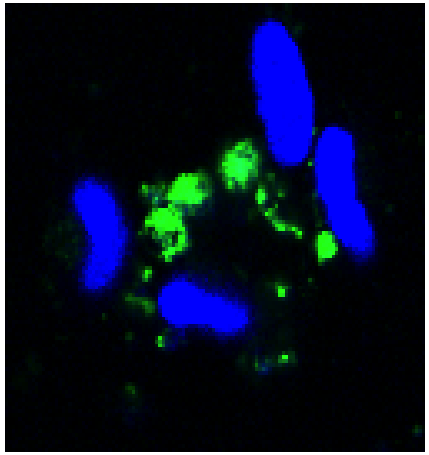
Effects of stress-induced aggregate particles on internalisation by DC

	Untreated	Shake	Metal Catalyst
30min			
60min			

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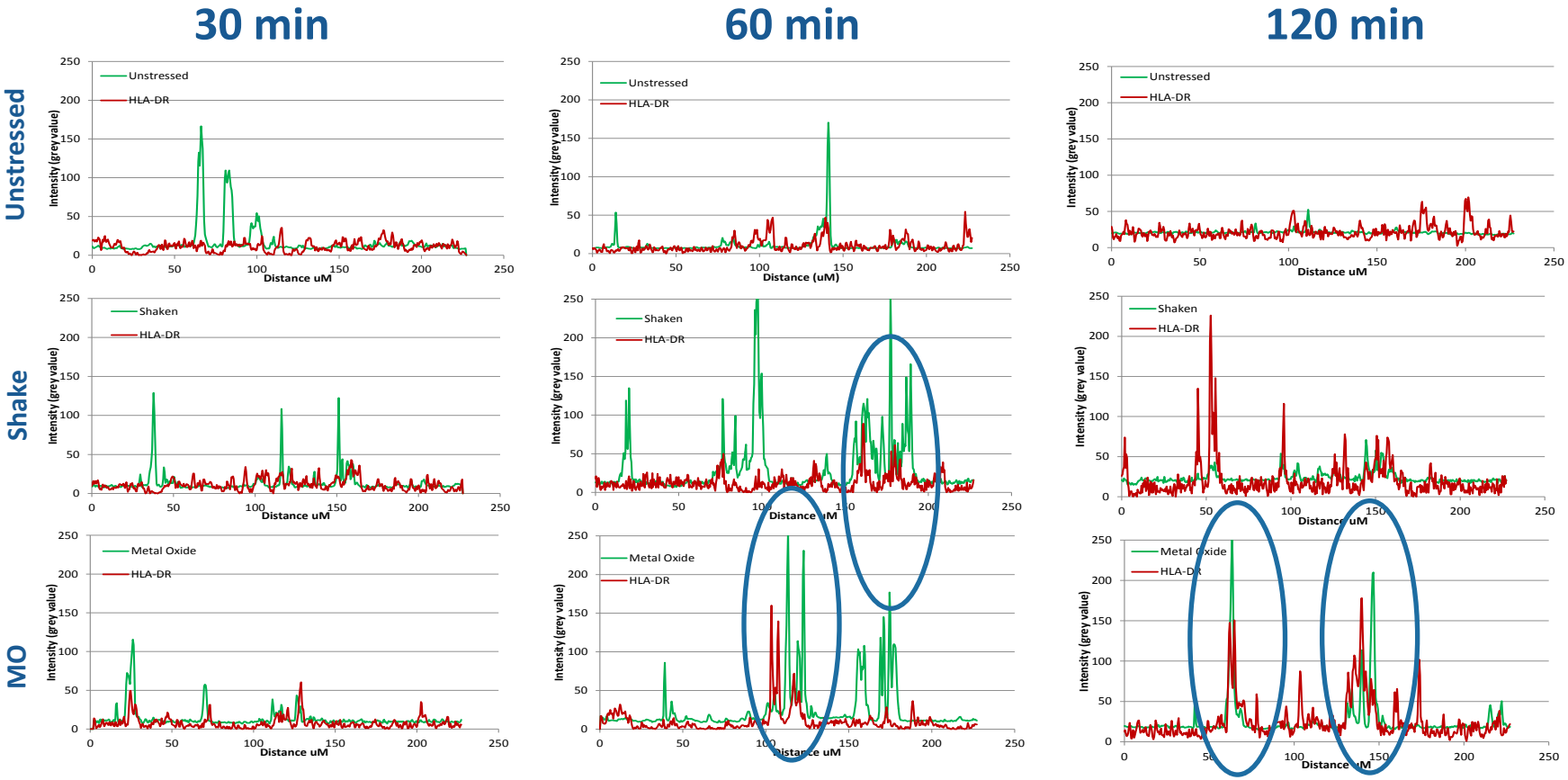
Differences in uptake and stability of stress-induced aggregates



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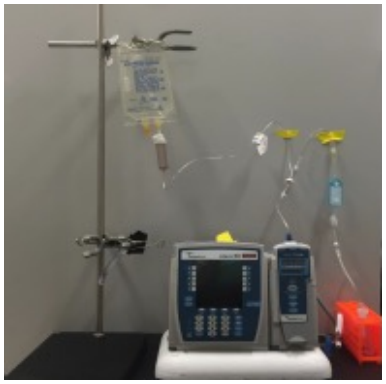
Variations in administration of biologics may potentially increase immunogenicity

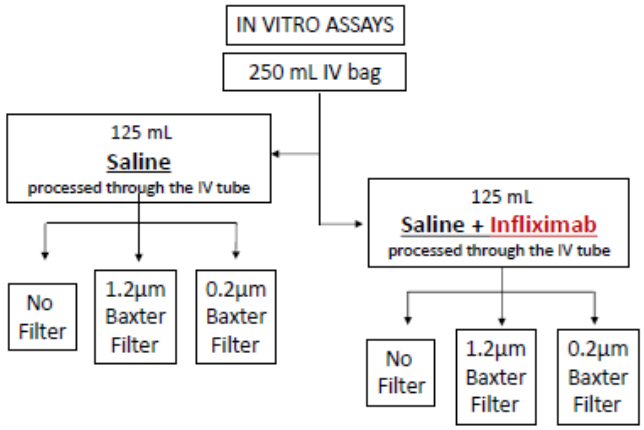
Manufactured biologics are tightly regulated from all aspects of manufacturing process:

- Product quality
- Batch variation
- Labelling
- Shipping

<u>The regulation is less stringent at the point of delivery of the product to the patient</u>

Infliximab





1. PBMC cultures
- Multiplex cytokine
- Proliferation
- Phenotyping

2. DC activation
- Phenotyping

3. TLR reporter
- QuantiBlue

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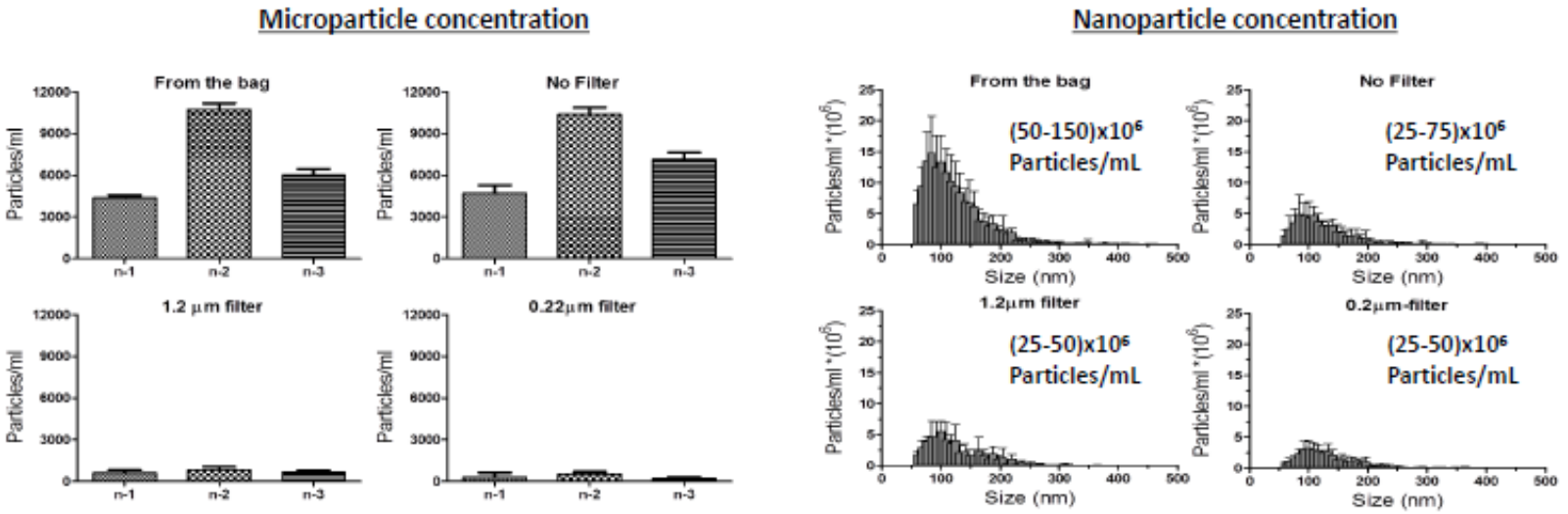
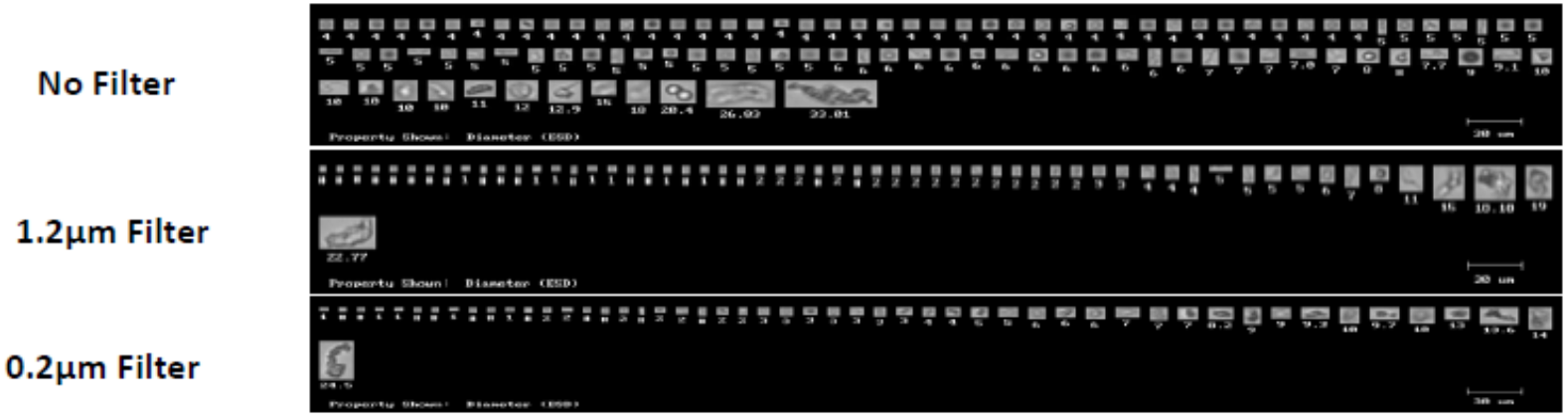
Characterisation of infliximab infusion bags





Nanoparticles persist even with in line filters

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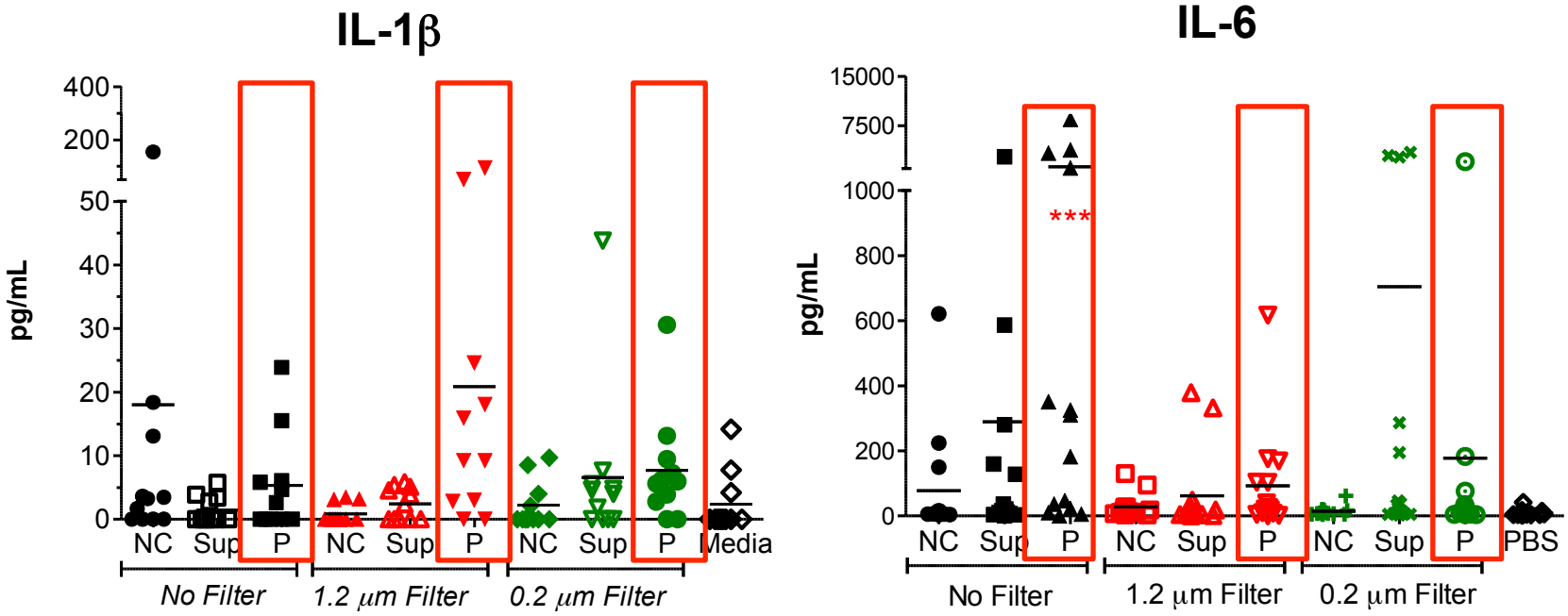
PBMC cytokine responses against infusion bag preparations of infliximab



Pellet samples = increased CD80, CD83 and CD40 on mDC

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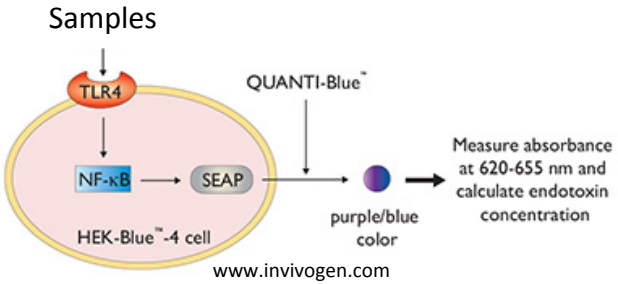
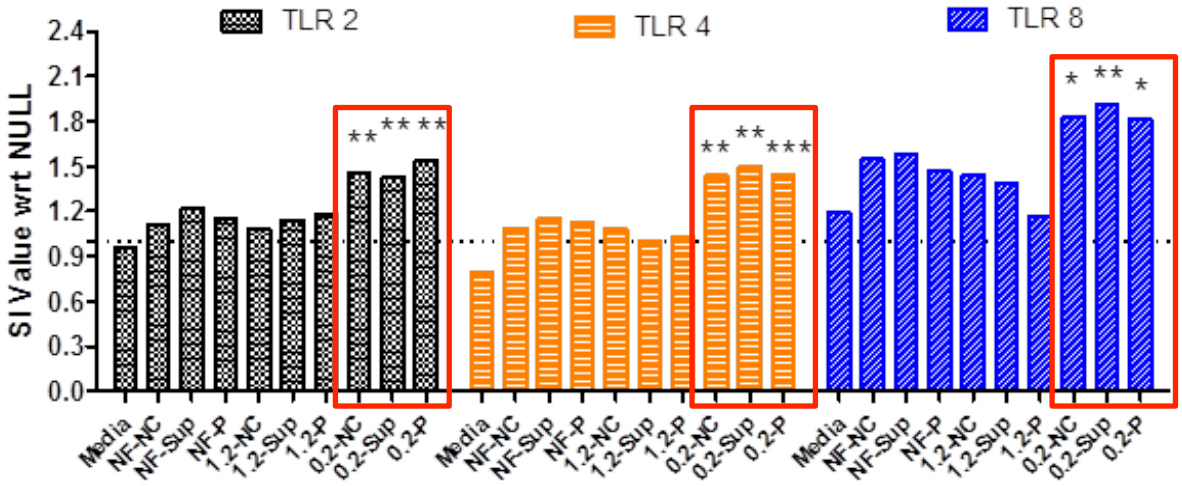
Use of in-line filters during infusion may enhance innate immune response





Addition of in line 0.2µ filter results in TLR signalling:
Particulates <0.2µ?
Other leachates?

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Summary

- Regardless of the immunogenicity risk of monomeric clinical therapeutics, the presence of small quantities (<3%) of aggregates can induce proliferation of PBMC
  - Shown for antibodies and insulin
  - T cell proliferation and cytokine production
- Low levels of particles/aggregates achieve this by activating the innate immune response
  - Detected by DC activation and cytokine secretion
- Aggregates are rapidly internalised by DC and associate with the endosomal pathway
  - Aggregates with certain properties may be more resistant to antigen processing
- Bystander activation of T cells may lead to increased stimulation of therapeutic-specific T cells
- Administration of protein drugs is not standardised or 'controlled' and particles can be produced that have a potential impact on the innate response
  - Maybe this is an area that needs better regulation?

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