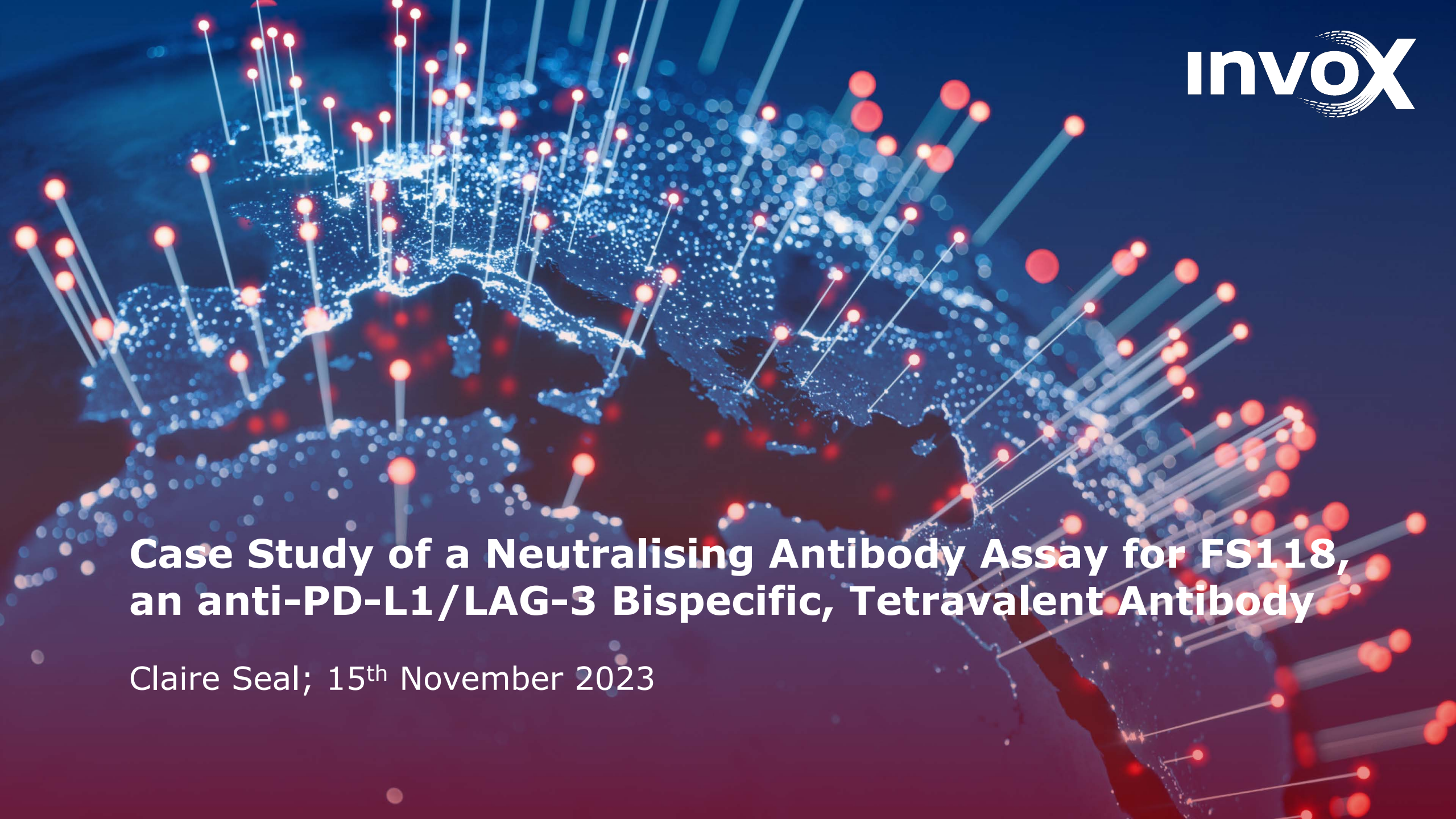
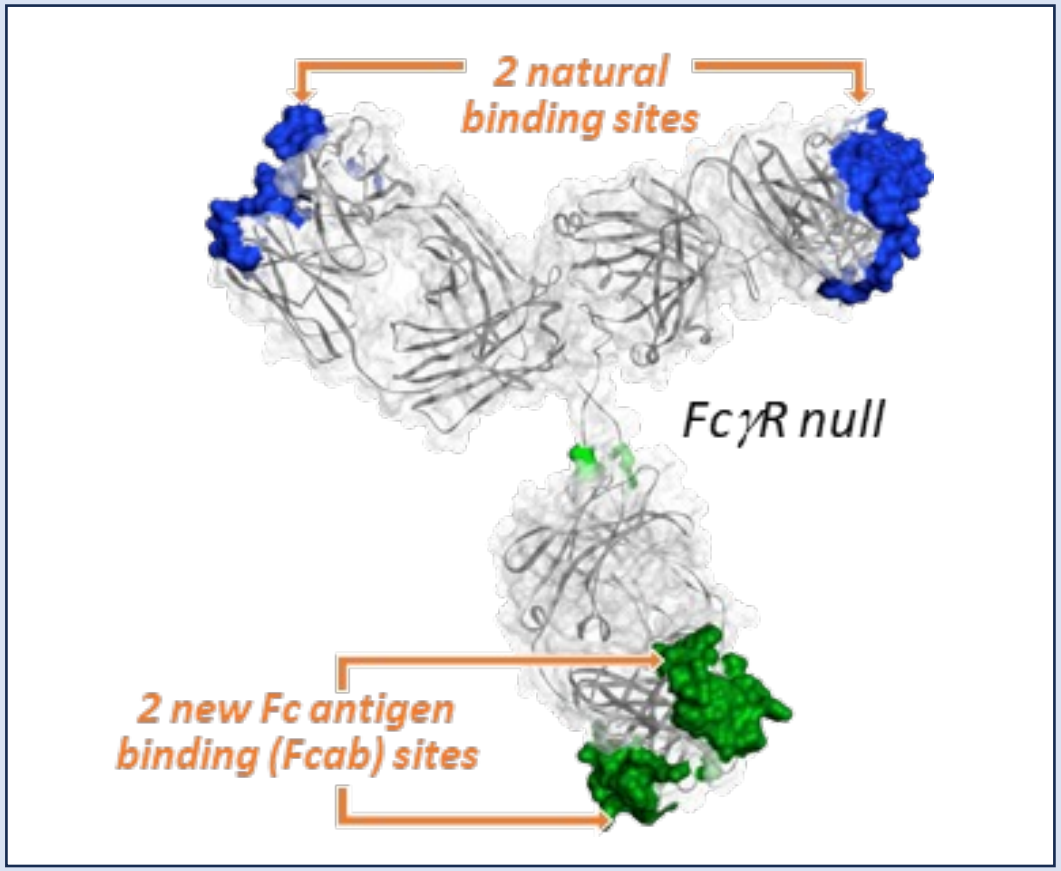


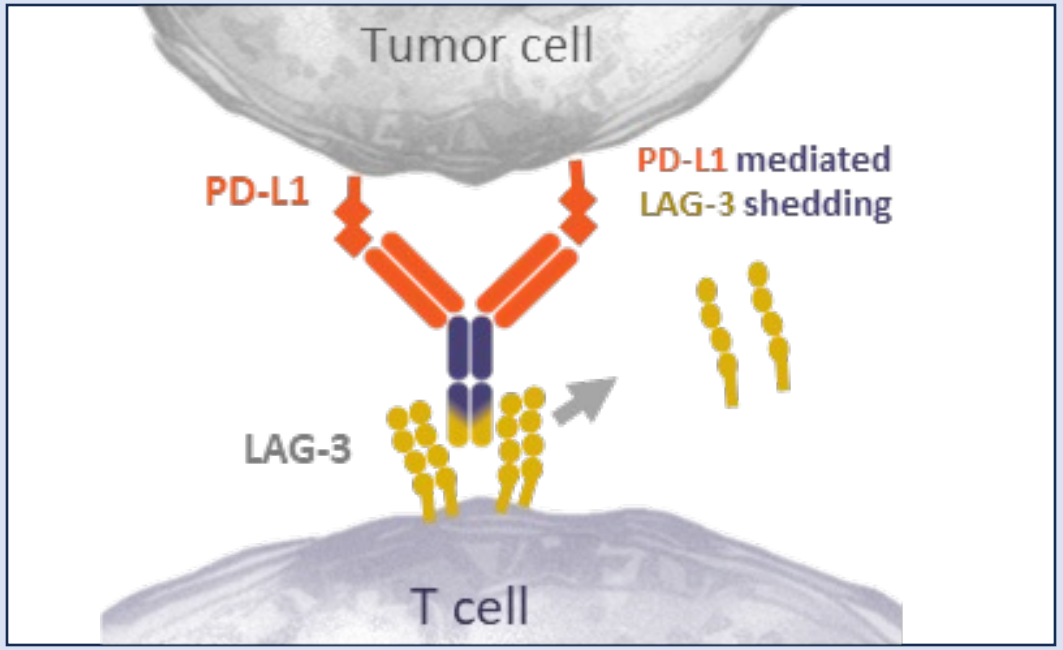
Case Study of a Neutralising Antibody Assay for FS118, an anti-PD-L1/LAG-3 Bispecific, Tetravalent Antibody

Claire Seal; 15th November 2023

FS118 – A Tetravalent Bispecific Antibody

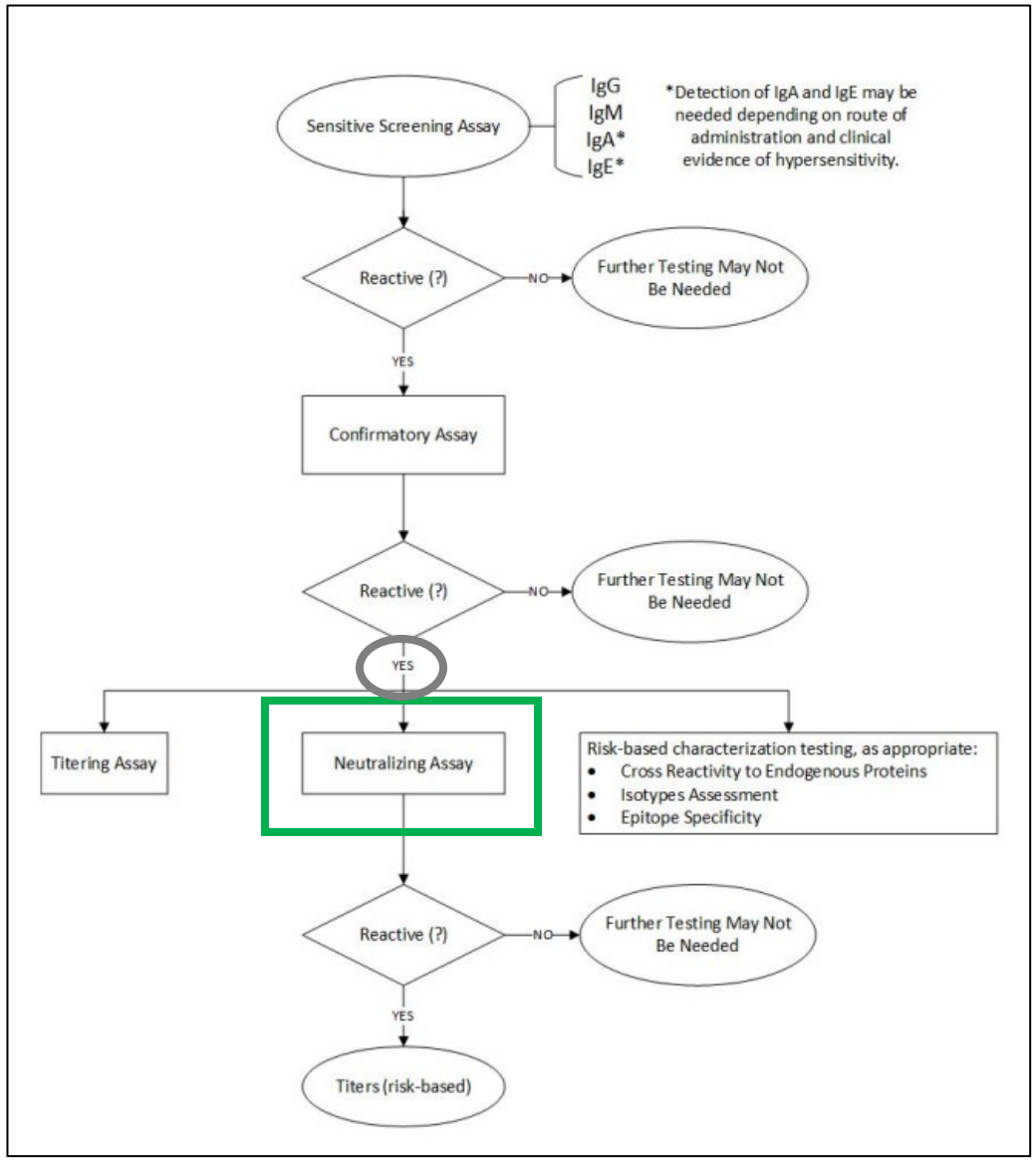


- Targets PD-L1 (Fab domain) and LAG-3 (Fcab® domain)



- Activate T cells and overcome immune suppression
- FS118 drives PD-L1-mediated **shedding** of LAG-3 on $CD4^+$ T cells

Anti-Drug Antibody – Testing Strategy



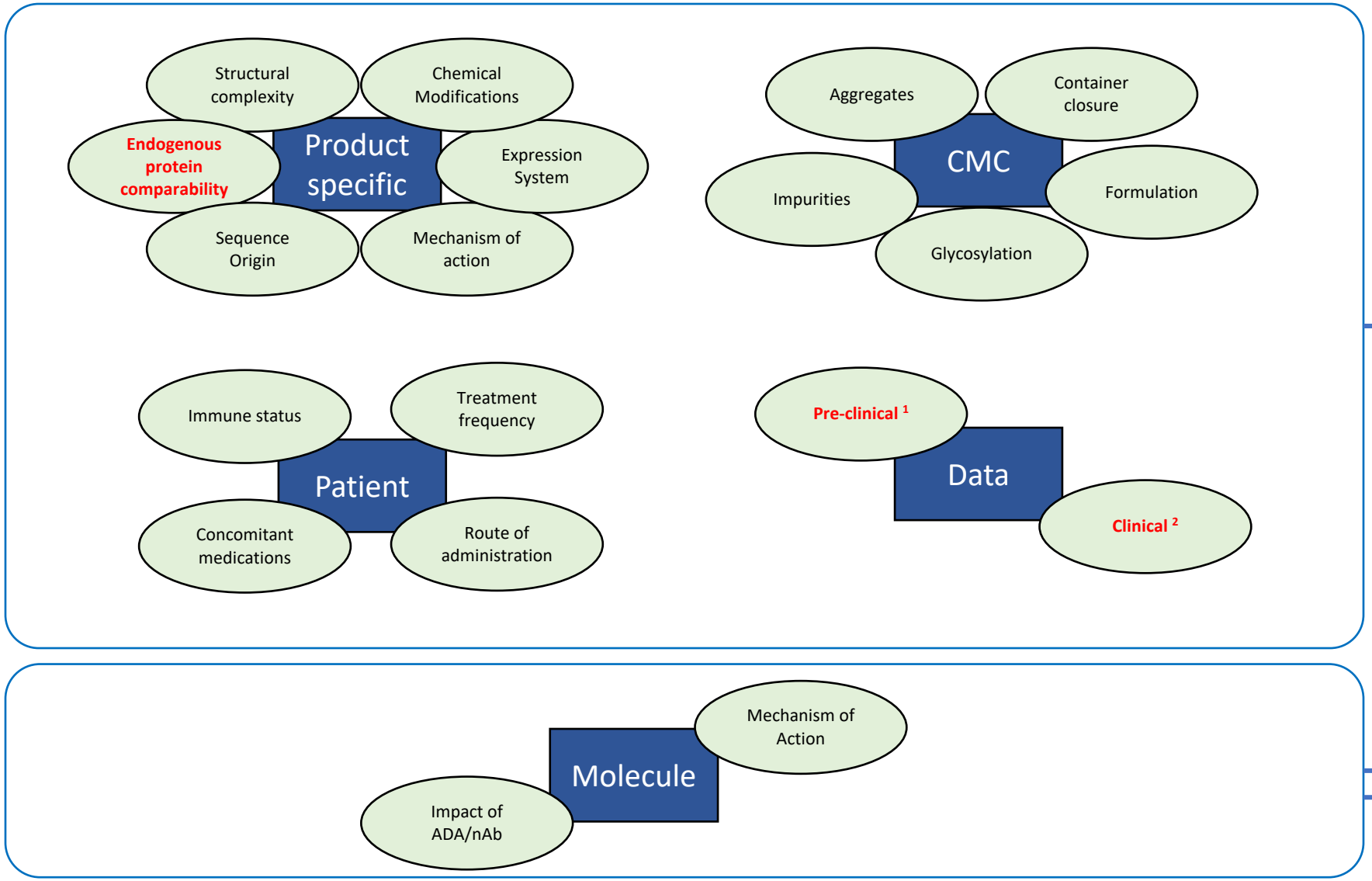
✓Therapeutics with a cell-based MOA should have a cell-based nAb assay

✓For antagonist therapeutics, a Competitive Ligand Binding Assay (CLBA) may be appropriate

✓Assay should be sensitive and drug tolerant

FDA Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection, 2019.
EMA Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use, 2012.

Immunogenicity Risk Assessment



FDA Guidance for Industry: Immunogenicity Assessment for Therapeutic Protein Products, 2014.
EMA Guidance on Immunogenicity Assessment of Therapeutic Proteins, 2017.

[1] *Pre-clinical ADA cannot predict clinical ADA. This relates to assessment of ADA linked to safety findings as opposed to incidence alone.*

[2] *For pre-clinical risk assessment, this is unknown. Clinical data can be included as it becomes available with link to safety*

Discussions with Regulatory Authorities

- Sponsor is responsible for design and conduct of clinical trials; global Regulatory Authorities review data package at key points for approval to progress to next stage

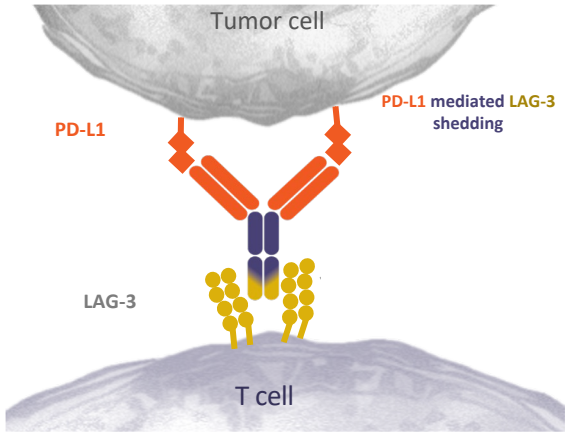
- Essential for Sponsor to understand their molecule and plan their programme with consideration of the regulatory guidelines to design appropriate strategy for each molecule

- Our approach is to engage with global Regulatory Authorities at appropriate points in the product development to align on strategy

- Interaction with Regulatory Authorities e.g. US FDA, EU authorities etc:
  - Type B meetings (IND, BLA)
  - Type C meetings; Scientific Advice meetings

We knew a cell-based potency assay was available for starting point of a nAb assay for our dual antagonist molecule

Actions of nAb on Dual Antagonist FS118



nAb binding the FS118 PD-L1 domain

PD-L1 associates with PD-1 on immune cells → **prevents immune cell activation** [1]

nAb binding the FS118 LAG-3 domain

LAG-3 binds to MHCII, preventing MHCII interaction with CD4 thus **preventing T cell activation** [1]

Action of nAb is to prevent FS118 binding to its targets, thereby targets can bind to their endogenous proteins.
Competitive ligand binding assay (CLBA) would demonstrate whether ADA prevent FS118 binding to targets.

[1] *Both PD-L1 and LAG-3 have additional binding partners that are less well characterised*

Competitive Ligand Binding Assay

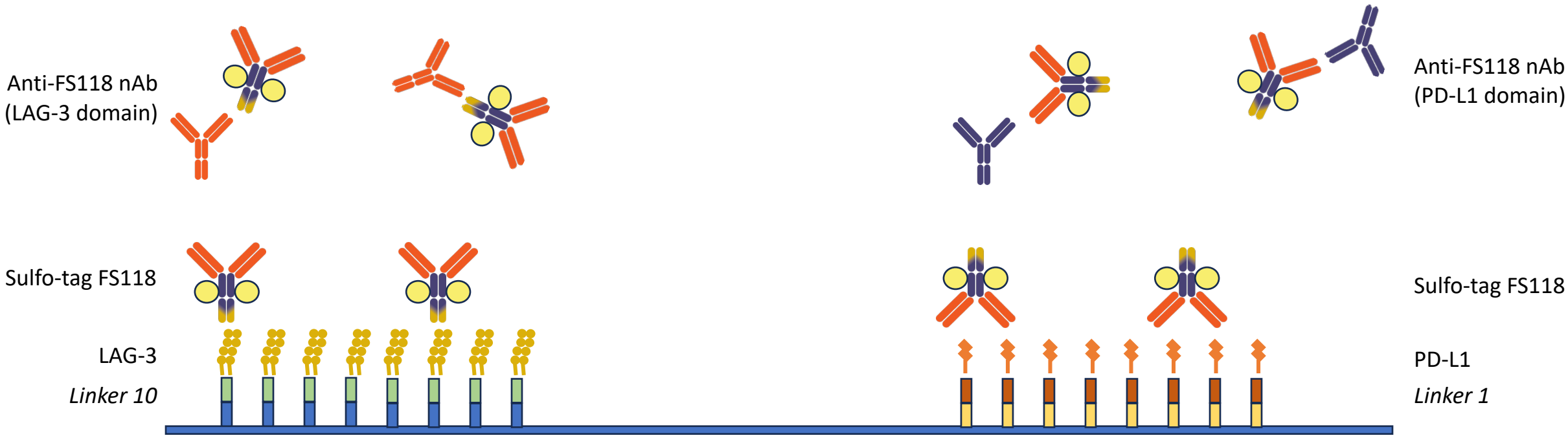
- MSD U-plex utilising Linker technology → both target proteins analysed in the same well per sample

- Both target proteins are added concurrently to each assay well.
- Addition of Sulfo-tag FS118 leads to generation of an assay signal.

https://www.mesoscale.com/en/products_and_services/assay_kits/u-plex_gateway/u-plex_technology

Competitive Ligand Binding Assay

- MSD U-plex utilising Linker technology → both target proteins analysed in the same well per sample

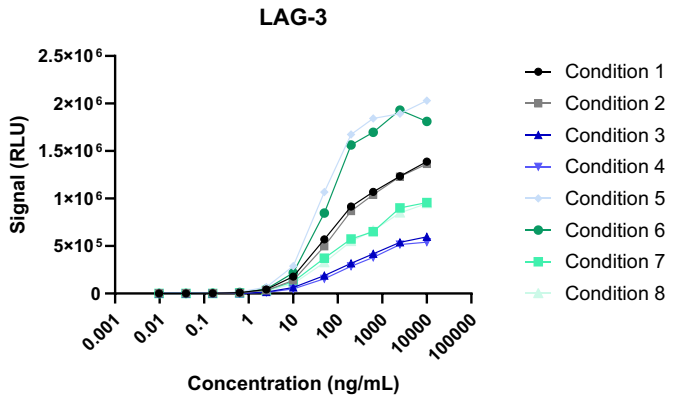


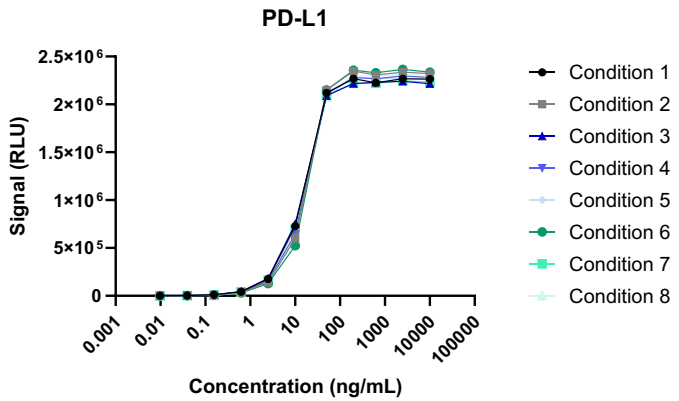
- Anti-FS118 nAb leads to decreased Sulfo-tag FS118 binding → decreased signal

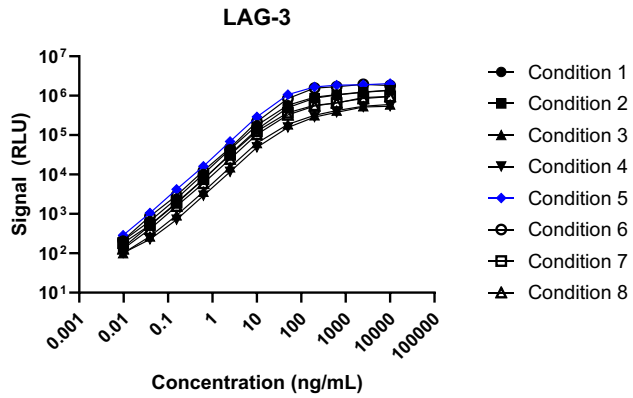
U-PLEX Plate with
10 specific linkers

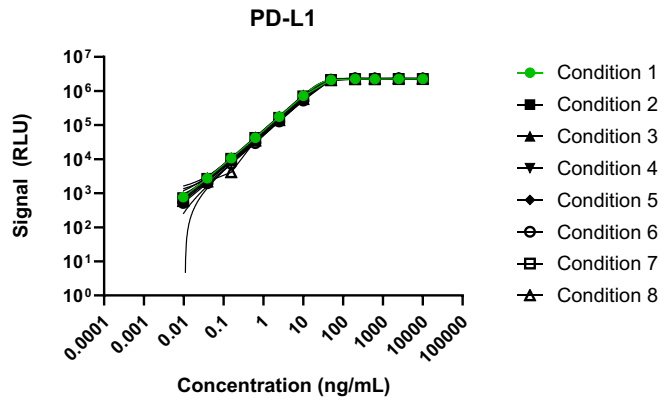
https://www.mesoscale.com/en/products_and_services/assay_kits/u-plex_gateway/u-plex_technology

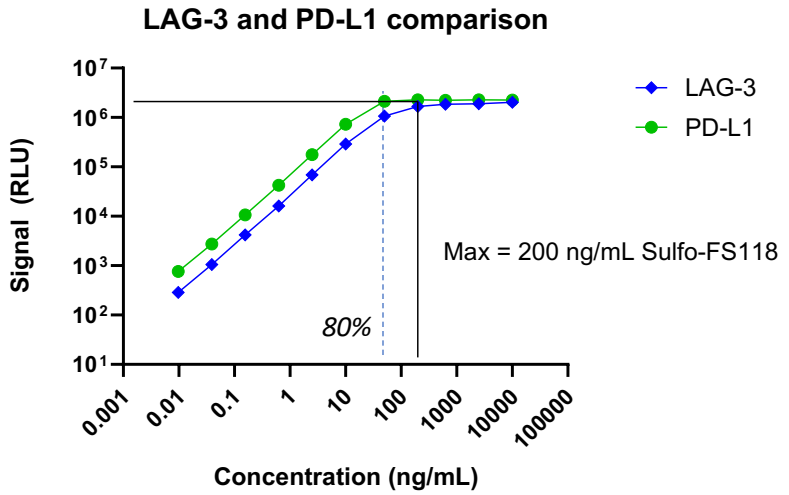
Dose-response Curve for FS118







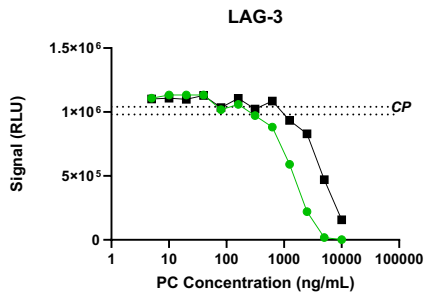


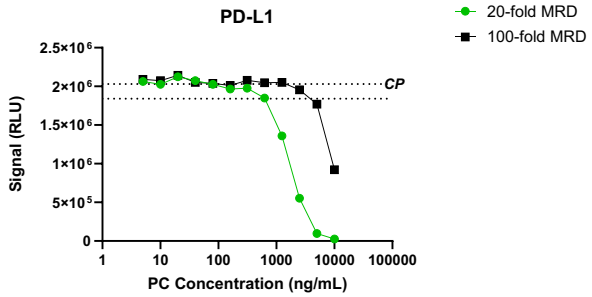


From dose-response curve, 80% maximal signal generated by ~40 ng/mL Sulfo-FS118

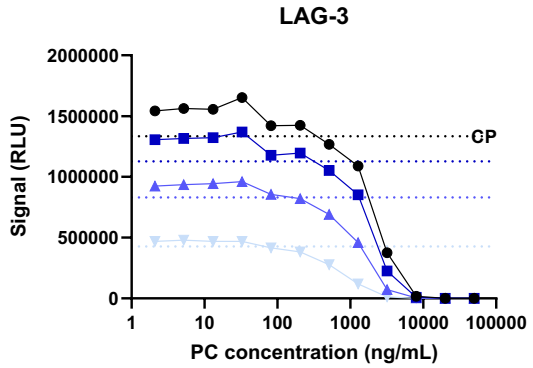
Preliminary Assay Sensitivity

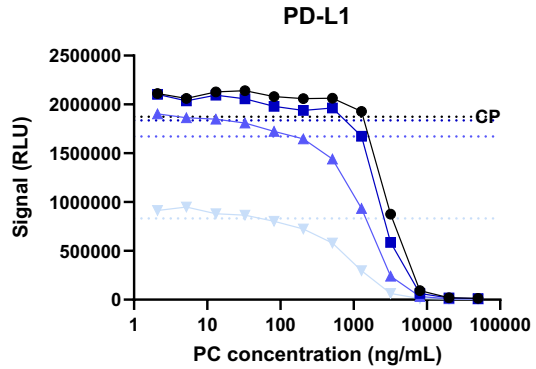
- Three polyclonal PC antibodies mixed equivalently
- All individually shown to be neutralising for both PD-L1 and LAG-3-binding domains (data not shown)

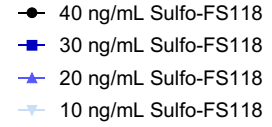




Constant Sulfo-FS118 = 40 ng/mL





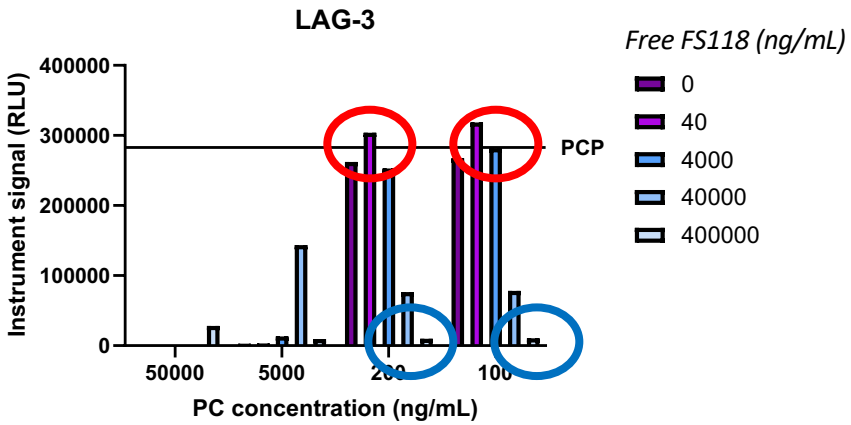


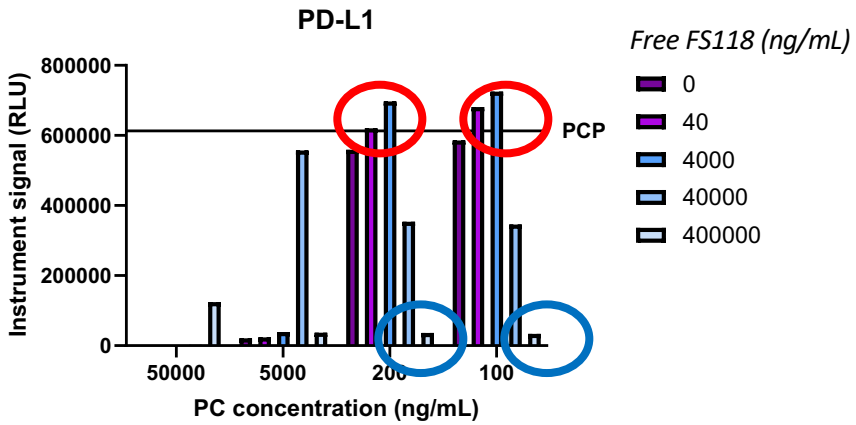
Sulfo-FS118 (ng/mL)	LAG-3 sensitivity	PD-L1 sensitivity
40	512	3200
30	512	1280
20	205	205
10	**81.9**	**81.9**

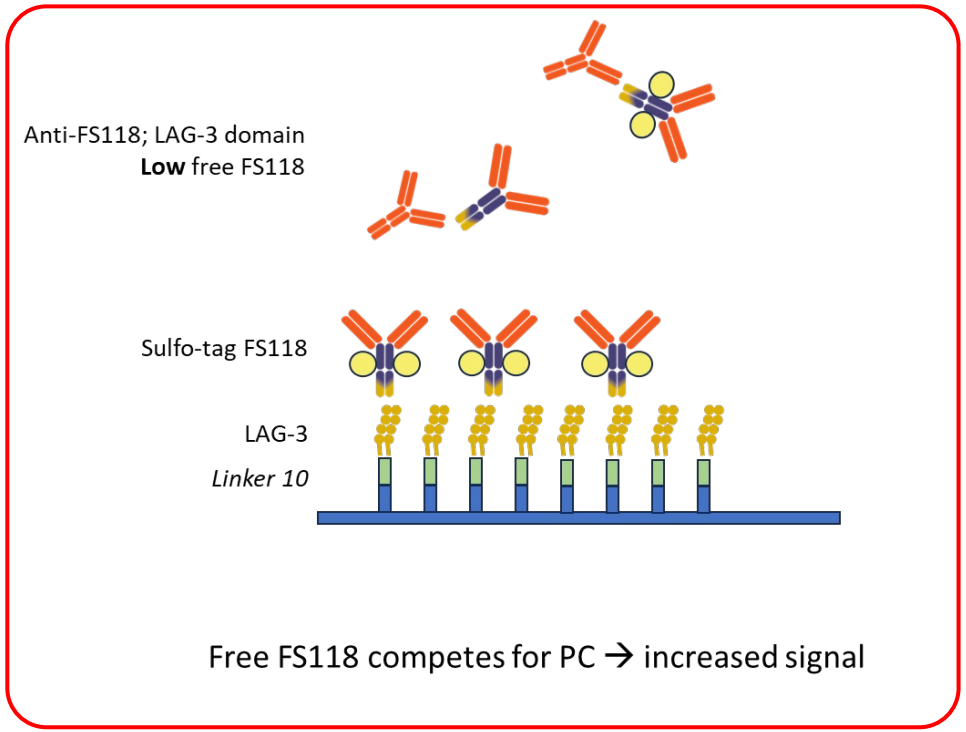
Sensitivity = 81.9 ng/mL. However what about drug tolerance?

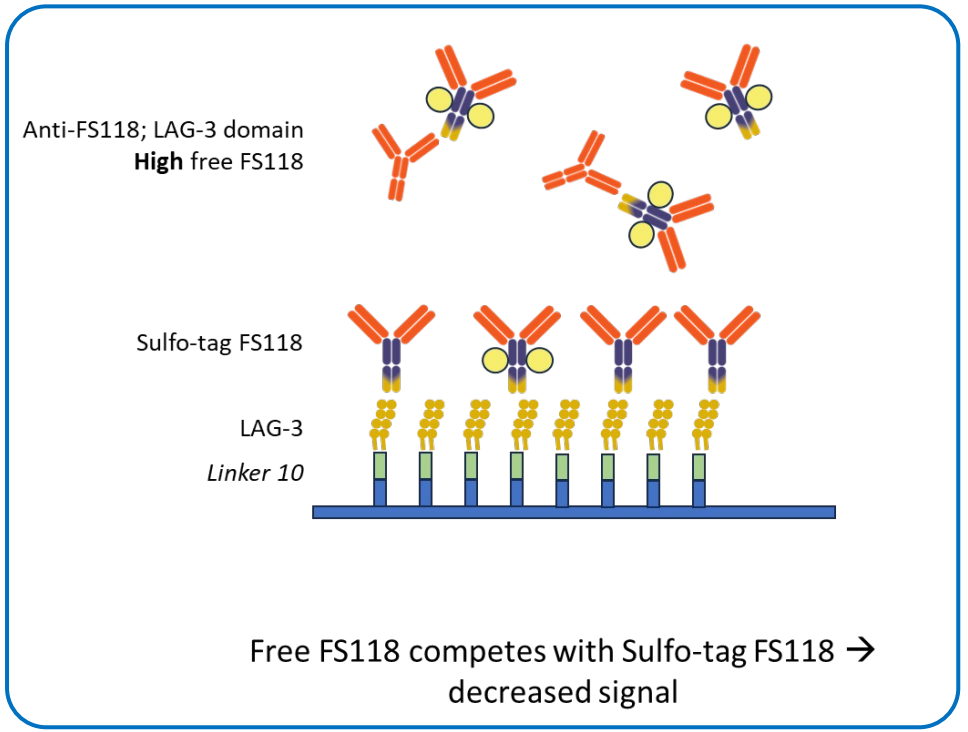
Drug Tolerance

- Drug tolerance requirement = ideally 40 µg/mL free FS118



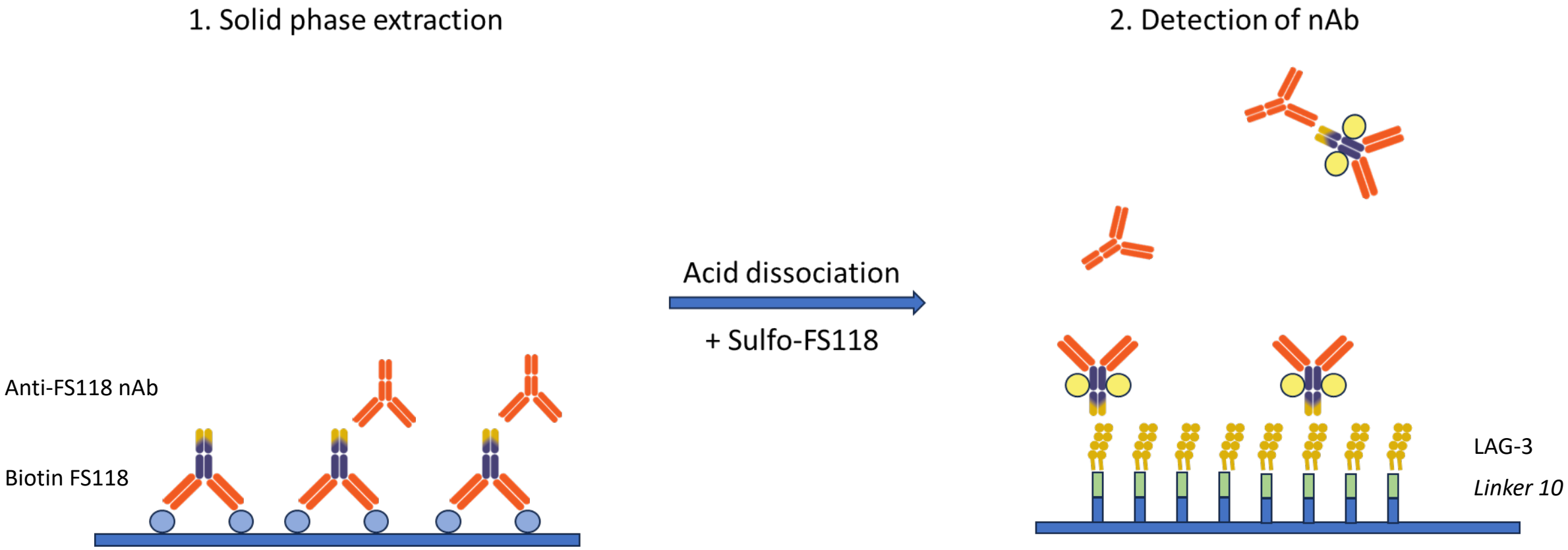




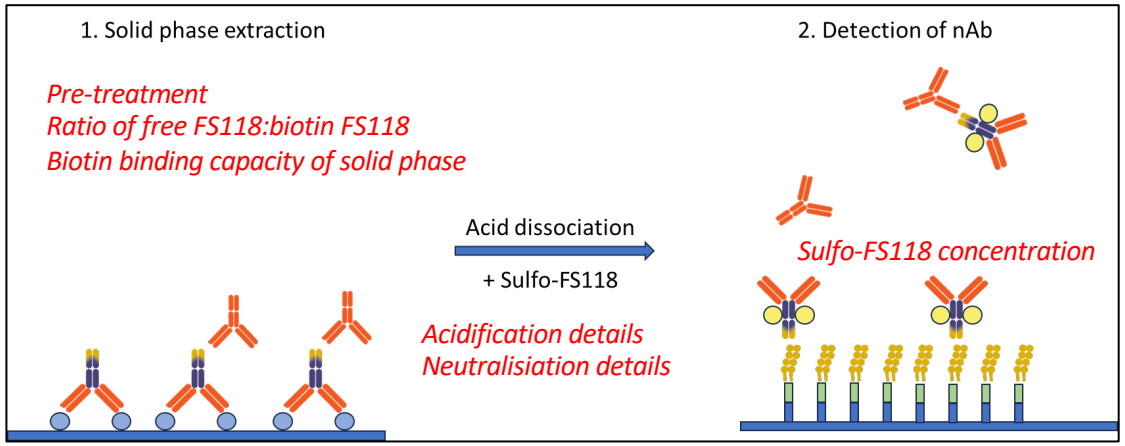


Improved Drug Tolerance

- Simple acidification → no improvement
- Proceed to SPEAD (solid phase extraction, acid dissociation)



Improved Drug Tolerance with SPEAD



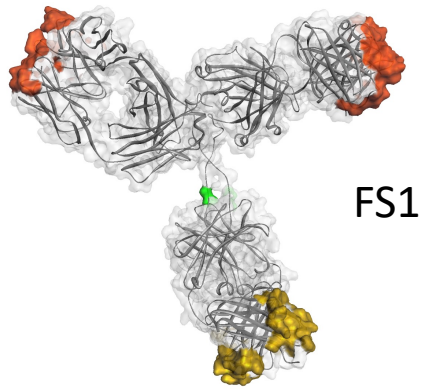
			SPEAD_Preliminary Optimisation			
		Detection	LAG-3		PD-L1	
ID	PC Conc. (ng/mL)	Free FS118 Conc.	Signal	ACP/BCP	Signal	ACP/BCP
PC1 + DT1	1000	40000	24946	BCP	122935	ACP
PC1 + DT2	1000	4000	17529	BCP	115312	BCP
PC1 + DT3	1000	400	11529	BCP	96784	BCP
PC1 + NC	1000	0	3280	BCP	78928	BCP
PC2 + DT1	500	40000	23973	BCP	117711	BCP
PC2 + DT2	500	4000	20349	BCP	111770	BCP
PC2 + DT3	500	400	19352	BCP	112385	BCP
PC2 + NC	500	0	12694	BCP	105344	BCP
PC3 + DT1	200	40000	27456	ACP	129428	ACP
PC3 + DT2	200	4000	24666	BCP	125083	ACP
PC3 + DT3	200	400	22627	BCP	118095	BCP
PC3 + NC	200	0	17058	BCP	104804	BCP
NC	0	0	29313	ACP	134086	ACP
		Mean NC	29313		134086	
		CPF (99%)	1.115		1.115	
		Plate Cut point	26290		120257	

- CLBA with SPEAD (preliminary optimisation) is promising
- Further optimisation needed once CLBA format decision is finalised

CLBA is Suitable for FS118 nAb Detection

- Reflects the dual antagonist MOA
- Sensitivity of the ADA assay (super-sensitive 10 ng/mL of PC):
  - CBA would struggle to achieve <1000 ng/mL of PC → disconnect between detecting ADA and assessing neutralising capability
  - CLBA can obtain a sensitivity closer to 10 ng/mL
- Drug tolerance in the CLBA has been shown to be just outside acceptability, with scope to optimise with BEAD approach
- CBA focuses on specific interactions, missing potential interactions with other binding partners
- Low level of immunogenicity in early patient population [1]
- Robustness of assay format at CRO

- Strategy for FS118 nAb assessment = CLBA for nAb assessment
- Imperative to understand your molecule, plan your position then be prepared (not scared) to defend your approach
- Continued dialogue and interaction with global regulatory agencies at appropriate stages in the development process is very important to ensure the Sponsor and agency(ies) are aligned



FS118

[1] *Yap et al, Clin Cancer Res, 29(5), 2023*

Conclusions

- Assessment of immunogenicity of each therapeutic is important
- Must be based on the risk of each individual molecule → first principles thinking
- For FS118, a CLBA format was suitable for assessment of nAb
  - Supported by preliminary development data demonstrating feasibility of approach
- Continued dialogue and discussion with Regulatory Agencies through the lifecycle of product development is essential for optimising success

Acknowledgements

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FS118 Project Team

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- Julia Hamlin