Pre-Existing Antibodies within Immunogenicity Testing

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INTRODUCTION

Monoclonal antibodies and next generation molecules such as antibody-drug conjugates (ADC) are being developed and moved into early phase clinical testing. These new molecules bring challenges for measuring immunogenicity within human serum samples.

Challenges:

- Therapeutic monoclonal antibodies could have structural regions which could contain ADA binding domains [Figure 1].
- ADC molecule has a mAb joined to a small drug by a linker region [Figure 2]. This may lead to a highly potent drug which is also a selective binder of a specific tumor antigen; however, the structure may also present a neoepitope to the immune system.
- Development of anti-drug antibody (ADA) assays to both mAb's and ADC's very commonly has demonstrated the presence of pre-existing antibodies (PEXA) in a small percentage of drug naïve normal human serum samples. It is currently unclear as to the evolutionary mechanism that has allowed PEXA to develop. Their presence may serve as a regulatory mechanism to suppress the immune system under certain conditions.

Approach:

- Regardless of how pre-existing antibodies may have developed, measuring an anti-drug antibody (ADA) response in serum samples may present additional challenges with these pre-existing molecules present. Here, we outline a strategy to determine where on the molecule the ADA reactivity is directed against.

METHOD

- PEXA detection used a homogenous MesoScale assay [Figure 3].
- Test samples and controls were diluted in either assay diluent (no drug) for screening, or assay diluent with drug or drug-regions added for confirmation.
- Samples were then incubated for 30 min. at R.T.
- During the confirmation assay, if endogenous molecule-specific PEXA are present, they will bind the added drug or drug regions.
- Samples were further diluted 1:1 in a master mix that contained biotinylated and TAG labelled drug, then incubated overnight at R.T.
- Next day samples were transferred onto MSD plate and incubated for 1 hr at R.T. allowing biotinylated drug to bind to the plate.
- The resulting luminescence signal was measured in ECL units.
- The intensity of the signal was directly proportional to the quantity of detected PEXA in the sample.
- For the initial screening assay, if PEXA are present they will bind and bridge the biotinylated and TAG labelled drug, producing a positive signal in the assay.

For the confirmation, PEXA if present will bind to drug or drug region during the initial 30 min. incubation and therefore not allow the labelled drug to form a bridge with PEXA. As a result, there will be a reduction in signal (% inhibition) compared to the sample that is not incubated with drug.

Figure 1. ADA to the Drug

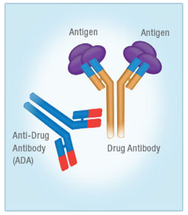

Figure 2. Example ADC Structure

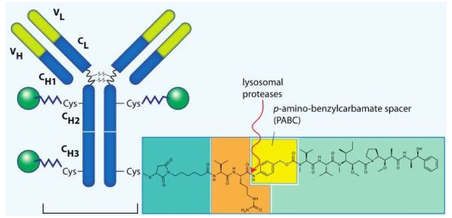

Figure 3. ADA Bridging Format

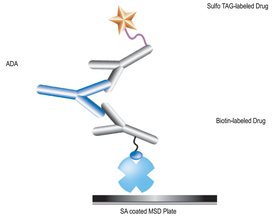

Figure 4. ADA screening data

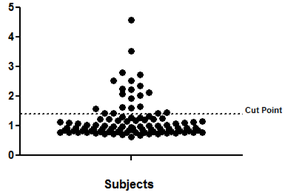

Figure 5. PEXA region-specific

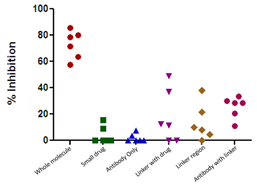

Figure 6. ADC molecule region-specific

a) Plate map b) Inhibition data

a)

	Without Drug			ADC			Total Ab			Payload with linker		
	1	2	3	4	5	6	7	8	9	10	11	12
A	HPC	LPC	NC	HPC	LPC	NC	HPC	LPC	NC	HPC	LPC	NC
B	HPC	LPC	NC	HPC	LPC	NC	HPC	LPC	NC	HPC	LPC	NC
C	Indi 01	Indi 02	Indi 03	Indi 01	Indi 02	Indi 03	Indi 01	Indi 02	Indi 03	Indi 01	Indi 02	Indi 03
D	Indi 01	Indi 02	Indi 03	Indi 01	Indi 02	Indi 03	Indi 01	Indi 02	Indi 03	Indi 01	Indi 02	Indi 03
E	Indi 04	Indi 05	Indi 06	Indi 04	Indi 05	Indi 06	Indi 04	Indi 05	Indi 06	Indi 04	Indi 05	Indi 06
F	Indi 04	Indi 05	Indi 06	Indi 04	Indi 05	Indi 06	Indi 04	Indi 05	Indi 06	Indi 04	Indi 05	Indi 06
G	Indi 07	Indi 08	Indi 09	Indi 07	Indi 08	Indi 09	Indi 07	Indi 08	Indi 09	Indi 07	Indi 08	Indi 09
H	Indi 07	Indi 08	Indi 09	Indi 07	Indi 08	Indi 09	Indi 07	Indi 08	Indi 09	Indi 07	Indi 08	Indi 09

b)

Data												
	11748	155	88	190	83	88	139	87	85	11180	158	83
	10995	158	87	191	82	87	138	87	81	10688	156	83
	80	85	86	86	82	90	84	88	83	87	85	84
	82	82	86	86	82	87	86	86	82	84	82	83
	72	87	76	68	83	84	76	84	79	81	86	76
	72	86	76	72	80	81	77	84	75	79	88	78
	77	82	77	76	76	85	80	83	78	88	79	80
	77	82	76	73	78	84	84	79	78	86	79	80

RESULTS & CONCLUSIONS

- ADA screening assays can be an effective tool to indicate if normal naïve serum panels contain PEXA's.
- Samples with ECL values above the cut point could potentially be PEXA positive. [Figure 4] is a naïve 100 sample serum population with a statistically generated cut point of 1.3 rECL shown. Here, 17 individuals demonstrated a rECL value above the cut point.
- Screening "multiple" serum panels can provide a more accurate determination when estimating percent of samples that contain the PEXA, as increased sample number will more closely represent naïve population.
- Once PEXA positive samples have been identified, they can be moved to the confirmatory assay. Using the whole molecule or molecular regions to spike the confirmatory assay, a percent inhibition can be generated determining the PEXA region of reactivity.
- [Figure 5] presents an example where the reactivity was associated with the linker. When the small drug or antibody-only were used within the confirmation assay, percent inhibition ranged from zero to less than 20%. When a linker containing region was used, increased inhibition was demonstrated. Whole molecule inhibition was at 80% and other linker containing regions were at 40%, or just below.
- [Figure 6] presents an example where the region of reactivity was associated with the antibody framework. The high and low positive controls (HPC, LPC) were inhibited by 98.3% and 47.3% respectively with the ADC. The total antibody inhibited the HPC and LPC at 98.7% and 44.4% respectively. The payload with linker inhibition was at 3.3% for the HPC and the LPC was not inhibited at all.
- This information can be used to pinpoint where the PEXA will bind on the molecule thereby allowing drug modification to eliminate PEXA reactivity prior to moving the drug into the clinic.
- PEXA's have the potential to affect the therapeutic drugs PK profile and thus early removal of these PEXA binding regions may eliminate subsequent issues associated with PEXA.
- Determining the region of PEXA reactivity may allow the new therapeutic to be modified to eliminate the reactivity.

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