Comparison of Generic Methods for the Quantification of Pembrolizumab Using Gyrolab™ and LC-MS/MS

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Project Aims

1. Investigate generic PK methods for quantification of mAbs in preclinical species

2. Use Pembrolizumab to compare generic LBA on Gyrolab™ with signature peptide quantification by LC-MS

3. Quantify the impact of Anti-Drug Antibodies on Gyrolab™ and LC-MS

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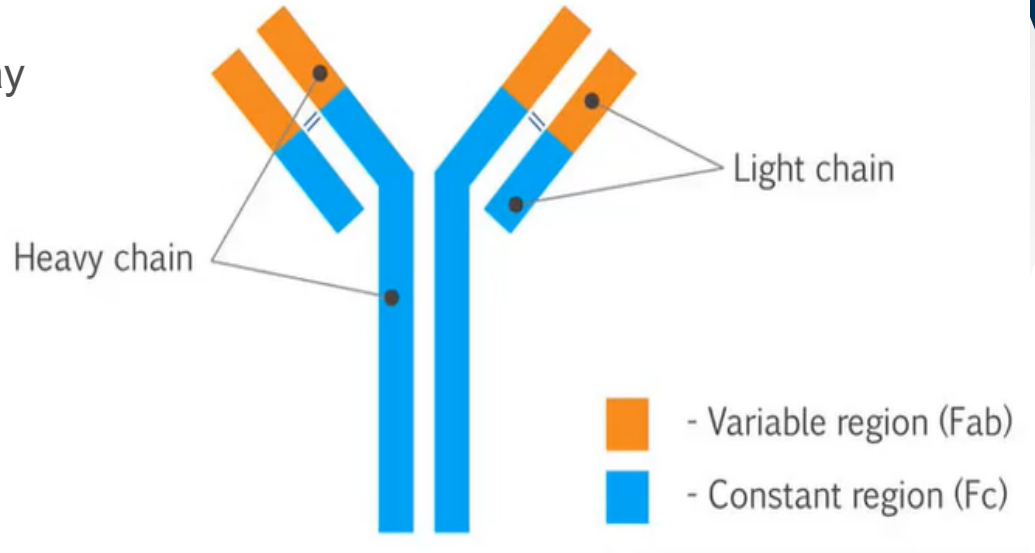
Why do we need a generic assay?

- Detects the constant region of the humanized mAb – allowing quantification of many different therapeutics with one generic assay
- Able to distinguish human antibodies dosed into a pre-clinical species
- Fit for purpose accuracy and precision – Very little MD/optimization – suited to a discovery group



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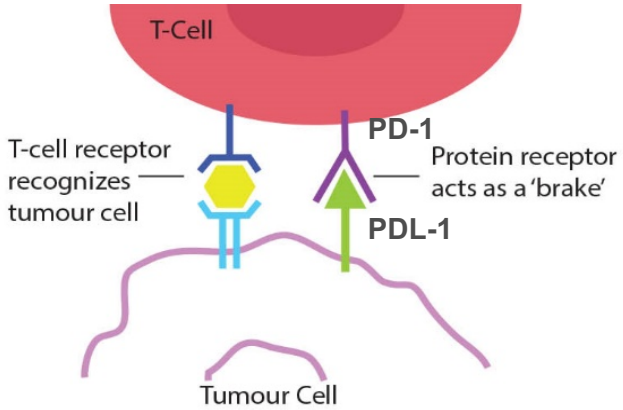
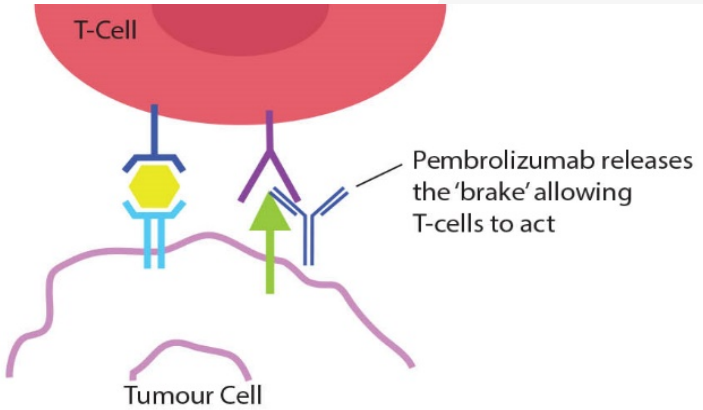
Pembrolizumab – Mode of Operation

- Humanized IgG4 monoclonal antibody
- Targets programmed cell death receptor (PD-1)
  - Binding to PD-1 on T cells to prevent binding to PDL-1 on tumor cell triggering programmed cell death
  - Therefore re-estabilishing T cell mediated anti-tumor response
- We want to prepare for next wave of combination pharmaceuticals used in conjunction with this therapy



Inhibition of T cell mediated anti-tumour response



Restoration of T cell mediated anti-tumour response

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Generic LC-MS Method Based on Literature Method

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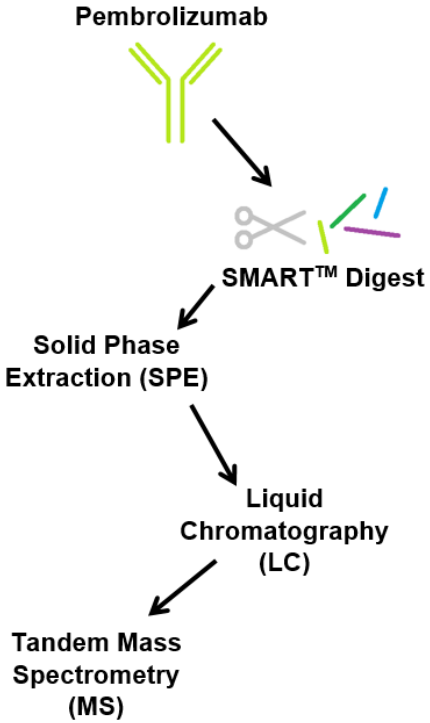
Method: LC-MS/MS

- Tryptic digestion and SPE, followed by LC with MS detection, SILu™Mab K1 as internal standard (stable label)
- Calibration standards (Cals) and quality controls (QCs) prepared in nonhuman primate (NHP) serum
- C18 column coupled with AB Sciex 6500+
- 15 µL sample volume to accommodate micro sampling



Method Development

1. Digestion and MS optimization of Pembrolizumab
2. Accuracy and precision
3. Immunogenicity interference test

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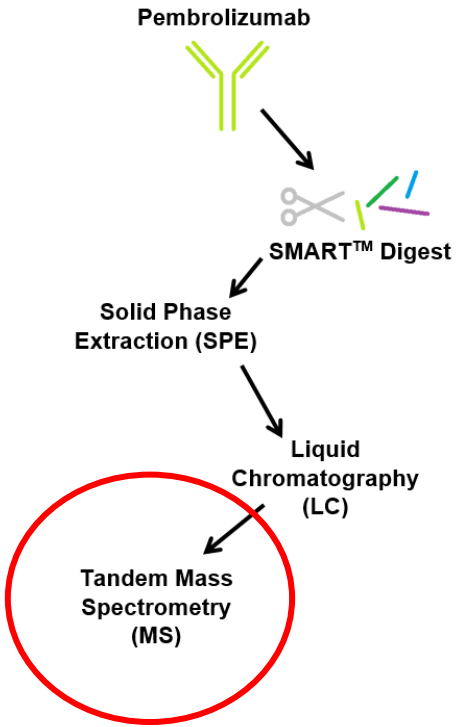
LC-MS/MS: Infusion of Pembrolizumab

Peptide Name	Sequence	IgG Subclass
Peptide 1	**VVSVLTVLHQDWLNGK**	IgG1, IgG4, IgG3
Peptide 2	GFYPSDIAVEWESNGQPENNYK	IgG1, IgG4
Peptide 3	**DSTYSLSSTLTLSK**	All
Peptide 4	**VDNALQSGNSQESVTEQDSK**	All



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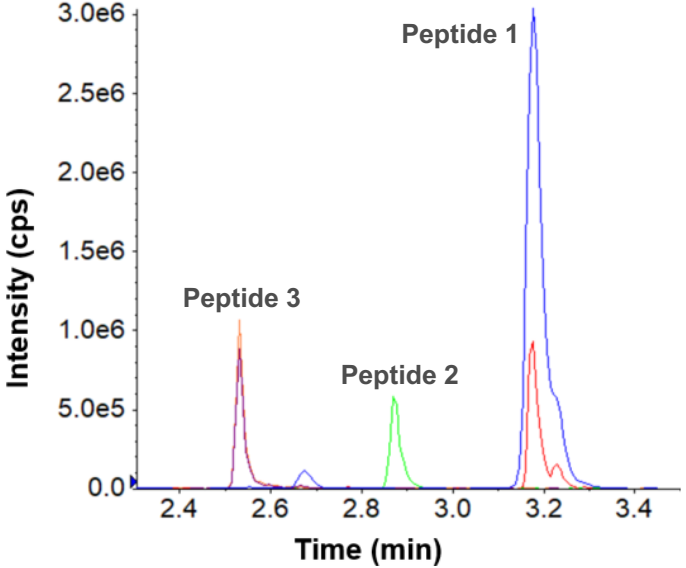
Signature peptide chromatography

A ULOQ 500,00 ng/mL



B LLOQ at 500 ng/mL

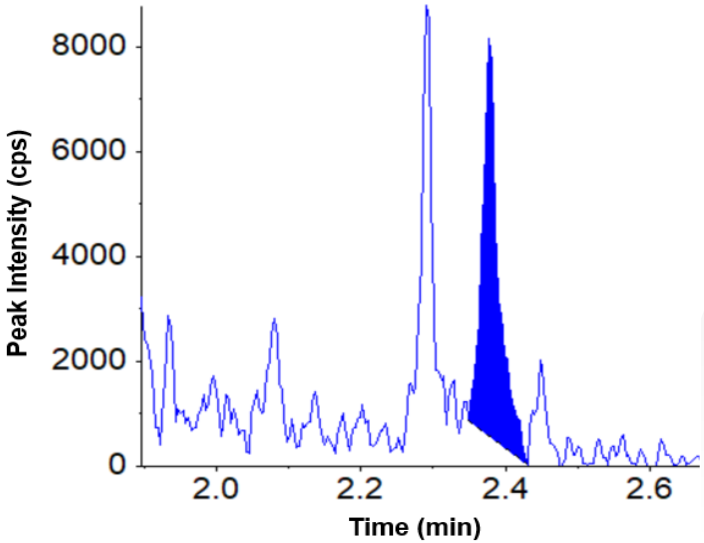


Figure: Representative chromatograms demonstrating peak intensities (cps) of 5 peptide transitions with highest peak intensities; (—) 603.7 → 806.0 (Peptide 1); (—) 603.7 → 712.8 (Peptide 1); (—) 849 → 764.4 (Peptide 2); (—) 752.0 → 836.5 (Peptide 3) and (—) 752.0 → 1036.6 (Peptide 3). In **(A)** peak intensities of transitions in the highest pembrolizumab calibration standard concentration of 500,000 ng/mL. In (**B**) Representative LLOQ chromatogram of Peptide 1 transition 603.7 → 806.0 following second LC-MS/MS A&P to determine LLOQ. Concentration of pembrolizumab was 500 ng/mL. S-T-N was >5: 1.

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LC-MS/MS: Accuracy and Precision (A&P) Run

What was done?

- Analysed 1 A&P Run 500 ng/mL to 500,000 ng/mL analysed in singlicate
- Six QC levels, each with 6 replicates: LLOQ – 500 ng/mL and ULOQ – 500 µg/mL

Intra-Assay Precision and Accuracy of Quality Control Sample Data

Replicate	LLOQ QC 500 ng/mL		LQC 2000 ng/mL		LMQC 20,000 ng/mL		MQC 200,000 ng/mL		HQC 400000 ng/mL	
	Concentration (ng/mL)	%Bias	Concentration (ng/mL)	%Bias	Concentration (ng/mL)	%Bias	Concentration (ng/mL)	%Bias	Concentration (ng/mL)	%Bias
1	545	9.0	1590	-20.5	17600	-12.0	185000	-7.5	344000	-14.0
2	515	3.0	1750	-12.5	19400	-3.0	-	-	361000	-9.8
3	488	-2.4	2030	1.5	19200	-4.0	185000	-7.5	373000	-6.8
4	508	1.6	1690	-15.5	19800	-1.0	189000	-5.5	368000	-8.0
5	511	2.2	2050	2.5	20100	0.5	199000	-0.5	422000	5.5
6	613	22.6	2180	9.0	22300	11.5	220000	10.0	427000	6.8
Precision (%)		8.4		12.6		7.8		7.6		8.9
Bias (%)		6.0		-6.0		-1.5		-2.0		-4.3

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Generic Gyrolab™ method using commercial kit by Gyros™

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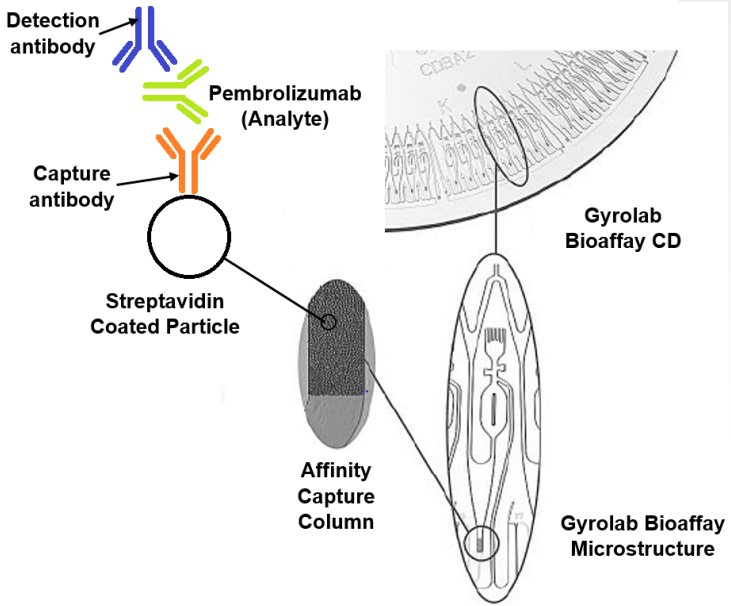
Method: Gyrolab™

- Used the Gyrolab™ generic PK kit
- Cals and QCs prepared in nonhuman primate (NHP) serum
- 10 µL sample volume used

Method Development

1. Reagent and assay range test
2. Accuracy and precision
3. Immunogenicity interference test



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Gyrolab™ PK Kit optimisation

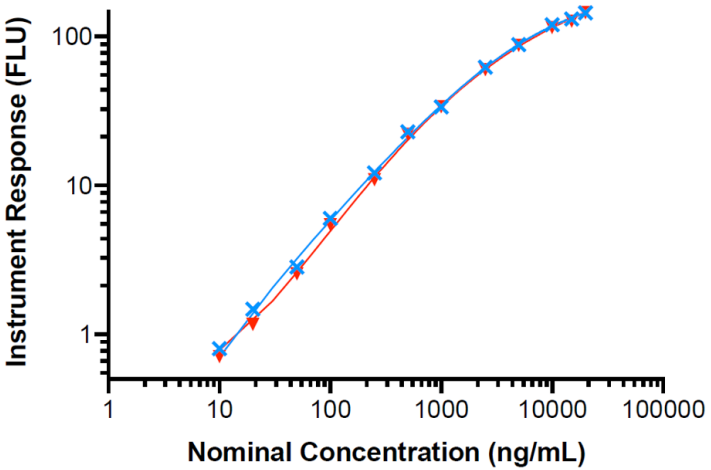

Figure: Calibration curves generated following Gyrolab™ analysis of calibration standards ranging from pembrolizumab concentrations of 10 ng/mL to 20,000 ng/mL. Standards were diluted in two different sample dilution buffers: Reagent E (-x-) and Reagent F (-▼-). The calibration standard at a concentration of 5 ng/mL was masked for both sample dilution buffers following failure of accuracy acceptance criteria (%bias was > 20%).

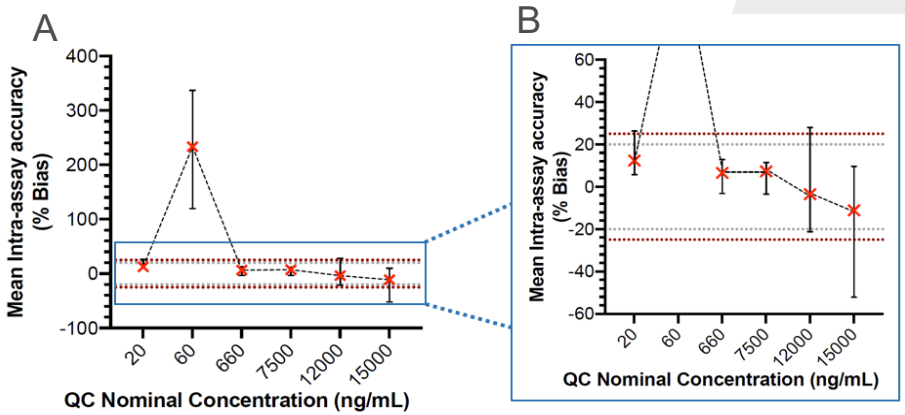

Figure: (A) Calculated intra-assay accuracies (%Bias) of QC concentrations in Gyrolab A&P investigation. Data is presented as mean % bias ± upper and lower % bias range of individual replicates. Intra-assay accuracy acceptance criteria for each QC concentration was a % bias less than ± 20% (····) and less than ± 25% at the LLOQ (20 ng/mL) and ULOQ (15,000 ng/mL) (····). In (B) the magnified view of intra-assay accuracy is shown. At each concentration n=6.

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Gyrolab™: Accuracy and Precision (A&P) Run

What was done?

- Analysed 3 A&P Runs 20 ng/mL to 15,000 ng/mL analysed in singlicate
- 6 QC levels, with 6 replicates each: LLOQ QC (20 ng/mL)

Inter-Assay Precision and Accuracy of Quality Control Sample Data						
	LLOQ	LQC	LMQC	MQC	HQC	ULOQ
	20 ng/mL	60 ng/mL	660 ng/mL	7500 ng/mL	10000 ng/mL	15,000 ng/mL
Mean (ng/mL)	24.51	58.41	757.64	7845.90	14727.49	14962.97
Precision (%)	17.57	18.74	9.32	14.04	24.24	20.00
Bias (%)	22.53	-2.65	14.79	4.61	5.10	-0.24

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How Do Anti-Drug Antibodies Affect the Bioanalysis?

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Immunogenicity Interference Test

Influence of anti-pembrolizumab antibodies on assay performance

Adding three different concentrations LQC & HQC samples

- Gyrolab™: ADA = big effect
- LC-MS = no interference

	Gyrolab		LC-MS/MS	
QC level (ng/mL)	LQC (450 ng/mL)	HQC (8,000 ng/mL)	LQC (2,000 ng/mL)	HQC (40,000 ng/mL)
pAb concentration (ng/mL)	**%Bias**			
0	12.5	3.0	-6.0	-4.3
FDA REQUIRED SENSITIVITY **100**	-13.0	-14.9	16.9	1.6
1,000	-54.3	11.1	13.7	-3.4
10,000	-98.1	-27.8	-17.1	-1.6

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Effect of ADA's on GYROLAB™

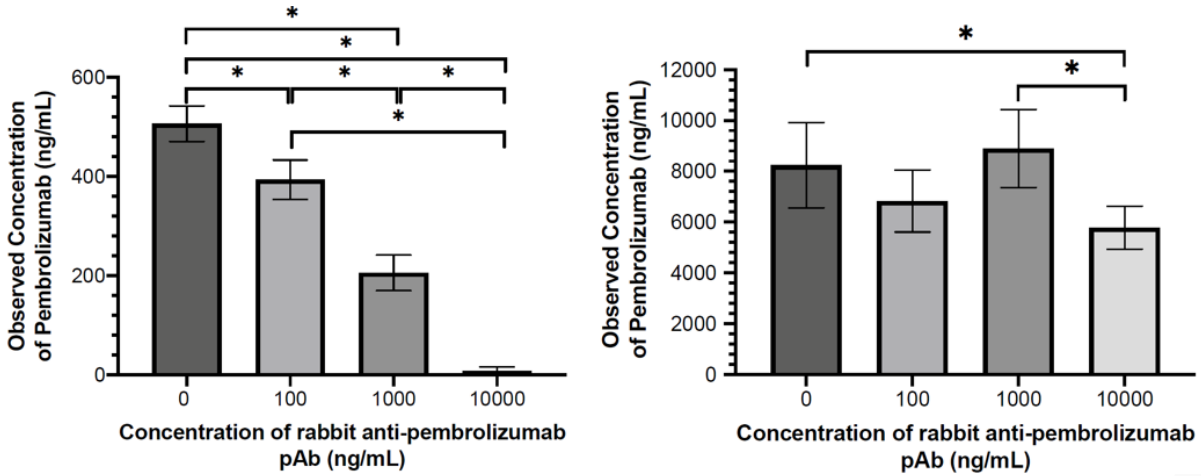

Figure 10: Effect of increasing concentrations of anti-pembrolizumab pAb on Gyrolab™ analysis. In (**A**) observed concentration of pembrolizumab in LQC samples following addition of 0 ng/mL, 100 ng/mL, 1000 ng/mL and 10,000 ng/mL anti-pembrolizumab pAb. Data is presented as mean ± standard deviation. At 0 ng/mL n=6, at 100 ng/mL and 1000 ng/mL n = 5 and at 10,000 ng/mL n=3 due to two replicates in this group being below the limit of detection on the Gyrolab™. In (**B**) observed concentration of pembrolizumab in HQC samples following addition of 0 ng/mL, 100 ng/mL, 1000 ng/mL and 10,000 ng/mL anti-pembrolizumab pAb. Data is presented as mean ± standard deviation. At 0 ng/mL n=6, at 100 ng/mL, 1000 ng/mL and 10,000 ng/mL n = 5. * **=** Significant difference ($p < 0.05$) between groups following one-way ANAVO and Tukey's post hoc test.

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Effect of ADA's on LC-MS/MS

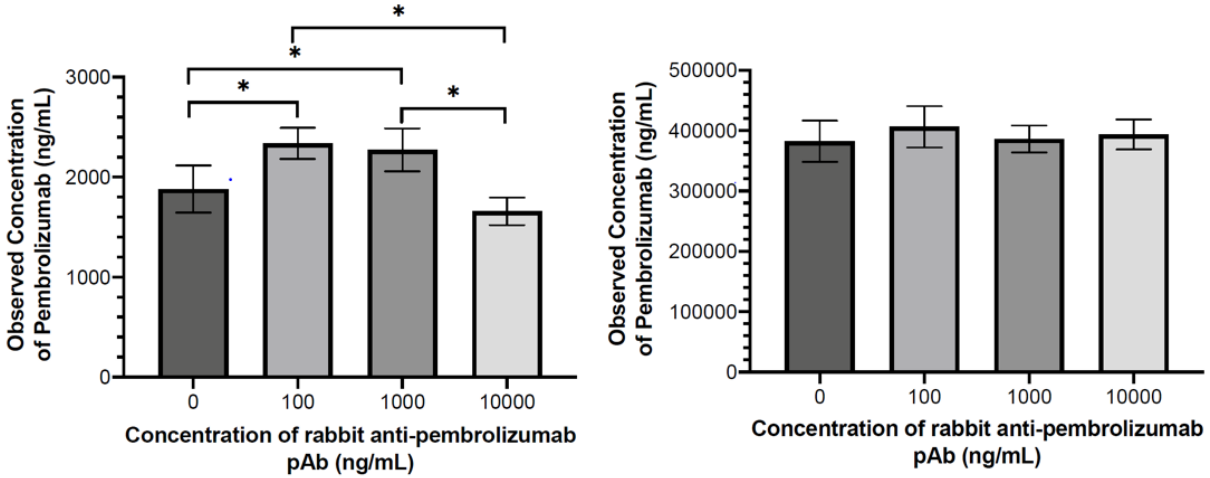

Figure 18: Effect of increasing concentrations of anti-pembrolizumab pAb on LC-MS/MS analysis. In (**A**) observed concentration of pembrolizumab in LQC samples following addition of 0 ng/mL, 100 ng/mL, 1000 ng/mL and 10,000 ng/mL anti-pembrolizumab pAb. Data is presented as mean ± standard deviation. At each anti-pembrolizumab pAb concentration n=6. In (**B**) observed concentration of pembrolizumab in HQC samples following addition of 0 ng/mL, 100 ng/mL, 1000 ng/mL and 10,000 ng/mL anti-pembrolizumab pAb. Data is presented as mean ± standard deviation. At each anti-pembrolizumab pAb concentration n=6. * = Significant difference ($p < 0.05$) between groups following one-way ANAVO and Tukey's post hoc test.

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Summary of Methods

Parameter	LC-MS/MS	Gyrolab™
Assay Sensitivity (ng/mL)	500	20
Assay Range (ng/mL)	500 – 500,000 (1000 fold)	20 – 15,000 (750 fold)
Accuracy and Precision	% CV < 13 % Bias < 6	% CV < 25 % Bias < 25
Sample Volume (µL)	15	10
Assay Time (h)	7.5	1.5
Influence of ADA	Low/None	High
Application	Toxicokinetics	Pharmacokinetics

Any Questions?

Project completed by Rebecca Taylor – University of Leeds Biopharmaceutical masters

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- Sarah Malpas
- Johannes Stanta

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