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

Robert Stewart
YSS Conference 25th September 2020

25th September 2020

COVANCE

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

COVANCE

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
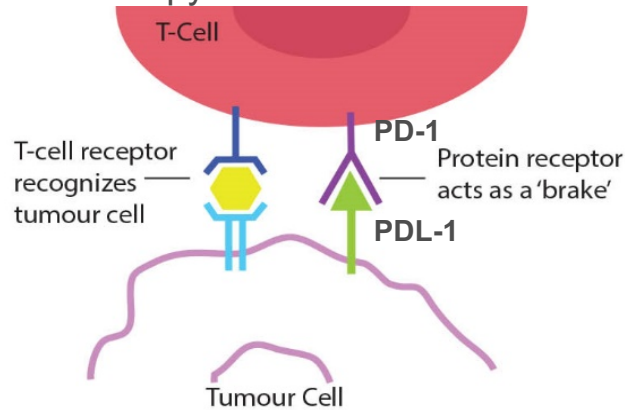
Inhibition of T cell mediated anti-tumour response
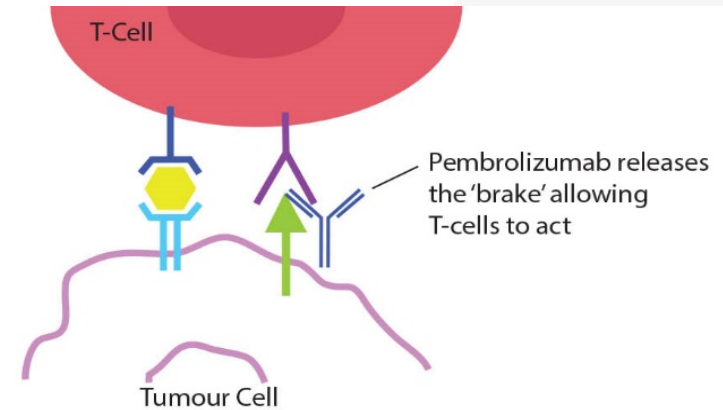
Restoration of T cell mediated anti-tumour response

COVANCE

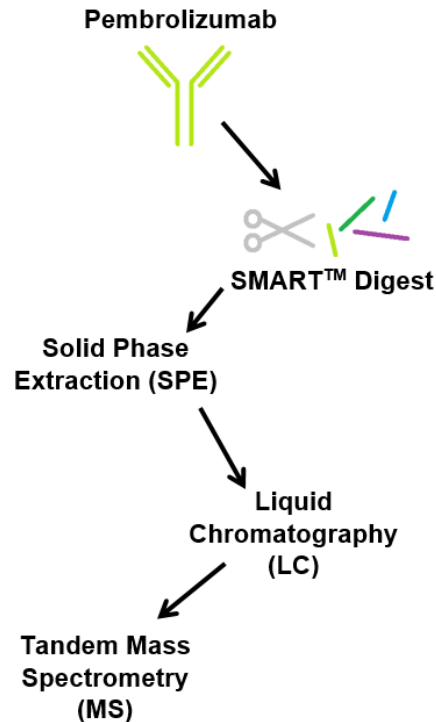# Generic LC-MS Method Based on Literature Method

© AB Sciex Pte. Ltd. – used with permission

COVANCE.

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**+**
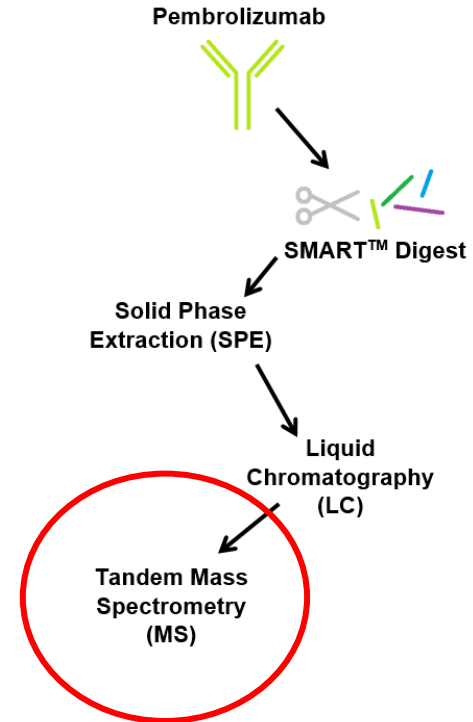
► 15 µL sample volume to accommodate micro sampling

Method Development

1. Digestion and MS optimization of Pembrolizumab

2. Accuracy and precision

3. Immunogenicity interference test

Pembrolizumab

↓

SMART™ Digest

↓

Solid Phase Extraction (SPE)

↓

Liquid Chromatography (LC)

↓

Tandem Mass Spectrometry (MS)

COVANCE

LC-MS/MS: Infusion of Pembrolizumab

Peptide Name	Sequence	IgG Subclass
Peptide 1	**VVSVLTVLHQDWL NGK**	IgG1, IgG4, IgG3
Peptide 2	GFYPSDIAVEWES NGQPENNYK	IgG1, IgG4
Peptide 3	**DSTYSLSSTLTLSK**	All
Peptide 4	**VDNALQSGNSQE SVTEQDSK**	All

Pembrolizumab

SMART™ Digest

Solid Phase
Extraction (SPE)

Liquid
Chromatography
(LC)

Tandem Mass
Spectrometry
(MS)

COVANCE

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 – 400 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	

Generic Gyrolab™ method using commercial kit by Gyros™
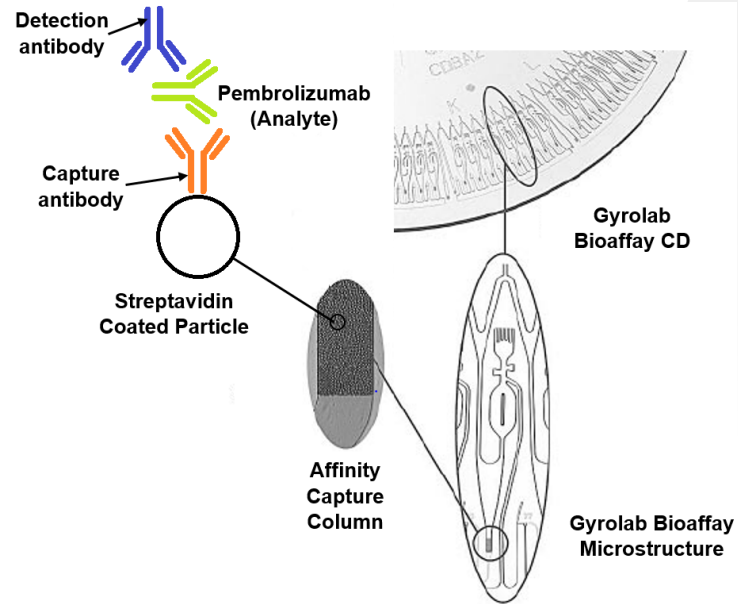
© Gyros – Picture used with permission

COVANCE

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

© Gyros – Picture used with permission

COVANCE

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

COVANCE.

How Do Anti-Drug Antibodies Affect the Bioanalysis?

COVANCE.

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

COVANCE.

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

COVANCE

Any Questions?

Project completed by Rebecca Taylor – University of Leeds Biopharmaceutical masters

Thanks to the following for their contributions to the project –

Rebecca Taylor – University of Leeds Biopharmaceutical masters

- Barry Hawthorne
- Gregory Bogle
- Emma Tipping
- Sam Willcox
- Sarah Malpas
- Johannes Stanta

COVANCE