Melanoma Institute Australia

PIPdx

Immunotherapy Response Biomarker Report

FOR THE PERSONALISED IMMUNOTHERAPY PLATFORM

REPORT CONTENT

PATIENT INFORMATION	
Surname:	
Given Name:	
Date of Birth:	
MRN:	
Sex:	
StudyID:	

SPECIMEN INFORMATION	
Specimen ID:	
Laboratory:	
Collection Date:	
Retrieval Date:	
Tissue Site:	

ORDERED BY	
Oncologist:	
Address:	
Request Date:	

A Translational Research Program of the Melanoma Institute Australia
Protocol Number: MIA2020/283. HREC reference number: 2020/ETH00426

1. REPORT SUMMARY

IMMUNOTHERAPY RESPONSE PREDICTION (CLINICAL MODEL)

Treatment	Response	Probability of response	Progression-free survival	Overall Survival

** Derived from the MIA Immunotherapy Outcomes Prediction Tool www.melanomarisk.org.au/IOCLand*

APPROVED BIOMARKERS

Biomarker	Result	Comments

** FDA approved pembrolizumab for the treatment of adult and paediatric patients with unresectable or metastatic tumour mutational burden-high [≥10 mutations/megabase (mut/Mb)] (Section 2.1)*

BIOMARKERS DERIVED FROM CLINICAL TRIALS

Biomarker	Result	Comments

** High IFNg is associated with increased response rates to immune checkpoint inhibitors (Section 2.2)*
*** Scores for gene expression signatures range from -0.5 to 0.5. High or low expression is determined based on the median, as calculated in a cohort of 156 retrospective patients with advanced melanoma.*

BIOMARKERS WITH POTENTIAL SIGNIFICANCE

Biomarker	Result	Comments

 # Inflamed immune cell distribution is associated with improved outcomes to immunotherapies. (Section 2.4)
PD-L1 expression is associated with improved outcomes. (Section 2.3)

EXPRESSED IMMUNOTHERAPY-BASED DRUG TARGETS WITHIN CLINICAL TRIALS

Biomarker	Result	Immunohistochemistry validation	Comments

** Immunohistochemistry cut-off for positivity is based on >1% of all cells within the tumour.*
***High or low expression is determined based on the median, as calculated in a cohort of 43 patients with advanced melanoma.*

1.1 Biomarkers Summary

The above figure displays the results for the patient tested within this report in comparison to the data generated from all patients tested via the PIPdx platform. The cut-off for high and low is the value that corresponds to the median of the cells.

2. REPORT BACKGROUND

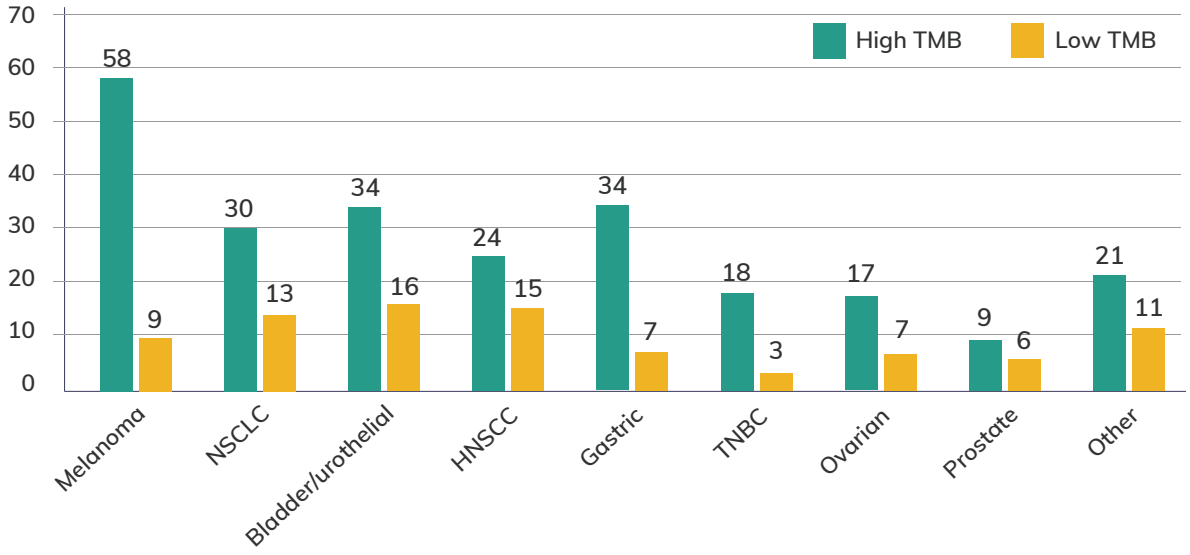
The **PERSONALISED IMMUNOTHERAPY PLATFORM** was developed by researchers at the Melanoma Institute Australia. The platform constitutes a suite of assays that generate clinical response predictions to anti-PD-1 monotherapy (nivolumab or pembrolizumab), or combination anti-PD-1 + anti-CTLA-4 (ipilimumab), for a specific cancer patient. The test is composed of a tissue immunohistochemical assay, gene expression assay and tumour mutation assay, the results from which are combined with the patient's clinical data to generate a prediction of the likelihood of responding to the aforementioned therapies.

This report provides a summary of the clinical, immune, genomic and transcriptomic features that contribute to the response prediction probability shown in Section 1.

2.1 Tumour Mutation Burden (TMB)

Somatic mutations in a cancer genome can result in the creation of novel antigens, neoantigens, which can be recognise by the patient's immune system [1,2]. A metric for the tumour mutation burden is the number of somatic mutations per a megabase of DNA assessed. Generally, **high mutation burden tumours have been associated with an increased immune recognition by tumour infiltrating lymphocytes, increased PD-L1 expression[3] and increased response rates to immune checkpoint inhibitors[4]**. Data from the the Keynote-158 study of 9 different advanced solid tumours, identified significantly higher response to pembrolizumab in the TMB-high versus TMB low patients (cut-off 10 mutations/megabase), which led to FDA approval of pembrolizumab for TMB high paediatric and adult solid tumours[5,6].

Response rates of advanced cancer patients from the Keynote-158 clinical trial, separated into tumour mutation burden high and low.



2.2 Gamma Gene Expression Signatures

Transcriptional signatures that relate to a pre-existing immune response against the tumour, such as the interferon gamma (IFN-g) signature have been associated with better responses to immunotherapies[7,8]. A set of landmark studies of anti-PD-1 therapy across multiple cancer types has identified a gene set, or collection of genes, which represents a T cell–inflamed microenvironment, which can be measured by the levels of IFN-g signalling, cytotoxic effector molecules, antigen presentation, and critical T cell active cytokines.Below are the response rates (RECIST complete response or partial response) from 22 tumour types from four KEYNOTE clinical trials per tumour mutation burden (TMB) and gene expression profile of IFN-g signature (GEP)[9].

PANCANCER

TMB high	14%	36%
TMB low	0%	17%
	IFNg low	IFNg High

Head and neck SCC

TMB high	13%	31%
TMB low	0%	24%
	IFNg low	IFNg High

Melanoma

TMB high	38%	54%
TMB low	10%	33%
	IFNg low	IFNg High

2.3 PD-L1 expression

Quantification of PD-L1 expression involves immunohistochemical staining of the PD-1 ligand protein (PD-L1). Studies have found the response rate for PD-L1 positive tumours in melanoma patients is higher compared to PD-L1 negative tumours. However, PD-L1 negative patients still respond (12-37%) and the assay may not be predictive in the setting of anti-CTLA-4[10]. The Dako 28–8 PD-L1 assay has an associated sensitivity of 58% and specificity of 49%, hence uptake in melanoma has been limited. While a useful tool, the single biomarker based PD-L1 assay is too simplistic to account for the range of factors that contribute to the anti-tumour response, and is not used by clinicians to select therapy in melanoma[11]. Below is a summary from the pancancer analysis of the KEYNOTE trials of anit-PD-1 immunotherapy with patients grouped into TMB high/low and PD-L1 high/low based on analysis of their pre-treatment cancer biopsy[9].

PANCANCER

TMB high	0%	35%
TMB low	0%	9%
	PD-L1 low	PD-L1 high

Head and neck SCC

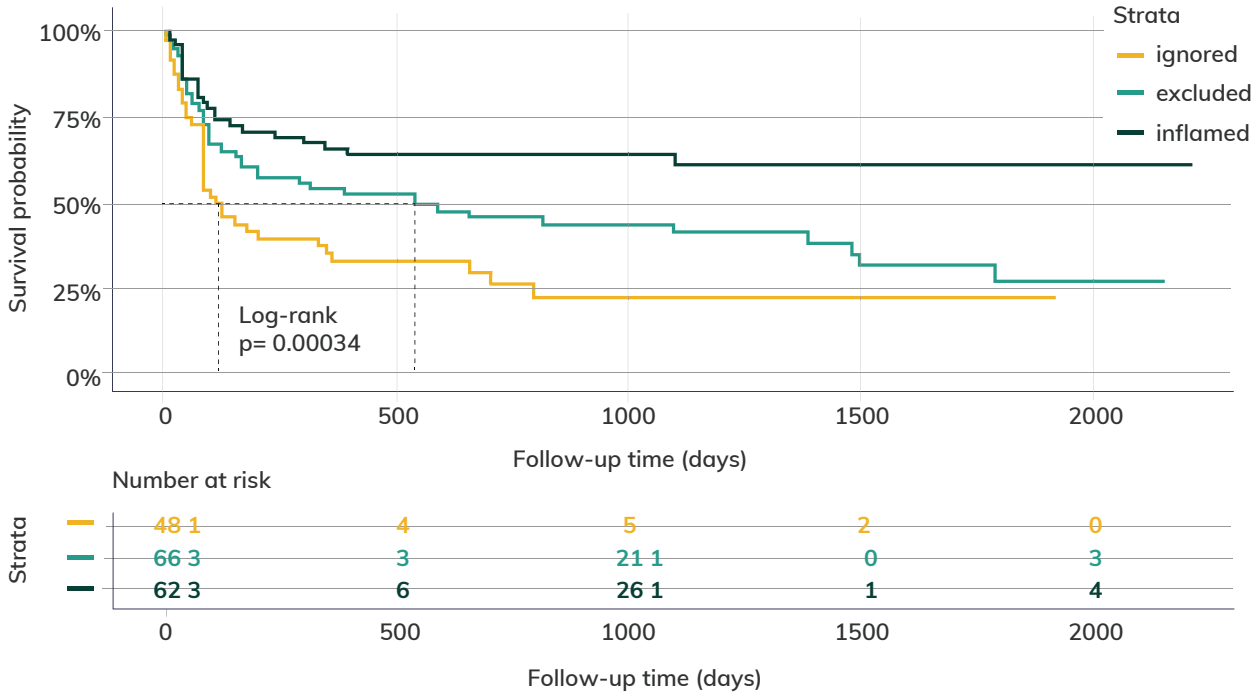
TMB high	14%	30%
TMB low	9%	12%
	PD-L1 low	PD-L1 high

Melanoma

TMB high	30%	57%
TMB low	0%	24%
	PD-L1 low	PD-L1 high

2.4 Tumour immune cell distribution

The degree and distribution of tumour infiltrating lymphocytes (TILs) within a pre-treatment tumour biopsy has been associated with response to immune checkpoint inhibitors[12], with increased of intratumoural lymphocytes penetrating the tumour being associating with higher response rates to immune checkpoint inhibition[12]. Tumour immune cell distribution can be generalised into classifications of; immune desert which are lacking TILs; immune excluded which contain TILs in the peritumour stroma but are excluded from the intratumour region, and inflamed/ immunogenic tumours which display dense infiltration of immune cells. Below is the data from the Personalised Immunotherapy Platform showing the progression-free survival of immunotherapy treated advanced melanoma patients stratified via their tumour immune cell distribution.



Progression-free survival of advanced melanoma patients treated with either anti-PD-1±CTLA-4 therapies separated by the immune infiltration into the tumour region. Patient pool consists of 190 patients as part of the Personalised Immunotherapy Platform.

2.5 Expression of immune drug targets current in clinical trials

The Personalised Immunotherapy Platform performs extensive gene expression and immunohistochemistry-based immunotherapy drug target assessment of all patients' tumours within the Platform. Patient's tumours are identified which are predicted to be unresponsive to standard treatments and maybe more likely to benefit from a clinical trial of a novel agent, and a two staged assessment of alternative immunotherapy or molecular drug targets which are active in that patient's tumour is performed.[13]

This process consists of two assessment methodologies; Firstly, patients' tumours undergo clinical immunohistochemistry for three drug targets currently available in clinical trials, including LAG3, TIGIT and TIM3. Table 2 provides a description of each drug target and current clinical trials in melanoma. The percentages of drug target positive cells in the melanoma biopsies are assessed via imaging software (Vectra 3 microscope, Akoya with Halo® image analysis software, Indica Labs) and results are confirmed by a clinical pathologist. Secondly, gene expression profiles of an extensive list of immunogenic agent receptor and ligand pairs that are within phase I-III clinical trials are assessed. The results of both of these assays are combined to validate and explore alternative treatment options for each patient.

Table 1. Immunotherapy drug targets and current clinical trials

Biomarker	Mechanism of Action	Drug Name	Clinical Trials
LAG3 (antilymphocyte activation gene 3)	LAG3 binds to its ligand, the MHC class II molecule to negatively regulate T cell activation and proliferation.	Relatlimab INCAGN02385 LAG525 LBL-007 XmAb®22841	NCT03743766 NCT04370704 NCT03484923 NCT04640545 NCT03849469
TIGIT (T-cell immunoreceptor with Ig and ITIM domains)	TIGIT is expressed on a subset of activated T cells and NK cells and works with PD-1/PD-L1 to inhibit effector CD8+ T cell function.	EOS-448 Vibostolimab AB-154	NCT05060432 NCT04305054 NCT05130177
TIM3 (T-cell immunoglobulin & mucin domain 3)	TIM-3 has been shown to suppress the activity of CD8+ cytotoxic T cells and Th1 helper cells and is associated with T cell exhaustion.	TSR-022 MBG453 INCAGN2390 LY3321367 BMS-986258	NCT04139902 NCT02608268 NCT04370704 NCT03099109 NCT03446040

Specific trials please visit https://melanoma.org.au/research/clinical-trials/

3. ASSAY BACKGROUND

3.1 Tumour Microenvironment Assessment (TME)

The TME assessment is a laboratory validated multiplex immunofluorescence assay which identifies Cytotoxic T-cell (CD8+), Macrophages (CD68+) and Melanoma cells (SOX10) in the patient's tumour biopsy[14]. The assay also assesses the expression of an immunotherapy drug target, Programmed Cell Death Ligand 1 (PD-L1) and immune phenotyping marker CD16 (described in Table 2). Our studies and others have shown that inflamed/immunogenic tumours with high cytotoxic T-cells respond to immunotherapies (anti-PD-1 and anti-CTLA-4) at a higher rate than tumours that lack immune cells[12]. The densities and spatial location of the cells in the tumour are assessed using quantitative pathology image analysis software and results are confirmed by a clinical pathologist for this report (Akoya with Halo® image analysis software, Indica Labs).

Table 2. Markers in predictive spatial pathology panel

Marker	Used to Define
CD8	Cytotoxic T-cells, attack tumour cells
PD-L1 (programmed cell death ligand 1)	Immune checkpoint, binds to PD-1
CD68	Pan-macrophage marker
CD16	Macrophages, natural killer cells, neutrophils, monocytes, and T-cells
SOX10	Melanoma cells

3.2 Gene Expression Assay

Gene expression of the potential biomarker genes is generated using the **Nanostring PanCancer IO 360™ gene expression panel** within the NATA accredited Ramaciotti Centre for Genomics. This assay covers a panel of 770 genes as well as a number of gene signatures designed to profile the immune and tumour microenvironment. A curated list of gene signatures, collections with known functional similarity are quantified across the tumours. This includes gene signatures such as the Interferon gamma signature[7], which measures pre-existing, peripherally suppressed adaptive immune responses in the tumour. In the clinical trial setting, signatures such as the IFNg signature have been identified and are therefore being studied as an Investigational Use Only (IUO) device as part of the Personalised Immunotherapy Platform.

Data is normalised to the Panel Standard in nSolver and analysed using the Nanostring IO 360 Data Analysis Service. The gene expression data is used to quantify the abundance of over 60 immune based drug targets within the tumour microenvironment.

3.3 Tumour Mutation Assay

Tumour mutational burden (TMB) is the total number of somatic mutations within a tumour genome. The tumour mutational burden is generated using the QIAseq Targeted DNA IO Panel (Qiagen, cat#333805) which covers 486 genes and 1.3mb of the genome. DNA and RNA are extracted from formalin fixed paraffin embedded routine tumour biopsies using the Qiagen AllPrep DNA/RNA FFPE kit. Libraries are generated using the QIAseq TMB Panel and sequencing performed on an Illumina Novaseq Instrument at the NATA accredited Ramaciotti Centre for Genomics. Variants are detected and tumour mutational burden derived using the predefined analysis pipeline , QIAseq Tumor Mutational Burden (TMB) workflow within the Biomedical Genomics Analysis of CLC Genomics Workbench (Qiagen, V20.0.0). Variants are annotated and clinically important variables identified using the QIAGEN Clinical Insights (QCI) pipeline where variants are tiered in terms of the clinical importance and association with known response to oncology therapeutics.

QIAseq TMB Panel Targets: covers exonic reqions of 486 genes

ABCB9	BARD1	CD70	**CTNNB1**	ERCC1	FH	HLA-B	JUN	MCM5	**MYD88**	PDIA3	PSMA4	PSMD4	RASA1	SIRT1	TCF7L2	WEE1
ABL1	BCL2	CD79A	CTSB	ERCC2	FIGF	HLA-C	KAT6A	MCM6	MYOCD	PDK1	PSMA5	PSMD5	**RB1**	SMAD2	TCP11L2	WT1
ABL2	BCL2L1	CD79B	CTSL	ERCC3	FKBP9	HLA-E	KDM5A	MCM7	NBN	PHF6	PSMA6	PSMD6	RBM10	**SMAD3**	TDG	XPO1
ACE2	**BCL6**	CD80	CTSS	ERCC4	FLCN	HLA-F	KDM5C	MDM2	NCOR1	PIK3C2B	PSMA7	PSMD7	REL	SMAD4	TERC	XRCC5
ACVR1B	BCOR	CD86	**CUL3**	ERCC5	FLT1	HLA-G	KDM6A	MDM4	**NF1**	**PIK3CA**	PSMA8	PSMD8	RET	**SMARCA4**	TERT	ZFHX3
AKT1	BCORL1	CDC27	CUL4B	ERG	FLT3	HMGB1	**KDR**	MED12	NF2	**PIK3CB**	PSMB1	PSMD9	RFC1	SMARCB1	TET2	ZNF217
AKT2	BLM	CDC73	CUX1	ERRFI1	FLT4	HMGN1	**KEAP1**	MEF2B	NFE2L2	PIK3CG	PSMB10	PSME1	RFC2	SMC1A	TGFBR2	
AKT3	**BRAF**	CDH1	CYLD	ESR1	**FOXA1**	HNF1A	KEL	MEN1	NFKBIA	**PIK3R1**	PSMB11	PSME2	RFC3	SMC3	TNF	
ALK	BRCA1	CDK12	DAXX	**ETV6**	FOXL2	**HRAS**	**KIT**	**MET**	NKX2-1	PIK3R2	PSMB2	PSME3	RFC4	SMO	TNFAIP3	
ALPK2	**BRCA2**	**CDK4**	DDR2	EWSR1	**FOXP1**	HSP90AA1	KMT2A	MICA	**NOTCH1**	PIM1	PSMB3	PSME4	RFC5	SOCS1	TNFRSF14	
AMER1	**BRD4**	CDK6	DDX3X	EXO1	FUBP1	ICOSLG	**KMT2C**	MICB	NOTCH2	PLCG2	PSMB4	PSMF1	RHEB	**SOS1**	TNFRSF9	
APC	BRIP1	CDK8	**DICER1**	**EZH2**	GABRA6	IDE	**KMT2D**	MITF	NOTCH3	PMS1	PSMB5	PSMG1	**RHOA**	SOX10	TNFSF14	
AR	BTK	CDKN1A	DIS3	FAM46C	GADD45A	IDH1	**KRAS**	MLH1	NOTCH4	PMS2	PSMB6	PSMG2	**RICTOR**	SOX17	TNFSF18	
ARAF	C10orf54	CDKN1B	DMD	FANCA	GATA1	IDH2	LGALS9	MLH3	NPEPPS	POLB	PSMB7	PSMG3	**RIT1**	SOX2	TNFSF4	
ARID1A	CALR	**CDKN2A**	DNER	FANCC	GATA2	IFI30	LGMN	MORC4	NPM1	POLD1	PSMB8	PSMG4	RNASEH2A	SOX9	TNFSF9	
ARID1B	CANX	CDKN2B	DNMT3A	FANCD2	GATA3	IGF1R	LIG1	MPL	**NRAS**	POLD2	PSMB9	PTCH1	RNF43	SPEN	TNKS	
ARID2	**CARD11**	CDKN2C	DOT1L	FANCE	GATA4	IGF2	LIG3	MR1	NRD1	POLD3	PSMC1	**PTEN**	ROS1	SPOP	TOP1	
ARID5B	**CASP8**	CEBPA	EED	FANCF	GATA6	IGF2R	LMO1	MRE11A	NSD1	POLD4	PSMC2	PTGS2	RPA1	SRC	**TP53**	
ASXL1	CBFB	CHD4	**EGFR**	FANCG	GLI1	IKBKE	LNPEP	MSH2	NTRK1	POLE	PSMC3	**PTPN11**	RPA2	SSBP1	TP53BP1	
ASXL2	**CBL**	CHEK1	EP300	FAS	**GNA11**	**IKZF1**	LPAR2	MSH3	NTRK2	POLE4	PSMC4	**PTPRD**	RPA3	STAG2	TP73	
ATM	**CCND1**	CHEK2	EPCAM	FAT1	GNA13	**IL7R**	LRP1B	MSH4	NTRK3	**PPP2R1A**	PSMC5	QKI	RPA4	STAT3	TPP2	
ATR	CCND2	CIC	**EPHA3**	**FBXW7**	**GNAQ**	INPP4B	LZTR1	MSH5	PALB2	PRDM1	PSMC6	**RAC1**	RPTOR	**STK11**	TREX1	
ATRX	CCND3	CNKSR1	EPHA5	FGF19	**GNAS**	**IRF4**	**MAP2K1**	MSH6	PARK2	PRKAR1A	PSMD1	RAD17	RUNX1	SUFU	TRRAP	
AURKA	CCNE1	COL5A1	**EPHA7**	FGF3	GRIN2A	IRF6	**MAP2K2**	**MTOR**	PARP1	PRKCG	PSMD10	RAD18	RUNX1T1	SUZ12	TSC1	
AURK	CD200	**CREBBP**	**EPHB1**	FGF4	GSK3B	IRS2	MAP2K4	MUC17	PAX5	PRKCI	PSMD11	RAD21	SDHA	SYK	TSC2	
AXIN1	CD274	CRKL	ERAP1	FGFBP1	H3F3A	ITGAV	MAP3K1	MUTYH	**PBRM1**	PRKCZ	PSMD12	RAD50	SDHB	TAP1	TSHR	
AXIN2	CD276	**CRLF2**	ERAP2	FGFR1	HERC1	ITGB3	MCL1	MYB	PCNA	PRKDC	PSMD13	RAD51	SDHC	TAP2	**U2AF1**	
AXL	CD40	CSF1R	**ERBB2**	FGFR2	HGF	JAK1	MCM2	MYC	PDCD1LG2	PSMA1	PSMD14	RAD51C	SDHD	TAPBP	VEGFA	
B2M	CD40LG	**CTCF**	ERBB3	FGFR3	**HIST1H3B**	JAK2	MCM3	MYCL	**PDGFRA**	PSMA2	PSMD2	**RAF1**	**SETD2**	TAPBPL	VHL	
BAP1	CD48	CTNNA1	ERBB4	FGFR4	HLA-A	JAK3	MCM4	MYCN	PDGFRB	PSMA3	PSMD3	RARA	**SF3B1**	TBX3	VTCN1	

4. DISCLAIMER, ABBREVIATIONS & REFERENCES

4.1 Disclaimer

This Immunotherapy Response Biomarker PIP Report ("PIP Report") has been developed by researchers associated with Melanoma Institute Australia and provides a summary of the clinical, immune, genomic and transcriptomic features that contribute to the response prediction probability in relation to a patient with metastatic melanoma.

A clinicians' use of the PIP Report is allowed on the following conditions:

1. The clinician acknowledges that it has been designed and is intended only for use in relation to patients with metastatic melanoma at the time of planning systemic immunotherapy treatment.
2. A clinician who uses the PIP Report in relation to a patient agrees and acknowledges that:
    a. The PIP Report is based on the MIA patient database population and may not perform reliably in relation to other populations.
    b. The PIP Report and associated predictions of patient response must be interpreted in relation to its clinical significance for the patient in question.
    c. The information provided by the PIP Report must only be used as a general guide for the patient in question.
    d. MIA gives no warranties, nor makes any representations to any clinician or patient regarding the accuracy, completeness, timeliness or usefulness of any patient response predictions generated by the PIP Report; and MIA will have no liability to the clinician or any patient arising from any treatment decision made - or any action taken or not taken - by the clinician or a patient following use of the data generated by the PIP Report.

4.2 Abbreviations

Term	Explanation
Analytic validity	Refers to the accuracy and robustness of an assay, establishes that an assay measures the intended analyte
Clinical validity	Refers to the test's ability to define or predict the disorder or characteristic of interest accurately and reliably
Non-responder	Patient that achieves a best response of partial response or stable disease for less than, or equal to, 6 months or progressive disease (per RECIST 1.1) within 6 months from the start of treatment
Negative predictive value	Negative predictive value (NPV) is defined as the percent of individuals in whom the test is negative and the disease is not present.
Positive predictive value	Positive predictive value (PPV) is defined as the percent of individuals in whom the test is positive and the disease is present.
Predictive biomarker	Predictive Biomarkers provides information about the favourable and unfavourable effect of therapeutic intervention. A predictive biomarker is a baseline characteristic which categorizes patients by their degree of response to treatment. A predictive biomarker is utilized to detect the treatment effect between a biomarker -positive and a biomarker negative group.
Predictive model	An integration of the melanoma tissue analyses (molecular and pathological) and clinical information (melanoma history and clinical laboratory data) that is used to calculate a prediction of response or resistance to immunotherapies.
Responder	Patient that achieves a complete response, partial response or stable disease (per RECIST 1.1) for greater than 6 months from the start of treatment
Sensitivity	Sensitivity of a test or marker is defined as the percentage of positive samples identified by a model as true positive. The false negative rate is the percent of patients with the disease for whom the test is negative.

Specificity	Specificity is defined as the percentage of negative samples (individuals without the disease) identified by a model as true negative. False positive is the number of individuals without the disease in whom the test is positive.
TEAM	Treat, Excise, Analyse Melanoma
AJCC	American Joint Committee on Cancer
AUC	Area under the curve
ctDNA	Circulating tumour DNA
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
H&E	Haematoxylin and eosin stain
IHC	Immunohistochemistry
MDT	Multidisciplinary team
MHC	Major histocompatibility complex
PD-1	Programmed cell death 1
PD-L1	Programmed death ligand 1
RNA-seq	Whole transcriptome sequencing
TILs	Tumour infiltrating lymphocytes
TMB	Tumour mutation burden

4.3 References

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