Strata Oncology Testing Summary

Patient MRN	Patient Name	Birth Date	Sex	Strata Case
---	---, ---	---	Male	---
Client Specimen ID	**Specimen Site**	**Part**	**Date of Collection**	**Date Received**
---	Liver	---	---	---
Client		**Ordering Physician**		
---		---		

Molecular Diagnosis and Tissue Specimen Information

Determined at time of testing

Cancer Type	Tumor Content
Renal Cell Carcinoma	65%
Cancer Subtype	**Surface Area**
Renal Clear Cell Carcinoma	$18mm^2$

Diagnosis Note. Although the StrataNGS test is not validated to determine 3p status, the presence of 3p loss of heterozygosity (along with the prioritized VHL and PBRM1 mutations), is consistent with clear cell renal cell carcinoma.

Pertinent Biomarker Summary

Strata Select	
Genomic Alterations	PBRM1 p.F379Lfs*25 (56% VAF), VHL exon 1 splice donor mutation (47% VAF)
Negative Genomic Findings	NTRK1, NTRK2, NTRK3, RET
Genomic Signatures	Microsatellite Stable, TMB Low (2 muts/Mb)
Immunotherapy Response Score	IRS High
Strata RNA Supplement	
Angio Treatment Response Score	Angio Low

Therapy Associations

Biomarker Results	Predicted Benefit	Therapies	Evidence Level
IRS High	Benefit	pembrolizumab +/- lenvatinib or axitinib, or nivolumab +/- cabozantinib or ipilimumab [1*,2*,L*]	1
Angio Low	Less benefit	sunitinib, cabozantinib, axitinib, lenvatinib, pazopanib [1, 2, L]	3

Therapy Rationale. Across pan-solid tumor validation cohorts, IRS High patients have significantly longer anti-PD-(L)1 monotherapy real world PFS and overall survival vs. IRS Low patients, independent of TMB status.

Strata Oncology Testing Summary

In a validation cohort of 90 renal cell carcinomas (29 clear cell and 61 non-clear cell) treated with single agent VEGFR TKI systemic therapy, Angio Low samples had significantly shorter real-world progression free survival vs. Angio High samples when controlling for clinical factors (median rwPFS 5.9 vs. 15.4 months, adjusted hazard ratio 1.82, p=0.015).

For all therapeutically relevant biomarkers, the predicted benefit of listed therapies based on the following levels of evidence in the reported tumor type is shown: Level 1: FDA recognized; 2: Standard of care biomarker recommended per NCCN; 3: Compelling clinical evidence; 4: Compelling biologic evidence. "Less benefit" predicts less clinical benefit compared to unselected patients. "Trial" indicates therapies available through partnered clinical trials. For therapies, superscripts 1/2/L/M denote line of therapy (first, second, later, maintenance); * denotes a NCCN preferred therapy. For tumor types where PD-(L)1 monotherapy is considered Level 1 or 2 evidence in the absence of a biomarker (or by PD-L1 immunohistochemistry), that level of evidence is maintained if the combination of MSI/TMB/IRS supports PD-(L)1 monotherapy benefit.

Strata Oncology Testing Summary Description

The Strata Oncology Testing Summary integrates all testing results performed on the indicated specimen to highlight the most clinically relevant information. Please review the following individual test reports for additional information, which may be informative for patient management. Additional descriptions of testing summarized herein are provided in the individual test descriptions. PD-L1 IHC testing, when ordered, is performed and interpreted by Mayo Clinic Laboratories; see the PD-L1 IHC report for additional information.

Treatment Decisions

The selection of any treatment or clinical trial suggested herein resides within the discretion and judgment of the treating physician and patient. Strata testing identified biomarkers and relevant treatment associations are shown and described in the 'Therapy Associations' section based on the final reported tumor type using the levels of evidence shown in that legend. Listed treatments and levels of evidence may not be applicable to the patient's specific cancer based on incorrectly reported tumor type (most commonly due to information not provided in the submitted pathology report), prior therapies, impact of other biomarkers not assessed by Strata testing, and specific indications requiring combination with non-targeted therapies. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical info and should not be based solely on the information contained in the Strata Oncology Testing Summary.

No Warranty or Guarantee of Clinical Benefit

This Summary does not make any promise or guarantee that a particular therapy or clinical trial will be effective or helpful in the treatment of disease in any patient.

Strata Select™

Patient MRN	Patient Name	Birth Date	Sex	Strata Case
---	---, ---	---	Male	---
Client Specimen ID	**Specimen Site**	**Part**	**Date of Collection**	**Date Received**
---	Liver	---	---	---
Client		**Ordering Physician**		
---		---		

Molecular Diagnosis and Tissue Specimen Information

Determined at time of testing

Cancer Type	Tumor Content
Renal Cell Carcinoma	65%
Cancer Subtype	**Surface Area**
Renal Clear Cell Carcinoma	18mm^2

Immunotherapy Response Score (IRS)

The Immunotherapy Response Score for this sample is High (defined as a score ≥54).

IRS High patients treated with anti-PD-(L)1 monotherapy have significantly greater real world progression free survival (rwPFS)* compared to IRS Low patients (>20 months vs <10 months) and better rwPFS compared to their prior line of treatment (>20 months vs <6 months).

Benefit from monotherapy was demonstrated independent of tumor mutation burden (TMB) status and across tumor types, including those already approved for anti-PD-1/L1 monotherapy, tumor types with mono vs combo therapeutic choice and tumor types not previously approved for anti-PD-1/L1 monotherapy.

* Data presented is aggregate and pan-tumor. Real world progression free survival can vary by tumor type, line of therapy, age and gender.

1. Tomlins, S.A., Khazanov, N.A., Bulen, B.J. et al. Development and validation of an integrative pan-solid tumor predictor of PD-1/PD-L1 blockade benefit. Commun Med 3, 14 (2023). https://doi.org/10.1038/s43856-023-00243-7

2. Bulen BJ, Khazanov NA, Hovelson DH, et al. Validation of Immunotherapy Response Score as Predictive of Pan-solid Tumor Anti-PD-1/PD-L1 Benefit. Cancer Res Commun 3, 7 (2023) https://doi.org/10.1158/2767-9764.CRC-23-0036

Genomic Signatures

Biomarker	Biomarker
MSS	TMB - Low
Microsatellite Stable	Mutations per MB: 2

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Prioritized Comprehensive Genomic Profiling Results

Biomarker	Biomarker
PBRM1 p.F379Lfs*25	VHL exon 1 splice donor mutation
NM_018313.4:c.1134del	NM_000551.3:c.340+2_340+6de...
Estimated variant allele frequency: 56%	Estimated variant allele frequency: 47%

Notes

- The specimen met all input requirements.

Strata Select™

Remaining Negative Comprehensive Genomic Profiling Results

The patient tested negative for all targeted genomic alterations in the following genes:

Prioritized hotspot mutations

AKT1	ALK	AR	ARAF	BRAF	CDK4	CTNNB1	EGFR	ERBB2	ERBB3	ERBB4
ESR1	EZH2	FGFR2	FGFR3	GNA11	GNAQ	HRAS	IDH1	JAK1	JAK2	JAK3
KIT	KRAS	MAP2K1	MAP2K2	MAP2K4	MAP2K7	MAPK1	MET	MTOR	MYD88	NRAS
NTRK1	PDGFRA	PIK3CA	POLE	RAF1	RET	RIT1	ROS1	SF3B1	SMO	SPOP
TERT										

Prioritized hotspot mutations or deleterious mutations

ATM	BRCA1	BRCA2	CDKN2A	MSH2	MSH6	PTEN	RB1	TP53		

Gene amplifications

AKT1	AKT2	AKT3	ALK	AR	AURKA	AXL	BRAF	CCND1	CCND2	CCNE1
CDK4	CDK6	CDK8	CRKL	DEK	DNMT3A	EGFR	ERBB2	ERBB3	ERBB4	ESR1
FGFR1	FGFR2	FGFR3	FGFR4	FOXP4	HIF1A	HRAS	IGF1R	IL6ST	KDR	KIT
KRAS	MAP2K1	MAPK1	MCL1	MDM2	MDM4	MET	MYB	MYC	MYCL	MYCN
NRAS	NTRK1	NTRK3	PDGFRA	PDGFRB	PIK3C2B	PIK3CA	PIK3CB	PPARG	RAF1	RET
ROS1	UBR5									

Deep gene deletions

APC	ARID1A	ARID2	ATM	ATR	ATRX	BAP1	BCL10	BCL11B	BLM	BMPR1A
BRCA1	BRCA2	BRIP1	CDC73	CDH1	CDK12	CDKN2A	CDKN2B	CDKN2C	CEBPA	CHEK1
CHEK2	CIC	CTNNA1	CYLD	DAXX	DCC	DICER1	ERCC2	ERCC3	ERCC4	FAM123B
FANCA	FANCC	FANCD2	FANCF	FANCG	FAS	FBXW7	FH	FLCN	FOXO1	KDM5C
KDM6A	KEAP1	LRP1B	MAP3K7	MEN1	MLH1	MLL	MLL2	MLL3	MRE11A	MSH2
MSH6	MUTYH	NBN	NF1	NF2	NOTCH1	NOTCH2	NOTCH4	NSD1	PALB2	PARP1
PBRM1	PHOX2B	PIK3R1	PIK3R2	PMS1	PMS2	POT1	PPP2R1A	PRDM1	PTEN	PTPRD
PTPRT	RAD50	RB1	RUNX1	SDHA	SDHB	SDHC	SDHD	SETD2	SH2D1A	SMAD2
SMAD4	SMARCA4	SMARCB1	STK11	SUFU	TCF3	TCF7L2	TET1	TET2	TGFBR2	TNFAIP3
TNFRSF14	TOP1	TP53	TSC1	TSC2	VHL	WRN	WT1	XRCC2		

Gene fusions

AKT2	ALK	AXL	BRAF	COL6A3	CSF1	DDIT3	EGFR	ERBB2	ERBB4	ERG
ESR1	ETV1	ETV4	ETV5	EWSR1	FGFR1	FGFR2	FGFR3	FGR	FLI1	FLT3
FN1	FOXO1	FUS	JAK2	KRAS	MET	MYB	MYBL1	NCOA1	NCOA2	NF1
NOTCH1	NOTCH4	NR4A3	NRG1	NTRK1	NTRK2	NTRK3	NUTM1	PDGFB	PDGFRA	PDGFRB
PIK3CA	PPARG	PRKACA	PRKACB	PTEN	RAD51B	RAF1	RELA	RET	ROS1	RSPO2
RSPO3	S100A10	SSX1	SSX4	STAT6	TERT	TFE3	USP6	WWTR1	YWHAE	YY1

No Calls

98.6% of tested positions yielded > 50x coverage. Specific positions not meeting usual coverage requirements are available upon request.

Variants of Unknown Significance

Non-synonymous, likely somatic

BAP1 | p.L14F

NM_004656.3

c.40C>T | 45% VAF

IRS2 | p.A663G

NM_003749.2

c.1988C>G | 32% VAF

NF2 | p.L505M

NM_000268.3

c.1513C>A | 33% VAF

These variants are included in case they are meaningful now or in the future. VAF values marked with asterisks are likely subclonal.

Strata Select™

• DNA Genes

Prioritized mutations and high-confidence somatic variants of unknown significance are reported for the following 417 genes from a wide panel and a high depth (genes indicated in bold) panel. Copy number alterations (amplifications for oncogenes and deep deletions for tumor suppressors) are reported for underlined genes. Genes indicated with an asterisk have targeted coverage for reporting of pre-specified hotspot mutations from the high depth panel only.

ABL1	BCL6	CDKN2B	EPHA3	FLI1	IGF2R	LTK	MTR	PALB2	PRDM1	SETD2	TGM7
ABL2	BCL9	CDKN2C	EPHA7	FLT1	IKBKB	MAF	MTRR	PARP1	PRKAR1A	**SF3B1**	THBS1
ACVR2A	BCR	CEBPA	EPHB1	FLT3	IKBKE	MAFB	MUC1	PAX3	PRKDC	SGK1	TIMP3
ADAMTS20	BIRC2	CHEK1	EPHB4	FLT4	IKZF1	MAGEA1	MUTYH	PAX5	PSIP1	SH2D1A	TLR4
AFF1	BIRC3	CHEK2	EPHB6	FN1	IL2	MAGI1	MYB	PAX7	PTCH1	SMAD2	TLX1
AFF3	BIRC5	CIC	**ERBB2**	FOXL2	IL21R	MALT1	**MYC**	PAX8	**PTEN**	SMAD4	TNFAIP3
AKAP9	BLM	CKS1B	**ERBB3**	FOXO1	IL6ST	MAML2	MYCL	PBRM1	PTGS2	SMARCA4	TNFRSF14
AKT1	BLNK	CMPK1	**ERBB4**	FOXO3	IL7R	**MAP2K1**	**MYCN**	PBX1	PTPN11	SMARCB1	TNK2
AKT2	BMPR1A	COL1A1	ERCC1	FOXP1	ING4	**MAP2K2**	**MYD88**	PDE4DIP	PTPRD	**SMO**	TOP1
AKT3	**BRAF**	CRBN	ERCC2	FOXP4	IRF4	**MAP2K4**	MYH11	PDGFB	PTPRT	SMUG1	**TP53**
ALK	**BRCA1**	CREB1	ERCC3	FZR1	IRS2	**MAP2K7***	MYH9	**PDGFRA**	RAD50	SOCS1	TPR
APC	**BRCA2**	CREBBP	ERCC4	G6PD	ITGA10	MAP3K7	NBN	PDGFRB	**RAF1**	SOX11	TRIM24
AR	BRD3	CRKL	ERCC5	GATA1	ITGA9	**MAPK1**	NCOA1	PER1	RALGDS	SOX2	TRIM33
ARAF*	BRIP1	CRTC1	ERG	GATA2	ITGB2	MAPK8	NCOA2	PGAP3	RARA	**SPOP***	TRIP11
ARID1A	BTK	CSF1R	**ESR1**	GATA3	ITGB3	MARK1	NCOA4	PHOX2B	**RB1**	SRC	TRRAP
ARID2	BUB1B	CSMD3	ETS1	GDNF	**JAK1**	MARK4	NF1	PIK3C2B	RECQL4	SSX1	TSC1
ARNT	CARD11	CTNNA1	ETV1	**GNA11**	**JAK2**	MBD1	NF2	**PIK3CA**	REL	STK11	TSC2
ASXL1	CASC5	**CTNNB1**	ETV4	**GNAQ**	**JAK3**	MCL1	NFE2L2	PIK3CB	**RET**	STK36	TSHR
ATF1	CBL	CYLD	EXT1	GNAS	JUN	**MDM2**	NFKB1	PIK3CD	RHOH	SUFU	UBR5
ATM	**CCND1**	CYP2C19	EXT2	GPR124	KAT6A	MDM4	NFKB2	PIK3CG	**RIT1***	SYK	UGT1A1
ATR	CCND2	CYP2D6	**EZH2**	GRM8	KAT6B	MEN1	NIN	PIK3R1	RNASEL	SYNE1	USP9X
ATRX	CCNE1	DAXX	FAM123B	GUCY1A2	KDM5C	**MET**	NKX2-1	PIK3R2	RNF2	TAF1	VHL
AURKA	CD79A	DCC	FANCA	HCAR1	KDM6A	MITF	NLRP1	PIM1	RNF213	TAF1L	WAS
AURKB	CD79B	DDB2	FANCC	HIF1A	KDR	MLH1	NOTCH1	PKHD1	**ROS1**	TAL1	WHSC1
AURKC	CDC73	DDIT3	FANCD2	HLF	KEAP1	MLL	NOTCH2	PLAG1	RPS6KA2	TBX22	WRN
AXL	CDH1	DDR2	FANCF	HNF1A	**KIT**	MLL2	NOTCH4	PLCG1	RRM1	TCF12	WT1
BAI3	CDH11	DEK	FANCG	HOOK3	KLF6	MLL3	NPM1	PLEKHG5	RUNX1	TCF3	XPA
BAP1	CDH2	DICER1	FAS	**HRAS**	**KRAS**	MLLT10	**NRAS**	PML	RUNX1T1	TCF7L1	XPC
BCL10	CDH20	DNMT3A	FBXW7	HSP90AA1	LAMP1	MMP2	NSD1	PMS1	SAMD9	TCF7L2	XPO1
BCL11A	CDH5	DPYD	**FGFR1**	HSP90AB1	LCK	MN1	**NTRK1**	PMS2	SBDS	TCL1A	XRCC2
BCL11B	CDK12	DST	**FGFR2**	ICK	LIFR	MPL	**NTRK3**	**POLE***	SDHA	**TERT***	ZNF384
BCL2	**CDK4**	**EGFR**	**FGFR3**	**IDH1**	LPHN3	MRE11A	NUMA1	POT1	SDHB	TET1	ZNF521
BCL2L1	**CDK6**	EML4	**FGFR4**	**IDH2**	LPP	**MSH2**	NUP214	POU5F1	SDHC	TET2	
BCL2L2	CDK8	EP300	FH	**IGF1R**	LRP1B	**MSH6**	NUP98	PPARG	SDHD	TFE3	
BCL3	**CDKN2A**	EP400	FLCN	IGF2	LTF	**MTOR**	PAK3	PPP2R1A	SEPT9	TGFBR2	

• RNA Genes

950 individual fusion isoforms involving the following 59 driver genes are targeted; additional fusion isoforms amplified from non pre-specified 5' and 3' primer pairs are also reportable.

AKT2	COL1A1	ERG	EWSR1	FLT3	MYB	NR4A3	NUTM1	PIK3CA	PTEN	ROS1	STAT6
ALK	CSF1	ESR1	FGFR1	FUS	MYBL1	NRG1	PAX3	PLAG1	RAD51B	RSPO2	TERT
AXL	EGFR	ETV1	FGFR2	JAK2	NF1	NTRK1	PAX7	PPARG	RAF1	RSPO3	TFE3
BRAF	ERBB2	ETV4	FGFR3	KRAS	NOTCH1	NTRK2	PDGFRA	PRKACA	RELA	SSX1	YWHAE
CAMTA1	ERBB4	ETV5	FGR	MET	NOTCH4	NTRK3	PDGFRB	PRKACB	RET	SSX4	

Strata Select™

Clinical Performance

The Strata Select test was developed and the performance characteristics determined by Strata Oncology. The test has neither been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has deemed that such clearance or approval is not necessary. Strata Oncology is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical laboratory testing. The Laboratory Director is Scott A. Tomlins, M.D., Ph.D

Strata Select Test Description

The Strata Select test is a 437 gene, pan-solid tumor, next generation sequencing (NGS) assay for FFPE tumor tissue, performed on co-isolated DNA and RNA[1,2]. In addition to simultaneous assessment of single-/multi-nucleotide variants (SNVs), splice site variants (SSVs), short (<40bp) insertions and deletions (indels), copy number alterations (CNAs; amplifications or deep deletions), microsatellite instability (MSI) status, gene fusions, and tumor mutation burden (TMB), Strata Select also reports an integrated Immunotherapy Response Score (IRS) by a Cox proportional hazard (CPH) model incorporating TMB with gene expression of four tumor/tumor microenvironment biomarkers (PD-L1 [CD274], PD-1 [PDCD1], ADAM12 and TOP2A; each normalized to the median of three housekeeping genes [HMBS, CIAO1 and EIF2B1]). Prioritized mutations, which include pre-defined mutations (activating SNVs, indels and SSVs for oncogenes; hot spot, loss of function SNVs, frame-shifting indels, and cancer-related pathogenic SNVs/SSVs for tumor suppressors) are reported separately from all other high-confidence somatic variants of unknown significance. Genes and alteration types assessed by the Strata Select test are shown in the DNA Genes and RNA Gene sections. The Strata Select test covers nearly the entire coding sequence for mutation reporting in 411 genes (median 96.1% coverage, IQR 90.7%-99.1%) with targeted coverage of 6 oncogenes; the specific regions not covered are available upon request. A negative result does not indicate that a gene is negative for any possible alteration, only the specific alterations assayed by the Strata Select test. The variant allele frequency (VAF) limit of detection for oncogenic mutations (SNVs or frame-preserving indels), other SNVs, indels, splice site variants, and single base indels at homopolymers from the bioinformatic pipeline are <1.1%-3.7%, 7.5%-8.3%, 4.7%-5.7%, 4.1%-5.4%, and 11.8%-14.9%, respectively. Orthogonally validated indels up to 15 bases were detected in the Strata Select validation, and well supported indels up to 82 bases have been detected and reported by Strata Select testing, however indel detection is impacted by the location in the covering amplicon(s). For gene fusions, only specific gene fusion partners are assayed for (e.g. EML4-ALK) so novel gene fusion partners will not be detected unless 5' and 3' primers are included in Strata Select. For copy number events, the cellularity adjusted whole copy number estimate is thresholded at 6 copies and the 90% confidence interval lower bound is thresholded at 5 copies for calling amplifications in known oncogenes. The copy number estimate is thresholded at 0.5 copies and the 90% confidence interval upper bound is thresholded at 1 copy for calling deep deletions (homozygous deletions, assuming diploid state) in known tumor suppressor genes. Estimated tumor content, variant allele frequencies, and copy number (with associated 90% confidence interval) are reported for informational purposes only. MSI status is determined by thresholding an MSI score derived from length variant allele counts observed at multiple microsatellite loci; specimens with an MSI score greater than 0.5 are considered microsatellite instability high (MSI-High). Tumor mutation burden includes non-coding (at highly characterized genomic loci) and coding, synonymous and non-synonymous, single and multi-nucleotide (two bases) variants present at variant allele frequency (VAF) > ¼ of molecularly-informed tumor content (TC); mutation rate per megabase (Mb) estimate and associated 90% confidence interval are calculated via the total number of positions with sufficient depth of coverage necessary for definitive assessment (maximum possible 1.7Mb). Quantitative TMB results are reported, along with TMB status (TMB-Low: <10 mutations per Mb; TMB-High: ≥10+ mutations per Mb). A complete list of all prioritized genomic variants and transcript annotations is available upon request. The tumor content (determined at the time of molecular review and signout) limit of detection for all Strata Select variant classes is ≥20%.

Strata Select assays for mutations in tumor tissue only, and therefore does not distinguish between somatic and germline mutations. Suspected germline variants with cancer relevance are highlighted and described in a "Germline Note" beneath the Pertinent Biomarker Summary in the Strata Oncology Testing Summary based on the Intermediate-permissive approach described by the ESMO Precision Medicine Working Group (PMID: 36529447), which recommend germline follow-up pan-tumor for Most/High-Actionable cancer susceptibility genes (CTNNA1 and NBN are considered High-Actionable by Strata Select) and germline follow-up on-tumor for Standard-Actionable genes. Per ESMO, germline analysis/follow-up for variants in APC, PTEN, RB1, TP53, CDKN2A and SMARCA4 should be restricted to tumors arising in those <30 years, and variants in these genes are not highlighted in the "Germline Note" but should be considered for analysis/follow-up as clinically indicated. Inclusion in "Germline Note" indicates recommended follow-up

analysis and/or confirmatory testing as clinically indicated, NOT that the alteration is definitively germline. Variants reported elsewhere may be true germline variants (including sub-gene deep deletions), but do not meet inclusion criteria for this Section; Strata Select testing is not inclusive of all known hereditary cancer genes and should not replace comprehensive genetic testing when clinically indicated.

Like TMB alone, IRS provides prognostic and predictive information to help inform pan-solid tumor systemic treatment decision making for PD-1 or PD-L1 (together PD-[L]1) monotherapy[3]. Feature selection for the IRS algorithm was performed by lasso-penalized CPH to predict time to next therapy (TTNT; a validated real world progression free survival treatment endpoint[4,5]) in 648 anti-PD-1 (pembrolizumab) treated patients from 26 tumor types. Individual sample IRS is reported by the following model, with IRS ≥0.873 (displayed on 0-100 scale as ≥54) reported as IRS High, and IRS <0.873 (displayed on 0-100 scale as <54) reported as IRS Low: IRS = 0.273758 * TMB + 0.112641 * PD-1 + 0.061904 * PD-L1 - 0.077011 * TOP2A - 0.057991 * ADAM12.

In the independent 248 patient (24 tumor types) validation cohort of patients treated with non-pembrolizumab PD-1 (n=194 [78%]) or PD-L1 (n=54 [22%]) monotherapy, IRS High vs. IRS Low patients had significantly longer TTNT (median 23.1 vs. 10.2 months; adjusted hazard ratio [aHR] 0.52, p=0.003) and overall survival (OS; median 40.4 vs. 21.4 months; aHR 0.49, p=0.005) when adjusted for age, gender, tumor type, PD-1/PD-L1 therapy, and line of therapy. Neither median TTNT nor OS significantly differed between IRS High/TMB High vs. IRS High/TMB Low patients (TTNT 21.0 vs. 28.2 months, p=0.31; median OS 40.4 months vs. Not Reached, p=0.53), confirming performance of IRS in both TMB High and TMB Low patients. Across 3,184 patients (23 tumor types) treated with a non-PD-(L)1 or CTLA4 containing first line systemic therapy, IRS status was not a significant predictor of TTNT (aHR 1.05, p=0.45), confirming the predictive nature of IRS.

In a second 352 patient anti-PD-(L)1 monotherapy validation cohort (31 tumor types; 82% treated with pembrolizumab), IRS-High vs. IRS-Low patients had significantly longer rwPFS (median 15.1 vs. 3.8 months, aHR 0.41, p<0.0001) and OS (41.0 vs. 11.7 months, aHR 0.47, p=0.0002). IRS significantly improved CPH associations with rwPFS and OS beyond microsatellite instability (MSI)/TMB status alone. In a 189 patient (10 tumor types) PD-L1 IHC comparison cohort, IRS, but not PD-L1 IHC nor TMB, was significantly associated with anti-PD-L1 rwPFS. In a 1,229 treatment-line cohort (from five relevant tumor types), rwPFS did not significantly differ in IRS-High patients treated with anti-PD-(L)1 vs. anti-PD-(L)1 + chemotherapy[6]. IRS associations were consistent across subgroups, including both Europeans and non-Europeans. IRS has been subsequently validated in additional studies under peer review.

IRS is not valid nor reported in samples failing either the TMB or gene expression component QC metrics, or in samples with <20% tumor content. IRS is not valid in patients who have already received anti-PD-(L)1 or CTLA4 therapy. We recommend submission of a current tissue specimen for the most relevant Strata Select results, particularly if the patient has been treated with targeted and/or hormonal therapy, or the tumor is suspected to have undergone transformation to small cell carcinoma.

Treatment Decisions

The selection of any treatment or clinical trial suggested by a biomarker resides within the discretion and judgment of the treating physician and patient. In the Strata Oncology Testing Summary, Strata Select identified biomarkers and relevant treatment associations are shown and described in the 'Therapy Associations' section based on the final reported tumor type using the following levels of evidence as determined by Strata: Level 1, FDA-recognized predictive biomarker in this indication; Level 2, NCCN or other professional guideline-recognized predictive biomarker in this indication; Level 3, Compelling predictive clinical evidence in this indication; Level 4, Compelling predictive biologic evidence in this indication. The biomarker associated benefit prediction ("Benefit", "Less Benefit", "Not indicated") is shown, with "Less Benefit" indicating less predicted clinical benefit compared to unselected patients. For tumor types where PD-(L)1 monotherapy is considered Level 1 or 2 evidence in the absence of a biomarker (or by PD-L1 immunohistochemistry), that level of evidence is maintained if the combination of MSI/TMB/IRS supports PD-(L)1 monotherapy benefit. Line of therapy (first, second, later, maintenance) are shown by superscript (* indicates a NCCN preferred therapy in that line). Listed treatments and levels of evidence may not be applicable to the patient's specific cancer based on incorrectly reported tumor type (most commonly due to information not provided in the submitted pathology report), prior therapies, impact of other biomarkers not assessed by Strata testing, and specific indications requiring combination with non-targeted therapies. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical info and should not be based solely on the individual Strata tests and information contained in the Strata Oncology Testing Summary.

Strata Select™

No Warranty or Guarantee of Clinical Benefit

This report does not make any promise or guarantee that a particular therapy or clinical trial will be effective or helpful in the treatment of disease in any patient.

References

1. Tomlins SA, Hovelson DH, Harms P, et al: Development and Validation of StrataNGS, a Multiplex PCR, Semiconductor Sequencing-Based Comprehensive Genomic Profiling Test. J Mol Diagn 23:1515-1533, 2021
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3. Tomlins SA, Khazanov N, Bulen BJ, et al: Development and Validation of an Integrative Pan-Solid Tumor Predictor of PD-1/PD-L1 Blockade Benefit. Communications Medicine, 7;3(1):14, 2023
4. Kehl KL, Riely GJ, Lepisto EM, et al: Correlation Between Surrogate End Points and Overall Survival in a Multi-institutional Clinicogenomic Cohort of Patients With Non-Small Cell Lung or Colorectal Cancer. JAMA Netw Open 4:e2117547, 2021
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Test Version

Laboratory workflow version: 4

Bioinformatics pipelines version: 4.10

CPT code: 81479

Test code: Z01Z0

Strata RNA Supplement

Patient MRN	Patient Name	Birth Date	Sex	Strata Case
---	---, ---	---	Male	---
Client Specimen ID	**Specimen Site**	**Part**	**Date of Collection**	**Date Received**
---	Liver	---	---	---
Client		**Ordering Physician**		
---		---		

Molecular Diagnosis and Tissue Specimen Information

Determined at time of testing

Cancer Type	Tumor Content
Renal Cell Carcinoma	65%
Cancer Subtype	**Surface Area**
Renal Clear Cell Carcinoma	18mm^2

Antibody Drug Conjugate Treatment Response Scores

Biomarker	Biomarker	Biomarker
FOLR1 TRS Low: 75	LIV-1 TRS Low: 46	Nectin-4 TRS Low: 23
HER2 TRS Low: 44	MET TRS High: 90	TF TRS Low: 28
HER3 TRS Low: 58	NaPi2b TRS High: 87	Trop2 TRS Low: 25

Angiogenesis Inhibitor Treatment Response Score

Angio TRS Low: 89

Gene Expression Biomarker Results

Biomarker	Biomarker	Biomarker	Biomarker
AR Low: 7.9 Percentile rank: 21.0%	EPHA2 Low: 8.4 Percentile rank: 21.2%	EPAS1 (HIF2A) High: 10.5 Percentile rank: 70.2%	CTAG1B (NY-ESO-1) Low: 3.3 Percentile rank: 24.0%
AXL High: 11.8 Percentile rank: 92.7%	ESR1 (ER) Negative: 9.9 Percentile rank: 48.8%	IL2RA High: 10.4 Percentile rank: 60.6%	SLC34A2 (NaPi2b) Low: 12.9 Percentile rank: 71.4%
NCAM1 (CD56) Low: 9.8 Percentile rank: 68.5%	FGF19 Low: 2.1 Percentile rank: 17.0%	LAG3 High: 13.6 Percentile rank: 97.9%	NECTIN4 (Nectin-4) Low: 0 Percentile rank: 4.7%
CLDN18.2 Low: 1.3 Percentile rank: 18.0%	FOLR1 Low: 9.7 Percentile rank: 51.6%	LY75 (CD205) Low: 9.6 Percentile rank: 42.2%	PGR (PR) Negative: 8.2 Percentile rank: 24.0%
DKK1 Low: 7.5 Percentile rank: 24.5%	GUCY2C Low: 2.6 Percentile rank: 1.0%	MET High: 11.2 Percentile rank: 73.8%	
DLL3 Low: 7.6 Percentile rank: 62.9%	ERBB2 (HER2) Negative: 8.3 Percentile rank: 13.9%		

Strata RNA Supplement

FOLH1 (PSMA) Low: 12.0 Percentile rank: 83.8%	ROR2 High: 10.4 Percentile rank: 58.7%	TIGIT High: 12.9 Percentile rank: 95.5%	Biomarker TACSTD2 (Trop2) Low: 0.9 Percentile rank: 2.3%

Additional Gene Expression Results

Biomarker	Biomarker	Biomarker
ALPG: 8.6 Pan-tumor percentile rank: 72.9%	FGFR2: 10.0 Pan-tumor percentile rank: 51.2%	Proliferation score: 9.6 Pan-tumor percentile rank: 38.5%
EGFR: 10.5 Pan-tumor percentile rank: 63.0%	FGFR3: 8.6 Pan-tumor percentile rank: 30.5%	
FGFR1: 10.1 Pan-tumor percentile rank: 51.7%	IGF1R: 9.5 Pan-tumor percentile rank: 33.4%	

Notes

- The specimen met all input requirements.

Strata RNA Supplement

Rationale

The Strata RNA Supplement is a laboratory-developed test performed in the Strata Oncology CLIA-certified laboratory. Selected gene expression results are reported from an analytically and clinically validated gene expression panel. The reported thresholds for determining low vs. high expression of each biomarker, as well as associations with IHC, have not been prospectively validated nor prospectively shown to associate with benefit or lack of benefit from any listed associated therapy. Clinical utility has not been established for this test. These test results are provided as they may be useful for correlating with treatment response to approved or investigational therapies, as well as other clinical, molecular, or pathological findings. Biomarker results may also confer eligibility for clinical trials.

Clinical Performance

The Strata RNA supplement test was developed and the performance characteristics determined by Strata Oncology. The test has neither been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has deemed that such clearance or approval is not necessary. Strata Oncology is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical laboratory testing. The Laboratory Director is Scott A. Tomlins, M.D., Ph.D

Strata RNA Supplement Test Description

The Strata RNA supplement generates quantitative gene expression data by multiplex PCR based next generation sequencing (NGS) in parallel with the RNA component of Strata Select testing (for gene fusions and Immunotherapy Response Score gene expression components)1. The current version of Strata RNA supplement reports 31 individual target gene biomarkers and 11 composite gene expression biomarkers (9 antibody drug conjugate (ADC) Treatment Response Scores, Angio Treatment Response Score and Proliferation Score).

Strata RNA supplement accuracy was validated by an expression survey of all individually reported targets, individual target components of composite biomarkers, and composite biomarkers vs. a reference set of pan-solid tumor gene expression, as well as by representative validation vs. quantitative reverse transcription PCR (qRT-PCR) and/or immunohistochemistry (IHC).

Individual target expression scores are determined in log2 units, representing target gene expression (normalized to the median expression of three pan-solid tumor stable housekeeping genes [HMBS, CIAO1 and EIF2B1]), then scaled to the distribution observed in a common control (in normalized reads per million [nRPM]), and quantile normalized per fixed, RNA panel/laboratory workflow. All individual targets and composite biomarkers are scaled such that the median score is 10.0 across the observed pan solid tumor range (>30,000 tumors at the time of database lock), with each unit increase representing a doubling from the median. In addition to an expression score, the pan-solid tumor percentile rank is also reported. Angio (multiple angiogenesis related target genes), proliferation (TOP2A and UBE2C) and HER2 (two distinct HER2 amplicons) composite biomarkers are determined by the average of the individual, quantile normalized, target gene values.The tumor content limit of detection for all individually reported and composite gene expression biomarkers is 20%.

In two separate validation cohorts of 141 and 218 evaluable breast cancer tissue samples, pre-specified Strata RNA supplement ER Positive (>13.684) vs. Negative (<11.934) status had 98.0% & 98.8% sensitivity and 100% & 98.2% specificity vs. ER status by clinical IHC (ER IHC >10% as Positive and IHC 0% as Negative; IHC 1-10% were excluded); samples with ER expression 11.934-13.684 are reported as ER Low and are considered inconclusive for ER (and hormone receptor) status and therapy associations, with deferral to clinical IHC in such cases recommended. In two separate validation cohorts of 122 and 174 evaluable breast cancer tissue samples, pre-specified Strata RNA supplement PR gene expression Positive (>13.2395) vs. Negative (<12.0) status had 95.7% & 93.4% sensitivity and 100% & 96.9% specificity vs. PR status by clinical IHC (PR IHC >10% as Positive and PR IHC 0% as Negative; PR IHC 1-10% were excluded); samples with PR expression 12.0-13.2395 are reported as PR Low and are considered inconclusive for PR status, with deferral to clinical IHC in such cases recommended.

Four HER2 categories are reported by the Strata RNA supplement: Negative (score ≤10.5935; breast 0-1+ IHC equivalent), Low (score 10.5935 to <12.50431; 1-2+ IHC equivalent), Moderate (score 12.50431-≤13.70431; 2+ IHC equivalent), Positive (score >13.70431; 3+ IHC equivalent). In two separate validation cohorts of 216 and 191 evaluable breast cancer tissue samples, pre-specified Strata

Strata RNA Supplement

RNA supplement HER2 gene expression Positive vs. Negative or Low status had 80.0% & 93.8% sensitivity and 100.0% & 100.0% specificity vs. HER2 status by clinical IHC (HER2 IHC 3+ as Positive and IHC 0-1+ as Negative; IHC 2+ were excluded). HER2 amplification status (by Strata Select) is used to make first line HER2 targeted therapy associations in Strata RNA supplement HER2 Moderate samples. Given the later line association of trastuzumab deruxtecan in HER2 IHC 1+ and 2+ (FISH/ISH negative) breast cancer, and the poor reproducibility of IHC distinction between 0+ and 1+ in routine practice2, the Strata RNA supplement HER2 Negative vs. Low expression threshold was set at the lower 25th percentile of IHC 1-2+ (non-ERBB2 amplified) samples in a 139 sample training cohort (where 39% of 0+ IHC samples were above this threshold) and applied to the 0+ IHC samples (n=124 and 91) in the two validation cohorts. In the two validation cohorts, 34% and 24% of 0+ IHC samples were above the Strata RNA supplement HER2 Negative threshold.

Antibody Drug Conjugate Treatment Response Scores for FOLR1, HER2, HER3, LIV-1, c-Met, NaPi2b, Nectin-4, TF, and TROP-2 are RNA expression based biomarkers integrating ADC target, proliferation, and cell adhesion. The TRS model better predicted objective response rates (ORR) across ADCs and tumor types than RNA expression of ADC target alone in a training cohort (n=16 observed ORRs [from 7 tumor types and 8 ADCs]) and a validation cohort (n=11 observed ORRs [from 9 tumor types and 3 ADCs]).

The Angiogenesis Treatment Response Score is a multi-gene expression algorithm that integrates multiple angiogenesis related target genes chosen based on co-expression patterns across VEGFR TKI responsive and resistant tumor types. The High/Low threshold (11.613), was set at the 42nd percentile of Strata tested clear cell renal cell carcinomas. In a validation cohort of 86 renal cell carcinomas (66 clear cell and 20 non-clear cell) treated with single agent VEGFR TKI systemic therapy, Angio High samples had significantly longer real-world progression free survival (rwPFS; by time to next therapy) vs. Angio Low samples when controlling for patient age, gender, line of systemic therapy, clear cell vs. non-clear cell histology, ISUP/WHO nuclear grade (incorporating sarcomatoid features), and TKI type (median rwPFS 15.8 vs. 5.6 months, adjusted hazard ratio 0.46, p=0.012).

In a validation cohort of 39 evaluate evaluable high grade serous ovarian/fallopian tube/primary peritoneal cancer tissue samples, pre-specified StrataEXP FOLR1 High (>=15.5) vs. StrataEXP FOLR1 Low (<15.5) status had 96.4% sensitivity and 100% specificity vs. the mirvetuximab soravtansine companion diagnostic FOLR1 IHC assay (VENTANA FOLR1 RxDx Assay; RxDx IHC >=75% 2-3+ positive as Positive and <50% 2-3+ positive as Negative; IHC 50-70% 2-3+ samples were excluded based on poor repeatability and reproducibility of this group in the FDA validation (https://www.accessdata.fda.gov/cdrh_docs/pdf22/P220006B.pdf). Of the 9 RxDx IHC 50-70% 2-3+ positive samples, 8 were StrataEXP FOLR1 High.

For remaining individual expression biomarkers, high/low biomarker thresholds were set based on pan-solid tumor or specific tumor type expression distributions observed in Strata RNA supplement tested tumor samples. Some biomarkers are reported without a threshold, and are equivalent to genomic variants of uncertain significance reported by Strata Select. Biomarker results may also confer eligibility for clinical trials. Strata RNA supplement results are provided as they may be useful for correlating with treatment response to associated or non-associated therapies or other clinical, molecular, or pathological findings. No Strata RNA supplement reported individual or composite biomarker thresholds have been shown to prospectively associate with benefit or lack of benefit from any therapy. When ordered as part of a Strata Oncology sponsored clinical trial or used to facilitate enrollment into a Strata Oncology sponsored clinical trial, and where applicable pursuant to the trial protocol, this test shall be considered subject to the following disclaimer: For Investigational Use Only. The Performance Characteristics of this Product Have Not Been Established.

No Warranty or Guarantee of Clinical Benefit

This Summary does not make any promise or guarantee that a particular therapy or clinical trial will be effective or helpful in the treatment of disease in any patient.

Treatment Decisions

The selection of any treatment or clinical trial associated with a biomarker resides within the discretion and judgment of the treating physician and patient. In the Strata Oncology Testing Summary, Strata RNA supplement identified biomarkers and relevant treatment associations are shown and described in the 'Therapy Associations' section based on the final reported tumor type using the following levels of evidence as determined by Strata: Level 1, FDA-recognized predictive biomarker in this indication; Level 2, NCCN or other professional guideline-recognized predictive biomarker in this indication; Level 3, Compelling predictive clinical evidence in this

Strata RNA Supplement

indication; Level 4, Compelling predictive biologic evidence in this indication. The biomarker associated benefit prediction (“Benefit”, “Less Benefit”, “Not indicated”) is shown, with “Less Benefit” indicating less predicted clinical benefit compared to unselected patients. Line of therapy (first, second, later, maintenance) are shown by superscript (* indicates a NCCN preferred therapy in that line). Listed treatments and levels of evidence may not be applicable to the patient’s specific cancer based on incorrectly reported tumor type (most commonly due to information not provided in the submitted pathology report), prior therapies, impact of other biomarkers not assessed by Strata testing, and specific indications requiring combination with non-targeted therapies. Whether expression profiles of tumors are maintained after progression or therapy is unclear, and we recommend submission of a current tissue specimen for the most relevant Strata RNA supplement results. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical info and should not be based solely on the individual Strata tests and information contained in the Strata Oncology Testing Summary.

References

1. 1. Tomlins SA, Khazanov N, Bulen BJ, et al: Development and Validation of an Integrative Pan-Solid Tumor Predictor of PD-1/PD-L1 Blockade Benefit. Communications Medicine, 7;3(1):14, 2023
2. 2. Fernandez AI, Liu M, Bellizzi A, et al: Examination of Low ERBB2 Protein Expression in Breast Cancer Tissue. JAMA Oncol 8(4): 1-4, 2022

Test Version

Laboratory workflow version: 4

Bioinformatics pipelines version: 4.10