PeptideXme Patient Report

pX_0001

Patient	HCC-2025-0042
Diagnosis	Hepatocellular Carcinoma (HCC)
Stage	Stage IIIB (BCLC C)
Report Date	2025-09-04
Institution	Massachusetts General Hospital — HCC Translational Lab
Sample Type	FFPE tumor tissue + matched blood
Turnaround	26 days

FOR RESEARCH USE ONLY — Not for use in diagnostic procedures

Executive Summary

147	72	38	10
TOTAL CANDIDATES	AS-DERIVED	MUTATION-DERIVED	MS-VALIDATED

Top Neoantigen: SLADTNSL

Source: Alternative splicing (SE event in TTN) | HLA: HLA-A*02:01 | IC50: 23.4 nM | Immunogenicity: 0.87

TME Classification

Immune-excluded | TMB: 4.2 muts/Mb (Low) | MSI: MSS (Microsatellite Stable)

Top Recommendation

Personalized peptide vaccine targeting top 15 AS-derived neoepitopes + anti-PD-L1 combination

Genomic Profile

TMB: 4.2 muts/Mb (Low) | MSI: MSS | Total mutations: 52 | Driver mutations: 7

Somatic Mutations

Gene	Variant	VAF	Consequence	Significance	Driver
TP53	c.733C>T (p.R248W)	34.0%	Missense	Pathogenic — dominant negative	True
CTNNB1	c.110T>C (p.S37P)	28.0%	Missense	Pathogenic — Wnt pathway activation	True
TERT	c.-124C>T (promoter)	41.0%	Promoter mutation	Pathogenic — telomerase reactivation	True
AXIN1	c.1582C>T (p.R528*	19.0%	Nonsense	Likely pathogenic — Wnt pathway	True
ARID1A	c.4891_4892del (p.G1631fs)	22.0%	Frameshift	Likely pathogenic — chromatin remodeling	True
ARID2	c.2386C>T (p.Q796*)	16.0%	Nonsense	Likely pathogenic — chromatin remodeling	False
KEAP1	c.607C>T (p.R203*)	31.0%	Nonsense	Pathogenic — oxidative stress response	True
NFE2L2	c.91_92insA (p.E31fs)	18.0%	Frameshift	Likely pathogenic — NRF2 activation	False
TSC2	c.1456C>T (p.R486*)	12.0%	Nonsense	VUS	False
VHL	c.250C>T (p.P84S)	8.0%	Missense	VUS	False

Copy Number Alterations

Gene	Type	CN	Log2
MYC	Amplification	8	2.1
CCND1	Amplification	6	1.5
CDKN2A/B	Homozygous deletion	0	-3.2
PTEN	Heterozygous loss	1	-0.9
FGF19	Amplification	5	1.3

Epigenetic Profile

Global hypomethylation (score: 0.62)

Key Differentially Methylated Regions

Gene	Change	Fold Change	Significance
RASSF1A	Hypermethylated	4.2x	Tumor suppressor silencing
CDKN2A	Hypermethylated	3.8x	Cell cycle checkpoint loss
GSTP1	Hypermethylated	5.1x	Detoxification pathway loss
APC	Hypermethylated	2.9x	Wnt pathway dysregulation

Splice-Regulatory Factor Expression

Gene	Mutation	log2FC	Note
SF3B1	None detected	1.2	Upregulated — may drive aberrant splicing
U2AF1	None detected	0.8	Mildly upregulated
SRSF2	None detected	1.5	Upregulated — correlated with AS-derived neoepitope burden
HNRNPA2B1	None detected	2.1	Strongly upregulated
RBFOX2	None detected	-1.3	Downregulated — loss of splicing fidelity

Transcriptomic Profile

Total AS events: 1284 | Tumor-specific: 342 | Peptide-generating: 72

Top Alternative Splicing Events

ID	Type	Gene	PSI Tumor	PSI Normal	Specificity
AS_001	SE (Skipped Exon)	**TTN**	0.12	0.89	0.95
AS_002	SE (Skipped Exon)	**MAGI2**	0.08	0.92	0.91
AS_003	A3SS (Alt 3' Splice Site)	**FHIT**	0.78	0.15	0.88
AS_004	RI (Retained Intron)	**DAB2**	0.65	0.05	0.93
AS_005	MXE (Mutually Exclusive Exons)	**PKM**	0.82	0.22	0.86
AS_006	SE (Skipped Exon)	**KIF1B**	0.15	0.85	0.89
AS_007	A5SS (Alt 5' Splice Site)	**MACF1**	0.72	0.18	0.87
AS_008	RI (Retained Intron)	**USP47**	0.58	0.08	0.92
AS_009	SE (Skipped Exon)	**FAT1**	0.2	0.88	0.9
AS_010	MXE (Mutually Exclusive Exons)	**CALD1**	0.75	0.25	0.85

Gene Fusions

ID	5' Partner	3' Partner	Reads	Frame	TPM	Peptide
FUS_001	**DNAJB1**	**PRKACA**	847	In-frame	156.3	True
FUS_002	**TFEB**	**CLTC**	312	In-frame	42.7	True
FUS_003	**ALK**	**EML4**	89	Out-of-frame	12.1	False

HLA Typing & Immunopeptidome

HLA Class I Typing

Locus	Allele	Confidence	LOH
HLA-A	**A*02:01**	0.99	False
HLA-A	**A*24:02**	0.98	False
HLA-B	**B*07:02**	0.99	True
HLA-B	**B*44:03**	0.97	False
HLA-C	**C*05:01**	0.98	False
HLA-C	**C*16:01**	0.96	False

HLA-B*07:02 shows loss of heterozygosity (LOH) in tumor — may impair antigen presentation on this allele

Immunopeptidome

Total peptides identified (MS): 8421 | MS-validated neoantigens: 10

Source	Count
Canonical	6892
AS-derived	892
Fusion-derived	47
Non-canonical	590

Neoantigen Candidates

Total: 147 | High confidence: 23 | MS-validated: 10

7a: Mutation-Derived Neoantigens

#	Peptide	Gene	HLA	IC50	Rank%	Imm	TCR	MS	TPM	Clonal
1	**KLDKVNSL**	TP53	HLA-A*02:01	45.2	0.15	0.82	0.71	False	89.3	Trunk
2	**STLPGVIAA**	CTNNB1	HLA-A*24:02	78.3	0.32	0.74	0.58	False	156.7	Trunk
3	**RMPGSPAA**	KEAP1	HLA-B*07:02	112.5	0.45	0.68	0.52	False	234.1	Trunk
4	**VTLNDEAA**	ARID1A	HLA-A*02:01	234.8	0.89	0.61	0.43	False	45.2	Subclonal
5	**YLDPSLTA**	AXIN1	HLA-B*44:03	189.3	0.72	0.65	0.47	False	67.8	Trunk

7b: AS-Derived Neoepitopes (PeptideXme Differentiator)

#	Peptide	Source	Gene	HLA	IC50	Rank%	Imm	TCR	MS	TPM	Clonal
1	**SLADTNSL**	AS-derived (SE)	TTN	HLA-A*02:01	23.4	0.12	0.87	0.78	True	312.5	Trunk
2	**MPEGTACV**	AS-derived (RI)	DAB2	HLA-A*02:01	34.7	0.18	0.84	0.72	True	198.3	Trunk
3	**WQRTYKPL**	AS-derived (SE)	MAGI2	HLA-A*24:02	56.1	0.25	0.79	0.65	True	145.2	Trunk
4	**GHNWAVTL**	AS-derived (A3SS)	FHIT	HLA-A*02:01	67.8	0.31	0.76	0.61	False	89.7	Subclonal
5	**KPFVYVLA**	AS-derived (MXE)	PKM	HLA-B*07:02	89.2	0.38	0.72	0.55	False	567.4	Trunk
6	**TNSLADTN**	AS-derived (SE)	KIF1B	HLA-A*02:01	112.3	0.44	0.69	0.51	False	78.9	Subclonal
7	**QVLPAGVA**	AS-derived (A5SS)	MACF1	HLA-A*24:02	134.5	0.52	0.66	0.48	False	123.4	Trunk
8	**HRLPAGSI**	AS-derived (RI)	USP47	HLA-A*02:01	156.7	0.61	0.63	0.44	False	56.2	Subclonal
9	**DLFSGTNV**	AS-derived (SE)	FAT1	HLA-B*44:03	178.9	0.68	0.6	0.41	False	34.5	Subclonal
10	**VTEPVLKA**	AS-derived (MXE)	CALD1	HLA-A*02:01	198.2	0.74	0.58	0.38	False	167.8	Trunk

7c: Fusion-Derived Neoepitopes

#	Peptide	Fusion	HLA	IC50	Rank%	Imm	TCR	MS	TPM	Clonal
1	**GPPVYNTC**	DNAJB1-PRKACA	HLA-A*02:01	41.3	0.19	0.81	0.69	True	156.3	Trunk
2	**KMEAFNRA**	TFEB-CLTC	HLA-A*24:02	67.8	0.33	0.75	0.57	False	42.7	Subclonal
3	**LPRGSPAA**	DNAJB1-PRKACA	HLA-B*07:02	89.4	0.41	0.7	0.5	False	156.3	Trunk

Immune Profile & TME

TCR Repertoire

Clonotypes: 12453 | Shannon diversity: 7.8 | Clonality: 0.42

Clonotype	Freq	Expansion	Specificity
TRBV9-TRBJ2-1_CASSDSNTGELFF	8.3%	High	Likely tumor-reactive
TRBV12-4-TRBJ2-7_CASSQAPRGTGELFF	5.6%	Medium	Unknown
TRBV20-1-TRBJ1-2_CASSLAPGATNEKLFF	4.1%	Medium	Likely tumor-reactive
TRBV6-4-TRBJ2-5_CASSDAGVSTDTQYF	3.2%	Low	Unknown
TRBV7-9-TRBJ2-3_CASSQETQYF	2.8%	Low	Unknown

TME Cell Composition

Cell Type	Fraction	Status
CD8+ T cells	8.0%	Low
CD4+ T cells	12.0%	Normal
Tregs	15.0%	High
MDSCs	18.0%	High
TAM (M2)	22.0%	High
NK cells	3.0%	Low
B cells	5.0%	Normal
Fibroblasts (CAF)	17.0%	High

Immune Signatures

Signature	Score	Status	Interpretation
Interferon-gamma	-1.2	Low	Reduced anti-tumor immune activation
T-cell inflamed	-0.8	Low	Cold TME — limited T-cell infiltration
Immune exclusion	1.5	High	T cells excluded from tumor core by stromal barrier
WNT/β-catenin	1.8	High	Associated with immune exclusion in HCC

Antagonistic Self-Peptides

Identified: 14

Peptide	HLA	Antagonism	Note
VLPTDLSV	HLA-A*02:01	0.78	Self-peptide with antagonistic TCR interaction — candidate for displacement
GMAKDSVAV	HLA-A*24:02	0.71	Self-peptide suppressing TCR activation
KLPQAGSV	HLA-B*07:02	0.65	Moderate antagonism — displacement candidate

Therapeutic Recommendations

Peptide Vaccine Candidates

#	Peptide	Source	Rationale	Confidence
1	**SLADTNSL**	AS-derived (TTN)	Highest immunogenicity + MS-validated + trunk clonality	High
2	**MPEGTACV**	AS-derived (DAB2)	MS-validated + high binding affinity + trunk	High
3	**GPPVYNTC**	Fusion (DNAJB1-PRKACA)	MS-validated + high expression + trunk	High
4	**WQRTYKPL**	AS-derived (MAGI2)	MS-validated + high tumor-specificity	High
5	**KLDKVNSL**	Mutation (TP53)	Driver mutation + high immunogenicity	Medium
6	**KPFVYVLA**	AS-derived (PKM)	High expression + trunk clonality	Medium
7	**STLPGVIAA**	Mutation (CTNNB1)	Driver mutation + trunk	Medium
8	**GHNWAVTL**	AS-derived (FHIT)	Good binding + tumor-specific	Medium

TCR-T Cell Therapy Targets

Peptide	HLA	Rationale	Confidence
SLADTNSL	HLA-A*02:01	Top candidate for TCR engineering — high TCR recognition probability (0.78)	High
MPEGTACV	HLA-A*02:01	MS-validated + high TCR recognition probability (0.72)	High
GPPVYNTC	HLA-A*02:01	Fusion-derived + MS-validated + trunk	High

Combination Strategy

Primary: Personalized peptide vaccine (top 15 AS-derived neoepitopes) + anti-PD-L1

Rationale: Immune-excluded TME with high PD-L1 expression suggests checkpoint blockade may release stromal barrier. AS-derived neoepitopes provide antigenic targets beyond mutation burden.

Secondary: Add self-peptide displacement peptides to counter antagonistic self-immunopeptidome

Monitoring: TCR repertoire tracking + ctDNA monitoring for response assessment

Self-Peptide Displacement Designs

Target	Displacing Peptide	Rationale
VLPTDLSV	**SLADTNSL (engineered variant)**	Higher MHC affinity displaces antagonistic self-peptide while maintaining TCR activation
GMAKDSVAV	**WQRTYKPL (engineered variant)**	Competitive displacement with enhanced immunogenicity

Technical Appendix

Methods & Software

Step	Tool	Version	Notes
DNA sequencing	Illumina NovaSeq 6000	WES 300X tumor / 150X normal	Augmented exome capture across 20,000 genes
Somatic variant calling	Mutect2 (GATK 4.4.0)	4.4.0.0	Tumor-normal mode, PoN filtering
CNV calling	CNVkit	0.9.10	Tumor-normal paired analysis
HLA typing	OptiType	1.3.2	4-digit resolution from WES
HLA-LOH	LOHHLA	1.0	Detected LOH at HLA-B*07:02
Long-read RNA-seq	PacBio Revio (Iso-Seq)	SMRT Link 13.0	Full-length isoform sequencing
Short-read RNA-seq alignment	STAR	2.7.11b	2-pass mode, GENCODE v44
Expression quantification	Salmon	1.10.1	Transcript-level TPM
AS event detection	rMATS + LongGF	4.1.2 / 1.3	Tumor vs normal paired, FDR < 0.05
Fusion detection	STAR-Fusion + Arriba	1.12.0 / 2.3.0	Consensus calls, ≥5 read support
Immunopeptidomics	MSFragger + FragPipe	19.0 / 21.0	1% FDR, customized database including AS/fusion peptides
MHC-I binding prediction	NetMHCpan-4.1 + MHCflurry-2.0	4.1 / 2.0	8-11mers, all patient HLA alleles
Immunogenicity prediction	DeepImmuno + PRIME	2.0 / 1.0	Deep learning-based
Structural modeling	AlphaFold2 + ColabFold	2.4 / 1.5.5	Peptide-MHC complex modeling
scRNA-seq processing	Cell Ranger + Scanpy	7.2 / 1.9.5	10x Chromium, 5,000 cells
TCR repertoire	MiXCR	4.0.0	TRB chain, productive rearrangements

QC Metrics

Metric	Value	Status
WES mean coverage (tumor)	312X	Pass
WES mean coverage (normal)	158X	Pass
RNA-seq mapped reads	87.3%	Pass
Long-read N50	2,847 bp	Pass
MS/MS peptide IDs (1% FDR)	8,421	Pass
scRNA-seq cell count	5,234	Pass
TCR productive rearrangements	12,453	Pass
Tumor purity (estimate)	0.72	Pass
Contamination check	None detected	Pass

Limitations

- Mock/demo data — not from a real patient. Values are programmatically generated for demonstration.
- Immunogenicity predictions are computational estimates requiring experimental validation.
- TCR recognition probabilities are model-based and require autologous T-cell assay confirmation.
- Self-peptide displacement designs are computational proposals requiring in vitro binding competition validation.

- TME deconvolution is estimated from bulk RNA-seq and scRNA-seq reference; absolute fractions may vary.

FOR RESEARCH USE ONLY — Not for use in diagnostic procedures. This report was generated by the PeptideXme computational pipeline for research purposes. All findings require experimental validation before any clinical application.