Detection and Targeting of Genomic Fusion Derived pMHC Neoantigens in Prostate Cancer

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Background: Prostate cancer remains unresponsive to immune checkpoint blockade in unselected patients. Tumor intrinsic factors such as low tumor mutational burden, limited PD-L1 expression, and tumor extrinsic components such as an immunosuppressive microenvironment contribute to an immune-cold phenotype. Current bispecific T-cell engager (BiTE) therapies for prostate cancer target tumor-associated antigens such as PSMA or PSCA, but their expression in normal tissues raises significant risks of on-target, off-tumor toxicity and limits clinical efficacy. There is a critical need for tumor-specific antigens with a broader therapeutic window.

Gene fusions are frequent genomic events in prostate cancer, present in over 80% of patients, and are known drivers of oncogenesis. These fusions generate novel peptide–MHC complexes (pMHCs) that are both highly immunogenic and tumor-specific. Fusion-derived pMHCs are absent from normal tissues, reducing the risk of toxicity, and are retained driver events, lowering the likelihood of immune escape. Thus, fusion pMHCs represent an ideal class of targets for antibody-based immunotherapy in prostate cancer.

Methods: To develop BiTEs against fusion pMHC-derived tumor specific neoantigens in prostate cancer we are leveraging (1) high-throughput, in vitro nanobody screens to specifically target pMHCs, (2) identifying candidate fusion pMHC from prostate cancer models by combining RNA-seq–based fusion detection with HLA-specific epitope prediction, and (3) validating predicted candidate fusions in prostate cancer models and patients using sequencing and mass spectrometry approaches.

Results: We have utilized a novel, cell-free, high-throughput ribosome display system to target the public neoantigen, p53 R175 in the globally-frequent allele, HLA-A*02:01. With a hit rate of 30% amongst E. coli expressible nanobodies, multiple candidate binders are currently being affinity matured and validated for pre-clinical efficacy (**Fig 1**). We have utilized transcriptomic and genomic sequencing to identify (a) HLA alleles present, (b) potential gene fusions and (c) peptides predicted to present on endogenous HLAs. (Fig 2). Comparing RNA-seq and Whole-genome sequencing pipelines has revealed divergent fusions (19 RNA fusions vs 266 Whole genome fusions for PRC3) indicating that many fusions are even more common, although not always expressed. Of potential binders, 17 are predicted to be weak while 7 are predicted to be strong binders, distributed across 6 different HLA – gene combinations.

Conclusions: pMHCs in prostate cancer represent an under-explored class of tumor specific antigens. They are targetable with new high-throughput binder screens. Furthermore, prostate cancers harbor high levels of genomic and expressed fusion proteins that are predicted to bind

to endogenous HLAs. We are now proceeding with empirically validating fusion derived pMHCs using HLA stabilization assays in prostate cancer and identifying fusion-pMHC specific binders.

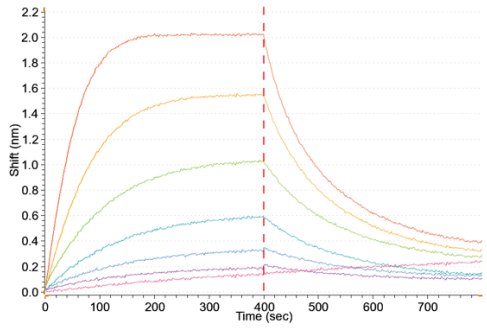

Figure 1: Biolayer interferometry of ribosome-display selected nanobody against HLA-A*02:01 p53 R175H. Example BLI data of candidate 23.

(A) Allele	**(B) RNA Detected Fusions**
HLA-A*01:196	SMAGP--TFCP2
HLA-A*24:44	SAMD8--ADK
HLA-B*55:01	KDM5B--LINC01351
HLA-B*56:60	MIER2--PPFIA3
HLA-C*06:02	CTNNA1--AC011405.1
HLA-C*01:180	PUS7--AC004917.1
	SIL1--AC034243.1
	CAMK2G--KIFBP
	PLOD2--IQCJ
	LINC02057--NDUFAF2
	SEPTIN7P2--PSPH
	PLEKHA6--ASAH2B
	HECTD4--RPL6
	PTEN--METTL18
	TVP23C--CDRT4
	RPTOR--AC079943.2
	TTC37--ARSK
	ERO1A--GNG2
	KRT81--KRT7

(C)

Fusion Name	HLA allele	Fusion Peptide Tested	Binding Category
CAMK2G--KIFBP_11	HLA-C06:02	KKSDGGVKL	WB
CAMK2G--KIFBP_11	HLA-A01:196	KSDGGVKLL	WB
CAMK2G--KIFBP_11	HLA-C06:02	KSDGGVKLL	WB
HECTD4--RPL6_17	HLA-A01:196	DLEDLWSSIEFLY	WB
HECTD4--RPL6_17	HLA-A01:196	LEDLWSSIEFLY	WB
PLOD2--IQCJ_12	HLA-B55:01	IPTGRTEKI	WB
PLOD2--IQCJ_12	HLA-B56:60	IPTGRTEKI	SB
PLOD2--IQCJ_12	HLA-B55:01	IPTGRTEKIA	WB
PLOD2--IQCJ_12	HLA-B55:01	KPSSIPTGR	WB
PLOD2--IQCJ_12	HLA-B55:01	KPSSIPTGRT	WB
SAMD8--ADK_2	HLA-B56:60	SPTRTAEGF	SB
SAMD8--ADK_2	HLA-B56:60	SPTRTAEGFF	WB
SAMD8--ADK_2	HLA-B56:60	SPTRTAEGFFL	WB
SAMD8--ADK_2	HLA-B55:01	TAEGFFLTV	WB
SAMD8--ADK_2	HLA-C06:02	TRTAEGFFL	SB
SAMD8--ADK_5	HLA-A01:196	RTAEEAATF	WB
SAMD8--ADK_5	HLA-B55:01	SPTRTAEEA	SB
SAMD8--ADK_5	HLA-B56:60	SPTRTAEEA	WB
SAMD8--ADK_5	HLA-B55:01	SPTRTAEEAA	SB
SAMD8--ADK_5	HLA-B56:60	SPTRTAEEAA	WB
SAMD8--ADK_5	HLA-B56:60	SPTRTAEEAATF	WB
SMAGP--TFCP2_1	HLA-B55:01	SPRGWCVQG	WB

SMAGP--TFCP2_1	HLA-B55:01	TPSPRGWCV	SB
SMAGP--TFCP2_1	HLA-B56:60	TPSPRGWCV	SB

Figure 2: HLA, fusion and peptide results for a sample prostate cancer cell line (PRC3) with (a) RNA-seq derived HLA detected alleles (b) RNA-seq derived fusion events (STAR -Fusion), (c) predicted binders to HLA alleles (MHC-Netpan 4.1)

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Conflicts of Interest Disclosure Statement:

I have no conflict of interest to report.