A personalized cancer vaccine approach to treat Lynch syndrome

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Background

Lynch Syndrome (LS), previously referred to as Hereditary Nonpolyposis Colorectal Cancer (HNPCC), is an inherited heterozygous autosomal dominant disorder, which predisposes affected individuals to 70-80% risk of developing colorectal cancer (CRC). Mutations in mismatch repair (MMR) genes, e.g. MLH1, MSH2, MSH3, PMS2 and EPCAM, impair DNA repair leading to a hypermutated tumor phenotype and microsatellite-instability (MSI) causing Lynch Syndrome. Tumor-derived immunogenic peptides arising from intracellular proteolytic processing of somatic mutations binds HLA Class I (MHC) proteins, and activates T-cell receptors (TCR) on naïve CD8+ T-cells. The activated T cells transform into cytotoxic T cells (CTLs) and secrete factors that mediate tumor lysis. Since almost all tumors in LS patients are hypermutated, they are likely to express large numbers of neoantigens, that could potentially elicit an antigen-specific cytotoxic T-cell response and aid tumor clearance.

Results

❖ **Identification of a common germline mutation in the MLH1 gene in two unrelated Lynch syndrome-affected families by NGS and Sanger sequencing.**

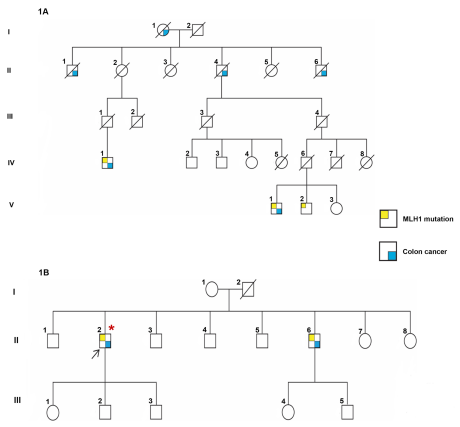

Figure 1: Pedigrees of Lynch syndrome affected families: A slash through the shape indicates a deceased member. Roman numerals indicate generations.

➢ ***Heterozygous mutation c.154delA; p.Glu53ArgfsTer found in MLH1 gene in three members of Family 1 and two members of Family 2. The families are unrelated.***

➢ ***Proband of Family 2 (II.6) was diagnosed with a relapsed colon adenocarcinoma.***

❖ **Neoepitope derived from the germline *MLH1* mutation using Oncopept*VAC*™ is non immunogenic.**

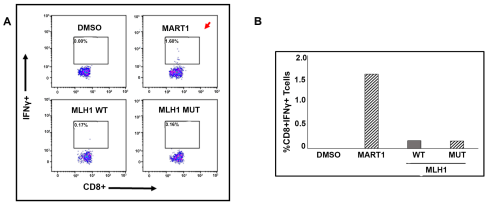

Figure 2: *MLH1*-germline mutation derived peptide tested in a CD8+ T cell activation assay in a Lynch Syndrome patient (Family 2; II.2, LS^{+}MLH1^{mut}).

Results

❖ **Neo-epitope prediction and prioritization using Oncopept*VAC*™ from somatic mutations found by exome sequencing of LS patient's tumor.**

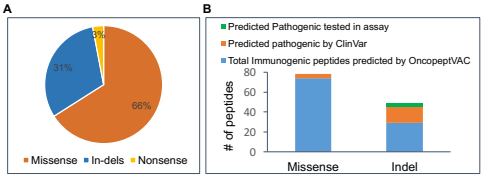

Figure 3: A. Subtypes of 959 Somatic mutations found in tumor of LS patient. B. Immunogenic peptides predicted by Oncopept*VAC*™.

Table 1: Oncopept*VAC*™ prediction of peptides derived from pathogenic somatic mutations

Gene	Variant	HLA	Wildtype peptide Affinity(nM)	Mutant peptide affinity (nM)	TCR binding Prediction	Mutation reported in COSMIC
MSH6	p.F1088LfsX5	A02:06	71.02	8.61	High	4
PIGO	p.T788LfsX25	A02:06	32787.64	10.3	High	6
AXIN2	p.G665AfsX24	A02:06	12860.25	425.67	High	26

➢ ***Out of 959 somatic mutations, only 21 were predicted to be pathogenic by Clinvar out of which 16 were found to be frameshift mutations (Indel).***

➢ ***Peptides were designed and immunogenicity was predicted by Oncopept*VAC*™.***

➢ ***Three mutations were found to be prevalent in various types of cancer (OncoMD and COSMIC) Wildtype and mutant peptides derived from these mutations were tested in in vitro assays.***

❖ **Neoepitopes derived from three somatic mutations identified in the tumor of LS patient are immunogenic.**

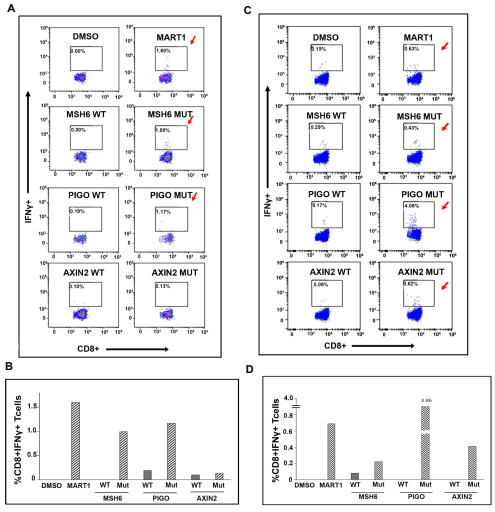

Figure 4. T cell activation assay: A. 2/3 mutant peptides were found to be activating CD8+ T cells (intracellular IFNγ) in PBMCs isolated from LS patient's blood. B. Graphical representation of the FACS data in 4A. C.3/3 mutant peptides showed upregulation in IFNγ+CD8+ T cells in PBMCs collected from a healthy donor with HLA-A02. D. Graphical representation of the FACS data in 4C.

➢ ***MSH6 and PIGO derived peptides elicited a robust CTL response in both the LS patient and healthy donors with HLA-A02 whereas AXIN2 peptide could elicit a response only in healthy donor.***

Results

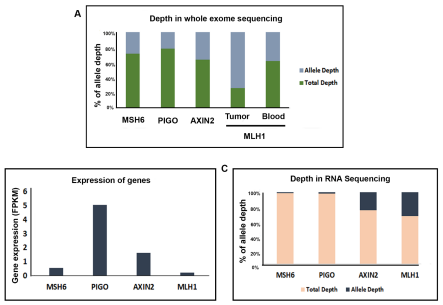

Figure 5. Expression analysis of four genes in the tumor of LS patient: A. Whole exome sequencing was performed with the tumor sample collected from LS patient. The bar diagram represents the percentage of wildtype and alternate allele depth of AXIN2, MSH6, PIGO and MLH1 genes (tumor and blood). B. Expression levels of four genes were determined using RNA Seq analysis (FPKM: Fragment per kilobase per million). C. Percentage of wildtype and mutant allele expression determined by RNA seq analysis.

➢ ***The alt RNA expression of the variant was found to be inversely proportional to the immunogenicity (CTL response in in vitro assay with LS patient PBMCs) of the mutant peptides designed from the corresponding variants.***

➢ ***Alt MLH1 allele depth in LS patient's tumor was double of MLH1 allele depth in LS patient's blood, which indicates that the second allele has undergone an MLH1 mutation in the tumor.***

❖ **Tumor Microenvironment analysis *of LS patient's tumor* using Oncopept*TUME* predicts the presence *of TILs and myeloid cells.***

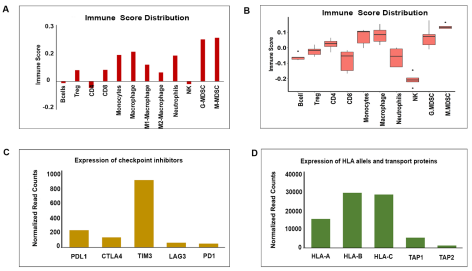

Figure 6. Tumor microenvironment analysis of sample collected from LS patient. A. RNA expression data analyzed in Oncopept*TUME* algorithm shows the presence of various tumor infiltrating cells based on the immune score distribution. CD8+T cell, B lymphocyte, Macrophage, Monocyte, Neutrophil, MDSC and Treg cell signature markers were found to be present in the tumor sample of LS patient. B. Oncopept*TUME* analysis on 5 MSI-H samples from TCGA database shows similar pattern like LS patient (6 A) C. RNA expression of checkpoint molecules like TIM-3 expression was observed, however PD-1, PD-L1, CTLA-4 and LAG-3 were found to be low in the tumor suggesting lack of complete T cell exhaustion. D. Expression of HLA alleles and transport protein TAP1 and TAP2 in the tumor of LS patient shows evidence of tumor escape mechanisms.

Conclusion

➢ Oncopept*VAC*™ derived immunogenic peptides form tumor of LS patient were found to be activating CD8+ T cells *in vitro* and can be used as vaccine candidates in conjunction with immune checkpoint inhibitors as a personalized cancer immunotherapy regimen.