Supplementary Online Content

Howitt BE, Shukla SA, Sholl LM, et al. Association of polymerase e–mutated and microsatellite instability endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1/PD-L1. *JAMA Oncol*. Published online July 9, 2015. doi:10.1001/jamaoncol.2015.2151

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Prediction of HLA type and neoantigen load

Inference of HLA type of POLE, MSI and MSS endometrial cancers was performed by applying the POLYSOLVER (POLYmorphic loci reSOLVER) tool[1] (Shukla *et al*, submitted) to whole-exome sequencing (WES) data generated from The Cancer Genome Atlas (TCGA) consortium as previously described[2]. In brief, this algorithm selects and aligns putative HLA reads to an imputed library of full-length genomic HLA allele sequences. The alignments then serve as a basis for the inference step that incorporates the number and base qualities of aligned reads, the empirical library insert size distribution and population-based allele frequencies. For prediction of neoantigen load, we used previously curated lists of somatic mutations (somatic single nucleotide variants and somatic insertions and deletions) for each of these samples (Sage Bionetworks' Synapse resource (http://www.synapse.org/#!synapse:syn1729383 and Lawrence et al[3])) from which individual-specific HLA-binding peptides were identified by a neoantigen prediction pipeline[1] that uses detected somatic mutations in the individual. Binding affinities of all possible 9 and 10-mer mutant peptides to the corresponding POLYSOLVER-inferred HLA alleles were predicted using NetMHCpan (v2.4)[4]. All predicted binders with an affinity < 500 nM were used to evaluate the neoantigen load.

Tumor samples

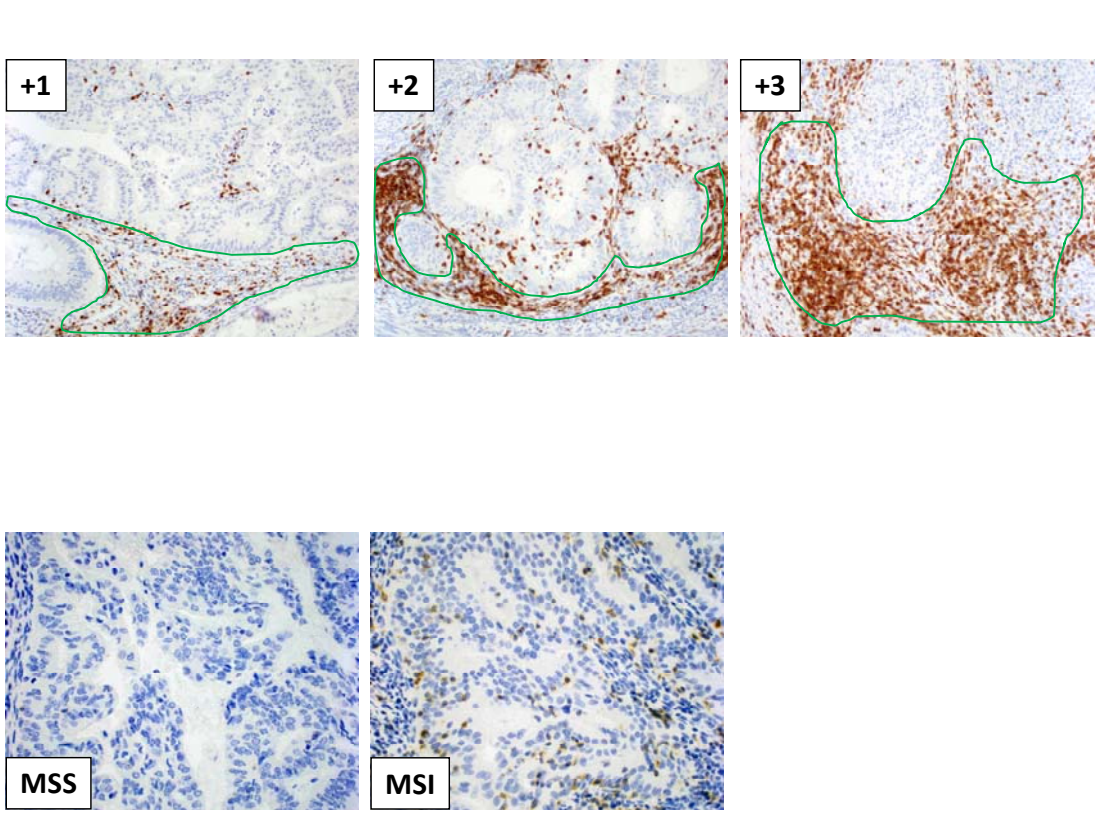
This study includes 63 cases retrieved from the archives of Brigham and Women's Hospital under institutional review board approved protocol and is comprised of three *POLE*-mutated (P286R), 28 MSI and 32 MSS primary endometrial tumors from patients who had not previously received any chemotherapy or radiation therapy. POLE mutated cases were identified by Sanger sequencing of two mutational hotspots (exon 9 and exon 13). MSI status was determined using MMR immunohistochemistry (see supplemental table 1).

Immunohistochemistry and evaluation of tumor associated lymphocytes

Immunohistochemistry (IHC) was performed for CD3, CD4, CD8, CD20, PD-1 and PD-L1 on formalin fixed paraffin embedded (FFPE) tissue samples using standard protocols (summarized in supplemental table 1). A photomicrograph of the area of maximum CD3+ intraepithelial lymphocytes was obtained (40X objective), and photomicrographs from the corresponding area were obtained for the additional stains. Counts were performed manually in Photoshop. For evaluation of TILs, we focused only on intraepithelial lymphocytes, i.e. lymphocytes located within the tumor epithelium, rather than in the peritumoral stroma. The number of CD3+ intraepithelial lymphocytes was manually counted in one high-power field (40X objective) of the highest density of TILs, blinded to the MSI status. Peritumoral lymphocytes (lymphocytes in the stroma immediately adjacent to the tumor epithelium) were scored using a semi-quantitative method (none (0), mild (1+), moderate (2+), marked (3+)).

Staining for PD-1 and PD-L1 occurred in our Center for Immuno-Oncology in our institution. For PD-L1 staining, we used a new clone rabbit monoclonal antibody offered by Cell Signaling Technologies E1L3N, which in our experience, is superior to all other commercial antibodies for PD-L1. It recognizes a cytoplasmic domain epitope of the protein and gives much less background than other commercial antibodies[5]. PD-1 is a standard marker offered by Cell Marque, and it has been used for several years now in our CLIA laboratory for routine diagnostic use for angioimmunoblastic T-cell lymphoma[6].

eFigure



A, Peritumoral lymphocytes: Examples of 1+, 2+, and 3+ semi-quantitative scoring of peritumoral CD3+ lymphocytes. Only the lymphocytes in the stroma adjacent to the tumor were scored (peritumoral stroma outlined in green). **B,** PD-1 Expression in TILs: Left panel: A MSS tumor shows no PD-1 expression in TILs. Right panel: An MSI tumor has a significant number of PD-1 positive TILs.

e**Table.** IHC Antibodies Used in This Study

Antibody	**Company**	**Clone**	**Dilution**	**Antigen Retrieval Buffer**
CD3	Dako	A0452 (polyclonal)	1:250	H2
CD4	Dako	M7310 (4B12)	1:80	H2
CD8	Dako	M7103 (C8/144B)	1:100	H2
CD20	Dako	M0755 (L26)	1:500	H1
PD-1	Cell Marque	315m-95 (NAT105)	1:300	H2
PD-L1	Cell Signaling	13684 (E1L3N)	1:200	H2
MSH2	Cal Biochem, EMD	NA27	1:200	EDTA
MSH6	BD Bioscience	PU29	1:50	citrate
MLH1	Novocastra	NCL-L-MLH1	1:75	citrate
PMS2	Cell Marque	MRQ-28	1:100	citrate

H1= citrate based pH 6.0 (Bond epitope retrieval solution)

H2= EDTA based pH 9.0 (Bond epitope retrieval solution)

PC= pressure cooker

CD3, CD4, CD8, CD20, PD-1, PD-L1 were stained on the Leica Bond automated stainer, and the Polymer Refine Detection Kit (DS9800)

MSH2, MSH6, MLH1, PMS2 were stained on a Dako autostainer and Envision detection system.

eReferences

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