

Systematic review

What Do We Have to Know about PD-L1 Expression in Prostate Cancer? A Systematic Literature Review. Part 6: Correlation of PD-L1 Expression with the Status of Mismatch Repair System, *BRCA*, *PTEN*, and Other Genes

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Table S1. Detailed results of the studies evaluating PD-L1 expression and microsatellite instability/mismatch repair system proteins status in patients with prostatic carcinoma.

Ref.	PD-L1 positivity rate, clone and score (IHC)(#)	Clinic-pathological features	Assays/Results (%)
[156]	206/206 (100%) (clone 22C3, CPS ≥ 1)	ST: NR GG: variable Stage: mCRPCs; T2-3 M0-1 Treatment: (PEM + ENZ) vs. PEM; no P-T	Assay: NR Results: 206/206 (100%) MSI-H (all the cases showed PD-L1 positivity and MSI-H according to the inclusion criteria).
[19]	0/33 (0%) (#) (clone SP142, $\geq 5\%$ of tumor cells)	ST: NR GG: variable Stage/Therapy: Atezolizumab to mCRPCs (22 with ≥ 3 P-T lines; 32 prior ENZ; 13 prior sipuleucel-T)	Assay: mutation analyses (FoundationOne assay T7 baitset, Foundation Medicine Inc.), tumor RNA expression analyses (RNASeq, Fluidigm technologies), WES. Results: 2/16 (12.5%) MSI, including - 1/14 cases (baseline biopsies) (7%): TMB 18 mutations/Mb, MSI-H, <i>MSH2</i> loss (no response to atezolizumab, survival time <6 months) (§) - 1 additional case (PR): <i>MSH2/MHS6</i> del; high TMB (30 mut/Mb); <i>BRCA2</i> E49*, <i>TP53</i> Y236D, <i>AR</i> W742C mutations; <i>MYC</i> amplification; <i>TMPRSS2-ERG</i> fusion, PD-L1 (IHC) (baseline: negative; post-treatment: positive in $\geq 5\%$ immune cells and <5% of tumor cells)

			- 1 additional case (PR): low TMB, microsatellite-stable, <i>BRCA2/ATM</i> (?) mutation (lymph node metastasis)
[9]	156/258 (60%) (clone 22C3, CPS ≥ 1)	ST: biopsies GG: variable Stage/Therapy: PEM to metastatic or locally confined CRPCs	Assay: WES, mSINGS assay (°°). Results: 0/6 (0%) responders with evaluable genomic data showed MSI (mSINGS), including 4/6 (67%) cases with somatic aberrations in ≥ 1 of 50 DDR genes (*): 1) <i>ATM</i> (chr11_108121426-108121426_A_) splice site acceptor del, <i>BRCA2</i> A1162V subs (VUS), <i>CDK12</i> G1461Afs* del, <i>FANCA</i> subs (chr16_89816312-89816312_T_C) (VUS), <i>FANCD2</i> R263H subs (VUS), <i>MLH3</i> T930Qfs*35 del, and <i>RAD54L</i> R511H subs (VUS); 2) <i>TP53</i> R273P subs; 3) <i>BRCA2</i> V1176Gfs*8 insertion; 4) <i>NBN</i> Q494P subs (VUS) and <i>TP53</i> S241F subs. A long responder (>2 years) with dMMR (IHC) below the cut-off for MSI-H by mSINGS, high TMB and high CD3+ intratumor infiltration. A long-responder with amplified <i>CD274/PDCD1LG2</i> locus.
[23,92]	0/28 (0%) (#) (clones E1L3N, 22C3; NR score)	ST: biopsies GG: 1-5 Stage: pT1c-3 N0-1 M1 Therapy: ENZ + PEM to CRPCs	Assay: PCR (BAT-25, BAT-26, NR-21, NR-24, MONO-27) and NGS (mSINGS) for MSI; WES, gene expression profiling, Knight Diagnostic Laboratory's GeneTrails Solid Tumor Panel, Personal Genome Diagnostics panel (Baltimore, MA, US). Results: 1/3 (33%) responders with MSI (PCR, mSINGS) and > 4000 mutations (also DRG, ≥ 1 heterozygous cancer-predisposing <i>ATM</i> variant) (WES) (§§); 0/13 (0%) non-responders with MSI: 1 with 1 marker of MSI (PCR; not MSI-H by mSINGS), 3 with heterozygous mutations in DRG (2 cancer-associated). PD-L1 was negative when performed in cases also tested for MSI (<i>n</i> = 13). No differences in PD-L1 expression or TMB (<i>n</i> = 10; <i>p</i> = 0.42) between responders and non-responders (gene expression profiling); higher number of neoepitopes among responders (679 vs. 405.5, <i>p</i> = 0.42).
[29,37]	29/220 (13%) (clone 22C3; mo/st ≥ 1% or we ≥ 10%)	ST: RP (TMA) GG: 1-5 Stage: pT2-3ab Therapy: Short-term ADT (DEG) + PEM + WPC to	Assay: IHC (clones G168–15, FE11, BC/44, A16–4) (positive: ≥ 1%). Results: 2/220 (0.9%) MLH1- (all with loss of other MMR); 6/220 (2.7%) MSH2- (all with <i>MSH6</i> loss); 37/220 (16.8%) MSH6-; 27/220 (12.3%) PMS2-; ≥ 1 MMR loss 50/220 (22.7%); ≥ 2 MMR loss 15/220 (6.8%); ≥ 3 MMR loss 5/220 (2.3%); 4 MMR loss 2/220 (0.9%). ≥ 2 MMR loss was associated with higher rate of PD-L1+ cancer cells (17.2% vs. 5.2%, <i>p</i> = 0.033) (@). 39 (17.7%) men with clinical recurrence or BCR after RP. No

		hormone-sensitive PCs	association of MSI status with age, family PC history, GS, pT/pN stage, and disease recurrence (ç), nor for PD-L1 positivity in cancer cells and clinical outcomes. Higher BCR-risk for ≥ 1 MMR loss and PD-L1+ TICs ($p = 0.045$). Significantly elevated preoperative PSA levels in dMMR PCs (not for ≥ 2 MMR loss).
[38]	20/91 (22%) acinar (13/50 HR, 7/41, 17% MPC) 1/27 (4%) ductal (**) (NR clone; $\geq 1\%$)	ST: RP, biopsies GG/Stage/Therapy: 50 HR; 41 MPC	Assay: IHC (NR). Results: 4/118 (3%) cases (2 MPC, 1 HR, 1 ductal PC) were dMMR (3 MSH2-/MSH6-, 1 PMS2-); only 1/4 (25%) cases was PD-L1+.
[43]	2/42 (5%) acinar 1/34 (3%) ductal (#) (clone SP263; $\geq 1\%$)	ST: RP, TURP (TMA) GG: 2-5 Stage/Therapy: NR	Assay: IHC (positive if at least some nuclei are stained). Results: 4/73 (5%) dMMR PCs (3/40, 8% acinar: 3 MSH6-/PD-L1-; 1/33, 3% ductal: 1 MSH2-/MSH6-/PD-L1- but PD-L1+ immune cells) ($p = 0.62$). 2 ductal PCs were indeterminate for MSH6 or MSH2/MSH6.
[80]	1/34 (3%) ductal (>10% ductal) 1/30 (3%) acinar (clone E1L3N, $\geq 1\%$)	ST: TMA (30 acinar, 6 ductal) + 28 non-TMA GG: NR Stage: 4/28 ductal N+ Therapy: NR	Assay: IHC (positivity for MMR+ $\geq 1\%$). Results: 1/34 (3%) ductal PCs were MLH1-/PMS2- (non-TMA), while 0/30 (0%) acinar PCs showed dMMR.
[53]	1/19 (5%) (***) (clone 22C3; NR score)	ST: RP (11), MTS (8) GG: 2-5 Stage: pN1 or pM1 Therapy: androgen therapy (9 CRPCs, 10 CSPCs)	Assays: OmniSeq Comprehensive®, FISH (copy number gain/loss), PCR (MSI), DNaseq (TMB). Results: 1/17 tested cases showed MSI (MSH2 copy number loss), as well as high TMB, PTEN, BRCA1/2, FBXW7, GATA3, SMO, TET2, TP53, and TSC1 mutations: it was the only PD-L1+ case (weak, in 5% cells; low rank for PD-L1: 26). No association with RNAseq rank of any gene, CRPC vs. CSPC status, or primary PC vs. metastases, nor between DNA mutational profile, CD3/8 or PD-L1 IHC status, RNA-seq CD8, or TMB and the expression profile of any genes.

[61]	9/51 (18%) (clone E1L3N; TPS)	ST: RP, biopsies, TURP GG/Stage: variable Therapy: adjuvant RT (some cases)	Assay: IHC for MMR (clones FE11, EP49, ES05, EP51) (positivity: nuclear positivity, regardless of intensity), PCR (Promega MSI Assay v1.2), NGS (targeted on MMR gene coding sequences, dMMR_MUT; or dMMR_MSINGS). Results: 10/124 (8.1%) dMMR/MSI mCRPC patients (biopsies) (more frequent false-positive, discordant results with Promega assay), also having higher mutational loads/MSINGS scores. Shorter median OS for dMMR/MSI cases (vs. non-dMMR/MSI; univariate/multivariate analysis; 3.8 vs. 7.0 years; aHR 4.09; 95% CI, 1.52–10.94; $p = 0.005$), while no differences for GS, PSA, age, and stage. dMMR in primary PC strongly associates with developing dMMR CRPCs. 5/85 (6%) matched HN and CRPC samples had dMMR (IHC) in primary PC: in mCRPC biopsies, 4/5 (80%) were diffusely dMMR (IHC), while 1/5 (20%) was pMMR (IHC). 2/5 HSPC and 1 CRPC showed heterogeneous staining. Higher likelihood of PD-L1 positivity in dMMR mCRPC (5/10, 50% vs. 4/41, 9.8%) (MELRM, OR 14; 95% CI, 2–84; $p = 0.005$). Some dMMR/MSI mCRPC have higher D-TIL levels than pMMR (°°°). Germline mutations ($n = 1$), non-synonymous somatic mutations ($n = 6$), or biallelic events ($n = 7$) in MMR genes (total $n = 14$) had higher dMMR-associated DNA mutational signature activity and mRNA expression signatures. Higher MSI-NGS scores correlated to dMMR mutational signatures. PCs with dMMR mutational signatures overexpressed a variety of immune transcripts, such as CD200R1, BTLA, PD-L1, PD-L2, ADORA2A, PIK3CG, and TIGIT. 5/10 (50%) dMMR mCRPCs were PD-L1+, as 4/41 (9.8%) pMMR
[68]	39/508 (8%) (clone SP263; ≥ 1%)	ST: RP, biopsies, autopsies (TMA) GG: 1-5 Stage: pT2-4 N0-1 Therapy: ADT in 57 mCRPCs	Assay: Microsatellite Instability Analysis System v1.2, MSI Multiplex Kit; Promega, Madison, WI; ABI 3130XL Genetic Analyzer, Applied Biosystems, Foster City, CA, US Results: 0/2 (0%) primary PD-L1+ PCs were MSI.
[56]	2/5 (40%) (clone E1L3N; ≥ 1%)	ST/GG: NR Stage: metastatic	Assay: Sequencing panel (500 genes). Results: 1/14 (7%) PMS2 frameshift indel in a BRCA2 mutated patient (intact second allele; no hypermutation phenotype).

		Therapy:	
		durvalumab +	
		olaparib to	
		mCRPCs (prior	
		ENZ +/- ABT)	
[77]	21/177 (12%):	ST: RP	Assay: IHC (score: 0 for "no loss", 1 for "complete loss in all
	18/130 (14%)	GG: 1-5	tumor cells").
	(HN); 3/44 (7%)	Stage: pT2/3b	Results: 1/21 (5%) PD-L1+ PCs was MSH2-/MSH6- (GS 9, 5+4,
	(AAPL)	Nx/0/1	pT3bN1, no prior neoadjuvant treatment; "interface pattern"
	(clones E1L3N,	Therapy: 44 AAPL;	of PD-L1+, TILs).
	SP142; ≥ 1%,	130 HN	
	mo/st)		

AAPL: neoadjuvant abiraterone Acetate + Prednisone and Leuprolide; ABT: abiraterone; ADT: androgen deprivation therapy; aHR: adjusted hazard ratio; BCR: biochemical recurrence; CPS: Combined positive score (number of PD-L1+ cells, including tumor cells, lymphocytes, and macrophages, divided by the total number of tumor cells, x 100); CI: confidence interval; CRPC: castration-resistant prostate cancer; CSPC: castration-sensitive prostate cancer; DDR: DNA damage repair genes; DEG: Degarelix; del: deletion; dMMR: deficient MMR; DRG: DNA repair genes; D-TILs: density of tumor-infiltrating lymphocytes; ENZ: enzalutamide; FISH: Fluorescent in situ hybridization; GG: Gleason score; GS: Gleason score; HN: hormone-naïve; HR: high-risk prostate cancer; HSPC: hormone treatment-naïve prostate cancer; IHC: immunohistochemistry; MELRM: mixed-effects logistic regression model; MMR: mismatch repair system proteins; mCRPC: metastatic castration-resistant prostate cancer; mo: moderate; MPC: metastatic prostatic cancer; MSI: microsatellite instability; MSI-H: high MSI; MTS: metastases; NGS: next-generation sequencing; NR: not reported; OR: odds ratio; PC: prostate cancer; PCR: Polymerase Chain Reaction analysis; PEM: pembrolizumab; pMMR: proficient MMR; PR: partial response; P-T: prior therapy; Ref: reference number; RP: radical prostatectomy; RT: radiation therapy; ST: sample type; st: strong; Subs: substitution; TILs: tumor-infiltrating immune cells; TILs: tumor infiltrating lymphocytes; TMA: tissue microarray; TMB: tumor mutation burden; TPS: Tumor proportion score; TURP: transurethral resection of the prostate; VUS: variant of unknown significance; we: weak; WES: whole exome sequencing; WPC: whole-prostate cryoablation. (#): the PD-L1 positivity rate refers to positivity in tumor cells. Petrylak et al. [19] found that 33/35 (94%) cases showed absent or <5% positivity in immune cells. Graff et al. [23] reported that 3/28 (11%) cases resulted PD-L1+ in TILs. Lindh et al. [43] described 10/34 (29%) ductal (1–20% cells) and 6/42 (14%) acinar (1–30% cells) cases with PD-L1+ immune cells. (°): only molecular features of patients with data concerning the MMR/MSI status are presented. Further information are provided in the original articles. (§): Other gene mutations (not limited to): *PTEN* (*n* = 1), *AR* (*n* = 4), *TP53* (*n* = 4), *SPOP* (*n* = 2), *BRCA2* (*n* = 3), *MYC* (*n* = 3), *ATM* (*n* = 4); it was unclear if there was another MSI-H case with *MSH6* mutations. (§§): DNA-repair genes examined included *BRCA2*, *ATM*, *FANCA*, *CHEK2*, *BRCA1*, *PALB2*, *HDAC2*, *RAD51*, *MLH3*, *ERCC3*, *MRE11*, *NBN*, *BARD1*, *BRIP1*, *CDK12*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*. (*): all the 6 patients were PD-L1+, except for case 2. DNA damage repair genes examined by Whole-Exome DNA Sequencing: *ATM*, *ATR*, *BAP1*, *BARD1*, *BLM*, *BRAP*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK12*, *CENPQ*, *CHEK1*, *CHEK2*, *EPCAM1*, *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC6*, *FAM175A*, *FAM175B*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *GEN1*, *HDAC2*, *MLH1*, *MLH3*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PIF1*, *PMS2*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*, *RDM1*, *TP53*, and *XRCC2*. (**): pure or mixed; (***) another case had an elevated rank (83) for PD-L1 by RNA-seq: only immune cells were PD-L1+. The mean ranks for PD-L1, PD-L2, and PD-1 by RNA-seq in all PC cases were 25, 27, and 18, respectively. (°°): *BRCA1/2* or *ATM* mutations (*n* = 19, 12%), mutations of other homologous recombination genes (*n* = 10, 7%), no homologous recombination gene mutations (*n* = 124, 81%). (°°°): 5/9 (55%) dMMR/MSI PCs in the upper quartile of TILs-density, and 3/5 (60%) showed >10 mutations (>90th percentile; 113-gene panel). None of pMMR samples in the upper quartile of TILs-density had pathogenic DNA repair defects (targeted NGS panel), while 2 pMMR

cases showed deleterious mutations in other pathways (*PIK3CA E542K*; *JAK1 E1051**). Some tumors without variants in MMR genes and with low MSINGS scores had dMMR-associated DNA mutation signatures (unclear significance). (@): not valid for: ≥ 1 MMR loss and PD-L1+ cancer cells (31.0% vs. 21.5%, $p = 0.340$); ≥ 1 MMR loss and PD-L1+ immune cells (18.2% vs. 23.5%, $p = 0.653$); ≥ 2 MMR loss and PD-L1+ immune cells (0% vs. 8%, $p = 0.135$). (ç): disease recurrence: MLH1, $p = 0.373$; MSH2, $p = 0.348$; MSH6, $p = 0.946$; PMS2, $p = 0.681$; ≥ 1 MMR loss, $p = 0.477$; ≥ 2 MMR loss, $p = 0.486$; ≥ 3 MMR loss, $p = 0.352$.