Concurrent RB1 and CDKN1A/p21(WAF1) truncating mutations (RW+) in bladder cancer show distinct genomic and immunological profiles suggestive of therapeutic strategies

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BACKGROUND

p53 target and cell cycle inhibitor CDKN1A/p21(WAF1) was initially not found to be mutated in cancer. TCGA analysis identified CDKN1A mutations are rare with frequencies of <1%, but much more common in bladder cancer (~8%). Truncating WAF1 mutations are associated with sensitivity to cisplatin and are associated with truncating Rb mutations in bladder cancer (RW+). We hypothesized RW+ bladder cancers may represent a unique subgroup with sensitivity to therapeutics.

METHODS

- A total of 1104 urothelial tumors underwent molecular profiling at Caris Life Sciences (Phoenix, AZ) utilizing NGS of DNA (592 Gene Panel, NextSeq, or WES, NovaSeq) and RNA (NovaSeq, WTS).
- Wilcoxon, Fisher's exact were used for statistical significance (p value without and q value with multi comparison correction).
- Immune cell fraction (QuanTIseq) and pathway analysis (ssGSEA) were assessed by mRNA analysis.
- Immune epitope prediction was performed using the NetMHCpan v4.0 method in the Immune Epitope Database.
- RSRD scores were calculated via mRNA expression per previously described and predicted the likelihood of a tumor's response to immunotherapy (Mcgrail et al., *Sci Transl Med*, 2021); HRD scores were assessed via incorporating BRCA1/2 mutational status and genomic scar score measured by WES (Sztupinszki et al., *npj breast cancer*, 2018).
- Tumors harboring only one RB1 or WAF1 mutation were excluded for further analysis.

RESULTS

- Concurrent truncating mutation (frameshift, nonsense) for RB1 and WAF1 were detected in 47 tumors (RW+, 4.25%) and tumors with wild-type status for both RB1 and WAF1 genes were classified as RW- group (54.08%).

Table 1. Association between RW+ alteration and clinicopathological features

	RW	RW+	q value
Count (N)	597	47	
Average Age (median)	71.5 (28 - >89)	70.7 (51 - >89)	0.576
Gender			
Male	72.7% (434/597)	80.9% (38/47)	0.2984
Female	27.3% (163/597)	19.1% (9/47)	
IO markers			
dMMR/MSI-H	2.2% (13/597)	0.0% (0/47)	0.7014
IHC-PD-L1 (22c3)	37.3% (214/574)	63.8% (30/47)	0.0024
TMB, median (mut/Mb)	8	11	0.002
TMB High	39.0% (232/595)	59.1% (26/44)	0.0232
Sites			
Primary	70.1% (312/445)	66.7% (30/45)	0.6314
Mets	29.9% (133/445)	33.3% (15/45)	

- When compared to RW- group, RW+ tumors showed lower mutation rate of TP53, ARID1A, and PIK3CA. Interestingly, RW+ was mutually exclusive with FGFR3 mutation.

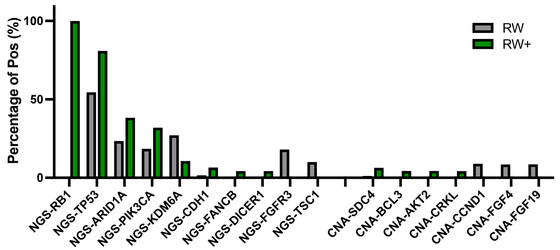

Figure 1. Association between RW+ alteration and clinicopathological features.

- E2F pathway (Normalized Enrichment Scores, NES: 0.89 vs 0.86, q<0.01) and DNA G2M checkpoint (NES: 0.89 vs 0.86, q<0.01) were found to be the most enriched in RW+ with respect to RW- group.

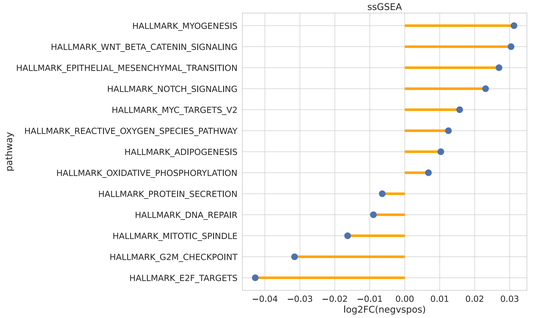

Figure 2. Differentially regulated pathways between RW+ subtype and RW- subtypes (logFC<0, enriched in RW+; logFC>0, enriched in RW).

- mRNA levels of FANCC/A, CHEK1, WEE1, CDC25A/C, PALB2 and BRCA1/2 were found to be overexpressed in RW+ group (q<0.05)

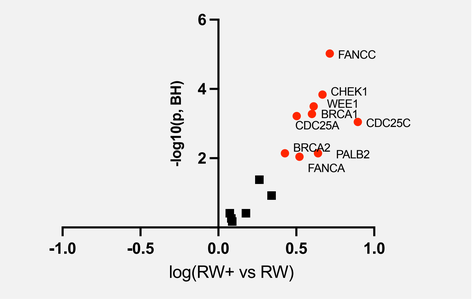

Figure 3. Differentially regulated genes in DNA damage/repair pathways between RW+ and RW subtypes.

- RW+ tumors also displayed a distinct immunological profile: They were associated with higher PD-L1 status, higher median TMB (Table 1) and with less frequent loss of heterozygosity for HLA-DPA1, with more high-binding-affinity neoantigen load to MHC proteins, consistent with the significantly more myeloid dendritic cells in RW+ group.

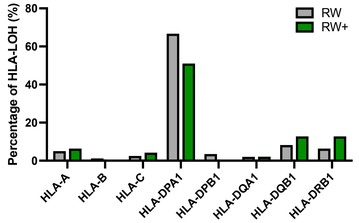

Table 2. Neoantigen load between RW+ vs RW (median displayed)

Binding Affinity	RW-	RW+	p value
High	3.90	4.78	<0.05
Intermediate	6.59	7.20	0.19
Low	17.04	19.09	0.09

Figure 4. Differential distribution of HLA subtypes between RW+ and RW.

- RW+ tumors had significantly more myeloid dendritic cell infiltrates and lower RSRD scores.

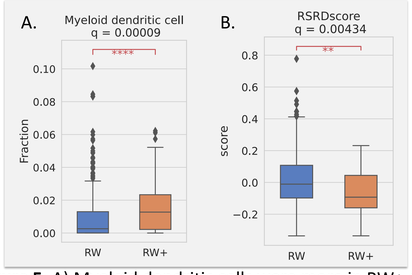

Figure 5. A) Myeloid dendritic cells were more in RW+ and B) RSRD scores was lower in RW+ subtype.

CONCLUSIONS

(RW+) bladder carcinomas have fewer p53, ARID1A, and PIK3CA mutations but are enriched for E2F targets, G2/M checkpoint genes, FANCC/A, CHEK1, WEE1, CDC25A/C, PALB2 and BRCA1/2 and have a distinct immunological profile.
The findings suggest therapeutic strategies for RW+ bladder cancers including Chk1/Wee1, PARP inhibitors, -/+ immunotherapy that may impact on clinical outcomes.