

DNA Damage Repair Pathway Alterations in Metastatic Clear Cell Renal Cell Carcinoma and Implications on Systemic Therapy

Supplementary materials

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eMethods:

- Studied Gene list for DNA Repair Pathways:
 1. Base Excision Repair: *MUTYH, PARP1, POLE*
 2. Checkpoint: *ATM, ATR, CHEK1, CHEK2, MDC1*
 3. Fanconi anemia: *BLM, BRIP1, FANCA, FANCC, PALB2, RAD51C*
 4. Homologous Recombination Repair: *BRCA1, BRCA2, MRE11, NBN, RAD50, RAD51, RAD51B, RAD51D, RAD52, RAD54L, RECQL4*
 5. Nucleotide Excision Repair: *ERCC2, ERCC3, ERCC4, ERCC5*
 6. Mismatch Repair: *MLH1, MSH2, MSH6, PMS1, PMS2*

Table S1. Details of biopsy sites used in the study					
Biopsy details	All patients (n=229)	I/O analysis (n=107)	TKI analysis (n=118)	Deleterious DDR (n=43)	DDR Wild type/VUS (n=186)
Pre-treatment	206 (90%)	94 (88%)	105 (89%)	37 (86%)	169 (91%)
Post-treatment	23 (10%)	13 (12%)	13 (11%)	6 (14%)	17 (9%)
Primary	140 (61%)	64 (60%)	75 (64%)	26 (61%)	114 (61%)
Metastatic site	89 (39%)	43 (40%)	43 (36%)	17 (39%)	72 (39%)
Abbreviations: DDR; DNA Damage Repair. I/O; Immune-Oncology. TKI; Tyrosine Kinase Inhibitor. VUS; Variants of Unknown Significance.					

Table S2. Details of deleterious DDR gene alterations

Gene	Alteration	Type of mutation	Germline or somatic	Pathway	FATHMM-MKL score for missense somatic mutation
<i>ATM</i>	S2394Qfs*12	Frameshift deletion	Somatic	Checkpoint	-
<i>ATM</i>	G1522A	Missense	Somatic	Checkpoint	0.98211
<i>ATM</i>	G2024R	Missense	Somatic	Checkpoint	0.99101
<i>ATM</i>	G2496*	Nonsense	Somatic	Checkpoint	-
<i>ATM</i>	K477Sfs*5	Frameshift deletion	Somatic	Checkpoint	-
<i>ATM</i>	L1194*	Frameshift deletion	Somatic	Checkpoint	-
<i>ATM</i>	N2646lfs*14	Frameshift deletion	Somatic	Checkpoint	-
<i>ATM</i>	Y1844*	Nonsense	Somatic	Checkpoint	-
<i>ATR</i>	I1096M	Missense	Somatic	Checkpoint	0.85245
<i>BLM</i>	K167R	Missense	Somatic	Fanconi Anemia	0.88656
<i>BRCA2</i>	K1964*	Nonsense	Somatic	Homologous Recombination Repair	-
<i>BRCA2</i>	N2781D	Missense	Somatic	Homologous Recombination Repair	1.0
<i>BRCA2</i>	S1982Rfs*22	Frameshift deletion	Germline	Homologous Recombination Repair	-
<i>CHEK1</i>	Q80H	Missense	Somatic	Checkpoint	0.95214
<i>CHEK2</i>	Fusion	Fusion	Germline	Checkpoint	-
<i>CHEK2</i>	I157T	Missense	Germline	Checkpoint	-
<i>CHEK2</i>	I157T	Missense	Germline	Checkpoint	-
<i>CHEK2</i>	K255Sfs*20	Frameshift deletion	Somatic	Checkpoint	-
<i>CHEK2</i>	K373N	Missense	Somatic	Checkpoint	0.92262
<i>CHEK2</i>	L280*	Frameshift deletion	Somatic	Checkpoint	-
<i>CHEK2</i>	S428F	Missense	Germline	Checkpoint	-
<i>CHEK2</i>	S428F	Missense	Germline	Checkpoint	-
<i>CHEK2</i>	T367Mfs*15	Frameshift deletion	Germline	Checkpoint	-
<i>CHEK2</i>	Y72*	Nonsense	Germline	Checkpoint	-
<i>ERCC4</i>	V510Afs*9	Frameshift deletion	Somatic	Nucleotide Excision Repair	-
<i>ERCC5</i>	L878V	Missense	Somatic	Nucleotide Excision Repair	0.97604

<i>ERCC5</i>	P925Lfs*57	Frameshift deletion	Somatic	Nucleotide Excision Repair	
<i>FANCA</i>	R880*	Nonsense	Somatic	Fanconi Anemia	-
<i>MLH1</i>	E199Dfs*4	Frameshift deletion	Somatic	Mismatch Repair	-
<i>MLH1</i>	G221*	Nonsense	Somatic	Mismatch Repair	-
<i>MSH2</i>	I794T	Missense	Somatic	Mismatch Repair	0.95504
<i>MSH6</i>	G652W	Missense	Somatic	Mismatch Repair	0.89919
<i>MSH6</i>	R911*	Nonsense	Germline	Mismatch Repair	-
<i>MSH6</i>	S280N	Missense	Somatic	Mismatch Repair	0.94347
<i>MSH6</i>	X152_splice	Splice variant	Somatic	Mismatch Repair	-
<i>MUTYH</i>	R56Gfs*21	Frameshift deletion	Germline	Base Excision Repair	-
<i>MUTYH</i>	G382D	Missense	Germline	Base Excision Repair	-
<i>MUTYH</i>	G382D	Missense	Germline	Base Excision Repair	-
<i>MUTYH</i>	G382D	Missense	Germline	Base Excision Repair	-
<i>NBN</i>	A713Gfs*29	Frameshift insertion	Somatic	Homologous Recombination Repair	-
<i>NBN</i>	G206Lfs*26	Frameshift insertion	Somatic	Homologous Recombination Repair	-
<i>NBN</i>	S240Cfs*8	Frameshift deletion	Somatic	Homologous Recombination Repair	-
<i>PALB2</i>	R975S	Missense	Somatic	Fanconi Anemia	0.58485
<i>PARP1</i>	G61D	Missense	Somatic	Base Excision Repair	0.84334
<i>RAD50</i>	R726C	Missense	Somatic	Homologous Recombination Repair	0.97082
<i>RAD51C</i>	M1?	Non-start	Somatic	Fanconi Anemia	-
<i>RECQL4</i>	P466R	Missense	Somatic	Homologous Recombination Repair	0.96481
<i>RECQL4</i>	X464	Missense	Germline	Homologous Recombination	-

				Repair	
Abbreviations: FATHMM-MKL; Functional Analysis Through Hidden Markov Models- Multiple Kernel Learning					

Type of DDR alteration	N (available RECIST)	CR	PR	SD	PD
Deleterious DDR	16	2 (12.5%)	4 (25%)	4 (25%)	6 (37.5%)
Wild type and VUS DDR	78	2 (2.6%)	22 (28.2%)	34 (43.6%)	20 (25.6%)

Abbreviations: CR; Complete Response. DDR; DNA Damage Repair. I/O; Immune-Oncology. PR; Partial Response. PD; Progressive disease. RECIST; Response Evaluation Criteria in Solid Tumors. SD; Stable Disease. VUS; Variants of Unknown Significance.

Type of DDR alteration	N (available RECIST)	CR	PR	SD	PD
Deleterious DDR	18	0 (0%)	6 (33.3%)	9 (50%)	3 (16.7%)
Wild type and VUS DDR	67	3 (4.5%)	28 (41.8%)	31 (46.2%)	5 (7.5%)

Abbreviations: CR; Complete Response. DDR; DNA Damage Repair. PR; Partial Response. PD; Progressive disease. RECIST; Response Evaluation Criteria in Solid Tumors. SD; Stable Disease. TKI; Tyrosine Kinase Inhibitor. VUS; Variants of Unknown Significance. VEGF; Vascular Endothelial Growth Factor.

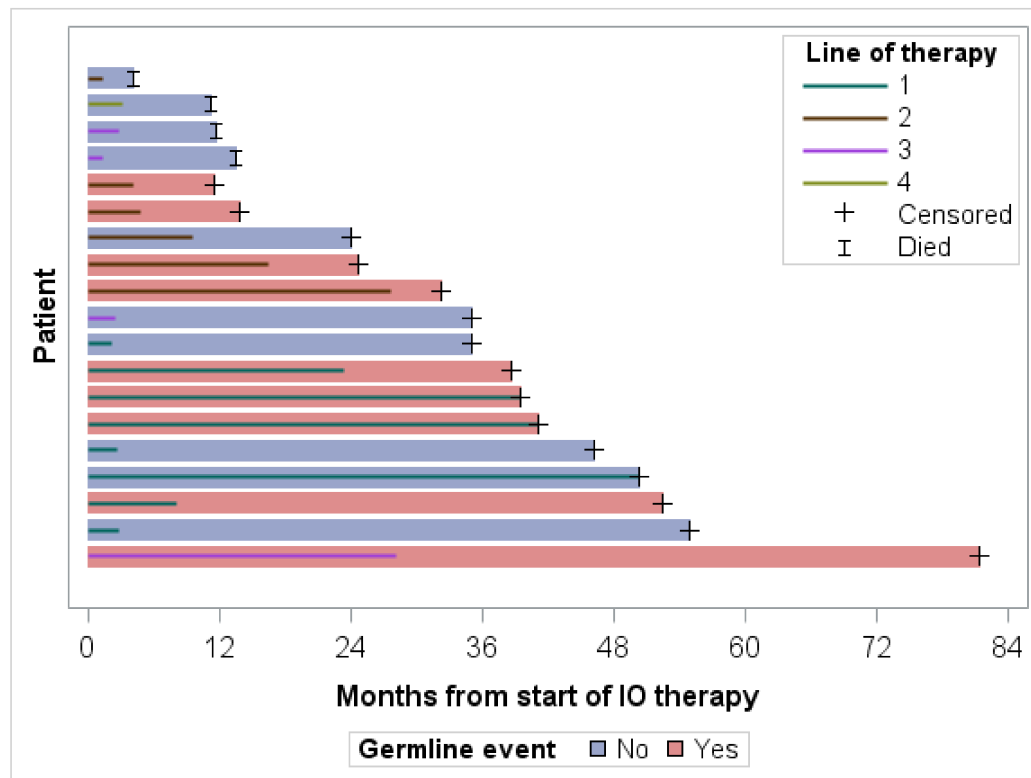


Figure S1. Swimmer's Plot depicting the time course of therapy for 19 patients with deleterious DDR alteration in the I/O therapy cohort.

Abbreviations: DDR; DNA Damage Repair. I/O; Immune-Oncology.

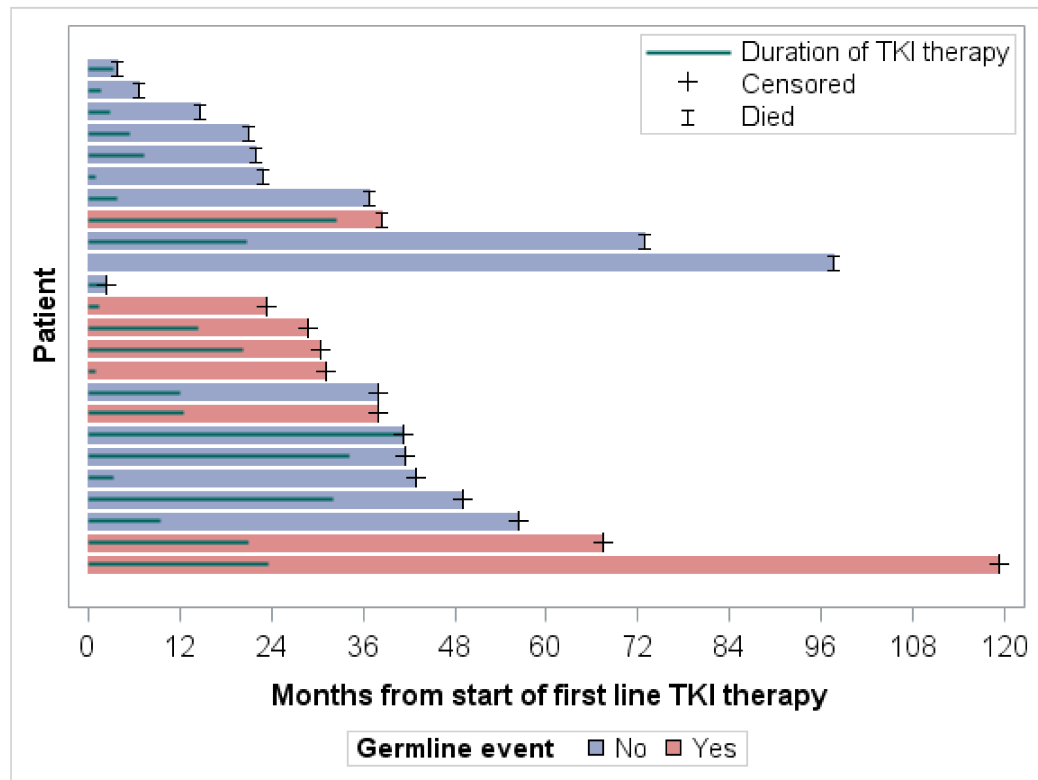


Figure S2. Swimmer's Plot depicting the time course of therapy for 24 patients with deleterious DDR alteration in the TKI therapy cohort.

Abbreviations: DDR; DNA Damage Repair. TKI; Tyrosine Kinase.

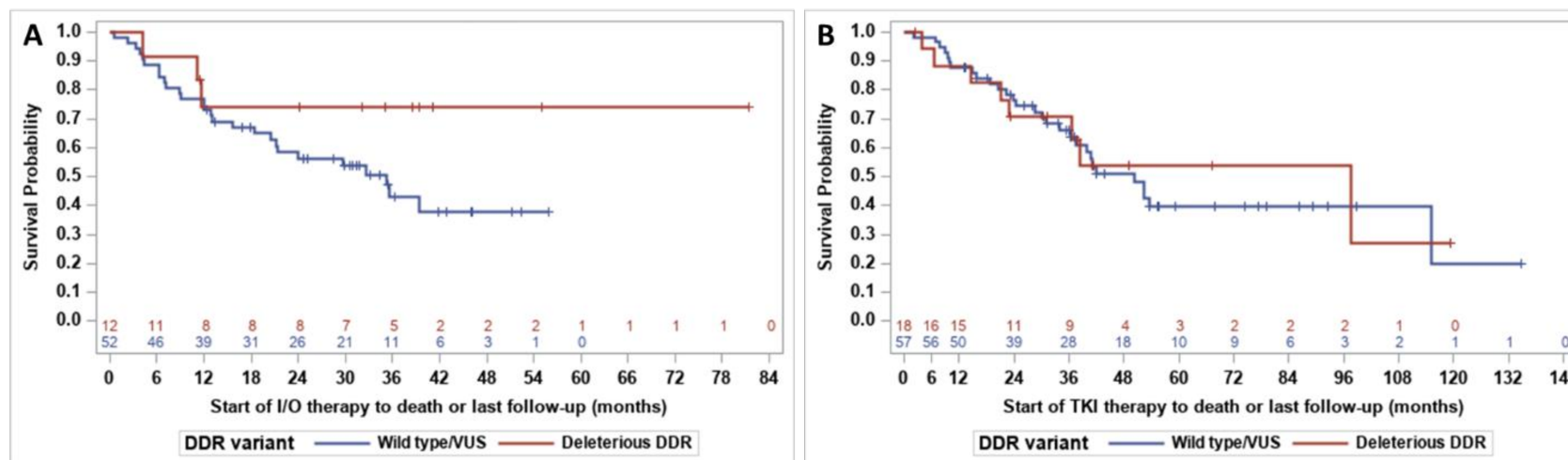


Figure S3. Kaplan-Meier estimate of OS in patients who underwent NGS of the primary tumor in (A) I/O-treated patients and (B) VEGF-TKI treated patients

Abbreviations: NGS; Next Generation Sequencing. I/O; Immune-Oncology. OS; Overall survival. VEGF; Vascular Endothelial Growth Factor. TKI; Tyrosine Kinase Inhibitor.

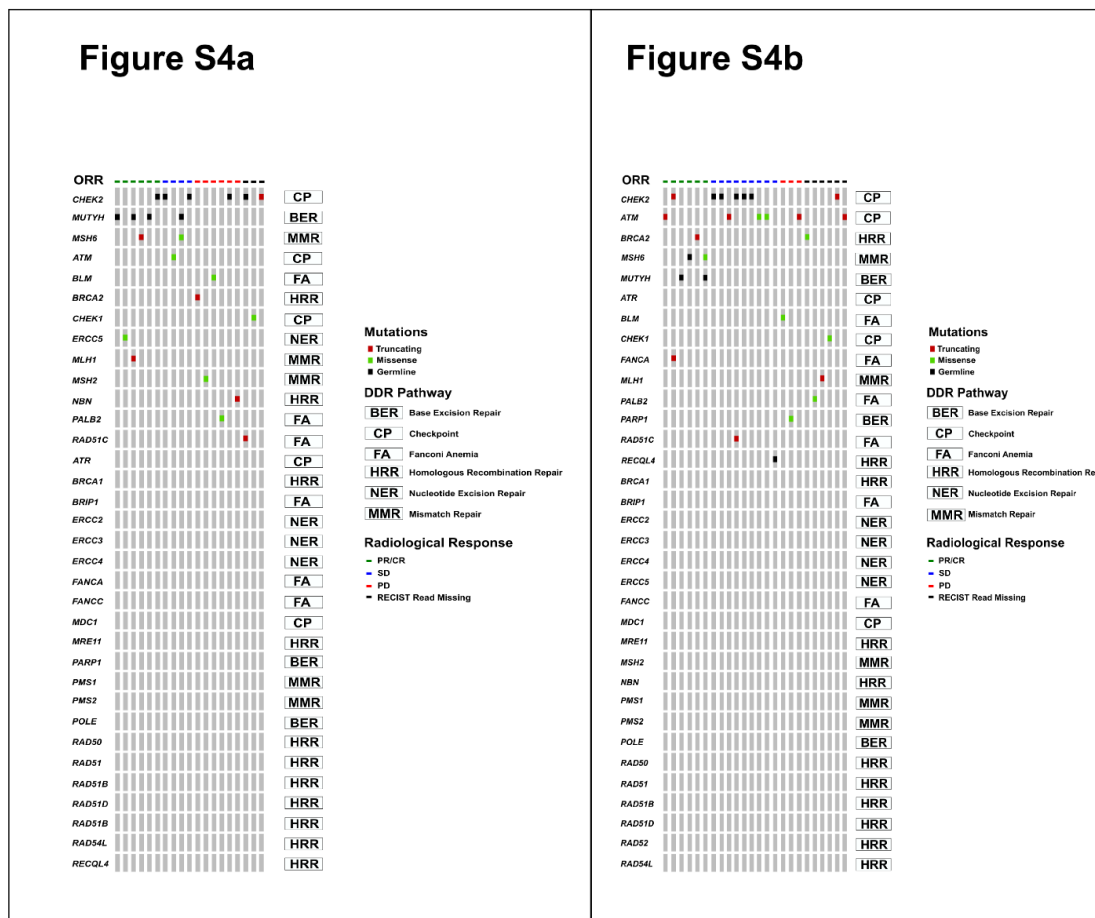


Figure S4. Co-mutation plot of deleterious DDR gene alterations and relevant pathways in the I/O (A) and TKI analysis (B) with the associated radiological responses. Each column represents an individual patient. Abbreviations: CR; Complete Response. DDR; DNA Damage Repair. I/O; Immune-Oncology. ORR; Objective Response Rate. PR; Partial Response. PD; Progressive Disease. RECIST; Response Evaluation Criteria in Solid Tumors. SD; Stable Disease. TKI; Tyrosine Kinase Inhibitor.

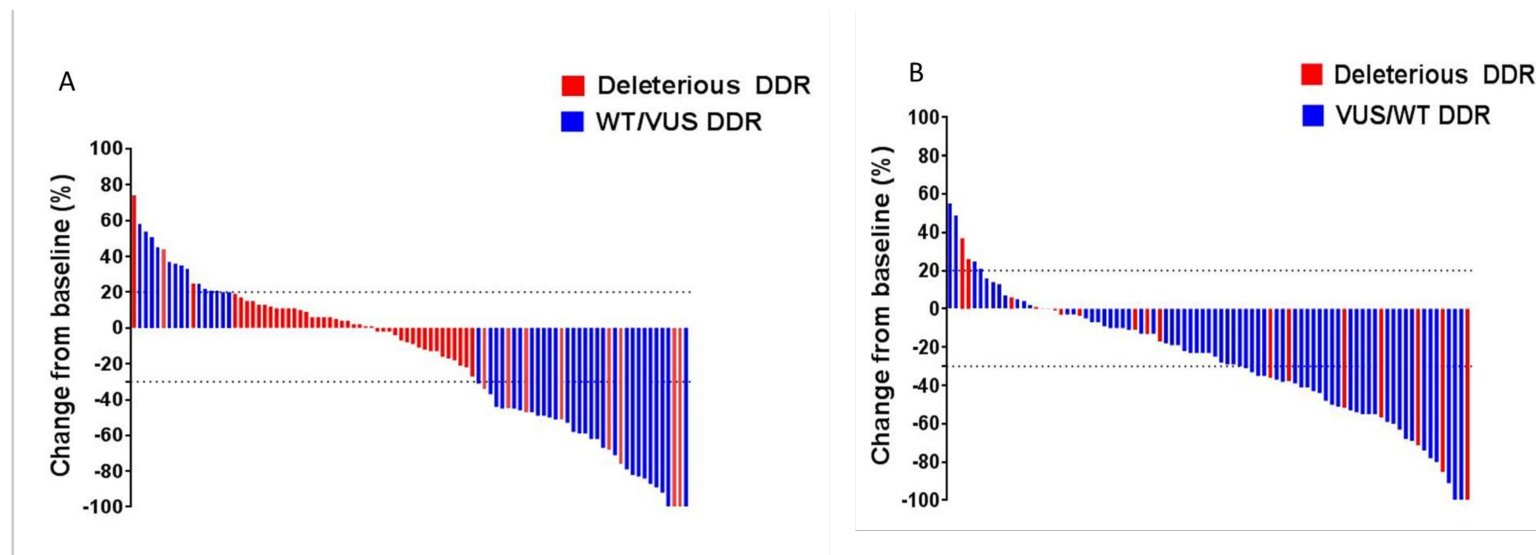


Figure S5. Waterfall Plot of ORR in patients treated with I/O (A) and VEGF-TKI (B) by underlying DDR gene alterations (Deleterious DDR vs. Wild type/VUS DDR). Abbreviations: DDR; DNA Damage Repair. I/O; Immune-Oncology. ORR; Objective Response Rate. TKI; Tyrosine Kinase Inhibitor. VEGF; Vascular Endothelial Growth Factor. VUS; Variants of Unknown Significance.