Hematological disorders after salvage PARPi treatment for ovarian cancer: cytogenetic and molecular defects and clinical outcomes

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Supplementary Materials and Methods

Ion Torrent Sequencing with the custom Myelo-panel

Targeted capture was carried out according to the manufacturer's protocols using the AmpliSeq kit with a custom panel, called the Myelo-panel, which analyzes 255 cancer-predisposing genes, as described in the main manuscript. Sequencing was performed with the Ion S5 system (ThermoFisher Scientific). Our sequencing libraries have a median read length of 215 bps.

Ion Torrent sequencing Variant analysis was performed with IonReporter software (ThermoFisher Scientific).

For identification of somatic variants from peripheral blood cells, buccal DNA samples were used as normal reference for each patient in order to subtract each individual germline variants. Moreover, we selected: i) variants in exonic regions of the genome and affecting the coding sequence: non-synonimous, stop-gain and stop/start loss SNVs, frameshift Indels; ii) variants with VAF of the alternative allele $<40\%$; iii) finally, in order to filter for germline polymorphisms, we removed any variant reported in any population database (ESP6500, ExAC, gnomAD, 1000 Genomes Phase 3, dbSNP) with a frequency >0.005.

For identification of germline variants from buccal DNA, we selected: i) variants in exonic regions of the genome and affecting the coding sequence: non-synonimous, stop-gain and stop/start loss SNVs, frameshift Indels; ii) we select variants with allelic depth (AD, reads supporting the alternative allele) $\geqslant 5$ reads and with VAF of the alternative allele $\geqslant 20\%$; iii) finally, in order to filter for germline polymorphisms, we removed any variant reported in any population database (ESP6500, ExAC, gnomAD, 1000 Genomes Phase 3, dbSNP) with a frequency >0.005.

Identified variants were annotated in terms of pathogenicity, using different computational tools, including ClinVar, Varsome and RENOVO.

Supplementary Tables

Table S1. List of genes included in the Oncomine Myeloid Research Assay

Hotspot genes (23)		Full genes (17)		Fusion driver genes (29)			Expression genes (5)	Expression control genes (5)
ABL1	KRAS	ASXL1	PRPF8	ABL1	HMGA2	NUP214	BAALC	EIF2B1
BRAF	MPL	BCOR	RB1	ALK	JAK2	PDGFRA	MECOM	FBXW2
CBL	MYD88	CALR	RUNX1	BCL2	KMT2A (MLL)	PDGFRB	MYC	PSMB2
CSF3R	NPM1	CEBPA	SH2B3	BRAF	MECOM	RARA	SMC1A	PUM1
FLT3	NRAS	ETV6	STAG2	CCND1	MET	RBM15	WT1	TRIM27
GATA2	PTPN11	EZH2	TET2	CREBBP	MLLT10	RUNX1		
HRAS	SETBP1	IKZF1	TP53	EGFR	MLLT3	TCF3		
IDH1	SF3B1	NF1	ZRSR2	ETV6	MYBL1	TFE3		
IDH2	SRSF2	PHF6		FGFR1	MYH11			
JAK2	U2AF1			FGFR2	NTRK3			
KIT	WT1			FUS				
DNMT3A								

Table S2. List of genes and pharmacogenomics single nucleotide polymorphisms (SNPs) included in our custom Myelo Panel

ABCB7	*CCND1*	*ERCC2*	*GNA11*	*MAPK8*	*PARN*	*REV7*	*SMARCB1*
ABL1	*CCND2*	*ERCC3*	*GNAQ*	*MASTL*	*PAX5*	*RFWD3*	*SMC1A*
ABL2	*CCND3*	*ERCC4*	*GP1BA*	*MDM2*	*PDGFB*	*RICTOR*	*SMC3*
ACBD5	*CCNE1*	*ERCC6L2*	*HAX1*	*MEF2B*	*PDGFRA*	*ROS1*	*SMO*
ACD	*CDAN1*	*ESR1*	*HDAC9*	*MEF2BNB-MEF2B*	*PDGFRB*	*RPL11*	*SMOX*
AK2	*CDK4*	*ETV6*	*HGF*	*MET*	*PDSS2*	*RPL15*	*SRC*
AKR1B1	*CDK6*	*EZH2*	*HNRNPK*	*MGMT*	*PGF*	*RPL23*	*SRP72*
AKT1	*CDKN1B*	*FANCA*	*HOXA11*	*MIR142*	*PGR*	*RPL26*	*SRSF2*
AKT2	*CDKN2A*	*FANCB*	*HRAS*	*MITF*	*PHF6*	*RPL27*	*STAG2*
AKT3	*CDKN2B*	*FANCC*	*HSP90AA1*	*MLH1*	*PIK3C2B*	*RPL31*	*STK11*
ALAS2	*CDKN2C*	*FANCD2*	*ID3*	*MMP2*	*PIK3CA*	*RPL35A*	*STK4*
ALK	*CEBPA*	*FANCE*	*IDH1*	*MPL*	*PIK3CB*	*RPL36*	*SYK*
ANKRD26	*CHEK2*	*FANCF*	*IDH2*	*MSH2*	*PIK3CD*	*RPL4*	*TAOK1*
AP3B1	*CHEK2 (CHK2)*	*FANCG*	*IGF1R*	*MSH6*	*PIK3R1*	*RPL5*	*TAOK2*
APC	*CREBBP*	*FANCI*	*IGF2*	*MST1R*	*PIK3R2*	*RPS10*	*TAZ*
AR	*CSF1R*	*FANCL*	*IKZF1*	*MTOR*	*PIP5K1A*	*RPS15*	*TCIRG1*
ARAF	*CSF3R*	*FANCM*	*JAGN1*	*MXRA5*	*PLCG2*	*RPS17*	*TEK*
ASXL1	*CTC1*	*FANCP*	*JAK1*	*MYC*	*PML*	*RPS19*	*TERC*
ATG2B/GSKIP	*CTNNB1*	*FANCQ*	*JAK2*	*MYCN*	*PMS2*	*RPS24*	*TERT*
ATM	*CXCR4*	*FBXW7*	*JAK3*	*MYD88*	*POT1*	*RPS26*	*TET2*
ATR	*DDR1*	*FGF3*	*KDR*	*MYH9*	*PRKCZ*	*RPS27*	*TINF2*
AURKA	*DDR2*	*FGF4*	*KIT*	*NAF1*	*PTCH1*	*RPS27A*	*TOP2A*
AURKB	*DDX41*	*FGFR1*	*KLF1*	*NBN (NBS1)*	*PTEN*	*RPS28*	*TP53*
AURKC	*DKC1*	*FGFR2*	*KMT2A*	*NCOR2*	*PTPDC1*	*RPS29*	*TSC1*
BAP1	*DNAJC21*	*FGFR3*	*KMT2D*	*NF1*	*PTPN11*	*RPS7*	*TSC2*
BCL2	*DNMT1*	*FGFR4*	*KRAS*	*NF2*	*PUS1*	*RTEL1*	*TYK2*
BCOR	*DNMT3A*	*FH*	*LAMTOR*	*NHP2*	*RAB27A*	*RUNX1*	*U2AF1*
BCR	*DOT1L*	*FKBP5*	*LCK*	*NOP10*	*RAC1*	*SAMD9*	*UBE2T*
BLM (RECQL3)	*EGFR*	*FLCN*	*LIG4*	*NOTCH1*	*RAC2*	*SAMD9L*	*USB1*
BRAF	*ELANE*	*FLT1*	*LYN*	*NOTCH2*	*RAD21*	*SBDS*	*VEGFA*
BRCA1	*EPCAM*	*FLT3*	*LYST*	*NOTCH3*	*RAD50*	*SEC23B*	*VEGFB*
BRCA2	*EPHA1*	*FLT4*	*MAP2K1*	*NOTCH4*	*RAD51*	*SF3B1*	*VPS13B*
BRIP1	*EPHA2*	*FUS*	*MAP2K2*	*NPM1*	*RAD51C*	*SF3B2*	*VPS45*
BTK	*EPHA3*	*FYN*	*MAP2K4*	*NRAS*	*RAF1*	*SH2B3*	*WAS*
C15orf41	*EPHA4*	*G6PC3*	*MAP3K1*	*NTRK1*	*RARA*	*SLC19A2*	*WRAP53*
CALR	*EPHB2*	*GATA1*	*MAP3K11*	*NTRK2*	*RBBP6*	*SLC25A38*	*WT1*
CBFB	*ERBB2*	*GATA2*	*MAP3K4*	*NTRK3*	*RBM8A*	*SLC37A4*	*XRCC2*
CBL	*ERBB3*	*GFI1*	*MAP4K1*	*PALB2*	*RECQL*	*SLTM*	*YES1*

CBX7	*ERBB4*	*GLRX5*	*MAPK1*	*PAPD5*	*RET*	*SLX4*	*rs1045642*
rs2032582	*rs9024*	*rs11572080*	*rs121434569*	*rs1138272*	*rs12948783*	*rs10981694*	*rs6755571*
rs1128503	*rs8133052*	*rs3892097*	*rs11568315*	*rs9274407*	*rs1042858*	*rs4795541*	*rs6431558*
rs246240	*rs9344*	*rs2070673*	*rs2227983*	*rs12654264*	*rs9937*	*rs4149015*	*rs3832043*
rs3740066	*rs2072671*	*rs2740574*	*rs712829*	*rs17583889*	*rs2898950*	*rs4149056*	*rs1801019*
rs717620	*rs532545*	*rs35599367*	*rs2234922*	*rs430397*	*rs1561876*	*rs2306283*	*rs25487*
rs2273697	*rs602950*	*rs776746*	*rs1136201*	*rs6313*	*rs1042919*	*rs11045585*	*rs1382368*
rs1051640	*rs60369023*	*rs17574269*	*rs11615*	*rs2075252*	*rs1130609*	*rs4880*	*rs8060157*
rs9561778	*rs3215400*	*rs2297595*	*rs3212986*	*rs1801133*	*rs5030743*	*rs2302948*	*rs20572*
rs16950650	*rs4646316*	*rs1801159*	*rs13181*	*rs1801131*	*rs1265138*	*rs10426377*	*rs4244285*
rs2231137	*rs9332377*	*rs17376848*	*rs2207396*	*rs1799983*	*rs1979277*	*rs1042522*	*rs121434568*
rs2494752	*rs4646*	*rs67376798*	*rs396991*	*rs2070744*	*rs9514091*	*rs1142345*	*rs518329*
rs7921977	*rs1048943*	*rs1801265*	*rs2297480*	*rs1800566*	*rs1051266*	*rs1800460*	*rs1695*
rs2227310	*rs1056836*	*rs55886062*	*rs1736557*	*rs1143684*	*rs11231825*	*rs12201199*	*rs885004*
rs12415607	*rs3745274*	*rs1801158*	*rs12613732*	*rs10932125*	*rs12210538*	*rs34489327*	*rs4148323*
rs4353229	*rs3211371*	*rs9981861*	*rs9679162*	*rs17626122*	*rs714368*	*rs34743033*	
rs1127687	*rs12721655*	*rs4444903*	*rs1806201*	*rs870995*	*rs316019*	*rs8175347*	

Table S3: Sequencing parameters for Ion Torrent Sequencing with the Oncomine Myeloid Research Assay (reference genome: GRCh37)

Sample ID*	Total Nr Reads	Nr mapped reads	On target (%)	Mean coverage (min-max)	Uniformity of coverage (%)	Percentage of targeted bases# with coverage ≥500X
1	3,088,528	3,087,305	97.46	5,981 (41-14,067)	97.69	99.37
2	3,419,776	3,408,433	97.51	6,533 (18-27,202)	98.66	99.45
3	1,803,589	1,799,444	97.37	3,482 (10-5,953)	98.85	99.41
4	1,075,496	1,070,929	98.30	2,009 (3-5,063)	96.87	96.04
5	4,376,463	4,373,835	97.76	8,472 (106-10,800)	96.48	99.45
6	1,972,494	1,967,232	98.42	3,847 (67-8,203)	98.09	99.22
7	2,544,926	2,543,588	96.74	4,834 (44-8,136)	98.35	99.42
8	1,688,031	1,684,264	98.14	3,249 (34-8,576)	98.18	98.61
9	2,385,941	2,379,140	98.36	4,660 (59-8,899)	98.81	99.22
10	ND	ND	ND	ND	ND	ND
11	4,030,479	4,021,159	99.24	7,971 (55-15,906)	98.34	99.23
12	1,696,798	1,693,474	98.12	3,265 (8-7,174)	98.71	99.01
13	1,789,935	1,785,899	97.75	3,469 (13-6,136)	98.94	99.44
14	3,102,671	3,095,967	98.02	6,016 (14-10,300)	98.87	99.44
15	ND	ND	ND	ND	ND	ND
16	ND	ND	ND	ND	ND	ND

*Sample ID according to Table 2 of the main manuscript.

#Targeted bases consist of bases amplified with the commercially available Oncomine Myeloid Research Assay

ND: not done.

Table S4: Sequencing parameters for Ion Torrent Sequencing with the custom Myelo-Panel (reference genome: GRCh38)

Sample ID*	Total Nr Reads	Nr mapped reads	On target (%)	Mean coverage (min-max)	Uniformity of coverage (%)	Percentage of targeted bases# with coverage ≥500X
1T	10,799,016	10,747,798	98.46	1,448 (1-4,013)	95.87	92.14
1N	4,310,982	4,288,725	74.09	437.1 (1-4,976)	55.15	54.88
2T	11,534,090	11,473,171	98.52	1,552 (1-4,059)	96.29	93.84
2N	6,886,552	6,850,150	86.71	817,3 (1-3,362)	95.15	73.36
3T	11,437,967	11,389,263	98.72	1,550 (1-4,154)	95.90	93.23
3N	7,320,156	7,286,553	92.28	925.2 (1-3,147)	95.51	80.13
4T	12,761,980	12,704,142	98.71	1,726 (1-4,619)	95.98	94.06
4N	7,916,747	7,875,735	91.86	898.9 (1-2,968)	95.55	83.57
5T	12,985,143	12,918,463	98.33	1,732 (1-5,034)	95.59	93.41
5N	9,476,323	9,401,385	60.76	778.9 (1-3,524)	95.02	70.60

*Sample ID according to Table 5 of the main manuscript.

#Targeted bases consist of bases amplified by 7296 custom Amplicons covering the coding regions of the 255 genes included in our custom Myeloid-panel

T: Tumor, peripheral blood; N: Normal, buccal DNA.