Rapid prediction of olaparib sensitivity for variants of unknown significance observed in homologous repair genes

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Background

The recent use of PARP inhibitors in clinical practice gives very interesting outcome for ovary tumors with *BRCA1* or *BRCA2* mutation but also in other tumors with homologous repair deficiency. Nevertheless, no hotspot mutations are present in these genes. Consequently, it is very difficult to classify the pathogenicity of a variant. Indeed, 80 to 85% of observed variants have unknown significance, blocking the use of PARP inhibitor.

Materials and methods

Population

At the Georges-François Leclerc Cancer Center, we analyzed a retrospective cohort of 27 patients treated with olaparib initiated between 2015 and 2017.

DNA extraction

DNA was isolated from archival tumor tissue using the Maxwell 16 FFPE Plus LEV DNA purification kit (Promega). DNA from whole blood (germline DNA) was isolated using the Maxwell 16 Blood DNA Purification Kit (Promega) following the manufacturer's instructions. Quantity of extracted genomic DNA was assessed by a fluorimetric method with the Qubit device.

Exome sequencing

Library preparation was done with the Agilent SureSelectXT All Exon v5 reagent kit (Agilent Technologies) by following manufacturer's instructions and normalized libraries were pooled and sequenced on an Illumina NextSeq500 device in 2*121bp,

Tumor DNA sequencing generated mean target coverages of 80X and a mean of more than 90% of the target sequence was covered with a read depth of at least 10X.

Exome analysis

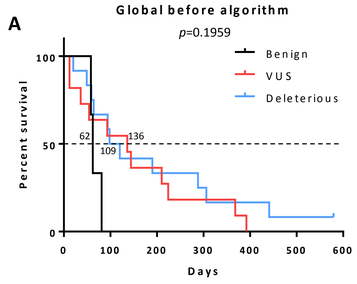
Raw DNA sequencing data were aligned to the hg19 genome build using the Burrows-Wheeler Aligner (BWA) version 0.7.15. Duplicates were marked with Picard version 2.5.0. Base quality score recalibration and variant calling were performed using GATK tools version 3.6.

For SNV (Single Nucleotide Variation), annotation was performed using the VariantStudio (V3) Illumina software. Filters of candidate variants included: coverage depth of 10X or greater and a variant nucleotide allelic fraction in tumor DNA greater than 10%.

Analysis of mutations

For homologous recombination genes, each mutation was manually reviewed by a molecular biologist and the mutation was interpreted as benign, pathogenous or likely pathogenous using ClinVar, ExPASy and dbSNP databases. For variants of unknown significance (VUS), PROVEAN software (http://provean.jcvi.org/seq_submit.php) and allelic frequency normalized with tumor cellular content were used to classify VUS. A VUS was classified as potentially benign (resistant to olaparib) when PROVEAN predicted NEUTRAL and allelic frequency revealed an enrichment of mutated allele present in less than 40% of the tumor. A VUS was classified as potentially deleterious (sensible to olaparib) when one of both predictions classified as deleterious (PROVEAN) and/or mutation enriched in more than 40% of tumor (ratio allelic frequency/HES > 0.70).

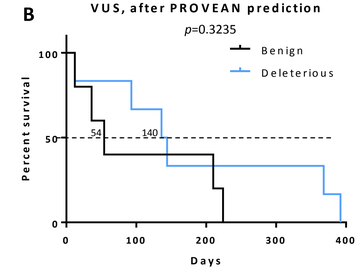
Results



On the 27 patients analyzed, 3 harbored a benign variant, 13 had a deleterious mutation, and 11 had VUS. The Progression Free Survival (PFS) analysis under olaparib (Figure A) showed that olaparib was not efficient on benign variant, whereas patients with a deleterious variant or VUS had a similar PFS, suggesting that VUS could be sensitive to olaparib.

Among the 11 patients with VUS, the PROVEAN website classified 5 patients as benign variant carriers and 6 as deleterious variant carriers (Table 1), with a median PFS of 54 days and 140 days (p=0.3235), respectively (Figure B).

With the prediction based on the allelic frequency, we classified 6 patients as benign variant carriers and 5 as deleterious variant carriers (Table 1), with a median PFS of 73.5 days and 210 days (p=0.29), respectively (Figure C).



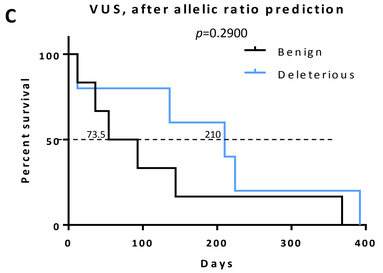
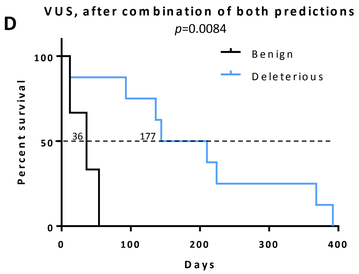
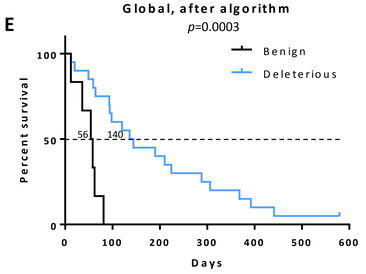


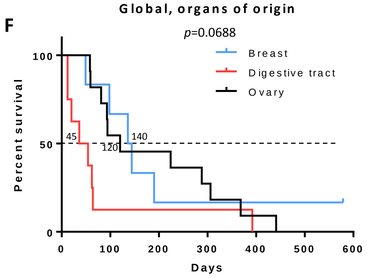
Table 1: Prediction of classification for 11 patients with VUS

Patient	Genes	Protein variation	PROVEAN prediction	Allelic ratio prediction	Final prediction	PFS (d)
#01	PALB2	p.Glu907Lys	Benign	Benign (0.67)	Benign	36
#02	PALB2	p.Asp219Gly	Benign	Deleterious (1.12)	Deleterious	224
#03	BRCA1 UIMC1	p.Arg841Trp p.Tyr564His	Deleterious Deleterious	Deleterious (1.48) Deleterious (0.91)	Deleterious	12
#04	BRCA1	p.Gly928Val	Deleterious	Deleterious (1.04)	Deleterious	136
#05	PALB2 RAD50 RAD51C	p.Pro811Ser p.Gln1014Arg p.Ala195Val	Benign Benign Benign	Benign (0.53) Benign (0.54) Deleterious (0.72)	Deleterious	210
#06	BRCA1	p.Met1689Leu	Benign	Benign (0.34)	Benign	54
#07	BRCA1	p.Glu1765Asp	Benign	Benign (0.27)	Benign	12
#08	BRIP1	p.Met1?	Deleterious	Deleterious (0.96)	Deleterious	392
#09	BRCA1	p.Ser915Phe	Deleterious	Benign (0.14)	Deleterious	368
#10	BRCA2 RAD51D	p.Leu1620Phe p.Asp90Asn	Deleterious Deleterious	Benign (0.44) Benign (0.16)	Deleterious	144
#11	BRCA2	p.Met990Lys	Deleterious	Benign (0.26)	Deleterious	93

As both predictions were discordant for 6 VUS (representing 5 patients), we decided to pool both prediction results and classify a VUS as deleterious when one of the 2 predictions classified as deleterious. Moreover, when a patient harbored VUS on 2 or 3 different genes, we classified a patient as carrier of a deleterious variation as soon as a deleterious prediction was observed whatever the prediction tool (Table 1).







Among the 11 patients with VUS, we finally classified 3 patients as carriers of benign variations and 8 patients as carriers of deleterious variants potentially sensitive to olaparib. The PFS analysis with the final prediction (Table 1) showed a significant difference between both groups (p=0.0084). Indeed, the median PFS of the benign group was 36 days, whereas it was of 177 days for the deleterious group (Figure D).

Finally, when we compiled the variants whose significance was known at the time of diagnosis with the VUS predicted thanks to our algorithm, we observed a significant difference in PFS between benign and deleterious groups (p=0.0003). Indeed, the median PFS were 56 days and 140 days for benign and deleterious group, respectively (Figure E).

It is nevertheless important to notice that our population of 27 patients was constituted by 12 ovary cancers (VUS #02, 09, 11), 6 breast cancers (VUS #04, 10), 5 pancreatic cancer (VUS #03, 06, 07, 08), 3 colorectal cancers (VUS #01), and 1 basal cell carcinoma (VUS #05). Probably due to the performance status at the time of treatment, digestive tract cancers presented a worse PFS (45 days) than ovary (120 days) and breast (140 days) cancers (Figure F), even if these median PFS differences were not statistically significant (p=0.0688) (Figure F).

Conclusion

This work showed that it is possible to classify VUS of homologous recombination genes observed at the tumor level to predict efficiency of olaparib. With these very enthusiastic results, we currently search partners to open a prospective clinical trial in order to test the efficiency of our algorithm in real life.

To go further, thanks to our algorithm of prediction and response to olaparib observed in treated patients, it may be possible to bring new arguments for the classification of germline VUS in the context of genetic predisposition to cancer.

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