Original Article

Transcriptome analysis reveals overlap in fusion genes in a phase I clinical cohort of TNBC and HGSOC patients treated with buparlisib and olaparib

Running Title: Transcriptome and fusion gene landscape in TNBC and HGSOC

Julia Eismann[1,2,3], Yujing J. Heng[2,4], Johannes M. Waldschmidt[2,5], Ioannis S. Vlachos[2,4], Kathryn Gray[2,6], Ursula Matulonis[2,5], Panos A. Konstantinopoulos[2,5], Charles J. Murphy[7], Sheida Nabavi[8], Gerburg Wulf[1,2]

Affiliations:

[1] Department of Hematology/Oncology, Beth Israel Deaconess Medical Center, Boston, MA
[2] Harvard Medical School, Boston, MA
[3] Department of Obstetrics and Gynecology, University Medical Center Freiburg, Freiburg, Germany
[4] Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA
[5] Department of Medical Oncology, Dana-Faber Cancer Institute, Boston, MA
[6] Biostatic Core, Dana Faber, Harvard Cancer Center, Boston, Massachusetts
[7] Institute for Computational Biomedicine, Weill Cornell Medical College, New York, New York
[8] Department of Computer Science and Engineering, Institute of System Genomics, University of Connecticut

Correspondence: Gerburg Wulf, MD, PhD, e-mail: gwulf@bidmc.harvard.edu

Key words: fusion gene, breast cancer, ovarian cancer, genomic profiling, RNA-seq

Formatted for
Journal of Cancer Research and Clinical Oncology

Supplementary Figures and Tables
(please see attached image files for high-resolution figures)

Supplementary Table 1. Distribution of fusions before correction for false-positives

Detected Fusions (applied filters)		**number**	**% of total**
Total number of fusions detected		**792**	**100%**
Fusion Description	Readthrough	17	2%
	Non-tumor-cell	324	41%
	Ribosomal	1	0.1%
	Adjacent	13	1.6%
	Pseudogene	4	0.5%
	Immunoglobulin	10	1.2%
	Breakpoint within homologous polyglutamine repeat regions	5	0.6%
Total number of fusions corrected for false-positives		**418**	**53%**
	Unique	156	20%
	Protein coding	47	6%
	No protein	109	14%

Supplementary Table 2. Distribution of the predicted effect of fusion transcripts across the study cohort

Predicted function	**n**	**% of total**
Total number of corrected fusion genes	156	100%
no CDS/ protein	109	68%
truncated	19	12%
in-frame	10	6%
out-of-frame	3	2%
other (intronic/CDS complete)	7	4%
unknown	8	5%

Supplementary Table 3. Overall frequency of detected fusion gene partners in study cohort (n=312 partner genes)

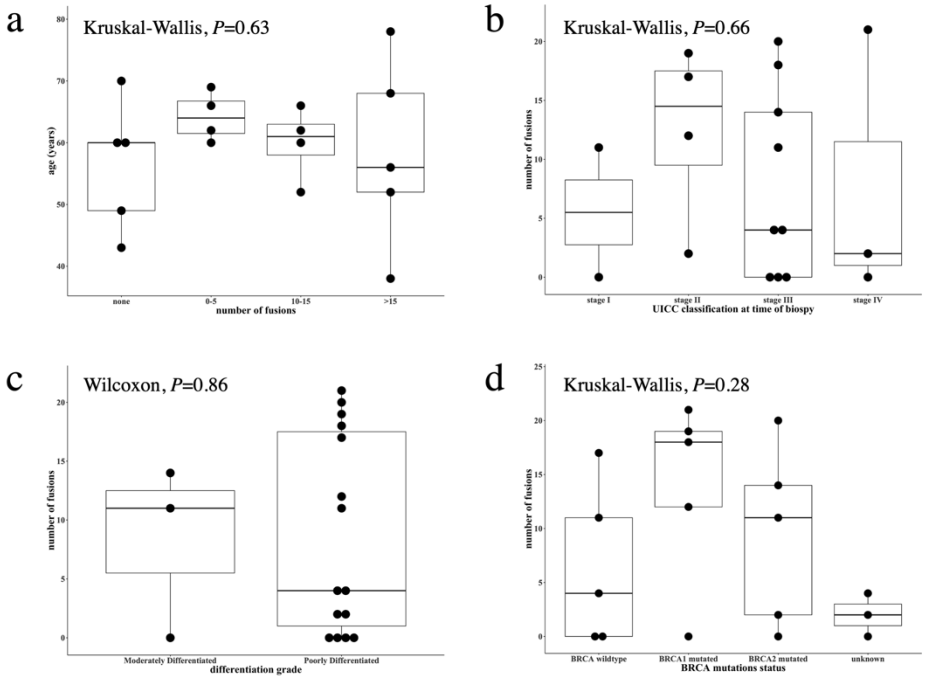
Gene partner	TNBC (n)	HGSOC (n)	Patients (n)
ACSL3	1		1
ACTB	3		1
AHNAK	1	2	3
AKAP13	1		1
ALK		2	1
ANKMY1		1	1
ANKRD11		1	1
ANTXR1		1	1
AP005135.2	1		1
APBB2	1		1
APP	2		2
ARMC9		1	1
ASH1L	1		1
ATM	1		1
ATP11B	2		2
ATXN3	4	1	3
BBX		1	1
BCL2	1		1
BIRC6	1		1
BMP2K		1	1
C19MC	1	1	2
C1ORF186		1	1
C2ORF81		1	1
CBWD2		1	1
CCDC102B	1		1
CCDC6		1	1
CD47		1	1
CD9		1	1
CDH1		1	1
CHD6		1	1
CHST11		1	1
CNOT1		1	1
COL11A1		2	1
COL14A1		1	1
COL1A1		1	1
COL1A2		1	1
COL3A1	1		1
COL6A2	1		1
DMD		1	1
DNM1L	1		1
DPYD	1		1
DST	2		2
EBF1	1	1	2
EEF1A1		2	2
EP300	1		1
ERP44		1	1
ETV6	2	2	3
FN1		1	1
FNDC3B	1		1
FOXO6		1	1
FOXP1	**5**	**1**	**5**
GAPDH		1	1
GSN		1	1
HIST1H2AI	1		1
HIST3H2A	1		1
HMGA1		1	1
HMOX1	1		1
HNRNPK	1		1
HUWE1		1	1
IGF1R	1		1
IGF2		1	1
ILF3	1		1
IQCK		1	1
IQGAP1	1		1
IRF2BPL		1	1
ITPR2	1		1
KIAA1217		1	1
KIDINS220		1	

Gene partner	TNBC (n)	HGSOC (n)	Patients (n)
KMT2C		1	1
LAMA4		1	1
LAMB1	1		1
LINC00578		1	1
LMO7		1	1
LPP		1	1
MACF1	3	1	4
MALAT1	**55**	**42**	**13**
MAML2		1	1
MAP4		1	1
MED12		1	1
MED13L	1		1
MLPH	1		1
MSI2	1		1
MTOR		1	1
MTR		1	1
MUC16		**19**	**3**
MYCBP2		1	1
MYH11	1		1
MYLK		1	1
NCKAP1L	1		1
NCL		4	2
NEAT1		1	1
NFIA		1	1
OGT		2	2
PAM		1	1
PAN3	1		1
PDIA3		1	1
PLCB1		1	1
PLEC		1	1
PLXDC2		1	1
POLA1		1	1
PRRC2C	1		1
PSD4		1	1
PSPC1	1		1
PUS3		1	1
RBM25		1	1
RBMS1	2		1
RMRP	2	1	2
RNF213	3	1	4
SETX		1	1
SF3B3	1		1
SMG1	4	1	4
SPTAN1	2		2
SPTBN1		1	1
SREBF2		1	1
STAG3	1	1	2
SULF1		1	1
SYNE1		1	1
SYNE2		1	1
TBC1D1		1	1
THAP11	4	1	3
THRAP3		1	1
TIMP3		1	1
USP9X	1	1	2
VMP1	1		1
VPS13B	3	2	4
WDR26	1		1
WDR43		1	1
WWOX	**4**	**2**	**4**
XIST	**3**	**3**	**4**
XXBAC		1	1
YBX1	1		1
YLPM1	1		1
ZNF124		1	1
ZNF664		1	1
ZNF843		1	1
ZNFX1	1		1

Supplementary Table 4. Patient characteristics in *FOXP1* fusion-positive versus - negative patients (n=18)

Variables	*FOXP1* fusion positive (n=5)	*FOXP1* fusion negative (n=13)
Age at diagnosis		
years (mean ± SEM [range])	55 ± 5.1 (44-70)	56 ± 2.9 (36-72)
Age at inclusion		
years (mean ± SEM [range])	60 ± 4.0 (49-70)	59 ± 2.9 (38-78)
Race		
White	5/5 (100%)	13/13 (100%)
Ethnicity		
Hispanic or Latino	1 (20%)	1 (8%)
non-Hispanic	3 (60%)	12 (92%)
unknown	1 (20%)	
Platinum status		
platinum resistant	2 (40%)	5 (38%)
platinum sensitive	1 (20%)	4 (31%)
unknown	2 (40%)	4 (31%)
Stage		
I	2 (40%)	3 (23%)
II	1 (20%)	7 (54%)
III	2 (40%)	3 (23%)
IV		
Histology		
adenocarcinoma	2 (40%)	2 (15%)
papillary serous		8 (62%)
transitional	1 (20%)	
others	2 (40%)	3 (23%)
Clinical Grade		
moderately differentiated	1 (20%)	2 (15%)
poorly differentiated	4 (80%)	11 (85%)
Prevalence of fusion genes mean ± SEM [range]	4.8 ± 2.6 (0-11)	9.9 ± 2.4 (0-21)
PFS		
months (median, 95% CI)	12.1 (0.3-2.6)	13.4 (0.4-3.4)
Reason for discontinuation		
progression by RECIST 1.1	5 (100%)	11 (85%)
unacceptable toxicity		2 (15%)
Overall survival		
years (median, 95% CI)	17.2 (0.8-10.2)	6.2 (0.1-1.3)

Supplementary Figure 1. Frequency of fusion genes relative to clinical characteristics and subtypes. Box plots were used to illustrate the number of fusions at initial diagnosis per age group (a), per UICC stage (b), per differentiation grade (c) and relative to *BRCA* mutation status (d). No significant correlation was noted for either of the latter categories (Kruskal-Wallis test P=0.63, P =0.66, P=0.86 and P =0.28, respectively)



Supplementary Figure 2. Correlation of *FOXP1* fusion genes with progression-free survival. Kaplan-Meier estimators were calculated to evaluate the progression-free survival (PFS, months) on buparlisib/ olaparib treatment in TNBC and HGSOC patients with as compared to patients without detectable *FOXP1* fusion gene. All patients had relapsed at the time of data analysis. No trend was observed for superior PFS in *FOXP1* fusion-positive patients (Mann-Whitney-Wilcoxon *P*=0.97).