Original Article

BRCA mutations detected by tumour next-generation sequencing in non-small cell lung cancer: impact on response to therapy and disease course

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Background: Data regarding the prevalence and clinical relevance of *BRCA* mutations in non-small cell lung cancer (NSCLC) is limited. Our objective was to evaluate the impact of pathogenic *BRCA* variants detected by tumour next-generation sequencing (NGS) on disease course and response to therapy.

Methods: We performed a retrospective analysis of all consecutive NSCLC patients with available NGS reports in a single institution between 01/2015 and 08/2020. Pathogenicity of identified mutations was determined according to American College of Medical Genetics (ACMG) guidelines. Log rank and cox regression analyses were used to determine the association between *BRCA* mutation status, overall survival (OS) and progression-free survival (PFS) under various front-line treatment modalities for advanced disease.

Results: Out of 445 patients with NGS data (54% tissue, 46% liquid), 109 (24.5%) patients had a documented *BRCA* variant; 5.6% (25/445) had a pathogenic/likely pathogenic variant (*pBRCA*). Forty percent (10/25) of *pBRCA* patients had no co-occurring NSCLC driver mutations. Patients with *pBRCA* NSCLC had a less prominent smoking history [mean 42.6 (29.2) *vs.* 25.7 (24.0) pack years; P=0.024]. Median PFS with first-line chemo-immunotherapy was significantly prolonged for *pBRCA* patients (n=7) compared with wild-type *BRCA* (*wtBRCA*) patients (n=30) (HR =0.279; P=0.021, 95% CI: 0.094–0.825).

Conclusions: *pBRCA*-mutated NSCLC can represent a specific subtype of pulmonary carcinoma. Patients whose tumours harbor *pBRCA* mutations present with a less prominent smoking history and exhibit prolonged PFS with chemo-immunotherapy combinations compared with *wtBRCA* controls. In a subset of these patients, *pBRCA* is the sole identifiable putative driver mutation, hinting at a significant role for *BRCA* loss in oncogenesis.

Keywords: Non-small cell lung cancer (NSCLC); BRCA; chemo-immunotherapy; next-generation sequencing (NGS); platinum-based therapy

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Submitted Sep 03, 2022. Accepted for publication Mar 16, 2023. Published online May 08, 2023.
doi: 10.21037/tlcr-22-594
View this article at: https://dx.doi.org/10.21037/tlcr-22-594

Introduction

Deleterious germline mutations in the *BRCA1* and *BRCA2* tumour-suppressor genes have long been implicated in the majority of hereditary breast and ovarian cancers (1). Individuals harbouring germline *BRCA* mutations are also at increased risk for pancreatic and prostate cancer, with a higher risk amongst *BRCA2* carriers (2-5). In these cancers, insight into the role of *BRCA* status has paved the way for genetic counselling programs, risk-reducing interventions (e.g., prophylactic bilateral salpingo-oophorectomy), and the development of therapeutic strategies. A heightened response to platinum-based therapy is a hallmark of *BRCA*-mutated breast and ovarian cancers, owing to their inherent inability to efficiently repair intra-stand DNA crosslinks induced by platinum agents (6). Alongside platinum-based therapy, poly (ADP-ribose) polymerase (PARP) inhibition is another important treatment strategy, currently approved as subsequent-line therapy for *BRCA*-mutated breast, ovarian and prostate cancers, and as maintenance therapy for ovarian and pancreatic cancer patients who have responded favourably to platinum-based chemotherapy (7-11).

In a recent pan-cancer analysis of 234,145 tumour samples by Sokol and colleagues, *BRCA1/2* alterations were observed in 3% of non-*BRCA1/2* associated cancers, suggesting a potentially broader landscape for *BRCA*-targeted therapy than is currently available (12). However, the phenotypic implications of *BRCA* status in these cancer types remains poorly defined.

For non-small cell lung cancer (NSCLC) patients, molecular profiling through next-generation sequencing (NGS) for the purpose of identifying targetable driver mutations (i.e., *EGFR*, *ALK*, *ROS1*, *MET*, *KRAS*, *BRAF*, *NTRK*) is considered the standard of care (13). Still, there is only limited data regarding the role of *BRCA* status as a prognostic and predictive biomarker in this setting. Tian *et al.* assessed the prevalence and distribution of pathogenic or likely pathogenic germline variants in 1764 Chinese patients with lung cancer (14). Pathogenicity was determined based on American College of Medical Genetics and Genomics (ACMG) guidelines. In this cohort, *BRCA2* was the most common site of germline mutation, with a prevalence of 0.79%, while *BRCA1* was the third-most common site, with a prevalence of 0.34%. Clinico-pathologic features of the *BRCA1/2* subgroup were not assessed.

More recently, the molecular profile of pulmonary tumours from 379 patients participating in the ongoing SAFIR02-Lung Trial was published (15). *BRCA1/2* mutations were identified in 5.3% of the cohort, with confirmed pathogenic variants identified in 2.1% of enrolled patients, of which 75% were somatic. Notably, the overall response rate (ORR) to platinum-based chemotherapy amongst the eight patients with pathogenic *BRCA* (either somatic or germline) was low (13%). This, together with a low concordance between *BRCA* pathogenicity and the patients' homologous recombination deficiency (HRD) score (a genomic instability score calculated based on copy number variations) lead the authors to doubt the predictive role of *BRCA* status in NSCLC.

In this study, we aimed to shed further light on the role of tumour *BRCA* mutation status in NSCLC by evaluating its impact not only on platinum-sensitivity, but on tumour response to chemo-immunotherapy, immunotherapy-only and platinum-based chemo-radiation regimens. We also sought to directly compare the clinico-pathological features and treatment response profile of patients with *BRCA*-mutated tumours against *BRCA*-wildtype counterparts. We present this article in accordance with the STROBE

Highlight box

Key findings

- This study identifies a subgroup of NSCLC tumours harbouring *BRCA* mutations.
- In a subset of these patients, *BRCA* is the sole identifiable putative driver mutation.

What is known and what is new?

- *BRCA*-mutated NSCLC tumours have previously been described.
- However, a novel observation in our study is that patients with *BRCA*-mutated NSCLC exhibit prolonged progression-free survival under treatment with chemo-immunotherapy, compared with wild-type *BRCA* controls.

What is the implication, and what should change now?

- Further prospective research is warranted to elucidate the therapeutic potential of targeting *BRCA* in this context.

reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-594/rc).

Methods

Study population

Cases were selected retrospectively. The study population consisted of all consecutive patients treated for stage III–IV NSCLC at the Rabin Medical Centre (RMC), for whom NGS was performed between January 2015 and August 2020 (Figure S1). This initial cohort was used for the purposes of genetic analyses. Treatments were assigned according to National Comprehensive Cancer Network (NCCN) guidelines: metastatic patients were treated with systemic therapy (chemotherapy, chemotherapy & immunotherapy combinations, or targeted oral therapy); stage III patients were treated with chemoradiation. Electronic medical records (EMR) were available for 396 (89%) out of the initial 445-patient cohort. This sub-cohort was used for the purposes of additional clinical analyses.

NGS

Commercial NGS was conducted in patients with advanced disease (stage III–IV) for the purpose of identifying targetable driver mutations. Sequencing was performed via one of two methods: tissue-based sequencing using FFPE samples (Foundation One©, TempusXT©, MyPG©, Genesort©), or liquid ctDNA-based sequencing (FoundationACT©, Foundation One Liquid©, Guardant360©).

Molecular characterization of tumour samples

Programmed death-ligand 1 (PD-L1) expression level in lung tumour samples was measured using the Dako PD-L1 immunohistochemistry (IHC) 22C3 pharmDx assay (16). Expression levels were reported as tumour proportion score (TPS) and classified as either negative (<1%), low-positive (1–49%) or positive (≥50%).

Variant classification

For the purpose of classifying *BRCA* variants by their pathogenic potential, we employed the scheme detailed in the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer, which includes the following categories: "variants of strong clinical significance", "variants of potential clinical significance", "variants of unknown clinical significance" and "benign or likely benign variants" (17).

For each *BRCA* variant, we performed search queries in both the Varsome and Clinvar databases (18,19). A consensus-based approach was used wherein only variants classified as having strong clinical significance or potential clinical significance in both databases were considered as such for downstream analysis. We termed these variants *pBRCA* (pathogenic/likely pathogenic).

Germline *BRCA* status was not evaluated in the study. Therefore, we were not able to reliably categorize any of the *pBRCA* mutations identified by NGS as being either a manifestation of a background germline *BRCA* mutation, or of purely somatic origin.

Curation of clinical data

Data were extracted from patients' EMR and includes the following: demographic details, smoking history, personal and family history of malignancy, tumour histology, disease stage and Eastern Cooperative Oncology Group (ECOG) score at presentation, tumour histology & level of differentiation, thyroid transcription factor-1 (TTF-1) status and PD-L1 status.

Clinical endpoints

The study's clinical endpoints included the prevalence of pathogenic or non-pathogenic *BRCA* mutations identified by NGS, response to platinum-based chemotherapy, response to treatment with chemotherapy & immunotherapy combinations, response to immunotherapy treatment, response to targeted oral therapy treatments, median progression-free-survival, and median overall survival (OS).

Response to various lines of systemic treatment was assessed and categorized as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), adapted from RECIST 1.1. Progression-free-survival (PFS) was defined as the time interval from treatment initiation to disease progression or death. OS was defined as the time interval between diagnosis and death.

Statistical analysis

All statistical analyses were performed using IBM© SPSS©

software version 25. A chi-square test of independence was performed to examine the relation between *BRCA* mutation status and various categorical clinico-pathologic variables. Frequencies are expressed as percentages. An independent samples *t*-test was used to examine the relationship between *BRCA* mutation status and various continuous clinico-pathologic variables. Results are expressed as mean ± standard deviation (SD). A log rank test was run to determine if there were differences in the survival distribution by *BRCA* mutation status, stratified by the treatment modality administered (platinum-based chemotherapy/chemoradiation, chemo-immunotherapy or immunotherapy). A Cox regression model was used to quantify the hazard ratio (HR) for disease progression under various treatment modalities, based on *BRCA* mutation status. P values of <0.05 were considered statistically significant.

Ethics

The study was approved by the Rabin Medical Center Institutional Review Board (IRB) (0391-14-RMC), and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written consent for the usage of NGS data, as well as demographics & disease characteristics for the purpose of research and/or publication, was provided by all participants.

Results

BRCA mutation status detected by tumour NGS

Of 445 reports included, 239 (53.7%) were from formalin-fixed paraffin-embedded (FFPE) samples and 184 (41.3%) were circulating tumour DNA (ctDNA)-based. For an additional 22 (5%) reports, there was no documentation of the sample source. Out of 445 patients in our cohort, 109 (24.5%) had a *BRCA1/2* variant, whereas in 336 (75.5%) no *BRCA* variants were reported (*wtBRCA*) (Figure S2). Of patients with a *BRCA* variant, 86 (78.9%) had a single variant, 16 (14.7%) had two different variants, 4 (3.7%) had three different variants and 3 (2.8%) had four different variants.

Amongst *BRCA*-mutated tumours, 25 had a confirmed pathogenic/likely pathogenic variant (*pBRCA*), which constitutes 5.6% of the entire study cohort (25/445), while 14/25 of *pBRCA* mutations were in *BRCA2* and 11/25 were in *BRCA1*. Notably, in 12 (48%) patients, the pathogenic variant was a well-established *BRCA* founder mutation, which might potentially reflect a germline origin. However, germline status was not routinely performed in the study, therefore none of the cases could be reliably classified as germline.

Demographics and disease characteristics

Three hundred and ninety-six out of 445 patients (89%) had EMR data available for analysis. Based on EMR data, demographic and disease characteristics were compared between *pBRCA* (n=24) and *wtBRCA* (n=292) patients (*Table 1*). The cumulative amount of smoking pack-years was lower in the *pBRCA* group [mean 42.6 (29.2) *vs.* 25.7 (24.0) pack years in the *wtBRCA* and *pBRCA* groups, respectively; P=0.024]. All other demographic and disease characteristics did not differ significantly between the groups.

Co-occurring driver mutations

Of the 25 patients with a *pBRCA* mutation identified by NGS, 15 (60%) had a co-occurring alteration in at least one of the established NSCLC driver genes (*EGFR*, *KRAS*, *ALK*, *BRAF*, *MET*, *ERBB2* or *ROS1*), including 13 patients with a characteristic driver mutation. Notably, 3 *pBRCA* patients had no identifiable driver mutation, and no history of smoking, suggesting a possible oncogenic role for *BRCA* in these patients.

Patient-level specifics for *pBRCA* patients, including co-occurring alterations, gender, smoking history, response to treatment (to either platinum-based therapy or single-agent immunotherapy) and PD-L1 expression levels, are shown in *Table 2*.

Disease control rate under platinum-based therapy

In total, 239 patients were treated with platinum-based therapy in the frontline setting for stage 3–4 disease (chemotherapy, chemo-radiation, or chemo-immunotherapy), including 175 *wtBRCA* patients and 17 *pBRCA* patients. In the *pBRCA* group, 10 patients were treated with chemotherapy/chemo-radiation, and 7 patients received a chemo-immunotherapy combination.

Overall disease control rates (PR, CR or SD) under the various platinum-based regimens, according to *BRCA* mutation status, are shown in *Table 3*. Disease control rates did not differ significantly between the *wtBRCA* and *pBRCA* groups.

Table 1 Demographics and disease characteristics according to tumour *BRCA* mutation status

Characteristics	*wtBRCA* (n=292)	*pBRCA* (n=24)	P value
Sex			0.842
Male	52.1% (n=152)	54.2% (n=13)	
Female	47.9% (n=140)	45.8% (n=11)	
Age (years) at diagnosis, mean (SD)	66.2 (11.2)	63.6 (8.0)	0.272
Smoking status			0.793
Never smokers	33.9% (n=99/260)	37.5% (n=9/22)	
Past/current smokers	55.1% (n=161/260)	54.2% (n=13/22)	
n/a	n=32	n=2	
Pack years, mean (SD)	42.6 (29.2)	25.7 (24.0)	0.024
Personal history of malignancy			0.53
No	77% (n=184/239)	82.6% (n=19/23)	
Yes	23% (n=55/239)	17.4% (n=4/23)	
n/a	n=53	n=1	
Family history of malignancy			0.804
No	72% (n=157/218)	69.6% (n=16/23)	
Yes	28% (n=61/218)	30.4% (n=7/23)	
n/a	n=74	n=1	
ECOG			0.549
0–1	82.5% (n=132/160)	88.2% (n=15/17)	
2–4	17.5% (n=28/160)	11.8% (n=2/17)	
n/a	n=32	n=7	
Stage at diagnosis			0.646
Early disease (1 to 2)	13.3% (n=35/263)	16.7% (n=4/24)	
Advanced disease (3 to 4)	86.7% (n=228/263)	83.3% (n=20/24)	
n/a	n=29	n=0	
Histology			0.224
Adenocarcinoma	75% (n=219)	75% (n=18)	
Squamous cell carcinoma	4.5% (n=13)	12.5% (n=3)	
Neuroendocrine	6.2% (n=18)	0	
Other/mixed/unknown	14.4% (n=42)	12.5% (n=3)	
TTF1 status			0.1
Negative	27.2% (n=44/162)	7.1% (n=1)	
Positive	72.8% (n=118/162)	92.9% (n=13)	
n/a	n=130	n=10	
PD-L1 status			0.87
Negative (<1%)	41.2% (n=49/119)	35.7% (n=5/14)	
Low positive (1–49%)	22.7% (n=27/119)	28.6% (n=4/14)	
Positive (≥50%)	36.1% (n=43/119)	35.7% (n=5/14)	
n/a	n=173	n=10	

wtBRCA, wild type *BRCA*; *pBRCA*, pathogenic/likely pathogenic *BRCA*; n/a, not applicable; SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; TTF1, thyroid transcription factor 1; PD-L1, programmed death-ligand 1.

Table 2 Co-occurring alterations & response to therapy in *pBRCA* patients

Patient ID	*BRCA 1/2*	Sex	Smoking Status	Response to platinum-based therapy	Response to single-agent immunotherapy	EGFR	KRAS	ALK	BRAF	MET	ERBB2	ROS1	PD-L1 expression
1	*BRCA2*	Male	Never smoker	PR	Not given	–	–	EML-4/ALK fusion	–	–	–	–	<1%
2	*BRCA2*	Male	Current smoker	SD	PD	–	KRAS G12A	–	–	–	–	–	1–49%
3	*BRCA2*	Male	Former smoker	PR	Not given	EGFR Q105H	–	–	–	–	–	–	<1%
4	*BRCA2*	Female	Former smoker	Not given	Not given	EGFR L858R	–	–	–	–	–	–	N/A
5	*BRCA2*	Female	Former smoker	Not given	Not given	EGFR amplification	–	–	–	MET exon 14 splice site	–	–	N/A
6	*BRCA2*	Male	Current smoker	PR	Not given	–	–	–	–	–	–	–	<1%
7	*BRCA1*	Female	Former smoker	PR	Not given	–	–	–	–	–	–	–	1–49%
8	*BRCA2*	Female	Never smoker	CR	Not given	–	–	–	–	MET exon 14 splice site	–	–	≥50%
9	*BRCA2*	Male	Former smoker	N/A	N/A	–	KRAS G12D 5.1%	–	–	–	–	–	≥50%
10	*BRCA1*	Female	Never smoker	PR	PD	–	–	–	–	–	–	–	N/A
11	*BRCA2*	Female	N/A	N/A	N/A	–	–	–	–	–	–	–	N/A
12	*BRCA1*	Male	Never smoker	PR	SD	–	–	–	–	MET exon 14 splice site	–	–	≥50%
13	*BRCA1*	Female	Current smoker	PR	Not given	–	–	–	–	–	–	–	N/A
14	*BRCA1*	Male	Current smoker	PR	Not given	–	–	–	–	–	–	–	1–49%
15	*BRCA2*	Male	Never smoker	PR	Not given	–	–	–	–	–	–	–	<1%

Table 2 *(continued)*

Table 2 *(continued)*

Patient ID	BRCA 1/2	Sex	Smoking Status	Response to platinum-based therapy	Response to single-agent immunotherapy	EGFR	KRAS	ALK	BRAF	MET	ERBB2	ROS1	PD-L1 expression
16	*BRCA2*	Female	Never smoker	PR	Not given	–	–	–	–	–	ERBB2 2.7% A775_G776insYVMA	–	N/A
17	*BRCA2*	Female	Former smoker	PD	PD	–	KRAS G12A	–	–	–	–	–	N/A
18	*BRCA2*	Male	Former smoker	Not given	CR	–	KRAS G12V	–	–	–	–	–	≥50%
19	*BRCA2*	Female	Never smoker	Not given	Not given	–	–	–	–	–	–	–	1–49%
20	*BRCA1*	Female	N/A	N/A	N/A	–	–	–	–	–	ERBB2: A775_G776insSVMA	–	N/A
21	*BRCA1*	Male	Current smoker	PR	PR	–	KRAS amplification	–	–	–	–	–	N/A
22	*BRCA1*	Male	Never smoker	PR	PD	–	–	–	–	–	ERBB2 A775_G776insYVMA	–	<1%
23	*BRCA1*	Male	Current smoker	SD	SD	–	–	–	–	–	–	–	N/A
24	*BRCA1*	Male	Never smoker	Not given	Not given	EGFR exon 19 deletion (E746_A750del) 0.21%	–	–	–	–	–	–	N/A
25	*BRCA1*	Female	N/A	Not given	Not given	–	–	–	–	–	–	–	1–49%

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; N/A, not available.

Table 3 Disease control rates with platinum-based therapy, according to tumour *BRCA* status*

Treatment regimen	Entire cohort			*wtBRCA*			*pBRCA*			*wtBRCA vs. pBRCA*
	Treated patients	Patients with response data available	Disease control rate	Treated patients	Patients with response data available	Disease control rate	Treated patients	Patients with response data available	Disease control rate	
Any platinum based regimen	n=239	n=192	141 (73.4%)	n=175	n=136	98 (72.1%)	n=17	n=16	15 (93.8%)	P=0.06
Chemotherapy/ chemoradiation	n=195	n =152	111 (73%)	n=144	n=107	79 (73.8%)	n=10	n=9	8 (88.9%)	P=0.316
Chemo-immunotherapy	n=44	n=40	30 (75%)	n=31	n=29	19 (65.5%)	n=7	n=7	7 (100%)	P=0.068

*, partial response (PR), complete response (CR) or stable disease (DS). wtBRCA, wild type BRCA; pBRCA, pathogenic/likely pathogenic BRCA.

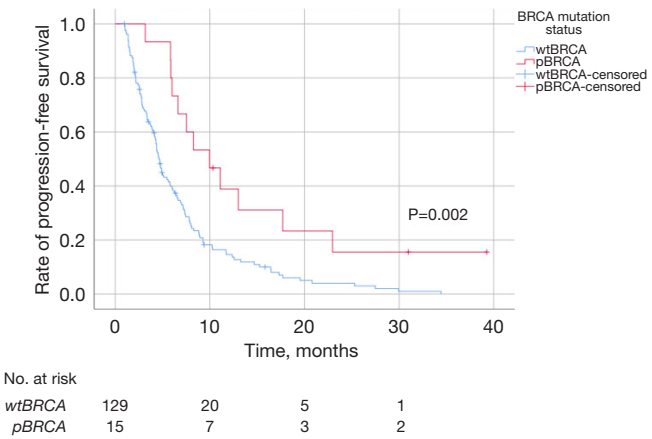

Figure 1 PFS with platinum-based therapy. wtBRCA, wild type BRCA; pBRCA, pathogenic/likely pathogenic BRCA; PFS, progression-free survival.

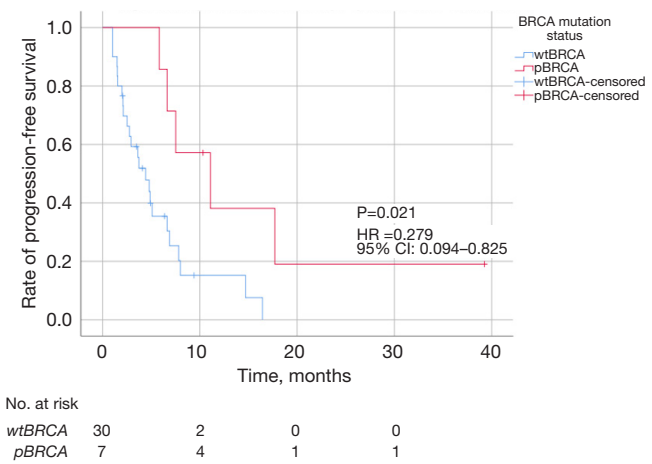

Figure 2 PFS with frontline chemo-immunotherapy. wtBRCA, wild type BRCA; pBRCA, pathogenic/likely pathogenic BRCA; PFS, progression-free survival.

PFS with platinum-based therapy

Mean time for the study cohort was 27 months. In a log-rank analysis, median PFS was significantly prolonged for *pBRCA* patients (n=15) compared with *wtBRCA* patients (n=129) treated with platinum-based therapy [10 (2.2) *vs.* 4.6 (0.3) months; P=0.002; *Figure 1*].

In a further breakdown by specific platinum-based regimens, median PFS was significantly prolonged for *pBRCA* (n=7) compared with *wtBRCA* (n=30) patients on chemo-immunotherapy [11.1 (3.7) *vs.* 4.4 (0.9) months; P=0.014; *Figure 2*]. Congruently, a univariate cox regression model showed that *pBRCA* status was associated with prolonged PFS under first-line chemo-immunotherapy (HR =0.279; P=0.021, 95% CI: 0.094–0.825).

Finally, median PFS with first-line platinum-based chemotherapy/chemo-radiation was prolonged for the *pBRCA* group (n=8) compared with *wtBRCA* (n=99), albeit not to a statistically-significant degree [8.3 (2.8) *vs.* 4.7 (0.5) months; P=0.062; *Figure 3*].

Response rate with single-agent immunotherapy

Eight patients with *pBRCA* mutated NSCLC were treated with single agent immune-checkpoint inhibition (one in the first-line setting and seven in the second-line setting). Four of them derived clinical benefit (one patient achieved CR, one patient achieved PR and two patients exhibited SD) (*Table 2*).

OS

A Log- Rank analysis was performed in order to assess the association between *BRCA* mutation status and OS, as

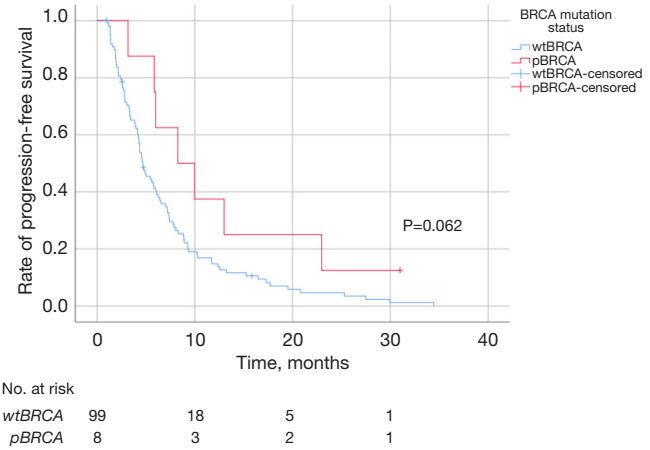

Figure 3 PFS with frontline chemotherapy/chemoradiation. wtBRCA, wild type BRCA; pBRCA, pathogenic/likely pathogenic BRCA; PFS, progression-free survival.

calculated from the time of being diagnosed with advanced disease (stage 3–4). Median OS did not differ significantly between the *wtBRCA* (n=266) and *pBRCA* (n=21) groups [21 (1.39) *vs.* 24.2 (3.1) months for *wtBRCA* and *pBRCA*, respectively; P=0.837; Figure S3].

Discussion

This study extends the limited literature concerning *BRCA* status in NSCLC.

The proportion of lung cancer tumour samples in our cohort harbouring a pathogenic *BRCA* mutation was 5.6%, which is higher than previously-reported rates, including in the SAFIR02-Lung trial (2.1%), and in a study by Jordan and colleagues comprising 860 metastatic lung adenocarcinoma patients (1.3%) (15,20).

Differences in the reported *pBRCA* prevalence rates might be due to variability in the way variants were classified as pathogenic: in our study, variants were stratified based on expert panel consensus in the Clinvar and Varsome© databases; Jordan *et al.* employed a four-tier system based on the level of evidence suggesting the variant in question might be targetable by standard or investigational therapies; lastly, in the SAFIR02-Lung trial variants were classified by a molecular geneticist as pathogenic, being of unknown pathogenicity or probably non-pathogenic based on an undisclosed classification scheme. Another putative factor is the high prevalence of germline *pBRCA* variants among Ashkenazi Jews (AJ), which are over-represented in this study (21,22). Indeed, in a comparable series of 248 Israeli patients with NSCLC from 2019, the rate of pathogenic germline *BRCA* mutation was 4.8%, which is closer to our own reporting (23). In both cohorts, the rate of *pBRCA* was higher than the background *BRCA* carrier frequency amongst AJs, which is estimated at maximally 2.5% (24).

For a detailed comparison of previous studies reporting on *BRCA* mutation carrier frequency amongst NSCLC patients, either somatic or germline (Table S1) (14-15,20,23,25).

Notably, 40% (n=1,025) of *pBRCA* tumours we identified did not have a co-occurring NSCLC driver mutation, hinting at a potentially significant role for *BRCA* loss in oncogenesis in these patients. This might be especially relevant in the 3 patients out of the 10 who have no prior history of smoking (*Table 2*).

In a direct comparison of *wtBRCA* and *pBRCA* patients, we found that *BRCA* positivity was associated with a longer PFS with platinum-based therapy as a whole, an effect which seems to be driven primarily by a favourable PFS under chemotherapy-immunotherapy regimens. While the median PFS for *pBRCA* patients treated with chemo-immunotherapy in our study was prolonged (11.1 months), even compared with the chemo-immunotherapy arm in the KEYNOTE-189 trial (8.8 months) (26), the number of patients who received chemo-immunotherapy in our study (n=7) is too small to draw a robust conclusion (24). Further research is warranted in order to establish the role of *BRCA* positivity as a determinant of response in this setting.

On the molecular level, there is mechanistic rationale for *BRCA*-deficient tumours to display a heightened sensitivity to immunotherapy. In these tumours, *BRCA*-mediated effects on the tumour-immune microenvironment, including increased neo-antigen load and the enrichment of distinct immune-cell subpopulations, result in greater immunogenicity (27,28). In the clinical setting, however, the extent of real-life efficacy of immunotherapy in *BRCA*-mutated tumours is variable. In breast cancer (BC), for example, it is likely dependent upon additional molecular underpinnings such as hormone receptor subtypes, loss of heterozygosity (LOH) and homologous-recombination deficiency (HRD) score (29). This is made evident by the reported ORR with single-agent immunotherapy for triple-negative BC, the most common hormonal classification in *BRCA*-mutated breast cancers, which is generally less than 20% (30-32). Similarly, in our study the ORR with single-agent immunotherapy in the *pBRCA* group was 25% (2/8).

This study has several limitations. First, small sample sizes in each of the analysed treatment arms warrant

caution when interpreting our findings, especially with regards to the efficacy of chemotherapy-immunotherapy combinations for *pBRCA* patients. Secondly, unaccounted-for imbalances between the *pBRCA* and wt*BRCA* groups might have contributed to the observed differences in PFS. This is especially true with regards to established predictive markers for immunotherapy response; there was a higher proportion of PD-L1 low-positive/positive patients in the *pBRCA* group compared with the *wtBRCA* group, albeit not to a statistically significant degree, while data regarding tumour mutational burden (TMB) in each of the groups is unfortunately lacking.

An additional limitation stems from the fact that prior to 2021, NGS in Israel was an out-of-pocket expense; as such, the study population excludes patients of lower socio-economic background and is therefore selective. In addition, response to treatment evaluation, as well as the genomic analyses, were de-centralized to several different oncology clinics and multiple commercial panels. On the other hand, variant pathogenicity was carefully determined in a centralized fashion by two of the authors: a medical geneticist (YG) and a medical oncologist (RT), using two separate and equally-validated databases. Since we relied on commercial tumour-directed NGS panels, we were unable to define the rate of germline *BRCA* mutations in this cohort.

Notwithstanding the above limitations, our findings do suggest that pulmonary tumours harbouring pathogenic *BRCA* mutations can represent a specific subtype of NSCLC, in which platinum-based therapy as a whole, and chemo-immunotherapy combinations in particular, is an effective strategy. Future subgroup analyses in large prospective trials can help better define this tumour subtype.

Conclusions

Our data suggests that *pBRCA*-mutated tumors may represent a distinct subtype of NSCLC. In a subset of these tumours, no additional driver mutations are identified, hinting at a potentially significant role for *BRCA* impairment in oncogenesis. The therapeutic yield of specifically targeting *BRCA* in this subset of patients remains to be validated through further prospective research.

Acknowledgments

The abstract has been presented at ESMO virtual scientific meeting 2020 (abstract 1351P).
Funding: None.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-594/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-594/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-594/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-594/coif). DAG has received institutional research funding from BMS, Merck and Janssen, and consulting fees from Vivio Health. He has also held an unpaid advisory and leadership role at Optimal Cancer Care Alliance. He holds personal stock at Viviohealth and TailorMed. ED has received institutional research funding from Astra Zeneca, and consulting/speaking fees from Roche, Astra Zeneca, Pfizer, Merck Sharpe & Dohme, Bristol Myers Squibb, Novartis, Takeda, Sanofi, Merck Serono, Medison Pharma, and Janssen Israel. She has also received support for attending meetings and/or travel from Merck Serono and Medison Pharma. In addition, ED participated in data safety monitoring boards at Roche, Astra Zeneca, Pfizer, Merck Sharpe & Dohme, Bristol Myers Squibb, Novartis, Takeda, Sanofi, Merck Serono, Medison Pharma, and Janssen Israel. ED also has occupied a chair position at the Israeli Society for Clinical Oncology and Radiotherapy (ISCORT), is an ESMO faculty member, and an IASLC mesothelioma committee member. NP has received advisory fees & honorarium from, and has performed research with AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, FoundationMedicine, Gaurdant^{360}, Merk, MSD Novartis, NovellusDx, Pfizer, Roche, and Takeda. He has also received support for attending meetings and/travel from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, FoundationMedicine, Gaurdant360, Merk, MSD, Novartis, NovellusDx, Pfizer, Roche, Takeda. AZ has received consulting fees from AstraZeneca, Oncohost, Steba MSD, Takeda Nixio and Medison. She has also received payments for lectures from BMS, Novartis,

Takeda, MSD, Pfizer and Roche. In addition, AZ received support for attending meetings and/or statements from Janssen, has participated on a data safety monitoring board at Beyond Cancer, and holds stock/stock options at Nixio. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Rabin Medical Center Institutional Review Board (IRB) (0391-14-RMC), and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written consent for the usage of NGS data, as well as demographics & disease characteristics for the purpose of research and/or publication, was provided by all participants.

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Cite this article as: Tschernichovsky R, Averbuch I, Goldstein DA, Mutai R, Dudnik E, Rotem O, Laufer-Geva S, Peled N, Goldberg Y, Zer A. *BRCA* mutations detected by tumour next-generation sequencing in non-small cell lung cancer: impact on response to therapy and disease course. Transl Lung Cancer Res 2023;12(5):1011-1022. doi: 10.21037/tlcr-22-594

Supplementary

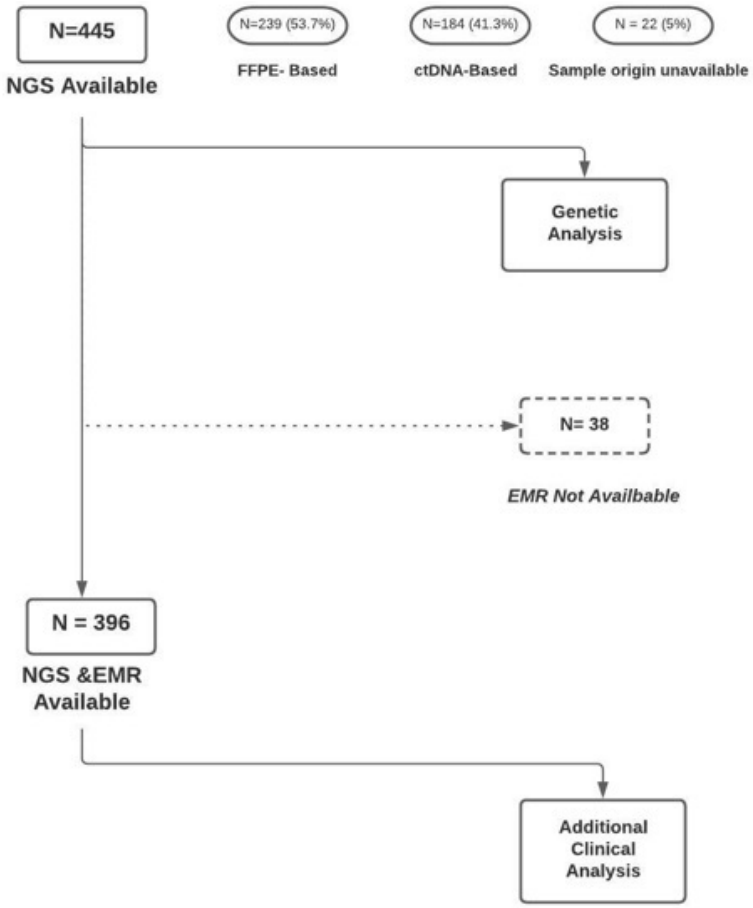

Figure S1 Study flowchart. NGS, next-generation sequencing; FFPE, formalin-fixed paraffin-embedded; ctDNA, circulating tumor DNA; EMR, electronic medical records.

https://dx.doi.org/10.21037/tlcr-22-594

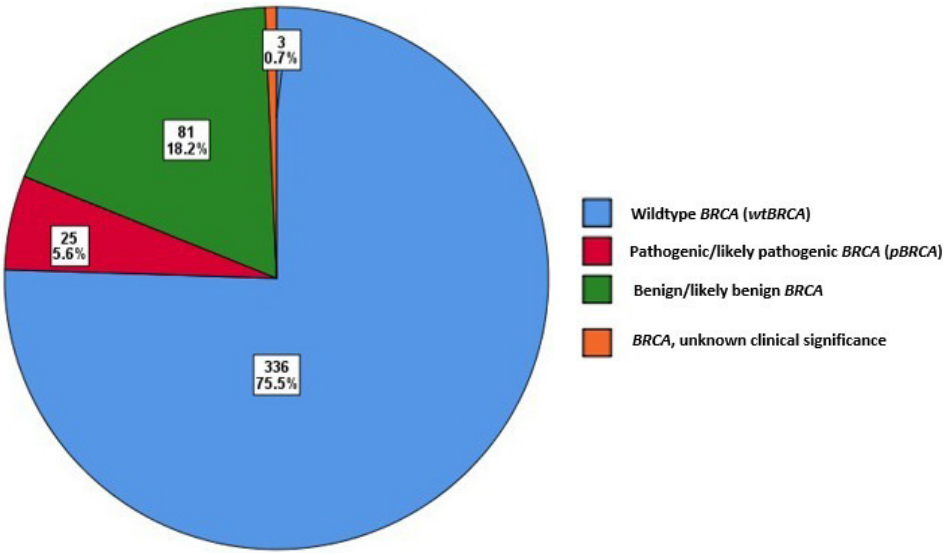

Figure S2 Patient distribution by tumor BRCA status. wtBRCA, wild-type BRCA; pBRCA, pathogenic/likely-pathogenic BRCA.

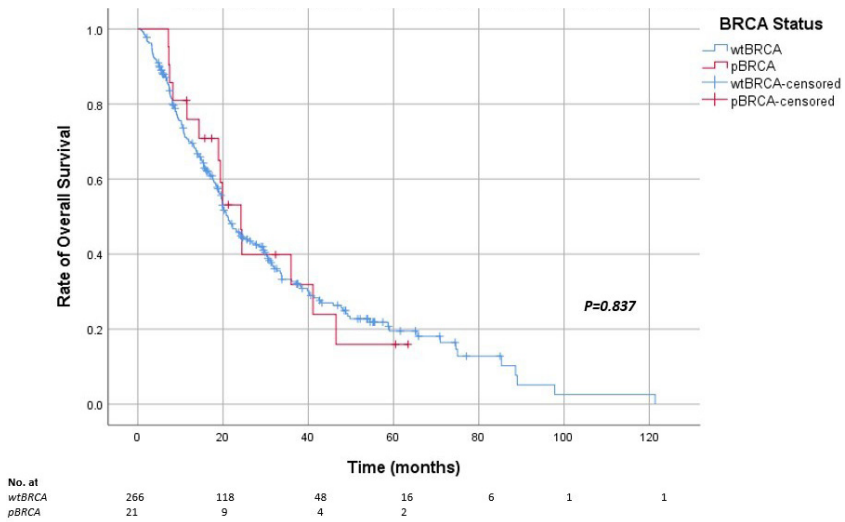

Figure S3 Overall survival from diagnosis of advanced disease. wtBRCA, wild-type BRCA; pBRCA, pathogenic/likely-pathogenic BRCA.

https://dx.doi.org/10.21037/tlcr-22-594

Table S1 Previously reported incidences of BRCA mutations in lung cancer patients

Study	Sample	Benign/likely benign/unknown clinical significance *BRCA*	Pathogenic *BRCA*
Variants identified in tumor tissue			
Remon 2020 (SAFIR02-Lung Trial)	n=379	n=12 (3.2%)	n=8 (2.1%)
Jordan 2017	n=860	Not reported	n=11 (1.3%)
Variants confirmed as germline			
Remon 2020 (SAFIR02-Lung Trial)	n=379	n=6 (1.6%)	n=2 (0.5%)
Kadouri 2019	n=248	n=1 (0.4%), BRCA1 Exon 6 delCTTT	n=12 (4.8%); BRCA1 185delAG PATH X2, BRCA1 5382insC PATH X2, BRCA2 6174delT PATH X7, BRCA2 IVS2+G>A PATH X
Tian 2020	n=1764	Not reported	n=20 (1.1%)
Hu 2019	n=6,220	Not reported	n=64 (1.03%)

https://dx.doi.org/10.21037/tlcr-22-594