Gynecologic Oncology 153 (2019) 304–311

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Impact of somatic molecular profiling on clinical trial outcomes in rare epithelial gynecologic cancer patients

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HIGHLIGHTS

- Somatic genotyping is feasible and expands the spectrum of therapeutic approaches in R-GYN.
- Clinical activity was seen in genotype-matched and unmatched trials across rare histologies.
- Potential Activity of MEK-based combination therapy in KRAS and/or NRAS mutant LGS was observed.

ARTICLE INFO

Article history:
Received 12 September 2018
Received in revised form 30 January 2019
Accepted 4 February 2019
Available online 18 February 2019

Keywords:
Rare gynecologic malignancies
Molecular profiling
Targeted therapies
Low Grade Serous ovarian cancer

ABSTRACT

Objectives. Conducting clinical trials in rare malignancies is challenging due to the limited number of patients and differences in biologic behavior. We investigated the feasibility and clinical utility of using genomic profiling for rare gynecologic malignancies.

Methods. Rare epithelial gynecologic cancer patients were analyzed for somatic variants through an institutional molecular profiling program using the Sequenom MassArray platform or the TruSeq Amplicon Cancer Panel on the MiSeq platform. Clinical trial outcomes by RECIST 1.1, and time on treatment were evaluated.

Results. From March 2012 to November 2015, 767 gynecologic patients were enrolled and 194 (27%) were classified as rare epithelial malignancies. At least one somatic mutation was identified in 72% of patients, most commonly in *TP53* (39%), *KRAS* (28%) and *PIK3CA* (27%). A total of 14% of patients were treated on genotype-matched trials. There were no significant differences in overall response rate between genotype-matched *versus* unmatched trials, nor in median time on treatment between genotype trials and the immediate prior systemic standard treatment. Among 13 evaluable Low Grade Serous ovarian cancer patients treated on genotype-matched trials with MEK inhibitor-based targeted combinations, there were four partial responses.

Conclusions. Somatic molecular profiling is feasible and enables the identification of patients with rare gynecologic cancers who are candidates for genotype-matched clinical trials. Genotype-matched trials, predominantly MEK-based combinations in *KRAS* and/or *NRAS* mutant Low Grade Serous ovarian cancer patients, and genotype-unmatched trials, have shown potential clinical activity. Prospective trials with integrated genotyping are warranted to assess the clinical utility of next generation sequencing tests as a standard clinical application in rare malignancies.

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https://doi.org/10.1016/j.ygyno.2019.02.005
0090-8258/

1. Introduction

Rare cancers, defined as an incidence of fewer than six per 100,000 people [1] make up over 50% of female genital tract tumors and 25% of all cancer deaths are represented by rare gynecologic tumors [1]. Novel subtypes due to improvements in our understanding of molecular pathogenesis also fit under the 'rare' category, and result in the further subdivision of common tumors [1,2]. Different factors contribute to the inferior outcomes of rare gynecologic malignancies in comparison to common histologies [1,3,4]. There are many rare histologies with limited information on evidence based treatment [1]. Rare tumors are often mis-diagnosed and/or there may be delays in diagnosis, which can impact timing of treatment [3]. Rare cancers can represent diverse biological behaviors with lack of established treatment guidelines [4]. Identifying patient who may be eligible for clinical trials remains challenging within this space and as such, there remains a need for disease specific trials and rare cancer registries [4]. Consequently, there is limited available evidence to support treatment recommendations and few available clinical trials focused on rare malignancies.

Genomic profiling through Next Generation Sequencing (NGS) has enabled unprecedented advances in our understanding of the molecular underpinnings of several malignancies, including gynecologic cancers [5]. Incorporation of NGS into routine care and clinical decision-making for rare gynecologic cancers (R-GYN) remains premature due to limited knowledge of clinical utility. Our group has previously reported that Low Grade Serous ovarian cancers (LGS) frequently harbor activating MAPK pathway gene mutations [6] that may be targeted with treatment combinations incorporating MEK inhibitors [7]. Similarly, NGS has allowed identification of other potentially druggable mutations across a range of R-GYN histologies such as mutations in the tumor suppressor gene *ARID1A* in clear cell and endometrioid ovarian cancers [8]; *KRAS* mutations in adenocarcinoma of the cervix [9]; *ERBB2* [10] in serous endometrial cancers; and *EGFR* mutations in vulvar cancers [11].

The need to increase our understanding of tumor biology has led to the establishment of several comprehensive large-scale molecular profiling initiatives in academic cancer centers. These initiatives have interrogated several tumor types, including gynecologic tumors. In general, broad genomic profiling programs have been shown to be feasible in routine practice, with a high proportion of "actionable" gene alterations identified [12–16]; although, as expected issues regarding cost and clinical utility of this systematic genomic assessment to enable precision oncology persist. Early results suggest that only a small proportion of patients (~5%–10%) are assigned to genotype-matched treatments based on molecular profiling data [12–16]. To date, the feasibility and utility of broad genomic profiling approaches for rare tumors has yet to be demonstrated. At the Princess Margaret Cancer Centre (PM), the Integrated Molecular Profiling in Advanced Cancers Trial (IMPACT) and Community Molecular Profiling in Advanced Cancers Trial (COMPACT) are prospective studies that provide molecular characterization data to oncologists to match patients with advanced solid tumors to clinical trials with targeted therapies [16]. Given the lack of data and therapeutic options in rare cancers, we aimed to evaluate the feasibility of conducting genomic characterization in a cohort of patients diagnosed with advanced epithelial rare gynecologic cancers enrolled in a comprehensive and prospective genomic profiling protocol at PM. We also investigated the frequency of actionable somatic mutations and assessed the clinical utility of delivering precision cancer medicine in this patient population. This also allowed us to investigate the potential challenges and barriers for implementation of broad genomic profiling in this setting.

2. Material and methods

2.1. Study population

Patients with advanced, rare epithelial gynecologic cancers (R-GYN) prospectively enrolled in the institutional review board–approved PM molecular profiling initiative between March 2012 and November 2015 were included. Eligible tumor histologies included: LGS (most were previously included in a report from our group [7]), transitional cell/Brenner ovarian cancer, ovarian squamous carcinoma, cervical adenocarcinoma or adenosquamous carcinoma, endometrial papillary serous or squamous tumors, vaginal and vulvar cancers, clear cell carcinoma, mucinous carcinoma, carcinosarcomas and small cell tumors of the gynecologic tract. To be eligible, patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤1 and available formalin-fixed paraffin-embedded (FFPE) archival tumor tissue (from either a primary or a metastatic site). Available FFPE tumor tissue was reviewed by a pathologist specialized in gynecological cancers to confirm histology and estimate tumor cellularity. This study was approved by the University Health Network Research Ethics Board and was registered on ClinicalTrials.gov [NCT01505400].

2.2. Sequencing analysis

DNA was extracted, purified, and quantified from sections of the most recent archival FFPE samples after tumor content review for sequencing; with minimum acceptable tumor cellularity of 10% defined. All procedures were performed in a laboratory accredited by the College of American Pathologists (CAP) and certified to meet Clinical Laboratory Improvement Amendments (CLIA). Tumor specimens underwent hotspot mutational testing on the basis of three molecular profiling assays: a custom multiplex genotyping panel on a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass-spectrometry platform (MassARRAY, Agena Bioscience, San Diego, CA, USA) to genotype 279 mutations within 23 genes, the TruSeq Amplicon Cancer Panel (TSACP, Illumina) on the MiSeq sequencer (Illumina) covering regions of 48 genes, and the Ion AmpliSeq Cancer Panel v2 (ASCP, ThermoFisher Scientific) on the Ion Proton sequencer (ThermoFisher Scientific) covering regions of 50 genes. Details of the molecular profiling assays have been previously described [16]. All genes included in the TSACP or MALDI-ToF analyses are listed in Supplementary Tables 1–3. The scheme of Sukhai et al. [17] was used for assessment and classification of variants. The molecular profiling report was included in the electronic medical record and returned to the treating oncologist. The clinical significance of profiling results was discussed with patients during a routine clinic visit by their treating oncologist. FFPE samples tested using the TSACP and ASCP panels included concurrent testing of matched blood samples for germline mutations. Patients were offered return of pathogenic germline results at the time of consent and asked to identify a family member delegate who could receive results on their behalf if required.

2.3. Data analysis

For each patient, baseline patient, tumor characteristics, profiling results and survival data were retrieved from medical records. Descriptive statistics were used to summarize patient characteristics, profiling results, and anti-tumor activity. The outcomes and comparisons between patients receiving standard treatment (pre- and post-molecular profiling) and clinical trials completed upon molecular profiling results were assessed using Wilcoxon's test. Genotype-matched trials were defined as studies with eligibility criteria restricted to patients with specific somatic mutations; those with a targeted drug with enriched clinical or preclinical activity in a patient's genotype; and/or those with a drug that inhibited a pathway directly linked to the somatic mutation. To facilitate trial accrual, multidisciplinary tumor board discussions, physician-directed email alerts with genotype-matched trial listings available at our institution, and individual physician summaries of profiling results were incorporated. Therapeutic outcomes were evaluated according to time on treatment, defined as date of trial enrollment until date of discontinuation of investigational treatment. RECIST v 1.1 was used to evaluate radiological responses, defined as Complete Response (CR), Partial response (PR), Stable Disease (SD) and Progressive

Disease (PD). Differences with p values of <0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

From March 2012 to November 2015, 767 patients with gynecologic cancers were enrolled onto the PM molecular profiling initiatives (IMPACT/COMPACT), and 720 (94%) were successfully profiled during the study period. Of 47 (6%) screen failures, 4% were for insufficient tissue or DNA and 2% for clinical deterioration or other reasons. Among these patients, a total of 194 (27%) patients were identified as R-GYN and selected for the purpose of our study (Fig. 1). The most prevalent histologies were LGS (54/194; 28%), clear cell (38/194; 20%), carcinosarcoma (32/194; 16%), uterine serous (24/194; 12%) and mucinous (17/194; 9%). Other very rare tumors accounted for almost 15% of the total (Table 1). At the time of registration for profiling, the median age was 59 (age range, 21–88), the majority of the patients had excellent performance status (69% ECOG PS = 0) and had received a median of 1 prior systemic treatment (range, 0–4). The median time from diagnosis to consent for profiling analysis was 18 months (range, 1–361). Genomic testing was on archival biopsy or surgical resection samples of the primary tumor for 68% and metastatic tumor biopsies for 32% (Table 1).

3.2. DNA sequencing results

A total of 194 patients with rare epithelial gynecologic cancers were successfully profiled. Fifty patients (26%) had samples tested by MALDI-TOF MS, 136 (70%) by TruSeq and 8 (4%) by ASCP (Fig. 1). Of the 194 patients profiled, 139 (72%) had one or more somatic mutations detected (range, 1–4), including 33 (24%) by MALDI-TOF MS, and 106 (76%) by TruSeq and ASCP. Fifty-five patients (28%) had two or more somatic mutations (Fig. 1). The most common somatic mutations identified were *TP53* (39%), *KRAS* (28%), *PIK3CA* (27%), *FBXW7* (7%) and *PTEN* (5%). Additional low incidence mutations (range of 1–5% frequency), including mutations in *NRAS*, *CTNNB1*, *BRAF* and *ERBB2*, among others, were also detected (Fig. 2). Details on the specific mutations identified in each histology are provided in Supplementary Fig. 1. For the mutation spectrum assessed, the percentage of patients with potentially actionable alterations (Class 1 or 2) was 34.5% (48/139). The proportion of patients with variants and class variants, and distribution by tumor type are shown on Supplementary Figs. 2A and 2B. We did not detect any patients with additional germline variants.

3.3. Clinical trials and patient outcomes

Of the 194 patients with molecular profiling results, 47 (24%) were subsequently treated in 56 therapeutic clinical trials, including 28 genotype-matched trials (14% of total population profiled). Of note, nine patients had two successive clinical trials after molecular profiling results. The number of lines of prior systemic therapy and duration of standard therapy prior to trial enrollment were similar between the genotype-matched and genotype-unmatched trial patient cohorts (Table 2). There was no difference in the proportion of patients treated on genotype-matched trials who underwent profiling using MALDI-TOF or a larger targeted NGS panel. The majority of genotype-matched trials were phase I trials (16/28; 57%). Genotype-matched trials commonly involved targeted drug monotherapies or targeted combinations without chemotherapy or immunotherapy (Table 2). Several reasons accounted for patients not being enrolled onto clinical trials following molecular profiling, including: deterioration in performance status (48 patients; 25%) and loss to follow-up or deceased (34 patients; 17%). Of note, 31 patients (16%) had no evidence of progression to prior line of treatment, and five patients (3%) completed therapy in another centre (Supplementary Fig. 3).

Twenty-seven of the 28 patients enrolled in genotype-matched were evaluable for efficacy. The key somatic alterations identified in the evaluable patients enrolled in genotype-matched trials were *KRAS* and *PIK3CA*, which accounted for 93% of genotype-matched trial enrollments. Among genotype-matched evaluable patients, 16/27 (59%) received MEK inhibitor-based targeted combinations and the majority were LGS (13/16, 81%). Other genotyped-matched therapies included single agents PI3K/AKT/mTOR inhibitors for patients with clear cell carcinoma, cervical adenocarcinoma, uterine serous carcinoma, and carcinosarcoma histologies. One patient with vaginal cancer harboring *PIK3CA* mutation completed two subsequent matched trials (single agent AKT and single agent PI3K inhibitors). Mutational status of genotype-matched *versus* unmatched patients is shown in Fig. 3A and B. A similar overall response rate (ORR) was observed among evaluable patients treated on genotype-matched trials (8/27 patients; 30%) *versus* those treated with genotype-unmatched trials (7/28 patients; 25%; p = 0.70) (Fig. 3A and B). All evaluable LGS patients treated on genotype-matched trials obtained clinical benefit from MEK inhibitor-based targeted combinations, including 4/13 patients with PR and 9/13 patients with SD as best response. However, patients with *PI3KCA*

Table 1
Characteristics of Rare Epithelial Gynecologic Cancer Patients enrolled into IMPACT/COMPACT (n = 194).

Characteristics	N (194)
Median age (range)	59 (21–88)
Median lines of prior treatment (range)	1 (0–4)
ECOG performance status (0/1)	69%/31%
Archival Primary lesion/Metastasic profiled	68%/32%
Tumor Histologies	
LGS	54 (28%)
Clear cell	38 (20%)
Ovarian	28 (14%)
Uterous	9 (4.5%)
Cervical	1 (0.5%)
Carcinosarcoma	32 (16%)
Ovarian	12 (6%)
Uterous	19 (9.5%)
Cervical	1 (0.5%)
Uterine Serous	24 (12%)
Mucinou	17 (9%)
Ovarian	7 (4%)
Cervical	10 (5%)
Vulvar	12 (6%)
Cervical adenocarcinoma/adenosquamous	11 (6%)
Vaginal	2 (1%)
Transitional ovarian	2 (1%)
Small cell (ovarian)	1 (0.5%)
Squamous ovarian	1 (0.5%)
Median time from diagnosis to consent for profiling (range, months)	18 (1–361)

Abbreviations: LGS: Low-Grade Serous ovarian cancers.

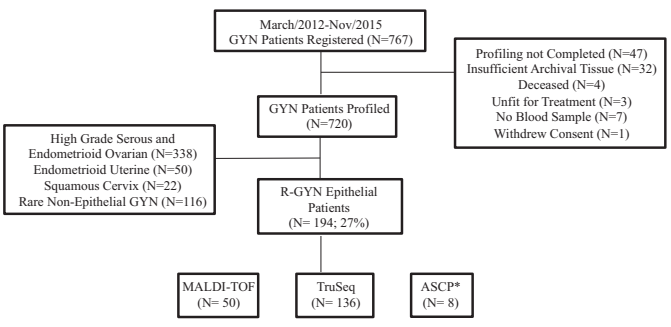

Fig. 1. Overview of outcomes for patients enrolled. *ASCP conducted for tumor specimens with low DNA quality. Abbreviations: R-GYN: Rare Gynecologic Malignancies; MALDI-TOF: matrix-assisted laser desorption/ionization time- of-flight; TruSeq: TruSeq Amplicon Cancer Panel; ASCP: The Ion AmpliSeq Cancer Panel.

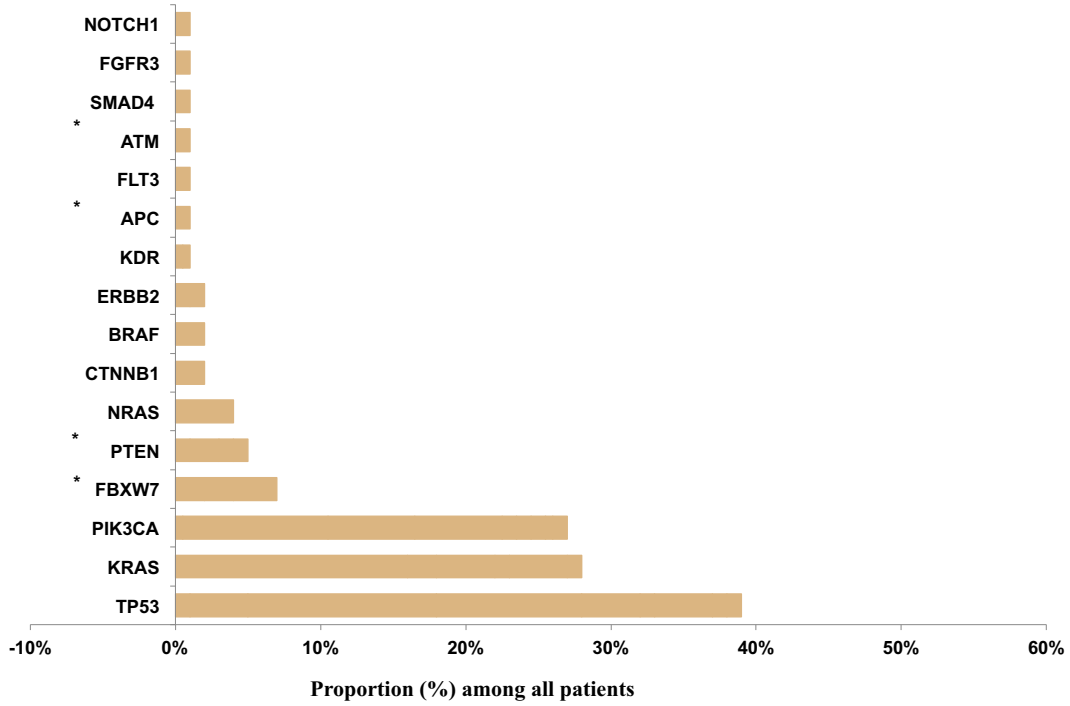

Fig. 2. Mutation frequency by gene, $n = 194$. Mutation frequency was calculated as the number of variant occurrences within each gene divided by the total number of patients* *Genes not included in MALDI-TOF panel.*

mutation treated with single agent PI3K/AKT/mTOR inhibitors had PD as best response. Genotype-matched trials did not significantly improve the likelihood to achieve a best response of any shrinkage in the sum of target lesions compared with patients on genotype-unmatched trial (78% *vs* 57%; $p = 0.16$). Waterfall plots for best-observed changes in target lesions for patients on genotype-matched and unmatched trials are summarized in Fig. 3A and B, respectively.

In 12/27 evaluable genotype-matched and 12/28 genotype-unmatched trial patients, comparison with time on treatment for the immediate prior systemic standard treatment was evaluated. For genotype-matched trials median time on treatment was 4.2 months (1.2–17) compared to 4.1 months (1.9–10) for the prior standard therapies ($p = 0.724$), whereas time on treatment for genotype-unmatched trials was 2.7 months (1–19) compared to 5 months (2–14) for immediate prior standard therapies ($p = 0.284$). Differences in time on treatment post-profiling for genotype-matched and unmatched trials compared to time on treatment on standard therapies for those patients that did not complete therapeutic trials were also assessed. We observed a statistically significant benefit in median time on treatment for genotype-matched treatments ($N = 24$) compared to both genotype-unmatched ($N = 25$) and standard therapy post-profiling ($N = 26$) [5.5 months (1.2–22) *vs* 2.7 months (0.7–18.9); $p = 0.03$); and 2.7 (0.3–14.6); $p = 0.02$, respectively].

Table 2
Characteristics of patients enrolled in therapeutic trials following molecular profiling.

Characteristics	Genotype-unmatched N = 28	Genotype-matched N = 28	*p value*
Median lines of prior treatment (range)	1 (0–4)	1 (0–3)	NS
Duration of Standard Therapy Prior to Molecular Profiling, weeks (range)	19 (4–327)	14 (7–31)	NS
Tumor type (number of patients)			
LGS	6	13	0.33
Clear cell	7	5	
Carcinosarcomas	3	1	
Uterine Serous	5	3	
Mucinous	3	1	
Vulvar	1	0	
Vaginal	0	2	
Cervical adenocarcinomas or adenosquamous	2	2	
Transitional/Brenner Ovarian	0	0	
Squamous ovarian	0	0	
Small cell	1	0	
Trial phase			
Phase I - N (trials)	11	16	0.31
Phase II - N (trials)	8	4	
Phase III - N (trials)	9	8	
Investigational agent(s)			
Targeted Monotherapy	14	18	<0.001
Targeted Drug Combination	0	13	
Chemotherapy Drug	8	0	
Targeted Drug + Chemotherapy	4	0	
Immunotherapy	2	0	

Abbreviations: LGS: Low-Grade Serous ovarian cancers.

4. Discussion

Establishing standard therapeutic approaches for patients with rare gynecologic cancers remains challenging due to low incidence, paucity of clinical activity data for cytotoxic or novel biologic agents, and difficulties of conducting clinical trials in this context. Over the last several years, the integration of genomic characterization in routine clinical care of different tumor types, such as melanoma or non-small lung cancer, has become standard; however, for rare tumors this remains investigational in nature. Our study demonstrates the feasibility of incorporating precision medicine in patients with rare gynecologic cancers who participated in a broad prospective genomic institutional profiling program. We also showed how genotyping or targeted NGS enabled the identification of patients with rare tumors and actionable mutations that could subsequently be candidates for genotype-

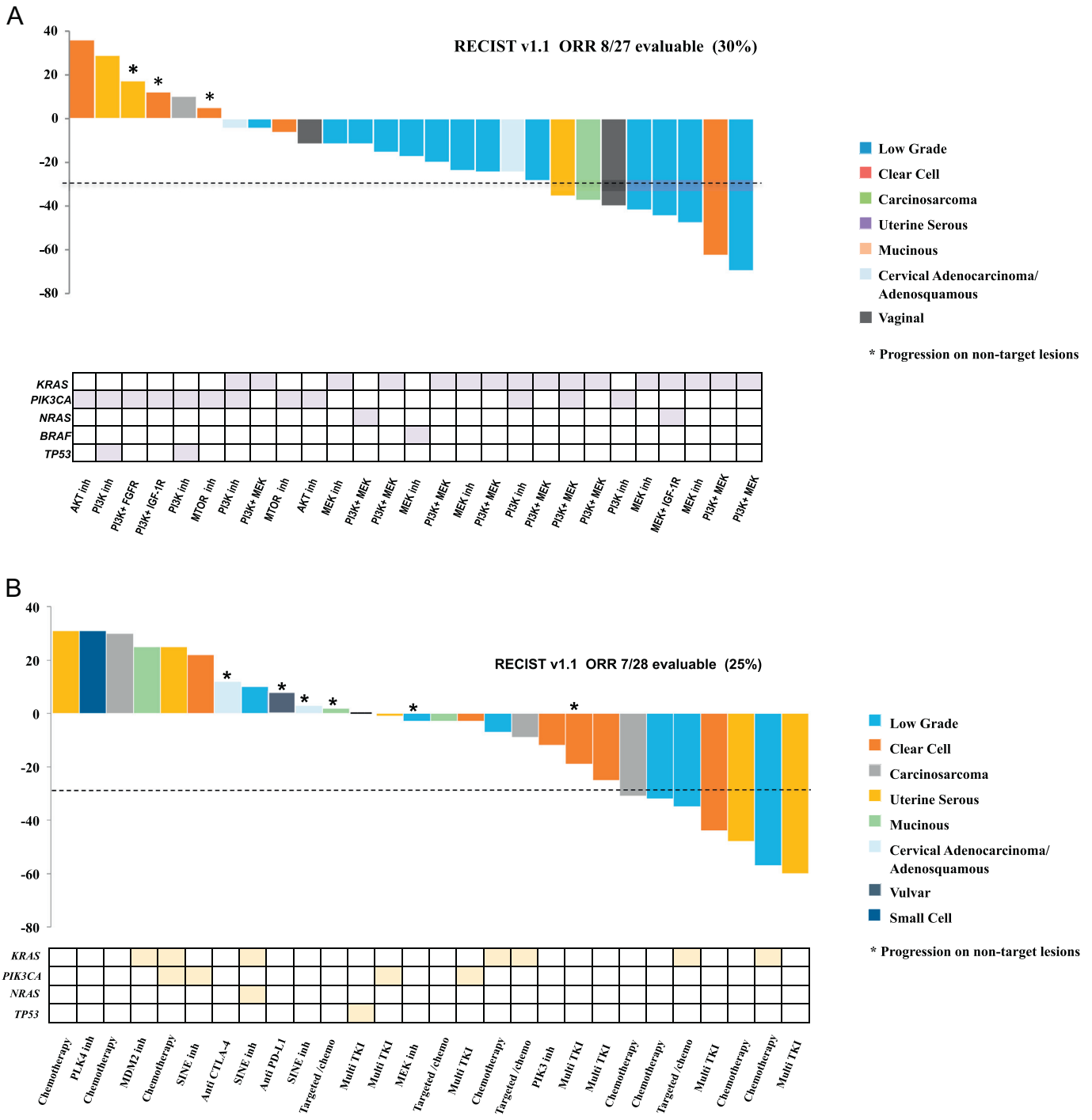

Fig. 3. A. Waterfall plots of best tumor shrinkage of target lesions by RECIST and mutational status for evaluable patients treated on (A) genotype-matched clinical trials ($n = 27$). Abbreviations: AKT: protein kinase B; PI3K: phosphoinositide 3-kinase; FGFR: fibroblast growth factor receptor; MTOR: Mammalian target of rapamycin (mTOR); IGF-1R: insulin-like growth gactor-1 receptor; MEK: mitogen-activated protein kinase inhibitor B. Waterfall plots of best tumor shrinkage of target lesions by RECIST and mutational status for evaluable patients treated on genotype-unmatched clinical trials ($n = 28$). Abbreviations: PLK4: polo-like kinase 4; MDM2: mouse double minute 2 homolog; SINE: selection inhibitor of nuclear exporter; CTLA-4: cytotoxic T lymphocyte antigen-4; PD-L1: Programmed Death-ligand 1; TKI: tyrosine kinase inhibitor; MEK: mitogen-activated protein kinase kinase; PI3K: phosphoinositide 3-kinase.

matched clinical trials, predominantly MEK-based combinations in *KRAS* and/or *NRAS* mutant LGS patients.

The gynecologic cancer patient population is well-represented among the total patient population enrolled on several large-scale institutional testing programs [12–16]. Other studies have specifically reported the adoption of genomic profiling and outcomes of gynecologic cancer patients enrolled on institutional genomic programs; however, it is important to note that the majority of patients enrolled had common histologies such as high grade serous ovarian or endometrioid uterine tumors [18–20]. This study focused on rare histologies, which represented 27% of the gynecologic population profiled at PM [16]. This rate, although significant, implies that there remains ample room for improvement in the implementation of genomic profiling for this subgroup of patients, considering that up to 50% of gynecological cancers are classified as "rare" [1]. Our study provides evidence of the utility of developing precision medicine for patients with rare gynecologic

cancers. Our rate of 14% of patients with rare histologies treated on genotype-matched clinical trials upon genomic results is higher than the 5% rate observed across multiple tumor types profiled at PM [16], and also higher than the 5–10% rate of matched therapy application reported among the gynecologic cancer population enrolled in other institutional programs [12–15]. This may reflect the high proportion of actionable somatic mutations (72%) identified in rare histologies. In contrast to common gynecologic tumors, such as HGSOC characterized by limited number of actionable mutations, our study noted a significant proportion of rare tumors having had at least one somatic mutation, particularly in targetable oncogenes such as *KRAS*/*NRAS*/*BRAF* or *PI3KCA* (Fig. 2). Our results also demonstrated that 34% of mutations detected were deemed to be clinically relevant (class 1–2). These findings could be essential for the allocation of a greater number of patients with rare gynecologic malignancies to genotype-matched trials.

In our study, all evaluable *KRAS* and/or *NRAS* mutant LGS patients treated on genotype-matched trials obtained clinical benefit from MEK inhibitor-based targeted combinations. Evidence from GOG-239 study demonstrated a 15% overall response rate and a disease control of 63% to MEK inhibition with Selumetinib, a non-ATP competitive inhibitor of MEK1/2 in LGS; although, no correlation was found between *BRAF* or *KRAS* mutation status and therapeutic response [21]. Development of the MEK inhibitor Binimetinib (MEK162) in the MILO study, a randomized phase III trial comparing MEK162 *versus* physician's choice of chemotherapy in LGS patients irrespective of RAS/RAF genotype, was discontinued for futility reasons after a pre-planned interim analysis, although molecular results of this study are yet to be reported [22]. The benefit derived with MEK-based combination treatments in KRAS/NRAS mutant histologies raises the question as to whether RAS mutations should be considered as a biomarker for MEK-based inhibitor therapies irrespective of the tumor subtype. A recent report from our institution has also confirmed this hypothesis but toxicity concerns have been raised for this class of drug [7] and further careful evaluation is necessary. In our study, none of the patients with *PI3KCA* mutation treated with single agent PI3K/AKT/mTOR inhibitors experienced clinical benefit. Although mutations in *PIK3CA* are common in gynecologic cancers, they have not been reliably predictive of clinical response to single agents targeting the PI3K/mTOR pathway [23–25]. Development of second-generation drugs, such as isotype-specific PI3K inhibitors, particularly PI3Kα inhibitors or combination strategies, has been associated with improved outcomes in patients with *PI3KCA*-mutated breast cancer treated in early phase clinical trials [26,27]. The drugs used during our study period may not have been optimal as the majority of genotype-matched clinical trials available did not involve alpha isoform-selective/specific PI3K inhibitors. The identification of a 40% rate of *TP53* mutations in our population together with encouraging results of Wee-1 inhibitors in *TP53* mutated tumors [28] or agents such as APR-246—converts mutant *TP53* to a form with wild type properties and is being investigated in *TP53* mutated ovarian cancer (NCT02098343)—could also provide more effective treatment strategies for rare tumors.

Whilst our study was enriched with a high proportion of LGS, targeted inhibition also provided some degree of clinical benefit in several other rare gynecologic cancer types, including: type I ovarian cancers, cervical adenocarcinoma, serous uterine and very rare tumors such as vaginal tumors. Given the limited numbers, our findings are hypothesis generating and may help support future directed investigations in rare histologies. They may be cautiously considered to be clinically meaningful, given the limited activity of conventional cytotoxic agents and the poor prognosis of this patient population.

Our study also identified anti-tumor activity across a variety of rare tumor histologies that underwent genotype-unmatched therapeutic strategies (either chemotherapy or targeted therapies). It is noteworthy that patients with LGS also experienced tumor shrinkage with chemotherapy trials, which is in line with literature [29,30] and suggests that although novel therapeutic approaches are warranted, chemotherapy remains a therapeutic option for recurrent LGS [29,30]. Other histologies, such uterine serous carcinoma or carcinosarcomas also benefited from genotype unmatched therapeutic strategies such as multi-tyrosine kinase inhibition. Encouraging activity has been reported with Cabozantinib in patients with advanced endometrial cancer, including serous carcinomas and carcinosarcomas, as separate cohorts [31]. Most recently, the irreversible pan-HER tyrosine kinase inhibitor Neratinib has shown promising activity across cohorts of patients with somatic mutations of *ERBB2* and *ERBB3*, including patients with cervical and serous uterine cancers, enrolled in a phase II molecularly driven basket trial [32]. Clear cell tumors, also resistant to standard chemotherapy [33] benefited from multi-TKI treatment in our study, probably in relationship with the key role of angiogenesis in this tumor type [33]. Activity data of single agent ENMD-2076 inhibition, an Aurora tyrosine kinase inhibitor, also with antiangiogenic effects, has been reported and interestingly, loss of *ARID1A* expression assessed by immunohistochemistry has been suggested as potential biomarker [34]; however, the signal was not sufficient to further investigation.

In our study, we did not observe statistical differences in terms of tumor response or differences in time on treatment with either genotype-matched or unmatched treatments compared with the immediate prior of standard therapy. This finding is in concordance with other studies focused on gynecologic cancers receiving targeted therapies through phases I clinical trials [18–20]. A striking feature of our study was the almost doubled time on treatment for genotype-matched treatments in comparison to either time on treatment for genotype unmatched or standard treatments completed after molecular profiling. It is important to note that these results should be interpreted with caution as can be biased by several factors, including: *i*) enrichment of LGS onto genotyped-matched trials; *ii*) limited number of patients included; and *iii*) numerical differences in inclusion onto phase I and phase II/III trials in genotype-matched or unmatched trials.

This study, conducted as a sub-analysis of a prospective institutional genomic program, provided us with valuable insights into the feasibility and clinical utility of using a genomically informed treatment decision-making approach to direct patients with rare cancer and limited therapeutic options to relevant clinical trials. A challenge unique to rare cancers is availability of clinical trials in the advanced setting, primarily due to low incidence and broad variety of histologies. Here, we have demonstrated that conducting biomarker-driven non-randomized trials, irrespective of histology or site of tumor origin is feasible and can overcome those challenges. Conducting broad genomic programs can be also exploited to improve the management of rare gynecologic malignancies and this approach may include: *i*) emphasizing the development of programs supporting access to off-label therapies, or access to biomarker-driven studies that are not dependent upon tumor site or histology [35]; *ii*) adopting genomic characterization at early stages of disease to help prevent performance status deterioration and the acquisition of cytotoxic drug resistance genomic alterations; and *iii*) establishing a pre-specified treatment algorithm depended upon genomic results. Importantly, the establishment of a multidisciplinary tumor board for rare tumors can be an efficient approach of not only interpreting molecular information, or the treatment decision-making process, but also to improving the delivery of precision medicine in rare gynecologic cancers [36].

There are several limitations of our study that warrant further examination. Although prospective in nature, our study is observational and includes different gynecological cancers and histologies. This is further punctuated by relatively small numbers of patients in each rare histology, which results in a degree of difficulty in making conclusions regarding differential therapeutic outcomes. We should note that very rare histologies such as non-epithelial and sarcomatoid tumors were not included in our analysis. In addition, this study did not comprehensively capture how testing results influenced the point-of-care decision-making process outside of clinical trial enrolment. Most of the genomic profiling was performed on samples obtained from archival tissue rather than fresh tumor biopsy which may have impacted the results

given that the level of evidence for targetability of cancer variants is dynamic, varying significantly over time [30]. There were a limited number of molecular events described in our population due to the sequencing approach chosen. With technologies improvement, different genomic tests were used over the study period but our genomic assessment was limited to hotspot point mutation or targeted sequencing of limited number of genes. Whilst our study captured common somatic alterations in gynecologic malignancies, such as *KRAS* and *PIK3CA* mutations, this did not include genomic alterations in DNA damage repair genes or measurement of tumor mutation burden, identification of microsatellite stability or instability, gene copy number alterations or recurrent translocations such *FGFR1* amplification or fusions, *NTRK* fusions, *PIK3CA* fusions and *ERBB2* fusions which may be identified in rare gynecological malignancies. Our study did not reveal any germline variant, likely due to the small panel used. With the evolution of omics and newer NGS panels including large panel of genes, deeper analysis will be available allowing further characterization of disease biology, first step for improving treatment strategy. As the knowledge on drivers and targetability is improving, it is critical to continuously update the resources used for clinical interpretation of cancer variants with emerging drugs.

5. Conclusions

This study demonstrates that precision medicine of patients with rare gynecologic cancers is feasible and enables the identification of patients who are candidates for genotype-matched clinical trials. Access to trial is key given the lack of treatment options for these patients. Despite this small study population size, genotype-matched treatments, particularly with MEK-based combinations, provided cautiously clinically meaningful results in specific patients. Additional studies are required to assess the clinical utility of NGS tests as a standard clinical application outside of clinical trials and to evaluate if this approach leads to improved clinical outcomes. Greater efforts should also be made to implement a more comprehensive and collaborative approach to expand opportunities for tailored treatment in patients with rare gynecologic malignancies.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.02.005.

Funding

This work was supported by the Princess Margaret Cancer Foundation; the Cancer Care Ontario Applied Clinical Research Unit [to LLS]; the University of Toronto Division of Medical Oncology Strategic Innovation [to PLB]; the Ontario Ministry of Health and Long-Term Care Academic Health Sciences Centre Alternate Funding Plan Innovation Award [to PLB] and the Spanish Society of Medical Oncology (SEOM), Spain [VRF].

Presentation

2016 ASCO annual meeting. Abstract no. 5509 Gynecologic Cancer. Oral presentation Clinical Science Symposium, Leveraging the Immuno-Molecular Landscape of Gynecologic Cancers.

Acknowledgments

The authors would like to thank all study participants and their families, medical oncologists, pathologists, laboratory technicians, clinical data coordinators, and correlative studies coordinators who participated in this research study.

Ethical standards

This study was approved by the University Health Network Research Ethics Board.

Informed consent

Informed consent was obtained for all participants in this study.

Author contribution

All authors participated in the acquisition, analysis, or interpretation of data. VRF, SL, VM and AMO drafted the manuscript for initial review by all authors. LW performed statistical analysis. All authors read and approved the final manuscript.

Conflict of interest statements

The authors have declared no conflicts of interest. Dr. Oza reports personal fees from Intas Pharmaceuticals, non-financial support from AstraZeneca, non-financial support from Tesaro, non-financial support from Clovis, outside the submitted work; Dr. Stockley reports grants and personal fees from AstraZeneca, during the conduct of the study; Dr. Butler reports personal fees from Merck, BMS, Novartis, EMD Serono, and from Immunovaccine, outside the submitted work; Dr. Bedard reports grants from Princess Margaret Cancer Foundation, grants from Ontario Ministry of Health and Long Term Care, grants from University of Toronto Division of Medical Oncology, Department of Medicine, during the conduct of the study.

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