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Original Research

Genetic heterogeneity after first-line chemotherapy in high-grade serous ovarian cancer

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Abstract ***Background:*** Most high-grade serous ovarian carcinoma (HGSOC) patients benefit from first-line platinum-based chemotherapy, but progressively develop resistance during subsequent lines. Re-activating *BRCA1* or *MDR1* mutations can underlie platinum resistance in end-stage patients. However, little is known about resistance mechanisms occurring after a single line of platinum, when patients still qualify for other treatments.
Methods: In 31 patients with primary platinum-sensitive HGSOC, we profiled tumours collected during debulking surgery before and after first-line chemotherapy using whole-exome sequencing and single nucleotide polymorphism profiling.
Results: Besides germline *BRCA1/2* mutations, we observed frequent loss-of-heterozygosity in homologous recombination (HR) genes and mutation spectra characteristic of HR-deficiency in all tumours. At relapse, tumours differed considerably from their primary counterparts.

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There was, however, no evidence of events reactivating the HR pathway, also not in tumours resistant to second-line platinum. Instead, a platinum score of 13 copy number regions, among other genes including *MECOM, CCNE1* and *ERBB2*, correlated with platinum-free interval (PFI) after first-line therapy, whereas an increase of this score in recurrent tumours predicted the change in PFI during subsequent therapy.

Conclusions: Already after a single line of platinum, there is huge variability between primary and recurrent tumours, advocating that in HGSOC biopsies need to be collected at relapse to tailor treatment options to the underlying genetic profile. Nevertheless, all primary platinum-sensitive HGSOCs remained HR-deficient, irrespective of whether they became resistant to second-line platinum, further suggesting these tumours qualify for second-line Poly APD ribose polymerase (PARP) inhibitor treatment. Finally, chromosomal instability contributes to acquired resistance after a single line of platinum therapy.

1. Background

Platinum resistance remains the major challenge in the treatment of recurrent high-grade serous ovarian carcinoma (HGSOC), which is the most frequent type of ovarian cancer associated with dismal prognosis.[1]. Sensitivity to first-line platinum-based chemotherapy in combination with debulking surgery is classified as refractory, resistant, partially-sensitive or sensitive, according to the interval between the last platinum-dose and relapse [2]. Since this platinum-free interval (PFI) correlates with the chance of secondary response to platinum, other treatment options are explored for platinum-resistant recurrences [3]. The mechanisms driving therapy resistance in HGSOC remain, however, poorly understood.

Although *BRCA1/2*-deficient tumours respond better to platinum-based chemotherapy, *BRCA1/2* alterations, which occur in ~30% of HGSOCs, only partially explain the spectrum of platinum sensitivity in primary HGSOC [4]. Secondary re-activating *BRCA1/2* mutations have been proposed to underlie platinum resistance of recurrent *BRCA1/2*-mutated HGSOC [5]. This was confirmed by Patch et al., who in addition to *BRCA1/2* reversal mutations, observed a decrease in *BRCA1* promoter methylation, as well as recurrent promoter fusion associated with overexpression of the drug efflux pump *MDR1* [6]. However, both studies compared primary platinum-sensitive biopsies to ascites derived from end-stage platinum-refractory patients that underwent multiple lines of platinum therapy, thereby comparing two extremes in the spectrum of platinum response. Although these observations are highly interesting, it would clinically be more relevant to assess genetic changes after the first-line of platinum therapy, because at this stage there is a much broader range of alternative therapies available, either as standard-of-care or in the context of clinical trials. For instance, it is important to explore whether these *BRCA1/2* reversion mutations emerge already after one line of platinum treatment, since this would influence response to poly APD ribose polymerase (PARP) inhibitors, which are currently approved as maintenance therapy for platinum-sensitive *BRCA1/2*-mutant recurrent HGSOC [7]. A better understanding of the mechanisms underlying platinum sensitivity and resistance arising during initial lines of platinum therapy could thus affect clinical decision-making for recurrent HGSOC.

Recent sequencing studies revealed a high degree of genetic heterogeneity within individual tumours and between their metastases [6,8–12]. Because of selective pressure exerted by the therapy, which eradicates treatment-sensitive tumour clones while selecting for resistant clones, this heterogeneity could inform us about genetic changes underlying therapeutic resistance [9]. In HGSOC, most studies report genetic heterogeneity through the analysis of clonal relationships between *spatially*-divided biopsies [8,10–12]. With respect to *temporal* heterogeneity, few patients were analysed and tumour DNA was either retrieved from ascites [10,12] or from tumour biopsies samples taken during neo-adjuvant chemotherapy [11,12]. Since surgery is rarely performed, and biopsies of recurrent HGSOCs are not routinely collected, almost no matched tumour pairs collected 'before' and 'after' a single line of platinum therapy have been analysed. As a result, little is known about temporal genetic heterogeneity and resistance to platinum occurring after the current standard-of-care for primary HGSOC.

2. Patients and methods

See online supplement for additional details.

2.1. Patients and tumours

We included patients with advanced (International Federation of Gynecology and bstetrics [FIGO] stage III–IV) HGSOC for which biopsies were obtained during debulking surgery, the first at initial diagnosis