REVIEW

Biomarkers of platinum resistance in ovarian cancer: what can we use to improve treatment

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Abstract

Ovarian cancer has poor survival rates due to a combination of diagnosis at advanced disease stages and disease recurrence as a result of platinum chemotherapy resistance. High-grade serous ovarian cancer (HGSOC), the most common ovarian cancer subtype, is conventionally treated with surgery and paclitaxel/carboplatin combination chemotherapy. Initial response rates are 60–80%, but eventually the majority of patients become platinum-resistant with subsequent relapses. Extensive research on individual biomarkers of platinum resistance has revealed many potential targets for the development new treatments. While this is ongoing, there are also epigenetic, DNA repair, genome and immune changes characterised in platinum-resistant HGSOC that can be targeted with current therapies. This review discusses biomarkers of platinum chemotherapy resistance in ovarian cancer with a focus on biomarkers that are targetable with alternative treatment combinations to those currently used. After decades of research focused on elucidating the biological cause of platinum resistance, future research needs to focus on using this knowledge to overcome resistance for patients with ovarian cancer.

Key Words
- ovarian cancer
- platinum chemotherapy
- cisplatin
- carboplatin
- resistance
- biomarkers

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Introduction

Ovarian cancer has an annual worldwide incidence of approximately 240,000 and annual mortality rate of 152,000 (Ervik *et al.* 2016). The high annual mortality rate is due to a combination of diagnosis at advanced disease stages and disease recurrence as a result of chemotherapy resistance. There are several subtypes of ovarian cancer with differing histology, anatomical origins and molecular profiles resulting in vastly different inherent sensitivities to chemotherapy. Herein, we review biomarkers of platinum chemotherapy resistance in ovarian cancer with a focus on biomarkers that are potentially targetable for future treatment combinations.

Ovarian cancer subtypes

There are 2 major subtypes of ovarian cancer that are determined by the tissue of origin and classified as either epithelial and non-epithelial. Non-epithelial ovarian cancers include sex cord stromal, germ cell and non-specified ovarian cancers. Epithelial ovarian cancers include transitional cell, mucinous, clear cell and serous ovarian

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cancer. The focus of this review is the epithelial ovarian cancer subtypes, in particular the most common, HGSOC.

Epithelial ovarian cancer

Epithelial ovarian cancer (EOC) is an umbrella term that covers a diverse groups of tumours that can be classified into different subtypes based on the 2 main pathways of tumorigenesis according to a unifying theory proposed by Kurman and Shih (Shih Ie & Kurman 2004, Kurman & Shih Ie 2010). Type 1 EOC are considered to be genetically stable, slow to develop and are usually contained within the ovary at presentation. Therefore, most are diagnosed at an early stage and respond well to mostly surgical treatment. They consist of low-grade serous, low-grade endometrioid, clear cell, mucinous and transitional or Brenner tumours that have developed from clearly recognised precursor lesions or borderline tumours (Kurman & Shih Ie 2010). Conversely, type 2 EOCs are highly invasive, grow quickly and are thus typically diagnosed at an advanced stage. These aggressive tumours consist mainly of high-grade serous, high-grade endometrioid, malignant mixed mesodermal tumours and undifferentiated carcinoma (Kurman & Shih Ie 2010).

Correct diagnosis of the subtype and stage of EOC is extremely important as each subtype responds differently to standard treatment options, and late-stage disease has poor survival rates. Despite these differences, almost all ovarian cancers are treated initially with surgical removal of tumour tissue termed 'debulking', followed by 6 courses of paclitaxel/carboplatin combination chemotherapy (in some cases with the addition of bevacizumab). However, upfront surgery is not appropriate for some patients, and neoadjuvant combination chemotherapy is administered prior to surgery. After debulking surgery, EOC is surgically staged based on the International Federation of Gynecologists and Obstetricians (FIGO) criteria (Prat *et al.* 2015). Most type 2 EOCs are diagnosed at stage IIIC or higher resulting in poor survival rates. Neoadjuvant chemotherapy regimes can affect the cellular architecture and morphological features of the tumour, thus making correct subtyping difficult if this treatment approach is used (McCluggage 2008).

Serous ovarian cancer

Serous ovarian cancer (SOC) is the most common subtype, accounting for ~70% of all ovarian cancers. SOC is not a single disease but is composed of high-grade serous ovarian cancer (HGSOC) and low-grade serous ovarian cancer (LGSOC). These are not two grades of the same neoplasm but rather distinctly different tumour types (O'Neill *et al.* 2005, McCluggage 2008) with vastly variable clincopathologic features and behaviours (Kurman 2013) derived from different pathogenetic pathways of formation (Malpica *et al.* 2007). HGSOC generally affects older women who present at a later FIGO stage. Even though there is an initial response to treatment, they become resistant over time and have an overall poorer prognosis. LGSOC is mainly diagnosed in younger women, is slow growing (McCluggage 2008), and more likely to be non-responsive to chemotherapy and ultimately has a better prognosis than HGSOC with a longer overall survival time (Ramalingam 2016).

HGSOC is characterised by a number of histological features that are not exclusive to the subtype, however are favoured by it. The cellular architecture is, predominantly and sometimes exclusively, papillary in nature across large sheets of cells and is associated with slit-like rather than round gland formations and psammoma bodies (Clements & Young 2008, Ramalingam 2016). For a diagnosis of HGSOC based on the two-tier grading system by Malpica and colleagues (Malpica *et al.* 2004), carcinomas must have nuclear atypia often in the form of multinucleated cells as well as >12 mitoses per 10 high-power fields. Solid variants do occur with minimal or no papillary or glandular differentiation making it difficult to determine morphologically if the tumour is HGSOC or an undifferentiated carcinoma. HGSOC is also characterised by p53 mutations (Vang *et al.* 2016), detected as aberrant p53 using immunohistochemistry (IHC) or by targeted next-generation sequencing (Cole *et al.* 2016).

There have been many theories on the origins of HGSOC. Originally, it was considered that HGSOC originates directly from the surface epithelium undergoing metaplastic changes. The 'incessant ovulation' hypothesis first proposed by Fathalla in 1971, described continual ovulation resulting in a repetitive cycle of damage and repair to the ovarian surface epithelium (OSE), leading to an increase in inflammation and hormonal level fluctuations resulting in oxidative DNA damage (Fathalla 1971). Humans are at an increased risk of this damage and repair cycle due to the high number of uninterrupted ovulation cycles compared to other animal models that have 'rest periods'. However, there has been an increase in pharmacologically induced non-ovulatory rest periods since the introduction of the oral contraceptive pill (OCP).

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The second theory was that it derives from cortical inclusion cysts (CIC) found within the ovary. These cysts are developed from the invagination of OSE that forms Müllerian type tissue and then is subjected to neoplastic transformation (Kurman 2013, Banet & Kurman 2015, Zeppernick *et al.* 2015). Although it has been speculated that HGSOC is of ovarian origin, there has been no definitive identification of a precursor legion. Therefore, a paradigm shift away from the ovary towards the epithelium of the fimbriated end of the fallopian tube developed (Kurman 2013, Zeppernick *et al.* 2015, Ramalingam 2016), wherein serous tubal intraepithelial carcinoma forms as a precursor to HGSOC. Although it is believed that 50–60% of HGSOC originate from the fallopian tube (Kroeger & Drapkin 2017), many are found to contain p53 mutations that are identical to those found in the corresponding serous tubal *in situ* carcinoma (STIC), thus suggesting a genetic connection between the tumour and the STIC (Kindelberger *et al.* 2007, Lee *et al.* 2007). This relationship has also been observed on the protein level, with HGSOC staining positive for PAX8, a Müllerian marker. However, staining negative for the mesothelial marker calretinin, indicating that HGSOC's expression profile is closer to that of the fallopian tube than that of the surface epithelium of the ovary (Zeppernick *et al.* 2015). Although there are several possible pathways and varied conceivable originating sites, the exact cell of origin of HGSOC has not been fully elucidated and requires further investigation to allow for a better understanding of this disease.

Chemotherapy

Platinum chemotherapy

Platinum chemotherapy was accidentally discovered in 1965 when it was first observed that a platinum compound was inhibiting cell division in *E. coli* (Rosenberg *et al.* 1965). The compound was later named 'cisplatin' and its effect on the division of cancer cells was confirmed in animal studies in 1970 (Rosenberg & VanCamp 1970). Clinical trials began soon after in 1972, and in 1978, cisplatin was approved in the USA by the Federal Drug Administration (FDA) for the treatment of testicular, bladder and ovarian cancer. The discovery was a turning point for the treatment of advanced ovarian cancer.

There are now 5 platinum chemotherapy analogues approved for use in the treatment of cancer: cisplatin, carboplatin, oxaliplatin, nedaplatin and lobaplatin.

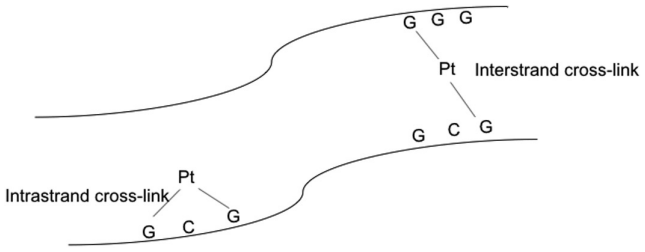

Figure 1
Mechanism of action for platinum chemotherapy. Platinum binds to the N-7 position of adjacent guanine (G) bases resulting in interstrand or intrastrand crosslinks.

The mechanism of action for the analogues used most commonly to treat ovarian cancer, cisplatin and carboplatin; is direct insertion of platinum into DNA to form crosslinks (Fig. 1). The resultant structural distortion of the DNA is either removed by specific DNA repair processes or it triggers a signalling cascade resulting in apoptosis.

Cisplatin or carboplatin monotherapy is used to treat some solid tumours, with testicular cancer obtaining cure rates of over 90% (Verhoeven *et al.* 2013). Platinum monotherapy is rarely used in HGSOC treatment (reviewed in Harter *et al.* 2010), it is only occasionally used for treatment of elderly patients where combination chemotherapy is not well tolerated.

Combination chemotherapy

HGSOC is most commonly treated with a combination of carboplatin and paclitaxel (Stuart *et al.* 2011), a tubulin target that blocks mitotic spindle assembly and halts cell division (Kampan *et al.* 2015). Many other agents are used in combination with platinum chemotherapies after relapse. These incude pegylated doxorubicin (TopII inhibitor, blocks replication) (Staropoli *et al.* 2014), gemcitabine (nucleoside analog, blocks DNA replication) (Pfisterer *et al.* 2006), trabectedin (transcription factor blocker) (D'Incalci & Galmarini 2010) and bevacizumab (angiogenesis inhibitor) (Oza *et al.* 2015).

Subtype-specific response to chemotherapy

Paclitaxel and carboplatin combination chemotherapy produces initial response rates in HGSOC of 60–80% (Selvakumaran *et al.* 2003), but eventually the majority of patients become platinum resistant with subsequent relapses. Clear cell, transitional cell, mucinous and LGSOC are predominantly resistant to platinum chemotherapy

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resulting in low-use of platinums in treatment regimes for these subtypes. The high level of resistance in these subtypes is problematic when assessing older studies that did not differentiate outcome analysis based on histological subtype. More recently, it has become standard practice to identify serous vs non-serous subtypes of ovarian cancer. In future studies, to assess new treatments or combinations, it would be ideal to segregate results of histological subtypes to ascertain accurate responses for each subtype.

Platinum chemotherapy resistance

Patients treated with platinum chemotherapy are categorized as either platinum sensitive or platinum resistant based on the amount of time from end of treatment to relapse, referred to as the platinum-free interval. The platinum-free interval is distinct from the progression-free interval (PFI) most commonly used to assess clinical trial outcomes (Davis *et al.* 2014). Davis *et al.* (2014) highlights this distinction in a 2014 review of platinum-resistant ovarian cancer; PFI is defined as the time from diagnosis to relapse, including the time undergoing first-line surgery and chemotherapy. The authors concluded that platinum-free interval is a more accurate way to categorize platinum response or sensitivity for ovarian cancer.

Platinum response is generally classified into refractory, resistant, partially-sensitive or sensitive. The Gynecologic Cancer InterGroup (GCOG) consensus statement recommended the following timelines for platinum response classifications: (1) Platinum-refractory: progression while receiving last line of platinum-based therapy or within 4 weeks of last platinum dose; (2) Platinum-resistant: progression-free interval since last line of platinum of less than 6 months; (3) Partially platinum sensitive: progression-free interval since last line of platinum of 6–12 months and (4) Platinum sensitive: progression-free interval since last line of platinum of more than 12 months (Stuart *et al.* 2011).

Platinum-sensitive ovarian cancer has a median survival of 2 years, with a range of 3 months to over 10 years. Platinum-resistant ovarian cancer has a median survival of 9–12 months and less than 15% respond to subsequent chemotherapy (Davis *et al.* 2014). Ultimately, almost all HGSOC patients become platinum resistant and succumb to the disease (Davis *et al.* 2014).

There has been a large body of research focused on identifying the mechanisms underlying HGSOC platinum resistance. The most studied mechanisms are within the cancer cells themselves, including p53 (Reles *et al.* 2001, Yang-Hartwich *et al.* 2015) and genomewide mutations (Patch *et al.* 2015), epigenetic changes (Wei *et al.* 2006, Vang *et al.* 2013, Chang *et al.* 2017) and dysfunctional DNA repair (Barakat *et al.* 2010). Possibly working together in concert, these genetic mechanisms lead to genomic instability that allows cancer cells to adapt and survive DNA damage caused by platinum chemotherapy. Although all these mechansisms have been associated with resistance, the exact mechanisms remain undefined.

Similarly the presence of cancer stem cells (CSCs) (Steg *et al.* 2012) and epithelial-to-mesenchymal transition (EMT) (Marchini *et al.* 2013, Chebouti *et al.* 2017) is associated with platinum resistance in HGSOC. Platinum chemotherapy is most effective on proliferating cells that make up the majority of rapidly growing cancer; therefore, it is hypothesized that populations of latent CSCs and mesenchymal-like cells are less likely to respond to platinum chemotherapy. In addition to changes to the genome and phenotype of HGSOC cancer cells, the tumour microenvironment, in particular, immune cell infiltration, angiogenesis and hypoxia have also been implicated in platinum chemoresistance.

To date, the complete set of mechanisms underlying platinum chemotherapy resistance and how they interact is not fully understood. The ultimate goal of establishing biomakers is to further this understanding and to assist clinicians and patients to make better informed treatment decisions. Some of the previously reported biomarkers have high potential for developing targeted therapies or for re-purposing non-traditional chemotherapies to improve treatment of platinum-resistant HGSOC. The main mechanisms of resistance and subtypes of biomarkers reported to date are reviewed in the following sections.

Mechanisms and biomarkers of resistance

Cancer stem cells

The cancer stem cells (CSCs) model of disease progression remains controversial as the process is still largely uncharacterized. CSCs are a relatively small subset of cancer cells that indefinitely self-renew, initiate and maintain tumour growth and may remain in quiescence for prolonged periods (Clevers 2011, Prasetyanti & Medema 2017). In HGSOC, they have been associated with platinum resistance and disease recurrence (Steg *et al.* 2012, Pylvas-Eerola *et al.* 2016). The mechanism of CSC

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associated platinum resistance is largely uncharacterised, but quiescence during chemotherapy remains the most likely mechanism. Quiescent ovarian CSCs are largely unaffected by chemotherapy as it relies on cell division to damage DNA and elicit an effect (Ottevanger 2017).

Markers of CSCs have been extensively studied in HGSOC, with ALDH and CD133 (Silva *et al.* 2011, Kryczek *et al.* 2012) being the most consistently replicated markers in both model systems and HGSOC tissue (Silva *et al.* 2011, Ruscito *et al.* 2017). Efforts have been made to identify CSCs markers for development of new treatments for HGSOC, from which the most promising so far is bone morphologenetic protein 2 (BMP2). BMP2 is upregulated in ovarian cancer cells (Le Page *et al.* 2006) and has been associated with poor prognosis (Le Page *et al.* 2009). An ovarian cancer cell hierarchical differentiation pattern in which BMP2 acts as a feedback mechanism promoting ovarian CSC expansion and suppressing progenitor proliferation was recently reported (Choi *et al.* 2015), but further studies to confirm this discovery in clinical populations is needed before directing new treatments towards this target. The evidence for HGSOC CSCs as targetable biomarkers of platinum resistance is compelling, but has yet to be translated into prognostic testing or development of targeted treatments.

Epithelial-mesenchymal transition

EMT is a process whereby cells undergo a series of changes that result in a transition from an epithelial cell phenotype to a mesenchymal cell phenotype (reviewed in Thiery & Sleeman 2006). The process is intricately linked to the presence of CSCs and many studies have focused on the role of CSCs in EMT resulting in cancer progression and treatment resistance. There is a substantial body of evidence that EMT is a vital component of cancer progression, particularly in HGSOCs (Takai *et al.* 2014). HGSOCs develop from epithelial cells but often display a mesenchymal phenotype, particularly if platinum resistant (Marchini *et al.* 2013, Chebouti *et al.* 2017).

Extensive molecular profiling of HGSOC has also identified a subgroup of HGSOC that exhibits a distinct mesenchymal gene expression profile (Yoshida *et al.* 2009, Cancer Genome Atlas Research 2011). Marchini and coworkers analysed gene expression profiles of 23 patient-matched treatment – naïve and platinum-resistant (after several lines of platinum therapy) HGSOC tumour samples. A resistance gene expression signature indicative of TGFβ-mediated EMT was identified and confirmed in a validation set of 52 EOCs (Marchini *et al.* 2013).

Despite the pivotal role EMT seems to play on HGSOC progression, development of therapeutics to specifically target and reverse EMT has proven difficult due to the complexity of the EMT process. Key components of the EMT process are also involved in apoptosis, metabolism, cell proliferation, angiogenesis and cell growth (Huang *et al.* 2012). PI3K-AKT-mTOR inhibitors are the most promising therapeutic targets for EMT reversal, but ascertaining if the disease control is a result of EMT reversal or suppression of the other processes previously mentioned will be difficult to achieve. Another approach to reversing EMT may be targeting the epigenetic alterations that drive the transition. These include HGSOC-specific microRNAs, DNA methylation and histone acetylation patterns.

miRNAs

MicroRNAs (miRNAs) are short (18–25 nucleotides) non-coding fragments of RNA that bind to and inhibit mRNA. There are over 1000 human miRNAs and most have been associated with regulation of mRNA in normal and disease processes. miRNAs can regulate multiple mRNAs and subesquent proteins that are pivotal for drug response, therefore, inhibiting specific miRNAs to overcome platinum resistance is appealing.

Several mechanisms to target miRNAs are currently in development for cancer treatment including expression vector 'miRNA sponges' (Ebert *et al.* 2007, Chen *et al.* 2014), antisense or mimic oligos (Trang *et al.* 2011) and small molecule inhibitors (SMIRs) (Watashi *et al.* 2010). SMIRs are the most promising therapeutic target for miRNAs, but significant barriers to delivery of these non-small-molecule agents and pharmacodynamic and pharmacokinetic properties are still major issues to overcome (Monroig Pdel *et al.* 2015). Several recent studies have focused on determining miRNAs involved in HGSOC platinum resistance (Table 1). The most promising targets to date are miR-622 (Choi *et al.* 2016), which targets the Ku pathway and downregulates NHEJ; miR-484 that targets VEGFB and VEGFR2 pathways and tumour vasculature (Vecchione *et al.* 2013); and a miRNA profile of 9 miRNAs that are involved in regulation of EMT and TGF/WNT signalling(Boac *et al.* 2016). Overexpression of miR-27a, miR-23a, miR-30c, Let-7g, miR-199a-3p (Eitan *et al.* 2009), miR-141-3p (Ying *et al.* 2015) and many others (reviewed in Yu *et al.* 2017) have also been associated with cisplatin resistance in HGSOC, therefore, determing which miRNAs are the best for miRNA targeted therapy development will be a challenge.

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Table 1 miRNAs associated with HGSOC platinum resistance.

miRNA	Action	Effect on platinum chemotherapy	Reference
Let-7b	Overexpression in HGSOC	Poor survival and resistance to chemotherapy	Tang *et al.* (2014)
miR-9	Downregulates BRCA1 High levels in SOC	Sensitizes to cisplatin Better response and longer PFS	Sun *et al.* (2013)
miR-21	Over expression in HGSOC from the TGCA Over expression in A2780 cisplatin-resistant cells regulates Programmed cell death 4, c-IAP2 and NAV3	Shorter PFS Cisplatin resistance	Chan *et al.* (2014) Pink *et al.* (2015)
miR-27a miR-23a miR-30c Let-7g miR-199a-3p	Overexpression in stage I and stage III HGSOC	Cisplatin resistance	Eitan *et al.* (2009)
miR-141-3p	Overexpression in OC cell lines	Cisplatin resistance	Ying *et al.* (2015)
miR-146a	Overexpression in SOC omental lesions	Cisplatin resistance	Vang *et al.* (2013)
miR-150	Overexpression in SOC omental lesions	Cisplatin resistance	Vang *et al.* (2013)
miR-181a	Over expression in SOC Suppresses Smad7 and mediates EMT	Cisplatin resistance, shorter OS and PFS	Pink *et al.* (2015)
miR-484	Low expression in SOC, targets VEGFB and VEGFR2 pathways and tumour vasculature	Poor chemotherapy response (stable or progressive disease) Does not mediate chemoresistance *in vitro*	Vecchione *et al.* (2013)
miR-622	Targets the Ku pathway and downregulates NHEJ	Mediates chemoresistance	Choi *et al.* (2016)
Profile of 9 miRNAs	Regulation of EMT and TGF/WNT signaling	Mediates chemoresistance	Boac *et al.* (2016)

Biomarkers that can be targeted using existing therapeutics

DNA methylation

DNA methylation is a key epigenetic regulator of gene expression. Aberrant DNA methylation has long been recognized as a contributing factor to the development of cancer. DNA methylation biomarkers have proven to be robust prognostic biomarkers of HGSOC. Wei and coworkers reported a set of 112 methylated loci that predicted progression-free survival after platinum chemotherapy with 95% accuracy (Wei *et al.* 2006). Progression-free survival after platinum chemotherapy was significantly shorter for patients with higher levels of methylation, suggesting that CpG island methylation is a strong biomarker to target to overcome chemotherapy resistance (Wei *et al.* 2006). Similarly, the Cancer Genome Atlas Network (Cancer Genome Atlas Research 2011) reported 168 genes as epigenetically silenced in HGSOC due to elevated DNA methylation and reduced tumour expression. The consistent methylation profiles reported for HGSOC (Gloss & Samimi 2014) have been a target for testing new combination treatment regimes (Fig. 4).

Cancer therapeutics to inhibit DNA methyltransferases have been successfully developed and approved for treatment. In 2004, azacitidine (DNA and RNA DNMT inhibitor) was approved for treatment of myelodysplastic syndrome (MDS), followed by decitabine (DNA-specific DNMT inhibitor) approval in 2006. DNMT inhibitors are cytotoxic when given at higher doses, but Fang *et al.* (2010) were the first to assess decitabine at repeated low dose to reduce DNA methylation and re-instate cisplatin sensitivity in a Phase 1 clinical trial for HGSOC. The combination was well tolerated with minimal adverse events. The follow-up Phase 2 trial reported 12/17 platinum-resistant patients had a complete response, partial response or stable disease after repeated low-dose decitabine followed by carboplatin (Matei *et al.* 2011, 2012, Fang *et al.* 2014). This is in contrast to Glasspool *et al.* (2014) who reported that the addition of a single-dose of decitabine 7 days before carboplatin reduced the efficacy of carboplatin in patients with partially platinum-sensitive HGSOC (relapsed 6–12 months after previous platinum therapy). The study authors concluded that assessment of patient selection strategies, different treatment schedules and alternative DNMT inhibitors should be considered.

Azacitidine and carboplatin combination treatment for platinum-resistant HGSOC was assessed in a phase 1b/2a clinical trial (Fu *et al.* 2011). Thirty patients received azacitidine for 5 days and carboplatin on day 2 every 28 days. The overall response rate (ORR) was

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13.8% (4/29; 95% CI, 10.1–17.5%): 1 patient achieved a clinical complete response, 3 patients achieved clinical partial response and 10 patients had stable disease, with a median survival of 14 months (Fu *et al.* 2011). The results of clinical trials that have combined demethylating agents and carboplatin indicate that repeated low-dose, patient selection or inclusion of additional agents is required to achieve clinical benefit from this combination, and it is a promising area for further studies.

Histone deacetylases

Histone acetylation is another component of epigenetic regualtion of gene expression. Histone deacetylases (HDAC) actively mediate the level of acetylation of histone structures, when high deacetylation is present the result is suppression of gene expression (reviewed in Ropero & Esteller 2007). Altered expression and mutations in HDACs have been reported in most cancers (Fraga *et al.* 2005), therefore, HDAC inhibitors were developed as promising cancer therapeutics.

Human HDACs are grouped into classes based on their homology to yeast HDACs. HDAC inhibitors that target the different structures of Class I, II and IV HDACs are made up of hydroxamic acids, carboxylic acids, benzamides, epoxides and cyclic peptides (Delcuve *et al.* 2012). The common mechanism of action for HDAC inhibitors is hyperacetylation of histones resulting in an open chromatin structure. The open chromatin structure is thought to allow better access for DNA-damaging therapies such as platinum chemotherapy, resulting in higher levels of apoptosis (Sato *et al.* 2006). DNA methyltransferase and HDAC inhibitors are synergistic in re-expression of epigenetically silenced genes (Cameron *et al.* 1999), therefore, combination therapies targeting epigenetic regulation of platinum resistance are of intense interest.

Falchook *et al.* (2013) assessed the safety and efficacy of combination DNMT inhibitor azacitidine, HDAC inhibitor valproic acid and carboplatin in a cohort of 32 patients with treatment-resistant solid tumours, including 10 platinum-resistant ovarian cancer patients. Among the patients with ovarian cancer, three (30%) achieved minor partial responses or stable disease lasting ≥4 months. Dose delays and reductions due to adverse events, including grade ≥3 fatigue and neutropenia in the majority of patients, made assessment of the combination difficult. The authors concluded that lower continuous treatment doses and patient selection by methylation status warrants further follow-up studies.

DNA repair

Homologous recombination repair

Homologous recombination repair (HR) repairs double-strand breaks that occur as a result of many DNA-damaging insults including ionizing radiation and chemotherapy (Powell & Kachnic 2003). The mechanistic role of HR in platinum chemotherapy response is to repair double-strand DNA breaks that occur at sites of platinum crosslinks during DNA replication. BRCA1 and BRCA2 are members of the (HR) repair pathway (Fig. 2) and have been associated with risk of developing HGSOC (reviewed in Powell & Kachnic 2003). The Cancer Genome Atlas network used integrated analysis of mRNA, miRNA, methylation and DNA copy number to determine that approximately 50% of HGSOC are HR deficient (Cancer Genome Atlas Research 2011), indicating that DNA repair deficiency is a key driver of HGSOC.

BRCA mutations

Germline mutations in *BRCA1* and *BRCA2* are the most established risk factor for HGSOC. The largest study of BRCA mutation incidence to date (n=1001), reported 14.1% of ovarian cancer patients have a germline BRCA mutation, with the highest incidence of 22.6% in HGSOC patients (Alsop *et al.* 2012). HR deficiency resulting from BRCA mutations leads to an accumulation of double-strand breaks after platinum chemotherapy, which in turn causes increased apoptosis and platinum sensitivity. It is well established that BRCA mutation carriers with HGSOC are more sensitive to platinum chemotherapy regimes and have longer overall survival than non-carriers (Vencken *et al.* 2011, Alsop *et al.* 2012, Rudaitis *et al.* 2014). Initially sensitive to platinum, BRCA mutation carriers eventually become resistant (Alsop *et al.* 2012) and attempts to inhibit other components of the HR pathway for further treatment have proven successful for some HGSOC patients, in particular, PARP inhibitors (reviewed in Scott *et al.* 2015).

PARP inhibitors are a synthetically lethal therapeutic cancers with DNA repair defects, particularly *BRCA1* or *BRCA2* mutations. In HR-deficient tumours, PARP inhibition blocks a downstream DNA repair process, which triggers apoptosis. Olaparib, is the first PARP inhibitor to be approved in most countries as maintenance treatment for patients with platinum-sensitive, relapsed ovarian cancer and a germline or somatic *BRCA1/2* mutation or as monotherapy for advanced ovarian cancer patients with a germline *BRCA1/2* mutation (Pujade-Lauraine *et al.* 2017).

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BRCAness

In addition to germline BCRA mutations, somatic BRCA mutations occur in HGSOCs. Moschetta and colleagues recently reviewed studies reporting somatic BRCA mutations and concluded 5–7% of HGSOC contain somatic BRCA mutations (Moschetta *et al.* 2016). Hypermethylation of the *BRCA1* promoter results in reduced expression of BRCA1 resulting in a HR-deficient phenotype in approximately 11% of HGSOCs (Geisler *et al.* 2002, Patch *et al.* 2015). Similarly, amplification of *EMSY*, which encodes a BRCA2-binding partner, leads to impairment of BRCA2 function (Wilkerson *et al.* 2011). Collectively, somatic BRCA mutations, hypermethylation of *BRCA1* promoter and *EMSY* amplification all result in HGSOC phenotypes and platinum chemotherapy response similar to germline BRCA mutation carriers and are referred to as HGSOC with 'BRCAness'.

In recent times, the BRCA 'wild-type' HGSOC subtype has not received as much attention as the BRCA mutant/BRCAness subtype with HR deficiency. The BRCA 'wild-type' or HR proficient subtype are more likely to be platinum resistant; therefore, it is a strong clinical subgroup to target for further development of platinum resistance biomarkers.

Intricate biological processes such as DNA repair rarely work in isolation. Most often, many of the proteins associated with a particular pathway have overlapping roles in multiple pathways and each process interacts with other similar processes. DNA repair is no exception, of the 6 DNA repair pathways HR interacts most closely with nucleotide excision repair (NER), the process responsible for recognizing platinum-induced DNA crosslinks as bulky adducts before double-strand breaks occur.

Nucleotide excision repair

The NER pathway consists of approximately 30 proteins that remove helix-distorting lesions such as platinum chemotherapy crosslinks via a step-wise process: damage recognition, unwinding of the DNA locally around damage, incision of damaged DNA by endonucleases and DNA resynthesis and ligation (Costa *et al.* 2003) (Fig. 2). There are two branches of damage recognition that converge on a common repair pathway: transcription coupled repair (TCR) and global genome repair (GGR). TCR is linked to active gene transcription and is initiated when RNA polymerase is stalled at DNA damage during transcription. GGR however is not dependent on transcription and scans

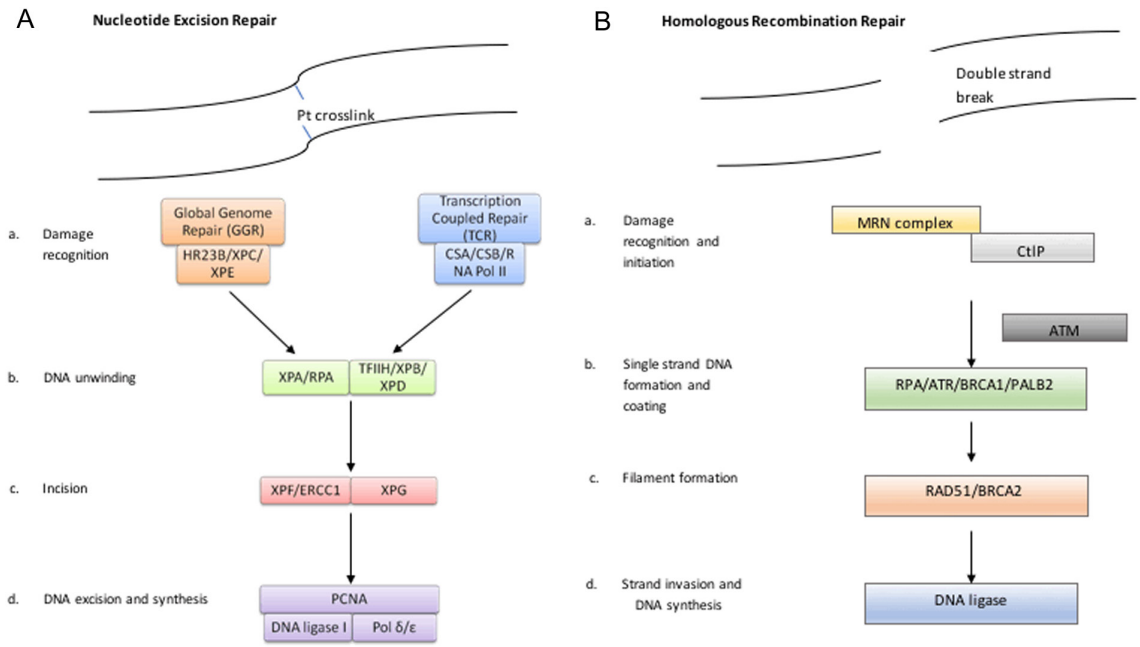

Figure 2

Nucleotide Excision Repair and Homologous Recombination Pathways. (A) Nucleotide excision repair recognises platinum-induced interstrand and intrastrand crosslinks and a co-ordinated process of DNA unwinding, incision, excision and synthesis follows. The process can result in a DNA double-strand break, which is recognised by homologous recombination repair. (B) Homologous recombination repair recognises double-strand breaks and initiates a process of single strand DNA formation, coating, filament formation, strand invasion and a final step of DNA synthesis.

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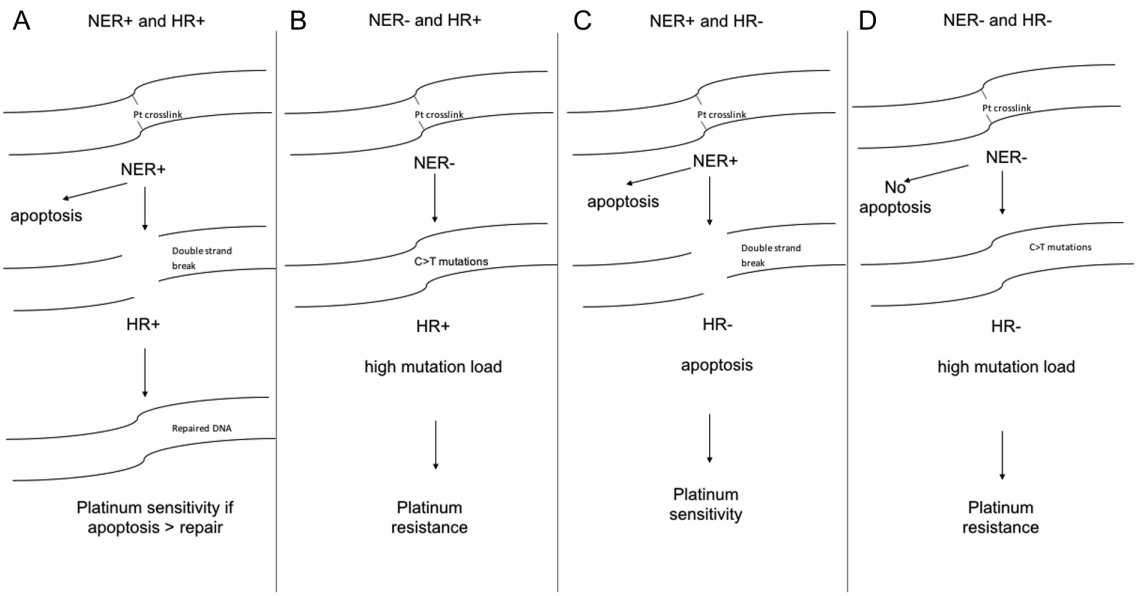

Figure 3
The role of DNA repair deficiencies in high-grade serous ovarian cancer (HGSOC) platinum chemotherapy resistance. (A) NER-and HR-proficient (NER+ and HR+) HGSOC is hypothesised to be sensitive to platinum chemotherapy if apoptosis is greater than DNA repair. (B) NER-deficient (NER–) and HR+ HGSOC is hypothesised to be platinum resistant due to lack of platinum cross-link recognition by NER. (C) NER+ and HR-deficient (HR–) HGSOC is hypothesised to be platinum sensitive due to NER triggering apoptosis and DNA double-strand breaks that are not repaired by HR. (D) NER– and HR– HGSOC is hypothesised to be platinum resistant due to lack of NER triggered apoptosis and a high mutation load.

the entire genome including both active and silent genes and non-transcribed regions using DNA damage-binding proteins XPC and UV-DDB (DDB1 and DDB2) (Nouspikel 2009). Early studies found an association between higher expression of NER mRNA (Dabholkar *et al.* 1992, 1994) before treatment with platinum resistance in ovarian cancer. This suggested over-active NER repairs platinum-induced DNA crosslinks before double-strand breaks can occur, indicating that NER expression could be a predictive biomarker of platinum response. This area has received little interest in the last 10 years, possibly due to the seemingly opposite discovery that when quantified after platinum chemotherapy, low NER expression has been associated with platinum resistance (Stevens *et al.* 2005, Barakat *et al.* 2010).

Recognition of excessive DNA crosslinks by the GGR portion of the NER pathway triggers apoptosis rather than attempting to repair the damage (Stoyanova *et al.*

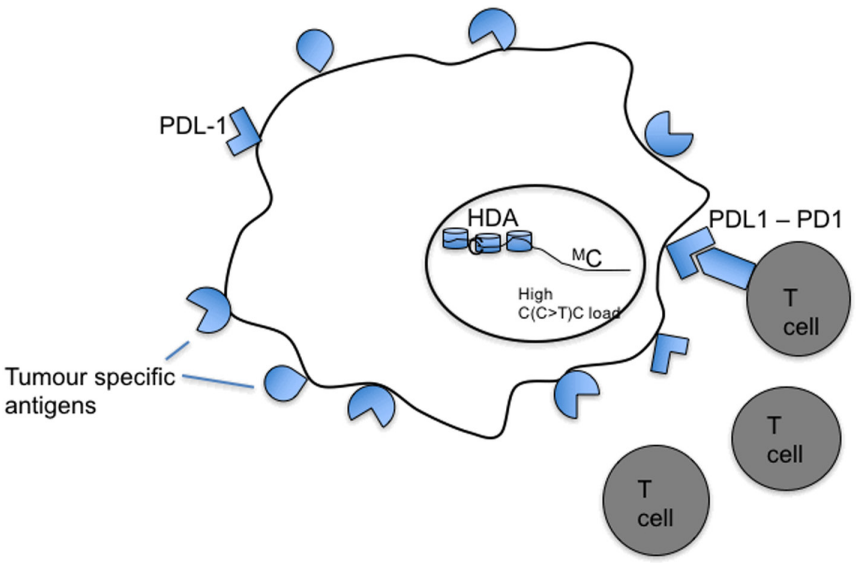

Figure 4
Therapeutic targets: epigenetic and immune biomarkers of HGSOC platinum chemotherapy resistance. Potential nuclear therapeutic targets/biomakers for platinum-resistant HGSOC are histone deactylases (HDA), DNA methylation (mC) and high C(C>T)C mutation load across the genome. Potential cell surface for platinum-resistant HGSOC are tumour-specific antigens and PDL-1 (PD-1 on T-cells).

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2009) (Fig. 3). Therefore, lack of induction of GGR after platinum chemotherapy results in a reduction of both cross-link repair by the NER pathway and apoptotic signalling, ultimately leading to limited or no response to platinum treatment. Lower levels of NER post-platinum chemotherapy have been confirmed in ovarian cancer studies including low DDB2 in platinum-resistant ovarian cancer cell lines (Barakat *et al.* 2010) and low XPA in platinum-resistant ovarian cancer tumours (Barakat *et al.* 2010). In addition, low NER and platinum resistance has been reported for several other cancer types including non-small-cell lung cancer, gastric cancer, colorectal cancer and melanoma (Bowden *et al.* 2010, 2013). NER proteins are a promising area in biomarker development for platinum resistance for use in real-time clinical settings after platinum treatment has concluded.

NER recognizes DNA crosslinks caused by platinum chemotherapy and converts the cross-link to a DNA double-strand break. HR is then required to repair the double-strand break to prevent apoptosis (Stergiou *et al.* 2011) (Fig. 3). For HGSOC with BRCA mutations or 'BRCAness' HR deficiency is already present. If deficient NER is also present, it may be the cause of the eventual platinum resistance seen in HR-deficient HGSOC. In addition, if deficient NER is not processing the DNA crosslinks into double-strand breaks, there is no requirement for HR to repair double-strand DNA breaks. Therefore, targeting HR deficiency with PARP inhibitors is ineffective, this requires further investigation but may be the underlying cause of PARPi failure in some patients (Fig. 3).

Platinum-resistant HGSOC with functional NER and HR deficiency is likely to be sensitive to trabectedin, a transcription factor inhibitor (Schoffski *et al.* 2011). Trabectedin shows decreased activity (2- to 8-fold) in NER-deficient cell lines, while cells deficient in HR are approximately 100 times more sensitive to the drug, indicating that trabectedin relies on DNA double-strand breaks (Herrero *et al.* 2006).

More studies into the role of NER and HR in HGSOC platinum resistance are required to understand the relationship and potentially develop ways to subtype HGSOC based on NER and HR proficiency.

Mutation load

The relationship between DNA repair dysfunction and increased mutation load across the cancer genome is well established (Alexandrov *et al.* 2013, Le *et al.* 2015). Ovarian cancer has a lower mutation load than cancer types such as melanoma and non-small-cell lung cancer, which have a high mutation load as a result of environmental and chemical carcinogen exposure (Alexandrov *et al.* 2013). The cancer types with high mutational load have historically been difficult to treat, but have shown exceptional response to immune checkpoint inhibitors (Le *et al.* 2015, Antonia *et al.* 2016, Ugurel *et al.* 2017). Patch and coworkers recently reported a significant C(C>T)C platinum chemotherapy imprint in the genome of platinum-resistant HGSOC (Patch *et al.* 2015). The platinum chemotherapy imprint is similar to the C>T UV-fingerprint consistently seen across the melanoma genome (Pleasance *et al.* 2010). Both platinum chemotherapy and UV-light DNA damage require NER and HR to either repair the damage or trigger apoptosis. If only one of the pathways is functional, it may compensate for the other when challenged with platinum chemotherapy.

Table 2 Biomarkers for alternative treatment for platinum-resistant HGSOC.

Biomarkers of platinum-resistant HGSOC	Alternative treatment	Evidence of response to alternative treatment	References
High mutation load	Checkpoint inhibitor Immunotherapy (anti-CTLA4 and anti-PD1)	Melanoma NSCLCs Mismatch repair deficient colorectal cancer	Takai *et al.* (2014), Tang *et al.* (2014), Thiery & Sleeman (2006)
CD8, PD-1 and PD-L1-expressing cells inside tumours	Checkpoint inhibitor Immunotherapy (anti-CTLA4 and anti-PD1)	Melanoma	Tumeh *et al.* (2014)
Low or absent NER and BRCA mutation/BRCAness	PARP inhibitors	Ovarian cancer	Reviewed in Reles *et al.* (2001)
Normal NER and and BRCA mutation/BRCAness	Trabectidin	Sarcoma	Stoyanova *et al.* (2009)
Methylation marker panel	Azacytidine and carboplatin	Ovarian cancer	Nouspikel (2009)
	Azacitidine, valporic acid (HDAC inhibitor) and carboplatin	Ovarian, prostate, cervical and colorectal cancer	Prasetyanti & Medema (2017)

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Table 3 Clinical trials using agents to target high-grade serous ovarian cancer platinum resistance biomarkers.

Agents	Biomarker target	Outcomes	Reference
Decitabine, repeated low-dose followed by cisplatin	DNA methylation	Phase 1: Minimal adverse events	Fang *et al.* (2010)
Decitabine, repeated low-dose followed by cisplatin	DNA methylation	Phase 2: 12/17 platinum-resistant patients had CR, PR or SD	Matei *et al.* (2011, 2012)
Azacitdine and carboplatin	DNA methylation	Phase 1b/2a: ORR 4/29 patients (1 CR, 3 PR, 10 SD), median survival of 10 months	Fu *et al.* (2011)
Azacitidine, valproic acid and carboplatin	DNA methylation and histone deactylation	Phase 2: 3/10 patients had PR or SD	Falchook *et al.* (2013)
Avelumab (anti-PD-L1 immune checkpoint inhibitor)	High mutation load (heavy platinum pretreatment)	Phase 1b: 41/75 patients had PR or SD	Disis *et al.* (2015)
Pembrolizumab (anti-PD-1 immune checkpoint inhibitor)	Expression of PD-1 and High mutation load (heavy platinum pretreatment)	Phase 1b: 9/26 patients has CR, PR or SD	Varga *et al.* (2015)
Nivolumab (anti-PD-1 immune checkpoint inhibitor)	High mutation load (heavy platinum pretreatment)	Phase 1b: 9/20 CR, PR or SD	Hamanishi *et al.* (2015)

This further supports the need for both NER and HR to be further developed as dual biomarkers of platinum resistance in HGSOC.

The increase in mutation load in platinum-resistant HGSOC is likely to be a strong predictor of response to immune checkpoint inhibitors, due to the increase in cancer-specific antigens (Fig. 4). Therefore, platinum resistance itself is a potential biomarker for predicting HGSOC responders to checkpoint inhibition. However, it has become clear from extensive clinical trial follow-up in melanoma that mutation load alone is not the strongest predictor of durable response to checkpoint inhibition. The extent and subtype of tumour-infiltrating lymphocytes (TILs), immune cell subsets in peripheral blood and the extent of disease are also strong predictors of response.

Immune cell subsets

The recent advancement of immune checkpoint inhibitors such as anti-PD1 (pembrolizumab, nivolumab and avelumab) and anti-CTLA4 (ipilumimab) monoclonal antibodies has led to biomarker development in relation to TILs (Tumeh *et al.* 2014) and circulating immune cell subsets (Huang *et al.* 2017). Pretreatment tumour samples obtained from patients that responded to anti-PD1 immunotherapy had higher numbers of CD8+, PD-1 and PD-L1-expressing cells at the invasive tumour margin and inside tumours (Tumeh *et al.* 2014). Several studies have performed similar analysis in HGSOC and found higher levels of CD8+ TILs in stromal tissue were associated with better overall survival. Lo *et al.* (2017) reported increased densities of CD3+ and CD8+ and PD-1+ T-cells in HGSOC after platinum chemotherapy. However, the increase in these T-cell subtypes was dependent on presence before treatment, indicating that platinum chemotherapy can induce a desired immune response, but only if the required T-cells are already present in the tumour (Tumeh *et al.* 2014). It is feasible that TILs present in platinum-resistant HGSOC before and after treatment could be used to predict response to checkpoint inhibitors (Fig. 4).

Conclusion

There is a suite of approved cancer therapeutics, with established safety and toxicity profiles, that should be assessed in the immediate future based on biomarkers of platinum-resistant HGSOC (Table 2). Several early phase clinical trials using methylation, HDAC and immunotherapy agents have already reported promising results (Table 3). Retrospective analysis of specific biomarkers in patient cohorts that received these therapies may shine a light on why a good response occurred in only a subset of patients, which will inform a new round of trials with selected patient populations.

Patient selection, dose selection, treatment timing and different combinations of the therapies listed in Table 2 will also be key to identifying effective treatments. Rather than focussing on single proteins, pathways or biological processes, as new therapeutics are developed the highly mutated, immunogenic and epigenetically altered platinum-resistant HGSOC phenotype should be exploited to overcome treatment resistance.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

B v Z and D T participated in writing the manuscript. N A B devised and oversaw the literature analysis and interpretation and writing the manuscript. All authors read and approved the final manuscript.

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