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Review

Targeting deficient DNA damage repair in gastric cancer

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Abstract

Introduction

Over recent years our understanding of DNA damage repair and defects associated with its various pathways has evolved. This has led to an expansion of the potential target population for therapies attempting to exploit DNA damage repair deficiencies across multiple solid tumour types. Gastric cancer has recently been identified as a tumour type where a subgroup of patients demonstrates deficiencies in the homologous recombination DNA repair pathway. This may provide a novel treatment approach for this poor prognosis disease.

Area Covered

This review seeks to provide an overview of DNA damage repair and how this has been targeted to date in other tumour types, particularly in ovarian and prostate cancer, exploiting the concept of synthetic lethality. This is followed by a discussion of how deficiencies in homologous recombination may be identified across different tumour types and then focuses on recent progress in targeting DNA repair deficiencies in gastric cancer, having outlined the current treatment paradigm for gastric cancer and explained the urgent unmet need for novel therapies.

Expert Opinion

Gastric cancer remains a difficult malignancy to treat and the possibility of targeting deficient DNA repair in a subgroup of patients with this disease is an exciting prospect. Future combinations with immunotherapy and radiotherapy are also appealing and appear to have a sound biological rationale. However, much work remains to be done to understand the

significance of the various genetic and epigenetic alterations involved, to elucidate the optimum predictive signatures or biomarkers and how they should be obtained and to consider means of overcoming treatment resistance.

Keywords

Gastric Adenocarcinoma

DNA Damage Repair

Homologous Recombination Deficiency (HRD)

BRCAness

Synthetic Lethality

PARP inhibitor

Ataxia Telangiectasia Mutated (ATM)

Article Highlights Box

- Developments in recent years have greatly expanded our understanding of DNA damage repair pathways and of defects affecting these pathways.
- Deficiencies in Homologous Recombination (HR) are seen across multiple tumour types, including gastric cancer, and various signatures have been proposed as a means to detect these deficiencies including multi-gene signatures, structural rearrangement signatures and transcriptional signatures.
- In gastric cancer dysfunction or loss of ATM protein appears to be an important cause of HRD and may be associated with microsatellite instability (MSI)
- The phase II Study 39 reported an improvement in overall survival in the second line treatment of advanced gastric cancer with paclitaxel in combination with the PARP inhibitor olaparib, particularly in patients with low ATM protein levels
- Future studies investigating therapies targeting DNA damage repair in the maintenance setting, in Western patients and in combination with immunotherapy, chemotherapy, other targeted agents and radiotherapy will establish whether this approach has the potential to significantly improve outcomes in gastric cancer
- The development of predictive signatures or biomarkers and means to overcome treatment resistance will prove vital to the success or otherwise of this approach

1. Introduction: Gastric Cancer and its current treatment

As the 5th most common cancer and the 3rd leading cause of cancer related death in 2012 gastric cancer remains a significant global health problem[1]. Despite modest improvements in overall survival over the last 4 decades the 5 year survival rate for gastric cancer remains low at 20%. Surgery provides the only curative treatment but two thirds of Western patients present with inoperable disease and, even with the addition of peri-operative therapy, the majority of patients treated with curative intent relapse within 5 years[2,3]. For patients with metastatic disease the prognosis is particularly bleak with median overall survival (mOS) of 3 months with best supportive care (BSC) and under a year with 1st line combination chemotherapy[4].

In the advanced setting chemotherapy remains the cornerstone of treatment. If performance status allows, the 1st line standard of care consists of a backbone of a platinum and fluoropyrimidine with the addition of either an anthracycline or a taxane[5,6]. The genomic landscape of gastric cancer is highly complex and defining predictive biomarkers has been particularly difficult, hampering the use of targeted therapies. However, for the 20% of gastric cancer patients who are HER-2 positive, trastuzumab may now be added to the platinum/fluoropyrimidine backbone[7] and ramucirumab, a vascular endothelial growth factor receptor 2 (VEGFR-2) inhibitor, may be considered in unselected patients as a single agent or in combination with paclitaxel in the second line setting[8,9]. In addition, apatanib, anotherVEGFR-2 inhibitor, has recently been reported to improve mOS in the third line setting in an Eastern population[10].

Worldwide second and even third line chemotherapy is increasingly used, with approximately half of patients who receive first line treatment being fit enough for second line treatment.

Weekly paclitaxel, docetaxel and irinotecan are all possible options but only improve mOS from approximately 3 months with BSC to between 4 and 5 months with chemotherapy[11,12,13].

Due to the limitations in current therapy for gastric cancer alternative strategies have been sought. Targeting damaged DNA repair is one such novel approach and is becoming an area of increased interest across multiple solid tumour types.

2. DNA damage repair

The human genome is constantly exposed to damage. This may be from endogenous factors such as oxidation, hydrolysis or alkylation of bases and errors in DNA replication or from exogenous factors including ultraviolet light, ionizing radiation and chemicals. The ability of the cell to repair this damage is vital for the maintenance of genomic integrity[14]. As such, organisms have evolved multiple pathways to repair DNA damage. Initially the cell must recognise the damaged DNA and activate the cell cycle checkpoints, to pause the cell cycle to allow the damage to be repaired. The repair pathway employed depends on the nature of the DNA damage. For single strand DNA damage the options include Mismatch Repair (MMR), Base Excision Repair (BER), Nucleotide Excision Repair (NER) and Direct Repair (DR) depending upon the type of damage present[15,16,17, 18]. If both DNA strands are severed, in a Double Strand Break (DSB), repair mechanisms used include Homologous Recombination (HR), classical or alternate Non- Homologous End Joining (NHEJ) and Single Strand Annealing (SSA)[19,20,21]. (Figure 1 and Table 1).

Malfunctions of these repair pathways have varied deleterious consequences and are frequently associated with cancer (Table 2). Patients with Lynch Syndrome for example, have inactivating mutations in one or more MMR genes (*MLH1, PMS2, MSH2* and *MSH6*) and are unable to correct errors in newly synthesized DNA. These errors often occur in repeated sequences known as microsatellites, which tend to be the same length throughout an

individual's genome. Deficiency of MMR results in variations in theses microsatellites known as microsatellite instability (MSI), a mutator phenotype and a propensity for cancer, particularly colon, gastric and endometrial cancers. MSI is also seen in sporadic cancer where it is thought to be caused by epigenetic modulation of the MMR genes, usually through promoter hypermethylation[22].

Another cancer associated with faulty DNA repair is hereditary breast/ovarian cancer. Here patients have a germline mutation in the tumour suppressor genes *BRCA1/2* and if they subsequently sustain a somatic mutation in the remaining wild type (WT) *BRCA1/2* allele they lose BRCA1/2 protein function. Cells without intact *BRCA1/2* are unable to repair DSB by HR as BRCA1/2 proteins are integral to this process[23]. The resulting HR deficiency (HRD) causes the cell to rely upon more error prone DNA repair mechanisms such as NHEJ with resulting genomic instability and oncogenesis. Although HRD is associated with the development of cancer it has also provided a novel approach to treating cancer, through exploiting synthetic lethality [24,25,26].

3. Targeting Deficiencies in DNA Damage Repair

3.1 Synthetic Lethality

The concept of synthetic lethality describes a situation where 2 separate genetic mutations are relatively harmless when they occur individually but lethal if they occur in combination. Utilising synthetic lethality is very appealing in cancer therapeutics, as targeting 1 such gene with a particular therapy in a patient whose tumour is known to already have a 2nd genetic mutation could result in tumour cell death with minimal toxicity to normal tissue, where cells do not carry the 2nd mutation. This theory has been elegantly validated in the treatment of *BRCA1/2* mutant cancers with Poly (ADP-ribose) polymerase (PARP) inhibitors.

3.2 Poly(ADP-ribose) polymerase (PARP) inhibition

The large PARP family of enzymes, particularly PARP1 and 2, has been implicated in a number of DNA repair mechanisms. PARP enzymes prevent the formation of DSB through their involvement in the repair of SSB in BER[27]. In addition PARP enzymes indirectly help repair DSB through their involvement in activating ataxia-telangiectasia mutation (ATM), involved in HR and in deactivating DNA-dependent protein kinase, involved in NHEJ[28].

PARP inhibitors were designed to competitively bind the NAD binding site of PARP enzymes and directly interfere with their DNA repair function. PARP inhibition therefore results in persistent DNA SSBs and subsequent stalling of DNA replication forks causing DSB formation. Employing the synthetic lethality concept, in normal cells such DSB would be repaired by HR but in patients with *BRCA1/2* mutations, tumour cells would be deficient in HR making this impossible. Instead the tumour cells would have to resort to the non-conservative repair mechanisms resulting in genomic instability and eventual cell death through apoptosis.

In 2005 a phase I study of olaparib (AZD2281, Lynparza), a selective and potent PARP1/2 inhibitor, in a population enriched for *BRCA1/2* mutants confirmed this approach had merit[29]. In this proof of concept study 19 heavily pre-treated patients with *BRCA1/2* mutations and breast, ovarian or prostate cancer demonstrated a disease control rate of 63% and an objective response rate of 47%. Further, observed toxicities were mild and, as predicted, there was no increase in toxicity in carriers of the *BRCA1/2* mutations versus non carriers. An expansion cohort in BRCA1/2 mutant ovarian cancer patients revealed a significant association between clinical benefit rate with olaparib and platinum sensitivity (platinum-sensitive 69%, resistant 45%, and refractory 23%)[30]. This has been attributed to the ability of platinum agents to stall replication forks through the formation of DNA cross-links, causing DNA damage which also requires repair by BRCA1/2 mediated HR.

Following on from these early trials, two phase II single agent olaparib studies demonstrated sustained responses in *BRCA1/2* mutant breast and ovarian cancers with response rates of 41% and 33% respectively[31,32]. A further phase II study of maintenance olaparib, following a response to platinum therapy in patients with familial or sporadic high grade serous ovarian cancer, failed to show a statistically significant increase in overall survival but increased progression free survival by approximately 7 months in those patients with germline or sporadic *BRCA1/2* mutations[33, 34].

Based on these results in December 2014, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) both approved olaparib monotherapy for the maintenance treatment of women with *BRCA1/2* mutant high grade serous ovarian cancer, fallopian tube or primary peritoneal cancer who have demonstrated a complete or partial response to platinum based chemotherapy.

More recently, in January 2016, the FDA also granted olaparib Breakthrough Therapy designation (BTD) for the monotherapy treatment of *BRCA1/2* or *ATM* mutated metastatic Castration Resistant Prostate Cancer (mCRPC) in patients who have received a prior taxane-based chemotherapy and at least one newer hormonal agent (abiraterone or enzalutamide). This was based on the results of the phase II TOPARP study where olaparib monotherapy demonstrated a response rate of 88% in patients with mCRPC and defects in DNA repair genes[35]. In this study the investigators hypothesized that in addition to prostate cancer patients with *BRCA2* defects, prostate cancer patients with mutations in other genes involved in DNA damage repair may also be sensitive to treatment with olaparib. Patients provided a baseline biopsy which was used to conduct exome and transcriptome sequencing to generate a biomarker suite including defects in *BRCA1/2, ATM, Fanconi's anaemia genes* and *CHEK2*. This phenomenon, where patients have sporadic mutations in DNA damage repair

genes evoking a phenotype similar to that seen in germline *BRCA1/2* mutation carriers, has previously been described as "BRCAness" or "BRCA-like" [36].

4. BRCA-like

The idea of a BRCA-like phenotype was first described over a decade ago to describe a group of patients who may benefit from PARP inhibition, in addition to *BRCA1/2* mutation carriers, who had defects in DNA repair which were not attributable to germline *BRCA1/2* mutations.

Germline mutations in *BRCA1/2 (gBRCAm)* are uncommon events. Only 5-10% breast cancers[37] and 10-15% ovarian cancers[38] are caused by an inherited mutation, most commonly in *BRCA1/2*. *BRCA* mutations are also seen in up to 7% pancreatic cancers[39] and 6% prostate cancers[40] but have rarely been described in gastric cancer.

If patients with alternative defects, germline or somatic, in the HR pathway genes could be identified, the scope for exploiting synthetic lethality through PARP inhibition or other means would be much wider, across multiple solid tumour types. To this end multiple groups have sought to establish deficiencies in HR present in different tumour types and to develop means of identifying patients with such deficiencies for treatment, suggesting various signatures of HRD.

5. Identifying Homologous Recombination Deficiency (HRD)

5.1 Gene Signatures

One method to identify HRD is to select an appropriate gene suite and look for defects, as demonstrated in the TOPARP study[35]. With advances in our understanding of DNA damage repair, a large number of genes which are important in HR and associated with malignancy have been identified[41]. These include *BRCA1/2*, *ATM*, Ataxia telangiectasia and Rad3-related (*ATR*), *RAD51*, Meiotic recombination 11 homologue A (*MRE11A*), Nijmegen breakage

syndrome protein 1(*NBS1*), Checkpoint Kinase 1/2 (*CHK1/2*), Cyclin-dependent protein kinase 12 (*CDK12*) and the Fanconi's anaemia gene family. Defects in other genes, distinct from those known to be intrinsically involved in HR, may also be important as predictive biomarkers for sensitivity to PARP inhibition such as *PTEN*[42], *ARID1A*[43] and *p53*[44].

Particular patterns of defects may be evident in different tumour types and much remains to be learnt about the importance of specific mutations within these genes, the significance of epigenetic modulation of these genes and the varying patterns of drug resistance which may develop depending on the genes involved.

Ongoing improvements in the affordability and speed of next generation sequencing make this a potentially valid approach in the future. Alternatively at present immunohistochemistry may be used to detect silencing of critical genes if validated assays are available.

5.2 Structural rearrangement signatures

Structural rearrangement signatures, looking at genomic scars, have also been investigated. This approach utilises the observation that structural rearrangements associated with defects in HR may take particular patterns. One such pattern of rearrangement is a high level of genomic loss of heterozygosity (LOH). Preliminary results from the ARIEL2 study, a phase 2 open label study of rucaparib in ovarian cancer, describe the use of single-nucleotide polymorphism (SNP) analysis to identify and quantify genomic LOH. Patients were divided into 3 groups based on this quantification and their BRCA status, *BRCA* mutant patients, *BRCA* wild-type (WT) patients with high LOH and *BRCA* WT patients with low LOH. Overall response rates to rucaparib in these 3 groups were 69%, 39%, and 11%, respectively, suggesting that high levels of genomic LOH may be used to identify a group of patients without BRCA mutations who may be more likely to respond to rucaparib[45]. This assay is currently being tested in the ARIEL3 study of rucaparib as switch maintenance following

platinum-based chemotherapy in patients with ovarian, primary peritoneal or fallopian tube cancer (NCT01968213).

Another example is "signature 3", a specific base-substitution signature seen in patients with germline or somatic *BRCA* mutant breast, ovarian and pancreatic cancers, described by Alexandrov et al in their analysis of 4,938,362 mutations from 7,042 cancers[46]. In this signature there are substantial numbers of deletions (of up to 50 base pairs in size) with overlapping microhomology at the breakpoint junctions. This pattern has been attributed to the use of error prone DNA repair pathways in the place of compromised HR. The investigators noted that signature 3 was seen in a number of patients who did not have BRCA mutations, suggesting the signature could be used to identify patients with alternative causes of HRD.

5.3 Mutational Burden

Perhaps a more straightforward approach would be to look at overall mutational burden within a tumour as a biomarker for being BRCA-like. High overall numbers of somatic exome mutations per genome have been demonstrated in *BRCA1/2* mutant ovarian cancer and within these patients, higher mutational load is associated with improved progression-free survival (PFS) and overall survival (OS) with platinum based chemotherapy. Interestingly in a study by Birkbak et al. using data from The Cancer Genome Atlas (TCGA) a substantial number of patients with *BRCA* mutated ovarian cancer but with low mutational burdens experienced relatively poor treatment outcomes, similar to those seen in patients with WT *BRCA*[47], suggesting knowledge of degree of a patient's mutational burden may be useful additional information when selecting treatment.

5.4 Transcriptional Signatures

As an alternative to investigating specific genomic defects or scars, transcriptional signatures of BRCAness have been derived, describing gene expression profiles using microarrays. In epithelial ovarian cancer, for example, Konstantinopoulos et al. used a publically available microarray dataset from patients with BRCA mutant and sporadic ovarian cancer to develop a 60 gene BRCAness profile[48]. This was then applied to tumour samples from patients with *gBRCAm* and sporadic disease. In the *gBRCAm* samples the BRCAness profile accurately distinguished between platinum sensitivity and resistance and in two patients the profile dynamically tracked the development of platinum resistance during treatment, associated with a return to functional *BRCA1*. Further, in the sporadic samples those patients with a *BRCA*-like profile had a better prognosis than those with a non-*BRCA*-like profile (overall survival 72 months vs. 41 months; log-rank P = 0.006). The investigators explained that they chose microarray gene expression profiling to develop a signature due to this technique having a broad based, non-mechanistic approach. They postulated this would have the highest chance of identifying the maximum number of patients with a BRCA-like phenotype, as this phenotype could have developed in a multitude of ways.

5.5 Proteomics

Moving downstream from a transcriptional signature, proteomics have also been considered. Proteomics analyses on RNAi knockdown breast cancer cell lines for key HR genes using 2 dimensional-difference gel electrophoresis identified 308 significant protein changes in pathways associated with cell death, post-translational modification and protein folding[49]. Exploratory proteomics analysis in an early phase study of olaparib and carboplatin in *BRCA1/2* mutated breast and ovarian cancer described eight proteins whose levels correlated with response to treatment. Differences in levels of pS209-eIF4E and FOXO3a statistically significantly contributed to a linear model predicting response duration, with FOXO3a staining being the more reproducible[50]. FOXO3a promotes phosphorylation of ATM and

activates ATM mediated apoptosis in response to DNA damage. This data requires prospective validation but theoretically the use of a simple IHC assay as a predictive biomarker is certainly appealing.

5.6 Functional assays

A different approach to developing signatures to determine the presence or absence of genes or proteins important in HR is to look at the ability of the cell to actually perform HR. However, developing a clinically relevant assay has been fraught with difficulty as DNA damage repair pathways are dynamic processes, requiring DNA damage for activation and as such are difficult to measure in pre-treatment biopsy samples.

One approach has been to look at RAD51 based functional assays, using immunohistochemistry to determine RAD51 nuclear localisation. RAD51 is an important DNA repair protein which is recruited to sites of DNA damage where it forms distinct nuclear foci when HR is active. The degree of foci formation can indicate if a cell is proficient in HR.[51] This technique is successful in vitro when cells are exposed to DNA damage and a number of groups have developed ex-vivo protocols where a RAD51 response is elicited by DNA damage to a fresh biopsy sample, using radiation[52] or the PARP inhibitor rucaparib[53]. It remains to be seen whether such an approach could realistically be transferred to the clinic.

At present all of the approaches to detect HRD listed above require substantial refinement and validation before being implemented in the clinic. What is clear is that a small but significant proportion of multiple tumour types may exhibit HRD or BRCA-like

characteristics even if we do not yet know how best to define these subgroups. Gastric cancer has recently been identified as a tumour type with such an HR deficient subgroup.

6. Homologous Recombination Deficiency in Gastric Cancer

Interest in targeting HRD in gastric cancer was generated when gastric cancer cell lines were found to be particularly sensitive to single agent olaparib treatment (IC_{50} <500nM vs.1300nM for ovarian cancer cell lines). This sensitivity was attributed to deficiencies in HR[54] and here *ATM* appears to play a key role.

6.1 *ATM* deficiency in gastric cancer

ATM, the tumour suppressor gene found on human chromosome 11q22-23 and mutated in the disorder Ataxia Telangiectasia, encodes a large multifunctional protein kinase (370kDa) [55]. This protein kinase is important in the cellular response to DNA DSBs, inducing cell cycle arrest via p53 and facilitating repair through the phosphorylation of numerous downstream targets[56].

In gastric adenocarcinoma, *ATM* mutation and ATM protein loss have been associated with older patients, distal tumours, larger tumours, and tumours of intestinal histological type[57]. Low levels of phosphorylated ATM have been correlated with a poor prognosis, as well as with poorly differentiated histology and lymph node metastases[58].

It has been established that low levels of ATM, assessed by IHC, correlate with gastric cancer cell line sensitivity to olaparib[59]. This sensitivity appears to be further heightened by co-existent p53 deficiency[60]. However, as *p53* alterations are seen more commonly in proximal tumours[61] in chromosomal unstable gastric cancers[62] and ATM alterations are found more often in distal tumours and in microsatellite unstable tumours there may be relatively few patients where co-alterations exist.

Mutations in *ATM* are relatively infrequent events in gastric cancer, with only approximately 10-15% patients' tumour samples having an alteration in *ATM* [58,63]. Germline ATM mutations are even rarer, found in only 2.7% gastric cancer patients[64]. ATM is a very large gene (66 exons spanning 150kb of genomic DNA) and there appear to be no particular mutational hot-spots, with the majority of the described alterations being single point mutations[58,63].

Despite this low frequency of *ATM* mutation, a comparatively high percentage of gastric tumour samples have been reported to have low ATM protein levels, between 21% and 65% in Eastern patients[65,58]. The causes of low ATM have not been fully elucidated but may include point mutations, epigenetic silencing, microRNA expression or intronic mutations associated with MSI[57,60]. In fact, in patients with gastric cancer and the MSI phenotype nearly 70% have an *ATM* intron mutation and over 50% have ATM protein loss[57]. It has been suggested that DNA repair genes may be a critical target for deficient MMR and MSI is seen in 15-30% of gastric cancers.

Based on the above observations, Study 39 (NCT01063517), a double-blind phase II study of paclitaxel/ olaparib versus paclitaxel/placebo in the second line treatment of advanced gastric adenocarcinoma, was conducted[66]. 124 patients were randomised to receive paclitaxel (80 mg/m^2 intravenously on days 1, 8, and 15 of every 28-day cycle) with olaparib (100mg twice a day continuously) or matched placebo, followed by maintenance monotherapy with olaparib (200mg twice a day) or placebo.

ATM status was assessed with a validated IHC assay, chosen as it demonstrated clear results with nuclear staining, and patients with low or undetectable levels of ATM protein were classified as ATM_{low}. Within this study only 14% of all patients screened were defined as

ATM_{low} but through patient selection the trial population was enriched such that 50% of the patients within the study were ATM_{low}.

The study did not meet its primary endpoint of improved progression free survival (PFS) with the addition of olaparib, although there was a trend towards improvement, particularly in the ATM_{low} group (median PFS 5.29 vs. 3.68 months, HR= 0.74). However, overall survival (OS) was significantly increased in both the overall population (median OS 13.1 vs. 8.3 months, HR= 0.56, 80% CI 0.41-0.75, p=0.005) and in the ATM_{low} group (median OS not reached vs. 8.2 months, HR =0.35, 80% CI 0.22-0.56, p=0.002).

The treatment with olaparib and paclitaxel was well tolerated with a safety profile consistent with the published literature. Neutropaenia was the most common grade 3/4 adverse event (56% paclitaxel/olaparib vs. 39% paclitaxel/placebo), although rates of febrile neutropaenia were low.

The investigators suggested a number of possibilities for the discrepancy between the OS and PFS results including the small sample size, a possible lack of correlation between PFS and OS, the effects of olaparib on long term colony formation or a post progression synergism of olaparib with irinotecan treatment[66]. The results from the follow on phase III GOLD study (NCT01924533), which has recently completed recruitment, are awaited and if the survival signal from study 39 is confirmed the results may be practice changing.

6.2 Alternative sources of HRD in gastric cancer

Although much has been made of the role of ATM loss leading to HRD in gastric cancer, as in other solid tumours, the full picture is no doubt more complicated and other HR factors may also be involved.

Using genomics data from the C.Bioportal[67,68] it is evident that many of the other genes involved in HR are also altered in gastric cancer, albeit at low frequencies (Figure 2 and Table 3). As with ATM deficiency, epigenetics and microRNA involvement may also result in reduced expression of these genes even if they are not mutated. It will be important to look in depth at the significance of mutations in these genes versus other causes of loss of expression. Considering hypermethylation in particular, it has been suggested that resistance to treatment may occur more quickly in patients with promoter hypermethylation rather than gene mutation as treatment may trigger promoter demethylation and reactivation of the gene in question[41]. This idea is supported by data from a study of ovarian cancer patients looking at BRCA1 promoter hypermethylation. The 15% of patients who had BRCA1 promoter hypermethylation had an earlier onset of disease but had no better survival with platinum based chemotherapy than the remaining patients with intact BRCA1 expression[69].

Translational work in progress within Study 39 and GOLD will hopefully provide further insights into causes of HRD in gastric cancer and may enable the development of specific multi-gene signatures for HRD in this tumour type, in addition to simply looking at low ATM protein expression.

One signature already in existence for HRD in gastric cancer is signature 3, the base-substitution signature described above, previously seen in BRCA mutated breast, ovarian and pancreatic cancer. A recent study where signature 3 was applied across 33 other tumour types demonstrated the presence of signature 3 in 12% gastric cancers but not in any other tumour type[70]. Further studies are required to generate clinical data on the presence of signature 3 and response to PARP inhibition or platinum treatment in gastric cancer.

7. Future Studies targeting faulty DNA damage repair in Gastric Cancer

The current interest in targeting HRD across various solid tumours has resulted in a multitude of clinical studies, many including patients with gastric cancer, a few of which we will highlight here.

The above mentioned GOLD study, a phase III study of olaparib in combination with paclitaxel, compared with placebo in combination with paclitaxel in patients with advanced gastric cancer who have progressed following first-line therapy, is expected to report in 2016 and the results have the potential to significantly impact this field. Patients in the GOLD study are from China, Japan, Korea and Taiwan. As recent clinical trials in oesophagogastric cancer have highlighted significant geographical variation in treatment response and outcomes, the OPERa study (EUDRACT: 2015-001605-14), currently in setup, will establish the frequency and significance of ATM alterations, other potential HRD signatures and PARP inhibition as a therapeutic strategy in gastric cancer in Western patients in the UK and Portugal, as much of the ATM data discussed above is from studies in Eastern patients.

As successfully demonstrated in ovarian cancer, the role of maintenance PARP inhibition, in the form of rucaparib, is to be investigated in gastric cancer in one of the arms of the UK PLATFORM study[71] (EUDRACT: 2014-002169-30).

In light of the current excitement over the potential of immunotherapy in gastric cancer[72] and elsewhere it is unsurprising that combining immunotherapy with agents targeting DNA damage repair is being considered. This has a sound biological rationale as a link has been established between HRD and increased immunogenicity in high grade serous ovarian cancer, with increased recruitment of tumour infiltrating lymphocytes, possibly due to hypermutated tumours harbouring high numbers of neoantigens[73]. In gastric cancer the link between MSI and ATM deficiency may also be important, as in colorectal cancer it has been demonstrated

that tumours with MMR defects are particularly susceptible to immune checkpoint blockade[74].

Another biologically sound approach is to combine radiotherapy with agents targeting DNA damage repair deficiencies as DNA DSBs represent the most biologically significant lesions induced by radiotherapy treatment and deficiencies in DSB repair lead to increased radiation sensitivity[75]. Trials in progress include the phase I PATRIOT study (NCT02223923) combining the ATR inhibitor AZD6738 with radiotherapy in solid tumours and the phase I ROCOCO (NCT 01460888) combining olaparib with radiotherapy in oesophageal adenocarcinoma or squamous carcinoma.

Moving on from PARP inhibition, yet other studies are looking to target other aspects of DNA damage repair using agents such as AZD0156, an ATM kinase inhibitor (NCT 02588105), AZD6738, an ATR inhibitor (NCT02264678) and AZD1775, a Wee1 inhibitor (NCT02511795).

Such clinical studies will add to the growing knowledge base of how best to target DNA damage repair. The translational work being conducted alongside these studies will also provide important insights; hopefully generate predictive biomarkers and yield information regarding mechanisms of resistance to treatment and optimal treatment combination.

8. Conclusion

In the past few decades our understanding of the pathways involved in DNA damage repair has substantially improved. This knowledge has generated a novel treatment strategy for cancers, such as gastric cancer, where a subgroup of patients appears to have deficient DNA

damage repair. This strategy has so far shown some early promise in gastric cancer but there are many unanswered questions and this approach requires further significant investigation and validation. Nevertheless, in this poor prognosis disease novel therapies supported by a clear biological rationale should be welcomed and it will be exciting to see how this field continues to develop over the next decade.

9. Expert Opinion

Gastric cancer is a challenging malignancy to treat. The genomic landscape is complex with no clear molecular driver and useful predictive biomarkers, with the exception of HER2, have remained elusive. Current treatment options are limited and novel approaches keenly required.

Targeting defective DNA damage repair in gastric cancer is appealing as a relatively substantial subgroup of patients may be suitable for such treatment, considering all of the various genetic alterations which may render a tumour sensitive. Establishing the significance of these gene alterations in gastric cancer and how best to define this sensitive subgroup remains a formidable task. We have discussed above the concern that hypermethylation of a gene's promoter region may have a different significance to mutation of that same gene and there is also the issue of heterogeneity, already reported as a problem when considering ATM IHC between primary tumours and sites of metastasis[65]. In an ideal scenario patients would be identified using a gene signature, ideally from a peripheral blood sample, possibly using circulating tumour DNA. A similar method would then be used to monitor patients on treatment, looking for the emergence of resistance. Unfortunately it is likely to be some considerable time before such an approach is available in clinic.

The ongoing translational work will provide key insights into the subtleties of DNA damage repair and its defects in gastric cancer and this will enable future rationale drug combinations

to increase efficacy and overcome resistance. As yet very little is known about resistance mechanisms to olaparib in gastric cancer where the HRD is caused by low ATM rather than defective BRCA1/2. Learning from the experience of PARP inhibition in ovarian cancer it seems likely that there will be multiple mechanisms, including secondary mutations and inactivation of other elements in the pathway to restore HR[41], but again the translational work alongside the large clinical studies is of paramount importance.

One treatment combination of particular interest is with immunotherapy. Immunotherapy represents a significant breakthrough in oncology and has shown promise in gastric cancer. One of the current challenges of immunotherapy is defining the patients who would most benefit from this approach and maximising the efficacy of treatment within those patients. The observation that HRD in ovarian cancer may result in increased numbers of tumour specific neoantigens, high numbers of CD8 +ve tumour infiltrating T cells and increased immunogenicity[73] is exciting and certainly warrants investigation in HRD gastric cancer. Various early phase clinical trials investigating the combination of immunotherapy and a drug targeting deficient DNA damage repair are underway and will hopefully continue to evolve the field.

Key Phrases in DNA repair (Glossary Box)

Synthetic lethality: Synthetic lethality refers to a situation where defects in 2 particular genes in combination are lethal but where each mutation on its own is compatible with viability. It is a concept exploited in cancer treatment with a view to sparing normal cells by targeting a cancer specific mutation.

Microsatellite Instability (MSI): MSI is characterised by the presence of variable lengths of short nucleotide repeats, microsatellites, in tumour DNA. Three levels of MSI are usually described- MSI high, MSI low and MSI stable. MSI is caused by defects in the MMR genes or in their transcription.

Chromosomal instability (CIN): CIN is characterised by abnormal numbers of chromosomes (copy number alterations, CNAs) and alterations in particular chromosomal regions (such as gene deletions, amplifications and loss of heterozygosity). These changes may result in oncogene activation and/ or loss of tumour suppressor gene function.

Genomic Instability: An almost ubiquitous characteristic of cancer (either through deficient repair or due to defects which drive the accumulation of mutations). Genomic instability includes chromosomal instability, microsatellite instability and other forms of instability characterized by increased numbers of base-pair mutations.

Western and Eastern: In this review these terms refer to patients of different ethnicities with Western patients coming from Europe, the Americas and Oceania and Eastern patients coming from Asia.

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Type of DNA damage	Repair Mechanism	Description	Key genes
Single Strand Break	Mismatch Repair (MMR)	MMR enzymes monitor recently synthesized DNA to detect and repair errors in copied DNA sequences (A-G or T-C mismatch), especially within repeated sequences called microsatellites.	*MSH2* *MLH1* *MSH6* *PMS2*
	Base Excision Repair (BER)	BER repairs simple DNA base lesions which do not distort DNA's helix structure, usually caused by endogenous damage.	*MUTYH*
	Nucleotide Excision Repair (NER)	NER repairs bulky DNA lesions which distort DNA's helix structure, usually caused by exogenous damage.	*XP genes*
	Direct Repair (DR)	DR involves the direct chemical reversal of a damaged base without excision or de novo synthesis	*MGMT*
Double Strand Break	Homologous Recombination (HR)	Preferred highly conserved, error free repair pathway. Active during S and G2 phase of the cell cycle. Here the undamaged sister chromatid is used as a homologous template to guide accurate repair. In addition to repairing DSB also involved in the repair of lesions which stall DNA replication forks.	*BRCA 1/2* *ATM* *ATR* *Fanconi Anaemia genes* *MRE11* *RAD51* *CHK1/2*
	Non-homologous end joining (NHEJ)	Rapid error prone pathway which is cell cycle independent and may lead to genomic instability. Here there is no homologous template. NHEJ can be divided into classical and alternative NHEJ which is associated with	*XRCC 5/6* *PRKDC* *DCLRE1C*

		insertions and deletions.	
	Single strand annealing (SSA)	Error prone pathway which repairs breaks between two repeat sequences. Here the repeat sequence is used as a template to guide repair but deletions result in the loss of genetic material.	RAD52 RAD59

Table 1: Types of DNA damage and repair

MSH2, mutS homolog 2; MLH1, mutL homolog 1; MSH6, mutS homolog 6; PMS2, PMS1 homolog 2 mismatch repair system component; MUYTH, MutY DNA glycosylase; XP, xeroderma pigmentosum; MGMT, O6-methylguanine DNA methyltransferase; BRCA 1/2, Breast Cancer 1/2; ATM, Ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; MRE11, Meiotic recombination 11 homolog; CHK1, Checkpoint Kinase 1; XRCC 5/6, X-ray Repair complementing defect gene; PRKDC, protein kinase DNA activated catalytic polypeptide; DCLRE1C, DNA cross-link repair 1C

Table 2: Syndromes associated with faulty DNA damage repair

DNA Repair Mechanism	Consequence of loss of function	Syndrome/ Associated Cancers
Mismatch Repair (MMR)	Microsatellite instability (MSI)	Lynch Syndrome (HNPCC)- colon, endometrial, ovarian, gastric, urinary tract cancers
Base Excision Repair (BER)	Single strand breaks resulting in double strand breaks	Loss may be embryonically lethal *MUTYH* mutation associated with colon cancer
Nucleotide Excision Repair (NER)	C to T mutations	Xeroderma pigmentosum (XP)- skin cancer
Homologous Recombination (HR)	Error prone DNA repair and failure of chromosome segregation at meiosis	Hereditary breast/ovarian/pancreatic cancer Ataxia Telangiectasia- leukaemia, lymphoma
Non-homologous end joining (NHEJ)	Error prone DNA repair as reliant upon alternative NHEJ such as microhomology mediated end joining (MMEJ)	LIG4 syndrome- leukaemia XLF-SCID syndrome XRCC4 defect embryonically lethal

HNPCC, Hereditary Non Polyposis Colon Cancer; LIG4, DNA ligase 4; SCID, severe combined immunodeficiency; XRCC4, X-ray Repair complementing defect gene

Table 3: Genetic alterations associated with HRD in gastric cancer

Gene	Frequency of gene alteration*
ATM	**4.5-12.5%**
ATR	**3.8-6.6%**
BRCA1	**2-9.1%**
BRCA2	**5.1-13.6%**
CDK12	**2.6-14.6%**
PALB2	**1-3.8%**
NBS1 (NBN)	**2-7%**
MRE11A	**1-9.4%**

ATM- ataxia telangiectasia mutated, ATR- ataxia telangiectasia and Rad3-related, CDK12- cyclin dependent kinase12, PALB2- partner and localiser of BRCA2, NSB1- Nijmegen breakage syndrome 1

*Percentages quoted from c Bioportal for cancer genomics[66,67]

Figure 1: DNA Damage Repair Pathways

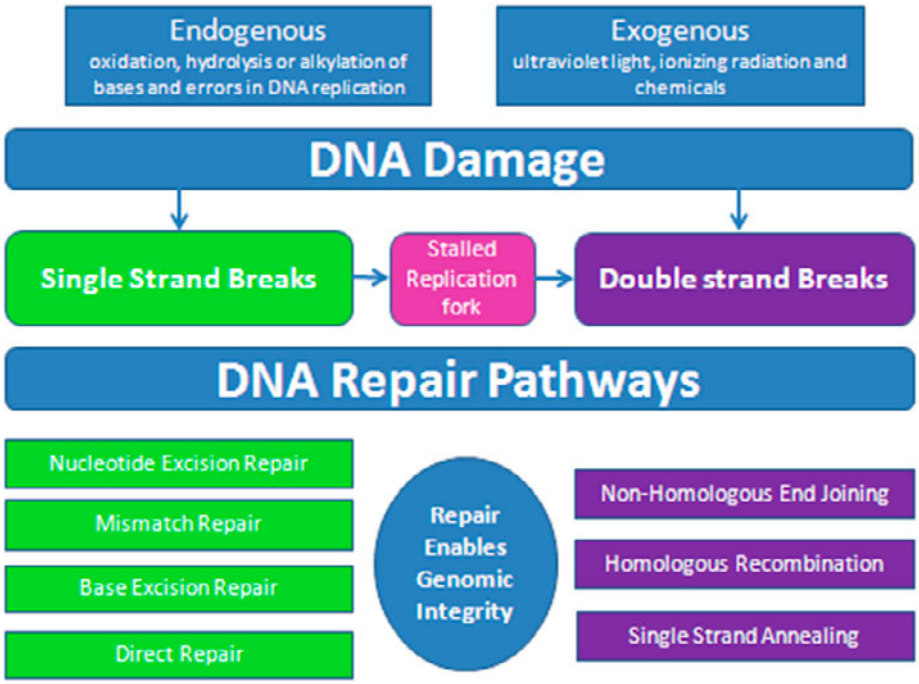

Figure 2. Cross-cancer alteration summary for ATM, ATR, BRCA1, BRCA2, CHEK1, CHEK2, FANCF, MDC1, MLH1, MSH2, PARP1, RAD51 (5 stomach adenocarcinoma studies / 12 genes)

Percentages quoted from c Bioportal for cancer genomics[66,67]

[Note the data from the UTokyo group was excluded as this only included patients with diffuse type gastric adenocarcinoma]