**Modulating the DNA Damage Response to Improve Treatment Response in Cervical Cancer**

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**Statement of Search Strategy**

The Pubmed database was searched for the term ‘cervical cancer’ in combination with the following search terms:

*DNA repair; DNA damage response; ATM; ATR; DNA-PK; Chk1; Chk2; Ku70; Ku80: PARP; Hyperthermia; EGFR; RNR; Proteasome Inhibition; homologous recombination repair; heat shock protein; NHEJ; HDAC; BARD1; BRCA; BRIP1; EXO1; RAD51; XRCC3; Fanconi anaemia; radiosensitivity DNA repair; chemosensitivity DNA repair.*

*And ’HPV’* in combination with the following search terms:

*DNA Repair; DNA damage response.*

The search was completed in December 2016 and the results (limited to those published in English language) were reviewed to identify relevant areas of investigation.

The clinical trials register ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) was also searched to identify relevant clinical trials.

**Abstract**

Cervical cancer is the 4th most common cause of cancer-related death in women worldwide and new therapeutic approaches are needed to improve clinical outcomes for this group of patients. Current treatment protocols for locally advanced and metastatic disease consist of ionising radiation and chemotherapy. Chemoradiation induces cytotoxic levels of DNA double strand breaks which activates programmed cell death via the DNA Damage Response (DDR). Cervical cancers are unique given an almost exclusive association with Human papillomavirus (HPV) infection; a potent manipulator of the DDR, with the potential to alter tumour sensitivity to DNA damaging agents and influence treatment response. This review highlights the wide range of therapeutic strategies in development which have the potential to modulate DDR and sensitise cervical tumours to DNA damaging agents in the context of HPV oncogenesis.

***Key Words***

Cervical Cancer; HPV; DNA Damage Response

**Abbreviations**

HPV – Human papillomavirus

DDR – DNA Damage Response

IR – Ionising Radiation

DSB – DNA double-strand break(s)

SSB – DNA single-strand break(s)

**Introduction**

Cervical cancer is the 4th most common cause of cancer-related death in women worldwide and as such represents a significant global health burden [1]. Locally advanced cervical cancer is treated by ionising radiation (IR) with platinum chemotherapy (cisplatin), which acts as a radiosensitiser and significantly improves treatment outcomes [2]. Many patients have residual disease or recurrence following chemoradiation with 5 year survival rates for stage IIB and IIIB disease at 61% and 44% respectively [2]. Therapeutic options for women with recurrent, persistent or metastatic disease are limited with platinum-containing doublet chemotherapy regimens resulting in response rates of 22-29% [3]. Some progress has been made with the addition of the anti-angiogenic agent bevacizumab in this setting, which increases overall survival by 3.7 months [4]. Further therapeutic advancements are needed and sensitising tumours to radiotherapy and chemotherapy is an area of considerable potential.

Chemoradiation induces multiple DNA lesions, the most toxic of which are DNA double strand breaks (DSB). DNA DSB can arise as a direct result of IR or indirectly from other types of damage such as interstrand cross-links induced by platinum chemotherapy and DNA single-strand breaks (SSB) caused by topoisomerase I inhibitors such as topotecan, particularly when these DNA lesions are encountered by DNA replication forks in S-phase. Cellular survival upon exposure to these treatments depends heavily on the cell’s ability to withstand and repair DNA DSB.

Cells respond to DNA damage by activating the DNA Damage Response (DDR) which halts cell cycle progression and facilitates repair of DNA lesions to prevent mutagenesis and genomic instability. In the context of overwhelming DNA damage in replicating cells the DDR activates programmed cell death, resulting in tumour regression [5]. Cancer cells can become resistant to this process by deregulation of DRR. This concept has particular relevance to cervical cancer, of which 99% are associated with high-risk Human Papillomavirus (HPV) infection (HPV 16, 18 31, 33) [6], acknowledged to manipulate the DDR at multiple junctures [7]. In order to facilitate HPV genome replication, HPV replication centres induce localised DDR activation [8, 9] and there is evidence that DDR proteins may be upregulated by HPV E7 protein and in response to HPV genomic integration [7, 10]. However, sustained activation of DNA repair promotes cell cycle arrest and pro-apoptotic mechanisms, which would be detrimental to the completion of the viral life cycle. HPV proteins abrogate these features of the DDR to enable viral replication, notably *via* E6-mediated degradation of p53 and E7-mediated degradation of retinoblastoma protein (Rb) [8]. The effect of HPV on the DDR is complex and further investigation is necessary to determine its role in mediating the sensitivity of HPV-associated tumours to DNA damaging agents *in vivo*. Transgenic mice expressing E6/E7 demonstrate an inhibition of DNA repair [11, 12] but these models lack HPV genomic integration sites, expression of other HPV proteins and have no representation of productive viral replication. In clinical practice, HPV positive cervical and oropharyngeal cancers are recognised as being more radiosensitive than their HPV negative counterparts [13, 14], but resistance to DNA damaging therapy still exists as evidenced by the poor survival outcomes in advanced HPV-positive cervical cancer. Therapeutic modulation of the DDR is an attractive strategy to increase treatment response and this review provides an overview of existing knowledge surrounding the development of such therapies for cervical cancer in the context of HPV-associated oncogenesis.

***The DNA Damage Response to Double Strand Breaks***

Several comprehensive reviews of the cellular response to DNA DSB have been published [15-17], which are outlined here and illustrated in Figure 1. There are two major pathways by which DNA DSB are repaired in mammalian cells; homologous recombination repair (HRR) and non-homologous end joining (NHEJ). HRR utilises the replicated sister chromatid as a template to accurately repair the lesion, and therefore is the method of choice during late S/G2 phase of the cell cycle. NHEJ, which occurs predominantly in G1, ligates the ends of DNA with minimal processing and is therefore more error-prone, and is consequently suppressed in S/G2 in favour of HRR.

The major sensors of DSB are the MRN complex (MRE11, RAD50, NBS1) and Ku70/Ku80 heterodimer which bind to the ends of DNA DSB. Identification of DSB activates the PI-3 kinase-like kinase ATM (ataxia telangiectasia mutated), a major signal transducer of DSB, which phosphorylates H2AX, Chk2 kinase and a variety of other proteins, resulting in the recruitment and activation of proteins involved in HRR, NHEJ and cell cycle arrest [18]. If HRR is to take place following the binding of DSB by the MRN complex, end resection occurs by MRE11, CtlP and exonuclease 1, which exposes a tail of 3’ ssDNA. Replication protein A (RPA) then binds and stabilises the ssDNA which is then further processed by HRR. Alternatively, another PI-3 kinase-like kinase, ATR (ataxia-telangiectasia and Rad3-related) is activated by ssDNA resulting from stalled or broken replication forks. This leads to Chk1 phosphorylation and the recruitment and activation of further proteins involved in DSB repair, HRR and cell cycle arrest [18]. HRR occurs when RAD51 is loaded onto the ssDNA by BRCA2, displacing RPA and forming a nucleofilament complex that identifies areas of sequence homology within the sister chromatid and facilitate strand invasion and extension of the 5’ end of DNA, branch migration and resolution into two fully intact sister chromatids. BRCA2 is required for RAD51 loading and multiple proteins including BRCA1, BARD1, XRCC3 are required for HRR with many, such as Fanconi anaemia protein FANCD2, playing accessory roles.

Alternatively in G1, or in S/G2 if HRR is absent or compromised, NHEJ take places. A synaptic complex including 53BP1 and Ku70/Ku80 is formed at the DSB to protect the DNA from end resection and recruit DNA dependent protein kinase (DNA-PK). Autophosphorylation of DNA-PK regulates the process of aligning and joining the two ends with ligase 4, XRCC4 and XLF.

Cellular selection of DSB-repair pathway (HRR or NHEJ) by the DDR is critical for maintaining cell survival whilst preserving genomic stability. Perturbation of this delicate balance may result in evasion of cell cycle checkpoints and apoptosis as evidenced by HPV infection. In the absence or reduction of HRR, illegitimate processing of DSB by NHEJ in S/G2 may allow increased cell survival, but leads to genomic instability through chromosomal rearrangements, which are characteristic of many cancers. Alternatively, disrupted DNA repair may offer therapeutic opportunities. In the absence of HRR, very high levels of DSB accumulate and their processing by erroneous NHEJ results in cell death.

**Pharmacological Inhibition of DNA Repair Proteins**

***ATM/ATR inhibition***

The recruitment and activation of ATM is necessary for HPV DNA amplification in differentiated cells [19]. High levels of ATM activation are associated with significantly worse loco-regional control and disease-specific survival in cervical cancer [20] and studies demonstrate a direct correlation between high baseline phospho-ATM in cervical cancer cell lines and resistance to IR [20]. Increased ATR expression has also been implicated in the pathogenesis of cervical cancer with an increased copy number found in cervical cancer specimens [21]. Pre-clinical studies demonstrate the radiosensitising effect of ATM/ATR inhibition *in vitro* [22-24] [25].

The development of ATR/ATM inhibitors in cervical cancer has been extensively reviewed [26]. Notably, pre-clinical observations have demonstrated that p53 deficient cells have increased sensitivity to ATR inhibitor VE-821 due to increased reliance on ATR-mediated checkpoint control following the loss of p53 [27] [28]. This may also be applicable to cells with abrogated p53 due to HPV infection. Several phase I studies are underway with ATR (AZD6738, VX-970) and ATM (AZD0156) inhibitors in advanced solid malignancies, however none to date specifically in cervical cancer. An inhibitor of Chk1 (a downstream kinase of ATR) has also been developed (LY2606368). A Phase I trial in squamous cell cancers suggest a favourable toxicity with this drug [29].

***DNA-PK Inhibition***

DNA-PK overexpression has been implicated in radio-resistant cervical tumours [30], and inhibition of this key NHEJ protein has been associated with increased chemosensitivity and radiosensitivity of cervical cell lines [24, 31]. The specific DNA-PK inhibitor NU7441 has demonstrated a radiosensitising effect in SiHa and HeLa cells lines and xenograft models [32] [33]. A phase I clinical study of the DNA-PK inhibitor MSC2490484A is underway in combination with radiotherapy in solid tumours (NCT02516813).

***Poly ADP Ribose Polymerase ((6)PARP) Inhibition***

The PARP1 protein has multiple functions in DNA repair and is a key factor in base excision repair of base lesions and SSB, which may be caused by IR. If unrepaired, these lesions can give rise to DSB resulting from replication fork collapse in dividing cells, and therefore loss of PARP1 function can lead to an increased requirement for HRR (figure 2.). This is exploited therapeutically in ovarian cancers with HRR deficiency resulting in ‘synthetic lethality’ as reviewed elsewhere [34]. There are studies which suggest a small number of cervical cancers have defects in HRR and therefore may be susceptible to this approach [11, 12, 35, 36].

PARP inhibitors may also be used to sensitise tumours to DNA damaging agents. Studies have suggested a role for PARP1 in chromatin remodelling by the NuRd complex at the site of DSB, as well promoting BRCA1 loading [37]. There is evidence that PARP-inhibition can reduce expression levels of BRCA1 and RAD51 by p130/E2F4 mediated promoter repression [38] although interestingly this study demonstrated a loss of p130-mediated repression in the presence of HPV E7 protein.

In support of the use of PARP inhibitors in cervical cancer, studies have demonstrated increased copy overexpression of PARP1 in cervical tumours [21, 39] and PARP1 levels post-irradiation are inversely correlated to radiation sensitivity of Caski cervical cancer cells [40, 41]. Increased PARP1 expression is found in a generated cisplatin-resistant HeLa cell line, the chemosensitivity of which is increased by PARP inhibition [42]. The PARP inhibitor veliparib has been studied in a cervical cancer xenograft model and resulted in the delayed the resolution of phosphorylated H2AX (a marker of DSB) and potentiated the cytotoxic effects of chemotherapeutic agents [43].

Early stage clinical trials are underway utilising PARP inhibitors in cervical cancer. A phase I-II study of topotecan and veliparib showed a low response rate of 7%, however this was in patients who had already received at least one line of systemic chemotherapy for recurrent or persistent disease [44]. Women whose initial tumour samples demonstrated low PARP1 expression were more likely to have a longer progression-free interval and survival after veliparib-topotecan therapy. Ongoing clinical studies with PARP inhibitors in cervical cancer are listed in Table 1.

**Indirect Pharmacological Modulation of the DNA Damage Response**

***Anti-angiogenics***

Sensitivity to DNA damaging therapy may also be conferred through indirect modulation of DDR activity by other cellular pathways (Figure 3). Antiangiogenic agents bevacizumab and cediranib have been trialled in combination with chemotherapy in advanced cervical cancer with improved survival outcomes [4] [45]. In addition to the metabolic effects of hypoxia on tumour cells, hypoxic conditions have been demonstrated to downregulate HRR gene expression including RAD51 [46], and therefore may also act by inhibiting the DDR. If the use of angiogenic agents can induce HRR deficiency in this manner, the combination of PARP inhibitors and anitangiogenics may prove to be an effective combination. A multicentre Phase II clinical trial to evaluate the combination of olaparib and cediranib as a maintenance therapy following chemotherapy for advanced/recurrent cervical cancer (COMICE) has been funded (R Lord; CI).

***Epidermal Growth Factor Receptor Inhibitors***

In addition to promoting cellular proliferation, it is recognised that the epidermal growth factor pathway is involved in the regulation of DSB repair following ligand-independent nuclear translocation of epidermal growth factor receptor (EGFR) and activation of DNA-PK [47, 48] [49, 50]. This process is activated by HPV E5 oncoprotein [51] and EGFR inhibition has been investigated as a means of sensitising cervical tumours to chemoradiation. *In vitro* cetuximab (a monoclonal antibody directed against EGFR) enhances the cytotoxicity of IR in cervical cell lines [52] [53] [54] however clinical trials to date have not demonstrated benefit from the addition of cetuximab to chemoradiation, although the EGFR tyrosine kinase inhibitor erlotinib shows more promise [55] [56]. Further work is underway to evaluate this group of drugs (Table 1), including newer monoclonal antibodies with increased specificity to EGFR (nimotuzumab, panitumumab). EGFR inhibition in combination with chemotherapy has largely demonstrated either lack of efficacy or excessive toxicity [57, 58] [59] except nimotuzumab which was well tolerated with cisplatin chemotherapy in a pilot study [60].

***Proteasome Inhibition***

Manipulation of DDR protein expression levels may be achieved through several non-specific cellular mechanisms which can be modified by pharmacological means. One such class of drugs are proteasome inhibitors which reduce the formation of DNA repair foci in cervical cell lines following IR and cisplatin, with evidence of reduced RAD51 activation and the inhibition of FANCD2 monoubiquitination [61, 62]. They also result in loss of E6-mediated degradation of p53, thereby promoting growth arrest and cell death in response to DDR activation [63, 64]. Proteasome inhibitors MG-132 and bortezomib are both active anticancer agents in cervical cell lines and xenograft experiments [65-68] and bortezomib has been evaluated in early trials in cervical cancer, however the results are yet to be reported (Table 1).

***Histone Deacetylase Inhibition***

The transcriptional regulation of DDR proteins can be altered by epigenetic modifications such as acetylation, which also plays a role in chromatin remodelling at sites of DNA damage [69]. There is evidence that HPV E7 directly interacts with histone deacetylases (HDACs) and influences gene transcription by directing HDAC activity to specific promoter sequences [70]. The use of HDAC inhibitors (HDACi) has been demonstrated to reduce the level of many proteins involved in DSB-repair (MRE11, BRCA1, BRCA2, RAD51, DNA-PK, Ku70/Ku80, ATM) and reduce BRCA1 foci formation with increased radiosensitivity of cancer cells [71] [72] and inhibition of growth in cervical cancer mouse xenografts [66].Clinical studies have utilised the anti-epileptic drug, magnesium valproate, which has been demonstrated to act as an HDAC inhibitor and repurposed as an anti-cancer agent in combination with hydralazine (a DNA methylation inhibitor) [73]. This combination can sensitise cervical cancer cells to both cisplatin and IR *in vitro* [74, 75], possibly by increasing p53 expression and stabilisation by acetylation [76, 77]. A small randomised phase III study demonstrated an increase in PFS with the use of this combination of epigenetic modulators with cisplatin/topotecan chemotherapy versus chemotherapy alone in advanced cervical cancer [78], and a further study is underway to assess the use of this combination with chemotherapy (NCT02446652). A phase III trial of hydralazine/valproate in combination with chemoradiation in patients with stage IIIB cervical cancer has demonstrated acceptable tolerability but efficacy was not assessed (83) [79].

***Heat Shock Protein Inhibition***

Heat shock proteins (HSPs) act as chaperones and stabilize protein structures during cellular stress. Hsp90 in particular has been implicated in regulating the activity of several DNA damage proteins including BRCA1, BRCA2, FANCA, FANCD2 and the MRN complex [80]. *In vitro* data demonstrates the cytotoxicity of Hsp90 inhibition on multiple cervical cancer cell lines [81], and pre-clinical studies show that Hsp90 antagonists reduce the level of ATR, phospho-ATR and Chk1 *via* a post transcriptional mechanism in HeLa cells, rendering the cells more susceptible to radiotherapy and DNA damage foci accumulation [82, 83]. Dote *et al* also demonstrated that Hsp90 inhibition reduced EGFR activation of DNA-PK [84]. Clinical evaluation of HSP inhibitors in cervical cancer has not yet occurred.

***Ribonucleotide Reductase Inhibition***

HPV-induced p53 degradation releases ribonuclease reductase (RNR) M2 and p53R2 subunits which are required for Ribonucleotide Reductase (RNR) activity, the rate-limiting step in deoxyribonucleotide production [85, 86]. Abundance of deoxyribonucleotides not only facilitates viral replication, but also HRR in response to IR. An association between over-expression of RNR subunits and increased risk of incomplete chemoradiation response, disease relapse and shortened disease-free survival has been observed in a small cohort using cervical biopsies [87] and pre-clinical studies demonstrates RNR inhibition resulting in increased radiosensitivity in cervical cancer cell lines [88] . Subsequent clinical evaluation (NCT00941070) of the RNR inhibitor 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) is promising, with a good clinical response rate, toxicity profile and long-term disease control when combined with chemoradiation in cervical and vaginal cancers [89] [90]. This drug is currently under further evaluation in randomised phase II trials (Table 1).

**Summary**

There is international consensus that further clinical trials are needed to develop treatments which improve clinical outcomes for advanced cervical cancer and enhance pelvic chemoradiation to reduce long and short term morbidity [91]. The identification of suitable targets within the DDR for therapeutic exploitation holds considerable promise and pre-clinical studies support the investigation of modulators of DDR in the clinical setting. Theoretical concerns over the systemic effects of pharmacological inhibitors of DDR proteins exist given the universally crucial nature of this process in protecting cells from chromosomal instability. Further clinical trial data is needed to determine whether this is a barrier to the introduction of such drugs. A greater understanding of the influence of HPV on DDR and treatment sensitivity of cervical cancer is desirable to aid these investigations, and perhaps facilitate the development of drugs with increased specificity of action.

Improved understanding of HPV-associated tumours arising in other sites (head and neck cancer, vulval cancer and anal cancer) may also benefit understanding of these complex interactions, and themselves benefit from translation of advances in cervical cancer management.

The potential use of the indirect modulators of DDR outlined in this review are of interest as they represent drug classes with clinical use in other indications and have well-recognised systemic effects to aid clinical trial design. Many of the drug classes anticipated to cause indirect modulation of DDR have other anti-cancer mechanisms of action, and therefore have the potential to increase tumour sensitivity *via* other cellular processes. Anti-angiogenic therapies are perhaps the most promising of these potential indirect modulators of DDR, having already demonstrated survival benefits in combination with chemotherapy in advanced cervical cancer, and will soon be evaluated in combination with PARP inhibition in a phase II UK clinical trial (COMICE).

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Conflicts of interest: none

**Figure 1**. A simplified schematic of the DDR in response to DSB demonstrating sites of direct pharmacological inhibition. Sensors of DNA DSB (MRN complex, Ku70/Ku80) activate DDR transducers ATM and ATR resulting in initiation of DNA repair (HRR or NHEJ), phosphorylation of H2AX, cell cycle arrest and apoptotic pathways. Direct inhibitors of ATM/ATR/Chk1 and DNA-PK may interrupt these processes.



**Figure 2**. PARP Inhibitor Mechanism of Action. PARP inhibition produces increased DNA DSB in replicating cells, which in the absence of sufficient HRR capacity leads to erroneous repair by NHEJ and/or activation of cell death. PARP inhibition may also contribute to HRR.



**Figure 3**. Indirect Modulation of the DNA Damage Response.



**Table 1**. Clinical trials of drugs with potential to modulate the DDR in cervical cancer

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target | Drug | Combination | Phase | Reference |
| PARP | Veliparib | Topotecan | I/II | [44] |
|  | Veliparib | Paclitaxel/Cisplatin | I/II | NCT01281852 |
|  | Veliparib | Topotecan | II | NCT01266447 |
|  | Olaparib | Carboplatin | I | NCT01237067 |
|  | Olaparib | Cediranib | II | [92] |
|  |  |  |  |  |
| Anti-angiogenics | Bevacizumab | Cisplatin/Paclitaxel, Topotecan/Paclitaxel  | III | [4] |
|  | Cediranib | Carboplatin/Paclitaxel | II | [45] |
|  | Cediranib | Olaparib | II | [92] |
|  |  |  |  |  |
| EGFR | Erlotinib | Chemoradiation | II | [56] |
|  | Cetuximab | Chemoradiation | II | [55], NCT00292955 |
|  | Cetuximab | Topotecan/Cisplatin | II | [57] |
|  | Cetuximab | Cisplatin | II | [58] |
|  | Cetuximab | Carboplatin/Paclitaxel | II | [59] |
|  | Panitumumab | Chemoradiation | II | NCT01158248 |
|  | Nimotuzumab | Chemoradiation | II | NCT02705612, NCT01301612 |
|  | Nimotuzumab | Cisplatin/Vinorelbine | III | NCT02083211 |
|  | Nimotuzumab | Carboplatin/Paclitaxel | II | NCT02039791 |
|  |  |  |  |  |
| Proteasome | Bortezomib | Chemoradiation | I | NCT00329589 |
|  | Bortezomib | Irinotecan | II | NCT00106262 |
|  |  |  |  |  |
| HDAC | Magnesium Valproate | Hydralazine/Cisplatin/Topotecan | III | [78] |
|  | Magnesium Valproate | Hydralazine/Carboplatin/Paclitaxel | III | NCT02446652 |
|  |  |  |  |  |
| Ribonucleotide Reductase  | Triapine | Chemoradiation | II | NCT00941070, NCT01835171, NCT02466971 |

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