Radiotherapy-PARP inhibitor combinations: progress to date.

Sarah Derby MBChB MRCP FRCR (1), Anthony J. Chalmers MBChB MRCP FRCR PhD (1), Ross Carruthers MBChB MRCP FRCR PhD (1)

1. Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow

Correspondence to:

Wolfson Wohl Cancer Research Centre,

Institute of Cancer Sciences,

Garscube Estate,

Glasgow,

G61 1QH

Email: Sarah.Derby@glasgow.ac.uk

Abstract

Radiation resistance remains a huge clinical problem for cancer patients and oncologists in the 21st century. In recent years the mammalian DNA damage response (DDR) has been extensively characterised and shown to play a key role in determining cellular survival following ionising radiation (IR) exposure. Genomic instability due to altered DDR is a hallmark of cancer, with many tumours exhibiting abnormal DNA repair or lack of redundancy in DDR. Targeting the abnormal DDR phenotype of tumour cells could lead to substantial gains in radiotherapy efficacy, improving local control and survival for patients with cancers that are refractory to current therapies. Poly(ADP-ribose) polymerase inhibitors (PARPi) are the most clinically advanced DDR inhibitors under investigation as radiosensitisers. Preclinical evidence suggests that PARPi may provide tumour specific radiosensitisation in certain contexts. In addition to inhibition of DNA single strand break (SSB) repair, PARP inhibitors may provide other benefits in combination treatment including radiosensitisation of hypoxic cells and targeting of alternative repair pathways such as microhomology mediated end joining which are increasingly recognized to be upregulated in cancer. Several early phase clinical trials of PARPi with radiation have completed or are in progress. Early reports have highlighted tumour specific challenges, with tolerability dependent upon anatomical location and use of concomitant systemic therapies; these challenges were largely predicted by preclinical data. This review discusses the role of PARP in the cellular response to IR, summarises preclinical studies of PARPi in combination with radiotherapy and explores current early phase clinical trials that are evaluating these combinations.

250 words

Introduction

Whilst radiotherapy is an important curative treatment modality for cancer, radiation resistance remains a major clinical problem in many tumour sites. Current radical radiotherapy regimens often rely upon combination with cytotoxic chemotherapeutic agents, which is a non-targeted approach that increases radiation induced DNA damage. Unfortunately it also exacerbates clinical toxicities and reflects a failure to appreciate the complexity of the biological pathways that govern DNA repair in cancerous and normal tissues. Although many factors in the tumour and normal cell microenvironment can influence responses to radiation, the network of DNA repair and cell cycle control mechanisms that we term the DNA damage response (DDR) which has the greatest influence on whether an irradiated cell perishes or endures. It is also becoming increasingly apparent that the DDR is commonly subverted during carcinogenesis, meaning that most cancer cells exhibit relative DDR defects in comparison to their non-malignant counterparts [1]. Compromised DDR in cancer cells may reflect an addiction to a specific repair pathway due to lack of redundancy when dealing with a particular type of DNA damage, or more significant defects in particular pathways. Such abnormal DDR phenotypes represent potential clinical levers by which to improve outcomes from radiotherapy in radioresistant cancers that are currently viewed as incurable.

Poly(ADP-ribose)polymerase-1 (PARP-1) has been subjected to extensive investigation with regard to its role in the DDR. PARP inhibitors (PARPi) are now standard of care in some BRCA deficient cancer groups however its potential as a radiosensitiser is less well appreciated. Preclinical studies have shown that PARPi act as radiosensitisers in many cancer types and show potential for tumour specific sensitisation under certain circumstances; this evidence has underpinned a number of early phase clinical trials. Here we will outline the biological rationale for PARP inhibition as a means of overcoming radioresistance and provide a comprehensive review of clincial trials, including both completed and ongoing studies.

The role of PARP

The PARP family encompasses at least 17 different proteins in humans whose functions are varied within the cell and include regulation of cell cycle progression, chromatin structure, mitochondrial activity and pro-inflammatory processes [2-4]. However, the most clinically relevant role of PARP is in the DDR.

The role of PARP-1 is best characterised in base excision repair (BER) which resolves the vast majority of radiation induced single stranded breaks (SSB) (see *fig 1.*) [5]. PARP-1 and to a lesser extent PARP-2 and PARP-3 are the proteins of greatest clinical interest due to their roles in BER and SSB repair [6]. The other PARP proteins make minimal contributions to the DDR and discussion of their roles and functions is outwith the scope of this review. Hence 'PARP' will primarily refer to PARP-1 in the rest of this manuscript.

The role of PARP in SSB repair

PARP senses and binds to SSBs facilitating recruitment of repair proteins involved in BER [7-9]. BER comprises two sub-pathways termed short and long patch repair, the utilisation of which depends upon the nature of the SSB. Short patch repair is generally used for repair of single nucleotide gaps whereas long patch is required for larger lesions involving up to ten nucleotides (see *fig 1*.).

The PARP protein consists of three functional domains: the N-terminus, the automodification domain and the C-terminus. The N-terminal domain contains zinc finger motifs that recognise and mediate binding of PARP to DNA SSBs [10, 11]. The central automodification domain allows for ADP-ribosylation of the PARP molecule itself and the C-terminal domain catalyses formation of poly(ADP-ribose) (PAR) polymer chains [12-14]. Upon binding of PARP to DNA, the C-terminal domain is stimulated to use donor nicotinamide adenine dinucleotide (NAD+) to catalyse and covalently bind multiple ADP-ribose units to form poly(ADP-ribose) (PAR) chains on the automodification domain of PARP (autoparylation) [15, 16]. This continues and forms chains up to hundreds of subunits long, formed in branching structures [17, 18]. PARP target proteins are numerous and include many DNA modifiers such as DNA ligases and polymerases. Parylation is a post-translational modification which alters the function of protein targets and their respective functional pathways [19, 20]. The negatively charged PAR polymer generated by autoparylation of PARP allows dissociation of PARP from the SSB (which is also negatively charged); this function is of importance when considering the action of PARPi, which blocks autoparylation and can lead to the phenomenon of 'PARP trapping' on DNA [18, 21, 22]. Dissociation of PARP from DNA facilitates access of X-ray repair cross-complementing protein 1(XRCC1) to the SSB site, acting as a scaffold for repair proteins DNA ligase III and DNA polymerase β (pol β) and facilitating SSB repair [23].

Figure 1. The role of PARP in SSB repair

<u>Rationale for combining radiotherapy and PARP inhibition</u>

Radiotherapy and PARP inhibition in replicating cancer cells

The mechanism of PARPi induced radiosensitisation is discrete from the synthetic lethality narrative described for tumours deficient in homologous recombination (HR) [24]. Whilst HR or other repair pathway deficiencies have been shown to increase the radiation sensitisation generated by PARPi, the vast majority proliferating cells exhibit radiosensitisation in response to PARPi and this effect is not dependent upon the integrity of HR. This is important to appreciate when considering both tumour response and normal tissue toxicity in the clinical scenario.

IR is believed to produce around 25 fold more SSBs than DSBs in mammalian cells [25]. Generally, IR induced SSBs are easily and efficiently processed by the cell and are not thought to contribute significantly to cell death. DSBs are the main lethal lesions generated by IR, and unrepaired DSBs have the potential to induce cell death via 'mitotic catastrophe' when an irradiated cancer cell attempts to progress through cell division. Some cancer cells are

susceptible to radiation induced cell death via apoptosis or other cell death mechanisms, but these pathways are often defective in the cancer setting.

PARPi radiosensitises by inhibiting the repair of SSBs generated by IR exposure, with consequent generation of potentially lethal single ended DNA DSBs via 'replication run off' as the irradiated cell traverses a subsequent S phase (see *fig 2.*) [26-28]. In effect, PARPi increase the burden of unrepairable DNA lesions by delaying BER and partly converting the usually inconsequential SSBs generated by IR into DSBs via the action of progressing and collapsing replication forks [29-31].

Dungey et. al. [28] demonstrated a replication-dependent increase in γH2AX and Rad51 foci in PARPi exposed glioblastoma (GBM) cell lines which implicated replication fork collapse in the aetiology of DSB generation in the context of radiation and PARPi. Additionally, PARPi-IR lesions arising at collapsed replication forks appeared to be more difficult to repair than endogenously caused fork collapses and affected cells continued to exhibit reduced proliferative capabilities up to and after 24 hours from exposure. The authors hypothesised that PARPi-IR lesions were exceptionally difficult for the GBM cells to repair either because of persistent PARP trapping at the site of SSBs/DSBs or via promotion of the NHEJ factor Ku binding to DSBs, which is thought to impede HR [32].

As well as PARPi-IR lesions resulting in collapsed replication forks, inhibition of PARP also appears to impact on replication fork regulation. PARP itself has a role in promoting fork progression and restart, either directly or indirectly, by opposing the role of F-box DNA helicase 1 (Fbh1), which negatively regulates HR, and by destabilising the key HR factor Rad51 at damaged replication forks [33, 34]. PARP also interacts with Mre11 at damaged replication forks. Mre11 is necessary for repairing damaged replication forks, and is thought to act by promoting HR-induced fork restart. PARP appears to maintain the presence of Mre11 at stalled replication forks thus promoting lesion resection, as reported by *Bryant et. al.* [35].

Figure 2. PARPi and replication fork collapse

The degree of radiosensitisation produced by different PARPi correlates with the extent of PARP trapping, a process whereby inhibition of autoparylation abrogates the electrostatic repulsion necessary to dislodge PARP from a SSB site, a process required for subsequent engagement of other SSB repair proteins. [22, 36]. Different PARPi vary comparatively in their ability to trap PARP (see table 1.). For example, *Laird et. al*. [37] demonstrated that talazoparib, a potent trapper of PARP, was a highly effective radiosensitiser and was associated with increased DSB generation compared to veliparib, a relatively less potent PARP trapper. *Pommier et. al.* [38] provide a more detailed discussion of PARP trapping.

Hypoxic cell radiosensitisation by PARPi

Radiation resistant hypoxic cells are a common characteristic of many cancer types. Several authors have explored the ability of PARP inhibitors to radiosensitise hypoxic cells *in vitro* [39, 40]. PARP is involved in the regulation of the hypoxic response and co-activates hypoxia inducible factor-1α (HIF-1α) gene expression [41]. HIF-1α expression is associated with poorer

prognosis in cancer patients and has been shown to promote tumour growth, new vessel formation and modulation of the surrounding microenvironment. PARP-inhibited cells display reduced gene expression and downregulated activity of HIF-1α [42, 43]. The relationship between HIF-1α and PARP provides a rationale for the proposed benefit of PARP inhibition in hypoxic tumours. In vitro, PARPi have been shown to radiosensitise hypoxic cells to a similar degree as non-hypoxic cells[39]. *Gani et. al.* [44] described effective radiosensitisation of human prostate cancer cell lines by olaparib under oxic, acute hypoxic and chronic hypoxic conditions. In a separate mechanism, PARPi share structural similarities with nicotinamide which is a vasodilatory agent that has been used as a radiosensitiser in bladder cancer [45]. PARPi induced vasodilation may reduce tumour hypoxia further and increase vulnerability to IR *in vivo* [46].

Potential Effects of PARPi on DNA DSB repair

The alternative DSB repair pathway microhomology-mediated end joining (MMEJ) is dependent on the XRCC1-DNA ligase III complex which requires PARP recruitment [47-49]. Predominantly acting in S phase, MMEJ has until recently been perceived as a repair process that provides backup when NHEJ or HR are dysfunctional. For example, deficiencies in the core NHEJ proteins XRCC4 and Ligase IV have been shown to result in increased use of MMEJ [50]. MMEJ is also utilised in HR deficient cells; both pathways process similar 3' overhang DSBs, although MMEJ requires microhomologies whereas HR requires a long 3' tail [51].

However recent data have shown that MMEJ activity is increased in cells suffering IR damage [52], and utilisation of MMEJ is increased in some cancer cells [53]. These new data indicate that targeting PARP might enhance radiosensitivity to a greater extent in cancer cells reliant on the MMEJ pathway by blocking DSB repair as well as SSB repair [48, 54].

MMEJ promotes genetic instability due to its highly error-prone mechanism (*see fig 3*.) [53, 55, 56]. MMEJ acts by finding microhomologies, which are short 5-25 base pair homologous sequences, on either side of a DSB to match these together and repair the damaged DNA [57]. The microhomologies are matched prior to ligation and the overhanging 3' ends are removed from both sides [51]. This method results in the loss of one of the sequences as well as any sequence from either side between both microhomologies. It can therefore generate genetic instability and risk gene knockouts as well as frameshift mutations [58].

Figure 3. MMEJ

<u>Bringing radiosensitising PARP inhibitors into the clinic</u>

In vivo studies of PARP inhibitors as radiosensitisers

There is a significant body of *in vivo* preclinical studies supporting a role for PARPi as radiation sensitisers. As previously mentioned *Gani et. al.* [44] demonstrated the ability of PARPi to radiosensitise hypoxic cells and went on to show increased clonogenic cell kill in a murine prostate cancer model using fractionated radiotherapy in combination with PARPi. Similarly, *in vivo* murine studies of GBM have shown the combination of PARPi-IR with temozolomide (TMZ) led to a delay in tumour growth [59]. Further studies in head and neck cancer and colon

cancer *in vivo* also show PARPi radiosensitise tumour cells and can significantly reduce tumour volume.

Biomarkers

Further studies have looked to identify approaches by which to enhance clinical application of PARPi through attempting to identify biomarkers that predict increased benefit. A recent study reviewed the anticorrelated relationship of MMEJ with transforming growth factor β (TGFβ) competency [60]. *Liu et. al.* showed that the loss of TGFβ function was present in cells using MMEJ and that these cells were more sensitive to radiotherapy and cytotoxic therapies providing a potential marker for sensitivity [60, 61]. Interestingly human papilloma virus-16 (HPV-16) positive cancers also appear to have increased levels of MMEJ and oncoprotein HPV-16 E7 is associated with NHEJ suppression thereby promoting MMEJ [62].

PARP is also implicated in maintaining Mre11 recruitment to damaged replication forks [35]. Mre11-low cancers are associated with lower cancer specific survival in radical radiotherapy, and may therefore act as a marker of suitability for radiotherapy [63]. Therefore the influence of PARPi in Mre11-low cells may merit investigation. Furthermore, BCL2-overexpressing cells are radiosensitised by PARPi and BCL2 may act as both a marker of radiation response and sensitivity to PARPi [64].

Other studies have shown that loss of SMAD4 gene expression is associated with downregulation of FANC/BRCA genes and can subsequently result in the "synthetically lethal" BRCA-deficient phenotype [65]. This study indicated that SMAD4 was lost in 35% of head and neck squamous cell carcinomas (HNSCCs) and that this inferred poorer response to treatment [66]. Treatment with PARPi-IR combinations *in vitro* showed comparatively higher levels of apoptosis in SMAD4-deficient cells compared with their SMAD4-proficient counterparts.

Imaging biomarkers for PARP are also under investigation with the use of Fluorine-18 (^{18}F)-radiolabelled PARPi in positron emission tomography (PET) imaging [67]. Preclinical work has indicated that use of ^{18}F-PARPi PET imaging could accurately predict response to PARPi treatment and may also be useful in determining radiation response and patient toxicity [68]. Radiolabelled PARPi could also aid radiotherapy planning by detection of deeply invasive cancers such as glioblastoma [69].

Current PARPi/radiotherapy combination trials

There are multiple early phase clinical trials investigating radiotherapy and PARP inhibitor combinations. PARP inhibitors currently undergoing investigation in clinical trials with radiotherapy are Olaparib, Veliparib, Rucaparib, Niraparib, Talazoparib and Pamiparib (trials listed on ClinicalTrials.gov and ISRCTN.com). Whilst all these compounds inhibit PARP they differ in terms of the cancer sites under investigation and areas of PARPi-IR research; the relevant trials are summarized in Table 1.

Current areas of Radiation and PARP inhibitor research

Head and Neck Squamous Cell Cancer (HNSCC)

PARPi-IR (Olaparib-69.3Gy in 33 fractions) has been trialed in HNSCC patients in combination with Cetuximab in patients with heavy smoking histories [70]. This phase I trial described an R2PD of 25mg twice daily and reported dose limiting toxicities (DLTs) of grade 4 dermatitis highlighting the challenges using concurrent cetuximab. Cetuximab is known to cause high levels of grade 3 and 4 dermatitis and mucositis and is generally only used in fit, cisplatin-ineligible patients [71]. Despite this, the patient group did have a promising 2 year survival of 72% though this must be taken in the context of the phase I design and small patient number. This study notes the challenges of Cetuximab-IR toxicity in combination with PARP inhibition however the preferred standard of care in HNSCC is concurrent treatment with cisplatin [72].

ORCA-2 is a phase I study combining Olaparib with cisplatin-based chemoradiotherapy in locally advanced HNSCC in patients [73]. Treatment comprises concurrent weekly cisplatin (35mg/m2) plus Olaparib starting 7 days prior to RT and given on days 1 to 3 of each week starting at 50mg twice daily. Primary completion date for this study was June 2020 and results are awaited. Further phase I studies include stage II and III laryngeal cancers as well as HPV negative oropharynx SCCs and combine single agent Olaparib with IR alone instead of cisplatin or cetuximab [74].

Thoracic cancers

Lung cancer remains a major clinical challenge with toxicity and treatment volume limiting radiation dose delivery. The combination of Olaparib with radiotherapy or chemoradiotherapy (66Gy in 24 fractions) with or without cisplatin 6mg/m^2 daily has been studied (Olaparib Dose Escalating Trial in Patients Treated With Radiotherapy With or Without Daily Dose Cisplatin for Locally Advanced Non-small Cell Lung Carcinoma) [75]. The trial reported the MTD of Olaparib without cisplatin to be 25mg once daily. Unfortunately radiosensitisation with PARP inhibition in combination with cisplatin could not be safely delivered, and resulted in haematological and late oesophageal DLTs. Across all groups there were significant pulmonary toxicities with 18% IR-associated pulmonary events resulting in death.

Two phase I studies investigating PARP inhibitors in combination with consolidation radiotherapy in extensive-stage small cell lung cancer (ES-SCLC) are ongoing using Talazoparib and Olaparib respectively [76, 77]. Both studies require at least stable disease after 4-6 cycles of palliative chemotherapy and give 10 fractions of consolidative radiotherapy. Balancing radiotherapy-PARPi toxicity may be less challenging to manage given that the ES-SCLC IR doses are low compared to radical dose NSCLC [78].

In addition to SCLC and NSCLC, radiotherapy concurrently with Olaparib is also being trialed as part of ROCOCO: **R**adiotherapy and **O**laparib in **Co**mbination for **C**arcinoma of the **O**esophagus (A Phase I trial) [79]. This phase I trial pairs Olaparib with radical radiotherapy (50Gy in 25 fractions) aiming to find the maximum tolerated dose of Olaparib in combination

with radiotherapy for those patients unsuitable for platinum base chemotherapies. Results of this study are currently awaited.

Pancreatic cancer

Early phase clinical studies have successfully demonstrated that PARP inhibition in pancreatic chemoradiation is deliverable, although the combination PARPi RP2D was relatively low. The VelGemRad trial (A Phase I Study of Veliparib (ABT-888) in Combination With Gemcitabine and Intensity Modulated Radiation Therapy in Patients With Locally Advanced, Unresectable Pancreatic Cancer) showed that 40mg of Veliparib twice daily could be safely delivered with 400mg/m^2 of gemcitabine concurrently with 36Gy in 15 fractions though noted an increased frequency of haematological toxicities [80].

Alternative chemotherapy-PARPi combinations in pancreatic cancer are also being investigated. The phase I PIONEER study investigates patients with locally advanced unresectable pancreatic cancer in combination with capecitabine 830mg/m^2 twice daily with the PARP inhibitor Olaparib concurrently with radiation (50.4Gy in 28 fractions) [81]. RP2D has been reported as 150mg twice daily for locally advanced pancreatic cancer (LAPC) patients and a dose expansion cohort of patients with borderline operable LAPC is under recruitment [82]. In contrast to gemcitabine, DLTs were grade 3 nausea, anorexia and fatigue.

Breast cancer

Veliparib has been trialed in a phase I study along with 50Gy to the chest wall and a 10Gy boost to macroscopic disease for inflammatory or locally recurrent breast cancer [83]. Whilst an MTD of 200mg twice daily dose level was reached there were two serious adverse events at the 150mg dose level with half of the patients experiencing late grade 3 skin toxicity and the final RP2D reported was 50mg twice daily. In contrast, the phase I RADIOPARP trial has investigated the olaparib-IR combination in triple negative inflammatory/locally advanced breast cancer and reached its maximum target dose of 200mg olaparib twice daily [84]. Using a 'run in' week, followed by 5 weeks of Olaparib with concurrent radiotherapy (50Gy in 25 fractions) no DLTs were observed during treatment or immediate follow up. The trial group reference the importance of assessing long term toxicity given the veliparib experience. Further trials evaluating PARPi in combination with radiotherapy for locally advanced breast cancer are underway (NCT02227082, NCT03945721, NCT03542175 and NCT01618357).

Rectal, peritoneal and gynaecological cancers

Phase I studies have also taken place in other cancer types including rectal, peritoneal and gynaecological cancers. 50.4Gy in 28 fractions with Capecitabine 825mg/m2 twice daily was investigated in locally advanced, resectable rectal cancer concurrent with Veliparib [85]. Resection by total mesorectal excision was performed 5-10 weeks following treatment. This combination proved tolerable with a reported RP2D of 400mg twice daily and the commonest side effects being nausea, diarrhoea and fatigue, which occurred in around half of patients. A pathological complete response was achieved in 29% of patients which appears higher than rates typically associated with conventional neo-adjuvant rectal chemoradiotherapy (~10-

20%) [86, 87]. The high R2PD with concurrent treatment is striking when compared to trials discussed earlier such as Veliparib-Gemcitabine-IR [80, 83].

A Phase I of Veliparib has been trialled combination with low dose fractionated whole abdominal radiation in patients with peritoneal carcinomatosis [88]. Whole abdominal radiotherapy has been evaluated over the years, predominantly in gynaecological cancer types, but has not shown superiority over chemotherapy [89]. In this study, veliparib was delivered for three cycles with low dose fractionated whole abdominal radiation on days 1 and 5 of weeks 1 to 3 for each cycle given twice daily up to 21.6Gy in 36 fractions delivering up to the maximum target dose of 160mg twice daily. However 7 of the 22 patients in the trial had to discontinue treatment due to multiple grade 2 toxicities as well as disease progression in 2 patients.

Brain metastases

Veliparib was investigated in combination with whole brain radiotherapy (WBRT, 30Gy in 10 fractions) in patients with non-small cell lung cancer (NSCLC) with unresectable cerebral metastases. This phase II study randomised 307 patients (1:1:1) to have WBRT plus Veliparib 50mg, Veliparib 200mg or placebo respectively [90]. No significant differences were observed between treatment arms in terms of overall survival (OS), response rate or time to progression, and there was no difference in adverse events. However, the benefit of WBRT for treatment of brain metastases is controversial with the recent QUARTZ trial demonstrating that WBRT in lung cancer does not improve OS or quality of life [91]. Therefore the hypothesis that a radiosensitising strategy could improve the efficacy of an already non-beneficial treatment may be flawed.

Glioblastoma

Glioblastoma (GBM) is a highly radiation resistant tumour, partly due to the existence of a population of glioblastoma cancer stem cells (GSCs) which exhibit increased levels of PARP expression and enhanced DNA DSB repair efficacy [92]. GBM cells are characterized by constitutive activation of the DDR and high proliferative rates whilst the surrounding normal brain tissue is essentially non dividing. Given the previous discussion on the replication dependency of PARPi radiosensitisation, GBM has been proposed as a site where PARPi could be given in combination with radiotherapy with relatively little increase in toxicity.

However, PARP inhibitors vary in their ability to cross the blood brain barrier (BBB) which poses a challenge to CNS combination treatment [93, 94]. Veliparib, Niraparib and Pamiparib, which are BBB penetrant according to *in vivo* data, are under currently investigation in phase I trials (NCT00770471, NCT03581292, NCT01514201, NCT04715620, NCT04614909 and NCT03150862) [93, 95]. The OPARATIC trial addressed specifically the concern of Olaparib penetration in GBM and successfully demonstrated drug penetration in tumour core and margin specimens [96]. This indicated that current preclinical models may fail to predict penetration of PARPi in patient tissue samples [93, 96, 97].

Building on the OPARATIC data, the phase I/II PARADIGM trial investigates the combination of Olaparib with 40Gy in 15 fractions in GBM (Ola**PA**rib And **RAD**iotherapy **I**n newly-diagnosed

Glioblasto**M**a) and is currently recruiting to a randomised phase II study with a RP2D of 200mg Olaparib twice daily with radiotherapy. Specifically PARADIGM targets patients with MGMT unmethylated GBM aged over 70 or those unsuitable for 6 weeks of combination radiotherapy with PARPi-IR, with a substudy of PARPi-IR and TMZ ongoing in MGMT methylated patients [98].

Similarly PARADIGM II (Ola**PA**rib and **RADI**otherapy or olaparib and radiotherapy plus temozolomide in newly diagnosed **G**lioblastoma stratified by **M**GMT status: 2 parallel phase I studies) is due to complete recruitment early this year and aims to identify the maximum tolerated dose of Olaparib in combination with IR (60Gy in 30 fractions) with or without TMZ in performance status 0-1 patients under 65 [99]. PARADIGM II stratifies by MGMT status so that MGMT methylated patients are treated with TMZ plus 60Gy in and escalating doses of Olaparib. Arguably, patients with unmethylated MGMT status do not benefit from concurrent TMZ and so in this arm escalating Olaparib doses are given without TMZ, allowing for higher doses to be given without the haematological toxicity of the TMZ-PARPi combination that has been seen in other trials [100, 101]. The phase I unmethylated arm has established an RP2D of 300mg twice daily (the full single agent dose) and is currently recruiting 30 patients to a dose expansion phase.

Emerging themes from early phase clinical studies of PARPi radiotherapy combinations

In Field Normal tissue toxicity

There are huge disparities in the MTDs identified by the various early phase clinical trials we have discussed, which may partly be explained by the different proliferative capacities of irradiated normal tissues within the radiotherapy volume. Based ln preclinical data we hypothesise that rapidly dividing cells of normal tissues that lie within the irradiated volume would suffer increased toxicity from the RT-PARPi combination compared to non-proliferating cells. This hypothesis is consistent with the low RP2D olaparib doses recommended in the phase I HNSCC and lung trials discussed above, since these tumour sites feature adjacent normal tissues with high proliferative rates [70, 75]. Similarly, the *Jagsi et. al.* study in breast cancer described significant skin toxicity, which is highly proliferative [83]. In contrast, very high RP2D's of Olaparib have been achieved in the PARADIGM and PARADIGM II studies, as well as the Veliparib brain metastasis studies where the irradiated neurological tissues consist of cells that are either quiescent or very slow to divide [90, 98, 99]. The addition of additional systemic agents such as cisplatin or cetuximab to PARPi-radiotherapy combinations may also create undesirable synergies that make dose escalation difficult. A further factor for consideration is radiation dose to be delivered, which varies widely depending on the cancer-specific standard regimen. Dose per fraction should also be considered as a potential contributor to toxicity, particularly given that some trials used up to 2.75Gy per fraction.

Haematological toxicity

Myelosuppression is commonly described in association with PARPi monotherapy but is generally relatively modest. It can be significantly exacerbated in combination with chemotherapy, however, and has been dose limiting in many studies [102]. Mechanistically,

DNA damaging chemotherapies such as platinums would be expected to result in highly sensitized tissue by PARPi. Clearly this is of importance when designing PARPi-IR chemotherapy regimens, as illustrated by the haematological toxicities reported in the Veliparib-Gemcitabine-IR pancreas and Olaparib-Cisplatin-IR lung trials [75, 80]. Of interest, PARPi-chemotherapy combinations are not universally myelosuppressive; for example the PARPi-capecitabine-IR combination was well tolerated in PIONEER study in pancreatic cancer (ref).

Predicting sensitivity and toxicity

Although most early phase clinical trials have adopted a conventional 'escalation to toxicity' approach when defining RP2D, there is evidence to suggest that PARPi are biologically effective in combination with radiation at lower doses than when used as monotherapy. This issue of predicting sensitivity to PARPi has been examined by *de Haan et. al.* [103]. By developing the radiation-enhanced-PAR (REP)-assay the authors were able to measure PARP inhibitory activity more accurately than previous methods. This enabled them to demonstrate that Olaparib was biologically effective as a radiosensitizer at very low doses (approximately 10-fold lower than required for single agent activity). This study adds confidence to the low R2PD values reported in some studies but also highlights the need for caution when escalating the intensity of combination treatments. Sensitivity to PARPi may also vary between individuals which further supports the concept of developing identifiers for PARPi-sensitive cancers [103, 104].

Conversely, several trials have reported that high PARPi doses can be safely delivered in combination with radiation, exceeding MTD expectations in pancreatic/rectal PARPi-Capecitabine-IR and PARPi-IR in CNS disease. Interestingly, preclinical research has demonstrated protective effects of PARPi in CNS and gastrointestinal pathologies. In particular, there is extensive evidence that PARP inhibitors have neuroprotective effects against ischaemic injury [105], and PARPi also improved chemotherapy-induced peripheral neuropathy *in vivo.* Similarly, pre-clinical murine models have showon that PARPi can reduce the severity of oxaliplatin-induced enteropathy, an effect that was associated with improved enteric neuronal survival [106, 107]. These protective effects may be related to the prevention of excessive PARP activation which would otherwise cause depletion of ATP and subsequent cell death through necrosis [108]. Henece PARP inhibition in key tissues prone to long-term neuropathic toxicities may provide some level of 'collateral radioprotection', even in combination with chemotherapy. Overall, it is clear that the toxicities associated with the use of PARP inhibitors as radiosensitisers vary in both intensity and type depending on the anatomical site.

Conclusion and Summary

Dysfunctional DDR is a hallmark of most cancers and a highly attractive target for drug development. PARP inhibitors are strong candidates as targeted radiosensitisers that act by increasing the lethal levels of DNA damage in cancer cells by delaying SSB, promoting

replication fork collapse, overcoming hypoxia and inhibiting MMEJ, which is a preferred DSB repair pathway in cancer cells. Since effects on non-proliferating cells are modest, targeting replicating cancer cells in combination with radiotherapy gives PARPi a targeted edge as a radiosensitiser.

Preclinical data support the use of PARPi in combination with IR as a potent radiosensitiser in particular tumour sites and biomarkers such as SMAD4 deficiency have been proposed that may enhance patient selection. Additionally radiolabelled PARPi has demonstrated promising results in use for PET imaging which may predict toxicity and response to treatment.

Clinical trials of PARPi-IR combinations are underway across multiple tumour sites. While some studies, for example in HNSCC and lung cancer, have reported low RP2D because of toxicity in highly proliferative normal tissues, others in CNS and rectal cancer have achieved high RP2Ds. Preclinical work using the 'REP-assay' has demonstrated that PARPi may radiosensitise at very low doses; this indicates that determining 'optimum biological dose' may be a more appropriate method than the historical MTD model. Conversely, parallel areas of PARP research have demonstrated neuroprotective effects on healthy CNS and GI tissues which indicate that CNS and gastrointestinal cancer sites may benefit from both radiosensitising effects and lower toxicity profiles.

Given this variability, identifying and validating predictive biomarkers will be important in determining which patients will most benefit from PARPi radiosensitisers. Clinical application of the REP assay could allow modulation of PARPi dose in order to ensure biologically sensitising doses are given to individual patients. Similarly, the development of radiolabelled PARPi for PET scanning could allow predictive toxicity to guide treatment approach.

Future investigation of biomarkers in apparently well tolerated cancer types are also needed to provide a fuller understanding of how different PARPi radiosensitise in different sites. However this must be considered in the wider perspective of PARP's role in cell processing and the protective properties of PARP inhibition. As research continues in PARPi-IR, the characterisation of PARP in cancer and in the wider cell processes must shape the role of PARPi as a radiosensitiser in the clinic.

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