Clinical Oncology 'Science in Focus' Editorial

Combining radiotherapy with inhibitors of the DNA damage response

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Introduction

Radiotherapy kills cells by damaging DNA and the immediate outcome after radiotherapy (does the cell die or survive?) is determined in large part by the ability of the cell to repair the DNA damage inflicted by radiation. The fact that radiotherapy is a useful treatment for cancer indicates that, in general, the cells of the normal tissues are better equipped to repair DNA damage than their malignant counterparts. In line with this general observation, there is increasing evidence that abnormalities in the DNA damage response (DDR) are a fundamental characteristic of cancer. This evidence base is now sufficiently robust for 'Genome Instability and Mutation' to feature as one of the two 'enabling characteristics' of cancer that were included in Hanahan and Weinberg's 'Hallmarks of Cancer: The Next Generation' in 2011 [1]. As well as contributing to carcinogenesis and malignant progression, DDR defects in cancer represent a promising therapeutic target, most famously illustrated by the sensitivity of BRCA-deficient breast and ovarian cancers to drugs that inhibit poly(ADP-ribose) polymerase (PARP) [2].

The cellular response to DNA damage comprises two main elements: cell cycle checkpoints and DNA repair [Figure 1]. Physical and biochemical repair of radiation induced DNA damage is executed by three main pathways: non-homologous end-joining (NHEJ) and homologous recombination (HR), which repair double stranded DNA breaks (DSB); and base excision repair (BER), which repairs single stranded DNA breaks (SSB) (reviewed in [3]). In the context of conventional external beam radiotherapy, DSB are generated in far fewer numbers than SSB but are highly mutagenic, and are cytotoxic if unrepaired. In contrast, the more numerous SSB are less cytotoxic, less mutagenic and more easily repaired. However, unrepaired SSB can be converted to DSB in the context of DNA replication and can interfere with important cellular processes such as gene transcription. Both pathways play important roles in the day-to-day maintenance of genomic integrity as well as the cellular response to genotoxic cancer treatments [4].

Cell cycle checkpoints have evolved to protect cells from the potentially catastrophic consequences of either replicating damaged DNA or attempting to undergo mitosis while carrying unrepaired DNA breaks. Inappropriate DNA replication is prevented by activation of the G1/S checkpoint, governed primarily by the ATM/p53/p21 signalling pathway, while entry into mitosis is guarded by the G2/M checkpoint under the control of ATM/ATR/Chk1/Chk2/Wee1 signalling. Additional cell cycle regulation is provided by intra-S phase and mitotic spindle checkpoints (reviewed in [5]).

In the context of cancer, defects in DNA repair result in the acquisition and accumulation of mutations that can drive carcinogenesis and malignant transformation, while dysfunctional cell cycle checkpoints are associated with an increase in the frequency and severity of chromosomal aberrations. Loss of cell cycle checkpoint integrity is a critical event in malignant progression: oncogenic stress in low grade tumours is associated with constitutive activation of cell cycle checkpoint proteins, including ATM, Chk2 and p53, which is significantly reduced in corresponding high grade tumours [6]. This somewhat counterintuitive observation led to the hypothesis that activated cell cycle checkpoints function as a 'brake' on malignant progression of low grade tumours. Consistent with this theory, acquisition of 'loss of function' mutations in these checkpoint proteins, most commonly p53, is associated with malignant progression. While not a universal phenomenon, the concept of the DDR as an 'anti-cancer barrier' is a plausible explanation for the high prevalence of DDR dysfunction in malignant disease, and identifies a family of attractive therapeutic targets (reviewed in [7]).

Exploiting DDR dysfunction to enhance responses to radiotherapy

When combining a novel agent with radiotherapy, clinical benefit is only achieved if the therapeutic ratio is widened, so it is vital to consider effects on both tumours and normal tissues. In the context of radical radiotherapy, radiation dose is limited primarily by the risk of causing irreversible damage to adjacent late-responding tissues such as the lung, heart, kidney, bowel, spinal cord or brain. As described above, tumour cell DNA damage responses differ from those of late responding normal tissues in several ways and thus have potential as tumour specific targets:

(1) Rapid cellular proliferation, compared with minimal proliferation in late responding tissues
(2) Elevated oxidative and replication stress
(3) Loss of function of the G1/S checkpoint and increased dependency on G2/M checkpoint integrity
(4) Defective DDR resulting from germline or somatic mutations in DDR genes and/or epigenetic or post-translational changes.

In parallel with the advances in cancer biology that revealed these potential targets, a range of potent and specific small molecule inhibitors of key DDR proteins have emerged, some of which have already entered the clinic. These dual developments make this an exciting and critical time in the evolution of individualised radiotherapy.

PARP inhibitors

Poly(ADP-ribose) polymerase (PARP) is a base excision repair enzyme that, upon sensing and binding to SSB, catalyses the addition of long, branching chains of the poly(ADP-ribose) polymer (PAR) to a variety of nuclear proteins including histones and other DDR proteins, thus facilitating SSB repair. Chemical inhibitors of PARP impede SSB repair and exert potent sensitising effects in combination with various cytotoxic drugs including alkylating agents (temozolomide, cyclophosphamide), topoisomerase inhibitors (irinotecan, topotecan) and cisplatin. In combination with radiation they exert modest sensitising effects on tumour cells but importantly this effect is only observed in actively replicating populations [8]. Radiopotentiating effects of PARP inhibitors (PARPi) have been demonstrated *in vivo* in a broad range of preclincial models and in most cases are more pronounced than in cellular studies. Together with the absence of radiosensitising effects in non-replicating cells, which predicts a lack of effect on late responding normal tissues, and the wealth of favourable clinical data for PARPi as single agents, these data have underpinned the emergence of PARPi as the first DDR targeting drugs to be tested in combination with radiotherapy in clinical trials. Additional mechanisms predicting tumour specificity have also been identified and are reviewed here: [9].

Several PARPi are now well characterised in clinical practice as single agents and clinical trials of PARPi/radiotherapy combinations are underway (Table 1). Various approaches have been adopted, ranging from intensive combinations of PARPi with radical chemoradiation regimes to more conservative evaluation of PARPi in combination with lower radiation dose schedules in populations of patients who are ineligible for radical treatment. Most combinations are currently undergoing phase I testing but the Abbvie PARPi veliparib has successfully progressed to randomised phase II evaluation in combination with whole brain radiotherapy for patients with brain metastases from non-small cell lung cancer [10].

DNA-PK inhibitors

Considering its role as a central component of NHEJ, the primary DSB repair pathway in mammalian cells, it is not surprising that downregulation of DNA-PK activity either genetically or through chemical inhibition is associated with dramatic increases in tumour radiosensitivity [11]. Since NHEJ is the only DSB repair pathway that functions during G1 phase of the cell cycle, however, there is a

significant risk that similar, or even greater, increases in radiosensitivity will be observed in late responding normal tissues. Toxicity studies in mice have to date revealed remarkably little evidence of this [unpublished data, personal communication], although it should be noted that these experiments have only addressed acute toxicity endpoints whereas the late responding normal tissues, consisting almost entirely of cells in G0 or G1 phase of the cell cycle, are theoretically at the greatest risk. A number of small molecule inhibitors of DNA-PK are in preclinical development and the Merck compound MSC2940484A has entered phase I clinical testing in combination with radiotherapy in patients receiving palliative radiotherapy for advanced solid tumours. The results of this study are eagerly awaited.

Cell cycle checkpoint inhibitors

The radiopotentiating effects of ATM inhibition are well established, and the potency of these compounds is thought to relate to the involvement of ATM in both cell cycle checkpoint activation and DNA repair (see Fig 1). Until recently, however, preclinical and clinical development of this class of DDRi has been hampered by adverse pharmacokinetic properties. Nonetheless, several research groups have demonstrated potent radiosensitising effects of ATM inhibitors including KU-55933 and KU-60019 both *in vitro* and *in vivo*, with particularly pronounced effects observed in glioblastoma (GBM) models [12]. The ability of ATM inhibition to overcome the innate and striking radioresistance of GBM stem-like cells has been documented by a number of authors and supports the potential therapeutic value of DDR inhibition in this currently incurable cancer [13]. The observation that p53 wild type cells appear to be relatively unresponsive to ATM inhibition indicates likely tumour specificity and also provides opportunities for patient selection [12].

Whereas ATM is acknowledged as the prime co-ordinator of the cellular response to radiation induced DSB, ATR is generally thought to function predominantly in response to DNA damage induced by non-ionising radiation such as ultraviolet light, and in enabling cells to tolerate DNA replication stress [5]. This clear division of duties is likely to represent an over-simplification, since there is clear evidence of at least partial redundancy between the two proteins, both of which are capable of phosphorylating a range of downstream DDR signalling proteins including Chk1, Chk2 and, indirectly, Wee1 [14]. In line with this, ATR inhibitors have shown exciting radiosensitising properties in a number of different tumour models [15, 16] and in the case of the Vertex compound VE-822 the enhancement of tumour growth delay occurred in the absence of any exacerbation of radiation damage to the intestine. The AstraZeneca compound AZD6738 is currently undergoing phase I evaluation in the context of a novel study design in which the combination with radiotherapy is evaluated as part of the First-In-Human study. Many of the functions of ATR are mediated through phosphorylation and activation of Chk1, and while Chk1 inhibitors have exhibited promising radiosensitising effects in preclinical studies, toxicity issues have hampered their clinical development. More recently, inhibition of Wee1, another kinase that promotes G2/M checkpoint activation in response to radiation, has emerged as a potential therapeutic strategy [17]. As with ATM inhibitors, a number of studies have indicated that the effects of ATR or Wee1 inhibitors are more pronounced in p53 defective contexts [18, 19].

Summary

Building on the success of PARP inhibitors as ‘synthetically lethal’ single agents in the treatment of HR deficient cancers, combining DDR inhibition with radiotherapy provides exciting opportunities to improve clinical outcomes for a much broader spectrum of solid tumours. Emerging data cataloguing the extent and patterns of DDR dysfunction that are a key feature of the malignant phenotype will facilitate a precision medicine approach in which the most appropriate DDR inhibitor/radiotherapy combination can be selected for each patient. Crucially, the preclinical data indicate that the radiopotentiating effects of many DDR inhibitors are likely to be tumour specific. Clinical trials are

now underway that will demonstrate whether this compelling scientific rationale translates into clinical benefit.

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Figure Legends

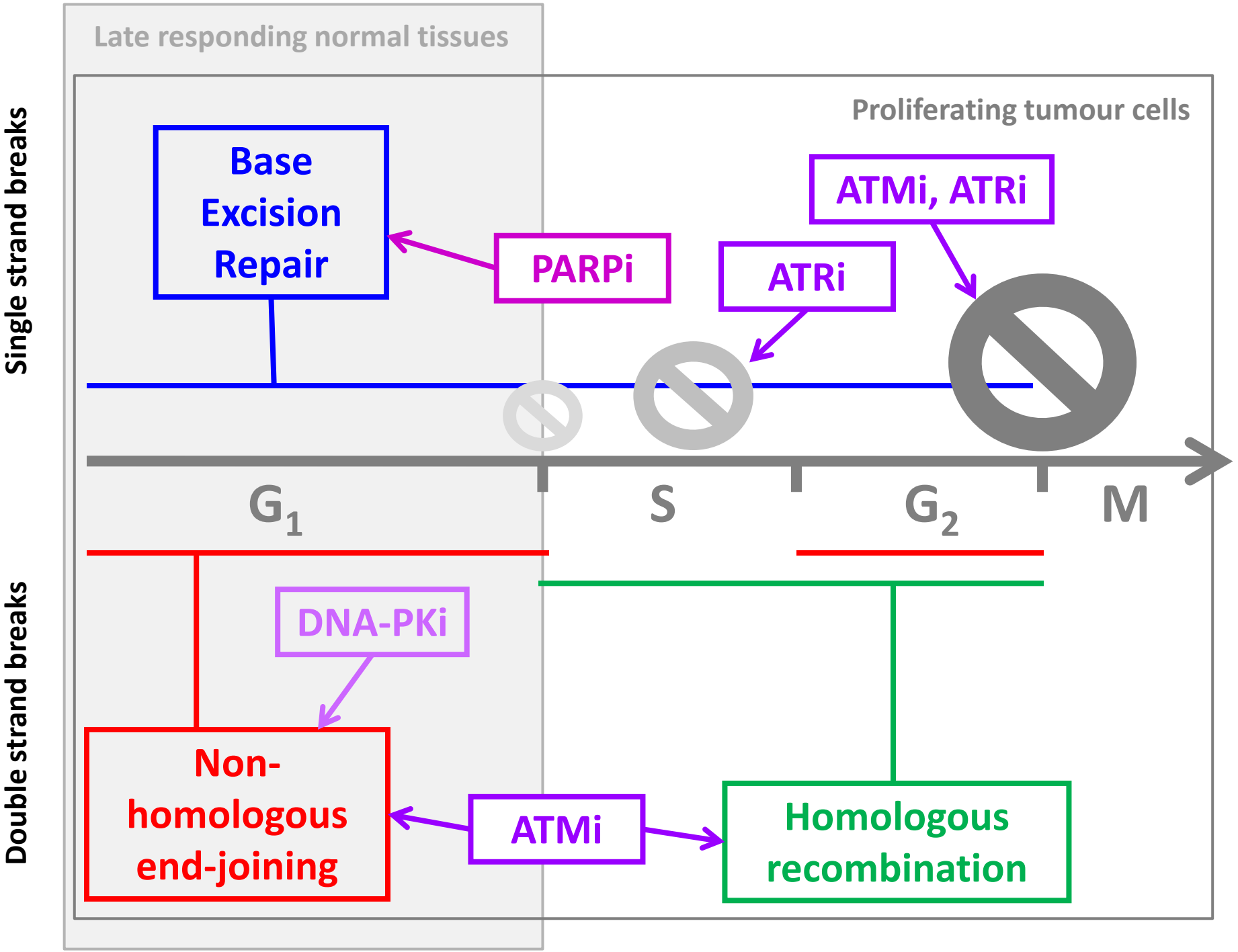
Figure 1:

Highly simplified diagram showing key components of the DDR, their relationships with the cell cycle and the sites of action of selected DDR inhibitors. The phases of the cell cycle are represented in

grey along the central axis of the figure. G1/S, intra-S and G2/M cell cycle checkpoints are illustrated by grey symbols, with relative size and colour representing the extent to which tumour cells are dependent on their function. The major repair pathways for radiation-induced DNA breaks are shown, with SSB repair pathways above and DSB repair pathways below the cell cycle axis. The relevance of these cell cycle dependent processes to effects of radiation-drug combinations on proliferating tumour cells and non-proliferating cells of late responding normal tissues are indicated by the large grey boxes. Tumour specificity of the radiosensitising effects of PARP inhibition is generated by the requirement for unrepaired single strand breaks to be converted to double strand breaks during DNA replication in S phase. Tumour specificity of inhibitors of ATM, ATR and Wee1 is predicted by the primary functions of their major targets taking place within S phase (ATR) and the G2/M checkpoint (ATM, ATR, Wee1).

DDR target	Agent	Tumour site	Venue	Phase	Radiation dose	Additional systemic agent	Clinical Trial ID	Status
PARP	Veliparib	Brain metastases	USA	Phase II	30 Gy in 10#	No	NCT01657799	Completed, results awaited
	Veliparib	Locally advanced rectal cancer	USA	Phase I	45 Gy in 25#	Capecitabine	NCT01589419	Completed, well tolerated
	Veliparib	Glioblastoma	USA	Phase I	60 Gy in 30#	Temozolomide	NCT00770471	Veliparib/temozolomide combination not tolerated
	Olaparib	Oesophagus Elderly patients	UK	Phase I	50 Gy in 25#	No	NCT01460888	Recruiting
	Olaparib	Head & neck cancer	USA	Phase I	69.3 Gy in 33#	Cetuximab 250 mg/m^2 weekly	NCT01758731	Recruiting
	Olaparib	Inoperable breast cancer	Netherlands	Phase I	61.8 Gy in 23#	No	NCT02227082	Recruiting
	Olaparib	Head & neck cancer	UK	Phase I	70 Gy in 35#	Cisplatin 35 mg/m^2 weekly	NCT02308072	Recruiting
	Olaparib	Glioblastoma Elderly patients	UK	Phase I	40 Gy in 15#	No	UKCRN18137	Recruiting
ATR	AZD6738	Solid tumours	UK	Phase I	20 Gy in 5# 30 Gy in 10#	No	NCT02223923	Recruiting
DNA-PK	MSC2490484A	Solid tumours	USA, Germany	Phase I	10-25 Gy, 1# 30 Gy in 10# 60 Gy in 30#	No	NCT02516813	Recruiting
Wee1	MK-1775*	Glioblastoma	USA	Phase I	60 Gy in 30#	Temozolomide	NCT01849146	Recruiting
	AZD1775*	Cervix	Canada	Phase I	25# then brachytherapy	Cisplatin weekly	NCT01958658	Recruiting

Table 1: Selected clinical trials evaluating DDR inhibitors in combination with radiotherapy. *Same agent.


Late responding normal tissues
Proliferating tumour cells
Single strand breaks
Base Excision Repair
PARPi
ATRi
ATMi, ATRi
G1
S
G2
M
Double strand breaks
DNA-PKi
Non-homologous end-joining
ATMi
Homologous recombination