Review Article

Targeting ataxia-telangiectasia mutated deficient malignancies with poly ADP ribose polymerase inhibitors

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Abstract: Small molecule inhibitors of poly ADP ribose polymerase (PARP) have shown considerable promise as therapeutic agents in human malignancies with disruption of the breast and ovarian cancer susceptibility genes *BRCA1* or *BRCA2*. Evidence is also accumulating that disruption of other genes involved in the detection and/or repair of DNA damage, in particular DNA double strand breaks (DSBs), may also have a synthetic lethal relationship with PARP. One of these is the DNA damage activated protein kinase, ataxia-telangiectasia mutated (ATM). Several studies have shown that ATM deficiency is common in a broad range of hematological and solid malignancies. Here, we discuss the potential for using PARP inhibitors in human cancers with mutation or disruption of *ATM*.

Key Words: Ataxia-telangiectasia mutated (ATM); malignancies; poly ADP ribose polymerase (PARP); PARP inhibitors

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Introduction

Poly(ADP-ribose) polymerase 1 (PARP1) is the founding member of the poly (ADP-ribose) polymerase (PARP) enzyme superfamily. It consists of an amino terminal DNA-binding domain which contains three zinc fingers, a central automodification domain that undergoes poly (ADP-ribosyl) ation, and a C-terminal catalytic domain which utilizes NAD^+ to generate poly (ADP-ribose) polymers on itself and its target proteins (1,2). PARP1 is activated in response to DNA damaging events, binds DNA strand interruptions and is involved in the detection and repair of DNA single strand breaks (SSBs) (1-3). The role of PARP in the detection of DNA damage led to the development of PARP inhibitors as chemo- and radiosensitizing agents (4,5). However, PARP1 inhibition as a potential major therapeutic strategy received a major boost in 2005 when studies from the Helleday and Ashworth laboratories revealed that small molecule inhibitors of PARP1 are cytotoxic in mammalian cells deficient for the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* (6,7). These observations were rapidly translated into the clinic. A small phase I clinical trial of patients with advanced ovarian, breast and other solid tumours treated with the PARP inhibitor olaparib (previously known as AZD2881) revealed anti-tumour activity in those with *BRCA1* or *2* mutations (8). This study was supported by subsequent phase 2 trials, which showed clinical benefit in women with BRCA1 or BRCA2 deficient breast and ovarian cancers (9,10). Subsequent trials showed increased progression free survival in patients with advanced ovarian cancer treated with olaparib (11,12).

Given that BRCA1 and BRCA2 are involved in the repair of DNA double strand breaks by the homologous recombination repair pathway (HRR), it was initially proposed that inhibition of PARP1 resulted in the accumulation of SSBs that, upon DNA replication, were converted to toxic DNA double strand breaks (DSBs) that required HRR for their repair (6,7). Thus,

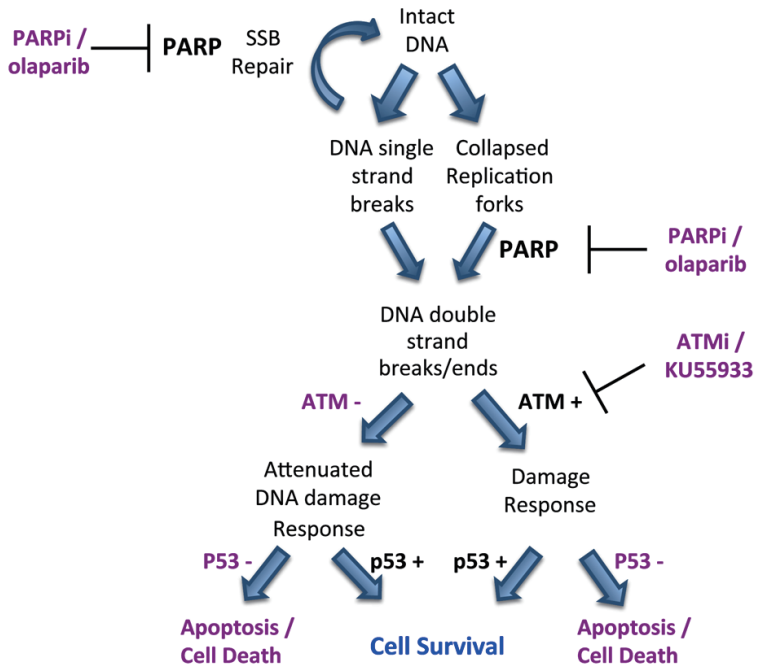

Figure 1 Models for the effects of PARP inhibitors on ATM-deficient cells [adapted from (13)]. Top left, endogenous SSBs are repaired by SSB repair pathways that involve PARP1. Inhibition of PARP (for example by olaparib), would be predicted to inhibit PARP-dependent SSB repair causing SSBs to be converted to DSBs during DNA replication; Top right, recent studies suggest that PARP is required for replication fork restart and that PARP inhibitors cause replication fork collapse, leading to toxic DSB ends that require ATM and components of HRR and Fanconi Anemia pathways for their repair; Lower right, in ATM-proficient cells, PARP-inhibitor induced damage is repaired regardless of p53 status; however, in ATM-deficient cell lines with mutant p53 or in ATM-deficient cells in which p53 function has been abrogated, PARP inhibitors induce cell death by apoptosis (lower left). In addition, inhibition of ATM kinase activity using KU55933 induces PARP-inhibitor dependent apoptosis in cells with inactivation or mutation of p53 (lower right). See text for details

cells compromised for HRR, for example by disruption of BRCA1 or BRCA2, were susceptible to PARP inhibition, a concept termed synthetic lethality (*Figure 1*). However, further studies revealed that many proteins, in addition to BRCA1 and BRCA2 are synthetic lethal or synthetic sick with PARP. These include not only proteins with critical roles in HRR, such as Rad51, Rad54, RPA and DSS1, but also proteins involved in DNA damage signaling in response to DSBs, such as Ataxia Telangiectasia Mutated (ATM), ATM and Rad3 related (ATR), cell cycle checkpoint kinase 1 (Chk1) and members of the Fanconi Anemia DNA damage response pathway (*Table 1*). Together, these studies suggest that the mechanism of PARP inhibitor cytotoxicity may be more complex than originally proposed (discussed further below) and that tumours with defects in DNA damage response genes other than *BRCA1* and *BRCA2* may also be targeted by PARP inhibitors. In this review, we will discuss the potential for PARP inhibitors in the treatment of human cancers characterized by deficiency in the DNA damage activated protein kinase, ATM.

ATM

ATM is a member of the phosphatidyl inositol 3 kinase like (PIKK) family of serine threonine protein kinases that phosphorylates its target proteins on serines or threonines that are followed by glutamines (SQ/TQ motifs) (23,24). ATM exists as an inactive dimer in the nucleus of mammalian cells and, upon DNA damage, is converted to an active monomer that is targeted to DSBs by the Mre11/Rad50/Nbs1 (MRN) complex, through interactions with the C-terminal region of Nbs1 (24-27). One of the hallmarks of ATM activation is autophosphorylation on serine 1981 (25), however, ATM undergoes DNA damage induced autophosphorylation at additional sites but whether autophosphorylation is a prerequisite for activation remains uncertain (28-31). Once activated, ATM phosphorylates multiple substrates resulting in regulation of cell cycle checkpoints and other cellular responses that together play critical roles in orchestrating the cellular response to DSBs. Some of the main targets of ATM in response to DNA damage include p53, Chk2, and

Table 1 Genes shown to be synthetic lethal or synthetic sick with PARP

Protein name	Protein symbol	Function	Ref.
Ataxia telangiectasia and Rad3 related	ATR	Cell cycle checkpoint signalling	(14,15)
Ataxia telangiectasia mutated	ATM	DSB damage signalling, repair of DSBs in heterochromatin	(14-16)
Breast and ovarian cancer susceptibility gene 1	BRCA1	Tumour suppressor, transcription, DSB signaling	(6)
Breast and ovarian cancer susceptibility gene 2	BRCA2	Tumour suppressor, HRR	(7)
Checkpoint kinase 1	CHEK1	Cell cycle arrest	(14,15)
Checkpoint kinase 2	CHEK2	Cell cycle arrest	(14)
Cyclin-dependent kinase 1	Cdk1	Cell cycle control	(17)
Cyclin-dependent kinase 5	CDK5	Neuronal cell cycle arrest and differentiation	(15)
Damage-specific DNA binding protein 1	DDB1	Nucleotide excision repair	(18)
Fanconi anemia, complementation group A	FANCA	DNA crosslink repair	(14)
Fanconi anemia, complementation group C	FANCC	DNA crosslink repair	(14)
Fanconi anemia, complementation group D2	FANCD2	DNA crosslink repair	(14)
Ligase 1	Lig1	DNA replication, base excision repair	(18)
Mitogen-activated protein kinase 12	MAPK12	Transduction of extracellular signal	(15)
Nibrin	NBS1	DSB repair	(14)
Nicotinamide phosphoribosyltransferase	NAMPT	Biosynthesis of nicotinamide adenine dinucleotide	(19)
Phosphatase and tensin homolog	PTEN	Dephosphorylates phosphatidylinositides	(20)
Polynucleotide kinase 3'-phosphatase	PNKP	Base excision repair, NHEJ	(15)
RAD51	RAD51	HRR	(14)
RAD54	RAD54	HRR	(14)
Replication protein A1	RPA1	SSB repair	(14)
Serine/threonine kinase 36	STK36	Possible role in sonic hedgehog (Shh) signaling	(15)
Split hand/foot malformation (ectrodactyly) type 1	DSS1	Limb development, HRR	(14)
Testis-specific serine kinase 3	STK22C	Germ cell differentiation	(15)
Ubiquitin specific peptidase 11	USP11	Deubiquitinating enzyme	(21)
X-ray repair complementing defective repair in Chinese hamster cells 1	XRCC1	SSB repair	(18)
XPA binding protein 2	XAB2	Transcription coupled repair, pre-mRNA splicing	(18)
X-ray repair complementing defective repair in Chinese hamster cells 2	XRCC2	HRR	(22)
X-ray repair complementing defective repair in Chinese hamster cells 3	XRCC3	HRR	(22)
RAD18	RAD18	Post replication repair	(22)
Ubiquitin-conjugating enzyme E2N	UBC13	Post replication repair	(22)

H2AX (23,24), however recent phosphoproteomics studies suggest that the total number of ATM substrates may be in the hundreds, if not thousands (32-34). Through phosphorylation of KAP-1, ATM also plays a critical role in the repair of DSBs that occur in heterochromatic regions (35,36). ATM is also the major sensor of reactive oxygen species (ROS) in the cell (37,38). Recent studies reveal that ATM also plays critical roles in mitochondrial integrity (39) and additional cytoplasmic roles have been reported (40). Thus, ATM plays critical roles in multiple cellular processes (41).

Table 2 Frequency of ATM mutation in various human cancers

Cancer type	Incidence (new cases per year)	Rate of *ATM* alteration given as % with reference source in parenthesis	Estimated number of potential *ATM*-deficient patients
Breast	22,900	3 (46)	687
Chronic lymphocyte Leukemia (CLL)	~546	9 (47)	50
Colorectal	23,300	7 (48)	1,631
Diffuse large B cell lymphoma (DLBCL)	~2,340	20 (49)	468
Gastric	3,330	6.7 (50)	223
Lung	25,600	7 (51,52)	1,792
MCL	~468	50 (53)	234
Ovarian	2,600	1.7 (54)	44
Pancreas	4,600	8 (55)	368
Total	85,684	n/a	5,497

Statistics for cancer incidence were taken from estimated new cases of cancer in 2012 provided by the Canadian Cancer Society (available on the Canadian Cancer Encyclopedia at http://info.cancer.ca/cce-ecc/default.aspx?Lang=E&toc=88). The incidences of MCL, DLBCL and CLL were estimated from the percentage of non-Hodgkin lymphoma cases reported by the Canadian Cancer Society (http://www.cancer.ca). The rate of alteration of the *ATM* gene was taken from the various publications cited and the total number of ATM deficient patients estimated from these values. The total population of Canada in 2011 was approximately 34 million (http://www.statcan.gc.ca/pub/12-581-x/2012000/pop-eng.htm), therefore the number of ATM-deficient patients in the USA is estimated to be approximately 10 fold higher than in Canada. Abbreviations: DLBCL, diffuse large B cell lymphoma; n/a, not applicable

Potential for targeting ATM-deficient malignancies with PARP inhibitors

The potential therapeutic relevance of ATM in this context can be inferred from a number of clinical and epidemiological observations. Mutation or deletion of both copies of the *ATM* gene result in Ataxia-Telangiectasia (A-T), a condition which becomes apparent in early infancy with the development of progressive cerebellar ataxia, but which is also characterized by radiation sensitivity, cancer predisposition and immune deficiencies (41-43). The latter three characteristics can be explained by an aberrant capacity to process DSBs while the ataxia is thought to be due to the toxic effects of ROS on Purkinje cells of the cerebellum in affected individuals. This cancer predisposition seems to extend to obligate A-T heterozygote blood relatives of A-T patients; indeed, ATM heterozygotes likely make up about 1% of the general population. Female A-T carriers have an increased risk of breast cancer, with some evidence of increased risk of colon and gastric cancers in men and women (44,45). Furthermore, recent evidence shows that a number of human malignancies have mutation or deletion of *ATM*. These include the B-cell lymphoid malignancies mantle cell lymphoma (MCL) and B-cell chronic lymphocytic leukemia (CLL), as well as carcinomas such as lung and gastric cancer (*Table 2*). The mechanism by which this arises is not defined (loss of heterozygosity is clearly a candidate), but the frequency with which *ATM* aberrations can be seen in tumours suggests that ATM deficiency is common enough to represent a numerically meaningful proportion of patients (*Table 2*).

Our laboratory has a long standing interest in ATM and other members of the PIKK family and their roles in the DNA damage response. Given that depletion of ATM using siRNA (14) confers sensitivity to PARP inhibitors and disruption of the genes for both *ATM* and *PARP-1* in mice is lethal (56), we hypothesized that human cancers with deficiencies in ATM might be sensitive to PARP inhibitors. To test this hypothesis, we assayed a panel of human MCL cell lines for ATM protein expression and function. Of the cell lines tested, two, Granta-519 and UPN2 were shown to have very low levels of ATM protein expression, low ATM serine 1981 phosphorylation, and low KAP-1 serine 824 phosphorylation, indicating defective ATM function. Moreover, Granta-519 and UPN2 were more sensitive to the PARP inhibitors PJ34 and olaparib than their ATM proficient counterparts. Olaparib also reduced tumour growth and enhanced survival in *RAG2*-deficient mice bearing xenografts of Granta-519 (57). Similar observations were reported by Stankovic and colleagues (58).

More recently we showed that ATM-deficient MCL

cell lines with inactivation or mutation of p53 were more susceptible to PARP inhibitors than those with wild type p53 (13) (*Figure 1*). Moreover, inhibition of ATM kinase activity using KU55933 enhanced olaparib sensitivity in MCL cells with mutant p53, opening the door to the possibility of sensitizing ATM-proficient human tumours with disruption of p53 to PARP inhibitors (13). This possibility is of particular interest given over 50% of human tumours have disruption or deletion of p53 (59). However, testing of this hypothesis will require the development of ATM inhibitors suitable for *in vivo* studies. It will also be interesting to determine whether other human cancers with deficiencies in ATM, such as gastric and lung, are also sensitive to PARP inhibitors, as these could account for many thousands of patients in Canada and North America alone (*Table 2*).

Mechanism of PARP inhibitor cytotoxicity

As discussed above, in early models it was proposed that PARP inhibitors induced the accumulation of SSBs that were converted to DSBs during replication and that the BRCA1/2-dependent HRR pathway was required for cellular survival. However, cells with depletion of many other genes, including those for ATM, PNKP and Fanconi Anemia -A, -D2 and -C were also shown to be susceptible to PARP inhibitors. Since these genes have no clear role in classical HRR, this suggested that the mechanism of PARP inhibitor induced cytotoxicity might be more complex than originally thought. Indeed, several studies have found that SSBs do not accumulate in PARP inhibitor treated cells [discussed in (60)], as predicted by the original model, and new studies suggest that PARP inhibitor synthetic lethality results, at least in part, from direct trapping of PARP at replication forks (22,60,61) (*Figure 1*). Resolution of these trapped DNA-PARP replication forks is proposed to require not only the traditional BRCA1/BRCA2-dependent HRR pathway, but also components of the Fanconi Anemia pathway, ATM, the FEN1 nuclease and DNA polymerase β (22).

Potential clinical application of PARP inhibitors in ATM-deficient malignancies

As discussed above, since the first demonstration of synthetic lethality between PARP and the BRCA genes in 2005, PARP inhibitors have rapidly advanced into the clinic with promising preliminary results. Indeed, over 85 clinical trials utilizing various PARP inhibitors are currently listed on the clinical trials registry ClinicalTrials.gov (http://clinicaltrials.gov/ct2/results?term=PARP+inhibitor&Search=Search). These ongoing trials cover a wide range of solid and hematopoietic malignancies, and utilize PARP inhibitors alone, in combination with other chemotherapeutic agents and with newer targeted agents (for example, bortezomib plus PARP inhibitor in multiple myeloma, NCT01495351). Although it is still early days, it seems likely that PARP inhibitors will have significant benefit to a subpopulation of cancer patients with defects in DNA damage response pathways (62,63).

However, clinical use of PARP inhibitors still faces several significant hurdles. One problem is the lack of an accurate predictive marker of PARP inhibitor utility. Experiments suggest that the genetic make up of the tumour will have confounding effects on susceptibility to PARP inhibitors. As discussed above, wild type p53 partially protects from olaparib sensitivity in ATM-deficient human cell lines (13), while depletion of 53BP1 confers resistance to PARP inhibitors (64). Moreover, the non-homologous end-joining (NHEJ) pathway has been shown to enhance PARP inhibitor sensitivity in HRR and ATM-deficient cells (13,65), suggesting that tumours with defects in NHEJ genes may influence PARP inhibitor sensitivity under some conditions. However, double deletion of Ku and PARP is lethal in mice (66), and the combination of inhibition of PARP and DNA-PKcs sensitize cells to the effects of IR (67) , so the relationship of PARP and NHEJ in DSB repair may be complex. Regardless, these observations point to the need for a thorough assessment of the molecular composition of human tumours prior to selection for PARP inhibitor treatment. However, determining how to make such an assessment is challenging. Immunohistochemical analysis may help delineate the presence or absence of the target (PARP), members of DNA repair pathways such as ATM and modulating factors such as p53 within the tumour. Interpreting the multiple potential combination of results, in contrast will be difficult. Determining functionality, likely the key predictive factor, is even more challenging. *Ex-vivo* FACS-based methodologies for assessing ATM functionally have been described (68,69), but their utility in the clinic for the management of solid tumors will be difficult unless applicable to viable circulating tumour cells. A second hurdle is that of acquired resistance. In the case of BRCA2, one way in which this can be induced is by mutations that revert tumours to wild type *BRCA2* (70). It has also been suggested that altered catalytic activity of PARP via, for example, small nucleotide polymorphisms, could decrease the effectiveness of PARP inhibition. Other potential problems include multidrug resistance via the efflux activity of p120 transporters as has been demonstrated

in vitro (64). Another significant hurdle, common to the personalized medicine approach in general, is intra-tumour heterogeneity, which will need to be addressed if personalized medicine is to reach its full potential (71,72).

In summary, PARP inhibition has shown promise in the treatment of cancers with deficiency in DNA repair pathways. Evidence suggests that ATM deficiency also renders cancer cells vulnerable to PARP inhibitors and that ATM deficiency is very common in a broad range of malignancies. The main hurdle to the translation of these observations to new clinical interventions are the lack of predictive markers and the potential evolution of resistance. Nevertheless, targeting DNA damage response deficient tumours, such as ATM deficient malignancies with PARP inhibitors and other interventions remains an exciting possibility and highly relevant topic for further investigation.

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Footnote

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