Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 30

The PARP story and a new strategy for cancer therapy.

Since most chemotherapy drugs damage DNA, the ability of cancer cells, as well as a patient's normal cells, to repair that damage had long been high in the minds of cancer researchers. Therefore, when it was discovered in the 1980's that poly(ADP-ribose)polymerase (PARP) had a role in DNA repair, cancer researchers began intensive studies of the effects of PARP inhibitors, which after many years led to useful new cancer therapy.

But what is PARP, and how was it discovered?

This chapter is about the PARP enzyme, and the polymer it produces, poly(ADPR). Both seemed unusual and strange at the time of their discovery, yet both turned out to be important players in many DNA repair processes, and PARP became an important target for novel cancer therapies. One of the first clues pointing to PARP inhibitors as potential anti-cancer drugs was that they increased the cell killing potency of alkylating agents (Durkacz et al., 1980) (Durkacz et al., 1981). But a major clinical success eventually came from the remarkable success of PARP inhibitors in patients whose cancers had inactivating mutations in a BRCA gene.

Discovery of a strange nucleic acid-like polymer, poly(ADPR).

The first time I heard about the newly discovered poly(ADPR) polymer and saw its chemical structure displayed on a poster at a conference in the 1960's, it seemed so bizarre that I had doubts about its existence. I was still imbued with the Watson-Crick lore of nucleic acid structures and could not believe that a chemical structure like poly(ADPR) had any right to exist in biology.

The discovery of this strange-seeming polymer traces back to 1963 to the Institut de Chimie Biologique in Strasbourg, France, where P. Chambon, J.D. Weill, P. Mandel and their coworkers noticed that there was an enzyme in cell nuclei that incorporated NAD (nicotinamide-adenine-dinucleotide, a prominent molecule in much of biochemistry) into an insoluble product that did not dissolve even in acid solvents (Chambon et al., 1966). I don't know whether the experiment that led to this observation was designed or whether it was an incidental observation. When they examined this phenomenon closely, they found that the enzyme's activity was greatly stimulated by DNA, but not by RNA. Chambon and colleagues might not have imagined that this observation of theirs was to develop into a body of knowledge of enormous importance in cell biology and cancer therapy.

Figure 30.1 depicts the structure of the new polymer, as originally conceived by Chambon and coworkers in 1966. It was noteworthy that the polymer was made up of NAD units, but without the nicotinamide part. A more up-to-date structure of poly(ADPR) and of its NAD building block is shown in Figures 30.2 and 30.3.

After so many years, culminating in so many important findings about its functions, this polymer no longer seems strange at all; in fact, it seems quite natural for it to exist and notable for all the things it does. It's remarkable how new knowledge can convert mystery into mundane experience; which is not to say that there is anything dull about poly(ADPR) or the enzyme that produces it, now known as PARP for poly(ADP-ribose) polymerase.

What at first seemed so bizarre about poly(ADPR) was how it violated the rules of ordinary DNA or RNA structures, as many of us conceived them in the 1960's. Although poly(ADPR) was composed of ordinary adenosine-ribose units, these units were linked to each other in a very non-nucleic acid-like manner (Figure 30.3). Instead of the regular head to tail order of nucleotide chains in DNA and RNA, the structural units of poly(ADPR) were linked via their diphosphates and, even stranger, they were also linked directly between their respective ribose units. Stranger yet, and very non-DNA or RNA-like, was that poly(ADPR) was a branched polymer. The branches come out of the second hydroxyl (OH) group of the ribose in the polymer chain. Ribose has two hydroxyl groups, one of which connects in the primary polymer chain, and the branches grow out of the other hydroxyl group on the riboses (Figure 30.3).

(As I write this, I am reminded of a conference I attended in 1951 or 1952 at Harvard during my senior year at the college. The speakers were excited by some of the first information about nucleic acid structure, which they obtained by using the then brand-new technique of ion-exchange chromatography. I recall their exciting conclusion that RNA was a branched molecule! I don't remember the evidence that led to that erroneous conclusion, but it was at least plausible in view of the apparently available extra hydroxyl group on the ribose parts of RNA. One might imagine that branched RNA molecules might once have existed during the early development of life on Earth, during what is called the RNA World, or that polymers of that kind will be found in life that remains to be discovered elsewhere in our Solar System. Linear RNA might have been required for replication. But primitive

RNA is thought also to have functioned as enzymes, which might be helped by a branched structure. The branches might have been added after the RNA chain was replicated.)

We can begin to understand the curious structure of poly(ADPR) from the fact that the building blocks from which the polymer was made differed from the ones from which DNA and RNA are made. DNA and RNA are composed of nucleotide units, assembled from nucleotide triphosphates, whereas poly(ADPR) is made from nicotine adenine dinucleotides (NAD). NAD had long been known to have essential roles in much of the cell's biochemistry. But to be used to make a biologically important polymer seemed bizarre. Moreover, the nicotinamide part of the NAD molecule was not even in the final polymer.

Now, to understand all that, we have to look more closely at the poly(ADPR) structure. We see (reading from right to left in Figure 30.3) that the polymer consists of chains of adenosine-phosphate-phosphate-ribose-adenosine-phosphate-phosphate-ribose- etc. (Ueda and Hayaishi, 1985).

So, what happened to the nicotinamide part of the NAD molecule that went into making poly(ADPR)? Why was it not in the polymer? Not only that, but it seemed strange to have one ribose bound to another ribose; I had never seen that before in any nucleic acid-like structure. Those questions whirled in my mind when I first saw the structure of poly(ADPR) displayed on the poster in the 1960's.

Soon the fog began to lift however when Hayaishi and coworkers noted that the bond holding the nicotinamide to the ribose is a high-energy bond (Nishizuka et al., 1969). That meant that the bond could easily break. Moreover, the energy released upon breakage of the bond drove the creation of the bond between the two ribose units that connected one unit of the polymer to the next. Thus, it was realized that the departure of the nicotinamide in fact drove the polymerization -- which was why there were no nicotinamide units in the polymer.

As already mentioned, the polymer had branches coming out of the ribose's second hydroxyl (OH) group. The polymer could grow a branch out of the second of the two ribose hydroxyls. The branched structure of poly(ADPR) was actually visualized in an electron microscope image in 1991 (Figure 30.4).

Next, we should talk about negative charge: The poly(ADPR) polymer has loads of it, because each phosphate bears a negative charge. Furthermore, the polymer's branched structure concentrates the negative charges into an even smaller volume of space. The poly(ADPR) molecule can be composed of hundreds of units and therefore can be quite large (Figure 30.4). When that happens, an extensive region of concentrated negative charge surrounds the chromatin region where poly(ADPR) polymers were later found to become attached. The concentrated negative charge would loosen the bonds between the DNA strands and between the DNA and its associated proteins, making it easier for the DNA repair machinery to access the sites of damage. That is getting ahead of the story, but it may be useful to have in mind where the story is heading -- particularly about the reason for the concentrated negative charge. The early picture was soon strengthened, when it was

discovered that poly(ADPR) polymers become attached to proteins, particularly the positively charged histones that are associated with DNA in chromatin (Hayaishi and Ueda, 1977).

The story, as it unfolded, was that, when DNA is damaged, PARP arrives at the scene, binds to the DNA at the damage site, and causes poly(ADPR) chains to grow from lysine amino acids of nearby histones. The large, branched polymer that hovers over the region of damage tends to make the region negatively charged, which would weaken the bonds holding together the DNA chains and their associated histones. Consequently, it would become easier for the DNA repair machinery to come in and do its job.

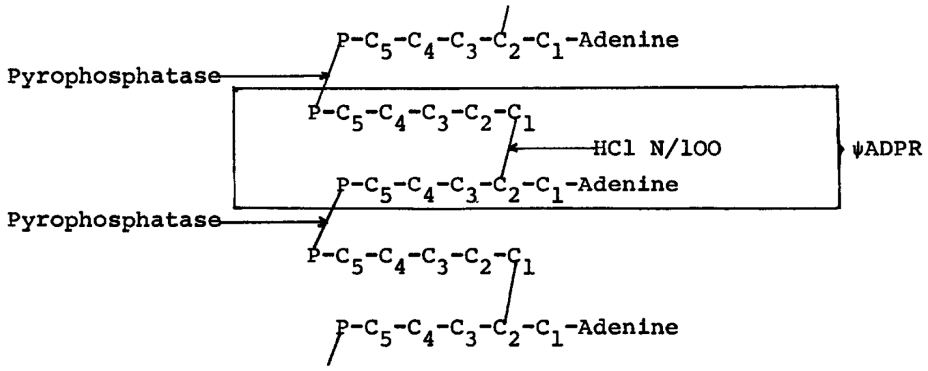


Figure 30.1. The structure of poly(ADPR) as originally conceived by Chambon and coworkers in 1966 (Chambon et al., 1966). The ribose parts are represented by carbon atoms C_1 through C_5. The repeated unit of the polymer is in the box.

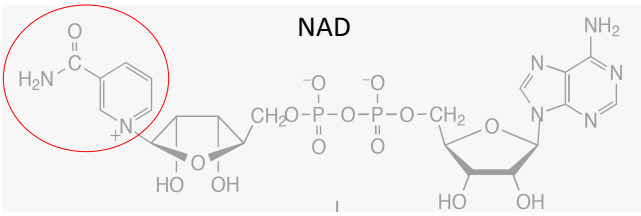


Figure 30.2. Chemical structure of NAD (nicotine-adenine-dinucleotide), the building block from which the Poly(ADPR) polymer is formed. The nicotinamide moiety within the red circle becomes cleaved away and does not become part of the polymer. The bond between the nicotinamide and the ribose is a high energy bond; breakage of that bond provides the energy for the polymerization of the poly(ADPR) polymer.

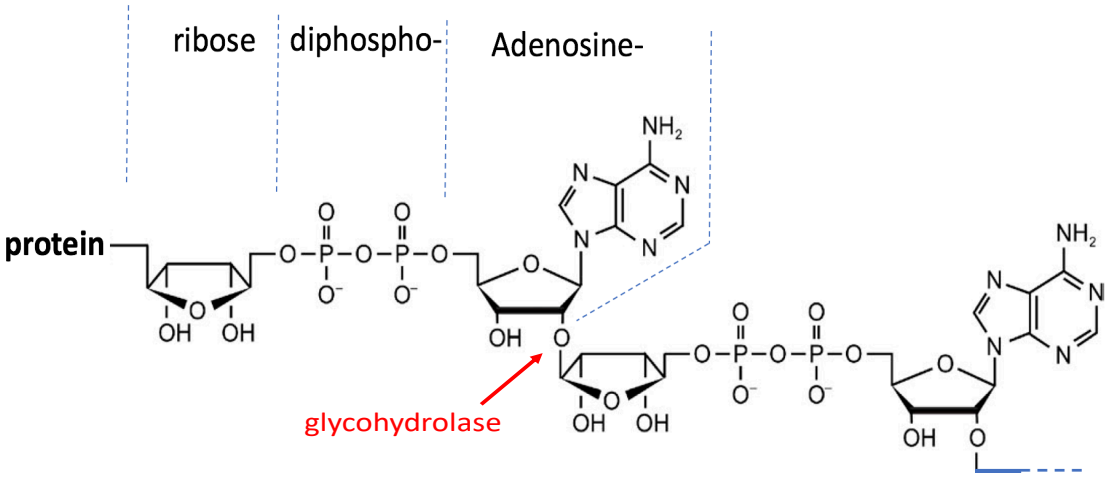


Figure 30.3. Chemical structure of adenosine-diphospho-ribose (ADPR) polymer, showing a chain of ADPR units (poly-ADPR) attached to a protein (left end in the diagram). Additional ADPR units may be connected via the dashed bond at the lower right. The enzyme that assembles the chain is poly(ADPR) polymerase (PARP). The negatively charged phosphates in the structure help to open the chromatin locally to allow repair enzymes to access the DNA at DNA damage sites. The remaining OH groups on the ribose units could attach to another adenosine-diphospho-ribose, leading to a branched polymer structure, which would concentrate the negative charge even more. The polymer can be broken apart by an enzyme (glycohydrolase), which breaks the bond between the ribose units (red arrow). Notice that there is no nicotinamide in the poly(ADPR) polymer. The cleavage of the bond to nicotinamide in NAD provided the energy for the assembly of the poly(ADPR) polymer.

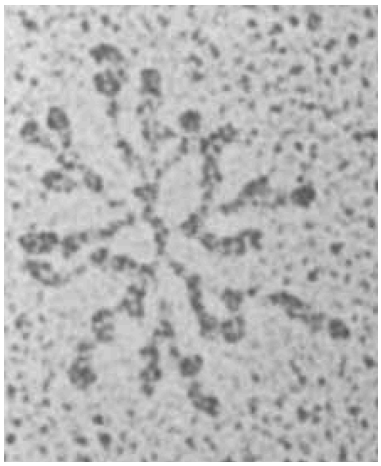


Figure 30.4. An electron microscope image of a large branched molecule of poly(ADPR) (de Murcia et al., 1991). The polymer spans a little over 0.1 microns, which could encompass about a hundred base-pairs or 10 turns of DNA.

Discovery of the poly(ADPR) polymer and the enzyme that produces it, PARP.

As mentioned above, the poly(ADPR) polymer was first observed in the 1960's by P. Chambon and coworkers, who found it as a product made from NAD in a reaction catalyzed by an enzyme in cell nuclei of chicken liver and beef spleen (Chambon et al., 1966). They remarked that, despite being made from NAD, the nicotinamide part of the NAD molecule was absent from the polymer. More information about the new poly(ADPR) polymer and how it is formed soon followed, largely from the laboratory of Osamu Hayaishi in Kyoto, Japan (Nakazawa et al., 1968; Nishizuka et al., 1968; Nishizuka et al., 1967; Nishizuka et al., 1969), who prepared it from rat liver. They noted that free nicotinamide was released from NAD coincident with the incorporation of the remainder of the NAD molecule into the polymer -- which was consistent with polymer assembly being driven by scission of the high energy bond to nicotinamide. The enzyme that catalyzed the polymerization, which became known as PARP, was located exclusively in the cell nuclei, where it grew poly(ADPR) chains onto histones in chromatin, as well as onto the PARP molecule itself. The role of PARP and poly(ADPR) in DNA repair, however, was not to emerge for several years. But first, we should talk about the PARP protein and its DNA-binding and enzyme activities.

The PARP1 protein and its domains of function.

Several proteins with PARP-like structure were discovered that eventually were considered to make up a family of at least 18 members. However, only PARP1 and PARP2 bound DNA, and PARP1 accounted for the great majority of the poly(ADPR) produced in the cell. Mouse embryos survived if either PARP1 or PARP2 was missing but died if both were missing (Ferraris, 2010; Schreiber et al., 2006).

An overview of the PARP protein, as diagrammed by (Schreiber et al., 2006) (Figure 30.5) shows the major regions ("domains") with the functions of each domain. The N-terminus of the amino acid sequence on the left and the C-terminus on the right. The amino acids are numbered from 1 to 1,014, starting from the N-terminus. A domain in the N-terminal region was found to be capable of binding DNA, and a domain near the C-terminus was where the PARP enzyme activity was located. In the central region, there was a region (an "automodification" domain) where the PARP1 protein's enzyme activity could grow poly(ADPR) polymer chains onto itself.

PARP1 was found to have a way of controlling its own enzyme activity. A region at the C-terminus was able to bend back on itself to bind and inhibit the adjacent enzyme activity region. When PARP1's N-terminal region binds to a DNA damage site, the bend is relieved, and the enzyme becomes highly active. In that way, PARP1 limits itself to generating poly(ADPR) chains only near sites of DNA damage.

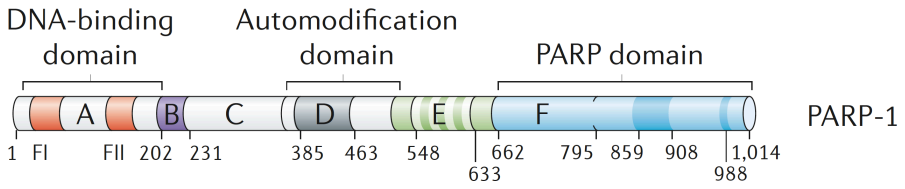


Figure 30.5. Diagram of the PARP1 amino acid chain, showing the locations of its functional parts (domains), as described by Schreiber et al in 2006. The N-terminal end of the molecule is on the left; and the C-terminus is on the right. The numbers are the amino acids, counting from the N-terminus. The N-terminal region was found capable of binding DNA, and the C-terminal region had the enzyme activity. The automodification domain near the center was where the PARP1 molecule attached poly(ADPR) polymer and onto itself (from (Schreiber et al., 2006)).

Discovery of PARP's role in DNA repair.

The discoveries that were to implicate PARP and poly(ADPR) in DNA repair, however, began long before anything was known about the polymer or the enzyme that makes it.

As often happens in break-through research, it all began with a puzzling observation. The first clue in the story goes back to 1956, with a curious observation by I. M. Roitt at the Courtauld Institute for Biochemistry in Middlesex Hospital, London (Roitt, 1956). When Roitt treated cells with an alkylating agent (triethyleneiminotriazine), he found that NAD (nicotinamide-adenine-dinucleotide), a major component in the cell's metabolic network, nearly disappeared. That was the first observation linking a DNA damaging agent with NAD, the building block that was later found to be used by PARP to make poly(ADPR).

However, it took an additional 20 years before the fall in NAD levels in DNA damaged cells was shown to be due to consumption of NAD during production of poly(ADPR) for linkage to histones in chromatin (Davies et al., 1977; Whish et al., 1975). In the meantime, there were over 100 reports about NAD depletion and the function of poly(ADPR), but all of them seem to have missed or ignored the relationship to DNA damage repair (Hayaishi and Ueda, 1977). There were just so many possibilities to consider among NAD's many functions in the cell that the DNA repair aspect apparently was not seriously considered.

The significance of Roitt's early observation in 1956 remained clouded until 1979, when Sidney Shall and his coworkers at the University of Sussex in England studied the effects of the DNA alkylating agent, dimethylsulfate, on mouse leukemia cells. They made a similar observation to that of Roitt in 1956: the alkylating agent caused a severe fall in the level of NAD in the cell. And as the NAD level fell, the PARP activity rose (Durkacz et al., 1980) (Figure 30.6.); PARP and its enzyme action were by then known, but their role in DNA

repair was just beginning to be revealed. It all suggested that perhaps the NAD was being used up to make a huge amount of poly(ADPR). They soon found out that PARP was, in fact, required to repair DNA strand breaks.

The Sidney Shall group had used dimethyl sulfate, which was known to methylate the N7-position of guanines in DNA, resulting in dissociation of the methylguanine followed by breakage of the DNA strand at the base-free site. They found that dimethyl sulfate caused NAD levels to fall and PARP activity to rise (Figure 30.6). In addition, they found that, when PARP was inhibited, the survival of the dimethylsulfate-treated cells was reduced, which indicated that PARP helped the cells survive DNA damage caused by the alkylating agent.

Then in 1982, Leonard Zwelling and Yves Pommier in my laboratory showed that PARP inhibitors impaired the ability of cells to repair x-ray induced DNA strand breaks (Zwelling et al., 1982) (Figure 30.7). They measured the DNA strand breaks using the alkaline elution technique that we had developed (see Chapter 9). In 1984, Mortimer Elkind and his coworkers then showed that a PARP inhibitor reduced the ability of cells to recover from DNA damage caused by x-rays (Ben-Hur et al., 1984) (Figure 30.8). Taken together, those three studies supported the idea that PARP helped to repair DNA damage produced by alkylating agents and x-rays.

Sixteen years later, in 2000, a research group in Newcastle upon Tyne led by Barbara Durkacz and David Newell reported that PARP inhibitors increased the killing of cultured human cancer cells when added to treatment with a topoisomerase I inhibitor (Figure 30.9) (Delaney et al., 2000) (see Chapter 11). The finding of synergy between PARP and a topoisomerase expanded the types of DNA damage whose cell killing was enhanced by PARP inhibitors and suggested that combining a PARP inhibitor with a topoisomerase inhibitor might be clinically beneficial.

However, the PARP inhibitors available at that time all had low potency and also inhibited enzymes other than PARP. Those early PARP inhibitors were used in many attempts to pin down an increased anti-cancer cell activity when combined with DNA damaging drugs. But the inhibitors were not good enough to create enthusiasm among clinical researchers. Further research into clinical applications therefore had to await the development of better PARP inhibitors.

To recapitulate to this point: After cells were exposed to radiation or alkylating agents, there was a marked reduction in the cell's content of a key molecule of metabolism, nicotinamide adenine dinucleotide (NAD). That was tied to another observation: the DNA damaging agents cause histones (the proteins around which DNA is wrapped in chromatin) to be modified by chains of adenosine-diphospho-ribose (ADPR) becoming stuck to them. The enzyme that catalyzed that reaction was poly(ADPR) polymerase (PARP), whose activity increased when there was DNA damage. It turned out, as inferred, that NAD became depleted because the molecule was used to make the poly(ADP-ribose) chains that were added onto the histone proteins, a process that was a required step in most DNA repair pathways. It seemed surprising that so much poly(ADPR) was made that it actually depleted the amount of NAD in the cell.

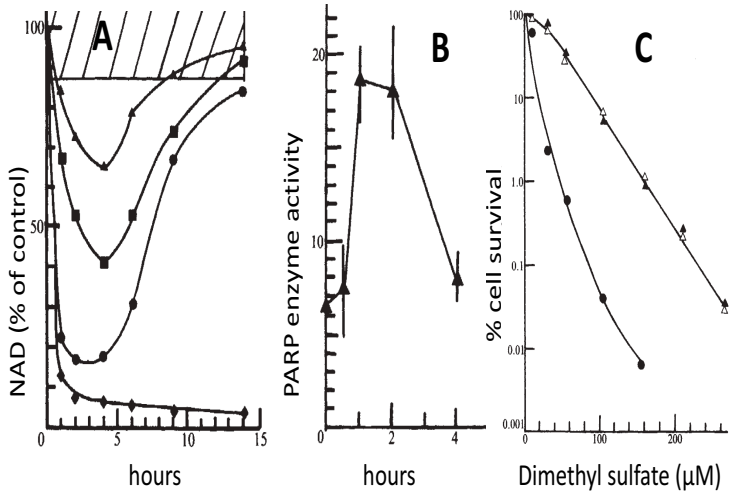


Figure 30.6. Treatment of mouse leukemia L1210 cells with dimethyl sulfate, which damages DNA by adding methyl groups at guanines-N7 positions, suppressed NAD levels (A), stimulated PARP enzyme activity (B), and impaired the viability of the cells (C) (Durkacz et al., 1980). The curves from top bottom in A are for increasing concentrations of dimethyl sulfate. The lower curve in C shows the increased cell killing when a PARP inhibitor (3-aminobenzamide) was added to the dimethyl sulfate treatment. The NAD suppression and PARP activity enhancement reverted after several hours, except at the highest dimethyl sulfate concentration.

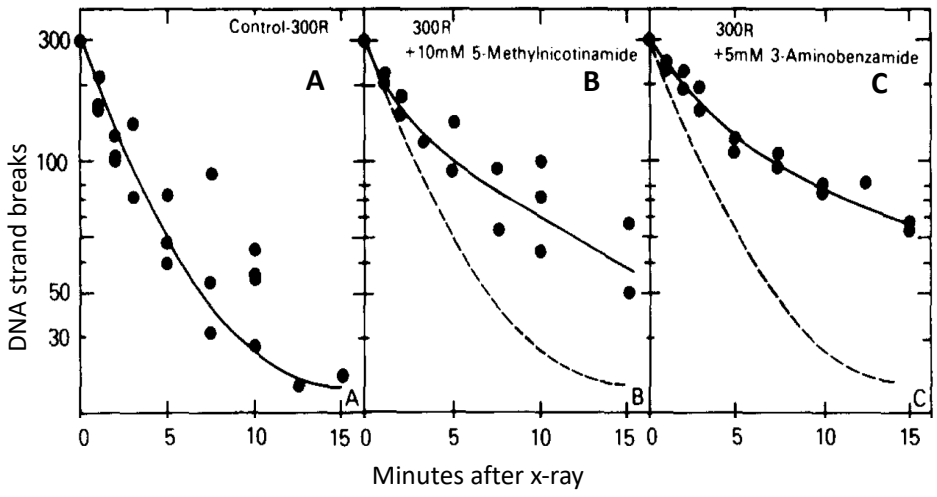


Figure 30.7. PARP inhibitors retarded the ability of cells to repair DNA single-strand breaks (Zwelling et al., 1982). Mouse leukemia L1210 cells growing in suspension culture were exposed to x-rays, which produced DNA strand breaks, mainly single-strand breaks. Panel A shows the rate at which the DNA strand breaks disappeared (were repaired) after exposure of cells to x-rays. Panels B and C showed that PARP inhibitors (5-methylnicotinamide and 3-aminobenzamide) reduced the rate of repair of x-ray-induced

strand break, compared with their rate of repair after x-ray alone (dashed curves). The DNA strand breaks were measured using the alkaline filter elution method we had developed (see Chapter 9). The PARP inhibitors used were among those available at the time, which had low potency and low specificity. They nevertheless indicated that PARP function was required for full effectiveness of the cell's ability to repair DNA strand breaks.

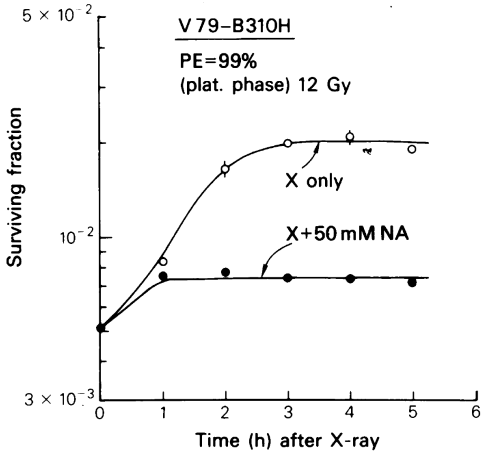


Figure 30.8. An experiment from Mortimer Elkind's laboratory that showed that a PARP inhibitor (nicotinamide, NA) suppressed the ability of cells to recover after x-radiation (Ben-Hur et al., 1984). The vertical axis is the fraction of the cells that were able to grow into colonies on an agar plate.

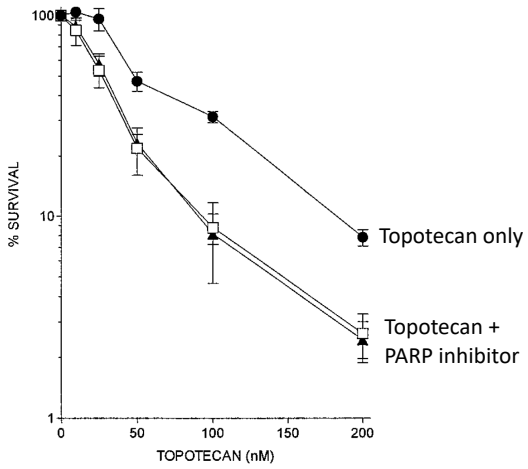


Figure 30.9. Synergy between a PARP inhibitor (NU1025) and a topoisomerase I inhibitor (topotecan) in killing a human colon cancer cell line grown in culture (Delaney et al., 2000). Cells were exposed to topotecan with or without a PARP inhibitor and then tested for survival of their ability to grow into colonies on an agar plate. The graph shows that the PARP inhibitor increased the killing of topotecan-treated cells, as measured by % survival of cells able to form colonies.

How did PARP assist in the repair of DNA damage, and what would happen if PARP's activity were blocked?

By the 1980's there was good reason to think that PARP's synthesis of poly(ADPR) helped to repair DNA strand breaks and to repair DNA damage whose repair path included DNA strand breaks along the way. An important discovery supporting that reasoning was that single-strand breaks in DNA induced PARP to synthesize poly(ADPR). Efforts were then made to find out how that happens and what its effects may be. To begin with, PARP was found to bind to DNA single-strand break sites. PARP binding was found to be the earliest response to at least some types of DNA damage. The binding of PARP to a DNA break site was actually caught in electron microscope images in 1994 (de Murcia and Menissier de Murcia, 1994) (Figure 30.10). The DNA binding activated PARP's enzyme domain, which then grew large branched poly(ADPR) chains (up to 200 ADPR units per branched chain) onto several chromatin proteins, including histones. It made sense that PARP would add these ADPR units to chromatin proteins near the DNA break site, where PARP became bound, and it seemed likely that these modifications of chromatin proteins in the vicinity of the DNA damage site would in some way prepare the damage for repair. In accord with that idea, several DNA-repair proteins were found to bind to the DNA-bound PARP. It was suspected also that the high concentration of negative charge conveyed by poly(ADPR) might be important. It all pointed to PARP being the first player to enter the DNA damage repair scene.

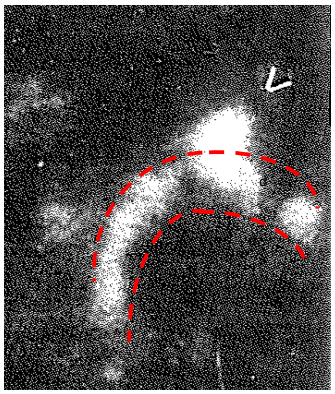


Figure 30.10. Electron microscope image of a segment of DNA that has a single-strand break (white patches between the dashed red lines) and a molecule of PARP bound to the break (white arrowhead). The break caused a bend in the DNA, which was accentuated by the bound PARP. (From (de Murcia and Menissier de Murcia, 1994), modified by addition of the dashed red lines.)

However, the cell had to limit the amount of poly(ADPR) allowed to accumulate, as well as the extent of the concurrent drop in NAD level that the cell could tolerate. In 1992, an enzyme (a glycohydrolase) was found that addressed that problem. The glycohydrolase

broke down the poly(ADPR) chains within minutes of their production (Althaus, 1992; Pieper et al., 1999), and was surmised to be important for balancing the production and removal of poly(ADPR) at the DNA damage sites. Also, the PARP molecule inactivated its own enzyme activity by growing poly(ADPR) onto itself, which was thought to cause PARP to dissociate from the DNA. A sufficiently large amount of DNA damage, together with the large amount of PARP that existed in the cell, could however overwhelm the glycohydrolase's capacity to eliminate the huge amount of poly(ADPR) that could be produced; the cell could die for lack of NAD.

The earliest model of how PARP works may have been that proposed by Tom Lindal and Masahiko Satoh in 1992 (Satoh and Lindahl, 1992) (Figure 30.11A). In 1995, Lindahl presented a somewhat more developed model (Lindahl et al., 1995) (Figure 30.11B). PARP was known to be divided into two separable parts, both of which were required for its ability to produce poly(ADPR) polymers. In addition, DNA was required for this activity (Nakazawa et al., 1968; Nishizuka et al., 1968; Nishizuka et al., 1967; Nishizuka et al., 1969). Lindahl's diagrams included much of what was then known. He depicted the PARP molecule as made up of a DNA-binding domain, connected by way of a middle segment to an enzyme domain that synthesized poly(ADPR) when the DNA-binding domain was bound to DNA. His diagrams showed poly(ADPR) chains becoming attached onto the PARP molecule itself. However, his diagrams did not show poly(ADPR) chains becoming attached to histones, evidence for which had already been reported by Nishizuka and coworkers in 1968 (Nishizuka et al., 1968). Lindahl may have been unaware of that older evidence, or perhaps thought it was not securely enough established to include in his model – or perhaps he felt that the reason for the histone binding was unknown and not relevant to what he wanted to show.

The steps of PARP's actions, as understood by Lindahl in 1992 and 1995, were: (a) PARP's DNA-binding domain binds to the site of damage, while DNA repair proteins wait in the wings; (b) PARP's enzyme domain adds poly(ADPR) chains onto itself, particularly onto the region that connects the two domains of the PARP molecule; (c) the PARP molecule is then repelled away from its binding site on the DNA, and repair proteins come in and bind to the vacated site. PARP adds poly(ADPR) chains to other molecules, such as histones, but only while it is attached to the DNA break.

Two decades later, the picture had filled out, as shown in Figure 30.12. In 2017, Lord and Ashworth (Lord and Ashworth, 2017) showed the elegant way that PARP1 efficiently administers the early steps in DNA repair. Here is their concept of how it works (the Roman numerals refer to the designations in Figure 30.12):
(i) The domain structure of PARP1 is shown from the N-terminus on the left to the C-terminus on the right. The diagram shows the Zn-fingers (ZnF) (the DNA-binding elements) at the N-terminus and the catalytic domain near the C-terminus.
(ii-iii) PARP1 recognizes and binds to a DNA strand break (accomplished by 3 Zn-finger structures at the N-terminus of the PARP1 molecule).
(iv) PARP1's catalytic domain near the C-terminus then binds a molecule of NAD (diamond shape in the Figure). The NAD concentration in the cell must be high enough for an NAD molecule to bind and allow poly(ADPR) polymer to assemble. This normally limits the rate

at which NAD is consumed, thereby tending to avoid reducing the NAD store to dangerously low levels.
(v) The catalytic domain, now activated by NAD, assembles poly(ADPR) chains onto histones in the vicinity of the break, as well as to proteins of the repair machineries and to PARP itself. The poly(ADPR) additions help to loosen DNA-bound proteins and thereby allow access of repair proteins to the DNA damage site.
(vi) The DNA-bound PARP1 adds poly(ADPR) chains onto itself! Neat! Because that causes the PARP1 molecule to release from the DNA and complete the cycle.
(vii) An inhibitor of PARP's enzyme activity would prevent the addition of poly(ADPR) chains to chromatin proteins as well as onto itself, which would prevent the PARP molecule from dissociating from the DNA. The inhibitor would thereby keep the PARP molecule trapped on the DNA, which would block DNA replication or RNA transcription attempting to pass through that point on the DNA, potentially killing the cell. This is where there was an opportunity to make potentially therapeutic inhibitors that would mimic NAD and bind PARP1 the way NAD does. An NAD mimic, if it binds PARP stably, would prevent future reaction steps, thereby locking PARP1 onto the DNA – which could be lethal to the cell, unless fixed by a complicated repair machinery.

It was estimated that there are typically about one million molecules of PARP in a mammalian cell, tightly bound to chromatin in the nucleus. This large number of PARP molecules scattered about in the chromatin was thought an efficient way for them to find DNA damage sites quickly, wherever they may be, as the first step in damage detection and setting in motion the DNA repair process. Binding to a DNA damage site would activate the poly(ADPR) production by PARP. But, if unchecked, this action might consume so much NAD that the cell could die for lack of that essential metabolite. The addition of poly(ADPR) polymer onto itself was a self-inhibitory action that reversed the PARP-DNA binding and stopped further PARP activity. The rapid breakdown of poly(ADPR) by glycohydrolase was another essential part of the PARP control mechanism.

The PARP trapping scenario was investigated further by Junko Murai, Yves Pommier and coworkers in our laboratory. They showed that, when bound to an inhibitor (niraparib or olaparib), PARP becomes trapped exactly at sites of DNA single-strand breaks (Murai et al., 2012). They found that, when PARP has bound to damaged DNA, it prevented the DNA strands at a single-strand break from swiveling around each other. They diagrammed their concept of how PARP becomes trapped at DNA single-strand breaks using the notation we had devised to make unambiguous molecular interaction diagrams (Figure 30.13).

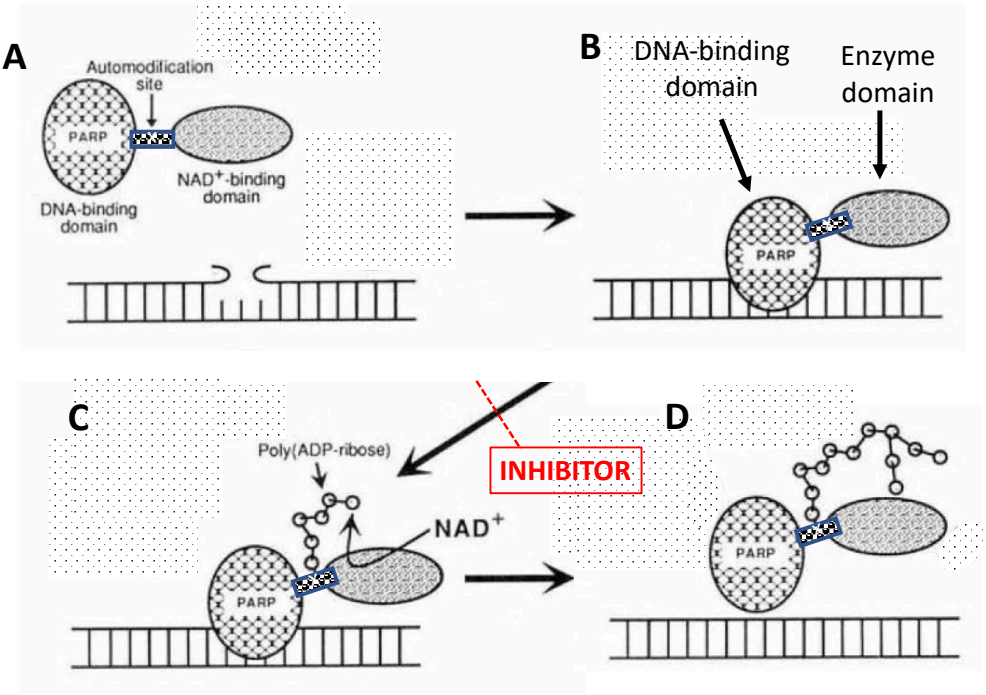


Figure 30.11A. The concept of PARP trapping, according to a PARP function scheme proposed by Satoh and Lindahl in 1992. The PARP molecule was considered to be made up of a DNA-binding domain, shown on the left in **A**, and a separate enzyme domain on the right. The region of the molecule between those two domains was where they thought PARP added poly(ADPR) onto itself. **A** shows the PARP molecule and a segment of DNA that has a strand break. **B** shows the PARP molecule bound to the DNA strand break by way of its DNA-binding domain. In **C**, the enzyme domain would normally grow a poly(ADPR) chain onto the intermediate region of the molecule, which would release the PARP molecule, shown in **D,** and allow repair of the strand break (not shown). A PARP inhibitor would block the enzyme domain's ability to make the polymer chain. Therefore, the PARP molecule would remain trapped at the DNA damage. (From (Satoh and Lindahl, 1992), modified and simplified.)

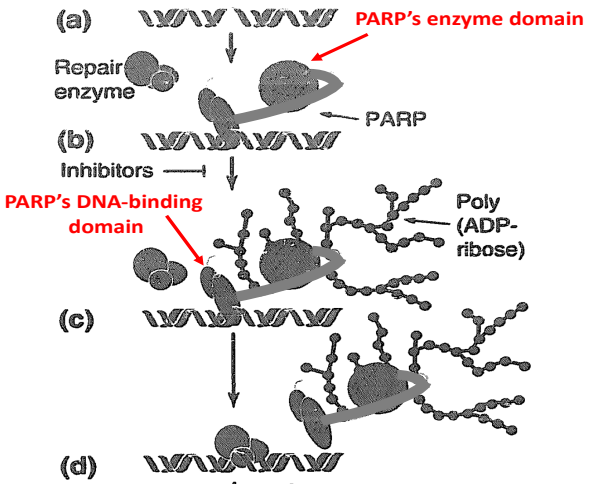


Figure 30.11B. A more developed view by Tom Lindahl in 1995 of the role of PARP and poly(ADPR) in DNA repair (From (Lindahl et al., 1995) with text in red added).

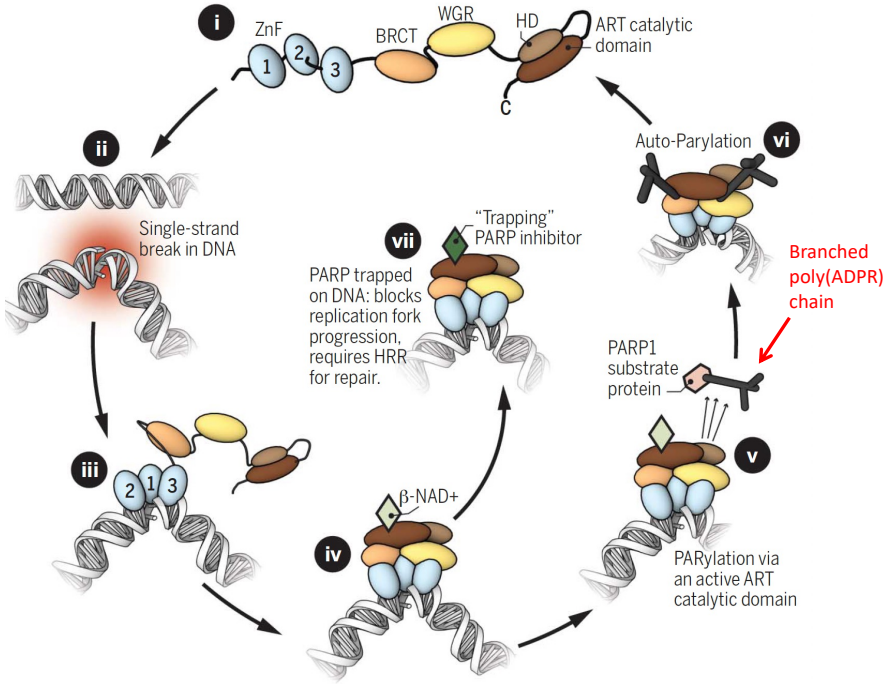


Figure 30.12. The beautiful way that PARP1 efficiently administers DNA repair, as depicted in 2017 by Lord and Ashworth. (From (Lord and Ashworth, 2017) with added label.) See text for explanation.

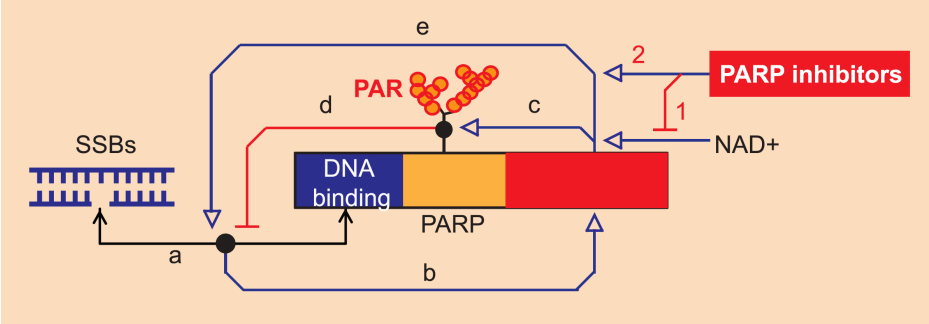


Figure 30.13. How PARP interacts at DNA breakage sites, as conceived by Murai, Pommier and coworkers (Murai et al., 2012). The interactions are diagrammed using the notation for molecular interaction maps (Kohn, 1999). The N-terminal end of the PARP molecule is at the left; the C-terminal end is at the right. The DNA-binding domain is indicated in blue; the enzyme domain is in red; the region where PARP can bind poly(ADPR) chains onto itself is in yellow. The diagram shows the main features of how PARP binds to DNA strand breaks and how PARP inhibitors can trap PARP on the DNA damage site. The main interactions are: (a) the DNA-binding domain of PARP binds to single-strand breaks in DNA; (b) when that binding has occurred, it stimulates the catalytic activity of the enzyme domain; (c) the enzyme domain then adds poly(ADPR) chains to the central region of the molecule; (d) those poly(ADPR) chains then release PARP from the DNA strand break. PARP inhibitors block the production of poly(ADPR) polymers, thereby preventing PARP from adding poly(ADPR) onto itself and preventing PARP from dissociating from the DNA. PARP is able to add poly(ADPR) to other molecules (not shown), but only while attached to the DNA break.

Finding better PARP inhibitors.

Much of the desire for PARP inhibitors was fueled by its role in DNA repair, and by early signs that combining PARP with a DNA-damaging drug enhanced the killing of treated cells (Smulson et al., 1977). As the PARP story developed, the number of research programs aiming to discover PARP inhibitors that could be used for treatment of patients mounted 4-fold during the 1990's (Ferraris, 2010).

Although at least 18 members of the PARP family of proteins were eventually discovered, only PARP1 and PARP2 bound DNA, and PARP1 accounted for the great majority of the poly(ADPR) produced in the cell (Ferraris, 2010). Therefore, inhibitors were sought primarily against PARP1. Then, in 2005, the stature of the search for better PARP inhibitors for cancer treatment increased enormously, because of an amazing discovery that wove PARP together with defects in certain genes. PARP-inhibitor drugs were found to be highly effective in patients with breast or ovarian cancers of a certain type, namely, cancers whose BRCA1 or BRCA2 genes were defective. The PARP-BRCA story is told later in this chapter. But first we focus on discoveries of PARP's role in DNA repair and therapeutics

independent of the BRCA status of the patient. However, it was first necessary to develop better PARP inhibitors.

The easiest route to an inhibitor was to target the enzyme site that binds NAD for use in making the polymer. An NAD-like molecule could bind to the enzyme site on the PARP molecule and prevent poly(ADPR) production. As already explained, Poly(ADPR) production would cease, and PARP would remain trapped on the DNA.

In other words, if the drug only inhibited PARP's enzyme site, its DNA-binding site could still bind to the DNA damage. Then, the PARP molecule would remain bound, because the drug would prevent PARP from growing poly(ADPR) chains onto itself to release the PARP molecule from the DNA (Figures 30.11A, 12, and 13). Furthermore, the trapped PARP would block normal events seeking to access or pass through that location on the DNA. The result would be an anticancer action by the inhibitor-bound PARP that would be trapped bound to its DNA-binding site, and the cell would be left with a difficult repair problem.

Taking advantage of the synergy between PARP inhibitors and DNA damaging drugs.

To review and expand on how the role of PARP in DNA repair was discovered: A major part of the story, as already mentioned, began in 1980 in Sidney Shall's laboratory at the University of Sussex, England, when they noticed that adding a PARP inhibitor increased the lethal effects of a DNA alkylating agent (Durkacz et al., 1980) (Nduka et al., 1980). It seemed that PARP helped cells withstand the lethal effects of an alkylating agent. Research following up on the 1980's findings about combining PARP inhibitors with DNA damaging agents were at first inconclusive, because of the low potency of the PARP inhibitors that existed at the time. At last, nearly 15 years later, organic chemists succeeded in synthesizing more potent inhibitors. Many studies then combined the new PARP inhibitors with temozolomide, an alkylating agent that adds methyl groups to the O6 position of guanine in DNA. Focus on this drug combination was driven by the use of temozolomide in the treatment of brain cancers, because temozolomide was one of the few anticancer drugs able to penetrate the blood-brain barrier (see Chapter 2). It was assumed that both drugs in the combination, both being uncharged, would be able to pass through the blood-brain barrier into the brain (de la Lastra et al., 2007).

Meanwhile, more evidence was obtained that the synergy between PARP inhibitors and DNA damaging agents was due to PARP being required for repair of the DNA damage. PARP was found to bind some of the proteins (XRCC1, DNA polymerase β, and DNA ligase III) that were part of the DNA base excision repair (BER) mechanism (Dantzer et al., 1999).

A knotty problem in chemotherapy was that cancer cells often migrated into the brain and caused brain metastases. To be fully effective when cancer had gone into the brain, the drugs must cross the blood-brain barrier. Many drugs bore a positive or negative charge, which usually blocked their ability to enter the brain. Temozolomide was one of the few

anticancer drugs that could pass into the brain, and it was therefore studied especially for its effect on brain cancers (see Chapter 2). Many PARP inhibitors also were devoid of charge and could pass into the brain. Hence, both drugs in the combination could enter the brain and potentially act on brain cancers or brain metastases. When a PARP inhibitor was combined with temozolomide, the 2-drug combination had a tremendous synergistic effect in prolonging the lives of mice bearing a lymphoma in the brain (Tentori et al., 2005) (Figure 30.14).

Then, in 2008, researchers reported that the most effective among many potential PARP inhibitors that they had up to that time synthetized was olaparib (originally called AZD2281) (Menear et al., 2008). A big advantage of olaparib, as well as PARP inhibitors discovered subsequently was the lack of a positive or negative charge, thereby allowing them to move into the brain and act on cancers there. The PARP-inhibitor plus temozolomide combination also had a striking effect on human colon cancers grown in immune deficient mice (xenografts) (Figure 30.15) (Menear et al., 2008).

PARP inhibitors approved for cancer treatment come onto the scene.

Those findings spurred the search for even better PARP inhibitors that would be effective in cancer treatment. The first PARP inhibitor to be approved for treatment of cancer patients was olaparib. It was the first of several structurally related drugs to become approved. Figure 30.16 shows the PARP inhibitors that were in clinical use by 2017, listed according to their potencies for trapping PARP at DNA stand breaks. The most potent was talazoparib, the least potent was veliparib, and olaparib had intermediate potency. Figure 30.17 shows the inhibitors according to their structural relatedness. They all had a structural feature (shown in red) resembling the nicotinamide part of NAD, which indicated that they all acted by binding and inhibiting the NAD site (the catalytic site) of PARP. But they differed in their strength of binding to DNA break sites as shown in Figure 30.16.

As of 2019, we still did not know which of the PARP inhibitors was best, because there had not yet been any clinical trials that compared them head-to-head (Mateo et al., 2019). This question however met a complication, when in 2014 Junko Murai and Yves Pommier in our laboratory and their colleagues noted that PARP inhibitors had two separable actions (Murai et al., 2014a). They compared olaparib, rucaparib, and talazoparib (BMN 673) for inhibition of enzyme activity, ability to trap PARP at DNA strand breaks, and killing of cancer cells. They found, remarkably, that, although all three drugs inhibited PARP enzyme activity with comparable potency, talazoparib was about 100 times as potent as the other two drugs when it came to potency of trapping on DNA. Also, talazoparib in combination with temozolomide was more potent in killing cancer cells compared to other PARP inhibitor-temozolomide combinations. It seemed that a major cell toxic effect of the inhibitors came from trapping PARP to DNA, not merely from inhibiting PARP enzyme activity.

A notable fact about talazoparib was that its stereoisomer, where the configurations of its sites labeled R and S in Figures 30.16 and 30.17 were reversed, was nearly inactive (Murai et al., 2014a). That meant that the 3-dimentional geometry of talazoparib was critical in allowing the drug to bind to its site on the PARP molecule; the mirror image of the inhibitor would not fit at the binding site.

Figure 30.18 shows an experiment that demonstrated the strong synergy between talazoparib and temozolomide (Shen et al., 2013).

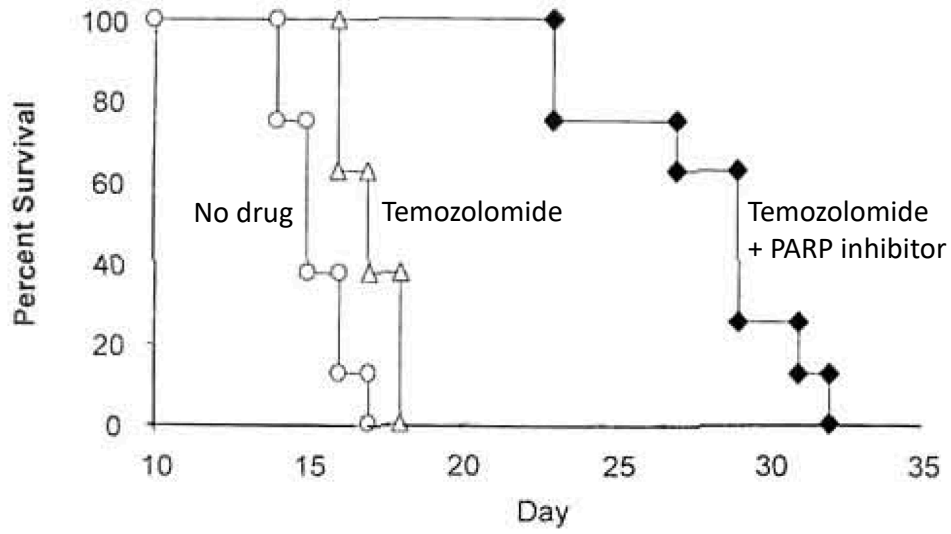


Figure 30.14. A PARP inhibitor (GPI 15427) together with temozolomide greatly increased the survival of mice that had a lymphoma in the brain. The PARP inhibitor by itself or temozolomide by itself had much less effect (Tentori et al., 2005). Both drugs were able to pass through the blood-brain barrier.

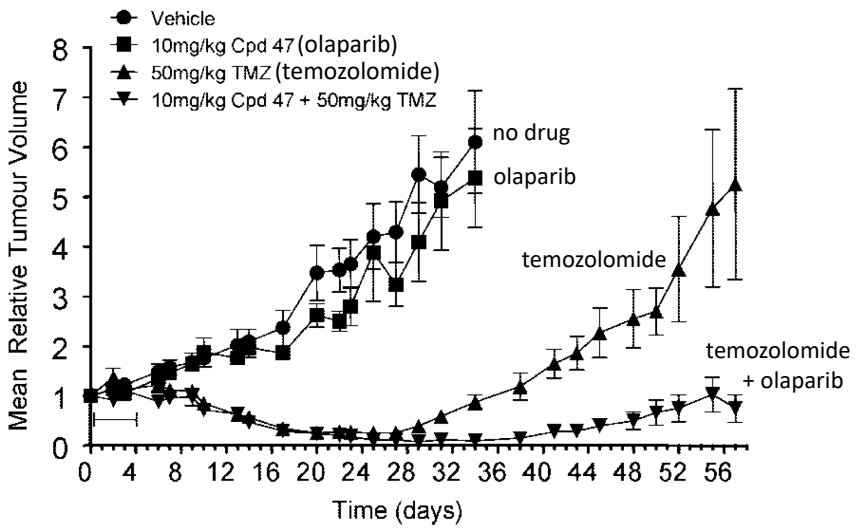


Figure 30.15. The PARP inhibitor, olaparib, together with temozolomide had an impressive synergistic effect in suppressing the growth of human colon cancer cells (SW620) in mice (Menear et al., 2008). The size of the tumor (vertical axis) is plotted against time (horizontal axis).

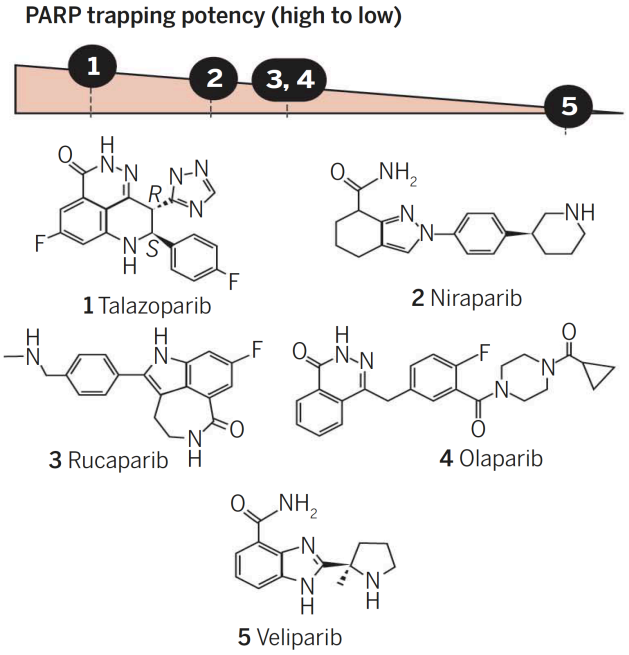


Figure 30.16. PARP inhibitors that were used in cancer treatment as of 2017, showing their relative potencies from 1 (most potent) to 5 (least potent) for trapping PARP on DNA, which correlated with potency for killing cells in culture (Lord and Ashworth, 2017).

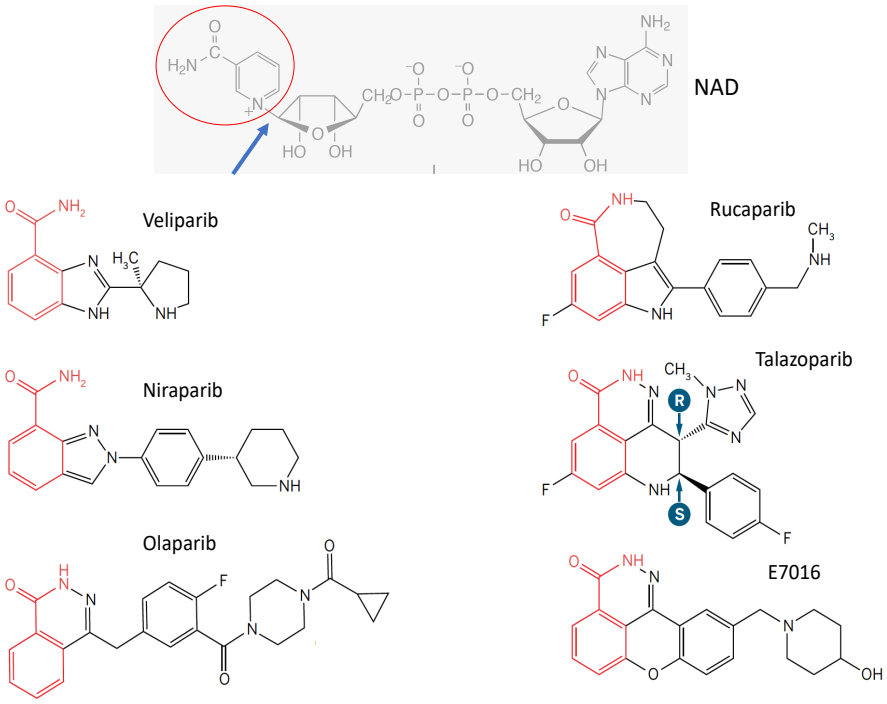


Figure 30.17. PARP inhibitor drugs showing their chemical structure relationships. A feature they had in common with NAD is shown in red (Pommier et al., 2016). This feature is like the nicotinamide part of the NAD molecule. It is the part of the molecule that binds to the active site of the PARP enzyme, which breaks its bond to the rest of the molecule. The cleavage of that bond drives the poly(ADPR) polymerization reaction (blue arrow in NAD structure). The nicotinamide-like part of the inhibitor structure is attached by multiple bonds to the rest of the structure and cannot be released. Therefore, when inhibitor binds to the enzyme at the nicotinamide-binding site, the inhibitor remains stuck there, because the reaction to release the nicotinamide-like part cannot occur.

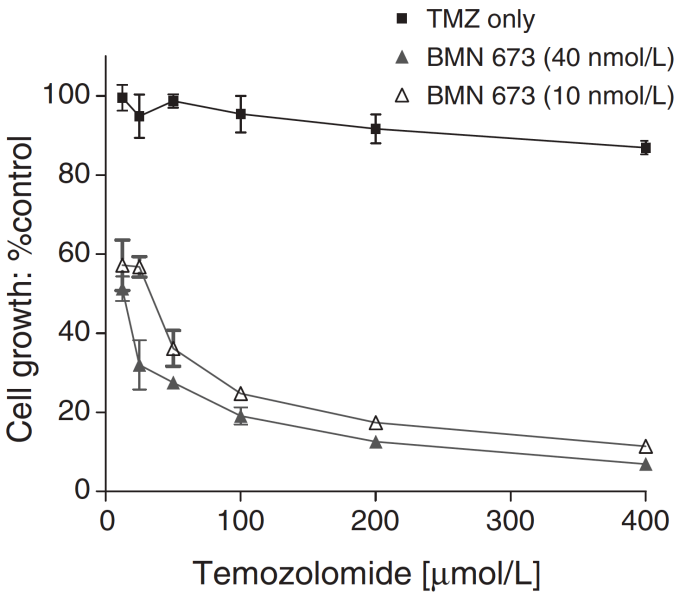


Figure 30.18. Synergy between the DNA alkylating agent, temozolomide, and the potent PARP inhibitor, talazoparib (originally called BMN 673) (Shen et al., 2013), in inhibiting the growth of a human colon cancer cell line, called LoVo. Temozolomide by itself had little effect (upper curve). Talazoparib by itself inhibited cell growth by about 40% (lower curves at zero temozolomide); when temozolomide was added at increasing concentrations, cell growth was inhibited up to 90%. That was a remarkable degree of synergy between the two drugs.

PARP has a major role in DNA repair.

The poly(ADPR) chains that PARP fixes onto histones in chromatin serve to recruit DNA repair enzymes to a variety of sites of DNA damage (Murai et al., 2012; van Wietmarschen and Nussenzweig, 2018). Evidence explained earlier in this chapter pointed to single-strand breaks and base-free sites as the DNA lesions where PARP comes into play during DNA repair.

It therefore became important to know which drugs produce such DNA lesions. For temozolomide, the DNA damage and repair scenario was described in Chapter 2. The most important damage it caused was methylation of guanine-O6 sites on DNA, in other words, the addition of a methyl groups to the O6 position of guanines, yielding a potentially toxic DNA product. A wonderful enzyme was discovered that efficiently plucked off the methyl group off and restored the normal DNA structure. The enzyme that did that was O6-methylguanine-methyl-transferase (MGMT) (see Chapter 2). Although that enzyme was highly efficient, it was not foolproof (no biological process is). Moreover, some cancer cells made little or no MGMT. As discussed in Chapter 2, that made those kinds of cancers vulnerable to treatment with drugs like temozolomide, because the cells did not have

enough MGMT to remove the methyl groups from all the methylated guanines. The remaining methylguanines then had to be repaired by a different mechanism: DNA base excision repair (BER), whose discovery was described in Chapter 24. BER plucks the bad base off the DNA, leaving a base-free site in the DNA chain: a deoxyribose unit without a base (adenine, guanine, thymine or cytosine) attached to it.

In the case of temozolomide, the BER mechanism removed the persistent O6-methylguanines *in toto* and left behind a base-free deoxyribose in the DNA. To PARP, the base-free site may look sufficiently like a single-strand break for it to bind there and bring in some of the needed DNA repair proteins. Those repair proteins, it seemed, removed the base-free deoxyribose and filled the resulting gap in the DNA strand, but left behind a single-strand break. It seemed that the PARP molecule would remain attached and continue to help in the repair.

Although the essentials may be correct, the mechanism may be more complicated than the relatively simple picture had suggested. A collaboration of several laboratories in 2017 enabled the use of new physics techniques to investigate in amazing detail some of the events during the repair of base-free sites at the level of individual molecules (Liu et al., 2017).

Their essential findings were:
(1) PARP1 and the endonuclease (APE1) that cleaves away the base-free deoxyribose both move around freely in search of base-free sites on a DNA molecule.
(2) A PARP1 molecule can bind to a single-strand break, base-free site, or DNA end.
(3) When PARP1 and APE1 are bound to the same base-free site, the PARP1 molecule is enabled to slide along the DNA while remaining close to the damage site; perhaps that opened the damage site for access to other repair proteins.
(4) Addition of poly(ADPR) chains onto itself allowed the PARP1 molecule to slide for greater distances along the DNA in the vicinity of the damage site.
(5) The PARP inhibitor, olaparib, did not affect the dissociation of PARP1 from the DNA, but increased the ability of PARP1 to slide along the DNA for greater distances away from the damage site.

A caveat of this investigation, however, was that the DNA they were able to study was bare, without any histones or other chromatin proteins. Nevertheless, they demonstrated a new technology for detailed investigation of how DNA repair works.

In general, drugs that caused DNA damage requiring base-excisions repair (BER) for good cell survival should be sensitive to PARP inhibitors, because BER entails the production of DNA single-strand breaks during the repair process. Aside from drugs like temozolomide that damage DNA bases directly, base analog drugs, such as thioguanine and 5-fluorouracil, become incorporated into DNA by the DNA replication machinery that is unable to distinguish the base analog nucleotide triphosphate from the normal nucleotide triphosphate. The DNA polymerase thus adds the bad base into the DNA chain. The bad base could then be removed by BER, leaving a base-free site and leading to a single-strand break where PARP would bind.

How do PARP inhibitors exert toxic effects on cells?

In 2014, as already mentioned, my colleagues Junko Murai and Yves Pommier, together with other researchers in our laboratory, gave us more insight into the workings of the PARP drug combinations. They measured the toxic effects on cells when a PARP inhibitor was combined with a DNA damaging drug (Murai et al., 2014b). Insight into the effects of PARP came from measuring what happens in cells whose PARP1 gene was deleted. Cells that had no functional PARP1 had increased sensitivity to temozolomide, as expected, since PARP was necessary for efficient DNA damage repair. Also as expected, PARP inhibitors had no effect on cells that had no functional PARP1 gene. But quite remarkably, cells having normal PARP genes were extremely sensitive to the combination of temozolomide and a PARP inhibitor. The inference was that, even though PARP itself helped to repair the DNA damage, the PARP-with-bound-inhibitor unit became trapped at temozolomide-induced DNA damage sites, resulting in extreme toxicity. It was all in accord with the idea that PARP inhibitors cause PARP to become trapped at sites of DNA damage, thereby producing severe toxicity to cells, possibly more severe against cells in a cancer than against critical normal cells in the body.

Hence, it seemed that PARP inhibitors had two different kinds of toxic effects on cells: (A) inhibition of poly(ADPR) production and (B) trapping of PARP at sites of DNA damage, particularly at strand breaks. The different PARP-inhibitor drugs had similar potencies for mechanism A, but different potencies for mechanism B. Moreover, the major toxic mechanism appeared to be mechanism B (Murai et al., 2014b).

In the same paper, Murai et al. asked what kinds of DNA damage would produce the extreme toxicity of PARP trapping. In other words, in what kinds of DNA damage did or did not PARP come in to help repair and at the same time become trapped on the DNA? They were able to infer answers from experiments such as those summarized in the previous paragraph.

Further studies of PARP looked to see when and where poly(ADPR) was produced (van Wietmarschen and Nussenzweig, 2018). Since poly(ADPR) is rapidly degraded by glycohydrolase, the researchers inhibited the enzyme, which allowed the polymer to accumulate at its sites of production where it could then be measured. They found that poly(ADPR) was produced only during DNA replication and at or near the replication sites. When they inhibited PARP, the gaps in the replication lagging strand (producers of Okazaki fragments) were not ligated. The gaps persisted into the next replication cycle, and then caused the replication fork to collapse. Repair of that problem required homologous recombination for which BRCA1 and BRCA2 were both needed (Gudmundsdottir and Ashworth, 2006). This gave some insight into the BRCA-PARP synthetic lethality strategy for cancer therapy described in a section at the end of this Chapter.

Topoisomerase 1 inhibitors produce another DNA damage scenario requiring PARP for repair.

A drug that produced DNA damage of a kind different from that of DNA base modifiers, such as temozolomide, was the topoisomerase inhibitor camptothecin (Chapter 11). In 2011, my colleagues led by Yves Pommier had, in the vein of our long interest in topoisomerase-targeted drugs, found that PARP inhibitors become trapped at the DNA sites of camptothecin action (Zhang et al., 2011). As explained in Chapter 11, camptothecin was known to produce transient single-breaks that allow swiveling of the DNA strands to relieve the torsional stress that accumulates as DNA is transcribed or replicated. Those single-strand breaks however open and close quickly in a controlled manner -- and do not qualify as DNA damage. Camptothecin however did induce DNA damage, because it bound and stabilized the open state during the topoisomerase I swivel cycle long enough to cause problems. Camptothecin nevertheless dissociated easily from this bound state. As described in Chapter 11, camptothecin induced DNA damage mainly while DNA was being replicated during S-phase of the cell cycle, which suggested that the damage happened when the DNA replication machinery collided with a camptothecin-blocked topoisomerase-I site. That idea held up and was extended by evidence that damage was induced, albeit to a lesser degree, by collisions involving RNA transcription. The essentials of an experiment showing these DNA damaging events are shown in Figure 30.19 (Zhang et al., 2011).

In general, DNA damaging drugs that required repair by BER, as well as drugs like camptothecin that impaired topoisomerase I, were synergized by PARP inhibitors. On the other hand, drugs like cisplatin that crosslink DNA or drugs that impair topoisomerase II, were repaired by mechanisms that did not require PARP, and these drugs were not synergized by PARP inhibitors (Zhang et al., 2011). A dual role of PARP1 and TDP1 in removing trapped Top1-DNA complexes on DNA is diagrammed in Figure 30.20.

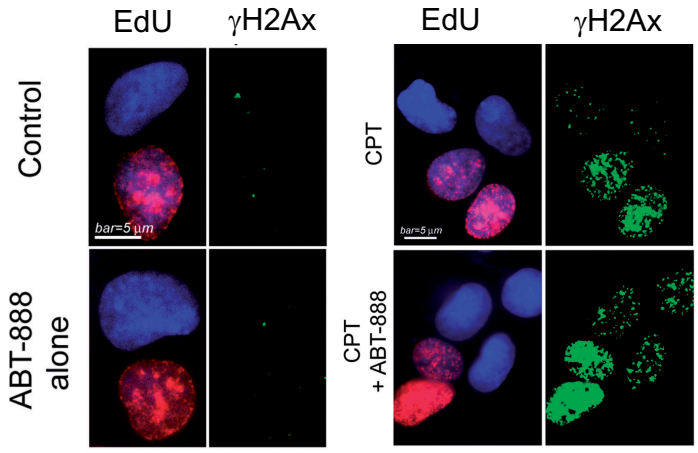


Figure 30.19. The PARP inhibitor, veliparib (ABT-888), combined with the topoisomerase 1 inhibitor, camptothecin, produced DNA damage, but mainly in cells that were undergoing

DNA synthesis (S-phase) (Zhang et al., 2011). In this experiment, human cancer cells (osteosarcoma U2OS) were treated with camptothecin (CPT) with or without veliparib (ABT-888). Cells undergoing DNA synthesis were labeled by adding a thymidine analog (EdU), which became incorporated into the newly synthesized DNA. The cells were then put on a slide and stained with two fluorescent antibodies that make cells that are undergoing DNA synthesis glow pink and cells that have DNA damage (where γH2Ax is bound) glow green. The left panel shows the results for cells that were treated with veliparib alone. Treatment with camptothecin alone showed some DNA damage (green in upper right of the right-hand panel). But there was much more green when camptothecin was combined with veliparib (lower right in the right-hand panel). Thus, camptothecin produced DNA damage mainly in S-phase cells (green), but the combination of camptothecin and veliparib produced a great deal more DNA damage (more green), mainly in S-phase cells, but to a smaller degree also in non-S-phase cells. (Modified from (Zhang et al., 2011).)

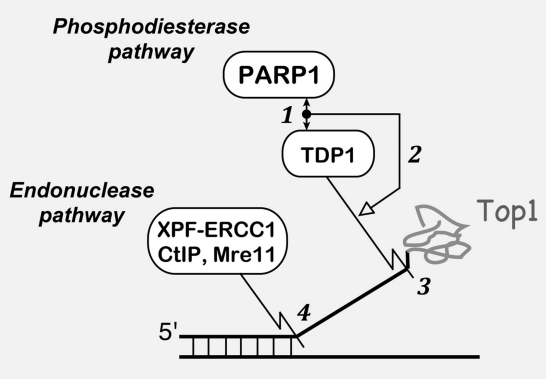


Figure 30.20. PARP1 works together with TDP1 and DNA repair enzymes to cleave away Top1 trapped as cleavage complexes on DNA) (Das et al., 2014). (See Figure 10.9 in Chapter 10. TDP1 acts similarly to remove trapped Top1 and Top2.) The diagram uses the molecular interaction map notation (Kohn, 1999). ***1.*** PARP1 binds TDP1. ***2, 3.*** The PARP1-TDP1 dimer stimulates TDP1 to cleave any remaining Top1 fragment away from the DNA ***4.*** DNA enzymes complete the repair. PARP1 may also serve to recruit the DNA repair enzymes to the site where they are needed (not shown in the diagram).

PARP inhibitors begin to be used to treat cancer patients.

The approved PARP inhibitor drugs, however, did not come easily. Far from it! A huge number of compounds were prepared and studied by several pharmaceutical companies to unravel the chemistry of exactly how the inhibitors worked and to prepare some of them for clinical trial.

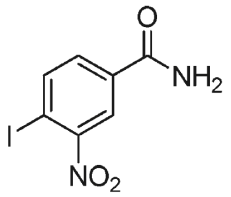
Going back to the early days of development of clinically approved PARP inhibitors, Figure 30.21 lists the PARP inhibitors that had been approved as of 2010 for testing in cancer patients, and the steps in development that each drug had passed (Ferraris, 2010). The first step was to obtain approval of the drug as an Investigational New Drug (IND) by the U.S. Food and Drug Administration (FDA). Approval of an IND depended on animal studies indicating that the drug's toxicities and therapeutic actions are well enough understood to allow preliminary testing in a small numbers of advanced cancer patients for whom there was no longer any approved therapy available. Next came Phase I, which aimed to determine the toxicity and safe dosage limits in a limited number of cancer patients, most of whom had relapsed after chemotherapy and for whom there was no approved therapy available. About 70% of the drugs went on from Phase I to Phase II, where several hundred patients with advanced cancers were treated with the aim of finding evidence that the drug was active against cancer, while continuing to monitor for untoward actions. If Phase II studies provided sufficient evidence of action against some types of cancer, the drug went on to Phase III, which aimed to determine whether the drug was better than the best previous treatment for some types of cancer. Several thousand patients were typically recruited for Phase III studies that were usually carried out in double-blind fashion.

Two drugs, at the top of the list in Figure 30.21, reached Phase III. The first was BSI-201, a small molecule whose structure is shown below the table. This structure was simpler than the structures of the drugs (Figures 30.17 and 30.18) that were later approved as drugs in the clinical armamentarium. BSI-201 did not pass beyond Phase III however and was dropped. The next Phase III drug in Figure 30.21, became "olaparib" and was the first PARP inhibitor to become an official clinical drug. The next in the list became "veliparib," and was in Phase II at the time that the list was compiled in 2010.

Table 1. Current Clinical Status of PARP-1 Inhibitors[a]

company	compd	IND	phase I	phase II	phase III	therapeutic indications
BiPar/Sanofi	BSI-201	*	*	*	*	triple negative breast cancer
KuDOS/AstraZeneca	KU 59436 (AZD8821, Olaparib)	*	*	*	*	metastatic breast cancer, advanced ovarian cancer
Abbott	ABT-888 (Veliparib)	*	*	*		metastatic breast cancer, metastatic melanoma, brain cancer
Pfizer	AG 14699 (PF-01367338)	*	*	*		metastatic breast cancer, advanced ovarian cancer
Inotek	INO-1001	*	*	*		malignant melanoma, heart/lung bypass surgery
Cephalon	CEP-9722	*	*			advanced solid tumors
Merck	MK-4827	*	*			advanced solid tumors
Mitsubishi	MP-124	*				cerebral ischemia
Guilford/MGI/Eisai	GPI 21016 (E7016)	*				glioblastoma

[a] The asterisk (*) indicates participation in the indicated stage.



64 (BSI-201)

Figure 30.21. PARP inhibitor drugs approved for clinical test as an Investigational New Drug (IND) as of 2010 (see text) (Ferraris, 2010).

The combination of a PARP inhibitor and the alkylating agent, temozolomide, began to be used to treat brain cancer patients, because both drugs were able to pass through the blood-brain barrier. Temozolomide, however, was expected to be most effective in cancers that had low levels of the DNA repair enzyme that removes methyl groups from the O6 position of guanine in DNA (DNA-O6-methylguanine-methyltransferase, MGMT) (see Chapter 2). Clinical trials of PARP-inhibitor plus temozolomide combination were under way at the time of this writing (Gupta et al., 2018). A response was already seen in a phase I/II trial (Figure 31.22).

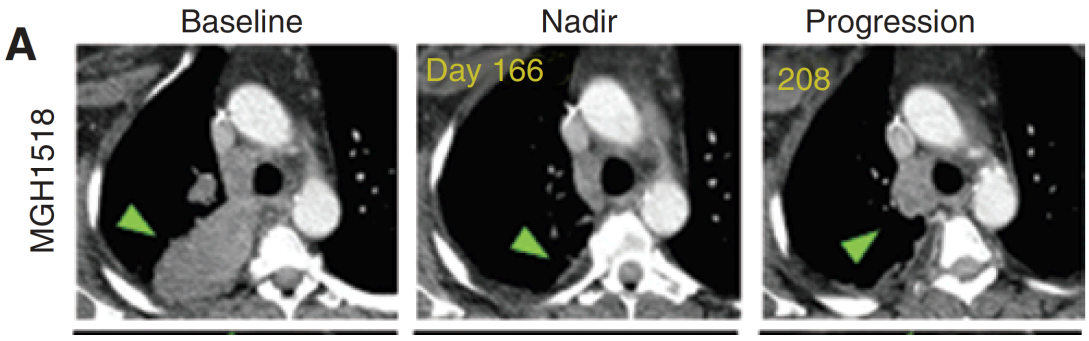


Figure 30.22. Response of a small-cell lung cancer (SCLC) tumor in the lung of a patient treated with olaparib plus temozolomide. The patient had relapsed after previous chemotherapy and was treated with the olaparib-temozolomide drug combination in a phase I/II trial. The green arrowheads point to the tumor. The middle panel showed that the tumor had shrunk almost to the point of invisibility. The tumor unfortunately grew again, as shown in the right panel (Farago et al., 2019).

A new paradigm for cancer treatment: synthetic lethality.

An unexpected finding by Alan Ashworth and his colleagues at Guy's Hospital, London, and Cambridge Science Park in 2005 led to a new strategy for cancer therapy, based on a concept called "synthetic lethality" (Farmer et al., 2005). Here is how their unexpected discovery came about: since BRCA enzymes were required for repair of DNA double-strand breaks, and PARP was required for repair of DNA single-strand breaks, they thought that a BRCA mutation together with a PARP inhibitor might make cells particularly sensitive to DNA-damaging agents. Unexpectedly, however, they found that cells whose BRCA function was inactive due to mutation were killed by PARP inhibitors, even without introducing a DNA-damaging drug! It was not clear exactly how PARP produced that effect, but it was nevertheless a clear case, they soon realized, of synthetic lethality that might have therapeutic potential (Figure 30.23.).

In the same issue of *Nature*, Thomas Helleday and his colleagues in the UK and Stockholm confirmed and gave more substance to the BRCA-PARP synthetic-lethality concept (Bryant et al., 2005). Both research teams found that PARP inhibition by itself generated DNA damage that required BRCA for repair and that, if the functions of both BRCA and PARP were lacking, cells died. Lack of BRCA1 or 2 function was not by itself lethal to cancer cells, nor did inhibition of PARP by itself kill the cells. However, the combination of a non-functioning BRCA and an inhibitor of PARP killed them. That was the essence of synthetic lethality (Figure 30.23).

Synthetic lethality, as first defined in 1946 by Dobazhansky (Dobzhansky, 1946) was a situation where the combination of 2 genetic changes was lethal, whereas either genetic change by itself was not. Figure 30.23 shows how synthetic lethality produced by combining a BRCA defect with a PARP inhibitor was a strategy for cancer therapy.

Body cells usually have two copies of each *BRCA* gene, one on each of two homologous chromosomes. In some families, one of the two copies (most commonly of the *BRCA1* of *BRCA2* gene) has an inherited mutation that destroys its function. As long as the second copy is ok, the cell has normal *BRCA* functions. However, a random mutation can inactivate the second copy and frequently occurs as an initiating event in cancer particularly of breast cancer. Family members who had inherited an inactivating mutation in one copy of a BRCA genes tended to have an unusually high incidence of breast cancer (see Chapter 26). They were vulnerable to developing cancer when a random mutation inactivated the second copy of the same *BRCA* gene. With BRCA1 or BRCA2 totally inactive, the cancer cells could not repair DNA by homologous recombination. For repair of DNA damage, such as double-strand breaks, the homologous recombination repair option would not be available to them. Such cancer cells could then be killed by PARP inhibitors that blocked the other major DNA repair pathways.

People whose *BRCA* genes were all normal had a much lower risk of breast cancer. However, a first inactivation of a *BRCA* gene could occur as a rare random mutation, for example in a normal breast cell from which a cancer could eventually develop. That was thought to be a route by which cancer could arise even when there was no family history of cancer.

In both the familial and the random circumstance, however – and this is the critical point – BRCA function would be defective in the cancer, but not in the normal cells. That difference was the basis for therapy: the cancer cells were vulnerable to PARP inhibitor, while the normal cells was not. It was a case of synthetic lethality with selectivity against cancer cells (Kaelin, 2005).

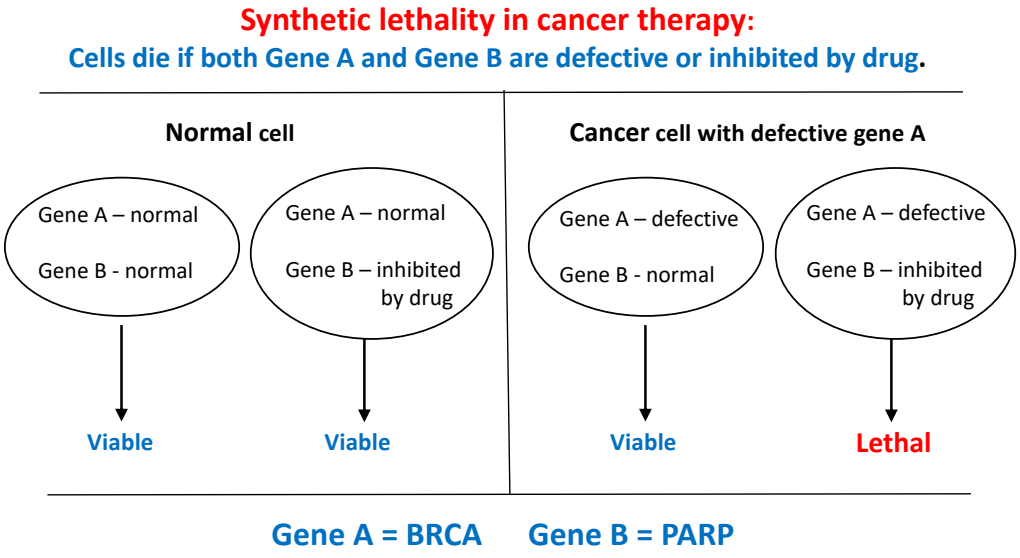


Figure 30.23. BRCA-defective cancer cells are killed by PARP inhibitor. This was the first therapy based on synthetic lethality, where cells die if an only if their Gene A and Gene B are both defective or drug-inhibited.

The BRCA-PARP synthetic-lethality strategy showed its promise against cancers in 2009 in a phase I study of ovarian cancer patients who had an inherited *BRCA1* or *BRCA2* mutation in one allele. Such patients were prone to develop cancer from cells that spontaneously acquired an inactivating mutation in the second allele. These cells lacked BRCA1 or BRCA2 function. Since both *BRCA* genes were needed for homologous recombination, the cells were unable to carry out this DNA repair function. Consequently, more mutations accumulated, leading to a high probability of cancer. Those cancers had a non-functional BRCA, making them vulnerable to PARP inhibitors, based on synthetic lethality when both of those genes were non-functional. Figure 30.24. shows the response to olaparib (an inhibitor of PARP) of an ovarian cancer in a patient who had a *BRCA1* gene mutation (Fong et al., 2009). Olaparib was approved in 2014 by the US Food and Drug Administration (FDA) for treatment of ovarian cancer in patients with a BRCA1 mutation. Another PARP inhibitor, talazoparib, was approved in 2018 for treatment of breast cancer patients who had a BRCA mutation (Turk and Wisinski, 2018; Zimmer et al., 2018).

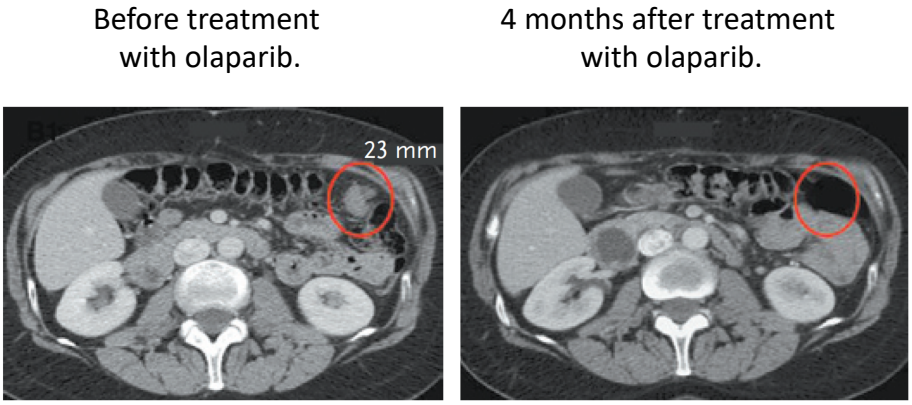


Figure 30.24. Regression of an ovarian cancer in response to treatment with the PARP inhibitor, olaparib; the patient had an inherited mutation of the *BRCA1* gene. The tumor disappeared completely in these computerized tomography (CT) scans of the abdomen (red circles) (Fong et al., 2009). Unfortunately, however, only about half of the *BRCA* mutant ovarian cancer patients responded to olaparib, the response was rarely complete, and the duration of response was 8 months at best (Fong et al., 2010).

To begin to understand how the remarkable cooperation between the *BRCA* and *PARP* genes led to new cancer drug therapy, we must go back to the story of PARP (see above). PARP is abundant in the cell nucleus, where it catalyzes the production of poly(ADP-ribose) polymers and attaches them to certain essential cell proteins, particularly histones, in chromatin. It is an essential part of the molecular machinery that repairs DNA single-strand breaks and other types of DNA damage. However, there is an effective backup mechanism to deal with DNA breaks that may remain unrepaired: homologous recombination repair of DNA double-strand breaks, which can form from unrepaired single-strand breaks, and requires both BRCA1 and BRCA2 (see Chapter 27A) (Gudmundsdottir and Ashworth,

2006). Therefore, if both PARP and either BRCA1 or BRCA2 are defective, then the cells die. That, in short, was the basis for the excitement engendered by prospects for therapy specific for cancers that have inactivating mutations of BRCA1 or BRCA2. Treatment of such cases with PARP-inhibiting drugs would cause the cancer cells to die.

More recently, Niek van Wietmarschen and Andre Nussenzweig at the National Cancer Institute reviewed evidence, some of it from our Laboratory (Murai et al., 2012), that suggested that the normal production of Okazaki fragments in the replication of the lagging DNA strand could lend itself to PARP-BRCA synthetic lethality (van Wietmarschen and Nussenzweig, 2018). The sealing of the fragments would be impaired by inhibition of PARP and would then rely on BRCA-dependent homologous recombination to deal with the difficulty. Consequently, this could be a of synthetic lethality mechanism.

The success of the PARP-BRCA therapy recently stimulated much thought and investigation of other potential therapy based on a synthetic lethality strategy (Ashworth and Lord, 2018; Setton et al., 2021).

Summary

The story developed in this chapter grew out of three roots: First, the discovery of a strange new polymer, poly(ADPR). Second, the unexpected observation that alkylating agents caused a fall in nicotinamide adenosine dinucleotide (NAD) level in the cell. Third, the discovery that inhibiting the enzyme activity of PARP (the enzyme that produces poly(ADPR)) impaired the ability of cells to repair DNA strand breaks and to survive DNA damage produced by alkylating agents and x-rays. The inference that PARP had a role in DNA repair then spurred investigation of how PARP worked and how to develop better PARP inhibitors that could become useful anti-cancer drugs. Later findings indicated that it was PARP's DNA binding, rather than inhibition of its enzyme activity *per se*, that was the main factor in killing cancer cells, at least at moderate dosage suited for treatment of patients. However, inhibitors of PARP's enzyme activity were effective, because the enzyme activity was required for addition of poly(ADPR) chains to the PARP molecule, which allowed PARP to dissociate from the DNA. The inhibition of PARP's enzyme activity thus tended to keep the PARP molecule attached to the DNA, thereby blocking DNA functions from passing through that point. Along with the development of clinically approved PARP inhibitors, focus was on combination of PARP inhibitors with DNA-damaging drugs, particularly the alkylating agent temozolomide. The drug combinations were highly effective against cancer cells in culture and against human tumors grown as xenografts in immune-deficient mice. Clinical trials of the drug combinations were then begun. Then, came the unexpected observation that PARP inhibitors were remarkably effective in cancers that had defects in BRCA gene function in DNA repair by homologous recombination. Cancer cells died if and only if both their BRCA and PARP functions were inactivated. This was the first case of synthetic lethality in mammalian cells and gave promise as a new strategy for cancer therapy.

References

Althaus, F.R. (1992). Poly ADP-ribosylation: a histone shuttle mechanism in DNA excision repair. Journal of cell science *102 (Pt 4)*, 663-670.

Ashworth, A., and Lord, C.J. (2018). Synthetic lethal therapies for cancer: what's next after PARP inhibitors? Nat Rev Clin Oncol.

Ben-Hur, E., Utsumi, H., and Elkind, M.M. (1984). Inhibitors of poly (ADP-ribose) synthesis enhance radiation response by differentially affecting repair of potentially lethal versus sublethal damage. Br J Cancer Suppl *6*, 39-42.

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., and Helleday, T. (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature *434*, 913-917.

Chambon, P., Weill, J.D., Doly, J., Strosser, M.T., and Mandel, P. (1966). On the formation of novel adenylic compounds by enzymatic extracts of liver nuclei. Biochemical and biophysical research communications *25*, 638-643.

Dantzer, F., Schreiber, V., Niedergang, C., Trucco, C., Flatter, E., De La Rubia, G., Oliver, J., Rolli, V., Menissier-de Murcia, J., and de Murcia, G. (1999). Involvement of poly(ADP-ribose) polymerase in base excision repair. Biochimie *81*, 69-75.

Das, B.B., Huang, S.Y., Murai, J., Rehman, I., Ame, J.C., Sengupta, S., Das, S.K., Majumdar, P., Zhang, H., Biard, D.*, et al.* (2014). PARP1-TDP1 coupling for the repair of topoisomerase I-induced DNA damage. Nucleic acids research *42*, 4435-4449.

Davies, M.I., Shall, S., and Skidmore, C.J. (1977). Poly(adenosine diphosphate ribose) polymerase and deoxyribonucleic acid damage [proceedings]. Biochem Soc Trans *5*, 949-950.

de la Lastra, C.A., Villegas, I., and Sanchez-Fidalgo, S. (2007). Poly(ADP-ribose) polymerase inhibitors: new pharmacological functions and potential clinical implications. Curr Pharm Des *13*, 933-962.

de Murcia, G., and Menissier de Murcia, J. (1994). Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends Biochem Sci *19*, 172-176.

de Murcia, G., Menissier-de Murcia, J., and Schreiber, V. (1991). Poly(ADP-ribose) polymerase: molecular biological aspects. BioEssays : news and reviews in molecular, cellular and developmental biology *13*, 455-462.

Delaney, C.A., Wang, L.Z., Kyle, S., White, A.W., Calvert, A.H., Curtin, N.J., Durkacz, B.W., Hostomsky, Z., and Newell, D.R. (2000). Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines. Clinical cancer research : an official journal of the American Association for Cancer Research *6*, 2860-2867.

Dobzhansky, T. (1946). Genetics of natural populations; recombination and variability in populations of Drosophila pseudoobscura. Genetics *31*, 269-290.

Durkacz, B.W., Irwin, J., and Shall, S. (1981). Inhibition of (ADP-ribose)n biosynthesis retards DNA repair but does not inhibit DNA repair synthesis. Biochemical and biophysical research communications *101*, 1433-1441.

Durkacz, B.W., Omidiji, O., Gray, D.A., and Shall, S. (1980). (ADP-ribose)n participates in DNA excision repair. Nature *283*, 593-596.

Farago, A.F., Yeap, B.Y., Stanzione, M., Hung, Y.P., Heist, R.S., Marcoux, J.P., Zhong, J., Rangachari, D., Barbie, D.A., Phat, S.*, et al.* (2019). Combination Olaparib and Temozolomide in Relapsed Small-Cell Lung Cancer. Cancer Discov *9*, 1372-1387.

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C.*, et al.* (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature *434*, 917-921.

Ferraris, D.V. (2010). Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. Journal of medicinal chemistry *53*, 4561-4584.

Fong, P.C., Boss, D.S., Yap, T.A., Tutt, A., Wu, P., Mergui-Roelvink, M., Mortimer, P., Swaisland, H., Lau, A., O'Connor, M.J.*, et al.* (2009). Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. The New England journal of medicine *361*, 123-134.

Fong, P.C., Yap, T.A., Boss, D.S., Carden, C.P., Mergui-Roelvink, M., Gourley, C., De Greve, J., Lubinski, J., Shanley, S., Messiou, C.*, et al.* (2010). Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. Journal of clinical oncology : official journal of the American Society of Clinical Oncology *28*, 2512-2519.

Gudmundsdottir, K., and Ashworth, A. (2006). The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene *25*, 5864-5874.

Gupta, S.K., Smith, E.J., Mladek, A.C., Tian, S., Decker, P.A., Kizilbash, S.H., Kitange, G.J., and Sarkaria, J.N. (2018). PARP Inhibitors for Sensitization of Alkylation Chemotherapy in Glioblastoma: Impact of Blood-Brain Barrier and Molecular Heterogeneity. Front Oncol *8*, 670.

Hayaishi, O., and Ueda, K. (1977). Poly(ADP-ribose) and ADP-ribosylation of proteins. Annual review of biochemistry *46*, 95-116.

Kaelin, W.G., Jr. (2005). The concept of synthetic lethality in the context of anticancer therapy. Nature reviews Cancer *5*, 689-698.

Kohn, K.W. (1999). Molecular interaction map of the mammalian cell cycle control and DNA repair systems. Mol Biol Cell *10*, 2703-2734.

Lindahl, T., Satoh, M.S., Poirier, G.G., and Klungland, A. (1995). Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. Trends Biochem Sci *20*, 405-411.

Liu, L., Kong, M., Gassman, N.R., Freudenthal, B.D., Prasad, R., Zhen, S., Watkins, S.C., Wilson, S.H., and Van Houten, B. (2017). PARP1 changes from three-dimensional DNA damage searching to one-dimensional diffusion after auto-PARylation or in the presence of APE1. Nucleic acids research *45*, 12834-12847.

Lord, C.J., and Ashworth, A. (2017). PARP inhibitors: Synthetic lethality in the clinic. Science *355*, 1152-1158.

Mateo, J., Lord, C.J., Serra, V., Tutt, A., Balmana, J., Castroviejo-Bermejo, M., Cruz, C., Oaknin, A., Kaye, S.B., and de Bono, J.S. (2019). A decade of clinical development of PARP inhibitors in perspective. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.

Menear, K.A., Adcock, C., Boulter, R., Cockcroft, X.L., Copsey, L., Cranston, A., Dillon, K.J., Drzewiecki, J., Garman, S., Gomez, S.*, et al.* (2008). 4-[3-(4-cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin- 1-one: a

novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. Journal of medicinal chemistry *51*, 6581-6591.

Murai, J., Huang, S.Y., Das, B.B., Renaud, A., Zhang, Y., Doroshow, J.H., Ji, J., Takeda, S., and Pommier, Y. (2012). Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. Cancer research *72*, 5588-5599.

Murai, J., Huang, S.Y., Renaud, A., Zhang, Y., Ji, J., Takeda, S., Morris, J., Teicher, B., Doroshow, J.H., and Pommier, Y. (2014a). Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. Molecular cancer therapeutics *13*, 433-443.

Murai, J., Zhang, Y., Morris, J., Ji, J., Takeda, S., Doroshow, J.H., and Pommier, Y. (2014b). Rationale for poly(ADP-ribose) polymerase (PARP) inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition. The Journal of pharmacology and experimental therapeutics *349*, 408-416.

Nakazawa, K., Ueda, K., Honjo, T., Yoshihara, K., Nishizuka, Y., and Hayaishi, O. (1968). Nicotinamide adenine dinucleotide glycohydrolases and poly adenosine diphosphate ribose synthesis in rat liver. Biochemical and biophysical research communications *32*, 143-149.

Nduka, N., Skidmore, C.J., and Shall, S. (1980). The enhancement of cytotoxicity of N-methyl-N-nitrosourea and of gamma-radiation by inhibitors of poly(ADP-ribose) polymerase. Eur J Biochem *105*, 525-530.

Nishizuka, Y., Ueda, K., Honjo, T., and Hayaishi, O. (1968). Enzymic adenosine diphosphate ribosylation of histone and poly adenosine diphosphate ribose synthesis in rat liver nuclei. The Journal of biological chemistry *243*, 3765-3767.

Nishizuka, Y., Ueda, K., Nakazawa, K., and Hayaishi, O. (1967). Studies on the polymer of adenosine diphosphate ribose. I. Enzymic formation from nicotinamide adenine dinuclotide in mammalian nuclei. The Journal of biological chemistry *242*, 3164-3171.

Nishizuka, Y., Ueda, K., Yoshihara, K., Yamamura, H., Takeda, M., and Hayaishi, O. (1969). Enzymic adenosine diphosphoribosylation of nuclear proteins. Cold Spring Harbor symposia on quantitative biology *34*, 781-786.

Pieper, A.A., Verma, A., Zhang, J., and Snyder, S.H. (1999). Poly (ADP-ribose) polymerase, nitric oxide and cell death. Trends Pharmacol Sci *20*, 171-181.

Pommier, Y., O'Connor, M.J., and de Bono, J. (2016). Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. Science translational medicine *8*, 362ps317.

Roitt, I.M. (1956). The Inhition of Carbohydrate Meatolism in Ascites Tumor Cells by Ethyleneimines. Biochem J *63*, 300-397.

Satoh, M.S., and Lindahl, T. (1992). Role of poly(ADP-ribose) formation in DNA repair. Nature *356*, 356-358.

Schreiber, V., Dantzer, F., Ame, J.C., and de Murcia, G. (2006). Poly(ADP-ribose): novel functions for an old molecule. Nature reviews Molecular cell biology *7*, 517-528.

Setton, J., Zinda, M., Riaz, N., Durocher, D., Zimmermann, M., Koehler, M., Reis-Filho, J.S., and Powell, S.N. (2021). Synthetic Lethality in Cancer Therapeutics: The Next Generation. Cancer Discov *11*, 1626-1635.

Shen, Y., Rehman, F.L., Feng, Y., Boshuizen, J., Bajrami, I., Elliott, R., Wang, B., Lord, C.J., Post, L.E., and Ashworth, A. (2013). BMN 673, a novel and highly potent PARP1/2 inhibitor for the treatment of human cancers with DNA repair deficiency. Clinical cancer research : an official journal of the American Association for Cancer Research *19*, 5003-5015.

Smulson, M.E., Schein, P., Mullins, D.W., Jr., and Sudhakar, S. (1977). A putative role for nicotinamide adenine dinucleotide-promoted nuclear protein modification in the antitumor activity of N-methyl-N-nitrosourea. Cancer research *37*, 3006-3012.

Tentori, L., Leonetti, C., Scarsella, M., Vergati, M., Xu, W., Calvin, D., Morgan, L., Tang, Z., Woznizk, K., Alemu, C.*, et al.* (2005). Brain distribution and efficacy as chemosensitizer of an oral formulation of PARP-1 inhibitor GPI 15427 in experimental models of CNS tumors. Int J Oncol *26*, 415-422.

Turk, A.A., and Wisinski, K.B. (2018). PARP inhibitors in breast cancer: Bringing synthetic lethality to the bedside. Cancer *124*, 2498-2506.

Ueda, K., and Hayaishi, O. (1985). ADP-ribosylation. Annual review of biochemistry *54*, 73-100.

van Wietmarschen, N., and Nussenzweig, A. (2018). Mechanism for Synthetic Lethality in BRCA-Deficient Cancers: No Longer Lagging Behind. Mol Cell *71*, 877-878.

Whish, W.J., Davies, M.I., and Shall, S. (1975). Stimulation of poly(ADP-ribose) polymerase activity by the anti-tumour antibiotic, streptozotocin. Biochemical and biophysical research communications *65*, 722-730.

Zhang, Y.W., Regairaz, M., Seiler, J.A., Agama, K.K., Doroshow, J.H., and Pommier, Y. (2011). Poly(ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells. Nucleic acids research *39*, 3607-3620.

Zimmer, A.S., Gillard, M., Lipkowitz, S., and Lee, J.M. (2018). Update on PARP Inhibitors in Breast Cancer. Current treatment options in oncology *19*, 21.

Zwelling, L.A., Kerrigan, D., and Pommier, Y. (1982). Inhibitors of poly-(adenosine diphosphoribose) synthesis slow the resealing rate of x-ray-induced DNA strand breaks. Biochemical and biophysical research communications *104*, 897-902.