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Evolution of Poly(ADP-ribose) Polymerase-1 (PARP-1) Inhibitors. From Concept to Clinic

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Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1[a]) has been an actively pursued drug discovery target for almost 3 decades. Often referred to as the "guardian angel of DNA",[1] this abundant nuclear enzyme has been the focus of over 20 medicinal chemistry programs in a wide range of therapeutic areas encompassing stroke, cardiac ischemia, cancer, inflammation, and diabetes (Figure 1).[2] Despite the great therapeutic potential for this target and the tremendous academic and industrial efforts dedicated to it, only recently have PARP-1 inhibitors made headway in clinical trials. Recent results from several PARP-1 inhibitors in phase II clinical trials for cancer therapy have attracted the attention of national media.[3] Of the several potential therapeutic indications for PARP-1 inhibitors, the two major areas that hold the most promise are ischemia and cancer. This review is structured to provide the readers with a brief summary of the rationale for PARP-1 as a therapeutic target, to explain the PARP-1 inhibitor pharmacophore, and to provide an update on the progress of the PARP-1 drug discovery programs. This Perspective will offer a historical account of the critical PARP-1 publications that instilled the interest of the biopharmaceutical industry in the late 1980s and early 1990s. Furthermore, I will discuss why PARP-1 received so much attention in the late 1990s and early 2000s followed by the slight decline in the medicinal chemistry efforts today (Figure 1). The major PARP-1 medicinal chemistry programs will be highlighted focusing on the lead generation, lead optimization, candidate selection, and clinical progress. Many aspects of the biological functions of PARP-1 fall outside the scope of this medicinal chemistry review. For this reason, the reader should refer to the following citations for a review of the PARP family of enzymes,[4−6] the biological functions of poly(ADP-ribose),[5] PARP-1 and intracellular signaling,[7] PARP and DNA repair,[8] PARP and epigenetics,[9] PARP and angiogenesis,[10] and the role of PARP-1 in inflammation.[11,12] The 30 years of medicinal chemistry on this topic have also afforded some excellent medicinal chemistry reviews, most of which predated the disclosure of clinical candidate structures and recent clinical trial results.[13−17]

PARP-1 Enzymatic Function

Poly(ADP-ribose) polymerase-1 (aka PARP-1, ADPRT, PARS: EC 2.4.2.30) is one of the most abundant and well characterized members of the PARP family of nuclear enzymes.[4] To date, 18 members of the PARP family have been identified and characterized, with PARP-1 being the most thoroughly studied and PARP-2 being its closest relative.[5] Despite the large number of enzymes in this family, PARP-1 accounts for >90% of the ADP-ribosylation within the cell and is highly evolutionarily conserved in all advanced eukaryotes.[17,18] PARP-2, the closest homologue to PARP-1, is the only other member of the PARP family with a DNA binding region indicating a potential functional redundancy within the genome. While PARP-1 knockout mice are viable, double PARP-1 and PARP-2 knockout mice are embryonically lethal[19] indicating the importance of these two family members in genomic integrity and cell survival. Because of the structural homology between PARP-1 and PARP-2, most PARP-1 inhibitors also inhibit PARP-2. One group has even described selective PARP-2 inhibitors as further tools to expound the role of this enzyme.[20] PARP-1, however, has been a well studied enzyme for over 40 years, and despite the new and exciting roles for other members of the PARP family such as PARP-2, the focus of this review will be on PARP-1 as a therapeutic target.

The PARP-1 enzyme is a 113 kDa protein with three major structural domains, a DNA binding domain with two zinc fingers, a 55 kDa catalytic domain, which utilizes nicotinamide adenine dinucleotide (NAD^+, outlined in gray, Figure 2) as a substrate to construct polymers of ADP-ribose on histones[21] and other nuclear acceptor proteins including the automodification domain of PARP-1 itself. It is generally accepted that the catalytic activity of PARP-1 is stimulated by DNA damage caused by peroxidation,[22] irradiation,[23] and DNA-damaging chemicals, e.g., chemotherapeutic agents.[24] Toward this end, the 42 kDa DNA binding domain with two zinc fingers binds damaged DNA and stimulates polymerization of ADP-ribose resulting in the unwinding of DNA from histones and exposing the damaged DNA for repair. The synthesis of these polymers is illustrated in Figure 2. This cartoon represents the linear chain polymerization reaction that PARP-1 catalyzes. The 2′-OH of an NAD^+ molecule

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[a] Abbreviations: PARP-1, poly(ADP-ribose)polymerase-1; PARG, poly(ADP-ribose)glycohydrolase; NAD^+, nicotinamide adenine dinucleotide; BRCA, breast cancer associated genes; XRCC1, X-ray repair cross-complementing 1; LIG-IIIα, deoxyribonucleic acid ligase III-α; 3-AB, 3-aminobenzamide; SSB, single strand breaks; DSB, double strand breaks; HR, homologous recombination; MTIC, 3-methyltriazen-1-yl)imidazole-4-carboxamide; MCAO, middle cerebral arterial occlusion; TMZ, temozolomide; TNBC, triple negative breast cancer; HER2, human epidermal growth factor receptor 2; MMS, methyl methane sulfonate; PBL, peripheral blood lymphocytes; PID, poly-(ADP-ribose)polymerase inhibitory dose; PBMC, peripheral blood mononuclear cells; PSA, polar surface area; HBD, hydrogen bond donors; HBA, hydrogen bond acceptors; PTEN, phosphatase and tensin homologue.

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binds to an intermediate oxonium ion that is generated by the dissociation of nicotinamide (blue, Figure 2) from the ribose ring. The critical amino acid residues (red, Figure 2) include Ser904 and Gly863 which form a hydrogen bonding network with the nicotinamide moiety. In addition, Tyr904 forms a planar surface which has a $\pi-\pi$-interaction with the nicotinamide group and stabilizes the oxonium ion. At the same time, Glu988 facilitates the proton transfer from the 2′-OH group before the glycosidic bond is formed.[25] PARP-1 can also catalyze the synthesis of branched polymers from the 2′-OH of the nicotinamide ribose.

PARP-1 has long been associated with DNA repair and maintenance of the integrity of genomic function.[26] Experiments have elucidated the role of PARP-1 in DNA repair as illustrated in Figure 3.[27] The first step of this cycle is DNA damage (oxidative, chemical, or *hν*) and PARP activation.

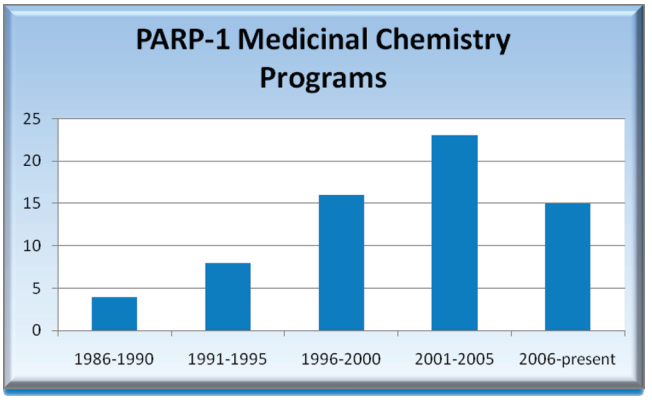

Figure 1. Active PARP-1 drug discovery programs based on patent and publication records.

During this step several events occur:[6] (1) PARP-1 is recruited to the site of damage and binds DNA by means of its two zinc fingers; (2) PARP-1 enzymatic activity is stimulated up to 500-fold upon DNA binding;[28] (3) poly(ADP-ribosyl)ation occurs on histone glutamate residues and upon PARP-1 itself within the automodification region of the enzyme;[21] (4) NAD^+, the substrate for PARP-1 is rapidly consumed in the generation of ADP-ribose polymers; (5) the negatively charged ADP-ribose chain results in a looser interaction between the histones and the DNA, making the site of damage more accessible for DNA repair enzymes; (6) PARP-1 upon being autopoly-(ADPribosyl)ated becomes down-regulated. Simultaneously, poly(ADP-ribose)glycohydrolase (PARG) rapidly breaks down the ADP-ribose polymers, leaving the site of DNA damage even more accessible for the DNA repair enzymes. At this stage, PARP-1 is also inactivated and dissociated from the damaged DNA. Finally, DNA repair occurs as the DNA repair enzymes such as X-ray repair cross-complementing 1 (XRCC1)[29] and DNA ligase III-α (LIG-IIIα) are recruited to the damaged site by (ADPribosyl)ated PARP-1.[30] This repair cycle is a dynamic process, and the total time for this cycle from beginning to end can be measured in minutes.[31]

PARP-1 as a Target for Oncology

The inhibition of PARP-1 has two potential therapeutic applications for drug discovery. The first application is as a chemopotentiator, since many anticancer therapeutics target DNA damage as a mechanism to destroy rapidly dividing cancer cells. Thus, the PARP-1 mediated repair pathway is one major mechanism for DNA repair by many cancerous cell types leading to drug resistance and continued tumor growth.[32] Hence, PARP-1 inhibition in combination with DNA damaging chemotherapeutics or radiation would

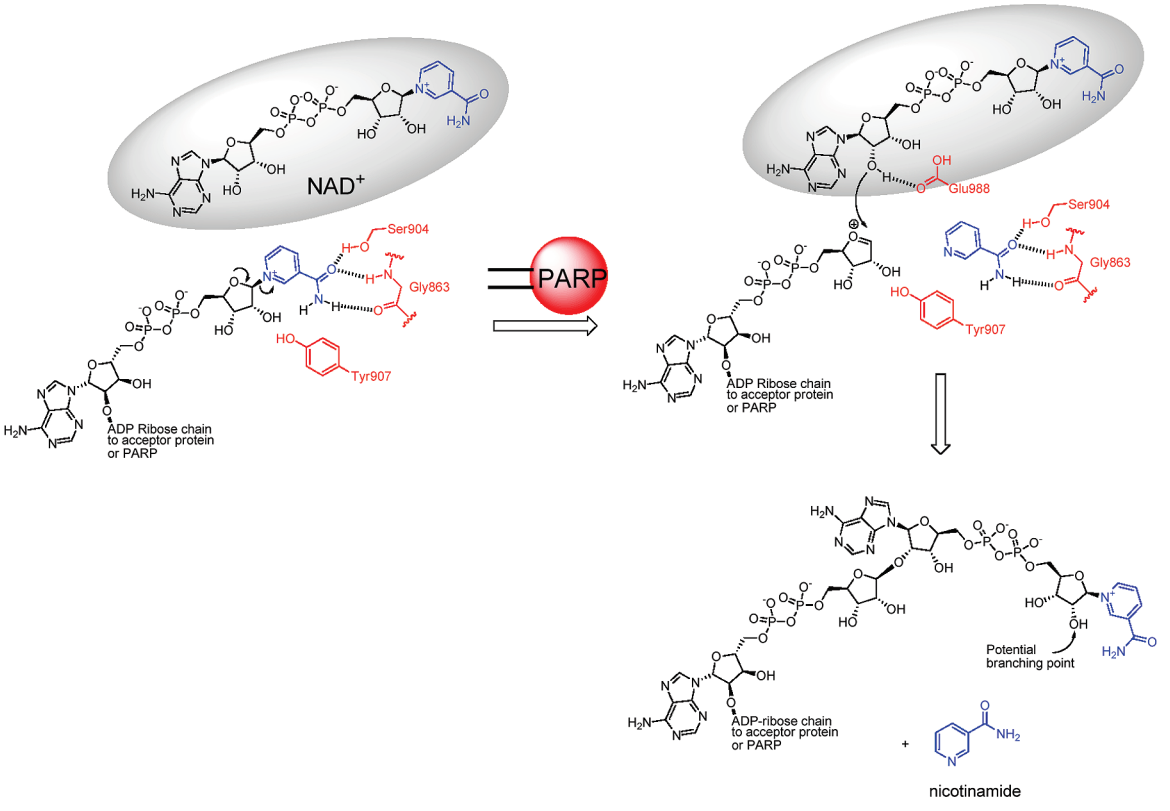

Figure 2. Poly(ADP-ribosyl)ation catalyzed by PARP-1.

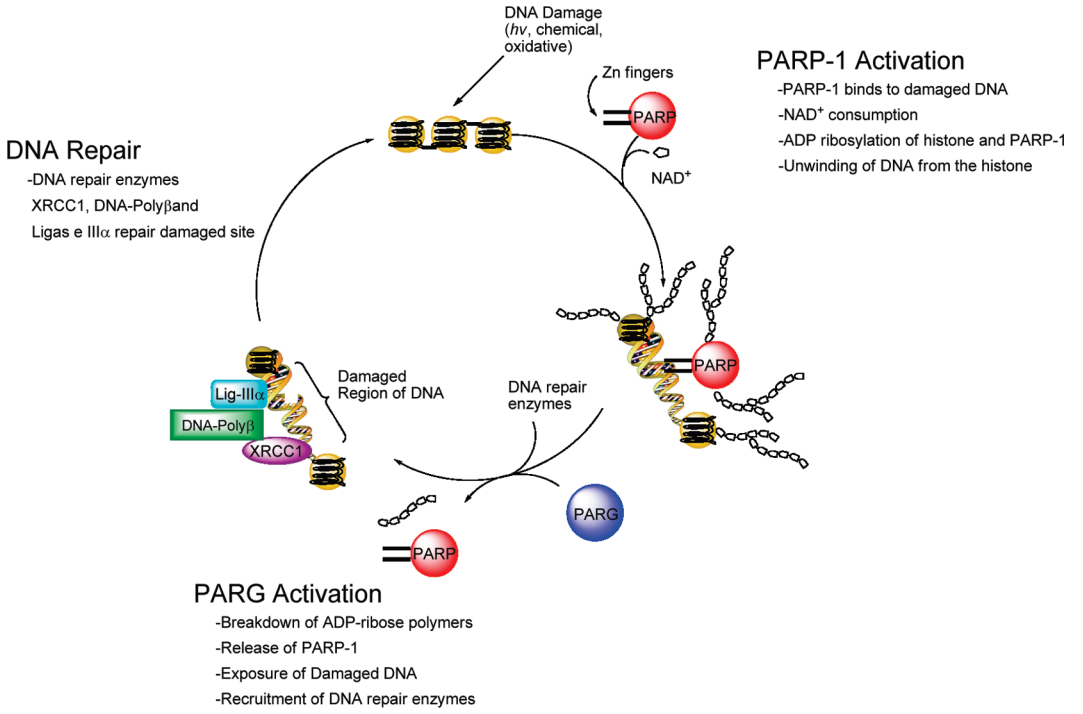

Figure 3. Role of PARP-1 in DNA repair.

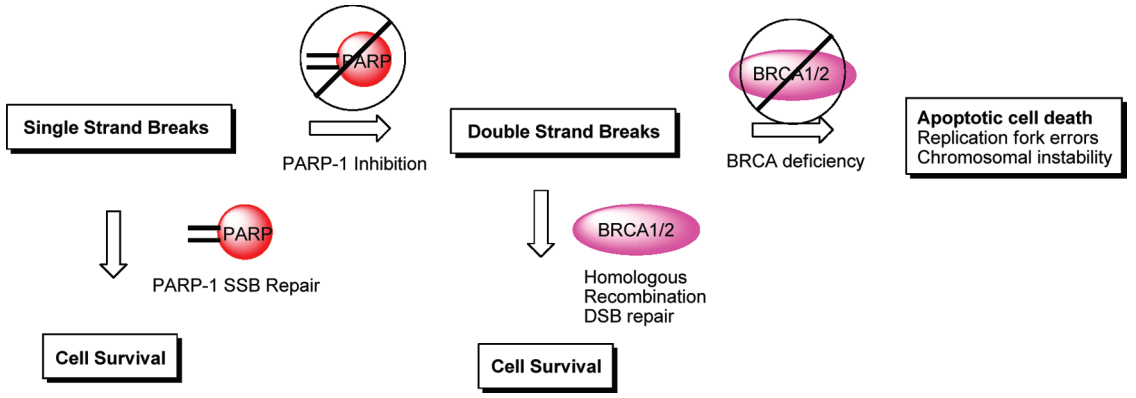

Figure 4. Synthetic lethality of cancer cells.

compromise the cancer cell DNA repair mechanisms, resulting in genomic dysfunction and cell death.[5] The second, a more recent discovery, is that PARP-1 can be used as a standalone therapy for tumor types that are already deficient in certain types of DNA repair mechanisms as discussed below.

Several pioneering publications vetting PARP-1 as a viable cancer target appeared in the 1980s. The earliest study demonstrated that 3-aminobenzamide (**2**, 3-AB, Figure 5),[33] first generation inhibitor of PARP-1 and close analogue of nicotinamide (**1**, Figure 5),[34] enhanced the cytotoxicity of DNA methylating agents in murine leukemia cells, providing a foreshadowing of PARP-1 inhibitors as chemopotentiators.[28] By this time, there were several compelling reasons for evaluating PARP-1 as an attractive therapeutic target for oncology: (1) much was known about the role of PARP-1 in DNA repair, providing a mechanism of action for PARP-1 inhibitors as chemopotentiators; (2) the biochemistry of the enzyme was well characterized providing a reasonable screening tool for small molecule inhibitors; (3) small molecule PARP-1 prototype inhibitors were known and shown to enhance the cytotoxicity of DNA damaging agents in cells;[28] (4) rodent xenograft models were commonplace and hence provided an in vivo screening tool for PARP-1 inhibitors. These factors led to a reasonable optimization paradigm that many of the first medicinal chemistry programs followed to discover clinical candidates.

The next major breakthrough in PARP-1 cancer research occurred in 2005 with two seminal *Nature* publications from independent groups regarding the sensitivity of BRCA1/2 deficient cell lines toward PARP-1 inhibitors.[35,36] This research supported the hypothesis that PARP-1 inhibitors could be used as single agents in cancer cell types with deficient DNA repair mechanisms. Breast cancer associated genes BRCA1 and BRCA2 have long been characterized as tumor suppressor genes that play an integral role in the repair of double strand breaks (DSB) in DNA through a process called homologous recombination (HR).[37] While PARP-1 inhibition will lead to an increase in single strand breaks (SSB), the preponderance of these SSBs will eventually lead to DSBs via replication fork collapse (Figure 4).[38] The increase of DSBs in the presence of HR deficient cell types leads to chromosomal aberrations and instability of the genome resulting in cell

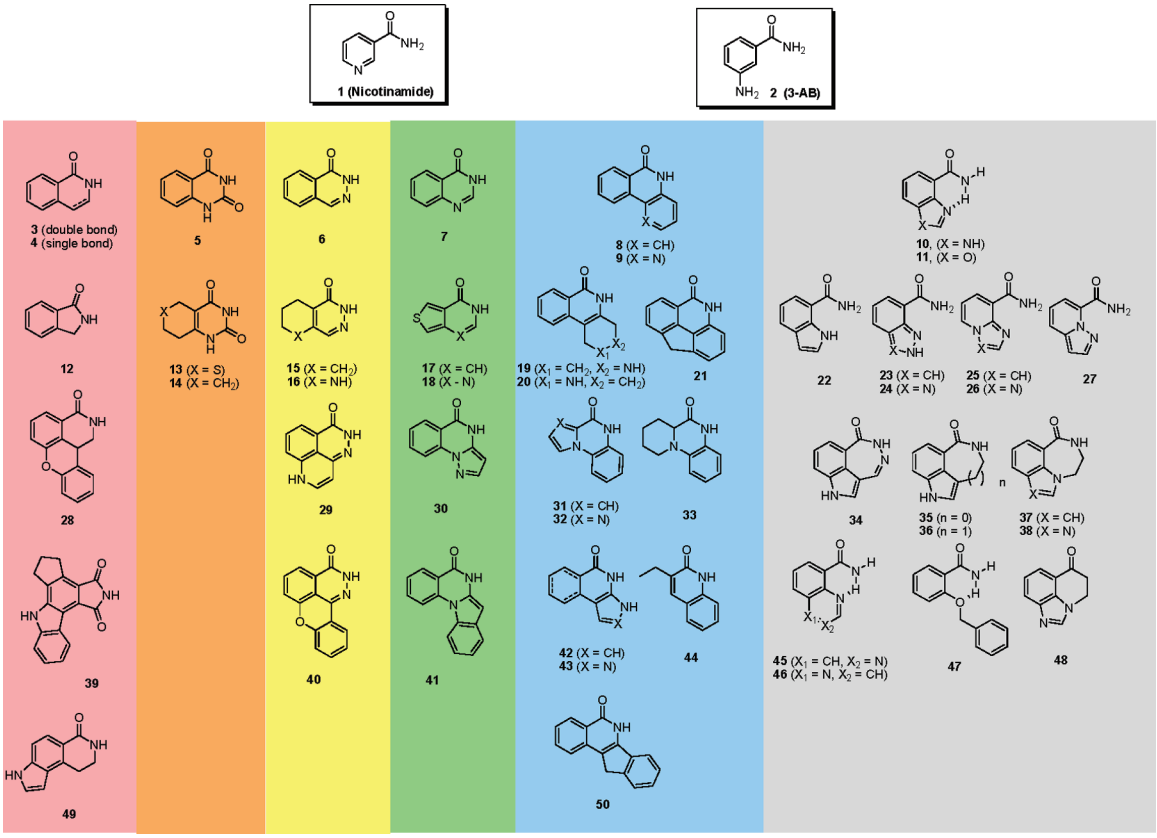

Figure 5. Evolution of PARP-1 Inhibitor Scaffolds.

death. This phenomenon is referred to as synthetic lethality;[39] namely, the loss of one gene function will result in cell susceptibility (i.e., loss of PARP-1 *or* BRCA1/2), but the loss of both is lethal (i.e., BRCA1/2 deficient cells *and* a PARP-1 inhibitor).

This discovery that PARP-1 inhibitors could be used as a single agent provided a new opportunity for the drug discovery programs. Now the path to a PARP-1 clinical candidate involved testing the inhibitor in cell lines deficient in DNA repair mechanisms such as BRCA1/2 (−/−) cells. In vivo, these cell lines could then be utilized in xenograft models to test PARP-1 inhibitors. In addition, any cell lines that lacked the ability for HR could be tested for PARP-1 inhibitor sensitivity. This so-called "BRCAness" has been used to describe tumor types with inactivated HR pathways.[40]

In addition to the changing research paradigms, now a promising new clinical pathway for PARP-1 inhibitors was apparent. Because of the substantial amount of evidence of BRCA1/2 mutations in hereditary breast and ovarian cancer, there was now a viable diagnostic screen to identify a patient population most likely to respond to PARP inhibition therapy. In addition, there seemed to be a significant overlap between BRCA1 mutation breast cancer and patients with so-called "triple negative" breast cancer (TNBC), i.e., deficiencies in estrogen receptor α (ER), progesterone receptor (PR) expression, and the HER2 gene. In fact, more than half of BRCA1 carriers have TNBC. In addition, patients with TNBC account for ~15% of the total breast cancer diagnoses and have a higher likelihood of recurrence and death.[41] Furthermore, the recent phase II clinical trials using a PARP-1 inhibitor[42] suggest that PARP-1 is up-regulated in TNBC patients (vide infra).

PARP-1 as a Therapeutic Target for Ischemia

In the mid-1990s, evidence was mounting that PARP-1 played a significant role in ischemic damage of cells. The link was made between oxidative DNA damage, (e.g., nitric oxide or peroxynitrite) and single strand breaks.[43] The hypothesis was that PARP-1 was overactivated under ischemia−reperfusion conditions where excess DNA damage is occurring. This overactivation led to rapid consumption of NAD^+ to form ADP-ribose polymers. Because the biosynthesis of NAD^+ is an ATP consuming process, the cellular level of ATP would be subsequently depleted and the ischemic cells would die from necrosis. Therapeutic PARP-1 inhibition, however, would still leave the cell in a damaged state, bringing into question the viability of this strategy. Indeed, the involvement of drug discovery efforts in ischemia did not start in earnest until the therapeutic benefit of PARP-1 inhibitors was firmly established. In 1997, the laboratories of Dawson and Snyder at Johns Hopkins University and Moskowitz at Massachusetts General Hospital discovered that genetic disruption of PARP-1 resulted in significant protective effects against ischemic events in vivo.[44,45] These groups demonstrated that PARP-1 knockout mice displayed profound protective effects (> 60% reduction in damaged tissue) in an animal model of stroke, middle cerebral arterial occlusion (MCAO). Both papers provided compelling evidence that PARP-1 inhibitors could potentially provide a therapeutic benefit to stroke patients by reducing the amount of damaged brain tissue. At the same time, further evidence started accumulating to provide additional support for the role of PARP-1 in cardiac ischemia and other forms of ischemic injury.[2] Later evidence confirmed that inhibition of PARP-1 in ischemic conditions elicited protective effects in two ways: (1) by preservation of the cellular

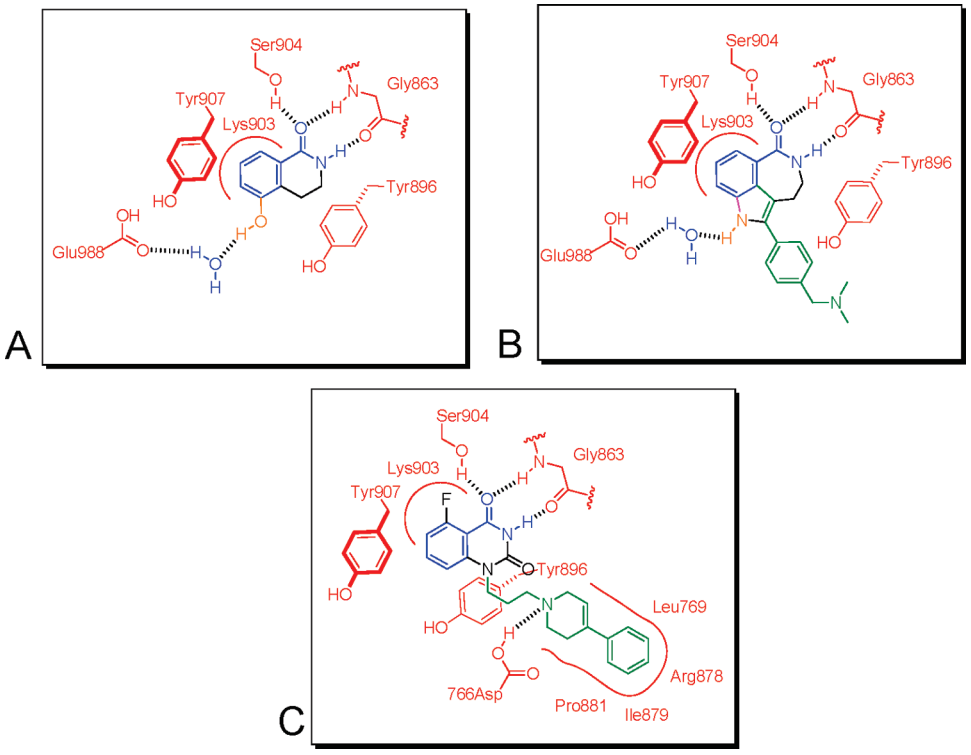

Figure 6. Binding modes of several PARP-1 inhibitors in the nicotinamide binding pocket: (A) dihydroisoquinolinone **51**; (B) indolobenzazepine, **52**; (C) indolinedione, **53**.

levels of NAD^+, and consequently ATP, the peripheral damaged cells, e.g., the penumbra in the brain, could utilize the energy to repair the damaged DNA if not too extensive;[2] (2) PARP-1 inhibition prevented the activation of certain inflammation pathways that could have contributed to further cellular damage via an immune response.[7]

By the late 1990s, PARP-1 became a drug discovery target for ischemia for several reasons: (1) specific, low MW scaffolds with nanomolar potency were already known, providing good starting points for medicinal chemistry optimization;[15] (2) very few pharmaceutical companies had PARP-1 drug discovery programs, leaving plenty of competitive space in the area of ischemia; (3) ischemic animal models were available with proof-of-concept data from knockout mice; (4) the unmet medical needs and potential indication for an ischemia drug was vast (e.g., stroke, cardiac ischemia, cardiac bypass, and sepsis). As compelling PARP-1 related ischemia data increased, a 3-fold increase in drug discovery programs from 1995 to 2005 resulted (Figure 1). This culminated in an explosion of intellectual property in the early 2000s and a refined pharmacophore for the next generation of nicotinamide based PARP-1 inhibitors.

PARP-1 Inhibitor Pharmacophore and Nicotinamide Binding Site

Figure 5 outlines 30+ PARP-1 inhibitor scaffolds that have afforded thousands of analogues providing a solid pharmacophore for NAD^+ competitive inhibitors. In the 1980s, two inhibitors provided the starting point for this pharmacophore, namely, nicotinamide (**1**, IC_{50} = 210 μM) and 3-aminobenzamide (**2**, IC_{50} = 30 μM).[15,33,34] These two inhibitors played important roles as proof of concept PARP-1 inhibitors albeit with suboptimal inhibitory potencies.[28] In an attempt to improve the potency of first generation PARP-1 inhibitors, Ueda and Banasik from Kyoto University screened over 100 compounds from several structural classes to discover multiple bicyclic and tricyclic lactams as submicromolar PARP-1 inhibitors. This work refined the PARP-1 pharmacophore by demonstrating that constraining the arylamide into another ring would restrict the degrees of freedom for the amide moiety, thus locking it into a geometry beneficial for PARP-1 inhibitory potency. The Banasik group introduced several bicyclic lactam derivatives with single digit micromolar potency such as the isoquinolinones (**3**),[46] dihydroisoquionlinones (**4**),[47] quinazoline diones (**5**), phthalazinones (**6**), quinazolinones (**7**), and phenanthridones (**8**, X = CH).[33] The groundbreaking work by Banasik and Ueda can be further appreciated by understanding the families of core structures that have evolved from, or bear close resemblance to, the basic scaffolds outlined in their publication (Figure 5). For example, the isoindolinones (**12**[48] and **39**),[49] thienopyridinones (**17**),[50] tetracyclic isoquinolinones (**28**),[51] and pyrroloisoquinolinones (**49**)[52] are closely related to isoquinolinones (pink, Figure 5). Likewise, the tetrahydroquinazoline diones (**13** and **14**)[53] are closely related to the quinazoline diones (orange, Figure 5). Phthalazinone based inhibitors (yellow, Figure 5) evolved into tetrahydrophthalazinones (**15** and **16**)[54] and tri- and tetracyclic phthalazinones (**29** and **40**).[55] Likewise, quinazolinone based inhibitors (light-green, Figure 5) developed into thienopyrimidinones (**18**),[50] pyrrazoloquinazolinones (**30**),[56] and indoloquinazolinones (**41**).[57] Because of the inherent potency of the phenanthridone core (**8**, $IC_{50} \approx 300$ nM),[58] many tricyclic and tetracyclic scaffolds evolved from it including the azaphenanthridones (**9**),[58] saturated phenanthridones (**19** and **20**[59] and **33**[60]), bridged phenanthridones (**21**),[61] heterocyclic phenanthridones (**31** and **32**,[60] **42** and **43**[62]), alkylquinolinones (**44**),[63] and indenoisoquinolinones (**50**).[64]

Another family of scaffolds appeared in the early 1990s from the laboratories of Roger Griffin and Bernard Golding at the University of Newcastle (gray, Figure 5). This group first introduced the imidazole and benzoxazole carboxamides (**10** and **11**),[65] in which the imidazole nitrogen acts as an intramolecular hydrogen bond acceptor to the amide NH. This intramolecular hydrogen bond forms a "pseudoring", locking the primary amide in the geometry most beneficial to PARP-1 binding, similar to the bicyclic and tricyclic lactams. The benzimidazole carboxamides core is perhaps one of the most potent core structures for PARP-1 inhibitors (K_i = 95 nM).[66] This inherent potency for such a small core spawned multiple subseries of compounds from which several clinical candidates derived (vide infra). These series include the indole carboxamides (**22**),[67] the indazole and triazole carboxamides (**23** and **24**),[68] imidazopyridine carboxamides (**25**, **26**, and **27**),[68,69] quinoxaline carboxamides (**45** and **46**),[70] alkoxybenzamides (**47**)[71] as well as several tricyclic series such as diazepinoindolones (**34**)[72] and [5,6,6]- and [5,6,7]tricyclicindole lactams (**35** and **36**)[73] and [5,6,7]tricyclicimidazole lactams (**37** and **38**).[74,75] Even tricyclic benzimidazoles (**48**)[76] evolved from this family, a series without the characteristic lactam prevalent in so many NAD^+ competitive inhibitors.

A distinct binding mode for several of these scaffolds was first established by Ruf and co-workers solving the first X-ray cocrystal studies with PARP-1 inhibitors and chicken PARP (cPARP), a close homologue (87%) of human PARP-1.[25] The common thread for each of the inhibitors was a hydrogen bonding network between the amide functionality of the inhibitor core and Ser904 and Gly863 of PARP-1. Indeed, as outlined in Figure 2, this is the same network of H-bonds that bind the nicotinamide portion of NAD^+. Lactam based inhibitors such as isoqunolinone NU1025 (**51**, Figure 6A) form three hydrogen bonds in the nicotinamide subsite of the NAD^+ pocket, two from Gly863 and one from Ser904. This network explained an improvement in potency observed with fused arylamides. This network of H-bonds also accounted for the fact that substituents close to the amide pharmacophore usually decrease enzymatic potency by disrupting these interactions. Other features of the nicotinamide pocket were the two aryl residues Tyr896 and Tyr907 forming a π-electron sandwich for the flat arylamide groups, explaining the improvement in potency often seen with arylamides versus saturated amides. The back wall of the nicotinamide subsite bordered by Ala898 and Lys903 formed a tight, small pocket just large enough for small substituents (e.g., CH_3, F, Cl) on the benzamide-containing ring (A-ring). In some instances, Glu988 also formed a hydrogen bond with the inhibitor either with or without a conserved water molecule mediating the H-bond. This glutamate residue explains why heteroatoms in certain positions on the A-ring increase the potency of inhibitors. The last feature of the nicotinamide binding pocket is the large hydrophobic pocket adjacent to the nicotinamide binding site. This pocket is often referred to as the adenine-ribose binding site (AD site),[77] and most series of PARP-1 inhibitors take advantage of this spacious pocket to improve potency, solubility, and other pharmaceutical properties of the series. Figure 6B and Figure 6C diagram the binding modes for two other PARP-1 inhibitors. The Pfizer/Agouron/Newcastle group published a cocrystal of a indolobenzazepine (**52**)[74] with the catalytic domain of cPARP. This structure bears all of the hallmarks of the isoquinolinone first published by Ruf,[25] indicating that this family of compounds shares the same binding mode as the bicyclic lactams. The Fujisawa group was one of the first groups to extensively study the X-ray cocrystal structures of their inhibitors with human PARP-1. The indolinedione (**53**)[78] formed the same network of hydrogen bonds within the nicotinamide pocket. Interestingly, however, the 4-phenyltetrahydropyridyl side chain depressed the back of the adenine diphosphate ribose binding pocket, forming a new hydrophobic subsite with residues Pro881, Ile879, Arg878, and Leu769. This tetrahydropyridyl nitrogen also forms a hydrogen bond with the carboxylate of Asp766, adding several orders of magnitude in binding potency. The Fujisawa compound exemplifies the increase in binding affinity with hydrophobic groups, 2° and 3° amines specifically, in the structures of many PARP-1 inhibitors.

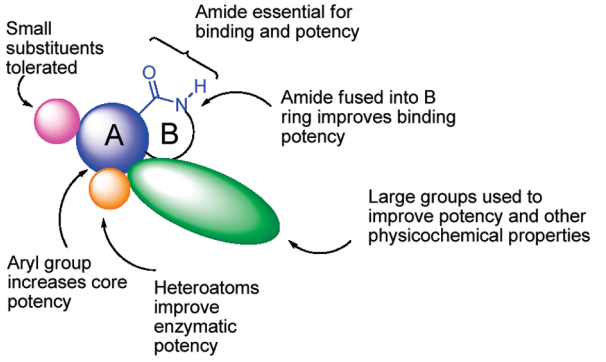

Figure 7. PARP-1 pharmacophore.

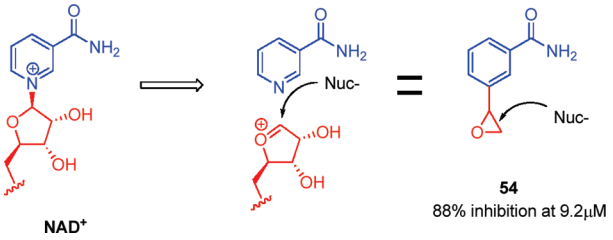

Figure 8. Mechanism based PARP-1 inhibitors designed by the Threadgill laboratories.

The X-ray cocrystal studies from a multitude of scaffolds provide ample support for the PARP-1 pharmacophore outlined in Figure 7. The PARP-1 pharmacophore includes one or more of the following structural elements contributing to the inhibitory potency: (1) an amide moiety fused within a bicyclic ring system or "pseudo bicyclic ring" (e.g., **10**, Figure 5) system as outlined in blue by rings A and B (Figure 7); (2) hydrogen bond donors and acceptors on the opposite side of the A-ring from the amide (orange, Figure 7); (3) small hydrophobic substituents on the A-ring, adjacent to the amide (pink, Figure 7); (4) large hydrophobic groups in the southeast portion of the pharmacophore (green, Figure 7).

PARP-1 Inhibitor Programs

University of Bath (Threadgill Laboratories). One of the earliest groups to explore PARP-1 as a therapeutic target was the laboratories of Michael Threadgill at the University of Bath in the early to mid-1990s. This group was the only one to specifically design mechanism based irreversible inhibitors,[46] mimicking the adenine based oxonium ion (Figure 2). Substituted benzamides and isoquinolin-1-ones with electrophilic groups were synthesized and tested for inhibition of PARP-1 at 10 μM. One of the most potent compounds in this series was the 3-substituted oxirane (**54**),[46] a compound that

situates an electrophilic carbon in a position similar to that of the ribose oxonium ion (Figure 8). The potency of this compound was reported as 88% inhibition at 9.2 μM ($IC_{50} \approx 1$ μM). However, the Threadgill group reported no PARP inactivation upon preincubation with the potential irreversible inhibitors, suggesting that these compounds were not acting as time dependent irreversible inhibitors. In this case, the nucleophile reacting with the epoxide is most likely on an adjacent acceptor protein or the automodification region of PARP-1, not a critical residue in the active site.

This group was also the first to design micromolar PARP-1 inhibitors with a heterocyclic A ring, namely, thienopyridinones (**17**) and thienopyrimidinones (**18**), derived from isoquinolones (**3**) and quinazolinones (**7**), respectively (Figure 9).[50] 6-Methylpyridinone (**55**) and the analogous 2-methylpyrimidinone (**56**) exhibited almost complete PARP-1 inhibition at 10 μM, demonstrating the feasibility of this core structure for optimization.[50]

This group was also one of the first groups to attempt to design prodrugs of PARP-1 inhibitors. The prodrug consisted of a nitroimidazole group as in compound **57** (red, Figure 10), capping the most prevalent feature of PARP-1 inhibitors, the amide NH.[79] Theoretically, this moiety will be reduced enzymatically under hypoxic conditions leading to the decomposition of the resultant aminoimidazole as outlined in Figure 10. This bioreductively triggered event would be most prevalent in areas with low oxygen such as hypoxic tumor tissue. The PARP-1 inhibitor, 5-bromoisoquinolinone (**58**, IC_{50} = 270 nM)[79] in this example, would then be released predominantly in the desired tissue because of this physiological difference. Reductive conditions similar to that of the physiological hypoxia were used to demonstrate this point. The Threadgill laboratories were able to probe some of the lesser studied aspects of PARP-1 inhibition, namely, mechanism based inhibitors, A-ring heterocycles, and amide based prodrugs.

University of Newcastle/Agouron/Pfizer: AG014699 (63, PF-01367338). One of the earliest groups dedicated to PARP-1 as a therapeutic target for cancer were the laboratories of Roger Griffin and Bernard Golding at the University of Newcastle in the early 1990s. At the time, a PARP-1 pharmacophore was starting to emerge from the bicyclic lactams (Figures 5 and 7). The focus of their efforts was on two core series, the quinazolinones (**7**, IC_{50} = 15.8 μM)[80] and, more importantly, benzimidazole carboxamides (**10**, K_i = 95 nM), a series that generated the clinical candidate several years later.

The quinazolinone core, originally discovered by Banasik and Ueda, was optimized by the Newcastle group via derivatization at the 2 and 8 positions of the bicyclic ring system. This optimization culminated in the discovery of compound **51** (IC_{50} = 0.44 μM, Figure 11), a quinazolinone based PARP-1 inhibitor 35 times more potent than the core structure. In addition, **51** was the subject of one of the first cPARP cocrystal structures (Figure 6A), providing a clear binding mode from which extensive SAR studies were carried out to identify some of the most potent PARP-1 inhibitors at the time (100−300 nM).[80] This compound also displayed cellular activity by moderately potentiating the cytotoxicity of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC, an active metabolite of temozolomide, TMZ) in L1210 leukemia cells. Compound **51** enhanced 90% cell killing (EF_{90}) of MTIC by 3.6-fold (EF_{90} = (IC_{90} of 200 mM MTIC)/(IC_{90} of 200 mM MTIC + 200 μM PARP-1 inhibitor)).[81] This result represented a 50-fold increase in potency over 3-AB in the same system. In vivo, **51** significantly enhanced the life span of tumor bearing mice (intracerebral neoplasias) when dosed intracerebrally (1 mg/mouse in PEG-400) in combination with 200 mg/kg TMZ.[82] Despite these promising results, **51** did not proceed into human trials, presumably suffering from a lack of solubility, a problem that plagued many early PARP-1 inhibitors.

A collaboration between the Newcastle group and Agouron Pharmaceuticals in the late 1990s spawned several subseries of compounds and preclinical leads as outlined in Figure 12. The benzimidazole carboxamides proved to be a strikingly potent core structure (K_i = 95 nM), the most potent core structure identified at the time.[66] Optimization of the benzimidazole carboxamides led to several 2-aryl derivatives with single digit nanomolar potency. Aryl groups substituted at the para position generally improved the potency of the core because of $\pi-\pi$ interactions with Tyr889 and Tyr907 in the nicotinamide binding site of PARP-1. The first lead compound that emerged from this series was NU1085 (**59**, K_i = 6 nM, Figure 12). In vitro, 10 μM **59** was determined to be as effective as 50 μM **51** at potentiating growth inhibition and cytotoxicity of TMZ and topotecan in several human cancer cell lines.[83] However, this compound still suffered from poor aqueous solubility, prompting an optimization pathway focused on better physicochemical properties.[66]

Agouron and Newcastle designed several new subseries of compounds derived from benzimidazole carboxamides in

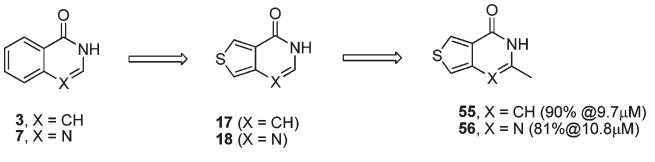

Figure 9. Design of thienopyridinones and thienopyrimidinones.

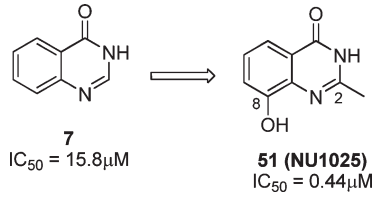

Figure 11. Newcastle's quinazolinone based PARP inhibitor.

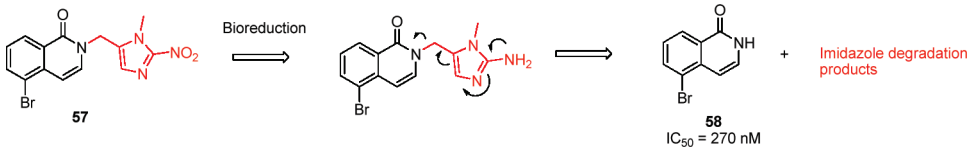

Figure 10. Nitroimidazole prodrug of PARP-1 inhibitors.

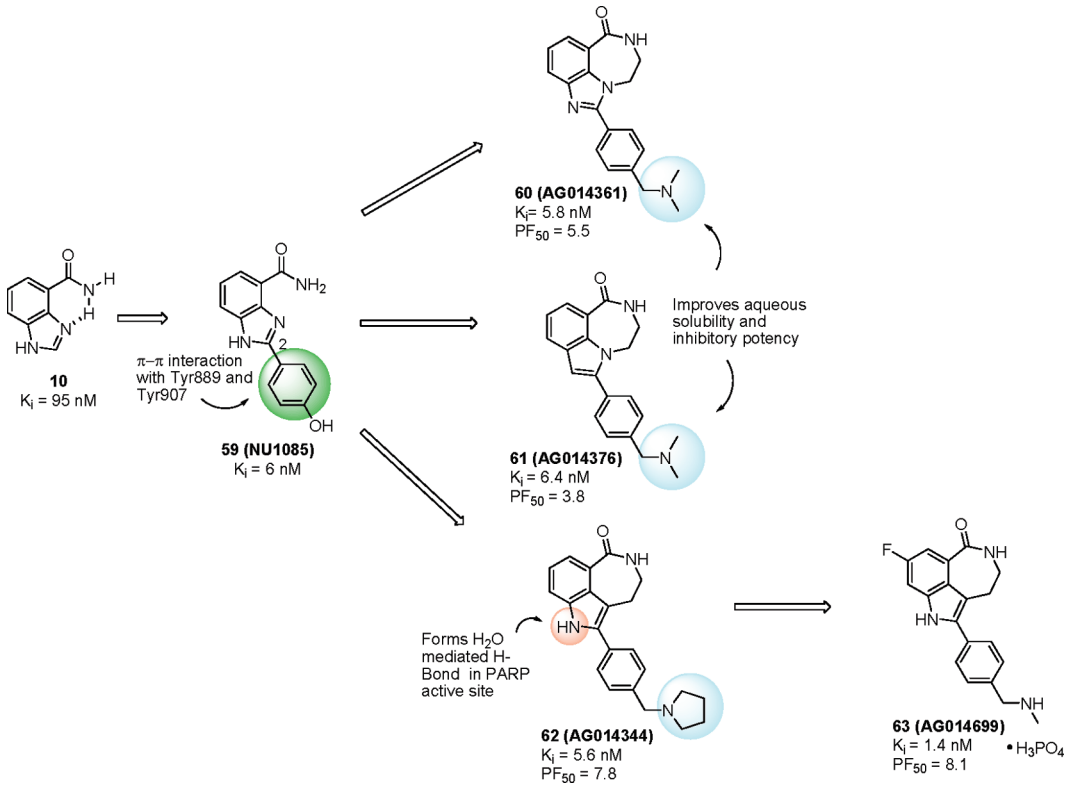

Figure 12. Design of several lead PARP-1 inhibitors from Newcastle/Agouron/Pfizer.

order to address the solubility issues and at the same time improve the structural novelty of their PARP-1 inhibitors. These groups surmised that the free carboxamide of **10** could be constrained within a six- or seven-membered ring, effecting the same result as an intramolecular H-bond. Three lead series emerged from this concept as outlined in Figure 12. One such series, the [5,6,7]tricyclicimidazole lactams (e.g., **38**, Figure 5) confirmed this hypothesis with several low nanomolar 2-substituted derivatives. Optimization of these tricyclic imidazoles led to the identification of AG014361 (**60**) as a lead PARP-1 inhibitor.[74] Compound **60** had a notable feature that started to become commonplace in many of the PARP-1 inhibitors, namely, a secondary or tertiary amine (light-blue, Figure 12) responsible for improving the aqueous solubility, inhibitory potency, and cell permeability of many of the arylamide cores. The high potency of **60** (K_i = 5.8 nM) led to increased antiproliferative activity of TMZ against LoVo cells (PF_{50} = 5.5 when **60** was tested at 0.4 μM). This lead PARP inhibitor also displayed in vivo efficacy by causing complete regression of SW620 xenograft tumors (ip daily at 5 or 15 mg/kg) in combination with TMZ (68 mg/kg po daily for 5 days).[84] Compound **60** served as the benchmark lead for the Agouron group as they designed other closely related series of PARP-1 inhibitors.

In an effort to improve the biological activity and identify a clinical candidate as a chemosensitizer, Agouron probed two other series of compounds. One such series was the [5,6,7]tricyclic indole lactams which was optimized in a similar manner to afford AG014376 (**61**, K_i = 6.4 nM).[85] However, **61** and many other derivatives with this indole core did not retain the in vitro chemopotentiation of TMZ (PF_{50} = 3.8 when tested at 0.4 μM) as the [5,6,7]tricyclic imidazoles. Another such series, the [5,6,6]- and [5,6,7]-tricyclic indole lactams (e.g., **35** and **36**, Figure 5), emerged with several examples of single digit nanomolar PARP-1 inhibitors. Optimization of this series led to compounds such as AG014344 (**62**, IC_{50} = 5.6 nM).[73] The binding mode for this series of inhibitors was confirmed by cocrystal data with cPARP (Figure 6B). The constrained amide formed an H-bond network with Ser904 and Gly863 similar to many of the early PARP-1 inhibitors. In addition, the indole NH formed a water mediated H-bond with Glu988 (orange, Figure 12), the aryl substituent formed a $\pi-\pi$ interaction with Tyr889, and the dimethylamino group interacted with Asp766. In addition, **62** (PF_{50} = 7.8 at 0.4 μM) demonstrated the ability to potentiate temozolomide cytotoxicity in LoVo cells with a greater effectiveness than **60**.

Agouron/Pfizer's clinical candidate, **63**, emerged from the series of [5,6,7]-tricyclic indole lactams.[86] The compound displayed better in vitro potency and in vivo efficacy than **60** and **62** (K_i = 1.4 nM and PF_{50} = 8.1 in LoVo cells). Because many of the [5,6,7]-tricyclic lactam PARP-1 inhibitors had similar potency and potentiation factors, the selection strategy for the clinical candidate assessed the potency of lead inhibitors in rodent xenograft studies in the presence of TMZ.[86] Compound **63**, when dosed at 0.15 (mg/kg)/day ip, exhibited a 50% increase in tumor growth delay as 1.5 (mg/kg)/day ip **60** in a 5 day xenograft study in conjunction with TMZ (68 (mg/kg)/day).[86] The clinical candidate (**63**) also displayed no toxicity alone or in combination with TMZ and no adverse effects on the PK of the coadministered anticancer agents. Gratifyingly, the group that had one of the oldest research interests in PARP-1 was the first group to test a PARP-1 inhibitor in human clinical trials as a chemopotentiator.

Because the most consistent chemopotentiation results were obtained in combination with TMZ, the phase I study of **63** was conducted in conjunction with TMZ in adults with

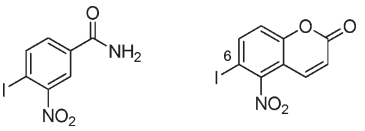

Figure 13. Lead PARP-1 inhibitors discovered by UCSF/Octamer/Bipar.

advanced solid tumors. This phase I study used pharmacodynamic analysis of PARP-1 activity in peripheral blood lymphocytes (PBLs) to assess the optimal PARP inhibitory dose (PID) for **63**. Patients then underwent a dose escalation of TMZ (up to 200 mg/m^2, MTD) with the outcome being a well tolerated combination of 200 mg/m^2 TMZ with 12 mg/m^2 **63** iv infusion. The drug possessed extensive tissue distribution (only 11% excreted in urine) and a terminal half-life of 9.5 h. These parameters translated into 74−97% PARP-1 inhibition in PBLs up to 24 h, indicating that PARP was inhibited throughout the treatment course of TMZ.[87] In 2005, phase II studies were conducted with TMZ in patients with malignant melanoma. The end point of this study was to increase the response rate of patients treated with 200 mg/m^2 TMZ and 12 mg/m^2 **63** iv infusion versus 200 mg/m^2 TMZ alone. Results from this phase II study indicated that the response rate for combination treatment was higher than TMZ alone, but **63** also enhanced the myelosuppression caused by TMZ more than what was expected from phase I data.[88]

In 2005, the Newcastle group in collaboration with the University of Stockholm and the University of Sheffield demonstrated that BRCA deficient cell lines were sensitive to stand-alone treatment with PARP-1 inhibitors, in particular **60** and **51**.[35] This seminal publication opened up a new avenue for the development of **63** and shifted the focus toward treating cancer patients with BRCA1/2 mutations. Currently, **63** (PF-01367338) is in a phase II trial in known carriers of BRCA1/2 with locally advanced metastatic breast cancer or advanced ovarian cancer (NCT 00664781).

UCSF/Octamer/BiPar/Sanofi-Aventis: BSI-201 (64). While the vast majority of PARP-1 drug discovery efforts focused on NAD$^+$ competitive inhibitors, a group from UCSF led by Ernest Kun in collaboration with Octamer Research Foundation identified two noncompetitive PARP-1 inhibitors (Figure 13) that interact with the zinc binding site of PARP-1 **64** and BSI-401 (**65**, Figure 13).[89,90] This unique interaction takes place on Arg34 as was determined by mutagenesis studies.[91] By disrupting the PARP-1 zinc finger/DNA interaction, these compounds would theoretically prevent PARP-1 activation and enhancement,[28] thus achieving a similar result as the NAD$^+$ competitive inhibitors. Clearly, these two compounds are unconventional PARP-1 inhibitors. The nitro group of **64** is known to be reduced in vivo to a nitroso group, the major metabolite that may covalently modify PARP-1 leading to inactivation even though this site has yet to be identified.[92] This covalent modification is a characteristic often mentioned when describing this compound. Regardless of the mechanism of action of these compounds, both display efficacy in several cancer cell lines at micromolar concentrations (50−200 μM) against Ovcar, Skov3, PC-3 cells as well as tumor reduction in xenograft models without signs of toxicity.[93] Because of the relatively modest PARP-1 inhibition (estimated at ∼50−200 μM based on analogous compounds),[90] it is quite possible that the these compounds interact with multiple targets, covalent or otherwise, the combination of which is necessary for anticancer activity. The UCSF/Octamer group provided the intellectual property for the BiPar Sciences PARP-1 program in the early to mid 2000s.[93] The most recent patent filings from BiPar include other 6-substituted benzopyrones, indicating an interest in exploring this unique mechanism of inhibition by medicinal chemistry optimization.[91]

The lead clinical candidate that evolved from BiPar's PARP-1 program is **64** (Figure 13).[93] This compound entered phase I human clinical trials in 2007 to assess the safety and tolerability of the drug when dosed iv in patients with advanced solid tumors. The dose levels were established using PARP inhibition in peripheral blood cell mononucleocytes (PBMCs). The drug inhibited PARP activity by greater than 50% at 2.8 mg/kg iv even though the compound was rapidly eliminated ($t_{1/2}$ = 4 min), indicating the participation of an active metabolite in PARP inhibition, most likely the 4-iodo-3-nitrosobenzamide.[94] Overall, the phase I study indicated that **64** was safe and well tolerated, and the pharmacodynamic end point established the relevant doses to achieve PARP inhibition. A phase IB study also established the safety and tolerability of iv **64** with topotecan, gemcitabine, carboplatin/paclitaxel, and TMZ.[95]

In 2009, Sanofi-Aventis acquired BiPar, and with the acquisition, the company achieved the rights to develop their clinical candidate. With the support of big pharma, the clinical development for **64** began to accelerate. Several phase II studies are underway with this drug, the most impressive of which was in triple negative breast cancer patients.[42] The iv administration of **64** in combination with gemcitabine/carboplatin (G/C) caused an increase in tumor response (48% patients with **64** + G/C vs 16% with G/C alone), progression free survival (211 days with **64** +G/C vs 87 days with G/C alone), and overall survival (> 254 days with **64** + G/C vs 169 days with G/C alone).[42] The phase III trial with **64** in patients with triple negative breast cancer started recruiting in July of 2009 (NCT00938652), preliminary results of which are expected in 2010 (estimated completion is June 2012). The story behind BiPar's clinical candidate is as intriguing and unconventional as its method of PARP-1 inhibition. All signs indicate that it will be one of the first PARP-1 inhibitors to seek approval by the FDA.

Inotek/Genentech: INO-1001 (68). The Inotek group led by Jagtap and Southan was one of the first biotech medicinal chemistry programs to aggressively pursue PARP-1 as a therapeutic target for ischemia in the late 1990s. Some of their earliest patent applications outlined a series of 2-substituted phenanthridone derivatives (Figure 14).[96] This series of phenanthridones was optimized by Inotek to improve the aqueous solubility of the core by addition of tertiary amines at the 2 position of the ring system. This optimization paradigm allowed the aqueous soluble salts of lead compounds to be tested iv in relevant models of ischemia. One such lead, PJ-34 (**66**, IC_{50} = 20 nM, EC_{50} = 35 nM),[7,97] so named for its inventor Prakash Jagtap, was used as a proof of concept compound for multiple indications.[7] Most notably, **66** demonstrated efficacy in multiple models of ischemic injury such as stroke,[98] cardiac ischemia,[99] and sepsis.[100] This compound, despite a plethora of in vivo data, never entered into human clinical trials.

The next series of inhibitors evolving from the phenanthridinones was the tetracyclic indenoisoquinolinones (**50**, Figure 14).[64] The insertion of a five-membered ring in the phenanthridone core served to slightly disrupt the planarity

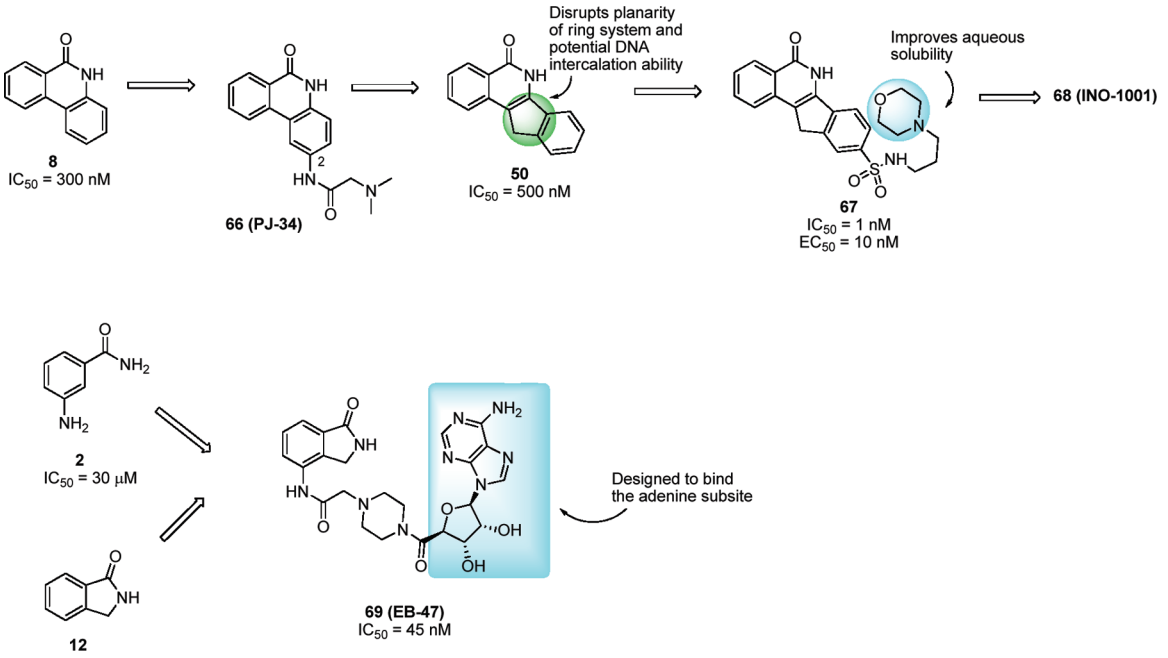

Figure 14. PARP-1 inhibitors designed by Inotek.

of the molecule (light-green, Figure 14), potentially alleviating the DNA intercalating ability of similar multicyclic ring systems.[101] Taking advantage of the large hydrophobic pocket of PARP-1, the Inotek group designed an aminopropylmorpholine derivative to improve the aqueous solubility of the core structure and to improve the enzymatic and cellular potency in peroxynitrite induced cell stress assay[64] (**67**, IC_{50} = 1 nM, EC_{50} = 10 nM).

Inotek also pursued a series of isoindolinones (**12**, Figure 14) and managed to take a rather weakly binding core and engineered the potency using an adenosine moiety in an attempt to bind the adenosine subsite of PARP-1. They achieved this goal with compound EB-47 (**69**),[48] which has an amidopiperazine linker joining the core and the adenosine. This derivative demonstrated good PARP-1 inhibitory potency (IC_{50} = 45 nM, Figure 14), a >600-fold improvement over the core structure. Extensive in vivo ischemia studies were performed with this compound. Compound **69** reduced the infarct volume in a rat transient MCAO model by 57% when dosed at 10 (mg/kg)/h prior to reperfusion. This compound also performed well in a cardiac reperfusion model by reducing the infarct size by 35% when dosing **69** at 20 mg/kg 10 min prior to reperfusion.[102]

Inotek's clinical candidate was the first PARP-1 inhibitor to enter the clinic as a potential therapy for cardiac ischemia, particularly ST-elevation myocardial infarction (STEMI).[103] While the structure of Inotek's clinical candidate (**68**) has not been disclosed, it is most likely a member of the indenoisoquinolinone family based on the known potency (< 10 nM) of the compound.[104] In a phase I trial, the safety, pharmacokinetics, and pharmacodynamics of **68** were evaluated in patients with STEMI undergoing primary percutaneous coronary intervention. The results indicated that **68** had a good terminal $t_{1/2}$ (7.5 h) and inhibited >90% of PARP-1 activity in cultured cells from patients at all doses tested (200, 400, and 800 mg iv bolus).[103] In addition, **68** reduced the level of inflammation markers IL-6 and C-reactive protein, indicating a decrease in neutrophil recruitment, preservation of tissue ATP levels, improved myocardial contractility, and potential reduction in infarct size. In 2005, Inotek started a phase II trial in patients undergoing heart lung bypass surgery to potentially alleviate some of the ischemia related side effects from this procedure. However, this trial is no longer recruiting patients (NCT00271176), and interim results have not been published. The end of Inotek's development of **68** for ischemia emphasizes the difficulty associated with this clinical path, a challenge that has faced many of the drug discovery groups in the PARP field to this day.

Like many lead PARP-1 inhibitors, however, **68** demonstrated the ability to be chemopotentiator preclinically by effectively prolonging life span and delaying tumor growth when dosed iv with TMZ in a glioma xenograft model.[105] In 2006, Genentech signed a licensing agreement with Inotek to pursue cancer chemopotentiation with **68** in combination with TMZ in malignant melanoma.[106] The results from this phase Ib trial were recently published indicating an increase in myelosuppression and liver enzymes at the dose limiting toxicities of **68.**[107] In March of 2009, Genentech terminated this phase Ib trial with **68**, returning all of the rights for this compound to Inotek. Currently, Inotek is pursuing treatment of retinal diseases for their PARP inhibitors and outlicensing opportunities for oncology indications with their more advanced PARP inhibitors such as **68** as indicated on the company's Web site.

Guilford/MGI/Eisai: GPI 21016 (74, E7016). Guilford Pharmaceuticals started a PARP-1 inhibitor program targeting ischemic injuries in the mid-1990s. The primary focus of the Guilford PARP-1 program was the treatment of neurodegenerative diseases, mainly stroke. Focusing on treating PARP-1 related CNS diseases, Guilford emphasized the necessity of brain penetration when designing their inhibitors. Indeed, the Guilford group routinely included tertiary amines on several PARP-1 core structures to improve the solubility and brain penetrability of the inhibitors.[108]

One series of compounds from the Guilford group included several 3-substituted phenanthridones such as compound **70** (IC_{50} = 14 nM, Figure 15).[109] Despite the relative success at discovering several soluble, low nanomolar PARP-1 inhibitors from this series, the structural similarity

to Inotek's phenanthridone series led to a shift in compound design. One alternative series was the saturated phenanthridones, a notable example being compound **71** (Figure 15)[59] with good inhibitory potency (IC_{50} = 25 nM) and good cellular potency in a peroxide induced cytotoxicity assay developed by the group[110] (EC_{50} = 30 nM). Despite achieving potency, solubility, and novelty within this series, the therapeutic limitation was a lack of brain penetrability.[59] To address this issue, the Guilford group designed the azaphenanthridones (e.g., **9**, Figure 5). The lead compound from this series, GPI 16539 (**72**, Figure 15), demonstrated comparable inhibitory potency to **71** as well as cellular potency in the same peroxide induced cytotoxicity assay (IC_{50} = 45 nM, EC_{50} = 125 nM).[110] The most important features of this compound, however, were high solubility in aqueous buffer and good brain penetration in rodents ($B/P \approx 10$). These two features of **72** allowed the compound to be dosed systemically for the treatment of stroke. In a transient MCAO model, **72** reduced infarct volume by 44% when dosed at 40 mg/kg prereperfusion, and in a permanent occlusion model it reduced infarct volume by 18% at the same dose

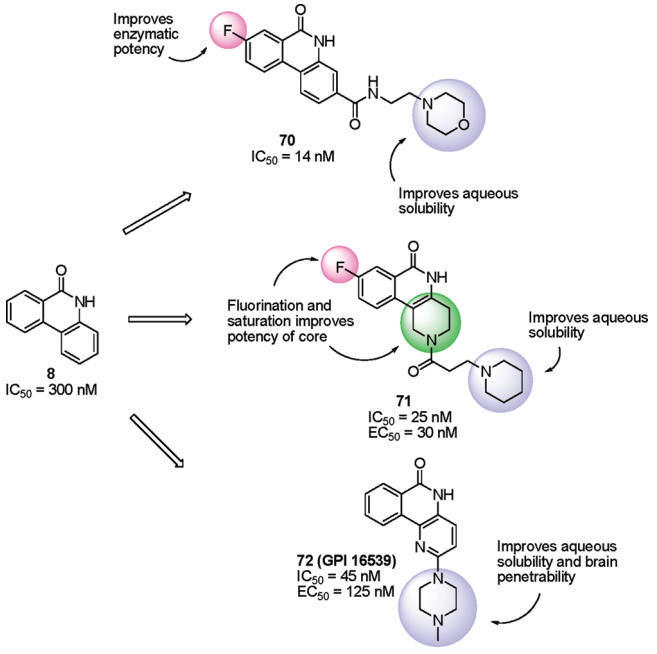

Figure 15. Lead series of phenanthridone based PARP-1 inhibitors designed by Guilford.

level.[58] This preclinical lead also displayed cardioprotective effects in a rat model of cardiac ischemia (25% reduction in infarct at 80 mg/kg).[58] Despite the promising ischemia data and therapeutic potential for **72**, this preclinical lead never advanced into human clinical trials for ischemia.

Another lead series designed by Guilford was the tetracyclic isoquinolones such as GPI-6150 (**28**, IC_{50} = 60 nM, Figure 16) derived from dihydroisoquinolinones.[51] This compound, despite its poor solubility, served as a proof of concept compound for many of the in vitro cellular models of ischemia and in vivo models of inflammation, myocardial infarction, colitis, and stroke.[7,111] In an effort to further improve the structural novelty and solubility of this series, the Guilford group designed several tri- and tetracyclic cores related to **28** such as tri- and tetracyclic phthalazinones (**29** and **40**).[55,112] Once again, their optimization strategy involved attaching a solubilizing group on the southeast portion of the core structure to take advantage of the large hydrophobic adenosine binding pocket of PARP-1. This strategy led to the discovery of GPI 15427 (**73**, IC_{50} = 31 nM), a tetracyclic phthalazinone (Figure 16).[55] Initially developed for ischemic reperfusion injury, **73** reduced total infarct volume in a rat transient MCAO model by 35% when dosed at 40 mg/kg iv prereperfusion. As seen from these in vivo results, the piperazine moiety conferred the aqueous solubility for this core as well as brain permeability. Leveraging this brain penetrability, this lead was also the first compound from Guilford to be used as a chemopotentiator with TMZ in brain cancer.[113] In an intracranial mouse glioblastoma xenograft model, **73**, when dosed systemically 40 mg/kg iv with 100 mg/kg TMZ for 3 days, increased life span by 32% over TMZ alone.[113] Despite the vast array of data and therapeutic potential for **73**, this preclinical lead never made it into human clinical trials for ischemia or cancer.

In late 2005, MGI Pharma, an oncology based company, acquired Guilford Pharmaceuticals. This acquisition provided the means and expertise to develop Guilford's latest preclinical lead (**74**) as a chemopotentiator with TMZ for brain cancer. Although the structure for this compound has not been disclosed, it is known to be structurally similar to **73**.[114] Compound **74** displayed significant chemopotentiating activity in a murine leukemia model in combination with cisplatin. When dosed 15 min pre-, 3 h post-, and 6 h postcisplatin,**74** (40 mg/kg ip) increased the life span by 160% compared to cisplatin alone. In addition to this chemopotentiating ability, the compound demonstrated

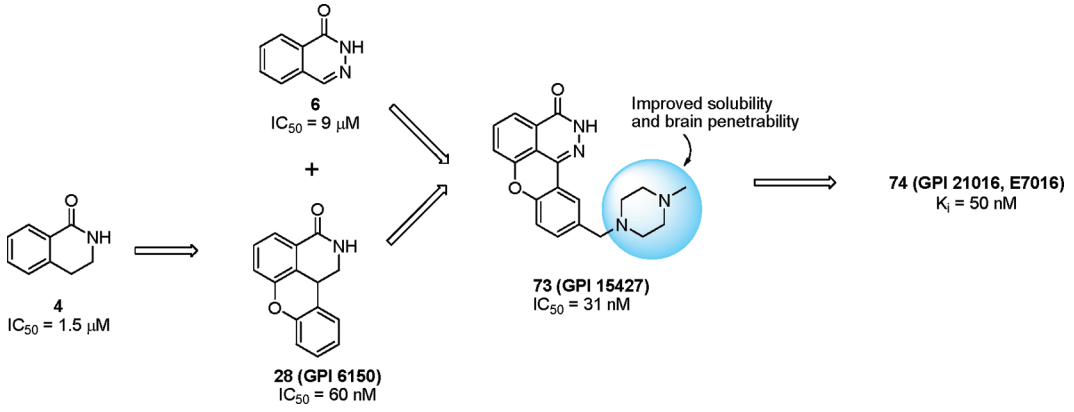

Figure 16. Tetracyclic PARP-1 inhibitors designed by Guilford.

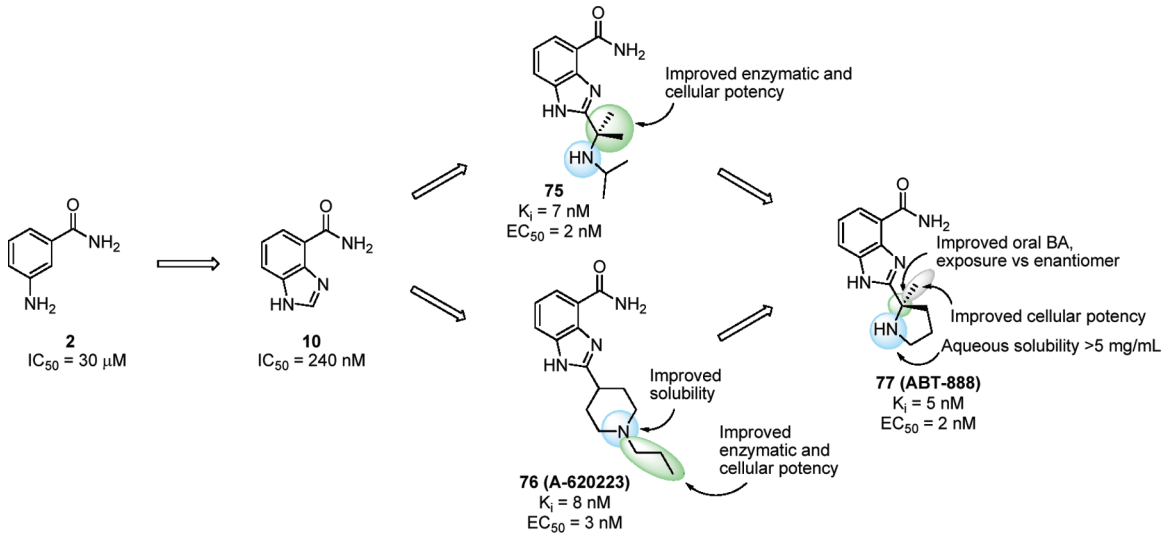

Figure 17. Discovery of Abbott's clinical candidate.

neuroprotective effects by restoring 82% of the nerve conduction velocity deficit caused by cisplatin.[115] A recent publication demonstrated the ability of **74** to act as a chemo- and radiosensitizer in vivo in a glioblastoma xenograft model.[114] Compound **74** (40 mg/kg po), when given in combination with radiation (4Gy) and TMZ (3 mg/kg), provided a 32% increase in life span over the TMZ/radiation group. This model verifies that **74** is effective in a system that is relevant to the current clinical standard of care for glioblastoma (i.e., TMZ plus radiation). These data indicate that the clinical direction for this compound will most likely be treatment of glioblastoma. In 2009, Eisai pharmaceuticals acquired MGI Pharma with plans to continue the development of **74**.

BASF/Abbott: ABT-888 (77, Veliparib). The BASF group, led by Wilfried Lubisch, entered into the PARP-1 field toward the end of the 1990s. This group's first PCT publications on PARP inhibitors were for the treatment of ischemic conditions covering a wide range of core structures including indole carboxamides (**22**),[67] phthalazines (**6**),[116] imidazopyridines (**25** and **26**),[117] and most importantly, 2-heteroaryl and 2-alkylamino benzimidazoles (**10**, X = NH),[118] the eventual series from which Abbott's clinical candidate evolved several years later. The BASF group made several hundred compounds culminating in four series of PARP-1 inhibitors and presenting several examples with single digit nanomolar inhibitory potency. Despite not having disclosed a defined clinical candidate from their research efforts, BASF managed to build a solid PARP-1 intellectual property position from which Abbott Laboratories could expand after the acquisition of BASF's pharmaceutical division in 2001.

The Abbott group, led by Thomas Penning has been perhaps one of the most efficient PARP-1 medicinal chemistry groups over the past 5+ years with the filing of over a dozen composition of matter patents with several hundred analogues as potent PARP-1 inhibitors. Attracted by the size and potency of the benzimiazole carboxamide core (**10**, IC_{50} = 240 nM, MW = 161), the Abbott group aggressively synthesized and characterized several hundred 2-alkylamino derivatives. Their screening paradigm selected compounds with <10 nM enzymatic potency and <10 nM cellular potency (C41 peroxide damaged cellular assay)[119] before advancing them into further in vivo studies. This strategy led to the identification of two closely related preclinical candidates **75** (K_i = 7 nM)[120] and A-620223 (**76**, K_i = 8 nM, Figure 17).[119]

The key discovery during the identification of **75** was that a tertiary carbon in the 2-position of the benzimidazole ring system was beneficial for both enzymatic potency and cellular efficacy. This feature would be utilized in the design of the clinical candidate. Compound **75** had a relatively short iv half-life across species (0.6 h in mice and monkeys to 2.8 h in dogs) and exhibited variable oral bioavailability over the same species (12.6% in monkeys to 82% in mice). Perhaps the variability in the PK parameters prevented this compound from advancing into human clinical trials, but the structural homology to the eventual clinical candidate is remarkable (Figure 17). A similar optimization strategy led to the discovery of **76**. As several other PARP-1 medicinal chemistry groups discovered, having a secondary or tertiary amine provided adequate solubility for the core structure (>5 mg/mL for **76**), and in this case, it improved the cellular penetration and potency in a peroxide induced DNA damage cellular assay (EC_{50} = 3 nM). Compound **76** displayed good oral bioavailability across species (32−82%) and terminal elimination half-lives of 1.2−2.7 h in the same species. This compound demonstrated chemopotentiation of TMZ (74−83% tumor growth inhibition vs 62% for TMZ alone) in a B16F10 melanoma model at 1 (mg/kg)/day over 14 days. In addition, this compound potentiated the effect of cisplatin in an MX-1 breast cancer tumor model albeit at higher doses.

The structure of Abbott's clinical candidate (**77**, K_i = 5 nM, EC_{50} = 2 nM), was disclosed in early 2007[121] followed by the medicinal chemistry summary in early 2009.[122] As this group discovered from previous series, the tertiary carbon adjacent to the benzimidazole ring was necessary for cellular efficacy and enzymatic potency. Consistently, compounds with this feature were 2−13 times more potent in the C41 cellular assay. While the enzymatic potency of **77** and its (*S*)-enantiomer were identical (K_i = 5 nM), stereochemistry played an important role in both the oral bioavailability and exposure of the compound leading to the selection of the (*R*)-enantiomer as the clinical candidate. This drug displayed excellent oral bioavailability across species (56−92%) and a comparable terminal half-life to their other preclinical leads **75** and **76** (1.2−2.7 h). In addition, **77** displayed moderate

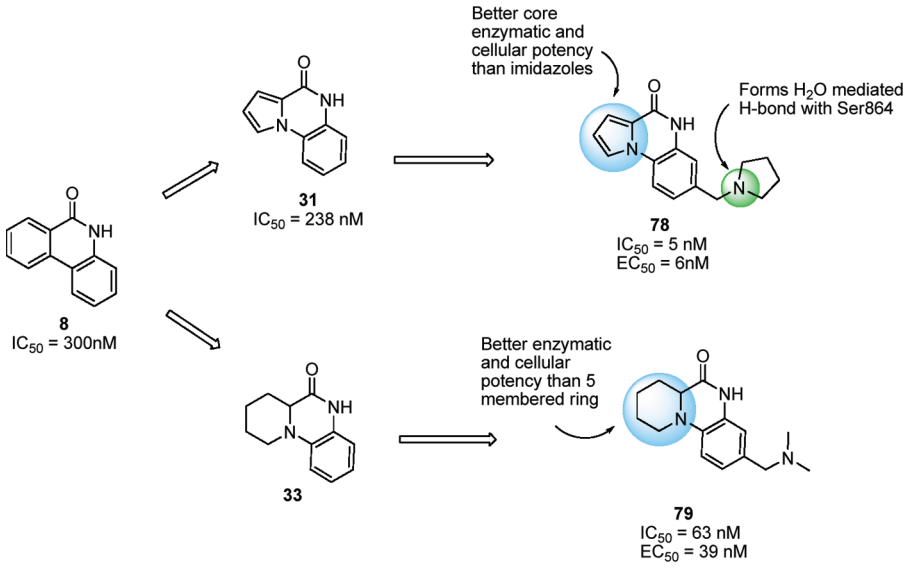

Figure 18. Current PARP-1 inhibitors from Abbott.

brain penetration (~1:3 brain/plasma ratio in rodents), a factor that influenced the eventual clinical path and its potential use in treating brain cancer. Compound **77** demonstrated excellent chemopotentiation in preclinical xenograft models. In a B16F10 melanoma model, **77**, administered orally in combination with TMZ, demonstrated a 43−64% tumor growth inhibition at 1, 5, and 12.5 (mg/kg)/day. Chemopotentiation was also observed in combination with carboplatin in an MX-1 breast cancer tumor model.[122]

In 2006, Abbott entered into the first ever phase 0 human clinical trial with **77**. Under the Exploratory Investigational New Drug Guidance of the FDA, a phase 0 study involves administering low, nontoxic doses for short periods to a limited numbers of patients. This type of trial requires less extensive toxicology and preclinical data than traditional phase I studies; hence, the first in human studies can be performed earlier than normal. The purpose of this study was to obtain biochemical, pharmacokinetic, and pharmacodynamic data to guide the design of subsequent phase I trials. The primary end point was inhibition of PARP in PBMCs and tumor biopsies. When dosed orally, **77** was rapidly absorbed (peak plasma levels at ~1 h) and a large quantity of the drug was cleared unchanged in the urine (~70% in 24 h). However, the drug demonstrated good oral bioavailability, was well tolerated, and provided statistically significant inhibition of PAR levels for up to 24 h in tumor biopsies and peripheral blood mononuclear cells at the highest doses tested (25 and 50 mg).[123] The study was completed 5 months after initiation and supported a twice daily schedule for administration in further clinical studies.

Abbott is currently involved in several phase I studies to assess the safety of **77** in combination with topotecan/carboplatin in leukemia (NCT00588991) and mitomycin C and cyclophosphamide in solid tumors (NCT00810966 and NCT 01017640). In addition, Abbott is supporting a phase I study assessing this lead PARP inhibitor in patients with BRCA1/2 mutations (NCT00892736). Leveraging the brain penetrability of this drug, Abbott is also currently supporting two phase I trials to treat brain cancer. One trial is testing the ability of TMZ/**77** combination to treat children with recurring brain tumors, and the other is testing the drug in combination with whole brain radiation therapy (NCT00994071) for patients with brain metastases (NCT00649207). In addition, **77** is in phase II studies with TMZ in metastatic breast cancer (NCT 01009788) and metastatic melanoma (NCT00804908, expected completion date of 2010).

The Abbott medicinal chemistry group is still active in the PARP-1 field as noted from their recent publications (Figure 18). A series of quinoxalinones (**31**, IC_{50} = 238 nM)[60] demonstrated that the classic benzamide core of phenanthidones could be replaced with heterocyclic arylamides. This strategy was attempted with moderate success by Bayer, Fujisawa, and Guilford (vide infra). The Abbott group demonstrated that this series bound to the PARP-1 nicotinamide subsite through cocrystal studies. The lead compound from this series was compound **78**[60] which displayed excellent enzymatic potency (K_i = 5 nM) and cellular (EC_{50} = 6 nM) potency due to an additional interaction between the pyrrolidine nitrogen and a water mediated hydrogen bond with Ser864 (green, Figure 18) contributing to the binding potency. Although unique, saturated derivatives of the A ring such as **79** (IC_{50} = 63 nM, EC_{50} = 39 nM)[60] were not as potent as the aromatic pyrrole **78**, a trend that was noted in a similar series of saturated phenanthridones.[59]

KuDOS/Maybridge/AstraZeneca: KU 59436 (82, AZD2281, Olaparib). The KuDOS/Maybridge group started their fruitful PARP collaborations in the early 2000s. With Maybridge's extensive compound collections and KuDOS medicinal chemistry group, the two companies discovered several lead series via HTS. Not surprisingly, these hits consisted of many of the usual bicyclic amides (Figure 19) discovered by Banasik in the early 90s. Many of these hits were validated by earlier work, and for this reason, KuDOS rapidly developed SAR by substitution on the positions most likely to lead to potent PARP-1 inhibitors. KuDOS/Maybridge filed a flurry of patents on 3- and 4-substituted isoquinolinones (**3**),[124] 4-substituted phthalazinones (**6**),[125] and 2-substituted quinazolinones (**7**),[126] from 2000 to 2003 (Figure 19).

The bicyclic ring systems were distilled down into a focused medicinal chemistry optimization of phthalazinones. Initial attempts at optimization of phthalazinones resulted in compounds with good PARP-1 inhibitory potency

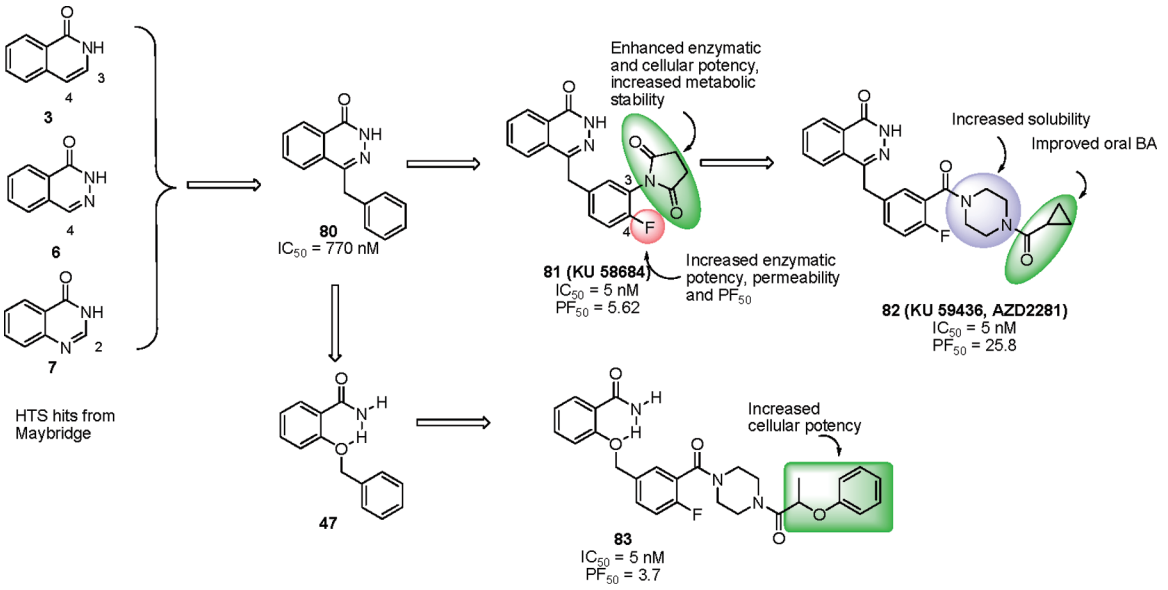

Figure 19. Evolution of KuDOS' clinical candidate.

but poor cellular activity. KuDOS was able to overcome this liability by optimization of benzyl substituted phthalazinones such as **80** with IC_{50} = 770 nM.[127,128] Within this series KuDOS discovered compounds with the desired balance of physicochemical properties, potency (< 10 nM), cytotoxicity potentiation in vitro (PF_{50} > 2), metabolic stability, pharmacokinetics (oral bioavailability), and the ability to potentiate the toxicity of various anticancer chemotherapies in vivo. All of these elements were addressed in the KuDOS screening paradigm over the course of their medicinal chemistry efforts. Initial improvement of this core was accomplished by addition of substituents in the 3 and 4 positions of the benzyl ring. The lead compound KU 58684 (**81**, Figure 19)[127] was the result of early optimizations. Structural features of this molecule include the fluorine and imide groups which together contributed to improved enzymatic potency (IC_{50} = 5 nM) and increased metabolic stability (human hepatic microsomes, Cl_i < 1 mL min^{-1} g^{-1}). In addition, **81** potentiated methyl methane sulfonate cell, killing 5.6 times (i.e., PF_{50} = 5.6 = (IC_{50} growth curve for MMS)/(IC_{50} growth curve of PARP-1 inhibitor plus MMS)).[127] As mentioned above, the KuDOS group was one of the first groups to establish the cytotoxic potential of PARP-1 inhibitors as single agents in cell lines that are deficient in certain DNA repair pathways. This preclinical candidate appeared in the seminal *Nature* paper describing the effects of PARP-1 inhibitors in cell lines deficient in BRCA1 and BRCA2.[36] In a BRCA2 deficient xenograft model, **81**, when dosed twice a day ip at 15 mg/kg, dramatically blocked tumor growth. This compound acted as a stepping stone for what would eventually be the clinical candidate (**82**).[129]

The necessity for oral bioavailability in the clinic took the medicinal chemistry efforts toward compounds that fit within "druglike" parameters predictive of oral bioavailability (MW < 550, PSA < 140 Å, rotatable bonds < 7, HBD and HBA < 10, solubility of > 0.1 mg/kg).[130] After an extensive effort, optimization of the benzylphthalazinone series culminated in **82** (Figure 19). The diacylpiperazine moiety of this molecule (light-blue) maintained the enzymatic potency (IC_{50} = 5 nM) and improved the cell kill potentiation (PF_{50} = 25.8) while improving the solubility over **81** (> 0.1 mg/mL).[129] The cyclopropyl group (light-green) conferred oral bioavailability in mice, rats, and dogs greater than the methyl, ethyl, or isopropyl analogues. The cellular potency of **82** was established in several BRCA deficient cell lines, confirming this group's earlier work. In addition to the in vitro effects as a single agent, **82** displayed significant efficacy in vivo as a potentiating agent for TMZ in mice xenografts equating to over 80% tumor growth inhibition throughout the terminal phase of the study. The drug was well tolerated in vivo and advanced into clinical trials in 2005. In early 2006, AstraZeneca acquired KuDOS Pharmaceuticals to strengthen its portfolio of anticancer therapies and to further accelerate the development of **82**. The phase I results for **82** indicated that the drug when dosed orally was rapidly absorbed and eliminated but still inhibited PARP in samples of PBMCs and tumor tissue. More importantly, $^1/_3$ of the patient population of this study were carriers of the BRCA mutation, and the majority of this subgroup (~60%) responded to treatment with this PARP inhibitor.[131] AstraZeneca has just completed two phase II studies for the treatment of BRCA positive advanced breast cancer (NCT00494234) and ovarian cancer (NCT00494442). The interim results from this phase II study indicate that there is a 33% response rate from BRCA1/2 carriers at the highest dose tested (400 mg/kg po). In addition, the clinical benefit rate (CBR = objective response rate and decline in CA-125 cancer biomarker) was determined to be 57.6% for this same group with mild toxicities. The results from these trials were recently reported at the latest ASCO meeting.[3,132,133]

In addition to treating patients with BRCA mutations, the AstraZeneca group has established an aggressive clinical program to use **82** as a chemopotentiator. AstraZeneca either has recently finished or is currently testing **82** in multiple phase I trials to assess its safety profile when dosed orally with various anticancer agents such as doxorubicin (NCT00819221), carboplatin (NCT00516724), gemcitabine (NCT00678132), dacarbazine (NCT00516802), topotecan (NCT00516438), and irinotecan (NCT00535353).

The KuDOS/AstraZeneca medicinal chemistry group recently published a new series of 2-benzyloxybenzamides (**47**, Figure 19) as a novel PARP-1 inhibitor scaffold.[71]

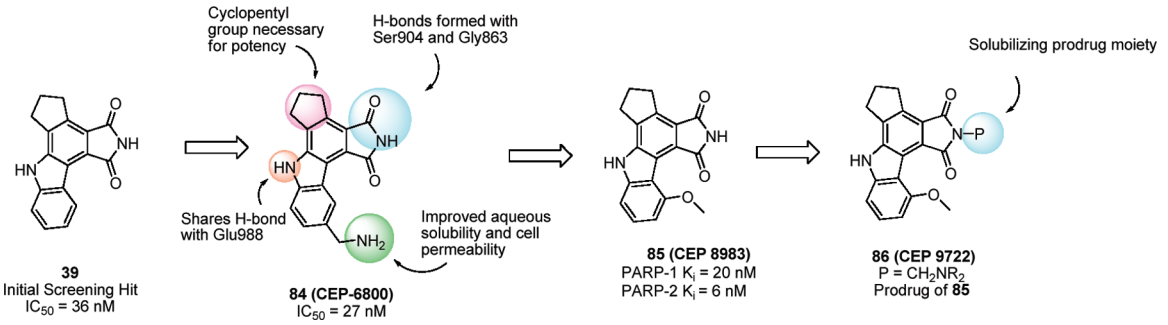

Figure 20. Discovery of CEP-9722.

The intramolecular hydrogen bond formed between the benzyloxy group and the primary amide forms a "pseudocycle" similar to benzimidazole carboxamides, locking the amide in the desired conformation for PARP-1 inhibition. Using knowledge accumulated from optimization of the phthalazinones, KuDOS conducted many of the same steps to arrive at their next lead compound. Compound **83** (IC_{50} = 5 nM)[71] bears many of the same moieties as **81**, with a notable exception of the phenoxyalkyl group (green, Figure 19). This group, however, was necessary to enhance the cellular potentiation factor (PF_{50} = 3.7) over other alkyl groups (i.e., cyclopropyl) that were successful in the phthalazinone series. Despite the relatively low potentiation factors for this series, the inherent PARP-1 inhibitory potency is promising and bodes well for the next generation of KuDOS compounds.

Cephalon: CEP-9722 (86). The first patent publications from Cephalon appeared in 2001. Similar to some of the earlier medicinal chemistry programs at the time (KuDOS/Maybridge, Fujisawa), this group conducted a high throughput screen to discover a structurally unique PARP-1 inhibitor core. The series of pyrrolocarbazole lactams (**39**, Figure 20) incorporates an arylamide core but one that is heavily substituted, a characteristic not seen among many of the first prototypic PARP-1 inhibitors. Compound **39** was very potent (IC_{50} = 36 nM, K_i = 5 nM) and much of the analoging actually diminished the enzymatic activity. Consequently, the size of the fused cyclopentane ring was critical for potency as the cyclohexyl, phenyl, and furyl groups caused a loss of inhibitory potency (pink, Figure 20).[49] The proposed binding mode for this series included the standard conserved web of H-bonds from Ser904 and Gly863 with the lactam (light-blue, Figure 20). In addition, the indole N−H formed an H-bond with Glu988 (light-orange, Figure 20). Further optimization of this core was performed by addition of an aminomethyl group (light green) to the 3-position of the indole (**84**, CEP-6800, Figure 20).[134] This substituent significantly improved the cellular potency in a PC12 cellular assay[135] as well as improved the aqueous solubility (0.1 mg/mL) of an otherwise insoluble core.

Compound **84** was tested extensively in vitro displaying a robust ability to potentiate TMZ, irinotecan, and cisplatin against U251MG glioblastoma, HT29 colon carcinoma, and Calu-6 small cell lung carcinoma cell lines, respectively.[136] The preclinical lead increased the amount and duration of DNA damage caused by these agents, further supporting the importance of PARP-1 in the DNA repair of these cell lines. This lead compound also performed well in vivo in xenograft models. The best results obtained were achieved by a combination of **84** (30 mg/kg sc, q.d., 5 days) and TMZ (34 mg/kg po, q.d., 5 days), displaying a 100% complete regression against U251MG tumors by day 28 versus 60% regression with TMZ alone. Despite the impressive package of preclinical data for this compound, **84** never made it to human clinical trials because of its myelotoxic effects.[137]

Cephalon further optimized this series to produce **85**, a 20 nM PARP-1 inhibitor from the same series (Figure 20).[134] The notable difference between **84** and **85** is the lack of solubilizing group, indicating the diminished aqueous solubility of **85**. Despite this fact, **85** was just as effective at sensitizing tumor cell lines (RG2, RH18, HT29, and NB1691) in vitro in combination with TMZ and camtothecin.[137] More importantly, **85** did not display any myelosuppresive effects alone nor did this compound exacerbate the myelosuppression of TMZ and camptothecin in a human bone marrow cell assay. The limiting factor for this PARP-1 inhibitor was its aqueous solubility. Many of the first and second generation PARP-1 inhibitors suffered from a lack of solubility, as would be expected from compounds with extended, fused aryl ring systems. Despite this problem, Cephalon was the first group to successfully derivatize a PARP-1 inhibitor core to a soluble prodrug (**86**), the eventual clinical candidate and a prodrug of **85**. While the structure of this compound has not been disclosed, one can intuit from Cephalon's latest patent filing that either the amide or the indole was modified with a methyldialkylamino group (Figure 20, P = CH_2NR_2).[138] A compound of this nature could form an amine salt, solving the aqueous solubility issue, while at the same time, it would be labile enough to chemical hydrolysis upon dosing/dissolution. Compound **86** demonstrated significant tumor growth inhibition in vivo when dosed sc (136 mg/kg/dose, q.d.) with TMZ (68 mg/kg/dose, po, q.d.) versus TMZ alone.[137] Unexpectedly however, **86** also showed some stand-alone efficacy against RG2 and HT29 tumors without TMZ. Cephalon hypothesized that **86** may interact with an angiogenesis pathway, accounting for the stand-alone efficacy. This antiangiogenesis effect has been noted with several other PARP-1 inhibitors including **66** and **77**.[12] While the molecular mechanism by which PARP-1 inhibitors are affecting angiogenesis is unclear, PARP-1 activity has the ability to modulate the expression of genes that are involved in angiogenesis, particularly hypoxia inducible factor-α (HIF-α).[139] It is generally accepted that the PARP-1 pathway must be intact in order to have proper angiogenic network development. Regardless of the mechanism of action, Cephalon initiated a phase I clinical trial with **86** in May 2009 to evaluate the safety, pharmacokinetics, and pharmacodynamics as single-agent therapy and as combination therapy with TMZ in patients with advanced solid tumors (NCT00920595).

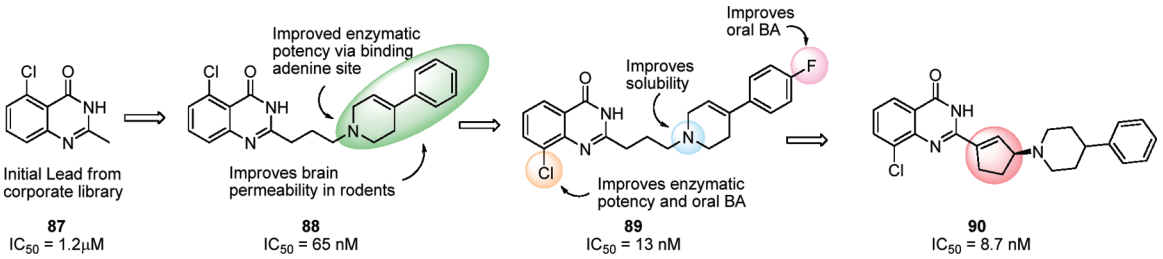

Figure 21. Lead PARP-1 inhibitors discovered by Fujisawa.

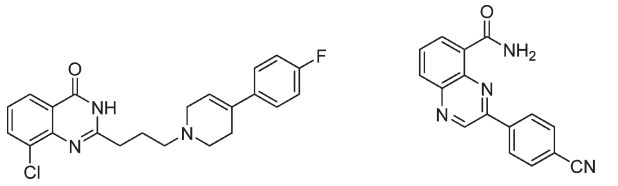

Figure 22. PARP-1 and PARP-2 selective inhibitors by Fujisawa.

Yamanouchi/Fujisawa/Astellas. Since the early 2000s, the Fujisawa group has pursued the neuroprotective effects of PARP-1 inhibitors. Similar to Guilford, the focus of their medicinal chemistry optimization efforts was brain penetration. Figure 21 outlines the evolution of Fujisawa's PARP-1 inhibitors starting with the chloroquinazolinone **87**, an HTS hit from their corporate library.[78] This quinazolinone core, however, did not possess acceptable PARP-1 potency or brain/plasma ratio (IC_{50} = 1.2 μM, B/P = 0.82). Optimization of this series of quinazolinones was carried out by substitution at the 2-position to build in the desired pharmacological characteristics. Optimization efforts led to compound **88** with 65 nM potency and a B/P ratio of 5.8 by using the 4-phenyl-1,2,3,6-tetrahydropyridine moiety as a lipophilic tail. As it turned out, this lipophilic amine not only provided adequate brain permeability in rodents when ligated to other PARP cores[140] but also had unique binding properties in the adenosine−ribose binding site similar to compound **53** (Figure 6C). The nitrogen atom in the tetrahydropyridine moiety forms a hydrogen bond to the carboxylate side chain of Asp766 within the AD site.[78] Further optimizations such as fluorination of the side chain aryl ring and moving of the chlorine atom led to an orally available, brain penetrable lead PARP-1 inhibitor (**89**, Figure 21). The side chain was further optimized to conformationally restrict the side chain and to improve the inhibitory potency while maintaining the B/P ratio (**90**, IC_{50} = 8.7 nM, $B/P \approx$ 2−3).[141]

The Fujisawa group has also dedicated some medicinal chemistry efforts to the selective inhibition of PARP-2.[142] As mentioned above, PARP-2 is the only other member of the PARP family with a DNA binding domain. The PARP-2 catalytic domain also has a high degree of homology with PARP-1. This fact implies that finding selective inhibitors for PARP-1 and PARP-2 is quite challenging. In fact, this group was the first to discover both selective PARP-1 and PARP-2 inhibtors with nanomolar potency and brain penetration as shown in Figure 22. Compound **89** from their quinazolinone series displayed a 39-fold selectivity over PARP-2 due mainly to the side chain interaction within the adenosine binding site of PARP-2 (i.e., Leu769 in PARP-1 is replaced with Gly314 in PARP-2). Consequently, compound **91** from the quinoxaline series displayed ~10-fold selectivity for PARP-2 over PARP-1.[142] While this selectivity is modest, further optimization of these series will certainly be useful in determining the relevance of PARP-2 in various disease states.

In 2005, Astellas Pharmaceuticals was formed by the union of Fujisawa and Yamanouchi Pharmaceuticals. This merger combined two companies with mature PARP-1 programs. Despite having several brain penetrable leads, Astellas has made no indication that they are developing a PARP-1 inhibitor nor does Astellas have any composition of matter patents or publications since 2007 on the subject.

Kyorin: KCL-440 (93). In the early 2000s the Kyorin group designed a series of isoquinolinone based PARP-1 inhibitors for the potential treatment of cerebral ischemia. The lead compound from this series was optimized from a screening hit **92** (IC_{50} = 890 nM) that was 10 times more potent than the isoquinolinone core. Similar to the Guilford and Fujisawa programs, optimization paradigms emphasized brain penetration and increased solubility of the core with a tertiary amine (light-blue, Figure 23). Preclinical candidate **93** evolved from this series with an IC_{50} value of 68 nM (K_i = 9.8 nM) against PARP-1 and an EC_{50} of 73 nM in the peroxide induced cytotoxicity assay in cortical cultures.[143] In rats, **93** displayed a brain/plasma ratio of 0.8 after a 6 h infusion of 10 (mg/kg)/h, an acceptable level to utilize in vivo. The Kyorin group designed their animal studies to replicate a realistic clinical scenario, paying particular attention to PARP activation over the course of the ischemic event. With this information the Kyorin group was able to obtain a therapeutic window for their lead candidate. In rats, the peak cerebral PARP activity was determined to be 6 h after the ischemic event, tapering off to control levels after 24 h as determined by immunostaining. In a transient MCAO model, **93** demonstrated protective effects similar to many of the lead candidates from other programs at the time (57% reduction at 3 (mg/kg)/h) when dosed preischemia. Reduction in infarct volume was also observed when **93** was dosed 2 h (50%) and 4 h (47%) after the ischemic event but not at 6 h.[144] In a photothrombotic MCA model of stroke, a more stringent model, **93** showed a significant decrease (~10−15%) in infarct volume when dosed 1 h after the ischemic event at several dose levels (0.1−3 (mg/kg)/h) but failed to show efficacy when dosed 2 or 3 h after the occlusion.[143] Despite having a brain penetrable preclinical lead showing efficacy in many relevant preclinical models, the Kyorin group never progressed into the clinic with their PARP program.

Mitsubishi: MP-124 (96). Along with Kyorin and Fujisawa, the Mitsubishi group's first PARP-1 patent applications

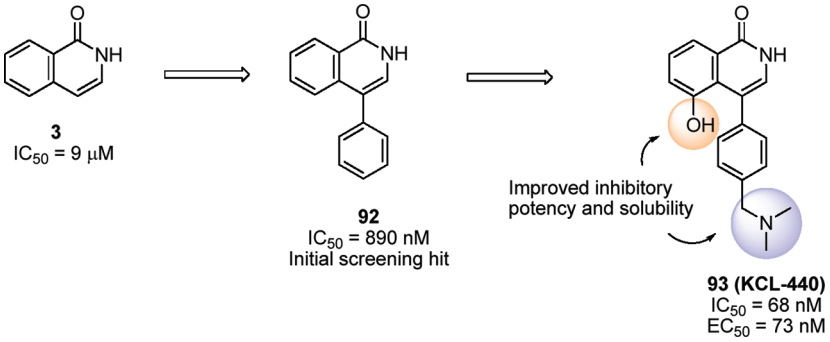

Figure 23. Design of Kyorin's preclinical candidate.

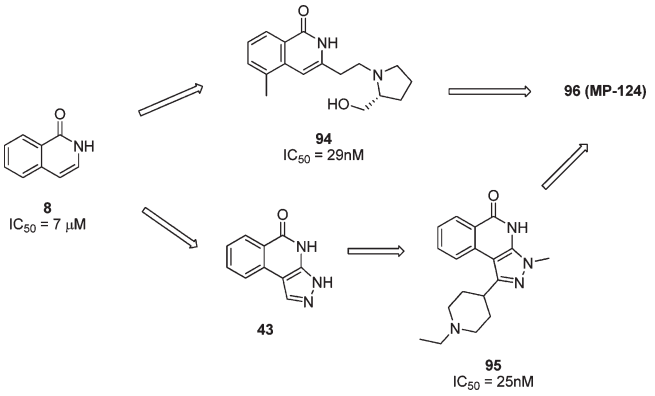

Figure 24. Lead PARP inhibitors designed by Mitsubishi.

appeared in the early mid-2000s. All of this group's medicinal chemistry focused on derivatives of isoquinolinones (Figure 24). The hydrophobic substituents such as the pyrollidino alcohol in **94**[145] improved the enzymatic potency of the core by over 2 orders of magnitude (IC_{50} = 29 nM). This group also discovered a series of pyrazoloisoquinolinones (**43**, X = N, Figure 24) such as lead compound **95** (IC_{50} = 25 nM).[62] After 7+ years of PARP-1 related research, Mitsubishi has one clinical candidate PARP-1 inhibitor (**96**, structure undisclosed) for cerebral ischemia. This compound has demonstrated efficacy in a primate model of MCAO (22−64% reduction in infarct volume when dosed iv at 0.3, 1, and 3 (mg/kg)/h). In addition, the primate study indicated that there is a significant therapeutic time window, as **96** displayed this reduction in infarct volume when dosed at 3 h and 6 h after the permanent occlusion. The lead PARP inhibitor also significantly improved many of the neurological deficits resulting from the ischemic event.[146] The company's pipeline indicates that **96** is in phase I clinical development, but no trials have been registered within the U.S. Should this phase I trial commence, Mitsubishi would be the only pharmaceutical company with a PARP-1 inhibitor in the clinic for ischemic injury and would be the first to initiate a stroke clinical trial with a PARP inhibitor.

Merck: MK-4827 (101). The first PARP patents and publications from Merck appeared in 2009. This group identified several novel series of PARP-1 inhibitors including the pyrrolodihydroisoquinolinones (**49**),[52] the pyrazoloquinazolinones (**30**),[56] and a series of indazole carboxamides (**23**) from which their clinical candidate (**101**) was derived.[68] This clinical candidate evolved from a series of heterocyclic benzamides related to benzimidazole carboxamides (Figure 25). An initial optimization paradigm selected the subseries with the best pharmacokinetics and enzymatic and cellular potency. Merck designed four closely related subseries from which to select: the triazolobenzimidazoles such as **97** (IC_{50} = 71 nM), the imidazo- and triazolopyridine carboxamides **98** (IC_{50} = 55 nM) and **99** (IC_{50} = 270 nM), and the indazole carboxamides **100** (IC_{50} = 24 nM).[68] Core compound **98** demonstrated better potency than any of the other series tested as well as cellular potency showing the ability to inhibit PAR polymers after induction of DNA damage by peroxide in HeLa cells (EC_{50} = 3.7 μM). In addition, **98** demonstrated moderate stability in rat and human microsomes and acceptable oral BA (41%) and terminal half-life (5.1 h) in rats. Optimization of the indazole core was accomplished by incorporating a solubilizing group on the para position of the aryl ring leading to general improvements in enzymatic and cellular potency. As part of Merck's screening paradigm, cellular potency was evaluated in BRCA1 silenced HeLa cells for their ability to inhibit cell growth by 50% (CC_{50}) versus BRCA1 wild type HeLa cells. The (*S*)-piperidine moiety of **101** afforded ~28-fold selectivity against BRCA1 silenced cells (CC_{50} = 33 nM) versus wild type (CC_{50} = 860 nM) over the (*R*)-enantiomer (~11-fold). The PK profile of **101** was acceptable with a high volume of distribution (Vdss = 6.9 L/kg), long terminal half-life ($t_{1/2}$ = 3.4 h), and excellent oral BA (65%) in rats. In vivo, **101** demonstrated tumor regression in a BRCA-1 mutant MDA-MB-436 xenograft model orally at 100 mg/kg q.d. or 50 mg/kg b.i.d. with no overt weight loss or signs of toxicity. Merck started a phase I study with oral **101** in 2008 to test the tolerability and PARP inhibitory activity in patients with advanced solid tumors (NCT00749502).

Other PARP-1 Inhibitor Programs. Several other groups have contributed to the field of PARP-1 medicinal chemistry, and some of their lead structures are outlined in Figure 26. In 2002, Novartis introduced indoloquinazolinone (**102**, IC_{50} = 12 nM), a compound with efficacy in a rabbit stroke model (60% reduction in infarct volume when dosed iv prereperfusion).[57] Ono Pharmaceuticals characterized a preclinical lead, ONO-1924H (**103**), a moderately potent PARP-1 inhibitor (IC_{50} = 210 nM) that demonstrated neuroprotective effects by significantly reducing cerebral damage when dosed postocclusion at 10 and 30 (mg/kg)/h.[147] Mochida introduced a series of tricyclic quinazolinones, one example of which is compound **104** with PARP-1 cocrystal data but little in vitro or in vivo data.[148] The Icos/Deltagen group recently published a series of pyrazolopyridinones such as compound **105** (IC_{50} = 7.3 nM) which displayed some submicromolar activity in a cell based chemosensitization assay.[149] Jannsen has recently filed several patents based on ethylquinolinones (**44**, Figure 5). This series

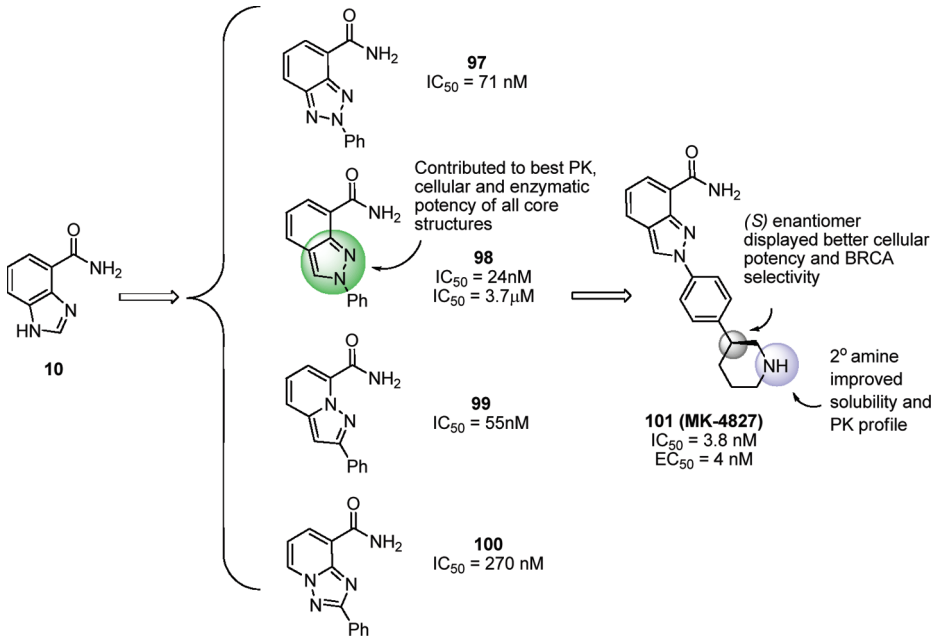

Figure 25. Discovery Merck's clinical candidate PARP inhibitor.

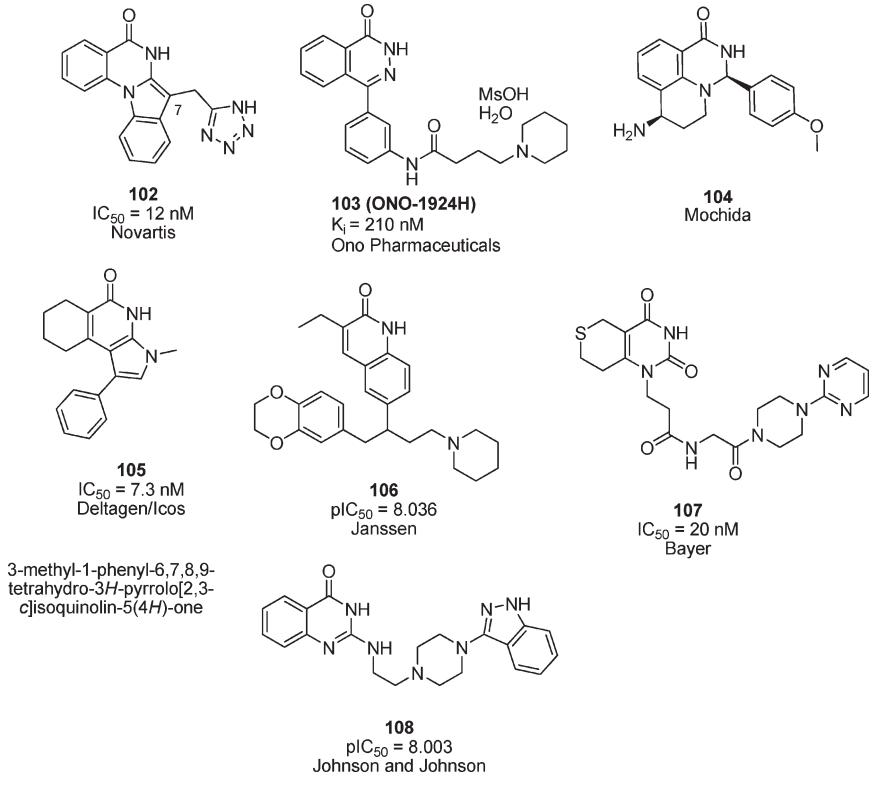

Figure 26. Lead structures from other PARP-1 inhibitor programs.

is a dramatic shift from the typical arylamide core. One such lead compound is derivative **106** (pIC_{50} = 8.036, Figure 26), presumably deriving much of its inhibitory effects from the side chain interactions rather than the ethylquinolinone core.[63] The Bayer group discovered a series of saturated quinazoline diones from which the lead compound **107** (IC_{50} = 20 nM) was derived.[53] Johnson & Johnson recently published a series of 2-substituted quinazolinones with elaborate side chains, the most potent of which was piperazine **108** (pIC_{50} = 8.003, Figure 26).[150]

Current Clinical Progress of PARP-1 Inhibitors and Future Directions. Of the ~15 current PARP-1 medicinal chemistry programs (Figure 1), most are focusing on optimization and development of PARP-1 inhibitors as anticancer agents. The slight decline in PARP-1 medicinal chemistry programs over the past few years can be explained by both corporate mergers (e.g., Astellas/Yamanouchi/Fujisawa and BiPar/Sanofi) and a waning interest in pursuing PARP-1 for ischemic injury or inflammation. Perhaps the most compelling reason that PARP inhibitors have not been pursued for

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	*	*	*		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.

inflammation is the potential long-term consequences of accumulation of double-strand DNA breaks in normal tissues of patients receiving chronic PARP-1 inhibitor therapy.[131] For the most part, PARP inhibitors have not been tested clinically for ischemia either, despite several promising preclinical lead compounds. The only group to test a PARP-1 inhibitor in humans for ischemic injury was Inotek for cardiac ischemia. However, they are no longer actively pursuing this indication. This is a testament to the difficulty of this clinical path. There are several challenges associated with development of an ischemia drug: (1) Preclinical animal models often provide a snapshot of ischemic benefit by tissue staining after animal sacrifice. These results may not accurately represent the final extent of the ischemic damage but just a slowing of the damage. (2) Despite the improvement in identification and modulation of clinical biomarkers, the translation into clinical benefit (i.e., reduced mortality or improved function) is lacking. (3) Imaging technology is still not sensitive enough to measure the extent of cell death and smaller infarcts in patients. (4) The patient study population is already in a high risk category for adverse events, increasing the likelihood for drug side effects. (5) Identification of suitable patients that are most likely to benefit from such therapy is difficult. (6) Demonstrating the benefit of a new class of therapeutics requires large sample sizes, as noted with prior clinical attempts at ischemia treatment.[151] These large sample sizes necessitate an enormous financial commitment and subsequently a lack of sponsors for late stage trials. The challenge associated with clinical design and outcome measurements make stroke and cardiac ischemia difficult pharmaceutical targets and account for the paucity of therapeutics available for such a debilitating conditions.[152] This high risk clinical path played a major role in guiding the decision making and clinical indications for many PARP-1 inhibitors that were initially designed for ischemia. Of the several groups with initial interest in ischemia treatment many have either changed therapeutic focus (i.e., Inotek, Guilford) or failed to advance a clinical candidate (i.e., Ono, Kyorin, Astellas, Novartis). Perhaps the last hope for PARP-1 inhibitors to treat ischemia is the Mitsubishi group who seems determined to proceed with a PARP inhibitor in cerebral ischemia. Should the Mitsubishi group succeed in their endeavor, it is likely that many of the PARP-1 inhibitors already in the clinic (or potentially approved) would shortly follow.

Currently, six groups have PARP-1 inhibitors in human clinical trials with two more on the verge of phase I studies (Table 1).[153] The most advanced of these programs are BiPar and KuDOS/Astrazeneca, both entering into phase III studies. Shortly following these groups will be Abbott and Pfizer with PARP-1 inhibitors in late phase II trials. Consequently, all of the current PARP-1 inhibitors are being tested as anticancer agents or chemopotentiators. In addition, the discovery that PARP-1 inhibitors have potential as single agents for tumors with compromised DNA repair machinery has provided a viable and exciting therapeutic course for patients with TNBC and BRCA carriers. This finding is especially important because effective therapy for TNBC patients is of paramount importance to the field.[41]

As cancer treatment moves into the future, clinicians and oncologists are becoming more aware of the genetic components of each patient and specific tumor type. Successes such as Imatinib (BCR-Abl) are a testament to understanding the genetic component of cancer and applying it to the future of cancer chemotherapy.[154] With more specific knowledge of the cancer genotypes, the doctors will be able to treat each patient accordingly. In the future, PARP-1 inhibitors may become the standard of care for patients who have identified tumor types deficient in DNA repair. Synthetic lethality would indicate that PARP-1 inhibitors will show efficacy against many tumors cell types with compromised DNA repair mechanisms, not just BRCA-1 and BRCA-2. While BRCA-1 and BRCA-2 are oncogenes most commonly associated with breast cancer, other tumor types have been described as displaying "BRCAness" as well.[40] Current research has identified the tumor suppressor gene PTEN (phosphatase and tensin homologue) as one of the most commonly mutated genes in cancer.[155] PTEN deficiency causes HR defects in human tumor cell lines leading to sensitivity toward PARP inhibitors. Findings such as this could dramatically expand the therapeutic utility of PARP-1 inhibitors and provide a more selective therapy for this debilitating disease.

Biography

Dana V. Ferraris received his Ph.D. degree in Chemistry from Johns Hopkins University in 1999 and his M.B.A. from Carey Business School in 2009. Over the past 10 years, Dr. Ferraris has held a variety of leadership positions in the pharmaceutical industry at Guilford Pharmaceuticals, MGI Pharma, and Eisai Research Institute. Dr. Ferraris has extensive drug discovery experience in oncology and CNS disorders. His research efforts have resulted in two investigational drugs currently in development for the treatment of cancer. Dr. Ferraris joined Johns Hopkins University as a Principal Scientist in 2009. He oversees medicinal chemistry activities at the Brain Science Institute's NeuroTranslational program with a primary mission of translating discoveries in basic science into novel therapeutics.

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