Enhancing tumor-targeting monoclonal antibodies therapy by PARP inhibitors

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ABSTRACT
Monoclonal antibodies (mAbs) have become a successful therapeutic approach in cancer. However, some patients do not achieve long-term clinical benefit and most mAbs only exert modest effects as monotherapies. Therefore, combinations with chemotherapy are currently being investigated. Emerging studies have shown a synergistic therapeutic effect of PARP inhibitors and mAbs in cancer. PARP enzymes catalytically cleave β-NAD⁺ and transfer the ADP-ribose moiety to acceptor proteins, modifying their function. In here, we update recent data about the therapeutic effect of the combination of PARP inhibitors with mAbs in cancer treatment and discuss the molecular mechanisms involved in this synergy.

INTRODUCTION
Tumor-targeting mAbs have proven to be one of the most successful therapeutic approaches in cancer acting through several effector mechanisms including steric inhibition and neutralization, complement activation, alteration of signaling downstream of their targets, activation of cell-mediated cytotoxicity, and blockade of immunologic checkpoints. Currently, around 20 mAbs have already been approved or are under review in the EU and USA for therapeutic use in cancer patients. However, in spite of remarkable clinical responses to mAb therapy, some patients do not obtain long-term clinical benefit and most mAbs only exert modest effects as monotherapies. Moreover, several tumors remain largely refractory to approved mAb therapies. To overcome these limitations, different approaches are being explored in pre-clinical and early-phase clinical trials, such as combinations strategies with radiotherapy and chemotherapy to improve antitumor effectiveness of mAbs. Emerging studies in human cancer cell lines, xenograft models and early clinical trials have shown a synergistic effect of tumor-targeting mAb treatment and poly(ADP-ribose) polymerase (PARP) inhibitors. The aim of this review is to update recent data on the potential therapeutic effect of the combination of PARP inhibitors with mAbs in cancer and discuss the molecular mechanisms that could be involved in this synergy. Understanding the biological impact of this therapeutic combination might provide invaluable clues to the rational development of new therapeutic approaches in cancer.

POLY(ADP-RIBOSYLATION): A TRANSIENT POST-TRANSLATIONAL MODIFICATION OF PROTEINS WITH PLEITROPIRIC BIOLOGICAL EFFECTS
Poly(ADP-ribosylation) (PARylation) is a post-translational modification of proteins in which ADP-ribose moiety from β-nicotinamide-adenine-dinucleotide (β-NAD⁺) molecules is transfer to acceptor amino acid residues of target proteins by members of the PARP family of enzymes, creating long chains of poly(ADP-ribose). This protein modification, first discovered over 50 years ago, is a dynamic process as indicated by the short half-life of the ADP-ribose polymer, which is rapidly exposed to degradation by the poly(ADP-ribose) glycohydrolase (PARG) and poly(ADP-ribose) hydrolases enzymes (Fig. 1). Even though the PARP family comprises 17 enzymes, currently only six of them (PARP-1, PARP-2, PARP-3, PARP-4, PARP-5A and PARP-5B), can be considered as “ bona fide” PARP proteins containing a conserved glutamate (Glu-988 in PARP-1) residue that defines the PARP catalytic activity, while the other family members function as mono(ADP-ribosyl) transferases or their enzymatic activity has not been yet characterized.

KEYWORDS
Apo2L/TRAIL; EGFR; HER2; monoclonal anti-body; PARP; VEGF

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Role of PARylation in the DNA damage response

Cells have developed mechanisms to fight DNA damage, collectively termed the DNA damage response (DDR), which include DNA lesions detection, signaling their presence and promote their repair. In response to DNA breaks, PARP-1, PARP-2 and PARP-3 become catalytically active, targeting mainly proteins involved in chromatin structure and DNA metabolism. This activation results in chromatin decondensation around damage sites, recruitment of repair machineries, and accelerate DNA damage repair. Accordingly, PARylation mediated by these PARP proteins plays a key role in DDR at different steps.

The contribution of PARP-1 and PARP-2 to the resolution of single-strand breaks as key players of the single strand break repair/base excision repair (SSBR/BER) pathway has long been recognized. However, the contribution of PARylation to double-strand breaks (DSB) repair, mediated by either homologous recombination (HR) or non-homologous end joining (NHEJ), is less well defined. PARylation may promote DSB repair working as a docking signal to mediate the quick recruitment of DSB repair proteins to the DSB sites and helping to stabilize and retain these proteins at the lesion sites. In addition to the previously mention mechanisms, PARylation mediated by PARP-1 and PARP-2 has also been linked to DDR by promoting genome stability through chromatin remodeling, chromosome segregation, and telomere integrity.

One of the most promising prospects for the future of cancer treatment is the exploitation of deregulated DDR. Accordingly, PARP inhibitors that compete with \( \beta\)-NAD\(^+\) at the highly conserved enzyme active site are arising as new potential therapeutic strategies as chemo- and radiopotentiation and for the treatment of cancers with specific DNA repair defects as single-agent therapies acting through the principle of synthetic lethality. This term describes the process by which defects in two different genes or pathways together result in cell death but independently do not affect viability. One of the best-known examples of exploitation of deregulated DDR by the synthetic lethality approach is based on the induced lethal effects of PARP inhibitors for BRCA1/2-deficient tumors. This proposal is based on the concept that PARP inhibition will increase SSB which eventually lead to DSB via replication fork collapse. The repair of these DSB will be compromised in tumor cells that have lost BRCA1 and BRCA2, critical components of the HR pathway, leading to chromosomal aberrations and instability of the genome resulting in cell death. Accordingly, several compounds targeting PARP have entered in clinical trials in different types of tumors. Although most of the PARP inhibitors are comparable at inhibiting PARP catalytic activity, the trapping of PARP to DNA strand breaks varies depending on the inhibitor, which may affects their toxicity upon cancer cells. Indeed, the PARP inhibitor Talazoparib (BMN-673) is approximately 100-fold more potent at trapping PARP-DNA complexes and, therefore, exhibited more cytotoxicity in vitro, used as single agent and in combination with DNA alkylating agents, than rucaparib or olaparib. Recently, the PARP inhibitor olaparib has been approved by FDA and the European Office of Medicines for the treatment of ovarian cancer with defect in BRCA1 and BRCA2 genes. Still, there is the possibility that PARP inhibitors might also be beneficial in other genetic context and by inducing a BRCAness phenotype which implies HR impairment by mechanisms other than BRCA mutations.

PARylation functions beyond DNA damage response

PARP proteins, through their physical association with, or by the PARylation of their partner proteins, have been shown to play biological functions beyond DDR (Fig. 1). These alternative roles may be exploited to expand the situations benefiting from treatment with PARP inhibitors in combination with other therapeutic interventions such as mAbs. One of the best examples is the role of PARylation in gene transcription regulation which may occur through different mechanisms like its role in chromatin remodeling, binding to specific gene enhancers and promoters, and direct regulation of transcription factors such as NF-kB, and NFAT, or as a combination of these processes.

PARP inhibitors also possess anti-angiogenic properties by decreasing the activity of critical pro-angiogenic factors such as hypoxia inducible factor-1\(\alpha\) (HIF-1\(\alpha\)), vascular endothelial growth factor (VEGF), transmembrane signaling protein syndecan-4 (SDC-4), and platelet/endothelial cell adhesion molecule (PECAM1/CD31). Moreover, PARP inhibitors abrogate cell migration in response to VEGF or placental growth factor (PGF) by inhibiting formation of tubule-like networks, and impaired angiogenesis in vivo. Furthermore, PARP-1 has also been shown to regulate epithelial-mesenchymal transition (EMT), a cellular process characterized by the loss of epithelial markers and acquisition of a mesenchymal phenotype which enables cancer cells to invade surrounding tissue and generate...
PARP-1 is regulating EMT by its regulatory effect on key inducers of this cellular conversion like vimentin and Snail1.29-31

Otherwise, different types of cell death processes such as apoptosis, parthanatos, necroptosis and autophagy have also been shown to be modulated by PARylation,32 but their full description lies beyond the limits of the current review. Furthermore, the impact of PARP inhibitors on mitochondrial respiration regulation and energy homeostasis may also have potential clinical implications in cancer.33,34

**Synergistics therapeutic effects of PARP inhibitors and monoclonal antibodies in cancer**

Recent preclinical evidence suggests that PARP inhibitors can be used in combinations with mAbs for synergistic therapeutic effects without overt toxicity. Accordingly, different clinical trials have already started including antibodies that modulated DNA repair pathways, or target angiogenesis, or induce death-signaling pathways in combination with PARP inhibitors (Table 1).

**PARP inhibitors and monoclonal antibodies targeting EGFR**

The epidermal growth factor receptor (EGFR) belongs to the ErbB family of tyrosine kinase protein receptors. This family includes four members: EGFR (HER1), HER2, HER3 and HER4.35 EGFR become activated upon ligand binding (either TGFα or EGF), leading to down-stream activation of different pathways that influence a variety of biological processes such as cell proliferation, apoptosis, angiogenesis, cell migration and transcription regulation.35 In addition, EGFR activation pathways have also been implicated in DNA repair (Fig. 2A). EGFR has been shown to regulate both HR through modulating BRCA1 function, and NHEJ dynamics through binding with DNA-PK.36,37 Moreover, EGFR modulates DNA synthesis and repair through Tyr phosphorylation of histone H4.38 Over-expression and increase activity of EGFR has been recognized as a significant factor in several epithelial cancers such as breast, pancreatic, colorectal and brain cancer as well as head and neck squamous cell carcinoma, and non-small cell lung cancer. Hence, targeting EGFR has been extensively studied as an antitumor strategy and has shown propitious clinical outcomes. Two approaches used either as monotherapy or in combination with additional chemotherapy and/or radiotherapy, have proven beneficial: (i) the use of small molecule tyrosine kinase inhibitors to block downstream activation pathways; and (ii) mAbs targeting the extracellular domain of EGFR.35

So far, two mAbs (cetuximab and panitumumab), targeting EGFR in an inhibitory manner, have already been approved and a third one (nectumumab) is under review by FDA. These mAbs have a high-affinity for EGFR and efficiently blocks ligand binding and therefore inhibit downstream effects of EGFR activation (Fig. 2A). In spite of clinical achievements, resistance mechanisms to the treatment with these mAb, either by intrinsic resistance or by the development of acquired resistance, are well recognized.35 These resistance mechanisms involve biological processes in which PARP proteins may play a substantial role, providing an excellent opportunity to combine cetuximab and panitumumab with PARP inhibitors in order to enhance their therapeutic value.

The PARP inhibitor veliparib enhanced the cytotoxicity of cetuximab in UM-SCC1, UM-SCC6, and FaDu head and neck squamous cell carcinoma cell lines.39 The underlying mechanism might involve cetuximab-mediated impairment of NHEJ and HR-mediated DSB repair. The subsequent persistence of DNA damage will make cancer cells sensitive to death upon PARP inhibition through the principle of synthetic lethality (Fig. 2A).39 Rely on these pre-clinical results, combination of the PARP inhibitor olaparib with radiation therapy and cetuximab is being currently evaluated in a phase I clinical trial in patients with advanced squamous cell carcinoma of the head and neck with a history of heavy smoking (NCT01758731). Furthermore, this strategy may also be feasible for other EGFR overexpressing tumors. Indeed, treatment of mice with anti-EGFR radioimmunotherapy combined with docetaxel, doxorubicin and the PARP inhibitor rucaparib eradicated triple-negative breast cancer orthotopic tumors and established metastases in a pre-clinical study.40

Mechanisms of resistance to EGFR-targeted antibodies may be overcome by combination with PARP inhibitors (Fig. 2A). Inhibition of angiogenesis has been identified as part of cetuximab anti-tumor effect 41 and resistance to this mAb might in part be explained because of the ability of cells to re-activate pro-angiogenic factors such as VEGF and VEGFR.46 Interestingly, PARP inhibitors have been shown to inhibit VEGF-induced migration, parthanatos, necroptosis and autophagy have also been shown to be modulated by PARylation.32 but their full description lies beyond the limits of the current review. Furthermore, the impact of PARP inhibitors on mitochondrial respiration regulation and energy homeostasis may also have potential clinical implications in cancer.33,34

**Table 1.** Pre-clinical and clinical studies combining PARP inhibitors and monoclonal antibodies to evaluate their efficacy in tumor

| Antibody name | Antibody target | PARP inhibitors | Tumor Indications | Study | References |
|---------------|-----------------|-----------------|-------------------|-------|------------|
| Cetuximab     | EGFR            | Olaparib        | Head and neck     | Pre-clinical | NCT01758731 |
| Cetuximab     | EGFR            | Veliparib       | Head and neck     | Pre-clinical | NCT01758731 |
| C225/17/Lu-anti-EGFR | EGFR               | Olaparib/Rucaparib | Pre-clinical |     |
| Trastuzumab   | HER2            | Olaparib/Rucaparib | Breast            | Pre-clinical | NCT01459380 |
| Bevacizumab   | VEGF            | Olaparib        | Advanced solid tumor | Clinical Phase I | NCT01459380 |
| Bevacizumab   | VEGF            | Niraparib       | Ovarian           | Clinical Phase II | NCT02354131 |
| Bevacizumab   | VEGF            | Veliparib       | Ovarian Fallopian tube Primary peritoneal | Clinical Phase I | NCT00989651 |
| Bevacizumab   | VEGF            | Veliparib       | Colorectal        | Clinical Phase II | NCT02350575 |
| Bevacizumab   | VEGF            | Veliparib       | Ovarian Fallopian tube Primary peritoneal | Clinical Phase | NCT01459380 |
| Bevacizumab   | VEGF            | Veliparib       | Pancreas          | Pre-clinical | NCT01217990 |
| TRA-8         | Apo2L/TRAIL Receptor (DR5) | Olaparib/Veliparib | Leukemia Lung Ovarian | Pre-clinical | NCT02121990 |
| TRA-8         | Apo2L/TRAIL Receptor (DR5) | Olaparib/Veliparib | Leukemia Lung Ovarian | Pre-clinical | NCT01217990 |

*ClinicalTrials.gov ID.
Figure 2. Sinergistic effects of PARP inhibitors and mAbs targeting EGF, HER2 and VEGF pathways. (A) Ligands binding to EGFR activates various intracellular signaling pathways including AKT, ERK and STAT pathways resulting in increased cell proliferation, migration, survival and expression of components of the HR pathway. HER2 activation requires heterodimerization with other (ligand-bound) EGFR-family receptors, most notably HER3 (left panel). These processes are limited by mAbs targeting these receptors (central panel). Interestingly, decrease HR components expression may generate a BRCAness situation that in the presence of PARP inhibitors result in synthetic lethality and cell death. Moreover, PARP inhibitors might overcome the resistance to mAbs targeting either EGFR or HER2 by different mechanisms (right panel). (B) Ligand binding to VEGF activates various intracellular signaling pathways including AKT, and ERK resulting in angiogenesis. mAbs against VEGF or VEGFR prevent the activation of VEGF-dependent signaling pathways and therefore limited angiogenesis. Mechanisms of resistance to mAbs targeting VEGF pathway comprise the activation of a hypoxia response including HIF induction, which in turn drives the release of additional angiogenic factors. PARP inhibitors dampen the expression and function of HIF. In addition, cells exposed to hypoxia have decreased HR response that in the presence of PARP inhibitors might leads to cell death by the principle of synthetic lethality.
proliferation and in vitro formation of tube structures in human umbilical vein endothelial cells (HUVECs) and in tumor models. Resistance to cetuximab may be also mediated by selecting for E-cadherinlow/vimentinhigh expressing cell population, a marker of EMT. Interestingly, we have demonstrated that PARP inhibition induced downregulation of vimentin expression, which may overcome the resistance to cetuximab treatment. An additional resistance mechanism might involve constitutive activation of EGFR downstream effector pathways such as upregulation of the PI3K/AKT pathway. Indeed, the constitutive activation of AKT due to the proteosomal degradation of PTEN induces resistance to cetuximab in the NSCLC cell line. Likewise, it has been reported that PARP inhibitors attenuate AKT activation through the upregulation of PHLPP1, which might account for the synergistic effect between PARP inhibitors and mAb targeting EGFR. However, this mechanism is controversial as other reports indicated that PARP inhibitors induced AKT activation, and prevent mTORC1 inhibition through its role in modulating AMP kinase pathways. Nonetheless, when kinet-ics of PARP inhibitors are taken into account, downregulation of AKT pathway is found when cells are treated for at least 3–4 h, revealing a second wave of interaction between PARylation and the AMPK/AKT/mTORC1 axis that counteract pro-survival pathways, probably related to shutting-off PAR synthesis. Discrepancies between studies could also be ascribed to the concomitant use or not of H2O2 or MNNG to achieve a maximal PAR activation. On the other hand, PARP-1 activation dramatically amplifies the ERK-signal pathways promoting cell growth, proliferation and differentiation. So, this ERK-signal amplification will be blocked in the presence of PARP inhibitors.

**PARP inhibitors and monoclonal antibodies targeting HER2**

HER2 differs from other members of the ErbB family in that it does not bind EGF-like ligands, relying instead on heterodimerization with other ligand-bound EGFR-family receptors for activation, most notably HER3. HER2 activation transmits downstream signaling leading to pathways that influence a variety of biological processes such as cell proliferation, apoptosis and cell survival (Fig. 2A). HER2 is over-expressed in approximately 25% of primary human breast cancer, and its overactivity is associated with poor clinical outcomes. So far, two mAbs, trastuzumab and pertuzumab targeting different domain of the extracellular portion of HER2, have already been approved by the FDA. These mAbs block HER2 dimerization and therefore inhibit downstream function of HER2 activation (Fig. 2A). In spite of demonstrated success of these mAbs, only a minority of patients whose tumor overexpress HER2 respond to trastuzumab as monotherapy, and almost all responders eventually develop resistance.

Recently, our group has demonstrated that PARP inhibition enhanced the antitumor activity of the anti-HER2 mAb trastuzumab in HER2 overexpressing breast cancer cell lines. Cells exposed to two different PARP inhibitors, either olaparib or rucaparib, in combination with trastuzumab, exhibited significantly decreased cell growth and had greater DNA damage than cells exposed to each agent alone. Moreover, combination treatment in the BT474 xenograft model resulted in significant growth inhibition, reduced tumor cell proliferation, and increased DNA damage and apoptosis. Mechanistic exploratory assays showed that trastuzumab down-modulated the HR protein proliferating cell nuclear antigen (PCNA). Olaparib did not affect PCNA expression and the combination did not further decrease PCNA compared to trastuzumab alone. PCNA down-modulation may impair HR which in turn may result in synthetic lethality in the presence of a PARP inhibitor (Fig. 2A). Further studies will be required to validate the clinical application of combining PARP inhibitors with mAb targeting HER2 such as trastuzumab, and pertuzumab. Nevertheless, similar mechanisms overcoming anti-EGFR resistance by PARP inhibitors may account as well for HER2 mAb treatment resistance (Fig. 2A). Moreover, PARP inhibitors have also been shown to potentiate small molecule tyrosine kinase inhibitors which block tyrosine kinase receptor mediated downstream pathways, maybe by similar mechanisms as described for the combination with mAb, and clinical trials are ongoing. Nonetheless, their full description lies beyond the limits of the current review.

**PARP inhibitors and monoclonal antibodies targeting VEGF pathway**

The VEGF family of proteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D and PGF) binds to VEGF receptors (VEGFR), leading to downstream activation of signaling pathways involved mainly in angiogenesis (Fig. 2B). A critical process for tumor growth. Therefore, angiogenesis inhibitors targeting VEGF signaling pathways are providing demonstrable therapeutic efficacy in many tumors types. Accordingly, two anti-angiogenesis mAbs (bevacizumab and ramucirumab) targeting the VEGF pathway have been approved for cancer treatment.

The mAb bevacizumab blocks angiogenesis and tumor growth by binding to VEGF-A and then preventing its interaction with its receptor (VEGFR2). Bevacizumab is licensed in combination with chemotherapy in different solid tumors such as metastatic colorectal cancer, renal cell cancer, and unresctable advanced non-squamous non-small cell lung cancer. However, initial tumor responses are followed by disease progression reflecting adaptive resistance. One mechanism of resistance by the tumor cells is induction of tolerance to hypoxia as a response to vessel regression caused by bevacizumab. The response to hypoxia is mediated by a family of transcription factors called the hypoxia-inducible factors (HIFs) being HIF-1α and HIF-2α the best characterized mem-

One of the best characterized members. To be transcriptionally active, either HIF-1α or HIF-2α needs to form a heterodimer during hypoxia with HIF-1β, which is constitutively active. The HIF-1α subunit is degraded during normoxia due to the hydroxylation of specific proline residues by the family of the prolyl hydroxylase domain-containing proteins (PHDs) that are only active in the presence of O2. This hydroxylation allows the von Hippel-Lindau factors (pVHL) to ubiquitin the HIF-1α subunit toward its degradation via proteasome. The induction of HIF expression in hypoxic tumor, endothelial and stromal cells drives the release of pro-angiogenic factors such as VEGF, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), angiopoietins (ANG) and SD1α (Fig. 2B). Different studies have proved that expression and function of HIF-1α and HIF-
2α is dampened after PARP inhibition, showing the anti-angiogenic function of PARP inhibitors. In addition, replicating cells exposed to severe hypoxia have decreased HR protein expression and function, a defect that is synthetically lethal with PARP inhibition. Interestingly, PARP inhibitors have been shown to reduce FGF-induced proliferation, migration and tube formation of HUVECs, suggesting an additional mechanism to overcome bevacizumab resistance.

Relying on these experimental data, combination of different PARP inhibitors with bevacizumab is being evaluated in an increasing number of clinical trials. A phase I clinical trial has shown that combination of olaparib and bevacizumab is well tolerated in patients with solid tumors and awaits phase II clinical studies to assess the therapeutic efficacy of this contextual synthetic lethal approach. In parallel, other clinical trials in phases I or II combining bevacizumab and PARP inhibitors are under evaluation (Table 1): (i) a phase II randomized study of niraparib and/or niraparib-bevacizumab combination against bevacizumab alone in platinum-sensitive ovarian cancer (NCT02354131); (ii) a phase I dose-escalation study of cisplatin, paclitaxel, bevacizumab and olaparib for newly diagnosed ovarian, primary peritoneal and Fallopian tube cancer (NCT012121990); (iii) a phase II randomized, multicenter study comparing veliparib plus FOLFIRI versus placebo plus FOLFIRI with or without bevacizumab in previously untreated metastatic colorectal cancer (NCT02305758); (iv) a phase I study of carboplatin, paclitaxel, bevacizumab and veliparib in treating patients with newly diagnosed stage II-IV ovarian epithelial, Fallopian tube, or primary peritoneal cancer (NCT00989651); (v) a phase I study of veliparib, pegylated liposomal doxorubicin hydrochloride, carboplatin and bevacizumab in treating patients with recurrent ovarian cancer, primary peritoneal cancer, or Fallopian tube cancer (NCT01459380).

The mAb ramucirumab targets, in an inhibitory way the VEGFR2, the main mediator of the VEGF-induced angiogenic signaling, blocking the VEGFR2-mediated signaling and activating pathways. Ramucirumab has been approved for treatment of advanced gastric or gastro-esophageal adenocarcinoma and is also under clinical investigation in several phase III trials of other tumors like colorectal cancer, hepatocellular carcinoma and breast cancer. The synergistic effect of PARP inhibitors with ramucirumab has not been tested yet, but is expected to enhance its therapeutic value by mechanisms similar to those described above with bevacizumab.

**PARP inhibitors and monoclonal antibodies targeting death-induced signaling pathways**

Binding of TNF-related apoptosis-induced ligand (Apo2L/TRAIL, TNFSF10) to death receptor 4 (DR4) (TNFRSF10A) or DR5 (TNFRSF10B) results in death-inducing signaling complex (DISC) formation which involves the recruitment and activation of the initiator caspases-8 and -10, via the adaptor protein FADD, that in turn activates the downstream effector caspase-3, -6 and -7 ultimately leading to cell death (Fig. 3). Hence, targeting DR4 and DR5 by using agonistic mAbs is emerging as a striking therapeutic strategy to promote apoptosis of tumor cells. Accordingly, agonist mAbs directed against DR4 or DR5 have been assessed in pre-clinical and early phase clinical studies either as single agents or in combination with chemotherapy or radiotherapy. However, many cancer cells develop resistance to these agents and no death receptor agonist therapies have moved into phase III trials. Therefore, there is considerable interest in improving sensitivity to these treatments.

Recently, several pre-clinical studies have shown a synergistic effect between PARP inhibitors with agonistic mAbs to Apo2L/TRAIL receptors. The first evidence, reported two years ago, showed that the PARP inhibitor PJ34 sensitizes pancreatic cancer cells to an agonistic mAb for DR5 (TRA-8)-induced apoptosis *in vitro* and enhancing the efficacy of the antibody TRA-8 therapy for pancreatic cancer in a mouse tumor xenograft model (Table 1). The authors of this study proposed a model whereby inhibition of PARP blocks PARylation of caspase-8 and, therefore decreases activation of downstream caspases resulting in apoptosis resistance. In the presence of PARP inhibitors, PAR is not produced, which permits full caspase-8 activation and in turn activation of downstream caspases leading to apoptosis.

By using the clinical approved PARP inhibitor olaparib, Wei Meng et al. extended this finding in a number of different cell types. They reported, by using two different PARP inhibitors (olaparib and veliparib) in combination with agonistic mAbs to TRAIL receptors, enhanced sensitivity of myeloid leukemia, non-small cell lung and ovarian cell lines as well as a majority of clinical acute myelogenous leukemia (AML) specimens. Mechanistic studies reveal that PARP inhibition increased both mRNA and cell surface expression of the death receptors Fas and DR5 at transcriptional level, without altering their trafficking to the cell surface. PARP inhibitors increased transcription of Fas and DR5 by enhancing binding of the transcription factor SP1 to the TNFR10B promoter. The enhanced expression of DR5 and Fas by PARP inhibition improved sensitivity of cancer cells to treatment with death ligands such as agonistic anti-Fas and anti-DR5 mAbs (Fig. 3).

Additional mechanisms may also be involved in the therapeutic synergy between PARP inhibitors and agonist mAb against Death Receptors. For instance, PARP inhibitors have
been shown to inhibit TRAIL-induced HMBG1 cytoplasmic translocation and subsequent HMBG1-BECN1 complex formation resulting in diminished autophagy, increased apoptosis, enhancing the anticancer activity of TRAIL in vitro and in a subcutaneous tumor model.\(^7\)

**Future perspectives**

Current PARP inhibitors target the catalytic site of PARP enzymes which is highly similar among PARPs family members and no isoform-specific PARP inhibitors are available. Hence, one major challenge in the PARP field is to understand the specific functions of PARP proteins to provide a basis for the rational development and exploitation of isoform-specific PARP inhibitors, the identification of new target molecules, and the design of new effective therapeutic approaches as single therapy or in combination with other agents such as mAbs. Furthermore, the identification of predictive individual biomarkers detecting patients with the greatest likelihood of response to PARP inhibitors in combination with tumor-targeting mAbs therapy will be of extremely valuable for clinical benefit. For instance, EGFR-activating mutations in lung cancer cells correlate with a Fanconi anemia-like cellular phenotype that includes PARP inhibitor sensitivity.\(^7\) A very important research area is related with immune markers that can provide useful predictive information and that increased clinical activity resulting in diminished autophagy, increased apoptosis, translocation and subsequent HMGB1-BECN1 complex formation has shown that PARP-1 deficiency significantly alters expression of genes involved in the Th1/Th2 balance.\(^8\) Prospective clinical trials will be required to determine how best to sequence these novel therapeutic strategies by combining mAbs and PARP inhibitors in cancer treatment.

**Disclosure of Potential Conflicts of Interest**

The authors declare that this article content has no conflicts of interest.

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