activate wild-type EGFR on other tumor cells (Inda et al., 2010). One could imagine that loss of IκBα in EGFR wild-type glioma could increase NF-κB activity and IL-6 levels, which can further activate EGFR.

In the tumors analyzed by Bredel et al. (2010), monoallelic, but not biallelic, loss of *NFKBIA* was observed. There are several potential interpretations of this result, again requiring further analysis. Loss of one copy of *NFKBIA* appears to be advantageous to the tumor, possibly for the reasons described above and/or for other reasons. However, it seems to be disadvantageous to lose both copies, as it was not observed in any of the datasets Bredel et al. utilized. Perhaps this speaks to the need to retain some degree of control and inducibility over the NF-κB pathway (Figure 1). Another possibility is that IκBα has roles besides its most well-studied function as an NF-κB inhibitor and this is critical for oncogenesis or survival of cells of this particular lineage.

Deletions of *NFKBIA* in glioblastomas reported by Bredel et al. (2010) add to the documented mutations in the NF-κB pathway. Given the percentage of tumors with *NFKBIA* deletion and the impact on patient survival, this event seems to be significantly involved in glioblastoma development, potentially providing new targets for therapy. Future work requires further analysis of the downstream effects, particularly on the NF-κB pathway. Comparison of *NFKBIA*-deleted and *EGFR*-amplified tumors will be important in determining whether these two alterations lead to a common phenotype or if they characterize two distinct subsets of glioblastoma. Potentially, the implication is that NF-κB signaling could be centrally involved in all gliomas, although the mutations responsible may vary between subsets.

REFERENCES

Annunziata, C.M., Davis, R.E., Demchenko, Y., Bellamy, W., Gabrea, A., Zhan, F., Lenz, G., Hanamura, I., Wright, G., Xiao, W., et al. (2007). Cancer Cell *12*, 115–130.

Bassères, D.S., Ebbs, A., Levantini, E., and Baldwin, A.S. (2010). Cancer Res. *70*, 3537–3546.

Boehm, J.S., Zhao, J.J., Yao, J., Kim, S.Y., Firestein, R., Dunn, I.F., Sjostrom, S.K., Garraway, L.A., Weremowicz, S., Richardson, A.L., et al. (2007). Cell *129*, 1065–1079.

Bredel, M., Scholtens, D.M., Yadav, A.K., Alvarez, A.A., Renfrow, J.J., Chandler, J.P., Yu, I.L.Y., Carro, M.S., Dai, F., Tagge, M.J., et al. (2010). N. Engl. J. Med., in press. Published online December 22, 2010. 10.1056/nejmoa1006312.

Courtois, G., and Gilmore, T.D. (2006). Oncogene *25*, 6831–6843.

Inda, M.M., Bonavia, R., Mukasa, A., Narita, Y., Sah, D.W.Y., Vandenberg, S., Brennan, C., Johns, T.G., Bachoo, R., Hadwiger, P., et al. (2010). Genes Dev. *24*, 1731–1745.

Karin, M. (2006). Nature *441*, 431–436.

Mayo, M.W., Wang, C.Y., Cogswell, P.C., Rogers-Graham, K.S., Lowe, S.W., Der, C.J., and Baldwin, A.S. (1997). Science *278*, 1812–1815.

Meylan, E., Dooley, A.L., Feldser, D.M., Shen, L., Turk, E., Ouyang, C., and Jacks, T. (2009). Nature *462*, 104–107.

Verhaak, R.G.W., Hoadley, K.A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M.D., Miller, C.R., Ding, L., Golub, T., Mesirov, J.P., et al. (2010). Cancer Cell *17*, 98–110.

PARP Inhibitors in Cancer Therapy: Promise, Progress, and Puzzles

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A recent article in the *New England Journal of Medicine* by O'Shaughnessy et al. provides evidence that a treatment strategy aimed at inducing DNA damage with chemotherapy while simultaneously disabling repair using a PARP inhibitor might offer hope for patients with a treatment-refractory form of breast cancer.

Many key genes inactivated in human cancer are involved in DNA damage and repair responses. Thus, specific DNA repair defects in tumor cells might be targeted selectively for therapeutic benefit in otherwise resistant malignancies. The emerging use of poly(ADP-ribose) polymerase (PARP) inhibitors in certain DNA repair-deficient cancers promises to fulfill this paradigm. Joyce O'Shaughnessy and colleagues recently demonstrated that treating patients with advanced "triple-negative" breast cancers using the PARP inhibitor iniparib in combination with DNA-damaging chemotherapy increased tumor responses and prolonged patient survival compared with chemotherapy treatment alone (O'Shaughnessy et al., 2011). Like many important clinical advances however, this study leaves many unresolved questions, and addressing these will be critical to realizing the substantial promise of PARP inhibitors in cancer therapy.

PARPs catalyze the NAD^+-dependent addition of poly(ADP-ribose) units to target proteins and regulate diverse cellular processes (Krishnakumar and Kraus, 2010). Most cellular PARP activity is attributable to PARP1, a ubiquitous and abundant nuclear protein that localizes to sites of DNA damage, leading to the recruitment of DNA repair proteins. Both PARP1 and PARP2 have been linked to base-excision repair, and *Parp1* null mice exhibit defective single-strand

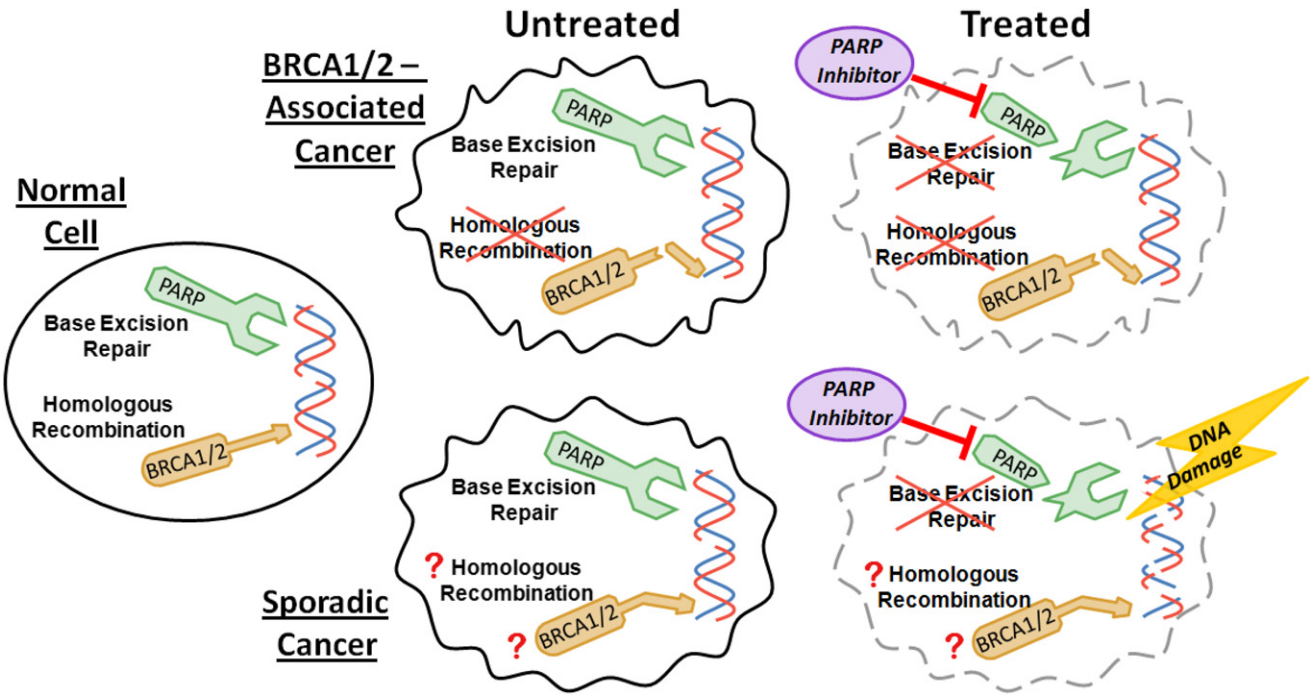

Figure 1. PARP Inhibitor Treatment of BRCA1/2-Associated and Sporadic Cancers

Normal cells have intact base excision repair and homologous recombination that are mediated by PARP and BRCA1/2-dependent pathways, respectively. In germline *BRCA1* or *BRCA2* mutation carriers, the capacity for HR is often lost during tumorigenesis due to the inactivation of the remaining wild-type allele. PARP inhibitor treatment disables the repair of spontaneous DNA damage in these tumor cells, leading to their death. Sporadic tumors including triple-negative breast cancer may have defects in BRCA1/2 expression and/or function. Treatment combining a PARP inhibitor with DNA-damaging chemotherapy may be an effective strategy for some of these tumors. (Illustration courtesy of Zachary Nash.)

break repair (Krishnakumar and Kraus, 2010). Nevertheless, PARP-deficient cells are able to carry out error-free DNA repair through homologous recombination (HR), a process mediated by large protein complexes whose components include proteins encoded by the breast cancer susceptibility genes *BRCA1* or *BRCA2*. This led to the hypothesis that following PARP inhibition, normal cells would effectively repair spontaneous DNA damage—most commonly single-strand breaks resulting in stalled replication forks—through HR. In contrast, BRCA1/2-deficient cells would be unable to carry out repair, ultimately leading to persistent double-strand breaks and cell death. In vitro studies confirmed the predicted "synthetic lethality" between PARP and BRCA1/2 pathways (Bryant et al., 2005; Farmer et al., 2005). Thus, an effective clinical strategy and a potentially large therapeutic window were suggested for the use of PARP inhibitors as single agents in patients with BRCA1/2-associated cancers, whose tumors have lost both copies of BRCA1/2 and are HR deficient but whose normal cells retain one functional copy and are HR competent (Figure 1).

These observations prompted the development of several PARP inhibitors that are now being tested in clinical trials. Olaparib (AZD2281) and veliparib (ABT888) are competitive inhibitors that mimic nicotinamide and compete for the catalytic domain of PARP1 and PARP2 (He et al., 2010). In keeping with its predicted mechanism, olaparib has been tested as a single agent in patients with advanced cancers, and significant tumor regression has been observed only in those with BRCA1/2-associated tumors (Fong et al., 2009; Tutt et al., 2010). Iniparib (BSI-201), the compound used in the O'Shaugnessy study, is, in contrast, a noncompetitive inhibitor of PARP1 that disrupts the interaction between PARP1 and DNA.

Notwithstanding the elegant development and validation of PARP inhibitors for BRCA1/2-associated tumors, these cancers are relatively uncommon. A more complicated question regards the rational use of PARP inhibitors in non-BRCA1/2-associated tumors. A case in point are triple-negative breast cancers, which lack expression of estrogen receptor (ER) and progesterone receptor (PR) as well as Her-2 amplification, and represent about 15% of all breast cancers (Foulkes et al., 2010). While 80% of BRCA1-associated breast cancers are triple-negative, the vast majority of triple-negative tumors arise sporadically in noncarriers, yet these two subsets share many distinct features (Foulkes et al., 2010). In the context of PARP inhibition, this begs the question whether BRCA1 and/or HR are functionally defective in both sporadic and BRCA1-associated triple-negative tumors. Many studies have reported that sporadic triple-negative tumors express low levels of BRCA1, owing to distinct mechanisms (Moskwa et al., 2011), and display various DNA damage response and repair defects. However, it has been challenging to demonstrate a profound defect in HR in sporadic triple-negative tumors. Furthermore, patients with such tumors have not demonstrated responses in small clinical studies using olaparib (Fong et al., 2009) or veliparib (Isakoff et al.,

2010). These experiences have prompted the testing of PARP inhibition in combination with DNA-damaging chemotherapeutics, which in theory could kill tumor cells by overwhelming the cellular DNA repair apparatus even in the absence of an intrinsic HR defect (Figure 1). An unresolved question, however, was how selective this approach would be for tumor versus normal cells.

In light of these concerns, the findings of O'Shaughnessy et al. (2011) are all the more remarkable. In this study, patients with advanced triple-negative breast cancer who were unselected for BRCA1/2 mutation status and who had received only one or two lines of previous chemotherapy were randomized to receive combination chemotherapy (gemcitabine, a nucleoside analog, and carboplatin, a DNA alkylating and crosslinking agent), either alone or together with iniparib. The addition of iniparib increased the tumor response rate from 32% to 52% of patients (O'Shaughnessy et al., 2011). Historically, even in the face of an improvement in response rates, it is uncommon to observe prolonged patient survival in such trials. This is because patients receive other therapies following completion of the trial, and, as in this trial, patients are allowed to "cross-over" and receive the study drug once their tumor begins to progress. Strikingly, however, the addition of iniparib in this trial increased the overall survival of these patients from 7.7 to 12.3 months. A larger (Phase III) clinical study that has already completed patient enrollment seeks to validate these findings.

How do we interpret the results of this trial in the context of the synthetic lethality between PARP and BRCA1/2-HR pathways? Although the number of BRCA1/2 carriers enrolled in this trial was not reported, based on population frequencies it can be assumed that the majority of patients were not, and therefore at least some of the clinical benefit occurred in noncarriers. Especially notable in this trial was the absence of significant combined toxicity with the addition of iniparib to chemotherapy. In particular, there was little or no additive bone marrow toxicity (a correlate of DNA damage), which contrasts with marked bone marrow toxicity reported when veliparib and chemotherapy were combined (Isakoff et al., 2010). As noted above, iniparib has a distinct mechanism of action. Further, in cell-based assays, iniparib exhibits potency in the micromolar range compared with the low nanomolar range displayed by other PARP inhibitors. Indeed, no maximally tolerated dose has been established for this agent (He et al., 2010). Thus, while iniparib represents an exciting compound from the clinical perspective, its precise mechanism of action remains to be defined.

Going forward, it is clear that PARP inhibitors will be an important component of therapy for cancers arising in BRCA1/2 carriers. Their application will likely extend to early-stage disease, with the hope that they might provide additional clinical benefits. PARP inhibition is also a plausible cancer preventative strategy in this setting. In theory, limited exposure to such an inhibitor might have little effect on normal cells but could eliminate nascent cancer cells that have undergone loss-of-heterozygosity for BRCA1/2. These potential benefits will have to be weighed against potential toxicities, given the established role of PARP in DNA repair, metabolism, and stem cell function (Krishnakumar and Kraus, 2010). In sporadic cancers, future clinical studies with additional PARP inhibitors are likely to further clarify the potential role of these agents, most likely in combination with DNA-damaging therapeutics. Parallel preclinical and translational studies to identify the most effective chemotherapy partners and the most relevant in vivo targets of the different PARP inhibitors will be essential to the rational design of future trials. Most exciting is the possibility that future work will uncover additional synthetic lethal relationships between PARP-dependent pathways and tumor-specific defects present in sporadic cancers (He et al., 2010). If successful, such studies may fulfill the promise of a rational synthetic lethal approach for many common cancers.

REFERENCES

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., and Helleday, T. (2005). Nature *434*, 913–917.

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., et al. (2005). Nature *434*, 917–921.

Fong, P.C., Boss, D.S., Yap, T.A., Tutt, A., Wu, P., Mergui-Roelvink, M., Mortimer, P., Swaisland, H., Lau, A., O'Connor, M.J., et al. (2009). N. Engl. J. Med. *361*, 123–134.

Foulkes, W.D., Smith, I.E., and Reis-Filho, J.S. (2010). N. Engl. J. Med. *363*, 1938–1948.

He, J.X., Yang, C.H., and Miao, Z.H. (2010). Acta Pharmacol. Sin. *31*, 1172–1180.

Isakoff, S. J., Overmoyer, B., Tung, N. M., Gelman, R. S., Giranda, V. L., Bernhard, K. M., Habin, K. R., Ellisen, L. W., Winer, E. P., and Goss, P. E. (2010). J. Clin. Oncol. *28*, suppl; abstr 1019.

Krishnakumar, R., and Kraus, W.L. (2010). Mol. Cell *39*, 8–24.

Moskwa, P., Buffa, F.M., Pan, Y., Panchakshari, R., Gottipati, P., Muschel, R.J., Beech, J., Kulshrestha, R., Abdelmohsen, K., Weinstock, D.M., et al. (2011). Mol. Cell *41*, 210–220.

O'Shaughnessy, J., Osborne, C., Pippen, J.E., Yoffe, M., Patt, D., Rocha, C., Koo, I.C., Sherman, B.M., and Bradley, C. (2011). N. Engl. J. Med. *364*, 205–214.

Tutt, A., Robson, M., Garber, J.E., Domchek, S.M., Audeh, M.W., Weitzel, J.N., Friedlander, M., Arun, B., Loman, N., Schmutzler, R.K., et al. (2010). Lancet *376*, 235–244.