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DNA Repair and Personalized Breast Cancer Therapy

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Abstract

Personalized cancer therapy is likely to be one of the next big advances in our search for a cure for cancer. To be able to treat people in an individualized manner, researchers need to know a great deal about their genetic constitution and the DNA repair status of their tumors. Specific knowledge is required regarding the polymorphisms individuals carry and how these polymorphisms influence responses to therapy. Researchers are actively engaged in biomarker discovery and validation for this purpose. In addition, the design of clinical trials must be reassessed to include new information on biomarkers and drug responses. In this review, we focus on personalized breast cancer therapy. The hypothesis we focus upon in this review is that there is connection between the DNA repair profile of individuals, their breast tumor subtypes, and their responses to cancer therapy. We first briefly review cellular DNA repair pathways that are likely to be impacted by breast cancer therapies. Next, we review the phenotypes of breast tumor subtypes with an emphasis on how a DNA repair deficiency might result in tumorigenesis itself and lead to the chemotherapeutic responses that are observed. Specific examples of breast tumor subtypes and their responses to cancer therapy are given, and we discuss possible DNA repair mechanisms that underlie the responses of tumors to various chemotherapeutic agents. Much is known about breast cancer subtypes and the way each of these subtypes responds to chemotherapy. In addition, we discuss novel design of clinical trials that incorporates rapidly emerging information on biomarkers.

Keywords

DNA repair; cancer; breast cancer; personalized gene therapy

INTRODUCTION

Standard cancer therapy involves the use of agents that damage DNA with the ultimate goal of killing the cell. For example, ionizing radiation (IR), anthracyclines, platinum compounds, and taxanes are used routinely to treat breast carcinoma. IR induces DNA double-strand (DSBs), single-strand (SSBs), and oxidized bases. Anthracyclines are topoisomerase II inhibitors and DNA intercalating agents, treatment with which leads to strand breaks, especially DNA DSBs. Platinum compounds are bifunctional alkylating agents that induce predominantly intra- and interstrand crosslinks (ICLs). Taxanes are mitotic inhibitors. Importantly, the

combination of molecular profiling of tumors and the results of clinical trials has significantly increased our understanding of the mechanisms of action of these drugs, especially with regard to breast cancer.

Damaging the DNA does not always result in cell kill, because cells have evolved elaborate DNA repair pathways that remove the damage from DNA, as described below. Recent research has suggested that the targeting of DNA repair pathways by specific agents, in addition to damaging the DNA, can result in effective killing of tumor cells. It is a well-known fact that tumors that appear to have similar diagnostic features, largely based upon histological characterization, do not always respond to treatment in the same way. This is likely due in part to differing molecular features among tumors, the varied repair capacity of the tumors, and the individuals harboring them. Thus, responses to cancer therapies will be impacted by germline or tumor-specific genetic alterations within DNA repair genes, as has been documented for several altered DNA repair genes including BRCA1 and 2 carriers treated with polyADP ribose1 inhibitors (see below). In addition to DNA repair genes, checkpoint proteins are also important players in the responses of tumors to cancer therapies, but these proteins are not the subject of this review.

In this review, we will briefly describe the molecular profiling and categorization of breast tumors. We touch on the features of various DNA repair pathways and their responses to the presence of DNA damage induced by IR and chemotherapeutic agents. Examples of targeting specific DNA repair pathways and enzymes for cancer therapy will be provided. Finally, we will discuss the design of clinical trials based upon the approach of personalized cancer therapy.

DNA REPAIR PATHWAYS

Cells sustain at least 20,000 oxidative DNA lesions per cell per day and even more when exposed to cancer therapies [Barnes and Lindahl, 2004]. Several DNA repair pathways have evolved to repair DNA adducts and function to prevent genomic instability and promote cell survival. However, during therapy, DNA repair can counteract the affect of chemo- and radiotherapy. Here, we briefly introduce DNA repair pathways that are likely to be critical players with the current breast cancer treatment modalities.

Nucleotide Excision Repair

A bulky, helix-distorting lesion is usually a substrate for nucleotide excision repair (NER) [Freidberg et al., 2006]. The lesion is first recognized and bound by the *Xeroderma pigmentosum* complementation group C-RAD23B-like (XPC-RAD23B) protein complex followed by the formation of a preincision complex. Proteins that surround the lesion at this time include replication protein A, *Xeroderma pigmentosum* complementation group A, *Xeroderma pigmentosum* complementation group G, and transcription factor IIH (RPA, XPA, XPG, and TFIIH). XPA interacts with and directs many of the NER proteins to the site of damage. TFIIH is a transcription factor that is important for transcription initiation but it also functions in NER. TFIIH is composed of 10 subunits and catalyzes helix opening during NER. Two proteins that are part of the TFIIH complex and are important for helix opening are the *Xeroderma pigmentosum* complementation group B and D (XPB and XPD) helicases. In the simplest form of NER, dual incision of the lesion takes place with XPG and excision repair cross-complementing group 1/*Xeroderma pigmentosum* complementation group F complex (ERCC1/XPF) cutting 3' and 5' to the lesion, respectively. The lesion is then released as a short 27-base oligonucleotide. The resulting gap is filled in by DNA polymerase, and the nick is then ligated. Transcription-coupled repair is a major pathway of NER, in which preferential repair of the transcribed strand occurs (for a review, see Hanawalt and Spivak [2008]).

Base Excision Repair

The base excision repair (BER) pathway is mainly responsible for the lesion-specific removal of nonbulky-damaged bases that arise from endogenous agents, such as reactive oxygen species produced by cellular respiration, and by exogenous agents, such as exposure to IR, alkylating drugs, or antimetabolites [Kinsella, 2009]. The most common form of BER is short-patch. A monofunctional DNA glycosylase initiates the pathway by recognizing and excising a damaged base, generating an abasic site [Freidberg et al., 2006]. AP endonuclease (APE1) converts the abasic site into a single nucleotide gap by incising the backbone 5' to the abasic site, leaving a 3'-OH and a 5'-deoxyribose (dRP) group. DNA polymerase β then catalyzes the removal of the dRP group and fills in the gap. Finally, the XRCC1-ligase III α complex seals the nick, completing repair. There are also bifunctional glycosylases that usually recognize and remove oxidized bases (for review, see Sweasy et al. [2006]). These glycosylases are able to catalyze the removal of the abasic site themselves, generating a 3'dRP and 5'phosphate. APE1 then catalyzes the removal of the 3'dRP leaving a 3'OH group. From here, Pol β can fill in the gap, and ligase III α seals the nick. An alternative BER pathway that does not depend on APE1 is used when the NEIL glycosylases initiate repair [Wiederhold et al., 2004]. NEIL 1, 2, and perhaps 3 catalyze excision of the damaged base via β,δ elimination, leaving a 3'phosphate and a 5'phosphate. The 3'phosphate is then removed by polynucleotide kinase (PNK). The other, less common BER pathway is long-patch (for a review, see Fortini and Dogliotti [2007]). This pathway involves the excision of 2–12 nucleotides, which are replaced by the actions of other DNA polymerase σ . This pathway requires additional proteins, including PCNA, RFC, FEN1, and DNA ligase I.

Single-Strand Break Repair

Single-strand breaks (SSBs) accumulate in cells in an indirect manner, via the action of DNA glycosylases and APE I during BER. They can also accumulate by a more direct route by damage to the sugar or by trapping of DNA by topoisomerase I, an enzyme that unwinds DNA during transcription (for an excellent review, see Caldecott [2008]). Poly(ADP-ribose) polymerase (PARP-1) detects the ends of SSBs, and this leads to the recruitment of additional repair factors. PARP is an abundant constitutively expressed nuclear enzyme that catalyzes the rapid synthesis of ADP-ribose polymers from the substrate NAD and thereby facilitates DNA repair, cell proliferation, and signaling to other critical cell-cycle proteins and oncogenes [Shall and de Murcia, 2000; Simbulan-Rosenthal et al., 2003; Audebert et al., 2004; Chang et al., 2004; Huber et al., 2004; Kim et al., 2004; Farmer et al., 2005]. Parp likely plays a critical role in the repair of gaps that form during BER and SSB repair. Parp binds to the breaks and becomes activated to form long poly(ADPribose) polymerase on acceptor proteins including itself. This creates a negatively charged area around the SSB, leading to the recruitment of repair proteins, including XRCC1. After the remodeling of the 5' and/or 3'termini by enzymes including Pol β , tyrosyl-DNA-phosphodiesterase 1, APE I, and PNK, DNA polymerases fill in the gap, and DNA ligase seals the nick.

Homology-Dependent Repair

This repair process is used for the repair of double-strand breaks (DSBs) that arise as a result of replication fork stalling or treatment with IR and drugs that induce DSBs including etoposide and bleomycin. There are essentially three different pathways that encompass HDR including the canonical DSB repair pathway, synthesis-dependent strand annealing, and single-strand annealing. HDR is a complex process that involves many proteins. The BRCA1 protein functions in the recruitment of mediators and enzymes to focal sites of recombination in cells. BRCA1 also functions in checkpoint control (for an excellent review, see Venkatarman [2009]). BRCA2 serves as a recombination mediator and is required for stabilization of RAD51 filaments on DNA (for a concise model, see San Filippo et al. [2008]). Several other

recombination mediators, including RAD52 and RAD54, and the RAD51 paralogs are also critical to homology-dependent repair, depending upon the specific cellular circumstance. RAD51 is a major cellular recombinase that catalyzes strand exchange. Resolvases act in the final step of HDR should a Holiday junction need to be resolved. Otherwise, helicases that have unwinding activity act in cases where there are noncrossover products (for an excellent review, see San Filippo et al. [2008]).

Nonhomologous End Joining

NHEJ is essentially ligation of DNA ends without a need for homology. This process can generate small insertions and deletions but can also be error free if direct ligation of the ends of a DSB occurs [Moore and Haber, 1996; Wilson et al., 1997]. In the case of incompatible ends, the NHEJ machinery uses microhomology to align the ends, followed by DNA synthesis and ligation, or by simple ligation. Several proteins, including MRE11, RAD50, NBS1, Ku70 and 80, DNA ligase IV, XRCC4, Artemis, and Lif1 participate in NHEJ (for a review, see Pardo et al. [2009]). If DNA synthesis is required, members of the X family of DNA polymerases, DNA polymerases lambda, and mu function in filling gaps within substrates with minimal homology [McElhinny et al., 2005]. Microhomology-mediated end joining can lead to large deletions and is likely an error-prone manner of repairing a DSB. Nonhomologous end joining or microhomology-mediated end joining between two different chromosomes can also lead to chromosomal aberrations.

ICL Repair

Interstrand crosslinks (ICLs) are highly cytotoxic to cells because they block DNA replication and transcription. Proteins that function in NER, HDR, and mismatch repair also function in this pathway. Multiple pathways exist, although, a description of each pathway is outside the scope of this review. Crosslink repair is initiated when the replication fork stalls at an ICL and the Fanconi Anemia (FA) proteins, including BRCA1, are recruited to the site (for review, see Bergstralh and Sekelsky [2008] and McCabe et al. [2009]). Should it be necessary, fork regression is likely mediated by Werner Syndrome protein, followed by cleavage by Mus81-EME1, creating a one-ended DSB, although other mechanisms are also possible. Another nick is created by incision by the mismatch repair machinery resulting in “unhooking” of the crosslink. After translesion synthesis occurs opposite of the unhooked crosslink, the NER machinery removes the unhooked crosslink. HDR proteins, including RAD51, and recombination mediators initiate strand invasion, resulting in restoration of the replication fork. This leads to a four-stranded DNA junction that must be resolved by a resolvase. It has been proposed that the XPF-ERCC1 NER nuclease can act as the resolvase in this process [Bergstralh and Sekelsky, 2008].

BREAST CANCER

Breast Cancer Subtypes

Clinical and molecular classification has clustered breast cancer into groups that seem to have biological significance. Among the categories emerging from these studies are estrogen receptor (ER)-and/or progesterone reception (PR)-positive tumors, HER2 gene-amplified tumors and ER/PR-negative and HER2-negative tumors. These subcategories were first defined by Perou and Botstein, using gene expression patterns from microarrays, and have been observed in independent studies from expression profiling of breast tumors [Perou et al., 2000; Gruvberger et al., 2001; Sorlie et al., 2001; West et al., 2001; van't Veer et al., 2002]. Despite differences in the platforms used for gene expression analysis, these subtypes can be identified using common genes from different datasets [Sorlie et al., 2003]. Although other categories of breast cancer exist (and will be added to the taxonomy), this classification reflects three major subgroups that we think of when treating patients with breast cancer.

From a therapeutic perspective, molecular classification is important, as it reduces the heterogeneity of patient groups and increases the likelihood of response to therapy [Martin et al., 2000]. There are two clear examples of this in breast cancer, from single-gene marker studies. The first, and perhaps most important finding in the biology of breast cancer, was the class distinction between ER-positive and -negative tumors. Across populations of breast cancer patients, it is clear that ER-positive tumors respond to antiestrogen therapy, whereas ER-negative tumors do not [MacGrogan et al., 1996; Colleoni et al., 2000]. A second example is that of HER2 gene-amplified tumors, which have been shown to respond preferentially to the anti-HER2 monoclonal antibody, trastuzumab (Herceptin¹) [Vogel et al., 2002]. It is gratifying, then, that expression profiling is able to identify these subgroups, across platforms, and has further distinguished sets of genes that define these tumor types. These subgroups are currently defined by cell lineage (luminal, basal) and the presence of the HER2 oncogene (HER2 enriched). The luminal tumors are divided into luminal A and luminal B. In addition, a new subtype, called “claudin low” has been added to the classification [Parker et al., 2009]. Although the initial study of Perou et al. [2000] used several hundred genes to classify the tumor subtypes, this group has recently distilled the classification to only 50 genes (termed the PAM50) [Parker et al., 2009]. In addition, they showed that the subtypes predict sensitivity to particular therapies. Furthermore, our recent analysis of a taxane monotherapy trial (CALGB 9842/9840) suggests that molecularly defined tumor subtypes differ in their response to this commonly used chemotherapy agent [Harris et al., 2009].

Responses of Breast Cancer Subtypes to Chemotherapy

Several lines of evidence suggest that different breast cancer subtypes respond differently to chemotherapy agents, as outlined in Table I.

HER2-Positive Tumors—The HER2 tumor subtype is more sensitive to anthracycline-containing chemotherapy and may be less sensitive to alkylating agents and agents which produce DNA crosslinks such as Cisplatin and Oxaliplatin [Arteaga et al., 1994; Harris et al., 2001; Gennari et al., 2008]. In addition, recent studies suggest that HER2 tumors are preferentially sensitive to taxanes [Hayes et al., 2007].

Anthracyclines are molecules that intercalate into DNA and interfere with DNA and RNA synthesis by blocking progression of topoisomerase II ahead of the replication fork [Rabbani et al., 2005]. Recent work supports this idea by showing that anthracyclines abrogate the action of etoposide by preventing formation of the cleavable complex [Treszezamsky et al., 2007]. The binding of anthracyclines to DNA, including daunomycin and adriamycin, has been characterized [Chaires et al., 1982, 1983, 1985; Fritzsche et al., 1982, 1987]. Interestingly, these drugs exhibit sequence context dependence in DNA binding, likely resulting from the structure of the DNA. The affinity of daunomycin for DNA increases as the GC content of DNA increases [Chaires et al., 1987, 1988, 1990]. Anthracyclines can also form covalent and bulky DNA adducts once they become intercalated into DNA. This occurs via a formaldehyde-mediated linkage. This adduct can then bind to the complementary strand of DNA forming a crosslink, although this is not believed to occur at high efficiency [Wang et al., 1991; Zeman et al., 1998]. Formation of the DNA adducts is also likely to involve the release of free radicals.

The underlying mechanism of the empiric observations that Her2-positive tumors respond to anthracyclines is not completely understood; however, there are potential explanations that are dependent upon the type of adduct formed in the DNA. Another possibility might be related to the mechanism of HER amplification itself.

Her2+ and Topo2A—Approximately 50% of HER2+ tumors are known to also carry an amplification of the TOP2A gene, which encodes topoisomerase II (topoII) alpha [Jarvin et al.,

1999; Di Leo et al., 2007]. Many of these tumors have increased expression of topoisomerase II alpha. Early clinical studies strongly suggest that tumors carrying amplifications of both the Her2 and topoII alpha genes respond to anthracycline-based therapy (for a review, see Di Leo et al. [2007]). However, the regulation of topoII protein expression is complicated, and it is not yet clear if protein levels can be used to predict therapeutic benefit. Importantly, this protein is the target of anthracycline-based therapy. Amplification of the TOP2A gene might result in increased levels of topoII alpha. At first glance, anthracycline treatment of tumor cells with large amounts of topoII alpha might be expected to result in little benefit, as only a portion of the topoII alpha molecules would likely be bound by drug. The molecules that are not bound by the drug would be expected to support DNA replication. However, it is also possible that large amounts of topoII alpha could bind to DNA, cleave it, and be trapped in a cleaved complex with an anthracycline drug, resulting in cell death, as shown in Table I. Amplification might also result in the production of a variety of splicing variants, some of which may be better protein substrates for anthracyclines. Much more needs to be done to provide mechanistic insight into the relationship between TOP2A amplification and sensitivity to anthracyclines.

Her2+ and NER—Sarasin and colleagues recently showed that cell lines with XPD mutations are more sensitive to doxorubin than cells with an intact NER system. This suggests that the bulky DNA adducts formed by anthracyclines are a substrate for NER proteins [Saffi et al., 2009]. Accumulation of these adducts in cells that are NER deficient is likely to lead to cell death due to inhibition of DNA synthesis, as shown in Table I. To the best of our knowledge, it is not known if HER2+ tumors are deficient in NER.

Her2+ and Aberrant DSB Repair—A second suggestion is that the HER2 amplification could have resulted from some type of repair deficiency, which might also be an underlying mechanism for sensitivity to anthracyclines and taxanes. It is known that many other classes of tumors exhibit hypersensitivity to DNA damage, suggesting that there is a connection between loss of DNA repair proficiency and tumorigenesis [Sweasy et al., 2005; Martin et al., 2008b]. For example, it is known that aberrant BER results in cellular transformation and also leads to sensitivity to alkylating agents [Lang et al., 2007]. A deficiency in DSB repair could be related to both the amplification of ERBB2 and the drug sensitivity. Her2+ tumors result from amplification of the Her2/ERBB2 locus, and this locus is surrounded by palindromic DNA sequences. Palindromic sequences are known to lead to stalling of the replication fork, which could lead to generation of a DSB. Should a DSB occur within the palindromic region, likely resulting from replication pausing or endogenous or exogenous DNA damage, it would need to be repaired. Aberrant HDR or NHEJ function, resulting from mutation of corresponding genes, could lead to aberrant or slow repair and result in gene amplification via the breakage-fusion-bridge mechanism either with or without single-strand annealing [Hanaka et al., 2007]. In addition, alterations in BER are known to lead to formation of DSBs (see for example work on Pol β variants from our lab [Lang et al., 2007]). If an increased frequency of break formation occurred in this palindromic region, ERBB2 amplification could result. Thus, it would not be surprising to observe a specific DNA repair landscape that is associated with Her2+ tumors. Should anthracycline therapy result in DSBs due to DNA intercalation and fork stalling, compromised DSB repair would be overloaded and eventually the cells would die, as shown in Table I. Should an increase in the overall level of oxidative DNA adducts result from anthracycline treatment, the BER system might be saturated, leading to an increase in the formation of DSBs. Cells with crippled HDR might not be able to repair all of the breaks, leading to increased cell death.

Taxanes, including paclitaxel, are microtubule-stabilizing agents. They act by stabilizing the GDP bound form of tubulin, leading to inhibition of mitosis [Weaver and Cleveland, 2005]. Recently, microarray profiling led to the identification of genes that were repressed in response to treatment of cells with taxanes. This same set of genes is overexpressed in tumors that exhibit

chromosomal instability (CIN) [Swanton et al., 2009]. Overexpression of these CIN survival genes correlates significantly with poor outcome [Sudo et al., 2004]. One of the CIN survival genes is BRCA1.

Taxanes and Top2A—The underlying mechanisms of the cellular responses to taxanes remain to be determined. Here, we discuss a few possibilities. TopoII alpha is associated with mitosis, which might link its genetic status in tumors to responses to taxanes. TopoII alpha functions in cell division by controlling the decatenation checkpoint [Luo et al., 2009]. This enzyme functions in the unwinding of intertwined chromatids before their segregation and must interact with the mediator of DNA damage checkpoint protein-1 for the activation of the checkpoint. TopoII alpha must also localize to centromeres before proper cell division can take place, and this is facilitated by its sumoylation [Dawlaty et al., 2008]. A defect in this function leads to CIN in mice. Should TopoII alpha be present in excess in tumor cells, such as HER2-positive tumors, a portion of it might not be sumoylated due to an imbalance in the sumoylation machinery and TopoII alpha. This could slow the overall rate of cell division, which would be exacerbated in the presence of taxanes, as shown in Table I. Should amplification of TOP2A lead to the preferential expression of variant isomers, some of these isomers might not be able to be sumoylated, once again leading to a slow rate of cell division that worsens in the presence of taxanes.

Taxanes and Downregulation of BRCA1—The observation of downregulation of CIN survival genes, including BRCA1, by taxanes may provide important insight into a possible mechanism of action. Downregulation of BRCA1 by paclitaxel would interfere with the formation of DSB repair complexes, leading to the repair of fewer DSBs significantly slowing repair of DSBs, as shown in Table I. Should HER2+ cells be defective in DSB repair, especially in a step that is downstream of DSB repair complex formation, DSBs would likely accumulate in cells and lead to increased cytotoxicity.

Luminal Tumors—Luminal tumors are driven, at least in part, by ER signaling. Estrogen metabolism can result in the production of reactive oxygen species that damage DNA (for review, see Yager and Davidson [2006]). This type of DNA damage is usually repaired by BER, and so variants in this pathway could be associated with ER+ breast subtypes. However, variants in HDR or NHEJ could also be associated with this subtype, as these pathways serve as a back up to BER.

Tumors in BRCA1 Carriers—Tumors from BRCA1 carriers, which are typically basal-like, are sensitive to cisplatin [Byrski et al., 2009; Jaspers et al., 2009]. Cisplatin and related compounds including carboplatin and oxaliplatin are bifunctional alkylating agents that induce predominantly intrastrand and ICLs but also induce monoadducts in DNA [Wang and Lippard, 2005]. ICLs induced by platin compounds are recognized by high-mobility group proteins and repaired by cross-link repair, which involves many of the FA and HDR proteins in addition to ERCC1/XPF (see above). Cisplatin monoadducts can be removed by the NER machinery [Wang and Lippard, 2005]. Recent studies have suggested that patients with very low levels of ERCC1 respond well to cisplatin therapy, likely because the adducts cannot be repaired completely via NER or cross-link repair (for a recent review see Martin et al. [2008a]). BRCA1 carriers are deficient in ICL repair and unlikely to be able to efficiently repair cisplatin-induced DNA damage, as shown in Table I.

Basal-Like Breast Tumors—A new subgroup of breast cancer, now termed the “basal-like” or “triple-negative” subtype, has emerged from microarray profiling studies. This tumor type is molecularly distinct from other breast cancers, expressing one or more of the basal cytokeratins (CK 5/6, CK 14, and CK903) and carries a worse outcome, with up to 50% of

these patients relapsing and dying of their breast cancer, even in early stages of disease [van't Veer et al., 2002; Perou et al., 2000; Sorlie et al., 2003; Sotiriou et al., 2003].

Unfortunately, the pathogenesis of these tumors is poorly understood, and it remains unclear what drives these tumor cells to proliferate and metastasize. These breast cancers are insensitive to receptor-directed inhibitors (tamoxifen and anti-HER2 therapy), which may explain the worse prognosis associated with this tumor type across multiple breast cancer datasets. However, the recently discovered “achilles heel” of this tumor subtype may lie in the fact that these tumors appear to be deficient in the HDR pathway, due to loss of BRCA1 or 2 activities [Bryant et al., 2005; Schneider et al., 2008; Fong et al., 2009]. Indeed, the tumors from BRCA1 carriers are nearly always triple negative (basal-like). Many groups are finding that basal breast tumors are more sensitive to agents that induce DSBs and/or crosslinks. These tumors are also sensitive to agents that, by synthetic lethality, knock-out two repair pathways (i.e., PARP inhibitors).

Recent studies suggest that BRCA1-deficient cells may be particularly sensitive to PARP inhibitors as these agents are able to disable the BER and SSB repair pathway; and this, coupled with the DSB repair defect in BRCA1/2-deficient tumors, leads to dramatic cytotoxicity [Bindra et al., 2005; Bryant et al., 2005].

The BRCA1 protein plays a role in numerous cellular processes, including DNA repair, cell-cycle checkpoint control, and transcription [Venkitaraman, 2002]. Although germ-line mutations in BRCA1 account for the majority of dominantly inherited breast cancers, sporadic breast carcinomas rarely show mutations in the BRCA1 gene [Futreal et al., 1994; Merajver et al., 1995]. Interestingly, decreased BRCA1 expression has been observed in sporadic breast cancers correlating with higher tumor grade and poor prognosis [Wilson et al., 1999; Beger et al., 2004; Ding et al., 2004]. In this case, BRCA1 loss may be a result of epigenetic silencing of the BRCA1 promoter [Olopade et al., 2008] or perhaps other mechanisms of BRCA1 downregulation. A novel mechanism of HDR loss has recently been shown by Glazer et al., of Yale Department Therapeutic Radiology [Bindra et al., 2005]. Specifically, it appears that hypoxia-induced downregulation of BRCA1 expression and consequently diminished homologous recombination may lead to genetic instability by shifting the balance between the high-fidelity homologous recombination and the error-prone NHEJ pathways of DSB repair. In support of this hypothesis, we have recently found that CAIX expression (a surrogate for hypoxia) is higher in triple-negative tumors and is inversely associated with levels of BRCA1 in these tumors (data not shown).

Hence, the loss of BRCA1 appears to be associated with this tumor subtype, further supporting the contention that triple-negative breast cancer is deficient in HDR, a fact that has been exploited clinically with the use of Cisplatin and PARP inhibitors (see below).

Translational Implications of Breast Cancer Therapy

Molecular Biology of Tumors and Clinical Trials—Microarray profiling of breast and other tumors, along with our increased understanding of how various types of tumors respond to cancer therapy based upon their molecular features, has and is likely to continue to make significant inroads in breast cancer management. As complete genome sequencing becomes cheaper, it is likely that the sequencing of breast and other tumors will become a diagnostic and treatment-planning tool in the near future. Therefore, the design of clinical trials for new modalities of treatment will have to be based at least partially on the molecular features of tumors and will likely be significantly different in the future. In the next section, we discuss various ideas regarding clinical trial design based upon molecular features of tumors.

Clinical Trial Design and Biomarker Development—The cost of developing cancer therapies is a growing concern. According to a survey of 681 cancer therapeutics in development, only 20% of them obtain final approval, with an average cost of \$1.04 billion per drug and a duration of 80 months [Adams and Brantner, 2006]. The high development costs of cancer therapy have been partly attributed to the highly heterogeneous cancer patient populations under treatment [Bell, 2010]. This translates into variable clinical treatment response among patients and, consequently, results in potential low overall treatment effectiveness. Therefore, large clinical trials enrolling many patients are needed to demonstrate the efficacy of the investigational therapeutic for approval. To meet such challenges, close attention has been paid to the development and utilization of biomarkers through the drug development process to deliver personalized health care [Frank and Hargreaves, 2003; Kelloff et al., 2006; Sawyers, 2008]. The ultimate goal of such efforts is to bring new therapies to appropriate patient populations as quickly as possible. Thus, clinical trial design and biomarker development need to go hand-in-hand to help realize the personalized medicine dream.

Three rough categories of biomarkers are pertinent in the discussion of improving clinical trial efficiency and providing tailored personalized medicine [George, 2008; Simon, 2008]. The greatest research focus of biomarker studies has been on prognostic biomarkers, where a biomarker is associated with clinical outcome. A surrogate biomarker is one that can be used in lieu of a primary clinical response endpoint. Predictive biomarkers could be used to direct patients to the most beneficial treatment option. Biomarkers can be simultaneously in more than one category. Clinical trial designs have been proposed for both discovering and validating predictive biomarkers [Simon, 2008; Tan et al., 2009].

The benefits of using biomarkers in clinical trials have been shown to improve trial efficiency by reducing the number of patients required to treat, unnecessary treatment of patients unlikely to benefit, and trial costs [Druker, 2002; Verweij et al., 2003; Romond et al., 2005]. However, an enrichment design of this nature is appropriate only when it is believed that patients tested positive for a particular biomarker are most likely to benefit from a particular therapy. In addition, to make trial accrual possible, the prevalence of the biomarker cannot be too low in the population, and screening methods for the biomarker must be robust and cost effective. In this design, the patients will be pretested for the biomarkers and only enrolled if they test positive.

Certain drugs such as trastuzumab (Herceptin) and imatinib (Gleevec) are prime successful examples of a biomarker enrichment strategy [Druker, 2002; Verweij et al., 2003; Romond et al., 2005]. However, not all markers have proven to be as useful in selecting patients for therapy, for example, EGFR in colon [Kwak et al., 2005]. In the case of DNA repair pathways, few markers have been developed to predict response in clinical settings [Powell and Bindra, 2009]. Perhaps some of the more interesting data comes from recent evidence that germline SNPs in the ERCC1 gene predict response to cisplatin in lung cancer [Olaussen et al., 2006].

Clinical trial designs are also available to demonstrate that the treatment is at least effective in test-positive sub-populations [Simon and Wang, 2006; Simon, 2008]. In such trials, efficacy tests will be performed first for the overall population; if no treatment effects can be demonstrated, then further tests are performed for the test-positive group only. Adaptive designs, which allow changing the enrollment criteria based on trial results and the status of the biomarker, have also been developed [Jiang et al., 2007; Wang et al., 2007; Simon, 2008]. One such trial design allows discontinuation of enrollment in the test-negative subgroup if preplanned interim analysis demonstrates that it is impossible to demonstrate efficacy in this group. If this type of result is obtained, the enrollment can be continued only for test-positive groups. High enough prevalence and a rapid response endpoint would be a prerequisite for such designs.

More progressive/controversial response-adaptive designs have been explored at a theoretical level for some time [Hu and Rosenberg, 2006]. Conceptually, these designs will allow patients to be allocated into a more favorable treatment arm according to evolving evidence. For example, in an active-control clinical trial, it is critical to investigate whether adding one or more new therapeutic components would be more beneficial than the standard therapy [Frei, 1972]. If a certain biomarker emerges to be indicative of preferable choice for a certain group of patients, such information will be allowed to be factored into modifying allocating probabilities of the incoming patient according to one's biomarker status. Currently, many obstacles hinder adopting such trial design in cancer trials [Sawyers, 2008; Bell, 2010]. Besides the ethnical and technical debates, the lack of suitable surrogate efficacy response biomarkers that could speed up the assessment of response in cancer clinical trials may render such design useless.

Impact of Personalized Therapy for Clinical Trials—Despite the fact that tremendous research efforts have been put into biomarker research, the biggest challenge is validating them in the clinical setting. Guidelines for biomarker discovery on popular platforms such as microarray have been developed, including rigorous reporting guidelines for biomarkers in clinical trials [McShane et al., 2005; Dupuy and Simon, 2007]. Anticipation of the broad utilization of pharmacogenetics and pharmacogenomics biomarkers in clinical trials related to various approval processes has led the FDA to issue submission guidelines for such data (March 2005). These efforts promote the standardization and rigorous validation process appropriate for usage of biomarkers.

To develop personalized medicine also calls for the redesign of clinical trials. The overall impact is multifold. The tremendous amount of genomics and proteomics information that now exists and the costs to obtain this information dictate the need for clinical trials to refine the patient population by putting this information to use [Singer, 2005]. If the therapeutic mechanism is well understood, simple tests could be done to enrich the population to reduce drug development costs and avoid unnecessary treatment, as we discussed in the cases of antiestrogen therapy and trastuzumab (Herceptin). The current clinical trial for PARP1 inhibitor in BRCA1/2 mutant ovarian and breast cancer, based on the discovery of synthetic lethality, is an example of using DNA repair pathway knowledge in both drug design and simultaneously refining the trial population [Martin et al., 2008b]. Such trials, in a sense, are the direct way to develop personalized medicine. Clinical trials could be used as vehicles to develop predictive markers for efficacy and safety and/or other prognostic markers concurrently with the drug-development process to facilitate the physician in the prioritization of treatments [Paik et al., 2004].

More recently, early clinical trials have begun to be used to validate the strategy and the underlying information-ranking algorithm to use individual molecular profiling to guide treatment choice in breast cancer. A trial of this type is currently recruiting; the trial aims to use molecular profiling by immunohistochemistry, fluorescence in situ hybridization, DNA microarrays, and reverse-phase protein microarrays of patients' tumors to select targets and provide treatment recommendations at the same time (www.clinicaltrials.gov, [Silvestri et al., 2010]). This latest approach is quite exciting if validated. If feasibility and cost are not the concern, all clinical trials could provide a standard validated set of genetic, genomic, proteomic, biochemical, and other patient characteristic information, and a therapeutic map could be built for each treatment to rapidly facilitate personalized medicine.

We would predict that a successful biomarker (or a set of markers) will be context specific, meaning specific to cancer type and certain patient populations. The key to the successes of these efforts would be the coordination, organization, and communication of many different talents such as basic research scientists, clinicians, statisticians, bioinformaticians, assay

developers among research institutes, pharmaceutical firms, and regulatory agencies. Translational research, the process that facilitates the translation of basic research results into concrete health care applications, has been promoted as the transformational driving force for the new century. Infrastructure that promotes translational research has begun to be established. We believe formulating and planning the DNA repair research in a translational research framework would further both basic and clinical application in this area.

DNA Repair Designer Drugs—Recently, synthetic lethality observed in tissue culture cells is being translated into the clinic. In this case, synthetic lethality refers to the inhibition of multiple repair pathways that tend to overlap with one another. This is based upon the initial discovery that BRCA-deficient cells are highly sensitive to Parp inhibitors. As mentioned earlier, Parp inhibitors likely interfere with BER and SSBR. This results in the accumulation of DSBs that cannot be repaired efficiently in BRCA-deficient cells.

Based on this concept, several groups have embarked upon treatment of the BRCA-deficient subtypes of breast cancer; that is, the BRCA1 and 2 carriers, and in triple-negative/basal breast cancer. Narod and coworkers [Byrski et al., 2009] conducted a study of preoperative cisplatin in BRCA1 and 2 carriers who were newly diagnosed with breast cancer. This study gave a striking result, with 90% complete response rate at the time of surgery—an unheard of level of activity of a single agent in any patient subgroup. This was confirmed in a subsequent study [Byrski et al., 2010]. In sporadic, triple-negative breast cancers, Garber et al. showed a single-agent pathologic complete response (pCR) rate of 30% in a preoperative trial of triple-negative/basal breast cancer. Although not as high as the Narod group, the single-agent pCR of 30% is higher than usually reported for preoperative therapy trials with combination therapy. In addition, a recent retrospective analysis of both early and metastatic breast cancer patients treated with cisplatin monotherapy showed that triple-negative breast cancers had a significantly higher response to cisplatin, 78% versus 51% in other subgroups [Sirohi et al., 2008]. This suggests that BRCA1 mutation carriers and sporadic triple-negative breast cancer may have similar DNA repair defects rendering their tumors sensitive to platin-containing compounds (see Table I). Cisplatin forms monoadducts and ICLs, as mentioned earlier. BRCA1 functions in the removal of crosslinks as part of the FA protein complex. Thus, tumors harboring dysfunctional BRCA1 variants would be expected to be sensitive to crosslinking agents including cisplatin.

BRCA and Parp Inhibitors—PARP inhibitors have been used in both BRCA carriers (as single agents) and in triple-negative breast tumors in combination with chemotherapy [O'Shaughnessy et al., 2009; Tutt et al., 2009]. In the former study, single-agent olaparib (Kudos/Asra-Zeneca) showed a 41% single-agent response rate in BRCA1 and 2 carriers with metastatic breast cancer, which is a remarkable effect for a single-agent-targeted therapy in breast cancer. Furthermore, O'Shaughnessy's group recently reported a doubling of overall response rate, and tripling of time to progression in triple-negative breast cancers treated with the new PARP inhibitor BSI-201 (BiPar/Sanofi-Aventis) in combination with chemotherapy. In addition, overall survival was improved with the addition of BSI-201, a benefit that is rarely seen in the metastatic setting and only preceded by the drug trastuzumab (Herceptin) in HER2-positive patients.

SUMMARY

Many chemotherapeutic agents exert their effects by damaging DNA. Evidence is emerging that the DNA repair landscape of individuals and their tumors impacts tumor development itself and the therapeutic responses of tumors. The concept of personalized cancer therapy with DNA-damaging agents could extend to other types of agents as well. Molecular profiling in combination with basic research and clinical trials has improved the treatment of breast cancer.

As we move toward whole genome sequencing, personalized cancer therapy is likely to emerge as a standard of care.

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REFERENCES

- Adams CP, Brantner VV. Estimating the cost of new drug development: Is it really 802 million dollars? *Health Aff (Millwood)* 2006;25:420–428. [PubMed: 16522582]
- Arteaga CL, Hurd SD, Dugger TC, Winnier AR, Robertson JB. Epidermal growth factor receptors in human breast carcinoma cells: A potential selective target for transforming growth factor α -pseudomonas exotoxin 40 fusion protein. *Cancer Res* 1994;54:4703–4709. [PubMed: 8062268]
- Audebert M, Salles B, Calsou P. Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. *J Biol Chem* 2004;279:55117–55126. [PubMed: 15498778]
- Barnes DE, Lindahl T. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet* 2004;38:445–476. [PubMed: 15568983]
- Beger C, Ramadani M, Meyer S, Leder G, Kruger M, Welte K, Gansauge F, Beger HG. Down-regulation of BRCA1 in chronic pancreatitis and sporadic pancreatic adenocarcinoma. *Clin Cancer Res* 2004;10:3780–3787. [PubMed: 15173085]
- Bell DW. Our changing view of the genomic landscape of cancer. *J Pathol* 2010;220:231–243. [PubMed: 19918804]
- Bergstralh DT, Sekelsky J. Interstrand crosslink repair: Can XPF-ERCC1 be let off the hook? *Trends Genet* 2008;24:70–76. [PubMed: 18192062]
- Bindra RS, Gibson SL, Meng A, Westermarck U, Jasin M, Pierce AJ, Bristow RG, Classon MK, Glazer PM. Hypoxia-induced down-regulation of BRCA1 expression by E2Fs. *Cancer Res* 2005;65:11597–11604. [PubMed: 16357170]
- Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005;434:913–917. [PubMed: 15829966]
- Byrski T, Huzarski T, Dent R, Gronwald J, Zuziak D, Cybulski C, Kladny J, Gorski B, Lubinski J, Narod SA. Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. *Breast Cancer Res Treat* 2009;115:359–363. [PubMed: 18649131]
- Byrski T, Gronwald J, Huzarski T, Grzybowska E, Budryk M, Stawicka M, Mierzwa T, Szwiec M, Wisnowski R, Slolek M, Narod SA, Lubinski J. Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant therapy. *J Clin Oncol* 2010;28:375–379. [PubMed: 20008645]
- Caldecott KW. Single-strand break repair and genetic disease. *Nat Rev Genet* 2008;9:619–631. [PubMed: 18626472]
- Chaires JB, Dattagupta N, Crothers DM. Studies on interaction of anthracycline antibiotics and deoxyribonucleic acid: Equilibrium binding studies on interaction of daunomycin with deoxyribonucleic acid. *Biochemistry* 1982;21:3933–3940. [PubMed: 7126524]
- Chaires JB, Dattagupta N, Crothers DM. Binding of daunomycin to calf thymus nucleosomes. *Biochemistry* 1983;22:284–292. [PubMed: 6824632]
- Chaires JB, Dattagupta N, Crothers DM. Kinetics of the daunomycin–DNA interaction. *Biochemistry* 1985;24:260–267. [PubMed: 3978073]
- Chaires JB, Fox KR, Herrera JE, Britt M, Waring MJ. Site and sequence specificity of the daunomycin–DNA interaction. *Biochemistry* 1987;26:8227–8236. [PubMed: 2831939]
- Chaires JB, Herrera J, Britt M, Fox KR, Waring MJ. Sequence specificity of the daunomycin–DNA interaction. *Biochem Pharmacol* 1988;37:1785–1786. [PubMed: 3377834]

- Chaires JB, Herrera JE, Waring MJ. Preferential binding of daunomycin to 5' ATCG and 5' ATGC sequences revealed by footprinting titration experiments. *Biochemistry* 1990;29:6145–6153. [PubMed: 2207063]
- Chang P, Jacobson MK, Mitchison TJ. Poly(ADP-ribose) is required for spindle assembly and structure. *Nature* 2004;432:645–649. [PubMed: 15577915]
- Colleoni M, Minchella I, Mazzarol G, Nole F, Peruzzotti G, Rocca A, Viale G, Orlando L, Ferretti G, Curigliano G, Veronesi P, Intra M, Goldhirsch A. Response to primary chemotherapy in breast cancer patients with tumors not expressing estrogen and progesterone receptors. *Ann Oncol* 2000;11:1057–1059. [PubMed: 11038046]
- Dawlaty M, Malureanu L, Jeganathan K, Sustmann C, Tahk S, Shuai K, FGrosschedl R, van Deursen J. Resolution of sister centromeres requires RanBP2-mediated SUMOylation of topoisomerase II α . *Cell* 2008;133:103–115. [PubMed: 18394993]
- Di Leo A, Claudino WM, Pestrin M, Licitra S, Biganzoli L. Using specific cytotoxics with a targeted mind. *Breast* 2007;16:S120–S126. [PubMed: 17720501]
- Ding SL, Sheu LF, Yu JC, Yang TL, Chen BF, Leu FJ, Shen CY. Abnormality of the DNA double-strand-break checkpoint/repair genes ATM, BRCA1 and TP53, in breast cancer is related to tumour grade. *Br J Cancer* 2004;90:1995–2001. [PubMed: 15138484]
- Druker BJ. STI571 (Gleevec) as a paradigm for cancer therapy. *Trends Mol Med* 2002;8(4 Suppl):S14–S18. [PubMed: 11927282]
- Dupuy A, Simon RM. Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. *J Natl Cancer Inst* 2007;99:147–157. [PubMed: 17227998]
- Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434:917–921. [PubMed: 15829967]
- Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–134. [PubMed: 19553641]
- Fortini P, Dogliotti E. Base damage and single-strand break repair: Mechanisms and functional significance of short- and long-patch repair subpathways. *DNA Repair (Amst)* 2007;6:398–409. [PubMed: 17129767]
- Frank R, Hargreaves R. Clinical biomarkers in drug discovery and development. *Nat Rev Drug Discov* 2003;2:566–580. [PubMed: 12838269]
- Frei E 3rd. Combination cancer therapy: Presidential address. *Cancer Res* 1972;32:2593–2607. [PubMed: 4118763]
- Freidberg, EC.; Wood, RD.; Walker, GC.; Siede, W. *DNA Repair and Mutagen*. ASM Press; Washington, DC: 2006.
- Fritzsche H, Triebel H, Chaires JB, Dattagupta N, Crothers DM. Studies on interaction of anthracycline antibiotics and deoxyribonucleic acid: Geometry of intercalation of iremycin and daunomycin. *Biochemistry* 1982;21:3940–3946. [PubMed: 7126525]
- Fritzsche H, Wahnert U, Chaires JB, Dattagupta N, Schlessinger FB, Crothers DM. Anthracycline antibiotics. Interaction with DNA, nucleosomes and inhibition of DNA synthesis. *Biochemistry* 1987;26:1996–2000. [PubMed: 3297138]
- Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y. BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 1994;266:120–122. [PubMed: 7939630]
- Gennari A, Sormani MP, Pronzato P, Puntoni M, Colozza M, Pfeffer U, Bruzzi P. HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: A pooled analysis of randomized trials. *J Natl Cancer Inst* 2008;100:14–20. [PubMed: 18159072]
- George SL. Statistical issues in translational cancer research. *Clin Cancer Res* 2008;14:5954–5958. [PubMed: 18829473]
- Gruvberger S, Ringner M, Chen Y, Panavally S, Saal LH, Borg A, Ferno M, Peterson C, Meltzer PS. Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. *Cancer Res* 2001;61:5979–5984. [PubMed: 11507038]

- Guidance for Industry Pharamcogenomic Data. UDS DHHS, FDA, CDER, CBER, CDRH. 2005
- Hanaka T, Cao Y, Bergstrom D, Kooperberg C, Tapscott S, Yao M-C. Intrastrand annealing leads to the formation of a large DNA plandrome and determines the boundaries of genomic amplification in human cancer. *Mol Cell Biol* 2007;27:1993–2002. [PubMed: 17242211]
- Hanawalt PC, Spivak G. Transcription-coupled DNA repair: Two decades of progress and surprises. *Nat Rev Mol Cell Biol* 2008;9:958–970. [PubMed: 19023283]
- Harris, L.; Parker, J.; Broadwater, G.; Schulz, V.; Halligan, K.; Lezon-Geyda, K.; Seidman, A.; Berry, D.; Winer, E.; Hudis, C.; Krasnitz, A.; Hicks, J.; Tuck, D.; Perou, C. Genome-Wide Profiling of Archived Material from CALGB 9840 and 9342 for Paclitaxel (P) and Trastuzumab (T) Response Biomarkers Using Gene Expression and Copy Number Analysis; Proceedings of the CTRC-AACR San Antonio Breast Cancer Symposium; San Antonio, TX. Philadelphia, PA: AACR; 2009. Abstract nr 4032
- Harris LN, Yang L, Liotcheva V, Pauli S, Iglehart JD, Colvin OM, Hsieh TS. Induction of topoisomerase II activity after ErbB2 activation is associated with a differential response to breast cancer chemotherapy. *Clin Cancer Res* 2001;7:1497–1504. [PubMed: 11410482]
- Hayes DF, Thor AD, Dressler LG, Weaver D, Edgerton S, Cowan D, Broadwater G, Goldstein LJ, Martino S, Ingle JN, Henderson IC, Norton L, Winer EP, Hudis CA, Ellsi MJ, Berry D. HER2 and response to paclitaxel in node-positive breast cancer. *N Engl J Med* 2007;357:1496–1506. [PubMed: 17928597]
- Hu, F.; Rosenberg, WF. The theory of response-adaptive reandomization in clinical trials. Wiley-Interscience; Hoboken, NJ: 2006.
- Huber A, Bai P, de Murcia JM, de Murcia G. PARP-1, PARP-2 and ATM in the DNA damage response: Functional synergy in mouse development. *DNA Repair (Amst)* 2004;3:1103–1108. [PubMed: 15279798]
- Jarvin T, Tanner M, Barlund M, Borg A, Isola J. Characterization of topoisomerase IIa gene amplification and deletion in breast cancer. *Genes Chromosomes Cancer* 1999;26:142–150. [PubMed: 10469452]
- Jaspers JE, Rottenberg S, Jonkers J. Therapeutic options for triple-negative breast cancers with defective homologous recombination. *Biochim Biophys Acta* 2009;1796:266–280. [PubMed: 19616605]
- Jiang W, Freidlin B, Simon R. Biomarker-adaptive threshold design: A procedure for evaluating treatment with possible biomarker-defined subset effect. *J Natl Cancer Inst* 2007;99:1036–1043. [PubMed: 17596577]
- Kelloff GJ, Lippman SM, Dannenberg AJ, Sigman CC, Pearce HL, Reid BJ, Szabo E, Jordan VC, Spitz MR, Mills GB, Papadimitrakopoulou VA, Lotan R, Aggarwal BB, Bresalier RS, Kim J, Arun B, Lu KH, Thomas ME, Rhodes HE, Brewer MA, Follen M, Shin DM, Parnes HL, Siegfried JM, Evans AA, Blot WJ, Chow WH, Blount PL, Maley CC, Wang KK, Lam S, Lee JJ, Dubinett SM, Engstrom PF, Meyskens FL Jr. O'Shaughnessy J, Hawk ET, Levin B, Nelson WG, Hong WK, AACR Task Force on Cancer Prevention. Progress in chemoprevention drug development: The promise of molecular biomarkers for prevention of intraepithelial neoplasia and cancer—A plan to move forward. *Clin Cancer Res* 2006;12:3661–3697. [PubMed: 16778094]
- Kim MY, Mauro S, Gevry N, Lis JT, Kraus WL. NAD₊-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. *Cell* 2004;119:803–814. [PubMed: 15607977]
- Kinsella T. Understanding DNA damage response and DNA repair pathways: Applications to more targeted cancer therapeutics. *Semin Oncol* 2009;36:S42–S51. [PubMed: 19393835]
- Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, Harris PL, Driscoll DR, Fidias P, Lynch TJ, Rabindran SK, McGinnis JP, Wissner A, Sharma SV, Isselbacher KJ, Settleman J, Haber DA. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci USA* 2005;102:7665–7670. [PubMed: 15897464]
- Lang T, Dalal S, Chikova A, DiMaio D, Sweasy JB. The E295K DNA polymerase beta gastric cancer-associated variant interferes with base excision repair and induces cellular transformation. *Mol Cell Biol* 2007;27:5587–5596. [PubMed: 17526740]
- Luo K, Yuan J, Chen JG, Lou Z. Topoisomerase II α controls the decatenation checkpoint. *Nat Cell Biol* 2009;11:204–210. [PubMed: 19098900]

- MacGrogan G, Mauriac L, Durand M, Bonichon F, Trojani M, de Mascarel I, Coindre JM. Primary chemotherapy in breast invasive carcinoma: Predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. *Br J Cancer* 1996;74:1458–1465. [PubMed: 8912545]
- Martin KJ, Kritzman BM, Price LM, Koh B, Kwan CP, Zhang X, Mackay A, O'Hare MJ, Kaelin CM, Mutter GL, Pardee AB, Sager R. Linking gene expression patterns to therapeutic groups in breast cancer. *Cancer Res* 2000;60:2232–2238. [PubMed: 10786689]
- Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: The role of DNA repair pathways. *Clin Cancer Res* 2008a;14:1291–1295. [PubMed: 18316546]
- Martin SA, Lord CJ, Ashworth A. DNA repair deficiency as a therapeutic target in cancer. *Curr Opin Genet Dev* 2008b;18:80–86. [PubMed: 18343102]
- McCabe KM, Olson SB, Moses RE. DNA interstrand crosslink repair in mammalian cells. *J Cell Physiol* 2009;220:569–573. [PubMed: 19452447]
- McElhinny SAN, Havener JM, Garcia-Diaz M, Juarez R, Bebenek K, Kee BL, Blanco L, Kunkel TA, Ramsden DA. A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining. *Mol Cell* 2005;19:357–366. [PubMed: 16061182]
- McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Cancer Inst* 2005;97:1180–1184. [PubMed: 16106022]
- Merajver SD, Pham TM, Caduff RF, Chen M, Poy EL, Cooney KA, Weber BL, Collins FS, Johnston C, Frank TS. Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. *Nat Genet* 1995;9:439–443. [PubMed: 7795652]
- Moore JK, Haber JE. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1996;16:2164–2173. [PubMed: 8628283]
- O'Shaughnessy J, Osborne C, Pippen J, Yoffe M, Patt D, Monaghan G, Rocha C, Ossovskaya V, Sherman B, Bradley C. Efficacy of BSI-201, a poly (ADP-ribose) polymerase-1 (PARP1) inhibitor, in combination with gemcitabine/carboplatin (G/C) in patients with metastatic triple-negative breast cancer (TNBC): Results of a randomized phase II trial. *J Clin Oncol (Meeting Abstr)* 2009;27(18S): 3.
- Olaussen KA, Dunant A, Fouret P, Brambilla E, Andre F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC, IALT Bio Investigators. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 2006;355:983–991. [PubMed: 16957145]
- Olopade O, Grushko T, Nanda R, Huo D. Advances in breast cancer: Pathways to personalized medicine. *Clin Cancer Res* 2008;14:7988–7999. [PubMed: 19088015]
- Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–2826. [PubMed: 15591335]
- Pardo B, Gomez-Gonzalez B, Aguilera A. DNA repair in mammalian cells: DNA double-strand break repair: How to fix a broken relationship. *Cell Mol Life Sci* 2009;66:1039–1056. [PubMed: 19153654]
- Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009;27:1160–1167. [PubMed: 19204204]
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature* 2000;406:747–752. [PubMed: 10963602]
- Powell SN, Bindra RS. Targeting the DNA damage response for cancer therapy. *DNA Repair (Amst)* 2009;8:1153–1165. [PubMed: 19501553]
- Rabbani A, Finn RM, Ausio J. The anthracycline antibiotics: Anti-tumor drugs that alter chromatin structure. *Bioessays* 2005;27:50–56. [PubMed: 15612030]

- Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;353:1673–1684. [PubMed: 16236738]
- Saffi J, Agnoletto MH, Guecheva TN, Batista LF, Carvalho H, Henriques JA, Stary A, Menck CF, Sarasin A. Effect of the anti-neoplastic drug doxorubicin on XPD-mutated DNA repair-deficient human cells. *DNA Repair (Amst)* 2009;9:40–47. [PubMed: 19926538]
- San Filippo J, Sung P, Klein H. Mechanism of eukaryotic homologous recombination. *Annu Rev Biochem* 2008;77:229–257. [PubMed: 18275380]
- Sawyers CL. The cancer biomarker problem. *Nature* 2008;452:548–552. [PubMed: 18385728]
- Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, Sledge GW, Carey LA. Triple-negative breast cancer: Risk factors to potential targets. *Clin Cancer Res* 2008;14:8010–8018. [PubMed: 19088017]
- Shall S, de Murcia G. Poly(ADP-ribose) polymerase-1: What have we learned from the deficient mouse model? *Mutat Res* 2000;460:1–15. [PubMed: 10856830]
- Silvestri A, Colombatti A, Calvert VS, Deng J, Mammano E, Belluco C, De Marchi F, Nitti D, Liotta L, Petricoin EF, Pierobon M. Protein pathway biomarker analysis of human cancer reveals requirement for upfront cellular-enrichment processing. *Lab Invest* 2010;2010:1–10.
- Simbulan-Rosenthal CM, Rosenthal DS, Luo R, Samara R, Espinoza LA, Hassa PO, Hottiger MO, Smulson ME. PARP-1 binds E2F-1 independently of its DNA binding and catalytic domains, and acts as a novel coactivator of E2F-1-mediated transcription during re-entry of quiescent cells into S phase. *Oncogene* 2003;22:8460–8471. [PubMed: 14627987]
- Simon R. The use of genomics in clinical trial design. *Clin Cancer Res* 2008;14:5984–5993. [PubMed: 18829477]
- Simon R, Wang SJ. Use of genomic signatures in therapeutics development in oncology and other diseases. *Pharmacogenom J* 2006;6:166–173.
- Singer E. Personalized medicine prompts push to redesign clinical trials. *Nat Med* 2005;11:462. [PubMed: 15875041]
- Sirohi B, Arnedos M, Popat S, Ashley S, Nerurkar A, Walsh G, Johnston S, Smith IE. Platinum-based chemotherapy in triple-negative breast cancer. *Ann Oncol* 2008;19:1847–1852. [PubMed: 18567607]
- Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lønning P, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;98:10869–10874. [PubMed: 11553815]
- Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003;100:8418–8423. [PubMed: 12829800]
- Sotiropoulos C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, Martiat P, Fox SB, Harris AL, Liu ET. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci USA* 2003;100:10393–10398. [PubMed: 12917485]
- Sudo T, Nitta M, Saya H, Ueno NT. Dependence of paclitaxel sensitivity on a functional spindle assembly checkpoint. *Cancer Res* 2004;64:2502–2508. [PubMed: 15059905]
- Swanton C, Nicke B, Schuett M, Eklund AC, Ng C, Li Q, Hardcastle T, Lee A, Roy R, East P, Kschischo M, Endesfelder D, Wylie P, Kim SN, Chen JG, Howell M, Ried T, Habermann JK, Auer G, Brenton JD, Szallasi Z, Downward J. Chromosomal instability determines taxane response. *Proc Natl Acad Sci USA* 2009;106:8671–8676. [PubMed: 19458043]
- Sweasy JB, Dalal S, Sun KW, Lai C-C, DiMaio D, Lang T. Expression of DNA polymerase beta mutants in mouse cells results in cellular transformation. *Proc Natl Acad Sci USA* 2005;102:14350–14355. [PubMed: 16179390]
- Sweasy JB, Lang T, DiMaio D. Is base excision repair a tumor suppressor mechanism? *Cell Cycle* 2006;5:250–259. [PubMed: 16418580]

- Tan DS, Thomas GV, Garrett MD, Banerji U, de Bono JS, Kaye SB, Workman P. Biomarker-driven early clinical trials in oncology: A paradigm shift in drug development. *Cancer J* 2009;15:406–420. [PubMed: 19826361]
- Treszezamsky AD, Kachnic LA, Feng Z, Zhang J, Tokadjian C, Powell SN. BRCA1- and BRCA2-deficient cells are sensitive to etoposide-induced DNA double-strand breaks via topoisomerase II. *Cancer Res* 2007;67:7078–7081. [PubMed: 17671173]
- Tutt A, Robson M, Garber JE, Domchek S, Audeh MW, Weitzel JN, Friedlander M, Carmichael J. Phase II trial of the oral PARP inhibitor olaparib in BRCA-deficient advanced breast cancer. *J Clin Oncol* (Meeting Abstr) 2009;27(18S):CRA501.
- van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530–536. [PubMed: 11823860]
- Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 2002;108:171–182. [PubMed: 11832208]
- Venkitaraman AR. Linking the cellular functions of BRCA genes to cancer pathogenesis and treatment. *Annu Rev Pathol Mech Dis* 2009;4:461–487.
- Verweij J, van Oosterom A, Blay JY, Judson I, Rodenhuis S, van der Graaf W, Radford J, Le Cesne A, Hogendoorn PC, di Paola ED, Brown M, Nielsen OS. Imatinib mesylate (STI-571 Glivec, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target. Results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study. *Eur J Cancer* 2003;39:2006–2011. [PubMed: 12957454]
- Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002;20:719–726. [PubMed: 11821453]
- Wang AH, Gao YG, Liaw YC, Li YK. Formaldehyde cross-links daunorubicin and DNA efficiently: HPLC and X-ray diffraction studies. *Biochemistry* 1991;30:3812–3815. [PubMed: 2018756]
- Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 2005;4:307–320. [PubMed: 15789122]
- Wang SJ, O'Neill RT, Hung HM. Approaches to evaluation of treatment effect in randomized clinical trials with genomic subset. *Pharm Stat* 2007;6:227–244. [PubMed: 17688238]
- Weaver BA, Cleveland DW. Decoding the links between mitosis, cancer, and chemotherapy: The mitotic checkpoint, adaptation, and cell death. *Cancer Cell* 2005;8:7–12. [PubMed: 16023594]
- West M, Blanchette C, Dressman H, Huang E, Ishida S, Spang R, Zuzan H, Olson JA Jr, Marks JR, Nevins JR. Predicting the clinical status of human breast cancer by using gene expression profiles. *Proc Natl Acad Sci USA* 2001;98:11462–11467. [PubMed: 11562467]
- Wiederhold L, Leppard JB, Kedar P, Karimi-Busheri F, Rasouli-Nia A, Weinfeld M, Tomkinson AE, Izumi T, Prasad R, Wilson SH, Mitra S, Hazra TK. AP endonuclease-independent DNA base excision repair in human cells. *Mol Cell* 2004;15:209–220. [PubMed: 15260972]
- Wilson CA, Ramos L, Villaseñor MR, Anders KH, Press MF, Clarke K, Karlan B, Chen JJ, Scully R, Livingston D, Zuch RH, Kanter MH, Cohen S, Calzone FJ, Slamon DJ. Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. *Nat Genet* 1999;21:236–240. [PubMed: 9988281]
- Wilson TE, Grawunder U, Lieber MR. Yeast DNA ligase IV mediates non-homologous DNA end joining. *Nature* 1997;388:495–498. [PubMed: 9242411]
- Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N Engl J Med* 2006;354:270–282. [PubMed: 16421368]
- Zeman SM, Phillips DR, Crothers DM. Characterization of covalent adriamycin-DNA adducts. *Proc Natl Acad Sci USA* 1998;95:11561–11565. [PubMed: 9751705]

TABLE I**Molecular Aspects of Breast Cancer Therapy**

Breast tumor subtype	Chemotherapy agent	Molecular target	Mechanism of action	Outcome
HER2+	Anthracyclines	Amplification of TOP2A	Direct binding to topoisomerase II, leading to accumulation of DNA breaks	Large accumulation of DNA breaks leads to cell death
		Formation of bulky anthracycline-DNA adducts in NER-deficient cells	Fewer adducts removed	DNA synthesis is inhibited leading to cell death
		Formation of oxidative lesions in cells with compromised DSB repair	Saturation of BER/SSBR leading to formation of DSBs	Accumulation of DSBs leads to cell death
	Taxanes	Stabilization of microtubules in cells with amplified TOP2A	Accumulation of topoII that is not sumoylated results in deficient cell division that is enhanced in presence of taxanes	Enhancement of defective cell division leads to cell death
		Downregulation of BRCA1 in cells already compromised for DSB repair	Complete or nearly complete deficiency in DSB repair	Accumulation of DSBs leads to cell death
Basal-like BRCA1 carriers	Platins	Formation of crosslinks in BRCA1-deficient cells	Crosslink repair deficiency	Accumulation of crosslinks leads to cell death
Basal-like/triple negative	Parp inhibitors	Synthetic lethality with defect in HDR	SSB and DSB repair deficiency	Accumulation of breaks leads to cell death