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Fine tuning chemotherapy to match BRCA1 status

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Abstract

Targeted cancer therapies have been primarily directed at inhibiting oncogenes that are overexpressed or constitutively active in tumors. It is thought that as the cell's circuitry gets rewired by the constitutive activation of some pathways it becomes exquisitely dependent on this activity. Tumor cell death normally results from inhibiting constitutively active pathways. The dependence of tumor cells on the activity of these pathways has been called oncogene addiction. Approaches that aim to exploit loss of function, rather than gain of function changes have also become a powerful addition to our arsenal of cancer therapies. In particular, when tumors acquire mutations that disrupt pathways in the DNA damage response they rely on alternative pathways that can be targeted pharmacologically. Here we review the use of *BRCA1* as a marker of response to therapy with a particular focus on the use of Cisplatin and PARP inhibitors. We also explore the use of *BRCA1* as a marker of response to microtubule inhibitors and how all these approaches will bring us closer to the goal of personalized medicine in cancer treatment.

Keywords

BRCA1; DNA damage; synthetic lethality; cancer; tumor suppressor; chemotherapy

I. INTRODUCTION

Genomic instability is a hallmark of cancer and it is thought to underlie the cancer cell's ability to acquire a more aggressive character over time as well as to develop drug resistance. It provides copious genetic variation on which selection acts to provide the tumor with the ability to ignore tissue controls and evade chemotherapy. The acquisition of genomic instability depends on the disruption of the cell's fail-safe mechanisms to prevent the passing of potentially harmful mutations to daughter cells. These mechanisms are collectively called the cellular DNA Damage Response (DDR). The DDR is better visualized as a large network of redundant processes, rather than a series of specific pathways, that sense and locate damaged DNA, coordinate cell cycle progression by activating cell cycle checkpoints, and promote repair.

Cancer cells carrying activated oncogenes are prone to replication stress, including stalled replication forks that eventually collapse and lead to the formation of double stranded DNA

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breaks. Premature termination of replication in these cells leads to a constitutive activation of the DDR and may result in activation of cell cycle checkpoints, induction of cell death or senescence [1–3]. Thus, it has been proposed that the DDR constitutes an earlier barrier to tumorigenesis, and full blown cancer can only develop by transposing this barrier [1;2]. Indeed, in early lesions markers of an activated DDR, such as phosphorylated forms of ATM, Chk2, p53, and H2AX reach maximal levels and precede allelic imbalance and mutations that disrupt cell cycle checkpoints [1–3]. Although in some cases disruption of these signaling pathways leads to lethality, in many other cases there is compensation by other redundant pathways.

For cancer cells, the flip side of this Faustian arrangement is manifested as an increased sensitivity to DNA damaging agents such as chemotherapy drugs or radiotherapy. In addition, as cancer cells become inevitably over dependent on specific pathways, pharmacologic targeting of these already overburdened processes result in tumor cell death (Figure 1). Detailed understanding of signaling pathways and their status in cells will allow the efficient exploitation of the tumor's increased sensitivity and its overdependence on certain pathways. We have made significant progress in the last twenty years on both of these fronts. Intensive research has now provided a coherent, albeit incomplete, view of the DDR [4]. Conceptual and technological improvements now allow us to start building a framework for personalized medicine.

II. CLONING *BRCA1*

The notion of an inherited predisposition to breast cancer is an old one [5;6], but it had been difficult to tease apart the contributions of genetic factors from those of shared environment. The identification of one large kindred with 668 individuals from Utah was instrumental to exclude the role of shared environment in the predisposition [7]. This family, called kindred 107, presumably shared the same environment and diet but only one branch of the family displayed a disproportionate incidence of breast cancers [7]. Despite this significant advance it was not until the late 1980's that the nature of the genetic determinants began to be more clearly delineated. The evidence from a large number of cluster families strongly suggested that the predisposition to breast and ovarian cancer was best explained by a dominant single locus [8]. This was quickly followed by the mapping of *BRCA1* to the long arm of chromosome 17 [9;10]. The identification of probes mapping proximal to the *BRCA1* locus provided the ability to show that *BRCA1* was a tumor suppressor gene conforming to the two-hit hypothesis with loss of the wild type allele in the tumor [11]. The Breast Cancer Linkage Consortium further refined its location by combining families from across the globe [12].

BRCA1 was positionally cloned in 1994 ending one of the most competitive races in science [13;14]. Cloning of *BRCA2*, which increases the risk of male breast cancer as well, came right after *BRCA1* and was important to allow the development of comprehensive genetic testing to identify individuals at increased risk for breast cancer [15–17].

III. *BRCA1* AS A MARKER FOR PROGNOSIS

With the development of genetic tests several groups started to look into how breast or ovarian cancer patients fared after the onset of disease depending on their *BRCA* (*BRCA1* or *BRCA2*) mutations had a more favorable clinical outcome than sporadic cancers [18–26] although more subtle differences or even opposite results have also been recorded [27;28]. These studies have different designs, patient population, tumor stratification, and endpoint measures which complicate the interpretation of these results. Yet the studies suggest a trend in which ovarian tumors arising in *BRCA* carriers tend to have a better outcome. Because

virtually all patients diagnosed with ovarian cancer are treated with platinum-based chemotherapy, the favorable outcomes may be directly related to an increased sensitivity of *BRCA*-linked tumors to platinum.

Because it was estimated that only approximately 10% of unselected epithelial ovarian cancers are attributable to germline mutations in *BRCA1* or *BRCA2*, and somatic mutations were thought to be rare in sporadic cancers [29–34] the implications of these results to ovarian cancer in general was thought to be limited. However, it is becoming increasingly clear that sporadic ovarian cancers inactivate *BRCA1* using different mechanisms. Reduced expression of *BRCA1* in ovarian cancers has been found in 13–90% of tumors suggesting that they might also respond well to Cisplatin-based therapy (reviewed in [35]).

The picture seems to be more complicated in the context of breast cancer. Retrospective studies on clinical outcomes of Ashkenazi Jewish women with breast cancer showed that *BRCA* mutation carriers had a poorer prognosis than non-*BRCA* mutation carriers [36;37]. Importantly, *BRCA1* mutation carriers had a worse overall survival if they did not receive adjuvant chemotherapy or adjuvant hormonal therapy [37]. Another study comparing *BRCA*-linked versus sporadic breast cancers found a trend towards a worse survival for *BRCA1*-associated ductal breast cancer [38]. A poorer prognosis for *BRCA1*-linked cancers was also found when comparing *BRCA1*-linked to other familial breast cancers [39]. However, in some cases no significant difference was noted [40;41]. Although there is some discordance in the results from these trials, which have the same caveats as the ones listed for ovarian cancer studies, there is evidence to suggest that *BRCA1* can be used as a prognostic marker.

IV. A ROLE FOR BRCA1 IN THE DNA DAMAGE RESPONSE

In the last fifteen years the role of *BRCA1* has come to a sharper focus. *BRCA1* functions in at least two cellular processes: the regulation of transcription and in DNA repair (reviewed in [42–48]). Both functions seem to be central to its role in the DNA Damage Response (DDR). These discoveries provide a clear rationale to frame the use of *BRCA1* not only as a prognostic marker but also as a predictor of clinical outcomes in response to therapy.

BRCA1 plays an important role in repair of double stranded breaks (DSBs) via homologous recombination (HR) [43;49]. DSB repair via homologous recombination occurs in late S or G2 phase when a newly synthesized sister chromatid can be used as a template [50;51]. Physically, *BRCA1* can be visualized at DSB sites as foci by immunofluorescence, colocalizing with histone H2AX phosphorylated at Serine 139, also known as γ -H2AX [52]. H2AX is phosphorylated by ATM following DNA damage in large megabase regions that spread from the location of the break [53;54]. *BRCA1* is involved in recruiting RAD51 to the DSB sites [55–57]. The RAD51 recombinase promotes strand invasion to use the sister chromatid DNA as a template to fix the DSB [58]. Underscoring the network structure of the DDR, *BRCA1* has been shown to be required for multiple cell cycle checkpoints (reviewed in [59]). In addition, at least partially, *BRCA1*'s tumor suppressor functions come from its other role as a transcription regulator for genes involved in DNA repair [60–69].

The participation of *BRCA1* in DNA repair processes at multiple levels provides a solid rationale for using *BRCA1* as a potential biomarker of response to genotoxic drugs. Data from in vitro and in vivo studies suggest that *BRCA1* status may be particularly suited to predict response to Cisplatin and to PARP inhibitors. Interestingly, sensitivity to a different class of drugs, microtubule-interfering drugs such as taxanes and vinca alkaloids, depends on *BRCA1* expression as well. Thus, *BRCA1* has been proposed to be a differential modulator of response to chemotherapy [70]. Below, we discuss the relationship between *BRCA1* status and response to these three classes of drugs.

V. TAXANES

Microtubule-interfering agents, such as taxanes and vinca alkaloids, bind to microtubules and disrupt the dynamic polymerization and depolymerization of tubulin, needed for the appropriate segregation of chromosomes during mitosis [71].

When Taxol binds to microtubules it prevents their depolymerization which leads to arrest in mitosis [72–74]. Taxanes currently used for chemotherapy are Paclitaxel (Taxol) and Docetaxel (Taxotere). After treatment with Paclitaxel cells go through an abnormal mitotic exit with multi-nucleated cells, followed by apoptosis [74;75]. Paclitaxel preferentially kills transformed cells rather than non-transformed cells, which was the basis for the initial enthusiasm for the drug in cancer treatment. The difference in sensitivity did not originate from an intrinsic property of the transformed state but rather from the fact that transformed cell populations have a higher number of mitotic cells, which are more susceptible to the drug's action than non-mitotic cells [74]. Also, non transformed cells resume normal proliferation after being released from the mitotic block by removal of Paclitaxel but transformed cells go into an abortive cell cycle with an abnormal chromosomal number and/or apoptosis [76]. By stabilizing microtubules, Paclitaxel disrupts mitotic spindle assembly and triggers the spindle checkpoint [77]. Defects in the spindle checkpoint have been associated with resistance to taxanes [78;79]. However, the picture is probably more complex as recent data have demonstrated that individual cells present a wide range of variation in their response to microtubule inhibitors and analysis of cell populations does not allow for the discrimination of these different responses [80;81].

Taxanes have been incorporated in the treatment of several cancers with promising results but neurotoxicity and bradycardia are both common side effects of Paclitaxel treatment [82]. Many patients suffer serious side effects caused by these drugs without experiencing a significant disease response. Thus, the emergence of BRCA1 as a possible predictor of response (reviewed in [83]) has sparked interest.

VI. BRCA1 AND TAXANES

Recently, a large body of in vitro, pre-clinical, and clinical data has highlighted a possible requirement for BRCA1 in the response to taxanes and other microtubule-interfering drugs. In 2001, two independent papers implicated BRCA1 as a mediator of the cellular response to microtubule-interfering drugs [84;85]. A series of experiments using different methods and a panel of breast cancer cell lines have confirmed that low levels of BRCA1 correlate with resistance to taxanes and to Vinca alkaloids [70;84–88](reviewed in [89]). Paclitaxel treatment leads to BRCA1-mediated activation of p38/MAPK and JNK/SAPK pathways [87;90]. BRCA1 promotes taxane-induced apoptosis but has the reverse effect on apoptosis induced by Cisplatin [70].

Evidence from mouse models (reviewed in [83]) and clinical studies also supports the role of BRCA1 in taxane sensitivity. Recently, several papers have explored the potential use of BRCA1 in lung cancer as a prognostic marker and as marker of clinical outcome after treatment. Results from these studies are variable but they clearly indicate that this is a promising line of research. Rosell et al. provided evidence that *BRCA1* levels could be used as a prognostic marker [91]. Overexpression of *BRCA1* mRNA was strongly associated with poor survival in chemo-naïve stage I, II, and IIIA NSCLC patients [91]. High *BRCA1* expression was also found to be a prognostic factor for both median and disease-free survival in stage IB and IIB surgically resected NSCLC patients [92].

Taron et al. performed the first study on whether *BRCA1* could be used as an indicator of chemoresistance in lung cancer. In a study of patients treated with Gemcitabine/Cisplatin in

a neoadjuvant setting, tumors with low levels of *BRCA1* mRNA presented with a better outcome than those with high levels [93]. This is in line with the idea that low *BRCA1* levels correlate with increased sensitivity to DNA damaging agents. The authors suggested, based on their study and on preclinical data [70], that patients with low levels of *BRCA1* would benefit from single agent Cisplatin whereas patients with higher levels would benefit from single agent Docetaxel [93]. On the other hand, a study of patients with NSCLC treated with Gemcitabine/Cisplatin or Epirubicin/Gemcitabine failed to see any correlation between levels of *BRCA1* in tumors and response [94].

Interestingly, Wang et al. found a significant positive correlation between *BRCA1* expression and sensitivity to Docetaxel in malignant pleural effusions of NSCLC patients [95]. A negative correlation with Cisplatin sensitivity was also found in line with previous studies [93;95]. A study with 102 patients with advanced NSCLC treated with Gemcitabine plus Docetaxel showed that as levels of *BRCA1* mRNA increased the probability of response increased and risk of progression decreased [96]. A negative correlation between low levels of *BRCA1* and longer time to progression was found in a subset of 31 patients receiving Cisplatin-based second-line therapy [96].

In summary, there is a growing body of exciting clinical evidence showing that low levels of *BRCA1* confers sensitivity to platinum [70;85;97;98] and resistance to microtubule drugs such as Paclitaxel, Docetaxel, and Vinorelbine [70;85;97]. *BRCA1* seems to be involved in the inverse relationship between genotoxic agents and microtubule-interfering drugs [99]. Thus, caution will need to be exercised when interpreting data from combination (*e.g.* Cisplatin and Docetaxel) therapy. In any event, exploration of *BRCA1* as a biomarker in lung cancer will potentially lead to better prediction of single agent chemotherapy in the context of neoadjuvant or second-line treatment.

VII. CISPLATIN: TEACHING NEW TRICKS TO AN OLD DOG

Cisplatin targets cellular DNA creating inter- and intra-strand cross links which distort the DNA and inhibit DNA replication, recombination and transcription [100]. Several pathways are involved in repairing these cross link lesions but primarily they are repaired by the Nucleotide Excision Repair (NER) pathway (reviewed in [101]). During Nucleotide Excision Repair a single stranded DNA break (SSB) intermediate is formed. If this SSB is not repaired before replication it leads to replication fork collapse and the generation of a double stranded break (DSB)[102]. DSBs can then be repaired by HR or by Non-homologous end joining (NHEJ). While HR repair is limited to late S-phase and G2, NHEJ repair of DSBs occur in all phases of the cell cycle [50]. NHEJ is the most utilized mechanism in mammalian cells but is an error-prone process that may lead to reduced survival [103;104]. Cell lines derived from patients with *Xeroderma Pigmentosum* and *Fanconi Anemia* are NER-deficient and HR-deficient, respectively, and are extremely sensitive to Cisplatin treatment [105].

Cisplatin does not specifically target cancer cells and can be extremely toxic to normal tissue causing irreversible damage. The dose limiting toxicity of Cisplatin is mainly nephrotoxicity, neurotoxicity, and myelosuppression [106;107]. Despite its toxicity, Cisplatin is widely used in cancer treatment (reviewed in [107]). The identification of tumors which are more likely to respond (for example, those with disruption of *BRCA1*) is likely to result in a broader therapeutic range for Cisplatin.

It is possible that lessons learned with *BRCA1* and response to Cisplatin may also be extrapolated to other genotoxic agents. Many pre-clinical and clinical studies have found that *BRCA1*^{-/-} cells are sensitive to a wide spectrum of DNA damaging drugs [108]. Cells deficient for *BRCA1* are more sensitive to doxorubicin, an anthracycline that targets

topoisomerase II and is used in breast cancer treatment [109;110]. Drugs that interfere with topoisomerases I or II produce double stranded breaks when the replication machinery encounters the DNA lesions, Breast cancer clinical studies with anthracyclines in the neoadjuvant setting showed the same trend in which *BRCA1/2* carriers had a better clinical response rate than did non-carriers [111]. Along similar lines, *BRCA1*^{-/-} cells are also more sensitive to topotecan, an alkaloid that inhibits topoisomerase I and is used for treatment of ovarian cancer [109]. Further clinical studies will be needed to clarify the role of *BRCA1* in the response to these drugs in the clinical setting.

VIII. RESTORATION OF BRCA PROFICIENCY BY BACK MUTATION: A NOVEL MECHANISM FOR RESISTANCE

Early experiments showed that mouse *Brcal*-defective cells were sensitive to Cisplatin [55]. Conversely, reconstitution of *BRCA1*-deficient cells with full length *BRCA1* leads to increased resistance to Cisplatin [88]. Upon treatment with Cisplatin tumors arising in *Brcal*^{-/-};*Tp53*^{-/-} mice displayed an increase in cell death, a decrease in the number of mitotic cells, and no RAD51 foci formation when compared to *Tp53*^{-/-} tumors [112]. Human breast cancer xenografts deficient in *BRCA1* responded better to in vivo Cisplatin treatment than xenografts of tumors formed with *BRCA1*-reconstituted cells [113].

There is mounting clinical evidence suggesting that Cisplatin-based treatment may be effective in treating *BRCA1*-associated cancers [114–116]. Similarly encouraging results were obtained with triple-negative breast tumors in the neoadjuvant setting [117]. Triple-negative tumors, which do not express estrogen or progesterone receptors, and do not contain amplification of *HER2/Neu* shared phenotypic features with *BRCA1*-linked tumors, although the two groups may not overlap completely. Interestingly, among the factors associated with platinum response was a low *BRCA1* mRNA expression or *BRCA1* promoter methylation, suggesting that *BRCA1* status could be used to predict response in sporadic breast cancers as well [117].

Over the years several different mechanisms of platinum resistance have been indentified, mostly using in vitro models (reviewed in [118]). However, there has been considerable debate about whether the mechanisms identified in cell lines operate in human tumors [118]. Recently, a novel mechanism of resistance to platinum drugs and PARP inhibitors was identified in *BRCA*-deficient tumors and cell lines, respectively [119–122]. In these cases, emergence of resistance was linked to restoration of HR through reversion mutation (reviewed in [123]). While most of the available data pertains to restoration of HR by reversion of *BRCA2* mutations [119–121], reversion of *BRCA1* mutations have also been observed [122]. Although the mechanisms have not been fully elucidated these mutations restore the reading frame of the protein and consequently its HR function [118]. A detailed knowledge of these mechanisms will be instrumental to improve chemotherapy results.

IX. PARP INHIBITORS: ADAPTING SYNTHETIC LETHALITY TO FIGHT CANCER

The concept of synthetic lethality originated with studies in *Drosophila* and in yeast [124–128]. Two genes can be defined as synthetic lethal when deletion (or markedly decrease in expression) of either in isolation does not affect viability but the combination leads to lethality. The current use of PARP (Poly ADP ribose Polymerase) inhibitors in patients with *BRCA*-related tumors is based on the concept of synthetic lethality.

The idea to use PARP inhibitors in *BRCA*-deficient cells stemmed from observations made in cells derived from *Parp1*^{-/-} mice [129–133]. PARP1 is an enzyme that recognizes SSBs,

is involved in base excision repair, and interacts with other base excision repair proteins such as XRCC1, DNA ligase III, and DNA polymerase beta to repair lesions in single stranded DNA [134]. Mice deficient in PARP are viable, fertile, and do not develop early onset tumors [131;135]. In the absence of PARP1, SSBs remain present for longer periods of time before being repaired [136]. In S-phase, the retention of unrepaired SSBs increases the likelihood of replication fork collapse and generation of a DSB. Although DSB can be repaired by HR or NHEJ [137–140], HR is the preferred mode during S-phase. The importance of HR-mediated repair in *Parp1*^{-/-} was revealed by a significant increase in RAD51 foci and in sister chromatid exchange when compared to *Parp1*^{+/+} cells [133]. These experiments led to the notion that PARP-deficient cells might be over dependent on HR.

As *BRCA1* and *BRCA2* have been shown to be important for HR [49;141;142] it was predicted that cells deficient in these genes might have increased sensitivity to PARP inhibitors [129;143]. As predicted, inhibition of PARP by pharmacologic compounds or by siRNA led to a decrease in clonogenic survival of *Brca1*- and *Brca2*-deficient mouse ES cells when compared to *Brca1*- and *Brca2*-proficient ES cells, with an increase in the formation of γ -H2AX foci, a marker for DSBs [129]. Along similar lines, *BRCA2*-deficient cells and their xenografts were also shown to be hypersensitive to PARP inhibitors [143].

The efficacy of a PARP inhibitor (AZD-2281) has also been verified in *Brca1*^{-/-};*Tp53*^{-/-} mice with mammary adenocarcinomas [144]. Importantly, there is also evidence that PARP inhibitors in combination with Cisplatin have even greater effect than the any of the two drugs alone [144]. In NSCLC and breast carcinoma xenografts treated with the PARP inhibitor and Cisplatin displayed significantly reduced tumor volume compared to Cisplatin monotherapy [145;146].

Results from a Phase I clinical trial using Olaparib (AZD-2281) in a cohort enriched in carriers of *BRCA1* or *BRCA2* mutations with refractory disease was recently reported [147]. Although not designed to assess efficacy, this trial reported durable objective responses in confirmed carriers of *BRCA1* or *BRCA2* mutations [147]. Of the nineteen *BRCA* mutation carriers treated solely with the PARP inhibitor 63% had a clinical benefit [147]. An important characteristic about the PARP inhibitors is that wild type cells are relatively unaffected by the drug and therefore toxicity should be low. This trial also confirmed these predictions as there was little to no toxicity reported even in mutation carriers [147].

PARP inhibitors currently under investigation are competitive inhibitors of NAD⁺, the substrate of PARP. Some of the first PARP inhibitors were nicotinamides and benzamides but the specificity to the PARP enzyme rather than other enzymes that uses NAD⁺ as a substrate was relatively low [148;149]. Chemical modifications based around these original structures led to more potent, specific, and soluble drugs that are going through clinical trials today [150].

Recently, it was also found that PTEN deficiency leads to a defect in HR. Similar to the case involving *BRCA* mutations, the HR defect caused by PTEN deficiency sensitizes cells to PARP inhibitors [151]. In addition, the use of siRNA screens to systematically look for synthetic lethal interactions with specific drugs is also likely to provide a wealth of potential targets [152]. The proof of principle for the use of synthetic lethality approaches provided by the encouraging results with PARP inhibitors will certainly add impetus to the search for synthetic lethal interactions that can be used in the clinic [101;153;154].

X. CONCLUSION: IS IT THE DAWN OF PERSONALIZED MEDICINE?

The accumulation of evidence that *BRCA*-deficient tumors respond well to Cisplatin and/or PARP inhibitors opens the possibility of fine tuning therapy to achieve maximal results with minimal side effects. Translating this knowledge into common practice clinical treatment is likely to be a smooth process as there are already genetic tests to reliably identify carriers of germline mutations. More importantly, the information from lung cancer trials using *BRCA1* levels as a predictor to drug response in tumors arising in individuals that are not mutation carriers suggests that the concept of tailoring therapy to the status of *BRCA1* could be extended to a larger group of patients [91–96]. In addition, the successful application of the synthetic lethality concept indicates that the identification of effective chemotherapy drugs for individual tumors is a clear possibility.

Certainly, several hurdles still have to be transposed. For example, we know very little about how tumor heterogeneity influences drug responses. Solid tumors are often an agglomerate of tumor and stromal cells of different origins such as fibroblasts, inflammatory and endothelial cells with a complex network of paracrine interactions. Knowledge of how these interactions can be harnessed to improve response will be one of the main challenges ahead. Likewise, tumor cells are also genetically and epigenetically heterogeneous. It is conceivable that certain subpopulations in the tumor respond different to certain drugs allowing for the emergence of resistance. Understanding how different tumors vary in their composition will help us to identify combination therapies and to tailor scheduling to obtain maximum efficacy.

Although these are issues that we are just now beginning to understand it is clear that just as the discovery of the *BRCA1* gene led the way for finding predisposition genes for hereditary cancer and a detailed understanding of DNA repair, the discovery of therapies tailored to specific genetic mutations can lead to a wide array of novel approaches in personalized medicine.

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Abbreviations

DDR	DNA Damage Response
DSB	double stranded break
HR	homologous recombination
NER	nucleotide excision repair
NHEJ	non-homologous end joining
NSCLC	non-small cell lung carcinoma
PARP	poly ADP ribose polymerase
siRNA	small interfering RNA
SSB	single stranded break

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