

## Temozolomide in the Era of Precision Medicine

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### Abstract

In the January 1, 2017, issue of *Cancer Research*, Nagel and colleagues demonstrate the value of assays that determine the DNA repair capacity of cancers in predicting response to temozolomide. Using a fluorescence-based multiplex flow cytometric host cell reactivation assay that provides simultaneous readout of DNA repair capacity across multiple pathways, they show that the multivariate drug response models derived from cell line data were applicable to patient-derived xenograft models of glioblastoma. In this commentary, we first outline the mechanism of activity and current clinical application of temozolomide, which, until now, has been largely limited to glioblastoma. Given the challenges of clinical application of

functional assays, we argue that functional readouts be approximated by genomic signatures. In this context, a combination of MGMT activity and mismatch repair (MMR) status of the tumor are important parameters that determine sensitivity to temozolomide. More reliable methods are needed to determine MGMT activity as DNA methylation, the current standard, does not accurately reflect the expression of MGMT. Also, genomics for MMR are warranted. Furthermore, based on patterns of MGMT expression across different solid tumors, we make a case for revisiting temozolomide use in a broader spectrum of cancers based on our current understanding of its molecular basis of activity. *Cancer Res*; 77(4); 823–6. ©2017 AACR.

In the January 1, 2017, issue of *Cancer Research* (1), Nagel and colleagues demonstrate that a combined measure of several DNA repair pathways using fluorescence-based multiplex host cell reactivation (FM-HCR) assays was able to better predict response to temozolomide in patient-derived xenograft (PDX) models of glioblastoma than methylguanine-DNA methyltransferase (MGMT) activity alone, which is the current standard. Temozolomide is an alkylating antineoplastic drug that underwent clinical testing in the 1990s and is now approved for treatment of patients with glioblastoma multiforme. It is rapidly and completely absorbed after oral administration and binds minimally to plasma proteins, resulting in limited interactions with concurrently administered drugs (2). Being a small lipophilic molecule, temozolomide penetrates the blood–brain barrier and is therefore one of the few drugs with central nervous system (CNS) activity. Dose modifications are not needed for liver and renal dysfunction as cytochrome P450 enzymes and kidney are not involved in temozolomide metabolism. Adverse events are predictable and toxicities are usually reversible and not severe. Despite its antitumor activity, favorable pharmacokinetic profile, and tolerability, current use of temozolomide is limited to a subset of CNS cancers (3).

Temozolomide is converted to the active metabolite 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) by nonenzymatic chemical conversion. Among the DNA lesions produced by MTIC, the most common is methylation at the N7 position of guanine, followed by methylation at the N3 position of adenine and the O6 position of guanine. Although the least frequent,

methylation of guanine at O6 ( $O^6$ -MeG) is critical for temozolomide cytotoxicity (Fig. 1A; ref. 4). In normal cells, direct repair of  $O^6$ -MeG by the enzyme MGMT effectively removes the methyl adduct, restoring guanine.  $O^6$ -MeG that persists in MGMT-deficient cells mispairs with thymine instead of cytosine during DNA replication. This alerts the DNA mismatch repair (MMR) pathway, which exclusively recognizes the mispaired thymine on the daughter strand and excises it, while the  $O^6$ -MeG persists in the template strand. The futile cycles of thymine reinsertion and excision result in extensive DNA resection and ultimately apoptosis. MMR-deficient cells do not detect alkylation adducts and hence are resistant to temozolomide even when they lack MGMT (5). Temozolomide is therefore most cytotoxic in cells with low levels of MGMT and intact MMR. Methylation of guanine at N7 is readily repaired by PARP (Fig. 1A) and is not cytotoxic unless the base excision repair (BER) pathway, and especially PARP, is inactivated, explaining the synergistic effect of temozolomide with PARP inhibitors.

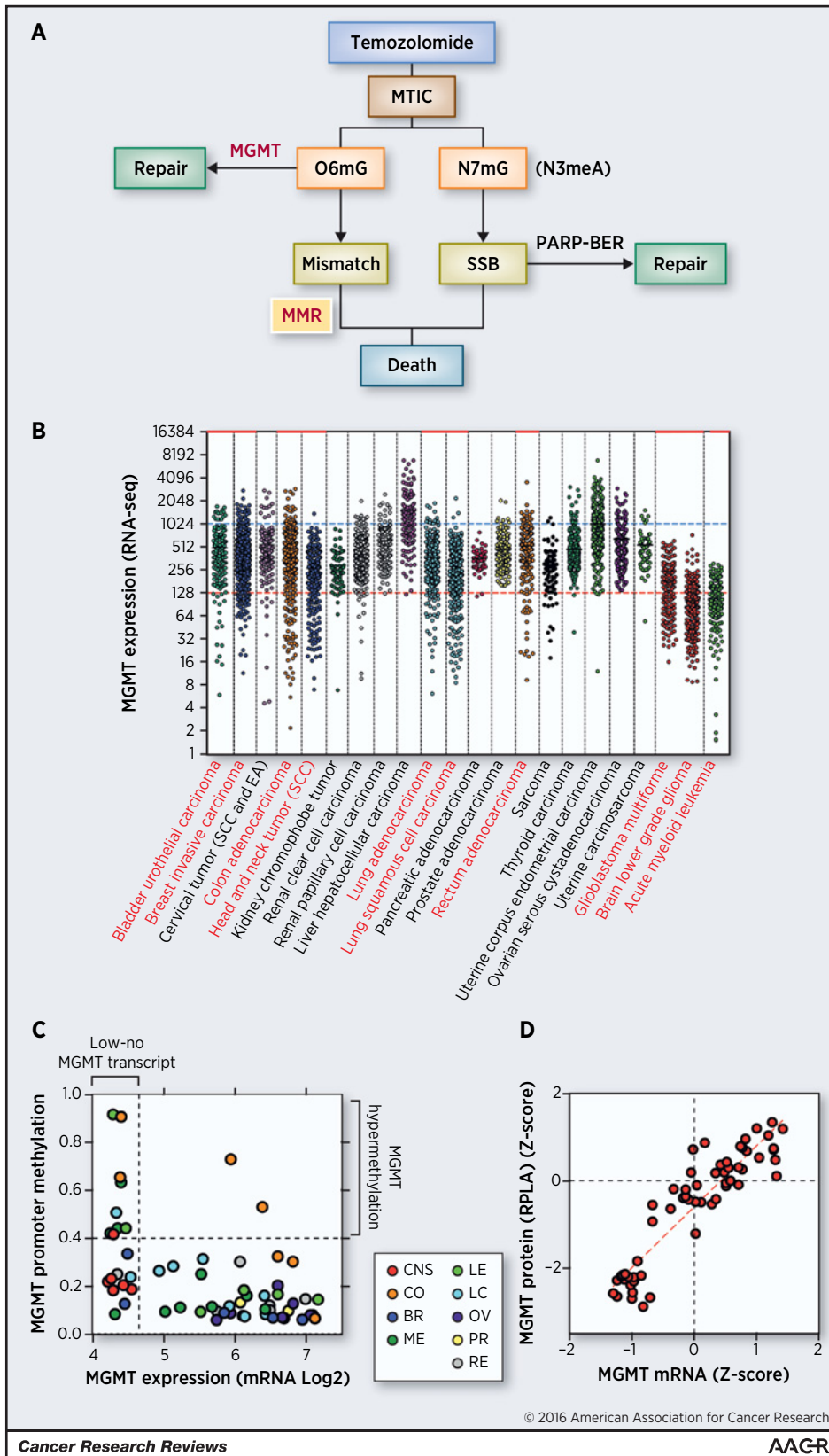
Nagel and colleagues used an FM-HCR assay (1, 6) to derive a functional assessment of several key DNA repair pathways, including MMR, homologous recombination (HR), nucleotide excision repair (NER), nonhomologous end joining, and MGMT, in human lymphoblastoid cell lines exposed to alkylating agents. The assay was then validated in PDX models of glioblastoma, where it was able to predict temozolomide sensitivity based on MGMT activity and even more reliably by adding MMR and HR to MGMT. MGMT is transcriptionally silenced in glioblastomas where MGMT promoter is methylated, and they tend to benefit from temozolomide compared with tumors with unmethylated MGMT (7). MGMT promoter methylation status has therefore remained in use clinically for several years now as a prognostic biomarker and as a selection criterion for temozolomide, especially for elderly patients with glioblastoma, but given the role of additional DNA repair mechanisms in determining temozolomide sensitivity, it is imperative to use multiparameter signatures that measure multiple DNA repair pathways to more precisely identify tumors that are more likely to benefit from

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**Figure 1.**

**A**, Schematic representation of mechanisms of cytototoxicity of temozolomide. **B**, mRNA expression (RNA-seq, RNA sequencing) of MGMT in the The Cancer Genome Atlas dataset. Data are from log<sub>2</sub> (RPKM) from RNA sequence, where RPKM is reads per kilobase of exon per million mapped reads. **C**, The comparison of MGMT mRNA expression and mean percentage of methylation in NCI-60 panel of cancer cell lines. mRNA expressions are determined from log<sub>2</sub> intensity values from Affymetrix microarrays. Methylation values are expressed as a beta (β) value (between 0 and 1) for each CpG site representing a continuous measurement from 0 (completely unmethylated) to 1 (completely methylated). **D**, High correlation between MGMT transcript and protein expression in the NCI-60 (<https://discover.nci.nih.gov/cellminer>). O6mG, O<sup>6</sup>-methyl guanine; N7mG, N7-methyl guanine; SSB, single-stranded break.

temozolomide. In this regard, the study by Nagel and colleagues is a step in the right direction. It is also encouraging that the multivariate drug response models based on DNA repair status can be extended from cell line data to PDX models.

Although patient-derived tumors could be an efficient way to functionally profile DNA repair pathways to direct therapeutic choices for individual cancer patients, there are several challenges to implementing this approach in clinic at the present time. First, it often takes several months to develop fully established cell lines from patient tumors, a time frame that precludes real-time applicability of the results (8). Newer technologies that would permit more rapid screening of cancer cells might circumvent this issue in the future. Approaches using explant models derived from patients are also limited by difficulties in obtaining viable tumor biopsies, especially in patients with progressive cancer. Models derived from circulating tumor cells that mirror the genomics and treatment response of patient tumors may provide tractable systems to evaluate DNA repair pathways (9). Current limitations of this approach include the extremely low frequency of circulating tumor cells in many cancers and the related issues of reliable detection and isolation. Second, in such assays, it is important to weigh the relative importance of each of the DNA repair pathways; clearly, MGMT and MMR are more useful in predicting temozolomide sensitivity than are HR and NER. Furthermore, it is important to note that there are additional mechanisms beyond DNA repair, including drug efflux pumps and DNA damage signaling, that affect chemotherapy resistance, highlighting the need to develop multiparametric measures that incorporate these factors. Assessing broader genomic signatures predictive of response might be easier and more effective than implementing a functional assay limited to DNA repair in clinical samples.

It is notable that DNA repair defects that predict sensitivity to temozolomide are prevalent in a broad spectrum of cancers beyond glioblastoma (10). MGMT deficiency primarily due to promoter hypermethylation has been reported in approximately 40% of colorectal cancers (10). MGMT gene promoter methylation is a frequent event in non-small cell lung cancer (11), small-cell lung cancer, gastric cancer (12), and pancreatic cancer (13). In The Cancer Genome Atlas, low MGMT expression is observed in a number of cancers beyond glioblastoma, including subsets of lung squamous cell carcinoma, breast-invasive carcinoma, colon adenocarcinoma, acute myeloblastic leukemia, and bladder carcinoma (Fig. 1B). Although defects in MMR have been characteristically described with hereditary nonpolyposis colon cancer (HNPCC) and cancers in the HNPCC spectrum, including colon and rectum, uterine endometrium, stomach, and ovaries, such defects are common in a broader spectrum of cancers (14). It is important to determine the MMR status of MGMT-deficient cancers because MMR inactivation would preclude the activity of temozolomide.

There are several key considerations in using MGMT and MMR status as predictive biomarkers. Although MGMT promoter methylation testing is the most commonly used technique to assess MGMT activity, its use to stratify patients for treatments is challenging (15). Methylation-specific PCR is the most commonly used diagnostic method for promoter methylation, owing to its simplicity and cost-effectiveness. However, identification and interpretation of methylation, which unlike mutations that are either present or absent, occur in patterns that are relevant for inhibition of expression, are challenging. There are other methods to assess MGMT promoter methylation status, each with its

unique benefits and drawbacks, but standardization of measurement and determining the optimal cutoff for outcome prediction is challenging. Another issue is the status of MGMT promoter methylation that can be unstable or heterogeneous and may change over time and with chemotherapy and could differ between primary and metastatic site (16–18). In addition, although MGMT promoter methylation is the major mechanism of MGMT gene silencing, it is not the only one; MGMT gene body methylation, histone modifications and aberrant expression or dysfunction of transcriptional activators or repressors, and miRNAs are others. Accordingly, a significant number of cancer cells that do not express MGMT do not show promoter hypermethylation, and other cancer cells show methylation but still express MGMT. In the cancer cell line panel of the NCI (Bethesda, MD), the NCI-60, DNA promoter methylation levels above 40% appear to affect MGMT expression levels, but result in background levels of expression for only a portion (81.2%) of those cell lines (Fig. 1C). In addition, DNA promoter methylation levels less than 40% occur in only a portion (81.8%) of expressed cell lines. Thus MGMT methylation is a useful but incomplete indicator of MGMT expression. Yet, MGMT expression is a good indicator of MGMT protein expression (Fig. 1D). Finally, even with MGMT inactivation, MMR deficiency confers resistance to temozolomide, indicating that it is imperative to measure both parameters. MMR is usually detected using a combination of immunohistochemical and molecular analysis for the occurrence of microsatellite instability. MMR protein IHC panel consisting of four proteins (MLH1, PMS2, MSH2, and MSH6) is scored on the basis of the presence or absence of nuclear expression of these proteins within the tumor cells (19). Recent studies have used mutational load detected by next-generation sequencing assays as an alternative means of screening for MMR deficiency (20). MMR status is critical not only for patient stratification for temozolomide, but it is increasingly becoming part of the standard assessment of patients with colorectal cancers where MMR status predicts clinical benefit of immune checkpoint blockade (21).

Although technical limitations preclude immediate clinical translation, the study reported by Nagel and colleagues (1) to identify functional assays to measure DNA repair capacity is an important step toward personalizing temozolomide use. Genomic signatures that provide an approximation of functional status of the DNA repair machinery could provide a more easily applicable clinical tool to select appropriate patients for temozolomide. Two important parameters to be assessed in this context are MGMT activity and MMR status of the tumor. Low MGMT activity and a functional MMR system are prerequisites for the cytotoxicity of temozolomide. We posit that, based on our current knowledge of cancer genetics and molecular mechanisms of drug activity, it is time to strategically reexamine the role of temozolomide in a broader spectrum of cancers in the era of precision medicine.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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