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ATM Is Required for the Repair of Oxaliplatin-Induced DNA Damage in Colorectal Cancer

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Genotoxic chemotherapy kills cancer cells at concentrations that do not kill normal cells in cancers that contain an abnormally large or unstable genome that replicates and divides more frequently, resulting in increased DNA damage induction. This scenario also applies to cancers with acquired mutations that limit DNA repair capacity. An appropriate strategy to improve clinical outcomes is to treat patients with a genotoxic agent that induces lesions in cancer cells, but not normal cells, that cannot be repaired as a consequence of an acquired mutation. In this issue of *Clinical Colorectal Cancer*, Sundar and colleagues[1] from the United Kingdom have identified an interesting association between oxaliplatin-based chemotherapy and superior overall survival (49 vs. 32 months) in patients with ataxiaetelangiectasia mutated (ATM)-deficient metastatic colorectal cancer (mCRC). In contrast, irinotecan-based therapy is not associated with improved overall survival in ATM-deficient mCRC but rather appears have a negative impact on clinical outcome in this particular subgroup of patients (24 vs. 33 months). Because the impact of an abnormally large or unstable genome is anticipated to affect DNA damage induction by oxaliplatin and irinotecan to a similar extent, the simplest explanation of these data is that ATM is essential for the repair of DNA lesions induced by oxaliplatin, but not for the repair of DNA lesions induced by irinotecan in colorectal cancer (CRC). The clinical implication of these findings is that CRC should be screened for ATM expression and/or ATM kinase function, and that ATM-deficient CRC should be treated with oxaliplatin-based therapy in the first-line setting rather than later-line settings.

ATM is a DNA damage signaling kinase activated at DNA double-strand breaks (DSBs).[2] ATM phosphorylates several thousand substrates that collectively affect cell cycle progression, DNA replication and repair, transcription, translation, and metabolism.[3] Above a poorly defined threshold of DNA damage, ATM kinase signaling can lead to either apoptosis or senescence, and accordingly, ATM loss may provide cancer cells with a selective advantage for growth and survival. ATM reduction and/or loss has been reported in

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537 (59%) of 908 of CRC tumors, and this was associated with worse disease-free survival.[4] In the present study, Sundar and colleagues reported ATM loss in 17 (8%) of 223 CRC tumor samples. The same anti-ATM antibody[5] was used in both studies; however, the scoring was more stringent in the Sundar study, in which ATM loss was not prognostic for survival. The frequency of ATM loss in CRC is clearly sufficient to impact clinical practice.

Genotoxic cytotoxic chemotherapy agents used to treat CRC include 5-fluorouracil (5-FU), irinotecan, and oxaliplatin, and each of these agents induce different DNA lesions that are accordingly repaired by different DNA repair mechanisms. 5-FU inhibits thymidylate synthase, and when cellular deoxythymidine triphosphate is exhausted, DNA replication forks stall. 5-FU can also be incorporated into DNA, thereby generating a lesion that is repaired by base excision repair, a DNA repair mechanism that does not involve cleavage of the phosphodiester backbone. Stalled replication forks are unstable, and when replication proteins dissociate from the replisome, so-called collapsed forks are generated. Collapsed forks are susceptible to exonuclease attack, which generates DNA double-strand breaks (DSBs). Irinotecan is a topoisomerase 1 poison that irreversibly inhibits the enzyme after single-strand cleavage. When a replication fork collides with a poisoned topoisomerase 1 complex, a DSB with a single free end is generated. Collapsed replication forks and DSBs at replication forks, including those induced by 5-FU and irinotecan, are repaired by homologous recombination, a DNA repair mechanism that uses the sister chromatid as a template for DNA synthesis to repair a DSB. Because the current study did not identify an association between irinotecan-based therapy and overall survival in ATM-deficient cancers, these findings strongly suggest that ATM is not essential for homologous recombination in CRC. For those ATM-proficient cancers, ATM inhibitors have been demonstrated to potentiate response to irinotecan-resistant tumors.[6]

Oxaliplatin is a third-generation bifunctional platinum analog that can react with 2 different nucleophilic centers in DNA. If the 2 sites are on opposite polynucleotide strands, interstrand DNA cross-links result, and if the 2 sites are in the same polynucleotide strand, intrastrand cross-links result. The major lesion generated by oxaliplatin is 1,2-intrastrand linkages between N7 positions of adjacent guanines, which are repaired by nucleotide excision repair (NER). The minor lesions generated by oxaliplatin are interstrand cross-links, which are repaired by the coordinated action of Fanconi anemia (FA), NER, and homologous recombination proteins. Because oxaliplatin-based therapy is associated with superior overall survival in ATM-deficient colorectal cancers, ATM is essential for the repair of intrastrand or interstrand cross-links, and presumably at an FA or NER step before homologous recombination, which functions to repair irinotecan-induced DSBs. NER removes a wide range of structurally unrelated bulky DNA lesions, including intrastrand and interstrand cross-links caused by oxaliplatin. Intra-strand cross-links are removed by NER, which is a cut-and-patch DNA repair mechanism; a string of nucleotides including the lesion are removed between 2 exonuclease cleavage events and then replaced by a DNA polymerase. The human disease yeroderma pigmentosum is caused by mutations in genes encoding NER proteins, and these mutations result in an inability to repair ultra-violet radiationeinduced thymidine dimers and 6,4-photoproducts. Interstrand cross-links are a complex lesion to repair, and nucleotides must be removed on both strands of the DNA. The human disease FA is caused by mutations in genes encoding FA proteins, which result in an

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inability to repair interstrand cross-links. Cells derived from FA patients show extreme sensitivity to cross-linking agents. It appears that ATM is essential for interstrand cross-link repair in colorectal cancers.

The immunohistochemical (IHC) assay used in the current study is a "presence or absence" assay, without the ability to differentiate among high, moderate, and low levels of ATM expression. For the remaining 92% of colon cancers with ATM protein, it remains unknown whether basal expression of ATM plays a role in the cancer's response to DNA damaging therapy. While ATM is activated (phosphorylation on Ser1981) after such therapies, the translational meaning of this observation to the patient is unclear. Higher induction of p-ATM may represent significant damage to the tumor DNA, thus suggesting a significant therapeutic response or rapid DNA repair leading to decreased therapeutic responses. Likewise, lower p-ATM induction may represent inefficient repair, genetic mutations within the repair pathway, or simply a lack of drug exposure to the tumor for pharmacologic reasons. To more specifically address these questions, a truly quantitative assay is necessary for total ATM protein expression and for the degree to which ATM is activated or phosphorylated.

With this in mind, our group has developed a quasiquantitative assay to determine the fold change of ATM phosphorylation (with respect to total ATM expression) after DNA damage therapy.[7] Detection of ATM activation requires specimens from the patient while active DNA damage responses are occurring. Obtaining multiple samples from patients via biopsy is not typically feasible. Thus, surrogate tissues (skin punches, peripheral blood mononuclear cells, circulating tumor cells are being utilized for such analyses. Mutational analysis by next-generation sequencing was also performed in this study. However, the mutational status of ATM did not seem to play a major role in its protein expression, as only 4 of 15 ATM IHC-loss specimens had ATM mutations. Thus, additional molecular analyses, such as DNA promoter methylation and RNA sequencing, may be necessary to further define ATM expression and its correlation with therapeutic response.

Oxaliplatin-containing combination chemotherapy, including FOLFOX (folinic acid, fluorouracil, and oxaliplatin) and XELOX (capecitabine and oxaliplatin), is a well-established standard-of-care option for the adjuvant therapy of early-stage colon cancer and for the treatment of mCRC. De Gramont et al[8] showed that the addition of oxaliplatin to infusional 5-FU (LV5FU2) in the first-line treatment of metastatic CRC significantly improved the overall response rate (22.3% vs. 50.7%; $P = .0001$) and progression-free survival (6.2 months vs. 9.0 months; $P = .0003$). To date, no validated biomarker has been identified to predict which patients have disease that is responsive to oxaliplatin-based chemotherapy for mCRC patients. The report by Sundar and colleagues raises an intriguing possibility that the significant additional tumor response with the addition of oxaliplatin to LV5FU2 in patients with mCRC may be due to an ATM defect, leading to a hypothesis that ATM deficiency, as determined by IHC, may be predictive for clinical response to oxaliplatin-containing combination chemotherapy in mCRC. This hypothesis could be evaluated retrospectively by analyzing available tumor tissues from prior phase 3 trials of oxaliplatin-containing combination chemotherapy trials. Furthermore, the validity of ATM deficiency as a predictive biomarker for clinical response to oxaliplatin could be tested

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prospectively by a randomized trial of FOLFOX/bevacizumab versus FOLFIRI (leucovorin, 5-FU, irinotecan, and oxaliplatin)/bevacizumab in the first-line treatment of patients with ATM-deficient mCRC defined by IHC, as reported by Sundar and colleagues. It is critical to develop a predictive biomarker for oxaliplatin therapy because oxaliplatin-containing chemotherapy is an effective systemic therapy. However, oxaliplatin is associated with a significant level of treatment-related toxicities, including oxaliplatin-induced cumulative peripheral neuropathy, myelosuppression, and gastrointestinal toxicity in the form of diarrhea and/or mucositis. For this reason, a predictive biomarker could help patients avoid ineffective and potentially toxic systemic chemotherapy.

This hypothesis is also relevant in the adjuvant setting because oxaliplatin-containing combination chemotherapy is a standard-of-care options for patients with stage III colon cancer. In the adjuvant setting, the addition of oxaliplatin to 5-FUebased adjuvant chemotherapy for patients with stage III colon cancer increases 5-year overall survival by an absolute risk reduction of 4% (cumulative risk of death by year 5, 22.1% in oxaliplatin/5-FU vs.26.3% in 5-FU alone).[9] A significant predictive correlation between ATM deficiency and survival benefit of oxaliplatin in the adjuvant setting could avoid oxaliplatin-induced severe toxicities by limiting oxaliplatin-containing adjuvant chemotherapy only to patients with ATM-deficient colon cancer. This hypothesis could be tested retrospectively by analyzing tumor tissues from prior randomized adjuvant trials of oxaliplatin-containing regiments, FOLFOX or XELOX, including MOSAIC, NSABP C-07, and NO16968 (XELOXA).[10–12] This could open the door for a personalized approach to administering conventional oxaliplatin-containing chemotherapy for patients with CRC by targeting ATM-deficient tumors, enhancing antitumor efficacy while avoiding oxaliplatin-induced toxicities.

DNA damage response inhibitors may potentiate the ATM-deficient CRC cell killing by oxaliplatin. ATR (ataxia telangiectasia and Rad 3-related) is an essential DNA damage signaling kinase activated at stalled and collapsed replication forks and resected DSBs that is similar in structure and function to ATM. Because the lesions that activate ATR are converted to lesions that activate ATM during DNA repair, and because the lesions that activate ATM are converted to lesions that activate ATR during DNA repair, both kinases are generally activated by genotoxic chemotherapy. Because ATM and ATR phosphorylate a broad and largely overlapping catalog of substrates, ATM loss may be compensated in part by ATR kinase signaling. Consistent with this premise, ATR kinase inhibitors, including M6620 and AZD6738, have been shown to synergize with cisplatin to kill ATM-deficient cancer cells in vitro and to have potent antitumor activity in in vivo xenograft models of ATM-deficient CRC.[13–16] ATM deficiency may therefore be another predictive biomarker for ATR inhibitor therapy and/or other agents targeting DNA damage response pathways.[17] These preclinical data suggest a hypothesis that the addition of ATR inhibitor to a FOLFOX/bevacizumab combination chemotherapy may represent a highly effective therapy for patients with ATM-deficient mCRC.[18] This hypothesis could be tested in a randomized phase 2/3 study of FOLFOX/bevacizumab with or without an ATR inhibitor in the first-or second-line treatment of patients with ATM-deficient mCRC.

ATM deficiency in CRC may now represent another treatment option for personalized medicine, as these patients have disease that may be responsive to ATR inhibitor-containing

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systemic therapy. Such a strategy would then expand personalized medicine approaches for patients with mCRC, which to date include testing for the presence of mutations in *RAS* and *BRAF*, as well as determining the status of DNA mismatch repair deficiency and/or microsatellite instability—high.

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