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Mutational burden in gliomas and implications for immune checkpoint immunotherapy

Immune checkpoint inhibition therapy, such as blockage of PD-1/PD-L1 interaction, has proven effective in many types of cancers. The mechanism underlying this therapy is postulated to involve "disinhibition" of tumor-infiltrating lymphocytes that respond to neoantigens expressed by tumor cells. In theory, the larger the number of neoantigens expressed, the greater the immune response resulting from disinhibition. Extension of this reasoning leads to the conclusion that a large number of tumor mutations and, consequently, a large number of neoantigens may be indicative of a better response to immune checkpoint inhibition. Therefore, assessments that measure tumor mutational load (TML) or mechanisms that result in a high level of mutations, such as mismatch repair (MMR) defects, increasingly are used as markers of responsiveness to immune checkpoint inhibition. The frequency of these biomarkers in gliomas has not been well studied. The authors conducted a study in which they profiled the frequency of shared biomarker phenotypes in gliomas. They studied 327 consecutive glioma patients for whom TML data were available. The cohort comprised predominantly grade 4 glioblastomas but also smaller numbers of grades 1, 2, and 3 gliomas. High, moderate, and low TML was defined as more than 20, 11 to 20, and 10 or fewer nonsynonymous missense mutations per 1.4 Mb, respectively. Immunohistochemistry was used to assess the number of CD8+ tumor-infiltrating lymphocytes as well as expression of PD-1 on such lymphocytes and PD-L1 on tumor cells for a subset of the cases. Immunohistochemistry and next-generation sequencing were used to assess expression and mutational status of the MMR system—*MLH1*, *MSH2*, *MSH6*, and *PMS2*. The authors found that a high TML was present only in high-grade gliomas and in only a small percentage of those tumors (15 of 327; 4.6 percent). Of those 15 gliomas with a high TML, 40 percent (six of 15) were newly diagnosed and 60 percent (nine of 15) were recurrent. The presence of a high TML was associated with loss of expression of MLH1, MSH2, MSH6, and PMS2. All cases with high TML showed loss of at least one of the four proteins analyzed. However, no association was found between high TML and the presence of one or more mutations in the MMR genes, which the authors suggest may be due to the inability of mutations to always alter protein activity. Interestingly, although a high TML and the likely increased neoantigen number might be expected to result in a greater influx of tumor-infiltrating lymphocytes, neither the gliomas with moderate nor high TML showed a significant association with CD8+ T-cell influx. Similarly, no association was found between a high TML and PD-1 or PD-L1 expression. The authors pointed out that previous studies of lung tumors have demonstrated that neoantigens induced via chemotherapy exposure are poor indicators of response to immune checkpoint inhibition. This may also be true for recurrent gliomas, which accounted for a significant percentage of the samples in this study, as radiation and chemotherapy may induce subclonal mutations that are less effective at stimulating an immune response. The authors concluded that it is unlikely that a single biomarker will predict responsiveness to immune checkpoint inhibition. Although additional studies with outcome information are needed to fully gauge the effectiveness of immune checkpoint blockade in gliomas, this study suggests that only a small subset of these tumors may show a durable response.

Hodges TR, Ott M, Xiu J, et al. Mutational burden, immune checkpoint expression, and mismatch repair in glioma: implications for immune checkpoint immunotherapy [published online ahead of print March 24, 2017]. *Neuro-oncol.* doi:10.1093/neuonc/nox026.

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Cell-free DNA sequence analysis as a source of genomic information in multiple myeloma

A bone marrow aspirate is the specimen of choice for the diagnosis and therapeutic monitoring of multiple myeloma. Because patient monitoring, in particular, may require repeated sampling, significant limitations of this approach include patient discomfort and technical challenges with collecting adequate sample. Similar sampling challenges complicate the study of solid tumors. In the latter setting, cell-free tumor DNA (cfDNA) from blood has shown promise as an alternative source of tumor-specific genomic information. The authors of this study suggest that cfDNA may also serve as a reliable source of genomic information for multiple myeloma patients. They used a hybrid capture-based next-generation sequencing assay to sequence the coding regions of *KRAS, NRAS, BRAF, EGFR*, and *PIK3CA* in 64 cfDNA specimens obtained from the plasma of 53 multiple myeloma patients (11 newly diagnosed and 42 relapsed, of which 13 were enrolled in a clinical trial of the MEK inhibitor trametinib). The authors found that the yield of cfDNA from these patients was higher than they had observed in specimens from solid tumor patients. To improve the ability of their bioinformatics process to distinguish somatic mutations from germline variants, they first used training and validation groups of cfDNA specimens for which sequence information from matched bone marrow aspirate samples was available. These studies demonstrated a 96 percent concordance between mutations detected in cfDNA and matched bone marrow (26 of 27 bone marrow mutations detected in cfDNA). A *KRAS* mutation present at an allele fraction of 1.3 percent in a bone marrow specimen (found only by ultra-deep sequencing of the marrow) was not detectable in the corresponding cfDNA sample. The absence of this mutation in the cfDNA sample was confirmed by digital droplet PCR. Because this mutation was present at a level below the reportable range of the laboratory's clinical assay—that is, 10 percent allele fraction—the overall concordance between the cfDNA assay and the clinical assay used for bone marrow analysis was 100 percent. Overall, mutant allele fractions were found to be highly concordant between cfDNA and bone marrow. The potential utility of this sampling approach was demonstrated by the ability to obtain sequence information from cfDNA samples for which corresponding bone marrow aspirate samples failed to yield adequate material for analysis (n=7). Furthermore, all 13 patients enrolled in the clinical trial of trametinib were correctly identified as biomarker positive or negative using the cfDNA assay. Although this study used an assay that is limited with regard to the scope of genes interrogated, it presents intriguing evidence supporting the value of a liquid biopsy approach to the diagnosis and monitoring of multiple myeloma patients.

Kis O, Kaedbey R, Chow S, et al. Circulating tumour DNA sequence analysis as an alternative to multiple myeloma bone marrow aspirates. *Nat Comm.* 2017;8:15086. doi:10.1038/ncomms15086.

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