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Ipilimumab and nivolumab for pituitary carcinoma

Marked response of a hypermutated adrenocorticotrophic hormone-secreting pituitary carcinoma to ipilimumab and nivolumab

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Context:
Pituitary carcinoma is a rare and aggressive malignancy with a poor prognosis and few effective treatment options.

Case:
A 35-year-old woman presented with an aggressive adrenocorticotrophic hormone-secreting pituitary adenoma that initially responded to concurrent temozolomide and capecitabine prior to metastasizing to the liver. Following treatment with ipilimumab and nivolumab, the tumor volume of the dominant liver metastasis reduced by 92%, while the recurrent intracranial disease regressed by 59%. Simultaneously, her plasma ACTH level decreased from 45,550 pg/ml to 66 pg/ml.

Molecular evaluation:
Both prospective clinical sequencing with MSK-IMPACT and retrospective whole-exome sequencing were performed to characterize the molecular alterations in the chemotherapy-naïve pituitary adenoma and the temozolomide-resistant liver metastasis. The liver metastasis harbored a somatic mutational burden consistent with alkylator-induced hypermutation that was absent from the treatment-naïve tumor. Resistance to TMZ treatment, acquisition of new oncogenic drivers, and subsequent sensitivity to immunotherapy may be attributed to hypermutation.

Conclusion:
Combination treatment with ipilimumab and nivolumab may be an effective treatment in pituitary carcinoma. Clinical sequencing of pituitary tumors that have relapsed following treatment with conventional chemotherapy may identify the development of therapy-induced somatic hypermutation, which may be associated with treatment response to immunotherapy.

Pituitary carcinomas may respond to ipilimumab and nivolumab; alkylator-induced hypermutation may contribute to malignant transformation and sensitivity to immunotherapy.

Introduction

Cushing disease (CD) is a rare endocrine condition associated with an adrenocorticotrophic hormone (ACTH)-secreting pituitary tumor causing excess adrenal cortisol production. While the vast majority of pituitary tumors are benign, approximately 0.1-0.2% are classified as carcinomas, which are defined by the presence of noncontiguous craniospinal or systemic...
metastasis. Pituitary carcinomas with systemic metastases are particularly aggressive malignant with a median survival of one year.

Treatment of aggressive and malignant pituitary tumors relies on a combination of surgical resection, radiation therapy (RT), and medical therapies which are few in number and often ineffective. The only tumor-directed medical therapies available for ACTH-secreting tumors are cabergoline, a dopamine-2 receptor agonist, which reduces circulating cortisol values in 25-40% of CD patients, and pasireotide, a somatostatin analogue which normalizes 24-hour urinary free cortisol values in about 13-25% of CD patients. These therapies are used for benign disease, but provide only modest reduction in tumor size, and have shown very limited success in the treatment of aggressive and malignant tumors. Temozolomide (TMZ) is an alkylating agent approved for the treatment of glioblastoma that has shown modest activity in pituitary carcinomas, especially when used in combination with capecitabine. Many patients either do not respond or escape control; hence, there remains a large unmet therapeutic need in this patient population.

The checkpoint inhibitors ipilimumab and nivolumab are effective in the treatment of a number of solid tumor types. A common adverse effect of anti-PD1 and CTLA-4 therapy is the development of hypophysitis, suggesting pituitary endocrine cell susceptibility to these agents. To date, the response of patients with pituitary tumors to immunotherapy has not been reported. Here, we report a patient with an immunotherapy-refractory aggressive ACTH-secreting pituitary carcinoma with sellar, contiguous dural involvement and hepatic metastases that responded to checkpoint inhibitors and we investigate the molecular basis for this response. This case suggests a potential role for immunotherapy in the treatment of pituitary carcinoma.

Case Report

A 35-year-old woman presented for management of a right third cranial nerve palsy, hirsutism, and weight gain in the fall of 2011. The patient was found to have a pituitary macroadenoma, elevated 24-hour urine-free cortisol values, and elevated plasma ACTH levels, consistent with CD. She underwent two consecutive subtotal transsphenoidal resections. Over the next year, the residual adenoma enlarged in size and she received fractionated radiotherapy (5,040 cGy in 28 fractions). When the tumor grew within months following RT, she underwent a third and a fourth transsphenoidal resection, 27 and 39 months following initial diagnosis.

Due to further growth and incomplete hormonal control despite pasireotide, ketoconazole, and ketoconazole in combination with cabergoline, she was treated with concurrent TMZ and capecitabine (CAPTEM); treatment was discontinued after 4 cycles due to thrombocytopenia and poor tolerance. She had a biochemical and radiographic response to treatment (a 41% decrease in tumor volume and her ACTH level decreased from 266 to 80 pg/ml). Though the pituitary adenoma remained stable in size over the next two years, she suffered from worsening hypercortisolemia-induced comorbidities including diabetes, hypertension, deep vein thrombosis, and pulmonary embolism. Treatment with mifepristone and metyrapone was unsuccessful due to refractory hypokalemia and inability to achieve eucortisolemia; hence, she underwent evaluation for a bilateral adrenalectomy with body computed tomography (CT), which revealed a liver lesion suspicious for malignancy 68 months following initial diagnosis. A biopsy revealed a high-grade neuroendocrine neoplasm that was focally positive for ACTH and exhibited a mitotic index of up to 50%. MGMT immunohistochemistry on this liver specimen demonstrated retained nuclear staining.
Following bilateral adrenalectomy, the patient experienced resolution of the diabetes, hypertension, obesity, and corticosteroid-induced psychosis, but developed worsening of the right third nerve palsy. Magnetic resonance imaging (MRI) of the sella and brain demonstrated extension of the pituitary tumor along the tentorium, suggestive of Nelson’s syndrome, and the post-operative MRI of the abdomen showed rapid progression of disease in the liver (Figure 1AB). She received two additional cycles of CAPTEM and was reimaged, revealing both intracranial and extracranial progression of disease. The original liver metastasis measured larger, and she was found to harbor additional liver metastases. Following progression on two cycles of carboplatin and etoposide and RT to the intracranial component, she consented to investigational treatment with combination ipilimumab (3 mg/kg every 3 weeks) and nivolumab (1 mg/kg every 3 weeks).

Five days after her first infusion of ipilimumab and nivolumab, she developed a fever to 40ºC and a mild transaminitis that resolved with high dose glucocorticoid treatment. Within one week of starting ipilimumab and nivolumab, ACTH levels dropped 10-fold, from 45,550 to 4,764 pg/ml. Following five treatments with ipilimumab and nivolumab, the dominant hepatic metastasis regressed by 92% (415 to 34 cm$^3$), the recurrent intracranial component decreased by 59% (3.9 to 1.6 cm$^3$), and her plasma ACTH level decreased to 66 pg/ml (Figure 1AB). Between cycles 4 and 5 of ipilimumab and nivolumab, two of the smaller satellite liver metastases enlarged in size, but then stabilized or shrank on subsequent imaging, consistent with pseudoprogression from immunotherapy. Clinically, there was a reversal in the right third nerve palsy. Of note, while the patient was hypopituitary prior to starting immunotherapy, serum TSH values decreased after immunotherapy, with free-T4 remaining in the normal range on replacement, suggesting additional hypophysitis-induced hypopituitarism may have occurred with her treatment. The patient has received 5 cycles of concurrent ipilimumab and nivolumab, is receiving maintenance nivolumab, and continues to respond without additional immunologic adverse reactions at 6 months of follow-up with an ACTH of 59 pg/ml at the time of this report.

Materials and Methods

MSK-IMPACT and exome sequencing
All clinical sequencing included in this report was performed via a research protocol that was approved by the Memorial Sloan Kettering Cancer Center Institutional Review Board. The patient provided written informed consent for tumor sequencing and review of medical records (NCT01775072). Prospective clinical sequencing on MSK-IMPACT (a targeted sequencing platform covering 468 cancer-associated genes and select intronic regions for detection of recurrent gene-fusion events) was performed in CLIA-certified environments, as previously described, for the following patient specimens: (1) the TMZ-naïve pituitary tumor from the final transphenoidal resection and (2) the TMZ-exposed liver lesion. All variants detected by MSK-IMPACT were manually reviewed. Additionally, the analysis of germline variants was performed in a subset of 76 genes, with manual variant review by members of the clinical genetics service.

For research purposes, the cDNA libraries from aforementioned assay were subjected to exome sequencing as previously described. Briefly, after target capture using SureSelect Human All Exon V6 (Agilent Technologies) and sequencing on HiSeq 2500 (Illumina) to generate paired-end 125-bp reads, reads were aligned to genome assembly b37 using BWA-MEM (v0.7.5a) and subsequently processed using GATK (GATK suite v3.3-0, Picard tools v2.9) best practices. The mean target coverage in the tumor specimens was 129× and 179×,
respectively, and \(2^x\) in the normal sample. Somatic SNVs were called with MuTect (v1.14),\(^{16}\) and Pindel (v0.2.5a7)\(^{17}\) and VarDict (v.1.5.1, www.github.com/AstraZeneca-NGS/VarDict) were used for detection of insertions/deletions. Variants were annotated with VEP (v88) using vcf2maf (v1.6.14, www.github.com/mskcc/vcf2maf). Filtering of false-positive mutation calls was performed using a set of filters (www.github.com/mskcc/ngs-filters).

Genomic variants were classified as oncogenic or likely oncogenic based on annotation with OncoKB (www.github.com/oncokb/oncokb-annotator, accession May 23, 2018). Mutational burden was estimated as the total number of mutations per megabases of targeted regions.

**Immunohistochemistry**

Immunohistochemistry for MSH6 (clone 44, ready-to-use, Ventana) was performed on the Ventana platform. Loss of staining was defined as no nuclear labeling in any of the tumor cells examined.

Immunohistochemistry for PD-L1 (clone E1L3N, dilution 1:250; Cell Signaling Technology) was performed on the Leica Biosystems’ Bond III platform. Membranous staining in either the tumor cells or the tumor-associated immune cells was counted as positivity.

For both stains, appropriate internal and external controls were applied.

**Radiology Response Review**

Radiographic response was determined by a board-certified radiologist who manually performed volumetric analysis using iNtuition 4.4.13 (TeraRecon, Foster City, CA) while blinded to the clinical history.

**Results**

Prospective sequencing of the sellar tumor using MSK-IMPACT revealed no somatic mutations, a focal amplification of \(CCND3\), and a homozygous deletion of \(PTPRD\). This tumor was typical of previously sequenced pituitary adenomas, which tend to harbor few mutations.\(^{18}\) MSK-IMPACT testing of the liver metastasis, demonstrated the same amplification of \(CCND3\) and homozygous deletion of \(PTPRD\), but also 105 somatic mutations that were not present in the pre-treatment sellar tumor.

MSK-IMPACT interrogates 468 cancer-associated genes and select intronic regions. To explore the clonal relatedness of these tumors in greater detail, we performed whole-exome sequencing on both tumor specimens along with a matched blood normal. Exome sequencing revealed that the sellar tumor and liver metastasis shared 15 somatic mutations, revealing their common origin and clonal relationships (Figure 2A). Notably, neither tumor harbored a mutation in \(USP8\), a recurrently mutated gene in ACTH-secreting pituitary adenomas.\(^{19,20}\) The TMZ-naïve sellar tumor harbored 93 mutations that were private to that specimen, indicating ongoing subclonal evolution. The TMZ-exposed liver metastasis was hypermutated, harboring 5,275 mutations (93 mutations/Mb) that were not detected in the pre-treatment specimen. TMZ is an alkylating agent that causes C>T/G>A transitions primarily at CpC and CpT dinucleotides. Indeed, mutational signature decomposition analysis revealed that 76% of the mutations in the liver metastasis had the signature of TMZ-induced hypermutation (Figure 2B). Moreover, no pathogenic or likely pathogenic allele was identified from germline analysis in this patient that could explain the somatic hypermutation identified.

Consistent with prior work exploring the mechanisms of TMZ resistance and TMZ-induced hypermutation in gliomas,\(^{21}\) we identified a truncating homozygous \(MSH6\) mutation present in the majority of cancer cells in the TMZ-hypermutated liver metastasis absent in the treatment-
naïve sellar tumor. Immunohistochemistry on the two specimens confirmed MSH6 loss in the hypermutated liver metastasis whereas the TMZ-naïve sellar tumor demonstrated retained expression of MSH6. Additionally, the liver metastasis was evaluated for PDL1 expression by immunohistochemistry, demonstrating <1% staining.

The sequenced liver metastasis reveals activation of pathways that were quiescent in the sellar tumor. In addition to developing additional cell cycle alterations, including a deletion at the CDKN2A/B locus, the liver metastasis demonstrated pathway activation of the PI3K pathway via a subclonal PIK3CA G1050D hotspot mutation, which is directly attributed to mutagenesis induced by alkylator chemotherapy (Figure 2AC).

Discussion:
Clinical experience with immunotherapy suggests that the pituitary cells may be susceptible to checkpoint inhibitors. In patients treated with ipilimumab for a non-pituitary neoplasm the rate of hypophysitis ranges from 4-15%.\textsuperscript{22-25} It has been postulated that this immunogenicity is in part mediated by ectopic expression of CTLA-4 on pituitary endocrine cells, leading to complement activation and the development of anti-pituitary antibodies.\textsuperscript{26} The addition of nivolumab to ipilimumab appears to potentiate the development of hypophysitis.\textsuperscript{25} We hypothesize that the susceptibility of pituitary cells to checkpoint inhibitors, in part explains our patient’s robust radiographic and biochemical response, and that checkpoint inhibition should be a treatment consideration, especially in tumors that have developed resistance to TMZ.

TMZ is an alkylating chemotherapy that creates O6-methylguanine adducts and induces apoptosis in the presence of a functional DNA repair system.\textsuperscript{27} Treatment with TMZ can induce a mutation in a MMR gene resulting in the accumulation of genetic lesions, also known as hypermutation.\textsuperscript{28} Aggressive pituitary adenomas that initially respond to TMZ may become resistant as seen in this case. In gliomas, it has been suggested that hypermutation can lead to the acquisition of new oncogenic drivers resulting in a more aggressive tumor clone.\textsuperscript{21} This may be reflected by the subclonal PIK3CA G1050D mutation in this patient’s liver metastasis as it is a known hotspot mutation. It has been previously shown that mutational burden correlates with treatment response to checkpoint inhibitors in metastatic melanoma, possibly due to the creation of neoantigens.\textsuperscript{29} TMZ-induced hypermutation may make pituitary adenomas, which are already uniquely immunogenic, further sensitive to treatment with immunotherapy.

The generalizability of this patient’s treatment response to other patients with pituitary tumors requires further study. This patient’s response to ipilimumab and nivolumab should prompt a clinical trial to better define the patient population with pituitary tumors that would most benefit from checkpoint inhibition.

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MP reports the he is on the scientific advisory board and has received honoraria from Bristol-Myers Squibb. The authors have no additional financial relationships to disclose.

References


**Figure 1:** A) Volumetric measurements of the intracranial tumor and liver metastasis across the patient’s clinical timeline and corresponding measurements of ACTH shows response to treatment with immunotherapy. B) MRI scans of the intracranial and liver sites before and after combination treatment with ipilimumab and nivolumab, mirroring response in (A). Abbreviations: ACTH (adrenocorticotropic hormone); TMZ (temozolomide); CAPTEM (capecitabine/temozolomide); Carbo/Etop (carboplatin/etoposide); and Ipi/Nivo (ipilimumab/nivolumab).

**Figure 2:** A) Sample tree showing clonal relationship of the pre-TMZ primary specimen and the post-treatment liver metastasis, with numbers in circles indicating mutations acquired from previous branch point. The two tumors share 15 mutations. The post-TMZ sample acquired a large number of private mutations, typical of therapy-induced hypermutation. Mutations considered likely oncogenic are highlighted. B) The substitution types (colored labels) and trinucleotide context (vertical labels) of SNVs in both samples, as a fraction of total mutations.
An enrichment for C>T/G>A transitions, characteristic of alkylator-induced hypermutation, was present in the post- but not pre-treatment sample. Numbers above bars indicate absolute number of mutations in that bin. C) Estimates of fraction of cancer cells harboring mutations in both samples, represented as a 3D density plot, showing the large number of mutations acquired in the post-TMZ sample. Whereas the mutation in $MSH6$ was nearly clonal in the liver specimen, the acquired mutation in $PIK3CA$ was found in a minority of the cell population.