NGS panel aids in diagnosis of rare collision tumor

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing’s important role in diagnosis, prognosis, and treatment. The following report comes from Columbia University Medical Center. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

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Collision tumors are thought to represent the chance encounter and temporospatial interaction of independent tumors, and their accurate classification may have significant prognostic and therapeutic implications. Here we showcase a next-generation sequencing panel used to analyze both components of a potential collision tumor and render the correct diagnosis.

Case presentation and history. The patient was an 87-year-old woman and former smoker who presented with hematochezia and dyspnea on exertion. She denied a history of gynecologic malignancy, uterine fibroids, or vaginal bleeding. Computed tomography of the abdomen and pelvis demonstrated a 6-cm right colonic mass without mesenteric lymphadenopathy. She was found to be severely anemic, with a plasma hemoglobin level of 5.5 g/dL, and to have serum carcinoembryonic antigen level elevated at 46.7 ng/mL. Diagnostic colonoscopy was aborted after the patient developed atrial fibrillation with a rapid ventricular response. Therefore, the patient underwent ileocollectomy.

Results. Gross examination showed a single 6.6-cm fungating mass extending through the muscularis propria into pericolic adipose tissue. Sectioning revealed an ill-defined firm, white area measuring approximately 3 cm. Histologically, the tumor was a composite of two interdigitating patterns. First, an adenocarcinoma with well-to-moderate differentiation and mucinous features arose from an adenoma with focal high-grade dysplasia. Immunohistochemical analysis demonstrated that adenocarcinoma cells expressed pan-cytokeratin (CK), CK20, and caudal-related homeobox protein-2 (CDX2) but not CK7. Second, the firm, white area identified grossly consisted of a spindle cell proliferation with fascicular architecture, focal necrosis, and high mitotic rate (up to 17 mitoses in 10 high-power fields). IHC analysis demonstrated that malignant spindle cells expressed desmin and smooth muscle actin but not pan-CK, CK7, CK20, CDX2, or markers of gastrointestinal stromal tumor, gynecologic malignancy, and neural and mesothelial origin, suggestive of smooth muscle differentiation. The differential diagnoses included carcinosarcoma and collision of adenocarcinoma and leiomyosarcoma. The carcinoma and sarcoma were staged separately as pT3N0 and pT1bN0, respectively.

IHC analysis also demonstrated loss of expression of MLH1 and PMS2 in the adenocarcinoma component, consistent with a DNA mismatch repair-deficient tumor, whereas expression of all mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2) was preserved in the sarcomatous component. Accordingly, microsatellite instability testing demonstrated high-frequency MSI in the adenocarcinoma but not in the sarcoma.

Mutational analysis was performed separately on the two tumor components by next-generation sequencing with a cancer gene panel. The area of carcinoma demonstrated the following mutations in cancer genes:
• c.1799T>A missense mutation (NM_004333.4, p.Val600Glu) in the BRAF gene with 22 percent variant allelic fraction;
fraction; fraction; and of all three markers. Both com-
primary rectal carcinoma with reten-
gastric carcinoma colliding with a
microsatellite markers in a metastatic
loss of heterozygosity in two of three
colorectal collision tumor included
ponents. A previous report of a
carcinomatous and sarcomatous com-
to analyze a collision tumor with
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nocarcinoma and leiomyosarcoma of
tract, and collision of mucinous ade-
sion tumor with pulmonary adeno-
carcinoma and malignant mesothe-
illioma components included copy
alysis on a limited, ampli-
number analysis by single nucleotide
lioma components included copy
sarcoma recur or metastasize, the
at 200× (inset, 400×).
Fig. 1. Ileocollectomy specimen with (A) grossly
identifiable fungating mass—outlined in white—
ating into pericolic adipose tissue and centrally
located firm, white area. (B) Microscopically, an
adenocarcinoma interdigitates with a malignant
spindle cell proliferation. Photomicrograph was taken
at 200× (inset, 400×).

Discussion. Leiomyosarcoma of the
intestinal tract is a rare tumor, and the finding of adenocarcinoma
closely associated with a high-grade
spindle cell proliferation raises two
main differential diagnoses: carcino-
sarcoma, either primary or metastatic
from a site such as the gynecologic
tract, and collision of mucinous ade-
ocarcinoma and leiomyosarcoma of
the colon. The combined evaluation
of IHC patterns and molecular find-
ings in our case include distinct MSI
status of the two separate tumor comp-
ents and distinct mutational pro-iles. It is likely that these lesions
orinate from different clones and
ifferent molecular pathways,
and thus represent a collision tumor
rather than carcinosarcoma.

This case represents the most ex-
tensive genetic analysis of a collision
tumor of the colon yet reported, and
it is the first report of NGS employed
to analyze a collision tumor with
carcinomatous and sarcomatous
ponents. A previous report of a
colorectal collision tumor included
loss of heterozygosity in two of three
microsatellite markers in a metastatic
gastric carcinoma colliding with a
primary rectal carcinoma with reten-
tion of all three markers. Both comp-
ponents of the collision tumor dem-
strated microsatellite stability.1 A
previous analysis of a thoracic colli-
sion tumor with pulmonary adeno-
carcinoma and malignant mesothe-
illioma components included copy
alysis by single nucleotide
opolymorphism microarray and mu-
tational analysis on a limited, ampli-
con-based NGS platform. Despite
significant differences in genomic
regional copy number, the NGS panel
was too limited to identify a driver
mutation in either tumor.2 The more
extensive NGS panel used in our case
allowed for identification of driver
mutations in both tumors. Despite
substantial evidence of mismatch
repair deficiency in the adenocarcin-
oma, no loss-of-function mutation
was identified in any mismatch repair
gene. In such cases, mismatch repair
deficiency may be caused by MLH1
gene promoter hypermethylation.
Although the MLH1 promoter was
not specifically interrogated in the
current case, a tight association be-
tween activating BRAF mutation and
MLH1 promoter hypermethylation
is well established.3,4 As an activating
mutation in BRAF was identified by
NGS in the current case, MLH1 pro-
moter hypermethylation is the likely
cause of mismatch repair in the cur-
rent case.

Therapy for collision tumors may
need to be individualized for each
tumor component. In this case, where
neither component showed evidence
of metastatic disease, surgical therapy
alone was curative. Follow-up at one
year after surgery did not show evi-
dence of recurrence or metastatic
disease. Thus, no further therapy was
required.

In clinical practice NGS is used to
provide predominantly predictive
and prognostic information in most
cancer cases. In this case, should the
carcinoma metastasize, its MSI status
would make the patient potentially
eligible for immune checkpoint inhi-
bition. Due to the presence of BRAF
V600E mutation, the patient would
be potentially eligible for trials of
BRAF inhibitors and alternative
therapies. However, in this case of a
possible collision tumor, it is espe-
ially important to note that extended
gene panel sequencing provided di-
gnostic information, in addition to
predictive information, complement-
ing morphologic and immunohisto-
chemical analysis.

Methods. MSI testing was per-
formed using the Promega (Madison,
Wis.) MSI system according to the
manufacturer’s instructions. Briefly,
a polymerase chain reaction using
fluorescently labeled primer ampli-
fies seven microsatellite markers—
five mononucleotide (NR-21, BAT-
26, BAT-25, NR-24, and MONO-27)
and two tetrancleotide (PENTA C
and PENTA D) repeats.
NGS was performed using the Columbia Comprehensive Cancer Panel. The CCCP targets exonic and intronic sequences in 467 cancer-associated genes using DNA from formalin-fixed, paraffin-embedded tissue using Custom Agilent (Santa Clara, Calif.) SureSelect capture and Illumina (San Diego, Calif.) HiSeq 2500 sequencing. Sequences were aligned and nucleotide variants were called using NextGENe (SoftGenetics, State College, Pa.) software. Nucleotide variants were manually curated and classified after filtering with an in-house pipeline.


**Test yourself**

Here are three questions taken from the case report. Answers are online now at [www.amp.org/casereviews](http://www.amp.org/casereviews) and will be published next month in CAP TODAY.

1. Which of the following are included in the differential diagnosis of spindle cell lesions of the gastrointestinal tract?
   a) Sarcomatoid carcinoma
   b) Gastrointestinal stromal tumor
   c) Metastatic leiomyosarcoma
   d) Sarcomatoid mesothelioma
   e) All of the above

2. Which of the following is not a gene that encodes for a mismatch repair protein?
   a) MLH1
   b) MSH2
   c) MSH6
   d) BRCA2
   e) PMS2

3. Activating mutation in the *BRAF* gene in colorectal carcinoma correlates most closely with which of the following?
   a) Hypermethylation of the *MLH1* gene promoter
   b) Somatic loss-of-function mutation in the *MLH1* gene
   c) Germline loss-of-function mutation in the *MLH1* gene
   d) Loss of heterozygosity at the *MLH1* gene locus