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Molecular Case Studies

RESEARCH REPORT

Fluorouracil sensitivity in a head and neck squamous cell carcinoma with a somatic *DPYD* structural variant

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Abstract Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers worldwide and represents a heterogeneous group of tumors, the majority of which are treated with a combination of surgery, radiation, and chemotherapy. Fluoropyrimidine (5-FU) and its oral prodrug, capecitabine, are commonly prescribed treatments for several solid tumor types including HNSCC. 5-FU-associated toxicity is observed in ~30% of treated patients and is largely caused by germline polymorphisms in *DPYD*, which encodes dihydropyrimidine dehydrogenase, a key enzyme of 5-FU catabolism and deactivation. Although the association of germline *DPYD* alterations with toxicity is well-described, the potential contribution of somatic *DPYD* alterations to 5-FU sensitivity has not been explored. In a patient with metastatic HNSCC, in-depth genomic and transcriptomic integrative analysis on a biopsy from a metastatic neck lesion revealed alterations in genes that are associated with 5-FU uptake and metabolism. These included a novel somatic structural variant resulting in a partial deletion affecting *DPYD*, a variant of unknown significance affecting *SLC29A1*, and homozygous deletion of *MTAP*. There was no evidence of deleterious germline polymorphisms that have been associated with 5-FU toxicity, indicating a potential vulnerability of the tumor to 5-FU therapy. The discovery of the novel *DPYD* variant led to the initiation of 5-FU treatment that resulted in a rapid response lasting 17 wk, with subsequent relapse due to unknown resistance mechanisms. This suggests that somatic alterations present in this tumor may serve as markers for tumor sensitivity to 5-FU, aiding in the selection of personalized treatment strategies.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers worldwide and represents a heterogeneous group of tumors originating from the squamous epithelium of the oral cavity, oropharynx, larynx, and hypopharynx. Human papillomavirus (HPV) infection is associated with 60%–70% of head and neck cancers. HPV-negative HNSCC tumors, however, tend to have a worse prognosis and response to treatment compared with HPV-positive tumors (Berman and Schiller 2017; Fung et al. 2017). Irrespective of

HPV status, the majority of HNSCC patients are treated with a combination of surgery, radiation, and chemotherapy (Adelstein et al. 2017). Fluoropyrimidine (5-FU) and its prodrug capecitabine are a frequently prescribed systemic therapy in the treatment of several solid tumor types including HNSCC (Diasio and Harris 1989). Importantly, 5-FU-related toxicity is observed in ~30% of treated patients (Meulendijks et al. 2015). Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme of 5-FU catabolism and deactivation (Diasio and Harris 1989). Consequently, DPD activity moderates response to 5-FU, and DPD deficiency as a result of germline polymorphism is considered a major cause of 5-FU-associated toxicity (van Kuilenburg 2004). Deleterious variants in *DPYD*, the large gene encoding DPD, have been described as significantly impacting enzymatic activity (Etienne-Grimaldi et al. 2017; van Kuilenburg et al. 2017; Henricks et al. 2018).

To date, few studies have fully characterized the somatic or germline genomic landscape of *DPYD* in cancer patients (Etienne-Grimaldi et al. 2017). Here, we present the case study of a patient with HNSCC who had a biopsy that underwent in-depth genomic and transcriptomic integrative analysis. We identified a number of alterations that are associated with 5-FU uptake and metabolism, including a novel somatic structural variant resulting in a partial deletion affecting the *DPYD* gene as well as a homozygous variant of unknown significance affecting *SLC29A1* and homozygous deletion of *MTAP*. There was no evidence of deleterious germline polymorphisms that have been associated with 5-FU toxicity. Given the well-described toxicity to 5-FU associated with deleterious germline variants affecting *DPYD*, we hypothesized that the somatic *DPYD* structural variant may have rendered the tumor to be sensitive to 5-FU and report the subsequent response to treatment.

RESULTS

Clinical Presentation

The patient was a 69-yr-old gentleman of East-Asian extraction who presented with a 4 mo history of oral discomfort and was found to have an ulcer on his right maxillary palate. The patient was a lifelong never-smoker, drank alcohol approximately once per month, and had no other significant medical history. Pathology evaluation of the resected ulcer (with a right hemimaxillectomy and right-sided neck dissection) identified a 3.5-cm well-differentiated squamous cell carcinoma involving the alveolar ridge and maxillary sinus with bone invasion; level I and II (but not III/IV) nodes were involved. Perineural and lymphovascular invasion was noted. He underwent adjuvant radiation therapy with 6000 cGy over 6 wk, which included the adjacent lymph nodes. Initially, he made a good recovery, but unfortunately after 3 mo of follow-up he presented with a 4-cm neck mass and a fine needle aspirate–confirmed cancer recurrence. The mass was considered surgically unresectable and was within the previous radiation field. Thus, he was referred for palliative systemic therapy.

At that time he was consented for participation in the Personalized Oncogenomics (POG) study at BC Cancer in Vancouver, British Columbia (see Methods), and a biopsy of his neck mass was taken for genomic and transcriptomic analysis as per study protocol. He was also enrolled on a clinical trial of avelumab plus an OX40 agonist as his first systemic therapy. After 3 mo of treatment, he had markedly progressed (Fig. 1), and this treatment was discontinued. Based on the findings of the POG analysis, treatment with weekly 5-FU (500 mg/m^2) and leucovorin (20 mg/m^2) was initiated. He had a rapid response (Fig. 1) both clinically and radiographically that was sustained for 17 wk. He did require a dose modification because of side effects (specifically mucositis and hand–foot syndrome) and was maintained on 80% dose receiving a total of 14 wk of treatment. A treatment break was initiated, but 3 wk following treatment cessation, a small cancer nodule reappeared. A repeat biopsy was taken for POG sequencing and analysis that did not reveal marked changes in the genome or

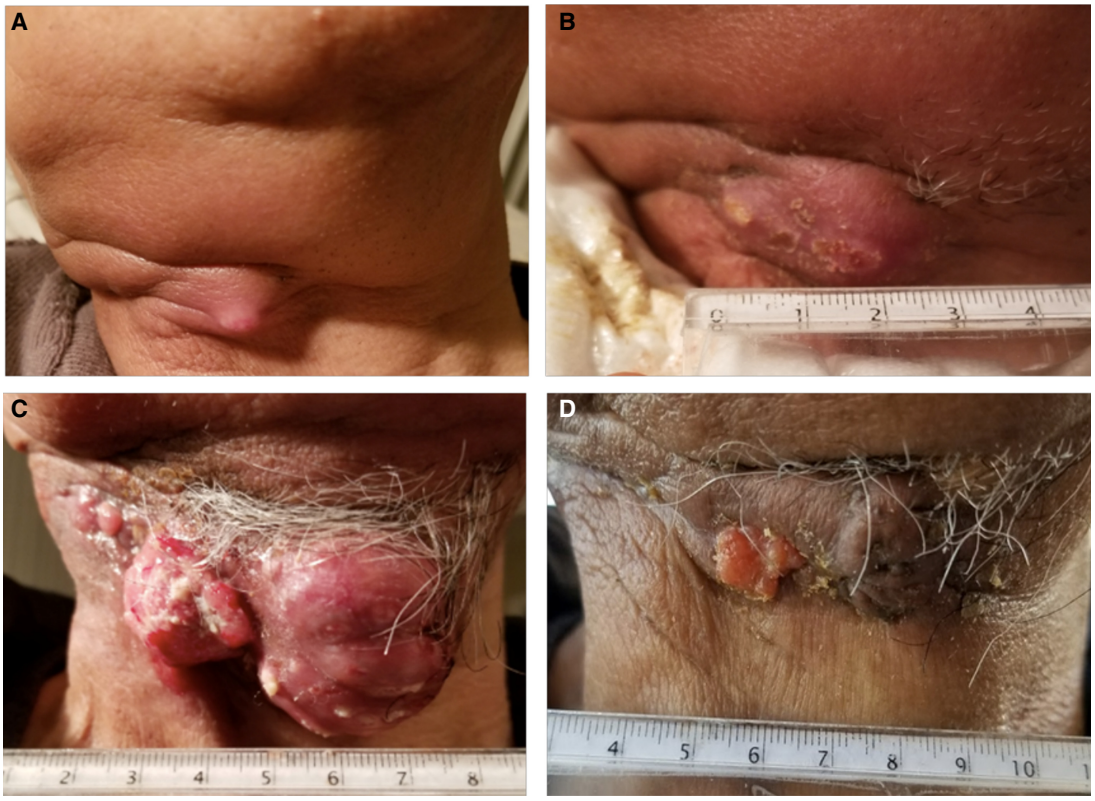

Figure 1. Clinical images of the metastatic deposits of this squamous cell carcinoma, originally surgically resected from the alveolar ridge. Images are organized chronologically starting from the baseline image (*A*) when the metastatic nodule presented clinically and the biopsy for the POG analysis was taken. Following this, *B* demonstrates the interim growth and representing the baseline image for the initiation of the clinical trial protocol of avelumab plus an OX40 agonist. *C* depicts the growth of the cancer despite this systemic therapy. Finally, *D* demonstrates the marked improvement after only 4 wk of weekly 5-FU/folinic acid therapy.

transcriptome compared with the initial biopsy. The patient was started on capecitabine (oral 5-FU), but unfortunately he did not respond to the reinitiation of this line of therapy. The mechanisms underlying the acquired resistance remain unclear. He was subsequently treated with cisplatin (progression as best response) and palbociclib (mixed response) on a clinical trial; unfortunately, he passed away because of tumor rupture and massive bleeding after 9 wk on trial.

Genomic Analysis

HPV genetic material was not detected in this tumor, further confirming the HPV-negative status identified in pathology. All relevant mutations and variants identified in the initial biopsy were confirmed in both the initial and repeat biopsies.

Single-Nucleotide Variants

We identified 85 somatic nonsynonymous protein-coding single-nucleotide variants (SNVs) from the sequencing data (Supplemental Table 1). Although none of these were deemed to be clinically actionable, three were of biological interest (Table 1). A homozygous promoter mutation (g.1295228G>A) at a recurrent hotspot was detected in the human telomerase reverse transcriptase (*TERT*) gene, an enzyme that maintains telomere length and genomic

Table 1. Somatic nonsynonymous single-nucleotide variant (SNV) and indels

Gene	Chr	Position	Ref	Alt	Type	HGVS CDS	HGVS protein	Genotype	Predicted effect	dbSNP
KEAP1	19	10602878	G	A	SNV	c.700C>T	p.Arg234Trp	Heterozygous	Missense	—
SLC29A1	6	44195070	C	T	SNV	c.20C>T	p.Pro7Leu	Homozygous	Missense	—
TERT	5	1295228	C	T	SNV	c.−124C>T	—	Homozygous	—	rs1242535815

(HGVS CDS) Human Genome Variation Society coding sequence, (dbSNP) Single Nucleotide Polymorphism Database.

integrity. Routine comparative analysis of gene expression revealed the *TERT* gene was moderately overexpressed compared with the Cancer Genome Atlas (TCGA) HNSCC data set (78th percentile). *TERT* promoter mutations occur in patients with oral cavity squamous cell carcinoma (SCC) at a high frequency and may be associated with aggressive disease (Killela et al. 2013; Zhao et al. 2015; Barczak et al. 2017; Chang et al. 2017). Additionally, there was a heterozygous variant (p.R234W) in *KEAP1*, a gene that is recurrently inactivated and associated with reduced survival in HNSCC (Network CGA 2015). Finally, a novel homozygous variant (p.P7L) was identified in nucleoside transporter *SLC29A1* (hENT1). The functional and clinical impact of this mutation has not been characterized. However, hENT1 is important in 5-FU transport, and low levels of mRNA expression have been associated with response to 5-FU in pancreatic cancer cell lines (Tsujie et al. 2007). In this case, the somatic variant was also associated with a low level of *SLC29A1* expression (5th percentile compared with TCGA HNSCC).

Copy-Number Variants

Based on the tumor/normal sequencing ratio and loss of heterozygosity, the tumor content was estimated to be 78% and a triploid model was used to describe the observed copy-number changes. Of particular interest, the genome-wide copy-number analysis revealed a homozygous *CDKN2A/CDKN2B/MTAP* codeletion. *CDKN2A* (p16) is a tumor suppressor that regulates the cell cycle and is the second most commonly inactivated gene in HNSCC (Beck and Golemis 2016). Disruption of p16 allows for activation of *CDK4* and *CDK6* and phosphorylation of *RB1*, leading to cell cycle progression (Asghar et al. 2015). *MTAP* is also thought to function as a tumor suppressor and is a key enzyme in the formation of adenine, influencing response to 5-FU therapy (Tang et al. 2012).

DPYD Structural Variant

Structural variants were identified using de novo sequence assembly followed by variant detection. A somatic fusion deletion event in the *DPYD* gene predicted to result in an in-frame deletion of exons 11–19 was detected in the genome and transcriptome (Fig. 2). The expression of the *DPYD* gene was moderate compared with the TCGA HNSCC data set (35th percentile). To our knowledge, our case is the first description of a somatic multi-exon deletion in *DPYD* in a HNSCC cancer patient.

Evaluation of normal DNA revealed that the patient did not harbor any of the three SNP alleles associated with 5-FU toxicity (rs55886062; rs67376798; rs3918290) nor evidence of germline deletion in *DPYD*.

DISCUSSION

Here, we describe a case study of a patient with HNSCC who demonstrated a notable response to 5-FU. Alterations affecting multiple genes that play a role in nucleotide transport and metabolism were identified in this case study and may have contributed to the response

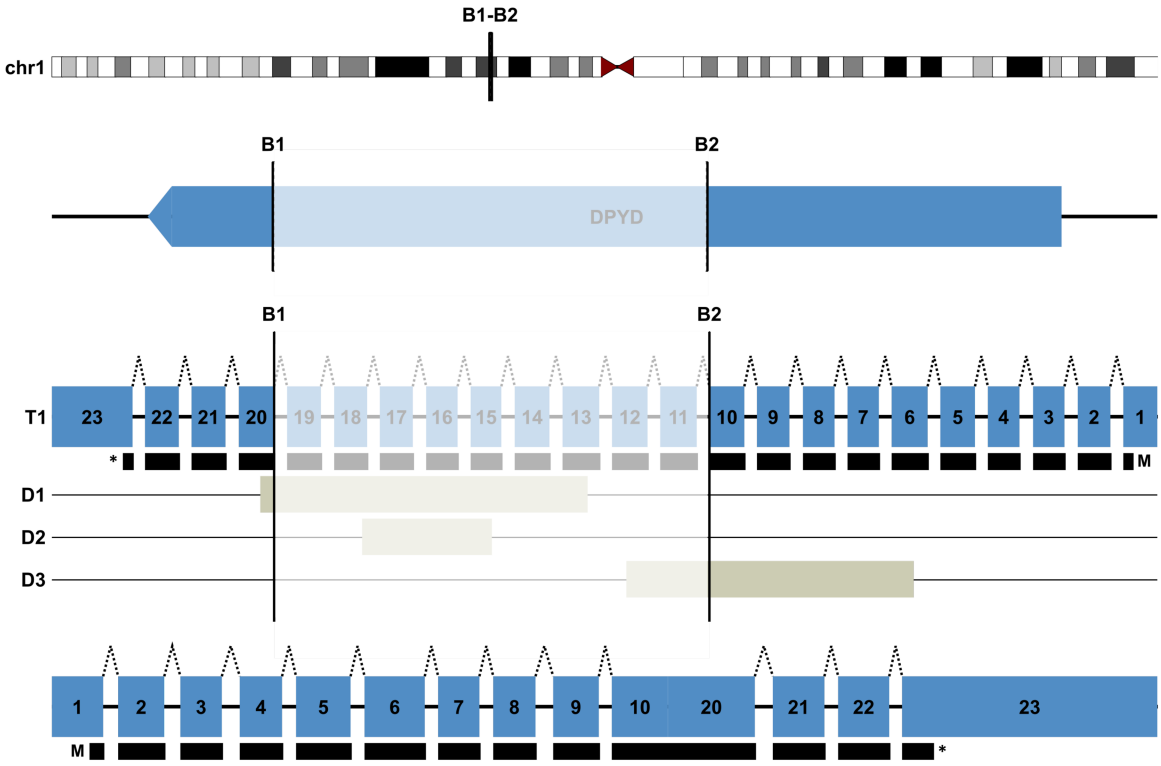

Figure 2. Somatic fusion deletion event in the *DPYD* gene. The plot depicts the breakpoints affecting the *DPYD* gene that was predicted to result in an in-frame deletion of exons 11–19. (*Top*) Deletion breakpoints B1 and B2 shown in relation to the chromosome. (*Middle*) Model of *DPYD* transcript ENST00000370192 (T1) depicting exons 1–23. *DPYD* contains three Pfam domains, which are depicted by D1 (PF01180: dihydro-orotate dehydrogenase), D2 (PF01207: TRNA-dihydrouridine synthase), and D3 (PF07992: Pyridine nucleotide-disulphide oxidoreductase). (*Bottom*) Resulting variant DPYD transcript lacking exons 11–19. Black blocks below the exons depict the protein-coding region of the transcript.

to therapy, including a novel somatic structural variant affecting *DPYD*. Discrete germline *DPYD* polymorphisms and deletions have been identified in cancer patients with severe 5-FU-associated toxicity (van Kuilenburg 2004; Etienne-Grimaldi et al. 2017; van Kuilenburg et al. 2017; Henricks et al. 2018). Thus, testing for *DPYD* variants known to affect DPD enzyme activity is becoming more prominent in patients undergoing 5-FU chemotherapy (Deenen et al. 2016). Our case was found to be negative for described germline alterations associated with 5-FU toxicity. However, the somatic structural variant analysis resulted in the identification of a rearrangement in the *DPYD* gene leading to a partial in-frame deletion of exons 11–19. This region of the gene contains part of the FAD-binding domain and the majority of the FMN/pyrimidine binding domain (van Kuilenburg 2004), which likely results in the translation of a nonfunctional protein that is unable to metabolize 5-FU. We, therefore, speculated that the tumor may be sensitive to 5-FU/capecitabine because of the somatic *DPYD* structural variant. Indeed, this patient demonstrated a rapid and dramatic reduction in tumor size following initiation of treatment with 5-FU, which lasted for 17 wk. This notable response was of particular interest as his cancer was resistant to other standard treatments. In support of the hypothesis that the somatic *DPYD* loss may contribute to 5-FU response, another study that conducted a retrospective analysis of triple-negative breast cancer patients revealed patients with somatic *DPYD* copy-number variants (CNVs) demonstrated a trend for a longer time to progression on 5-FU (Gross et al. 2013).

Unfortunately, the patient relapsed within 3 wk of ending 5-FU therapy and was subsequently reinitiated on oral 5-FU, capecitabine, but did not respond. Repeat biopsy prior to initiation of capecitabine did not reveal any further alterations of note, and the mechanism of resistance remains unclear.

Additional genomic findings that may also contribute to 5-FU response include a variant of unknown significance affecting *SLC29A1* and homozygous deletion of *MTAP*. High expression of *SLC29A1* has been associated with resistance to 5-FU in pancreatic cancer cell lines (Tsujie et al. 2007) by either facilitating bilateral transport of 5-FU or by preferential transport of nucleosides over 5-FU into cells (Wang et al. 2014). Although the low expression of *SLC29A1* in the tumor has been associated with a clinical response to 5-FU (Tsujie et al. 2007; Phua et al. 2013), the homozygous single nucleotide variant (p.P7L) has not been observed in any public databases to date and is located on the amino terminus of the protein with no associated functional domain. Therefore, the precise clinical impact of this variant on 5-FU response remains unclear. Additionally, *MTAP* deletion is commonly found in a number of different cancer types (Tang et al. 2012) including oral SCCs (Chen et al. 2004). Adenine derived from *MTAP* activity could compete with purine analogs such as 5-FU for phosphoribosyl-5-pyrophosphate substrate utilization, thereby decreasing the amount of toxic nucleotide produced (Tang et al. 2012). Thus, the deletion of *MTAP* could result in increased amounts of toxic analog. In line with this hypothesis, several in vitro studies have shown that the deletion of *MTAP* in various cell lines resulted in the enhanced cytotoxicity in response to 5-FU treatment (Lubin and Lubin 2009; Lubin and Lubin 2010; Tang et al. 2012). However, there is no clinical evidence of its impact on 5-FU treatment to date. Altogether, the genomic findings point to the inability of the tumor to catabolize and deactivate 5-FU and therefore increase its therapeutic efficacy.

Structural variants and inactivating mutations in *DPYD* are rare events, with only nine cases cataloged in TCGA and COSMIC databases (cBioportal; Cerami et al. 2012; Gao et al. 2013). *DPYD* alterations were found in a variety of different cancer types, highlighting the potential impact that somatic mutations can have on treatment choices and outcomes. By sequencing the whole genome, we were positioned to find novel variants, including the *DPYD* structural variant described here, that informed personalized patient management that may otherwise be missed in targeted sequencing approaches.

CONCLUSION

Comprehensive genomic and transcriptomic analysis of a metastatic HNSCC was performed. A somatic structural variant affecting *DPYD* was detected that lead to the patient receiving 5-FU therapy. The patient had a rapidly growing cancer that was resistant to radiation, immunotherapy, and cisplatin and yet he demonstrated an impressive clinical response to 5-FU therapy, which we hypothesize to be in part due to the *DPYD* structural variant. This analysis shows for the first time that somatic lesions in *DPYD* may be used as a potential biomarker in prospectively evaluating for somatic variants that point to treatment sensitivity in patients with HNSCC and other cancer types in which 5-FU is commonly used.

METHODS

Sample Collection and Processing

The patient was enrolled in the ongoing POG project at BC Cancer in Vancouver, Canada (NCT02155621). This study was approved by the University of British Columbia Research Ethics Board (REB#H14-006817). Patient identity is de-identified for the research team

Table 2. Sequencing coverage

Sample	Tumor DNA coverage (WGS)	Normal DNA coverage (WGS)	Tumor RNA coverage (RNA-seq)
HNSCC case	101×	39×	360M reads

and information is communicated to the clinicians through unique patient identifiers. Patients consent to the potential publication of findings. Raw sequence data, analytics, and clinical data are maintained in secure computing environments.

As per study protocol, each patient undergoes a study-specific biopsy. In this case, an ultrasound-guided core-needle biopsy of the neck and blood samples were collected for paired-end whole-genome sequencing (WGS) and transcriptome sequencing. RNA and DNA libraries were prepared and sequenced on the Illumina platform as previously described (Grewal et al. 2017).

Sequencing Data Analysis

In total, 150 bp paired-end normal and tumor reads were aligned to the human reference genome (hg19) using the Burrows–Wheeler alignment tool (Li and Durbin 2010) (v0.7.6a). Somatic point mutations and small insertion and deletions were detected using Strelka (v1.0.6) (Saunders et al. 2012). Somatic copy-number alterations that were present in the tumor DNA but not in the germline were identified using CNAseq (v0.0.6) (Jones et al. 2010) and loss of heterozygosity was determined using APOLLOH (v0.1.1) (Ha et al. 2012). De novo assembly of genomic and transcriptomic data using ABySS v1.3.4 (Simpson et al. 2009) and TransABySS (v1.4.10) (Birol et al. 2009; Simpson et al. 2009) was carried out to detect rearrangements. Mutations were annotated to Ensembl v69 (Flicek et al. 2014) using SNPEff (v4.1) (Cingolani et al. 2012). Tumor content and sequencing coverage are outlined in Table 2.

RNA-seq reads were aligned using Jaguar (v2.0.3) (Butterfield et al. 2014) to the human reference (hg19) with a database of exon junctions based on Ensembl v69 (Flicek et al. 2014), and normalized expression levels were computed in reads per kilobase per million mapped reads (Mortazavi et al. 2008). Publicly available transcriptome sequencing data from HNSCC from TCGA (https://tcga-data.nci.nih.gov/tcga/; Network CGA 2015) and a compendium of adjacent normal tissue samples from the Illumina Human BodyMap 2.0 project (www.illumina.com; ArrayExpress ID: E-MTAB-513) were used to explore the expression profile of human genes and transcripts.

ADDITIONAL INFORMATION

Database Deposition and Access

The whole-genome and transcriptome sequencing data for this case are available as .bam files from the European Genome-phenome Archive (EGA; www.ebi.ac.uk/ega/home) as part of the study EGAS00001001159, accession ID EGAD00001004934. The *DPYD* structural variant and variants affecting *KEAP1* and *SLC29A1* are deposited in ClinVar (https:www.ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV000996023, SCV000996021, and SCV000996022, respectively.

Ethics Statement

The patient provided written informed consent for metastatic biopsies, sequencing, and publication of results as part of the Personalized Oncogenomics Program of British Columbia (NCT02155621, University of British Columbia Clinical Research Ethics Board approval no. H14-006817).

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Competing Interest Statement

The authors have declared no competing interest.

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