

A diagnostic challenge of KIT p.V559D and BRAF p.G469A mutations in a paragastric mass

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Abstract

A patient with gastrointestinal stroma tumor (GIST) and KIT p.V559D and BRAF p.G469A alterations was referred to our institutional molecular tumor board (MTB) to discuss therapeutic implications. The patient had been diagnosed with B-cell chronic lymphocytic leukemia (CLL) years prior to the MTB presentation. GIST had been diagnosed 1 month earlier. After structured clinical annotation of the molecular alterations and interdisciplinary discussion, we considered BRAF/KIT co-mutation unlikely in a treatment-naïve GIST. Discordant variant allele frequencies furthermore suggested a second malignancy. NGS of a CLL sample revealed the identical class 2 BRAF alteration, thus supporting admixture of CLL cells in the paragastric mass, leading to the detection of 2 alterations. Following the MTB recommendation, the patient received imatinib and had a radiographic response. Structured annotation and interdisciplinary discussion in specialized tumor boards facilitate the clinical management of complex molecular findings. Coexisting malignancies and clonal hematopoiesis warrant consideration in case of complex and uncommon molecular findings.

Key words: GIST; lymphocytic leukemia; hematopoiesis; KIT p.V559D; BRAF p.G469A.

Key Points

- Complex molecular profiles represent a challenge for clinical management.
- Structured analysis and multidisciplinary discussion of complex findings in molecular tumor boards are essential.
- Coexisting malignancies or clonal hematopoiesis should be considered as a differential diagnosis in complex molecular profiles.
- Discordant variant allele frequencies should be diagnostically considered.
- Zanubrutinib and imatinib combination therapy was safe and effective.
- Zanubrutinib was effective in CLL with class II BRAF mutation.

Patient story

A 71-year-old male patient was referred to the institutional molecular tumor board (MTB) after co-occurring KIT

p.V559D and BRAF p.G469A alterations had been identified by NGS panel sequencing in a gastrointestinal stromal tumor (GIST). In 2020, the patient had initially been diagnosed with

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a monoclonal B-cell lymphocytosis. He first presented to our institution in May 2023, with a diagnosis of B-cell chronic lymphocytic leukemia (CLL) Binet stage B, because a progressing testicular mass was found during follow-up visits. The patient underwent hemiorchiectomy, and resected tissue showed CLL infiltration without signs of transformation. Repeated computed tomography of the abdomen then revealed a progressing paraesophageal mass with otherwise stable retroperitoneal lymph nodes. Endosonography-guided biopsy of the paraesophageal mass was performed and a CD117-positive GIST was diagnosed in September 2023. After multidisciplinary discussion, neoadjuvant treatment with imatinib for UICC stage Ib GIST was initiated. Next-generation sequencing of the GIST sample revealed an activating KIT mutation and a BRAF alteration (Table 1). The patient was referred to the MTB in October 2023.

Molecular tumor board

Genotyping results and interpretation of molecular results

Identified molecular alterations underwent a standardized clinical annotation process prior to MTB presentation, as described previously¹: the KIT p.V559D variant is a well-described activating alteration in GIST.² Exon 11 alterations—such as the p.V559D variant—are the most common molecular alterations described in 55%-80% of GIST,³ thus supporting the diagnosis. BRAF p.V600E alterations have also been observed in GIST, albeit at a frequency of less than 10%.⁴ Additionally, reported BRAF alterations were activating p.V600E alterations. The BRAF p.G469A belongs to activating class 2 BRAF alterations and has been described in 2 patients with soft-tissue sarcomas.⁵ BRAF alterations, including the p.G469A mutation, have been previously described in CLL.⁶⁻⁸ Co-occurring alterations of BRAF and KIT in treatment-naïve GIST are considered extremely rare.

Functional and clinical significance

KIT p.V559D is an established predictive biomarker for the efficacy of several approved tyrosine kinase inhibitors, including imatinib.⁹ BRAF p.G469A alterations have been associated with resistance to vemurafenib in individual patients.^{10,11} However, case reports have documented clinical benefits with dabrafenib/trametinib therapy and prolonged stable disease with trametinib.^{12,13} Co-occurring KIT and BRAF p.V600E alterations have been described as a resistance mechanism to KIT-inhibition in GIST.^{4,14,15}

Potential strategies to target the pathway and implications for clinical practice

We discussed these data in our multidisciplinary tumor board. Since BRAF and KIT co-alterations are extremely rare

in treatment-naïve GIST, the occurrence of a BRAF/KIT co-mutation in the absence of prior targeted therapy was considered unlikely. Discrepant variant allele frequencies between BRAF and KIT alterations were additionally thought to represent 2 distinct cell populations. Since class II BRAF alterations are rare in GIST but have been previously described in B-CLL, sequencing results were considered to reveal a mixture of GIST and CLL DNA in the tumor sample. Panel-based sequencing of the histologically confirmed testicular manifestation of CLL was performed, following an MTB recommendation, and revealed the previously identified BRAF p.G469A alteration (Table 1). The concurrent alterations in the initial GIST sample were therefore considered to represent GIST with activating KIT alteration and an admixture of BRAF-altered CLL. Immunohistochemical analysis of the paraesophageal biopsy sample revealed an infiltration of CLL in addition to the GIST (Figure 1). A recommendation to continue imatinib therapy was made.

Patient update

Computed tomography after 3 months of imatinib treatment revealed a reduction in tumor size (Figure 2). The patient developed progression of CLL to Binet C, CLL-IPI 4 (high risk) with leukocytosis, anemia, fatigue, and night sweats in January 2024, 3 months after MTB presentation. Zanubrutinib treatment was initiated in a reduced dose (80mg 1-0-1) additionally to the standard dose imatinib. Treatment was well tolerated and B-symptoms and leukocytosis resolved within a few weeks (Figure 3). Subsequent surgery for the GIST is planned and pending.

In summary, we here report a case of a patient with 2 tumor entities and distinct oncogenic alterations, both identified in a paraesophageal tumor. Admixture of blood cells in solid tumor samples has been previously reported as a reason for erroneous interpretation of molecular results in as many as 5% of patients undergoing broad tumor-only genomic profiling.¹⁶ Although BRAF alterations were not reported in this study, other alterations associated with clonal hematopoiesis were commonly misinterpreted. Since BRAF alterations, including atypical class 2 or class 3 alterations, are usually encountered in solid tumors, the concurrent hematologic malignancy represented an additional challenge in this patient. A structured analysis of available data for the 2 identified molecular alterations revealed an unlikelihood of BRAF/KIT co-altered GIST but favored an underlying second primary malignancy, which was known at the time of tumor board presentation. Differences in variant allele frequency and an adequate assessment of tumor cell content are additionally required to raise suspicion of second malignancies.

In order to definitely differentiate between molecularly defined coexisting malignancies, co-occurring mutations, or

Table 1. Results from NGS (OncoPrint Focus Assay, Thermo Fisher Scientific; IonTorrent Platform, Thermo Fisher Scientific) performed on the paraesophageal mass (sample 1) and the orchiectomy sample (sample 2).

Gene name	Reference sequence	Exon	c.HGVS; p.HGVS, sample 1	Allele frequency, sample 1	c.HGVS; p.HGVS, sample 2	Allele frequency, sample 2
BRAF	NM_004333	11	c.1406G>C; p.G469A	11	c.1406G>C; p.G469A	17
KIT	NM_000222	11	c.1676T>A; p.V559D	28		

The GIST—tumor cell content (sample 1) was approximately 45%.

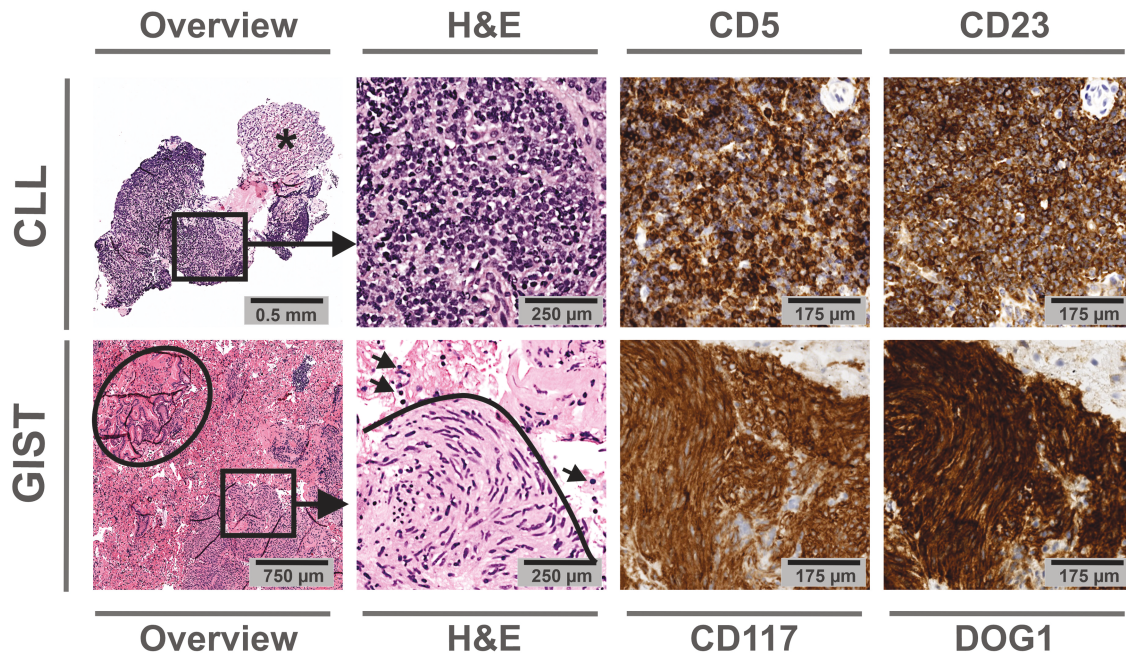


Figure 1. Histopathology of the corresponding gastric biopsy. The upper row shows mucosa from the gastric antrum-corpus transition area with monomorphic B-cell aggregates that show strong membranous expression of CD5 and CD23, compatible with the diagnosis of chronic lymphocytic leukemia (CLL). The mucosa is marked with an asterisk, CLL infiltrates are outlined by the rectangle. The lower row shows antral mucosa (encircled) and gastric muscular wall with infiltrates of a mainly spindled cell lesion below the black line in the H&E panel). The spindled appear bland, comprise elongated nuclei without atypia or mitoses, and show a strong membranous expression of CD117 and a strong cytoplasmic expression of DOG1 (discovered on GIST-1), compatible with a gastrointestinal stromal tumor (GIST). The spindled lesion is intermixed with fibrin and small lymphocytes as correlate for concomitant CLL infiltration (smaller arrow in H&E panel).

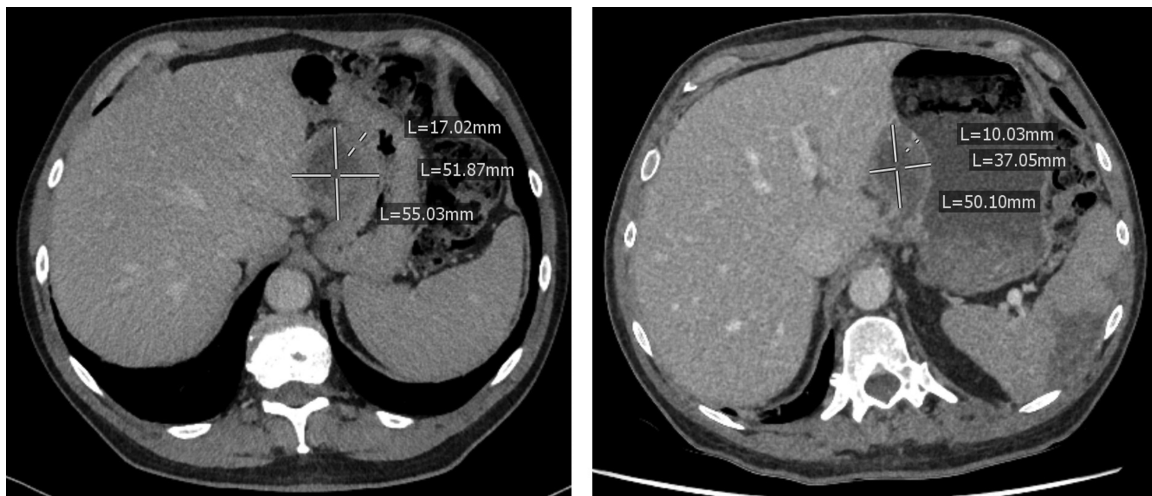


Figure 2. Computed tomography staging of the GIST prior to therapy (left panel) and after 3 months of imatinib treatment (right panel) documented a reduction in tumor size.

subclonal disease, cases like these might benefit from the integration of single-cell and/or spatial sequencing techniques. We believe that the identification of coexisting clinically relevant molecular alterations could represent an important use case to develop these research technologies into clinical-grade diagnostic tools.

BRAF-mutant CLL represents a rare subgroup with unknown clinical implications. Previous work suggests an unfavorable prognosis in CLL patients with RAS-RAF-pathway alterations, in line with the more aggressive clinical course and unusual presentation in the here presented patient.⁷

The impact of these alterations on therapeutic response with available CLL treatments is unknown.¹⁷ Despite concerns for BRAF alterations as a potential downstream resistance mechanism to BTK-inhibition, zanubrutinib treatment was initiated and was well tolerated in combination with imatinib and led to a rapid response in B-symptoms and leukocyte count. This report and available data therefore do not yet suggest different clinical management of BRAF-altered CLL.

In conclusion, this case report highlights the importance of a structured annotation of molecular findings to adequately inform clinical management. Second primary malignancies or

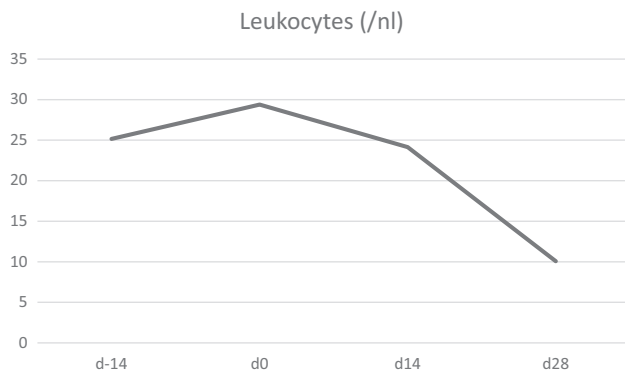


Figure 3. Blood leukocyte count before and after the initiation of zanubrutinib (d0) revealed a marked decrease corresponding to a rapid improvement in B-symptoms over time (d = day).

clonal hematopoiesis are a potential differential diagnosis for complex molecular findings.

Author contributions

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Conflicts of interest

M.S. reports advisory work for Fosanis GmbH. D.T.R. reports having received honoraria/speaker fees/travel support and/or advisory board compensation from Bayer, BMS, Roche, Lilly

and BeiGene. The other authors report no potential conflicts of interest with regard to this work.

Data availability

Data supporting this article are provided in the text and figures. Additional information is available from the authors upon reasonable request.

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