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Use of Colony-Stimulating Factors With Chemotherapy: Opportunities for Cost Savings and Improved Outcomes

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Myeloid colony-stimulating factors (CSFs) decrease the risk of febrile neutropenia (FN) from high-risk chemotherapy regimens administered to patients at 20% or greater risk of FN, but little is known about their use in clinical practice. We evaluated CSF use in a multiregional population-based cohort of lung and colorectal cancer patients (N = 1849). Only 17% (95% confidence interval [CI] = 8% to 26%) patients treated with high-risk chemotherapy regimens received CSFs, compared with 18% (95% CI = 16% to 20%) and 10% (95% CI = 8% to 12%) of patients treated with intermediate- (10%–20% risk of FN) and low-risk (<10% risk of FN) chemotherapy regimens, respectively. Using a generalized estimating equation model, we found that enrollment in a health maintenance organization (HMO) was strongly associated with a lower adjusted odds of discretionary CSF use, compared with non-HMO patients (odds ratio = 0.44, 95% CI = 0.32 to 0.60, $P < .001$). All statistical tests were two-sided. Overall, 96% (95% CI = 93% to 98%) of CSFs were administered in scenarios where CSF therapy is not recommended by evidence-based guidelines. This finding suggests that policies to decrease CSF use in patients at lower or intermediate risk of FN may yield substantial cost savings without compromising patient outcomes.

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Neutropenia is a potentially serious complication of chemotherapy that increases the risk of life-threatening infections because of an abnormally low number of neutrophils in the blood. Myeloid colony-stimulating factors (CSFs), when given prophylactically, substantially decrease the risk of febrile neutropenia (FN) (1,2) and are widely used in clinical practice since their approval by the Food and Drug Administration (FDA) in the early 1990s (2–5). CSFs now used in practice include granulocyte-CSF (G-CSF; filgrastim), pegylated G-CSF (pegfilgrastim), and granulocyte-macrophage-CSF (GM-CSF; sargramostim). The American Society of Clinical Oncology (ASCO) first introduced guidelines on the use of CSFs in 1994, which recommend primary prophylaxis (ie, with the first cycle of chemotherapy) with a CSF when the anticipated risk of FN associated with chemotherapy is 40% or higher (changed to ≥20% in 2006) (6,7). The National Comprehensive Cancer Network (NCCN) guidelines also recommend CSFs when FN risk is high (≥20%) and recommend consideration of CSFs when there is an intermediate risk (10%–20%) of chemotherapy-induced FN (8,9). The NCCN guidelines also suggest consideration of CSFs for secondary prophylaxis (ie, in patients who experience FN in a previous chemotherapy cycle) if dose reduction may compromise survival. None of the above guidelines recommend using CSFs to maintain chemotherapy dose and schedule.

Although CSFs are an important therapeutic advance for patients at high risk of neutropenic complications, little is known about their use in general clinical practice. The only population-based study conducted thus far examined the discretionary use of CSFs in the region of western Washington and suggested that they are most likely overused (10). Given the high costs of CSFs ($2000 per chemotherapy cycle), understanding the multiple factors associated with their use may have important implications for optimizing the use of CSFs in clinical practice.

In this study, we examined the patterns of CSF use in a population-based, observational, multiregional cohort of lung and colorectal cancer patients receiving care in diverse health-care settings and assessed the association of clinical factors and type of insurance with discretionary CSF use outside of the ASCO and NCCN guidelines. Study subjects were patients aged 21 years or older who were diagnosed with lung or colorectal cancer from September, 2003, through December, 2005, and enrolled by the Cancer Care Outcomes Research and Surveillance Consortium (CanCORS) (11). CanCORS patients have been shown to be representative of all cancer cases reported by National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program (12). Data collection included surveys of patients or their surrogates approximately 4 months after diagnosis. Data on chemotherapy regimens, CSF use, cancer stage and histology, and comorbidities (13) were abstracted from medical records through 15 months after diagnosis. We used information from four of the seven CanCORS data collection sites, which collected complete information on CSF use. Three sites were population-based cancer registries, and the fourth was a system of health maintenance organizations (HMOs). These sites obtained local institutional review board approval and patient consent to participate.

We classified chemotherapy regimens received by FN risk as follows: low (<10% risk), intermediate (10%–20% risk), or high (≥20%) using NCCN guidelines (14). Primary prophylaxis was defined as receiving CSF within the first 5 days of initiating a new chemotherapy regimen. Because each patient could have received CSFs over multiple chemotherapy regimens, a generalized estimating equation regression model was used with multiple observations

CONTEXT AND CAVEATS

Prior knowledge

Use of colony-stimulating factors (CSFs) with chemotherapy regimens decreases the risk of febrile neutropenia (FN) in patients who are at high risk. CSFs are expensive ($2000 per cycle of chemotherapy), but not much is known about whether it is under- or overused in clinical practice.

Study design

Associations between CSF use outside of the American Society of Clinical Oncology and National Comprehensive Cancer Network guidelines and multiple sociodemographic and clinical factors, chemotherapy regimens (high-risk, intermediate-risk, and low-risk), and health maintenance organization (HMO) enrollment were assessed in 1785 lung or colorectal cancer patients enrolled by the Cancer Care Outcomes Research and Surveillance Consortium (CanCORS).

Contribution

Only 17% patients receiving high-risk chemotherapy regimens (≥20% risk of FN) received CSFs compared with 18% intermediate- (10–20% risk of FN) and 10% low-risk (<10% risk of FN) regimens. Factors that showed a strong association with CSF use included intermediate-risk regimens, severe comorbidity, having small-cell lung cancer, and non-HMO enrollment. Overall 96% of CSF use occurred outside the current evidence-based American Society of Clinical Oncology and National Comprehensive Cancer Network guidelines.

Implications

Lower discretionary use of CSFs in HMO plans (financial compensation has no incentives for physicians), suggests that financial interests could be driving the higher discretionary use of CSFs in non-HMO plans, and decreasing the unnecessary use of CSFs may reduce costs without compromising the quality of treatment.

Limitations

CSF use may have changed in patients since the time they were treated (2004–2006), and data on white blood cell counts or diagnoses of FN episodes were not obtained. The analysis may have underestimated the use of CSF.

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(ie, chemotherapy regimens) per patient, thus accounting for autocorrelation effects (clustering of regimens within patients). The model was used to examine associations between CSF use (yes or no) and independent sociodemographic (age, race or ethnicity, sex), clinical (cancer type, stage, histology, comorbidities, FN), insurance type (HMO vs non-HMO), and chemotherapy regimen (based on risk of FN) covariates. We performed two-sided Wald χ^2 tests to assess statistical significance of the association with covariates, and all *P* values less than .05 were considered to be statistically significant.

Overall, we identified 1849 patients who received chemotherapy that formed our study cohort. Only 64 of these patients received a "high-risk" chemotherapy regimen, identified by the inclusion of the drug topotecan, which is linked with a high risk (≥20%) of FN (14). Among these 64 high-risk patients, 11 received CSFs (17%; 95% confidence interval [CI] = 8% to 26%). This represented 4% (95% CI = 2% to 7%) of all patients who received CSFs (n = 268) in the study cohort. This finding suggested that given the strong evidence base and guidelines for CSF prophylaxis, interventions to increase CSF use was warranted in these patients.

Because so few patients received high-risk chemotherapy regimens, we focused our analyses on the factors that are associated with CSF use among patients receiving low- and intermediate-risk regimens, where all CSF use is discretionary. Table 1 shows the distribution of the sociodemographic, clinical, and health-care setting characteristics of these patients, after exclusion of 64 high-risk patients (n = 1785). A total of 982 of 1785 (55%; 95% CI = 53% to 57%) patients received at least one chemotherapy regimen classified as intermediate-risk of FN (10%–20%) based on NCCN guidelines. The unadjusted crude rates of the receipt of CSFs for each patient covariate along with odds ratios (ORs) of receipt of CSFs adjusted for all other covariates are also shown in Table 1.

Overall, 9% (95% CI = 7% to 11%) of patients diagnosed with colorectal cancer, 14% (95% CI = 12% to 16%) of patients diagnosed with non–small cell lung cancer, and 33% (95% CI = 27% to 40%) with small cell lung cancer ever received a CSF (Table 1). Also, 10% (95% CI = 8% to 12%) of the low-risk regimen patients received CSFs compared with 18% (95% CI = 16% to 20%) of the intermediate-risk regimen patients. Most CSF use was not for primary prophylaxis: 13% of small cell lung cancer (95% CI = 9% to 18%), 4% of non–small cell lung cancer (95% CI = 3% to 6%), and less than 1% (95% CI = 0.3% to 2%) of colorectal cancer patients received CSFs with the first cycle of a chemotherapy regimen (data not shown). Neither FN during a previous cycle (indicating secondary prophylaxis use) nor stage was associated with increased odds of receiving CSFs. Although the ASCO and NCCN guidelines suggest consideration of CSF, prophylaxis in patients older than 65 years was not associated with increased odds of receiving CSF. Factors that were statistically significantly associated with receipt of CSF included treatment with an intermediate-risk regimen (OR = 1.44, 95% CI = 1.04 to 1.99, *P* = .03), having small cell lung cancer vs colorectal cancer (OR = 3.10, 95% CI = 2.05 to 4.69; *P* < .001), and having severe vs no comorbidity (OR = 1.77, 95% CI = 1.16 to 2.70; *P* = .01). The odds of receiving CSFs were lower in women (OR = 0.76, 95% CI = 0.58 to 0.99; *P* = .04) and patients enrolled in group or staff HMO plans (OR = 0.44, 95% CI = 0.32 to 0.60; *P* < .001).

In this population-based, observational, multiregional cohort of lung and colorectal cancer patients, only 17% of patients at high risk of FN received a CSF, much lower than the percentage reported by previous studies conducted in either academic centers (17) or in selected community oncology practices (2,4,5). Most CSF use was neither for primary or secondary prophylaxis of FN as recommended by the guidelines but instead appeared to be reactive (ie, in response to neutropenia), most likely to maintain dose and schedule. Although we were unable to explicitly distinguish reasons for discretionary CSF use, the low incidence of FN among those receiving CSF in our sample of patients not treated with high-risk chemotherapy regimens suggested that only a small percentage of the discretionary use of CSF was in response to FN. However, cancer stage was not associated with CSF use, suggesting that the intent of therapy (curative vs palliative) did not appear to influence the decision to use CSFs. We did not find an association with age, despite recent evidence suggesting that older lung cancer patients may be at increased risk for FN (18). Discretionary CSF use was strongly associated with whether care

Table 1. Rates and associations of multiple factors with receipt of colony-stimulating factors (CSFs) in intermediate-risk and low-risk patients*

Covariate	No. of patients (%)†	No. of CSF recipients (%)‡	Adjusted OR (95% CI)§	*P*||
Age at diagnosis, y				
<65 (reference)	876 (49)	113 (13)	1.0 (referent)	
65–74	548 (31)	86 (16)	1.21 (0.89 to 1.64)	.22
>75–82	361 (20)	58 (16)	1.12 (0.79 to 1.58)	.53
Race or Ethnicity				
White (reference)	1295 (73)	189 (15)	1.0 (referent)	
Non-Hispanic Black	168 (9)	25 (15)	1.21 (0.77 to 1.91)	.42
Hispanic	122 (7)	18 (15)	1.18 (0.70 to 1.99)	.54
Other Race	200 (11)	25 (13)	1.35 (0.86 to 2.11)	.19
Sex				
Men (reference)	768 (43)	126 (16)	1.0 (referent)	
Women	1017 (57)	131 (13)	0.76 (0.58 to 0.99)	.04
Cancer type				
Colorectal cancer (reference)	654 (37)	58 (9)	1.0 (referent)	
NSCLC	921 (52)	129 (14)	0.88 (0.59 to 1.31)	.53
SCLC	210 (12)	70 (33)	3.10 (2.05 to 4.69)	<.001
Stage at diagnosis¶				
Stage I–III (reference)	1090 (61)	137 (13)	1.0 (referent)	
Stage IV	695 (39)	120 (17)	1.23 (0.93 to 1.61)	.14
Comorbidity (ACE-27)#				
None (reference)	424 (24)	50 (12)	1.0 (referent)	
Mild	743 (42)	104 (14)	1.24 (0.86 to 1.80)	.25
Moderate	330 (18)	40 (12)	1.25 (0.81 to 1.91)	.31
Severe	288 (16)	63 (22)	1.77 (1.16 to 2.70)	.01
Regimen risk for FN**				
Low (reference)	803 (45)	81 (10)	1.0 (referent)	
Intermediate	982 (55)	176 (18)	1.44 (1.04 to 1.99)	.03
FN				
No (reference)	1661 (93)	230 (14)	1.0 (referent)	
Yes	124 (7)	27 (22)	1.39 (0.88 to 2.20)	.16
HMO enrollment				
No (reference)	1174 (66)	207 (18)	1.0 (referent)	
Yes	611 (34)	50 (8)	0.44 (0.32 to 0.60)	<.001
Total No. (%)	1785 (100)	257 (17)		

* All patients included in this study were recruited at the following CanCORS data collection centers that collected data on the use of CSF: HMO Cancer Research Network covering the states of Alabama and Iowa, and Los Angeles County of California. ACE= Adult Comorbidity Evaluation; AJCC = American Joint Committee on Cancer; CanCORS = Cancer Care Outcomes Research and Surveillance Consortium; CI = confidence interval; FN =Febrile Neutropenia; HMO = Health Maintenance Organization; NSCLC = non–small cell lung cancer; OR = odds ratio; SCLC = small cell lung cancer.

† Numbers and percentages of patients in respective categories of patient characteristics (covariates). Percentage denominators were entire sample of 1785 patients receiving either low- or intermediate-risk chemotherapy regimens.

‡ Numbers and percentages of CSF recipients in respective categories of patient covariates. Percentage denominators were the combined numbers of both recipients and nonrecipients of CSFs that belong to a particular covariate.

§ Adjusted odds ratio estimates were computed as the natural antilogarithm of estimates of the logarithm of the odds ratios (log odds). Similarly, 95% confidence intervals for odds ratio estimates were computed as the antilogarithm of confidence intervals of the log odds ratio estimates. Log odds estimates were computed using a generalized estimating equation model.

|| *P* values were calculated using a two-sided Wald χ^2 test for testing the null hypothesis of no association (ie, the odds ratio = 1).

¶ AJCC staging system (15) was used.

Comorbidity was measured using the ACE-27 index which categorizes each patient as having no, mild, moderate, or severe comorbidity based on medical chart review of the presence and severity of 27 specific medical conditions (16). Patients are assigned to one of the categories based on the highest level of severity detected across all 27 conditions.

** Risk of febrile neutropenia can vary by regimen for patients receiving more than a single chemotherapy regimen.

was delivered within an HMO, where physician compensation is generally not directly affected by drug administration, vs outside an HMO. This suggests that financial incentives may be driving the largely discretionary use of CSFs, at least in part. Other factors, such as a more uniform, system-wide approach to care may help explain our finding of lower discretionary use of CSFs in HMO plans. The HMO plans included in our study did not place any specific restrictions on CSF use during the study period.

Our study has a few limitations. This included possible changes in CSF use since 2003–2005 when these patients were diagnosed and first treated, inability to collect data on white blood cell counts or diagnoses of FN episodes, and possible underestimation of CSF use because of errors in medical record abstraction or inability to access the treating oncologist's chart.

Despite these limitations, our study had several strengths. It is among the first to

describe variations in practice patterns for CSF use in a population-based cohort of patients of all age groups. Other strengths of our study were its population-based design, which reflects a range of healthcare delivery settings; diverse patient population with respect to age, race or ethnicity, and socioeconomic status; and a standardized medical record abstraction component linked with registry data and patient surveys.

Overall, 96% (95% CI = 93% to 98%) of CSFs in lung and colorectal cancer patients were administered in clinical situations where CSF therapy is not recommended by current evidence-based guidelines. This finding suggests that policies to decrease CSF use in lower-risk patient subsets may yield substantial cost savings without compromising patient outcomes. Finally, research is needed to better guide patient selection, including determination of whether CSFs reduce FN risk among specific patient subgroups defined by age, comorbidities, treatments received, or other prognostic risk factors.

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Notes

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SDHA Loss-of-Function Mutations in *KIT–PDGFRA* Wild-Type Gastrointestinal Stromal Tumors Identified by Massively Parallel Sequencing

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Approximately 10%–15% of gastrointestinal stromal tumors (GISTs) in adults do not harbor any mutation in the *KIT* or *PDGFRA* genes (ie, KIT/PDGFRA wild-type GISTs). Recently, mutations in *SDHB* and *SDHC* (which encode succinate dehydrogenase subunits B and C, respectively) but not in *SDHA* and *SDHD* (which encode subunits A and D, respectively) were identified in KIT/PDGFRA wild-type GISTs. To search for novel pathogenic mutations, we sequenced the tumor transcriptome of two young adult patients who developed sporadic KIT/PDGFRA wild-type GISTs by using a massively parallel sequencing approach. The only variants identified as disease related by computational analysis were in *SDHA*. One patient carried the homozygous nonsense mutation p.Ser384X, the other patient was a compound heterozygote harboring a p.Arg31X nonsense mutation and a p.Arg589Trp missense mutation. The heterozygous nonsense mutations in both patients were present in germline DNA isolated from peripheral blood. Protein structure analysis indicates that all three mutations lead to functional inactivation of the protein. This is the first report, to our knowle dge, that identifies *SDHA* inactivation as a common oncogenic event in GISTs that lack a mutation in *KIT* and *PDGFRA*.

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract and arise from the interstitial cells of Cajal. In approximately 85% of GISTs, gain-of-function mutations in either the *KIT* gene (which encodes a receptor for stem cell factor) or the platelet-derived growth factor receptor, alpha polypeptide (*PDGFRA*) gene are the oncogenic events that lead to tumor development, resulting in the constitutive ligand-independent activation of the receptor tyrosine kinases encoded by these genes and their downstream signaling pathways (1). Approximately 10% of GISTs in adult patients do not harbor a mutation in either gene (defined as KIT/PDGFRA wild-type). Notably, approximately 85% of GISTs that arise in children are KIT/PDGFRA wild-type and are often associated with a cancer syndrome (2). KIT/PDGFRA wild-type GISTs are often localized to the stomach, are multicentric in origin, and can have an indolent clinical course (3,4). The introduction of inhibitors of the KIT and PDGFRA tyrosine kinases to the therapeutic armamentarium has dramatically changed the medical treatment of GIST patients; as has been widely demonstrated, treatment response with these inhibitors strictly depends on the mutation status of *KIT* and *PDGFRA* (5–10). For example, in the metastatic setting, KIT/PDGFRA wild-type GISTs are more resistant to imatinib and more sensitive to sunitinib than GISTs with a mutant kinase (eg, GISTs with a *KIT* exon 11 mutation) (7–10). KIT/PDGFRA wild-type GISTs also differ from KIT/PDGFRA mutant GISTs in their clinical behavior and underlying genomic background and thus represent a distinct molecular subtype of GIST. Gene expression and gene copy number profiles of KIT/PDGFRA wild-type GISTs differ from those of mutant GISTs (11–15). For example, among GISTs that arise in children and young adults, insulin-like growth factor 1 receptor overexpression is commonly observed in those that are KIT/PDGFRA wild-type but not in those with either mutant kinase (11–13). Moreover, KIT/PDGFRA wild-type GISTs in children and young adults have minimal genomic copy number changes compared with kinase-mutant GISTs, which frequently have gross chromosomal copy number changes, including complete or partial deletion of chromosome arm 14 and deletion of chromosome arms 1p and 22q (14,15). Except for rare case reports of activating mutations in *BRAF* (16) and a recent report of mutations in *SDHB* and *SDHC* (which encode subunits B and C, respectively, of succinate dehydrogenase [SDH]) (17), to our knowledge, no pathogenic mutations have been identified in nonsyndromic GISTs that are *KIT* and *PDGFRA* mutation negative. We searched for novel pathogenic mutations by performing whole-transcriptome next-generation sequencing of sporadic KIT/PDGFRA wild-type GISTs that arose in two young adult patients (GIST_07 and GIST_10). Next generation RNA sequencing is the only approach that allows the complete and thorough identification of all the possible genetic alterations (point mutations, insertions, deletions, and rearrangements) for all genes expressed in a pathological sample. This study was approved by the local institutional ethical committee of Azienda Ospedaliero-Universitaria Policlinico S. Orsola-Malpighi (approval number 113/2008/U/Tess). All patients provided written informed consent.

Poly(A) RNA was isolated from each tumor and subjected to whole-transcriptome paired-end sequencing with the use of a Genome Analyzer IIx system

CONTEXT AND CAVEATS

Prior knowledge

Approximately 10% of gastrointestinal stromal tumors (GISTs) in adults and 85% of GISTs in children do not carry a gain-of-function mutation in the receptor tyrosine kinase–encoding *KIT* or *PDGFRA* genes. KIT/PDGFRA wild-type GISTs differ from KIT/PDGFRA mutant GISTs in their response to treatment with kinase inhibitors, clinical behavior, and underlying genomic background.

Study design

Whole-transcriptome next-generation sequencing was used to search for novel pathogenic mutations in sporadic KIT/PDGFRA wild-type GISTs that arose in two young adult patients. Computational analysis was used to determine the likelihood that a mutation is disease related or not.

Contribution

The only variants identified as disease related by computational analysis were in *SDHA*, the gene encoding succinate dehydrogenase subunit A. One patient carried a homozygous nonsense mutation, the other patient was a compound heterozygote harboring a nonsense mutation and a missense mutation. The heterozygous nonsense mutations in both patients were present in germline DNA isolated from peripheral blood.

Implications

SDHA inactivation may be a common oncogenic event in GISTs that lack a mutation in *KIT* and *PDGFRA*.

Limitations

Only two KIT/PDGFRA wild-type GISTs were sequenced.

From the Editors

(Illumina, San Diego, CA) as described in detail in Supplementary Methods (available online), yielding an average of 202 779 378 reads that aligned onto the human reference genome for both patients. Alignments were processed according to a routine technical procedure for calling single-nucleotide variants, which were filtered to exclude known polymorphisms that are annotated in the National Center for Biotechnology Information dbSNP (http://www.ncbi.nlm.nih.gov/snp) and the 1000 Genomes databases, leaving 173 617 and 183 159 putative novel variants for GIST_07 and GIST_10, respectively (Supplementary Table 1, available online). The two patients had nonsynonymous mutations in the coding sequences of the same 261 genes, for a total of 582 novel variants, which we sorted according to a threshold confidence value (Supplementary Methods, available online). This procedure identified nine candidate genes that were mutated in the tumors of both patients (Supplementary Table 2, available online). The mutations in these nine genes were analyzed with SNPs&GO (18), a method for computing the likelihood of a mutation being disease related or not depending on the protein sequence and its functional annotation. This method predicted that only the three mutations in the coding sequence of *SDHA*, which encodes subunit A of SDH, are disease related with a high reliability index (Supplementary Table 2, available online). Massively parallel sequencing analysis revealed that GIST_07 had a C to G transversion at nucleotide 1151 in exon 9 of *SDHA*, a nonsense mutation resulting in the replacement of serine with a stop codon at residue 384 of SDHA, which causes truncation of the peptide chain at residue 383 (p.Ser384X). GIST_10 had two mutations in *SDHA*: 1) a C to T transition at nucleotide 91 in exon 2, a nonsense mutation resulting in the replacement of arginine with a stop codon at residue 31 (p.Arg31X) and 2) a C to T transition at nucleotide 1765 in exon 13, a missense mutation resulting in the replacement of arginine at residue 589 with tryptophan (p.Arg589Trp; Table 1).

SDH (also known as complex II) consists of four subunits: SDHA, SDHB, SDHC, and SDHD (19). Mutant SDH results in dysfunction of complex II of the electron transport chain in mitochondria and, consequently, defective oxidative phosphorylation, which mediates a pseudohypoxic response (ie, the abnormal stabilization of hypoxia-inducible factors [HIFs] under normoxic conditions). Patients with the Carney–Stratakis syndrome, who are predisposed to developing paragangliomas and GISTs, have germline mutations in *SDHB*, *SDHC*, and *SDHD* (20–22). Although SDHA forms a complex with SDHB, SDHC, and SDHD, to our knowledge, no mutations in *SDHA* have been reported in patients with the Carney–Stratakis syndrome or in patients who develop sporadic KIT/PDGFRA wild-type GISTs.

To validate our results and discriminate whether the detected *SDHA* mutations were present in the germline or somatic, we performed targeted exon sequencing of DNA isolated from tumor and peripheral blood of both patients (Supplementary Methods, available online). Patient GIST_07 carried c.1151C>G as a heterozygous germline mutation in blood and as a homozygous mutation in the tumor (Figure 1, A). Single-nucleotide polymorphism array analysis (15) and quantitative polymerase chain reaction analysis of tumor and peripheral blood DNA revealed that there was no statistically significant difference in copy number at the *SDHA* locus between the tumor DNA and the matched peripheral blood DNA, which suggests that the mutation in the tumor was present in homozygosis (Supplementary Figure 1, available online). Quantitative polymerase chain reaction analysis of cDNA synthesized from tumor RNA revealed that the homozygous nonsense mutation in the GIST_07 tumor was associated with a

Table 1. Succinate dehydrogenase subunit A (*SDHA*) mutations

Gene ID	Gene name	Uniprot ID	Genomic coordinate*	Exon	Patient	Allele mutation	Residue mutation†
SDHA	Succinate dehydrogenase (ubiquinone) flavoprotein subunit A	P31040	chr5:288345	9	GIST_07	C>G	S384X
			chr5:276624	2	GIST_10	C>T	R31X
			chr5:304554	13	GIST_10	C>T	R589W

* As in National Center for Biotechnology Information v36.1.

† Residue substitutions are shown with their position in the protein chain.

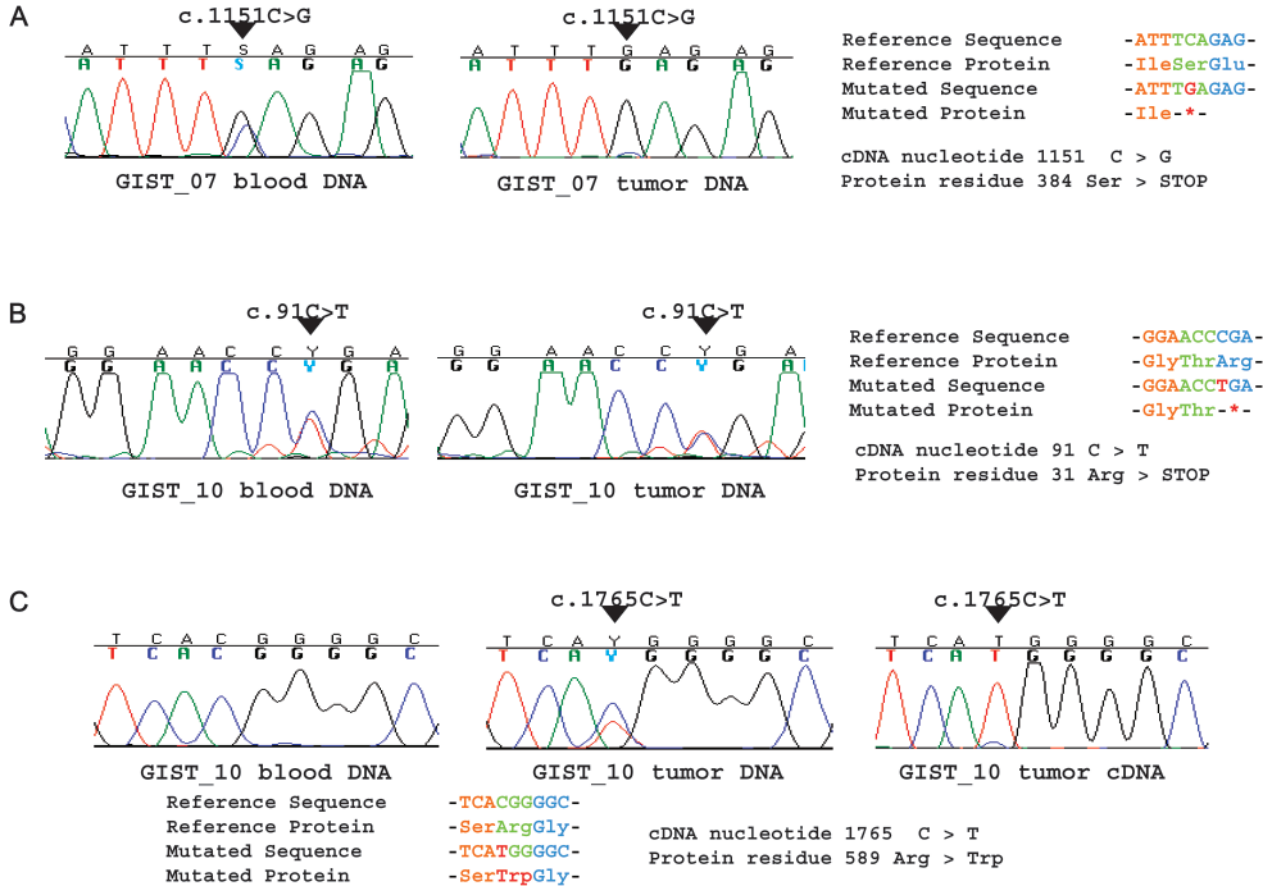

Figure 1. Sequence chromatograms of DNA isolated from two patients with sporadic KIT/PDGFRA wild-type gastrointestinal stromal tumors. **A**) Region harboring the c.1151C>G mutation (p.Ser384X) in patient GIST_07. *Left,* heterozygous mutation in blood DNA; *Right,* homozygous mutation in tumor DNA. **B**) Region harboring the c.91C>T mutation (p.Arg31X) in patient GIST_10. *Left,* heterozygous mutation in blood DNA; *Right,* heterozygous mutation in tumor DNA. **C**) Region harboring the c.1765C>T mutation (p.Arg589Trp) carried by GIST_10 patient. *Left,* wild-type sequence in GIST_10 blood DNA; *Middle,* heterozygous mutation in GIST_10 tumor DNA; *Right,* predominance of expression of the mutated allele in cDNA synthesized from GIST_10 tumor RNA.

marked reduction in the level of SDHA mRNA compared with that in the 14 of 17 KIT/PDGFRA mutant GIST samples for which there was sufficient RNA available for analysis (mean normalized SDHA expression, GIST_07 vs 14 mutant GISTs = 0.89 vs 6.08; fold difference = 6.8, 95% CI = 4.5 to 12.2; $P < .001$, Student t test, two-tailed) (Supplementary Figure 2, available online). A KIT/PDGFRA wild-type GIST from a pediatric patient (GIST_24) had essentially the same fold reduction in SDHA mRNA compared with KIT/PDGFRA mutant GISTs as did GIST_07 (Supplementary Figure 2, available online).

Patient GIST_10 carried c.91C>T as a heterozygous nonsense mutation in both blood and tumor (Figure 1, B), indicating that this patient had a germline genetic alteration of the *SDHA* gene. A second hit that affected *SDHA* in the tumor of this patient was compound heterozygosity for an independent somatic mutation (c.1765C>T), resulting in the Arg589Trp mutation in the mature protein (Figure 1, C). Sequence analysis of cDNA synthesized from tumor RNA revealed that the mutant allele was predominantly expressed in the tumor (Figure 1, C). To understand the effect of the Arg589Trp mutation in the mature SDHA, we computed a three-dimensional model of the mutated subunit by adopting as a template the structure of its porcine counterpart, which is known at atomic resolution (Supplementary Figure 3, available online). Protein structure analysis highlights that, in the wild-type protein, Arg 589 is located in the flavin adenine dinucleotide-binding domain, which is critical for SDHA function. The Arg589Trp mutation results in a side-chain substitution that promotes misfolding of this domain (and, as a consequence, functional inactivation of SDHA) by destabilizing the local polar environment of the wild-type Arg 589 (Supplementary Figure 4, available online).

Together, these results indicate that patients GIST_07 and GIST_10 each carried a first-hit germline mutation in *SDHA*, represented by two different single-base changes, which introduced a stop codon that resulted in a truncated mature protein. A SDHA heterozygous nonsense mutation in the germline of both patients may indicate a neoplastic syndrome that includes KIT/PDGFRA mutation–negative GISTs and potentially other cancers. It would be interesting to know the cancer history of long-term survivors of GISTs that are *PDGFRA* and *KIT* mutation negative.

Recently, Janeway et al. (17) found germline mutations in *SDHB*, *SDHC*, or *SDHD* in six of 38 KIT/PDGFRA wild-type GISTs from pediatric patients with no family history of paraganglioma and, moreover, the loss of SDHB protein expression and complex II activity in KIT/PDGFRA wild-type GISTs with no *SDHB*, *SDHC*, or *SDHD* mutations or deletions from 13

pediatric patients. These findings support our view that loss of SDH function plays a role in the pathogenesis of KIT/PDGFRA wild-type GISTs and together with our findings suggest that children or young adults with KIT/PDGFRA wild-type GISTs should be screened for germline or de novo mutations in all four subunits of SDH complex. Disruption of the SDH complex leads to increased expression of HIF-1 alpha and may cause GIST or paraganglioma through similar molecular pathways as seen in renal cell cancers that display loss of von Hippel–Lindau tumor suppressor function (23).

The exact role of mutant SDHA in tumor initiation is poorly understood. Recently, Burnichon et al. (24) identified a germline *SDHA* mutation resulting in p. Arg589Trp that was associated with loss of heterozygosity in a catecholamine-secreting abdominal paraganglioma and suggested that *SDHA* may work as a tumor suppressor gene. The authors showed that this mutant SDHA was associated with loss of enzymatic activity of the SDH complex in tumor tissue and in a yeast model, and, like mutations in *SDHB*, *SDHC*, and *SDHD*, resulted in pseudohypoxia and increased angiogenesis and cell proliferation in vitro. In this study, we found that *SDHA* gene mutations are present in PDGFRA/KIT wild-type GISTs, supporting the hypothesis that SDHA may act as a tumor suppressor in these tumors.

A limitation of this study is the small sample size. A larger number of KIT/PDGFRA wild-type GISTs needs to be evaluated for *SDHA* mutations to reach definitive conclusions about the role of this gene in the development of sporadic KIT/PDGFRA wild-type GISTs. To our knowledge, this is the first report describing germline and somatic loss-of-function mutations in *SDHA* that are linked to the development of sporadic KIT/PDGFRA wild-type GISTs. The finding that inactivation of the SDH complex seems to be an event shared by sporadic and syndromic GISTs that lack mutations in *PDGFRA* and *KIT* may open new avenues for pharmacologic treatments.

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