HHS Public Access

Author manuscript

Urology. Author manuscript; available in PMC 2021 July 13.

Published in final edited form as:

Urology. 2020 October ; 144: 111–116. doi:10.1016/j.urology.2020.07.027.

RAS/MAPK Pathway Driver Alterations Are Significantly Associated With Oncogenic *KIT* Mutations in Germ-cell Tumors

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Abstract

OBJECTIVE—To report the mutational profile and clinical outcomes of a cohort of patients with *KIT*-mutant seminomas and nonseminomatous germ-cell tumors (SGCT/NSGCTs).

PATIENTS AND METHODS—Retrospective cohort study of all patients with *KIT*-mutant GCTs sequenced at Memorial Sloan Kettering between March 2014 and March 2020. Tumors were assessed with MSK-IMPACT, a DNA next-generation sequencing assay for targeted sequencing of up to 468 key cancer genes.

RESULTS—Among 568 patients with GCTs, 8.1% had somatic *KIT* mutations, including 28 seminomas and 18 mixed/NSGCTs. Exons 17 (67.3%), 11 (22.4%), and 13 (6.1%) were most commonly affected. *KIT*-mutant cases were enriched for oncogenic RAS/MAPK pathway alterations compared to *KIT*-wildtype cases (34.8% vs 19.2%, $P = .02$). Among *KIT*-mutant cases, concurrent mutations were noted in *KRAS* (21.7%), *RRAS2* (11.8%), *CBL* (6.5%), *NRAS* (4.3%), *MAP2K1* (2.2%), and *RAC1* (2.2%). Mutations in *KRAS*, *RRAS2*, and *NRAS* were mutually exclusive. In all, 73.9% of patients developed metastases and 95.7% received chemotherapy. No patients received KIT-directed tyrosine kinase inhibitors (TKIs). Classification as a NSGCT rather than a SGCT was associated with an increased risk of death (hazard ratio 9.1, 95% confidence interval 1.1–78.4, $P = .04$) while the presence of a concurrent RAS/MAPK pathway alteration was not (hazard ratio 0.8, 95% confidence interval 0.1–4.3, $P = .76$).

CONCLUSION—Mitogenic driver alterations can co-occur with activating KIT mutations, which may explain the lack of efficacy of KIT-directed TKIS in 0070rior trials. Novel KIT-directed TKIS that target exon 17 mutations may benefit chemotherapy-refractory patients with KIT-mutant GCTs without RAS/MAPK alterations. Dual MEK/KIT inhibitor therapy in KIT-mutant GCTs with concurrent RAS/MAPK alterations could also be a plausible therapeutic strategy.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urology.2020.07.027.

A subset of seminomas and nonseminomatous germ-cell tumors (SGCT/NSGCTs) is characterized by activating mutations in *KIT*, which encodes the 21-exon cell surface receptor tyrosine kinase protein KIT (CD117) that drives downstream signaling through the RAS/MAPK pathway.[1,2] Such mutations have potential therapeutic relevance, as patients with *KIT*-mutant gastrointestinal stromal tumors experience durable clinical benefit from tyrosine kinase inhibitors (TKIs).[3,4] However, a phase 2 clinical trial of imatinib treatment in KIT-expressing, chemotherapy-refractory GCTs showed no evidence of significant antitumor activity.[5] That trial enrolled patients based on KIT expression by immunohistochemistry (IHC) rather than the presence of *KIT* mutations by sequencing. This lack of efficacy has therefore not been rigorously explored by considering underlying mutations in *KIT* or concurrent downstream genomic alterations in the RAS/MAPK pathway that could potentially explain the inactivity of TKIS in GCTs. Herein, we report the mutational profiles and clinical outcomes in a cohort of patients with *KIT*-mutant GCTs.

PATIENTS AND METHODS

Design, Setting, and Participants

We performed a retrospective cohort study of all patients with *KIT*-mutant GCTs sequenced between March 2014 and March 2020 at Memorial Sloan Kettering Cancer Center (MSK) in New York, NY. The study was approved by the MSK Institutional Review Board and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Case Selection and Diagnostic Criteria

All patients with *KIT*-mutant GCTs were included in the study. *KIT*-wildtype cases sequenced during the same period were selected for comparison. Cases were diagnosed by experienced subspecialized genitourinary pathologists according to the criteria specified in the 2016 World Health Organization Classification of Tumors of the Urinary System and Male Genital Organs.

DNA-based Molecular Analyses

To assess for the presence of *KIT* mutations and other concurrent molecular alterations, tumors and matched normal blood samples were analyzed with the MSK-IMPACT DNA next-generation sequencing platform that targets up to 468 genes and select introns to produce data on single nucleotide variants, small insertions and deletions, copy number changes, and structural variants.[6] Variants were classified as oncogenic based on their curation in the MSK Precision Oncology Knowledge Base (OncoKB).[7] Tumors that did not meet minimum requirements for tumor content or sequencing coverage were excluded.

Clinicopathologic and Survival Data

Clinicopathologic data were extracted from the electronic medical record, including data on age, sex, date of initial diagnosis, pathologic diagnosis including results of KIT IHC, survival time, and treatment history with chemotherapy or targeted therapy. Overall survival was defined as the time from initial pathologic diagnosis until the time of death due to any cause.

Statistical Analyses

Differences among categorical variables were assessed using Fisher's exact test. Those among continuous variables were assessed using the nonparametric Mann-Whitney-Wilcoxon test. Clinicopathologic variables were examined in Cox proportional hazards models for associations with overall survival. Genomic data were accessed using the internal MSK cBioPortal for Cancer Genomics,[8] PathwayMapper,[9] and ProteinPaint.[10] Statistical analyses were performed using R version 3.6.2 (R Foundation for Statistical Computing). Statistical tests were 2-sided and used a significance threshold of $P < .05$. Reported P values were not adjusted for multiple testing.

RESULTS

Samples Included in the Study

Among 568 patients with GCTs, 8.1% (46/568) had somatic *KIT* mutations curated in OncoKB as likely oncogenic alterations that may respond to TKIs in other tumor types, including 23 men (of 381 men) with testicular GCTs, 15 men (of 71 individuals) with mediastinal GCTs, 7 women (of 66 women) with ovarian GCTs, and 1 man (of 4 men) with a pineal germinoma. Mediastinal GCTs were more likely to have *KIT* mutations than were GCTs from other sites (21.1% [15/71] vs 6.2% [31/497], $P < .001$). The median age at initial pathologic diagnosis of patients with *KIT*-mutant GCTs was 33.4 years (range, 7.2–58.9 years) and 15.2% (7/46) were female. Patients with *KIT*-mutant GCTs did not differ from those with *KIT*-wildtype GCTs with respect to age ($P = .62$) or sex ($P = .99$). The histologic classification (eg, SGCT vs NSGCT), presence or absence of a seminomatous component (for NSGCTs), specimen sequenced (eg, primary vs metastatic lesion), clinical details, and associated genetic alterations for each *KIT*-mutant ease are shown in Figure 1. Specific pathologic diagnoses, as well as additional data on the molecular alterations presented below, are provided in the Supplementary Data.

Spectrum of KIT Mutations

Among the 46 *KIT*-mutant cases, 95.6% (44/46) had 1, 2.2% (1/46) had 2, and 2.2% (1/46) had 3 *KIT* mutations. The spectrum of *KIT* mutations identified among the samples is shown in Figure 2. Exons 17 (67.3% [33/49]), 11 (22.4% [11/49]), and 13 (6.1% [3/49]) were most commonly affected. The exonic distribution did not differ by sex ($P = 1.0$), primary site ($P = .91$), or presence of a seminomatous component ($P = .56$). The most frequently mutated codons overall were D816 and N822, both part of the exon 17 tyrosine kinase II activation loop, in 30.6% (15/49) and 20.4% (10/49) of cases, respectively. The most frequently mutated codons in the exon 11 juxtamembrane domain were L576 (8.2% [4/49]) and W557 (8.2% [4/49]). The most frequently mutated codon in the exon 13 tyrosine kinase I domain was N655 (4.1% [2/49]). Individual cases with mutations in exons 9 and 18 were also identified (K5091 and A829P, respectively). Among the 32.6% (15/46) of eases for which KIT IHC was reported, 93.3% (14/15) exhibited positive staining.

Prevalence of Concurrent RAS/MAPK Pathway Alterations

Cases with *KIT* mutations were significantly enriched for oncogenic RAS/MAPK pathway mutations compared to *KIT*-wildtype cases (34.8% [16/46] vs 19.2% [100/522], $P = .02$) (Supplemental Table 1). Among *KIT*-mutant cases, concurrent mutations were noted in *KRAS* (21.7% [10/46]), *RRAS2* (11.8% [2/17]), *CBL* (6.5% [3/46]), *NRAS* (4.3% [2/46]), *MAP2K1* (2.2% [1/46]), and *RACI* (2.2% [1/46]). *PDGFRA* amplification (6.5% [3/46]) and *NF1* homodeletion (2.2% [1/46]) were also rarely noted. Of note, *PDGFRA* and *KIT* are adjacent to one another on 4q12, and all 3 *PDGFRA*-amplified cases exhibited co-amplification of *KIT*. Alterations in *KRAS*, *RRAS2*, *NRAS*, *PDGFRA*, and *NF1* were mutually exclusive. Copy-number gains in 12p (including *KRAS*, among other genes) were seen in 47.8% (22/46) of cases, suggesting isochromosome 12p. The spectrum of *KRAS*, *RRAS2*, and *NRAS* mutations identified is shown in Figure 3. A pathway diagram summarizing the frequency of RTK/RAS/MAPK alterations among is eases is shown in Figure 4.

Clinical Outcomes

The median follow-up time among the 46 patients with *KIT*-mutant GCTs was 6.0 years (interquartile range, 3.3–9.4). In all, 73.9% (34/46) had metastasis at any point and 95.7% (44/46) received chemotherapy. One patient, a 16-year-old man with a multiply refractory (ie, high-dose chemotherapy resistant) primary mediastinal yolk sac tumor with widespread metastases, received the PD-L1 inhibitor atezolizumab and then the MEK inhibitor cobimetinib after chemotherapy failed, with minimal response. No patients received KIT-directed TKIs, although the majority (n = 36) were cured by other treatments and thus not considered for TKI therapy. At follow-up, 73.9% (34/46) of patients were free of disease, 13.0% (6/46) had disease, and 13.0% (6/46) had died due to disease, including the patient who received cobimetinib. On multivariable Cox proportional hazards regression, classification as a NSGCT rather than a SGCT was associated with an increased risk of death (hazard ratio 9.1, 95% confidence interval 1.1–78.4, $P = .04$) while the presence of a concurrent RAS/MAPK pathway alteration was not (hazard ratio 0.8, 95% confidence interval 0.1–4.3, $P = .76$), although the number of events was too small to draw firm conclusions.

COMMENTS

This retrospective cohort study of 568 patients with GCTs from multiple sites demonstrated that 8.1% (46/568) had potentially clinically actionable somatic *KIT* mutations, of which 34.8% (16/46) exhibited concurrent oncogenic mutations in RAS/MAPK pathway genes, an observation that could potentially explain the lack of efficacy of *KIT*-directed TKIs in prior case reports and clinical trials.[5] In addition, it is known that exon 17 *KIT* mutations, the most frequent site of mutations in GCT, are not sensitive to the majority of available KIT-directed TKIs including imatinib and sunitinib.[11,12] No patients received KIT-directed TKIs in this cohort, so potential reasons for the previously demonstrated resistance to KIT-directed TKIs could not be directly explored.

The 8.1% prevalence of *KIT* mutations among GCTs identified in this report and their relative distribution between the kinase and juxtamembrane domains are broadly concordant with the results of previously published studies. A 2015 whole-exome sequencing study of 42 testicular GCTs identified *KIT* mutations in 14.3% (6/42) of cases, 83.3% (5/6) in the exon 17 tyrosine kinase domain II activation loop and 16.7% (1/6) in the exon 11 juxtamembrane domain.[1] However, in contrast to the present study, only a single *KIT*-wildtype case harbored a KRAS mutation. A larger 2018 analysis of 137 primary testicular GCTs demonstrated somatic mutations in *KIT*, *KRAS*, and *NRAS* exclusively in samples with seminoma components.[2] *KIT* mutations were noted in 18.2% (25/137) of cases, including in the exon 17 kinase activation loop (74.1% [20/27]), the exon 11 juxtamembrane domain (22.2% [6/27]), and the exon 13 protein tyrosine kinase I domain (3.7% [1/27]). Concurrent *KRAS* and *NRAS* mutations were noted in 16.0% (4/25) and 8.0% (2/25) of the *KIT*-mutant cases, respectively, and were mutually exclusive of one another.

In a 2018 study of 24 ovarian GCTs, 16.7% (4/24) had oncogenic *KIT* mutations in exons 11, 13, or 17, including a case of pure dysgerminoma (synonymous to seminoma) with a concurrent *NF1* mutation.[13] All the *KIT*-mutant cases were dysgerminomas or mixed forms with a dysgerminomatous component. In all, 8.3% (2/24) had *KRAS* mutations, although again contrasting with the present study, none had alterations in *KIT*. Last, in a 2014 study of intracranial GCTs, 25.8% (16/62) had mutations in *KIT*, including 2 cases with concurrent mutations in the negative RAS pathway regulator *CBL*,[14] similar to the present study. All the *KIT*-mutant cases had seminomatous components. Cases with *KRAS* (14.5% [9/62]), *NRAS* (4.8% [3/62]), and *INF1* (3.2% [2/62]) mutations were also identified; however, none had concurrent mutations in *KIT*.

In contrast to prior reports, our study revealed that *KIT* and RAS/MAPK pathway alterations also co-occurred in NSGCTs lacking seminomatous components and that RAS/MAPK mutations were more common in *KIT*-mutant compared to *KIT*-wildtype tumors. It also confirmed the increased frequency of exon 17 compared to exon 11 mutations in GCTs, contrary to the pattern seen in GISTs, where exon 11 alterations predominate.[15] Further, concurrent RTK/RAS/MAPK pathway alterations were noted not only in *KRAS*, *NRAS*, *CBL*, and *NF1*, but also in *RRAS2*, *MAP2K1*, *RAC1*, and *PDGFRA*. Mutual exclusivity among concurrent RAS/MAPK alterations was noted not just for *KRAS* and *NRAS*, but also for *RRAS2*, *PDGFRA*, and *NF1*.

The identification of activating *RRAS2* mutations that were mutually exclusive with *KRAS* and *NRAS* in the present study merits special consideration. *RRAS2*, also known as TC21, is a RAS superfamily oncogene[16,17] recently recognized as a rare cause of the RASopathy Noonan syndrome.[18] The literature on its role in cancer is relatively sparse, although reports have linked *RRAS2* alterations to ovarian and breast carcinomas and radiotherapy-associated gliomas.[19–21] *RRAS2* encodes a 6-exon protein with significant homology to *KRAS* and *NRAS*.[10] Specifically, *RRAS2* amino-acid positions 20–25 (VGGGGV) have a sequence similar to *KRAS* and *NRAS* positions 9–14 (VGAGGV), including a recurrent G23 hotspot variant homologous to the G12 hotspot variant in *KRAS* and *NRAS*. No specific RRAS2 inhibitor is available for clinical use, although research into multivalent small molecule pan-RAS inhibitors is ongoing.[22]

Identification of oncogenic *KIT* mutations is of potential clinical relevance given that it has been successfully targeted in other solid tumors, GIST being the most notable example.[3,4] However, KIT has yet to be successfully targeted in GCTs. A phase 2 clinical trial published in 2006 of imatinib treatment in KIT-expressing, chemotherapy-refractory GCTs showed no evidence of significant antitumor activity. That trial enrolled patients based on KIT expression by IHC rather than the presence of *KIT* mutations by sequencing. Since IHC has not been demonstrated to be a predictive biomarker for response to KIT inhibitors, the lack of efficacy could thus be explained by possible absence of KIT mutations in this cohort. In addition, most available KIT inhibitors primarily target exon 11 or 13 mutations with limited efficacy against exon 17 mutations, which are more prevalent in GCT. Finally, co-mutations in downstream pathways such as RAS/MAPK could serve to limit TKI activity.

The potential of RAS/MAPK alterations to induce resistance to TKIs like imatinib is relevant given ongoing trials of MEK inhibitors such as trametinib, cobimetinib, and binimetinib.[23–25] Interestingly, in melanomas and GISTs where *KIT* is recurrently mutated, activating *KIT* mutations represent mitogenic drivers that are generally mutually exclusive with other activating mutations in the RAS/MAPK pathway.[26,27] In contrast, our study demonstrates that activating mutations in *KIT* and RAS/MAPK genes commonly co-occur in GCTs. Although these alterations may seem redundant, these co-mutations may reflect the unique tissue-specific dependencies of germ-cell differentiation and development. Importantly, these data suggest that dual inhibition of KIT and the RAS/MAPK pathway may provide a therapeutic strategy for patients with these co-mutations. Such a strategy could potentially yield successes similar to those noted for dual RAF/MEK inhibition in colorectal adenocarcinoma and malignant melanoma, where synergistic inhibition of both kinase pathways overcomes resistance to therapy with BRAF kinase inhibitors alone.[28,29]

Since the advent of cisplatin-based chemotherapy for treating patients with testicular cancer, the combination of surgery and chemotherapy can be expected to cure >90% of patients with GCTs.[30] Therefore, at present, the possibility of utilizing targeted therapy in patients with GCTs is relevant only to those with multiply relapsed or refractory disease. The clinical outcomes of the *KIT*-mutant cases in the present study mirrored the generally favorable prognosis of GCTs overall, with 73.9% of patients cured of disease and 13.0% of patients whose treatment was ongoing at the time of this writing. As expected, patients with NSGCTs had worse outcomes than those with SGCTs. The presence of a concurrent RAS/MAPK pathway alteration was not associated with a better or worse outcome in this patient population, although the number of adverse outcomes (ie, persistent disease or death) was low, and no patients received KIT-directed therapy.

This study has limitations. Not all patients at MSK with clinical stage I GCTs are subjected to sequencing, thus patients with metastatic disease and with extragonadal GCTs are overrepresented in this study. Although 46 patients with GCTs with somatic *KIT* mutations were identified, the presence of *RRAS2* alterations was only interrogated in 32.6% (15/46) of patients since it was only recently added to the MSK-IMPACT assay design. Further, the presence of isochromosome 12p was inferred indirectly by copy-number analysis and the sensitivity of the assessment could be adversely affected by low tumor content and the level of genomic instability, thus its prevalence could be underestimated. Last, no patients

received KIT-directed TKIS in this cohort, thus future studies will be required to test the hypothesis that ascertaining the mutational status of both *KIT* and RAS/MAPK pathway genes could successfully inform therapy in patients with chemotherapy-refractory disease. Studies of KIT inhibitors targeting tumors with exon 17 mutations are ongoing (eg, NCT02508532 and NCT03673501).

CONCLUSION

Next-generation sequencing analysis of GCTs can uncover potentially TKI-responsive *KIT* mutations and associated genomic alterations. *KIT* mutations occur most commonly in exon 17 and are associated with RAS/MAPK alterations, which may explain the lack of efficacy of TKIS in prior trials. Novel KIT-directed TKIS that target exon 17 mutations may benefit patients with *KIT*-mutant GCTs without RAS/MAPK alterations. Alternately, dual MEK/KIT inhibitor therapy in *KIT*-mutant GCTs with concurrent RAS/MAPK alterations may be a plausible therapeutic strategy.

DATA SHARING STATEMENT

Clinical and genetic data are available for download in the provided Supplementary Data file.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment.

The authors thank the members of the Molecular Diagnostics Service in the Department of Pathology and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology for the establishment of the MSK-IMPACT dataset.

Funding Sources: *This research was supported in part by the National Cancer Institute of the National Institutes of Health (P30 CA008748).*

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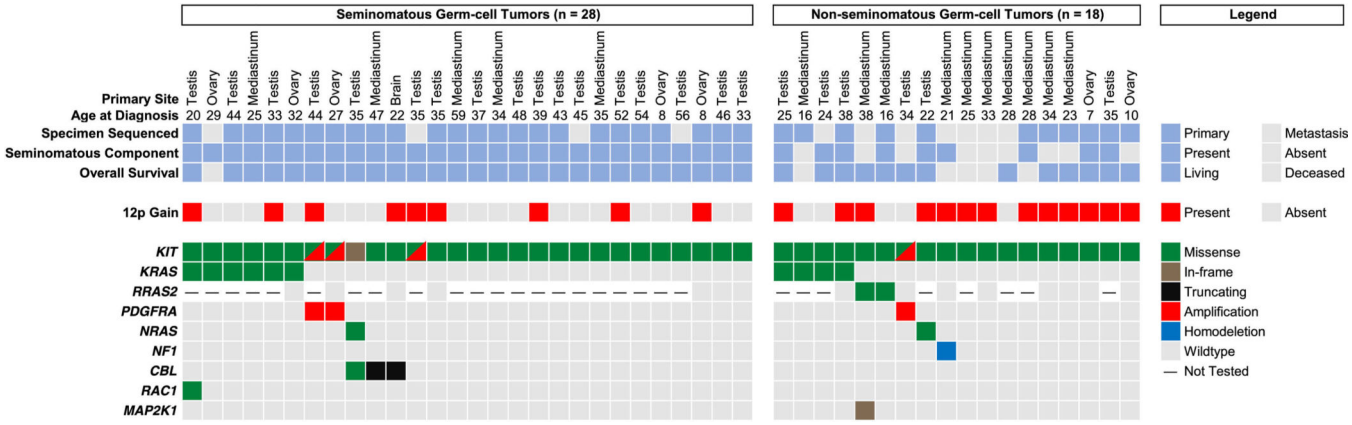

Figure 1.

Clinical, histologic, and genetic features of patients with *KIT*-mutant germ-cell tumors. The specimens are stratified according to histologic classification (ie, seminomatous vs nonseminomatous germ-cell tumor). Of the 11 metastatic samples sequenced, 3 were obtained prior to chemotherapy, 1 was obtained prior to chemotherapy but after radiotherapy, and 7 were obtained after chemotherapy.

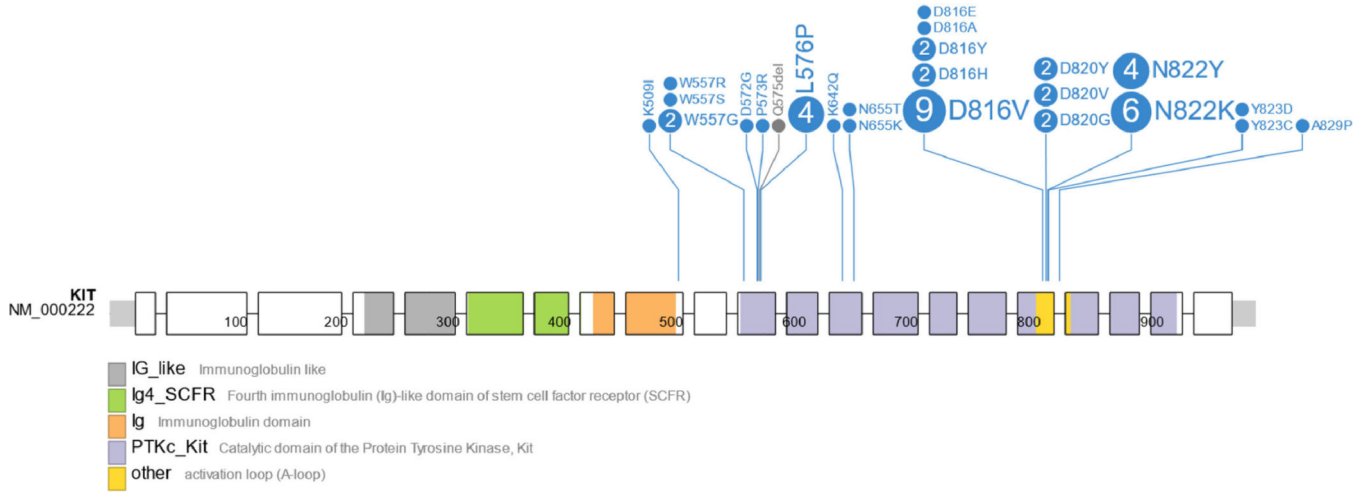

Figure 2.

Spectrum of somatic mutations in *KIT*. Exons 1–21 are represented from left to right by the enclosed boxes with superimposed amino acid positions. Missense mutations are shown in in the lollipop plot in blue; deletions are in gray. The number within each circle corresponds to the number of times that mutation was identified. Selected protein domains are indicated by the colors shown below the figure.

Figure 3.

Spectrum of somatic mutations in *KRAS*, *NRAS*, and *RRAS2*. Missense mutations are shown in the lollipop plots in blue. The number within each circle corresponds to the number of times that mutation was identified. Selected protein domains are indicated by the colors shown below the diagrams. The amino acid sequences and positions are superimposed.

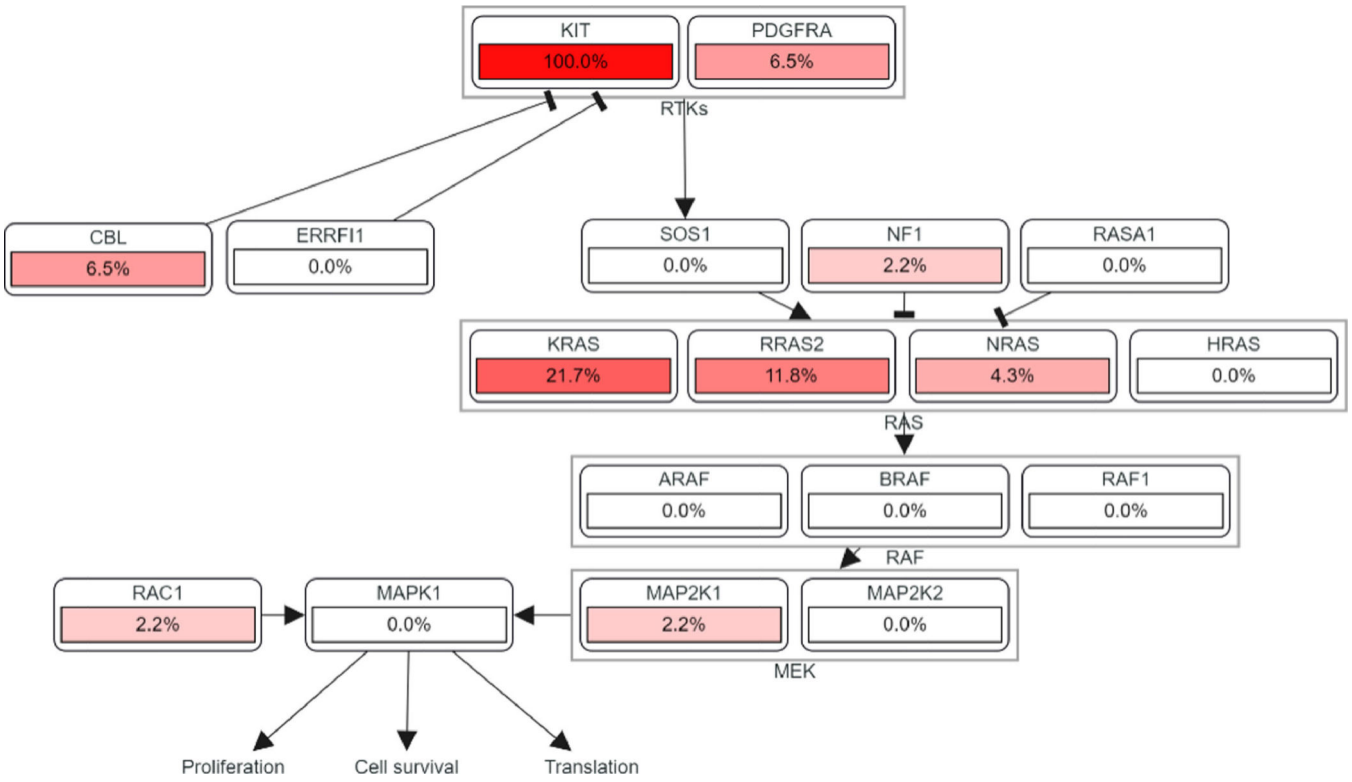

Figure 4.

RTK/RAS/MAPK pathway alterations in *KIT*-mutant germ-cell tumors. The values shown represent the percentage of *KIT*-mutant samples (n = 46) with oncogenic alterations in the listed genes.