Tumor and Constitutional Sequencing for Neurofibromatosis Type 1

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abstract

PURPOSE NF1 variants in tumors are important to recognize, as multiple mechanisms may give rise to biallelic variants. Both deletions and copy-neutral loss of heterozygosity (LOH) are potential mechanisms of NF1 loss, distinct from point mutations, and additional genes altered may drive different tumor types. This study investigates whether tumors from individuals with neurofibromatosis type 1 (NF1) demonstrate additional gene variants and detects NF1 second hits using paired germline and somatic sequencing. In addition, rare tumor types in NF1 may also be characterized by tumor sequencing.

MATERIALS AND METHODS Sequences of 529 cancer driver genes were analyzed in 6,381 tumors, yielding 391 NF1-mutated tumors in which NF1 LOH analysis was performed. Driver genes were evaluated by tumor type including malignant peripheral nerve sheath tumors and gliomas.

RESULTS NF1 LOH was seen in 133 of 391 tumor samples in the cohort. Individuals with NF1 had more prevalent copy-neutral LOH ($P < .0001$), suggesting somatic intrachromosomal recombination. Osteosarcoma in NF1 also had NF1 LOH and additional p53 alteration. NF1 second hit data from tumors were informative for inferring deleteriousness of missense variants that were conflicting in ClinVar, potentially helping to add to NF1 annotation. Although criteria for evaluating germline and somatic variants are different, deleterious effects on NF1 function may be shared.

CONCLUSION Sequencing of NF1-associated tumors demonstrated a spectrum of second hits in NF1 and the prevalence of copy-neutral LOH. Future work may be aimed at further understanding of LOH mechanisms and strategies to mitigate tumor risk.

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common condition occurring in approximately 1 in 3,000 individuals.1 Individuals with NF1 have an increased risk for tumor development with a predilection for specific subtypes compared with those without.2,3 Some tumors, however, can be phenotypically similar. Malignant peripheral nerve sheath tumor (MPNST) is a well-known malignancy associated with NF1, but MPNST can also be unrelated to NF1.4 NF1 variants are seen in malignancies such as gliomas, but NF1 variants may also lead to tumors that are not classically described in NF1.5,6 Molecular characterization of tumors is therefore key to elucidating genetic origins underlying tumors. Loss of heterozygosity (LOH) occurs in NF1-related tumors and can occur via a deletion mechanism or through mitotic recombination, resulting in copy-neutral LOH.7,8 Copy-neutral LOH has been seen in manifestations associated with NF1 including dystrophic scoliosis, tibial pseudarthrosis, juvenile myelomonocytic leukemia, and glomus tumors, suggesting that the somatic change has widespread implications.9-13 Several unanswered questions remain, despite significant work in the field: What underlies the LOH observed? Are there strategies to mitigate second hits in NF1 or to understand the role of comutated genes? To characterize tumor-related genes in NF1-related tumors, large multigene sequencing platforms can be used to identify mutated NF1 and possible homozygosity, suggesting potential opportunities to identify NF1 loss and alterations in other oncogenes/tumor suppressors in tissue. If tumor sequencing and normal sequencing are performed simultaneously, variants detected can be reasonably identified in their sequence of occurrence.14-16 Applied to a cohort of tumors, such sequencing can yield variants, genes, and potentially mutation mechanisms underlying tumor pathophysiology, particularly in tumors with limited burden of pathogenic variants. The data may also help with inferring pathogenicity attributed to variants in tumor and germline.17-20 The study presents paired sequencing from samples from individuals with NF1, analyzes types of tumors

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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Individuals with neurofibromatosis type 1 (NF1) are at increased risk for tumor development. Multiple mechanisms may lead to biallelic inactivation that drives these tumors. This study analyzed somatic and germline samples to determine the landscape of variation and the spectrum of second hits in NF1.

Knowledge Generated

Data from multigene sequencing demonstrated that the most prevalent type of second hit in tumors from individuals with constitutional NF1 was copy-neutral loss of heterozygosity. NF1 variant annotation may benefit from analysis of paired normal-tumor data, including for osteosarcoma, a tumor rarely seen with NF1.

Relevance

Understanding of the mechanism of second hit acquisition in NF1 will be important for devising therapeutic and prophylactic strategies. With expanded use of tumor-normal sequencing, large amounts of data can be leveraged to understand pathogenesis and to interpret germline variants with conflicting or uncertain pathogenicity.

MATERIALS AND METHODS

Genetic Evaluation of Tumors

Tumors were evaluated from 2015 to 2021, including a total of 6,168 tumors. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue. Capture-based next-generation sequencing was performed at the UCSF Clinical Cancer Genomics Laboratory using an assay (UCSF500) that targets the coding region of 529 cancer-related genes and selects introns from approximately 40 genes. Copy number variants were detected and visualized using Integrated Genome Viewer and Nexus Copy Number. Tumor samples contained at least 25% of tumor in samples with a standard quality read depth of 500x. Three hundred ninety-one tumors had a pathogenic or likely pathogenic (P/LP) NF1 variant including nonsense, frameshift insertion or deletion, and splice donor/acceptor variants, and these were reviewed manually and compared with COSMIC,20 ClinVar,21 and gnomAD.22 Missense variants were additionally annotated using several prediction tools including Combined Annotation-Dependent Depletion (CADD) scores.23 Matched tumor-normal samples were paired from tumor and peripheral blood or buccal swab including 130 cases with matched tumor and normal samples, including 21 cases with germline NF1 and 109 without germline NF1. Two hundred sixty-one cases were tumor-only submissions, and these tumors had NF1 variants without immediate germline information. Nineteen additional cases were added on the basis of clinically diagnosed NF1 cases or cases where another genetic test demonstrated a P/LP NF1 germline alteration. The median variant allele frequency (VAF) in tumor for NF1 germline cases was 0.77 ± 0.19. In addition to NF1, comutated genes were also required to be P/LP with interpretation on the basis of standard laboratory criteria. Comutated variants had a median VAF of 0.35 ± 0.20. The Cancer Genome Atlas (TCGA) was examined for variant and tumor type comparisons.23-25

Mutation/LOH

Data corresponding to NF1 status were obtained from medical and genetic testing and the laboratory reports. Cases were analyzed for LOH at NF1 (NM_001042492) and for other variants in any of the 529 genes. Copy-neutral LOH was assessed on the basis of the allelic ratio of polymorphic single nucleotide polymorphisms (SNPs) across 17q by the clinical laboratory team. The 133 cases that had LOH were categorized as LOH because of deletion or copy-neutral LOH, and these had higher median VAF compared with samples without LOH (P < .0001, Mann-Whitney U). Copy-neutral LOH status was determined according to technical laboratory guidance.26 The status was also verified by a second laboratory genetic review of allele frequency and copy number.

NF1 germline cases were also analyzed for somatic tumor missense NF1 variants; somatic variants were compared with ClinVar annotation. Eight missense variants in NF1 were identified for review; four variants in tumor-only samples could not be further analyzed. D2632G carries a likely pathogenic interpretation on the basis of one submission in ClinVar with a germline R192*; the variants were too far apart to determine phase. I2615V was not annotated in ClinVar and was seen in a tumor with copy-neutral LOH and a germline splice variant. G629R and S82F were second NF1 hits in tumor. The VAF in tumor for G629R and S82F was 0.27 and 0.82, respectively.
Data and Statistics

Genetic data were visualized using the ComplexHeatmap package in R. Analyses were performed using R version 4.0.3.

Ethics Declaration

This study was reviewed and approved by the University of California—San Francisco Institution Review Board (IRB, 20-33093). Informed consent was not required. It was not practicable to contact all the cases with tumors in this retrospective study. The research data were deidentified.

RESULTS

NF1 Alterations in Tumor Samples

Of 6,168 tumor samples, 391 tumors harbored P/LP NF1 variants. Forty tumors were from individuals with constitutional NF1. The nervous system and skin were the most common anatomic sites for the NF1-altered tumors (Appendix Tables A1 and A2). There were 369 unique coding variants leading to 294 protein alterations. 85.4% were nonsense, frameshift insertion/deletion, or splice acceptor/donor variants, and 11.7% were pathogenic missense variants. Recurrent mutated sites affected NF1 residues Y2285, n = 19, and F1247, n = 18, which we confirmed as recurrent sites in the TCGA database. One hundred sixty-two NF1 variants were not previously reported in variant databases.

Sequencing of Tumors from Individuals With NF1 Also Demonstrates Osteosarcoma

Tumors from individuals with NF1 included known tumor types, including MPNST, glioma, and gastrointestinal stromal cell tumor; however, a case of osteosarcoma was also observed. Osteosarcoma in individuals with NF1 has been reported infrequently (Table 1). Furthermore, sequencing of the osteosarcoma tumor demonstrated somatic copy-neutral LOH of NF1, in addition to somatic TP53 and NOTCH3 variants. The germline splice acceptor NF1 VAF was 0.48 in the germline and 0.82 in tumor, consistent with biallelic mutation in tumor.

Commutations seen in these tumors included CDKN2A/2B deletion (37.5% of cases), TP53 mutation (17.5%), and ATRX (12.5%). Three cases had PTEN pathogenic variants. One case that did not have an identifiable second NF1 variant had pathogenic variants in TP53 and PTEN. Few instances of pathogenic variants in genes downstream of NF1 in the RAS signaling pathway (PIK3CA, PTPN11, PIK3R1, and RAF1) were noted.

Copy-Neutral LOH is Common in Tumors From Individuals With NF1

Patterns of NF1 loss were observed including copy-neutral LOH and NF1 focal deletion/chromosomal loss. 76% of individuals with constitutional NF1 had tumors that displayed copy-neutral LOH, and 24% had loss/deletion. The results suggested a predominance of copy-neutral LOH in the NF1 cohort here. For tumors that did not have NF1 germline, 24% of tumors had copy-neutral LOH and 76% had copy loss—deletion (Table 2). Of 351 nongermline-altered NF1 tumors, 204 had a second hit documented. Small protein-altering NF1 variants represented the second hit in 13 of 38 germline cases, and 96 of 204 nongermline cases had two or more NF1 protein-altering variants without a copy number variant (P = .2, two-proportion z test).

TABLE 1. Reports of Patients With NF1 and Osteosarcoma

| Year/Citation | Age, Years | Sex | NF1 | Site of Osteosarcoma | Other Tumors | Treatment Notes for Other Tumors |
|---------------|------------|-----|-----|----------------------|--------------|---------------------------------|
| 201339        | 17         | Male | +   | R distal femur       | None         | NA                              |
| 201330        | 17         | Male | +   | Femur                | MPNST        | s/p surgery                     |
| 200931        | 37         | Male | +   | Distal femur         | None         | NA                              |
| 200931        | 21         | Male | +   | Proximal humerus     | None         | NA                              |
| 200931        | 34         | Female | +  | Proximal tibia       | None         | NA                              |
| 200931        | 25         | Female | +  | Distal femur         | Spindle cell | s/p chemotherapy, amputation     |
| 200632        | 29         | Female | +  | Proximal femur       | MPNST        | s/p chemotherapy, surgery       |
| 200233        | 50         | Female | +  | Ramus of left mandible | Parathyroid adenoma | NA |

Abbreviations: MPNST, malignant peripheral nerve sheath tumor; NA, not available; s/p, status post.
**TABLE 2.** Patterns of Second Hit in NF1-Altered Tumors

| Type of Second Hit                  | NF1 Germline Cases (n = 38), No. (%) | NF1 Nongermline Cases (n = 204), No. (%) |
|------------------------------------|--------------------------------------|-------------------------------------------|
| Copy-neutral LOHa                   | 19 (48)                              | 26 (13)                                   |
| Loss—deletionb                     | 6 (15)                               | 82 (40)                                   |
| Small protein-altering variantc     | 13 (37)                              | 96 (47)                                   |

Abbreviations: LOH, loss of heterozygosity; NF1, neurofibromatosis type 1.

aTwo-sided \( P < .0001 \), two-proportion \( z \) test.
bTwo-sided \( P = .007 \), two-proportion \( z \) test.
cTwo-sided \( P = .9 \), two-proportion \( z \) test.

**MPNST NF1-Altered and Non-NF1-Altered**

MPNST was also analyzed, and LOH was examined (Fig 2). Eleven MPNSTs were observed; five had constitutional NF1, four did not have NF1 variants identified, and two were unknown. LOH was commonly observed in MPNST associated with constitutional NF1 (57% \( v \) 0% in non-NF1 MPNST). NF1 comutations included CDKN2A/2B deletion and TP53 mutation. Non-NF1 MPNST had one case with CDKN2A/2B deletion and one case with a TP53 structural rearrangement, in support of the role of these genes in MPNST.34,35 The PRC2 genes including EED and SUZ12 were sporadically altered, as has been described.36 Non–NF1-related MPNST did not have any recurrent variants. Three of the seven NF1-related MPNST cases lacked a second hit NF1.

**Tumors From Individuals With NF1 Occur at Younger Age**

Among NF1-altered tumors, we could compare tumors from individuals with constitutional NF1 germline versus those without. In individuals with germline NF1, tumors tended to have fewer pathogenic mutations compared with tumors from individuals who did not have germline NF1 (3.3 ± 1.4 pathogenic mutations versus 5.3 ± 3.7 pathogenic mutations). The mean age at which tumors were sent for sequencing was 22.7 ± 16.5 years for NF1 germline cases versus 51.9 ± 22.0 years. The total number of pathogenic mutations was not correlated with age in germline or somatic cases overall (\( P = .89 \)). Ki67 staining correlated with higher-grade tumors, but did not correlate with LOH status.

**Second Hit Missense Variants in NF1 Tumors May Provide Support for Deleteriousness**

Although the majority of NF1 variants are predicted to lead to loss of function, missense variants are more difficult to predict. In the context of an NF1-related tumor with relatively limited pathogenic comutations, somatic missense NF1 variants may represent a driver event or stochastic coincidence. For tumor missense variants found in combination with an NF1 germline variant, there were four somatic missense variants in the data. In agreement with ClinVar, G629D had multiple annotations of pathogenicity with a CADD score of 32, suggesting germline and tumor deleteriousness. The variant was seen in combination with a germline splice acceptor variant. Two other missense variants, D2632G and I2615V, were of unclear significance after similar analyses. S82F demonstrates conflicting evidence of pathogenicity in ClinVar although the two recent entries suggest that the variant is pathogenic. The S82F variant was seen in a tumor with a germline NF1 deletion and had a CADD score of 32. This missense variant with the germline deletion supports the pathogenic role of S82F. Should these variants be found as germline variants, they may similarly confer deleteriousness and be associated with tumor risk.

**DISCUSSION**

Previous large sequencing efforts with NGS technology of tumor cohorts have revealed germline variants; however, cases of NF1 were rare, with fewer than 10 cases in each of these studies.37-39 In this study, we present data on tumors...
from 40 individuals with NF1 profiled on a multigene sequencing platform. Past sequencing efforts on tumors in NF1 have described specific tumor types, such as atypical neurofibroma (ANF) and MPNST. CDKN2A/2B deletion appears to be as a molecular event differentiating ANF and MPNST, and biallelic deletions were more common in MPNST compared with ANF.36 TP53 variants have also been noted in MPNST, and in vivo data support a synergistic role for such mutations.34,35 More recently, the molecular landscape of glioma in individuals with NF1 has been described and often involves genetic alterations in TP53; also, lesions in CDKN2A/2B were noted to be prevalent.40 The cohort of NF1 tumors in this study further supports the importance of TP53 and CDKN2A/2B in the tumorigenesis.

LOH was prevalent, and our data from tumors in NF1 support that copy-neutral LOH is more common compared with deletion. Copy-neutral LOH is thought to involve mitotic recombination, a process that is known to occur in tumors but that may occur in the general population. Studies investigating the landscape of structural genomic variation in degradation have noted that copy-neutral uniparental disomy represented 48% of mosaic events detected in a control population with increasing prevalence with age and most common on chromosomes 9 and 14.41-43 80% of the events were telomeric on the p or q arm. Only three events were detected in NF1. In studies of NF1, mitotic recombination in NF1 tended to be centromeric; although copy-neutral LOH is likely seen in the general population, the specific location does not seem to mirror the profile in patients with NF1. The mechanism driving copy-neutral LOH has not been fully elucidated. Studies have suggested that BRCA2, DNA-protein kinase, and POLQ may suppress inter-homolog recombination although these findings were not specific to copy-neutral LOH at the NF1 locus.44 Biallelic inactivation of homologous recombination repair genes has further been linked to genome-wide LOH.45 Copy-neutral LOH has also been documented in tumor suppressors including TSC1/2, BRCA1/2, and TP53 in other tumors as well, emphasizing this mechanism in tumorigenesis.46,47

The tumor subtypes represented tumors commonly associated with NF1. Rare tumors such as osteosarcoma in NF1 are of interest as osteosarcoma has been reported in NF1 and can be challenging to treat.29-33 Of eight cases of osteosarcoma, two were postsurgery and chemotherapy at a site of a previous malignancy. None of the literature cases had genomic characterization of tumors to our knowledge. The DKFZ Pediatric Pan-Cancer collection of genomic characterization has 42 cases of osteosarcoma, but none of these have NF1 alteration. One study estimated an increased prevalence of osteosarcoma in individuals with NF1 compared with control; however, further data are needed to make further estimates.3 Therefore, the molecular data presented here may represent a unique report of NGS from osteosarcoma, NF1 alteration and those without.

Among cases of MPNST, two subcategories are evident: those with NF1 alteration and those without. TP53, EED, SUZ12, and CDKN2A2B deletions were seen in the data set. Furthermore, among NF1-altered MPNST, not all cases demonstrated a second hit in NF1. Compared with TCGA...
data, this study has a similar number of NF1-altered MPNST cases (8 of 11 vs 6 of 14 on TCGA). In the TCGA data set, 3 of 6 demonstrated two NF1 lesions. Previous analysis of germline and somatic variations in NF1 in MPNST revealed large deletions including the NF1 locus in 91% of their cohort. Their data set also had 2 cases with NF1 germline without evidence of a second hit.50 and early studies had identified TP53 loss in some cases that lacked a second NF1 hit.51 Although MPNST is a key risk lesion for patients with NF1, a second hit in NF1 is not always identified.

NGS may also contribute to the interpretation of NF1 variant deleteriousness. For example, missense variants can be challenging to annotate (eg, ClinVar has 293 conflicting and 3,106 VUS in NF1). The study here evaluated NF1 variants that occurred somatically as second hits in the context of germline NF1 variants. Could this information potentially be applied to the variant’s germline variant classification criteria? Current American College of Medical Genetics and Genomics classification allows for determination of pathogenicity of variants on the basis of functional studies.52 The increasing information from genomic studies of paired tumor and normal samples may represent an avenue of assessment of variants given a proper context, that is, if a change can be considered a potential driver and phenomena such as hypermutation are absent.

At this time, tumor-only testing is more common compared with paired germline and tumor testing, in part due to costs and insurance. Tumor-only testing has advantages but has limitations in terms of inferring germline status from VAF.53 The data presented here highlight another potential benefit of paired testing: improving variant interpretation of not only somatic but also corresponding germline variation.

This study has a number of limitations. As data are from a tertiary center, the cohort may be enriched for specific types of tumors or cases that may harbor certain types of genetic changes. Brain tumors represent the most common tumors in the data set, which could also affect the variants observed. Although the platform has a sequencing depth of > 500x, it is largely limited to coding regions, and therefore, intron or promoter variants that could affect the gene are not specifically detected. The resolution of copy number calling is also limited by probe density. NF1 variants were also unphased, which is a limitation to interpretation of small variants, eg, whether these are in cis or trans. Age in our study was time of panel sequencing, which may be delayed relative to surgical resection or diagnosis.

The results add to the data on NF1-altered tumors, particularly tumors that arise in the context of individuals with NF1. The sample size and analysis of LOH emphasize the importance of copy-neutral heterozygosity in these tumors. Osteosarcoma, a tumor type not traditionally thought to be NF1-related, appears to be mechanistically related to double hits in NF1. This tumor in addition to others reported in the cohort here shows a spectrum of second alterations in the context of NF1 germline that favor copy-neutral LOH rather than deletion in lesions with limited genetic variation. Future studies on paired normal tissue and tumor may be able to provide further guidance on mechanisms to target in potential therapeutic strategies.

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**DATA SHARING STATEMENT**

All data are available upon request. Further methods are also available by contacting the laboratory.

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**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

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## APPENDIX

### TABLE A1. Distribution of Cancer Types Among UCSF500 Samples

| Anatomic Category | No. of Tumors (n = 6,168) | Tumors With NF1 Pathogenic or Likely Pathogenic Alteration (n = 391) |
|-------------------|---------------------------|-------------------------------------------------|
| CNS               | 2,152                     | 226                                            |
| Skin              | 379                       | 39                                             |
| Unknown primary   | 585                       | 22                                             |
| Lung              | 342                       | 17                                             |
| Uterus            | 142                       | 15                                             |
| Peripheral nerve  | 81                        | 14                                             |
| Others            | 771                       | 13                                             |
| Ovarian/fallopian | 172                       | 13                                             |
| Soft tissue       | 154                       | 8                                              |
| Bladder/urinary tract | 120               | 5                                              |
| Breast            | 119                       | 4                                              |
| Head and neck     | 58                        | 3                                              |
| Lymphoid/myeloid  | 181                       | 3                                              |
| Bowel             | 86                        | 2                                              |
| Thyroid           | 91                        | 2                                              |
| Ampulla of vater  | 6                         | 1                                              |
| Bone              | 47                        | 1                                              |
| Pancreas          | 82                        | 1                                              |
| Stomach           | 5                         | 1                                              |
| Stomach/esophagus | 52                        | 1                                              |
| Adrenal           | 11                        | 0                                              |
| Appendix          | 5                         | 0                                              |
| Biliary           | 26                        | 0                                              |
| Cervix            | 10                        | 0                                              |
| Eye               | 46                        | 0                                              |
| Germ              | 7                         | 0                                              |
| Kidney            | 90                        | 0                                              |
| Liver             | 24                        | 0                                              |
| Pleura            | 6                         | 0                                              |
| Prostate          | 79                        | 0                                              |
| Thymus            | 3                         | 0                                              |
| Unknown           | 234                       | 0                                              |
| Vagina            | 2                         | 0                                              |

Abbreviation: NF1, neurofibromatosis type 1.
| Pathologic Category                          | NF1-Altered Tumors (n = 391) |
|---------------------------------------------|-------------------------------|
| Glioblastoma                               | 79                            |
| Glioma                                     | 72                            |
| Astrocytoma                                | 40                            |
| Melanoma                                   | 33                            |
| Others                                     | 26                            |
| Unknown primary                            | 20                            |
| Lung adenocarcinoma                        | 15                            |
| Gliosarcoma                                | 10                            |
| Uterine cancer                             | 9                             |
| Diffuse intrinsic pontine glioma           | 8                             |
| Malignant peripheral nerve sheath tumor    | 7                             |
| Ovarian cancer                             | 7                             |
| Neurofibroma                               | 5                             |
| Breast cancer                              | 4                             |
| Bladder urothelial carcinoma               | 4                             |
| Sarcoma                                    | 3                             |
| Rosette-forming glioneuronal tumor of the fourth ventricle | 3 |
| Spindle cell neoplasm                      | 3                             |
| Head and neck squamous cell carcinoma      | 2                             |
| Ganglioglioma                              | 2                             |
| Endometrial carcinoma                      | 2                             |
| Dysembryoplastic neuroepithelial tumor     | 2                             |
| Colorectal cancer                          | 2                             |
| Atypical nevus                             | 2                             |
| Adenocarcinoma, NOS                        | 2                             |
| Urachal adenocarcinoma                     | 1                             |
| Subependymoma                              | 1                             |
| Poorly differentiated thyroid cancer       | 1                             |
| Plexiform neurofibroma                     | 1                             |
| Pleomorphic xanthoastrocytoma              | 1                             |
| Pheochromocytoma                           | 1                             |
| Ovarian epithelial tumor                   | 1                             |
| Osteosarcoma                               | 1                             |
| Oligodendroglioma                          | 1                             |
| Ocular melanoma                            | 1                             |
| Neuroepithelial tumor                      | 1                             |
| Myeloproliferative neoplasms               | 1                             |

(Continued in next column)

| Pathologic Category                          | NF1-Altered Tumors (n = 391) |
|---------------------------------------------|-------------------------------|
| Mucosal melanoma of the urethra             | 1                             |
| Mixed phenotype acute leukemia, T/myeloid, NOS | 1 |
| Miscellaneous neuroepithelial tumor         | 1                             |
| Malignant neurocristic neoplasm             | 1                             |
| Lung squamous cell carcinoma                | 1                             |
| Large cell neuroendocrine carcinoma        | 1                             |
| High-grade serous fallopian tube cancer     | 1                             |
| GIST                                        | 1                             |
| Embryonal rhabdomyosarcoma                  | 1                             |
| B-cell lymphoma                            | 1                             |
| B-cell acute lymphoid leukemia              | 1                             |
| Atypical teratoid/rhabdoid tumor            | 1                             |
| Anaplastic thyroid cancer                   | 1                             |
| Anaplastic oligodendroglioma                | 1                             |
| Anaplastic meningioma                       | 1                             |
| Anaplastic ependymoma                       | 1                             |
| Ampullary carcinoma                         | 1                             |

Abbreviations: GIST, gastrointestinal stromal cell tumor; NOS, not otherwise specified.