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The Pan-Cancer Landscape of Coamplification of the Tyrosine Kinases KIT, KDR, and PDGFRA

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Key Words. amplification • tyrosine kinase inhibitor • KIT • PDGFRA • genomic profiling • sarcoma

ABSTRACT

Purpose. Amplifications of receptor tyrosine kinases (RTKs) are therapeutic targets in multiple tumor types (e.g. HER2 in breast cancer), and amplification of the chromosome 4 segment harboring the three RTKs *KIT*, *PDGFRA*, and *KDR* (4q12amp) may be similarly targetable. The presence of 4q12amp has been sporadically reported in small tumor specific series but a large-scale analysis is lacking. We assess the pan-cancer landscape of 4q12amp and provide early clinical support for the feasibility of targeting this amplicon.

Experimental Design. Tumor specimens from 132,872 patients with advanced cancer were assayed with hybrid capture based comprehensive genomic profiling which assays 186–315 genes for all classes of genomic alterations, including amplifications. Baseline demographic data were abstracted, and presence of 4q12amp was defined as 6 or more copies of *KIT/KDR/PDGFRA*. Concurrent alterations and treatment outcomes with matched therapies were explored in a subset of cases.

Results. Overall 0.65% of cases harbored 4q12amp at a median copy number of 10 (range 6–344). Among cancers with >100 cases in this series, glioblastomas, angiosarcomas, and osteosarcomas were enriched for 4q12amp at 4.7%, 4.8%, and 6.4%, respectively (all $p < 0.001$), giving an overall sarcoma ($n = 6,885$) incidence of 1.9%. Among 99 pulmonary adenocarcinoma cases harboring 4q12amp, 50 (50%) lacked any other known driver of NSLCC. Four index cases plus a previously reported case on treatment with empirical TKIs monotherapy had stable disease on average exceeding 20 months.

Conclusion. We define 4q12amp as a significant event across the pan-cancer landscape, comparable to known pan-cancer targets such as NTRK and microsatellite instability, with notable enrichment in several cancers such as osteosarcoma where standard treatment is limited. The responses to available TKIs observed in index cases strongly suggest 4q12amp is a druggable oncogenic target across cancers that warrants a focused drug development strategy.

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Implications for Practice: Coamplification of the receptor tyrosine kinases (rtks) *KIT/KDR/PDGFR* (4q12amp) is present broadly across cancers (0.65%), with enrichment in osteosarcoma and gliomas. Evidence for this amplicon having an oncogenic role is the mutual exclusivity of 4q12amp to other known drivers in 50% of pulmonary adenocarcinoma cases. Furthermore, preliminary clinical evidence for driver status comes from four index cases of patients empirically treated with commercially available tyrosine kinase inhibitors with activity against *KIT/KDR/PDGFR* who had stable disease for 20 months on average. The sum of these lines of evidence suggests further clinical and preclinical investigation of 4q12amp is warranted as the possible basis for a pan-cancer drug development strategy.

INTRODUCTION

Matching treatment strategy to tumor biology is the central tenet of precision medicine, and ectopic activation of receptor tyrosine kinases (RTKs) is a pervasive theme in oncogenesis [1]. Oncogenic fusions involving the anaplastic lymphoma kinase (ALK) and ROS proto-oncogene1 (ROS1) RTKs and small deletions in epidermal growth factor receptor (*EGFR*) are well established as predicting patient benefit from target-matched tyrosine kinase inhibitors (TKIs), particularly in the context of non-small cell lung cancer (NSCLC) [1, 2]. Amplification of wildtype RTK genes, such as *ERBB2* (encoding HER2) in breast and gastroesophageal cancers, also serve as oncogenic drivers; however, the underlying oncogenic mechanism is less well understood [3, 4].

The coamplification of three distinct RTK encoding genes—*KIT*, *KDR*, and *PDGFR*—on chromosome 4q12 (4q12amp) may similarly be an oncogenic driver and thus target for therapy. Limited series in glioblastoma, lung cancer, and malignant peripheral nerve sheath tumor identified 4q12amp by fluorescence in situ hybridization or immunohistochemistry [5–7]. Preclinical studies support the druggability of this amplicon with the use of approved, but promiscuous, TKIs that are variously active against some of the encoded genes [5, 8]. A recent clinical trial of axitinib in treatment described the best responding patient as harboring 4q12amp as also profiled with the platform in this study [9]. Based on the preclinical rationale and pilot clinical data, we sought to further delineate the pan-cancer landscape of 4q12amp in a large series of advanced cancer patients assayed with genomic profiling during clinical care. Herein, we present the identification of recurrent 4q12amp across tumor types and provide additional data supporting clinical actionability.

SUBJECTS, METHODS, AND METHODS

Comprehensive genomic profiling (CGP) was performed in a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited laboratory (Foundation Medicine, Inc., Cambridge, MA). Approval for this study, including a waiver of informed consent and a HIPAA (Health Insurance Portability and Accountability Act) waiver of authorization, was obtained from the Western Institutional Review Board (protocol no. 20152817). The pathologic diagnosis of each case was confirmed on routine H&E-stained slides, and all samples forwarded for DNA extraction contained a minimum of 20% tumor nuclear area, compared with benign nuclear area. In brief, ≥ 50 ng DNA was extracted from 40 microns of 132,872 cancer cases in formalin-fixed, paraffin-embedded tissue blocks. The samples were assayed by CGP using adaptor ligation,

and hybrid capture was performed for all coding exons from 186 (version 1) to 287 (version 2) to 315 (version 3) cancer-related genes plus select introns from 14 (version 1) to 19 (version 2) to 28 (version 3) genes frequently rearranged in cancer [10]. A subset of samples also were interrogated for gene rearrangements in 265 genes via RNA sequencing.

Sequencing of captured libraries was performed using the Illumina HiSeq technology to a mean exon coverage depth of $>500\times$, and resultant sequences were analyzed for genomic alterations, including short variant alterations (base substitutions, insertions, and deletions), copy number alterations (focal amplifications and homozygous deletions), and select gene fusions or rearrangements, as previously described [10, 11]. Germline variants documented in the dbSNP database (dbSNP142; <http://www.ncbi.nlm.nih.gov/SNP/>), with two or more counts in the ExAC database (<http://exac.broadinstitute.org/>), or recurrent variants of unknown significance that were predicted by an internally developed algorithm to be germline were removed, except for known driver germline events (e.g., documented hereditary BRCA1/2 and deleterious TP53 mutations) [12]. Known confirmed somatic alterations deposited in the Catalog of Somatic Mutations in Cancer (COSMIC, v.62) were highlighted as biologically significant. All inactivating events (i.e., truncations and deletions) in known tumor suppressor genes were also called as significant. To maximize mutation detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a $\geq 5\%$ mutant allele frequency (MAF), indels with a $\geq 10\%$ MAF with $\geq 99\%$ accuracy, and fusions occurring within baited introns/exons with $>99\%$ sensitivity [10, 11].

Each tumor sample was analyzed alongside an internally validated mixture of 10 heterozygous diploid HapMap control samples, which use custom algorithms to normalize the sequence coverage distribution across baited targets. Normalized coverage data for exonic, intronic, and single-nucleotide polymorphism (SNP) targets accounting for stromal admixture are plotted on a logarithmic scale and minor allele SNP frequencies are concordantly plotted across the genome. Further cluster groupings of targets and minor allele SNPs were used to define upper and lower bounds of genomic segments. Empirical Bayesian algorithms employ a distribution of parameters including purity and base ploidy, and probability matrices are derived using different statistical sampling methodologies to fit these data and generate copy number alteration variant calls; all computational models were reviewed by expert analysts for each sample. Given that each copy number model was dynamically generated for each individual sample, credibility and confidence

intervals vary with sample data. As previously described, copy number calling achieves high performance (sensitivity was 99% with positive predictive value >99%) within a range of 30%–75% tumor content and for high level amplifications (copy number ≥ 8), with slight reduction in sensitivity (>80%) at lower sample purities (20%–30%) and low level amplifications but still called above threshold of 6 (copy number 6–7). We used a prespecified cutoff of 6 or more predicted copies for all three genes of the 4q12 amplicon (*KIT*, *KDR*, *PDGFRA*) as being on a chromosomal segment less than 20Mb in length to define 4q12amp. Supplemental online Table 5 describes the number of cases that harbor two or one but not three amplified genes on the 4q12 amplicon. Descriptive statistics and Fisher exact test with false discovery rate correction were used to compare 4q12amp among tumor types.

To explore the frequency of *KIT*, *KDR*, and *PDGFRA* amplification co-occurrence, we mined the publicly available The Cancer Genome Atlas (TCGA) using established methods on the cBioPortal (www.cbioportal.org) [13, 14]. Specifically, from samples with available mutation and copy number data ($n = 44,697$), we identified all cases harboring concurrent amplification of the 4q12 kinases *KIT*, *KDR*, and *PDGFRA* using an iterative search process. Anatomic tumor type and clinicopathologic features were also abstracted [13, 14].

RESULTS

Among 132,872 consecutive advanced cancer specimens analyzed, 0.65% (857) harbored 4q12amp of *KIT*, *KDR*, and *PDGFRA*. The median 4q12 copy number was 10 (range, 6–344) within the 4q12amp population (Fig. 1A). Patients with tumors harboring 4q12amp were almost evenly divided between women (48.5%, $n = 416$) and men (51.5%, $n = 441$) and had a median age of 60 years (range, 4–87). The cases harboring 4q12amp had a low median tumor mutation burden (TMB; defined as mutations [mut] per Mb) of 5.00 mut per Mb, with the 75th percentile being 10.81 mut per Mb (supplemental online Fig. 1, supplemental online Table 1). Across the cancer landscape, recurrent (>100 cases in our series) 4q12amp was observed primarily in central nervous system (CNS) neoplasms and sarcomas (supplemental online Table 1). Specifically, 4q12amp was detected in 3.7% (211 of 5,689 cases) of primary intracranial neoplasms and enriched in glioblastomas relative to the occurrence of this alteration across cancers (4.7%, 172 of 3,620 cases; $p < .001$; Fig. 2A, supplemental online Table 1). 4q12amp was also detected in 1.9% (129 of 6,885; $p < .001$) sarcoma cases, with angiosarcoma 4.8% (10/208; $p < .001$) and osteosarcoma (6.4%; $p < .001$) demonstrating enrichment relative to all cancers (Fig. 2B). In addition, 4q12amp is present in cancers that can arise in multiple anatomic sites, including 3.3% of mucosal melanomas (6/182 cases; $p < .05$) and 2.7% of adenoid cystic carcinoma (AdCC; 21 of 767 cases; $p < .001$; supplemental online Table 1). Cases with one or two of the three 4q12amp genes were also observed. Cases harboring a single gene amplification were most prevalent, whereas cases harboring two of the three RTKs were less common than 4q12amp (supplemental online Fig. 2). Landscapes for these permutations were unique from the 4q12amp landscape suggesting amplification of all three genes occurs in a unique biological context (supplemental online Table 2).

Among the major lung cancer types, 4q12amp was enriched in squamous cell carcinoma and present in small cell carcinoma, non-small cell carcinoma not otherwise specified, and lung adenocarcinoma at frequencies of 1.5% ($p < .001$), 1.4%, 1.0%, and 0.6%, respectively (Fig. 2C). To assess the co-occurrence of 4q12amp with known oncogenic drivers in NSCLC, the genomic profiles of 99 cases of adenocarcinoma harboring 4q12amp (0.6%) were reviewed (Fig. 2C). Within the 4q12amp lung cancers, 50% of cases did not have other oncogenic drivers consistent with established driver alterations in NSCLC (Fig. 2D and E). Of the 50% of cases with coexisting likely drivers, half (25/50) harbored driver alterations as follows: *EGFR* sensitizing alterations L858R, G779F, and G719S (16.2%, 16/99); *ALK* fusions (1.01%, 1/99); *RET* fusions (1.02%, 1/99); *ERBB2*-activating alterations including amplification and S310F (3.03%, 3/99); *MET* exon 14 skipping mutations (1/99, 1.01%); and *MET* amplification (5/99, 5.1%); but not *ROS1* fusions or *BRAF* V600E substitutions (Fig. 2E). It should be noted that three of the cases with *EGFR*-sensitizing alterations also harbored mutations in either *KRAS* or *MET* (codon 12, exon 14 skipping, and amplification, respectively). The other half of such driver co-occurring 4q12amp cases (26/50) harbored *KRAS* mutations but no other drivers, >95% of which occurred as *KRAS* G12x or G13x (total greater than 50 because of the three cases with other drivers). Complete clinical histories of these cases for assessing treatment with prior therapies was not available.

We further explored the TCGA database inclusive of cell line data and identified *KDR* amplification in 459/44,697 (1%) of cases, *KIT* amplification in 542 cases (1.2%), and *PDGFRA* amplification in 568 or 1.3% of TCGA cases. Among *PDGFRA*-amplified cases, 80.5% (457/568) harbored concurrent *KIT* amplification, and among cases with both *PDGFRA* and *KIT* amplification, 84.5% (386/457) harbored amplification of *KDR* (TCGA sample list can be found in supplemental online Table X). Thus, we identified concurrent *KDR*, *KIT*, and *PDGFRA* in 0.86% of sampled TCGA cases. Recurrent 4q12-contained kinase amplification was seen in glioblastoma, thoracic neoplasms, and sarcomas at frequencies similar to those in the series here (data not shown).

To explore the targetability of 4q12amp in advanced cancer by treatment with a TKI, we explored several lines of evidence. First, to rule out a prognostic effect of harboring 4q12amp, we analyzed glioblastoma, sarcoma, and head and neck squamous cell carcinoma TCGA datasets for cases harboring 4q12amp to compare to the remaining cases of the same tumor type and observed no difference in survival (data not shown). A study of axitinib in incurable AdCC by Ho et al. reported stable disease in 75% of patients and partial responses in 9.1% of patients. Notably, the patient harboring 14 copies of 4q12 (also profiled by this platform used in the series) had the longest duration of benefit from axitinib at 21.8 months of disease control (progression-free survival; PFS), which is four times greater than the median of 5.7 months PFS for the overall study [9]. We also sought to find other TKI-treated cases from this series of patients in this study. On obtaining treatment histories of a subset of patient within this series, two patients were found to be treated with imatinib, and five patients did not receive a TKI. These two

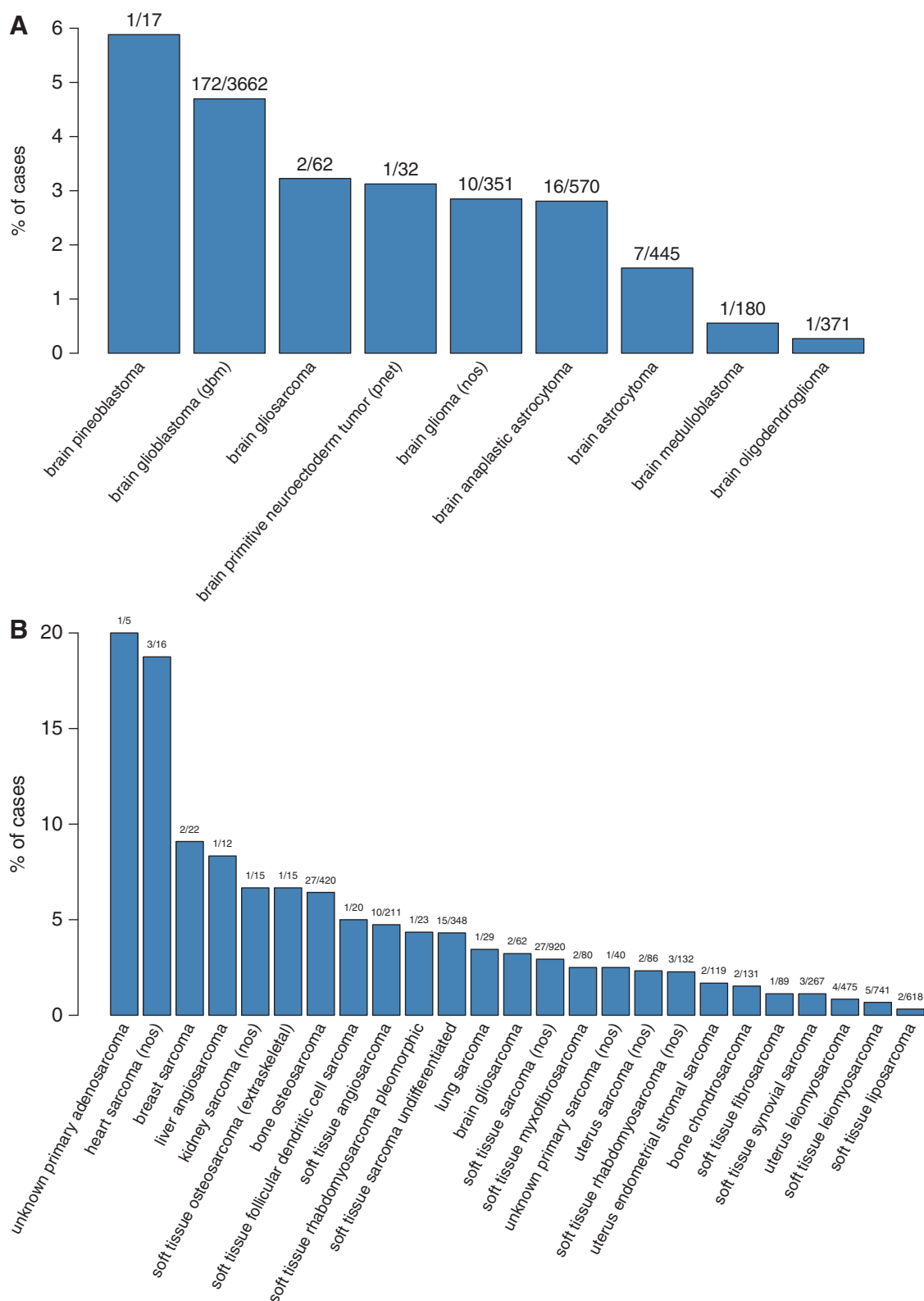


Figure 2. Frequency of 4q12amp in diseases that display a notable enrichment. **(A):** Frequency in primary brain tumors. **(B):** Frequency in sarcomas. **(C):** Frequency in lung carcinoma. **(D):** Pie chart of 4q12amp and known drivers in lung adeno. **(E):** Pie chart of co-occurring drivers in lung adenocarcinoma cases harboring 4q12amp. Abbreviation: NOS, not otherwise specified.

feasibility of pan-cancer genomic biomarkers such as the recent clinical successes of the anti-NTRK larotrectinib for NTRK fusion positive and anti-HER2 neratinib for ERBB2/3

mutated cancers [15, 16]. Here we present evidence of recurrent 4q12amp across anatomically and morphologically disparate tumor types and provide pilot clinical data suggesting

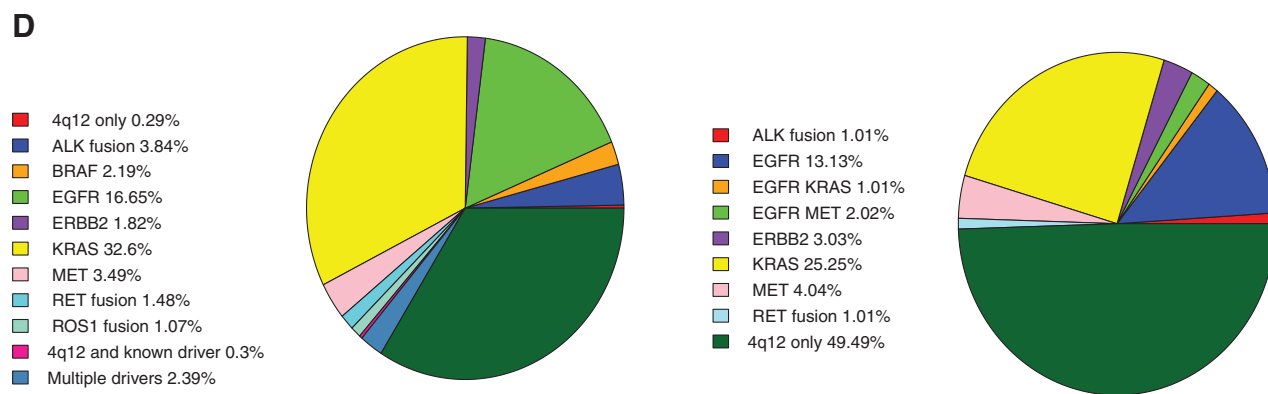
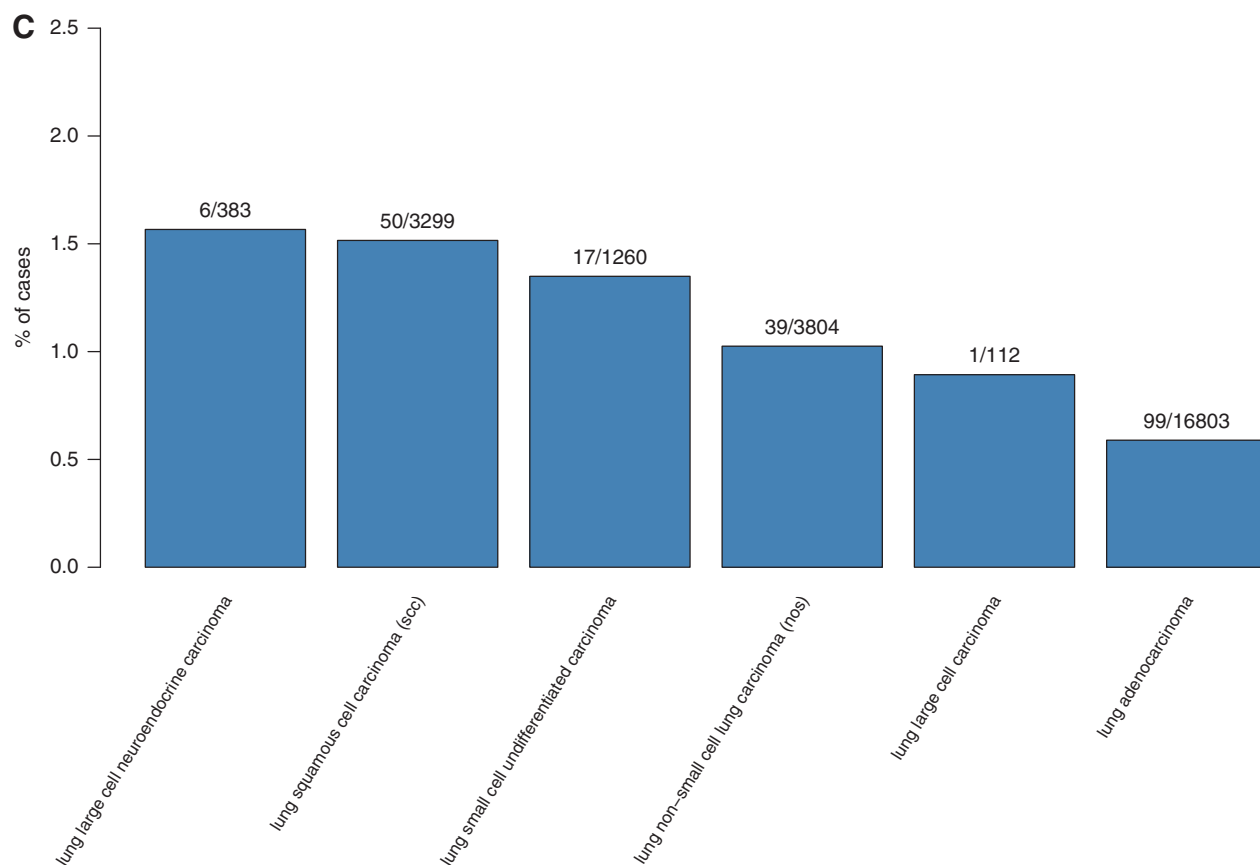


Figure 2. (Continued)

actionability regardless of histology. Although rare in absolute terms, the frequencies observed for 4q12amp are aligned with other uncommon but highly targetable oncogenic kinase alterations like NTRK fusions [15]. In addition to confirming recurrent 4q12 in glioblastoma, we significantly expand upon the available data and identify recurrent 4q12amp across sarcomas, particularly osteosarcoma and angiosarcoma [5–7].

Sarcomas represent a heterogeneous mix of tumors with diverse anatomic origin with few well established and rationally matched therapies. In this series, 4q12amp was enriched at 6.4% in osteosarcoma, in contrast to previous whole exome and whole genome sequencing studies that defined mutated PI3K and mTOR pathway genes as a possible therapeutic targets but not 4q12amp [17]. The discrepancy may be due to the lower

depth of coverage (100x or less) of the whole exome and whole genome sequencing platform, which makes detection of amplifications difficult [10]. Among the 4q12amp osteosarcoma cases ($n = 27$), the median age and TMB were similar relative to the overall osteosarcoma population, and nonspecific genomic features were enriched (data not shown). Similarly, angiosarcomas lack standard evidence-based therapies for recurrent disease and are enriched for 4q12amp [18]. Clinical history was not available for our angiosarcoma cases, but orthogonal support for 4q12amp actionability has been seen in a previously reported case treated with pazopanib on the basis of *KDR* and *FLT4* (*VEGFR3*) amplification and subsequent durable response [19]. Given the lack of rationally matched therapies for advanced sarcoma of all stripes, we believe that defining

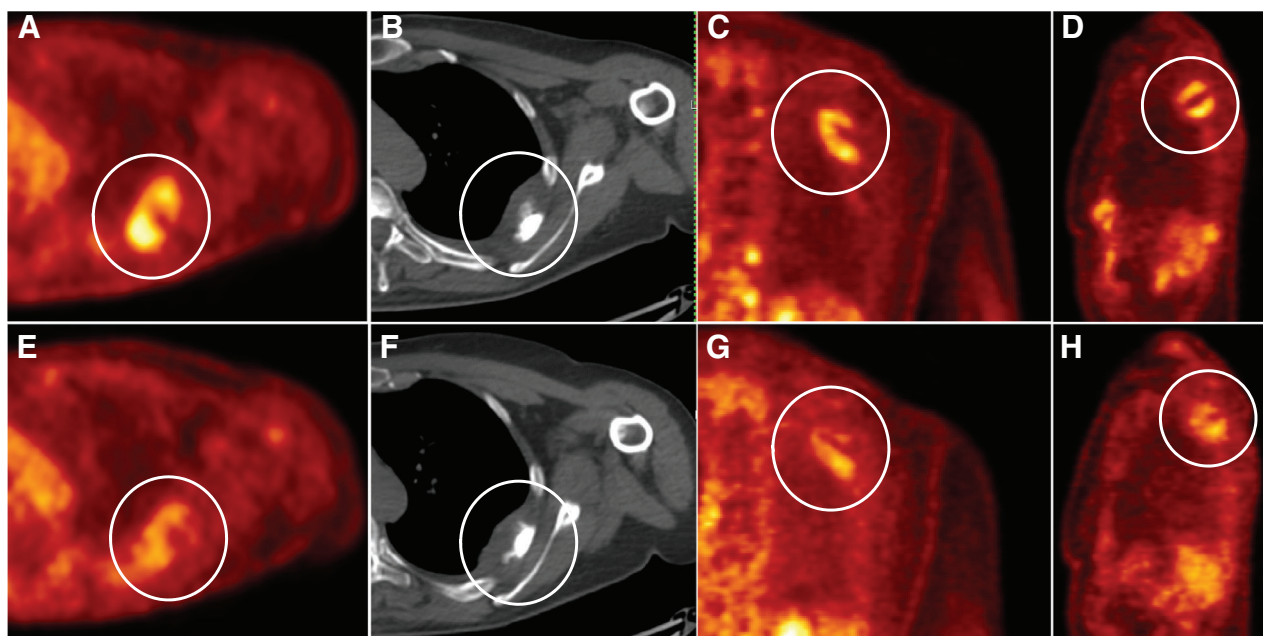


Figure 3. Index case no. 1, MPNST with durable clinical benefit to pazopanib. Baseline axial (A), coronal (C), and sagittal (D) FDG-PET CT attenuation corrected and axial CT (B) images demonstrate left intercostal muscle FDG AVID mass with corresponding decreased FDG activity on corresponding 3-month follow-up (E–H), white circles. Abbreviations: CT, computed tomography; FDG, fluorodeoxyglucose; MPNST, malignant peripheral nerve sheath tumor; PET, positron emission tomography.

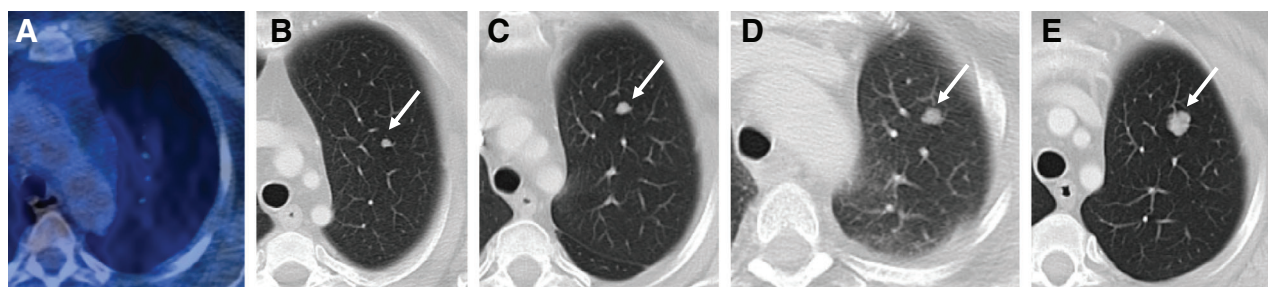


Figure 4. Index case no. 2, amelanotic melanoma with response to imatinib. (A): FDG-PET CT. (B): Three months later, chest CT. (C): Nine months later, chest CT. (D): One year later, chest CT. (E): One year and 7 months. Chest CT demonstrating left upper lobe pulmonary nodule (white arrows), not visible on initial study, and enlarging over subsequent follow-up studies. Abbreviations: CT, computed tomography; FDG, fluorodeoxyglucose; PET, positron emission tomography.

4q12amp subset of sarcomas is an initial step in evaluating the targetability of this alteration for the benefit of these patients.

Beyond sarcomas, 4q12amp was enriched in some types of carcinoma, tumors of epithelial origin, and other cancers of other histogenetic origins. We observed 4q12amp in AdCC, a slow-growing but ultimately lethal carcinoma pathognomonically associated with fusions of *MYB* or *MYB1* [20, 21]. The previously reported 4q12amp AdCC case with durable benefit from axitinib was also positive for *MYB* rearrangement and demonstrates that even within tumors with well-known oncogenic drivers, co-occurring 4q12amp can be targeted for clinical benefit [9]. Similarly, our finding of 4q12amp in noncutaneous melanoma is broadly consistent with previous work describing the presence of amplified *KIT* in melanoma, although these studies did not assess *KDR* and *PDGFRA* and as such could not definitively presence of 4q12amp [22–26]. This might suggest that examination of 4q12amp by CGP in *KIT*-overexpressing melanoma is

warranted as to best delineate which TKIs, beyond anti-*KIT* TKIs, may best benefit these patients [22–26].

In NSCLC, oncogenic drivers predominantly occur mutually exclusive fashion (i.e., an EGFR mutant lung cancer will not also harbor an ALK fusion) [27, 28]. Interestingly, however, 50% lung adenocarcinomas harboring 4q12amp have no other drivers, but the remaining 50% do (Fig. 2). Similarly, within the TCGA lung adenocarcinoma data set, three specimens (1.3%) harbored concurrent *KIT*/*KDR*/*PDGFRA* amplification, and among these cases, one of three had *ERBB2* amplification, with no putative drivers in two of three (data now shown). Moreover, the frequency of 4q12amp is lowest in lung adenocarcinoma and higher in other lung carcinoma types, which is the opposite of known oncogenic drivers in NSCLC which are typically most frequent in adenocarcinoma.

However, the index cases presented here and previously reported suggest that despite not neatly fitting our conception of an oncogenic driver, 4q12amp in advanced cancers

can be targeted for patient benefit. In the trial of axitinib for recurrent AdCC, the single longest responder (almost four times longer than the median PFS for the rest of the study cohort) was the 4q12amp patient profiled on the platform used in this study [9]. Axitinib has IC_{50} in the nanomolar range for VEGFR1–3, PDGFRA, and KIT [29]. This specific benefit of axitinib for this patient suggests that matching a TKI to 4q12amp can benefit the patient. The index cases here had similar durable clinical benefit exceeding 20 months of stable disease from TKIs with known anti-PDGFRA and KIT activity. The aggregate of these observations and the lack of impact of 4q12amp on prognosis per analysis of the TCGA suggest strongly that 4q12amp can be targeted for clinical benefit.

Which TKI Would Be Most Effective for Patients with Advanced Cancer Harboring 4q12amp?

Imatinib, pazopanib and axitinib have all benefitted the patients in this series, but each has different potencies against KIT, KDR, and PDGFRA (supplemental online Table 5). In tandem with clinical investigation, laboratory studies are needed to develop an understanding of which TKI may be superior for targeting 4q12amp and, equally importantly, which kinase, if one can be singled out, may be the critical oncogenic driver. A recent laboratory study of 4q12amp in a thyroid cancer derived cell line suggested that levatinib, recently approved in hepatocellular carcinoma, may also be an effective agent [30].

Importantly, reviewing the therapeutic strategies for metastatic breast cancer gives a relevant insight as to optimizing therapy for patients with harboring 4q12amp and suggests that monotherapy with a TKI alone, no matter how well matched, may be insufficient. For HER2-amplified breast cancer, trastuzumab alone, an anti-HER2 targeted monoclonal antibody, yielded modest benefit for HER2-amplified metastatic breast cancer, but the combination of trastuzumab and a chemotherapy backbone was very beneficial to patients and is approved in the metastatic setting [31, 32]. By analogy, the combination of a TKI and a chemotherapy backbone may be more effective for 4q12amp patients than a TKI alone and increase magnitude of response. Posing this question in a clinical trial is an important next step for this class of patients with cancer.

The size of the database of patient genomic profiles ($n = 132,872$ cases) is a clear strength of our study and allows reliable detection of rare variants such as 4q12amp, beyond that observed in the TCGA. However, we acknowledge limitations of our findings, largely owing to lack of complete clinical annotation. In this vein, we acknowledge that broad conclusions on clinical activity cannot be definitively drawn from our limited cases. However, a genomic biomarker-driven treatment paradigm is both feasible and associated with improved patient outcomes, and potentially lower toxicities, across multiple basket trials and meta-analyses [15, 33–36]. Large collaborative efforts including the NCI-MATCH and ASCO TAPUR trials may be the optimal forum to explore 4q12amp clinical actionability, although 4q12 is not currently included as a drug match [37].

CONCLUSION

We identify recurrent 4q12amp across tumor types with specific enrichment in sarcomas as a whole, several sarcoma

types, CNS neoplasms, and several cancers that arise in multiple anatomic sites. Beyond this demonstration of significant frequency and selective enrichment, the index cases with durable patient benefit presented here further buttress the targetability of this amplicon by matched TKIs. However, the identification of optimally effective TKI remains to be systematically investigated, both clinically and in the laboratory setting. As such, 4q12amp can be characterized as an oncogenic contributor either in conjunction with other oncogenic drivers or by itself, as suggested by studies in lung adenocarcinoma. Ongoing clinical trials with diverse TKIs may further define the extent of 4q12amp targetability and should be linked to an assessment of whether TKI treatment with a chemotherapy backbone may maximize patient benefit.

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DISCLOSURES

Umut Disel: Foundation Medicine, Inc. (RF); **Russell Madison:** Foundation Medicine, Inc. (E); **Jon H. Chung:** Foundation Medicine, Inc. (E); **Sally E. Trabucco:** Foundation Medicine, Inc. (E); **Asli O. Matos:** Foundation Medicine (E, OI); **Garrett M. Frampton:** Foundation Medicine, Inc. (E); **Lee A. Albacker:** Foundation Medicine, Inc. (E); **Venkataprasanth Reddy:** Foundation Medicine (E); **Jennifer Webster:** Foundation Medicine, Inc. (E); **Ruben Cabanillas:** Foundation Medicine, Inc. (H); **Breelyn Wilky:** Merck, Pfizer, Agenus (RF), Springworks, Eli

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