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LETTER TO THE EDITOR

KIT mutations in primary mediastinal B-cell lymphoma

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Primary mediastinal B-cell lymphoma (PMBL) is a specific subtype of diffuse large B-cell lymphoma with a distinctive clinical course and a specific genetic profile.[1,2] Several prior studies have provided key molecular–genetic aberrations;[1,3] however, a targeted next-generation sequencing-based study of relevant oncogenes in all currently available PMBL cell lines has not been performed. Here we report previously unrecognized mutations in known hot-spot regions of the *KIT* oncogene in ~12.5% of PMBL tumors. Interestingly, activating mutations in the proto-oncogene *KIT* have already been described in a variety of malignancies, including mast cell leukemia, acute myelogenous leukemia, gastrointestinal stromal tumors, as well as several other solid tumors,[4] where they carry, in part, therapeutic relevance due to clinically effective inhibitors.[5,6]

We performed next-generation sequencing-based screening of all three available PMBL cell lines using a panel of 50 relevant oncogenes (see Supplementary material and Supplementary Table 1). The full list of sequence alterations is provided in Supplementary Table 2. Notably, we found *KIT* mutations in two of three PMBL cell lines that we confirmed by Sanger sequencing (Figure 1). Given that these alterations were present at previously described hot spots, we further determined the mutation frequency in primary tumor samples using our PMBL cohort.[7] We found three missense mutations in 30 tumor samples (10%). One of these samples (PMBL.1) was the parental tumor, from which we derived the Med-B1 cell line,[8] thereby confirming that the *KIT* mutation is indeed present in the primary tumor and is not a result of genomic cell culture permutation. DNA analysis from micro-dissected non-tumor tissue in two of the mutated tumor samples showed wild-type sequence and confirmed the mutations as somatic.

Regarding the prevalence of *KIT* mutations in PMBL, we had to take the duplicate sample Med-B1 and parental tumor (PMBL.1) into account; we found a total of five mutations in 32 tested samples and determined a mutation frequency of ~15.6% in our cohort. When further restricting these calculations to missense mutations, we excluded sample PMBL.29 that harbored only a sense mutation (c.2394 C>T; p.I798I); the overall frequency of missense *KIT* mutations in PMBL (4 of 32 different samples) surmounts to ~12.5% (Figure 1). Besides this relatively high prevalence of *KIT* mutations, interestingly, all detected *KIT* missense mutations in PMBL are rare. Three mutations have been previously reported in other tumors (Table 1), and affect amino acids located in the activation loop (p.809–823) of the kinase domain (Supplementary Figure 1). One mutation (p.C788Y) is located in the kinase domain and has not been reported before. Notably, all four *KIT* missense mutations in PMBL are classified as 'damaging' by several *in silico* prediction tools and have therefore a possible impact on protein function (Table 1). This is also suggested by previous functional studies investigating missense mutations at these genetic hotspot regions of *KIT*.[9–11] Thus, our findings collectively suggest the possibility of specific diagnostic, prognostic and therapeutic implications of *KIT* mutations in PMBL.

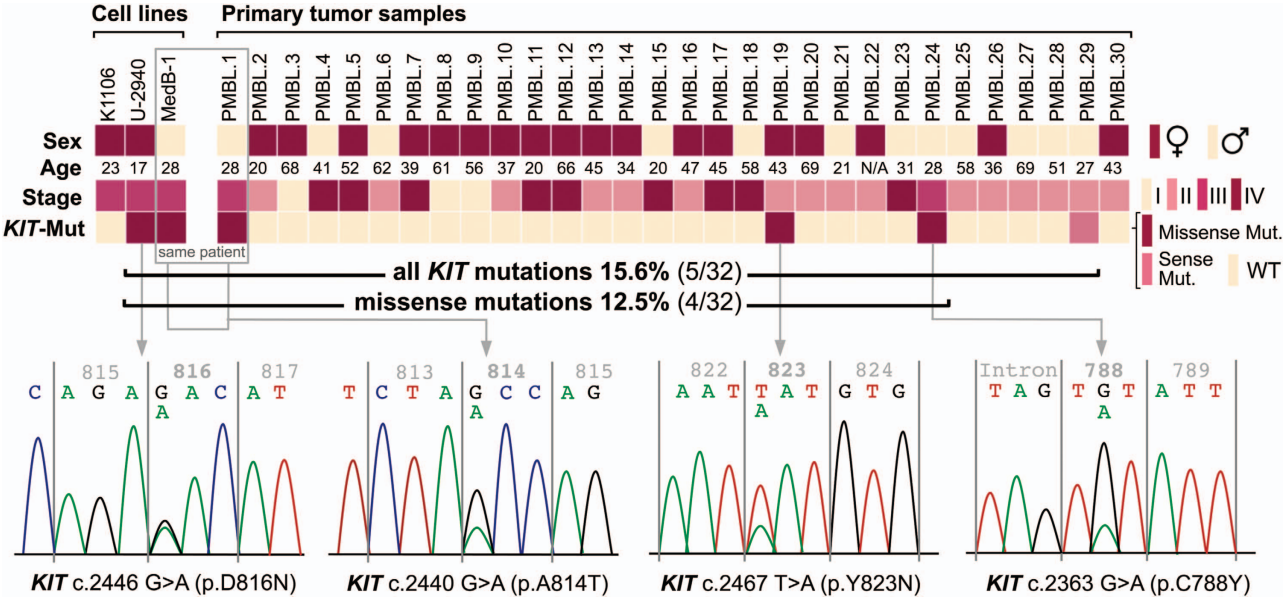

Figure 1. *KIT* mutations in primary mediastinal B-cell lymphoma. Key clinico-pathological features of individual samples (columns) along with genotyping result from the next-generation oncopanel and traces from confirmational Sanger sequencing. Mut., mutant; WT, wild type.

Table 1. *KIT* mutations in primary mediastinal B-cell lymphoma

	K1106	*U-2940*		*MedB-1* [a]	*PMBL.19*	*PMBL.24*	*PMBL.29*
Nucleotide substitution	—	c.2446G > A	c.1621A > C	c.2440G > A	c.2467T > A	c.2363G > A	c.2394C > T
Amino-acid change	—	p.D816N	p.M541L	p.A814T	p.Y823N	p.C788Y	p.I798I
SIFT score	—	Damaging 0.1	Tolerated 0.6	Damaging 0	Damaging 0	Damaging 0	—
PolyPhen-2	—	Probably damaging	Benign	Probably damaging	Probably damaging	Probably damaging	—
COSMIC	—	NK-T cell lymphoma ($N = 1$)	$N = 31$	Mast cell neoplasm ($N = 1$)	Colorectal cancer ($N = 2$); germ cell tumor ($N = 1$)	—	$N = 12$
EVS	—	—	AA = 5327, CA = 1110, CC = 66	—	—	—	CC = 6190, TC = 303, TT = 10

Abbreviations: COSMIC, catalogue of somatic mutations in cancer (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic, last accessed 30 March 2014); Dam., damaging; EVS, exome variant server (http://evs.gs.washington.edu/EVS), containing a data set comprising 2203 African-American and 4300 European-American unrelated individuals; PolyPhen-2, prediction of functional effects of an amino-acid substitution (http://genetics.bwh.harvard.edu/pph2); Prob. dam., probably damaging; SIFT, sorts intolerant from tolerant (http://sift.jcvi.org/); Tol., tolerated. [a]Note that the mutation in the MedB-1 cell line was also present in the parental tumor that the cell line was derived from (PMBL.1; see also Figure 1).

Notably, a recent genome-wide study delineated the mutational landscape in PMBL,[3] but missed the striking frequency of over 10% recurrent *KIT* mutations in PMBL. Arguably, this genome-wide study was performed according to very high standards, sufficient coverage and expert bioinformatics analysis. Nonetheless, their approach was to initially sequence two PMBL patient samples, and further experiments—including targeted sequencing of one identified gene in a larger number of samples—were made based on the findings in these two patient samples. Thus, despite the advents of genome-wide analyses, the imposed sampling bias precluded identification of recurrent *KIT* mutations in PMBL. Pointing this out should in no way compromise the importance of revealing the mutational spectrum of PMBL; however, our data paradigmatically prove the existence of false negatives in these large-scale data sets.[12–14] Furthermore, our data—albeit at a much smaller sequencing scale—emphasize targeted re-sequencing as an effective approach to perform high-throughput genotyping in tumor samples.[14,15] In summary, we report for the first time that ~12.5% of PMBL samples harbor mutations in the *KIT* oncogene.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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