logical abnormalities were observed in Pten$^{-/-}$ embryos (Supplementary Figure 2g). Flow cytomery analysis on fetal liver cells revealed that overall fetal hematopoiesis in Pten$^{-/-}$ mice was normal, except a skewed lymphoid/myeloid development at the progenitor level. There was no reduction in the hematopoietic stem cell-enriched lin^{-} sca-1^{+} c-kit^{+} population. However, there was an increased percentage of common myeloid progenitor population (Figures 2f and h) as well as a decreased percentage of common lymphoid progenitor (Figures 2g and h). This is consistent with previous report on adult bone marrow cells after Pten deletion.[13]

In conclusion, we have evaluated the effects of Kras^{G12D} activation or Pten deletion during fetal murine hematopoiesis side by side, and have observed dramatically different phenotypes. Kras^{G12D} mutation introduced by Vav-iCre during development leads to fetal lethality, suggesting that this murine model cannot be used to assess the leukemogenic ability of Kras *in utero*. On the contrary, Pten deletion during early fetal hematopoietic development leads to T-ALL in adult mice. The T-ALL phenotype observed in the Pten$^{-/-}$ mice has a high penetration/prevalence, and therefore, can be used as an excellent non-viral-mediated murine model to study this disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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OPEN

Subclonal evolution involving *SF3B1* mutations in chronic lymphocytic leukemia

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Mutations in genes encoding the spliceosome machinery recently have been described in hematological malignancies,[1,2] particularly in myelodysplastic syndrome (MDS) and chronic lymphocytic leukemia (CLL). These mutations can occur in genes encoding different components of the spliceosome.[3] However, the most frequent of such mutations observed in CLL occurs in the gene-encoding splicing factor 3 subunit 1 (*SF3B1*), a core component of the RNA splicing machinery,[1,4–6] and is found in 5–17% of CLL patients.[5–7] *SF3B1* mutations have been associated with a relatively poor prognosis in CLL,[6–8] but appear associated with a relatively good prognosis in MDS.[2,9–11] Although initially proposed to represent a driver mutation in CLL,[4] a recent study noted two cases in which the CLL cells appeared to have acquired *SF3B1* mutations during clonal evolution.[7]

In this report, we investigated the progression of *SF3B1* mutations in the CLL B cells over time in an attempt to elucidate whether there exists subclonal evolution involving *SF3B1* mutations in CLL. Accumulation of CLL cells harboring mutations in *SF3B1* suggests that such subclones have some competitive advantage, which might account for accelerated progression of the disease in some patients over time. Alternatively, subclones of CLL cells might be selected during therapy, similar to what has been observed in mutations involving *TP53* in CLL cells of patients treated with standard chemotherapy.

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Table 1. Twenty cases with *SF3B1* mutations examined serially

Patient number	*Prior Tx*	*SF3B1 mutation*	*IGHV status*	*ZAP-70 status*	*FISH abnormality*	*Initial ratio (%)*[a]	*Increase over time (%)*	*First to last SC (months)*	*Increase/ month (%)*	P-*value*
1	Yes	K700E	UM	Pos	del(17p)	30.1	45.5	7.9	5.8	0.0091
2	No	E622D	MU	Neg	del(13q)	37.0	63.1	12.9	4.9	0.0095
3	No	K700E	MU	Neg	None	16.3	161.2	40.2	4.0	0.0022
4	No	G740E	UM	Pos	None	23.9	62.1	23.6	2.6	0.0096
5	Yes	G740E	UM	Pos	None	39.0	23.1	9.8	2.4	0.0111
6	Yes	R625C	UM	Pos	None	30.8	34.2	16.6	2.1	0.0464
7	Yes	K700E	MU	Pos	None	30.5	32.3	27.3	1.2	0.0017
8	No	K700E	UM	Pos	del(13q)	36.1	27.7	24.1	1.1	0.1472
9	No	K700E	UM	Pos	None	33.0	17.8	16.1	1.1	0.2104
10	No	K666E	UM	Neg	None	45.4	6.8	6.3	1.1	0.0726
11	No	K666E	UM	Pos	del(11q)	46.9	7.3	11.1	0.7	0.1641
12	No	K666N	UM	Neg	trisomy 12	49.9	10.4	23.8	0.4	0.1643
13	Yes	K666Q	UM	Pos	del(11q)	20.9	3.0	11.0	0.3	0.0750
14	No	E622D	UM	Pos	del(11q)	61.6	6.8	36.1	0.2	0.1413
15	Yes	K700E	MU	Neg	del(13q)	46.3	1.2	10.3	0.1	0.7482
16	Yes	K700E	UM	Pos	None	45.9	−0.3	35.7	0.0	0.7762
17	Yes	K700E	UM	Pos	del(11q)	48.7	−2.3	61.5	0.0	0.7643
18	No	N626Y	UM	Pos	del(13q)	52.5	−1.9	23.8	−0.1	0.4690
19	Yes	K741T	MU	Neg	del(13q)	63.7	−4.7	52.0	−0.1	0.1531
20	Yes	K700E	MU	Neg	del(13q)	49.2	−11.7	26.7	−0.4	0.2838

Abbreviations: FISH, fluorescence *in situ* hybridization; IGHV, immunoglobulin heavy chain variable; MU, mutated; neg, negative; pos, positive; SC, sample collection ; UM, unmutated. The column marked 'Prior Tx' indicates whether the patient did (yes) or did not (no) have prior therapy for CLL. The column marked '*SF3B1* mutation' provides the site (middle number) and amino-acid residue of the wild-type and mutant allele, using standard single-letter amino-acid nomenclature. The column marked 'IGHV status' provides the mutation status for the expressed *IGHV* gene, which was considered UM when it had $\geqslant$98% identity with the germ-line *IGHV* or MU when it had $<$98% identity. The column marked 'ZAP-70 status' indicates whether the CLL sample was pos or neg for ZAP-70, as assessed via flow cytometry. The column marked 'FISH abnormality' provides the abnormal FISH finding (if any) for the initial sample. The column marked 'Initial ratio (%)' provides the proportionate representation of the *SF3B1*-mutant allele in this initial sample. The column labeled 'increase over time (%)' provides the percent increase in the proportionate representation of the mutant *SF3B1* allele, which was calculated with the formula $100 \times$ [(mutant allele ratio of the last SC - mutant allele ratio of the first SC)/mutant allele ratio of the first SC]. The column marked 'First to last SC (months)' provides the number of months between the last SC and first SC. The column labeled 'Increase/months (%)' was calculated by dividing the value in the column marked 'Increase over time' by the value in the column labeled 'First to last SC'. The *P*-values were calculated using the Student's *t*-test when two time points were available or using the one-way analysis of variance when data from three or more time points from the same case were available. [a]Initial proportionate representation of the *SF3B1*-mutant allele according to the formula $100 \times$ (M peak intensity/(M peak intensity + WT peak intensity)).

To address these questions, we examined for *SF3B1* mutations in the CLL cells of 545 patients. The CLL samples of 448 patients were acquired before any therapy, and 97 following treatment for CLL. We investigated for changes in the proportionate representation of subclones harboring *SF3B1* mutations over time, with or without therapeutic intervention.

Thirty-six cases (6.6%) had CLL cells with detectable mutations in the 545 cases examined. In all, 20 (56%) of these 36 cases had K700E and 6 had K666E/Q/N/T (6/36, 17%). As noted in another study,[7] the proportion of cases that had *SF3B1* mutations was significantly higher in the samples of patients who had received prior therapy (12.4%, 12/97) than in samples from patients with no prior therapy (5.4%, 24/448, $P = 0.02$) (Supplementary Figure 1A). Cases found to have *SF3B1* mutations more commonly were found to have unfavorable prognostic features than did cases without *SF3B1* mutations, such as expression of unmutated immunoglobulin heavy chain variable region genes (75%, 27/36, $P = 0.0002$), as reported previously.[5] Furthermore, a higher proportion of cases with *SF3B1* mutations also expressed ZAP-70 (75%, 27/36, $P < 0.0001$) or CD38 (58%, 21/36, $P = 0.0007$) than did cases without *SF3B1* mutations (Supplementary Figure 1B). Patients with CLL cells harboring *SF3B1* mutations were found to have a significantly shorter median treatment-free survival (3.0 versus 6.0 years, $P < 0.0001$) and overall survival (OS, 11.4 versus 21.0 years, $P = 0.0021$) than did patients with CLL cells lacking detectable *SF3B1* mutations (Supplementary Figures 1C and D).

Fluorescence *in situ* hybridization (FISH) data were available for 362 (71%) out of 509 CLL patients with leukemic cells without *SF3B1* mutations and 33 (92%) out of 36 CLL patients with leukemic cells with *SF3B1* mutations. A higher proportion of the *SF3B1*-mutated cases lacked detectable chromosomal abnormalities than did those cases without *SF3B1* mutations (15/33, 45% versus 88/362, 24%, $P = 0.012$). Regarding the genetic abnormalities detected by FISH (del17p, del11q, +12 and del13q), we did not observe any one genetic abnormality associated with cases that did or did not have *SF3B1* mutations (Supplementary Figures 2A and B).

We then examined serial samples from the patients identified as having *SF3B1* mutations. Of 36 cases identified to have *SF3B1* mutations, 20 cases were included in these analyses (Table 1) with time intervals between sample collections ranging from 6 to 61.5 months (median 23.7 months). We measured the height of the peak corresponding to the wild-type or mutant *SF3B1* and calculated the proportionate representation of the height for the mutant allele of samples collected at different times (Figure 1). For some of the samples, this value exceeded 50% (for example, cases 2,12, 14 and 19). None of these samples had the most common *SF3B1* mutation (for example, K700E), making it unlikely that these samples had the same mutation on both alleles. Rather it is more likely that each of these samples had cells harboring a mutation in *SF3B1* and a deletion of the wild-type allele. Prior studies had identified uncommon cases of CLL that had deletions in 2q (the chromosomal location for *SF3B1*), which were not detected by conventional cytogenetics.[12] For seven cases (no. 1–7), we noted significant increases in the proportionate representation of the *SF3B1*-mutant allele over time (Table 1). For three out of the seven cases (cases 2, 3 and 4), all serial samples were collected before therapy; for the other four cases, all serial samples were collected after therapy (Supplementary Table 1). These data indicate that the proportion of subclones harboring *SF3B1* mutations can increase spontaneously over time independent of any therapeutic intervention.

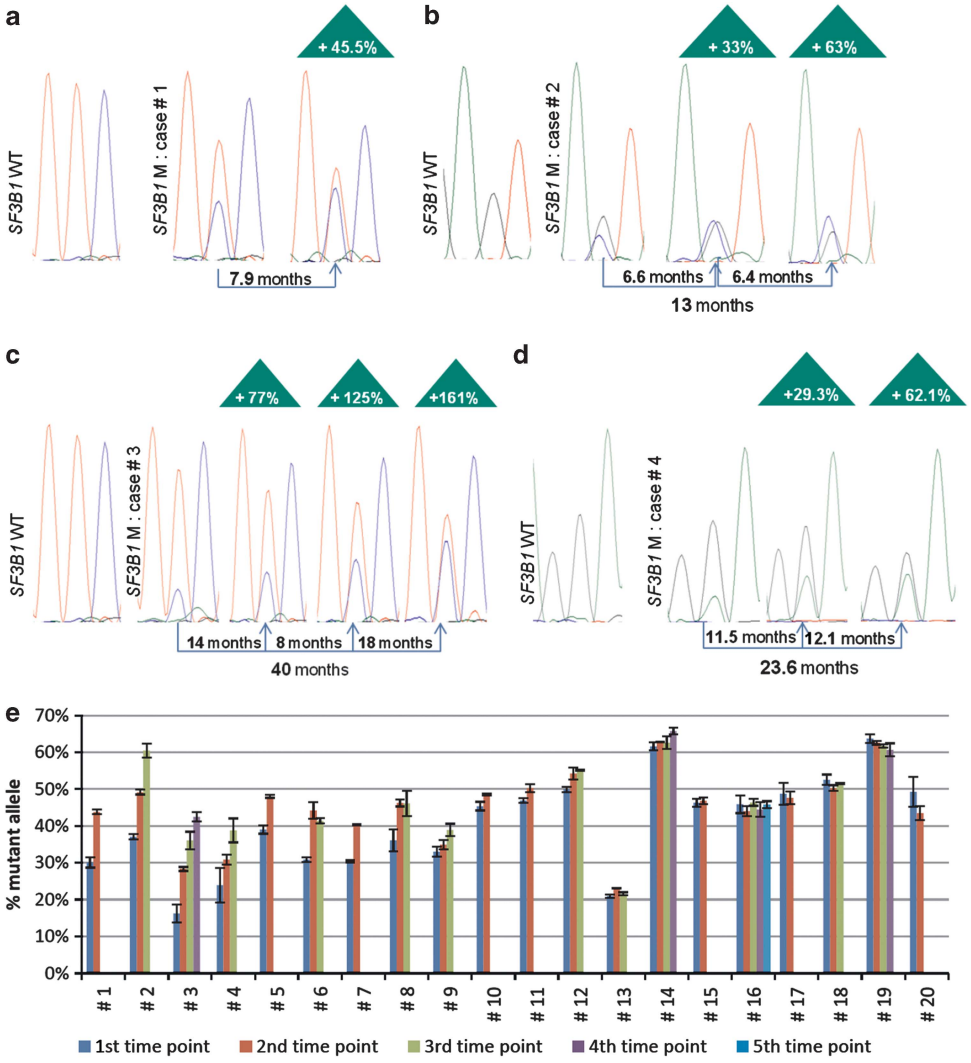

Figure 1. Evolution of *SF3B1* mutations over time. (**a**–**d**) Representation of the serial sample sequence chromatograms for patient no. 1, 2, 3 and 4, respectively. The time intervals between each time point are indicated as well as the overall time span studied. The percentage of increase over time of the *SF3B1*-mutant allele is indicated in the triangles and is calculated by comparison with the first time point. (**e**) Bar graph representing the percentage of *SF3B1*-mutant allele measured at each time points for all the 20 *SF3B1*-mutant cases included in the serial analysis. The 20 cases are ranked by their increase/months (%), according to Table 1. Each time point has been sequenced in duplicate, and the error bars represent the mean ± s.d.

We studied p53 function in two out of these four cases (no. 1 and 6, Supplementary Figure 3), for which CLL cells were available at the second sample collection and after treatment. To evaluate p53 function, we monitored for expression of p53 and p21 after γ-irradiation via flow cytometry. We found γ-irradiation-induced p53 and p21 in each of these two samples, indicating functional *TP53*. As such, subclonal expansion of cells harboring mutations in *SF3B1* can occur in cells that have not lost p53 function.

For 19 cases out of the 20 cases analyzed serially, we conducted FISH analyses on the serial samples. We did not observe any changes between the serial samples for 15 (79%) cases, including 4 that had the largest increases in the proportionate representation of the *SF3B1* allele over time in association with concurrent disease progression (Supplementary Table 1). However, in four cases, we observed one or two new cytogenetic abnormalities in the second sample (for example, for case 11, there was acquisition of del13q, for cases 17 and 18, there was acquisition of del17p and for case 14, there was acquisition of del13q and del17p).

Patient no. 1 was treated with fludarabine, cyclophosphamide and rituximab in July 2004. The patient relapsed after achieving a partial response, developing progressive disease. Between sample collections, the patient's absolute lymphocyte count (ALC) increased from 13 to 102×10^9/l, which was associated with an increase in the proportionate representation of the *SF3B1* allele from 30 to 44%. Similarly, between sample collections of patient no.2, the ALC increased from 67 to 250×10^9/l, which was associated with an increase in the proportionate representation of the *SF3B1* allele from 37 to 60%. Patient no. 3 experienced increases in ALC from 87 to 197×10^9/l between sample collections, for which we detected an increase in the proportionate representation of the *SF3B1* allele from 16 to 43%. Finally, the ALC increased in patient no. 4 from 8 to 47×10^9/l between sample collections, for which we noted an increase in the proportionate representation of the *SF3B1* allele from 24 to 39%. These data imply that subclones of CLL cells harboring mutant *SF3B1* had either a higher growth rate and/or lower death rate than subclones lacking mutations in *SF3B1*, this potentially contributing to disease progression.

For four cases with *SF3B1* mutations (cases 6, 13, 16 and 17), we examined samples before and after therapy, which resulted in

>50% reduction in ALC. For each of these cases, however, we did not observe a significant change in the proportionate representation of the mutant *SF3B1* allele, indicating that such treatments did not have selective activity or inactivity for subclones with *SF3B1* mutations.

Taken together, this study reveals subclonal evolution involving cells with *SF3B1* mutations in CLL. Our data indicate that the proportionate representation of cells harboring *SF3B1* mutations can increase independent of therapy or loss of functional p53. Finally, the data presented here suggest that subclones with *SF3B1* mutations do not necessarily have a selective advantage or disadvantage in the setting of effective cytoreductive therapy. Nevertheless, the prevalence of *SF3B1* mutations appears higher among patients who already have undergone therapy. This might reflect the fact that treated patients more commonly have had longer disease histories, potentially providing greater time for emergence and subclonal evolution of CLL cells harboring *SF3B1* mutations. Further studies with additional cases will be required to address the therapeutic implications of the *SF3B1* mutations and subclonal evolution in this disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

MS, EG, LR and TJK designed, analyzed the data and wrote the manuscript. LZR and TJK provided patients samples and clinical data. MO performed DNA extractions and edited the paper. MLD'A examined the cytogenetics. JFF analyzed the data and edited the paper.

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Call for case histories of BMT in patients with coincident schizophrenia

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Recently, the case for an immune component in the etiology of schizophrenia has regained support,[1,2] leading to randomized controlled trials to explore treatment with immunosuppressants and anti-inflammatory drugs.[3] Both *post-mortem*[4] and *in-vivo* studies[5,6] provided indications for an increased pro-inflammatory status in the brain of patients with recent-onset schizophrenia. A common characteristic of most if not all autoimmune diseases (AD) is their favourable response to immunoablation and rescue with bone marrow transplantation. It was established in radiation chimaeras more than 50 years ago that the immunological and hematological systems have a common stem cell. In the wake of this discovery came a strain of investigations into the role of the bone marrow in AD demonstrating that both hereditary AD and

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