The role of constitutive activation of FMS-related tyrosine kinase-3 and *NRas/KRas* mutational status in infants with *KMT2A*-rearranged acute lymphoblastic leukemia

Constitutive activation of the FMS-related tyrosine kinase-3 (FLT3) by mutations or high expression levels is common in acute leukemias.[1] Aberrant FLT3 signaling is mediated via the RAS/MAPK and PI-3-Kinase/AKT pathways leading to proliferation, survival, and therapy resistance.[1] Infants and children with *KMT2A* (*MLL*)-rearranged acute lymphoblastic leukemia (*KMT2A*r-ALL) have an unfavorable prognosis. Activating *FLT3* tyrosine kinase domain mutations (TKDs) and *N/KRAS* mutations are frequently identified in *KMT2A*r-ALL.[2-6] This subgroup is also characterized by exceptionally high FLT3 expression associated with ligand-independent signaling.[7,8] In order to investigate the prognostic impact of a constitutive activation of FLT3 in infants and children, we analyzed *FLT3* transcription levels and common mutations in *FLT3* and *NRAS/KRAS*. We show that in infants, high FLT3 activation correlates with a superior prognosis. Moreover, we show that *RAS* mutations alter the positive effect of constitutive FLT3 activation and increase relapse risk. High FLT3 activation surprisingly inhibits proliferation of human *KMT2A*r-ALL samples in patient-derived xenografts (PDX). Small molecule FLT3 inhibition was efficient in *FLT3*^{high} PDX without an activating *FLT3* mutation suggesting that FLT3 expression, regardless of mutations, may play a role for novel therapies in the future.

Analyses were performed on samples from infants and children with *KMT2A*r-ALL from trials ALL-BFM 86, 90, 95, 2000, AEIOP-BFM ALL 2009 and German patients enrolled in Interfant-99/-06 (*Online Supplementary Methods, Online Supplementary Table S1*). We detected mutations in the TKD or the juxtamembrane domain (JMD) of *FLT3* in 15/167 (9%) patients (Table 1). In children (patients >1 year, n=72), only one *FLT3* aberration (TKD-D835H) was found (1/72, 1.4%). In infants (n=95), *FLT3*-TKD mutations were identified in 12/95 (12.6%) patients. Seven of these had D835 substitutions and 4 had I836 deletions, all of which are activating.[9,10] One infant had a novel 12-base pair (bp) deletion and 3-bp insertion involving codons D835 to S838 similar to the mutation reported by Taketani *et al.*[3] Notably, of the 12 infants with a *FLT3*-TKD mutation, only 3 suffered from relapse; 3-year cumulative incidence of relapse (CIR) 26±14%. In only 2/95 (2.1%) infant patients, alterations in the JMD of *FLT3* were detected, namely one uncharacterized duplication, and one novel Y589_F594 deletion. Both patients relapsed.

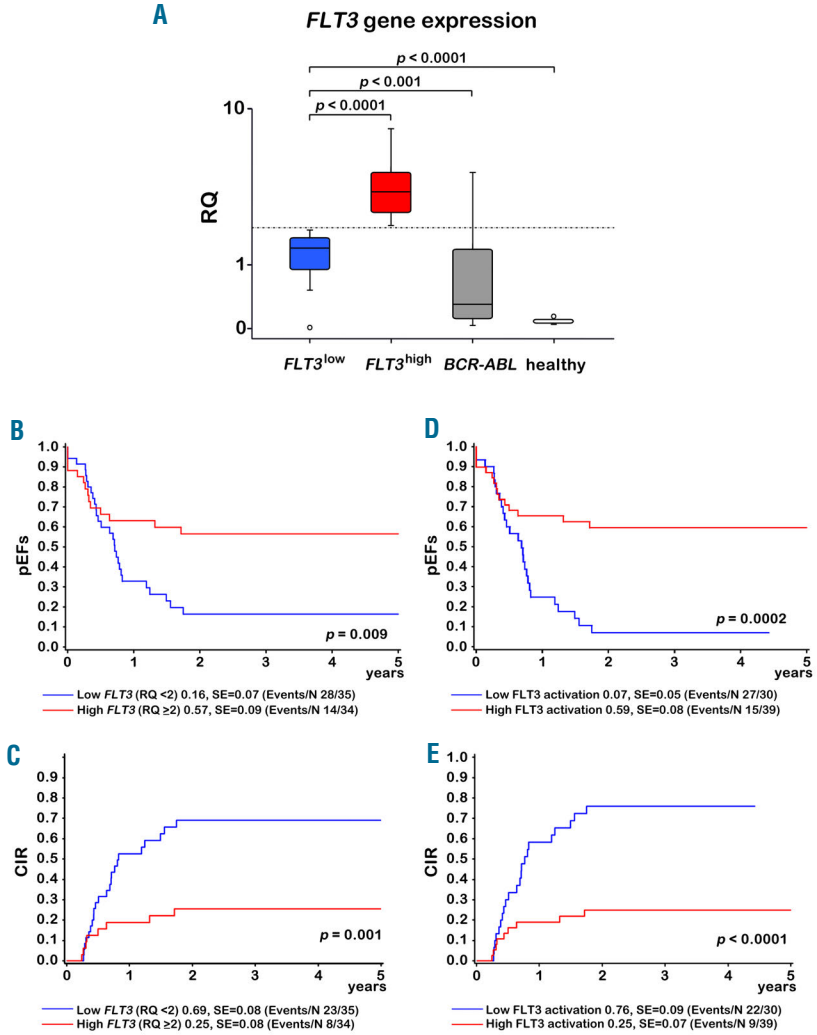

Figure 1. Prognostic impact of *FLT3* transcription and FLT3 activation in infant *KMT2A*r-ALL. A: Box plot with FLT3 expression levels in *FLT3*^{high}, *FLT3*^{low}, BCR-ABL1 positive patients and in healthy bone marrow. B-E: Kaplan-Meier estimates for probability of event-free survival (pEFS) (B and D) and for cumulative incidence of relapse (CIR) (C and E) at 3 years. Plots for low *vs.* high levels of *FLT3* transcription (RQ < or ≥2) are shown in B and C, and plots for low *vs.* high *FLT3* activation are shown in D and E. High or low activation of FLT3 was derived from *FLT3* transcription level (RQ < or ≥2) and *FLT3* mutational status (*FLT3*$^{high/mut}$, *FLT3*$^{high/non-mut}$, *FLT3*$^{low/mut}$ *vs.* *FLT3*$^{low/non-mut}$/*FLT3*^{mut} but no transcription).

We further identified 39 non-synonymous *N/KRAS* mutations in 21/95 (22.1%) infants and 10/72 (13.9%) children (Table 1). *N/KRAS* 12/13 mutations were detected in 28/167 patients. Seven patients had more than one *N/KRAS* 12/13 mutation. One of these had a *KRAS* A11D and a G12C mutation. Another patient had a 3-bp insertion leading to a *KRAS* 10A11 mutation. *NRAS* 61 mutations were found in 2 patients. No *KRAS* 61, but one *KRAS* E63K mutation, previously described in Philadelphia chromosome-like ALL,[11] was identified. Of 8 available relapse samples, 3 had *RAS* mutations present at diagnosis. Of the 5 relapse specimens that did not have *RAS* mutations at relapse, only 1 had a *RAS* mutation at diagnosis, suggesting a subclonal mutation. None of the 8 patients had a *FLT3* mutation, neither at diagnosis nor at relapse. In infants, the presence of *RAS* mutations correlated with a lower 3-year probability of event-free survival (3y-pEFS mut 16±8% *vs.* wt 43±6%, *P*=0.04), and a

Table 1. Clinical features and *FLT3* transcription level of infants and children with *MLL*-rearranged ALL and mutations in the *FLT3, NRAS*, or *KRAS* genes.

	Trial	Age at Dx (years)	Diagnosis	Cyto-genetics	Remission status	RG	RG 06	SCT in 1st CR	RQ *FLT3*	FLT3-JMD	FLT3-TKD	NRAS 12/13	NRAS 61	KRAS 12/13	KRAS 63
	Infants														
1	Interfant-99	0.3	Pro-B ALL	t(4;11)	Relapse	HR	HR	no	*ND*	Y589_F594Δ					
2	Interfant-06	0.6	B-ALL	t(9;11)	Relapse	IR	IR	no	0.56	Duplication*					
3	Interfant-06	0.3	cALL	t(11;19)	Relapse	HR	HR	yes	*ND*		D835H				
4	Interfant-06	0.9	cALL	t(4;11)	Death	IR	IR	yes	7.81		D835H				
5	Interfant-06	0.2	Pro-B ALL	t(4;11)	CCR	HR	HR	yes	1.87†		D835H				
6	Interfant-06	0.1	Pro-B ALL	t(11;19)	Relapse	HR	HR	no	1.48†		D835H			G12D	
7	Interfant-06	0.9	Pro-B ALL	t(11;19)	CCR	IR	IR	no	0.79†		D835E				
8	Interfant-06	0.9	Pro-B ALL	t(1;11)	Relapse	IR	IR	yes	<0.01		D835_S838 DIMSinsL				
9	Interfant-99	0.1	Pro-B ALL	t(4;11)	CCR	HR	HR	yes	3.85		D835E + D835Y				
10	Interfant-06	0.2	Pro-B ALL	t(4;11)	CCR	IR	IR	no	0.99†		D835Y + D835H				
11	Interfant-06	0.6	Pro-B ALL	t(4;11)	CCR	IR	IR	no	1.87†		I836				
12	Interfant-06	0.7	Pro-B ALL	t(4;11)	CCR	IR	IR	yes	5.38		I836				
13	Interfant-99	0.5	Pro-B ALL	t(11;19)	CCR	HR	IR	no	*ND*		I836 subclonal				
14	Interfant-99	0.5	Pro-B ALL	t(4;11)	Death	SR	IR	no	*ND*		I836 subclonal			G12S	
15	Interfant-99	0.2	cALL	t(10;11)	Relapse	HR	HR	yes	2.07					11A12	
16	Interfant-99	0.4	Pro-B ALL	t(4;11)	Relapse	SR	IR	no	3.46			G12D		G12D‡	
17	Interfant-06	0.6	Pro-B ALL	t(4;11)	CCR	IR	IR	no	*ND*			G12A		G13D	
18	Interfant-06	0.3	Pro-B ALL	t(4;11)	Relapse	IR	IR	no	2.45			G12C + G12D		G13D‡	
19	Interfant-06	0.1	Pro-B ALL	t(6;11)	Death	HR	HR	yes	0.62			G12S			
20	Interfant-06	0.1	Pro-B ALL	t(4;11)	Relapse	HR	HR	no	1.9			G13D			
21	Interfant-06	0.3	cALL	t(10;11)	Relapse	HR	HR	no	0.52			G13D			
22	ALL-BFM 90	0.1	Pro-B ALL	t(4;11)	Relapse	IR	HR	no	*ND*			G12D		G12D	
23	Interfant-99	0.3	Pro-B ALL	t(11;19)	CCR	SR	IR	no	*ND*					G12D	
24	Interfant-99	0.8	Pro-B ALL	t(9;11)	CCR	SR	IR	no	*ND*					G12D	
25	Interfant-99	0.4	Pro-B ALL	t(4;11)	Relapse	SR	HR	no	*ND*					G12D	
26	Interfant-99	0.3	Pro-B ALL	t(4;11)	Relapse	HR	HR	yes	*ND*					G13D	
27	Interfant-99	0.3	cALL	t(11;19)	Death	HR	HR	yes	*ND*					G12V	
28	Interfant-06	0.2	Pro-B ALL	t(4;11)	Relapse	HR	HR	yes	2.42					G12S	
29	Interfant-06	0.7	Pro-B ALL	t(4;11)	CCR	IR	IR	no	2.92					G12S	
30	Interfant-99	0.1	Pro-B ALL	t(9;11)	Relapse	HR	HR	no	1.79				Q61K		
31	Interfant-06	0.1	Pro-B ALL	t(4;11)	Relapse	IR	IR	yes	1.93				Q61K‡		
32	Interfant-06	0.4	Pro-B ALL	t(4;11)	Relapse	HR	HR	no	3.66					G12S	
33	Interfant-99	0.1	Pro-B ALL	t(4;11)	LFU	SR	HR	no	2.32					G12D + G12V	
	Children														
34	ALL-BFM 2000	1.7	cALL	t(9;11)	CCR	SR	*NA*	no	*ND*						E63K
35	ALL-BFM 2000	12.4	Pro-B ALL	t(1;11)	CCR	IR	*NA*	no	3.4					G12A	
36	ALL-BFM 2000	1.3	cALL	t(9;11)	Death	IR	*NA*	no	1.48			G12C			
37	ALL-BFM 2000	1.6	Pro-B ALL	t(9;11)	Relapse	IR	*NA*	no	1.59		D835H subclonal	G12S			

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38	ALL-BFM 2000	15.7	Pro-B ALL	t(4;11)	CCR	HR	*NA*	yes	2.27	G12D	G13D
39	AIEOP-BFM ALL 2009	17.6	Pro-B ALL	t(4;11)	Death	HR	*NA*	yes	3.1	G12D	
40	AIEOP-BFM ALL 2009	12.0	Pro-B ALL	t(4;11)	CCR	HR	*NA*	yes	3.62		G12S
41	AIEOP-BFM ALL 2009	3.0	Pro-B ALL	t(4;11)	CCR	HR	*NA*	no	4.17		G12D
42	AIEOP-BFM ALL 2009	1.4	Pro-B ALL	t(9;11)	CCR	SR	*NA*	no	2.08		A11D + G12C
43	AIEOP-BFM ALL 2009	14.7	Pre-T ALL	t(9;11)	Relapse	HR	*NA*	yes	0.17		G12D

Dx indicates diagnosis; RG, risk group of original trial; RG '06, risk group according to Interfant-06 trial; SCT, stem cell transplantation; RQ, relative quantification ($2^{-\Delta\Delta CT}$); FLT3-JMD, FLT3 juxtamembrane domain mutation; FLT3-TKD, FLT3 tyrosine kinase domain mutation; *ND*: not determined (no RNA available); cALL, common ALL; CCR, continuous complete remission; and LFU, lost to follow up; SR, standard risk; IR, intermediate risk; HR, high risk; NA, not applicable. *uncharacterized duplication of unknown size; †Patients with low *FLT3* transcription level but activating *FLT3* mutation; ‡mutation present at relapse.

higher 3y-CIR (mut 69±11% *vs.* wt 40±6%, *P*=0.01).

Next, we analyzed *FLT3* transcription by qRT-PCR in 124 patients (69 infants, 55 children). When we separated the infant cohort into 2 groups according to the median RQ value (< or ≥2, Figure 1A), a low *FLT3* transcription level significantly correlated with a low pEFS ($FLT3^{low}$ 16±7% *vs.* $FLT3^{high}$ 57±9%, *P*=0.009) and a high CIR ($FLT3^{low}$ 69±8% *vs.* $FLT3^{high}$ 25±8%, *P*=0.001) (Figure 1B-C). Moreover, the median *FLT3* transcription level was low in all 8 relapse samples analyzed (initial 1.85 *vs.* relapse 1.46, *P*=0.263). It is important that median *FLT3* transcription levels in the $FLT3^{low}$ group of infants was 16-fold higher than in bone marrow of healthy controls ($FLT3^{low}$ n=35 *vs.* healthy n=8, *P*<0.0001) and 4.6-fold higher than in BCR-ABL-positive ALLs ($FLT3^{low}$ n=35 *vs.* BCR-ABL n=15, *P*=0.009). In multivariate analysis, the prognostic impact of *FLT3* transcription levels in infants was independent of age (< *vs.* ≥6 months), initial white blood cell count (< *vs.* ≥300,000/µl), prednisone response (poor *vs.* good), and non-remission on day 33: in a Cox regression analysis for EFS and relapse incidence with these covariables, $FLT3^{low}$ resulted in a hazard ratio of 3.80 (95% confidence interval (CI) 1.71-8.44, *P*=0.001) for EFS and 6.68 (95% CI 2.37-18.8, *P*<0.0001) for relapse incidence (*Online Supplementary Table S2*). No prognostic relevance of *FLT3* transcription was observed for children with B- or biphenotypic ALL (3y-pEFS $FLT3^{low}$ 71±11% *vs.* $FLT3^{high}$ 65±10%, *P*=0.73, and 3y-CIR $FLT3^{low}$ 12±8% *vs.* $FLT3^{high}$ 12±7%, v=0.93). Children with T-ALL were excluded as they showed very low *FLT3* transcription (B-cell/biphenotypic ALL, n=46, median 3.17 *vs.* T-ALL, n=9, median 0.17, *P*<0.00001). Of the 6 infants with low *FLT3* transcription, but with a *FLT3*-TKD mutation, two had a relapse. Importantly, in one of these two patients (patient 8), a *FLT3*-TKD mutation (new del/ins with unknown effects) was present, but the *FLT3* gene was not transcribed at all. We next analyzed infants with a presumed high *FLT3* activation ($FLT3^{high}$/mut, $FLT3^{high}$/non-mut, $FLT3^{low}$/mut) compared to those with a low *FLT3* activation ($FLT3^{low/non-mut}$/$FLT3^{mut}$ but no transcription) and found the CIR to be further reduced and the pEFS to be further increased in the group with high *FLT3* activation (Figure 1D-E). The other relapsed patient with a *FLT3*-TKD mutation (patient 6) had, in addition, a *RAS* mutation (Table 1). Likewise, most patients with a high *FLT3* transcription level that suffered from relapse also had *RAS* mutations, suggesting that *RAS* mutations alter the positive effect of FLT3 activation. A Cox regression analysis of relapse incidence in infants with the same variables as above plus $FLT3^{low}$ *vs.* $FLT3^{high}$, *RAS* mutation *vs.* wildtype and the interaction of $FLT3^{high}$ and *RAS* wildtype resulted in a hazard ratio of 0.04 (95% CI 0.01-0.37, *P*=0.005) for the interaction (*Online Supplementary Table S3*).

In order to clarify the protective mechanism of high FLT3 activation, we performed xenotransplantation assays of 19 *KMT2A*r samples into NOD.Cg*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice. When these patients were separated according to *FLT3* activation ($FLT3^{high/mut}$, $FLT3^{high/non-mut}$, $FLT3^{low/mut}$ *vs.* $FLT3^{low/non-mut}$/$FLT3^{mut}$ but no transcription), a high FLT3 activation correlated with prolonged PDX survival, confirming the clinical data (*P*=0.0054, Figure 2A). *In vivo* bromodeoxyuridine (BrdU) assays in a subset of 3 $FLT3^{high}$ and 3 $FLT3^{low}$ PDX bearing different patients revealed significantly slower proliferation rates in human leukemic cells from $FLT3^{high}$ mice (Figure 2B-C). Human PDX cells from six situations ($FLT3^{high/mut}$, $FLT3^{high/non-mut}$, $FLT3^{low/mut}$, $FLT3^{low/non-mut}$, $FLT3^{high/Ras-mut}$, $FLT3^{low/Ras-mut}$) revealed an activation of p-Erk in $FLT3^{high}$, $FLT3^{mut}$ and *RAS*-mutated, but not in $FLT3^{low/non-mut}$ samples (Figure 2D) supporting that $FLT3^{high/mut}$, $FLT3^{high/non-mut}$ and $FLT3^{low/mut}$ correspond to high FLT3 activation. High p-Akt was detected in $FLT3^{high}$, but not in $FLT3^{low}$ samples without *RAS*-mutations. No changes were detected in *RAS*-mutated samples, implying a minor role of p-Akt in that situation. Interestingly, CDKN1A/p21 indicating cell cycle arrest was higher in $FLT3^{high}$ patients (Figure 2D), supporting our BrdU data. In order to test if $FLT3^{high}$ ALLs are targetable by FLT3 inhibitors irrespective of *FLT3* mutations, we performed experiments in $FLT3^{high/non-mut}$ compared to $FLT3^{low/non-mut}$ PDX. Treatment with Lestaurtinib resulted in a small but significant prolongation of median survival in $FLT3^{high/non-mut}$ xenografts (57 *vs.* 54 days, *P*<0.0001) but not in $FLT3^{low/non-mut}$ animals (Figure 2E). When Lestaurtinib was combined with a regimen mimicking standard induction chemotherapy in humans (vincristine, dexamethasone, PEG-asparaginase), survival could be increased by ≈30% as compared to chemotherapy only in $FLT3^{high/non-mut}$ animals

(113 *vs.* 86 days, *P*=0.0003), whereas this effect was only marginal in the *FLT3*$^{low/non\text{-}mut}$ setting (63 *vs.* 60 days, *P*<0.0001) (Figure 2E). These data support the relevance of FLT3 expression irrespective of *FLT3* mutations.

In summary, we report that FLT3 activation is associated with a good prognosis in *KMT2A*r infant ALL, which is in contrast to several studies.[12-14] Chillon *et al.*[13] analyzed a cohort of 17 *KMT2A-AF4*-positive ALL cases including only 4 infants. Kang *et al.*[14] used *FLT3* transcription levels to separate infant *KMT2A*r and *KMT2A*-

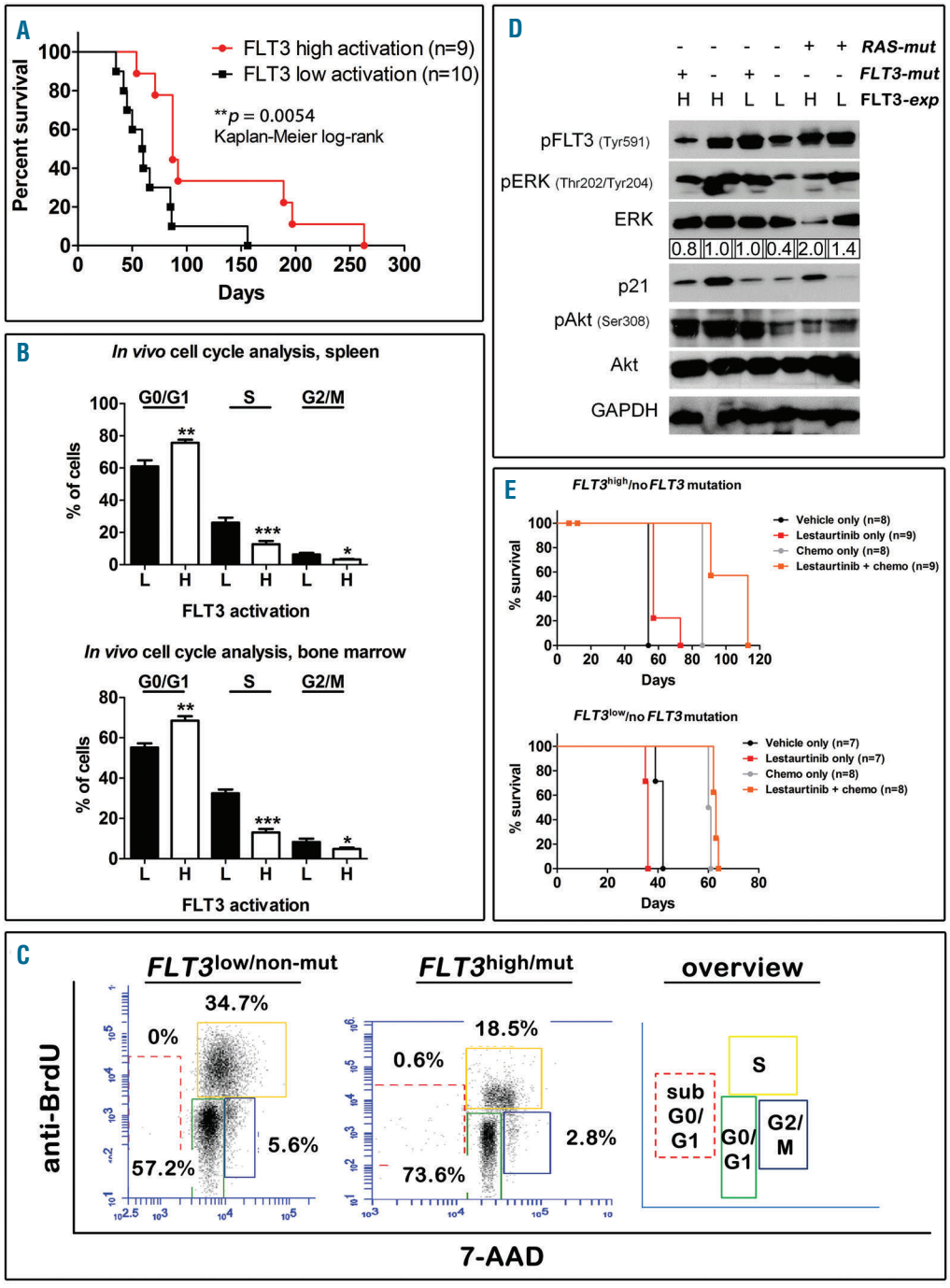

Figure 2. High FLT3 activation inhibits the growth of *KMT2A*r-ALL cells *in vivo*. Cells from patients with a high and a low FLT3 activation status as indicated were injected into NSG mice and mice were sacrificed upon detection of leukemic symptoms. A: Survival prolongation in mice bearing patients with a high FLT3 activation status (*FLT3*$^{high/mut}$, *FLT3*$^{high/non\text{-}mut}$, *FLT3*$^{low/mut}$), Kaplan-Meier log-rank test. B: A subgroup of 3 mice from each group, were fed with BrdU before euthanasia. Human leukemic cells were recovered from the spleens (upper panel) and the bone marrow (lower panel), stained with an anti-BrdU antibody and 7-AAD and FACS analysis was performed. The percentages of cells in the different cell cycle phases are depicted. H = high FLT3 activation status; L = low FLT3 activation status; **P*<0.05; ***P*<0.01; ****P*<0.001. C: Representative plots of cell cycle distributions of two samples from both groups as indicated and schematic overview. D: Human leukemic cells were recovered from fully engrafted xenograft spleens. Cells were lysed and subjected to Western Blotting experiments for the markers indicated. H = high FLT3 expression; L = low FLT3 expression. Quantification of the p-Erk/Erk ratio was determined using Image J software. E: Xenograft mice (FLT3$^{high/non\text{-}mut}$, upper panel and FLT3$^{low/non\text{-}mut}$, lower panel) were subjected to treatments with vehicle only, Lestaurtinib, chemotherapy only (dexamethasone, vincristine and PEG-asparaginase), or a combination of chemotherapy and Lestaurtinib, as indicated. Survival curves, Kaplan-Meier log-rank test.

germline ALL. The latter were characterized by low *FLT3* transcription and a good prognosis. Stam *et al.*[12] analyzed a smaller cohort of *KMT2A*r infants and identified a small group of *FLT3*^{high} patients with a low 1y-EFS, but the level of significance was also low. In accordance with a previous study, we confirmed that *RAS* mutations correlate with a poor prognosis in *KMT2A*r-ALL,[4] abolishing the positive effect of high FLT3. Conversely, low FLT3 expression is associated with an inferior prognosis, regardless of *RAS*. Our *in vivo* studies confirm our clinical data as high FLT3 inhibits cell cycle and delays leukemic outgrowth in mice, suggesting pro-survival functions. Distinction of high/low FLT3 levels remains artificial, and the lack of a validation cohort and small sample sizes should be addressed in the future. Also, we cannot exclude that mutations in other genes may interfere with our findings. A recent clinical trial was unable to show a benefit from adding Lestaurtinib to chemotherapy in *KMT2A*r infant ALL patients, even though 95% confidence intervals were very large.[15] Further studies are needed to confirm potential effects in subgroups, depending on FLT3 expression and *FLT3* and *RAS* mutational status.

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Acknowledgments: we thank Birthe Fedders, Katrin Timm-Richert and Katrin Neumann for excellent technical assistance. We thank the Institute of Clinical Molecular Biology in Kiel for providing Sanger sequencing as supported in part by the DFG Cluster of Excellence "Inflammation at Interfaces" and "Future Ocean". We thank Sandra Greve, Tanja Henke and Stefanie Arndt for technical support.

Funding: this work was supported by the European Union's Seventh Framework Program (FP7/2007-2013) under the project European Network for Cancer Research in Children and Adolescents (ENCCA), grant agreement HEALTH-F2-2011-261474. J. S. is supported by the Madeleine Schickedanz-Kinderkrebs-Stiftung. D. M. S. is supported by a Max-Eder Junior Research Grant by the Deutsche Krebshilfe e. V. and by the Wilhelm-Sander Stiftung.

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doi:10.3324/haematol.2017.169870

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Annesley CE, Brown P. The biology and targeting of FLT3 in pediatric leukemia. Front Oncol. 2014;4:263.
2. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. Cancer Cell. 2003;3(2):173-183.
3. Taketani T, Taki T, Sugita K, et al. FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. Blood. 2004;103(3):1085-1088.
4. Driessen EM, van Roon EH, Spijkers-Hagelstein JA, et al. Frequencies and prognostic impact of RAS mutations in MLL-rearranged acute lymphoblastic leukemia in infants. Haematologica. 2013;98(6):937-944.
5. Liang DC, Shih LY, Fu JF, et al. K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. Cancer. 2006;106(4):950-956.
6. Andersson AK, Ma J, Wang J, et al. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330-337.
7. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30(1):41-47.
8. Stam RW, den Boer ML, Schneider P, et al. Targeting FLT3 in primary MLL-gene-rearranged infant acute lymphoblastic leukemia. Blood. 2005;106(7):2484-2490.
9. Yamamoto Y, Kiyoi H, Nakano Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood. 2001;97(8):2434-2439.
10. Grundler R, Thiede C, Miething C, Steudel C, Peschel C, Duyster J. Sensitivity toward tyrosine kinase inhibitors varies between different activating mutations of the FLT3 receptor. Blood. 2003;102(2):646-651.
11. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005-1015.
12. Stam RW, Schneider P, de Lorenzo P, Valsecchi MG, den Boer ML, Pieters R. Prognostic significance of high-level FLT3 expression in MLL-rearranged infant acute lymphoblastic leukemia. Blood. 2007;110(7):2774-2775.
13. Chillon MC, Gomez-Casares MT, Lopez-Jorge CE, et al. Prognostic significance of FLT3 mutational status and expression levels in MLL-AF4+ and MLL-germline acute lymphoblastic leukemia. Leukemia. 2012;26(11):2360-2366.
14. Kang H, Wilson CS, Harvey RC, et al. Gene expression profiles predictive of outcome and age in infant acute lymphoblastic leukemia: a Children's Oncology Group study. Blood. 2012;119(8):1872-1881.
15. Brown PK, Wang C, Dreyer Z, et al. Addition of FLT3 inhibitor lestaurtinib to post-induction chemotherapy does not improve outcomes in Mll-rearranged infant acute lymphoblastic leukemia (ALL): AALL0631, a Children's Oncology Group Study. Pediatr Blood Cancer. 2016;63(S5):S321.