The *EBF1-PDGFRB* T681I mutation is highly resistant to imatinib and dasatinib *in vitro* and detectable in clinical samples prior to treatment

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Supplemental Data

Table S1. The full spectrum of kinase domain mutations identified in *in vitro* saturation mutagenesis screens of *EBF1-PDGFRB* with imatinib and dasatinib.

	Table 1: In-Vitro Saturation Mutagenesis Screens				
KD mutations / TKIs	Incidence (n)				
	Imatinib 5uM	**Imatinib 10uM**	**Dasatinib 25nM**	**Dasatinib 50nM**	**Dasatinib 100nM**
Recurrent single KD mutations	**T681I** (65/67)	**T681I** (168/178)	**T681I** (85/112) **N666S** (11/112)	**T681I** (160/209) **N666S** (7/209)	**T681I** (93/95)
Single KD mutations			**I654T** (1/112) **L667P** (1/112) **D850N** (1/112) **D854G** (1/112) **G861S** (1/112) **F864P** (1/112)		**D850V** (1/95)
Non-T681I compound KD mutations			**N666S/G846S** (1/112) **G687R/M909I** (1/112)	**M741I/D850Y** (1/209)	
Recurrent T681I compound KD mutations		**T681I/S731Y** (4/178) **T681I/E943K** (2/178)		**T681I/G687E** (2/209) **T681I/A764T** (3/209)	

KD: kinase domain; TKIs: tyrosine kinase inhibitors

Table S2. Baseline characteristics and outcomes of *EBF1-PDGFRB* patients stratified by subclonal T681I mutation status.

	All patients (n=23)	T681I-positive (n=3)	T681I-negative (n=20)	p-value
Median age at diagnosis, years	12 [8-16]	14 [12-17]	12 [7.5-16]	0.4703
Median WBC at diagnosis, x10^9/L	39.0 [17.0-80.7]	13.4 [5.0-26.0]	45.4 [23.3-91.4]	0.0889
CR rate, %	60.0	100.0	52.9	0.2421
EOI MRD>1%, %	84.2	100.0	84.2	1.000
HSCT, %	52.5	33.3	55.0	0.5901
Relapse, %	43.5	100.0	35.0	0.0678
Median time from diagnosis to relapse, months	31 [27-39]	28 [18-39]	31.5 [27-40]	0.7086
Mortality, %	34.8	33.3	35.0	1.0000
5-year EFS, %	30.4	0	35.0	0.4467
5-year OS, %	64.9	66.7	64.6	0.8472

WBC: white blood cell; CR: complete remission; EOI MRD: end of induction minimal residual disease; HSCT: hematopoietic stem cell transplantation; EFS: event-free survival; OS: overall survival.

Figure S1. Comparative structure models of PDGFRB. (**A,B)** Cartoon representation of the ATP binding site indicating the predicted pose of dasatinib (shown as sticks with a transparent molecular surface). **A**. The gatekeeper residue T681 (shown as sticks in green) is predicted to form a hydrogen bond (shown as a black line) with the amide nitrogen on dasatinib. **B**. The mutant T681I (shown as sticks in orange) is predicted to be sterically incompatible (shown by red lines) with dasatinib binding. (**C,D**) Cartoon representation of the loop between helix alphaC and strand beta4. **C**. The N666 residue (shown as sticks in green) is predicted to form the basis of a network of hydrogen bonds (shown as black lines) stabilizing the loop region. **D.** The mutant N666S (shown as sticks in orange) is predicted to lack the ability to form the equivalent set of favorable interactions (shown by the absence of hydrogen bonds).