Fig. S1. Pim kinase inhibitor reduces cell surface MET expression. PC3-LN4, H1993, or BT474 cells were treated with AZD1208 (3 μM) for 24 hours. Levels of cell surface MET were visualized by flow cytometry using anti-MET antibody and PE-conjugated secondary antibody.

Fig. S2. Wound-healing/scratch assay. Mouse prostate epithelial cells with (WFU12) and without (WFU8) Pim-1 expression were treated with HGF (100 ng/ml) for 24 h. SMI-4a (10 μM) or PHA665752 (1 μM) were added. The moving fronts are indicated.

Fig. S3. eIF4B regulates MET expression. (A) Predicted secondary structure of the MET 5'UTR as determined using the MFOLD algorithm (http://mfold.rna.albany.edu/). The overall folding energy (ΔG) of the most stable structure = -239.30 kcal/mol. (B) HeLa cells were treated with eIF4B siRNA (sieIF4B) or a control siRNA (siC) for 72 h. Cell lysates were analyzed by immunoblot assays using indicated antibodies. (C) HeLa and U2OS cells were transfected with pR-MET-F construct together with a nontargeting control (siC), or eIF4B (sieIF4B), or eIF4A (sieIF4A) siRNA. Ratios of firefly/*Renilla* luciferase activities are shown. The average +/-S.D. of four independent experiments with duplicates in each experiment are shown. $p < 0.05$, siC Vs sieIF4B.

Fig. S4. Pim-1 phosphorylates eIF4B at S406. (A) Tandem mass spectra of HPSWRSEETQER, the trypsin cleavage fragment 404-415 of eIF4B (upper panel), and SRTGSESSQTGTSTTSSR, the trypsin cleavage fragment 418-435 of human eIF4B (lower panel). The amino acid sequence was determined via a Sequest HT search with phosphorylation confirmed at S406 (**s)**. The site of phosphorylation was identified with 89% localization probability using the PhosphoRS 3.0 algorithm and confirmed by a

manual review of the data. PC3-LN4 (B) and BT474 (C) cells were treated with Pim-1 siRNA for 72 h. Cell lysates were analyzed by immunoblot assays using the indicated antibodies. PC3-LN4 (D) and BT474 (E) cells were treated with Pim-1 siRNA for 48 h, then cells were serum-starved for 24 h before stimulated with insulin (1 μg/ml) or 20% FBS for additional 30 min. Cell lysates were analyzed by immunoblot assays using indicated antibodies.

Fig. S5. Phosphorylation of eIF4B S406 and S422 are controlled by Pim and PI3K/AKT/mTOR pathways, respectively. PC3-LN4 (A) and BT474 (B) cells were treated with various inhibitors for 3 h: MK2206 (MK, 1 μM), Rapamycin (Rapa or Ra, 100 nM), PP242 (1 μM), BEZ235 (0.5 μM), UO126 (UO, 15 μM), BI-D1870 (BI, 10 μM), GNE-652 (1 μM), AZD1208 (3 μM). Cell lysates were analyzed by immunoblot assays using the indicated antibodies. PC3-LN4 (C) and BT474 (D) cells were serum-starved for 24 h and pretreated with inhibitors as in (A) and (B) before stimulating with insulin (1 μg/ml) for 30 min. Cell lysates were analyzed by immunoblot assays using the indicated antibodies.

Fig. S6. eIF4B S406 phosphorylation is important for MET translation. (A) PC3-LN4 or BT474 cells were treated with AZD1208 (3 μM), BEZ235 (0.5 μM), cycloheximide (CHX, 100 μg/ml) for 3 h prior to labeling new protein synthesis with ^{35}S. Newly synthesized MET and ERK were immunoprecipitated and separated by SDS-PAGE, and visualized by autoradiography. (B) U2OS cells were transfected with pR-MET-F construct together with eIF4B or its mutants. After 24 h luciferase assays were performed. Relative ratios of firefly/*Renilla* luciferase activities are shown. The ratio for the vector control was set

as 1. The average +/-S.D. of these measurements are shown. $p < 0.05$, WT Vs Vector; $p < 0.02$, WT Vs S406A or S406/422A.

Fig. S7. Pim-1 regulates the protein translation of INSR and c-Myc through the phosphorylation of eIF4B S406. (A) Cell lysates from U2OS and PC3-LN4 cells treated with Pim-1 siRNA or MEFs (WT and TKO) were analyzed by immunoblot assays using the indicated antibodies. (B) Real-time PCR assays were carried out using total RNA extracted from PC3-LN4 cells treated with Pim-1 or MEFs (WT and TKO). (C) Polysome profiling assays were performed in MEFs (WT and TKO). 18 fractions (0.6 ml/fraction) were collected. RNA gels were shown (middle panel). Semi-quantitative RT-PCR assays were carried out using pooled fractions as indicated. (D) U2OS cells were transfected with a control vector, or plasmids expressing wild-type eIF4B (WT) and its mutants for 48 h. Cell lysates were analyzed by immunoblot assays using indicated antibodies.

Table S1. Phase I clinical trial of AZD1208 patient dosing and sample collecting information.

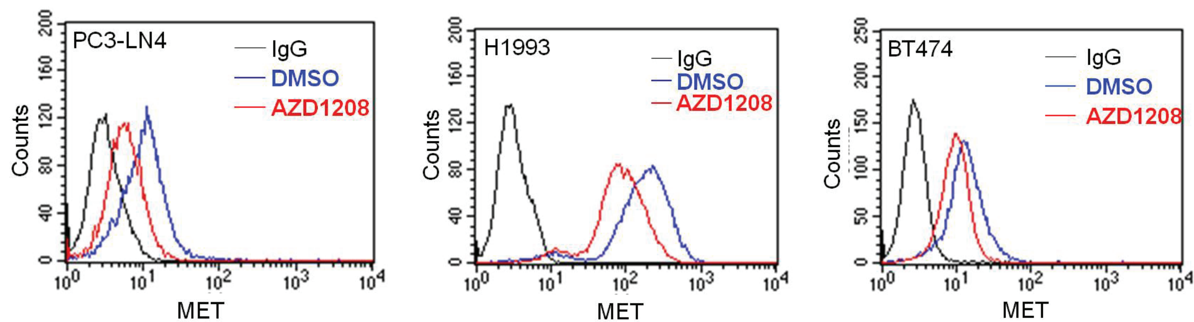


Fig. S1

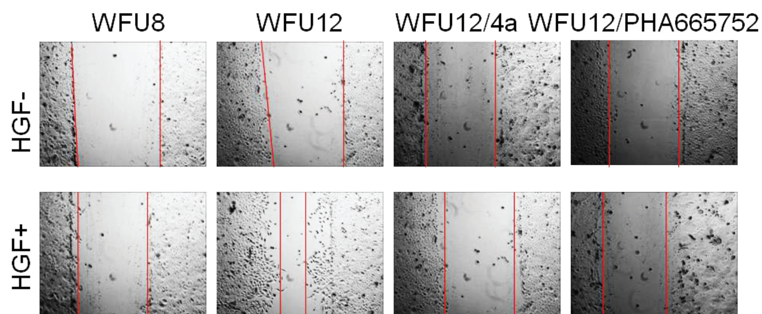
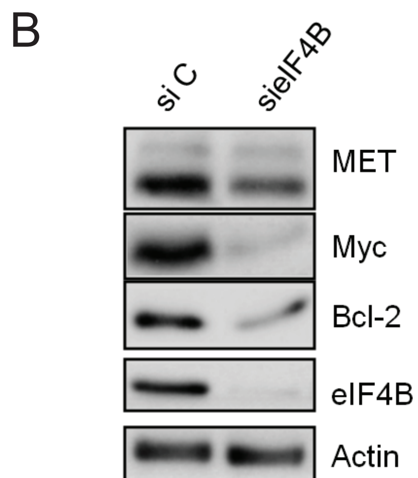


Fig. S2

A

dG = -239.30 met

B



C

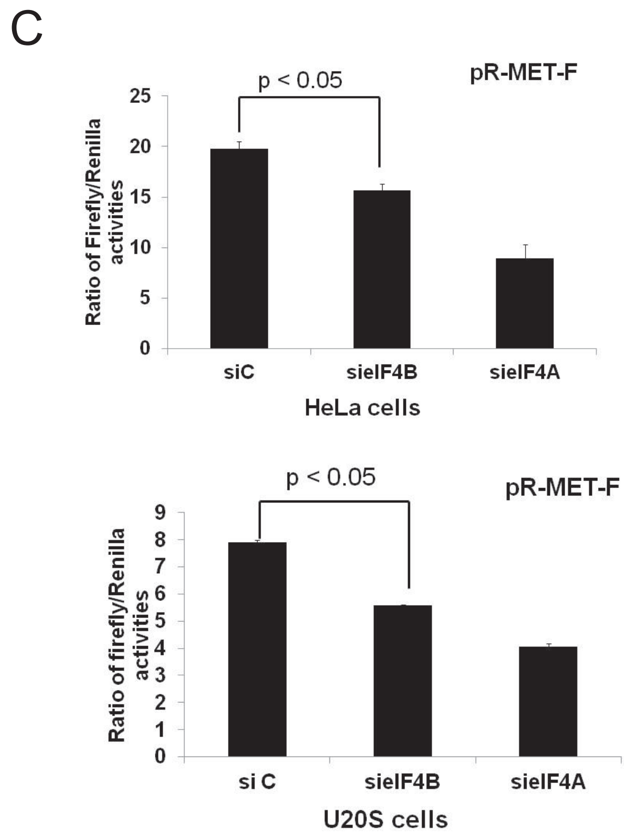
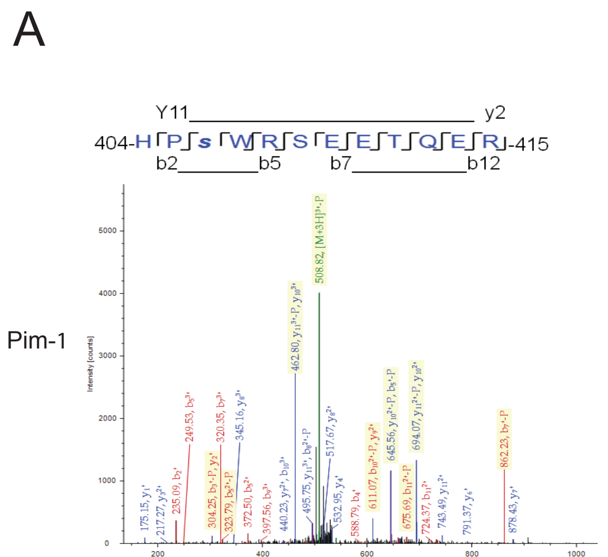
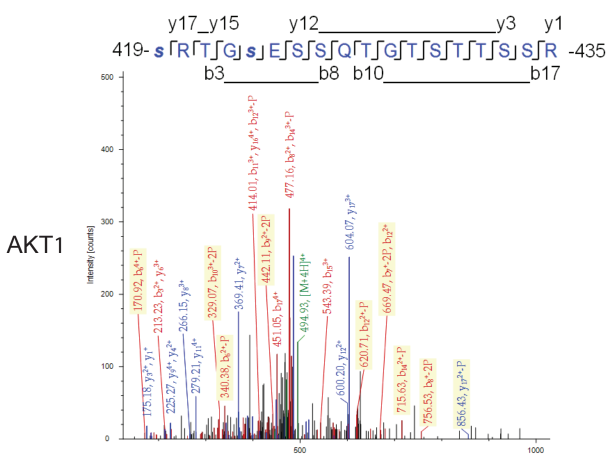
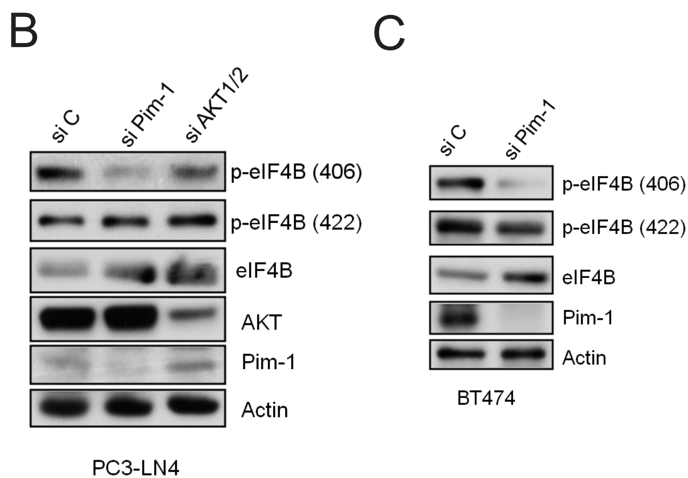
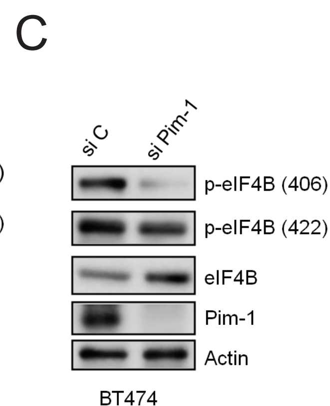


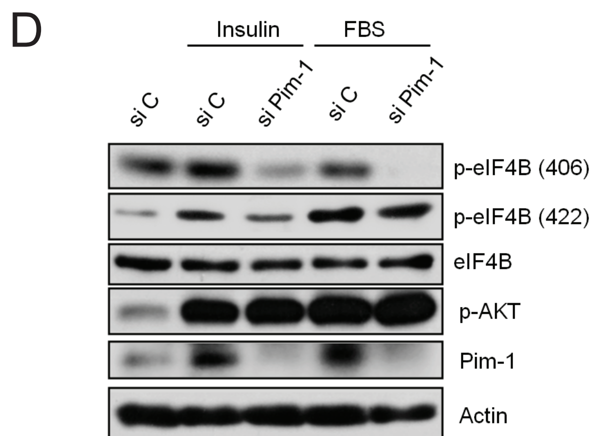
Fig. S3











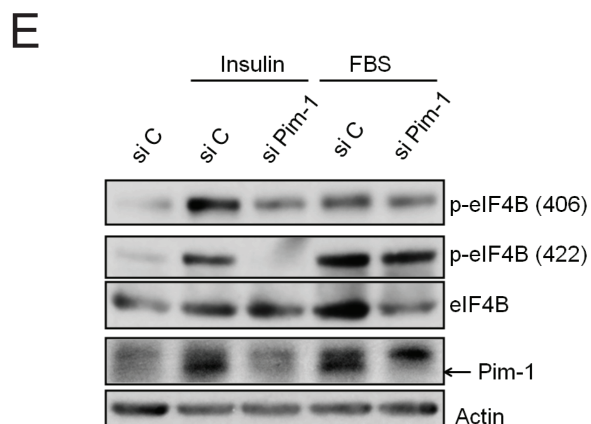
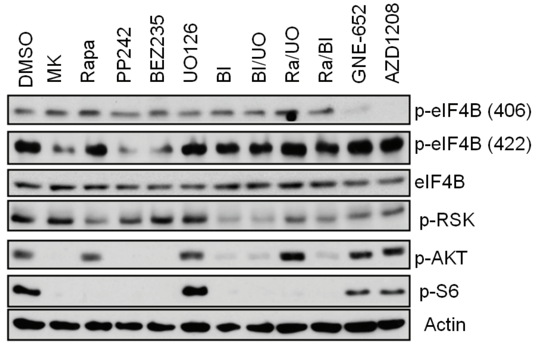
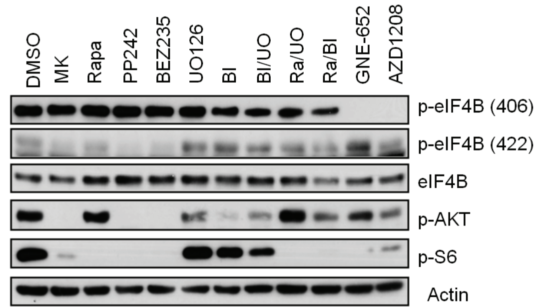
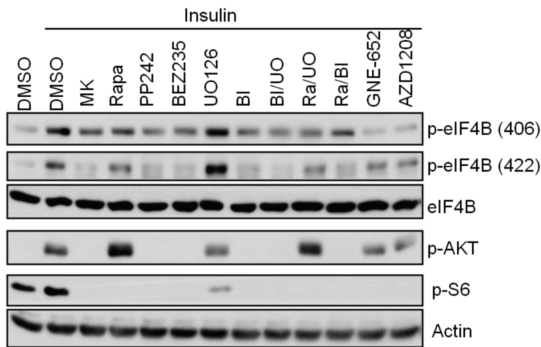


Fig. S4







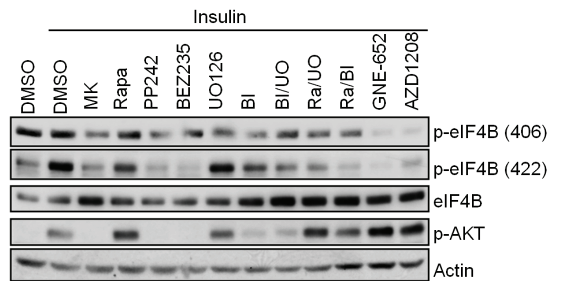


Fig. S5

A

DMSO AZD1208 BEZ235 CHX

IP: MET ^{35}S

IP: ERK ^{35}S

Cell lysates ^{35}S

Cell lysates Coomassie blue

PC3-LN4

DMSO AZD1208 BEZ235 CHX

IP: MET ^{35}S

Cell lysates ^{35}S

Cell lysates Coomassie blue

BT474

B

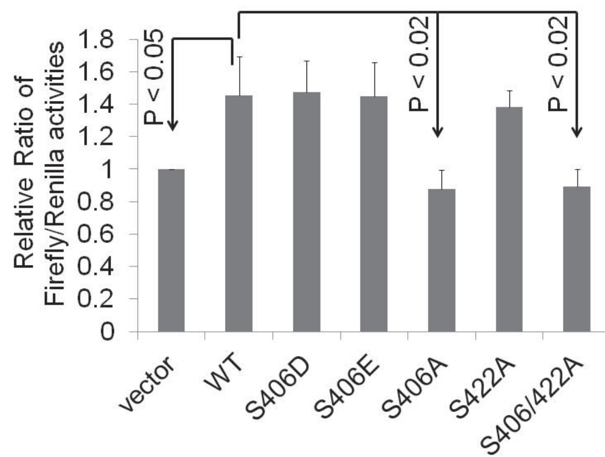


Fig. S6

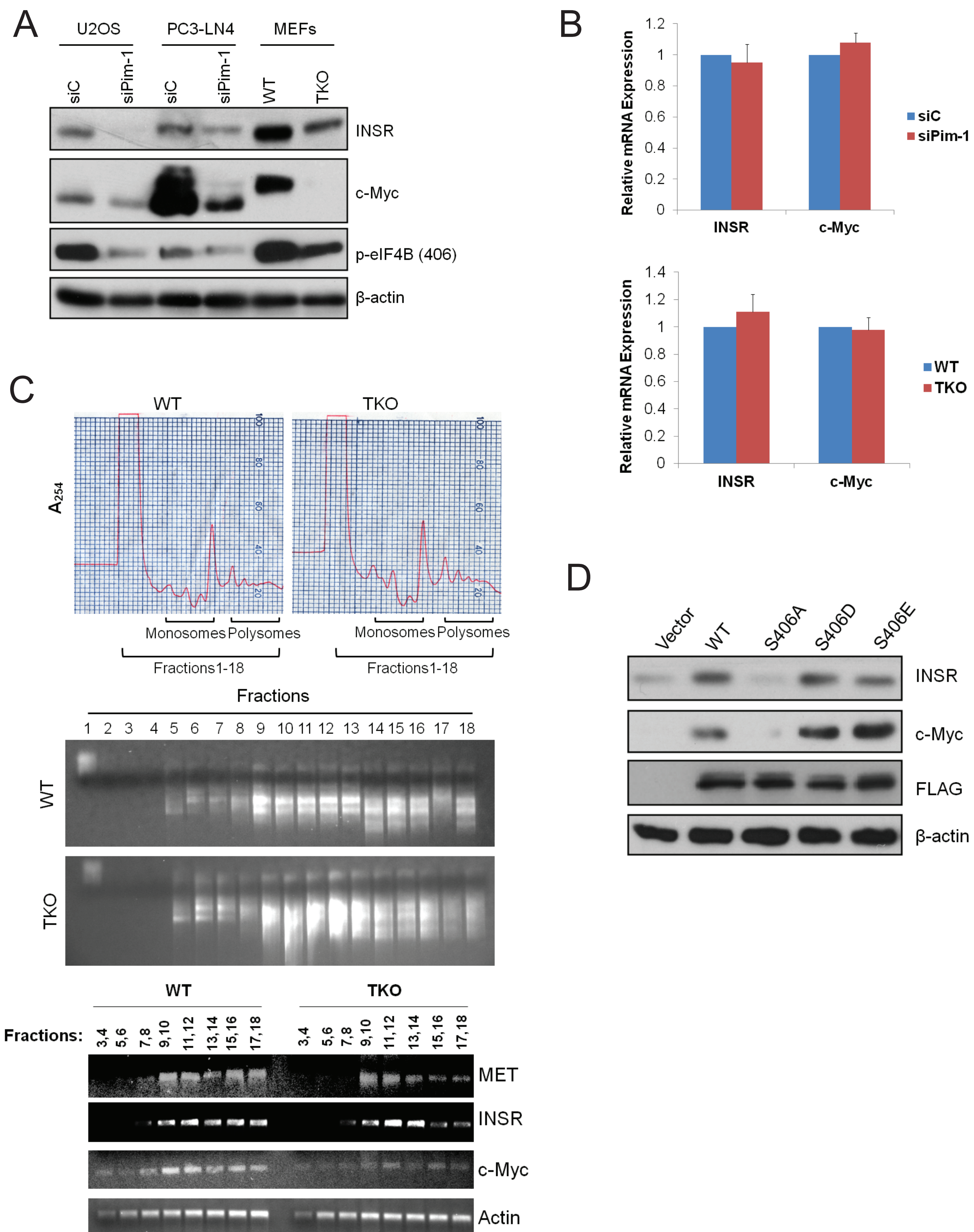


Fig. S7

Phase I Clinical Trial of AZD1208 (NCT01489722)
Patient Information

Patient	Drug Dose (mg)	Sample Blast Count	
		Bone Marrow	Peripheral Blood
1009	480	unknown	unknown
2003	240	89% blasts	17×10^9/L
2012	900	unknown	8×10^9/L
2013	900	41% blasts	17×10^9/L
2016	700	75% blasts	$<1 \times 10^9$/L
3002	240	80% blasts	22×10^9/L
3010	700	unknown	unknown

Table S1