RNA-seq identifies clinically relevant fusion genes in leukemia including a novel MEF2D/CSF1R fusion responsive to imatinib.

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Letter to the Editor

To date, more than 500 fusion genes have been described in hematological malignancies, yet only a small fraction of these are sufficiently common, and with a known clinical impact, to justify routine clinical analysis.[1] For this small subgroup, however, their presence can provide crucial information for diagnosis, prognosis and treatment. For example, the presence of fusion genes such as *BCR/ABL1* in chronic myeloid leukemia (CML) and *PML/RARA* in acute promyelocytic leukemia warrants treatment specifically targeting the fusion proteins.[2,3] Other gene fusions, for which there might not exist targeted therapies, are used to risk stratify leukemias into different treatment groups; for example *ETV6/RUNX1*, *TCF3/PBX1*, and various *MLL*-fusions in childhood B-cell acute lymphoblastic leukemia (ALL).[4] The proportion of cases harboring known clinically relevant fusion genes varies substantially between hematological malignancies, but in ALL and acute myeloid leukemia (AML) between 25-45% of the cases are positive for any of the clinically relevant fusion genes.[4,5] To what extent the remaining cases harbor novel fusions, perhaps also suitable for targeted treatment, is not known, largely because the process of identifying new gene fusions has been laborious.

Here, we describe how RNA sequencing (RNA-seq) can be used as a reliable tool to identify known clinically relevant fusion genes, in addition to novel fusions. Fifteen leukemic cell lines that were derived from either ALL, AML, or CML containing a known prognostically relevant fusion gene were included in the study (Table 1). In addition to these, one primary preB-ALL, negative for *BCR/ABL1*, *TCF3/PBX1*, and *ETV6/RUNX1*, but harboring a t(1;5)(q21;q33), was studied (for a detailed case history, see Supplementary Materials and Methods). Massively parallel sequencing was performed on mRNA libraries from all samples, generating between 12 and 21 million read

pairs for each library (Table S1 and Supplementary Materials and Methods). A list of 42 candidate fusion genes was identified using chimerascan[7] followed by a filtering approach. Of these, 31 (74%) could be verified as genuine gene fusions using RT-PCR and Sanger sequencing (Table 1). Notably, this included all fifteen clinically relevant fusion genes previously known to be present in the cell lines. Seven of the remaining verified fusions were reciprocal to previously known fusions and nine were novel. Of the nine novel fusions, seven were expected to produce non-functional proteins or no protein, either because the translated regions were fused out of frame or because of fusion to untranslated regions. However, these non-functional fusions contained genes such as *RUNX1*, *NUP214*, and *EBF* (Table 1) that are known to be involved in leukemogenesis in several types of aberrations, for example in-frame fusions.[1] Hence, it seems as if the establishment of truncating fusion genes is an alternative way to impair the function of important genes during leukemogenesis. Similar out-of-frame fusion genes were recently shown to be present in 31/200 AMLs (16%).[8]

In the patient sample, a novel in-frame *MEF2D/CSF1R* fusion consistent with the described t(1;5)(q21;q33) could be identified. Interestingly, a clinical FISH analysis with a commercially available probe targeting *PDGFRB* (Abbott Molecular, Des Plaines, IL, USA) had previously indicated that *PDGFRB* was the gene rearranged on 5q33. However, *CSF1R* and *PDGFRB* are located only 500 bp apart and the commercial FISH probe would not differentiate between a *CSF1R* and a *PDGFRB* rearrangement, further illustrating the importance of unbiased RNA sequencing in finding this fusion.

The *MEF2D*/*CSF1R* transcript, fusing exon 7 of *MEF2D* to exon 12 of *CSF1R*, represents a novel fusion whose constituent genes have been described in a single fusion gene each prior to this.[1] The predicted fusion protein retains the MADS-box (a DNA-binding and dimerization domain) from MEF2D and the tyrosine kinase domain of CSF1R (Figure 1A). In analogy with other oncogenic fusion genes containing receptor tyrosine kinases (such as *ETV6/PDGFRB* and *ZMYM2/FGFR1*), it seems plausible that dimerization of the fusion protein through the MADS-box

domain renders a constitutively active tyrosine kinase.[9]

Normal CSF1 signaling through CSF1R is known to regulate macrophage development and was recently shown to instruct myeloid lineage fate decisions in hematopoietic stem cells.[10] Although *CSF1R* mutations were initially reported to be common in AML and MDS,[11] this has not been confirmed in later large scale sequencing studies,[8] indicating that the hybridization-based technique used in the initial study might have had a high false-positive rate. However, two functionally validated *CSF1R* alterations have been described in hematological malignancies – both in myeloid neoplasms.[12,13] Hence, the *MEF2D/CSF1R* fusion gene is the first described *CSF1R* alteration in a lymphoid neoplasm.

To verify that the novel *MEF2D/CSF1R* fusion gene encodes a constitutively active tyrosine kinase that is necessary for the leukemic cells, we examined the *in vitro* sensitivity of patient-derived leukemic bone marrow cells to two TKIs: imatinib, which is a clinically available TKI known to inhibit several tyrosine kinases including CSF1R, and GW2580, an inhibitor with a specific activity against CSF1R (Figure 1B).[14,15] This revealed that imatinib impaired cell growth of the leukemic cells carrying *MEF2D/CSF1R* to a similar degree as that of leukemic cells from a CML carrying *BCR/ABL1*. Treatment with GW2580 effectively impaired cell growth of the leukemic cells with *MEF2D/CSF1R* while CML cells did not respond. Considering that GW2580 specifically inhibits CSF1R, this demonstrates that the leukemic cells are dependent on the activity of the novel MEF2D/CSF1R fusion protein, identifying the fusion gene as a genuine driver mutation. However, while GW2580 is not available for clinical use, the sensitivity to imatinib suggests that this compound could constitute a viable treatment alternative.

In conclusion, we have demonstrated that unbiased mRNA sequencing can identify a number of known clinically relevant fusion genes as well as novel fusion genes in cell lines and clinical leukemia samples. The identification of a novel *MEF2D/CSF1R* in a preB-ALL, sensitive to *in vitro* treatment with imatinib and the CSF1R specific inhibitor GW2580, also demonstrates that

RNA-seq may have a direct clinical impact for treatment selection.

Supplementary information is available at the journal's website.

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Conflict of interest

The authors declare no conflict of interest.

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References

1. Mitelman F, Johansson B, Mertens F. Mitelman database of chromosome aberrations in cancer. 2013. http://cgap.nci.nih.gov/Chromosomes/Mitelman
2. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001; **344**: 1038–1042.
3. Warrell RP, Frankel SR, Miller WH, Scheinberg DA, Itri LM, Hittelman WN, et al. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoic acid). *N Engl J Med* 1991; **324**: 1385–1393.
4. Pui C-H, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med* 2004; **350**: 1535–1548.
5. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. *Blood Reviews* 2004; **18**: 115–136.
6. Chase A, Ernst T, Fiebig A, Collins A, Grand F, Erben P, et al. *TFG*, a target of chromosome translocations in lymphoma and soft tissue tumors, fuses to *GPR128* in healthy individuals. *Haematologica* 2010; **95**: 20–26.
7. Iyer MK, Chinnaiyan AM, Maher CA. ChimeraScan: a tool for identifying chimeric transcription in sequencing data. *Bioinformatics* 2011; **27**: 2903–2904.
8. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* 2013; **368**: 2059–2074.
9. Toffalini F, Demoulin J-B. New insights into the mechanisms of hematopoietic cell transformation by activated receptor tyrosine kinases. *Blood* 2010; **116**: 2429–2437.
10. Mossadegh-Keller N, Sarrazin S, Kandalla PK, Espinosa L, Stanley ER, Nutt SL, et al. M-CSF instructs myeloid lineage fate in single haematopoietic stem cells. *Nature* 2013; **497**: 239–243.
11. Ridge SA, Worwood M, Oscier D, Jacobs A, Padua RA. *FMS* mutations in myelodysplastic, leukemic, and normal subjects. *Proc Natl Acad Sci U S A* 1990; **87**: 1377–1380.
12. Gu T, Mercher T, Tyner JW, Goss VL, Walters DK, Cornejo MG, et al. A novel fusion of *RBM6* to

CSF1R in acute megakaryoblastic leukemia. *Blood* 2007; **110**: 323–333.

13. Chase A, Schultheis B, Kreil S, Baxter J, Hidalgo-Curtis C, Jones A, et al. Imatinib sensitivity as a consequence of a CSF1R-Y571D mutation and CSF1/CSF1R signaling abnormalities in the cell line GDM1. *Leukemia* 2008; **23**: 358–364.
14. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr–Abl positive cells. *Nat Med* 1996; **2**: 561–566.
15. Conway JG, McDonald B, Parham J, Keith B, Rusnak DW, Shaw E, et al. Inhibition of colony-stimulating-factor-1 signaling *in vivo* with the orally bioavailable cFMS kinase inhibitor GW2580. *Proc Natl Acad Sci U S A* 2005; **102**: 16078–16083.

Table 1. Fusion genes detected by RNA sequencing

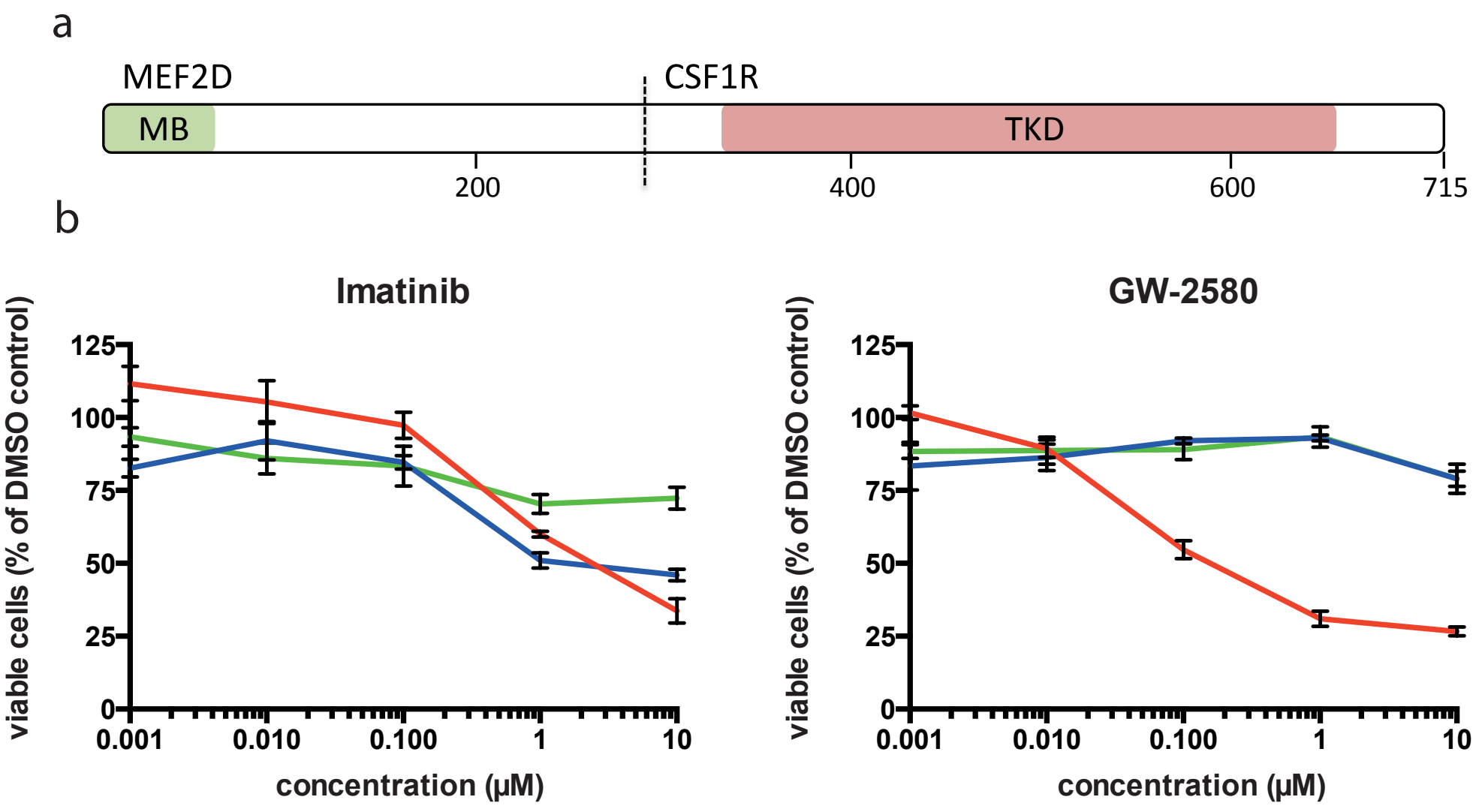
Sample	Disease	Detected fusion genes
REH cell line	ALL	***ETV6/RUNX1*** *RUNX1/PRDM7*[a]
AT-1 cell line	ALL	***ETV6/RUNX1*** *RUNX1/ETV6* *PVT1/EBF1*[a]
RCH-ACV cell line	ALL	***TCF3/PBX1***
697 cell line	ALL	***TCF3/PBX1***
ML2 cell line	AML	***MLL/MLLT4***
Mono Mac 6 cell line	AML	***MLL/MLLT3*** *MLLT3/MLL* *RUNX1/ATP8A2*[a]
THP-1 cell line	AML	***MLL/MLLT3*** *CSNK2A1/DDX39B*[a] *DDX39B/CSNK2A1*[a] *SNAPC3/MLL*[a]
MV 4;11 cell line	AML	***MLL/AFF1*** *AFF1/MLL*
SEM cell line	ALL	***MLL/AFF1***
RS 4;11 cell line	ALL	***MLL/AFF1*** *AFF1/MLL* *TFG/GPR128*[b]
SD-1 cell line	ALL	***BCR/ABL1* p190**
TOM-1 cell line	ALL	***BCR/ABL1* p190** *ABL1/BCR*
SUPB15 cell line	ALL	***BCR/ABL1* p190** *ABL1/BCR*
BV-173 cell line	CML	***BCR/ABL1* p210** *ABL1/BCR*
K562 cell line	CML	***BCR/ABL1* p210** *NUP214/XKR3*[a]
Patient sample	ALL	*MEF2D/CSF1R*

Fusion genes previously known to be present in the sample are indicated in **bold type**.
[a]Out-of-frame fusion gene.
[b]Previously described to be a normal germline variant.[6]

Figure Legends

Figure 1. Structure of MEF2D/CSF1R and sensitivity to tyrosine kinase inhibitors

(**a**) The predicted protein structure of MEF2D/CSF1R. The MADS-box (MB) from MEF2D and the tyrosine kinase domain (TKD) from CSF1R are predicted to be retained in the fusion protein. (**b**) *In vitro* sensitivity to tyrosine kinase inhibitors. Bone marrow derived cells representing ALL (with *MEF2D/CSF1R*), CML (with *BCR/ABL1*), and normal bone marrow were cultured in the presence of imatinib or GW2580 at the indicated concentrations. Corresponding cultures containing only DMSO, in concentrations matching those of the tyrosine kinase inhibitor solutions, were used for normalization. Imatinib hampered the growth of both ALL and CML cells, whereas the CSF1R-specific inhibitor GW2580 had a marked effect only on the ALL cells carrying the *MEF2D/CSF1R* fusion gene.


a
MEF2D
CSF1R
MB
TKD
200
400
600
715
b
Imatinib
GW-2580
viable cells (% of DMSO control)
125
100
75
50
25
0
0.001
0.010
0.100
1
10
concentration (μM)
ALL with MEF2D/CSF1R
CML with BCR/ABL1 (p210)
Normal bone marrow cells

RNA-seq identifies clinically relevant fusion genes in leukemia including a novel *MEF2D/CSF1R* fusion responsive to imatinib

Henrik Lilljebjörn *et al*.

Supplementary information

Supplementary Materials and Methods

Patient case history

A 22-year-old female was diagnosed with a pre-B acute lymphoblastic leukemia (ALL) in August 2011. Peripheral blood (PB) cell counts were: WBC, 19 x 10^9 /l; platelets 90 x 10^9 /l, and Hb 90 g/l. A bone marrow aspirate showed maximal cellularity with a dominance (91%) of blast cells displaying the following immunophenotype, consistent with a pre-B ALL: CD45+/CD34+/CD19+/CD10+/CD20+/CD22+/CD123+/TdT+/cCD79a+. Cytogenetic characterization revealed the following karyotype: 46,XX,t(1;5)(q21;q33)[17]/42-48,idem,+8[cp2]/48,idem+der(5)t(1;5)(q21;q33),+21[2]/46,XX[3]. Given the breakpoint at 5q32-33, in which the *PDGFRB* gene is localized, fluorescent in situ hybridization (FISH) analysis was performed using a break-apart probe (LSI *PDGFRB* Dual Color Break Apart Rearrangement Probe, Abbott Molecular). FISH-analysis revealed a split signal, suggestive of a rearrangement of *PDGFRB.* However, RNA sequencing (see "Results and discussion") of the diagnostic sample, performed when the patient had entered complete remission, detected a fusion of the *MEF2D* gene at 1q22 and the *CSFIR* gene located only 500 bp centromeric of *PDGFRB* in 5q32-33. Subsequent RT-PCR analysis confirmed the presence of a *MEF2D/CSF1R* fusion transcript. Notably, given that the commercial probe set used to detect a *PDGFRB*-rearrangement also covers the *CSF1R* locus, it was concluded that initial FISH-analysis had yielded a false-positive result of a *PDGFRB*-rearrangement. The patient received treatment according to the NOPHO 2008 intermediate risk (IR) protocol (NOPHO-ALL 2008, Version 3a, EudraCT number: 2008-003235-20) and a bone marrow aspirate at Day 15 showed 19% remaining blasts by immunophenotypic analysis and 9% using a

PCR-based assay for clonal IGH rearrangements. A bone marrow aspirate on day 28 showed 3% blasts by FACS and 2% by PCR, while cytogenetic analysis revealed a normal karyotype. During the course of treatment, dose reductions in the high-dose methotrexate and mercaptopurine were needed because of side effects, but otherwise the treatment was given according to the protocol. However, in August 2012, approximately one year after start of treatment, an asymptomatic relapse in CNS was diagnosed with 26 x 10^6 CD34+/CD19+/CD10+ cells/L in the liquor. At this stage, no bone marrow relapse could be verified. Intrathecal treatment with dexamethason, cytarabine and methotrexate was started and thereafter an intravenously relapse treatment with rituximab + cytarabine, betamethasone, cyclophosphamide, daunorubicin, and vincristine (R-ABCDV) was given. CNS remission and continued bone marrow remission was seen. Consolidation treatment with vincristin, cytarabin, betamethason and amsacrine (VABA) was given in October 2012. The patient was allografted in December 2012 with 6 x 10^6 CD34+ cells from her sister after a myeloablative conditioning with total body irradiation and high dose cyclophosphamid. She developed a grade IV mucositis, a capillary leak syndrome and an upper gastro-intestinal graft-versus-host-disease that was treated with low dose Prednisolon. Full donor chimerism was seen and a continued bone marrow remission. In July 2013 she developed a severe headache and a new CNS-relapse with >1000 x 10^6 CD34+/CD19+/CD10+ leukemic cells could be seen in the liquor. No bone marrow relapse was seen. Treatment with imatinib was considered, but due to its poor penetration of the blood brain barrier,[1] only intrathecal chemotherapy was given. At the last follow-up in September 2013, the patient was in continued remission. Should a bone marrow relapse be seen, treatment with imatinib will be reconsidered. This study was approved by the Research Ethics Committee of Lund University.

mRNA sequencing

RNA from fifteen cell lines and one patient sample was prepared for sequencing using the Truseq RNA sample preparation kit v2 (Illumina, San Diego, CA, USA). In brief, 500 ng of total RNA was

enriched for poly-A tailed RNA using magnetic oligo-dT beads. The poly-A tailed enriched RNA was diluted in the "Elute, Prime, Fragment" buffer (part of the Truseq RNA sample preparation kit) and subjected to thermal fragmenting at 94 ºC for 10s to generate fragments with a median size of 200 nucleotides. Single stranded cDNA was synthesized from the fragmented RNA using Superscript II reverse transcriptase (Life Technologies, Carlsbad, CA, USA). A second DNA strand was produced using DNA polymerase I and RNase H. The DNA ends were repaired and the 3' ends were adenylated. Indexed sequencing adapters were ligated to the double stranded cDNA fragments. The adapter-bound fragments were purified and enriched using a 15-cycle PCR with primers complementary to the adapters. The RNA libraries were sequenced on a HiscanSQ (Illumina) generating paired end 2x100 bp reads.

Bioinformatic analysis

Base calling of the sequence data was performed using Casava 1.8.2 (Illumina). Candidate fusion transcripts were identified using chimerascan 0.4.5.[2] Only candidate fusions with 1) at least one read covering the fusion breakpoint, and 2) a fusion junction not matching a known expressed sequence or normal human genomic region, as determined using BLAST (http://blast.ncbi.nlm.nih.gov/), were considered. Candidate fusion transcripts involving adjacent genes or genes within the same 100 kb region were discarded unless the region had a copy number change as determined by single nucleotide polymorphism array (SNP-array) copy number profiling. All candidate fusion transcripts were validated using RT-PCR and Sanger sequencing. To estimate the number of supporting reads for the known fusion genes, a "virtual" genome containing the inferred fusion transcripts as well as normal transcripts for each affected gene was constructed. All reads were mapped to this genome using Bowtie2,[3] and read pairs mapping uniquely to a fusion transcript with a mapping quality above 30 were considered as supporting the presence of the fusion gene.

RT-PCR and sequencing

The presence of candidate fusion transcripts were validated using reverse transcriptase polymerase chain reaction (RT-PCR). In brief, complementary DNA was produced from 500 ng RNA using M-MLV reverse transcriptase (Life Technologies) and random primers (Life Technologies). An inner and outer primer pair were designed for each candidate fusion transcript using primer3 (http://frodo.wi.mit.edu/). RT-PCR for the candidate fusion transcripts were performed using Accuprime taq polymerase system (Life Technologies) according to the manufacturer's specifications.

SNP array analysis

DNA from all cell lines was analyzed using Affymetrix genome-wide human SNP 6.0 arrays in accordance with the manufacturers instructions. In brief, 500 ng DNA was divided into two equal parts where one was digested using StyI and the other digested using NspI (New England Biolabs, Ipswich, MA, USA). Each digested DNA sample was ligated to oligonucleotide adaptors using T4 DNA ligase (New England Biolabs) and amplified by PCR using adaptor-specific primers. The NspI and StyI digested DNA were pooled and purified using Ampure XP beads (Beckman Coulter Genomics, Danvers, MA, USA). The purified amplification product was fragmented using DNase I (Affymetrix, Santa Clara, CA, USA) and labeled with Labeling reagent (Affymetrix). The labeled DNA was injected into genome-wide human SNP 6.0 arrays and hybridized at 50 °C for 16–18 h while the arrays where rotating at 60 rpm. The arrays were washed and stained in a Fluidics station 450 (Affymetrix) and scanned in a Genechip scanner 3000 7G (Affymetrix). CEL files containing raw signal intensities were exported from the Genechip operating software (Affymetrix). The CEL files were imported into Genotyping console (Affymetrix) to produce genotype calls using the "birdseed-v2" algorithm. The raw signal intensities and genotype calls were imported into dChip,[4] and the raw signal intensities were normalized to a baseline level using an invariant set of probes. In dChip, the "expression value" of each SNP was calculated from the raw signal intensities using

model based expression[5] with only perfect match probes. Changes in copy number were calculated using median smoothing with a 11 SNP window. A reference copy number was calculated from all samples by trimming the 25% extreme values in both ends.

Tyrosine kinase in vitro sensitivity assay

Primary bone marrow mononuclear cells from the *MEF2D/CSF1R*-positive patient, one CML patient, and one healthy volunteer were thawed and resuspended in serum-free Stemspan SFEM medium (StemCell Technologies Inc) with 100 units/mL penicillin and 100 g/mL streptomycin. The medium was supplemented with the following cytokines (Peprotech, Rocky Hill, NJ, USA): 100 ng/mL stem cell factor, 50 ng/mL trombopoietin, 100 ng/mL Flt-3-ligand, 25 ng/mL IL-3, and 10 ng/mL IL-6. Cells were plated in 48-well tissue culture treated plates at a density of 100000 cells per well. Tyrosine kinase inhibitors (TKI) Imatinib (Selleck Chemicals, Houston, TX, USA) and GW2580 (LC Laboratories, Woburn, MA, USA) were dissolved in DMSO to 10 μM concentration and stored at -80°C. At experiment start, TKIs were further diluted in PBS before addition to the wells to reach the final working concentrations of 0,001 – 10 μM. Cells were cultured in three replicates at each concentration, and a corresponding amount of DMSO was used as control. The plates were incubated for 72 hours, after which the cells were harvested and counted using Countbright beads (Life Technologies) in a FACS Canto II (Becton Dickinson, Franklin Lakes, NJ, USA), according to the manufacturer's instructions.

Supplementary Tables

Table S1. RNA sequencing data.

Sample	Total number of read pairs	Clinically relevant fusion gene	Supporting read pairs (identified by chimerascan)	Supporting read pairs (mapping against known transcript)
REH cell line	14 000 000	*ETV6/RUNX1*	11	19
AT-1 cell line	13 000 000	*ETV6/RUNX1*	24	28
RCH-ACV cell line	16 000 000	*TCF3/PBX1*	104	156
697 cell line	18 000 000	*TCF3/PBX1*	37	65
ML2 cell line	15 000 000	*MLL/MLLT4*	18	34
Mono Mac 6 cell line	19 000 000	*MLL/MLLT3*	5	16
THP-1 cell line	15 000 000	*MLL/MLLT3*	5	13
MV 4;11 cell line	17 000 000	*MLL/AFF1*	4	10
SEM cell line	15 000 000	*MLL/AFF1*	8	19
RS 4;11 cell line	14 000 000	*MLL/AFF1*	4	10
SD-1 cell line	14 000 000	*BCR/ABL1* p190	3	5
TOM-1 cell line	17 000 000	*BCR/ABL1* p190	29	37
SUPB15 cell line	14 000 000	*BCR/ABL1* p190	25	38
BV-173 cell line	14 000 000	*BCR/ABL1* p210	14	29
K562 cell line	12 000 000	*BCR/ABL1* p210	12	24
Patient sample	21 000 000	*MEF2D/CSF1R*	120	245

References

1. Takayama N, Sato N, O'Brien SG, Ikeda Y, Okamoto S-I. Imatinib mesylate has limited activity against the central nervous system involvement of Philadelphia chromosome-positive acute lymphoblastic leukaemia due to poor penetration into cerebrospinal fluid. *Br J Haematol* 2002; **119**: 106–108.
2. Iyer MK, Chinnaiyan AM, Maher CA. ChimeraScan: a tool for identifying chimeric transcription in sequencing data. *Bioinformatics* 2011; **27**: 2903–2904.
3. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Meth* 2012; **9**: 357–359.
4. Lin M, Wei L-J, Sellers WR, Lieberfarb M, Wong WH, Li C. dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. *Bioinformatics* 2004; **20**: 1233–1240.
5. Li C, Wong WH. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc Natl Acad Sci U S A* 2001; **98**: 31–36.