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(54) Title: METHOD FOR DIAGNOSING ACUTE BASOPHILIC LEUKEMIA

(57) Abstract: The present invention relates to a method for diagnosing acute basophilic leukemia in a subject comprising the step of detecting the presence or absence in a biological sample obtained from said subject of a MYB-GATA1 fusion wherein the presence of said MYB-GATA1 fusion is indicative that said subject suffers from acute basophilic leukemia.



Method for diagnosing acute basophilic leukemia

FIELD OF THE INVENTION

The present invention relates to a method for diagnosing acute basophilic leukemia and to a method for producing granulocytic cells.

BACKGROUND OF THE INVENTION

Acute myeloid leukemia (AML), a hematopoietic disorder characterized by abnormal proliferation and blockage of myeloid progenitors at various stages of differentiation, accounts for 15-20% of cases of childhood leukemia (Aquino, 2002). Acute basophilic leukemia (ABL) is a rare subtype of acute leukemia, classified as AML not otherwise specified in the last World Health Organization (WHO) classification (Swerdow et al., 2008). Clinical features are close to those of poorly differentiated AML with, in some cases, symptoms related to hyperhistaminemia due to excessive expansion of basophil compartment (Duchayne et al., 1999). The diagnosis of such disease is associated with the detection of basophilic blast cells.

However, the lineage identity of blast cells is not easy to establish since these cells often appear poorly differentiated, explaining that a part of these leukemia are classified as AML with minimal differentiation.

AML are often associated with translocations that create chimeric genes encoding oncogenic proteins (Martens and Stunnenberg, 2010). To date, due to the rarity of ABL cases, no recurrent cytogenic abnormality has been described. Rare cases of t(X;6)(p11;q23) translocations have been reported but these were sporadic and were not investigated molecularly (Chessels et al., 2002; Dastugue et al., 1997).

This difficulty to diagnose ABL both by classical clinical and by cytogenic approaches could lead to an underestimation of the real incidence of ABL.

Therefore, there is a need for a reliable and easy to carry out method for diagnosing ABL.

SUMMARY OF THE INVENTION

The present invention relates to a method for diagnosing acute basophilic leukemia in a subject comprising the step of detecting the presence or absence in a biological sample obtained from said subject of a MYB-GATA1 fusion wherein the presence of said MYB-GATA1 fusion is indicative that said subject suffers from acute basophilic leukemia.

The present invention also relates to a MYB-GATA1 fusion protein.

The invention also provides a method for producing granulocytic cells comprising the step of transforming lineage negative (lin-) cells with a *MYB-GATA1* fusion gene.

DETAILED DESCRIPTION OF THE INVENTION

5 Definitions

MYB is a well-known transcriptional regulator. MYB contain three functional key domains, an N-terminal DNA-binding domain comprised of three tandem 50 amino acid myb repeats that specifically bind to the sequence PyAACG/TG, a centrally located transcription activation domain and a C-terminal negative regulatory domain involved in transcriptional repression (Lieu, et al. (2009) ; Oh IH et al., (1999) ; Ramsay RG et al., (2008)).

GATA1 also named erythroid transcription factor or GATA-binding factor 1 is a zinc finger (zf) transcription factor(Cantor AB et al. (2002)). It plays a central role in erythroid development. It was first identified by its ability to bind functionally important DNA regulatory sequences found in globin genes (Tsai et al., 1989; Evans and Felsenfeld, 1989).

15 Since then, GATA-binding motifs ((T/A)GATA(A/G)) have been identified in the promoters and/or enhancers of virtually all erythroid and megakaryocytic-specific genes studied (Orkin, 1992; Weiss and Orkin, 1995a). GATA-1 contains two zinc fingers, both of the Cys-X2-Cys-X17-Cys-X2-Cys configuration. The carboxyl terminal zinc finger is responsible for high-affinity DNA binding, whereas the amino terminal zinc finger stabilizes the interaction
20 (Martin and Orkin, 1990).

As used herein, the term “MYB-GATA1 fusion” refers to a *MYB-GATA1* fusion gene, a MYB-GATA1 fusion mRNA or a MYB-GATA1 fusion protein resulting from the fusion of a portion of the *MYB* gene encoding the N-terminal DNA binding domain and trans-activation
25 domain of MYB and a portion of the *GATA1* gene encoding the c-terminal zinc finger of GATA1.

The *MYB-GATA1* fusion gene is the gene resulting from this fusion.

The *MYB-GATA1* fusion gene comprises:

a) at least exons 1 to 8 of *MYB*, preferably at least exon 1 to a part of exon 9, even more
30 preferably exons 1 to 9 of *MYB*
fused to

b) at least a part of exon 5 and exon 6 of *GATA1*, preferably exons 5 to 6 of *GATA1*.

Even more preferably, *MYB-GATA1* fusion gene comprises exons 1 to 9 of MYB fused to exons 5 to 6 of *GATA1*.

MYB-GATA1 fusion gene is transcribed into MYB-GATA1 fusion mRNA.

MYB-GATA1 fusion mRNA is translated into MYB-GATA1 fusion protein.

MYB-GATA1 fusion protein comprises:

a) at least amino acids 1 to 285, preferably at least amino acids 1 to 295, preferably at least amino acids 1 to 305 preferably at least amino acids 1 to 315, even more preferably at least amino acids 1 to 327 of MYB, that is called hereafter the MYB portion

fused to

b) at least amino acids 282 to 413, preferably at least amino acids 262 to 413, preferably at least amino acids 272 to 413, even more preferably amino acids 282 to 413 of GATA1 –that is called hereafter the GATA1 portion-.

Basophils are a type of bone marrow derived granulocytes that represent less than 1% of the circulating leukocytes. Their nuclei are less heterochromatic than those of other granulocytes. These cells contain large irregularly shaped specific granules that stain with basophilic dyes. These specific granules are similar to granules of mast cells in that they contain histamine and heparin-like compounds.

Lineage negative cells (lin⁻ cell) are cells that doesn't express "lin" specific antigens comprising CD2, CD3, CD4, CD5, CD8, NK1.1, B220 TER-119, GR-1 or express them at very low levels. Monoclonal antibody cocktails directed against these lineage markers are used to remove cells expressing these antigens from source tissues (for example, bone marrow, umbilical cord blood, mobilized peripheral blood, or fetal liver). This negative selection procedure yields a population of cells that is enriched for primitive hematopoietic stem cells or very early progenitor cells or precursor cells that do not (yet) express these markers.

Diagnostic method

The present invention relates to a method for diagnosing acute basophilic leukemia in a subject comprising the step of detecting the presence or absence in a biological sample obtained from said subject of a MYB-GATA1 fusion wherein the presence of said MYB-GATA1 fusion is indicative that said subject suffers from acute basophilic leukemia.

Indeed, the inventors have demonstrated that t(X;6)(p11;q23) translocation reported by Chessels et al. generates surprisingly a MYB-GATA1 fusion.

More precisely, the inventors have demonstrated that it is not only a translocation that is involved in ABL but a translocation and an inversion. Indeed, *MYB* and *GATA1* having

opposite orientations only a translocation could not lead to a functional *MYB-GATA1* fusion gene able to express a MYB-GATA1 fusion protein.

In one embodiment, the biological sample is a bone marrow sample or a blood sample.

In a preferred embodiment, the biological sample is a bone marrow sample.

- 5 The bone marrow sample may be collected using standard medical practices, for example, using bone marrow biopsy or using bone marrow aspiration (B J Bain , 2005).

In a preferred embodiment, the step of detecting the presence or absence of a MYB-GATA1 fusion comprises the step of detecting a MYB-GATA1 fusion mRNA.

- 10 Fusion mRNA may be detected using a variety of techniques well known in the art.

For example, nucleic acids contained in the sample are extracted according to standard methods (e. g. using lytic enzymes or chemical solutions or extracted by nucleic acid binding resins following the manufacturer's instructions). Then, the extracted mRNA are detected by hybridization (e. g. Northern blot) and/or amplification (e. g. RT-PCR) methods.

15

In a preferred embodiment, fusion mRNA is detected by RACE-PCR.

For example, after extraction with Trizol method (Invitrogen), total RNA are reverse-transcribed into cDNA by using an oligo dT-anchor primer (5'/3' RACE kit, 2nd generation; Roche). This cDNA product is amplified by PCR using the PCR anchor primer having the
20 nucleotide sequence as set forth in SEQ ID NO 1 and a *MYB*-specific forward primer, for example MYB-1F having the nucleotide sequence as set forth in SEQ ID NO 2 or MYB-8F having the nucleotide sequence as set forth in SEQ ID NO 3 or a *GATA1*-specific forward primer, for example GATA1-2R having the nucleotide sequence as set forth in SEQ ID NO 4.

- 25 In another embodiment, fusion mRNA may be detected by RT-PCR. A *MYB*-specific reverse primer or a *GATA1*-specific reverse primer may be used as primer for reverse transcription and reverse primer and respectively a *MYB*- or a *GATA1*- specific forward primer when reverse primer was a *GATA1*- or *MYB* specific reverse primer for PCR.

- 30 Above cited primers are cited as examples they should not be interpreted in any way as limiting the scope of the present invention. A person skilled in the art will easily design suitable *GATA1* and *MYB* reverse or forward primers.

Other amplification methods include but are not limited to transcription mediated amplification (TMA), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA).

- 5 The present invention also relates to a kit for diagnosing acute basophilic leukemia comprising means for detecting in a biological sample the presence or absence of a MYB-GATA1 fusion.

10 In one embodiment, the means for detecting the presence or absence of said MYB-GATA1 fusion comprise a *MYB* forward primer and/or a *GATA1* forward primer.

The kit may also include, for example, PCR buffers, enzymes, positive control sequences and reaction control primers.

- 15 In another preferred embodiment, the step of detecting the presence or absence of said MYB-GATA1 fusion comprises the step of detecting a MYB-GATA1 fusion protein.

MYB-GATA1 fusion protein may be detected using a variety of methods well known in the art.

These methods include but are not limited to Western blot and ELISA.

20

Binding partner(s) capable of selectively interacting with a MYB-GATA1 fusion protein or a fragment thereof may be suitable for use in these methods.

- 25 In one embodiment, a binding partner used for detecting a MYB-GATA1 fusion protein is specific for a MYB-GATA1 fusion protein and does not interact with MYB or GATA1 alone. Thereafter, unless otherwise indicated, that binding partner is called “anti-MYB-GATA1 fusion portion”.

- 30 In another embodiment, a binding partner used for detecting MYB-GATA1 fusion protein is specific for the MYB portion of MYB-GATA1 fusion protein or a fragment thereof. Thereafter, unless otherwise indicated, that binding partner is called “anti-MYB”.

In another embodiment, a binding partner used for detecting MYB-GATA1 fusion protein is specific for the GATA1 portion of MYB-GATA1 fusion protein or a fragment thereof. Thereafter, unless otherwise indicated, that binding partner is called "anti- GATA1".

- 5 The binding partner may be generally an antibody that may be polyclonal or monoclonal, preferably monoclonal.

Polyclonal antibodies directed against MYB-GATA1 fusion protein or a fragment thereof can be raised according to known methods by administering the appropriate antigen or epitope to a host animal selected, e.g., from pigs, cows, horses, rabbits, goats, sheep, and mice, among
10 others. Various adjuvants known in the art can be used to enhance antibody production. Although antibodies useful in practicing the invention can be polyclonal, monoclonal antibodies are preferred.

Monoclonal antibodies against MYB-GATA1 fusion protein or a fragment thereof can be prepared and isolated using any technique that provides for the production of antibody
15 molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique originally described by Kohler et al. Nature. 1975;256(5517):495-7; the human B-cell hybridoma technique (Cote et al Proc Natl Acad Sci U S A. 1983;80(7):2026-30); and the EBV-hybridoma technique (Cole et al., 1985, In Monoclonal Antibodies and Cancer Therapy (Alan Liss, Inc.) pp. 77-96). Antibodies useful in
20 practicing the present invention also include binding partners against MYB-GATA1 fusion protein or a fragment thereof including but not limited to F(ab')₂ fragments, which can be generated by pepsin digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments.

The binding partners of the invention may be labelled with a detectable molecule or
25 substance, such as a fluorescent molecule, a radioactive molecule or any others labels known in the art. Labels are known in the art that generally provide (either directly or indirectly) a signal.

As used herein, the term "labeled", with regard to the binding partner, is intended to encompass direct labeling of the binding partner by coupling (i.e., physically linking) a
30 detectable substance, such as a radioactive agent or a fluorophore (e.g. fluorescein isothiocyanate (FITC) or phycoerythrin (PE) or Indocyanine (Cy5)) to the binding partner, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. A binding partner of the invention may be labeled with a radioactive molecule by any method

known in the art. For example radioactive molecules include but are not limited radioactive atom for scintigraphic studies such as I123, I124, In111, Re186, Re188.

In a preferred embodiment, MYB-GATA1 fusion protein is detected by Western blot.

- 5 Western blot technique is well known technique. It is in particular described in Burnette WN., Analytical Biochemistry, vol. 112, no 2, Avril 1981, p. 195-203.

Typically, cells from biological sample are lysed, for example with RIPA, and then sonicated. Then total proteins from biological sample are separated using gel electrophoresis, for example a SDS-PAGE. The proteins are transferred out of the gel and onto a membrane,
10 typically polyvinylidene difluoride or nitrocellulose, where they are probed using a labeled binding partner capable of selectively interacting with MYB-GATA1 fusion protein or a fragment thereof.

In a preferred embodiment, the binding partner is an anti-MYB or an anti-GATA1.

In another embodiment, the binding partner is an anti-MYB-GATA1 fusion portion.

15

In another embodiment, MYB-GATA1 fusion protein is detected by ELISA.

Typically, cells from biological sample are lysed, sonicated and microcentrifuged. After suitable dilution, the supernatant is added to wells of a microtiter plate that are coated with a set of primary antibodies.

- 20 In one preferred embodiment, these antibodies are anti-MYB. Then, after a period of incubation sufficient to allow the formation of antibody-antigen complexes, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule, anti-GATA1, added. The plate washed and the presence of the secondary binding molecule detected using methods well known in the art.

- 25 In another preferred embodiment, primary antibody is anti-GATA1 and detectably labeled secondary binding molecule is anti-MYB.

In another embodiment, primary antibody or detectably labeled secondary binding molecule is anti-MYB-GATA1 fusion portion.

- 30 In one embodiment, the means for detecting the presence or absence of MYB-GATA1 fusion of the kit according to the invention comprise a binding partner capable of selectively interacting with MYB-GATA1 fusion protein.

In one embodiment, the means for detecting the presence or absence of said MYB-GATA1 fusion comprise a binding partner capable of selectively interacting with MYB-GATA1 fusion portion.

- 5 In another embodiment, the means for detecting the presence or absence of MYB-GATA1 fusion of the kit according to the invention comprise a binding partner specific of MYB portion of MYB-GATA1 fusion protein or a fragment thereof and with a binding partner specific of GATA1 portion of MYB-GATA1 fusion protein.
- 10 The present invention also relates to the use of MYB-GATA1 fusion as a diagnostic marker of acute basophilic leukemia.

MYB-GATA1 fusion protein and nucleic acid thereof

- 15 The present invention relates to a MYB-GATA1 fusion protein.
- Studying acute basophilic leukaemia, the inventors have discovered a MYB-GATA1 fusion protein comprising the DNA binding domain and trans-activation domain of MYB and c-terminal zinc finger of GATA1.
- 20 A further object of the invention relates to a polynucleotide comprising a sequence encoding said fusion protein.
- Typically, said polynucleotide is a DNA or a RNA molecule, which may be included in any suitable vector, such as plasmid, cosmid, episome, artificial chromosome, phage or viral vector.
- 25 The term "vector" mean the vehicle by which a DNA or a RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.
- So, a further object of the invention relates to a vector comprising a polynucleotide of the invention.
- 30 Such vectors may comprise regulatory elements, such as a promoter, an enhancer, a terminator and the like. These vectors may be any expression vector so long as a gene encoding MYB-GATA1 fusion protein is can be inserted and expressed.
- Examples of such vectors are pMIE or pcDNA3.

A further object of the present invention relates to a host cell which has been transformed by a polynucleotide and/or a vector according to the invention.

The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence into a host cell, so that the host cell will express the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed". As the term "transformation" is used therein, a transfection or an infection by a foreign gene leading to the expression of this gene is a transformation.

Typically, said host cell may be a mammal cell such as a rodent, a feline, a canine, or a primate cell and more particularly a human cell.

Typically, host cells may be lineage negative cells (lin⁻ cells) such as hematopoietic stem cells or lin⁻ CD34⁺ cells.

For example, lineage negative cells may be isolated from bone marrow, umbilical cord blood or placenta by using methods well known in the art (Hideo E. et al, (2007)).

After lysis of red blood cells, lin⁻ cells are isolated by depletion of lin⁺ cells using for example the lineage cell depletion kit (Miltenyi Biotec) or by positive selection of lin⁻ cells. Then, obtained cells are cultured in suitable medium and transformed using a vector according to the invention.

Typically, suitable medium consists of a base medium containing nutrients (a source of carbon and amino acids), a pH buffer and salts, which can be supplemented with serum of human or other origin and/or growth factors and/or antibiotics. For example, suitable medium may be Stempsan (StemCell Technologies), 10% Fetal Bovine Serum (FBS) supplemented with IL3 and IL6.

Method for producing cells

Whereas basophils are known for more the 100 years, their rarity and the lack of useful analytical tools such as model animals has make their study difficult. Recent studies have shown that they play critical roles in systemic anaphylaxis and chronic allergic inflammation (Mukai K et al. (2009)) In particular, they are involved in histamine release.

So, there is a need for new tools in particular new models to study basophils.

Thus, the present invention also relates to a method for producing granulocytic cells comprising the step of transforming lineage negative (lin-) cells with a *MYB-GATA1* fusion gene.

5 Indeed, one symptom of ABL is an excessive growth of basophils in all stages of differentiation. The inventors have found that excessive growth was due to the presence of *MYB-GATA1* fusion gene and that transformation of lineage negative cells with *MYB-GATA1* fusion gene leads to the formation of more granulocytes and less granulomonocytes and monocytes than lineage negative cells without *MYB-GATA1* fusion gene.

10

In one embodiment, the granulocytic cells produced according to the present invention are basophils.

In one embodiment, the granulocytic cells produced according to the present invention are basophilic blast cells.

15

Indeed, the inventors have found that transforming lin- cells with *MYB-GATA1* fusion gene leads in particular to proliferation of basophilic blast cells. Furthermore, they have found that proliferation was a long-term proliferation.

20 In one embodiment, the lineage negative cells are hematopoietic stem cells or lin- CD34+ cells.

These cells are transformed with a *MYB-GATA1* fusion gene as above described, for example by using a pMIE wherein a *MYB-GATA1* fusion gene is cloned as vector.

25

The method according to the invention may comprise a step of culturing transformed cells. Thus, after transformation, cells are seeded in a suitable medium in order that granulocytic cells proliferate. Example of suitable medium is IMDM supplemented with FBS, IL3, IL6 and stem cell factor (SCF).

30

The method according to the invention may comprise a step of isolating granulocytic cells.

Granulocytic cells may be isolated from others cellular types by methods known in the art as depletion of lineage specific markers or by the positive selection of marker.

The present invention also relates to granulocytic cells obtained by a method according to the invention.

5 The granulocytic cells produced according to the present invention provide a new tool to study the role of basophils in immune response. In particular, they provide a new model to investigate histamine release.

Thus, the present invention also provides a method for studying the effect of a molecule on histamine release comprising the steps of transforming lineage negative cell(s) with a *MYB-*
10 *GATA1* fusion gene to produce granulocytic cells, contacting said granulocytic cells with molecule to be tested and measuring the histamine release.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the
15 present invention.

FIGURE LEGENDS

Figure 1: Structure of MYB-GATA1 - Schematic representation of the MYB-GATA1 fusion protein. The fusion between the MYB and GATA1 proteins is indicated by the black arrow.
20 R1, R2 and R3 are three imperfect 52-residue repeats that encompass the DNA-binding domain of MYB; AD is the transcription activation domain of MYB. FD is the c-terminal zinc finger of GATA1.

Figure 2: Expression and location of MYB-GATA1 fusion- Western blotting with anti-GATA1 antibody confirms the expression of MYB-GATA1 fusion protein in patient 2 (P2)
25 compared with HeLa cells transformed with pcDNA3 empty vector as negative control and pcDNA3 comprising MYB-GATA1 fusion gene as positive control.

Figure 3: MYB-GATA1 fusion expression commits cells to the granulocytic lineage and blocks their differentiation. A: Counting of CFUs at day 12. Each graph represents one type of CFU, as indicated. B: Identification of various types of cells by staining with MGG.
30 Immature granulocytic cells correspond to undifferentiated blasts, myeloblasts and promyelocytes; mature granulocytic cells correspond to myelocytes, metamyelocytes and neutrophils.

Figure 4: FACS analysis of cells transformed with MYB–GATA1 fusion and collected from methylcellulose at each plating. Cells remained CD11b+/GR-1+ throughout. Each graph represents the mean values $\pm$ SEM of 4 independent assays. Magnification x 630.

Figure 5: Effects of MYB–GATA1 fusion on proliferation and survival - Number of colonies counted after twelve days of differentiation in methylcellulose in MYB–GATA1 fusion-transformed lin- cells compared to controls.

Figure 6: Effects of MYB–GATA1 fusion on proliferation and survival - In vitro immortalization assay of transformed lin- cells. At each plating, colonies were counted. Cells transformed with MYB–GATA1 fusion continued to form colonies throughout the five platings.

EXAMPLE

Material and methods

Patients

Fresh and thawed samples from two patients (P1 and P2) have been obtained after informed consent and stored at the HIMIP collection, an INSERM malignant hemopathy collection declared to the Ministry of Higher Education and Research (DC 2008-307 collection 1).

Samples from two other patients (P3 and P4) have been obtained from the tumor bank of the Centre Hospitalier Universitaire Bordeaux. (Declared to the Ministry of Higher Education and Research).

Fluorescence in situ hybridization (FISH)

Cytogenetic analysis of diagnostic bone marrow samples was performed by standard methods as previously described (Coyaud E. et al.). As probes for the *MYB* and *GATA1* genes, the BAC and fosmids clones were selected by using of the Human Genome Browser Gateway (<http://genome.ucsc.edu/cgi-bin/hgGateway/>). These BAC and fosmids are shown in Table 1. The probes were labeled by nick translation with dUTP-SpectrumOrange or dUTP-SpectrumGreen (Abbott), as previously described (Coyaud E. et al.). The breakpoints on chromosome X and 6 were mapped by using high-resolution multicolour banding (mBAND) FISH according to the manufacturer's instructions (Metasystems).

Table 1 : Fosmids and BACs used to refine the karyotype of patients

Gene, band, clone	Position in relation to the gene	Fluorochrome
MYB, 6q23		
G248P8806E9	5'	Spectrum green
G248P84467F3	3'	Spectrum orange
RP11-104D9	Span	Spectrum orange
RP11-905P20	Span	Spectrum green
GATA1, Xp11		
G248P85531H11	Telomeric	Spectrum orange
G248P87328H10	Span	Spectrum green
RP11-416B14	Span	Spectrum green

Rapid Amplification of 3' cDNA ends by PCR (RACE-PCR)

Total RNA was extracted according to the Trizol method (Invitrogen). One microgram of total RNA from each of patients was reverse-transcribed into cDNA by using an oligo dT-anchor primer (5'/3' RACE Kit, 2nd Generation; Roche). This cDNA product was used as a template for PCR by using the BD Advantage 2 PCR Enzyme System (BD Biosciences), the PCR anchor primer having the nucleotide sequence as set forth in SEQ ID NO 1 and a *MYB*-specific forward primer, MYB1F having the nucleotide sequence as set forth in SEQ ID NO 2. PCR was carried out in a GeneAmp PCR system 9600 (Perkin Elmer) with the following cycling parameters: 94°C initial denaturation for 1 min, 1 cycle of 94°C for 30 s, 64°C for 30 s, 68°C 40 min, 35 cycles of 94°C for 30 s, 64°C for 30 s, 68°C for 5 min and a final extension of 68°C for 10 min. The PCR product was purified by using the QIAquick Gel Purification Kit (Qiagen) and sequenced with the ABI Prism Dye Terminator Kit (Perkin-Elmer, Applied Biosystems).

Screening of mutations

The mutational status of eight genes (*FLT3*, *NPM1*, *WT1*, *CEBPa*, *K-RAS*, *N-RAS*, *IDH1*, *IDH2*) was analyzed by high-resolution melting (HRM) PCR. DNA mutation screening of *FLT3*-TKD exon 20, *IDH1* and *IDH2* exon 4, *N-RAS* and *K-RAS* exons 2 and 3, *WT1* exons 7 and 9 was performed by HRM with the use of LightCycler 480 (Roche Applied Science) in High Melting Resolution Master Mix 1X (Roche Applied Science) with 10 ng of genomic DNA, 0.1 µmol/L of suitable primer, and 25 mmol/L MgCl₂. High-resolution melting-PCR cycling conditions were: initial denaturation at 95°C for 10 min, followed by 50 cycles at

95°C for 10 s, 63°C for 15 s, and 72°C for 25 s. The melting curve was measured from 72°C to 95°C with 25 acquisitions per degree centigrade. *FLT3* exon 13, *ITD* and *NPM1c* mutation screening was performed by multiplex PCR using Gold Taq DNA polymerase (Applied Biosystems). PCR products were analyzed on a sequencer by using sizing fragment analysis.

5 *CEBPA* screening was performed according to Pabst et al..

Western blotting

Cells were lysed with RIPA and then sonicated. Total proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then
10 electrotransferred onto nitrocellulose membranes. The membranes were blocked with a solution of 10% milk in PBST (0, 1% Tween 20 in PBS) and probed with anti-GATA1 antibody (sc-1234, Santa Cruz) or anti β -actin antibody (Sigma Aldrich) and secondary antibodies diluted in PBST 1 %. The immunoreactive bands were visualized using the enhanced chemiluminescence (ECL) lighting system (Perkin Elmer).

15

Immunolocalization

24 h post-transformation with lipofectamine according to the manufacturer's instructions, HeLa cells were fixed with 3% paraformaldehyde for 10 minutes at room temperature. Cells were permeabilized for 10 minutes with 0.2% Triton X-100 in phosphate-buffer saline (PBS)
20 containing 10 mM HEPES, 3% bovine serum albumin (BSA). Nonspecific sites were saturated with 3% BSA and 3% FCS in the same buffer for 1 hour. The cells were incubated for 1 hour at room temperature with anti-GATA1 antibody (sc-1234, Santa Cruz). After 3 washings with PBS containing 10 mM HEPES and 3% BSA, cells were incubated with the appropriate secondary antibodies (Alexa Fluor 546 anti-goat antibody) for 1 hour at a dilution
25 of 1:300 in the same buffer. Samples were mounted ProLong® Gold antifade reagent with DAPI (Invitrogen).

Cloning of MYB-GATA1 fusion

Full-length *MYB-GATA1* cDNA was amplified with Advantage 2 Polymerase Mix (Clontech)
30 using MYB-8F primer having the nucleotide sequence as set forth in SEQ ID NO 3 and GATA1-2R primer having the nucleotide sequence as set forth in SEQ ID NO 4 and cloned in the pCR®2.1 vector (TA cloning kit, Dual Promoter, Invitrogen). After cleavage of the PCR product with EcoRI or BstXI, *MYB-GATA1* fusion gene was cloned into the pMSCViresEGFP (pMIE) retroviral vector (pMIE-MG) and pcDNA3 respectively.

Preparation of lineage negative (lin-) cells

Bone marrow cells were harvested from C57BL/6 mice aged 6–12 weeks by flushing their femurs, tibias and hips with IMDM, 2% FBS (Invitrogen). After lysis of red blood cells with ammonium chloride–potassium (ACK) buffer and filtration through a 0.70 μ m filter, the cells were washed and counted. Lin- cells were obtained after two rounds of separation using the Lineage Cell Depletion Kit (Miltenyi Biotec) to remove mature hematopoietic cells and their committed precursor, according to the manufacturer's instructions. Cells obtained were seeded 24 h before transduction in untreated plates at a density of 5×10^5 cells/mL in Stemspan (StemCell technologies), 10% Fetal Bovine Serum (FBS) (PAN Biotech) supplemented with 10 ng/mL of IL3, 20ng/mL of IL6 and 40 ng/mL of SCF (Peprotech).

Retrovirus production and transduction.

Cells of the packaging cell line Phoenix-Eco were seeded at a density of 1.6×10^6 cells/60 cm plate the day before transformation. The cells were transiently transformed with 6 μ g of retroviral expression vector by using Lipofectamine 2000 Transfection Reagent (Invitrogen). The next day, cells were treated for 6 h with 10 mM sodium butyrate and then incubated for 24 h at 32°C in IMDM containing 10% fetal bovine serum (FBS). The virus-containing supernatant was collected and filtered through a 0.45 μ m filter and incubated for 15 min with 8 μ g/mL of polybrene (Sigma Aldrich). Lin- cells were re-suspended in this supernatant supplemented with 10 ng/mL of IL3, 20ng/mL of IL6 and 40 ng/mL of FBS at a final density of 10^6 cells/mL. The cells were plated and centrifuged for 2 h at 3000 rpm and 32°C and the medium was replaced by Stemspan, 10% FBS supplemented with 10 ng/mL of IL3, 20ng/mL of IL6 and 40 ng/mL of SCF. The next day, the cells were transduced a second time.

Colony-forming cell assay

Lin- cells were transduced with: pMIE or pMIE-MG. The transduced cells were then seeded at a density of 1.5×10^4 cells in 3mL of methylcellulose (M3434; StemCell Technologies) and 1mL of this mixture was plated in 35 mm dishes (n=3). Twelve days later, the colonies were counted. For in vitro transforming assays described previously (Lavau C. et al.; 1997), cells were plated at the same density as previously reported. Colonies were scored and cells were harvested and re-plated at the same density every 7 days. Cell samples were also harvested for further analyses (FACS analysis and MGG staining).

FACS

Cells were stained with anti-mouse CD11b PE-Cy7, anti-mouse Ly-6G (Gr-1) APC, anti-Mouse Fc epsilon Receptor I alpha (FcεR1) and anti-mouse CD117 (c-Kit) PE-Cy5 (eBioscience) and analyzed with a BD FACSCalibur Flow Cytometer (BD Biosciences).

- 5 Long-term cell culture. After the fifth plating of the in vitro immortalization assay, cells were pooled and propagated in IMDM containing 15% FBS, 100nM β-mercaptoethanol, 10 ng/mL of IL3, 10 ng/mL of IL6 and 50 ng/mL of SCF. After a few weeks, IL6 and SCF were removed.

10 Statistical analyses

Statistical analyses were made using paired t test, for counting of CFUs, cells and colonies and two-way ANOVA for in vitro immortalization assay, *P<0.05, **<0.005 and ***P<0.001.

15 Results

- Acute myeloid leukemias, hematopoietic disorders characterized by abnormal proliferation and blockage of myeloid progenitors at various stages of differentiation, are often associated with chromosomal translocations that create chimeric genes encoding oncogenic proteins (Martens, J. H. et al.; 2010). The four patients (P1, P2, P3, P4) reported here presented at
20 diagnosis with similar clinical signs, consistent with a hyperhistaminemia syndrome and acute basophilic leukemia (ABL) associated with a t(X;6)(p11;q23) chromosomal translocation. Patients P1 and P2 have been reported previously (Dastugue, N. et al.; 1997).

- In all patients, the karyotype at diagnosis was simple (≤ 3 unrelated abnormalities) with a
25 recurrent t(X;6)(p11;q23) translocation.

- The involvement of the *MYB* gene, located on 6q23 locus, was investigated by fluorescence in situ hybridization (FISH), using bacterial artificial chromosomes (BAC) spanning this gene (RP11-104D9 and RP11-905P20). The hybridization showed delocalization of the probe spanning the 5' part of *MYB* on the derivative chromosome X. The presence of a fusion gene
30 involving *MYB* was investigated by rapid amplification of 3' cDNA ends (RACE) on three patients for whom nucleic acids were available (P2, P3 and P4). Sequence analysis of a 1.5 kb RACE product revealed in-frame fusion of the 5' part of *MYB* (up to exon 9) to the 3' part of *GATA1* (from exon 5 to the end). In the absence of material for mRNA extraction FISH, carried out on cells from P1, confirmed co-localization of *MYB* and *GATA1* on the derivative

chromosome X. Due to the mechanisms of the translocation, the reciprocal *GATA1-MYB* fusion gene could not be detected. Since *GATA1* is on chromosome X and all patients were males, there is a loss of *GATA1* expression. A simple t(X;6) translocation would not result in a functional *MYB-GATA1* fusion gene because the two genes have opposite orientations. At least, two events (a translocation and an inversion of one fragment) are needed to generate this product. These structural abnormalities were found for P1, P3 and P4 (no material was available for P2) by using FISH (with BACs, fosmids and mBAND probes). The karyotype was interpreted as der(X)(6qter→6q24::6q23::Xp11→Xq13::6q22q23::Xp11→Xp22::Xq24::Xp22→Xpter) and der(6)(6pter→6q22::Xq28→Xq25::Xq23→Xq13::Xqter) for P1 with a loss of 5' *GATA1*, as der(X)(12qter→12q23::6q22→6q23::Xp11→Xq28::Xp11→Xpter) and del(6)(q21q23) for P3 who had a complex rearrangement between chromosomes X, 6 and 12 and as der(X)(6qter→6q24::6q23::Xp11→Xq23::Xq25→Xqter) and der(6)(6pter→6q23::Xp11→Xp21::Xq24::Xp21→Xpter) for P4. The FISH revealed complex and unbalanced chromosomal rearrangements. The mutational status of 8 genes frequently found mutated in acute myeloid leukemia: *FLT3*, *NPM1*, *WT1*, *CEBPa*, *K-RAS*, *N-RAS*, *IDH1*, *IDH2* was tested in two cases (P2 and P3) for whom DNA was available. P2 displayed a single *K-RAS* exon 2 (G12S) mutation. Since there were 80% of blasts, the weak intensity of the mutation signal on sequence curves suggested that it likely represented a secondary event (data not shown).

The predicted fusion protein is composed of the DNA-binding domain and trans-activation domain of *MYB* fused to the c-terminal DNA-binding domain of *GATA1* (Fig. 1). Western blotting detected a 53 kDa fusion protein in the bone marrow cells of one patient (Fig. 2); no material was available from the other patients for protein analysis. Because of the lack of cellular material from the patients, the nuclear location of the *MYB-GATA1* fusion protein was confirmed by transformation experiments in HeLa cells.

MYB is a master regulator of haematopoiesis (Lieu Y. K. Et al.; 2009) and its expression, which is largely restricted to progenitor cells, decreases as cells differentiate. Except for rare cases of T-cell acute lymphoblastic leukemia with t(6;7)(q23;q34) translocation (Clappier E. et al.; 2007) and a sporadic case of acute myeloid leukemia with t(6;7)(q23;q36) translocation (Nagel S. ; 2005), *MYB* has not been observed in chromosomal translocations in other hematological cancers, although recently a *MYB* fusion protein, *MYB-NFIB*, was found in breast and head and neck carcinomas (Persson M. et al.; 2009). By contrast, *GATA1*, which encodes a key transcription factor of erythroid differentiation (Pevny et al.; 1991), has

not previously been found in fusion genes. It is known, however, that *GATA1* knockout in mice causes a profound block of erythroid differentiation (Fujiwara Y.; 1996), and its mutation is associated with various types of leukemia and hematopoietic disorders, some of them being X-linked¹¹.

- 5 The effects of MYB-GATA1 FUSION on myeloid differentiation were investigated by transducing hematopoietic progenitors. Lineage negative cells (lin-) transduced with MYB-GATA1 FUSION or with a control vector were seeded in methylcellulose containing cytokines (IL6, IL3, SCF and EPO) to compare the formation of different myeloid lineage colony-forming units (CFUs). On day 12, MYB-GATA1 FUSION expressing cells formed
- 10 significantly more CFU-G (granulocyte), but less CFU-M (monocyte) and CFU-GM (granulomonocyte) colonies than control cells (Fig. 3A), suggesting that expression of MYB-GATA1 commits these cells to the granulocytic lineage. A concomitant decrease in the number of monocytes was confirmed by May Grunwald Giemsa (MGG) staining of cells harvested at day 7 of differentiation (Fig. 3B).
- 15 In vitro immortalization assays involving serial plating of the cells in methylcellulose allowed us to study the effects of MYB-GATA1 FUSION on differentiation and proliferation. Before each plating, cells were collected and stained with MGG stain. At plating 1 (day 7), the proportion of immature granulocytic cells was higher in cells transduced with *MYB-GATA1* than in controls (Fig. 3B). From the second plating to the fifth, cells expressing MYB-
- 20 GATA1 had features of immature granulocytes (i.e. basophilic cytoplasm and some granules) but lacked features typical of basophils (toluidine blue-, FcεRIα-). FACS analysis of these cells through the serial platings showed that they expressed granulocytic markers (CD11b+/GR-1+, c-KIT-) and kept this phenotype over time (Fig. 4). After the fifth plating, cells were pooled and propagated in suspension culture. They remained GFP-positive,
- 25 CD11b+/GR-1+, but the myeloperoxidase test, performed on long-term culture cells, was negative. These morphologic and phenotypic characteristics were maintained over time, indicating a blockage at immature stages of granulocytic differentiation. The total number of colonies in the colony forming cell assays increased ($p=0.05$ paired t test) when cells were transduced with *MYB-GATA1* when compared to the control, suggesting an effect on self-
- 30 renewal of progenitor cells (Fig. 5). Moreover, the CFU-Gs observed in methylcellulose were more dense and compact than normal CFU-G colonies showing an effect of MYB-GATA1 on proliferation of these cells. This effect of MYB-GATA1 was confirmed by in vitro immortalization assays. When the cells were transduced with *MYB-GATA1*, they continued to

form colonies throughout five platings (Fig. 6) and were further maintained in culture with IL3 (for more than 6 months) suggesting a transforming effect of the fusion protein.

In conclusion, these experiments strongly show the fact that MYB–GATA1 behaves like an oncogene in hematopoietic cells by promoting proliferation of granulocyte precursors and profoundly blocking differentiation in this lineage. The *MYB–GATA1* translocation and loss of *GATA1* is the primary events in the process of leukemogenesis in these cases, as, at least in two patients, either no other mutation was found, or the one mutation found it was considered to be secondary. It is worth noting that GATA1 has been shown to be a negative regulator of *MYB* (Takahashi T. et al.; 2000). In reported here patients, the *MYB* promoter is no longer repressed by wild type *GATA1*, as *GATA1* is disrupted, which may contribute to the disease phenotype. Indeed, acute basophilic leukemia with *MYB–GATA1* fusion gene occurring in infant boys represents a distinct molecular and cytogenetic entity.

REFERENCES

Throughout this application, various references describe the state of the art to which the invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

B J Bain. Bone marrow biopsy morbidity: review of 2003. J Clin Pathol. 2005 April; 58(4): 406–408.

Cantor AB, Orkin SH. Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene. 2002 May 13;21(21):3368-76.

Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, Jin XL, Tang W, Li XS, Xong SM, Shen ZX, Sun GL, Ma J, Zhang P, Zhang TD, Gazin C, Naoe T, Chen SJ, Wang ZY, Chen Z., In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As₂O₃) in the treatment of acute promyelocytic leukemia: As₂O₃ induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood. 1996 Aug 1;88(3):1052-61.

Chessells, J.M. et al. Clinical features, cytogenetics and outcome in acute lymphoblastic and myeloid leukaemia of infancy: report from the MRC Childhood Leukaemia working party.

Leukemia 16, 776-84 (2002).

Ciovacco, W.A., Raskind, W.H. & Kacena, M.A. Human phenotypes associated with GATA1 mutations. Gene 427, 1-6 (2008).

- Clappier, E. et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. *Blood* 110, 1251-61 (2007).
- Coyaud, E. et al. Wide diversity of PAX5 alterations in B-ALL: a Groupe Francophone de
5 Cytogenetique Hematologique study. *Blood* 115, 3089-97.
- Dastugue, N. et al. Acute basophilic leukaemia and translocation t(X;6)(p11;q23). *Br J Haematol* 98, 170-6 (1997).
- Duchayne, E., Demur, C., Rubie, H., Robert, A. & Dastugue, N. Diagnosis of acute basophilic leukemia. *Leuk Lymphoma* 32, 269-78 (1999).
- 10 Fujiwara, Y., Browne, C.P., Cunniff, K., Goff, S.C. & Orkin, S.H. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA1. *Proc Natl Acad Sci U S A* 93, 12355-8 (1996).
- Galli SJ. Mast cells and basophils. *Curr Opin Hematol* 2000; 7:32-9.
- Hideo Ema, Yohei Morita¹, Satoshi Yamazaki, Azusa Matsubara, Jun Seita, Yuko Tadokoro,
15 Hiroyoshi Kondo, Hina Takano & Hiromitsu Nakauchi; Adult mouse hematopoietic stem cells: purification and single-cell assays; *Nature Protocols* 1, - 2979 - 2987 (2007).
- Mukai K, Obata K, Tsujimura Y, Karasuyama H. ; New insights into the roles for basophils in acute and chronic allergy ; *Allergol Int.* 2009 Mar;58(1):11-9. Epub 2009 Jan 25.
- Lavau, C., Szilvassy, S.J., Slany, R. & Cleary, M.L. Immortalization and leukemic
20 transformation of a myelomonocytic precursor by retrovirally transduced HRX-ENL. *Embo J* 16, 4226-37 (1997).
- Lieu, Y.K. & Reddy, E.P. Conditional c-myb knockout in adult hematopoietic stem cells leads to loss of self-renewal due to impaired proliferation and accelerated differentiation. *Proc Natl Acad Sci U S A* (2009).
- 25 Martens, J.H. & Stunnenberg, H.G. The molecular signature of oncofusion proteins in acute myeloid leukemia. *FEBS Lett* 584, 2662-9 (2010).
- Nagel, S., Kaufmann, M., Scherr, M., Drexler, H.G. & MacLeod, R.A. Activation of HLXB9 by juxtaposition with MYB via formation of t(6;7)(q23;q36) in an AML-M4 cell line (GDM-1). *Genes Chromosomes Cancer* 42, 170-8 (2005).
- 30 Oh IH, Reddy P. The myg gene family in cell growth, differentiation and apoptosis. *Oncogene* 1999; 18:3017-33.
- Pabst, T. et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. *Nat Genet* 27, 263-70 (2001).

Persson, M. et al. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. *Proc Natl Acad Sci U S A* 106, 18740-4 (2009).

Pevny, L. et al. Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA1. *Nature* 349, 257-60 (1991).

- 5 Prussin C, Metcalfe DD. 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003; 111: S486-4.

Takahashi, T. et al. Inhibitory interaction of c-Myb and GATA1 via transcriptional co-activator CBP. *Oncogene* 19, 134-40 (2000).

- Ramsay RG, Gonda TJ. MYB function in normal and cancer cells. *Nat Rev Cancer*
10 2008;8:523-34.

CLAIMS

1. A method for diagnosing acute basophilic leukemia in a subject comprising the step of detecting the presence or absence in a biological sample obtained from said subject of a MYB-GATA1 fusion wherein the presence of said MYB-GATA1 fusion is indicative that said subject suffers from acute basophilic leukemia.
2. The method according to claim 1 wherein the step of detecting the presence or absence of said MYB-GATA1 fusion comprises the step of detecting a MYB-GATA1 fusion mRNA.
3. The method according to claim 1 wherein the step of detecting the presence or absence of said MYB-GATA1 fusion comprises the step of detecting a MYB-GATA1 fusion protein.
4. A kit for diagnosing acute basophilic leukemia comprising means for detecting in a biological sample the presence or absence of a MYB-GATA1 fusion.
5. The kit according to claim 4 wherein said means for detecting the presence or absence of said MYB-GATA1 fusion comprise a *MYB* forward primer and/or a *GATA1* forward primer.
6. The kit according to claim 4 wherein said means for detecting the presence or absence of said MYB-GATA1 fusion comprise a binding partner capable of selectively interacting with MYB-GATA1 fusion protein.
7. A MYB-GATA1 fusion protein.
8. A polynucleotide comprising a sequence encoding the fusion protein according to claim 7.
9. A vector comprising a polynucleotide according to claim 8.
10. A host cell transformed by a polynucleotide according to claim 8 and/or a vector according to claim 9.
11. A method for producing granulocytic cells comprising the step of transforming lineage negative (lin-) cells with a *MYB-GATA1* fusion gene.

12. The method according to claim 11 wherein the lin- cells are hematopoietic stem cells or lin- CD34+ cells.

13. The method according to claim 11 or 12 wherein the granulocytic cells are basophils.

14. The method according to claim 11 or 12 wherein the granulocytic cells are basophilic blast cells.

15. The method according to any of claims 11 to 14 comprising a step of culturing transformed cell(s) and/or a step of isolating granulocytic cells.

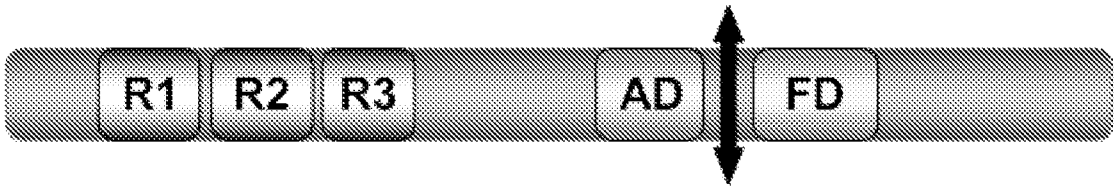


FIG. 1

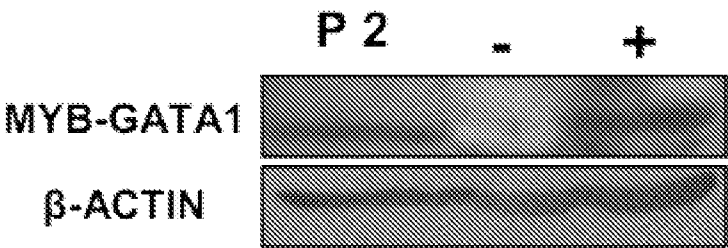


FIG. 2

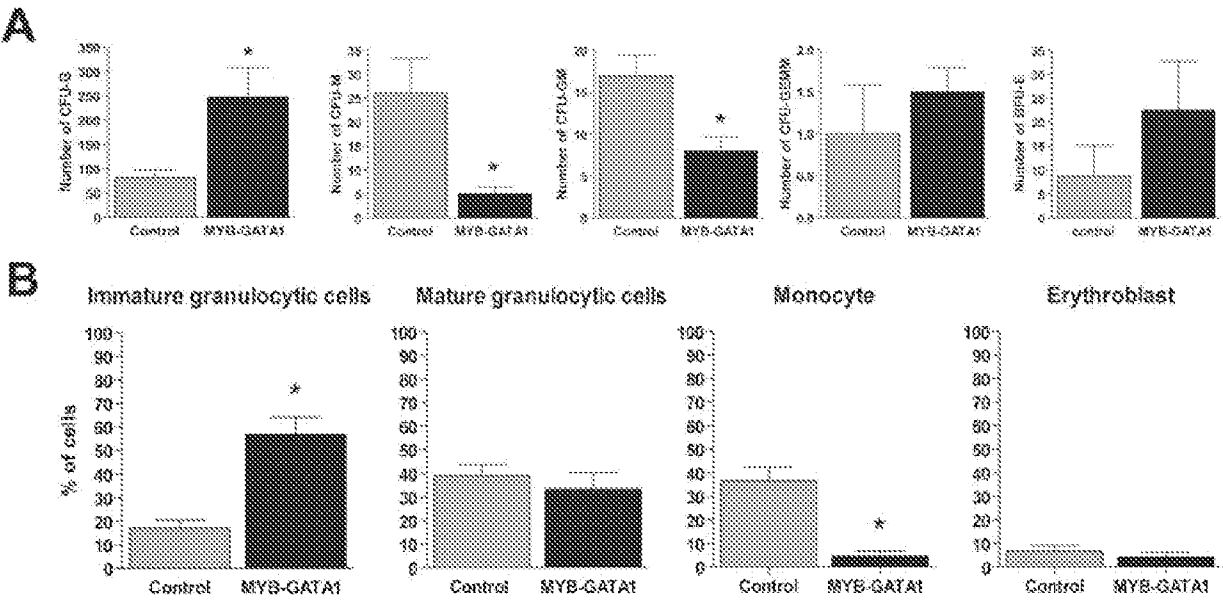


FIG. 3

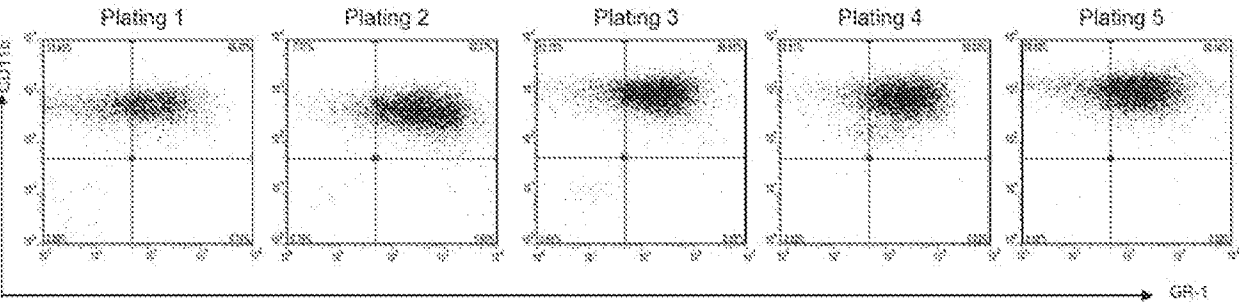


FIG. 4

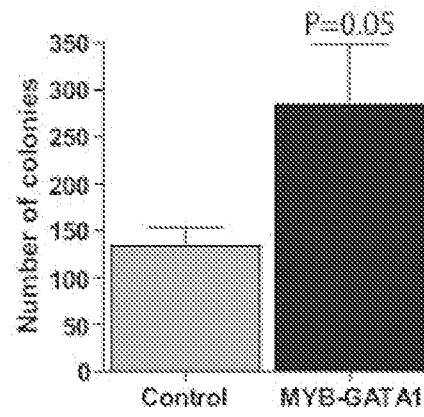


FIG. 5

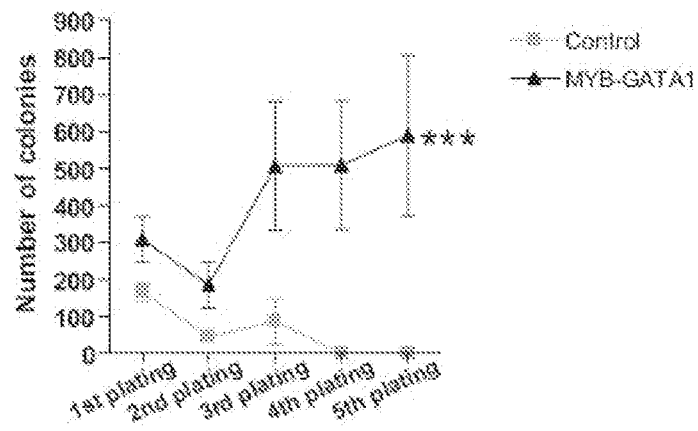


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/000790

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BELLONI E ET AL: "In vivo expression of an aberrant MYB-GATA1 fusion induces leukemia in the presence of GATA1 reduced levels.", LEUKEMIA : OFFICIAL JOURNAL OF THE LEUKEMIA SOCIETY OF AMERICA, LEUKEMIA RESEARCH FUND, vol. 25, no. 4, 21 January 2011 (2011-01-21), pages 733-736, XP002663468, ISSN: 1476-5551 the whole document -/--	1-15

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

18 November 2011

Date of mailing of the international search report

09/12/2011

Name and mailing address of the ISA/

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NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Hoff, Céline

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2011/000790

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	-& BELLONI E ET AL: "Supplementary Materials and Methods: In vivo expression of an aberrant MYB-GATA1 fusion induces leukemia in the presence of GATA1 reduced levels.", LEUKEMIA : OFFICIAL JOURNAL OF THE LEUKEMIA SOCIETY OF AMERICA, LEUKEMIA RESEARCH FUND, 21 January 2011 (2011-01-21), pages 1-6, XP002664387, ISSN: 1476-5551 the whole document	1-15
T	----- QUELEN CATHY ET AL: "Identification of a transforming MYB-GATA1 fusion gene in acute basophilic leukemia: a new entity in male infants.", BLOOD, vol. 117, no. 21, 26 May 2011 (2011-05-26), pages 5719-5722, XP002663469, ISSN: 1528-0020 the whole document	1-15
A	----- CHESSELLS J M ET AL: "Clinical features, cytogenetics and outcome in acute lymphoblastic and myeloid leukaemia of infancy: Report from the MRC Childhood Leukaemia working party", LEUKEMIA, vol. 16, no. 5, May 2002 (2002-05), pages 776-784, XP002663470, (BASINGSTOKE) ISSN: 0887-6924 the whole document	1-15
A	----- STAAL-VILIARE A ET AL: "A case of de novo acute basophilic leukaemia: diagnostic criteria and review of the literature", ANNALES DE BIOLOGIE CLINIQUE, vol. 64, no. 4, July 2006 (2006-07), pages 361-365, XP002663471, ISSN: 0003-3898 the whole document	1-15
A	----- DASTUGUE NICOLE ET AL: "Acute basophilic leukaemia and translocation t(X;6)(p11;q23)", BRITISH JOURNAL OF HAEMATOLOGY, vol. 98, no. 1, 1997, pages 170-176, XP002663472, ISSN: 0007-1048 the whole document ----- -/--	1-15

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2011/000790

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SETH TULIKA ET AL: "Acute basophilic leukemia with t(8;21).", LEUKEMIA & LYMPHOMA, vol. 45, no. 3, March 2004 (2004-03), pages 605-608, XP009154028, ISSN: 1042-8194 the whole document	1-15
A	----- DUCHAYNE E ET AL: "Diagnosis of acute basophilic leukemia.", LEUKEMIA & LYMPHOMA, vol. 32, no. 3-4, January 1999 (1999-01), pages 269-278, XP009154027, ISSN: 1042-8194 the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2011/000790

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☐

 on paper
 - ☒

 in electronic form
 - b. (time)

☒

 in the international application as filed
 - ☐

 together with the international application in electronic form
 - ☐

 subsequently to this Authority for the purpose of search
2.

☐

 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: