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(54) Title: METHOD TO PREDICT AML OUTCOME

(57) Abstract: The present invention relates to a method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML). In this study, the inventors used conditional *Jam-3*-deficient mice crossed with iMLL-AF9 leukemia model. They found that *Jam-3*-deficiency rewired the transcriptional program of leukemia initiating cells (LIC) with upregulation of genes belonging to AP-1/TNF- α pathways. Transposition of results to human allowed to determine a new prognosis score called ATIC for AP-1/TNF- α Initiating Cells, complementary and distinct from the LSC17 score. Thus, the invention relates to a method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) based on the determination in a sample obtained from the patient of the expression levels of at least 7 genes selected in the group consisting in: JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUNE, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GASS, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSE, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN and F2RL2.

METHOD TO PREDICT AML OUTCOME

FIELD OF THE INVENTION:

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The invention relates to a method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML).

BACKGROUND OF THE INVENTION:

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Acute Myeloid Leukemia (AML) is a heterogeneous disease that originates from genetic alterations and clonal expansion of Hematopoietic Stem and Progenitor Cells (HSPC). The organization of leukemic cells in AML is similar to normal haematopoiesis with Leukemic Stem Cells (LSC) at the apex that can reconstitute the clonal heterogeneity and propagate AML disease in xenograft experiments¹. LSCs are thought to be involved in AML relapse and enriched within, but not restricted to the CD34⁺/CD38⁻ phenotypic compartment^{2,3}. Additional LSCs markers have thus been described including CD123, CD44, CLL1, CD96, CD47, TIM-3, CD32, CD25, IL1RAP, CD123, CD33, CD93, CD98, CD99, CD117, GPR56/ADGRG1 and JAM-C³⁻¹⁸. However, none of them, used alone or in combination, is necessary and sufficient to identify pure population of cells with leukemic initiating activity within- or across- patient samples, merely reflecting heterogeneity of LSC in AML disease^{19,20}. This prompted several teams to search for proxys reflecting the abundance of cells with leukemia initiating activity that may predict disease outcome. Several gene expression signatures have been associated with poor prognosis²¹⁻²³ and some of them relied on increased frequencies of primitive quiescent leukemia stem cells in AML^{12,24-26}. Among them, the LinClass-7 subscore established with 7 genes of the leukemic stemness LSC17 score has been shown to predict drug response suggesting that cellular hierarchy influences the overall characteristics of AML²⁷. Other experimental approaches to identify hallmarks of LSC consisted in deciphering cellular heterogeneity of AML disease using single cell RNA sequencing²⁸⁻³⁰. This allowed identification of differentially expressed genes in tumor-derived HSC-like cells as compared to more mature leukemic cells, some of which (MMRN1, CD34, SOCS2, SMIM24, FAM30A, CDK6) being also included in the leukemic stemness LSC17 score. Other experimental approaches consisted to model AML disease in mouse in order to identify features of leukemia initiating cells³¹. Since the pioneering study by D. Baltimore's team³², a number of studies have

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used retroviral transduction of mouse hematopoietic stem and progenitor cells (HSPC) to express genetic alterations supposed to drive leukemic initiation. Among them, the MLL-AF9 fusion efficiently induced transformation of HSPC which were able to reconstitute the disease after expansion in methyl-cellulose³³. Further studies using the same experimental model or 5 *MLL-AF9* heterozygous knock-in mice³⁴ demonstrated that the most aggressive leukemia originated in HSC rather than in more mature granulocyte/monocyte progenitors (GMP)³⁵⁻³⁷. More recently, an inducible model for MLL-AF9 expression (iMLL-AF9) based on the reverse tetracycline-controlled transactivator (rtTA) was described³⁸. The MLL-AF9 fusion was inserted in the *Hprt* locus (Chr X) downstream of the tetracycline responsive element. In this 10 model, leukemia originated from LT-HSC-like and GMP-like compartments with more aggressive diseases when leukemia derived from HSC transformation. Gene expression study revealed that aggressiveness was correlated to leukemic initiation in the LT-HSC-like compartment expressing high levels of *Evi1* (*Mecom*) and *Erg* as well as genes related to epithelial mesenchymal transition or cell migration and adhesion such as *Zeb1*, *Tcf4*, *Trsp1*, 15 *Itga6*, *Alcam*, *Ceacam*. This is consistent with our previous findings that human LSC (CD45^{dim}CD34⁺CD38^{low}CD123⁺) expressing the adhesion molecule JAM-C (encoded by *JAM3*) also co-expressed high levels of *ALCAM* or *ITGA6* and that high frequency of JAM-C-expressing cells at diagnosis was associated with poor disease outcome⁴. These results suggest, but do not prove, that LSC adhesion to surrounding bone marrow microenvironment may play 20 a role in AML disease initiation and outcome.

SUMMARY OF THE INVENTION:

In this study, the inventors used conditional *Jam-3*-deficient mice crossed with iMLL-AF9 leukemia model. They found that *Jam3*-deficiency rewired the transcriptional program of 25 leukemia initiating cells (LIC) with upregulation of genes belonging to AP-1/TNF- α pathways. Transposition of results to human allowed to determine a new prognosis score called ATIC for AP-1/TNF- α Initiating Cells, complementary and distinct from the LSC17 score.

Thus, the invention relates to a method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML).

30 Particularly, the invention is defined by its claims.

DETAILED DESCRIPTION OF THE INVENTION:

The inventors, thanks to methods like RNA sequencing from HSC-like (hematopoietic stem cells-like) and GMP-like (granulocyte/monocyte progenitors-like) cells isolated from leukemic mice revealed a first signature of 53 genes useful to predict the survival time of a patient suffering from an Acute Myeloid Leukemia (AML). Particularly, using machine learning algorithm, the inventors show that at least 7 genes from the signature of 53 genes can be useful to predict the survival time of an AML patient.

Thus, a first aspect of the invention relates to a method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) i) determining in a sample obtained from the patient the expression levels of at least 7 genes selected in the group consisting in: JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN and F2RL2 ii) comparing said expression levels determined at step i) with their predetermined reference values and iii) providing a good prognosis when the expression levels of the genes determined at step i) are lower than their predetermined reference values, or providing a bad prognosis when the expression levels of the genes determined at step i) are higher than their predetermined reference values.

The method of the invention is an *ex vivo* method.

In a particular embodiment the expression level of 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53 genes of the invention is determined for the method of the invention.

In a particular embodiment, the expression levels of the 7 following genes JAM3, H2BC8, SLFN12L, NR4A1, GAS5, RPS13 and MYCN are determined to implement the method of the invention.

In a particular embodiment, the expression levels of the 8 following genes SLFN12L, GAS5, RPS13, ZNF699, HEY1, GIMAP4, MYCN and F2RL2 are determined to implement the method of the invention.

In a particular embodiment, the expression levels of the 9 following genes JAM3, TPT1, TAF7, SLFN12L, GAS5, SNORA31, FOS, HEY1 and MYCN are determined to implement the method of the invention.

5 In a particular embodiment, the expression levels of the 10 following genes H2BC8, SLFN12L, GAS5, RPS13, MYL9, ZNF699, GIMAP4, ITGA2B, MYCN and F2RL2 are determined to implement the method of the invention.

In a particular embodiment, the expression levels of the 10 following genes TPT1, BTG2, JUNB, GAS5, EGR3, ZNF699, HEY1, GIMAP4, PF4 and MYCN are determined to implement the method of the invention.

10 In a particular embodiment, the expression levels of the 10 following genes H2BC8, SNORA28, TPT1, SLFN12L, GAS5, ZNF699, FOS, HEY1, GIMAP4 and CCL3 are determined to implement the method of the invention.

In a particular embodiment, the expression levels of the 11 following genes DUSP1, TPT1, SLFN12L, GAS5, EGR3, RPS13, ZNF699, RPP25L, HEY1, GIMAP4 and MYCN are
15 determined to implement the method of the invention.

In a particular embodiment, the expression levels of the 12 following genes JAM3, DUSP2, TPT1, GAS5, SNORA31, RHOB, ZNF699, HEY1, GIMAP4, EFCAB11, MYCN and F2RL2 are determined to implement the method of the invention.

20 In a particular embodiment, the expression levels of the 12 following genes JAM3, DUSP1, H2BC8, TPT1, KLF2, SLFN12L, GAS5, RPS13, ZNF699, GIMAP4, EFCAB11 and MYCN and F2RL2 are determined to implement the method of the invention.

In a particular embodiment, the expression levels of the 14 following genes JAM3, DUSP1, RGS1, H2BC8, NFKBID, ZFP36, SLFN12L, GAS5, RPP25L, HEY1, GIMAP4, EFCAB11, CCL4 and MYCN are determined to implement the method of the invention.

25 In a particular embodiment, the at least 7 genes of the 53 genes of the invention comprise at least the gene GAS5.

According to the invention, the method of the invention can also be useful to stratify patients in function of the gravity of their AML. As used herein, the term “stratify” consists in,
30 depending on the level of expression of the genes according to the invention, classifying the patients with a good or bad prognosis between them and in adapting the treatment to the patient according to their biological characteristics.

Thus the invention also relates to a method for stratifying a patient suffering from an Acute Myeloid Leukemia (AML) i) determining in a sample obtained from the patient the

expression level of at least 7 genes selected in the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN, F2RL2 ii) comparing said expression levels determined at step i) with their predetermined reference values and iii) providing a good prognosis when the expression level of the genes determined at step i) are lower than their predetermined reference values, or providing a bad prognosis when the expression level of the genes determined at step i) are higher than their predetermined reference values.

According to the invention, the method of the invention can also be useful for monitoring a patient's response to a therapy. As used herein, the term "monitoring" consists in, depending on the level of expression of the genes according to the invention, determining if the patients will respond or not to the therapy.

Thus the invention also relates to a method for monitoring patient's response to a therapy against an Acute Myeloid Leukemia (AML) i) determining in a sample obtained from the patient the expression levels of at least 7 genes selected in the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN, F2RL2 ii) comparing said expression levels determined at step i) with their predetermined reference values and iii) providing that the patient will response to the therapy when the expression levels of the genes determined at step i) are lower than their predetermined reference values, or providing that the patient will not response to the therapy when the expression levels of the genes determined at step i) are higher than their predetermined reference values.

According to this particular embodiment, the therapy can be a chemotherapy like azacytidine or ivosidenib or the venetoclax.

According to the invention, the method of the invention can also be useful for predicting the relapse of an AML in a patient. As used herein, the term "relapse" consists in, depending

on the level of expression of the genes according to the invention, determining if the patients will again or not suffer from an AML.

Thus the invention also relates to a method for predicting the relapse of an Acute Myeloid Leukemia (AML) in a patient i) determining in a sample obtained from the patient the expression levels of at least 7 genes selected in the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN, F2RL2 ii) comparing said expression levels determined at step i) with their predetermined reference values and iii) providing that the patient will response to the therapy when the expression levels of the genes determined at step i) are lower than their predetermined reference values, or providing that the patient will not response to the therapy when the expression levels of the genes determined at step i) are higher than their predetermined reference values.

In a particular embodiment, the Acute Myeloid Leukemia (AML) can be a relapsed Acute Myeloid Leukemia, the evolution of a myelodysplastic syndrome (MDS), an AML with myelodysplastic related changes, a resistant AML, or a resistant acute myeloid leukemia (AML).

In a particular embodiment, the resistant AML is a resistant cytogenetically normal AML (CN-AML) or a resistant acute myeloid leukemia with trisomy 8.

According to this particular embodiment, the sample can be blood, peripheral-blood, serum, plasma or tumoral circulating cells.

As used herein, the term “patient” denotes a mammal, such as a rodent, a feline, a canine, and a primate. Particularly, the patient according to the invention is a human and more particularly, the patient is an human with AML according to the invention.

As used herein, the term “survival time” denotes the percentage of people in a study or treatment group who are still alive for a certain period of time after they were diagnosed with or started treatment for a disease, such as AML (according to the invention). The survival time rate is often stated as a five-year survival rate, which is the percentage of people in a study or treatment group who are alive five years after their diagnosis or the start of treatment.

As used herein and according to the invention, the term “survival time” can regroup the term OS.

As used herein, the term “Overall survival (OS)” denotes the time from diagnosis of a disease such as AML (according to the invention) until death from any cause. The overall survival rate is often stated as a two-year survival rate, which is the percentage of people in a study or treatment group who are alive two years after their diagnosis or the start of treatment.

Gene symbol	Gene name	Entrez (NCBI) Gene
JAM3	Junctional adhesion molecule 3	83700
DUSP1	Dual specificity protein phosphatase 1	1843
JUN	Transcription factor Jun	3725
IER2	Immediate Early Response Gene 2	9592
DUSP2	Dual Specificity Protein Phosphatase 2	1844
RGS1	Regulator of G-protein signaling 1	5996
H2BC8	H2B Clustered Histone 8	8339
PTGS2	Prostaglandin-endoperoxide synthase 2	5743
NFKBID	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta	84807

PPP1R15A	Protein Phosphatase 1 Regulatory Subunit 15A	23645
NFKBIZ	NF-kappa-B inhibitor zeta	64332
ZFP36	zinc finger protein 36 homolog	7538
SNORA28	Small Nucleolar RNA, H/ACA Box 28	677811
TPT1	Tumor Protein, Translationally- Controlled 1	7178
KLF2	Krüppel-like Factor 2	10365
BTG2	BTG family member 2	7832
JUNB	Transcription factor jun-B	3726
JUND	Transcription Factor Jun-D	3727
ATF3	Activating Transcription Factor 3	467
UBC	Polyubiquitin-C	7316
SKIL	SKI Like Proto- Oncogene	6498
TAF7	TATA-Box Binding Protein Associated Factor 7	6879
SLFN12L	Schlafen family member 12 like	100506736
NR4A1	Nuclear receptor 4A1	3164

CHST2	Carbohydrate Sulfotransferase 2	9435
GAS5	Growth arrest- specific 5	60674
SNORA31	small nucleolar RNA, H/ACA box 31	677814
HES1	Hes Family BHLH Transcription Factor 1	3280
EGR3	Early Growth Response 3	1960
RPS13	Ribosomal Protein S13	6207
PMAIP1	Phorbol-12- Myristate-13- Acetate-Induced Protein 1	5366
RHOB	Ras Homolog Family Member B	388
MYL9	Myosin Light Chain 9	10398
ZNF699	Zinc Finger Protein 699	374879
ZNF101	Zinc finger protein 101	94039
FOS	Fos Proto-Oncogene	2353
FJX1	Four-Jointed Box Kinase 1	24147
RPP25L	Ribonuclease P/MRP Subunit P25 Like	138716
HEY1	Hairy/enhancer-of- split related with	23462

	YRPW motif protein 1	
PTMA	Prothymosin Alpha	5757
GIMAP4	GTPase IMAP family member 4	55303
EFCAB11	EF-Hand Calcium Binding Domain 11	90141
FOSB	FosB Proto- Oncogene	2354
CD14	CD14 Molecule	929
CCL4	Chemokine (C-C motif) ligands 4	388372
CCL3	C-C Motif Chemokine Ligand 3	6348
PF4	Platelet factor 4	5196
OSM	Oncostatin M	5008
CD69	CD69 Molecule	969
ITGA2B	Integrin Subunit Alpha 2b	3674
VWF	Von Willebrand Factor	7450
MYCN	N-myc proto- oncogene protein	4613
F2RL2	Coagulation Factor II Thrombin Receptor Like 2	2151

Table1: list of the 53 genes of the invention use as prognostic markers (here “the genes of the invention” or “the biomarkers of the invention”).

Measuring the expression level of the genes of the invention (see the table 1) can be done by measuring the gene expression level of them or by measuring the level of the protein of these genes and can be performed by a variety of techniques well known in the art.

Typically, the expression level of a gene may be determined by determining the quantity of mRNA. Methods for determining the quantity of mRNA are well known in the art. For

example the nucleic acid contained in the samples (e.g., cell or tissue prepared from the patient) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (e. g., Northern blot analysis, in situ hybridization) and/or amplification (e.g., RT-qPCR). Particularly, nanostring assay can be used.

Other methods of Amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of interest herein find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments, it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization.

Typically, the nucleic acid probes include one or more labels, for example to permit detection of a target nucleic acid molecule using the disclosed probes. In various applications, such as in situ hybridization procedures, a nucleic acid probe includes a label (e.g., a detectable label). A “detectable label” is a molecule or material that can be used to produce a detectable signal that indicates the presence or concentration of the probe (particularly the bound or hybridized probe) in a sample. Thus, a labeled nucleic acid molecule provides an indicator of the presence or concentration of a target nucleic acid sequence (e.g., genomic target nucleic acid sequence) (to which the labeled uniquely specific nucleic acid molecule is bound or hybridized) in a sample. A label associated with one or more nucleic acid molecules (such as a probe generated by the disclosed methods) can be detected either directly or indirectly. A label can be detected by any known or yet to be discovered mechanism including absorption, emission and/ or scattering of a photon (including radio frequency, microwave frequency, infrared frequency, visible frequency and ultra-violet frequency photons). Detectable labels include colored, fluorescent, phosphorescent and luminescent molecules and materials, catalysts (such as enzymes) that convert one substance into another substance to provide a detectable difference (such as by converting a colorless substance into a colored substance or vice versa, or by producing a precipitate or increasing sample turbidity), haptens that can be detected by antibody binding interactions, and paramagnetic and magnetic molecules or materials.

Particular examples of detectable labels include fluorescent molecules (or fluorochromes). Numerous fluorochromes are known to those of skill in the art, and can be selected, for example from Life Technologies (formerly Invitrogen), e.g., see, The Handbook—A Guide to Fluorescent Probes and Labeling Technologies). Examples of particular fluorophores that can be attached (for example, chemically conjugated) to a nucleic acid molecule (such as a uniquely specific binding region) are provided in U.S. Pat. No. 5,866,366 to Nazarenko et al., such as 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid, acridine and derivatives such as acridine and acridine isothiocyanate, 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid (EDANS), 4-amino-N-[3 vinylsulfonyl]phenyl]naphthalimide-3,5 disulfonate (Lucifer Yellow VS), N-(4-anilino-1-naphthyl)maleimide, antlIranilamide, Brilliant Yellow, coumarin and derivatives such as coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcoumarin (Coumarin 151); cyanosine; 4',6-dianilindino-2-phenylindole (DAPI); 5',5"-dibromopyrogallol-sulfonephthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino] naphthalene-1-sulfonyl chloride (DNS, dansyl chloride); 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives such as eosin and eosin isothiocyanate; erythrosin and derivatives such as erythrosin B and erythrosin isothiocyanate; ethidium; fluorescein and derivatives such as 5-carboxyfluorescein (FAM), 5-(4,6-dichloro-2-pyridinyl)aminofluorescein (DTAF), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), fluorescein, fluorescein isothiocyanate (FITC), and QFITC Q(RITC); 2',7'-difluorofluorescein (OREGON GREEN®); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbelliferone; ortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthalaldehyde; pyrene and derivatives such as pyrene, pyrene butyrate and succinimidyl 1-pyrene butyrate; Reactive Red 4 (Cibacron Brilliant Red 3B-A); rhodamine and derivatives such as 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride, rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, rhodamine green, sulforhodamine B, sulforhodamine 101 and sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; rosolic acid and terbium chelate derivatives. Other suitable fluorophores include thiol-reactive europium chelates which emit at approximately 617 nm (Heyduk and Heyduk, Analyt.

Biochem. 248:216-27, 1997; J. Biol. Chem. 274:3315-22, 1999), as well as GFP, LissamineTM, diethylaminocoumarin, fluorescein chlorotriazinyl, naphthofluorescein, 4,7-dichlororhodamine and xanthene (as described in U.S. Pat. No. 5,800,996 to Lee et al.) and derivatives thereof. Other fluorophores known to those skilled in the art can also be used, for example those
5 available from Life Technologies (Invitrogen; Molecular Probes (Eugene, Oreg.)) and including the ALEXA FLUOR® series of dyes (for example, as described in U.S. Pat. Nos. 5,696,157, 6, 130, 101 and 6,716,979), the BODIPY series of dyes (dipyrrometheneboron difluoride dyes, for example as described in U.S. Pat. Nos. 4,774,339, 5,187,288, 5,248,782, 5,274,113, 5,338,854, 5,451,663 and 5,433,896), Cascade Blue (an amine reactive derivative
10 of the sulfonated pyrene described in U.S. Pat. No. 5,132,432) and Marina Blue (U.S. Pat. No. 5,830,912).

In addition to the fluorochromes described above, a fluorescent label can be a fluorescent nanoparticle, such as a semiconductor nanocrystal, e.g., a QUANTUM DOTTM (obtained, for example, from Life Technologies (QuantumDot Corp, Invitrogen Nanocrystal
15 Technologies, Eugene, Oreg.); see also, U.S. Pat. Nos. 6,815,064; 6,682,596; and 6,649, 138). Semiconductor nanocrystals are microscopic particles having size-dependent optical and/or electrical properties. When semiconductor nanocrystals are illuminated with a primary energy source, a secondary emission of energy occurs of a frequency that corresponds to the bandgap of the semiconductor material used in the semiconductor nanocrystal. This emission can be
20 detected as colored light of a specific wavelength or fluorescence. Semiconductor nanocrystals with different spectral characteristics are described in e.g., U.S. Pat. No. 6,602,671. Semiconductor nanocrystals that can be coupled to a variety of biological molecules (including dNTPs and/or nucleic acids) or substrates by techniques described in, for example, Bruchez et al., Science 281 :20132016, 1998; Chan et al., Science 281:2016-2018, 1998; and U.S. Pat. No.
25 6,274,323. Formation of semiconductor nanocrystals of various compositions are disclosed in, e.g., U.S. Pat. Nos. 6,927, 069; 6,914,256; 6,855,202; 6,709,929; 6,689,338; 6,500,622; 6,306,736; 6,225,198; 6,207,392; 6,114,038; 6,048,616; 5,990,479; 5,690,807; 5,571,018; 5,505,928; 5,262,357 and in U.S. Patent Publication No. 2003/0165951 as well as PCT Publication No. 99/26299 (published May 27, 1999). Separate populations of semiconductor
30 nanocrystals can be produced that are identifiable based on their different spectral characteristics. For example, semiconductor nanocrystals can be produced that emit light of different colors based on their composition, size or size and composition. For example, quantum dots that emit light at different wavelengths based on size (565 nm, 655 nm, 705 nm, or 800 nm

emission wavelengths), which are suitable as fluorescent labels in the probes disclosed herein are available from Life Technologies (Carlsbad, Calif.).

Additional labels include, for example, radioisotopes (such as ^3H), metal chelates such as DOTA and DPTA chelates of radioactive or paramagnetic metal ions like Gd^{3+} , and liposomes.

Detectable labels that can be used with nucleic acid molecules also include enzymes, for example horseradish peroxidase, alkaline phosphatase, acid phosphatase, glucose oxidase, beta-galactosidase, beta-glucuronidase, or beta-lactamase.

Alternatively, an enzyme can be used in a metallographic detection scheme. For example, silver in situ hybridization (SISH) procedures involve metallographic detection schemes for identification and localization of a hybridized genomic target nucleic acid sequence. Metallographic detection methods include using an enzyme, such as alkaline phosphatase, in combination with a water-soluble metal ion and a redox-inactive substrate of the enzyme. The substrate is converted to a redox-active agent by the enzyme, and the redoxactive agent reduces the metal ion, causing it to form a detectable precipitate. (See, for example, U.S. Patent Application Publication No. 2005/0100976, PCT Publication No. 2005/003777 and U.S. Patent Application Publication No. 2004/0265922). Metallographic detection methods also include using an oxido-reductase enzyme (such as horseradish peroxidase) along with a water soluble metal ion, an oxidizing agent and a reducing agent, again to form a detectable precipitate. (See, for example, U.S. Pat. No. 6,670,113).

Probes made using the disclosed methods can be used for nucleic acid detection, such as ISH procedures (for example, fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH) and silver in situ hybridization (SISH)) or comparative genomic hybridization (CGH).

In situ hybridization (ISH) involves contacting a sample containing target nucleic acid sequence (e.g., genomic target nucleic acid sequence) in the context of a metaphase or interphase chromosome preparation (such as a cell or tissue sample mounted on a slide) with a labeled probe specifically hybridizable or specific for the target nucleic acid sequence (e.g., genomic target nucleic acid sequence). The slides are optionally pretreated, e.g., to remove paraffin or other materials that can interfere with uniform hybridization. The sample and the probe are both treated, for example by heating to denature the double stranded nucleic acids. The probe (formulated in a suitable hybridization buffer) and the sample are combined, under conditions and for sufficient time to permit hybridization to occur (typically to reach

equilibrium). The chromosome preparation is washed to remove excess probe, and detection of specific labeling of the chromosome target is performed using standard techniques.

For example, a biotinylated probe can be detected using fluorescein-labeled avidin or avidin-alkaline phosphatase. For fluorochrome detection, the fluorochrome can be detected
5 directly, or the samples can be incubated, for example, with fluorescein isothiocyanate (FITC)-conjugated avidin. Amplification of the FITC signal can be effected, if necessary, by incubation with biotin-conjugated goat antiavidin antibodies, washing and a second incubation with FITC-conjugated avidin. For detection by enzyme activity, samples can be incubated, for example, with streptavidin, washed, incubated with biotin-conjugated alkaline phosphatase, washed
10 again and pre-equilibrated (e.g., in alkaline phosphatase (AP) buffer). For a general description of in situ hybridization procedures, see, e.g., U.S. Pat. No. 4,888,278.

Numerous procedures for FISH, CISH, and SISH are known in the art. For example, procedures for performing FISH are described in U.S. Pat. Nos. 5,447,841; 5,472,842; and 5,427,932; and for example, in Pir1kel et al., Proc. Natl. Acad. Sci. 83:2934-2938, 1986; Pinkel
15 et al., Proc. Natl. Acad. Sci. 85:9138-9142, 1988; and Lichter et al., Proc. Natl. Acad. Sci. 85:9664-9668, 1988. CISH is described in, e.g., Tanner et al., Am. J. Pathol. 157:1467-1472, 2000 and U.S. Pat. No. 6,942,970. Additional detection methods are provided in U.S. Pat. No. 6,280,929.

Numerous reagents and detection schemes can be employed in conjunction with FISH,
20 CISH, and SISH procedures to improve sensitivity, resolution, or other desirable properties. As discussed above probes labeled with fluorophores (including fluorescent dyes and QUANTUM DOTS®) can be directly optically detected when performing FISH. Alternatively, the probe can be labeled with a nonfluorescent molecule, such as a hapten (such as the following non-limiting examples: biotin, digoxigenin, DNP, and various oxazoles, pyrazoles, thiazoles,
25 nitroaryls, benzofurazans, triterpenes, ureas, thioureas, rotenones, coumarin, coumarin-based compounds, Podophyllotoxin, Podophyllotoxin-based compounds, and combinations thereof), ligand or other indirectly detectable moiety. Probes labeled with such non-fluorescent molecules (and the target nucleic acid sequences to which they bind) can then be detected by contacting the sample (e.g., the cell or tissue sample to which the probe is bound) with a labeled
30 detection reagent, such as an antibody (or receptor, or other specific binding partner) specific for the chosen hapten or ligand. The detection reagent can be labeled with a fluorophore (e.g., QUANTUM DOT®) or with another indirectly detectable moiety, or can be contacted with one or more additional specific binding agents (e.g., secondary or specific antibodies), which can be labeled with a fluorophore.

In other examples, the probe, or specific binding agent (such as an antibody, e.g., a primary antibody, receptor or other binding agent) is labeled with an enzyme that is capable of converting a fluorogenic or chromogenic composition into a detectable fluorescent, colored or otherwise detectable signal (e.g., as in deposition of detectable metal particles in SISH). As indicated above, the enzyme can be attached directly or indirectly via a linker to the relevant probe or detection reagent. Examples of suitable reagents (e.g., binding reagents) and chemistries (e.g., linker and attachment chemistries) are described in U.S. Patent Application Publication Nos. 2006/0246524; 2006/0246523, and 2007/ 01 17153.

It will be appreciated by those of skill in the art that by appropriately selecting labelled probe-specific binding agent pairs, multiplex detection schemes can be produced to facilitate detection of multiple target nucleic acid sequences (e.g., genomic target nucleic acid sequences) in a single assay (e.g., on a single cell or tissue sample or on more than one cell or tissue sample). For example, a first probe that corresponds to a first target sequence can be labelled with a first hapten, such as biotin, while a second probe that corresponds to a second target sequence can be labelled with a second hapten, such as DNP. Following exposure of the sample to the probes, the bound probes can be detected by contacting the sample with a first specific binding agent (in this case avidin labelled with a first fluorophore, for example, a first spectrally distinct QUANTUM DOT®, e.g., that emits at 585 nm) and a second specific binding agent (in this case an anti-DNP antibody, or antibody fragment, labelled with a second fluorophore (for example, a second spectrally distinct QUANTUM DOT®, e.g., that emits at 705 nm). Additional probes/binding agent pairs can be added to the multiplex detection scheme using other spectrally distinct fluorophores. Numerous variations of direct, and indirect (one step, two step or more) can be envisioned, all of which are suitable in the context of the disclosed probes and assays.

Probes typically comprise single-stranded nucleic acids of between 10 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are “specific” to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency hybridization conditions (corresponding to the highest melting temperature T_m , e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate).

The nucleic acid primers or probes used in the above amplification and detection method may be assembled as a kit. Such a kit includes consensus primers and molecular probes. A

preferred kit also includes the components necessary to determine if amplification has occurred. The kit may also include, for example, PCR buffers and enzymes; positive control sequences, reaction control primers; and instructions for amplifying and detecting the specific sequences.

In a particular embodiment, the methods of the invention comprise the steps of
5 providing total RNAs extracted from cumulus cells and subjecting the RNAs to amplification and hybridization to specific probes, more particularly by means of a quantitative or semi-quantitative RT-PCR (or q RT-PCR).

In another preferred embodiment, the expression level is determined by DNA chip analysis. Such DNA chip or nucleic acid microarray consists of different nucleic acid probes
10 that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. A microchip may be constituted of polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, or nitrocellulose. Probes comprise nucleic acids such as cDNAs or oligonucleotides that may be about 10 to about 60 base pairs. To determine the expression level, a sample from a test subject,
15 optionally first subjected to a reverse transcription, is labelled and contacted with the microarray in hybridization conditions, leading to the formation of complexes between target nucleic acids that are complementary to probe sequences attached to the microarray surface. The labelled hybridized complexes are then detected and can be quantified or semi-quantified. Labelling may be achieved by various methods, e.g. by using radioactive or fluorescent labelling. Many
20 variants of the microarray hybridization technology are available to the man skilled in the art (see e.g. the review by Hoheisel, Nature Reviews, Genetics, 2006, 7:200-210).

Expression level of a gene may be expressed as absolute expression level or normalized expression level. Typically, expression levels are normalized by correcting the absolute expression level of a gene by comparing its expression to the expression of a gene that is not a
25 relevant for determining the cancer stage of the patient, e.g., a housekeeping gene that is constitutively expressed. Suitable genes for normalization include housekeeping genes such as the actin gene ACTB, ribosomal 18S gene, GUSB, PGK1, TFRC, GAPDH, TBP and ABL1. This normalization allows the comparison of the expression level in one sample, e.g., a patient sample, to another sample, or between samples from different sources.

30 According to the invention, the level of the proteins of the genes of the invention (here “the proteins of the invention”) may also be measured and can be performed by a variety of techniques well known in the art. For measuring the expression level of the proteins of the

invention, techniques like ELISA (see below) allowing to measure the level of the soluble proteins are particularly suitable.

In the present application, the “level of protein” or the “protein level expression” or the “protein concentration” means the quantity or concentration of said protein. In another embodiment, the “level of protein” means the level of the proteins fragments. In still another embodiment, the “level of protein” means the quantitative measurement of the proteins of the invention expression relative to a negative control.

According to the invention, the proteins of the invention may be measured at the surface of the tumor cells or in an extracellular context (for example in blood or plasma).

Typically protein concentration may be measured for example by capillary electrophoresis-mass spectroscopy technique (CE-MS) or ELISA performed on the sample.

Such methods comprise contacting a sample with a binding partner capable of selectively interacting with proteins present in the sample. The binding partner is generally an antibody that may be polyclonal or monoclonal, preferably monoclonal.

The presence of the protein can be detected using standard electrophoretic and immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays. Such assays include, but are not limited to, Western blots; agglutination tests; enzyme-labeled and mediated immunoassays, such as ELISAs; biotin/avidin type assays; radioimmunoassays; immunoelectrophoresis; immunoprecipitation, capillary electrophoresis-mass spectroscopy technique (CE-MS).etc. The reactions generally include revealing labels such as fluorescent, chemiluminescent, radioactive, enzymatic labels or dye molecules, or other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith.

The afore mentioned assays generally involve separation of unbound protein in a liquid phase from a solid phase support to which antigen-antibody complexes are bound. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e. g., in membrane or microtiter well form); polyvinylchloride (e. g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidene fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

More particularly, an ELISA method can be used, wherein the wells of a microtiter plate are coated with a set of antibodies against the proteins to be tested. A sample containing or suspected of containing the marker protein is then added to the coated wells. 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 is added. The secondary binding molecule is allowed to react with any captured sample marker protein, the plate is washed and the presence of the secondary binding molecule is detected using methods well known in the art.

5 Methods of the invention may comprise a step consisting of comparing the proteins and fragments concentration in circulating cells with a control value. As used herein, "concentration of protein" refers to an amount or a concentration of a transcription product, for the proteins of the invention. Typically, a level of a protein can be expressed as nanograms per microgram of tissue or nanograms per milliliter of a culture medium, for example. Alternatively, relative units
10 can be employed to describe a concentration. In a particular embodiment, "concentration of proteins" may refer to fragments of the proteins of the invention. Thus, in a particular embodiment, fragment of the proteins of the invention may also be measured.

 In a particular embodiment, the detection of the level of the proteins of the invention can be performed by flow cytometry.

15

Predetermined reference values

 Predetermined reference values used for comparison of the expression levels may comprise "cut-off" or "threshold" values that may be determined as described herein. Each reference ("cut-off") value for the genes of the invention level may be predetermined by
20 carrying out a method comprising the steps of:

- a) providing a collection of samples from patients suffering of a LAM;
- b) determining the level of the genes of the invention for each sample contained in the collection provided at step a);
- c) ranking the tumor tissue samples according to said level
- 25 d) classifying said samples in pairs of subsets of increasing, respectively decreasing, number of members ranked according to their expression level,
- e) providing, for each sample provided at step a), information relating to the actual clinical outcome for the corresponding AML patient;
- f) for each pair of subsets of samples, obtaining a Kaplan Meier percentage of survival
30 curve;
- g) for each pair of subsets of samples calculating the statistical significance (p value) between both subsets
- h) selecting as reference value for the level, the value of level for which the p value is the smallest.

For example the expression level of the genes of the invention has been assessed for 100 AML samples of 100 patients. The 100 samples are ranked according to their expression level. Sample 1 has the best expression level and sample 100 has the worst expression level. A first grouping provides two subsets: on one side sample Nr 1 and on the other side the 99 other samples. The next grouping provides on one side samples 1 and 2 and on the other side the 98 remaining samples etc., until the last grouping: on one side samples 1 to 99 and on the other side sample Nr 100. According to the information relating to the actual clinical outcome for the corresponding AML patient, Kaplan Meier curves are prepared for each of the 99 groups of two subsets. Also for each of the 99 groups, the p value between both subsets was calculated.

The reference value is selected such as the discrimination based on the criterion of the minimum p value is the strongest. In other terms, the expression level corresponding to the boundary between both subsets for which the p value is minimum is considered as the reference value. It should be noted that the reference value is not necessarily the median value of expression levels.

In routine work, the reference value (cut-off value) may be used in the present method to discriminate AML samples and therefore the corresponding patients.

Kaplan–Meier curves of percentage of survival as a function of time are commonly used to measure the fraction of patients living for a certain amount of time after treatment and are well known by the man skilled in the art.

The man skilled in the art also understands that the same technique of assessment of the expression level of a protein should of course be used for obtaining the reference value and thereafter for assessment of the expression level of a protein of a patient subjected to the method of the invention. Particularly, other methods like median thresholding”, “max-stat” and “Youden’s J statistic can be used to determine a threshold.

Such predetermined reference values of expression level may be determined for any at least 7 genes of the invention defined above.

Kit

A further object of the invention relates to kits for performing the methods of the invention, wherein said kits comprise means for measuring the expression level of the genes of the invention in the sample obtained from the patient.

The kits may include probes, primers macroarrays or microarrays as above described. For example, the kit may comprise a set of probes as above defined, usually made of DNA, and that may be pre-labelled. Alternatively, probes may be unlabelled and the ingredients for

labelling may be included in the kit in separate containers. The kit may further comprise hybridization reagents or other suitably packaged reagents and materials needed for the particular hybridization protocol, including solid-phase matrices, if applicable, and standards. Alternatively the kit of the invention may comprise amplification primers that may be pre-labelled or may contain an affinity purification or attachment moiety. The kit may further comprise amplification reagents and also other suitably packaged reagents and materials needed for the particular amplification protocol.

The present invention also relates to the genes of the invention as biomarkers for outcome of AML patients.

Computer program product and ATIC score

In another aspect, the invention relates to a computer program product for use in conjunction with a computer having a processor and a memory connected to the processor, the computer program product comprising a computer readable storage medium having a computer mechanism encoded thereon, wherein the computer program mechanism may be loaded into the memory of the computer and cause the computer to carry out the method of the invention.

Particularly, the invention relates to a computer implemented product for predicting the survival time of a patient suffering from an AML comprising: (a) a means for receiving values corresponding to expression levels of a patient in a patient sample; (b) a database comprising reference expression levels representing a control, wherein the patient expression levels and the reference expression levels each have at least one value representing the expression level of at least 7 genes selected from the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN, F2RL2; wherein the computer implemented product compares the reference expression levels to the patient expression levels, wherein a difference in the expression profiles is used to provide a bad prognosis or a good prognosis.

As used herein, the term "control" denotes a specific value or dataset that can be used to prognose or classify the value e.g expression level or reference expression profile obtained from the test sample associated with an outcome class.

In a particular aspect, the invention relates to a computer readable medium having stored thereon a data structure for storing the computer-implemented product described herein.

In a particular aspect, the invention relates to a computer system comprising (a) a database including records comprising a reference expression levels of at least 7 gene selected from the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN, F2RL2 for a cohort of patients; (b) a user interface capable of receiving a selection of expression levels of the at least 7 genes for use in comparing to the reference expression levels in the database; (c) an output that displays a prediction of outcome wherein a difference in the expression profiles is used for predicting the survival time of a patient suffering from an AML.

In a particular aspect, the invention relates to a computer program product for use in conjunction with a computer having a processor and a memory connected to the processor, the computer program product comprising a computer readable storage medium having a computer mechanism encoded thereon, wherein the computer program mechanism may be loaded into the memory of the computer and cause the computer to carry out the method described herein.

In another particular aspect, the invention relates to a computer implemented product for predicting the survival time of a patient suffering from an AML comprising: (a) a means for receiving values corresponding to a patient expression levels in a subject sample; (b) a database comprising a reference expression levels representing a control, wherein the patient expression levels and the reference expression levels each have at least one value representing the expression level of at least 7 gene selected from the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN and F2RL2; wherein the computer implemented product compares the reference expression profile to the patient genes expression levels, wherein a difference in the expression profiles is used to predict the survival time of a patient suffering from an AML.

In the context of the invention, the inventors determined a score thanks to machine learning algorithm tool like “Least Absolute Shrinkage and Selection Operator” (LASSO) and statistical algorithm tool like “median thresholding”, “max-stat” or “Youden’s J statistic” to
5 classify patients and predict their survival times. They called the score “ATIC score” for AP-1/TNF- α Initiating Cells score. Notably, the ATIC score is obtained by weighted summing the expression level of at least 7 genes of the invention.

Thus, in a particular embodiment, the computer implemented product calculates an
10 ATIC score comprising the sum expression levels of each of the at least 7 genes of the invention. Optionally, classification of the patient into a low or high-risk group is based on a high ATIC score in comparison with a control cohort of AML patients or with a threshold.

Particularly, the ATIC score is the weighted sum expression of each of the at least 7
15 genes of the 53 genes of the invention. The weight of the genes will depend on their relevance.

Thus, in a particularly embodiment, the invention relates to a method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) i) determining in a sample obtained from the patient the expression levels of at least 7 genes selected in the group consisting in: JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID,
20 PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN and F2RL2 ii) performing an algorithm tool from the expression levels determined at step i) determining a
25 selection of the most relevant genes, iii) weight and sum the expression levels of the selected genes obtained at the step ii) to calculate an ATIC score, vi) comparing said ATIC score determined at step iii) with a predetermined reference value and v) providing a good prognosis when the score determined at the step iii) is lower than a predetermined reference value, or providing a bad prognosis when the score determined at the step iii) is higher than a
30 predetermined reference value.

According to the invention, the term “algorithm tool” denotes machine learning algorithm tool like “Least Absolute Shrinkage and Selection Operator” (LASSO) and the machine learning method described below and statistical algorithm tool like “median thresholding”, “max-stat” or “Youden’s J statistic”. According to the invention, the machine

learning algorithm tool and statistical algorithm tool like can be combined or not to determine the selection of the most relevant genes and to then calculate the ATIC score.

Depending on the algorithm tool and notably the statistical algorithm tool used in the method of the invention, the selection of the most relevant genes and their weight to calculate the ATIC will be different.

For example and in a particular embodiment, when the inventors use the median method, the ATIC score is obtained as the weighted sum of the following 14 genes of the invention: $(-0.02537109 \times \text{JAM3}) + (-0.03654864 \times \text{DUSP1}) + (-0.007206288 \times \text{RGS1}) + (0.008025696 \times \text{H2BC8}) + (-0.01143364 \times \text{NFKBID}) + (-0.00423489 \times \text{ZFP36}) + (-0.05511406 \times \text{SLFN12L}) + (-0.3608987 \times \text{GAS5}) + (0.1012163 \times \text{RPP25L}) + (0.05303331 \times \text{HEY1}) + (-0.01449147 \times \text{GIMAP4}) + (0.09348158 \times \text{EFCAB11}) + (-0.03832157 \times \text{CCL4}) + (-0.01028905 \times \text{MYCN})$.

For example and in a particular embodiment, when the inventors use the median method, the ATIC score is obtained as the weighted sum of the following 12 genes of the invention: $(\text{JAM3} \times -0.005866212) + (\text{DUSP2} \times -0.019479537) + (\text{TPT1} \times -0.153814998) + (\text{GAS5} \times -0.276975301) + (\text{SNORA31} \times -0.008822175) + (\text{RHOB} \times -0.004605374) + (\text{ZNF699} \times -0.067213843) + (\text{HEY1} \times 0.020546724) + (\text{GIMAP4} \times -0.078907542) + (\text{EFCAB11} \times 0.021803546) + (\text{MYCN} \times -0.039450311) + (\text{F2RL2} \times -0.011360306)$.

For example and in a particular embodiment, when the inventors use the median method, the ATIC score is obtained as the weighted sum of the following 10 genes of the invention as followed: $(\text{TPT1} \times -0.043205893) + (\text{BTG2} \times -0.010484238) + (\text{JUNB} \times -0.040442332) + (\text{GAS5} \times -0.295826023) + (\text{EGR3} \times -0.022279824) + (\text{ZNF699} \times -0.148198814) + (\text{HEY1} \times 0.008165923) + (\text{GIMAP4} \times -0.059185952) + (\text{PF4} \times -0.010480130) + (\text{MYCN} \times -0.019247414)$.

For example and in a particular embodiment, when the inventors use the median method, the ATIC score is obtained as the weighted sum of the following 10 genes of the invention: $(\text{H2BC8} \times 0.0020822620) + (\text{SLFN12L} \times -0.0308366591) + (\text{GAS5} \times -0.1096235372) + (\text{RPS13} \times -0.1086035387) + (\text{MYL9} \times 0.0047290321) + (\text{ZNF699} \times -0.0888478416) + (\text{GIMAP4} \times -0.0414516464) + (\text{ITGA2B} \times 0.0006137659) + (\text{MYCN} \times -0.0362358633) + (\text{F2RL2} \times -0.0741935733)$.

In another particular embodiment, when the inventors use the Max-Stat method, the ATIC score is obtained as the weighted sum of 10 genes of the invention as followed: $(\text{H2BC8} \times 0.003869971) + (\text{SNORA28} \times 0.082143034) + (\text{TPT1} \times -0.180191515) + (\text{SLFN12L} \times -$

0.032713592) + (GAS5 x -0.230689244) + (ZNF699 x -0.189028531) + (FOS x -0.043514705) + (HEY1 x 0.021512705) + (GIMAP4 x -0.016145838) + (CCL3 x -0.023873930).

In another particular embodiment, when the inventors use the Max-Stat method, the ATIC score is obtained as the weighted sum of 12 genes of the invention as followed: (JAM3 x -0.018362735) + (DUSP1 x -0.061571565) + (H2BC8 x 0.007759048) + (TPT1 x -0.067574035) + (KLF2 x 0.017460986) + (SLFN12L x -0.048092254) + (GAS5 x -0.184702865) + (RPS13 x -0.092093286) + (ZNF699 x -0.067291813) + (GIMAP4 x 0.034121423) + (EFCAB11 x 0.004327896) + (MYCN x -0.022981704).

In another particular embodiment, when the inventors use the Max-Stat method, the ATIC score is obtained as the weighted sum of 11 genes of the invention as followed: (DUSP1 x -3.948514e-02) + (TPT1 x -1.476539e-01) + (SLFN12L x -8.091971e-02) + (GAS5 x -2.437514e-01) + (EGR3 x -7.153055e-05) + (RPS13 x -2.351622e-04) + (ZNF699 x -6.893090e-02) + (RPP25L x 3.724891e-02) + (HEY1 x 3.152983e-03) + (GIMAP4 x -4.195367e-02) + (MYCN x -2.829746e-02).

In another particular embodiment, when the inventors use the Youden method, the ATIC score is obtained as the weighted sum of the following 9 genes of the invention: (JAM3 x -0.008561100) + (TPT1 x -0.196057762) + (TAF7 x -0.009083842) + (SLFN12L x -0.125831666) + (GAS5 x -0.144287204) + (SNORA31 x -0.082700716) + (FOS x -0.047705059) + (HEY1 x 0.013346800) + (MYCN x -0.047514761).

In another particular embodiment, when the inventors use the Youden method, the ATIC score is obtained as the weighted sum of the following 8 genes of the invention: (SLFN12L x -0.1732423421) + (GAS5 x -0.0691438523) + (RPS13 x -0.3087526357) + (ZNF699 x -0.0009789303) + (HEY1 x 0.0180139152) + (GIMAP4 x -0.0055809215) + (MYCN x -0.0499696473) + (F2RL2 x -0.0028135476).

In another particular embodiment, when the inventors use the Youden method, the ATIC score is obtained as the weighted sum of the following 7 genes of the invention: (JAM3 x -0.0401441653) + (H2BC8 x 0.0001428465) + (SLFN12L x -0.0779526478) + (NR4A1 x 0.0108135109) + (GAS5 x -0.0555269849) + (RPS13 x -0.1266144431) + (MYCN x -0.0664972573).

According to the invention, machine learning method of the invention may be chosen from Adaboost, GradientBoosting, MRMR, neural network methods, decision trees, k-nearest neighbors method, carrier vector machines, algorithm based on a linear model (like LASSO), a

generalized linear model discriminant, a factor regression model, a partial least square model, a factor analysis, a support vector machine, a support vector regression, a graphical model, a tree-based model, a random forest model, a random ferns model, a naive Bayes model, a linear discriminant analysis, a quadratic linear discriminant analysis, a perceptron model, a neural network model, nearest neighbor model, a nearest prototype model, an ensemble model, a prototype-based supervised algorithm, a bagged model, a Bayesian model, a regularized linear model, a polynomial model, a rule -based model, a Gaussian process model, a mixture discriminant model, a regression spline model, a rule induction method, a prototype model, a quantile regression model, a relevance vector machine, a soft independent modelling of class analogies model, a principal component-based model, an independent-based model, a self-organizing map model.

The methods of the present invention can be performed by one or more programmable processors executing one or more computer programs to perform functions by operating on input data and generating output. The algorithm can also be performed by, and apparatus can also be implemented as, special purpose logic circuitry, e.g., an FPGA (field programmable gate array) or an ASIC (application-specific integrated circuit). Processors suitable for the execution of a computer program include, by way of example, both general and special purpose microprocessors, and any one or more processors of any kind of digital computer. Generally, a processor will receive instructions and data from a read-only memory or a random access memory or both. The essential elements of a computer are a processor for performing instructions and one or more memory devices for storing instructions and data. Generally, a computer will also include, or be operatively coupled to receive data from or transfer data to, or both, one or more mass storage devices for storing data, e.g., magnetic, magneto-optical disks, or optical disks. However, a computer need not have such devices. Moreover, a computer can be embedded in another device. Computer-readable media suitable for storing computer program instructions and data include all forms of non-volatile memory, media and memory devices, including by way of example semiconductor memory devices, e.g., EPROM, EEPROM, and flash memory devices; magnetic disks, e.g., internal hard disks or removable disks; magneto-optical disks; and CD-ROM and DVD-ROM disks. The processor and the memory can be supplemented by, or incorporated in, special purpose logic circuitry. To provide for interaction with a user, embodiments of the invention can be implemented on a computer having a display device, e.g., in non-limiting examples, a CRT (cathode ray tube) or LCD (liquid crystal display) monitor, for displaying information to the user and a keyboard and a pointing device, e.g., a mouse or a trackball, by which the user can provide input to the

computer. Other kinds of devices can be used to provide for interaction with a user as well; for example, feedback provided to the user can be any form of sensory feedback, e.g., visual feedback, auditory feedback, or tactile feedback; and input from the user can be received in any form, including acoustic, speech, or tactile input. Accordingly, in some embodiments, the algorithm can be implemented in a computing system that includes a back-end component, e.g., as a data server, or that includes a middleware component, e.g., an application server, or that includes a front-end component, e.g., a client computer having a graphical user interface or a Web browser through which a user can interact with an implementation of the invention, or any combination of one or more such back-end, middleware, or front-end components. The components of the system can be interconnected by any form or medium of digital data communication, e.g., a communication network. Examples of communication networks include a local area network ("LAN") and a wide area network ("WAN"), e.g., the Internet. The computing system can include clients and servers. A client and server are generally remote from each other and typically interact through a communication network. The relationship of client and server arises by virtue of computer programs running on the respective computers and having a client-server relationship to each other.

Another object of the present invention is a computer-program product comprising code instructions for executing the method described above, when it is implemented by a computer.

Combination of the ATIC score with the LSC17 score

In a particular embodiment, the ATIC score can be used in combination with the LSC17 score as described in the patent application WO2017132749.

The LSC17 is based on the determination of the expression level of at least 3 genes selected in the group consisting in DNMT3B, ZBTB46, NYNRIN, ARHGAP22, LAPTM4B, MMRN1, DPY5L3, KIAA0125, CDK6, CPX 1, SOCS2, SMIM24, EMP1, NGFRAP1, CD34, AKR1C3, GPR56.

Therapeutics applications

In a further aspect, the invention also relates to a method for treating an AML in a patient with a bad prognosis as described above comprising the administration to said patient of an anti-cancer agent.

Anti-cancer agent can be selected in the group consisting in venetoclax, cytarabine, anthracyclines, fludarabine, gemcitabine, capecitabine, methotrexate, taxol, taxotere, mercaptopurine, thioguanine, hydroxyurea, cyclophosphamide, ifosfamide, nitrosoureas,

platinum complexes such as cisplatin, carboplatin and oxaliplatin, mitomycin, dacarbazine, procarbazine, etoposide, teniposide, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, L-asparaginase, doxorubicin, epimbicm, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, 5 irinotecan, estramustine, etoposide, nitrogen mustards, BCNU, nitrosoureas such as carmustme and lomustine, vinca alkaloids such as vinblastine, vincristine and vinorelbine, imatimb mesylate, hexamethylnelamine, topotecan, kinase inhibitors, phosphatase inhibitors, ATPase inhibitors, tyrphostins, protease inhibitors, inhibitors herbimycm A, genistein, erbstatin, and lavendustin A. In one embodiment, additional anticancer agents may be selected from, but are 10 not limited to, one or a combination of the following class of agents: alkylating agents, plant alkaloids, DNA topoisomerase inhibitors, anti-folates, pyrimidine analogs, purine analogs, DNA antimetabolites, taxanes, podophyllotoxin, hormonal therapies, retinoids, photosensitizers or photodynamic therapies, angiogenesis inhibitors, antimitotic agents, isoprenylation inhibitors, cell cycle inhibitors, actinomycins, bleomycins, MDR inhibitors and 15 Ca²⁺ ATPase inhibitors.

Additional anti-cancer agent may be selected from, but are not limited to, cytokines, chemokines, growth factors, growth inhibitory factors, hormones, soluble receptors, decoy receptors, monoclonal or polyclonal antibodies, mono-specific, bi-specific or multi-specific antibodies, monobodies, polybodies.

20 Additional anti-cancer agent may be selected from, but are not limited to, growth or hematopoietic factors such as erythropoietin and thrombopoietin, and growth factor mimetics thereof.

In the present methods for treating cancer, additional therapeutic active agent can be added like an antiemetic agent. Suitable antiemetic agents include, but are not limited to, 25 metoclopramide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acethylleucine monoemanolamine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dunenhydrinate, diphenidol, dolasetron, meclizme, methallatal, metopimazine, nabilone, oxypemdy, pipamazine, scopolamine, sulpiride, tetrahydrocannabinols, 30 thiephylperazine, thioproperazine and tropisetron. In a preferred embodiment, the antiemetic agent is granisetron or ondansetron.

In another embodiment, the further therapeutic active agent can be an hematopoietic colony stimulating factor. Suitable hematopoietic colony stimulating factors include, but are not limited to, filgrastim, sargramostim, molgramostim and epoietin alpha.

In still another embodiment, the other therapeutic active agent can be an opioid or non-opioid analgesic agent. Suitable opioid analgesic agents include, but are not limited to, morphine, heroin, hydromorphone, hydrocodone, oxymorphone, oxycodone, metopon, apomorphine, nomioipine, etoipbine, buprenorphine, mepedidine, lopermide, anileddine, 5 ethoheptazine, piminidine, betaprodine, diphenoxylate, fentanil, sufentanil, alfentanil, remifentanil, levorphanol, dextromethorphan, phenazodine, pemazocine, cyclazocine, methadone, isomethadone and propoxyphene. Suitable non-opioid analgesic agents include, but are not limited to, aspirin, celecoxib, rofecoxib, diclofinac, diflusinal, etodolac, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, indomethacin, ketorolac, meclofenamate, mefanamic acid, 10 nabumetone, naproxen, piroxicam and sulindac.

In yet another embodiment, the further therapeutic active agent can be an anxiolytic agent. Suitable anxiolytic agents include, but are not limited to, buspirone, and benzodiazepines such as diazepam, lorazepam, oxazepam, chlorazepate, clonazepam, chlordiazepoxide and alprazolam.

15 In yet another embodiment, the further therapeutic active agent can be a checkpoint blockade cancer immunotherapy agent.

Typically, the checkpoint blockade cancer immunotherapy agent is an agent which blocks an immunosuppressive receptor expressed by activated T lymphocytes, such as cytotoxic T lymphocyte-associated protein 4 (CTLA4) and programmed cell death 1 (PDCD1, 20 best known as PD-1), or by NK cells, like various members of the killer cell immunoglobulin-like receptor (KIR) family, or an agent which blocks the principal ligands of these receptors, such as PD-1 ligand CD274 (best known as PD-L1 or B7-H1).

Typically, the checkpoint blockade cancer immunotherapy agent is an antibody.

In some embodiments, the checkpoint blockade cancer immunotherapy agent is an 25 antibody selected from the group consisting in anti-CTLA4 antibodies, anti-PD1 antibodies, anti-PDL1 antibodies, anti-PDL2 antibodies, anti-TIM-3 antibodies, anti-LAG3 antibodies, anti-IDO1 antibodies, anti-TIGIT antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies, anti-BTLA antibodies, and anti-B7H6 antibodies.

In yet another embodiment, the further therapeutic active agent can be a cellular 30 immunotherapeutic agent such as CAR-T, CAR-NK, etc... cell (see PMID: 37345050).

The invention also relates to a pharmaceutical composition comprising an anti-cancer treatment for use in the treatment of an AML in a subject with a bad prognosis as described above.

Any therapeutic agent of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc.

The pharmaceutical compositions of the invention can be formulated for a topical, oral, intranasal, parenteral, intraocular, intravenous, intramuscular, intrathecal or subcutaneous administration and the like.

Particularly, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment.

In addition, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently can be used.

Typically the anti-cancer agent according to the invention are administered to the subject in a therapeutically effective amount.

By a "therapeutically effective amount" the anti-cancer agent is meant a sufficient amount of the anti-cancer agent for treating cancer at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the anti-cancer agent will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the

disorder; activity of (the specific) the anti-cancer agent employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific the anti-cancer agent employed; the duration of the treatment; drugs used in combination or coincidental with the specific anti-cancer agent employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the anti-cancer agent at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the the anti-cancer agent for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the anti-cancer agent, preferably from 1 mg to about 100 mg of the anti-cancer agent. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a particular embodiment, the anti-cancer agent may be used in a concentration between 0.01 μ M and 20 μ M, particularly, the anti-cancer agent may be used in a concentration of 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 μ M.

According to the invention, the anti-cancer agent is administered to the subject in the form of a pharmaceutical composition. Thus, the invention also relates to a therapeutic composition comprising the anti-cancer agent for use in the treatment of a cancer in a subject in need thereof.

Typically, the anti-cancer agent may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel

capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

5 Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized
10 water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists.
15 It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising the NDPK-D protein or fragment thereof and/or an agent for NDPK-D protein expression of the invention or the anti-cancer agent of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as
20 hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The NDPK-D protein or fragment thereof and/or an agent for NDPK-D protein expression of the present invention or the anti-cancer agent of the invention can be formulated into a composition in a
25 neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric
30 hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle

size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

5 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating
10 the various sterilized agent of the present inventions into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the typical methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the anti-cancer agent of the invention plus any additional desired ingredient from a previously
15 sterile-filtered solution thereof. The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are
20 easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular,
25 subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

30
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 present invention.

FIGURES:

Figure 1. JAM-C is expressed by a subset of GPR56-expressing cells in AML samples at diagnosis.

5 **A:** Histogram showing the frequency of cells expressing JAM-C (left panel) or GPR56 (right panel) within the indicated phenotypic compartment defined by CD34 and CD38 expression. **B:** Histogram showing the frequency of cells expressing JAM-C within the indicated phenotypic compartment defined by combination of GPR56 and CD34 expression as described in Pabst et al ¹⁷. **C:** Expression of transcripts encoding JAM-C (*JAM3*, left panel) or
10 GPR56 (right panel) in patient samples stratified according to median LSC17 score as measured using nCounter nanostring. n = 67. Data are represented with mean ± SEM. ns: not significant, * p<0.05, ** p<0.01, *** p<0.001.

Figure 2. *Jam-C*-deficiency results in up-regulation of AP-1/TNF-α transcriptional network in HSPC

15 Venn diagram showing differentially expressed genes (DEGs) identified in the present study, in the LSC17 core signature ²⁴ or in genes defining the CD34⁺/AP-1^{High} cluster in the scRNA sequencing study by Velten and collaborators ²⁸.

Figure 3. Genes from the LSC17 score and AP-1/TNF-α signature belong to different clusters of co-regulated genes in human

20 **A:** Correlation plot showing expression of genes identified in the present study and selected from Ng et al²⁴ and Stavropoulou et al³⁸ across samples from the TCGA cohort (n= 173). Color and size represent the direction and the magnitude of the correlation. Only correlations with P < 0.05 are shown. Two gene expression correlation clusters are underlined and conserved across cohorts. One cluster encompass genes related to cell adhesion or
25 migration (ITGA6, ZEB1, JAM3) and genes from the LSC17 score (Cluster I). The second cluster contains number of genes up-regulated in HSPC isolated from JAM3-deficient leukemic mice (Cluster II). **B:** Heatmap showing unsupervised patient sample clustering according to Z-score of genes belonging to LSC17 and DEGs from the present study across samples from assembly of TCGA, OHSU and Leucegene cohorts representing 887 AML samples at
30 diagnosis. Four groups of samples can be visualised expressing respectively high or low levels of LSC17 genes and high or low levels of DEGs identified in the present study (AP-1/TNF-α signature).

Figure 4 : **A:** Histogram showing the accuracy of patient stratification using the ten best models with median thresholding. Classification occurrence of individual patients as ATIC^{Low}

or $ATIC^{High}$ is quantified as the number of times that a patient belongs to $ATIC^{Low}$ or $ATIC^{High}$ groups with values set to 0 when a patient is predicted to belong to one or the other group with half of the models. B: Same as in A, using the ten best models with maximally selected rank and statistics thresholding (Max-Stat). Note the increased dissymmetry in patient classification using Max-Stat as compared to median thresholding.

Figure 5. A: Kaplan-Meier survival curves of patients from the validation cohort (n=435, half of the mixed OHSU (n=571), Leucegene (n=263) and TCGA (n=173) cohorts) in $ATIC^{Low}$ and $ATIC^{High}$ groups. Groups are defined by the median ATIC score value calculated as the weighted sum of 14 genes expression. B: Kaplan-Meier survival curves in $LSC17^{Low}$ and $LSC17^{High}$ group on the validation cohort (n=435). Groups are defined by the median LSC17 score. C: Kaplan-Meier survival curves of patients from the validation cohort according to combined stratification using LSC17 and ATIC scores (n=435). D: Kaplan-Meier survival curves of patients from the HOVON/SAKK cohort (ArrayExpress, E-MTAB-3444) in $ATIC^{Low}$ and $ATIC^{High}$ groups (n= 600). E: Same as D according to LSC17 score. F: Kaplan-Meier survival curves of patients from the HOVON/SAKK cohort according to combined stratification using LSC17 and ATIC scores. Curve comparison p-values are calculated by Log-Rank test.

EXAMPLE:

Material & Methods

Human samples

All biological samples were collected with informed consent according to the procedure approved by our institutional review board at Institut Paoli Calmettes (IPC). Vials were thawed in hot bath water and incubated 10min in 15mL of pre-heated RPMI 30% FCS + 1% Pen/strep + 100U/mL DNase and 10U/mL of heparin and centrifugated 10min at 1600 RPM. Cell pellets were washed with pre-heated RPMI 10% FCS/1% Pen-Strep/ 100U/mL DNase/ 10U/mL heparin and centrifugated for 10min at 1600 RPM. Dead cells were removed by layering 1ml of cell suspension onto 2ml of Ficoll and centrifugated 20min at 2000 RPM without brake. After centrifugation living cells were recovered into RPMI 10% FCS + 1% Pen/strep and washed.

Mice experiments

iMLL-AF9 mice were obtained from J.Schwaller lab (Basel) and crossed with Mx1-Cre Jam3fl/fl mice. All experiments were performed in compliance with the laws and protocols approved by animal ethics committees. Baseline WBC count was assessed on day 0 in IMLL

Jam3^{fl/fl} mice and Jam3 gene deletion was induced by three intraperitoneal injections of 200µg poly (I:C) (InvivoGen) on Day 1, Day 3, and Day 5. Doxycycline (400µg/mL) (Sigma) was provided in water supplemented with 5% sucrose nine days after the last poly (I:C) injection. Leukemic burden was monitored each week by following white blood cell count (WBC) using
5 IDEXX ProCyt Dx Haematology Analyzer (IDEXX Laboratories) starting 4 weeks after Doxycycline induction.

Flow cytometry and cell sorting

Human samples: single cell suspensions were incubated with antibodies against CD33, CD45, CD38, CD34, CD41, GPR56 and JAM-C in PBS/0.5mM EDTA/2% FCS for 30min at
10 4°C, washed and processed for analysis. Mouse samples: BM cells were recovered from femur and tibia, red blood cells were lysed using 1X RBC lysing buffer (eBioscience) and stained with a biotinylated lineage cocktail containing: CD4, CD8, CD3, CD19, CD11c, DX5, Ter119, CD11b, B220 and Gr1. All antibodies used for flow cytometry are described in Supplemental Table 1. FACS analysis was performed on a FACS LSR II (BD Biosciences) and cell sorting on
15 a FACS Aria III (BD Biosciences). Data were analyzed using DIVA V.8.01 (BD Biosciences) or OMIQ (Omiq Inc).

RNA sequencing

HSCs and GMP were directly sorted in RLT buffer from mRNA purification kit using the RNeasy micro kit (QIAGEN). Samples were sent to the GenomEast platform
20 (<http://genomeast.igbmc.fr/> Illkirch, France). Libraries were paired-end sequenced (2 x 100bp) on a HiSeq4000 system (Illumina).

Nanostring assay

Total mRNA from patient samples was extracted using the RNeasy mini kit (QIAGEN) according to manufacturer recommendations. The custom nCounter Nanostring Code Set
25 containing 61 probes (Supplemental Table 2) was used according to manufacturer instructions. After quantification, results were normalized using nSolver software (Version 4.0) and Log2 transformed values were used for LSC17 score calculation.

Statistical analysis

Statistical analysis was performed using GraphPad 6 software and error bars represent
30 the mean ± SEM. Normality was assayed using D'Agostino & Pearson omnibus normality test and sample were compared with a Mann-Whitney test when normality was not reached. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Publicly available datasets used for model training and validation

Publicly available mRNA sequencing datasets from the TCGA and OHSU cohort (also known as BEAT AML cohort³⁹) were retrieved from cBioportal using the CGDS-R package. The Leucegene dataset was retrieved from GEO data repository under accession number GSE67040. The three cohorts were assembled in a single matrix of gene expression, scaled, centered and half-split in training and validation cohorts to establish a Cox regression model using the least absolute shrinkage selector operator (LASSO) algorithm. The gene expression dataset from the HOVON/SAKK cohort (662 adult AML cases) was retrieved from the ArrayExpress database (www.ebi.ac.uk/arrayexpress, Acc# E-MTAB-3444). All bioinformatic pipelines are available upon request.

Results

JAM-C identifies a subset of leukemic stem cells

To address the phenotypic heterogeneity of AML cellular compartments enriched in LSC, we conducted a correlation study of “LSC markers” gene expression across three cohorts. GPR56/JAM-C and CD93/CD32 correlations were systematically found, while correlation between other “LSC markers” varied from cohort to cohort (data not shown). Since CD93 is selectively expressed in the CD34⁺CD38⁻ fraction of *MLL*-rearranged AML but not in non-*MLL* diseases¹⁵, we focused on GPR56 and JAM-C (data not shown). Both markers were tested by flow cytometry in combination with Live Dead, CD34, CD38, CD41, CD123, CD33 and CD45 on 62 blood AML patient samples collected in the frame of the NCT02320656 clinical trial (Table 1). Since JAM-C is expressed by human platelets, CD41⁺ events were excluded from analysis. Uniform Manifold Approximation and Projection (UMAP)⁴⁰ analysis revealed that most of the JAM-C-expressing cells belonged to the CD34⁺/GPR56⁺/CD38^{-low} compartment (data not shown). GPR56⁺ cells expressed various levels of CD34, CD38 and JAM-C (data not shown), but higher frequencies of JAM-C or GPR56 expressing cells were found in the CD34⁺ compartment (Figure 1A). JAM-C expressing cells represented less than 1% of GPR56 expressing cells (Figure 1B). Since *GPR56* gene expression is contributing to the LSC17 score, we tested whether *JAM3* expression was associated with LSC17 score. To this end, JAM-C expression and LSC17 score were measured using nCounter Nanostring in samples processed by flow-cytometry. Results showed that *JAM3* and *GPR56* expression were significantly higher in samples belonging to the LSC17^{High} group (Figure 1C), suggesting that JAM-C may identify a subset of cells contributing to the leukemic stem cell transcriptional program quantified by the LSC17 score.

Genetic deletion of Jam-C before leukemic onset alters imbalanced HSPC expansion driven by MLL-AF9 expression

To test whether JAM-C plays an active role in the maintenance of leukemic stem cell transcriptional program, we established a mouse model in which conditional knock-out of *Jam3* (encoding Jam-C) can be achieved before leukemic onset. To this end, inducible iMLL-AF9 mice were crossed with Mx1-Cre/*Jam3^{fl/fl}* mice resulting in iMLL-AF9/Mx1-Cre/*Jam3^{fl/fl}* mice (called iMLL *Jam3^{fl/fl}* thereafter). Specific deletion of *Jam3* in hematopoietic cells was induced upon polyI:C injection and AML initiation was driven by MLL-AF9 expression under the control of the reversed tetracycline transactivator (rtTA)³⁸. Leukemia burden was measured by white blood cells count (WBC) and experiments were stopped when a value of 30 000 cells/ μ l was reached (data not shown). As expected, efficient deletion of Jam-C in long-term HSC (LT-HSC), short-term HSC (ST-HSC) and multipotent progenitors 3 (MPP3) was observed upon polyI:C treatment (data not shown). Exposition to doxycycline (DOX) led to similar WBC increase in leukemic mice lacking Jam-C expression in hematopoietic cells (iMLL *Jam3^{ko/ko}*) or not (iMLL *Jam3^{fl/fl}*) as compared to non-leukemic animals (data not shown). Increase in red blood cells distribution width (RDW) values, which preceded that of WBC as described in human⁴¹, confirmed a similar leukemia progression in *Jam-C*-proficient and -deficient leukemic mice (data not shown).

Previous reports have shown that leukemic initiating cells (LIC) upon MLL-AF9 expression are found in LT-HSC-like and GMP-like compartments^{33,38,42}. Early haematopoiesis in non-leukemic and leukemic iMLL *Jam3^{fl/fl}* and iMLL *Jam3^{ko/ko}* mice was thus analysed by flow cytometry using the SLAM markers and a gating strategy⁴³. As previously reported³⁸, we found a twofold reduction in the frequency of LSK cells in leukemic mice as compared to healthy animals regardless of *Jam-C* expression (data not shown). Within the LSK compartment, the twofold expansion of the LT-HSC-like compartment in leukemic mice as compared to healthy animals appeared to be Jam-C-dependent (data not shown). In contrast, expansion of the ST-HSC-like observed in leukemic animals was even further increased in *Jam-C*-deficient leukemic mice, reaching more than 60% of the LSK compartment (data not shown). This was at the expense of the multipotent progenitors, MPP-2, -3 and -4 (data not shown). The twofold expansion of the GMP-like compartment observed in Jam-C-proficient leukemic mice as compared to healthy animals was abolished in *Jam-C*-deficient leukemic mice (data not shown). These results were confirmed by UMAP analysis showing that LT-HSC-like, ST-HSC-like and GMP-like expansion observed in iMLL-AF9 *Jam3^{fl/fl}* mice was replaced by expansion of a CMP-like compartment in *Jam-C*-deficient leukemic mice (data not shown).

Jam-C deletion rewires AP-1/TNF- α /NF κ B transcription network

To identify the specific molecular mechanisms by which JAM-C regulates HSC-like expansion and commitment in leukemic mice, we performed bulk mRNA sequencing on HSC-like and GMP-like isolated from the bone marrow of leukemic *Jam-C*-proficient and *Jam-C*-deficient mice. All animals were treated with poly I:C prior to leukemia initiation using doxycycline in order to exclude an effect of the poly I:C on differentially expressed genes. Fifty-three genes were upregulated in HSC-derived AML cells isolated from *Jam-C*-deficient leukemic mice, while only eleven genes, including *Jam3*, were significantly downregulated with a p-adjusted value below 0.05 (data not shown). In GMP-derived AML cells isolated from *Jam-C*-deficient leukemic mice, we also observed number of upregulated genes even though GMP-like did not express Jam-C (data not shown)^{44,45}. Gene set enrichment analysis (GSEA) revealed enrichment of pathways related to cell-cell adhesion, TNF- α signalling via NF κ B and Activation Protein-1 (AP-1) transcription factor in HSC-like isolated from leukemic *Jam-C*-deficient mice (data not shown). The comparison of differentially expressed genes (DEGs) between *Jam-C*-deficient and -proficient leukemic mice (log2 relative abundance above 1.2) highlighted upregulation of AP-1 transcription factors (*Jun*, *Fos*, *Junb*, *Jund*, ...) in leukemic HSC-like and GMP-like cells isolated from *Jam-C*-deficient mice (data not shown). We then questioned whether genes affected by Jam-C deletion in HSC-like and GMP-like cells may identify specific LSC compartments in humans. None of the DEGs identified in HSC-like and GMP-like cells were present in the LSC17 gene list (data not shown). In contrast, 25 out of the 56 DEGs found in our study were over-expressed by CD34⁺ Blasts AP-1^{High} previously identified by single-cell transcriptomic of AML patient samples (Figure 2)²⁸. We concluded that JAM-C-expression repress transcription of genes from AP-1 and TNF- α pathways that have been involved in the early steps of pre-leukemic to leukemic transition^{46,47,48}.

The AP-1/TNF- α signature identifies poor prognosis AML patient

To test whether the AP-1/TNF- α signature identified a different cell-of-origin than those identified by the LSC17 or the EMT-related gene expression signature of LT-HSC-derived AML of iMLL-AF9 model, gene expression correlation was carried out with three independent AML cohorts (TCGA, Leucegene and OHSU). Irrespective of the cohort, two major clusters of co-regulated genes were identified and named respectively cluster I and cluster II (Figure. 3A). Cluster I contained *JAM3*, *ZEB1*, *ITGA6*, *ERG* and genes from the LSC17 score (*GPR56*, *DNMT3B*, *NYNRIN*...). In contrast, most of the genes belonging to the AP-1/TNF- α signature

were found in cluster II, suggesting that cluster I and cluster II of coregulated genes may reflect different features of patient samples. Gene expression datasets from the three cohorts were thus assembled in a single mixed dataset representing 871 patient samples to perform unsupervised clustering according to expression of genes from the LSC17 score and AP-1/TNF- α signatures.

- 5 Four groups of patients expressing inverse, high or intermediate levels of the two gene expression signatures were identified (Figure. 3B). This raised the question whether patients stratified by the LSC17 score may be sub-stratified by the AP-1/TNF- α signature. To do so, the mixed dataset was equally splitted in training and validation cohorts in order to define the ATIC score (AP-1/TNF- α Initiating Cells). We used Least Absolute Shrinkage and Selection
- 10 Operator (LASSO) algorithm to relate expression of genes from the AP-1/TNF- α signature to patient survival in the training cohort using age and LSC17 scores as offsets. LASSO was run on two thousand drawings of the training cohort. Weighted sum of gene expression obtained from the LASSO was trained in a cox model to define a threshold for the ATIC score using either the maximally selected rank and statistics (Max-Stat) method or the median value of the
- 15 ATIC score. Quality of the different models was then evaluated according to the area under the curve (AUC) and accuracy of the ten best models with respect to median or Max-Stat thresholding was then tested. We found that most of the patients were classified as high or low irrespective of the model (Figure 4, Classification Occurrence = 10). Only few patients were not repeatedly classified as high or low and had classification occurrence different from 10.
- 20 Using Median thresholding two patients were classified five times as high and five time as low resulting in Classification Occurrence value of 0 (Fig. 4A), while four patients belonged to the classification occurrence class 0 using Max-Stat thresholding (Fig. 4B). We thus retained models using median thresholding as the most robust method to calculate the ATIC score. The model with the highest AUC using genes detectable across platforms (RNAseq, nCounter
- 25 Nanostring and Affymetrix) allowed defining the ATIC score as the weighted sum of 14 genes: $((-0.02537109 \times \text{JAM3}) + (-0.03654864 \times \text{DUSP1}) + (-0.007206288 \times \text{RGS1}) + (0.008025696 \times \text{H2BC8}) + (-0.01143364 \times \text{NFKBID}) + (-0.00423489 \times \text{ZFP36}) + (-0.05511406 \times \text{SLFN12L}) + (-0.3608987 \times \text{GAS5}) + (0.1012163 \times \text{RPP25L}) + (0.05303331 \times \text{HEY1}) + (-0.01449147 \times \text{GIMAP4}) + (0.09348158 \times \text{EFCAB11}) + (-0.03832157 \times \text{CCL4}) + (-0.01028905 \times \text{MYCN}))$.
- 30 High ATIC scores were strongly associated with poor overall survival with respective median survival values of 7,9 and 33 months for ATIC^{High} and ATIC^{Low} patients (Figure. 5A). Stratification of the same validation cohort according to the LSC17 score yielded respective median survival values of 10,5 and 27 months for LSC17^{High} and LSC17^{Low} patients (Figure. 5B). Combination of ATIC and LSC17 scores on identified patients with a median survival not

reached after eight years of follow-up in the ATIC^{Low}/LSC17^{Low} arm (medians: 7, 10.5, 21, NA, Figure. 5C), whereas no significant difference was observed between patients belonging to the ATIC^{High}/LSC17^{High} and ATIC^{High}/LSC17^{Low} arms. To confirm these results, we tested an independent cohort of 662 adult AML cases for which gene expression was measured using Affymetrix (HOVON cohort). ATIC and LSC17 scores remained significantly associated with disease outcome and respective median survival of : 17.1 months [14.4; 21.1] for ATIC^{High} , 31.1 months [21.4; 59.4] for ATIC^{Low} , 12.3 months [10.6; 15.2] for LSC17^{High} and 132 months [56.1; NA] for LSC17^{Low} (Figure. 5D-E). Despite the excellent predictive value of the LSC17 score on this cohort, combination of the ATIC score with LSC17 allowed to reclassifying 139 cases with ATIC^{High} score in each of the LSC17 groups (Figure. 5F). ATIC^{High}/LSC17^{High} and ATIC^{Low}/LSC17^{Low} scores were strongly associated with disease outcome with respective median survival values of 10,6 months and not reached after 200 months. Together, these results demonstrate that combination of scores reflecting cellular heterogeneity at diagnosis are invaluable biomarkers for risk stratification in AML.

Example on other ATIC signatures generated thanks to the method of the invention

Thanks to the initial signature of 53 genes and the methods of the invention, the inventors generated different ATIC signatures and showed that these signatures allow to identify poor or good prognosis AML patients (data not shown). More, combined with the LSC17 score the ATIC signatures are more efficient to identify poor or good prognosis AML patient (data not shown).

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

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CLAIMS:

1. A method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) i) determining in a sample obtained from the patient the expression levels of at least 7 genes selected in the group consisting in: JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN and F2RL2 ii) comparing said expression levels determined at step i) with their predetermined reference values and iii) providing a good prognosis when the expression levels of the genes determined at step i) are lower than their predetermined reference values, or providing a bad prognosis when the expression levels of the genes determined at step i) are higher than their predetermined reference values.
2. A computer implemented product for predicting the survival time of a patient suffering from an AML comprising: (a) a means for receiving values corresponding to expression levels of a patient in a patient sample; (b) a database comprising reference expression levels representing a control, wherein the patient expression levels and the reference expression levels each have at least one value representing the expression level of at least 7 genes selected from the group consisting in JAM3, DUSP1, JUN, IER2, DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN, F2RL2; wherein the computer implemented product compares the reference expression levels to the patient expression levels, wherein a difference in the expression profiles is used to provide a bad prognosis or a good prognosis.
3. A method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) i) determining in a sample obtained from the patient the expression levels of at least 7 genes selected in the group consisting in: JAM3, DUSP1, JUN, IER2,

DUSP2, RGS1, H2BC8, PTGS2, NFKBID, PPP1R15A, NFKBIZ, ZFP36, SNORA28, TPT1, KLF2, BTG2, JUNB, JUND, ATF3, UBC, SKIL, TAF7, SLFN12L, NR4A1, CHST2, GAS5, SNORA31, HES1, EGR3, RPS13, PMAIP1, RHOB, MYL9, ZNF699, ZNF101, FOS, FJX1, RPP25L, HEY1, PTMA, GIMAP4, EFCAB11, FOSB, CD14, CCL4, CCL3, PF4, OSM, CD69, ITGA2B, VWF, MYCN and F2RL2 ii) performing an algorithm tool from the expression levels determined at step i) determining a selection of the most relevant genes, iii) weight and sum the expression levels of the selected genes obtained at the step ii) to calculate an ATIC score, vi) comparing said ATIC score determined at step iii) with a predetermined reference value and v) providing a good prognosis when the score determined at the step iii) is lower than a predetermined reference value, or providing a bad prognosis when the score determined at the step iii) is higher than a predetermined reference value.

4. A method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) according to the claims 1 to 3 wherein the expression levels of the 14 following genes JAM3, DUSP1, RGS1, H2BC8, NFKBID, ZFP36, SLFN12L, GAS5, RPP25L, HEY1, GIMAP4, EFCAB11, CCL4 and MYCN are determined.
5. A method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) according to the claims 1 to 3 wherein the expression level of gene GAS5 is at least determined.
6. A method for predicting the survival time of a patient suffering from an Acute Myeloid Leukemia (AML) according to the claims 3, 4 or 5 wherein the ATIC score is combined with the LSC17 score.
7. A method for treating an AML in a patient with a bad prognosis according to the claims 1 to 6 comprising the administration to said patient of an anti-cancer agent.

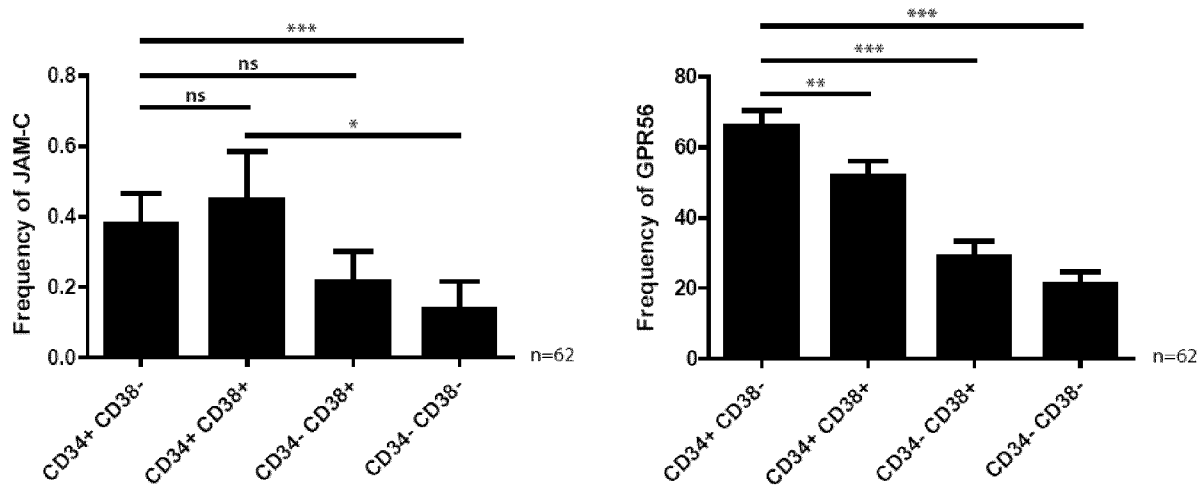


Figure 1A

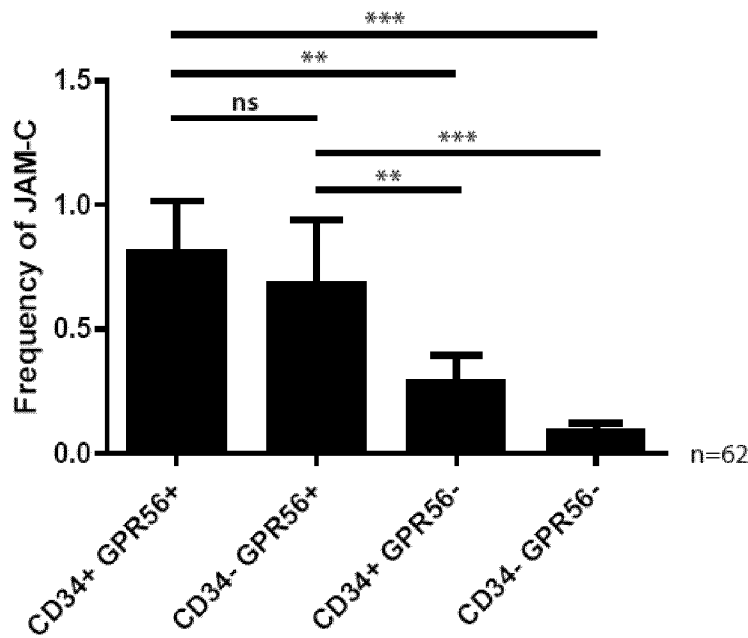


Figure 1B

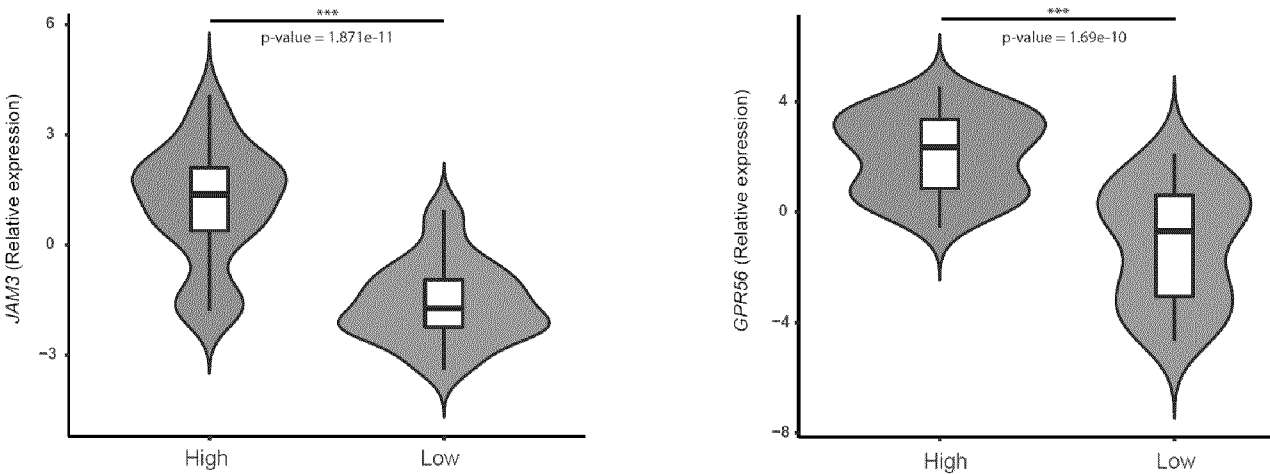


Figure 1C

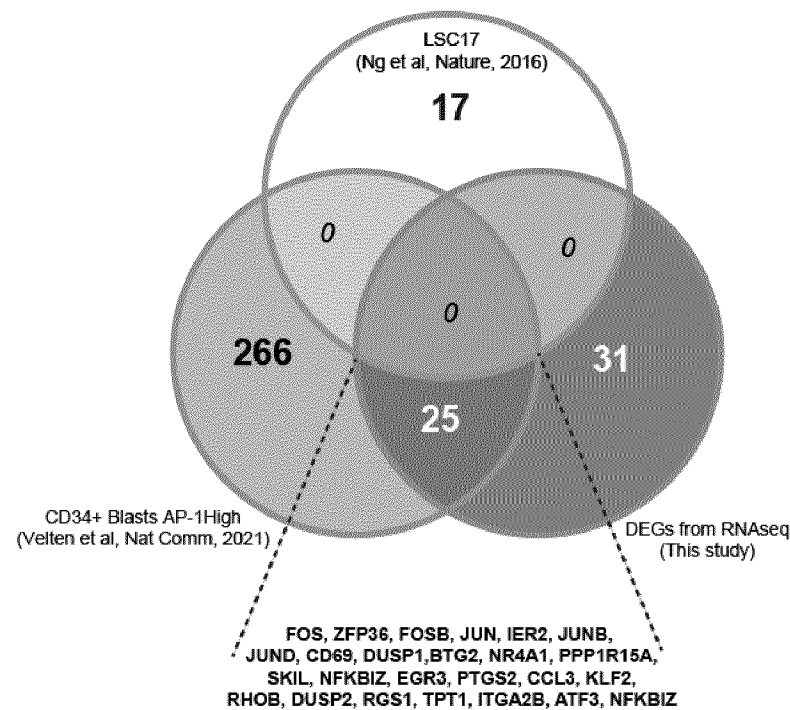


Figure 2

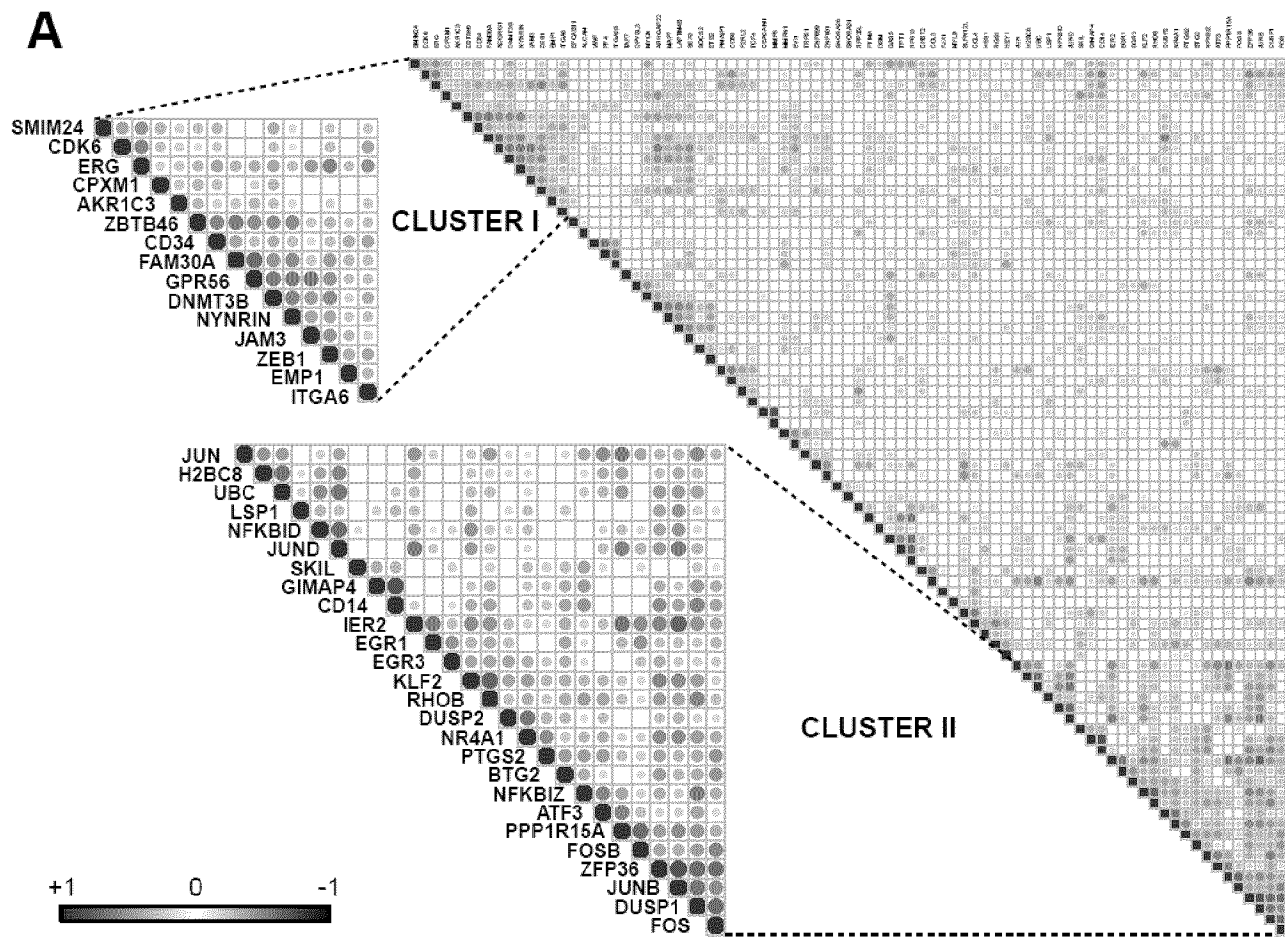


Figure 3A

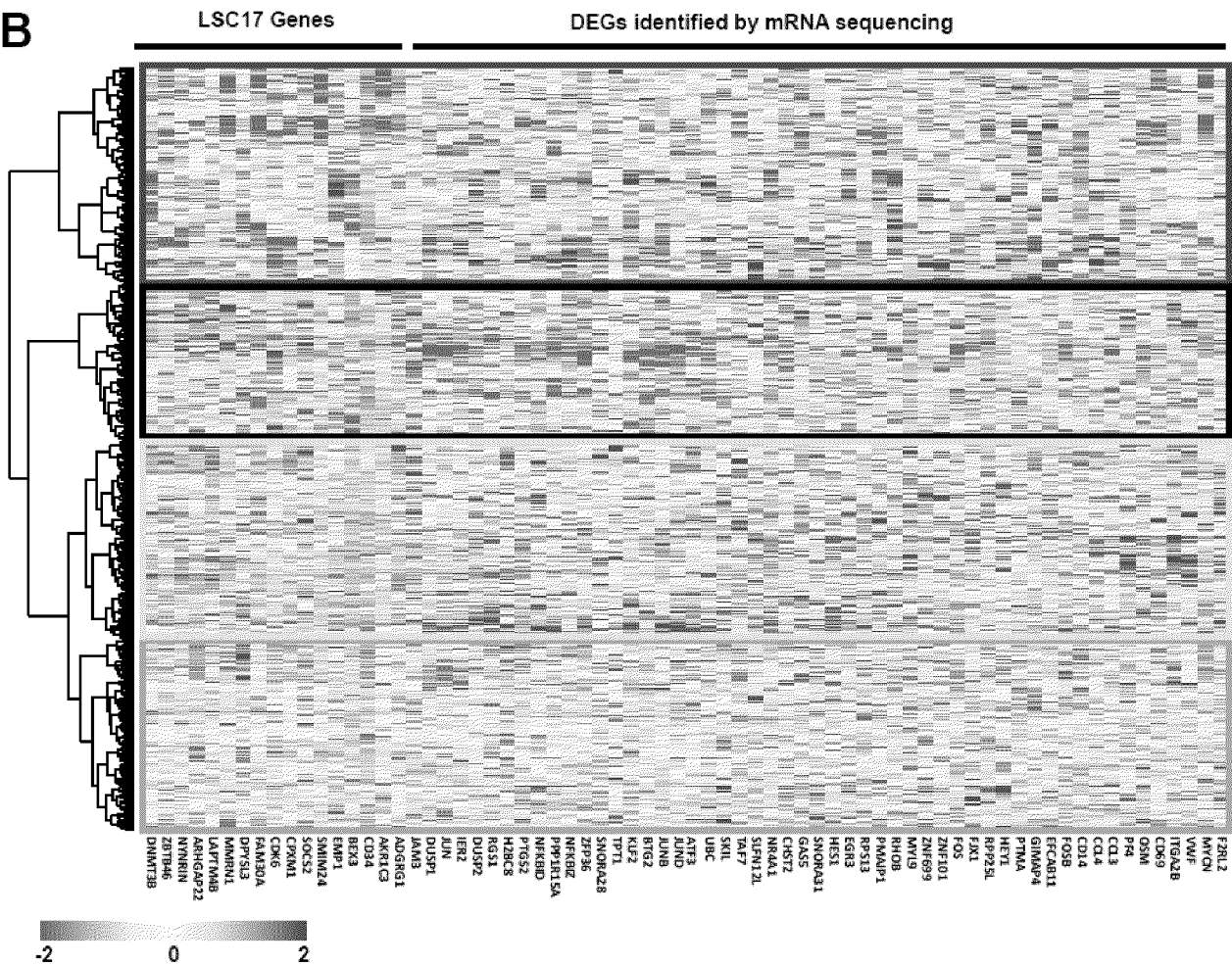


Figure 3B

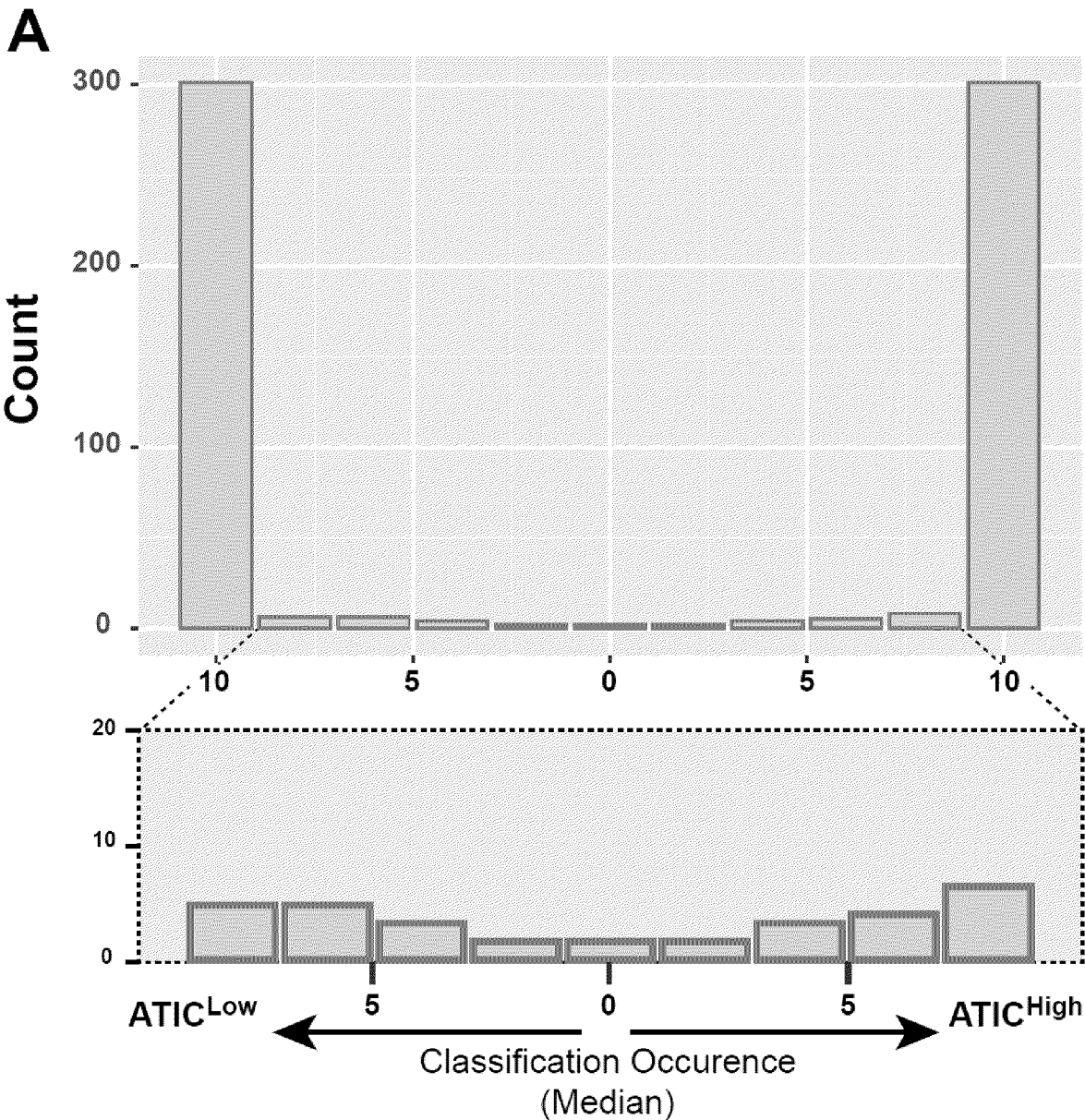


Figure 4A

B

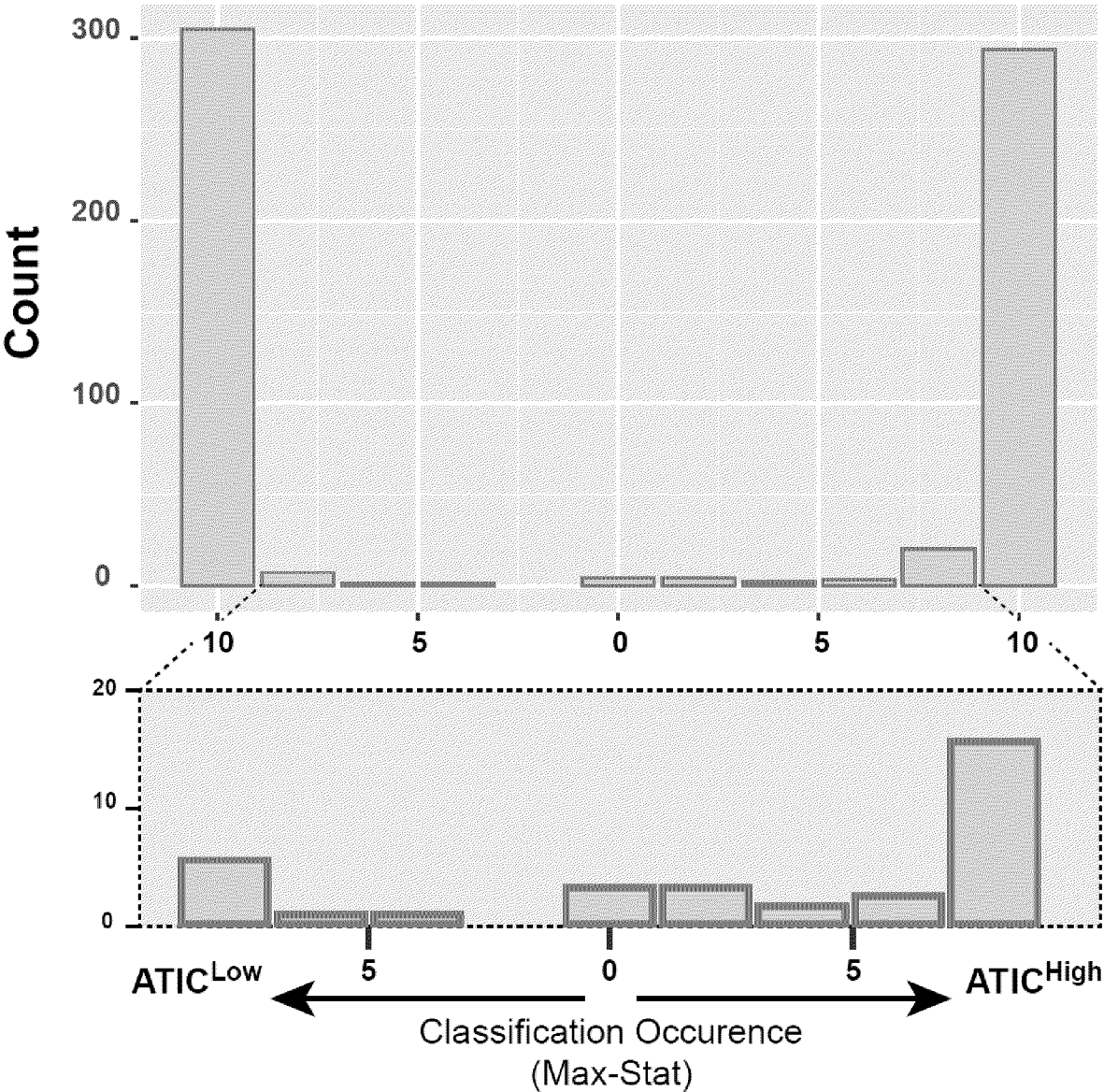


Figure 4B

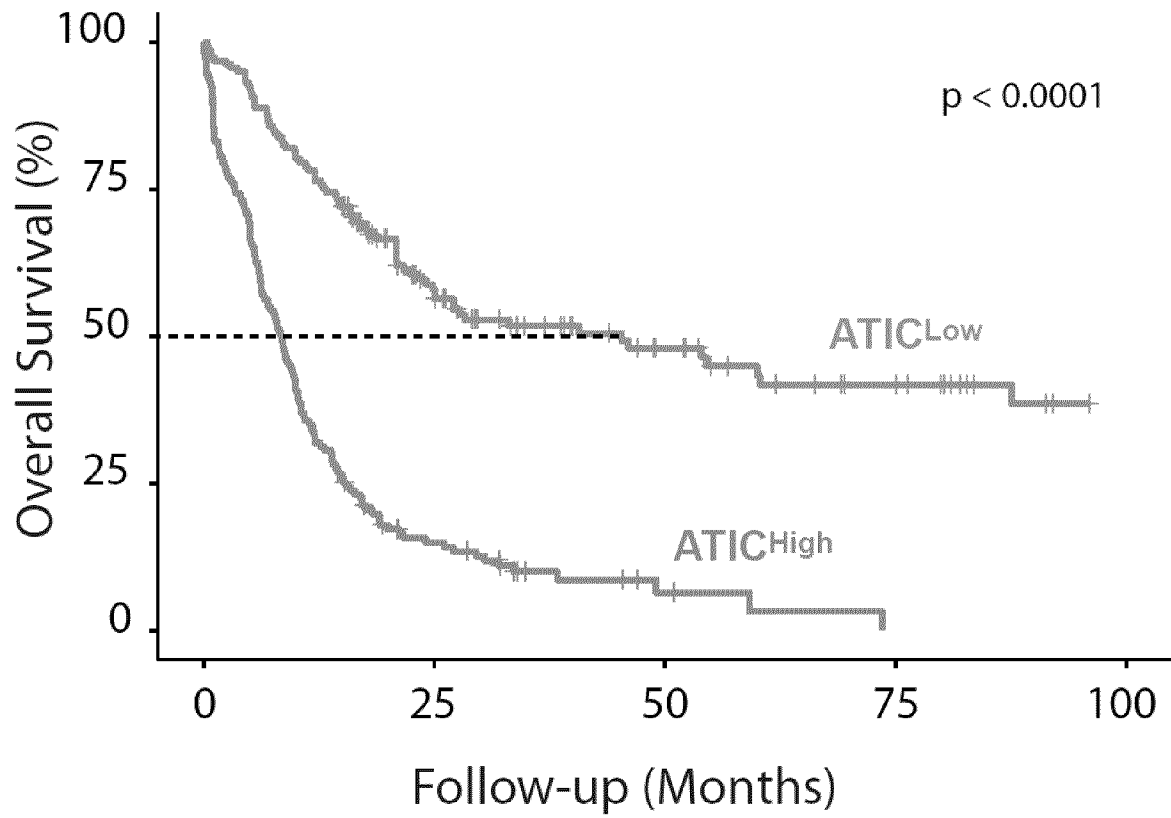


Figure 5A

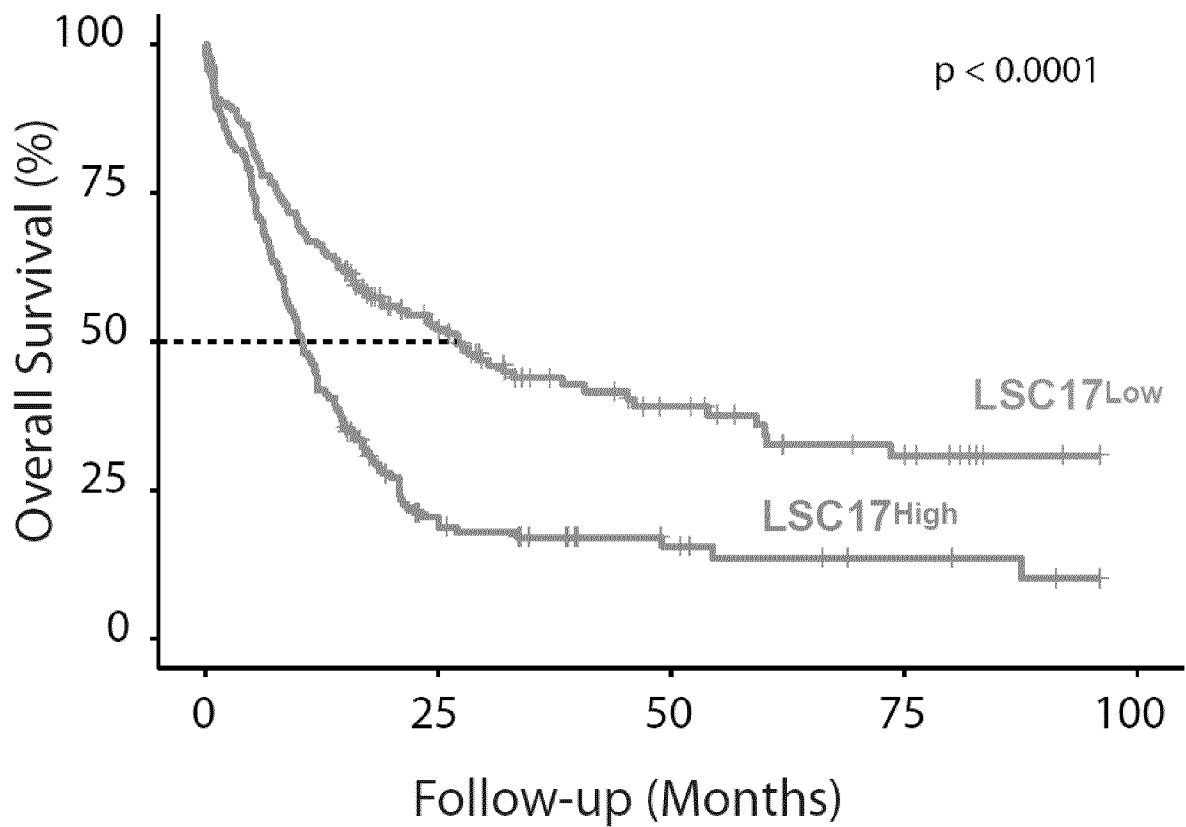


Figure 5B

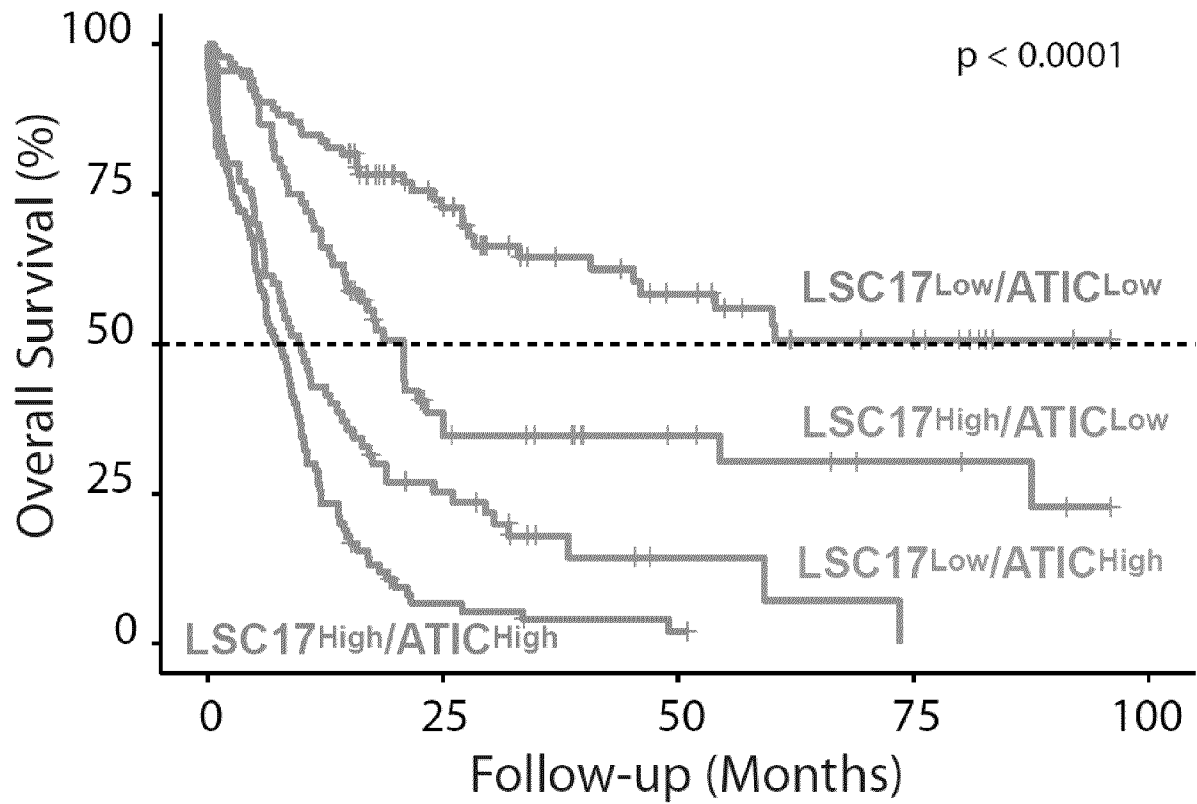


Figure 5C

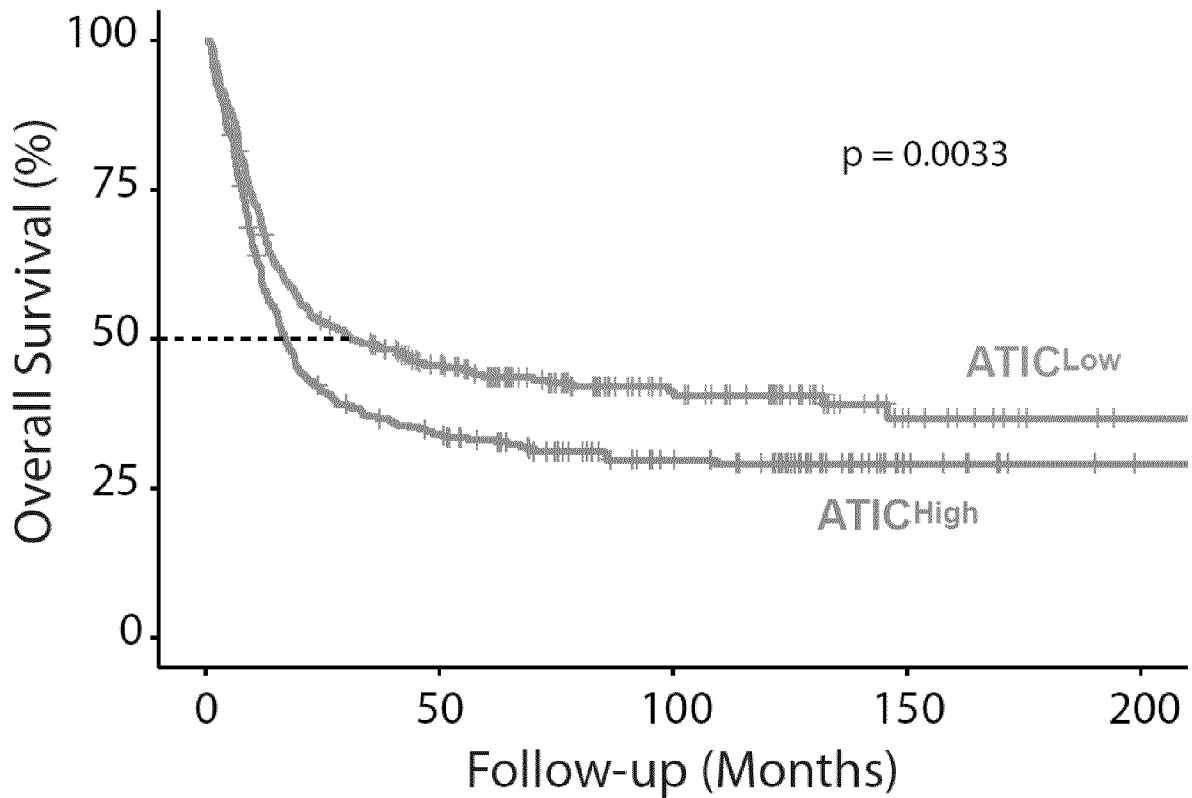


Figure 5D

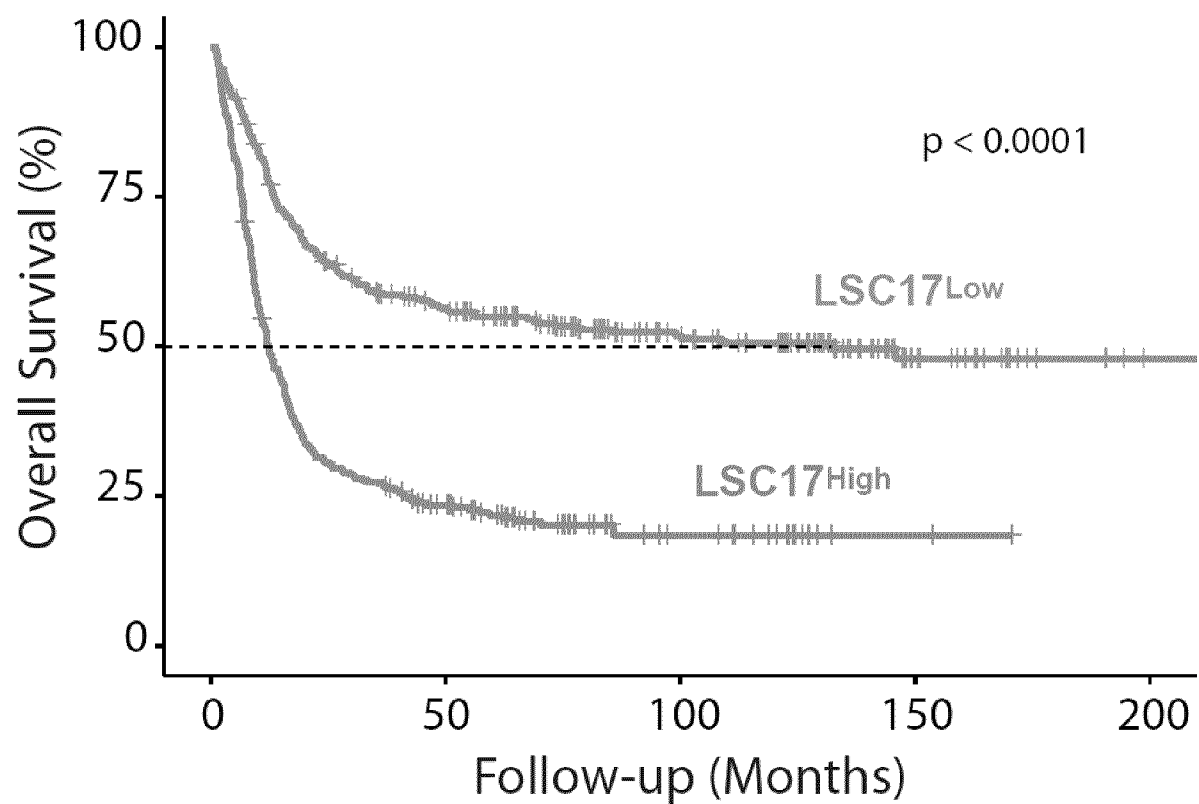


Figure 5E

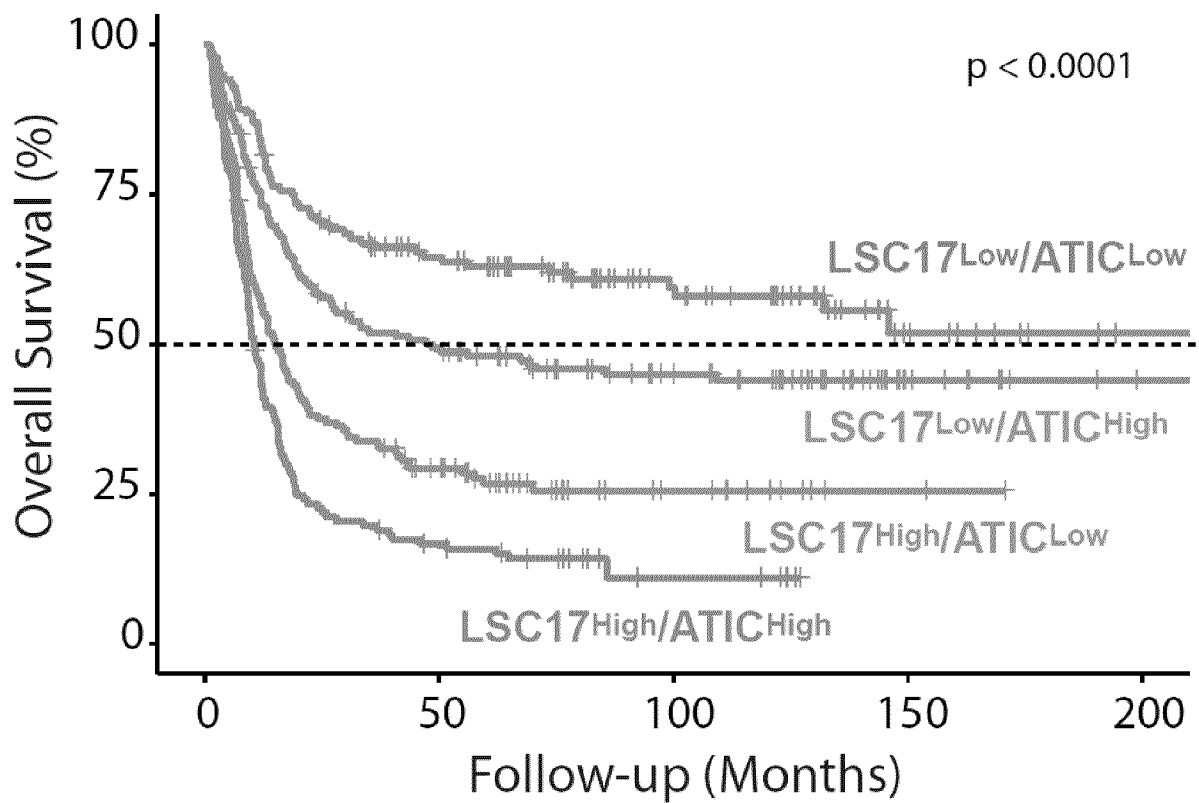


Figure 5F

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/077010

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/6886

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)

C12Q

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 practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, 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.
A	US 2018/105882 A1 (AURRAND-LIONS MICHEL [FR] ET AL) 19 April 2018 (2018-04-19) the whole document -----	1 - 7
A	STAVROPOULOU VAIA ET AL: "MLL-AF9Expression in Hematopoietic Stem Cells Drives a Highly Invasive AML Expressing EMT-Related Genes Linked to Poor Outcome", CANCER CELL, CELL PRESS, US, vol. 30, no. 1, 23 June 2016 (2016-06-23), pages 43-58, XP029636913, ISSN: 1535-6108, DOI: 10.1016/J.CCELL.2016.05.011 the whole document ----- - / - -	1 - 7



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

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Date of the actual completion of the international search

6 January 2025

Date of mailing of the international search report

22/01/2025

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/077010

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ASSI SALAM A ET AL: "Subtype-specific regulatory network rewiring in acute myeloid leukemia", NATURE GENETICS, NATURE PUBLISHING GROUP US, NEW YORK, vol. 51, no. 1, 12 November 2018 (2018-11-12), pages 151-162, XP036661393, ISSN: 1061-4036, DOI: 10.1038/S41588-018-0270-1 [retrieved on 2018-11-12] the whole document -----	1-7
A	ZENG ANDY G X ET AL: "A cellular hierarchy framework for understanding heterogeneity and predicting drug response in acute myeloid leukemia", NATURE MEDICINE, NATURE PUBLISHING GROUP US, NEW YORK, vol. 28, no. 6, 26 May 2022 (2022-05-26), pages 1212-1223, XP037898389, ISSN: 1078-8956, DOI: 10.1038/S41591-022-01819-X [retrieved on 2022-05-26] the whole document -----	1-7
A	SAITO Y ET AL: "Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells", INTERNET CITATION, 3 February 2010 (2010-02-03), pages 1-22, XP002696543, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3005290/ the whole document -----	1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/077010

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2018105882 A1	19-04-2018	EP 3277841 A1	07-02-2018
		US 2018105882 A1	19-04-2018
		WO 2016156400 A1	06-10-2016
