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(54) **Title**: PHARMACEUTICL COMPOSITIONS USEFUL FOR THE TREATMENT OF CANCERS, IN PARTICULAR ACUTE MYELOID LEUKEMIA AND ACUTE PROMYELOCYTIC LEUKEMIA

(57) **Abstract**: The present invention relates to new pharmaceutical compositions useful for the treatment of cancers, in particular acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL). The compositions comprise retinoic acid or a related compound in combination with at least one phosphodiesterase inhibitor or at least one agent enabling to increase the cellular content of cAMP. The compositions may further comprise an arsenic derivative.

PHARMACEUTICAL COMPOSITIONS USEFUL FOR THE TREATMENT OF CANCERS, IN PARTICULAR ACUTE MYELOID LEUKEMIA AND ACUTE PROMYELOCYTIC LEUKEMIA

The present invention relates to new pharmaceutical compositions useful for the treatment of cancers, in particular acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL).

Acute promyelocytic leukemia (APL) is characterized by a differentiation blockage at the promyelocytic stage and a specific t(15,17) translocation, which encodes a PML/RARA fusion protein. PML/RARA is a potent transcriptional repressor with both gains of function and dominant-negative properties, resulting in transcriptional repression of retinoic acid (RA) or non-RA target genes (de Thé, H., *et al.* The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell*, 66, 675-684 (1991); Kamashev, D.E., Vitoux, D. & De Thé, H. PML/RARA-RXR oligomers mediate retinoid- and rexinoid- /cAMP in APL cell differentiation. *J. Exp. Med.* 199, 1-13. (2004); van Wageningen, S., *et al.* Gene transactivation without direct DNA binding defines a novel gain-of-function for PML-RAR (alpha). *Blood* 111, 1634-1643 (2008)).

Gene silencing involves enhanced recruitment of nuclear receptor corepressors, the polycomb complex or Daxx, resulting in changes in chromatin organization and DNA-methylation (Minucci, S., *et al.* Oligomerization of RAR and AML1 transcription factors as a novel mechanism of oncogenic activation. *Mol. Cell* 5, 811-820 (2000); Lin, R. & Evans, R. Acquisition of oncogenic potential by RAR chimeras in acute promyelocytic leukemia through formation of homodimers. *Molecular Cell* 5, 821-830. (2000); Di Croce, L., *et al.* Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* 295, 1079-1082. (2002) ; Zhu, J., *et al.* A sumoylation site in PML/RARA is essential for leukemic transformation. *Cancer Cell* 7, 143-153 (2005); Zhou, J., *et al.* Dimerization-induced corepressor binding and relaxed DNAbinding specificity are critical for PML/RARA-induced immortalization. *Proc Natl Acad Sci U S A* 103, 9238-9243 (2006); Villa, R., *et al.* Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. *Cancer Cell* 11, 513-525 (2007)).

What makes APL a unique model in cancer biology is the existence of two therapeutic agents, retinoic acid and arsenic that target PML/RARA and whose association cures many patients (Shen, Z.X., *et al.* All-trans retinoic acid/As_2O_3 combination yields a

high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci U S A* 101, 5328-5335. (2004); Estey, E., *et al.* Use of all-trans retinoic acid plus arsenic trioxide as an alternative to chemotherapy in untreated acute promyelocytic leukemia. *Blood* 107, 3469-3473 (2006)).

RA binds PML/RARA, turns it into a transcriptional activator and triggers its degradation (Raelson, J.V., *et al.* The PML/RAR alpha oncoprotein is a direct molecular target of retinoic acid in acute promyelocytic leukemia cells. *Blood* 88, 2826-2832 (1996); Nervi, C., *et al.* Caspases mediate retinoic acid-induced degradation of the acute promyelocytic leukaemia PML/RARalpha fusion protein. *Blood* 92, 2244-2251 (1998); Zhu, J., *et al.* Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RAR alpha) and oncogenic RAR alpha fusion proteins. *Proc. Natl. Acad. Sci. USA* 96, 14807-14812 (1999)). Similarly, arsenic activates kinases targeting PML/RARA or its obligatory RXR partner, modulates PML and PML/RARA sumoylation and triggers their degradation (Zhu, J., *et al.* A sumoylation site in PML/RARA is essential for leukemic transformation. *Cancer Cell* 7, 143-153 (2005); Lallemand-Breitenbach, V., *et al.* Role of Promyelocytic Leukemia (PML) Sumoylation in Nuclear Body Formation, 11S Proteasome Recruitment, and As_2O_3-induced PML or PML/Retinoic Acid Receptor alpha Degradation. *J Exp Med* 193, 1361-1372. (2001); Mann, K.K., *et al.* Arsenic trioxide inhibits nuclear receptor function via SEK1/JNK-mediated RXRalpha phosphorylation. *J Clin Invest* 115, 2924-2933 (2005) ; Zhu, J., *et al.* RXR is an essential component of the oncogenic PML/RARA complex in vivo. *cancer cell* 12, 23-35 (2007)).

Both drugs induce to varying extend leukemia differentiation, making APL the first, and yet only, example of differentiation therapy (Warrell, R., de Thé, H., Wang, Z. & Degos, L. Acute promyelocytic leukemia. *New Engl. J. Med.* 329, 177-189 (1993)). Yet, while the two drugs sharply synergize for APL eradication, this does not seem to reflect enhanced differentiation ((Shen, Z.X., *et al.* All-trans retinoic acid/As_2O_3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci U S A* 101, 5328-5335. (2004); Estey, E., *et al.* Use of all-trans retinoic acid plus arsenic trioxide as an alternative to chemotherapy in untreated acute promyelocytic leukemia. *Blood* 107, 3469-3473 (2006); Shao, W., *et al.* Arsenic trioxide as an inducer of apoptosis and loss of PML/RARalpha protein in acute promyelocytic leukemia cells. *J. Natl. Cancer Inst.* 90, 124-133 (1998); Rego, E.M., He, L.Z., Warrell, R.P., Jr., Wang, Z.G. & Pandolfi, P.P. Retinoic acid (RA) and As_2O_3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the

leukemogenic process induced by the PML-RARalpha and PLZF-RARalpha oncoproteins. *Proc. Natl. Acad. Sci. U S A* 97, 10173-10178 (2000); Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999)). The respective contributions of drug-induced differentiation, apoptosis, transcriptional activation and PML/RARA degradation to APL cure, have been a matter of debate.

Nuclear receptors harbour two distinct transcriptional activation domains, AF1 and AF2. The later overlaps the ligand-binding domain (LBD) and is under the direct control of ligand-induced conformational changes. In contrast, AF-1 is inlaid within the variable N-terminal domain and is regulated by phosphorylation, but its contribution to nuclear receptor signaling has never been established *in vivo*. In the case of RARA, AF1 is phosphorylated by the cdk7/cyclin H sub-complex of TFIIH (Rochette-Egly, C., Adam, S., Rossignol, M., Egly, J.-M. & Chambon, P. Stimulation of RAR alpha activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7. *Cell* 90, 97-107 (1997)), pending on the docking of cyclin H, itself controlled by phosphorylation of a cAMP-PKA site S369, within the LBD (Bour, G., *et al.* Cyclin H binding to the RARalpha activation function (AF)-2 domain directs phosphorylation of the AF-1 domain by cyclin-dependent kinase 7. *Proc Natl Acad Sci U S A* 102, 16608-16613 (2005); Gaillard, E., *et al.* Phosphorylation by PKA potentiates retinoic acid receptor alpha activity by means of increasing interaction with and phosphorylation by cyclin H/cdk7. *Proc Natl Acad Sci U S A* 103, 9548-9553 (2006)). Both APL differentiation and PML/RARA transactivation are enhanced by cAMP signaling, and some RA-resistant APL cell-lines differentiate upon cAMP exposure (Kamashev, D.E., Vitoux, D. & De Thé, H. PML/RARA-RXR oligomers mediate retinoid- and rexinoid- /cAMP in APL cell differentiation. *J. Exp. Med.* 199, 1-13. (2004) ; Guillemin, M.C., *et al.* In Vivo Activation of cAMP Signaling Induces Growth Arrest and Differentiation in Acute Promyelocytic Leukemia. *J Exp Med* 196, 1373-1380. (2002)).

Recent evidence has demonstrated that not all cancer cells are identical. In particular, only a small number of cells have the ability to regenerate new tumours and hence control transplantability and metastasis development (Wang, J.C. & Dick, J.E. Cancer stem cells: lessons from leukemia. *Trends Cell Biol* 15, 494-501 (2005)). These leukemia-initiating cells (LIC) have been invoked as a major source of therapy failure, because they do not cycle or actively extrude many drugs. PML/RARA promotes stem cell self-renewal and allows immortalization of subsequent progenitors (Zhu, J., *et al.* A

sumoylation site in PML/RARA is essential for leukemic transformation. *Cancer Cell* 7, 143-153 (2005) ; Zheng, X., *et al.* Arsenic but not all-trans retinoic acid overcomes the aberrant stem cell capacity of PML/RARalpha-positive leukemic stem cells. *Haematologica* 92, 323-331 (2007)).

RA, arsenic derivatives or a combination thereof are able to suppress the differentiation blockage at the promyelocytic stage leading thus to a remission of a patient having AML or APL disease but are unable to eradicate the LIC.

Another experimental approach suggests that cyclic AMP (cAMP, adenosine 3'-5' cyclic monophosphate), or its derivatives, could also be viewed as a drug of choice for the induction of differentiation. Indeed, *ex vivo*, activation of the cAMP signal transduction pathway differentiates many acute myeloid leukemia cell-lines and strongly synergizes with other differentiating agents (Olsson, I.L., and T.R. Breitman. 1982. Induction of differentiation of the human histiocytic lymphoma cell line U-937 by retinoic acid and cyclic adenosine 3':5'-monophosphate- inducing agents. *Cancer Res* 42:3924-3927; Olsson, I.L., T.R. Breitman, and R.C. Gallo. 1982. Priming of human myeloid leukemic cell lines HL-60 and U-937 with retinoic acid for differentiation effects of cyclic adenosine 3':5'-monophosphate-inducing agents and a T-lymphocyte-derived differentiation factor. *Cancer Res* 42:3928-3933; Ruchaud, S., E. Duprez, M.C. Gendron, G. Houge, H.G. Genieser, B. Jastorff, S.O. Doskeland, and M. Lanotte. 1994. Two distinctly regulated events, priming and triggering, during retinoid-induced maturation and resistance of NB4 promyelocytic leukemia cell line. *Proc. Natl. Acad. Sci. USA* 91:8428-8432.), reviewed in (Roussel, M.J., and M. Lanotte. 2001. Maturation sensitive and resistant t(15;17) NB4 cell lines as tools for APL physiopathology: nomenclature of cells and repertory of their known genetic alterations and phenotypes. *Oncogene* 20:7287-7291). Yet, a number of acute toxicities have precluded or severely limited *in vivo* trials using cAMP, or its derivatives (Langdon, S.P., A.A. Ritchie, M. Muir, M. Dodds, A.F. Howie, R.C. Leonard, P.K. Stockman, and W.R. Miller. 1998. Antitumour activity and schedule dependency of 8-chloroadenosine-3',5'- monophosphate (8-ClcAMP) against human tumour xenografts. *Eur J Cancer* 34:384-388), and its potential benefits in the treatment of cancers have never been soundly assessed.

Thus, pharmaceutical compositions comprising at least one compound activating the cAMP signal transduction pathway, in particular theophylline in association with a cell-differentiation factor such as RA and an apoptose inducer such as As_2O_3, useful for the treatment of cancers, have been described (WO 2004/026319).

However, said compositions only acts on the differentiation of cells inducing only remission of the disease, not on the eradication of LIC leading to complete healing of the disease.

WO 2004/062671 relates to PDE IV inhibitors such as N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) alone or in combination with differentiation inducing agents such as RA or As_2O_3 for the treatment of neoplasm of lymphoid cells.

In the following of this description, the chemical name or INN name of piclamilast will be employed and will refer to the same molecule. Likewise, the chemical name or INN name of roflumilast will be employed and will refer to the same molecule.

An object of the present invention is to provide new pharmaceutical compositions for the treatment of cancers, in particular AML and APL, allowing to prevent the reemergence of the disease from remaining stem cells after cessation of the classical treatments and thus eradicate the disease, or to treat RA resistant patient.

Another object of the present invention is to provide a method for screening drugs liable to be used for the manufacture of medicaments intended for the eradication of LIC and in particular for the treatment of pathologies such as AML or APL.

The present invention relates to the use of a composition comprising retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) or with an arsenic derivative, or with at least one phosphodiesterase inhibitor (PDEI) and an arsenic derivative, for the manufacture of a drug intended to suppress the differentiation blockage of cancer cells such as neuroblastoma cells or leukaemia cells, and to eradicate cancer stem cells such as neuroblastoma initiating cells (NIC) or leukaemia initiating cells, for the treatment of pathologies such as cancer, in particular neuroblastoma, acute myelocytic leukaemia (AML) including acute promyelocytic leukaemia (APL).

By "retinoic acid" is meant all-trans retinoic acid or cis derivatives of ATRA such as 9-cis retinoic acid or 13-cis retinoic acid, vitamin A (retinol), carotene or rexinoids or pharmacologically acceptable salts thereof.

The expression "related compound thereof" means an analogue of retinoic acid, i.e. compound able to bind to and activate nuclear receptors, for instance retinoids. There are six known retinoid receptors, the retinoic acid receptors: RAR α, β and γ and the retinoid X receptors: RXR α, β and γ.

In this description, RAR α or RARA are used independently and have the same meaning.

Examples of retinoid but without being limited to, can be found in Beard et al. (*Handbook of experimental pharmacology, retinoids the biochemical and molecular of vitamin A and retinoid action;* Nau, H., Blaner W. S., Eds.; Springer: Berlin Heidelberg, 1999; Vol. 139, p185) or in Beard et al (Bioorg. Med. Chem. Lett. 12 (2002), 3145-3148).

PDEI are drugs that blocks one or more of the five subtypes of the enzyme phosphodiesterase (PDE), therefore preventing the inactivation of the intracellular second messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), by the respective PDE subtype(s).

The different subtype inhibitors, but without being limited to them, are listed below:

-non selective inhibitors such as caffeine, theophylline, aminophylline, isobutylmethyl xantine,

- PDE1-selective inhibitors such as vinpocetine,

- PDE2-selective inhibitors such as EHNA,

- PDE3-selective inhibitors such as enoximone and milrinone,

- PDE4-selective inhibitors such as mesembrine, rolipram, ibudilast, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast), methoxyquinazoline,

- PDE5-selective inhibitors such as sildenafil, tadalafil and vardenafil, udenafil and avanafil, zaprinast.

Arsenic derivative is a common naturally occurring substance that can exist under three inorganic forms: red (arsenic disulfide referred to as realgar, pararealgar or sandacara), yellow (arsenic trisulfide referred to as arsenikon, aurum pigmentum or orpiment) and white (arsenic trioxide).

The expression "suppress the differentiation blockage of..." means that the composition is able to eliminate the blockage of the differentiation of the cells, in particular at their promyelocytic stage for the leukaemia cells and thus trigger the differentiation of the cells (figure 1).

FACS analysis allows distinguishing differentiated cells from undifferentiated cells. Indeed, the lin- cells obtained in figure 2A are completely undifferentiated and have no differentiation markers (Gr-1$^-$, CD-11b$^-$, lower left zone delimited by 10^1 on the x and y axis of the square fig 3A). After growing on MC, cells acquire the differentiation marker

GR-1 and become only partially differentiated ($Gr\text{-}1^+$, $CD\text{-}11b^-$, zone (a) of the square (fig 3A)). The left part of the square (zones (a) and (b) corresponds therefore to promyelocytes and cells in figures 3A are constituted of promyelocytes only.

After using the composition of the invention, the cells acquire the differentiation marker CD-11b and are constituted of granulocytes only ($Gr\text{-}1^+$, $CD\text{-}11b^+$, zone (c) of the square and $Gr\text{-}1^-$, $CD\text{-}11b^+$, zone (d), (fig 3C)). The right part of the square corresponds therefore to granulocytes.

Neuroblastoma is the most common extracranial solid cancer in childhood and the most common cancer in infancy.

In cancers, a small number of cells with stem-like properties have the ability to regenerate new tumors. In AML or APL, these cells with stem-like properties are called leukaemia initiating cells (LIC).

In this description, the expressions "initiating cells", "progenitor cells", "stem cells" or "clonogenic cells" have the same meaning.

By the term "eradicate" is meant a complete disappearance of the clonogenic cells.

Eradication can be evaluated according to the protocol described in example 6 or 7.

Example 5 and 7 shows that RA treatment alone, whatever the treatment length, only relapse but never eradicate APL. Indeed, there is a complete dissociation between efficient RA-induced differentiation, leading to a remission of the disease but not a complete treatment of the disease, and eradication of LIC.

The complete treatment of the APL necessitates thus both suppression of differentiation blockage **and** eradication of LIC.

Therefore, one of the advantages of the present invention is to provide a composition comprising RA or a retinoid in combination with at least a PDEI and/or an arsenic derivative, said composition being advantageously synergic and able to **both suppress the differentiation blockage and at the same time to eradicate the LIC.**

The composition of the invention can comprise at least two compounds (RA or related compound and at least one PDEI or an arsenic derivative), or at least three compound (RA or related compound and at least one PDEI and an arsenic derivative).

In a preferred embodiment, the composition defined above comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI), and possibly an arsenic derivative.

The composition of the invention can thus comprise at least two compounds (RA or related compound and at least one PDEI, or at least three compounds (RA or related compound and at least one PDEI and an arsenic derivative).

In a preferred embodiment, the composition defined above comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with an arsenic derivative.

The composition of the invention comprises two compounds (RA or related compound and an arsenic derivative).

In a preferred embodiment, the invention relates to the use of a composition comprising retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP and an arsenic derivative,

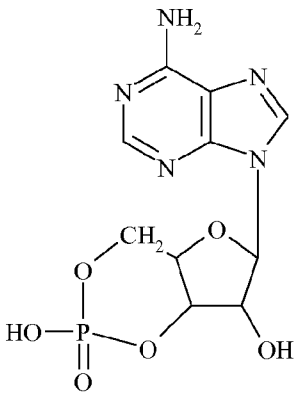
or with at least one phosphodiesterase inhibitor (PDEI) or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP, for the manufacture of a drug intended to suppress the differentiation blockage of cancer cells such as neuroblastoma cells or leukaemia cells, and to eradicate cancer stem cells such as neuroblastoma initiating cells (NIC) or leukaemia initiating cells, for the treatment of pathologies such as cancer, in particular neuroblastoma, acute myelocytic leukaemia (AML) including acute promyelocytic leukaemia (APL).

Thus in this embodiment, the composition comprises:

-RA or a retinoid, in combination with a PDEI or one agent enabling to increase the cellular content of cAMP and an arsenic derivative, or,

- RA or a retinoid, in combination with a PDEI or one agent enabling to increase the cellular content of cAMP, without an arsenic derivative.

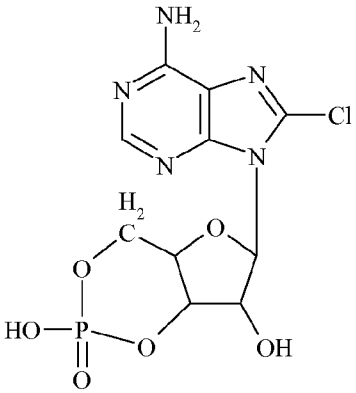
cAMP corresponds to the following formula:



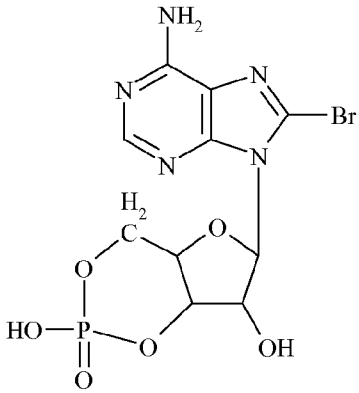
cAMP

The derivatives of cAMP are well known to the man skilled in the art, they notably comprise 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP and dibutyryl-cAMP, or pharmacologically acceptable salts thereof.

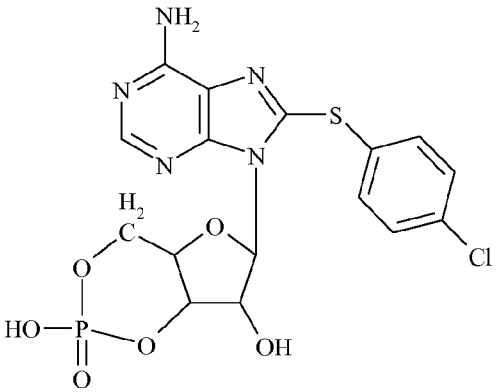
The formulae of several derivatives of cAMP are shown below:



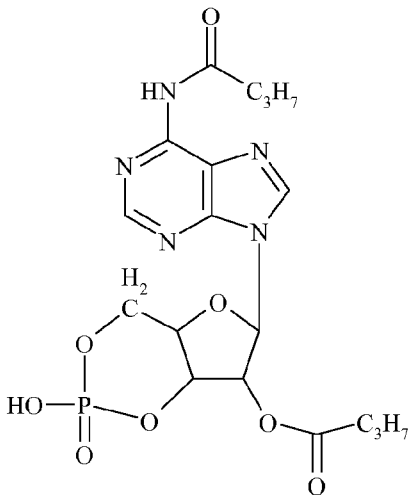
8-Cl-cAMP



8-Br-cAMP



8-CPT-cAMP



dibutyryl-cAMP

The "originally present cellular content of cAMP" relates to the cAMP content of cells prior to the addition to said cells of any compound liable to modify the cellular concentration of cAMP.

The cellular content of cAMP, or of its derivatives, can be measured according to methods well known to the man skilled in the art.

The cAMP content of a cell results from an equilibrium between two opposite reaction types, i.e. reaction concurring to the synthesis of cAMP, such as reactions catalyzed by adenylate cyclases, and reactions concurring to the degradation of cAMP, such

as reactions catalyzed by phosphodiesterases (PDE). Consequently, a rise in the cellular content of cAMP can be observed following addition of compounds either activating cAMP synthesis or inhibiting cAMP degradation. Thus, an "agent enabling to increase the cellular content of cAMP or derivatives thereof", can be for instance, cAMP or a derivative thereof in itself, or an agent activating the intracellular synthesis of cAMP, or an agent inhibiting the intracellular degradation of cAMP or derivatives thereof, provided it is added to cells in an amount sufficient to lead to an increase of the cAMP content of said cells.

In a preferred embodiment, the invention relates to the use of the composition defined above for the manufacture of a drug intended to degrade PML-RARA of leukaemia cells for the treatment of pathologies such as leukaemia, in particular acute promyelocytic leukaemia (APL).

The expression "degrade PML-RARA" means that the PML/RARA fusion protein encoded by the specific t(15,17) translocation characteristic of APL is degraded. In the case of arsenic, the catabolism of PML/RARA is produced by a conjugation to the ubiquitin-like peptide SUMO that triggers the degradation of the fusion protein.

In the case of RA or compounds that activate PKA, the catabolism of PML/RARA is produced by a phosphorylation of the S873 RARA PKA site (see examples 10 et 11 showing that PML/RARA S873 phosphorylation is essential for RA-induced loss of LIC self-renewal), leading in both cases to the eradication of LIC.

As already discussed, the complete treatment of the APL or AML necessitates both suppression of differentiation blockage **and** eradication of LIC.

Example 8 shows that a proteasome inhibitor (Velcade®) delays the APL regression, blocks the restoration of normal haematopoiesis and antagonizes loss of LIC triggered by RA/arsenic association. PML/RARA degradation is thus a critical molecular determinant of LIC eradication.

Therefore, another advantage of the invention is that the compositions are able to both **suppress the differentiation blockage and at the same time to degrade** the PML/RARA fusion protein encoded by the specific t(15,17) translocation, by sumoylation and/or by S873 phosphorylation and thus **eradicate** the LIC.

In a preferred embodiment, the invention relates to the use of a composition defined above wherein said related compound of RA is a compound able to degrade PML/RARA.

In a preferred embodiment, the present invention relates to the use of the composition defined above, wherein said composition comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one

phosphodiesterase inhibitor (PDEI) said PDEI being selected from the list consisted of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and an arsenic derivative.

Thus in this embodiment, the composition comprises RA or a retinoid, in combination with piclamilast or roflumilast and an arsenic derivative.

In a preferred embodiment, the present invention relates to the use of one of the above defined compositions wherein the drug is for administration of a RA dose in the range from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2.

RA is usually used at a dosage of about 10 mg/m^2, but when used alone, RA at this dose is able to promote the differentiation but its concentration is too low to complete the PML/RARA catabolism. Much higher RA concentrations are required for full PML/RARA catabolism than for efficient target gene activation (example 11, figure 21).

At a dose above 200 mg/ m^2, the toxicity of RA is too high to be administrated to an animal or a human being.

Therefore, still another advantage of the invention is to provide compositions wherein high RA doses, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, more preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, that allow to both **suppress the differentiation blockage and at the same time to degrade** the fusion protein and thus **eradicate** the LIC.

In another preferred embodiment, the present invention relates to the use of one of the above defined compositions wherein the drug is for administration of a RA dose in the range from 100 mg/m^2 to 46 mg/m^2, preferably from 90 mg/m^2 to 50 mg/m^2, more preferably from 80 mg/m^2 to 50 mg/m^2, more preferably from 70 mg/m^2 to 50 mg/m^2, more preferably from 60 mg/m^2 to 50 mg/m^2, in particular 50 mg/m^2.

In another preferred embodiment, the present invention relates to the use of one of the above defined compositions wherein the drug is for administration of a RA dose in the

range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2.

The expression "less than 20 mg/m^2", used here and in the following of the description, means that the RA dose cannot be equal to 20 mg/m^2 but can be at the most equal to 19.99 mg/m^2.

As stated above, RA is usually used at a dosage of about 10 mg/m^2, but when used alone, RA at this dose is only able to promote the differentiation but not a complete catabolism.

Nevertheless, examples 10 and 11 show that activation of cAMP signalling by analogs of cAMP or phosphodiesterase inhibitors synergizes with RA to induce degradation.

The synergic effect is represented in figure 20 wherein the survival of secondary transplant recipient of primary APL mice treated with cAMP taken alone is about 20 days, and 32 days for the mice treated with RA 10 mg/m^2 taken alone, while it is 68 days for the combination of cAMP and RA.

It is also represented in figure 23 wherein the degradation of PML/RARA with an association PDEI /RA 10^{-7}M is greater than each compound taken alone.

Therefore, another advantage of the invention is to provide compositions wherein classic RA doses in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2 are synergized with PDEI and allow to both **suppress the differentiation blockage and at the same time to degrade** the fusion protein and thus **eradicate** the LIC.

In a preferred embodiment, the present invention relates to the use of one of the above defined compositions, wherein said composition comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) said PDEI is selected from the list consisted of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).Thus in this embodiment, the composition comprises RA or a retinoid, in combination with piclamilast or roflumilast, without an arsenic derivative.

In a more preferred embodiment, the present invention relates to the use of one of the above defined compositions wherein the drug is for administration of a RA dose in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2.

The expression "less than 10 mg/m^2", used here and in the following of the description, means that the RA dose cannot be equal to 10 mg/m^2 but can be at the most equal to 9.99 mg/m^2.

RA is usually used at a dosage of more than 10 mg/m^2 but presents serious side effects over a long term use, such as mucocutaneous toxicity, hypertriglyceridémie and headache (Agnish N. D.; Kochar, D. M., In *Retinoid and clinical practice*; Korean, G., Ed.; Mercel Dekker: New york, 1992; p47; Standeven, A. M.; Johnson, A. T.; Escobar, M.; Chandraratna, R. A. S. *Toxicol. Apl. Pharmacol*, 1996, *138*, 169).

Low plasma RA concentrations constitute a major cause of clinical RA-resistance. Unexpectedly, Example 6 shows that a RA dosage reproducing this situation, i.e. a RA dosage of 1.5 mg/m^2, suppresses the differentiation blockage but not the LIC eradication.

Example 9 shows that cyclic AMP or PDEI synergizes with suboptimal RA dosage to induce LIC loss and in opposition to RA taken alone, the combination allows suppressing the differentiation blockage and the LIC eradication. Nevertheless, the synergic effect is only seen for the eradication not for the differentiation confirming the complete uncoupling between differentiation and eradication.

The synergic effect is represented in figure 16 wherein the survival of secondary transplant recipient of primary APL mice treated with a PDEI: (N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) taken alone is about 23 days, and 23 days for the mice treated with RA 1.5 mg/m^2 taken alone while it is 39 days for the combination PDEI and RA.

The synergy is dramatically raised with a combination As_2O_3 and RA 1.5 mg/m^2 that results in 165 days of survival.

The combination of RA 1.5 mg/m^2, piclamilast and As_2O_3 leads to further improved synergy reaching more than one year of survival, which is shown in Example 9 (figure 16).

Another advantage of the present invention is therefore to use a composition comprising RA at a dosage (1.5 mg/m^2) exhibiting lowered side effects while maintaining **the differentiation blockage and at the same time the degradation of** the fusion protein and thus **eradication** of LIC.

The composition of the invention can therefore comprise at least two compounds (RA or related compound and at least one PDEI), or at least three compound (RA or related compound and at least one PDEI, and an arsenic derivative).

In a preferred embodiment, the present invention relates to the use of one of the above defined compositions wherein the drug is for administration of a RA dose selected from the group consisting of 2 mg/m^2, 2.5 mg/m^2, 3 mg/m^2 or 3.5 mg/m^2.

In a preferred embodiment, the present invention relates to the use of one of the above defined compositions, wherein RA is selected from the group consisting of all-trans retinoic acid (ATRA) or 9-cis retinoic acid or 13-cis retinoic acid, in particular all-trans retinoic acid.

9-cis retinoic acid or 13-cis retinoic acid binds to both RXR and RAR with high affinity.

In another preferred embodiment, the present invention relates to the use of one of the above defined compositions, wherein said retinoid is a stable analogue of retinoic acid, in particular a RARA agonist poorly sensitive to CYP26A1 degradation.

Retinoid can exhibit different selectivity relative to the RARα, RAR RAR β and γ, for instance an improved RARα binding selectivity relative to RAR β and γ.

Distinct RAR subtypes are expressed in different tissues. For example, the most abundant receptor in the skin is RAR γ and studies strongly suggest that the mucocutaneous toxicity caused by retinoids is trough activation of RAR γ (Bioorg. Med. Chem. Lett. 12 (2002), 3145-3148).

Therefore, another advantage of the present invention is to provide a composition having lowered side effects, in particular the mucocutaneous toxicity, while maintaining suppression of **the differentiation blockage and at the same time the degradation of** the fusion protein and thus **eradication** of LIC.

CYP26A1 is a retinoic acid metabolising enzyme produced by a gene which is normally activated in response to RA in mouse APL.

The expression of this gene leads therefore to the degradation of RA.

Thus, the use of a stable analogue of RA, in particular a RARA agonist poorly sensitive to CYP26A1 degradation, i.e. RARA agonists resistant to the degradation or the catabolism caused by CYP26A1, allows treating patients who are resistant to retinoic acid and thus wherein RA treatment to trigger the differentiation is no more possible.

In a preferred embodiment, the present invention relates to the use of one of the above defined composition, wherein said pathologies such as AML and APL are resistant to conventional leukaemia treatments against leukaemia such as radiotherapy, chemotherapy, or retinoic acid administration.

Some patients are resistant to retinoic acid and thus RA treatment taken alone to trigger the differentiation is no more possible.

Other patients do not respond to conventional treatments such as radiotherapy, chemotherapy.

Therefore, it is another advantage of the present invention to provide a composition, wherein RA is

at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

at a classic RA dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, able to circumvent the problems of non response to conventional treatment, or of resistance to RA, allowing triggering again the **differentiation blockage and at the same time the degradation of** the fusion protein and thus **eradication** of LIC.

In a more preferred embodiment, the present invention relates to the use of the above defined composition comprising retinoic acid (RA) at a RA dose in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or at a RA dose, in the range from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or a related compound thereof such as a retinoid in combination with at least one phosphodiesterase inhibitor (PDEI), or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof, and possibly an arsenic derivative, wherein said PDEI is selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-

methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

Therefore, in this embodiment, the composition comprises a low or high dosage of RA as above defined or a retinoid, in combination with a PDEI or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof, as above defined and an arsenic derivative or not.

In a preferred embodiment, the present invention relates to the use of the above defined composition comprising retinoic acid (RA) or a related compound thereof such as a retinoid in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast), and an arsenic derivative.

Therefore, in this embodiment, the composition comprises RA at a classic dosage, i.e. from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, in combination with N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and an arsenic derivative.

In a more preferred embodiment, the present invention relates to the use of the above defined composition comprising retinoic acid (RA) or a related compound thereof such as a retinoid in combination with an arsenic derivative, or with at least one phosphodiesterase inhibitor (PDEI) and an arsenic derivative, wherein said arsenic derivative is selected from the group consisting of arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

Thus, in this embodiment, the composition comprises RA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

at a classic RA dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage as above defined, in combination with an arsenic derivative such as As_2O_3 or As_4S_4, or in combination with any PDEI defined above and an arsenic derivative such as As_2O_3 or As_4S_4

In a more preferred embodiment, the present invention relates to the use of the above defined composition wherein the active substance of the composition consists in retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof.

Thus in this embodiment, the composition comprises RA at a high dosage, i.e. at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage, as above defined, in combination with any PDEI or one agent enabling to increase the cellular content of cAMP or derivatives thereof.

In another aspect, the present invention relates to a pharmaceutical composition comprising as active substance, retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) or with an arsenic derivative, or with at least one phosphodiesterase inhibitor (PDEI) or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP and an arsenic derivative, as defined above, in a pharmacologically acceptable vehicle.

Thus, in this aspect, the pharmaceutical composition comprises RA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

at a classic RA dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

at a low RA dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage as above defined,

in combination with any PDEI above defined or with an arsenic derivative above defined or in combination with any PDEI above defined and an arsenic derivative above defined.In a preferred embodiment, the present invention relates to the pharmaceutical composition above defined, comprising as active substance, RA or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the list consisted of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and an arsenic derivative.

Thus, in this aspect, the pharmaceutical composition comprises RA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

at a classic RA dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

at a low RA dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid, different from RA, whatever the dosage as above defined, from 1 mg/m^2 to 200 mg/m^2,

in combination with any PDEI above defined and an arsenic derivative above defined.

In another preferred embodiment, the dosage of RA in the above defined compositions is in the range from 100 mg/m^2 to 46 mg/m^2, preferably from 90 mg/m^2 to 50 mg/m^2, more preferably from 80 mg/m^2 to 50 mg/m^2, more preferably from 70 mg/m^2 to 50 mg/m^2, more preferably from 60 mg/m^2 to 50 mg/m^2, in particular 50 mg/m^2.

In a preferred embodiment, the present invention relates to the composition above defined, comprising as active substance, retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI), and possibly an arsenic derivative, in a pharmacologically acceptable vehicle.

Thus, in this embodiment, the pharmaceutical composition comprises RA at a 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably dosage, i.e. from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

at a classic RA dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

at a low RA dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage as above defined,

in combination with any PDEI above defined and possibly an arsenic derivative above defined.

In a preferred embodiment, the present invention relates to the composition above defined, comprising as active substance, retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with an arsenic derivative, in a pharmacologically acceptable vehicle.

Thus, in this embodiment, the pharmaceutical composition comprises RA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

RA at a classic dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

RA at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage as above defined,

in combination with an arsenic derivative above defined.

In a preferred embodiment, the present invention relates to the pharmaceutical composition above defined, comprising as active substance, RA or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the list consisted of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

Thus, in this embodiment, the pharmaceutical composition comprises RA at a classic dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

a retinoid, different from RA, whatever the dosage as above defined, from 1 mg/m^2 to less than 200 mg/m^2,

and piclamilast or roflumilast, without arsenic derivative.

In a preferred embodiment, the present invention relates to the composition above defined, wherein the RA dose is in the range comprised from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2 and possibly an arsenic derivative in combination with at least one PDEI, in a pharmacologically acceptable vehicle.

In a preferred embodiment, the present invention relates to the composition above defined, wherein the RA dose is in the range comprised from less than 20 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2 and possibly an arsenic derivative in combination with at least one PDEI, in a pharmacologically acceptable vehicle.

In a preferred embodiment, the present invention relates to the composition above defined, wherein the RA dose is in the range comprised from 1 mg/m^2 to less than 10 mg/m^2, preferably 1 to 5 mg/m^2, in particular 1.5 mg/m^2 and possibly an arsenic derivative in combination with at least one PDEI, in a pharmacologically acceptable vehicle.

Therefore, in this embodiment, the pharmaceutical compositions comprises a low dosage of RA or of ATRA or cis derivatives of ATRA, or a retinoid as above defined, in combination with any PDEI above defined and possibly an arsenic derivative.

In another preferred embodiment, the pharmaceutical compositions above defined are in a form appropriate for the administration of RA at a dose selected from the group consisting of 2 mg/m^2, 2.5 mg/m^2, 3 mg/m^2 or 3.5 mg/m^2.

In a preferred embodiment, the retinoic acid of one of the above defined pharmaceutical compositions, is selected from the group consisting of all-trans retinoic acid (ATRA) or 9-cis retinoic acid or 13-cis retinoic acid, in particular all-trans retinoic acid.

Thus, in this embodiment, the pharmaceutical compositions comprise ATRA or 9-cis retinoic acid or 13-cis retinoic acid at a 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably dosage, i.e. from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

ATRA or 9-cis retinoic acid or 13-cis retinoic acid at a classic dosage in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, or

ATRA or 9-cis retinoic acid or 13-cis retinoic acid at a low dosage from 1 mg/m^2 to less than 10 mg/m^2, preferably 1 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage as above defined,

in combination with any PDEI above defined and possibly an arsenic derivative above defined.

In a preferred embodiment, the present invention relates to the composition above defined, wherein said retinoid is a stable analogue of retinoic acid, in particular a RARA agonist poorly sensitive to CYP26A1 degradation.

Therefore, in this embodiment, the pharmaceutical composition comprises an stable analogue of retinoic acid, in particular a RARA agonist poorly sensitive to CYP26A1 degradation, in combination with any PDEI above defined and possibly an arsenic derivative.

In a preferred embodiment, the present invention relates to the composition above defined, wherein the active substance of the composition consists in said RARA agonist poorly sensitive to CYP26A1 degradation, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) and an arsenic derivative such arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

In another preferred embodiment, the pharmaceutical compositions above defined are in a form appropriate for the administration of RA at a low dose in the range comprised

from 1 mg/m^2 to less than 10 mg/m^2, preferably 1 to 5 mg/m^2, in particular 1.5 mg/m^2, or at a high dose in the range from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably 75 mg/m^2 to 20 mg/m^2, preferably 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or a retinoid in combination with at least one PDEI selected from the group consisting of methylxanthines such as caffeine or theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and possibly an arsenic derivative.

Thus, in this embodiment, the pharmaceutical compositions comprise RA at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

at a high dosage in the range from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2,

or a retinoid whatever the dosage,

and a PDEI above defined and possibly an arsenic derivative.

In a more preferred embodiment, the pharmaceutical composition above defined comprises as active substance, RA or a related compound thereof such as a retinoid, in combination with at least one PDEI selected from the group consisting of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof and possibly an arsenic derivative as defined above.

Thus, in this embodiment, the pharmaceutical composition comprises RA or ATRA or cis derivatives of ATRA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from

150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

RA or ATRA or cis derivatives of ATRA at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage,

in combination with N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

In a more preferred embodiment, the arsenic derivative of the pharmaceutical compositions above defined is selected from the group consisting of arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

In a preferred embodiment, the pharmaceutical compositions above defined are in a form appropriate for the administration of about 10 mg/m^2 to 45 mg/m^2 of RA, and of about 0.014 mg/kg/day to about 0.43 mg/kg/day of arsenic trioxide, and N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

Therefore, in this embodiment, the pharmaceutical composition comprises RA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2, preferably from 175 mg/m^2 to 20 mg/m^2, preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from about 20 mg/m^2 to about 45 mg/m^2, preferably from about 20 mg/m^2 to about 40 mg/m^2, more preferably from about 20 mg/m^2 to about 30 mg/m^2, in particular 30 mg/m^2, or RA at a classic dosage, i.e. from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2,

in combination with piclamilast or roflumilast and arsenic trioxide.

In a preferred embodiment, the arsenic trioxide dose of the pharmaceutical compositions above defined is comprised from about 0.014 mg/kg/day to about 0.43 mg/kg/day, preferably from about 0.014 mg/kg/day to about 0.25 mg/kg/day, preferably from about 0.014 mg/kg/day to about 0.14 mg/kg/day, more preferably from about 0.05 mg/kg/day to about 0.14 mg/kg/day, in particular about 0.014 mg/kg/day.

In another preferred embodiment, the pharmaceutical composition above defined is in a form appropriate for the administration of about 1 mg/m^2 to less than 10 mg/m^2 of RA, and of about 0.014 mg/kg/day to about 0.43 mg/kg/day of arsenic trioxide, and N-(3,5-

dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

Thus, in this embodiment, the pharmaceutical composition comprises a low dosage of RA dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or a retinoid, in combination with N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and arsenic trioxide.

In another preferred embodiment, the pharmaceutical composition above defined, comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

Thus, in this embodiment, the pharmaceutical composition comprises RA at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, or

RA at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or

a retinoid whatever the dosage as above defined,

in combination with a PDEI above defined.

In another aspect, the present invention relates to a product containing:

- a first pharmaceutical composition comprising as active substance:

* RA at a dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or a related compound thereof such as a retinoid, in combination with:

* a least one PDEI selected from the group consisting of methylxanthines such as caffeine or theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil,

vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof and,

* an arsenic derivative,

and,

-a second pharmaceutical composition comprising as active substance:

*retinoic acid (RA) at a dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or a related compound thereof such as a retinoid, in combination with:

*a least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast),

as a combined preparation for simultaneous, separate or sequential use in cancer therapy, in particular in APL , AML or lymphoïd leukemia.

Therefore, in this embodiment, the first pharmaceutical composition comprises RA at low dosage in association with an PDEI and an arsenic derivative.

The second pharmaceutical composition comprises RA at low dosage in association with piclamilaste or roflumilaste, without an arsenic derivative.

In case of separate treatment or therapy, the first pharmaceutical composition is intended for an induction treatment or therapy and the second pharmaceutical composition is intended for a maintenance treatment or therapy.

The induction treatment can be carried out during three to five months, and the maintenance treatment can be carried out during one to two years.

Maintenance treatment follows immediately after the end of the induction treatment.

In a preferred embodiment, the PDEI of said first pharmaceutical is selected from the list consisted of piclamilaste or roflumilaste.

In still another aspect, the present invention relates to a product containing:

- a first pharmaceutical composition comprising as active substance:

*retinoic acid (RA) at a high dosage, i.e. from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, in combination with:

* a least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast), and,

* an arsenic derivative,

and,

- a second pharmaceutical composition comprising as active substance:

* retinoic acid (RA) at a dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, or a related compound thereof such as a retinoid, in combination with:

* a least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast),

as a combined preparation for simultaneous, separate or sequential use in cancer therapy, in particular in APL , AML or lymphoïd leukemia.

Therefore, in this embodiment, the first pharmaceutical composition comprises RA at a high dosage in association with piclamilaste or roflumilaste, and an arsenic derivative.

The second pharmaceutical composition comprises RA at low dosage in association with piclamilaste or roflumilaste, without an arsenic derivative.

In case of separate treatment or therapy, the first pharmaceutical composition is intended for an induction treatment or therapy and the second pharmaceutical composition is intended for a maintenance treatment or therapy.

The induction treatment can be carried out during three to five months, and the maintenance treatment can be carried out during one to two years.

Maintenance treatment follows immediately after the end of the induction treatment.

In another aspect, the invention relates to a process of in vitro screening of molecules to test having the capacity to eradicate the leukaemia initiating cells comprising the step of contacting leukaemia initiating cells with a molecule to test in combination with a dose of retinoic acid in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2, and an PDEI.

In a preferred embodiment, the process above defined comprises the following steps:

1. culturing, in two different culture plates **A** and **B** containing the same culture medium, previously isolated bone marrow transformed primary haematopoietic progenitor cells of a mammal, in particular expressing PML/RARA,
2. contacting said cultured cells in culture plate **A** with a dose of RA of 1.5 mg/m^2 in association with an PDEI for a week as a control, and check the absence of small colonies,
3. contacting said cells cultured in culture plate **B** with a dose of RA of 1.5 mg/m^2 in association with a molecule to test, for a week,
4. checking the presence or absence of small colonies in the previous step and when the colonies are absent, selecting the molecule.

Culture medium are numerous and commercially available. For example, methyl cellulose, but without being limited to, can be used and is available from Stem Cell Technologies, Vancouver.

The mammal can be a mouse, a rat …

The expression "small colonies" refers to small size colonies constituted in small cells.

The absence of small colonies obtained in step 2. means that LIC have been eradicated.

Therefore, a molecule to test will be selected if there is also an absence of colonies in step 4. and further studied in particular by in vivo screening.

In another aspect, the invention relates to a process of in vitro screening of molecules to test having the capacity to eradicate the leukaemia initiating cells comprising the following steps :

1. culturing, in a culture plate containing a culture medium, previously isolated bone marrow transformed primary haematopoietic progenitor cells of a mammal, in particular expressing PML/RARA,
2. contacting said cultured cells in culture plate with molecule to test and check the phosphorylation of S873 PKA site,
3. selecting the molecules of step 2. giving rise to said phosphorylation.

The presence of the phosphorylation in step 2. means that PML//RARA has been degraded leading thus to the eradication of LIC.

Therefore, a molecule to test will be selected if there is phosphorylation in step 2. and further studied in particular by in vivo screening.

In another aspect, the present invention relates to a process of in vivo screening of molecules to test having the capacity to eradicate the leukaemia initiating cells comprising the step of injecting to a second mammal recipient cells isolated from bone marrow of a first mammal recipient treated with a molecule to test in combination with RA at a low dosage in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2,and As_2O_3.

In a preferred embodiment, the process of in vivo screening above defined comprising the following steps:

1. treating a first mammal recipient with a dose of RA of 1.5 mg/m^2 in association with a PDEI and As_2O_3 for a week,
2. isolating PML/RAR transformed primary haematopoietic progenitor cells from the bone marrow of said first mammal recipient,
3. injecting said isolated cells to a second mammal recipient,
4. measuring the time to death in second mammal recipient to obtain a control time,
5. treating another first mammal recipient with a dose of RA of 1.5 mg/m^2 in association with a molecule to test and As_2O_3 for a week,
6. isolating PML/RAR transformed primary haematopoietic progenitor cells from the bone marrow of said other first mammal recipient of step 5,
7. injecting said PML/RAR transformed primary haematopoietic progenitor cells of step 6 to another second mammal recipient,
8. measuring the time to death in said other second mammal recipient of step 7,

9. selecting the molecule for which the time to death of step 7 is statistically higher than the time to death of step 4.

By "mammal recipient" is meant a mouse, a rat …

Techniques to isolate cells from bone marrow are well known from the man skilled in the art.

A control can be obtained by making the same four first steps on another mammal recipient without treatment with RA of 1.5 mg/m^2 in association with a PDEI and As_2O_3 for a week.

Each first mammal recipient and second mammal recipient must be different (control, treated with RA of 1.5 mg/m^2 in association with a PDEI and As_2O_3 for a week, treated with RA of 1.5 mg/m^2 in association with a molecule to test and As_2O_3 for a week)

The time to death obtained in step 4 when treated with RA of 1.5 mg/m^2 in association with a PDEI and As_2O_3 for a week is statistically higher than the time to death obtained in control, because the leukaemia stem cells of the control have not been eradicated.

Therefore, an interesting molecule to select in step 9 is a molecule with statistically higher time to death than the time to death of step 4.

Brief description of the figures

Figures 1A and 1B represent the model for APL pathogenesis (Grignani; *Cell* 1993, Minucci, Lin, *Mol Cell* 2000).

The repression of RARA targets by RARA dimerisation-induced enhanced corepressor binding (**Figure 1A).** Treatment with retinoic acid 10-^{6}M causes the activation of RARA targets (**Figure 1B)** leading to the differentiation of the cells and to clinical remission.

Figures 2 to 4 show that the RA trigger APL cell differentiation and loss of clonogenic precursors are uncoupled:

Figure 2A, Figure 2B, Figure 2C, Figure 2D and Figure 2E represent the colonies obtained with or without treatment by RA.

Figure 2A represents the *ex vivo* PML/RARA-transformed primary haematopoietic progenitors grown in methyl-cellulose (MC). Briefly, mice are treated with 5-Fluoro-Uracyl and after 5 days, the bone marrow is isolated and treated by method well known by the man skilled in the art to remove lineage + (lin +), for example Gr-1$^+$, TER119$^+$, CD-11b$^+$ and

isolate lin - which are transformed by PML/RARA and grown on methyl cellulose (MC) medium to give colonies (mean of 5 independent experiments)

Figures 2B to 2D represent the exposition of colonies obtained in figure 2A to 10^{-6}M RA for a week (figure B) and regrown:

large colonies in MC in the absence of RA for another week (figure 2C),

small colonies and isolated cells in MC in the absence of RA for another week (figure 2D),

Figures 2E and 2F represent the schematic indication of the nature of the myeloid cells in RA-treated (E) or untreated colonies (F). Despite RA-triggered terminal differentiation, a significant number of clonogenic cells are recovered.

Figure 3A, Figure 3B, Figure 3C, Figure 3D, Figure 3E, Figure 3F, Figure 3G, and Figure 3H represent the comparative FACS analysis with two myeloid differentiation markers and May Grunwald Giemsia stain of the recovered cells of the figures 2A to F.

Figures 3A and B represent the FACS and May Grunwald Giemsia stain of the non treated cells (see figure 2A).

Figures 3C and D represent the FACS and May Grunwald Giemsia stain of the treated cells (see figure 2B).

Figures 3E and F represent the FACS and May Grunwald Giemsia stain of the non treated cells after regrowing of the large colonies (see figure 2C).

Figures 3G and H represent the FACS and May Grunwald Giemsia stain of the non treated cells after regrowing of the small colonies (see figure 2D).

RA treated cells lead to large colonies and small colonies which are regrowing without RA. Large colonies lead to a composition of differentiated and undifferentiated cells (Fig.3E and 3F) and small colonies lead to undifferentiated cells only (Fig. 3G and 3H) indicating that significant clonogenic cells are recovered.

Figure 4A, Figure 4B, Figure 4C, Figure 4D, Figure 4E, Figure 4F, Figure 4G, Figure 4H, and Figure 4I represent the morphologically bone marrow from RA-treated (RA 10 mg/m^2 (RA 10) for a week) APL mouse containing a large number of remaining leukaemia initiating cells (LIC).

Figure 4A represents the spleen weight of untreated or RA-treated APL mice for a week with dose RA pellets (RA10 for a week) at D7 (left histogram: control APL, middle histogram RA 10, right histogram: normal mice).

Figures 4B and C represent the bone marrow May Grunwald Giemsa (MGG) stains (B: control, C: RA 10 treatment for a week).

Figures 4D and E represent FACS analysis with two myeloid differentiation markers, CD-11b (Mac) and GR-1 (D: control, E: RA 10 treatment for a week).

Figure 4F represents the abundance of the PML/RARA gene determined by quantitative PCR (left histogram: control, right histogram: RA).

Figure 4G represents the peripheral white blood cells for the control, treated mice (RA 10 for a week) and normal mice (left histogram: control, middle histogram RA 10, right histogram: normal mice).

Figure 4H represents the treatment protocol of mice.

Ten millions bone marrow cells from RA-treated (7 days) or untreated animals were injected in secondary syngenic recipients.

Figure 4I represents the mean time to death in 6 independent experiments obtained after secondary transplant 7 days post treatment (upper histogram: RA, lower histogram: control).

Despite full differentiation and morphological clearance of the marrow, numerous LIC remain.

Figures 5 to 8 show further dissociation of differentiation and loss of clonogenic potential.

Figure 5A, Figure 5B, Figure 5C, Figure 5D, Figure 5E, and Figure 5F represent the MGG-stained bone marrows of mice treated with classic (RA10 mg/m^2) or lower (RA1.5 mg/m^2) retinoic acid concentrations for a length of time of D3 or D7.

Figure 5A and D represent the control at D3 and D7 respectively.

Figure 5B and E represent the RA 1.5 treatment at D3 and D7 respectively.

Figure 5C and F represent the RA 10 treatment at D3 and D7 respectively.

Figure 5G, Figure 5H, Figure 5I, Figure 5J, Figure 5K, and Figure 5L represent the FACS analysis of the corresponding bone marrows.

Figure 5G and J represent the control at D3 and D7 respectively.

Figure 5H and K represent the RA 1.5 treatment at D3 and D7 respectively.

Figure 5I and L represent the RA 10 treatment at D3 and D7 respectively.

Figure 6A, Figure 6B, and Figure 6C present the results obtained with the different treatments with RA.

Figure 6A represent the spleen weight of the mice (light grey D3 and dark grey D7; left histogram: control; middle histogram: RA1.5 mg/m^2 (RA 1.5), right histogram: RA 10)

Figure 6B represents PML/RARA content at day 7 (left histogram: control; middle histogram: RA 1.5, right histogram: RA 10).

Figure 6C represents LIC abundance, as assessed by secondary transplantation showing the time to death in secondary transplant 7 days post treatment (upper histogram: RA 10; middle histogram: RA 1.5, lower histogram: control).

Figure 7A, Figure 7B, Figure 7C, Figure 7D, and Figure 7E show that the PLZF/RARA murine APLs, another fusion protein similar to PML/RARA, terminally differentiate in response to RA, without any restoration of haematopoiesis or significant LIC loss. Treatments with dose RA pellets (RA10) were for 7 or 12 days.

Figures 7A and B represent the bone marrow May Grunwald Giemsa (MGG) stains showing complete terminal granulocytic differentiation at D12 (A: control, B: RA).

Figures 7C and D represent the FACS analysis at D12 (A: control, B: RA).

Figure 7E represents the peripheral white blood cells at D7 (left histogram: control, right histogram: RA 10).

Figure 8A, Figure 8B, Figure 8C represent present the results obtained with the treatment with RA at D7.

Figure 8A represents the spleen weight of untreated or RA-treated PZF/RARA APL mouse (left histogram: control APL, middle histogram RA 10, right histogram: normal mice).

Figure 8B represents abundance of the PLZF/RARA gene determined by quantitative PCR (left histogram: control, right histogram: RA 10).

Figure 8C represents mean time to death of syngenic animals injected with 10^7 bone marrow cells from RA-treated of untreated leukaemic animals (left histogram: RA 10, right histogram: control).

Leukaemia initiating cells are as abundant in the differentiated marrow as in the blastic one.

Figure 9 to 12 show that LIC eradication by the RA/arsenic association is dependent on active proteolysis.

Figure 9A, Figure 9B, Figure 9C, Figure 9D, Figure 9E, Figure 9F, Figure 9G, and Figure 9H show that the RA 10 mg/m^2/arsenic association allows differentiation and synergizes for LIC eradication.

Figure 9A, Figure 9B, Figure 9C, Figure 9D represents MGG stains of RA10- or arsenic-treated mice for 3 days (A: control , B: RA, C: As_2O_3, D: Ra + As_2O_3).

Figure 9E, Figure 9F, Figure 9G, Figure 9H represents the FACS of RA10- or arsenic-treated mice for 3 days (A: control , B: RA, C: As_2O_3, D: Ra + As_2O_3).

No significant synergy for differentiation can be noted.

Figure 10A represents spleen weights at day 7 with the different treatments (from left to right histograms: control, RA, As_2O_3, Ra + As_2O_3).

Figure 10B represents the luciferase imaging of APL mouse treated for 3 days (from left to right images: control, RA, As_2O_3, Ra + As_2O_3).

Figure 10C represents luciferase activity (arbitrary unit) of secondary transplants from the mice imaged in figure 10B.

Figure 10D represents survival of secondary transplants after 7 days treatment of the primary mice with RA, arsenic or the combination (mean of 3 independent experiments; from upper to lower histograms: Ra + As_2O_3, As_2O_3, RA, control).

Mice were sacrificed after a year and were negative for PML/RARA fusion gene, demonstrating that the RA/arsenic association had eradicated LICs in the primary mice.

Figure 11A, Figure 11B, Figure 11C, Figure 11D, Figure 11E, Figure 11F, Figure 11G, Figure 11H, Figure 11I, Figure 11J, Figure 11K, Figure 11L show that the RA/arsenic synergy is dependent on active proteolysis and represent the RA/arsenic association evaluation as above, in the presence or absence of Velcade®, a clinically used proteasome inhibitor.

Figure 11A, Figure 11B, Figure 11C, Figure 11D, Figure 11E, Figure 11F represent MGG stains after 6 days treatment (A: control, B: RA10, C: As_2O_3, D: Velcade®, E: Ra + As_2O_3, F: Ra + As_2O_3 + Velcade®).

Figure 11G, Figure 11H, Figure 11I, Figure 11J, Figure 11K, and Figure 11L represent FACS of the bone marrows after 6 days treatment (G: control, H: RA10, I: As_2O_3, J: Velcade®, K: Ra + As_2O_3, L: Ra + As_2O_3 + Velcade®).

The arrow on the RA10 + As_2O_3 + Velcade® FACS emphasizes the absence of recovery of normal haematopoiesis.

Figure 12A represents the PML/RARA DNA in the marrows after 3 days treatment of the primary mice (from left to right histograms: control, RA + As_2O_3, Velcade®, Ra + As_2O_3 + Velcade®).

Figure 12B represents the time to death in secondary transplant 3 days post treatment (from upper to lower histograms: RA + As_2O_3 + Velcade®, Velcade®, RA + As_2O_3, control).

While Velcade® induces significant differentiation on its own, it hampers leukaemia eradication, restoration of normal haematopoiesis and LIC loss.

Figure 13 to 16 show that activation of cAMP signalling dramatically synergizes with low-dose RA to induce LIC loss.

Figure 13A represents the spleen weights (A) at day 7 of mice treated with normal or low doses RA, in the presence or absence of cAMP (from left to right histograms: control, RA 10, RA 1.5, As_2O_3, cAMP, RA 1.5 + cAMP).

Figure 13B represents the light units (B) of mice treated with normal or low doses RA, in the presence or absence of cAMP at day -1 (white histogram), 3 (light grey histogram, 6 (dark grey histogram) (from left to right histograms: control, RA 1.5, RA 10, As_2O_3, cAMP, RA 1.5 + cAMP).

Figure 14A, Figure 14B represent the MGG stains of bone marrows of the treated animals with RA 10 at D3 (A) and D7 (B).

Figure 14C, Figure 14D represent the MGG stains of bone marrows of the treated animals with RA 1.5 at D3 (C) and D7 (D).

Figure 14E, Figure 14F represent the MGG stains of bone marrows of the treated animals with cAMP at D3 (E) and D7 (F).

Figure 14G, Figure 14H represent the MGG stains of bone marrows of the treated animals with RA 1.5 and cAMP at D3 (G) and D7 (H).

Figure 15A, Figure 15B, Figure 15C, Figure 15D represent the FACS analysis at day 3 post treatment (A: control, B: RA 1.5, C: RA 10, D: RA1.5 + cAMP). These figures demonstrate a similar induction of differentiation for RA1.5 in the presence or absence of cAMP.

Figure 16A represents the survival of secondary transplant recipients of primary APL mice treated at day 7 (from upper to lower histograms: RA 1.5 + As_2O_3 + Piclamilast, As_2O_3 + Piclamilast, RA 1.5 + Piclamilast, Piclamilast, RA 1.5 + As_2O_3, As_2O_3, RA 1.5, control).

Figure 16B represents the survival of secondary transplant recipients of primary APL mice treated at day 3 (from upper to lower histograms: RA 1.5 + As_2O_3 + Piclamilast, As_2O_3 + Piclamilast, RA 1.5 + Piclamilast, Piclamilast, RA 1.5 + As_2O_3, As_2O_3, RA 1.5, control).

In that case cAMP signalling was not activated by a stable analog, but by a phospho-diesterase inhibitor (PDEi: piclamilast). PDEi (piclamilast) exerts a dramatic effect to eradicate LICs. A representative experiment out of 3 is shown.

Figure 17 to 20 show that mutation of S873 in PML/RARA yields RA-resistant transgenic-derived APLs.

Figure 17 represents the spleen weights at day 7 of mice treated with RA or As_2O_3, in the presence or absence of cAMP (from left to right histograms: control, cAMP, RA 10, RA 10 + cAMP, As_2O_3, As_2O_3 + cAMP, RA 10 + As_2O_3).

Figure 18A, Figure 18B, Figure 18C, Figure 18D, Figure 18E, Figure 18F represent MGG stains after 6 days treatment (A: control, B: cAMP, C: RA10, D: RA 10 + cAMP, E: As_2O_3, F: As_2O_3 + cAMP, G: Ra + As_2O_3).

Figure 19A, Figure 19B, Figure 19C, Figure 19D, Figure 19E and Figure 19F represent the FACS analysis at day 3 post treatment (A: control, B: cAMP, C: RA10, D: RA 10 + cAMP, E: As_2O_3, F: As_2O_3 + cAMP). These figures demonstrate a similar induction of differentiation for RA1.5 in the presence or absence of cAMP.

Figure 20A represents the white blood cells content (WBC) obtained at D3 and D6 in presence (right histogram) or absence (left.histogram) of RA 10.

Figure 20B represents the PML/RARA abundance in presence (right histogram) or absence (left histogram) of RA 10.

Figure 20C represents the survival of secondary transplant recipients of primary APL mice treated at day 6 (from upper to lower histograms: control, RA 10, cAMP, As_2O_3, As_2O_3 + cAMP, RA 10 + As_2O_3).

Figures 21-23 show that the PKA phosphorylation site in RARA or PML/RARA is dispensable for RA-induced activation of target genes, but desensitizes PML/RARA to RA-induced degradation.

Figures 21 A and 21B represent the activation of *rarb* (A) and *cyp26a* (B) gene expression in RARA (black) or RARAS369A (grey)-transduced *rara,b,g* -/- MEFs after 6 hours at different RA doses.

Figure 22 represents the activation of *tgII*, *rarb* and *cyp26a* by PML/RARA or PML/RARA-S873A in mouse APLs (Tg), retroviraly transduced progenitors (BM) or *rara,b,g -/-* MEFs. Mean +/- standard deviation of 3 independent experiments.

Figure 23 represents the PML/RARA degradation in mouse APLs triggered by 15 hours exposure to the indicated compounds. The mutant APL is less sensitive to RA - induced degradation than the wild-type one. Also note the synergistic effects of RA (10^{-7} M) and the phospho-diesterase inhibitor (PDEI: piclamilast) in normal, but not mutant APLs. (representative experiment from 3 independent ones).

Figure 24 represents the white blood cells content (WBC/mm^3, full line) and the blast content (%, dotted line) obtained after two cycles of a high RA dose treatment (45 mg/m^2/day).

EXAMPLES

EXAMPLE 1

Cell culture and Retroviral Transduction:

Retroviruses were obtained following transient transfection of the Plat-E packaging cell line by the different pMSCV vectors. They were used to infect lineage-depleted murine hematopoeitic cells collected from donor mice that were given intraperitoneal injections of 5-Fluorouracil 5 days prior to BM isolation as described previously (Zhu, J., *et al.* A sumoylation site in PML/RARA is essential for leukemic transformation. *Cancer Cell* 7, 143-153 (2005)).

After spinoculation of these cells by centrifugation for 2 hours at 32°C, transduced cells were cultured in methylcellulose medium (Stem Cell Technologies, Vancouver) supplemented with 100 ng/ml stem cell factor (SCF) and 10 ng/ml of each of IL-3, IL-6 and GM-CSF (Abcys) in the presence of G418 selection. After a week, neomycin-selected cells were recovered from Methylcellulose and either analyzed or replated at a density of 10,000 cells/well in the presence or absence of Retinoic acid (RA) 1 μM (Sigma-Aldrich). Serial replating assays were performed in duplicate every 7 days.

Immortalized MEFs in which the three RARs were excised (Altucci, L., *et al.* Rexinoid-triggered differentiation and tumours selective apoptosis of AML by protein kinase-A-mediated de-subordination of RXR. *Cancer Res* 65, 8754-8765. (2005)) were retrovirally transduced with either RARA, PML/RARA or the corresponding mutants followed by G418 selection. Cells were cultured in DMEM containing 10% FBS and treated overnight with RA 1 μM.

For the ex vivo studies, bone marrow cells were collected from bilateral femurs and tibiae of control or treated leukemic mice by flushing marrow cavities with cRPMI through a 21-Gauge needle. Then cells were cultured ex vivo in cRPMI medium supplemented with IL-3, IL-6 and SCF in the presence or absence of RA.

EXAMPLE 2

Transgenic mice and in vivo animal studies:

Transgenic mice were obtained as previously described in the FVB strain, using the MRP8 expression cassette. All experiments including mice were repeated at least three times. Animal handling was done according to the guidelines of institutional animal treatment committees using approved protocols.

The transplantation model of APL was previously described (Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999)). Briefly, PML/RARA APLs were transplanted in syngenic female mice, while PLZF/RARA+RARA/PLZF APLs were serially transplanted in Nude mice. For transplantation of secondary recipients, bone marrow cells were isolated from the tibiae and femurs of control and treated mice. 10^7 cells were re-injected intravenously in syngeneic recipient mice and survival was monitored.

RA was administered to mice via subcutaneous implantation of a 21-day-release pellet containing 10 mg or 1,5 mg RA (Innovative Research of America), and cAMP by subcutaneous implantation of Alzet pumps (Guillemin, M.C., *et al.* In Vivo Activation of cAMP Signaling Induces Growth Arrest and Differentiation in Acute Promyelocytic Leukemia. *J Exp Med* 196, 1373-1380, (2002)). Arsenic was administered by daily intraperitoneal injections. Morphological differentiation was assessed by May-Grünwald-Giemsa-stained cytospin slides. Tissues and cells from autopsied mice were analyzed as before (Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999)). Leukemias were transduced by retroviral infection (kind gift of S. Kogan) to allow for luciferase expression. In vivo imaging was performed using a Xenogen IVIS100 facility according to the manufacturer's instructions.

EXAMPLE 3

Real-time PCR:

Total RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA). First-strand cDNA was synthesized using SuperScript II reverse transcriptase (Invitrogen). Quantitative real-time PCR was done using the LightCycler technology (Roche). Probes and primers for TaqMan assays for rarb and tg II genes were purchased from Applied Biosystems. YWHAZ was used as endogenous control to calibrate the amount of mRNA target in different samples.

PML/RARA and PLZF/RARA DNA was quantified by real-time quantitative PCR. DNA was isolated using the NucleoSpin DNA extraction kit (Macherey Nagel). PCR reactions were carried out on a 7500 Fast Real-Time PCR System (Applied Biosystems) using either the TaqMan (for PML/RARA) or SYBR Green (for PLZF/RARA) chemistries. Reactions were performed using The TaqMan Fast Universal PCR Master Mix (Applied

Biosystems) or the LIGHT Cycler FASTSTART DNA Master plus SYBR GREEN I (ROCHE) according to the manufacturer's instructions. 18S RNA was used an internal control.

EXAMPLE 4

Western blot analysis:

Protein lysates were prepared from treated and untreated cells. Total cellular proteins were loaded onto 7% acrylamide gels, subjected to electrophoresis and transferred onto nitrocellulose membranes. The blots were blocked for one hour at room temperature in 5% skimmed milk in TBS. The membranes were then probed overnight with primary antibody at 4°C. PML/RARA expression was evaluated using an anti-RARA rabbit serum (RP115) kindly provided by P. Chambon. Detection was performed with the chemiluminescent substrate SuperSignal WestPico (Pierce biotechnology). Loading of equal amounts of protein was assessed by reprobing membranes with an anti-β-actin antibody (Sigma-Aldrich)

FACS analysis :

For flow cytometric analysis, cellular Fc receptors were first blocked with normal rat Immunoglobulin G. Immunophenotypic analysis was then performed using fluorochrome-conjugated monoclonal antibodies to c-Kit (Clone 2B8), Mac-1 and Gr-1 (clone RB6-8-C5). Staining was performed at 4°C for 20 min. Cells were washed twice and resuspended in 0.5 µg/ml Propidium Iodide. Analysis was performed using the CellQuest software. Dead cells were gated out by high-Propidium Iodide staining and forward light scatter.

EXAMPLE 5

APL differentiation does not parallel loss of leukaemia initiating cells ex vivo and in vivo

PML/RARA expression in murine primary haematopoietic progenitor cells induces a sharp differentiation arrest and allows indefinite replating in methyl-cellulose cultures, less than 1% of transformed cells yielding colonies (Zhu, J., *et al.* A sumoylation site in PML/RARA is essential for leukemic transformation. *Cancer Cell* 7, 143-153 (2005)).

These cells express low levels of PML/RARA, comparable to those found in APL cell-lines or transgenics (data not shown). Whatever the passage, individual colonies contain on average $3{,}1x10^4$ undifferentiated cells and 100 clonogenic cells (figure 2A, 3).

RA (10^{-6}M) led to a 10 fold decrease in the number of colonies, which now contained 19,000 terminally differentiated granulocytes (Figure 2B, 3). Thus RA exerts a dual effect to inhibit colony formation and to induce the terminal differentiation of clones that arose.

Surprisingly, when RA-treated cells or individual colonies were reseeded in fresh methyl-cellulose without RA, colonies developed and replated essentially as if parental cells had not been exposed to RA (Figure 2C and 2D, 3). PML/RARA-transformed progenitors could be replated up to three times in RA-containing methyl-cellulose and recovered a promyelocytic phenotype upon re-growth in standard conditions (data not shown). Similar results were obtained for individual colonies derived from RA-containing methyl-cellulose, which contained on average 24 clonogenic cells among 19,000 granulocytes (Figure 2, 3).

Even when all visible colonies were removed from the methyl-cellulose prior to reseeding, a large number of clones developed, presumably deriving from those clonogenic cells that had failed to grow in the presence of RA (Figure 2, 3). It must be noted, however, that since RA decreases the total cell progeny, although the global proportion of clonogenic cells was only slightly diminished, their actual total number was significantly reduced upon RA exposure, even at the single colony level. Altogether, these *ex vivo* studies demonstrate that RA triggers two distinct events: an irreversible differentiation of the bulk of the colonies and a reversible inhibition of clonogenic cells self-renewal.

In an APL transplantation model (Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999)). derived from MRP8-PML/RARA transgenics, RA lead to a rapid differentiation, followed at day 7, by the complete disappearance of APL cells from the marrow, as assessed by morphology and quantitative PCR on the PML/RARA gene (Figure 4). RA normalized the spleen weights and the blood counts of treated mice. However, the ability of these morphologically normal RA-treated marrows to initiate APL development in secondary recipient mice was only modestly reduced, indicating that many APL LIC remained (Figure 4).

Longer RA-treatments never eradicated LIC, consistent with the previous observation that RA-treated mice always relapse (Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999)) and that RA-treatment alone never eradicates APL (Warrell, R., de Thé, H., Wang, Z. & Degos, L. Acute promyelocytic leukemia. *New Engl. J. Med.* 329, 177-189 (1993)).

Essentially similar results were obtained with arsenic trioxide (data not shown). Such immediate differentiation and delayed inhibition of LIC self-renewal *in vivo* are fully consistent with *ex vivo* observations and strengthen the demonstration of the uncoupling of these two events.

EXAMPLE 6

Sub-optimal RA concentrations trigger differentiation, but not LIC loss

Low plasma RA concentrations, linked to increased RA-induced RA catabolism, constitute a major cause of clinical RA-resistance in PML/RARA APLs (Muindi, J., *et al.* Continuous treatment with all-trans retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and retinoid "resistance" in patients with acute promyelocytic leukemia. *Blood* 79, 299-303 (1992). It has been thus attempted to reproduce this situation in mice APL by delivering only 15% of the standard dose. A sharp initial differentiation of murine APL was nevertheless observed, virtually identical to the one induced by full dose RA (Figure 5, 6).

However, after one week, both differentiated and blastic promyelocytes were still present in the marrow, the size of the spleen never normalized, normal haematopoiesis was not restored, and PML/RARA DNA remained abundant in the marrow (Figure 5, 6). Within the liver, multiple foci of very actively proliferating APL cells (assessed by Ki67 labelling, data not shown), remained, contrasting with APL clearance by standard RA doses (Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999); Guillemin, M.C., *et al.* In Vivo Activation of cAMP Signaling Induces Growth Arrest and Differentiation in Acute Promyelocytic Leukemia. *J Exp Med* 196, 1373-1380. (2002)).

Thus, with sub-optimal RA concentrations a rapid initial differentiation is triggered, but LICs continue to proliferate.

These observations, which accurately reflect the ones made in RA-resistant patients (Guillemin, M.C., *et al.* In Vivo Activation of cAMP Signaling Induces Growth Arrest and Differentiation in Acute Promyelocytic Leukemia. *J Exp Med* 196, 1373-1380. (2002)), imply that only RA 10 concentrations affect self-renewal of leukemic progenitors, while lower RA concentrations suffice to trigger differentiation.

EXAMPLE 7

RA fully differentiates PLZF/RARA APLs without LIC loss

Patients with the t(11,17) translocation, encoding PLZF/RARA, do not respond clinically to RA (Licht, J.D., *et al.* Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). *Blood* 85, 1083-1094 (1995)) and double PLZF/RARA+RARA/PLZF mice develop a RA-resistant APL-like disease (He, L., *et al.* Two critical hits for promyelocytic leukemia. *Mol. Cell* 6, 1131-1141 (2000)). Unexpectedly, in a nude mice transplantation model of these APLs, it has been found that RA induced rapid, complete and terminal differentiation of the leukemic cells (Figure 7, 8).

Yet, in sharp contrast to PML/RARA-derived APLs, the spleen weight or blood counts remained elevated and normal haematopoietic cells never reappeared in the marrow, even after 2 weeks of treatment. Quantification of PLZF/RARA DNA by Q-PCR showed that despite terminal differentiation, the marrow remained fully leukemic. Finally, transplantation of the marrows of RA-treated mice into secondary recipients demonstrated that transplantation of RA-treated (granulocytic) or untreated (blastic) marrows consistently resulted in exactly the same kinetics of leukaemia development in recipient mice, even after prolonged RA therapy (Figure 7, 8).

This model thus exemplifies a complete dissociation between efficient RA-induced differentiation and complete absence of LIC loss, where RA essentially converts APL into a chronic myelogenous leukemia. Interestingly, in two patients with t(11,17) APL, RA also led to a significant decrease in the marrow blast counts, that was reversed upon RA-withdrawal (Figure 24).

EXAMPLE 8

The curative RA/arsenic association triggers an immediate LIC loss that requires active proteolysis

The RA/arsenic association eradicates mouse APLs (Lallemand-Breitenbach, V., *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* 189, 1043-1052 (1999); Rego, E.M., He, L.Z., Warrell, R.P., Jr., Wang, Z.G. & Pandolfi, P.P. Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RARalpha and PLZF-RARalpha oncoproteins.

Proc. Natl. Acad. Sci. U S A 97, 10173-10178 (2000)) a finding that has now been corroborated in patients (Shen, Z.X., *et al.* All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci U S A* 101, 5328-5335. (2004); Estey, E., *et al.* Use of all-trans retinoic acid plus arsenic trioxide as an alternative to chemotherapy in untreated acute promyelocytic leukemia. *Blood* 107, 3469-3473 (2006)).While in the primary recipient mice the two drugs do not synergize for differentiation at day 3, their association constantly showed a striking synergy for disease regression, as assessed by luciferase imaging. This dramatic acceleration in disease regression was due to a synergistic LIC loss after 3 or 7 days of treatment (Figure 9, 10), exemplifying yet another dissociation between differentiation and APL clinical course.

RA and arsenic share the ability to induce PML/RARA degradation through non-overlapping pathways, which may therefore cooperate to clear the fusion protein (reviewed in (Zhu, J., Lallemand-Breitenbach, V. & de The, H. Pathways of retinoic acid- or arsenic trioxide-induced PML/RARalpha catabolism, role of oncogene degradation in disease remission. *Oncogene* 20, 7257-7265. (2001)). To address the role of PML/RARA degradation in the biological response to these agents, mice were treated with the RA/arsenic association in the presence or absence of the proteasome inhibitor Velcade®. While the inhibitor induced terminal differentiation and APL cell loss on its own, when combined to the RA/arsenic association, it retarded APL regression and blocked the restoration of normal haematopoiesis (Figure 11, 12).

Strikingly, Velcade® sharply also antagonized loss of LIC triggered by the RA/arsenic association, implying that PML/RARA degradation is an important molecular determinant of LIC eradication by this association.

EXAMPLE 9

Cyclic AMP synergizes with suboptimal RA doses to induce LIC loss

Since activation of cAMP signalling dramatically sensitizes PML/RARA to RA-induced transcriptional activation and promotes differentiation of RA-resistant APL cell-lines, cAMP has been combined with sub-optimal RA doses in the APL mouse. Cyclic AMP alone induced a significant reduction in tumor mass, accompanied by apoptosis induction (Guillemin, M.C., *et al.* In Vivo Activation of cAMP Signaling Induces Growth Arrest and Differentiation in Acute Promyelocytic Leukemia. *J Exp Med* 196, 1373-1380.

(2002)). When cAMP was combined to low-dose RA, a dramatic synergy was observed for disease regression and restoration of normal haematopoiesis, but not immediate differentiation, as shown by FACS analysis of the bone marrows treated for 3 days (Figure 9-12).

To ensure that the cAMP analogs actually acted through activation of PKA signalling, its H89 inhibitor has been co-administered and indeed it has been observed that it completely reversed the effect of cAMP to enhance RA-induced disease regression (Figure 9-12). Most importantly, in transplantation experiments, cAMP or phospho-diesterase inhibitors, when associated with RA, arsenic, or with the RA/arsenic combination consistently demonstrated a synergistic reduction in LIC abundance (Figure 13-16).

Taken together, these experiments strongly suggest that cAMP in association with RA suppress the differentiation blockage and acts synergistically to dictate LIC fate.

EXAMPLE 10

A PML/RARA PKA site is essential for RA-induced LIC loss

The hypothesis was raised that PKA-triggered phosphorylation of PML/RARA might underlie the dramatic RA/cAMP synergy and generated MRP8-PML/RARA*S873A* transgenics, where the site corresponding to RARA S369 PKA site, was inactivated (Figure 17-20). S873 is indeed phosphorylated in NB4 cells, particularly upon RA exposure. One founder and its offspring eventually yielded 3 independent APLs that exhibited identical morphology, transplantability or PML/RARA expression, as the ones previously obtained with PML/RARA (data not shown). These APLs all showed a dramatically impaired response to RA 10 concentrations, without any APL regression. Blood counts remained elevated, normal hematopoiesis never reappeared and the marrow remained fully leukemic, strikingly resembling sub-optimal RA treatment. Yet, in sharp contrast to sub-optimal RA treatments, RA-resistance could not be rescued by cAMP administration. Similarly, the arsenic/cAMP association failed to induced any differentiation (Figure 17-20), in sharp contrast to wild-type APLs (Guillemin, M.C., *et al.* In Vivo Activation of cAMP Signaling Induces Growth Arrest and Differentiation in Acute Promyelocytic Leukemia. *J Exp Med* 196, 1373-1380, (2002)). cAMP still induced some reduction in tumour mass, consistent with the existence of PML/RARA-independent growth suppressive pathways (Altucci, L., *et al.* Rexinoid-triggered differentiation and tumours selective apoptosis of AML by protein kinase-A-mediated de-subordination of RXR. *Cancer Res* 65, 8754-8765. (2005)).

Altogether, these results demonstrate that PML/RARA S873 phosphorylation is essential for RA-induced loss of LIC self-renewal.

EXAMPLE 11

Distinct gene networks downstream of PML/RARA control differentiation and LIC loss

To test whether the altered *in vivo* response of these leukemias reflected altered RA-activated transcription, target genes activation was analyzed in different cellular backgrounds, including RARA- or PML/RARA-transduced mouse embryo fibroblasts (MEF) derived from *rara,b,g -/-* embryos (Zhou, J., *et al.* Dimerization-induced corepressor binding and relaxed DNAbinding specificity are critical for PML/RARA-induced immortalization. *Proc Natl Acad Sci U S A* 103, 9238-9243 (2006)).

Dose-response quantitative RT-PCR experiments show that the *rarb, cyp26a and tgII genes* are normally activated in response to RA in mouse APLs, tranduced progenitors or MEFs (Figure 21-22), demonstrating that absence of LIC loss does not merely reflect defective RA-dependent transcriptional activation.

Because LIC loss is, at least in part, dependent on PML/RARA proteolysis, it has been examined whether phosphorylation of S873 or PKA activation would influence PML/RARA catabolism. In *ex vivo*-cultured transgenic-derived mouse APL cells, it has been found that high dose (10^{-6}M) RA induced an almost complete degradation of the fusion protein, while that triggered by 10^{-7}M RA was less pronounced.

Mouse APLs bearing the S873A mutation were clearly less sensitive to RA-induced proteolysis (Figure 23). Importantly, cAMP signaling consistently synergized with 10^{-7}M RA to enhance degradation of the fusion, in PML/RARA, but not in S873A APLs (Figure 23).

Thus, PML/RARA phosphorylation at S873 regulates the RA-triggered degradation of the fusion, but not the activation of direct transcriptional targets.

EXAMPLE 12

Effect of a high RA dose (45 mg/m^2/day) on a patient diagnosed with t(11,17) and PZLF/RARA APL

A man diagnosed with t(11,17) and PLZF/RARA APL was treated with chemotherapy then with two cycles of RA 45 mg/m2/day, as indicated.

The patient did not achieve complete remission, but there was a significant and reversible decrease in the blast counts upon RA treatment. Marrow samples were repeatedly positive for t(11,17) during follow up, as indicated figure 24).

CLAIMS

1. Use of a composition comprising retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP and an arsenic derivative or with at least one phosphodiesterase inhibitor (PDEI) or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP, for the manufacture of a drug intended to suppress the differentiation blockage of cancer cells such as neuroblastoma cells or leukaemia cells, and to eradicate cancer stem cells such as neuroblastoma initiating cells (NIC) or leukaemia initiating cells, for the treatment of pathologies such as cancer, in particular neuroblastoma, acute myelocytic leukaemia (AML) including acute promyelocytic leukaemia (APL).

2. Use of a composition according to claim 1, for the manufacture of a drug intended to degrade PML-RARA of leukaemia cells for the treatment of pathologies such as leukaemia, in particular acute promyelocytic leukaemia (APL).

3. Use according to claim 1 or 2, wherein said composition comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) said PDEI being selected from the list consisted of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and an arsenic derivative.

4. Use according to claim3, wherein the drug is for administration of a RA dose in the range from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2.

5. Use according to claim3, wherein the drug is for administration of a RA dose in the range from less than 20 mg/m^2 to 10 mg/m^2, preferably from 15 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2.

6. Use according to claim3, wherein the drug is for administration of a RA dose in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2.

7. Use according to anyone of claims 1 to 6, wherein RA is selected from the group consisting of all-trans retinoic acid (ATRA) or 9-cis retinoic acid or 13-cis retinoic acid, in particular all-trans retinoic acid.

8. Use according to claim 1 to 2, wherein said retinoid is a stable analogue of retinoic acid, in particular a RARA agonist poorly sensitive to CYP26A1 degradation.

9. Use according to anyone of claims 1 to 8, wherein said pathologies such as AML and APL are resistant to conventional leukaemia treatments against leukaemia such as radiotherapy, chemotherapy, or retinoic acid administration.

10. Use according to claim 6 to 9, wherein the composition comprises retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof, and an arsenic derivative.

11. Use according to claim 10, wherein said arsenic derivative is selected from the group consisting of arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

12. Use according to claim 6 to 9, wherein the active substance of the composition consists in retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) or with at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof.

13. Pharmaceutical composition comprising as active substance, RA or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP and an arsenic derivative , or with at least one phosphodiesterase inhibitor (PDEI), according to claim 1 or 2, in a pharmacologically acceptable vehicle.

14. Pharmaceutical composition according to claim 13, comprising as active substance, RA or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the list consisted of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast) and an arsenic derivative

15. Pharmaceutical composition according to claim 14, wherein the RA dose is in the range comprised from 200 mg/m^2 to 20 mg/m^2 , preferably from 175 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20 mg/m^2 , preferably from 150 mg/m^2 to 20

mg/m^2, preferably from 100 mg/m^2 to 20 mg/m^2, preferably from 75 mg/m^2 to 20 mg/m^2, preferably from 45 mg/m^2 to 20 mg/m^2, preferably from 40 mg/m^2 to 20 mg/m^2, more preferably from 30 mg/m^2 to 20 mg/m^2, in particular 30 mg/m^2, in a pharmacologically acceptable vehicle.

16. Pharmaceutical composition according to claim 14, wherein the RA dose is in the range comprised from less than 20 mg/m^2 to 10 mg/m^2, in particular 10 mg/m^2, in a pharmacologically acceptable vehicle.

17. Pharmaceutical composition according to claim 13, wherein the RA dose is in the range comprised from 1 mg/m^2 to less than 10 mg/m^2, preferably 1 to 5 mg/m^2, in particular 1.5 mg/m^2 and possibly an arsenic derivative in combination with at least one PDEI, in a pharmacologically acceptable vehicle.

18. Pharmaceutical composition according to claim 13 to 17, wherein said retinoic acid is selected from the group consisting of all-trans retinoic acid (ATRA) or 9-cis retinoic acid or 13-cis retinoic acid, in particular all-trans retinoic acid.

19. Pharmaceutical composition according to claim 13, wherein said retinoid is a stable analogue of retinoic acid, in particular a RARA agonist poorly sensitive to CYP26A1 degradation

20. Pharmaceutical composition according to claim 19, wherein the active substance of the composition consists in said RARA agonist poorly sensitive to CYP26A1 degradation, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) and an arsenic derivative such arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

21. Pharmaceutical composition according to anyone of claims 17 to 19, comprising retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline, isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-

methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast), or at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof and an arsenic derivative.

22. Pharmaceutical composition according to claim 21, wherein said PDEI is selected from the group consisting of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

23. Pharmaceutical composition according to anyone of claims 22, wherein said arsenic derivative is selected from the group consisting of arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

24. Pharmaceutical composition according to claim 23, in a form appropriate for the administration of about 10 mg/m^2 to 45 mg/m^2 of RA, and of about 0.014 mg/kg/day to about 0.43 mg/kg/day of arsenic trioxide, and N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

25. Pharmaceutical composition according to claim 17, in a form appropriate for the administration about 1 mg/m^2 to less than 10 mg/m^2 of RA, and of about 0.014 mg/kg/day to about 0.43 mg/kg/day of arsenic trioxide, and N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

26. Pharmaceutical composition according to anyone of claims 17 to 19, comprising retinoic acid (RA) or a related compound thereof such as a retinoid, in combination with at least one phosphodiesterase inhibitor (PDEI) selected from the group consisting of methylxanthines such as caffeine, theophylline, aminophylline,

isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, methoxyquinazoline, N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (INN: piclamilast) or 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide (INN: roflumilast).

27. Product containing a first pharmaceutical composition according to claim 21, and a second pharmaceutical composition according to claim 26, as a combined preparation for simultaneous, separate or sequential use in cancer therapy, in particular in APL , AML or lymphoïd leukemia.

28. Product containing a first pharmaceutical composition according to claim 15, and a second pharmaceutical composition according to claim 26 wherein PDEI is selected from Piclamilaste or Roflumilaste, as a combined preparation for simultaneous, separate or sequential use in cancer therapy, in particular in APL, AML or lymphoïd leukemia.

29. Process of in vitro screening of molecules to test having the capacity to eradicate the leukaemia initiating cells comprising the step of contacting leukaemia initiating cells with a molecule to test in combination with a dose of retinoic acid in the range from 1 mg/m^2 to less than 10 mg/m^2, preferably from 1 mg/m^2 to 5 mg/m^2, in particular 1.5 mg/m^2 according to claim 5, and a PDEI.

30. Process according to claim 29, comprising the following steps:

1. culturing, in two different culture plates **A** and **B** containing the same culture medium, previously isolated bone marrow transformed primary haematopoietic progenitor cells of a mammal, in particular expressing PML/RARA,
2. contacting said cultured cells in culture plate **A** with a dose of RA of 1.5 mg/m^2 in association with a PDEI for a week as a control, and check the absence of small colonies,
3. contacting said cells cultured in culture plate **B** with a dose of RA of 1.5 mg/m^2 in association with a molecule to test, for a week,

4. checking the presence or absence of small colonies in the previous step and when the colonies are absent, selecting the molecule.

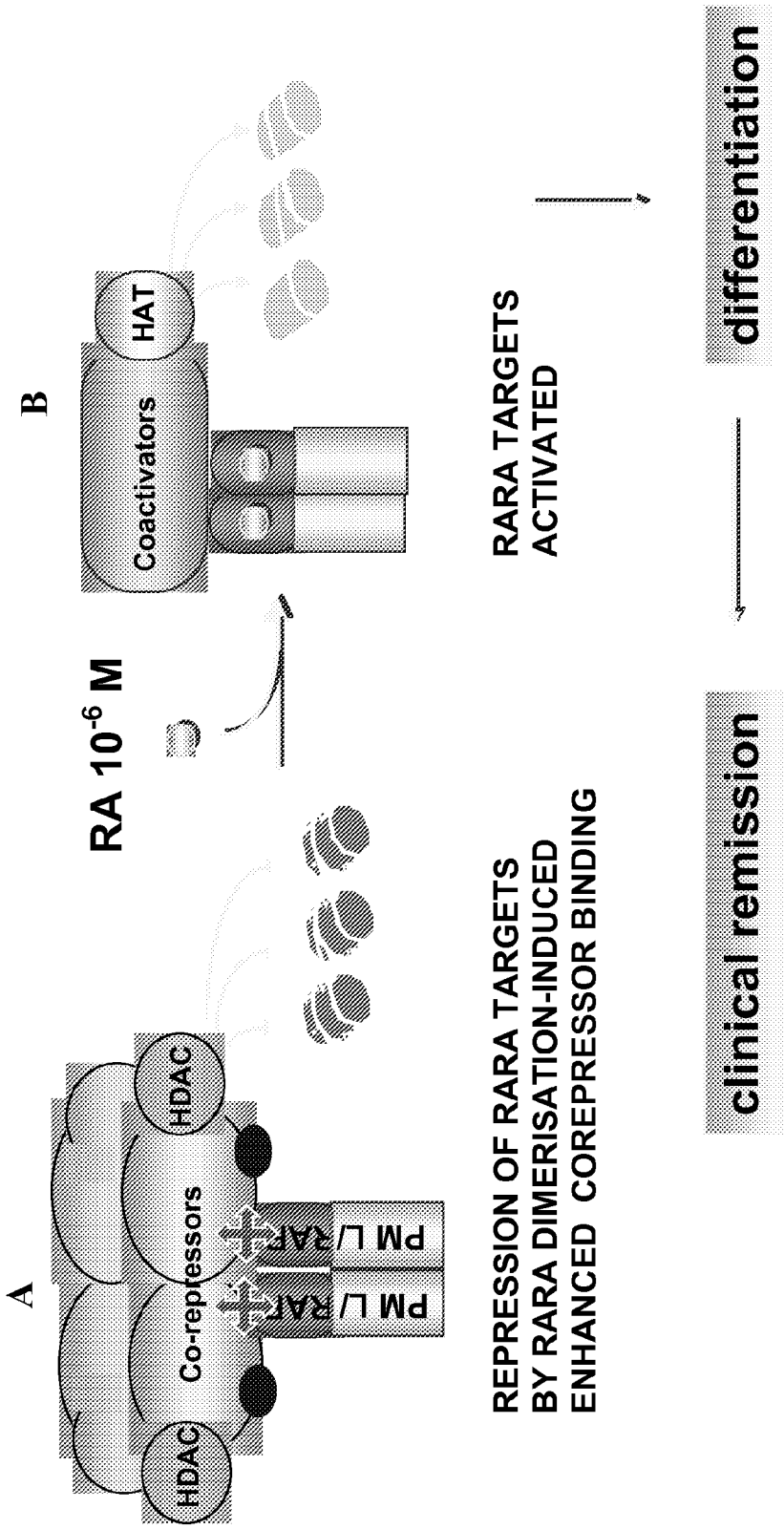

A
HDAC
Co-repressors
HDAC
PML/RAR
PML/RAR
RA 10-6 M
B
HAT
Coactivators
REPRESSION OF RARA TARGETS
BY RARA DIMERISATION-INDUCED
ENHANCED COREPRESSOR BINDING
RARA TARGETS
ACTIVATED
differentiation
clinical remission

FIGURE 1

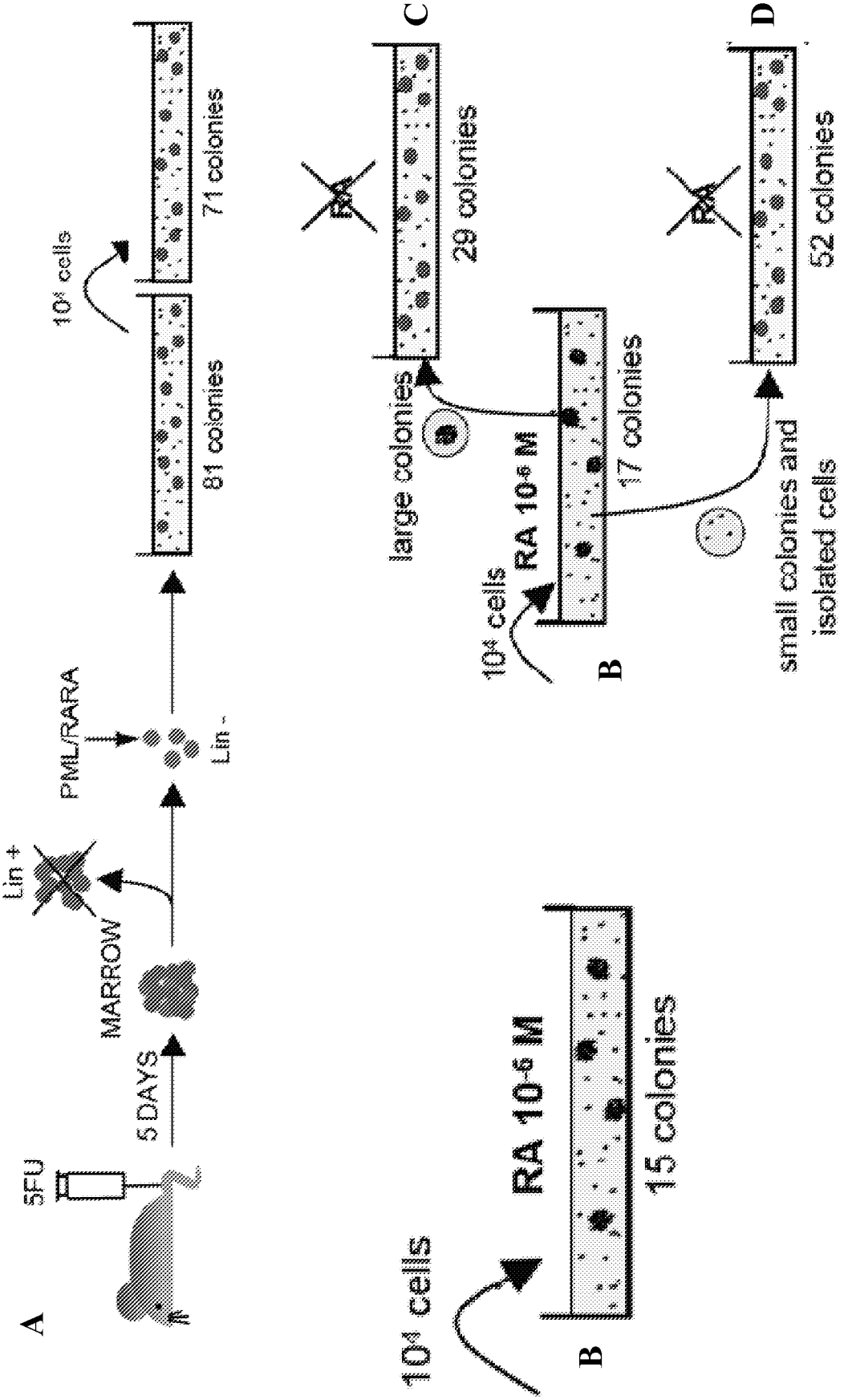

A
5FU
5 DAYS
MARROW
Lin +
PML/RARA
Lin -
10⁴ cells
81 colonies
71 colonies
B
10⁴ cells
RA 10⁻⁶ M
15 colonies
C
large colonies
29 colonies
RA
B
10⁴ cells
RA 10⁻⁶ M
17 colonies
D
small colonies and
isolated cells
52 colonies
RA

FIGURE 2

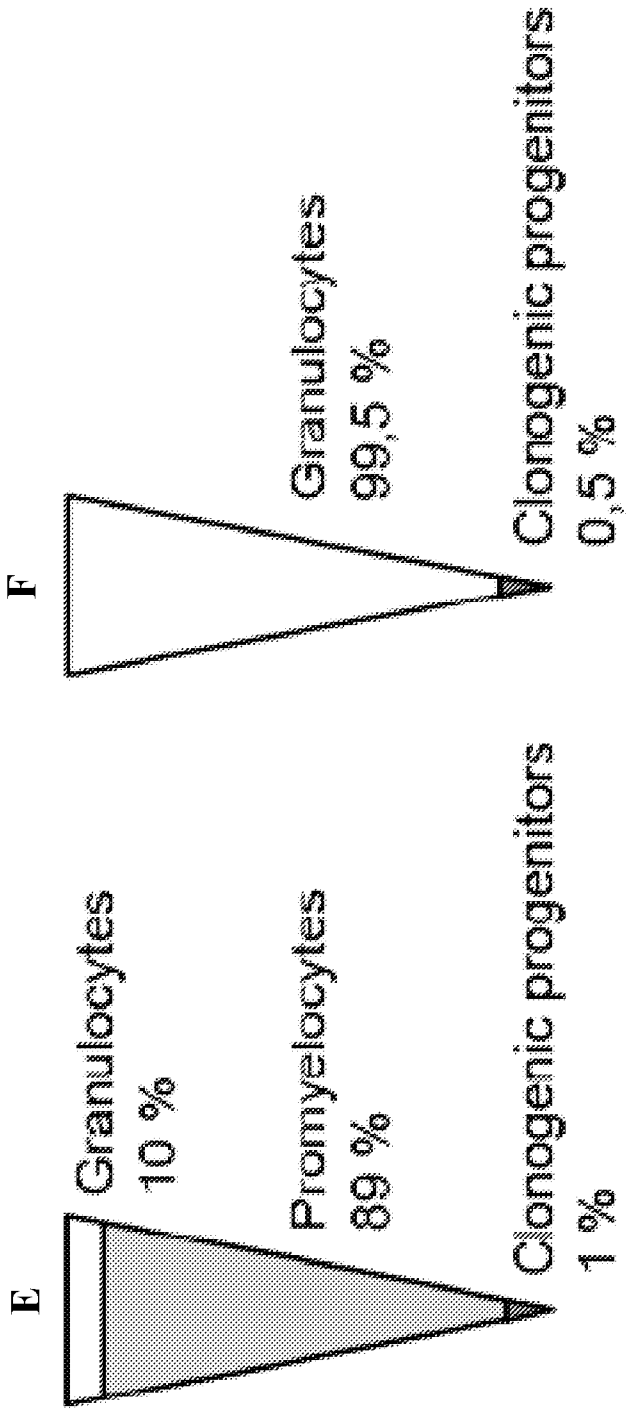


FIGURE 2 (continued)

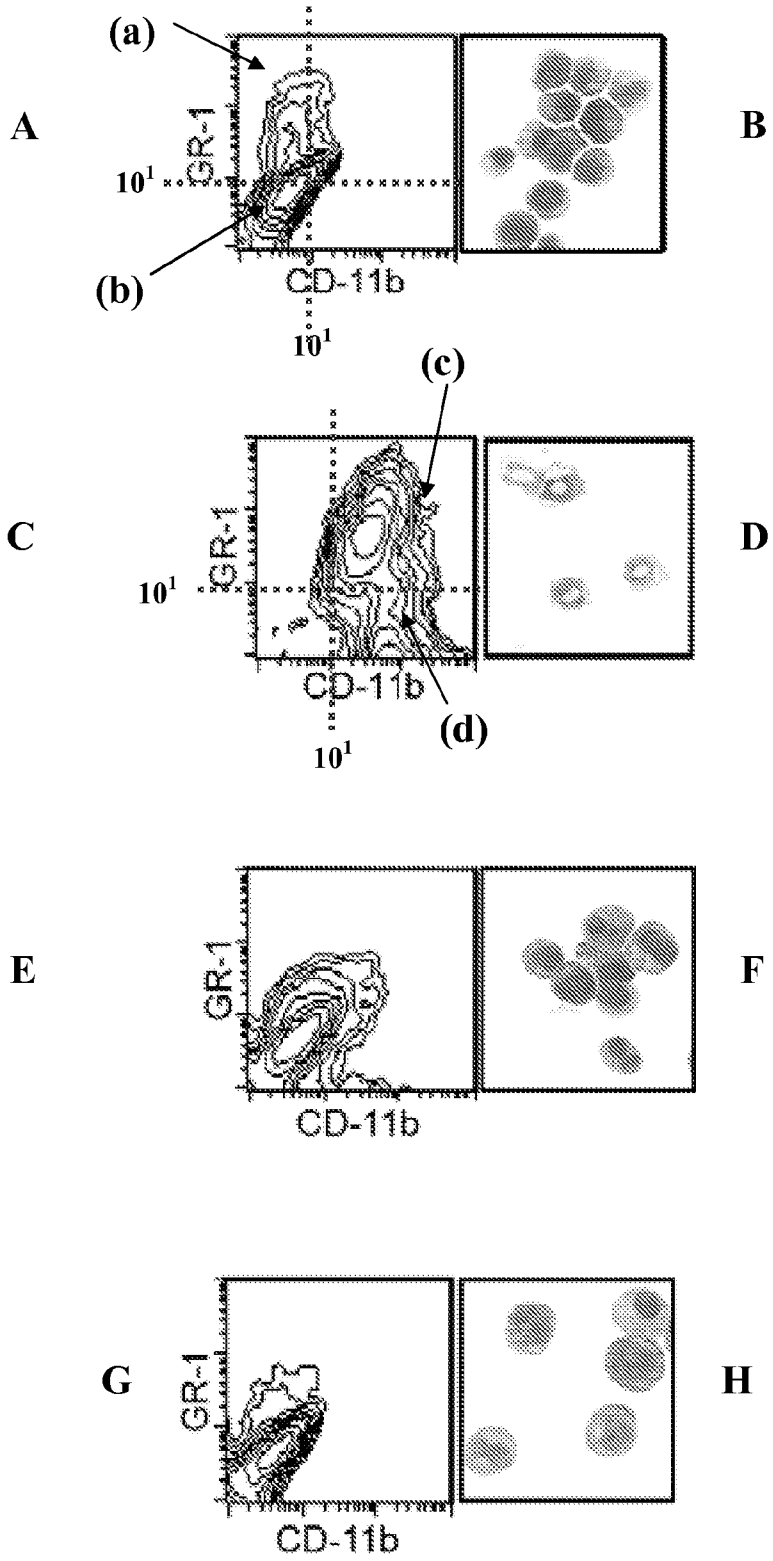


FIGURE 3

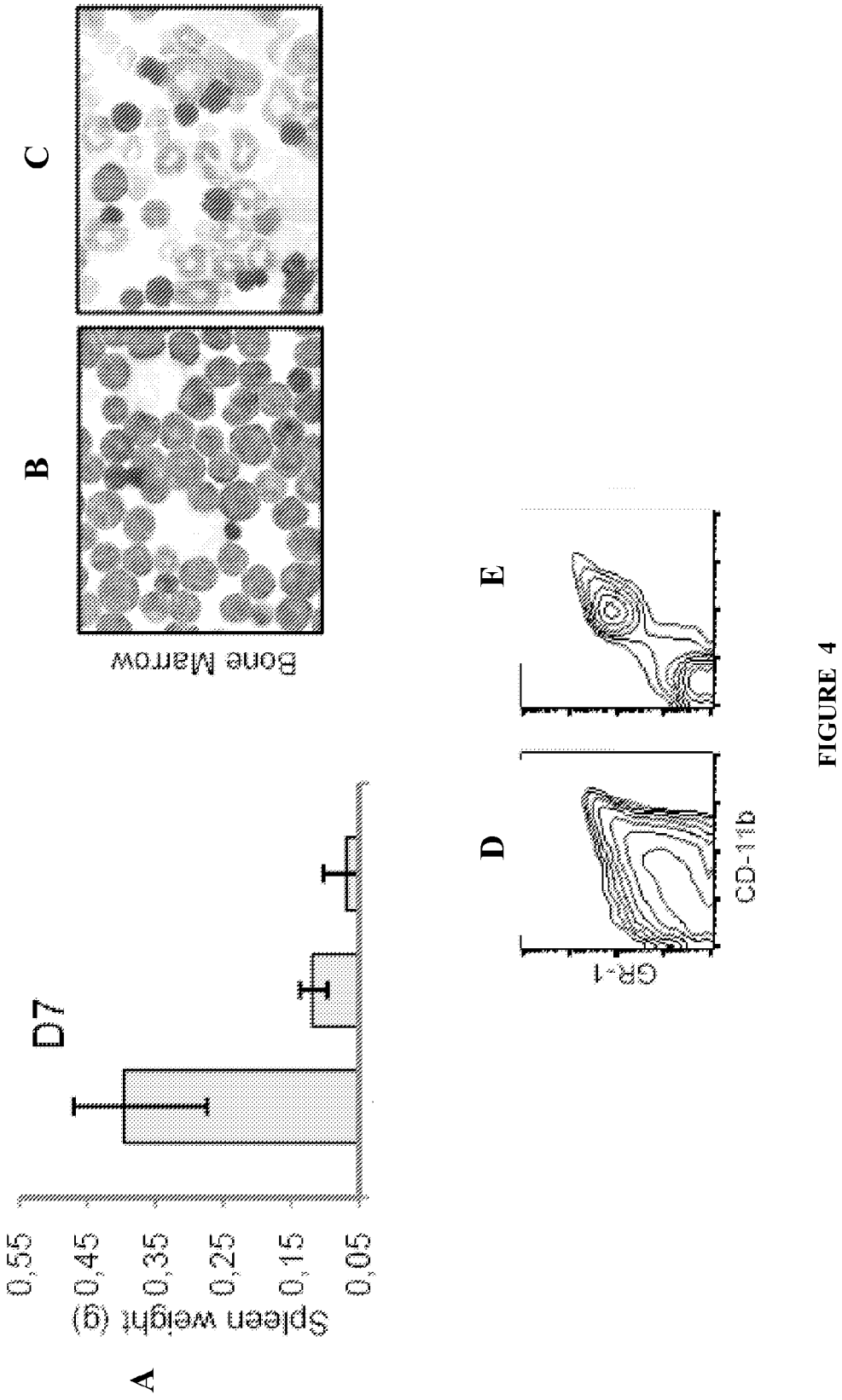

A
Spleen weight (g)
0,55
0,45
0,35
0,25
0,15
0,05
D7
B
C
Bone Marrow
D
GR-1
CD-11b
E

FIGURE 4

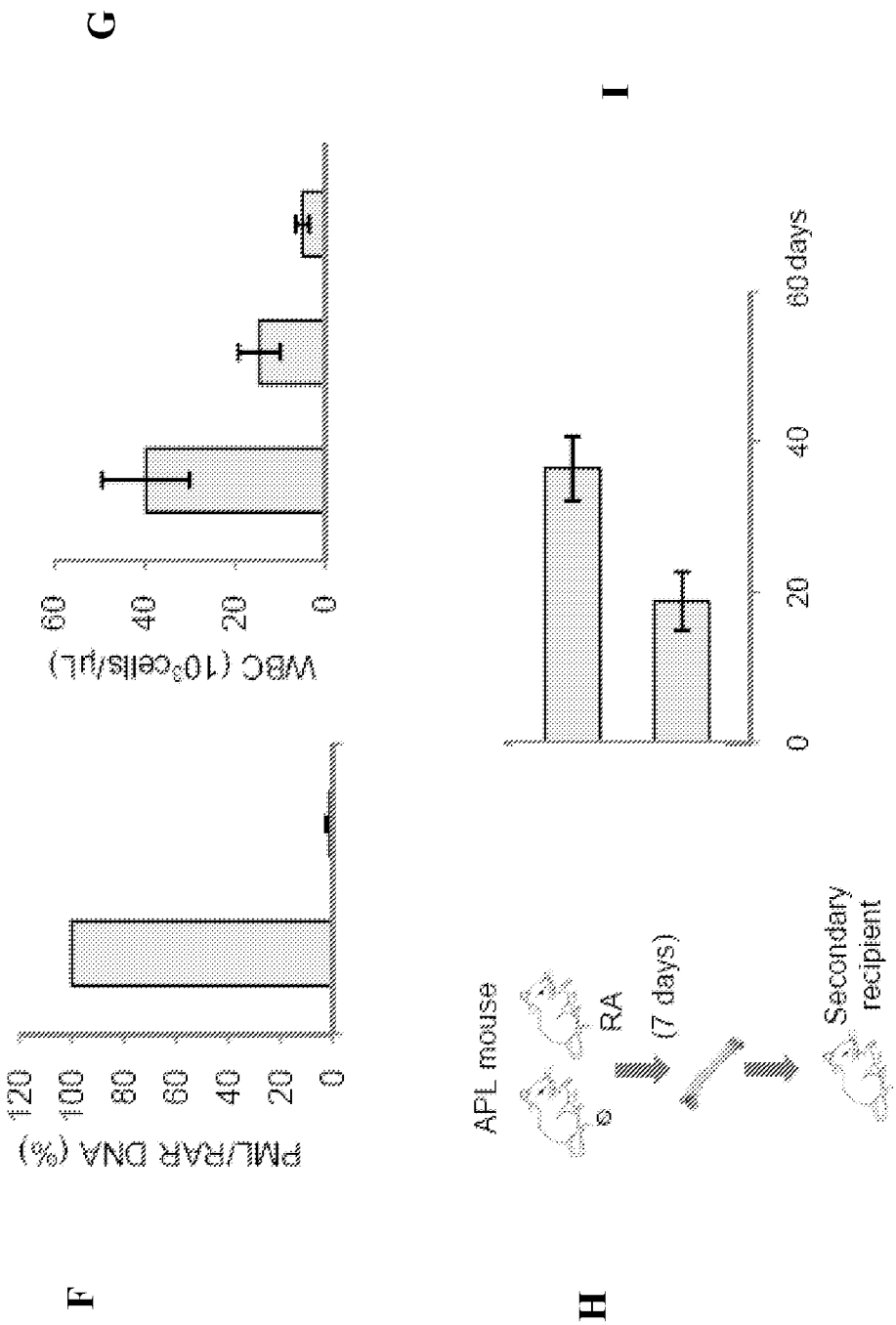
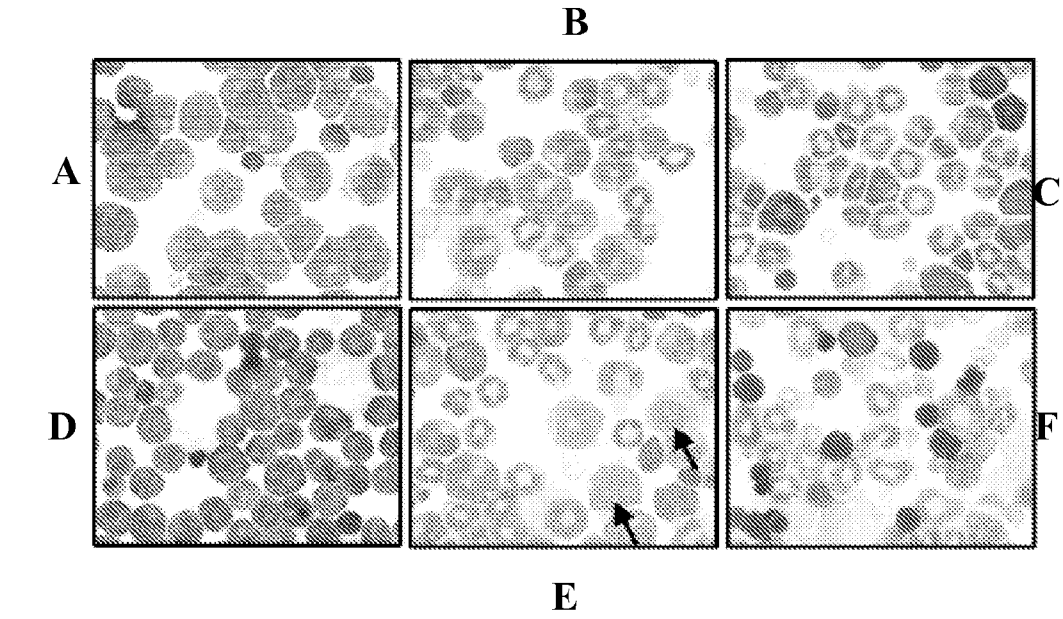


FIGURE 4 (continued)



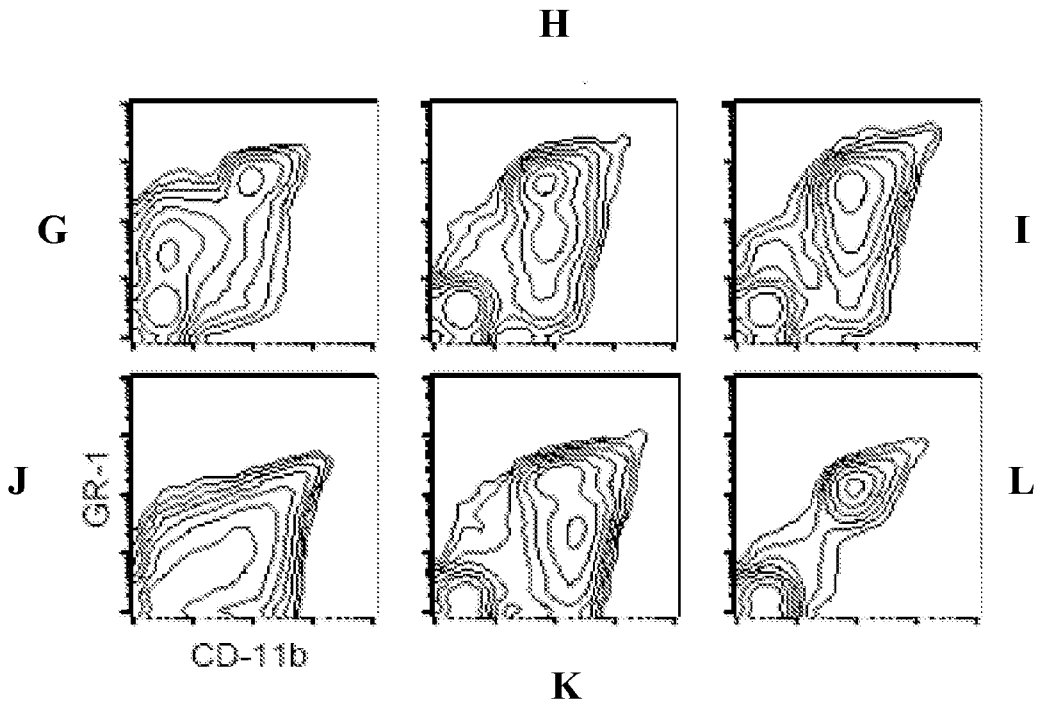


FIGURE 5

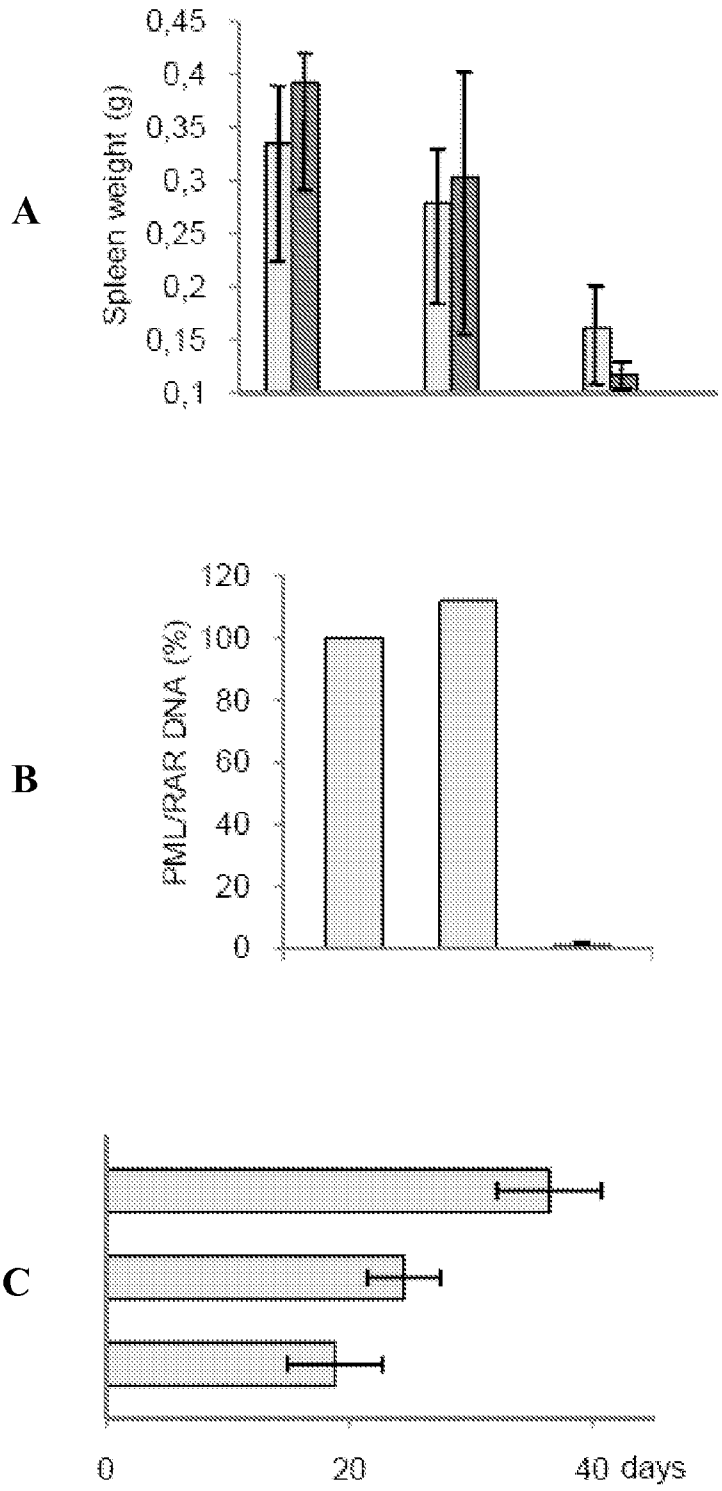

A
Spleen weight (g)
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
B
PML/RAR DNA (%)
120
100
80
60
40
20
0
C
0
20
40 days

FIGURE 6

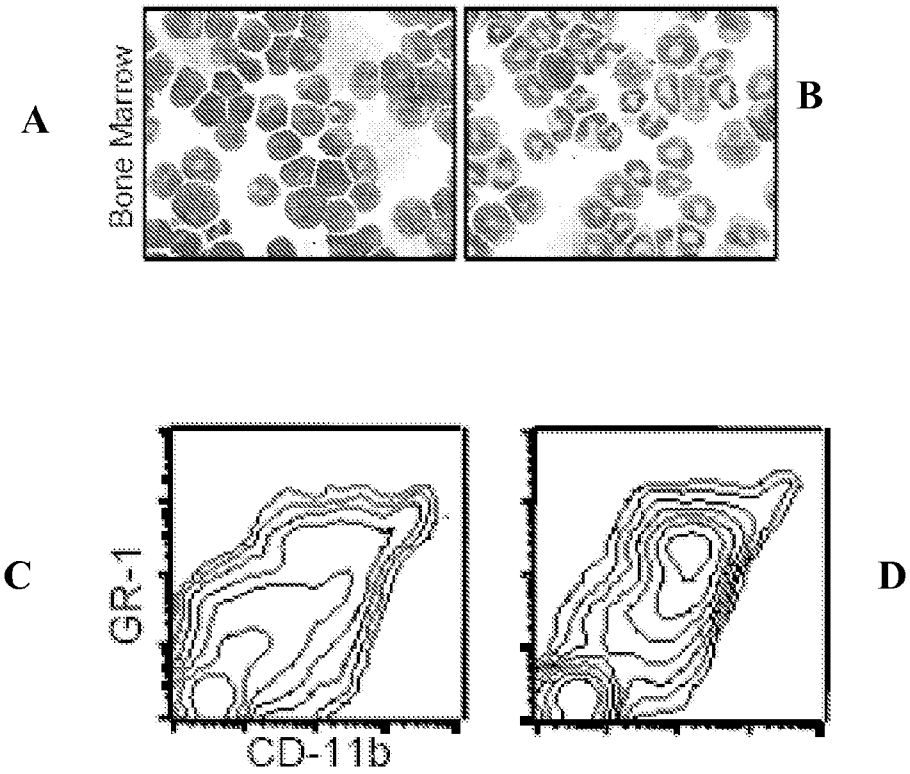
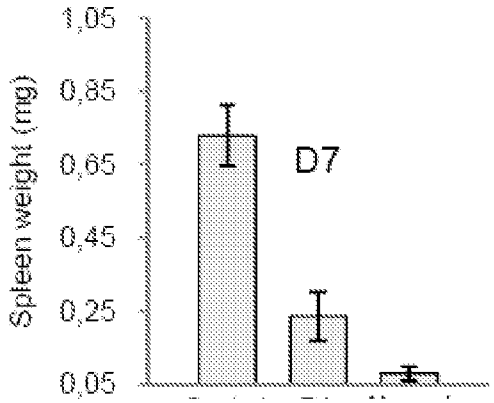
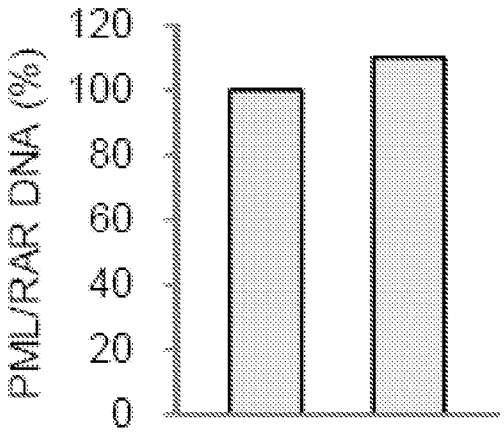


FIGURE 7

A


1,05
0,85
0,65
0,45
0,25
0,05
Spleen weight (mg)
D7

B


120
100
80
60
40
20
0
PML/RAR DNA (%)

C

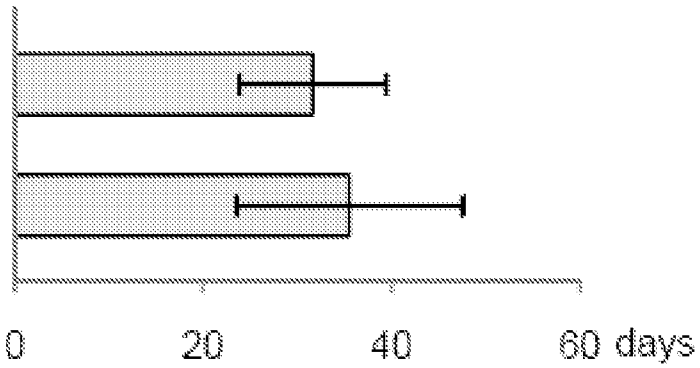

0
20
40
60 days

FIGURE 8

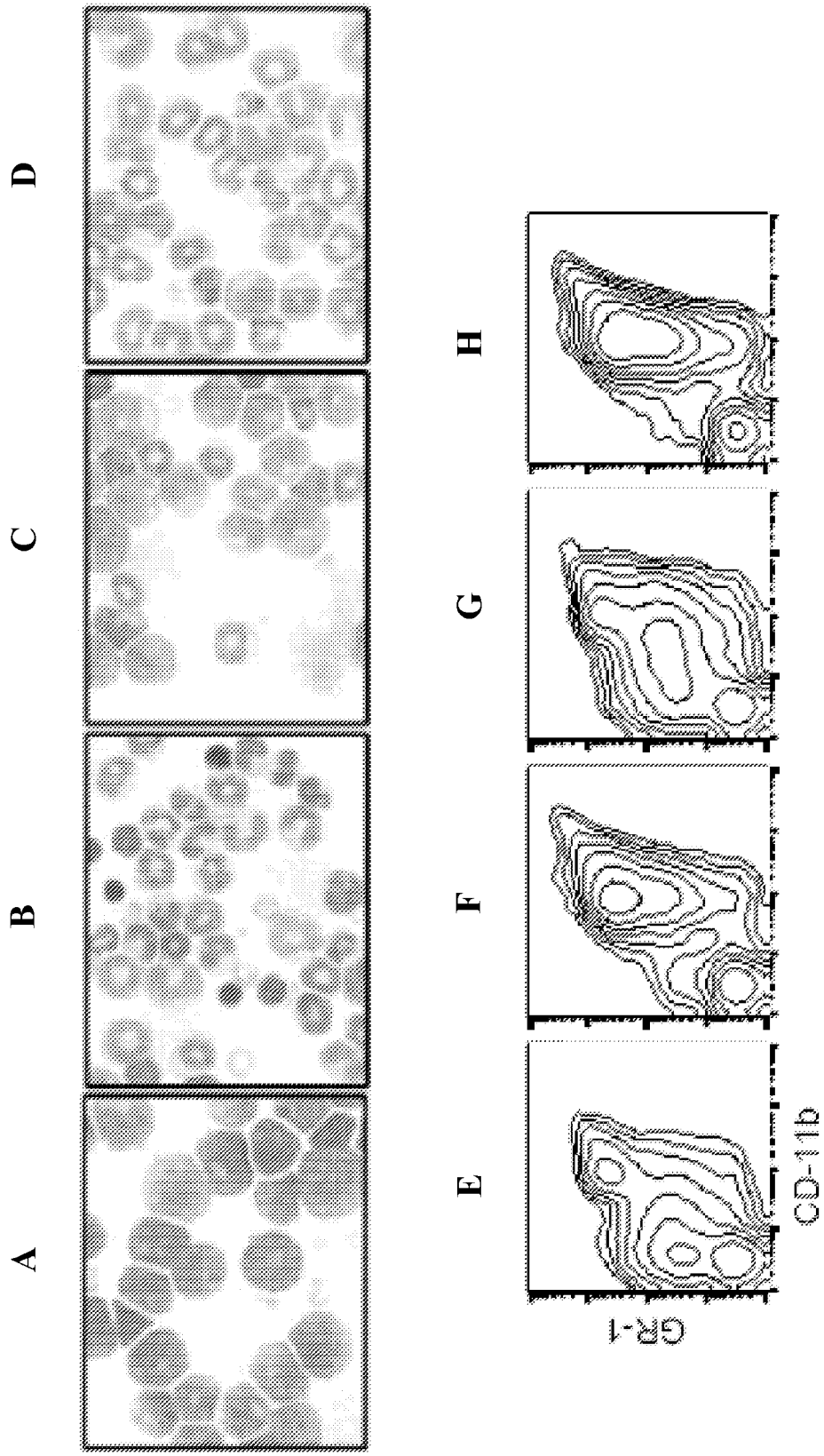


FIGURE 9

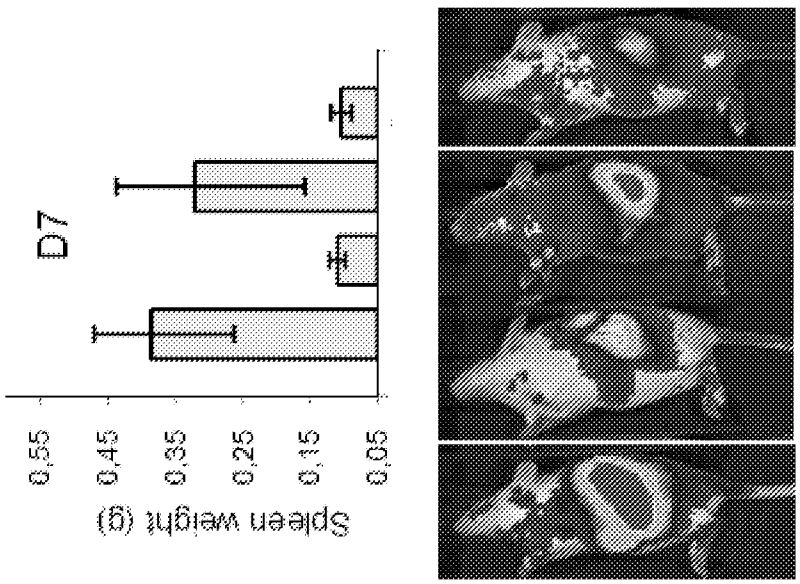
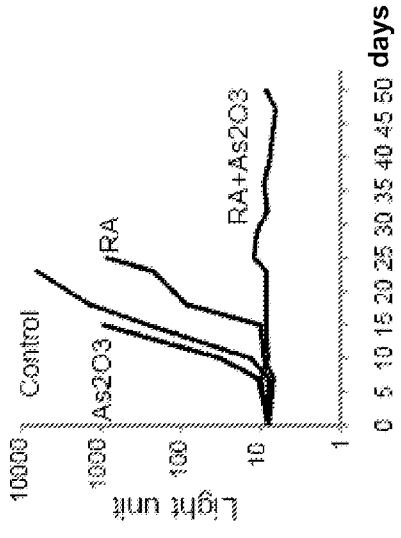


FIGURE 10



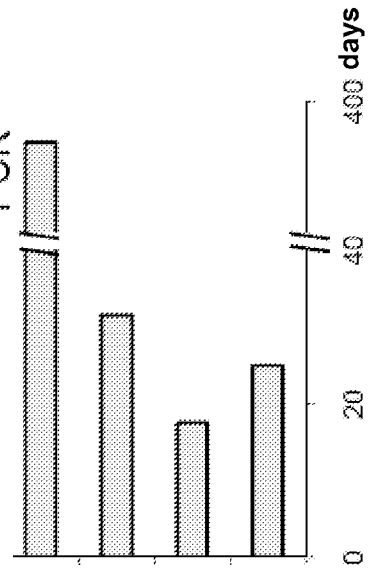


FIGURE 10 (continued)

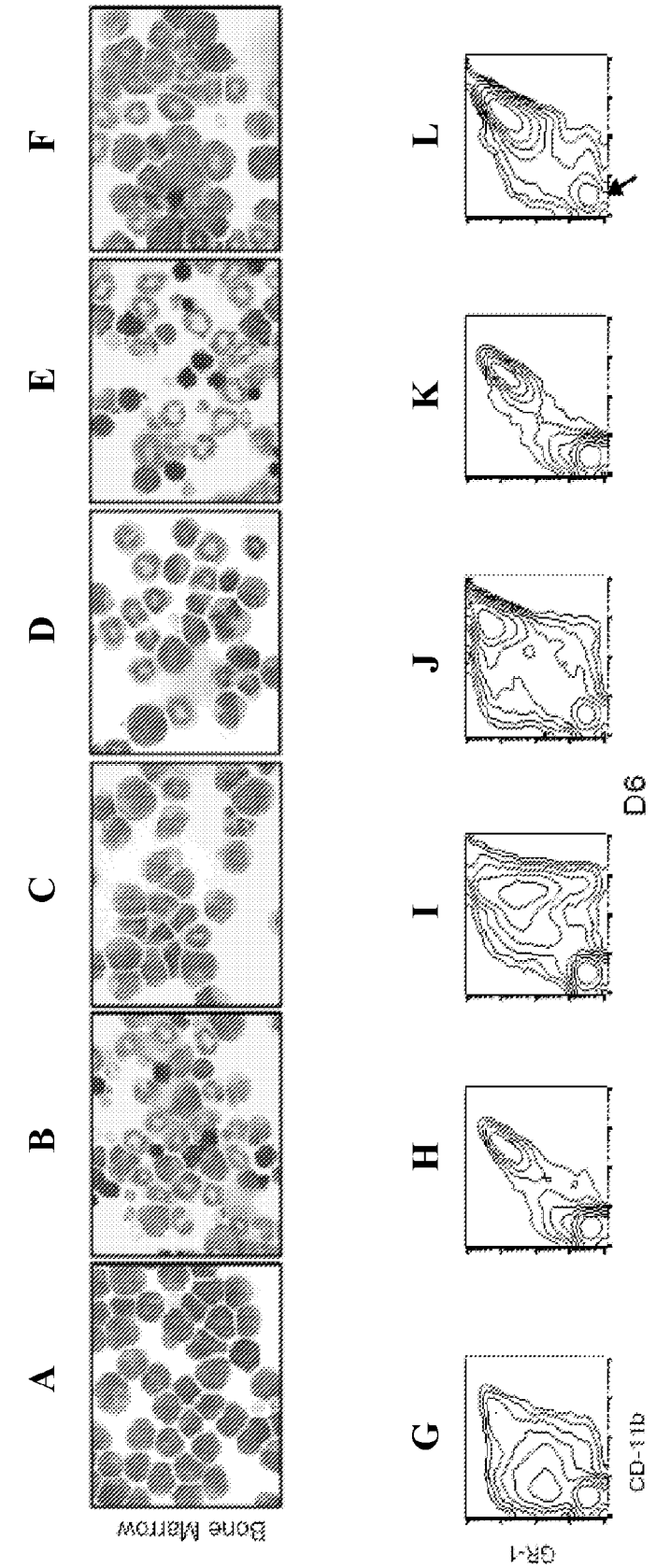


FIGURE 11

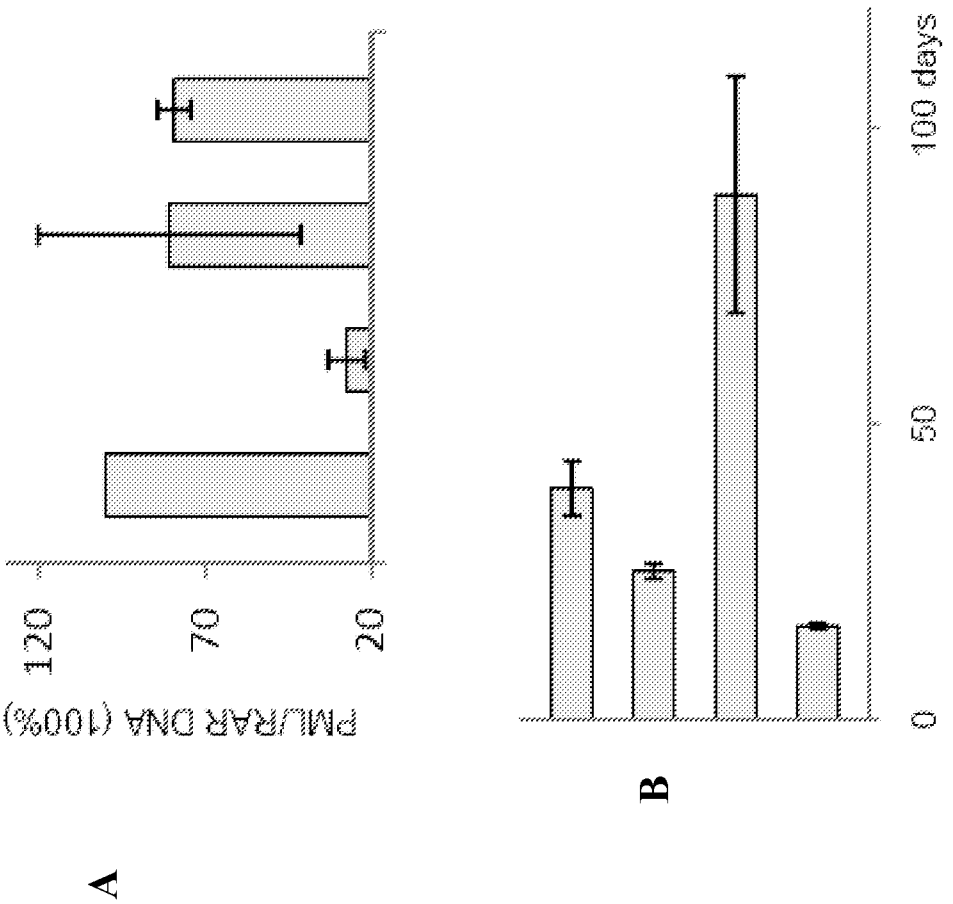


FIGURE 12

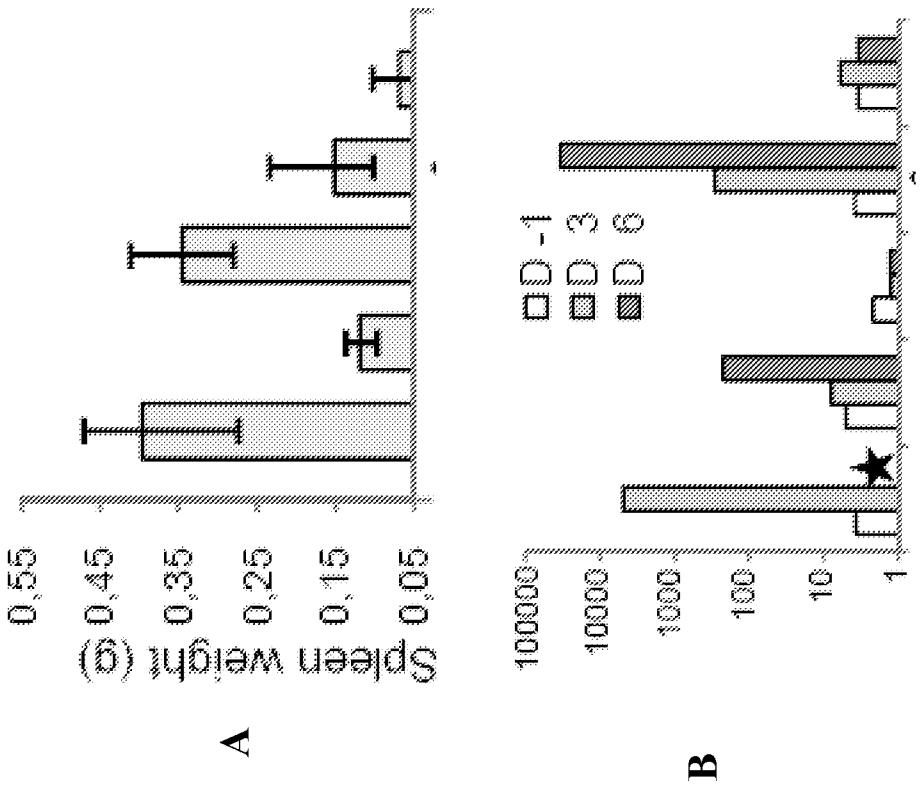

Spleen weight (g)
0,55
0,45
0,35
0,25
0,15
0,05
A
100000
10000
1000
100
10
1
D -1
D 3
D 6
B

FIGURE 13

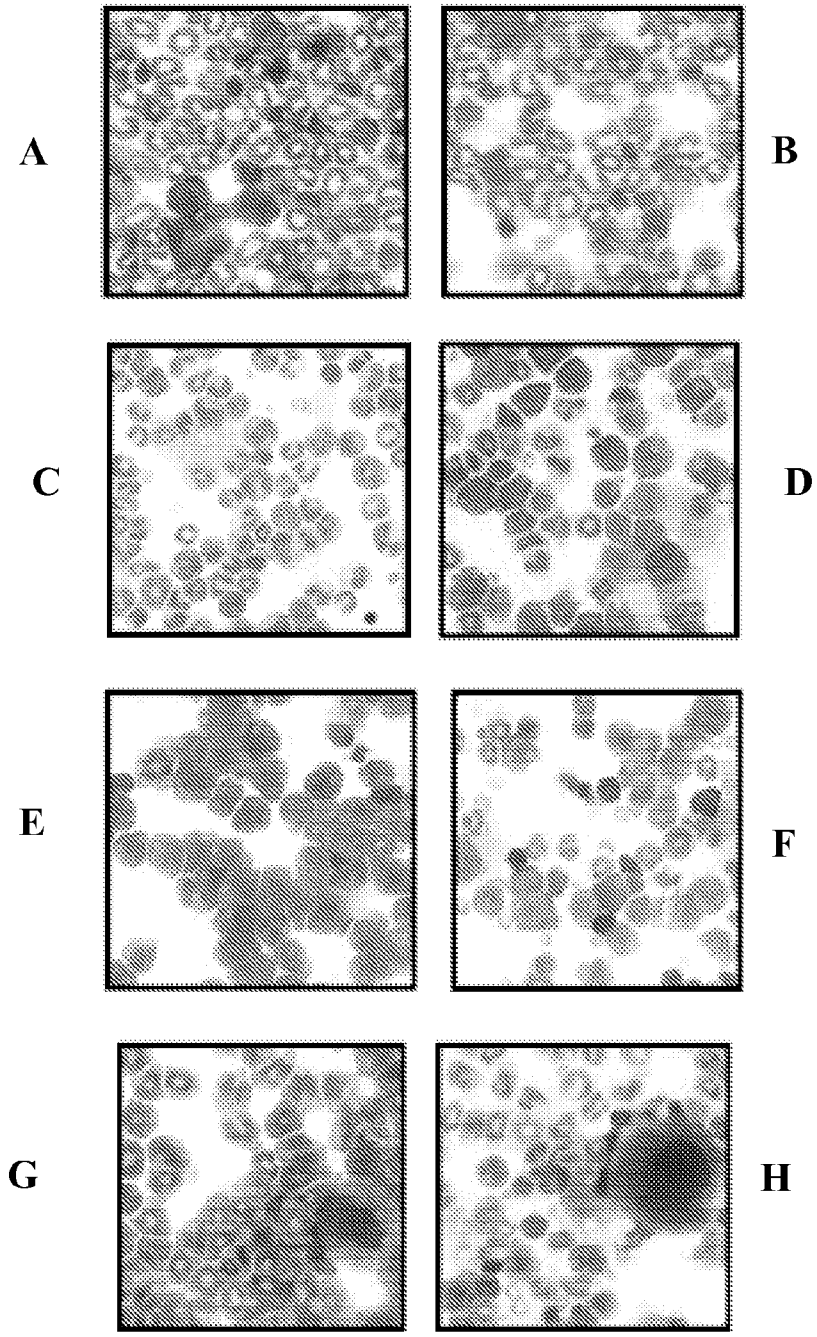
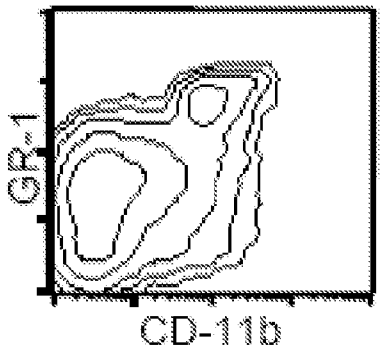
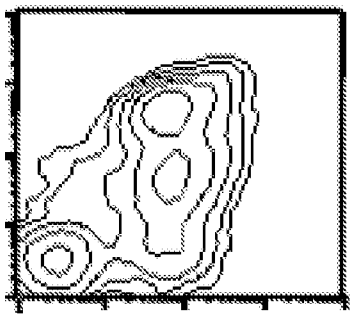


FIGURE 14

A



B



C

D

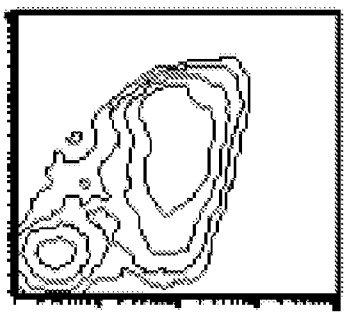


FIGURE 15

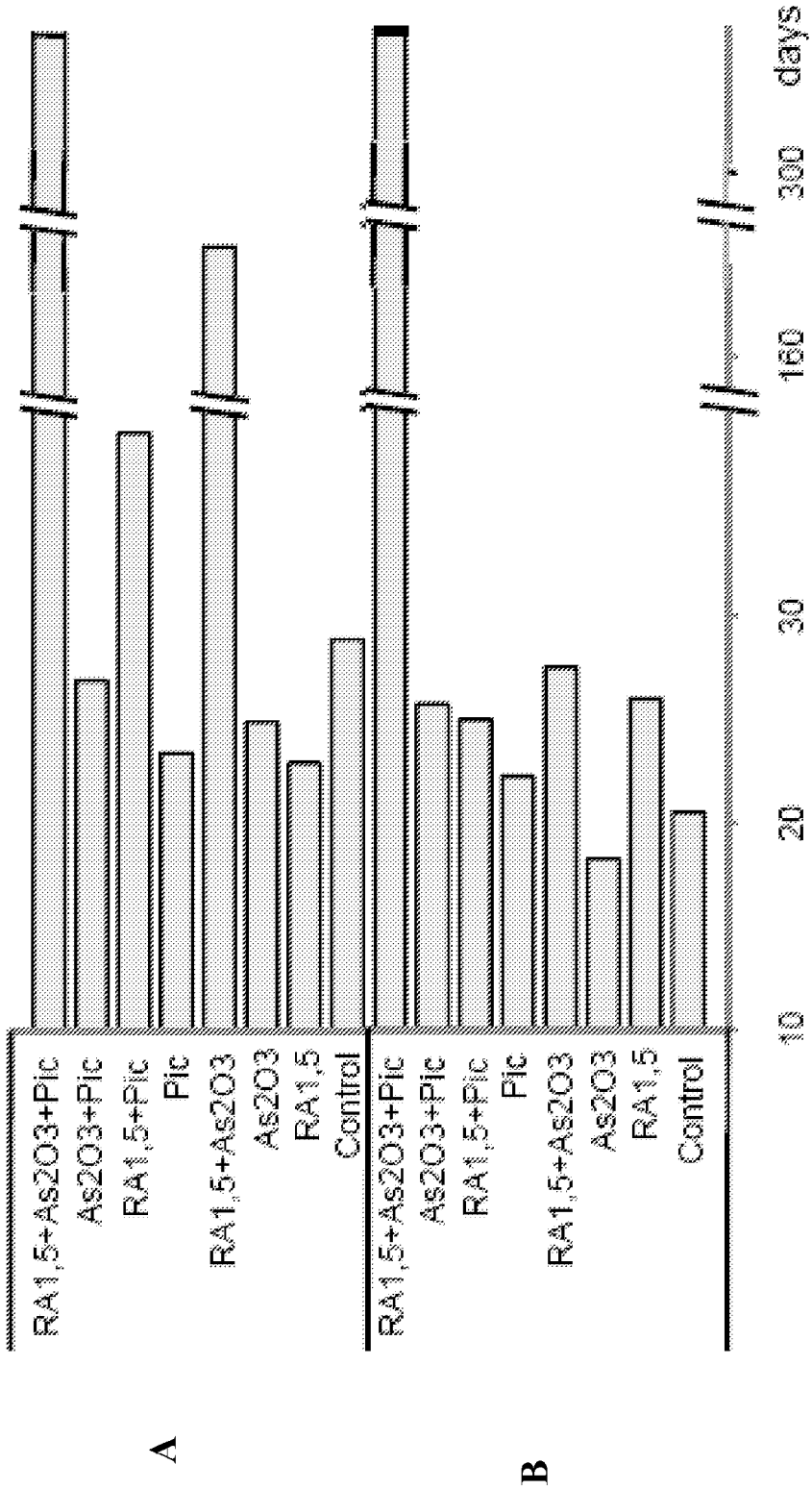

A
RA1,5+As2O3+Pic
As2O3+Pic
RA1,5+Pic
Pic
RA1,5+As2O3
As2O3
RA1,5
Control
B
RA1,5+As2O3+Pic
As2O3+Pic
RA1,5+Pic
Pic
RA1,5+As2O3
As2O3
RA1,5
Control
10
20
30
160
300
days

FIGURE 16

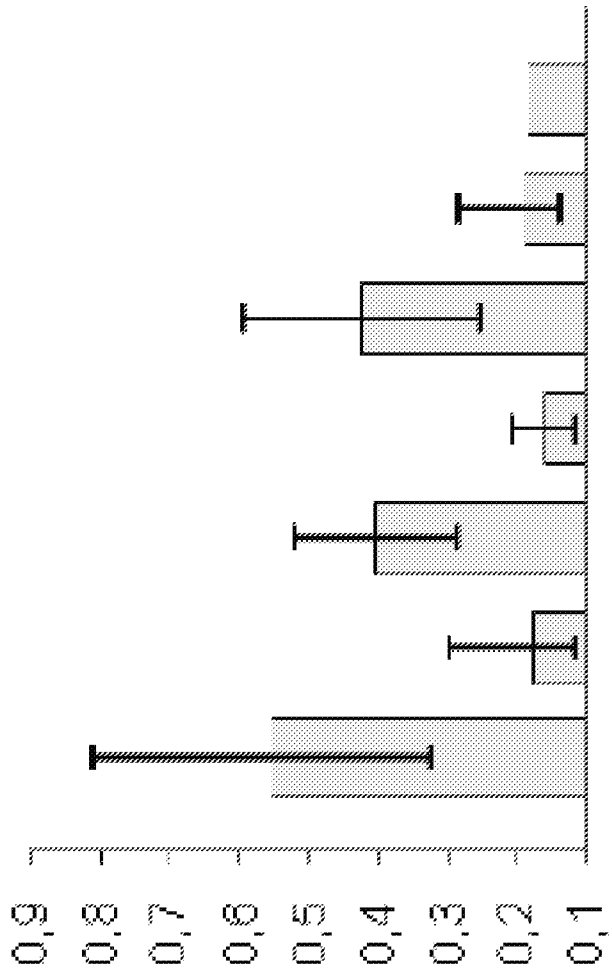


FIGURE 17

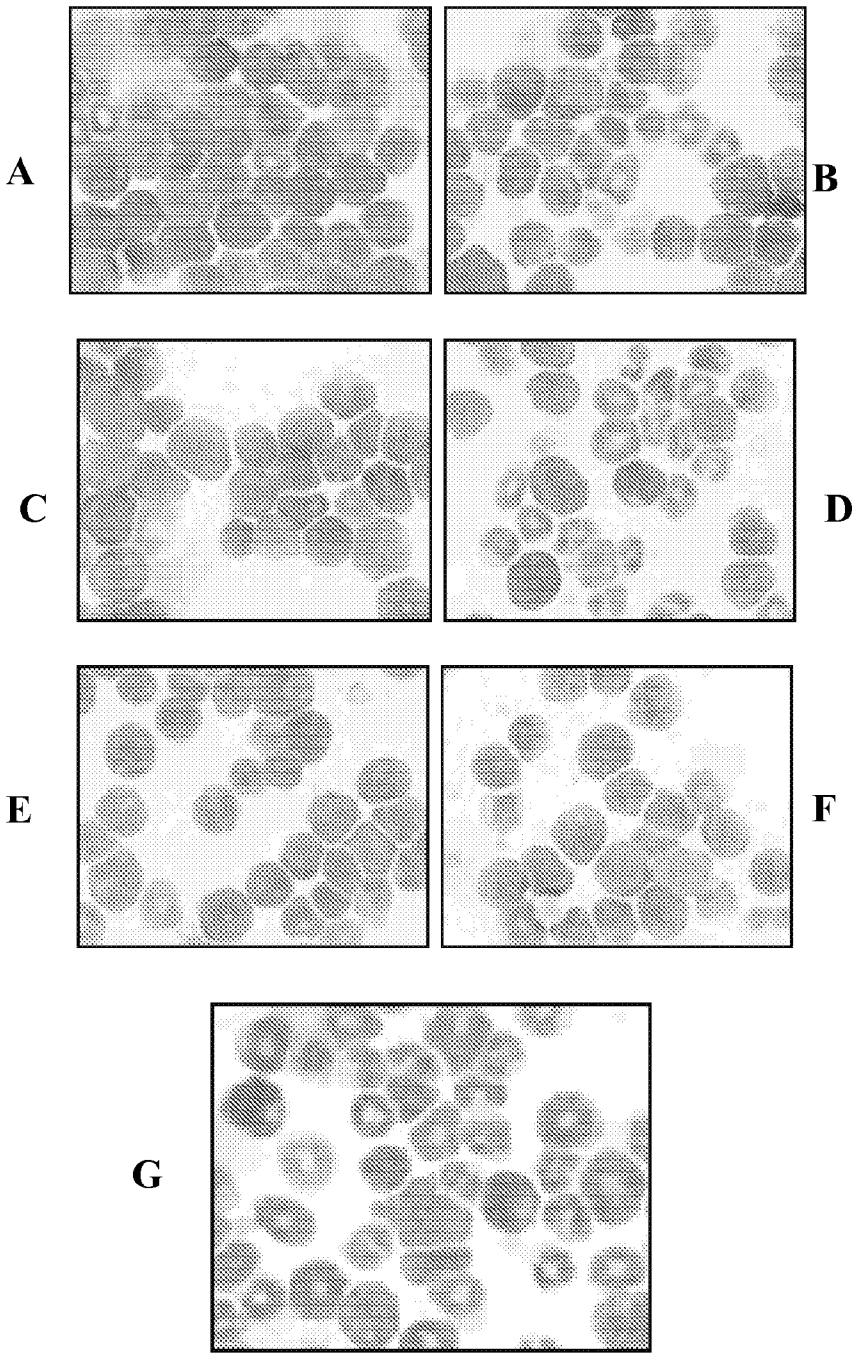


FIGURE 18

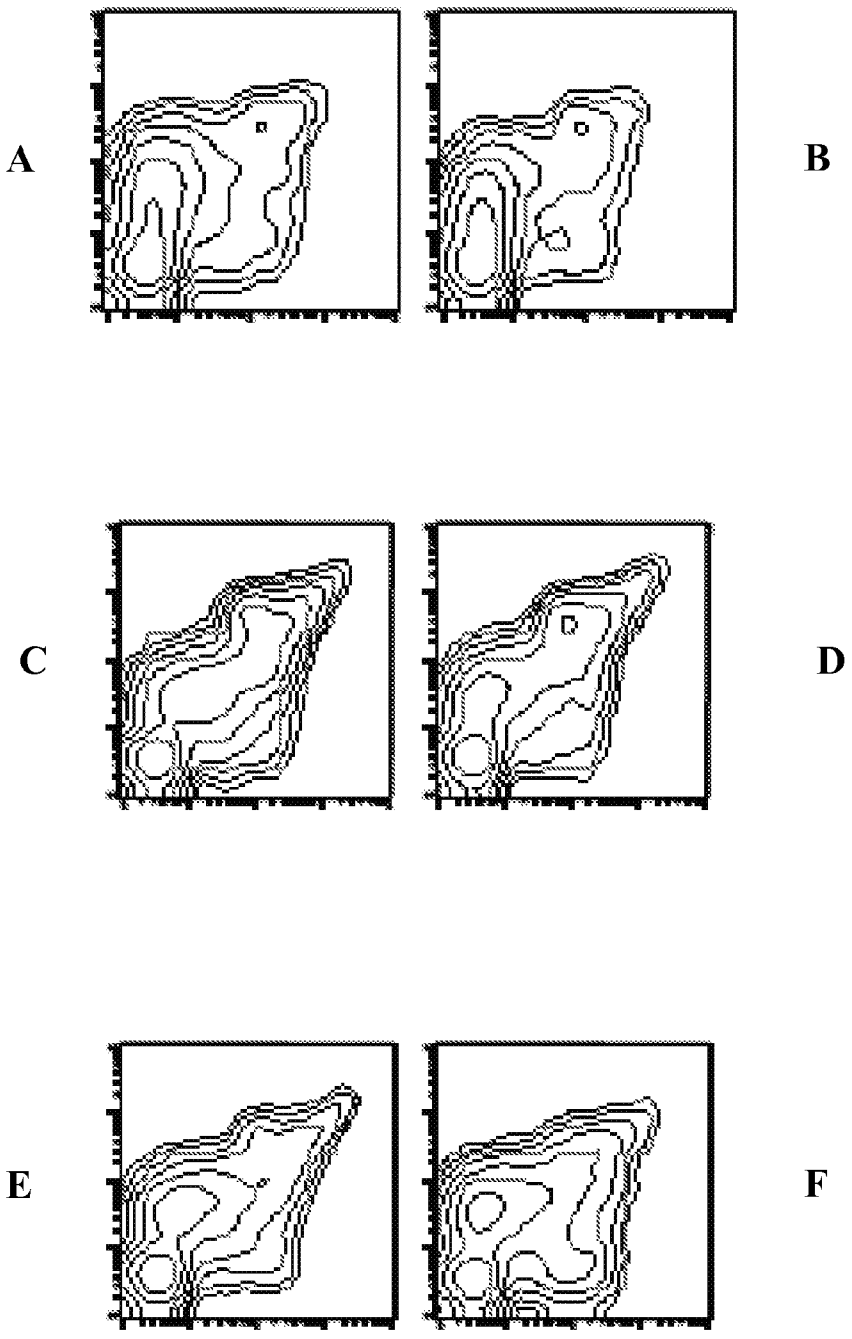


FIGURE 19

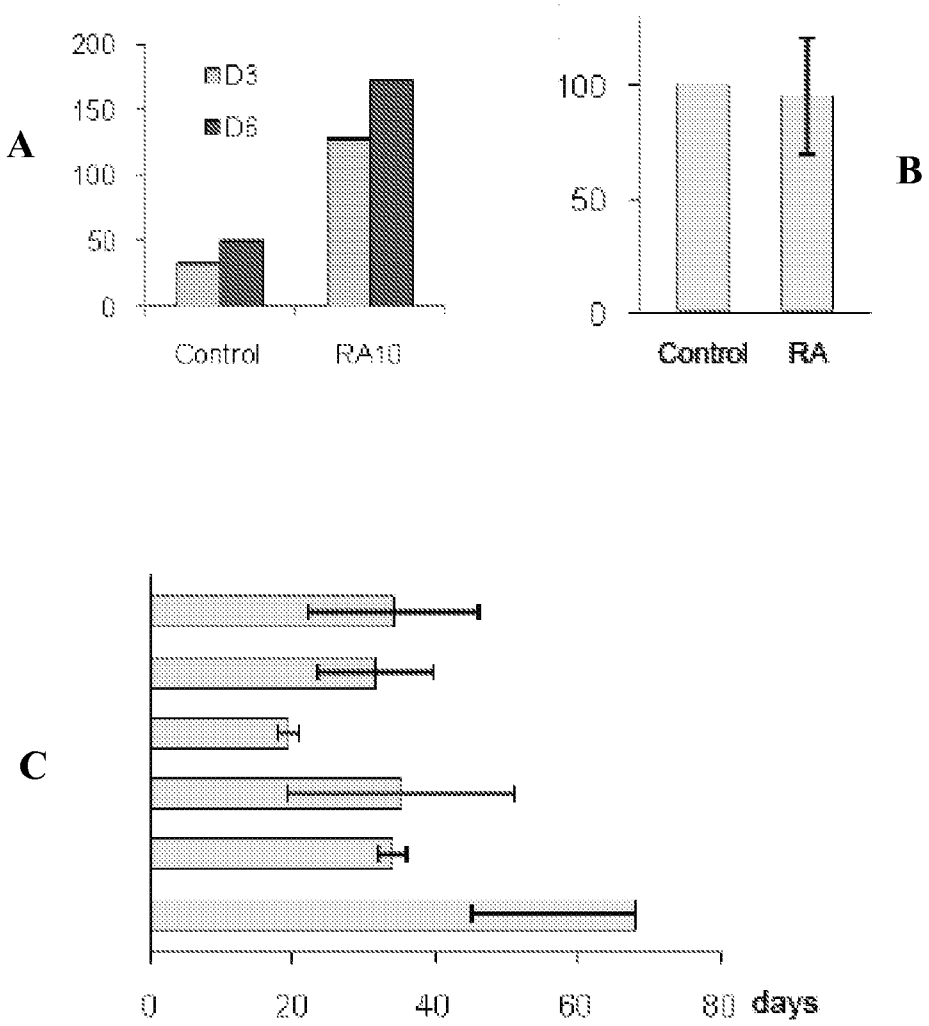
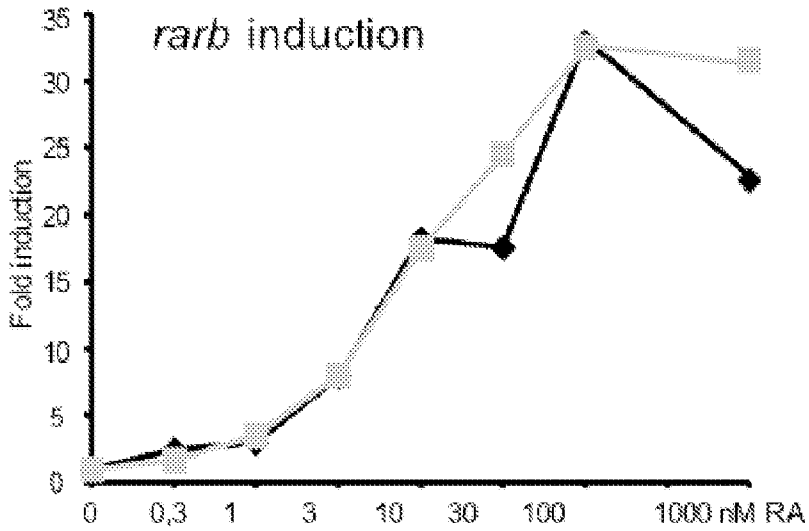

A
200
150
100
50
0
D3
D6
Control
RA10
B
100
50
0
Control
RA
C
0
20
40
60
80
days

FIGURE 20

A


rarb induction
Fold induction
35
30
25
20
15
10
5
0
0
0,3
1
3
10
30
100
1000 nM RA

B

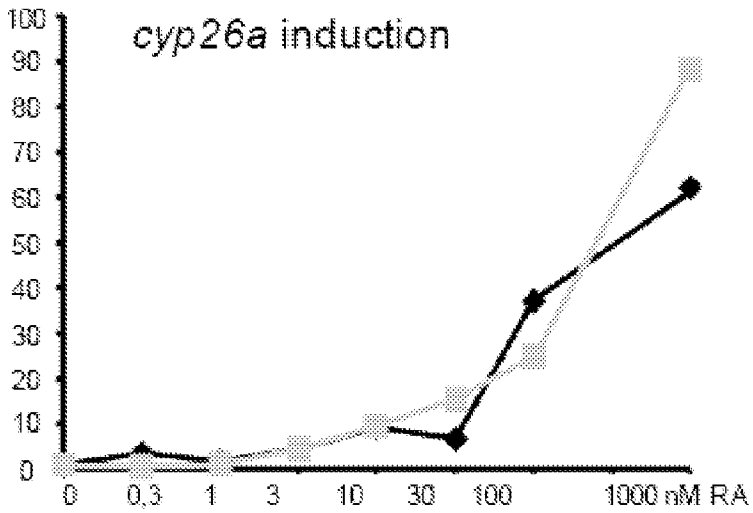

cyp26a induction
100
90
80
70
60
50
40
30
20
10
0
0
0,3
1
3
10
30
100
1000 nM RA

FIGURE 21

Fold induction	Tg	BM	MEF
tgII	13+/-3	19+/-6	6+/-4
tgII	66+/-27	26+/-12	7+/-3
rarb	376+/-519	26+/-21	11+/-7
rarb	98+/-127	14+/-11	3+/-2
cyp26a	6149+/-8045	195+/-270	5
cyp26a	18438+/-16805	297+/-289	6

PML/RARA

PML/RARA S873A

FIGURE 22

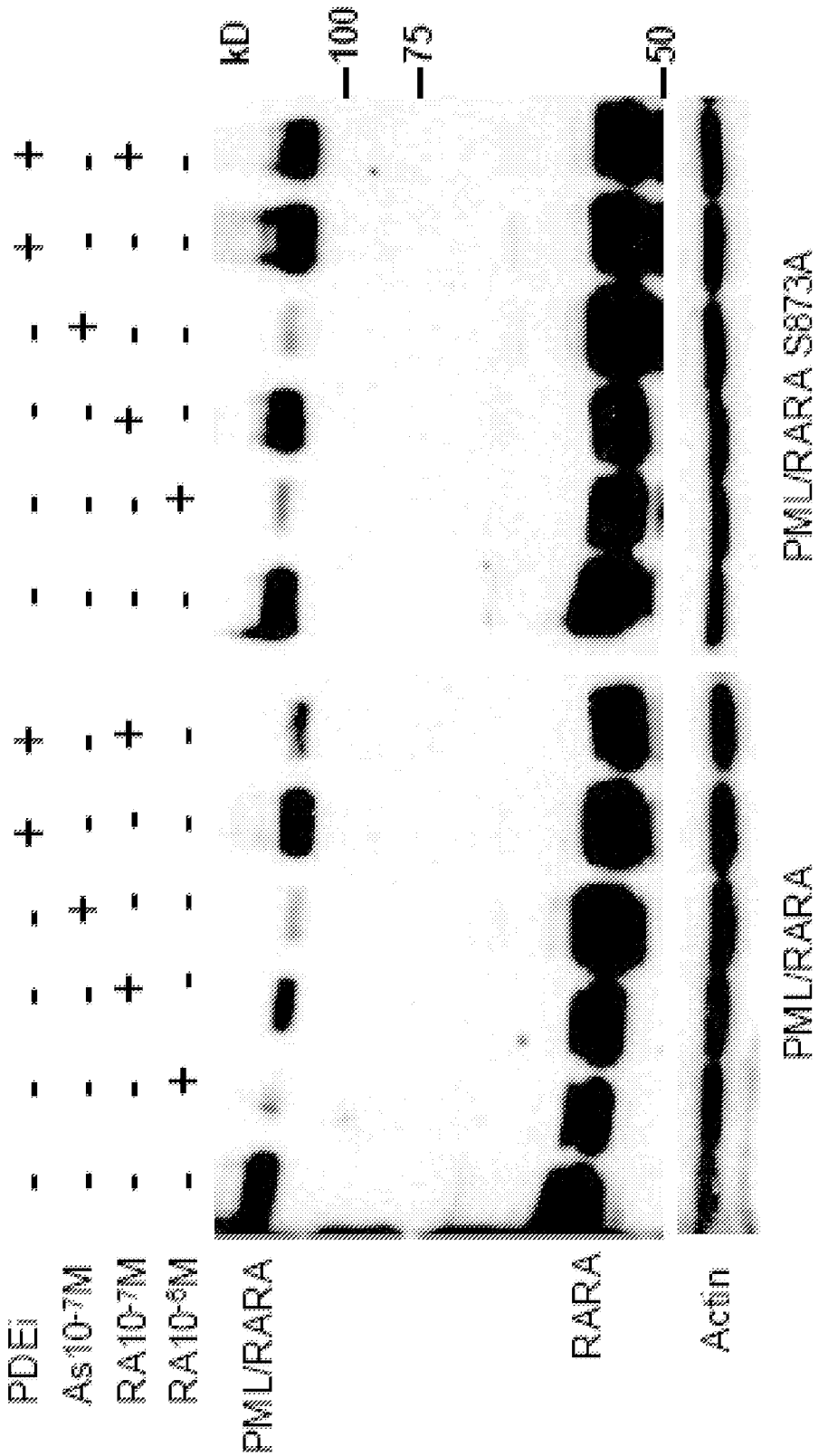

PDEi
As10-7M
RA10-7M
RA10-5M
kD
100
75
50
PML/RARA
RARA
Actin
PML/RARA
PML/RARA S873A

FIGURE 23

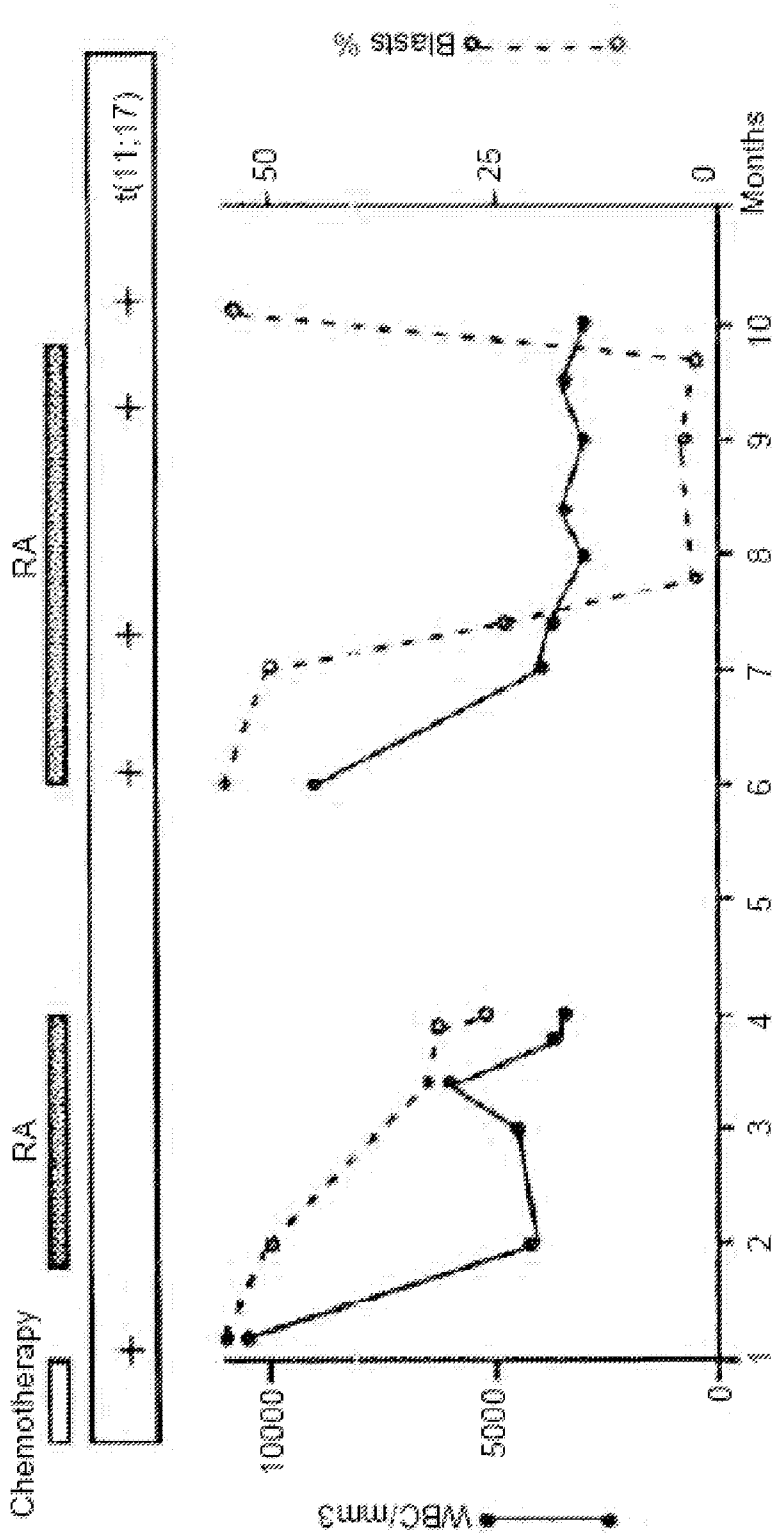

Chemotherapy
RA
RA
t(11;17)
WBC/mm3
10000
5000
0
Blasts %
50
25
0
Months
1
2
3
4
5
6
7
8
9
10

FIGURE 24

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/056798

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/203 A61K31/44 A61K31/519 A61K31/52 A61K33/36
A61K45/06 A61P35/00 A61P35/02 G01N33/569

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)
A61K A61P G01N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TSAI DONALD E ET AL: "A phase I trial of bexarotene, a retinoid x receptor agonist, in non-M3 acute myeloid leukemia: Evidence of myeloid differentiation and clinical activity." BLOOD, vol. 108, no. 11, Part 1, November 2006 (2006-11), page 553A, XP009107428 & 48TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; ORLANDO, FL, USA; DECEMBER 09 -12, 2006 ISSN: 0006-4971 abstract ----- -/--	1-28

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2009	27/10/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Langer, Astrid

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/056798

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHENG XIAOMIN ET AL: "Arsenic but not all-trans retinoic acid overcomes the aberrant stem cell capacity of PML/RAR alpha-positive leukemic stem cells" HAEMATOLOGICA-THE HEMATOLOGY JOURNAL, vol. 92, no. 3, March 2007 (2007-03), pages 323-331, XP009107429 ISSN: 0390-6078(print) 1592-8721(ele Abstract page 324, column 1, paragraph 1 - column 2, paragraph 2 page 327, column 1, paragraph 1 - column 2, paragraph 1	1-28
Y	DEGOS LAURENT ET AL: "All trans retinoic acid in acute promyelocytic leukemia" ONCOGENE, vol. 20, no. 49, 29 October 2001 (2001-10-29), pages 7140-7145, XP002501546 ISSN: 0950-9232 abstract	1-27
X	STAPNES CAMILLA ET AL: "Treatment with valproic acid, all-trans retinoic acid (ATRA) and theophyllamine for 9 days caused a persistent increase in peripheral blood platelet counts for a patient with acute myelogenous leukemia" ACTA ONCOLOGICA (STOCKHOLM), vol. 45, no. 3, April 2006 (2006-04), pages 346-349, XP009107432 ISSN: 0284-186X	1,2,7-9, 12
Y	abstract; table 1	1-30
X	GUILLEMIN M-C ET AL: "In vivo activation of cAMP signaling induces growth arrest and differentiation in acute promyelocytic leukemia" JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, JP, vol. 196, no. 10, 18 November 2002 (2002-11-18), pages 1373-1380, XP002267247 ISSN: 0022-1007 page 1375, paragraph 1 - page 1376, paragraph 2	1-30
X	WO 2006/091542 A (CEDARS SINAI MEDICAL CENTER [US]; BLACK KEITH [US]) 31 August 2006 (2006-08-31)	1,2,7-9, 12
Y	page 8, line 5 - page 9, line 11; claims	1-28

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/056798

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/062671 A (ALTANA PHARMA AG [DE]; BRAUNGER JUERGEN [DE]; SCHUDT CHRISTIAN [DE]) 29 July 2004 (2004-07-29) cited in the application page 16, paragraph 7 page 17, paragraph 8 - page 18, paragraph 1	1-30
X	WO 2004/026319 A (CENTRE NAT RECH SCIENT [FR]; INST NAT SANTE RECH MED [FR]; DE THE HUGU) 1 April 2004 (2004-04-01) cited in the application the whole document	1-30
X	ABEMAYOR E: "The effects of retinoic acid on the in vitro and in vivo growth of neuroblastoma cells." THE LARYNGOSCOPE OCT 1992, vol. 102, no. 10, October 1992 (1992-10), pages 1133-1149, XP009107433 ISSN: 0023-852X	1,2,7-9, 12
Y	abstract; figures	1-28
A	GARATTINI ENRICO ET AL: "Retinoids as differentiating agents in oncology: A network of interactions with intracellular pathways as the basis for rational therapeutic combinations" CURRENT PHARMACEUTICAL DESIGN, vol. 13, no. 13, 2007, pages 1375-1400, XP009107423 ISSN: 1381-6128 abstract	1-28
A	LANOTTE M ET AL: "On growth regulation of the rat promyelocytic leukemia (BNML): Growth inhibition and eradication of clonogenic cells by cholera toxin" LEUKEMIA RESEARCH, NEW YORK,NY, US, vol. 10, no. 11, 1 January 1986 (1986-01-01), pages 1319-1326, XP022922232 ISSN: 0145-2126 [retrieved on 1986-01-01] the whole document	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/056798

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DARWICHE N ET AL: "Retinoic acid dramatically enhances the arsenic trioxide-induced cell cycle arrest and apoptosis in retinoic acid receptor alpha-positive human T-cell lymphotropic virus type-I-transformed cells" HEMATOLOGY JOURNAL, MCMILLAN, BASINGSTOKE, GB, vol. 2, no. 2, 1 January 2001 (2001-01-01), pages 127-135, XP009113285 ISSN: 1466-4860 abstract; figures	1-28
X	VITOUX ET AL: "Acute promyelocytic leukemia: New issues on pathogenesis and treatment response" INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, EXETER, GB, vol. 39, no. 6, 1 January 2007 (2007-01-01), pages 1063-1070, XP022098676 ISSN: 1357-2725 page 1067, column 2, paragraph 1	1-30
Y	CHEN Z ET AL: "Arsenic trioxide and acute promyelocytic leukemia: clinical and biological." CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY 2007, vol. 313, 2007, pages 129-144, XP009113287 ISSN: 0070-217X page 130 - page 133	1-28
Y	GALAN A ET AL: "Uncoupling of apoptosis and Jun/AP-1 activity in human promonocytic cells treated with DNA-damaging and stress-inducing agents" EUROPEAN JOURNAL OF CELL BIOLOGY, WISSENSCHAFLICHE VERLAGSGESELLSCHAFT, STUTTGART, DE, vol. 79, no. 1, 1 January 2000 (2000-01-01), pages 1-9, XP004954619 ISSN: 0171-9335 abstract; figures	1-28

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/056798

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GUIDEZ FABIEN ET AL: "Rexinoid therapy bypasses the differentiation block associated with acute promyelocytic leukemia harboring the PLZF/RARalpha rearrangement" BLOOD, vol. 98, no. 11 Part 1, 16 November 2001 (2001-11-16), pages 766a-767a, XP009120984 & 43RD ANNUAL MEETING OF THE AMERICAN SOCIETY OF HEMATOLOGY, PART 1; ORLANDO, FLORIDA, USA; DECEMBER 07-11, 2001 ISSN: 0006-4971 abstract	1-30
P,X	NASR RIHAB ET AL: "Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation" NATURE MEDICINE, vol. 14, no. 12, December 2008 (2008-12), pages 1333-1342, XP002539938 ISSN: 1078-8956 the whole document	1-28
Y	WO 2006/113790 A (UNIV MICHIGAN [US]; YILMAZ OMER H [US]; MORRISON SEAN L [US]) 26 October 2006 (2006-10-26) claims	29,30
Y	TUTTER ANTONIN V ET AL: "EMBRYONIC STEM CELLS: A GREAT HOPE FOR A NEW ERA OF MEDICINE" CURRENT OPINION IN DRUG DISCOVERY AND DEVELOPMENT, CURRENT DRUGS, LONDON, GB, vol. 9, no. 2, 1 March 2006 (2006-03-01), pages 169-175, XP009075464 ISSN: 1367-6733 figures	29,30
Y	LALLEMAND-BREITENBACH VALERIE ET AL: "Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 189, no. 7, 5 April 1999 (1999-04-05), pages 1043-1052, XP002550631 ISSN: 0022-1007 Abstract page 1044, column 1	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2009/056798

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-28

Composition and its use for suppressing differentiation blockage of cancer cells and eradication of cancer stem cells

1.1. claims: Claims 1, 2, 7-9, 12 (in part), 3-6, 10, 11, 13-28

the composition comprising
- retinoic acid (RA) or a related compound thereof and
- a phosphodiesterase inhibitor (PDEI) or a compound increasing cAMP and
- an arsenic derivative

1.2. claims: Claims 1, 2, 7-9, 12 (in part)

the composition comprising
- retinoic acid (RA) or a related compound thereof and
- a phosphodiesterase inhibitor (PDEI) or a compound increasing cAMP.

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2. claims: 29, 30

Process for in vitro screening for the capacity to eradicate the leukaemia initiating cells comprising contacting those cells with a test molecule in combination with retinoic acid and a PDEI.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2009/056798

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2008188480 A1	07-08-2008
WO 2004062671 A	29-07-2004	AU 2004204355 A1	29-07-2004
		CA 2512819 A1	29-07-2004
		HR 20050699 A2	30-11-2006
		IS 7970 A	05-08-2005
		JP 2006515367 T	25-05-2006
		US 2006148804 A1	06-07-2006
WO 2004026319 A	01-04-2004	AU 2003270202 A1	08-04-2004
WO 2006113790 A	26-10-2006	NONE	