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(71) Applicant: UNIVERSITÉ LAVAL [CA/CA]; 2320 rue
des Bibliothèques, Québec, Québec G1V 0A6 (CA).(72) Inventors: TESSIER, Philippe; 3470 avenue Marcil,
Montréal, Québec H4A 2Z3 (CA). BARABÉ, Frédéric;
1041 Grande Allée Ouest, Québec, Québec G1S 1E1 (CA).
LAOUEDJ, Malika; Appt 2, 2834 rue Jules Dallaire,
Québec, Québec G1V 2J8 (CA).(74) Agent: NORTON ROSE FULBRIGHT CANADA
LLP/S.E.N.C.R.L., S.R.L.; Suite 2500, 1 Place Ville-
Marie, Montréal, Québec H3B 1R1 (CA).

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(54) Title: S100A9 PEPTIDE FOR TREATING LEUKEMIAS

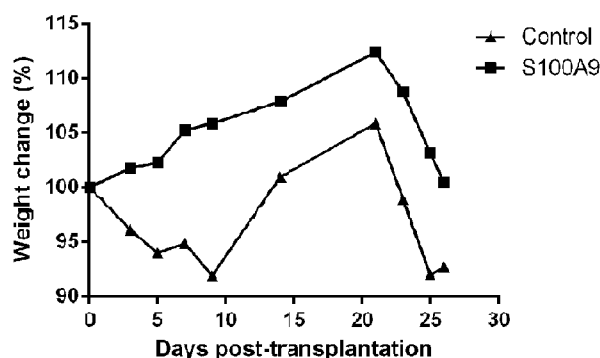


Fig. 2

(57) Abstract: The present description relates to a composition comprising a S100A9 peptide or a peptidomimetic thereof, and a carrier for treating leukemia.

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S100A9 PEPTIDE FOR TREATING LEUKEMIAS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority from U.S. provisional application serial no. 62/120,527 filed on February 25, 2014. The content of the priority application is herewith incorporated in its entirety.

TECHNICAL FIELD

[0002] The present description relates to a composition comprising S100A9 peptide for treating leukemias.

BACKGROUND ART

[0003] Acute leukemias are the result of a series of genetic and epigenetic events occurring in a stem or progenitor hematopoietic cell, giving rise to a clonal expansion of progenitors with an impaired capacity to differentiate. The past 20 years have been very fruitful in the identification of recurrent genetic lesions in acute leukemias. Improvement in leukemia-free survival has been mostly due to better risk stratification which allows for adjustment of treatment intensity and also to allogenic hematopoietic stem cell transplantation. However, drugs used in acute myeloid treatment (AML) are basically the same today as they were 25-30 years ago and prognosis remains poor. The 5-year survival rate is as low as 55% for children with AML and even worse for adults (30-40%) and elderly (> 65 yo) (<15%). Therefore, novel and innovative approaches need to be explored in order to improve leukemia-free survival of acute leukemia patients.

[0004] There is thus still a need to be provided with a composition or methodology for treating leukemia.

SUMMARY

[0005] In accordance with the present description there is now provided a composition comprising S100A9 peptide or a peptidomimetic thereof, and a carrier for treating leukemia.

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[0006] In an embodiment, the S100A9 peptide is human S100A9 protein.

[0007] In another embodiment, the peptide comprises the amino acid sequence set forth in SEQ ID NO: 1.

[0008] In a further embodiment, the peptide has at least 60%, at least 70% or at least 80%, at least 90% identical, or at least about 95% identity with SEQ ID NO: 1.

[0009] In a further embodiment, the peptide consists of the amino acid sequence set forth in SEQ ID NO: 1.

[0010] In an additional embodiment, the peptide or the peptidomimetic thereof comprises a chemically or enzymatically attached modification.

[0011] In another embodiment, the peptide or peptidomimetic thereof is pegylated, hesylated, pasylated, myristoylated, or glycosylated.

[0012] In a further embodiment, the composition described herein is formulated for an oral administration, an inhalation, a nasal administration, or an administration by injection.

[0013] In another embodiment, the injection is subcutaneously.

[0014] In a further embodiment, the composition described herein further comprises a chemotherapeutic agent.

[0015] In another embodiment, the chemotherapeutic agent is an alkylating/alkaloid agent, an antimetabolite, a hormone, a hormone analog, or an antineoplastic drug.

[0016] In an additional embodiment, the chemotherapeutic agent is cytarabine (cytosine arabinoside, Ara-C, Cytosar-U), carboplatin, carmustine, chlorambucil, dacarbazine, ifosfamide, lomustine, mechlorethamine, procarbazine, pentostatin, (2'deoxycoformycin), etoposide, teniposide, topotecan, vinblastine, vincristine, paclitaxel, dexamethasone, methylprednisolone, prednisone, all-trans retinoic acid, arsenic trioxide,

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interferon-alpha, rituximab (Rituxan®), gemtuzumab ozogamicin, imatinib mesylate, melphalan, busulfan (Myleran®), thiotepa, bleomycin, platinum (cisplatin), cyclophosphamide (Cytosan®), daunorubicin, doxorubicin, idarubicin, mitoxantrone, 5-azacytidine, cladribine, fludarabine, hydroxyurea, 6-mercaptopurine, methotrexate, 6-thioguanine, or any combination thereof.

[0017] In an embodiment, it is provided the use of the S100A9 peptide or peptidomimetic thereof, of the composition described herein, for treating leukemia in a subject or in the manufacture of a medicament for treating leukemia in the subject.

[0018] In another embodiment, it is also provided a method of treating leukemia in the subject comprising administering to said subject an effective amount of the S100A9 peptide or peptidomimetic thereof, of the composition described herein.

[0019] In an embodiment, the subject is a mouse or a human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Reference will now be made to the accompanying drawings.

[0021] Fig. 1 illustrates the secretion of S100A8/A9 in the serum of mice with primary leukemias, the primary leukemia generated by injecting mouse hematopoietic stem/progenitor cells transfected with a retrovirus expressing GFP, as well as genes HOXA9 and MEIS1 (3 different primary mice: AH13, AH15 and AH16), wherein peripheral blood was harvested from moribund or control mice, and the S100A8/A9 concentrations were measured by ELISA in serum, wherein the data are the mean $\pm$ sem of 3 independent measurements.

[0022] Fig. 2 illustrates the S100A9 protection of mice injected with HoxA9-MEIS1 leukemia.

[0023] Fig. 3: illustrates the S100A9 treatment increase of latency of HOXA9-MEIS1 leukemia in secondary mice.

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[0024] Fig. 4A illustrates a photographic depiction of the increased differentiation of AML cells in mice treated with S100A9.

[0025] Fig. 4B illustrates the increased differentiation of AML cells in bone marrow and blood (neutro, band cells, metamyelocytes, myelocytes, blast) in mice treated with S100A9.

[0026] Fig. 5 illustrates that S100A9 promotes the growth arrest of mouse leukemia cells.

[0027] Fig. 6 illustrates that S100A9 promotes the differentiation of human leukemia cells.

[0028] Fig. 7 illustrates that S100A9 induces differentiation of human AML cells of human patients.

[0029] Fig. 8A illustrates that S100A9 induces differentiation of human AML cells of a human patient by measuring the differentiation marker CD14.

[0030] Fig. 8B illustrates that S100A9 induces differentiation of human AML cells of a human patient by examining the cell cycle by propidium, iodide staining.

[0031] Fig. 9A illustrates that S100A9 promote differentiation of the AML cell line Mono-Mac-1 by measuring the expression of the differentiation marker CD14.

[0032] Fig. 9B illustrates that S100A9 promote growth arrests of the AML cell line Mono-Mac-1 by measuring the number of cells.

DETAILED DESCRIPTION

[0033] It is provided a composition comprising a peptide comprising the amino acid sequence set forth in SEQ ID NO: 1 and a carrier for treating leukemia.

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[0034] It is accordingly provided a composition comprising S100A9 peptide for treating leukemias, more particularly acute leukemias.

[0035] The S100 protein family comprises 22 members of small (10 to 14 kDa) acidic calcium-binding proteins named as S100A1, S100A2, and so on, according to the time of their discovery and on the chromosome on which they are found. These intracellular proteins are involved in the control of protein phosphorylation, enzymatic activities, Ca^{2+} homeostasis, and intermediate filaments polymerisation. S100A8, S100A9 and S100A12 belong to a subset called myeloid related proteins (MRPs) because they are predominantly expressed in neutrophils (30% of cytoplasmic protein) and monocytes, which derive from myeloid precursors.

[0036] S100A9, also known as calgranulin B and myeloid related protein-14 (MRP-14), is a calcium- and zinc-binding protein that belongs to the S100 protein family. S100A9 is highly expressed by the myeloid cell lineage and is found in the extracellular milieu during inflammatory conditions. S100A9 forms heterodimers with S100A8, another member of the S100 family. However, S100A9 may also form monomers which execute specific functions. Human S100A9 has a molecular mass of about 13 kDa and is composed of 114 amino acid residues.

Human S100A9

10	20	30	40	50
MTCKMSQLER	NIETIINTFH	QYSVKLGHPD	TLNQGEFKEL	VRKDLQNFLK
60	70	80	90	100
KENKNEKVIE	HIMEDLDTNA	DKQLSFEEFI	MLMARLTWAS	HEKMHEGDEG
110				
PGHHHKPGLG	EGTP			

(SEQ ID NO: 1)

[0037] S100A9 is expressed abundantly in neutrophils and monocytes. Epithelial, mesenchymal and endothelial cells can also express and secrete S100A9 under infectious or inflammatory conditions. S100A8 and S100A9 are arranged as noncovalently bonded homodimers. In addition, in the presence of

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calcium, S100A8 and S100A9 form a noncovalent heterodimer called S100A8/A9 or calprotectin, presumed to be involved in the cellular control of calcium concentrations.

[0038] It has been demonstrated that *S100a9*^{-/-} mice are resistant to adjuvant-induced arthritis and systemic lupus erythematosus, due at least in part to reduced CD8 T cell activation (Loser et al., 2010, *Nature medicine*, 16: 713-717). mAbs against S100A9 prevent inflammation and joint destruction in the collagen-induced arthritis model (Cesaro et al., 2012, *PloS one* 7: e45478). This is associated with reduced leukocyte migration to the inflammatory sites and secretion of TNF α and IL-6. Accordingly, it is known that S100A9 promotes inflammation by enhancing phagocyte migration and inducing the secretion of pro-inflammatory cytokines, as well as the release of tissue-degrading enzymes and ROS. These activities are presumably counterbalanced by oxidized S100A8 which acts as an anti-inflammatory factor.

[0039] S100A8 and S100A9 are presumed to bind to RAGE, the scavenger receptor (CD36), CD33 or the Toll-like receptor 4 (TLR4). Potential receptors for S100A8 and S100A9 on the surface of leukemic cells have never been investigated although TCGA data show TLR2, TLR4 and CD36, but not RAGE, are expressed at high levels (average RPKM values > 20) in AML patient samples. These receptors are also highly expressed on normal cells (monocytes, granulocytes), while S100A8 and S100A9 knockout mice seem to have normal hematopoiesis. These data suggest that targeting the S100A8/A9 complex is a better strategy than targeting the receptors to maximize the therapeutic effects and minimize the side effects on normal blood cell populations.

[0040] U.S. patents nos. 7,553,488 and 8,282,925 disclose the use of an inhibitor or antagonist of S100A9, such as an antibody, for modulating, reducing or treating inflammation. U.S. patent application no. 62/191,733 describes the use of a humanized anti-S100A9 antibody for reducing or inhibiting inflammation.

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[0041] European patent no. 1608397 describes the use of a composition comprising S100A9 protein for stimulating proliferation, differentiation and release of neutrophils in a patient.

[0042] The Cancer Genome Atlas (TCGA) Research Network performed whole genome/whole exome sequencing on 200 adult AML cases along with RNA, microRNA and DNA-methylation analysis. S100A8 and S100A9 were amongst the top 5 discriminatory genes for one important sub-group mainly composed of myelomonocytic and monocytic AML, with high RPKM levels (Cancer Genome Atlas Research, N., 2013, *The New England journal of medicine*, 368: 2059-2074). High concentrations of S100A8 and S100A9 proteins are found in the serum of patients with acute and chronic myeloid leukemia (Ivanov et al., 1996, *Immunology letters*, 49: 7-13) (AML and CML, Fig 1), and these concentrations correlate with growth-stimulating activity in these sera (Fig 2). In addition, S100A8 has been identified as a predictor of poor survival in *de novo* AML patients (Nicolas et al., 2011, *Leukemia: official journal of the Leukemia Society of America*, Leukemia Research Fund, U.K, 25: 57-65). Constitutive over-expression of S100A8/A9 is also associated with resistance to prednisone treatment in MLL-rearranged B-ALL, and forced expression of S100A8/A9 in MLL-rearranged B-ALL cells transform prednisone-sensitive into prednisone insensitive cells *in vitro* (Spijkers-Hagelstein et al., 2012, *Leukemia : official journal of the Leukemia Society of America*, Leukemia Research Fund, U.K, 26: 1255-1265. Taken together, these data confirm the expression of S100A8/A9 in acute leukemias.

[0043] Accordingly, it is disclosed for the first time a composition comprising a peptide comprising the amino acid sequence set forth in SEQ ID NO: 1 for treating leukemia.

[0044] The term peptide is used broadly to mean peptides, proteins, fragments of proteins and the like. The peptide disclosed herein may be synthesized as a peptide mimetic, mimicking the biological activity of the native peptide. Also encompassed is a peptidomimetic, meaning a peptide-like molecule that has the activity of the native peptide. Such peptidomimetic include

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a chemically-modified peptide, and a peptide-like molecule containing non-naturally occurring amino acids. A peptidomimetic can include amino acid analogs and can be a peptide-like molecule which contains, for example, an amide bond isostere such as a retro-inverso modification; reduced amide bond; methylenethioether or methylenesulfoxide bond; methylene ether bond; ethylene bond; thioamide bond; trans-olefin or fluoroolefin bond; 1,5-disubstituted tetrazole ring; ketomethylene or fluoroketomethylene bond or another amide isostere.

[0045] Accordingly, provided herein is a peptide comprising the amino acid sequence set forth in SEQ ID NO: 1, or a conservative substitute or a peptidomimetic thereof. In a preferred embodiment, the peptide consists of the amino acid sequence set forth in SEQ ID NO: 1, or a conservative substitute or peptidomimetic of the sequence. As used herein, a "conservative substitute" is an amino acid sequence in which a first amino acid is replaced by a second amino acid or amino acid analog having at least one similar biochemical property, which can be, for example, similar size, charge, hydrophobicity or hydrogen-bonding capacity. For example, a first hydrophobic amino acid alanine can be conservatively substituted with a second (non-identical) hydrophobic amino acid such as alanine (Ala), valine (Val), leucine (Leu), or isoleucine (Ile), or an analog thereof. Similarly, a first amino acid having an uncharged side chain (i.e., serine, threonine and glutamine) can be conservatively substituted with a second (non-identical) amino acid such as serine (Ser), threonine (Thr), asparagine (Asn) and glutamine (Gln), or an analog thereof.

[0046] In another embodiment, the peptide used in the compositions and methods described herein consists essentially of a peptide which is at least 60%, at least 70% or at least 80% identical to SEQ ID NO: 1. In another embodiment, the peptide is at least 90% identical to SEQ ID NO: 1. In another embodiment, the peptide is at least about 95% identical to SEQ ID NO: 1.

[0047] Also encompassed herein are further modified forms of the peptide and peptidomimetic described herein. Such modified peptide or peptidomimetic may comprise e.g. chemically or enzymatically attached modifications that

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render the peptide more stable, e.g. against protease degradation, that allow to provide the peptides or peptidomimetic as pharmaceutically acceptable salts, or which e.g. improve the biological properties of the peptide or peptidomimetic such as half-life. For example, but not limited to, such modifications include pegylated, hesylated, pasylated, myristoylated, glycosylated, and/or cyclic forms of the peptide and/or peptidomimetic, and in particular the peptide of SEQ ID NO: 1 or a peptidomimetic of this sequence.

[0048] In another embodiment, the peptide, peptidomimetic and/or composition described herein is formulated for oral administration, inhalation, nasal administration, or for an administration by injection such as subcutaneously.

[0049] In another embodiment, the peptide described herein is used in combination with a chemotherapeutic agent. Encompassed herein is a chemotherapeutic agent with therapeutic usefulness in the treatment of leukemias known in the art. Chemotherapeutic agents as used herein encompass both chemical and biological agents. Categories of chemotherapeutic agents include alkylating/alkaloid agents, antimetabolites, hormones or hormone analogs, and miscellaneous antineoplastic drugs. For example, but not limited to, the chemotherapeutic agent encompassed herein is cytarabine (cytosine arabinoside, Ara-C, Cytosar-U), carboplatin, carmustine, chlorambucil, dacarbazine, ifosfamide, lomustine, mechlorethamine, procarbazine, pentostatin, (2'deoxycoformycin), etoposide, teniposide, topotecan, vinblastine, vincristine, paclitaxel, dexamethasone, methylprednisolone, prednisone, all-trans retinoic acid, arsenic trioxide, interferon-alpha, rituximab (Rituxan®), gemtuzumab ozogamicin, imatinib mesylate, melphalan, busulfan (Myleran®), thiotepa, bleomycin, platinum (cisplatin), cyclophosphamide (Cytosan®), daunorubicin, doxorubicin, idarubicin, mitoxantrone, 5-azacytidine, cladribine, fludarabine, hydroxyurea, 6-mercaptopurine, methotrexate, 6-thioguanine, or any combination thereof.

[0050] As confirmed herein, S100A8/A9 concentrations are increased in the serum of patients with AML and CML (Fig. 1), correlate with growth activity (Fig.

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2) and blocking S100A8/A9 inhibits the proliferation of AML cells (Fig. 3). S100A8/A9 was reported to induce apoptosis in various tumor cell lines (Yui et al., 1997, Journal of leukocyte biology, 61: 50-57), but this effect has not been validated *in vivo*. This heterodimer also binds to and activates NF- κ B, and induces proliferation of colonic and prostate tumor cell lines (Hermani et al., 2006, Experimental cell research, 312: 184-197). Moreover, lymphoma and sarcoma growth is reduced in *S100a9*^{-/-} mice due to a reduction of myeloid-derived suppressor cell numbers and activity (Cheng et al., 2008, The Journal of experimental medicine, 205: 2235-2249). S100A8/A9 expression by lung phagocytes has also been linked to metastasis and migration to this organ (Hiratsuka et al., 2006, Nature cell biology, 8: 1369-1375).

[0051] As disclosed herein, murine hematopoietic stem/progenitor cells were transduced using retroviral vectors to express HOXA9-MEIS1 and injected into irradiated mice. HOXA9-MEIS1 expressing cells generated AML at a median of 60 days after transplantation with the same phenotype. An important increase of S100A8/A9 in the serum of the model leukemia was observed (Fig. 1), demonstrating that this model recapitulates the increase in S100A8/A9 concentration in the serum seen in patients. Interestingly, S100A8 and S100A9 were only expressed, but at high levels, in a fraction of leukemic cells (approximately 10%) in the bone marrow and may correspond to leukemic fraction with higher leukemia stem cell frequency as these cells also express levels of Ly6G and c-kit, 2 genes overexpressed in leukemia stem cells.

[0052] HOXA9-MEIS1 leukemic cells from murine bone marrow of primary recipients were collected and then injected into secondary mice. This strategy allows for a shorter and homogeneous latency in multiple mice using the same clonal disease. The therapeutic effect of recombinant S100A9 protein was tested on HOXA9-MEIS1 leukemia *in vivo*. Mice were intra-peritoneally (i.p.) injected with S100A9 (20 μ g/mouse) 3 times a week, starting on day 3 post-injection of leukemia cells. Treatment with S100A9 protein reduced weight loss associated with leukemia (Fig. 2), suggesting that S100A9 protein, reduces the proliferation of leukemia cells.

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[0053] The effect of S100A9 protein on the survival of mice with secondary HOXA9/MEIS1 leukemia was tested, using S100A8 protein as a control. Injection of S100A8 had no effect on the survival of mice with AML. 100,000 bone marrow cells from a HOXA9-MEIS1 primary leukemic mouse were injected in sub-lethally irradiated secondary mice, and recombinant S100A8 or S100A9 (20 µg/mouse) were injected 3 times a week from day 3 till day 35 (6 mice per group) (** $p \leq 0.01$, t-test) (Fig. 3). Injections of S100A9 prolonged survival of mice with AML (Fig. 3). Interestingly, 2 mice did not show any sign of leukemia at day 60 post-injection of leukemia cells, demonstrating that injection of S100A9 is sufficient to cure from leukemia. Histological analyses showed increased differentiation of leukemia cells in the bone marrow and blood of mice injected with S100A9 protein (Fig. 4A and B), demonstrating that injection of S100A9 promotes the differentiation of leukemia cells. Again 100,000 bone marrow cells from a HOXA9-MEIS1 primary leukemic mouse were injected in sub-lethally irradiated C57BL/6 mice. Recombinant S100A9 (20 µg/mouse) was injected 3 times a week from day 15 till day 35 (6 mice per group), wherein Wright-Giemsa staining of cytosins of bone marrow or blood cells at the time of sacrifice (>80% cells GFP+) showed increased differentiation of cells (Fig. 4A).

[0054] The effect of S100A9 on AML cell proliferation was measured. Murine HOXA9/MEIS1 leukemia cells were grown in presence or absence of murine S100A8 and S100A9 for 24h. Cell cycle was then examined by staining with propidium iodide. S100A8 protein did not affect the cell cycle of AML cells. In contrast, more cells in the G0/G1 phases were detected following stimulation with recombinant S100A9, demonstrating that S100A9 inhibits cell proliferation. Accordingly, S100A9 promotes the growth arrest of mouse leukemia cell (Fig. 5).

[0055] Taken together, these results indicate that injection of S100A9 protects mice from AML by promoting the differentiation of leukemia cells, resulting in reduced proliferation.

[0056] In order to evaluate impact of injection of S100A9 in a human patients, human cord blood cells transfected with the fusion gene MLL-AF4

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were stimulated with recombinant human S100A9 for 72h. Cell differentiation was examined using expression of CD14 as a marker of cell differentiation. S100A9 stimulates the expression of CD14 by these cells (Fig. 6), indicating an increased differentiation of the cells.

[0057] In addition, bone marrow cells from 5 M4 and M5 AML patients (03H031, 06H061, 07H006, 07H072, 07H138) were thawed, then cultured with 20 µg of S100A9 for 24 hours. Expression of the differentiation markers CD11b and CD14 was examined by FACS. As seen in Fig. 7, S100A9 induces differentiation of human AML cells.

[0058] Accordingly, as seen in mouse model described hereinabove, S100A9 induces differentiation and growth arrests of human AML cells (see Fig. 8A and B). Bone marrow cells from an AML patient were grown for 5 days. The cells were stimulated with 20 µg/ml of human S100A9. Expression of the differentiation marker CD14 was examined by FACS (Fig. 8A). Cell cycle was examined by propidium iodide staining (Fig. 8B).

[0059] As further seen herein, S100A9 promote differentiation of the AML cell line Mono-Mac-1, a cell line derived from the peripheral blood of a 65 year old patient with AML.

[0060] Mono-Mac-1 (200,000 cells per well) were grown for 3 days. The cells were stimulated with 20 µg/ml of human S100A9. Expression of the differentiation marker CD14 was examined by FACS (Fig. 9A). The cells were counted at the end of the experiment (Fig. 9B).

[0061] Thus, not only is it disclosed herein that treatment with S100A9 protein prolongs survival of mice with AML, stimulation of leukemia cell proliferation by S100A9 is observed in human AML. S100A9 as demonstrated herein induces differentiation and growth arrest of human AML cell lines and patient cells. Accordingly, a composition comprising S100A9 is disclosed herein for treating leukemia in human patient.

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[0062] The present disclosure will be more readily understood by referring to the following examples which are given to illustrate embodiments rather than to limit its scope.

EXAMPLE I

Bone marrow progenitor cell transduction and transplantation procedure

[0063] Bone marrow cells isolated from 5-fluorouracyl-treated C57BL/6 mice (8-12 week old female) were cultivated 2 days in DMEM with 15% fetal calf serum with 10 ng/ml IL-3, 10ng/ml IL-6 and 100ng/ml stem cell factor to promote cell cycle entry. Bone marrow cells were then co-cultured with irradiated GP+E86 cells transfected with MSCV-*hoxa9-ires-meis1 hpgk-EGFP* (virus producing cells) in presence of 5 µg/ml of polybrene for 2 days.

[0064] Primary leukemia recipient C57BL/6 mice were irradiated (7 Gy), then injected i.v. 24 h later with 4×10^5 infected bone marrow cells (expressing *Hoxa9*, *Meis1* and *Egfp*). Mice were followed every day for signs of leukemia. Peripheral blood was harvested every week to quantify the number of leukemia cells (expressing GFP) by flow cytometry. Muribund mice were sacrificed and the peripheral blood and bone marrow were harvested.

[0065] Secondary leukemia was induced by injecting 2×10^5 bone marrow cells from primary leukemia mice into sublethally irradiated (4Gy) recipient.

EXAMPLE II

S100A9 treatment

[0066] Leukemic cells isolated from bone marrow of primary leukemia mice were injected into sublethally irradiated (4Gy) recipient C57BL/6 mice. The presence of AML cells (EGFP+) in peripheral blood was evaluated by flow cytometry 14 days later. Mice were injected i.p three times per week with 20 µg/ml of recombinant S100A9 murine starting on day 3. Mice exhibiting signs of ill health were sacrificed and peripheral blood and bone marrow were harvested for histology and flow cytometry analyses.

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EXAMPLE III

Stimulation MLL-AF4

[0067] Human cord blood cells transfected with the fusion gene MLL-AF4 were cultivated in IMDM supplemented with 15% fetal calf serum, IL-6 and stem cell factor. The cells were plated 96 well microtiter plates (200 000 cells/well), then stimulated with 20 µg/ml of recombinant human S100A8 or S100A9, or PBS. After 72h, cells were collected and immunophenotyping was performed by flow cytometry following staining with anti- CD14.

EXAMPLE III

S100A9 induction of differentiation in patient cells

[0068] Leukemia cells from peripheral blood of 5 M4 and M5 AML patients (03H031, 06H061, 07H006, 07H072, 07H138) were thawed, then cultured with 20 µg of S100A9 for up to 5 days. Expression of the differentiation markers CD11b and CD14 was examined by FACS. Cell cycle was examined by flow cytometry following propidium iodide staining.

EXAMPLE IV

S100A9 induction of differentiation of AML cell line Mono-Mac-1

[0069] Mono-Mac-1 (200,000 cells) were cultured with 20 µg of S100A9 for 3 days. The cells were then counted and expression of CD14 was analyzed by flow cytometry.

[0070] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention, as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A composition comprising S100A9 peptide or a peptidomimetic thereof, and a carrier for treating leukemia.
2. The composition of claim 1, wherein the S100A9 peptide is human S100A9 protein.
3. The composition of claim 1 or 2, wherein the peptide comprises the amino acid sequence set forth in SEQ ID NO: 1.
4. The composition of claim 3, wherein the peptide has at least 60%, at least 70% or at least 80%, at least 90% identical, or at least about 95% identity with SEQ ID NO: 1.
5. The composition of claim 3 or 4, wherein the peptide consists of the amino acid sequence set forth in SEQ ID NO: 1.
6. The composition of any one of claims 1-5, wherein the peptide or the peptidomimetic thereof comprises a chemically or enzymatically attached modification.
7. The composition of claim 6, wherein the peptide or peptidomimetic thereof is pegylated, hesylated, pasylated, myristoylated, or glycosylated.
8. The composition of any one of claims 1-7, formulated for an oral administration, an inhalation, a nasal administration, or an administration by injection.
9. The composition of claim 8, wherein the injection is subcutaneously.
10. The composition of any one of claims 1-9, further comprising a chemotherapeutic agent.

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11. The composition of claim 10, wherein the chemotherapeutic agent is an alkylating/alkaloid agent, an antimetabolite, a hormone, a hormone analog, or an antineoplastic drug.

12. The composition of claim 10 or 11, wherein the chemotherapeutic agent is cytarabine (cytosine arabinoside, Ara-C, Cytosar-U), carboplatin, carmustine, chlorambucil, dacarbazine, ifosfamide, lomustine, mechlorethamine, procarbazine, pentostatin, (2'deoxycoformycin), etoposide, teniposide, topotecan, vinblastine, vincristine, paclitaxel, dexamethasone, methylprednisolone, prednisone, all-trans retinoic acid, arsenic trioxide, interferon-alpha, rituximab (Rituxan®), gemtuzumab ozogamicin, imatinib mesylate, melphalan, busulfan (Myleran®), thiotepa, bleomycin, platinum (cisplatin), cyclophosphamide (Cytosan®), daunorubicin, doxorubicin, idarubicin, mitoxantrone, 5-azacytidine, cladribine, fludarabine, hydroxyurea, 6-mercaptopurine, methotrexate, 6-thioguanine, or any combination thereof.

13. Use of a S100A9 peptide or a peptidomimetic thereof for treating leukemia in a subject.

14. Use of a S100A9 peptide or a peptidomimetic thereof in the manufacture of a medicament for treating leukemia in a subject.

15. The use of claim 13 or 14, wherein the S100A9 peptide is human S100A9 protein.

16. The use of any one of claims 13-15, wherein the peptide comprises the amino acid sequence set forth in SEQ ID NO: 1.

17. The use of claim 16, wherein the peptide has at least 60%, at least 70% or at least 80%, at least 90% identical, or at least about 95% identity with SEQ ID NO: 1.

18. The use of claim 16 or 17, wherein the peptide consists of the amino acid sequence set forth in SEQ ID NO: 1.

- 17 -

19. The use of any one of claims 13-18, wherein the peptide or the peptidomimetic thereof comprises a chemically or enzymatically attached modification.

20. The use of claim 19, wherein the peptide or peptidomimetic thereof is pegylated, hesylated, pasylated, myristoylated, or glycosylated.

21. The use of any one of claims 13-20, wherein said peptide or peptidomimetic thereof is formulated for an oral administration, an inhalation, a nasal administration, or an administration by injection.

22. The use of claim 21, wherein the injection is subcutaneously.

23. The use of any one of claims 13-22, further comprising using a chemotherapeutic agent.

24. The use of claim 23, wherein the chemotherapeutic agent is an alkylating/alkaloid agent, an antimetabolite, a hormone, a hormone analog, or an antineoplastic drug.

25. The use of claim 23 or 24, wherein the chemotherapeutic agent is cytarabine (cytosine arabinoside, Ara-C, Cytosar-U), carboplatin, carmustine, chlorambucil, dacarbazine, ifosfamide, lomustine, mechlorethamine, procarbazine, pentostatin, (2'deoxycoformycin), etoposide, teniposide, topotecan, vinblastine, vincristine, paclitaxel, dexamethasone, methylprednisolone, prednisone, all-trans retinoic acid, arsenic trioxide, interferon-alpha, rituximab (Rituxan®), gemtuzumab ozogamicin, imatinib mesylate, melphalan, busulfan (Myleran®), thiotepa, bleomycin, platinum (cisplatin), cyclophosphamide (Cytoxan®), daunorubicin, doxorubicin, idarubicin, mitoxantrone, 5-azacytidine, cladribine, fludarabine, hydroxyurea, 6-mercaptopurine, methotrexate, 6-thioguanine, or any combination thereof.

26. The use of any one of claims 13-25, wherein the subject is a mouse or a human.

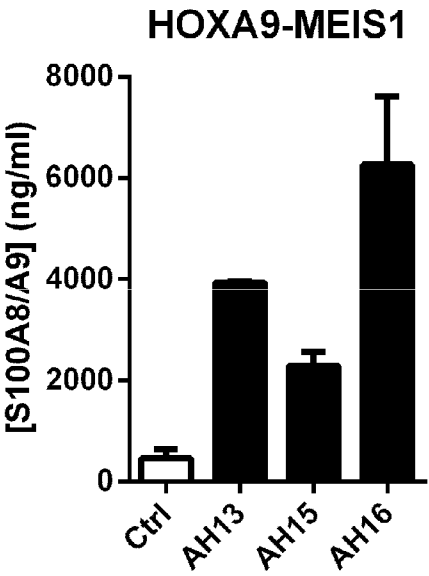


Fig. 1

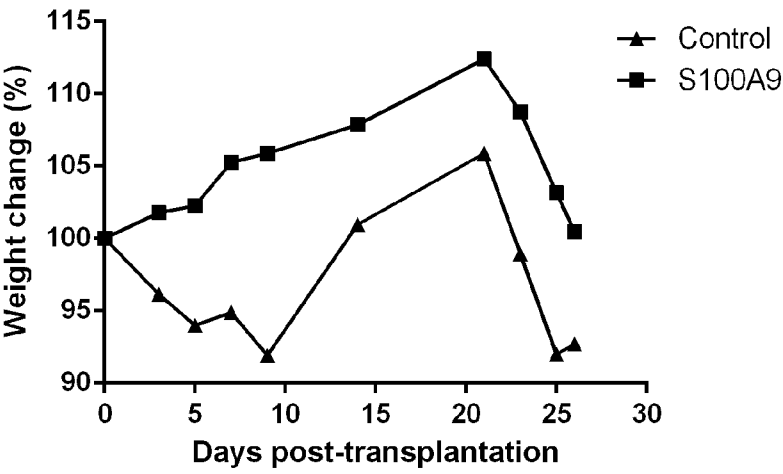


Fig. 2

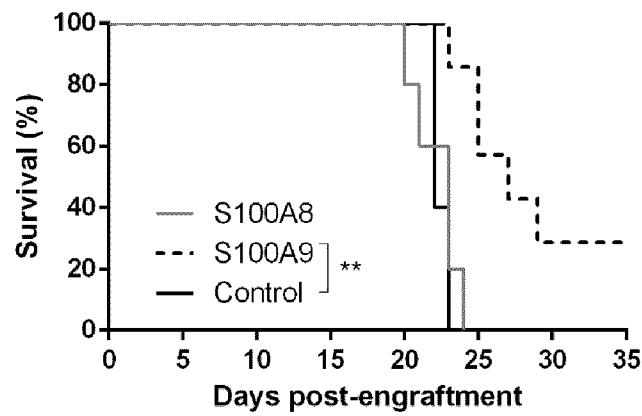


Fig. 3

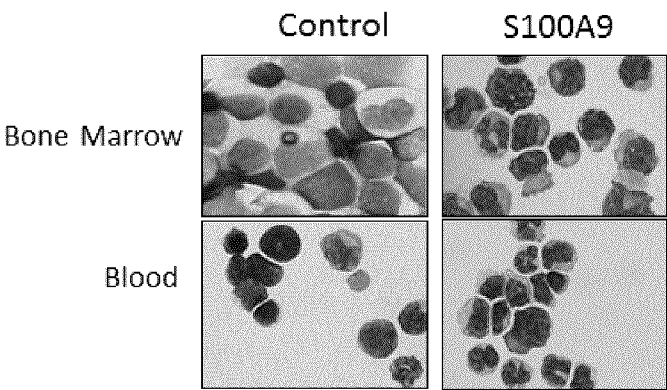


Fig. 4A

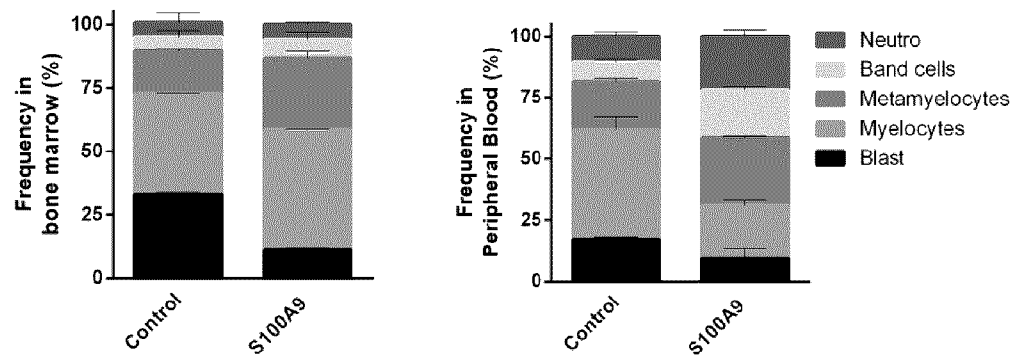


Fig. 4B

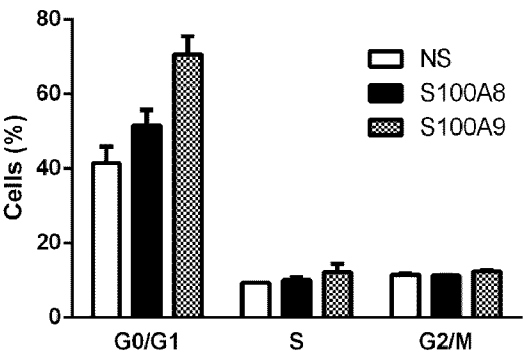


Fig. 5

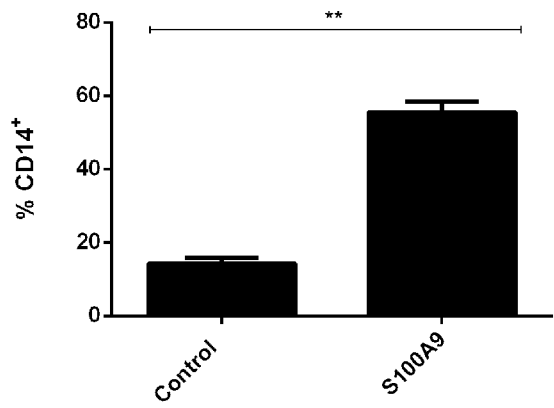


Fig. 6

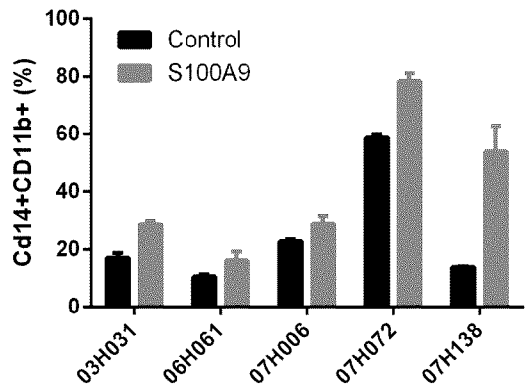


Fig. 7

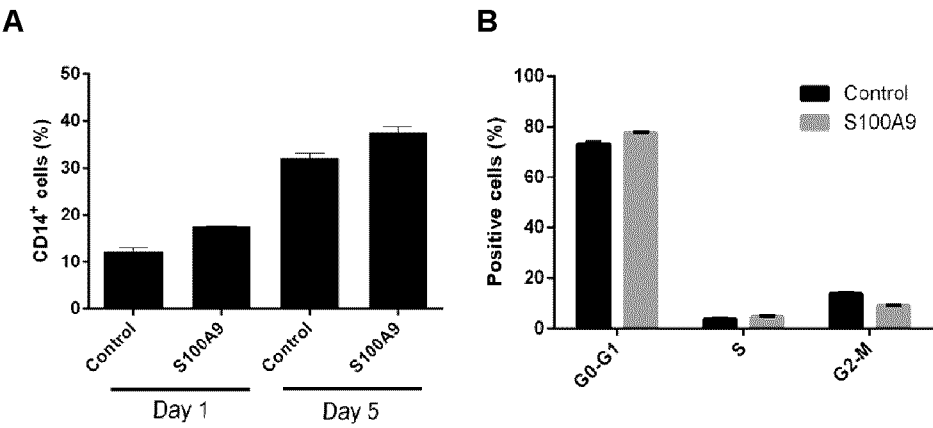


Fig. 8

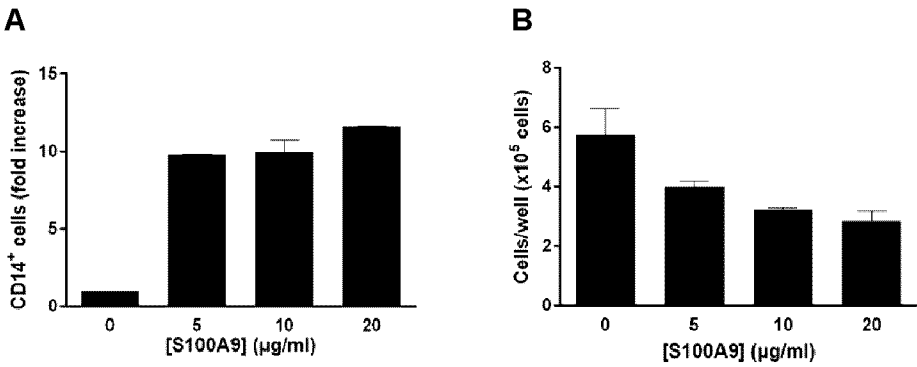


Fig. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/050177

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 38/17** (2006.01), **A61K 47/48** (2006.01), **A61P 35/02** (2006.01), **C07K 14/47** (2006.01)

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 38/17 (2006.01), **A61K 47/48** (2006.01), **A61P 35/02** (2006.01), **C07K 14/47** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: GenomeQuest (GQ-Gold Plus; GQ-Platinum; GenPept; Geneseq; ENSEMBLE, Translated ENSEMBL, Swiss-Prot; Ref-Seq), Canadian Patent Database; STN (Medline CaPlus, BIOSIS)

Keywords: SEQ ID NO: 1 (GenomeQuest, 65% identity cutoff), S100A9, MRP14, MRP 14, Calgranulin B, CalgranulinB, Acute Myeloid Leukemia, AML, chemo*, chemotherapy*, composition, Tessier, Barabe, Laouedj

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US20090305985A1 (PALEFSKY, J. and SROUSSI, H) 10 December 2009 (10-12-2009) see Abstract; paragraph [003]; Figure7B and SEQ ID NO: 4	1-9
X	WO2004084928A1 (TESSIER, P. et al.) 7 October 2004 (07-10-2004) see Abstract; page 8, lines 16-23; page 9, line 8-14; claim 13	1-12
Y	WO2006005186A1 TESSIER, P. et al. 19 January 2006 (19-01-2006) See page 5, lines 4-8; page 6, lines 11-14; page 7, lines 22-23; page 9, lines 4-9; page 13, lines 20-27; page 14, lines 13-25; page 17, lines 12-15 and Figure 5	1, 4, 6-14, 17 and 19-26

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date 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) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 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 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
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Date of the actual completion of the international search

Date of mailing of the international search report
25 May 2016 (25-05-2016)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Ralph Salvino (819) 639-7650

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/050177

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	BARABE, F. et al. Myeloid-Related Protein S100A9 induces cellular differentiation in Acute Myeloid Leukemia through TLR2 and TLR4 receptors; 57 th Annual Meeting of the American Society of Hematology, Orlando, Florida, December 2015, Poster 3858. [retrieved on 2016-04-12]. Retrieved from the Internet: <URL>:https://ash.confex.com/ash/2015/webprogram/Paper86520.html	1-26

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 1-26 (partially)
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:

See Extra Sheet

3. ☐ Claim 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:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying 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 report covers only those claims for which fees were paid, specifically claim 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 claim 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.

Box No. II (continuation)

The application provides clarity and support within the meaning of Article 6 and/or disclosure within the meaning of Article 5 only for certain products defined by the expression "S100A9 peptide or a peptidomimetic" and for only one type of leukemia. The scope of protection sought is large and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Specifically, a S100A9 peptide according to paragraph [044] is broadly meant to mean peptides, proteins, fragments of proteins and the like. It is unclear what is meant by "and the like". Furthermore, S100A9 peptidomimetics are not defined by sufficient technical terms nor are they disclosed with any particularity. In terms of the type of leukemia, leukemias can originate from myeloid elements, that are either immature or undifferentiated (acute myeloid leukemia) or mature and differentiated (chronic myeloid leukemia) or from lymphatic elements that are either immature or undifferentiated (acute lymphoblastic leukemia) or mature and differentiated (chronic lymphocytic leukemia). The present application discloses S100A9 protein differentiates immature myeloid cells leading to growth arrest. Consequently, the search has been established for the parts of which appear to be clear, supported and/or enabled, namely then S100A9 peptide can be SA100A9 protein or a fragment thereof (i.e. includes a peptide) and the disease is acute myeloid leukemia. In this respect, claims 1-26 are partially searched with respect to the disease being acute myeloid leukemia. As well, claims 2-5, 15-18 are fully searched and claims 1, 6-14 and 19-26 are partially searched wherein the product is an S100A9 protein or fragment thereof.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/050177

Patent Document Cited in Search Report	Publication Date	Family Member(s)	Publication Date
US2009305985A1	10 December 2009 (10-12-2009)	US2009305985A1 US2007123455A1 WO2004089292A2 WO2004089292A3	10 December 2009 (10-12-2009) 31 May 2007 (31-05-2007) 21 October 2004 (21-10-2004) 29 June 2006 (29-06-2006)
WO2004084928A1	07 October 2004 (07-10-2004)	WO2004084928A1 AT411038T CA2520515A1 CA2520515C DE602004017148D1 EP1608397A1 EP1608397B1 US2006281674A1 US7632802B2	07 October 2004 (07-10-2004) 15 October 2008 (15-10-2008) 07 October 2004 (07-10-2004) 28 August 2012 (28-08-2012) 27 November 2008 (27-11-2008) 28 December 2005 (28-12-2005) 15 October 2008 (15-10-2008) 14 December 2006 (14-12-2006) 15 December 2009 (15-12-2009)
WO2006005186A1	19 January 2006 (19-01-2006)	WO2006005186A1 CA2573596A1 EP1784216A1 EP1784216A4 US2007231317A1 US2015110774A1	19 January 2006 (19-01-2006) 19 January 2006 (19-01-2006) 16 May 2007 (16-05-2007) 03 June 2009 (03-06-2009) 04 October 2007 (04-10-2007) 23 April 2015 (23-04-2015)