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**SOURABH GHOSH ET AL: "Three-dimensional culture of melanoma cells profoundly affects gene expression profile: A high density oligonucleotide array study", JOURNAL OF CELLULAR PHYSIOLOGY, vol. 204, no. 2, 1 August 2005 (2005-08-01), pages 522-531, XP0055167052, ISSN: 0021-9541, DOI: 10.1002/jcp.20320**  
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# DESCRIPTION

## Field of Invention

**[0001]** The present invention relates to agents for use in the treatment and diagnosis of solid tumours, such as prostate cancer, breast cancer, lung cancer, colorectal cancer, melanomas, bladder cancer, brain/CNS cancer, cervical cancer, oesophageal cancer, gastric cancer, head/neck cancer, kidney cancer, liver cancer, lymphomas, ovarian cancer, pancreatic cancer, and sarcomas.

## Background

**[0002]** Drug resistance is a major factor that limits the effectiveness of chemotherapy in solid tumours. Such tumours may be intrinsically resistant prior to chemotherapy, or resistance may be acquired during treatment by tumours that are initially sensitive to chemotherapy.

**[0003]** Furthermore, in the process of acquiring resistance, the tumour may become cross-resistant to a range of chemotherapies and result in resistance, which ultimately leads to treatment failure in over 90% of patients with metastatic disease.

**[0004]** Balagurunathan et al. (2008) Mol. Cancer Ther. 7(9):3071-80 describes gene expression profiling-based identification of cell-surface targets for developing multimeric ligands in pancreatic cancer. Using this approach, the authors report the identification of gene combinations that are expressed at an efficient binding level in tumours but not in normal tissues, which may be used to develop multimeric ligands for the imaging and treatment of pancreatic cancer.

**[0005]** Accordingly, the present invention seeks to provide new agents and methods for use in the treatment and diagnosis of solid tumours.

## Summary of Invention

**[0006]** A first aspect of the invention provides an agent comprising or consisting of an antibody, or antigen-binding fragment thereof, with specificity for interleukin-1 receptor accessory protein (IL1RAP) for use in treating or preventing a cancer in a patient, wherein the cancer is a solid tumour having the cells express IL1RAP. Thus, the invention provides agents for use in treating or preventing a solid tumour in a patient.

**[0007]** By "interleukin-1 receptor accessory protein", "IL1RAP" and "IL1-RAP" we specifically include the human IL1RAP protein, for example as described in GenBank Accession No.

AAB84059, NCBI Reference Sequence: NP\_002173.1 and UniProtKB/Swiss-Prot Accession No. Q9NPH3-1 (see also Huang et al., 1997, Proc. Natl. Acad. Sci. USA. 94 (24), 12829-12832). IL1RAP is also known in the scientific literature as IL1R3, C3orf13, FLJ37788, IL-1RAcP and EG3556.

**[0008]** Advantageously, the antibody, or antigen-binding fragment thereof, is capable of binding selectively (*i.e.* preferentially) to IL1RAP under physiological conditions. The antibody, or antigen-binding fragment thereof, preferably has specificity for human IL1 RAP, which may be localised on the surface of a cell (*e.g.* the solid tumour cell).

**[0009]** By "cells associated with a solid tumour" we include solid tumour cells *per se*. In addition, such cells include pathological stem cells (*i.e.* cancer stem cells, or CSCs) and progenitor cells which are responsible, directly or indirectly, for the development of a solid tumour in an individual. Examples of CSCs are disclosed in Visvader & Lindeman, 2008, Nat Rev Cancer 8:755-768.

**[0010]** In one embodiment of the first aspect of the invention, the solid tumour is selected from the group consisting of prostate cancer, breast cancer, lung cancer, colorectal cancer, melanomas, bladder cancer, brain/CNS cancer, cervical cancer, oesophageal cancer, gastric cancer, head/neck cancer, kidney cancer, liver cancer, lymphomas, ovarian cancer, pancreatic cancer, and sarcomas. For example, the solid tumour may be selected from the group consisting of cancers of the prostate gland, breast, skin, colon, lung, urinary organs and uterus. In another embodiment, the solid tumour may be selected from the groups consisting of prostate cancer, melanomas, cervical cancer, oesophageal cancer, and head and/or neck cancer.

**[0011]** In a further embodiment of the first aspect of the invention, the solid tumour is a melanoma.

**[0012]** In relation to the therapeutic and prophylactic aspects of the invention, it will be appreciated by persons skilled in the art that binding of the agent to IL1RAP present on the surface of the cells associated with the solid tumour may lead to a modulation (*i.e.* an increase or decrease) of a biological activity of IL1RAP. However, such a modulatory effect is not essential; for example, the agents of the invention may elicit a therapeutic and prophylactic effect simply by virtue of binding to IL1RAP on the surface of the cells associated with the solid tumour, which in turn may trigger the immune system to induce cell death (*e.g.* by ADCC and/or by the presence within the agent of a cytotoxic/radioactive moiety).

**[0013]** By "biological activity of IL1RAP" we include any interaction or signalling event which involves IL1RAP on the cells associated with the solid tumour. For example, in one embodiment the agent is capable of blocking binding of one or more co-receptors to IL1RAP (such as IL1R1, ST2, C-KIT and/or IL1RL2).

**[0014]** Such inhibition of the biological activity of IL1RAP by an agent of the invention may be

in whole or in part. For example, the agent may inhibit the biological activity of IL1RAP by at least 10%, preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%, and most preferably by 100% compared to the biological activity of IL1RAP in cells associated with the solid tumour which have not been exposed to the agent. In a preferred embodiment, the agent is capable of inhibiting the biological activity of IL1RAP by 50% or more compared to the biological activity of IL1RAP in cells associated with the solid tumour which have not been exposed to the agent.

**[0015]** Likewise, it will be appreciated that inhibition of growth and/or proliferation of the cells associated with the solid tumour may be in whole or in part. For example, the agent may inhibit the growth and/or proliferation of the cells associated with the solid tumour by at least 10%, preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%, and most preferably by 100% compared to the growth and/or proliferation of cells associated with the solid tumour which have not been exposed to the agent.

**[0016]** In a further preferred embodiment, the agent is capable of killing the cells associated with the solid tumour. In particular, the agent may be capable of cell death by apoptosis or autophagy. For example, the agent may induce apoptosis by antibody-dependent cell-mediated cytotoxicity (ADCC).

**[0017]** As indicated above, the agents of the invention may comprise or consist of any suitable chemical entity constituting a binding moiety with specificity for IL1RAP.

**[0018]** Methods for detecting interactions between a test chemical entity and IL1RAP are well known in the art. For example ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods may be used. In addition, Fluorescence Energy Resonance Transfer (FRET) methods may be used, in which binding of two fluorescent labelled entities may be measured by measuring the interaction of the fluorescent labels when in close proximity to each other.

**[0019]** Alternative methods of detecting binding of IL1RAP to macromolecules, for example DNA, RNA, proteins and phospholipids, include a surface plasmon resonance assay, for example as described in Plant et al., 1995, *Analyt Biochem* 226(2), 342-348. Such methods may make use of a polypeptide that is labelled, for example with a radioactive or fluorescent label.

**[0020]** A further method of identifying a chemical entity that is capable of binding to IL1RAP is one where the protein is exposed to the compound and any binding of the compound to the said protein is detected and/or measured. The binding constant for the binding of the compound to the polypeptide may be determined. Suitable methods for detecting and/or measuring (quantifying) the binding of a compound to a polypeptide are well known to those skilled in the art and may be performed, for example, using a method capable of high throughput operation, for example a chip-based method. New technology, called VLSIPS™, has enabled the production of extremely small chips that contain hundreds of thousands or

more of different molecular probes. These biological chips have probes arranged in arrays, each probe assigned a specific location. Biological chips have been produced in which each location has a scale of, for example, ten microns. The chips can be used to determine whether target molecules interact with any of the probes on the chip. After exposing the array to target molecules under selected test conditions, scanning devices can examine each location in the array and determine whether a target molecule has interacted with the probe at that location.

**[0021]** Another method of identifying compounds with binding affinity for IL1 RAP is the yeast two-hybrid system, where the polypeptides of the invention can be used to "capture" proteins that bind IL1RAP. The yeast two-hybrid system is described in Fields & Song, Nature 340:245-246 (1989).

**[0022]** By "antibody" we include substantially intact antibody molecules, as well as chimaeric antibodies, humanised antibodies, human antibodies (wherein at least one amino acid is mutated relative to the naturally occurring human antibodies), single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen binding fragments and derivatives of the same.

**[0023]** By "antigen-binding fragment" we mean a functional fragment of an antibody that is capable of binding to IL1RAP.

**[0024]** Preferably, the antigen-binding fragment is selected from the group consisting of Fv fragments (e.g. single chain Fv and disulphide-bonded Fv), Fab-like fragments (e.g. Fab fragments, Fab' fragments and F(ab)<sub>2</sub> fragments), single variable domains (e.g. V<sub>H</sub> and V<sub>L</sub> domains) and domain antibodies (dAbs, including single and dual formats [*i.e.* dAb-linker-dAb]).

**[0025]** The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

**[0026]** Also included within the scope of the invention are modified versions of antibodies and antigen-binding fragments thereof, e.g. modified by the covalent attachment of polyethylene glycol or other suitable polymer (see below).

**[0027]** Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries (Orlandi. et al, 1989. Proc. Natl. Acad. Sci. U.S.A. 86:3833-3837; Winter et al., 1991, Nature 349:293-299) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the

Epstein-Barr virus (EBV)-hybridoma technique (Kohler et al., 1975. Nature 256:495-497; Kozbor et al., 1985. J. Immunol. Methods 81:31-42; Cote et al., 1983. Proc. Natl. Acad. Sci. USA 80:2026-2030; Cole et al., 1984. Mol. Cell. Biol. 62:109-120).

**[0028]** Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", J G R Hurrell (CRC Press, 1982).

**[0029]** Likewise, antibody fragments can be obtained using methods well known in the art (see, for example, Harlow & Lane, 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, New York). For example, antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

**[0030]** It will be appreciated by persons skilled in the art that for human therapy or diagnostics, human or humanised antibodies are preferably used. Humanised forms of non-human (e.g. murine) antibodies are genetically engineered chimaeric antibodies or antibody fragments having preferably minimal-portions derived from non-human antibodies. Humanised antibodies include antibodies in which complementary determining regions of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non human species (donor antibody) such as mouse, rat or rabbit having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported complementarity determining region or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody (see, for example, Jones et al., 1986. Nature 321:522-525; Riechmann et al., 1988, Nature 332:323-329; Presta, 1992, Curr. Op. Struct. Biol. 2:593-596).

**[0031]** Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones et al., 1986, Nature 321:522-525; Reichmann et al., 1988. Nature 332:323-327; Verhoeyen et al., 1988, Science 239:1534-1536; US 4,816,567) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised

antibodies are chimaeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

**[0032]** Human antibodies can also be identified using various techniques known in the art, including phage display libraries (see, for example, Hoogenboom & Winter, 1991, J. Mol. Biol. 227:381; Marks et al., 1991, J. Mol. Biol. 222:581; Cole et al., 1985, In: Monoclonal antibodies and Cancer Therapy, Alan R. Liss, pp. 77; Boerner et al., 1991. J. Immunol. 147:86-95).

**[0033]** Once suitable antibodies are obtained, they may be tested for activity, for example by ELISA.

**[0034]** In addition to the binding moiety, the agents of the invention may further comprise a moiety for increasing the *in vivo* half-life of the agent, such as but not limited to polyethylene glycol (PEG), human serum albumin, glycosylation groups, fatty acids and dextran. Such further moieties may be conjugated or otherwise combined with the binding moiety using methods well known in the art.

**[0035]** Likewise, it will be appreciated that the agents of the invention may further comprise a cytotoxic moiety.

**[0036]** For example, the cytotoxic moiety may comprise or consist of a radioisotope, such as astatine-211, bismuth-212, bismuth-213, iodine-131, yttrium-90, lutetium-177, samarium-153 and palladium-109.

**[0037]** Alternatively, the cytotoxic moiety may comprise or consist of a toxin (such as saporin or calicheamicin).

**[0038]** In a further alternative, the cytotoxic moiety may comprise or consist of a chemotherapeutic agent (such as an antimetabolite).

**[0039]** Likewise, it will be appreciated that the agents of the invention may further comprise a detectable moiety.

**[0040]** For example, the detectable moiety may comprise or consist of a radioisotope., such as technetium-99m, indium-111, gallium-67, gallium-68, arsenic-72, zirconium-89, iodine-12 or thallium-201.

**[0041]** Alternatively, the detectable moiety comprises or consists of a paramagnetic isotope, such as gadolinium-157, manganese-55, dysprosium-162, chromium-52 or iron-56.

**[0042]** Cytotoxic and detectable moieties may be conjugated or otherwise combined with the



binding moiety using methods well known in the art (for example, the existing immunoconjugate therapy, gemtuzumab ozogamicin [tradename: *Mylotarg®*], comprises a monoclonal antibody linked to the cytotoxin calicheamicin).

**[0043]** Also described herein is a pharmaceutical composition comprising an effective amount of an agent as defined in relation to the first aspect of the invention together with a pharmaceutical acceptable buffer, diluent, carrier, adjuvant or excipient.

**[0044]** Additional compounds may also be included in the compositions, including, chelating agents such as EDTA, citrate, EGTA or glutathione.

**[0045]** The pharmaceutical compositions may be prepared in a manner known in the art that is sufficiently storage stable and suitable for administration to humans and animals. For example, the pharmaceutical compositions may be lyophilised, e.g. through freeze drying, spray drying, spray cooling, or through use of particle formation from supercritical particle formation.

**[0046]** By "pharmaceutically acceptable" we mean a non-toxic material that does not decrease the effectiveness of the IL1RAP-binding activity of the agent of the invention. Such pharmaceutically acceptable buffers, carriers or excipients are well-known in the art (see Remington's Pharmaceutical Sciences, 18th edition, A.R. Gennaro, Ed., Mack Publishing Company (1990) and handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed., Pharmaceutical Press (2000)).

**[0047]** The term "buffer" is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilising pH. Examples of buffers are Trizma, Bicine, Tricine, MOPS, MOPSO, MOBS, Tris, Hepes, HEPBS, MES, phosphate, carbonate, acetate, citrate, glycolate, lactate, borate, ACES, ADA, tartrate, AMP, AMPD, AMPSO, BES, CABS, cacodylate, CHES, DIPSO, EPPS, ethanolamine, glycine, HEPPSO, imidazole, imidazolelactic acid, PIPES, SSC, SSPE, POPSO, TAPS, TABS, TAPSO and TES.

**[0048]** The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the agent in the pharmaceutical preparation. The diluent may be one or more of saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, corn oil, peanut oil, cottonseed oil or sesame oil).

**[0049]** The term "adjuvant" is intended to mean any compound added to the formulation to increase the biological effect of the agent of the invention. The adjuvant may be one or more of zinc, copper or silver salts with different anions, for example, but not limited to fluoride, chloride, bromide, iodide, thiocyanate, sulfite, hydroxide, phosphate, carbonate, lactate, glycolate, citrate, borate, tartrate, and acetates of different acyl composition. The adjuvant may also be cationic polymers such as cationic cellulose ethers, cationic cellulose esters, deacetylated hyaluronic acid, chitosan, cationic dendrimers, cationic synthetic polymers such as poly(vinyl imidazole), and cationic polypeptides such as polyhistidine, polylysine, polyarginine, and peptides containing these amino acids.

**[0050]** The excipient may be one or more of carbohydrates, polymers, lipids and minerals. Examples of carbohydrates include lactose, glucose, sucrose, mannitol, and cyclodextrines, which are added to the composition, e.g., for facilitating lyophilisation. Examples of polymers are starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate, polyethyleneglycol/polyethylene oxide, polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone, all of different molecular weight, which are added to the composition, e.g., for viscosity control, for achieving bioadhesion, or for protecting the lipid from chemical and proteolytic degradation. Examples of lipids are fatty acids, phospholipids, mono-, di-, and triglycerides, ceramides, sphingolipids and glycolipids, all of different acyl chain length and saturation, egg lecithin, soy lecithin, hydrogenated egg and soy lecithin, which are added to the composition for reasons similar to those for polymers. Examples of minerals are talc, magnesium oxide, zinc oxide and titanium oxide, which are added to the composition to obtain benefits such as reduction of liquid accumulation or advantageous pigment properties.

**[0051]** The agents of the invention may be formulated into any type of pharmaceutical composition known in the art to be suitable for the delivery thereof.

**[0052]** In one embodiment, the pharmaceutical compositions may be in the form of a liposome, in which the agent is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids, which exist in aggregated forms as micelles, insoluble monolayers and liquid crystals. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Suitable lipids also include the lipids above modified by poly(ethylene glycol) in the polar headgroup for prolonging bloodstream circulation time. Preparation of such liposomal formulations is can be found in for example US 4,235,871.

**[0053]** The pharmaceutical compositions may also be in the form of biodegradable microspheres. Aliphatic polyesters, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), copolymers of PLA and PGA (PLGA) or poly(carpolactone) (PCL), and polyanhydrides have been widely used as biodegradable polymers in the production of microspheres. Preparations of such microspheres can be found in US 5,851,451 and in EP 0 213 303.

**[0054]** In a further embodiment, the pharmaceutical compositions are provided in the form of polymer gels, where polymers such as starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polyvinyl imidazole, polysulphonate, polyethyleneglycol/polyethylene oxide, polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone are used for thickening of the solution containing the agent. The polymers may also comprise gelatin or collagen.

**[0055]** Alternatively, the agents may simply be dissolved in saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, corn oil, peanut oil, cottonseed oil or sesame oil), tragacanth gum, and/or various buffers.

**[0056]** It will be appreciated that the pharmaceutical compositions may include ions and a defined pH for potentiation of action of the active agent. Additionally, the compositions may be subjected to conventional pharmaceutical operations such as sterilisation and/or may contain conventional adjuvants such as preservatives, stabilisers, wetting agents, emulsifiers, buffers, fillers, *etc.*

**[0057]** The pharmaceutical compositions may be administered via any suitable route known to those skilled in the art. Thus, possible routes of administration include parenteral (intravenous, subcutaneous, and intramuscular), topical, ocular, nasal, pulmonar, buccal, oral, parenteral, vaginal and rectal. Also administration from implants is possible.

**[0058]** In one preferred embodiment, the pharmaceutical compositions are administered parenterally, for example, intravenously, intracerebroventricularly, intraarticularly, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are conveniently used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

**[0059]** Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

**[0060]** Thus, the pharmaceutical compositions are particularly suitable for parenteral, e.g. intravenous, administration.

**[0061]** Alternatively, the pharmaceutical compositions may be administered intranasally or by inhalation (for example, in the form of an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoro-methane, dichlorotetrafluoro-ethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas). In the case of a

pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active polypeptide, *e.g.* using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.* sorbitan trioleate.

**[0062]** Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

**[0063]** The pharmaceutical compositions will be administered to a patient in a pharmaceutically effective dose. A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent. In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art. The administration of the pharmaceutically effective dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administrations of subdivided doses at specific intervals. Alternatively, the dose may be provided as a continuous infusion over a prolonged period.

**[0064]** The polypeptides can be formulated at various concentrations, depending on the efficacy/toxicity of the compound being used. Preferably, the formulation comprises the active agent at a concentration of between 0.1  $\mu\text{M}$  and 1 mM, more preferably between 1  $\mu\text{M}$  and 500  $\mu\text{M}$ , between 500  $\mu\text{M}$  and 1 mM, between 300  $\mu\text{M}$  and 700  $\mu\text{M}$ , between 1  $\mu\text{M}$  and 100  $\mu\text{M}$ , between 100  $\mu\text{M}$  and 200  $\mu\text{M}$ , between 200  $\mu\text{M}$  and 300  $\mu\text{M}$ , between 300  $\mu\text{M}$  and 400  $\mu\text{M}$ , between 400  $\mu\text{M}$  and 500  $\mu\text{M}$  and most preferably about 500  $\mu\text{M}$ .

**[0065]** It will be appreciated by persons skilled in the art that the pharmaceutical compositions may be administered alone or in combination with other therapeutic agents used in the treatment of solid tumours, such as antimetabolites, alkylating agents, anthracyclines and other cytotoxic antibiotics, vinca alkaloids, etoposide, platinum compounds, taxanes, topoisomerase I inhibitors, antiproliferative immunosuppressants, corticosteroids, sex hormones and hormone antagonists, and other therapeutic antibodies (such as trastuzumab).

**[0066]** Also described herein is a kit comprising an agent as defined in relation to the first aspect of the invention or a pharmaceutical composition thereof.

**[0067]** Also described herein is the use of an agent as defined in relation to the first aspect of the invention in the preparation of a medicament for inducing cell death and/or inhibiting the growth and/or proliferation of cells associated with a solid tumour, wherein the cells express IL1RAP.

**[0068]** Also described herein is the use of an agent as defined in relation to the first aspect of the invention for detecting cells associated with a solid tumour, wherein the cells express IL1RAP.

**[0069]** In the above use aspects, the solid tumour is selected from the group consisting of cancers of the prostate cancer, breast cancer, lung cancer, colorectal cancer, melanomas, bladder cancer, brain/CNS cancer, cervical cancer, oesophageal cancer, gastric cancer, head/neck cancer, kidney cancer, liver cancer, lymphomas, ovarian cancer, pancreatic cancer, and sarcomas. For example, the solid tumour may be selected from the group consisting of cancers of the prostate gland, breast, skin, colon, lung, urinary organs and uterus. In another embodiment, the solid tumour may be selected from the groups consisting of prostate cancer, melanomas, cervical cancer, oesophageal cancer, and head and/or neck cancer.

**[0070]** In a further embodiment, the solid tumour is a melanoma.

**[0071]** Also described herein is a method for inducing cell death and/or inhibiting the growth and/or proliferation of cells associated with a solid tumour in an individual, comprising the step of administering to the individual an effective amount of an agent as defined in relation to the first aspect of the invention, or a pharmaceutical composition thereof, wherein the cells express IL1RAP.

**[0072]** Thus the method is for the treatment of solid tumours. By 'treatment' we include both therapeutic and prophylactic treatment of the patient. The term 'prophylactic' is used to encompass the use of a polypeptide or formulation described herein which either prevents or reduces the likelihood of a solid tumour in a patient or subject.

**[0073]** In one embodiment of the above method, the solid tumour is selected from the group consisting of prostate cancer, breast cancer, lung cancer, colorectal cancer, melanomas, bladder cancer, brain/CNS cancer, cervical cancer, oesophageal cancer, gastric cancer, head/neck cancer, kidney cancer, liver cancer, lymphomas, ovarian cancer, pancreatic cancer, and sarcomas. For example, the solid tumour may be selected from the group consisting of cancers of the prostate gland, breast, skin, colon, lung, urinary organs and uterus. In another embodiment, the solid tumour may be selected from the groups consisting of prostate cancer, melanomas, cervical cancer, oesophageal cancer, and head and/or neck cancer.

**[0074]** In a further embodiment of, the solid tumour is a melanoma.

**[0075]** Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

**Figure 1. P210 *BCR/ABL1* expression induces IL1RAP expression in cord blood CD34<sup>+</sup> cells**

Flow cytometric analysis confirms that IL1RAP expression is induced upon retroviral P210 *BCR/ABL1* expression in cord blood CD34<sup>+</sup> cells, three days post transduction. CD34<sup>+</sup>GFP<sup>+</sup> cells were gated according to the gates in the dot plots. The histogram shows the expression of IL1RAP for negative control staining (white), MIG control (light gray) and MIG-P210 (dark gray). The numbers in the dot plots show the percentage of cells within individual gates/quadrants. A representative experiment out of three is shown.

**Figure 2. IL1RAP is upregulated in primitive CML cells**

FACS analysis on CD34<sup>+</sup> cells from five CML patients and from 2 normal bm samples. FACS dot plot showing gating for CD34<sup>+</sup>CD38<sup>+</sup> or CD34<sup>+</sup>CD38<sup>-</sup> cells in a representative CML patient **(A)**. Histogram showing IL1RAP expression within CD34<sup>+</sup>CD38<sup>+</sup> cells **(B)**. Histogram showing IL1RAP expression within CD34<sup>+</sup>CD38<sup>-</sup> cells **(C)**. White represent control stained samples and gray represent IL1RAP stained samples. The sorting gates for CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> and CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> cells are outlined in the histograms. The numbers in the dot plot and histograms show the percentage of cells within individual gates/quadrants.

**Figure 3. IL1RAP expression distinguishes Ph<sup>+</sup> from Ph<sup>-</sup> CML cells within the CD34<sup>+</sup>CD38<sup>-</sup> cell compartment**

Flow-drop-FISH on CML CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> and CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> cells from 5 CML patient samples revealed an almost complete separation between BCR/ABL1<sup>-</sup> and BCR/ABL1<sup>+</sup> cells, respectively. Black bars represent BCR/ABL1 negative cells and white bars represent BCR/ABL1 positive cells. Outlined at the top of each bar is the number of Ph<sup>+</sup> cells of the total nuclei scored.

**Figure 4. IL1RAP expression distinguishes Ph<sup>+</sup> CML stem cells from normal HSC**

Number of LTC-CFC derived from CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> and CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> cells **(A)**. Black bars represent IL1RAP<sup>-</sup> cells and white bars represent IL1RAP<sup>+</sup> cells. Interphase FISH on LTC-CFC **(B)**. Black bars represent BCR/ABL1 negative cells and white bars represent BCR/ABL1 positive cells. Outlined at the top of each bar is the number of Ph<sup>+</sup> cells of the total nuclei scored.

**Figure 5. Killing of a CML cell line by antibody targeting of IL1RAP**

Histogram showing IL1RAP expression on KU812 cells derived from a CML patient and containing a Philadelphia chromosome, compared to expression on KG-1 cells lacking a Philadelphia chromosome **(A)**. White show control stained samples and gray show KMT-1 stained samples. The leukemic cell line KG-1 was devoid of IL1RAP expression, whereas

KU812 express IL1RAP (B). As a consequence, low level of antibody induced cell death was observed in KG-1, while a dose-dependent ADCC effect was observed using KMT-1 on KU812 cells (B). As a control for unspecific ADCC effects, a rabbit IgG antibody was also used in the experiments. The graph shows the average and standard deviation of antibody induced cell death from three independent experiments.

#### **Figure 6. Killing of CML stem cells by antibody targeting of IL1RAP**

By using KMT-1, normal bone marrow CD34+CD38- cells stained negative for IL1RAP, whereas CML CD34+CD38+ and CD34+CD38- cells expressed IL1RAP. Histograms on CML-1 are shown from a representative experiment (A). White show control stained samples and gray show KMT-1 stained samples. In line with the level of IL1RAP expression, no obvious ADCC effect was seen using normal bone marrow CD34+CD38-cells, whereas KMT-1 induced a strong dose-dependent ADCC effect in both CML CD34+ and CD34+CD38- cells (B). As a control for unspecific ADCC effects, a rabbit IgG antibody was also used in the experiments. The graph shows the average and standard deviation of antibody induced cell death from three independent experiments using CML-1, CML-3, CML-4, and four normal bone marrow samples.

#### **Figure 7. IL1RAP is expressed also on primary ALL and AML stem cells**

Acute myeloid leukemia (AML) cells were received from patients at diagnosis. IL1RAP expression on CD34+CD38- and CD34+CD38+ cells from a representative AML patient is presented (A). The AML cell line MONO-MAC-6 and the ALL cell line REH express IL1RAP (B). Acute lymphoid leukemia (ALL) cells were received from patients at diagnosis. IL1RAP expression on CD34+CD38- and CD34+CD38+ cells from a representative Ph+ ALL patient is presented (C). White show control stained samples and gray show IL1RAP stained samples.

#### **Figure 8. Killing of AML and ALL cell lines by antibody targeting of IL1RAP**

In the ADCC assay, a KMT-1 dose dependent cell death was induced in both the MONO-MAC-6 and the REH cell line, suggesting that IL1RAP targeting antibodies may have a broader therapeutic window than just CML. As a control for unspecific ADCC effects, a rabbit IgG antibody was also used in the experiments. The graph shows the average and standard deviation of antibody induced cell death from three independent experiments.

#### **Figure 9. Killing of AML and ALL stem cells by antibody targeting of IL1RAP**

In the ADCC assay, a KMT-1 induced cell death was observed in both primary AML CD34+CD38- (A) and ALL CD34+CD38- (B) cells, confirming that IL1RAP targeting antibodies also have a therapeutic effect in AML and ALL with upregulation of IL1RAP on their cell surface. As a control for unspecific ADCC effects, a rabbit IgG antibody was also used in the experiments. The graph shows the specific antibody induced cell death.

#### **Figure 10. IL1RAP is expressed on leukemic stem cells from MPD and MDS patients.**

Contour plots showing IL1RAP expression in CD34+CD38- cells of two MPD patients (MPD-1 and MPD-2), with and without the JAK2 mutation (A). Histogram showing IL1RAP expression in an MDS patient progressed into AML (B). White show control stained samples and gray shows a sample stained with anti-IL1RAP antibodies.

**Figure 11. IL1RAP is expressed on the surface of cancer cells from solid tumours.**

Different cell lines derived from human solid tumours were stained with anti-human IL-1 RAcP/IL-1 R3-APC (cat no FAB676A, R&D system) (black lines) and isotype control (gray lines). Flow cytometry analysis show expression of IL1RAP on COLO829 (malignant melanoma), HCC1954 (breast ductal carcinoma), NCI-8228 (lung adenocarcinoma), NCI-H716 (colon cancer), OV-90 (ovarian adenocarcinoma), H716 (colon cancer), H2228 (lung adenocarcinoma), SH-4 (melanoma), SR (lymphoma) and SW 1783 (astrocytoma).

**Figure 12. IL1RAP is expressed on the surface of cancer cells from solid tumours**

Histogram from flow cytometry analysis on cells from four different human cancer cell lines labeled with mab81.2, an antibody against IL1RAP, showing IL1RAP expression on H716 (colon cancer), H2228 (lung adenocarcinoma), HCC1954 (breast ductal carcinoma), and SH-4 (melanoma).

**Figure 13. Antibody targeting of IL1RAP directs human NK-cells to ADCC on human cancer cells**

Graphs showing the degree of specific cell death induced by the anti-human IL1RAP antibody mab81.2, and human NK-cells in an ADCC assay. As isotype control, a non-specific human IgG1 antibody was included in the experiments.

**Figure 14. Effect of the mAb 81.2 on the in vivo growth of SK-MEL-5 melanoma cell line.**

MAb 81.2 was administered at 10mg/kg body weight intraperitoneally twice weekly. Control mice were treated with equivalent volumes of PBS. Each experimental group contained ten mice. Results are presented as average tumour volume (mm<sup>3</sup>); error bars represent Standard Error of the Mean (SEM).

**EXAMPLE 1****[0076] *IL1RAP is a cell surface biomarker for chronic myeloid leukemia stem cells*****Summary**

**[0077]** Therapeutic strategies for chronic myeloid leukemia (CML) aiming at achieving a permanent cure of the disorder, will require a full eradication of the CML stem cells. The CML stem cells, sharing the capacity to self-renew with normal hematopoietic stem cells (HSCs), represent a small population of leukemic cells that so far have been indistinguishable from normal (HSCs) using cell surface markers. One strategy to target the CML stem cell would be to identify a cell surface biomarker for CML stem cells, to which future therapeutic antibodies could be directed. In this study, we identified IL1RAP as commonly upregulated both in primitive CML CD34<sup>+</sup> cells and as a consequence of ectopic P210 BCR/ABL1 expression using global gene expression analyses. We further show that IL1RAP expression divides the rare



CD34<sup>+</sup>CD38<sup>-</sup> cell population, harboring both CML and normal HSCs, into two fractions; one having low/absent expression, the other having higher IL1RAP expression. After establishing a protocol, allowing detection of BCR/ABL1 by FISH in small numbers of sorted cells, we observed that within the CML CD34<sup>+</sup>CD38<sup>-</sup> cells; the IL1RAP<sup>+</sup> cells were BCR/ABL1<sup>+</sup>, whereas IL1RAP<sup>-</sup> cells were almost exclusively BCR/ABL1<sup>-</sup>. By further performing long term culture- initiating cell (LTC-IC) assays on the two cell populations, we found that candidate CML stem cells and normal HSC could be prospectively separated. This study thus identifies IL1RAP as the first cell surface biomarker distinguishing CML stem cells from normal HSC and opens up new avenues for therapeutic and diagnostic strategies in CML as well as in related disorders such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myeloproliferative disorders (MPDs) and myelodysplastic syndrome (MDS).

## **Introduction**

**[0078]** To identify a cell surface biomarker for CML stem cells, we performed global gene expression analyses and identified the interleukin 1 receptor accessory protein (IL1RAP) as the top candidate, being upregulated both in primitive CML patient cells and as a consequence of ectopic P210 *BCR/ABL1* expression. Upon development of an assay for detecting *BCR/ABL1* in low numbers of sorted cells, we show that the IL1RAP expression enables prospective separation of primitive leukemic and normal cells. Through long-term culturing- initiating cell assays, we further show that IL1RAP is a cell surface biomarker for CML stem cells, for the first time allowing prospective separation of CML stem cells from normal HSC.

## **Material and Methods**

### ***Collection of CML patient cells***

### ***Isolation and transduction of cord blood CD34<sup>+</sup> cells***

**[0079]** Blood and occasionally bone marrow samples from CML patients were obtained at diagnosis before treatment was initiated after informed consent according to a protocol approved by the local ethical board. Samples were received both from the Department of Hematology at Lund University Hospital, Sweden and from Rigshospitalet, Copenhagen, Denmark. Mononuclear cells (MNCs) were separated using Lymphoprep™ (Axis-Shield PoC AS, Oslo, Norway) according to the manufacturer's instructions and CD34<sup>+</sup> cells were enriched using the CD34<sup>+</sup> cell isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany) as previously described<sup>22</sup>, on a regular basis, this yielded a purity of CD34<sup>+</sup> cells above 95%. A subfraction of mononuclear cells was viably stored in liquid nitrogen before antibody staining was initiated.

CD34<sup>+</sup> cells were split in two fractions; one fraction was washed in PBS and resuspended in Trizol and frozen in -80 °C, whereas the other fraction was frozen in liquid nitrogen. As reference samples, bone marrow samples from healthy volunteers were obtained after informed consent at the Lund University Hospital, followed by CD34-cell isolation as described above.

### ***Microarray analysis***

**[0080]** Microarray analysis was performed using oligonucleotide slides from the Swegene DNA Microarray Resource Center at Lund University, Sweden. Hybridizationss were performed using the Pronto Universal Hybridization kit (Corning Inc, Corning, NY). The RNA isolation and microarray analysis was performed essentially as previously described<sup>23</sup>. Data visualization was performed using the software Qlucore Omics Explorer 2.0 (Qlucore, Lund, Sweden).

### ***Flow cytometric analysis***

**[0081]** Flow cytometric analyses were performed in a FACS Canto and flow cytometric cell sorting was done in a FACS Aria (both from BD). Prior to cell staining, CD34<sup>+</sup> cells were thawed according to standard procedures and washed once in PBS containing 2 % FCS (washing medium). Biotin-labeled goat anti-human IL1RAP polyclonal antibody (batch 667, R&D Systems, Abingdon, UK) was used at a 1:100 dilution for staining the cells for 30 min on ice. Subsequently, the cells were washed and PE-conjugated streptavidin was used at a 1:200 dilution for 30 min. The APC-conjugated anti-CD34 and FITC-conjugated anti-CD38 monoclonal antibodies were used for co-staining (except IL1RAP all antibodies used were purchased from Beckton-Dickinson Immunocytometry Systems, Mountain View, CA). Before cell sorting, cells were washed twice to avoid unspecific binding of PE-conjugated streptavidin. Isotype matching control antibodies were used as negative controls.

### ***Cell sorting and interphase FISH***

**[0082]** Glass slides were treated with 0.01 % poly L-lysine (Sigma-Aldrich, Stockholm, Sweden) for two hours while kept in a moist chamber, washed once in water, and dried on a hot plate at 37 °C until dry. Subsequently, a hydrophobic pen (Daido Sangyo Co., Ltd. Tokyo, Japan) was used to draw circles with a 96-well tissue culture plate as template. Prior to cell sorting, but after at least two hours drying in room temperature, 25 µL PBS was applied to the rings to form drops. During cell sorting, 30 to 3000 cells were sorted simultaneously directly into two drops. To allow attachment of the cells to the surface and to avoid drying of the drops, slides were maintained in a moist chamber on ice for 30 min before cells were fixed in methanol:acetic acid (3:1) for 10 min. Subsequently, slides were incubated in a 70 °C oven over night, followed by

FISH. Dual color probes for *BCR/ABL 1* (Abbot, Wiesbaden, Germany) were used.

#### ***Long term culture- initiating cells (LTC-IC)***

**[0083]** M<sub>2</sub>10B<sub>4</sub> stroma cells were cultured in RPMI-1640 medium supplemented with 10 % FCS as previously described<sup>24,25</sup>. Two days prior to cell sorting, stroma cells were seeded into wells of a 96-well plate at density of 50,000 cells per mL in 200 µL Myelocult medium (Stem Cell Technologies, Vancouver, Canada) containing 10<sup>-6</sup> M Hydrocortisone (Sigma-Aldrich, Stockholm, Sweden). Twenty-four hours before cell sorting, stroma cell were irradiated with 1000 Rad. During cell sorting, 100-500 cells were sorted directly into the stroma-precoated wells in duplicate and 100 µL medium was exchanged 3 h later. Once per week, the exchange of 100 µL culture medium was repeated. After 5-6 weeks culture, cells were washed and plated in methylcellulose medium (MethoCult H44435; Stem Cell Technologies) in a 24-well plate. Two weeks later, the number of colonies was scored. Colonies from individual wells were pooled, washed, applied to PBS drops on slides, and followed by FISH analysis as described above.

#### ***P210 BCR/ABL 1 expression in cord blood CD34<sup>+</sup> cells***

**[0084]** Umbilical cord blood samples were collected from normal deliveries after obtaining informed consent according to a protocol approved by the local ethical board. CD34<sup>+</sup> cells were enriched as previously described<sup>22</sup>, yielding a purity of CD34<sup>+</sup> cells above 95%. The RD114 pseudotyped MSCV-IRES-GFP (MIG) and MIG-P210 viral vectors were used in this study<sup>23</sup>. CD34<sup>+</sup> cells were cultured and transduced in SFMM medium (Stem Cell Technology, Vancouver, Canada) supplemented with thrombopoietin (TPO; 50 ng/mL), stem cell factor (SCF; 100 ng/mL), and Flt-3-ligand (FL; 100 ng/mL) as previously described<sup>23</sup>.

### **Results and Discussion**

#### ***Global gene expression analysis identifies IL1RAP as upregulated on CML CD34<sup>+</sup> cells***

**[0085]** Much effort has been put into investigations aimed at identifying a cell surface biomarker for Ph<sup>+</sup> CML stem cells (reviewed by C Eaves<sup>14</sup>). Leukemic and normal cells can rather easily be identified retrospectively in CML following detection of the leukemia specific *BCR/ABL 1* fusion gene by FISH, making it an ideal disorder for evaluating attempts to prospectively separate leukemic and normal cells. However, so far, no cell surface marker has

been identified that allows prospective separation of CML stem cells from normal HSC. Global gene expression analyses have proven to be a powerful strategy in searching for new HSC markers such as the SLAM receptors distinguishing hematopoietic stem and progenitor cells<sup>15</sup>. To search for upregulated genes encoding candidate cell surface biomarkers for CML stem cells, the transcriptional profiles of CD34<sup>+</sup> cells from 11 CML patient samples and 5 normal bone marrow (bm) samples were compared. The identified upregulated genes in CML were matched to the Gene Ontology (GO) category "integral to plasma membrane" that had been manually curated to include all known CD molecules (see Material and Methods for details). In total, 13 upregulated genes in CML CD34<sup>+</sup> cells matched to the integral to plasma membrane gene category (data not shown). To further link the upregulated genes more directly to P210 *BCR/ABL1* expression, we in parallel generated a list of upregulated genes as a consequence of P210 *BCR/ABL1* expression in cord blood CD34<sup>+</sup> cells. This analysis resulted in 23 upregulated genes matching to the same GO category gene list (data not shown). Interestingly, only one gene, the Interleukin 1 receptor accessory protein (IL1RAP), showed a strong upregulation both in CD34<sup>+</sup> CML cells and in cord blood CD34<sup>+</sup> cells as a consequence of P210 *BCR/ABL1* expression. The findings that IL1RAP was present on both gene lists suggest that its upregulation on primitive CML cells is closely coupled to the P210 *BCR/ABL1* expression and indicate that IL1RAP is a novel leukemia-associated antigen on primitive CML cells.

***IL1RAP is upregulated on CD34<sup>+</sup>CD38<sup>-</sup> cells from CML patients and is induced as a consequence of ectopic P210 *BCR/ABL1* expression***

**[0086]** IL1RAP is a member of the Toll-like receptor superfamily and is a well-known co-receptor to Interleukin 1 receptor type 1 (IL-1R1)<sup>16</sup>. IL1RAP is thus crucial in mediating the effect of the pro-inflammatory cytokine IL-1, but it is also involved in mediating the signal of IL-33, a cytokine that activates T-cells and mast cells through binding its receptor ST2, which subsequently dimerizes with IL1RAP<sup>17</sup>. IL-1R1 activation has previously been shown to stimulate colony growth of interferon sensitive CML cells<sup>18</sup>, however, IL1RAP has to our knowledge not previously been linked directly to CML.

**[0087]** As P210 *BCR/ABL1* is present in CML cells as a hallmark of the disease, ideally, a reliable cell surface biomarker in CML, should be directly coupled to the presence and expression of P210 *BCR/ABL1*. In agreement with the microarray data, IL1RAP expression was indeed upregulated on the cell surface on CB CD34<sup>+</sup> cells following retroviral P210 *BCR/ABL1* expression (Fig 1). This suggests that P210 *BCR/ABL1* regulates IL1RAP expression, either directly or through an indirect effect, strengthening its candidature as a CML biomarker.

**[0088]** We next investigated the cell surface IL1RAP expression on CML CD34<sup>+</sup>CD38<sup>+</sup> cells, representing the majority and more mature CD34<sup>+</sup> cells. In this cell population, an upregulation

of IL1RAP was observed compared to the expression in corresponding normal bm cells (Fig 2A, B). The normal CD34<sup>+</sup>CD38<sup>+</sup> cells displayed a lower IL1RAP expression that partially overlapped with the expression on CML cells. We then turned to the CD34<sup>+</sup>CD38<sup>-</sup> cell compartment of normal cells, containing the HSCs. In agreement with a previous study, this population displayed a low/absent IL1RAP expression (Figure 2C)<sup>19</sup>. Strikingly, the CD34<sup>+</sup>CD38<sup>-</sup> cells from CML patients, harboring both Ph<sup>+</sup> CML stem cells and normal HSCs were divided into two populations; one having low/absent IL1RAP expression, the other having higher IL1RAP expression (Fig 2C). In the peripheral blood (PB) of five CML patients, the IL1RAP positive cell fraction constituted between 75 % and 95 % of the CD34<sup>+</sup>CD38<sup>-</sup> cells (n = 5). Based on these findings, we speculated that the IL1RAP expression might distinguish normal and leukemic cells within the CD34<sup>+</sup>CD38<sup>-</sup> cell compartment in CML. As all CML stem cells and normal HSC exclusively are found within the CD34<sup>+</sup>CD38<sup>-</sup> cells, such separation between normal and leukemic cells, would allow a prospective separation of CML stem cells from normal HSC.

***Flow-drop-FISH shows that IL1RAP expression separates normal and leukemic cells within CML CD34<sup>+</sup>CD38<sup>-</sup> cells***

**[0089]** To test whether the IL1RAP expression distinguishes normal (Ph<sup>-</sup>) and leukemic (Ph<sup>+</sup>) cells within the CML CD34<sup>+</sup>CD38<sup>-</sup> cell compartment, we established a new protocol for doing fluorescent in situ hybridization (FISH) on small numbers of sorted cells (see Material and Methods). The first steps in this protocol is partly based on a method for sorting cells into drops on slides followed by single cell immuno-staining<sup>20</sup>. By applying this new protocol involving cell sorting directly into drops on slides followed by FISH, hereafter referred to as Flow-drop-FISH, we sorted as few as 30 cells into a drop, from which 15 nuclei were successfully scored by FISH (CML-5, Figure 3). Interestingly, we found by Flow-drop-FISH that the CML CD34<sup>+</sup>CD38<sup>-</sup> IL1RAP<sup>+</sup> cells were *BCR/ABL*1<sup>+</sup>, whereas CML CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> cells were almost exclusively Ph<sup>-</sup> (n = 5, Fig 3). These data show that IL1RAP expression separates leukemic and normal cells within the CML CD34<sup>+</sup>CD38<sup>-</sup> cell compartment, indicating that CML stem cells and normal HSC can be prospectively separated.

***CML stem cells are CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> whereas normal HSC are CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>low</sup>***

**[0090]** Studies on chronic phase CML stem cells has so far relayed on access to rare CML patients in which the stem cells compartment have been dominated by leukemic cells following long-term assays<sup>14</sup>. As CML stem cells generally show poor engraftment in immuno-deficient

mice, the long-term culture initiating cell (LTC-IC)- assay is widely used as a surrogate assay for detection of candidate CML stem cells. To test whether CML CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> and CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>low</sup> uniquely contain candidate CML stem cells and normal HSC, respectively, we tested the two cell populations in the LTC-IC assay. For bone marrow CD34<sup>+</sup> cells from normal controls, long term culture- colony forming cells (LTC-CFC) were found at an >100-fold higher frequency among CD34<sup>+</sup>CD38<sup>-</sup> IL1RAP<sup>-</sup> cells compared to CD34<sup>+</sup>CD38<sup>-</sup> IL1RAP<sup>+</sup> cells (Fig 4A, n= 2), indicating that normal CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> are hierarchically on top of CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> cells. In CML, we observed on average a 3.6-fold higher frequency of LTC-CFC within the CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> cells compared to the CD34<sup>+</sup>CD38<sup>-</sup> IL1RAP<sup>+</sup> cells (n= 5, Fig 4A), suggesting that CML CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> cells are more enriched for primitive cells. Importantly, although a higher number of LTC-IC were found among CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> cells than within CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> cells from both CML patient samples and from normal controls, FISH on CML LTC-colonies revealed an almost complete discrimination between Ph<sup>-</sup> and Ph<sup>+</sup> cells in the two groups (Fig 4B). CML LTC-colonies derived from CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>-</sup> cells were almost exclusively Ph<sup>-</sup>, whereas CD34<sup>+</sup>CD38<sup>-</sup>IL1RAP<sup>+</sup> were almost exclusively Ph<sup>+</sup>. These data suggest that IL1RAP is a novel cell surface biomarker that can be used to separate CML stem cells from normal HSC.

**[0091]** Herein, we identified through global gene expression analysis a novel cell surface antigen, IL1RAP, that following challenging in multiple assays fulfilled the criteria for being a novel cell surface biomarker for Ph<sup>+</sup> CML stem cells. Based on this discovery, future directed therapies in CML could be designed to target the CML stem cells while preserving normal HSC by using a therapeutic antibody directed towards IL1RAP. In addition, an antibody cocktail containing anti-CD34, anti-CD38 and anti-IL1RAP antibodies can be used for diagnostic purposes and for follow-up studies of CML patients under different treatments. Importantly, a prospective separation of normal and CML stem cells will enable future mechanistic studies of these two cell populations. Moreover, we here also show that Flow-drop-FISH could serve as a useful method in characterizing genetic aberrations in small numbers of sorted cells, such as leukemic stem cells, a cell type that has been purified to increasingly smaller and purer cell populations<sup>21</sup>. For future studies, this method would for example allow detection of genetical aberrations in various small leukemic stem and progenitor cell populations, findings that are likely to provide novel insights into which orders the various aberrations have been acquired, key knowledge to understand leukemogenesis. In addition, Flow-drop-FISH could be used to monitor therapeutic effects on leukemic stem cells during treatment. Importantly, we here identified by using Flow-drop-FISH that IL1RAP is the first cell surface biomarker that distinguishes CML stem cells from normal HSCs, a finding that opens up new therapeutic opportunities for CML and other neoplastic hematologic disorders associated with upregulation of IL1RAP on stem cells and/or progenitor cells.

## **References**

[0092]

1. 1. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. *Blood*. 2000;96:3343-3356.
2. 2. Fialkow PJ, Denman AM, Jacobson RJ, Lowenthal MN. Chronic myelocytic leukemia. Origin of some lymphocytes from leukemic stem cells. *J Clin Invest*. 1978;62:815-823.
3. 3. Kavalierchik E, Goff D, Jamieson CH. Chronic myeloid leukemia stem cells. *J Clin Oncol*. 2008;26:2911-2915.
4. 4. Jiang X, Zhao Y, Smith C, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. *Leukemia*. 2007;21:926-935.
5. 5. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006;107:4532-4539.
6. 6. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med*. 2006;12:1167-1174.
7. 7. Tavor S, Petit I, Porozov S, et al. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. *Cancer Res*. 2004;64:2817-2824.
8. 8. Jin L, Lee EM, Ramshaw HS, et al. Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. *Cell Stem Cell*. 2009;5:31-42.
9. 9. Majeti R, Chao MP, Alizadeh AA, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell*. 2009;138:286-299.
10. 10. Hosen N, et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. *Proc Natl Acad Sci U S A*. 2007;104:11008-11013.
11. 11. van Rhenen A, van Dongen GA, Kelder A, et al. The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. *Blood*. 2007;110:2659-2666.
12. 12. Eisterer W, Jiang X, Christ O, et al. Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease. *Leukemia*. 2005;19:435-441.
13. 13. Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci U S A*. 1997;94:5320-5325.
14. 14. Jiang X, Zhao Y, Forrest D, Smith C, Eaves A, Eaves C. Stem cell biomarkers in chronic myeloid leukemia. *Dis Markers*. 2008;24:201-216.
15. 15. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005;121:1109-1121.
16. 16. Subramaniam S, Stansberg C, Cunningham C. The interleukin 1 receptor family. *Dev Comp Immunol*. 2004;28:415-428.

17. 17. Ali S, Huber M, Kollewe C, Bischoff SC, Falk W, Martin MU. IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proc Natl Acad Sci U S A*. 2007;104:18660-18665.
18. 18. Estrov Z, et al. Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity. *Blood*. 1991;78:1476-1484.
19. 19. Hystad ME, Myklebust JH, Bo TH, et al. Characterization of early stages of human B cell development by gene expression profiling. *J Immunol*. 2007;179:3662-3671.
20. 20. Ema H, Morita Y, Yamazaki S, et al. Adult mouse hematopoietic stem cells: purification and single-cell assays. *Nat Protoc*. 2006;1:2979-2987.
21. 21. Dick JE. Stem cell concepts renew cancer research. *Blood*. 2008;112:4793-4807.
22. 22. Nilsson M, Karlsson S, Fan X. Functionally distinct subpopulations of cord blood CD34+ cells are transduced by adenoviral vectors with serotype 5 or 35 tropism. *Mol Ther*. 2004;9:377-388.
23. 23. Jaras M, Johnels P, Agerstam H, et al. Expression of P190 and P210 BCR/ABL1 in normal human CD34(+) cells induces similar gene expression profiles and results in a STAT5-dependent expansion of the erythroid lineage. *Exp Hematol*. 2009;37:367-375.
24. 24. Hogge DE, et al.. Enhanced detection, maintenance, and differentiation of primitive human hematopoietic cells in cultures containing murine fibroblasts engineered to produce human steel factor, interleukin-3, and granulocyte colony-stimulating factor. *Blood*. 1996;88:3765-3773.
25. 25. Castor A, Nilsson L, Astrand-Grundstrom I, et al. Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia. *Nat Med*. 2005;11:630-637.

## EXAMPLE 2

***Antibody-targeting of IL1RAP on leukemia stem and progenitor cells cause antibody-dependent cell-mediated cytotoxicity (ADCC)***

### Summary

**[0093]** Therapeutic strategies for leukemias aimed at achieving a permanent cure will require a full eradication of the leukemia stem cells. The leukemia stem cells, representing a small population of leukemic cells, have so far have been indistinguishable from normal hematopoietic stem cells (HSCs) using cell surface markers. A new concept for targeting leukemia stem cells would be to identify a cell surface biomarker for leukemia stem cells, to which future therapeutic antibodies could be directed (see Example 1).



**[0094]** In this study, we generate an anti-IL1RAP antibody and provide proof of concept that anti-IL1RAP antibodies targeting chronic myeloid leukemia (CML) stem cells, Acute myeloid leukaemia (AML) stem cells, and Acute lymphoblastic leukaemia (ALL) stem cells can be used to induce antibody-dependent-cell-mediated cytotoxicity (ADCC), whereas no cytotoxic effect was observed on normal HSC. Furthermore, we demonstrate a dose-dependent IL1RAP targeting ADCC in the IL1RAP positive cell lines KU812 (CML), MONO-MAC-6 (acute myeloid leukemia; AML) and REH (acute lymphoblastic cell line; ALL). We also demonstrate that MDS and MPD stem cells have increased IL1RAP expression, indicative that future therapeutic anti-IL1RAP targeting antibodies will be effective also in these disorders.

**[0095]** This study thus opens up for a novel therapeutic opportunity in CML, AML, ALL, MDS, and MPD by antibody targeting of IL1RAP on leukemic stem cells.

### **Materials and Methods**

#### ***Generation of KMT-1; a polyclonal rabbit anti-human IL1RAP antibody***

**[0096]** Rabbits were immunized with the extracellular domain of IL1RAP. Serum from rabbits were purified according to standard procedures, except that an additional step was added, in which antibodies binding to the immunoglobulin domain, present on the immunizing protein for increased half-life, was discarded through binding to immunoglobulin loaded columns. Purified antibodies were confirmed in ELISA to bind the extracellular domain of IL1RAP and to be devoid of antibodies binding the human immunoglobulin domain. When used in flow cytometry, a PE-conjugated goat anti-rabbit IgG antibody was used as secondary reagent.

#### ***ADCC assay***

**[0097]** The ADCC assay was based on a protocol previously described<sup>1</sup>. In brief, target cells were labelled with PKH26 (Sigma-Aldrich, St Louis, Missouri) according to manufacturer's instructions and either cells were put directly into wells of a 96-well plate, or seeded into the wells following sorting of CD34<sup>+</sup>CD38<sup>-</sup> cells. The KU812 and KG-1 cell lines and primary CD34<sup>+</sup> cells were seeded at 10,000 cells per well, whereas primary CD34<sup>+</sup>CD38<sup>-</sup> cells were seeded at 2,000-3,000 cells per well. Subsequently, antibodies were added to wells in different concentrations and incubated for 20 min before 100,000 NK- effector cells were added to each well. NK-cells were extracted from healthy volunteers after informed consent by using a NK-cell negative cell isolation kit according to manufacturer's instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). Rabbit IgG antibodies purified from a non-immunized rabbit was used as control antibody in the experiments (R&D Systems Abingdon, UK). 7-AAD positive cells for detection of cell death were measured using a FACS CANTO flow cytometer (BD). The

average and standard deviation of antibody induced cell death was calculated according to the following equation: (Percentage 7-AAD<sup>+</sup> cells at defined antibody concentration - Percentage 7-AAD<sup>+</sup> cells without antibody) / (0.01 x Percentage 7-AAD<sup>-</sup> cells without antibody) from at least three independent experiments (except Fig 9; 1 experiment only).

**[0098]** Samples from eleven AML patients and two Ph<sup>+</sup> ALL patients were received from Lund University hospital and the expression of IL1RAP was analyzed in the CD34<sup>+</sup>CD38<sup>+</sup> and CD34<sup>+</sup>CD38<sup>-</sup> cell populations using the same settings as for the analysis of CML cells. The AML cell line MONO-MAC-6 and the ALL cell line REH were also tested in ADCC assays using the same setup as the for the KG-1 and KU812 cell lines.

## **Results**

### ***Antibody-targeting of IL1RAP on CML stem and progenitor cells but also on a CML cell line directs NK-cells to ADCC***

**[0099]** Antibody-dependent-cell-mediated cytotoxicity (ADCC) is a conserved mechanism of the innate immune system, through which several therapeutic antibodies, such as Rituximab directed against CD20, are believed to at least partially exert their therapeutic effect<sup>2</sup>. To test whether ADCC could be achieved using IL1RAP as a target, we generated a polyclonal rabbit anti-human IL1RAP antibody hereafter referred to as KMT-1, as the Fc domain of rabbit antibodies in contrast goat antibodies are recognized by cells of the human immune system.

**[0100]** As expected, low levels of ADCC were observed in the IL1RAP negative/low leukemia cell line KG-1, even at high KMT-1 concentrations (Fig 5 A, B). In contrast, in the CML cell line KU812 expressing IL1RAP, a natural killer (NK)-cell mediated ADCC was observed in the presence of KMT-1 (Fig 5 A, B), demonstrating that KMT-1 has the potential to induce ADCC by recruiting cytotoxic immune cells to IL1RAP<sup>+</sup> target cells.

**[0101]** On primary cells from CML patients and from normal controls, KMT-1 showed a slightly weaker, but similar staining pattern as the previously used polyclonal goat antihuman IL1RAP antibody (Example 1, Fig. 6A). Immature cells from CML-1, CML-3 and CML-4 (no more cells remained from CML-2 and CML-5) were tested in ADCC assays in parallel to cells from healthy control samples. In CML CD34<sup>+</sup> cells, the binding of KMT-1 resulted in ADCC at higher levels than in normal CD34<sup>+</sup> control cells, correlating to the expression level of IL1RAP, in particular at lower antibody concentrations (Fig 6B). More strikingly, among the stem cell enriched CD34<sup>+</sup>CD38<sup>-</sup> cells, KMT-1 did not induce ADCC of normal CD34<sup>+</sup>CD38<sup>-</sup> cells, whereas a clear dose dependent ADCC effect was observed in CML CD34<sup>+</sup>CD38<sup>-</sup> cells (Fig 6 B), again showing strong correlation to the expression pattern of IL1RAP on these cell types.

***Antibodies targeting IL1RAP on AML and ALL cells direct NK-cells to ADCC***

**[0102]** IL1RAP expression was observed in AML CD34<sup>+</sup>CD38<sup>-</sup> cells in 9 out of 11 tested samples (Fig 7A). In the CD34<sup>+</sup>CD38<sup>+</sup> cell population, a similar IL1RAP expression pattern was observed (Fig 7A). In addition, IL1RAP was expressed in the AML cell line MONO-MAC-6 and the ALL cell line REH (Fig 7B). IL1RAP expression was also observed in Ph<sup>+</sup> ALL CD34<sup>+</sup>CD38<sup>-</sup> cells in 2 out of 2 tested samples (Fig 7C). Using IL1RAP as target, the MONOMAC-6 and REH cell lines were also tested in ADCC assays. In both these cell lines, a dose dependent IL1RAP targeting ADCC effect was observed (Fig 8), demonstrating that therapeutic anti-IL1RAP targeting antibodies have a broader application than just CML.

**[0103]** We also performed ADCC experiments on primary AML and ALL CD34<sup>+</sup>CD38<sup>-</sup> cells and demonstrated proof of principle that also in these disorders, an increased cell death could be achieved using KMT-1 (Fig. 9).

**[0104]** In addition, CD34<sup>+</sup>CD38<sup>-</sup> cells from one MDS patient at progression into AML and two MPD patients (one of them JAK2 mutation<sup>+</sup>) were stained with an IL1RAP targeting antibody. An increased IL1RAP expression was observed in comparison to normal bone marrow CD34<sup>+</sup>CD38<sup>-</sup> cells (Fig. 10, Fig 2C).

**Discussion**

**[0105]** In the present study, we have identified IL1RAP as the first cell surface biomarker that distinguishes candidate CML stem cells from normal HSCs and used this knowledge to induce an antibody-dependent cell killing of CML stem cells. Further, we identified IL1RAP as upregulated on AML stem cells, ALL stem cells, MPD stem cells and MDS stem cells and showed that both AML and ALL stem cells can be killed using an IL1RAP-targeting antibody, whereas normal stem cells were unaffected. Based on the finding that CML, ALL and AML stem cells can be killed by IL1RAP targeting antibodies, it is expected that also MPD and MDS stem cell would be killed in the ADCC assay. These findings opens up a new concept for treatments of leukemia patients by direct targeting of the leukemia stem cells, a concept that is distinct from the tyrosine kinase inhibitors currently used, which preferentially target cells downstream of the CML stem cells<sup>3,4</sup>.

**[0106]** The reason why CML stem cells are resistant to drugs such as Glivec is partially unclear, but factors that may contribute are features such as quiescence and relatively high level of *BCR/ABL1* expression, but also combinatorial expression of specific membrane transporter proteins in these cells<sup>3,5,6</sup>. Given these features of the CML stem cells, it is highly desirable to find novel treatment approaches to ultimately eradicate the CML stem cells. An antibody-based therapy directly targeting CML stem cells would serve in such a strategy as the

antibodies mode of action is independent of the known resistant mechanisms causing CML stem cells to be unresponsive to kinase inhibitor treatments. The major limitations for such developments have been the complete lack of a cell surface receptor distinguishing CML Ph+ from normal, healthy (Ph-) stem cells. We herein identified IL1RAP as such a target from global gene expression analyses and importantly linked its expression to *BCR/ABL1* expression (see Example 1 above).

**[0107]** Importantly, by generation of an antibody targeting IL1RAP, we here, for the first time, provide proof of concept that candidate CML stem cells can be targeted while preserving normal HSC. Importantly, as the antibodies mode of action in ADCC is to direct immunological cells to target cell killing, the therapeutic mechanisms is independent of the known mechanisms causing kinase inhibitor resistance in CML using current treatments. Hence, antibody targeting of CML stem cells has the capacity to eradicate CML stem cells, either alone or in combination with current regimens, ultimately leading to a permanent cure for CML patients.

**[0108]** Interestingly, we also observed that IL1RAP targeting antibodies can cause ADCC of AML stem cells; the most common type of acute leukemia among adults having a poor prognosis, and also ALL stem cells; the most common type of childhood leukemia. Collectively, the finding of IL1RAP expression on leukemic stem cells having a CD34<sup>+</sup>CD38<sup>-</sup> immunophenotype in CML, AML, ALL, MDS, and MPD, and the ADCC experiments demonstrating cell killing in an IL1RAP dependent manner, indicates that these disorders can be treated with anti-IL1RAP therapeutic antibodies.

**[0109]** In the ADCC experiments presented herein, a polyclonal anti-human IL1RAP antibody was used (which is essentially a mixture of several different monoclonal antibodies). However, it will be appreciated by persons skilled in the art that individual monoclonal antibodies targeting IL1RAP can also be identified which have ADCC potential.

## **References**

**[0110]**

1. 1. Wilkinson RW, Lee-MacAry AE, Davies D, Snary D, Ross EL. Antibodydependent cell-mediated cytotoxicity: a flow cytometry-based assay using fluorophores. J Immunol Methods. 2001;258:183-191.
2. 2. Morris JC, Waldmann TA. Antibody-based therapy of leukaemia. Expert Rev Mol Med. 2009;11:e29.
3. 3. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. Blood. 2006;107:4532-4539.
4. 4. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+

CML cells. Blood. 2007;109:4016-4019.

5. 5. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to ST1571 in vitro. Blood. 2002;99:319-325.
6. 6. Jiang X, Zhao Y, Smith C, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia. 2007;21:926-935.

### **EXAMPLE 3 - Gene expression on solid tumours**

#### **Materials and Methods**

[0111] Using the Oncomine search engine ([www.oncomine.org](http://www.oncomine.org)), we identified all data sets containing various cell lines established from different tumour types. The largest data set identified was the data set "Wooster Cell Line2". This data set contains 308 cancer cell lines, representing 20 different tumour types. The query term used was "IL1RAP" with the reporter setting "205277\_at".

#### **Results**

[0112] In total, we identified 17 different solid tumour types that were represented by cell lines meeting our criteria for an upregulated expression of IL1RAP (see Table 1). The percentage of cell lines within each tumour type showing upregulated IL1RAP ranged from 4% (colorectal cancer) to 67% (melanoma, prostate cancer). Among the tumour types, we identified some of the most common cancer entities in humans, including malignancies from breast, colon, lung, prostate and bladder. In addition, some tumour types associated with poor clinical outcomes, such as melanoma and brain tumours displayed highly upregulated expression of IL1RAP.

#### **Conclusions**

[0113] We conclude that several different tumour entities show an upregulated gene expression level of IL1RAP.

[0114] These data indicate that treatment with antibodies directed against IL1RAP will provide a new therapeutic avenue in several different human cancer types.

#### **Table 1**

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Upregulation of IL1 RAP in 308 cancer cell lines representing different tumour types*	
Tumour type	Number of tumours displaying upregulation of IL1RAP**
Bladder Cancer	3/9 (33%)
Brain and CNS Cancer	7/16 (44%)
Breast Cancer	4/19 (21%)
Cervical Cancer	4/7 (57%)
Colorectal Cancer	1/23 (4%)
Esophageal Cancer	3/4 (75%)
Gastric Cancer	1/5 (20%)
Head and Neck Cancer	3/6 (50%)
Kidney Cancer	1/8 (12%)
Liver Cancer	3/9 (33%)
Lung Cancer	14/73 (19%)
Lymphoma	2/38 (5%)
Melanoma	8/12 (67%)
Ovarian Cancer	2/5 (40%)
Pancreatic Cancer	3/9 (33%)
Prostate Cancer	2/3 (67%)
Sarcoma	5/13 (38%)
<p>*The Wooster data set on 308 cancer cell lines was searched using Oncomine (<a href="http://www.oncomine.org">www.oncomine.org</a>). The query term used was "IL1RAP" with the reporter setting "205227_at". The platform used was Human Genome U133 Plus 2.0 Arrays (Affymetrix Inc.)</p> <p>**Only tumour cell lines displaying an equal or higher expression level of IL1RAP than in the Philadelphia-positive cell line KU812 were scored as "upregulated". KU812 has previously been shown by us to have an upregulated protein expression of IL1RAP at the cell surface (Järås et al., 2010, PNAS 107(14): 16280-5).</p>	

#### EXAMPLE 4 - Analysis of IL1RAP expression on human cell lines by flow cytometry

##### Materials and methods

##### **Reagents**

[0115]

- Fc-receptor blockers from BD Biosciences
  - anti-human CD16 (cat no 555404)
  - anti-human CD32 (cat no 555447)
- APC-mouse IgG1 k Isotype control (cat no 555751) from BD Biosciences
- Anti-human IL-1 RAcP/IL-1 R3-APC (cat no FAB676A) from R&D system.

**Cell lines**

[0116]

Table 2

Cell line	Description	ATCC/DSMZ Catalog No.
KG-1	Human acute myeloid leukemia (used as a negative control)	ACC 14
KU-812	Human chronic myeloid leukemia in myeloid blast crisis (used as a positive control)	ACC 378
NCI-H2228	Lung Adenocarcinoma	CRL-5935
NCI-H716	Colon Cancer	CCL-251
HCC1954	Breast Ductal Carcinoma	CRL-2338
SR	Lymphoma	CRL-2262
OV-90	Ovarian Adenocarcinoma	CRL-11732
COLO 829	Malignant Melanoma	CRL-1974
SH-4	Melanoma	CRL-7724
SW 1783	Astrocytoma	HTB-13

[0117] The cell lines were cultured under standard conditions in medium recommended by the suppliers.

**FACS analysis**

[0118] Cells (350 000) were resuspended in 2 ml FACS buffer (PBS without calcium and

magnesium supplemented with 0.5% BSA), and centrifuged for 4 min at 300 x g. The supernatant was discarded and Fc-receptors were blocked by incubating cells with anti-CD16/CD32 mAbs at a concentration of 3 µg/ml in a volume of 30 µl for 5 minutes at room temperature. Then, 55µl FACS buffer and 4 µl APC-labeled isotype antibody or 5 µl APC-labeled monoclonal antibody directed against human IL1RAP were added to the cells and incubated for 30 minutes at +4°C. The cells were washed with 3 ml FACS buffer, centrifuged for 4 minutes at 300 x g and the supernatant was discarded. Cells were finally resuspended in 200 µl FACS buffer and flow cytometric analysis was performed according to standard settings on a FACS Cantoll flow cytometer (BD Biosciences).

## **Results**

[0119] IL1RAP expression levels on the solid tumour cell lines tested are shown in Table 3 below and in Figure 11.

**Table 3**

Expression of IL1RAP on different human cell lines.			
Values represent mean fluorescence intensity.			
Cell line	Blank	Isotype	Anti-IL1 RAP
KG-1	46	52	113
KU-812	62	69	451
NCI-H2228	80	96	587
NCI-H716	60	96	2043
HCC1954	112	119	410
SR	51	54	2257
OV-90	78	89	1921
COLO 829	77	82	3732
SH-4	40	51	5189
SW 1783	119	153	341

## **Conclusions**

[0120] Expression of IL1RAP was observed on the solid tumour cell lines NCI-H2228, NCI-H716, HCC1954, SR, OV-90, COLO 829, SH-4 and SW 1783. The expression on these cell lines was comparable or higher than that on the human chronic myeloid leukemia cell line KU-812.

**EXAMPLE 5 - Antibody-targeting of IL1RAP on solid tumour cells causes antibody-**



***dependent cell-mediated cytotoxicity (ADCC)*****Materials and Methods**

**[0121]** *Development and production of the chimeric monoclonal antibody 81.2 hlgG1.*

**[0122]** A murine hybridoma cell line which was secreting monoclonal antibodies specific to the extracellular part of human IL1RAP was generated by standard procedures. Briefly BALB/c mice were immunized with a fusion protein consisting of the extra cellular part of IL1RAP and the Fc-part of human IgG1 (Pro100-Lys330). Splenocytes were fused with the mouse myeloma cell line Sp2/0 and clones producing antibodies directed against the extracellular part of IL1RAP were isolated by screening with the fusion protein used for the immunisations and counter-screened with human IgG1.

**[0123]** The antibody produced by the hybridoma cell line clone 81.2 was of IgG1/kappa type and was found to have a high specificity to IL1RAP-positive cells and the recombinant protein human IL1RAP (21-367). From this cell line, total RNA was isolated and cDNA representing the variable regions of the heavy and light chains, VH and VK, were amplified by PCR, cloned and sequenced.

**[0124]** The genetic element coding for the murine VK in frame with the constant part of human kappa gene was synthesised and cloned in to a plasmid mammalian expression vector.

**[0125]** The PCR fragment coding for the murine VH were combined with the constant parts of human IgG1 and cloned in to a plasmid mammalian expression vector.

**[0126]** HEK 293 cells were co-transfected with both plasmids and the cells were cultured in serum-free medium supplemented with 100 ng/ml kifunensine. The chimeric antibody 81.2 hlgG1 was purified from the culture medium by Protein G chromatography.

***Flow cytometry***

**[0127]** Cells from four different human solid cancer cell lines, H2228 (adenocarcinoma; non-small cell lung cancer), H716 (colorectal adenocarcinoma), HCC1954 (ductal breast carcinoma), and SH-4 (melanoma), were harvested and stained with mab81.2, an anti-human IL1RAP antibody (Cantargia AB, Lund, Sweden). For detection, cells were stained with a secondary anti-human IgG PE-conjugated antibody (Thermo-Fisher, Waltham, MA), and cells were analyzed using a FACS CANTO flow cytometer (BD Immunocytometry Systems, Mountain View, CA).

**ADCC assay**

**[0128]** The ADCC assay was based on a protocol previously described (see Example 2 above). In brief, target cells were labeled with PKH26 (Sigma-Aldrich, St Louis, MO) according to manufacturer's instructions, and seeded into a 96-well plate at a density of 10,000 cells per well. Subsequently, antibodies were added to wells in different concentrations and incubated for 30 min before 100,000 NK- effector cells were added to each well. NK-cells were extracted from healthy volunteers after informed consent by using a NK-cell negative cell isolation kit according to manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). A non-specific human IgG1 antibody was used as control in the experiments (Eureka Therapeutics, Emeryville, CA).

**[0129]** The degree of cell death was assessed by detection of 7-AAD positive cells using a FACS CANTO flow cytometer (BD). The level of antibody induced cell death was calculated according to the following equation: Percentage 7-AAD+ cells at defined antibody concentration - Percentage 7-AAD+ cells without antibody.

**Results**

***An antibody against human IL1RAP labels human non-leukemic cancer cells in flow cytometry, and directs NK-cells to ADCC resulting in killing of human cancer cells***

**[0130]** We have shown that KMT1, a polyclonal antibody against human IL1RAP, could direct NK-cells to ADCC, and induce cell death of the IL1RAP-high expressing CML cell line KU812, but not on IL1RAP-low expressing KG1 cells (see Example 2 above).

**[0131]** The results from the present study show that not only leukemic cells are sensitive to ADCC mediated by IL1RAP, but also cells from solid human cancers. Four different human cancer cell lines, representing four different solid human cancer types, were studied, and all showed expression of IL1RAP on the cell surface (Fig. 12).

**[0132]** All four cell lines tested were also shown to be sensitive to ADCC mediated by mab81.2, an antibody against human IL1RAP, in what seems to be a dose-dependent way (Fig. 13).

**Conclusion**

**[0133]** The present study confirms that IL1RAP is expressed on the cell surface of several human cancer types, including lung cancer, colon cancer, breast cancer, and malignant

melanoma.

**[0134]** Using an antibody directed against IL1RAP, the cells of all four cell solid tumour lines tested were shown to be targeted by specific NK-mediated killing in an ADCC-assay.

**EXAMPLE 6 - *Efficacy of monoclonal antibody 81.2 in vivo in a human melanoma SK-MEL-5 xenograft mouse model***

**Materials and Methods**

**[0135]** *The development and production of the mouse monoclonal antibody 81.2 of IgG1 and IgG2a isotype.*

**[0136]** A murine hybridoma cell line which was secreting monoclonal antibodies specific to the extracellular part of human IL1RAP was generated by standard procedures. Briefly BALB/c mice were immunized with a fusion protein consisting of the extra cellular part of IL1RAP and the Fc-part of human IgG1 (Pro100-Lys330). Splenocytes were fused with the mouse myeloma cell line Sp2/0 and clones producing and antibodies directed against the extracellular part of IL1RAP were isolated by screening with the fusion protein used for the immunisations and counter-screened with human IgG1.

**[0137]** The antibody produced by the hybridoma cell line clone 81.2 was of IgG1/kappa type and was found to have a high specificity to IL1RAP-positive cells and the recombinant protein human IL1RAP (21-367). From this cell line, total RNA was isolated and cDNA representing the variable regions of the heavy and light chains, VH and VK, were amplified by PCR, cloned and sequenced.

**[0138]** The genetic element coding for the murine VK in frame with the constant part of murine kappa gene was synthesised and cloned in to a plasmid mammalian expression vector.

**[0139]** The PCR fragment coding for the murine VH were combined with the constant parts of murine IgG2a and cloned in to a plasmid mammalian expression vector.

**[0140]** HEK 293 cells were co-transfected with both plasmids and the cells were cultured in serum-free medium. The mouse antibody 81.2 of IgG2a isotype was purified from the culture medium by Protein G chromatography.

***Flow cytometry***

**[0141]** In order to confirm the IL1RAP expression on the human malignant melanoma cell line,

SK-MEL-5, and compare expression to the human CML cell line KU812, both cell lines were cultured according to standard procedures and maintained in logarithmic growth phase. At cell harvest  $3.5 - 5.0 \times 10^5$  cells/mL were labeled with the mouse IgG1 81.2 monoclonal antibody at 1 - 50µg/mL. An IgG1 isotype control antibody was used as control. The staining was analyzed using the Accuri C6 Flow Cytometer.

#### ***Drugs and Treatment***

[0142]

**Table 4**

Group	n	Drug/Testing Agent			
		Agent	mg/kg	Route	Schedule
Control	10	Vehicle (PBS)	-	ip	biwk x 6
Treated	10	mAb 81.2	10	ip	biwk x 6

#### ***In vivo administration of human IL1RAP specific mAb 81.2 or vehicle***

[0143] Eight to 12-week-old female CD.17 SCID mice were injected with  $1 \times 10^7$  SK-MEL-5 tumour cells in 50% Matrigel per animal, subcutaneously in the flank. Treatment was started approximately one week after melanoma cell injection when tumours had reached a size of 108 - 128mm<sup>3</sup>. A paired match of tumour size in 20 animals was done giving 10 mice each in the two treatment groups.

[0144] 81.2, a mouse IgG2a monoclonal antibody, was prepared at a dose of 10mg/kg and with a volume of 10mL/kg in PBS. Control animals were given equal volumes of PBS. Treatments were given via the intra-peritoneal route. Tumour volume by calliper measurement and total weights were monitored twice weekly.

[0145] The endpoint of the study is tumour growth delay.

#### **Results**

#### ***Flow Cytometry***

[0146]

**Table 5**

Expression of IL1RAP on SK-MEL-5 human melanoma cell line and human CML cell line KU812. Values represent mean fluorescence intensity			
Cell line	Sample Name	Concentration	IL1RAP expression
	No label	N/A	473
SK-MEL-5	81.2	1 µg/mL	501
	81.2	10 µg/mL	19010
	81.2	50 µg/mL	17560
	Isotype Control	10 µg/mL	605
	Isotype Control	50 µg/mL	548
	Secondary Only	1 µg/mL	497
	No label	N/A	291
KU812	81.2	1 µg/mL	1188
	81.2	10 µg/mL	2156
	81.2	50 µg/mL	1868
	Isotype Control	10 µg/mL	715
	Isotype Control	50 µg/mL	463
	Secondary Only	1 µg/mL	309
	No label	N/A	

#### ***In vivo activity of exemplary mAb 81.2***

[0147] Analysis of the study at day 33 from start of dosing showed a statistically significant delay in tumour growth in the treatment group compared to the control group on days 22 ( $p < 0.05$ ), 26 and 29 ( $p < 0.001$ ) and day 33 ( $p < 0.0001$ ) (see Figure 14).

#### **Conclusion**

[0148] Expression of IL1RAP was confirmed by flow cytometry on the melanoma tumour cell line SK-MEL-5 and showed an expression which was higher than that on the human chronic myeloid leukemia cell line KU-812.

[0149] The in vivo data indicate that the human IL1RAP specific monoclonal antibody, 81.2, administered twice weekly at a dose of 10mg/kg, caused inhibition in tumour cell growth of the IL1RAP expressing human melanoma cell line, SK-MEL-5.

## **REFERENCES CITED IN THE DESCRIPTION**

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

#### Patent documents cited in the description

- [US4816567A](#) [0031]
- [US4235871A](#) [0052]
- [US5851451A](#) [0053]
- [EP0213303A](#) [0053]

#### Non-patent literature cited in the description

- **BALAGURUNATHAN et al.** Mol. Cancer Ther., 2008, vol. 7, 93071-80 [0004]
- **HUANG et al.** Proc. Natl. Acad. Sci. USA., 1997, vol. 94, 2412829-12832 [0007]
- **VISVADERLINDEMAN** Nat Rev Cancer, 2008, vol. 8, 755-768 [0009]
- **PLANT et al.** Analyt Biochem, 1995, vol. 226, 2342-348 [0019]
- **FIELDSSONG** Nature, 1989, vol. 340, 245-246 [0021]
- **ORLANDI et al.** Proc. Natl. Acad. Sci. U.S.A., 1989, vol. 86, 3833-3837 [0027]
- **WINTER et al.** Nature, 1991, vol. 349, 293-299 [0027]
- **KOHLER et al.** Nature, 1975, vol. 256, 4950497- [0027]
- **KOZBOR et al.** J. Immunol. Methods, 1985, vol. 81, 31-42 [0027]
- **COTE et al.** Proc. Natl. Acad. Sci. USA, 1983, vol. 80, 2026-2030 [0027]
- **COLE et al.** Mol. Cell. Biol., 1984, vol. 62, 109-120 [0027]
- **H ZOLA** Monoclonal Antibodies: A manual of techniques CRC Press 1988 0000 [0028]
- **J G R HURRELL** Monoclonal Hybridoma Antibodies: Techniques and Applications CRC Press 1982 0000 [0028]
- **HARLOWLANE** Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory 1988 0000 [0029]
- **JONES et al.** Nature, 1986, vol. 321, 522-525 [0030] [0031]
- **RIECHMANN et al.** Nature, 1988, vol. 332, 323-329 [0030]
- **PRESTA** Curr. Op. Struct. Biol., 1992, vol. 2, 593-596 [0030]
- **REICHMANN et al.** Nature, 1988, vol. 332, 323-327 [0031]
- **VERHOEYEN et al.** Science, 1988, vol. 239, 1534-1536 [0031]
- **HOOGENBOOMWINTER** J. Mol. Biol., 1991, vol. 227, 381- [0032]
- **MARKS et al.** J. Mol. Biol., 1991, vol. 222, 581- [0032]

- **COLE et al.**In: Monoclonal antibodies and Cancer Therapy Alan R. Liss 1985 000077- [0032]
- **BOERNER et al.**J. Immunol., 1991, vol. 147, 86-95 [0032]
- Remington's Pharmaceutical Sciences Mack Publishing Company 1990 0000 [0046]
- handbook of Pharmaceutical Excipients Pharmaceutical Press 2000 0000 [0046]
- **DEININGER MW GOLDMAN JMMELO JV.**The molecular biology of chronic myeloid leukemia Blood, 2000, vol. 96, 3343-3356 [0092]
- **FIALKOW PJ DENMAN AM JACOBSON RJ LOWENTHAL MN.**Chronic myelocytic leukemia. Origin of some lymphocytes from leukemic stem cells J Clin Invest., 1978, vol. 62, 815-823 [0092]
- **KAVALERCHIK E GOFF DJ AMIESON CH**Chronic myeloid leukemia stem cells J Clin Oncol., 2008, vol. 26, 2911-2915 [0092]
- **JIANG X ZHAO Y SMITH C et al.**Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies Leukemia, 2007, vol. 21, 926-935 [0092] [0110]
- **COPLAND M HAMILTON A ELRICK LJ et al.**Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction Blood, 2006, vol. 107, 4532-4539 [0092] [0110]
- **JIN L HOPE K J ZHAI Q SMADJA-JOFFE F DICK JE.**Targeting of CD44 eradicates human acute myeloid leukemic stem cells Nat Med., 2006, vol. 12, 1167-1174 [0092]
- **TAVOR S PETIT I POROZOV S et al.**CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice Cancer Res., 2004, vol. 64, 2817-2824 [0092]
- **JIN L LEE E M RAMSHAW H S et al.**Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells Cell Stem Cell, 2009, vol. 5, 31-42 [0092]
- **MAJETI R CHAO M PALIZADEH AA et al.**CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells Cell, 2009, vol. 138, 286-299 [0092]
- **HOSEN N et al.**CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia Proc Natl Acad Sci U S A., 2007, vol. 104, 11008-11013 [0092]
- **VAN RHENEN A VAN DONGEN G AKELDER A et al.**The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells Blood, 2007, vol. 110, 2659-2666 [0092]
- **EISTERER W JIANG X CHRIST O et al.**Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease Leukemia, 2005, vol. 19, 435-441 [0092]
- **BHATIA M WANG J KAPP U BONNET D DICK JE.**Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice Proc Natl Acad Sci U S A., 1997, vol. 94, 5320-5325 [0092]
- **JIANG X ZHAO Y FORREST D SMITH C EAVES A EAVES C.**Stem cell biomarkers in chronic myeloid leukemia Dis Markers, 2008, vol. 24, 201-216 [0092]
- **KIEL M J YILMAZ O HIWASHITA T TERHORST C MORRISON SJ.**SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for

stem cells *Cell*, 2005, vol. 121, 1109-1121 [0092]

- **SUBRAMANIAM SSTANSBERG CCUNNINGHAM C.**The interleukin 1 receptor family *Dev Comp Immunol.*, 2004, vol. 28, 415-428 [0092]
- **ALI SHUBER MKOLLEWE CBISCHOFF SCFALK WMARTIN MU.**IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells *Proc Natl Acad Sci U S A.*, 2007, vol. 104, 18660-18665 [0092]
- **ESTROV Z et al.**Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity *Blood*, 1991, vol. 78, 1476-1484 [0092]
- **HYSTAD MEMYKLEBUST JHBO TH et al.**Characterization of early stages of human B cell development by gene expression profiling *J Immunol.*, 2007, vol. 179, 3662-3671 [0092]
- **EMA HMORITA YYAMAZAKI S et al.**Adult mouse hematopoietic stem cells: purification and single-cell assays *Nat Protoc.*, 2006, vol. 1, 2979-2987 [0092]
- **DICK JE.**Stem cell concepts renew cancer research *Blood*, 2008, vol. 112, 4793-4807 [0092]
- **NILSSON MKARLSSON SFAN X.**Functionally distinct subpopulations of cord blood CD34+ cells are transduced by adenoviral vectors with serotype 5 or 35 tropism. *Mol Ther.*, 2004, vol. 9, 377-388 [0092]
- **JARAS MJOHNELS PAGERSTAM H et al.**Expression of P190 and P210 BCR/ABL1 in normal human CD34(+) cells induces similar gene expression profiles and results in a STAT5-dependent expansion of the erythroid lineage *Exp Hematol.*, 2009, vol. 37, 367-375 [0092]
- **HOGGE DE et al.**Enhanced detection, maintenance, and differentiation of primitive human hematopoietic cells in cultures containing murine fibroblasts engineered to produce human steel factor, interleukin-3, and granulocyte colony-stimulating factor *Blood*, 1996, vol. 88, 3765-3773 [0092]
- **CASTOR ANILSSON LASTRAND-GRUNDSTROM I et al.**Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia *Nat Med.*, 2005, vol. 11, 630-637 [0092]
- **WILKINSON RWLEE-MACARY AEDAVIES DSNARY DROSS EL.**Antibody-dependent cell-mediated cytotoxicity: a flow cytometry-based assay using fluorophores *J Immunol Methods*, 2001, vol. 258, 183-191 [0110]
- **MORRIS JCWALDMANN TA.**Antibody-based therapy of leukaemia *Expert Rev Mol Med.*, 2009, vol. 11, e29- [0110]
- **JORGENSEN HGALLAN EKJORDANIDES NEMOUNTFORD JCHOLYOAKE TL.**Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells *Blood*, 2007, vol. 109, 4016-4019 [0110]
- **GRAHAM SMJORGENSEN HGALLAN E et al.**Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro *Blood*, 2002, vol. 99, 319-325 [0110]
- **JÄRÅS et al.**PNAS, 2010, vol. 107, 1416280-5 [0114]



**Patentkrav**

1. Middel omfattende eller bestående af et antistof, eller et antigen-bindende fragment deraf, hvor antistoffet er specifikt for interleukin-1 receptor-accessory-protein (IL1RAP) til anvendelse til behandling eller forebyggelse af kræft hos en patient, hvor kræften er en fast tumor med celler, der udtrykker IL1RAP.
2. Middel til anvendelse ifølge krav 1, hvor den faste tumor er valgt fra gruppen bestående af prostatakkræft, brystkræft, lungekræft, kolorektal kræft, melanomer, urinblærekræft, hjerne-/CNS-kræft, livmoderhalskræft, spiserørskræft, mavekræft, hoved- og halskræft, nyrekræft, leverkræft, lymfomer, kræft i æggestokkene, kræft i bugspytkirtlen og sarkomer.
3. Middel til anvendelse ifølge krav 1 eller 2, hvor den faste tumor er valgt fra gruppen bestående af lungekræft, tyktarmskræft, brystkræft, lymfomer, kræft i æggestokkene, kræft i bugspytkirtlen, melanomer og astrocytomer.
4. Middel til anvendelse ifølge et hvilket som helst af de foregående krav, hvor midlet er i stand til at dræbe cellerne associeret med den faste tumor.
5. Middel til anvendelse ifølge krav 4, hvor midlet er i stand til at inducere apoptose af cellerne associeret med den faste tumor.
6. Middel til anvendelse ifølge krav 4, hvor cellerne dræbes ved at inducere antistof-afhængig celle-medieret cytotoxicitet (ADCC).
7. Middel til anvendelse ifølge et hvilket som helst af de foregående krav, hvor midlet er i stand til at blokere binding af en eller flere co-receptorer til IL1RAP.
8. Middel til anvendelse ifølge et hvilket som helst af de foregående krav, hvor midlet omfatter eller består af et intakt antistof.
9. Middel til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor midlet omfatter eller består af et antigen-bindende fragment af et antistof.

**10.** Middel til anvendelse ifølge krav 9, hvor det antigen-bindende fragment er valgt fra gruppen bestående af Fv-fragmenter (f.eks. enkeltkæde-Fv, disulfid-bundet Fv og domæne-antistoffer) og Fab-lignende fragmenter (f.eks. Fab-fragmenter, Fab'-fragmenter og F(ab)2-fragmenter).

5

**11.** Middel til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet eller det antigen-bindende fragment deraf er humant eller humaniseret.

**12.** Middel til anvendelse ifølge et hvilket som helst af de foregående krav,  
10 endvidere omfattende en del for at forhøje *in vivo*-halveringstiden af midlet.

**13.** Middel til anvendelse ifølge et hvilket som helst af de foregående krav, endvidere omfattende en cytotoxisk del.

15

# DRAWINGS

FIGURE 1

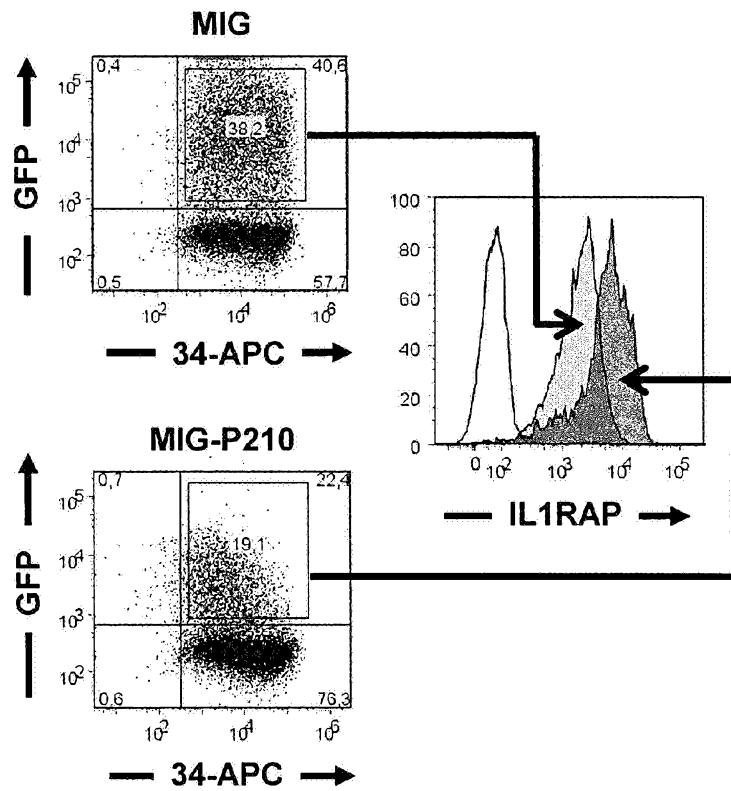


FIGURE 2(A)

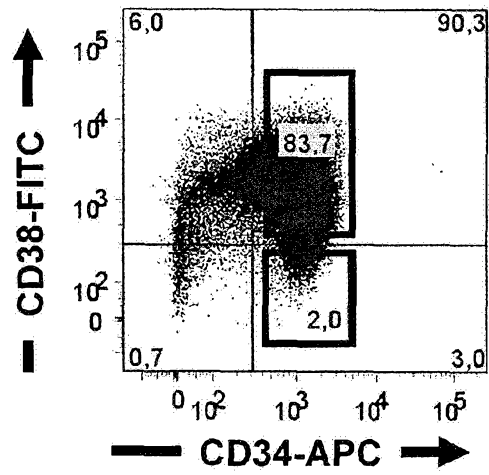


FIGURE 2(B)

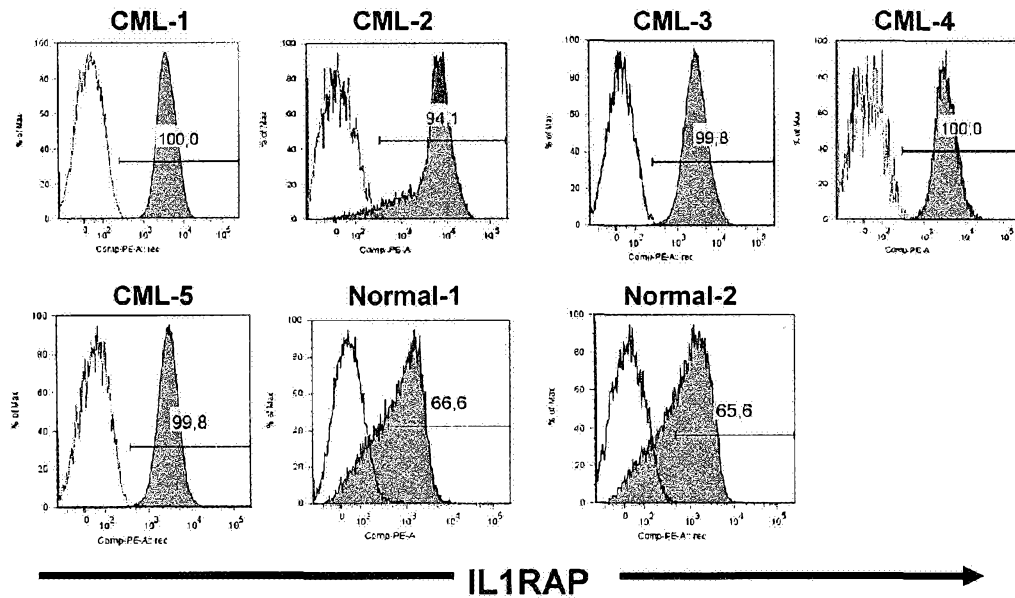
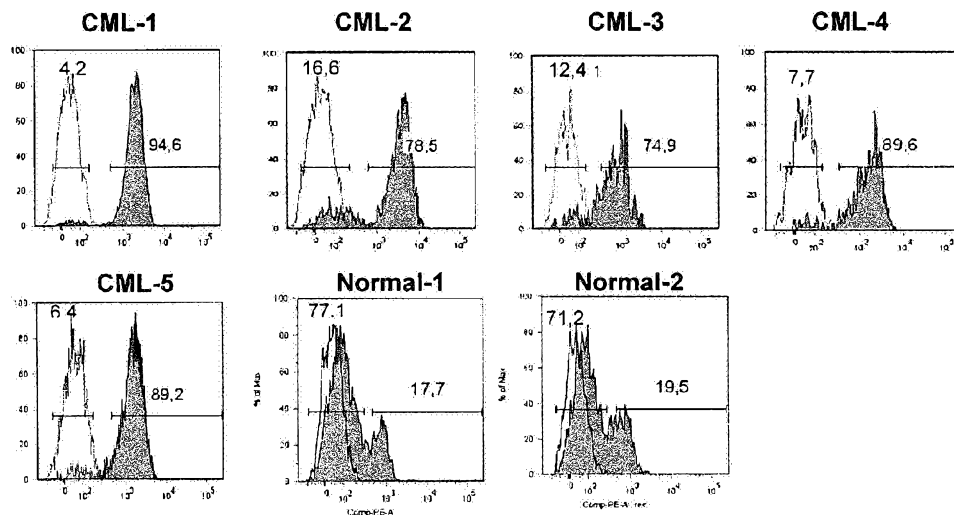
**CD34<sup>+</sup>CD38<sup>+</sup>**

FIGURE 2(C)

**CD34<sup>+</sup>CD38<sup>-</sup>**

IL1RAP

FIGURE 3

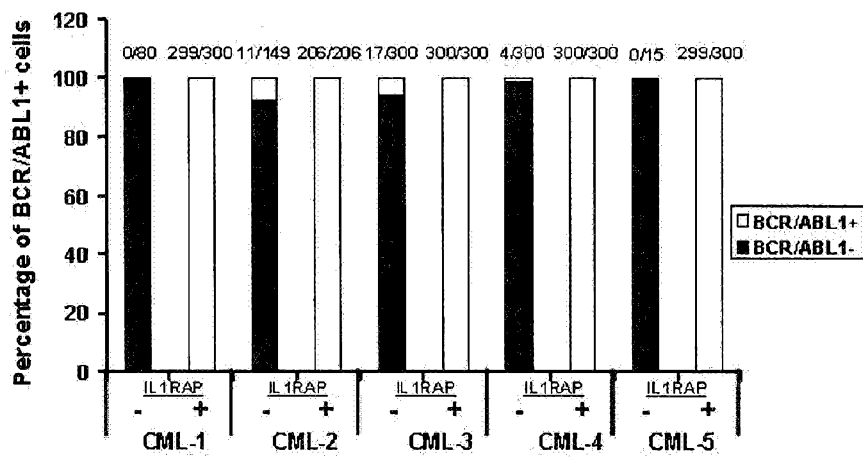


FIGURE 4(A)

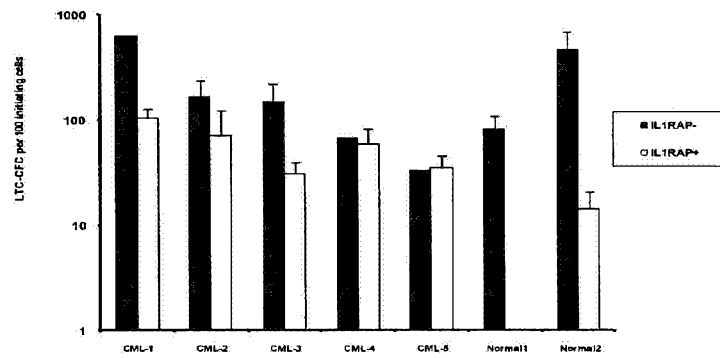




FIGURE 4(B)

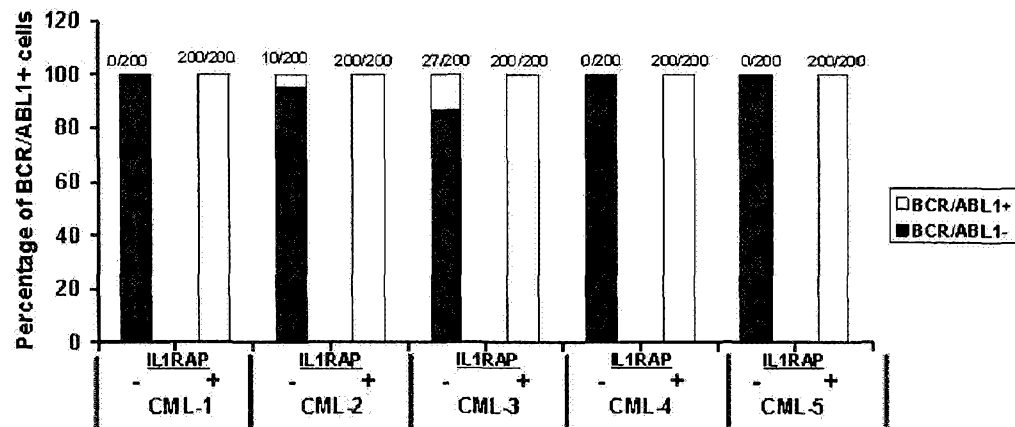


FIGURE 5

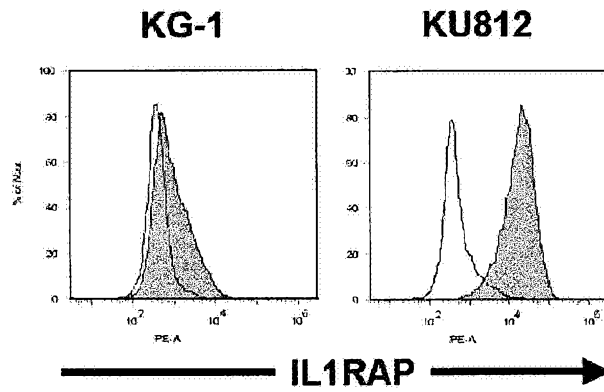
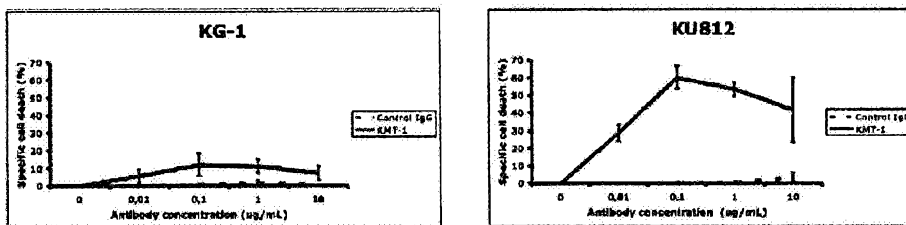
**A****B**

FIGURE 6(A)

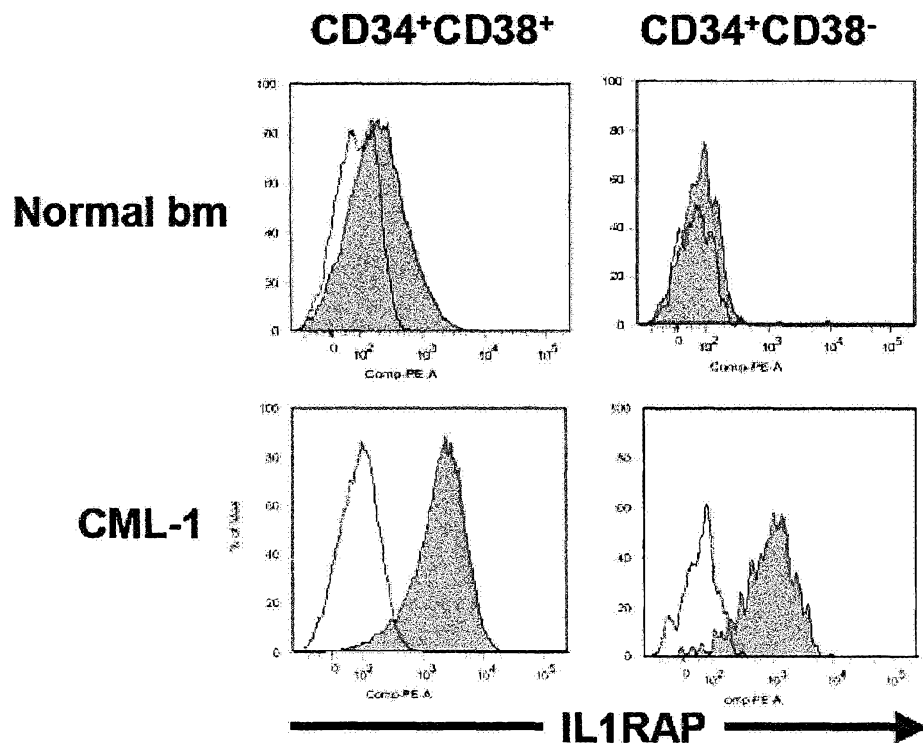


FIGURE 6(B)

CD34<sup>+</sup>CD38<sup>-</sup>

CD34<sup>+</sup>

Normal  
bm

CML

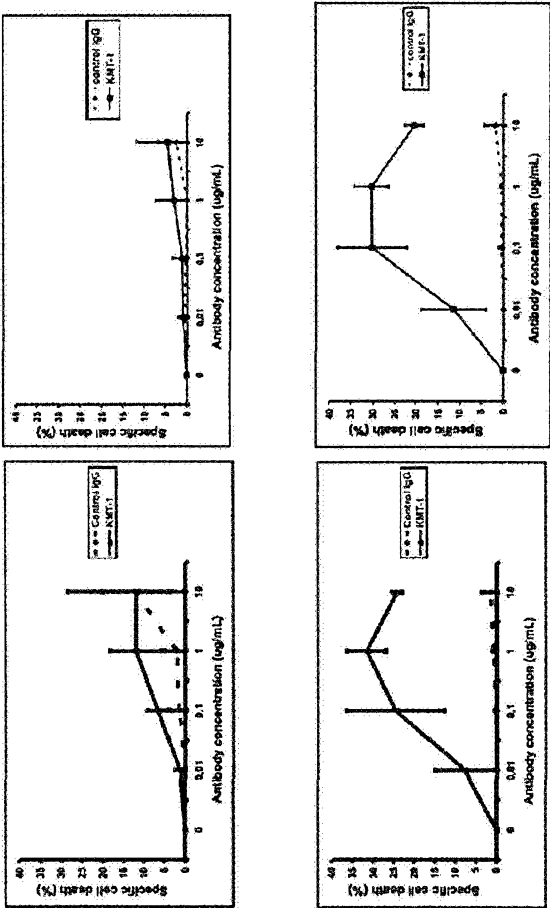


FIGURE 7(A &amp; B)

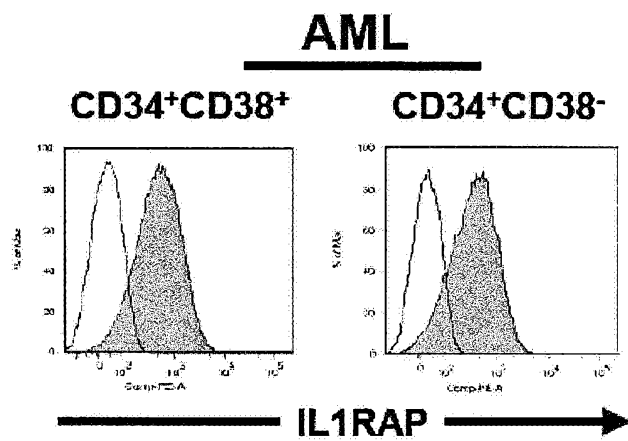
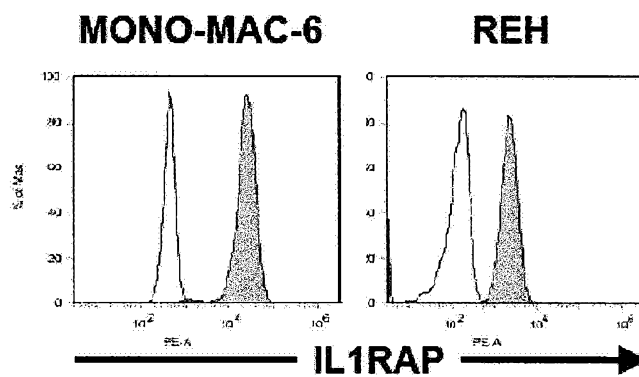
**A****B**

FIGURE 7(C)

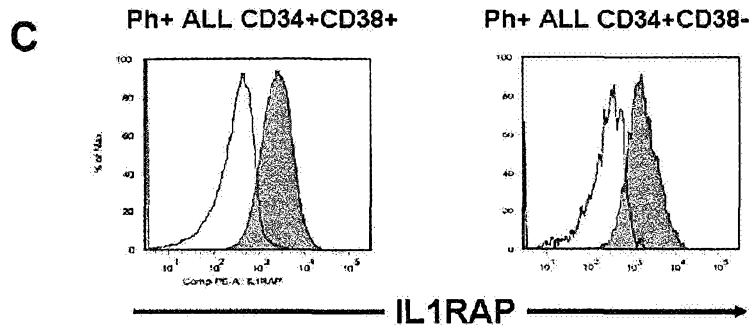


FIGURE 8

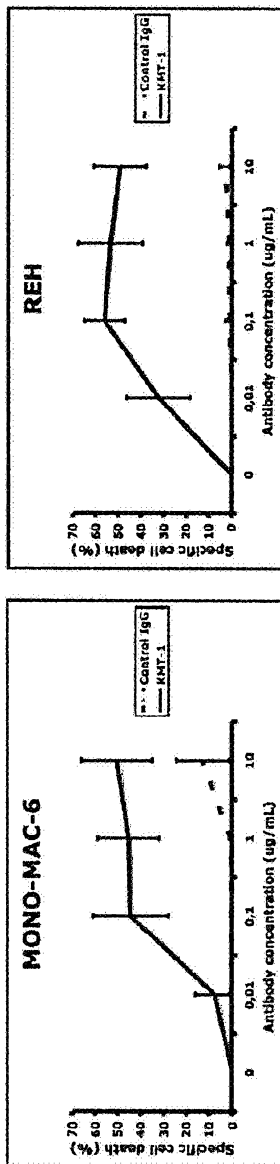
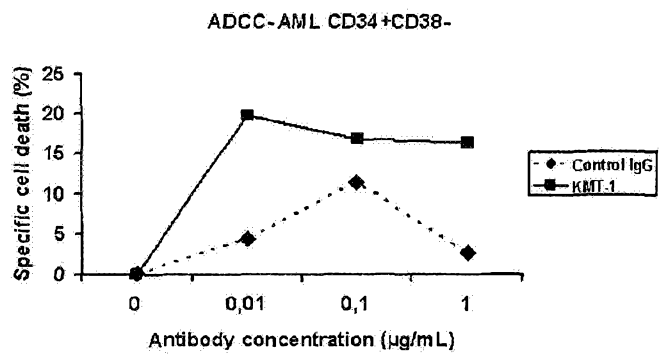


Figure 9 (A &amp; B)

A



B

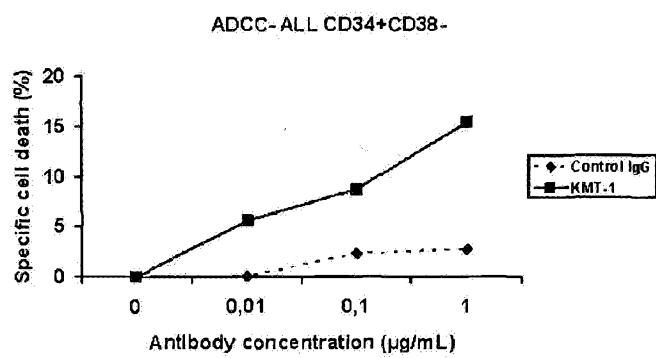
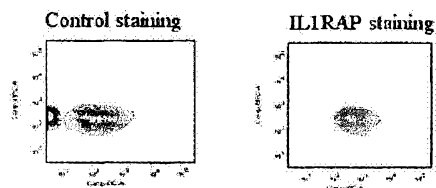




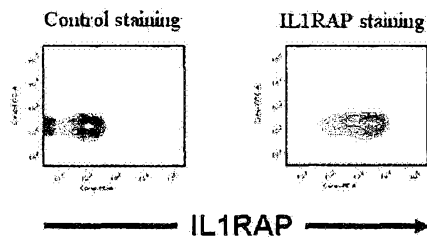
Figure 10 (A &amp; B)

A

MPD-1 (JAK2 mutation+) CD34+CD38-



MPD-2 (JAK2 mutation-) CD34+CD38-



B

MDS/AML CD34+CD38-

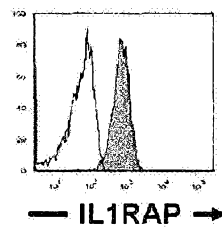


FIGURE 11

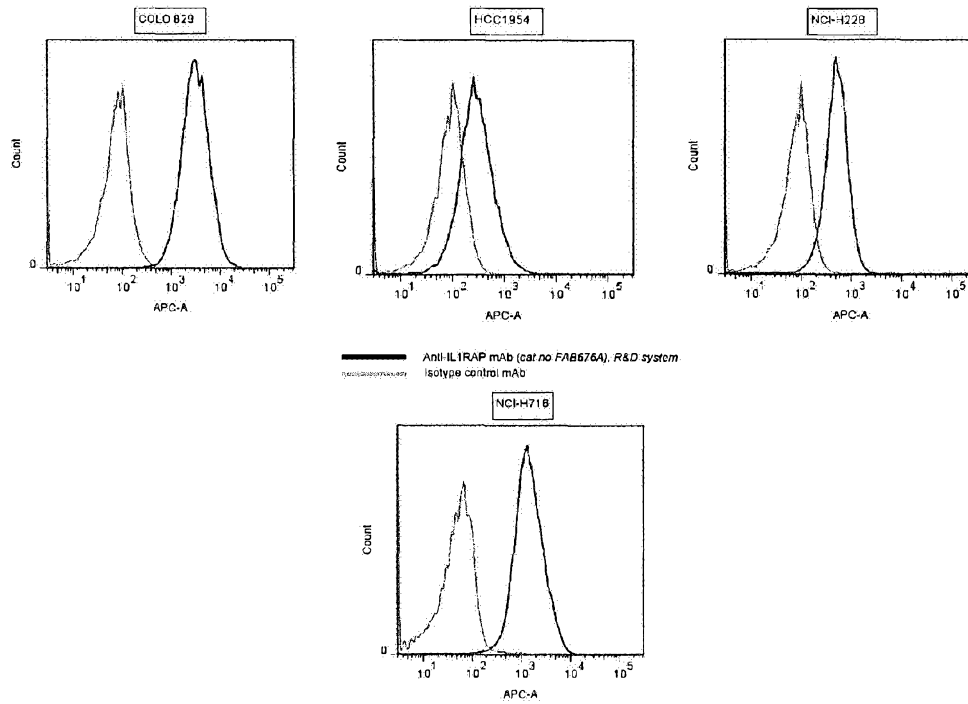


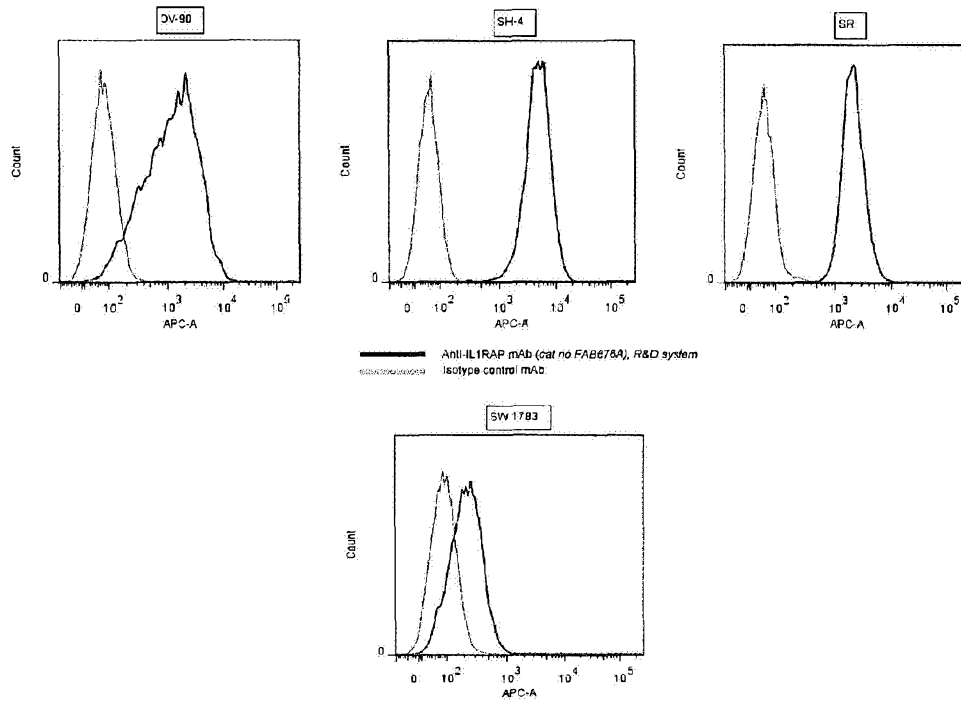
FIGURE 11 – *continued*

FIGURE 12

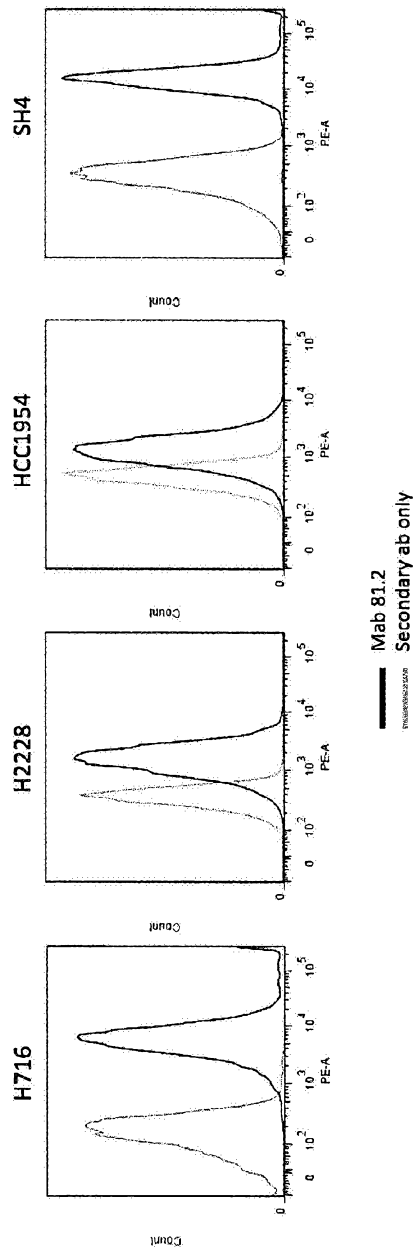


FIGURE 13

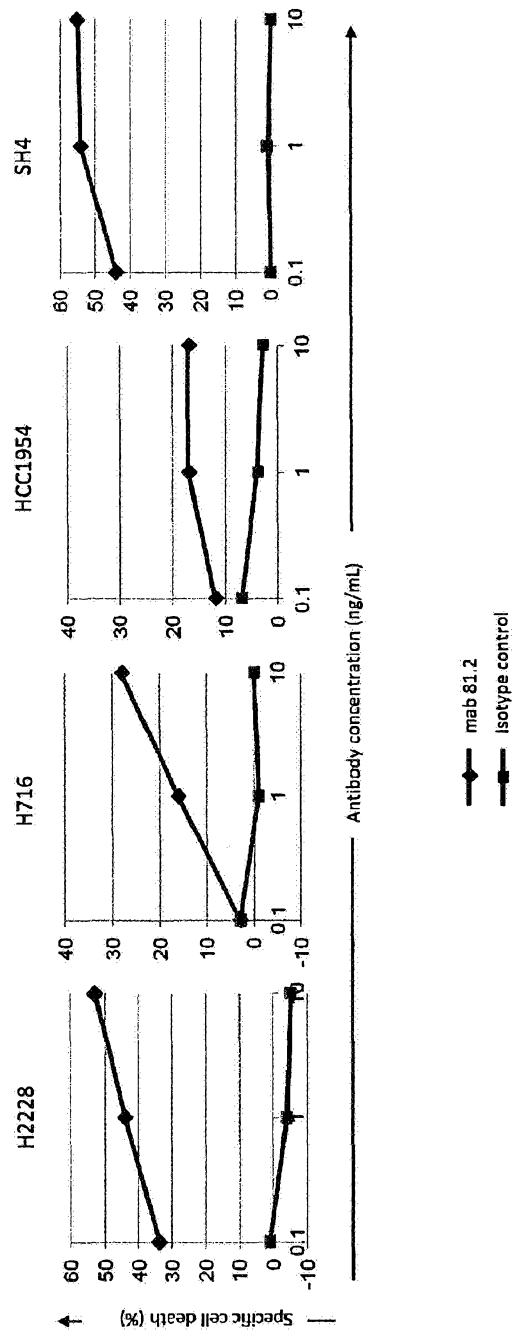


FIGURE 14

