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(74) Agent: CABINET REGIMBEAU; 129 Rue Servient,
F-69326 Lyon Cedex 03 (FR).

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(71) Applicant (*for all designated States except US*): APOXIS SA [CH/CH]; 22, Chemin Des Croisettes, CH-1066 Epalinges (CH).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): DUPUIS, Marc [CH/CH]; Bains de l'Alliaz, CH-1807 Blonay (CH). DEMOTZ, Stéphane [CH/CH]; Chemin de l'Eglise 7, CH-1066 Epalinges (CH). GREANEY, Peter [GB/CH]; Impasse de Chevrine 5, CH-1034 Boussens (CH). ATTINGER, Antoine [CH/CH]; Chemin des Ormeaux 20, CH-1066 Epalinges (CH). PERROD, Séverine [CH/CH]; Avenue du Grey 68, CH-1018 Lausanne (CH).

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(54) Title: MULTIMERIC SOLUBLE FAS LIGAND FOR ELIMINATING ALLOREACTIVE T LYMPHOCYTE IN ALLOGENIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION

(57) Abstract: The present invention concerns novel approaches for eliminating activated allo-reactive T lymphocyte in vivo and in vitro, preventing GVHD development with a sufficient amount of a multimeric form of the soluble portion of FasL as defined below.



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Method for eliminating alloreactive T lymphocyte in allogenic haematopoietic stem-cell transplantation

Allogenic hematopoietic stem-cell transplantation (HSCT) is the treatment of choice for many hematological malignancies and inherited disorders. However Graft-versus-host disease (GVHD) represents a major complication of allogenic HSCT, where immunocompetent donor allo-reactive T cells react against the recipient's cells. Acute GVHD develops within 3 months after allogenic HSCT and affects mainly the skin, liver and gastrointestinal tract. When pharmacologic immunosuppression is used in GVHD prophylaxis, moderate to severe acute GVHD occurs in 25% to 60% of MHC matched related donor transplant recipients, and up to 45% to 70% in MHC unrelated donor recipients.

Immunosuppressive agents for GVHD prophylaxis and therapy include methotrexate MTX, corticosteroids, cyclosporine A (CsA), tacrolimus (FK506), sirolimus (Rapamycin), mycophenolate mofetil (MMF). Antibodies for immunosuppression include polyclonal anti-T cell globulin (ATG) as well as monoclonal antibodies directed against CD3 (muromonab, OKT3), CD52 (alemtuzumab), CD25 (basiliximab, daclizumab, Ontak), CD154, CD40 ligand and TNF-alpha (infliximab) (reviewed in 1).

The present invention concerns a 3 novel approaches for eliminating activated allo-reactive T lymphocyte in vivo and in vitro, preventing GVHD development with a sufficient amount of a multimeric form of the soluble portion of FasL as defined below.

In the method according to the invention, MegaFasL can be used in combination with other known means of treatment. Such "means of treatment" comprise other molecules or compositions used in oncology (such as steroids or other immunosuppressive drugs). Such other molecules or compositions suitable are well known in the art, such as any of the molecules or compositions listed under the heading "Cancerologie" in the Dictionnaire Vidal (2003 ed.), in the Merk Index or in the Physician Desk Reference, including doxorubicin, platinum salts like cisplatin and velcade.

The present invention thus concerns a new method for preparation of donor cells substantially devoid of alloreactive T lymphocytes, comprising the step of treating a sample of donor cells in the presence or absence of irradiated host cells with a sufficient amount of soluble MegaFasL, to eliminate alloreactive lymphocytes without substantially

affecting the non-alloreactive T lymphocytes which will be transplanted, as disclosed above.

The present invention concerns a first method for preventing GVHD for allogenic HSCT prior transplantation, comprising the steps of culturing human peripheral blood mononuclear cells (PBMC) from a donor in an appropriate medium comprising an effective amount of a multimeric form of the soluble portion of FasL.

In a particular embodiment of the invention, the human PBMC from a donor are first cultured in presence of irradiated donor cells prior culture in a medium with a multimeric form of the soluble portion of FasL.

10 A preferred concentration of multimeric form of the soluble portion of FasL in the medium is comprised between 10 and 200 ng/ml.

The culture in the medium comprising a multimeric form of the soluble portion of FasL is maintained for at least 1 hour, preferably between 3 to 5 hours and up to 24 hours.

The present invention also concerns a second method for the prevention and treatment of GVHD for allogenic HSCT after transplantation, wherein the patient is treated by administration of an effective amount of a multimeric form of the soluble portion of FasL.

Administration of the effective amount of the multimeric form of the soluble portion of FasL is done by any suitable method of administration known in the art, preferably by injection, more preferably by intravenous injection.

The multimeric form of the soluble portion of FasL is generally administered in a form of a pharmaceutical composition suitable for the chosen administration route, preferably in the form of a solution and/or a suspension in an acceptable carrier.

Suitable carriers, adjuvant, preservatives, etc., used prepare pharmaceutical compositions, are well-known to those in the art (Gennaro (ed.), Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company 1995)).

As a preferred embodiment of the present invention, the pharmaceutical composition comprises from 0.1 to 100 weight % of multimerized forms of ligands according to the invention, based on the total weight of the pharmaceutical composition, more preferably from 2.5 to 100 %. When the composition according to the invention comprises 100 % multimerized forms of ligands, it is preferably in a lyophilized form.

A preferred dose of multimeric form of the soluble portion of FasL is comprised between 0,001 and 0,020 mg/kg. Administration is done in one or several times, the dose per 24 hour being preferably below 0,020 mg/kg.

Multimeric forms of the soluble portion of FasL are known in the art. Multimeric forms of the soluble portion of FasL are preferably defined below.

The multimerized form of the soluble portion of FasL comprise at least four, globular soluble extracellular fractions of the ligands of the TNF family, preferably at least five, more preferably at least six, even more preferably six globular soluble extracellular fractions of FasL bounds to a multimerization moiety.

In a preferred embodiment of the invention, the multimerized form of FasL is an hexamer comprising six monomers, assembled together, each of the monomers comprising a polypeptide of formula (I):



wherein

L represents a C-terminal ligand moiety, comprising the soluble extracellular fraction of FasL, and

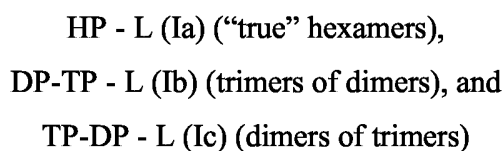
H represents a N-terminal hexamerization moiety.

According to the present invention, the ligand moiety L includes the "full length" of the soluble extracellular fraction of FasL and biologically functional fragments of the same fraction. "Biologically functional fragments" are fragments of a soluble extracellular fraction of FasL conserving their ability to bind to the same receptor(s), with substantially the same affinity.

L is preferably comprises the full length extracellular soluble fraction of FasL, more preferably comprising amino acids Glu 139 to leu 281 of hFasL.

Hexamers according to the invention are either "true" hexamers, dimers of trimers or trimers of dimers. In the first case, H is a hexamerization polypeptide HP. In the latter cases, H comprises two moieties, a first moiety consisting of a dimerization polypeptide (DP) and a second moiety consisting of a trimerization polypeptide (TP).

The polypeptides according to the present invention comprise a polypeptide represented by one the following formulas (Ia), (Ib) and (Ic):



wherein L, HP, DP and TP are defined above and below.

Examples of HP, TP and DP are well known in the art and comprise isolated peptide fragments of natural hexameric, trimeric or dimeric polypeptides, the said isolated fragments being responsible for the hexamerization, dimerization or trimerization of the said natural hexamers, dimers or trimers.

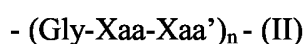
Such molecules are well known in the art and comprises polypeptides of the collectin family, such as the ACRP30 or ACRP30-like proteins (WO96/39429, WO 99/10492, WO 99/59618, WO 99/59619, WO 99/64629, WO 00/26363, WO 00/48625, WO 00/63376, WO 00/63377, WO 00/73446, WO 00/73448 or WO 01/32868), apM1 (Maeda et al., Biochem. Biophys. Res. Comm. 221: 286-9, 1996), C1q (Sellar et al., Biochem. J. 274: 481-90, 1991), or C1q like proteins (WO 01/02565), which proteins comprise "collagen domains" consisting in collagen repeats Gly-Xaa-Xaa'.

Other oligomerized polypeptides are known in the art, including polypeptides with a "coiled-coil" domains (Kammerer RA, Matrix Biol 1997 Mar;15(8-9):555-65; discussion 567-8; Lombardi & al., Biopolymers 1996;40(5):495-504; <http://mdl.ipc.pku.edu.cn/scop/data/scop.1.008.001.html>), like the Carilage Matrix Protein (CMP) (Beck & al., 1996, J. Mol. Biol., 256, 909-923), , or polypeptides with a dimerization domain, like polypeptides with a leucine zipper or osteoprotegerin (Yamaguchi & al., 1998).

According to a specific embodiment of the invention, HP comprises the hexamerization domains of A, B or C chains of polypeptides of the C1q family.

TP are known in the art and comprise the trimerization domains (C-terminal moiety) of CMP (i.e. GeneBank 115555, amino acids 451-493) or the trimerization domain of ACRP30 and ACRP30-like molecules. According to a preferred embodiment of the present invention, TP comprises a stretch of collagen repeats.

According to the invention, a "stretch of collagen repeats" consists in a series of adjacent collagen repeats of formula (II):



wherein Xaa and Xaa' represents independently an amino acid residue, and

n represents an integer from 10 to 40.

Xaa and Xaa' are preferably selected independently among natural amino acids such as Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

Xaa preferably represents independently an amino acid residue selected among Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Met, Pro or Thr, more preferably Arg, Asp, Glu, Gly, His or Thr.

Xaa' preferably represents independently an amino acid residue selected among Ala, Asn, Asp, Glu, Leu, Lys, Phe, Pro, Thr or Val, more preferably Asp, Lys, Pro or Thr.

When Xaa' represents a Pro residue, the collagen repeat Gly-Xaa-Pro is designated to be a "perfect" collagen repeat, the other collagen repeats being designated as "imperfect".

According to a preferred embodiment of the invention, the stretch of collagen repeats comprises at least 1 perfect collagen repeat, more preferably at least 5 perfect collagen repeats.

According to a preferred embodiment of the invention, n is an integer from 15 to 35, more preferably from 20 to 30, most preferably 21, 22, 23 or 24.

According to the present invention, the stretch of collagen repeat may comprise up to three "non collagen residues" inserted between two adjacent collagen repeats. These "non collagen residues" consist in 1, 2 or 3 amino acid residues, provided that when the "non collagen residue" consists in 3 amino acids residues, the first amino acid is not Gly.

According to a preferred embodiment of the invention, TP consists in an uninterrupted stretch of 22 collagen repeats. More preferably, TP consists in the stretch of 22 collagen repeats of SEQ ID NO 1, corresponding to amino acids 45 to 110 of mACRP30, as represented in SEQ ID NO 2 of WO 96/39429:

Gly Ile Pro Gly His Pro Gly His Asn Gly Thr Pro Gly Arg Asp Gly Arg Asp Gly Thr Pro Gly Glu Lys Gly Glu Lys Gly Asp Ala Gly Leu Leu Gly Pro Lys Gly Glu Thr Gly Asp Val Gly Met Thr Gly Ala Glu Gly Pro Arg Gly Phe Pro Gly Thr Pro Gly Arg Lys Gly Glu Pro Gly Glu Ala

According to another preferred embodiment of the invention, TP consists in the stretch of 22 collagen repeats corresponding to amino acids 42 to 1107 of hACRP30, as represented in SEQ ID NO 7 of WO 96/39429:

DP are known in the art and comprises dimerization fragments of immunoglobulins (Fc fragments), the C-terminal dimerization domain of osteoprotegerin (Receptor: δ N-OPG; amino acids 187-401), or polypeptides sequences comprising at least 6, preferably 8 to 30 amino acids and allowing dimerization. These peptides generally comprise at least a cysteine residue allowing the formation of disulfide bonds. Other polypeptides useful as

DP according to the invention are peptides designated as "leucine zippers" comprising a Leucine residue being present every seventh residue.

Examples of such peptides comprising at least a cysteine residue comprise the following peptides:

- 5 - Val Asp Leu Glu Gly Ser Thr Ser Asn Gly Arg Gln Cys Ala Gly Ile Arg Leu
- Glu Asp Asp Val Thr Thr Thr Glu Glu Leu Ala Pro Ala Leu Val Pro Pro Pro Lys
 Gly Thr Cys Ala Gly Trp Met Ala
- Gly His Asp Gln Glu Thr Thr Thr Gln Gly Pro Gly Val Leu Leu Pro Leu Pro Lys
 Gly Ala Cys Thr Gly Trp Met Ala.

- 10 The second sequence above corresponds to amino acids 17 to 44 of mACRP30 as represented in SEQ ID NO 2 of WO 96/39429, and the third sequence above corresponds to amino acids 15 to 41 of SEQ ID NO 7 of WO 96/39429.

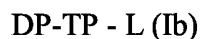
- 15 Other peptides comprising at least one cysteine residue, can be found in amino acid sequences upstream the stretch of collagen repeats of molecules having a structure analogous to ACRP30 (ACRP30-like) as disclosed in WO 99/10492, WO 99/59618, WO 99/59619, WO 99/64629, WO 00/26363, WO 00/48625, WO 00/63376, WO 00/63377, WO 00/73446, WO 00/73448 or WO 01/32868.

- 20 Leucine zippers are well known in the art and can be found in natural proteins and eventually identified using bioinformatics tools available to the one skilled in the art (<http://www.bioinf.man.ac.uk/zip/faq.shtml>; <http://2zip.molgen.mpg.de/>; Hirst, J.D., Vieth, M., Skolnick, J. & Brooks, C.L. III, *Predicting Leucine Zipper Structures from Sequence*, Protein Engineering, 9, 657-662 (1996)).

- 25 The constitutive elements L, H, HP, TP and/or DP in the polypeptides of formula I, Ia, Ib or Ic, according to the invention, are assembled by peptides bonds. They may be separated by "linkers" which will not affect the functionality of the polypeptide according to the invention, its ability to form hexamers and to bind with the receptor corresponding to the ligand L. Such linkers are well known in the art of molecular biology.

- 30 The polypeptide according to the invention may also comprise peptide sequences on its N-terminus and/or C-terminus, which will not affect the functionality of the polypeptide according to the invention. These peptides may comprise affinity tags, for purification or detection of the polypeptide according to the invention. Such affinity tags are well known in the art and comprise a FLAG peptide (Hopp et al., Biotechnology 6: 1204 (1988)) or a Myc-His tag.

According to a preferred embodiment of the invention, H comprises a dimerization polypeptide (DP) and a trimerization polypeptide (TP), and is most preferably represented by the following formula:



5 wherein R, DP and TP are defined above and below.

More preferably, DP and TP represent together amino acids 17 to 110 of mACRP30 as represented in SEQ ID NO 2 of WO 96/39429 or amino acids 15 to 107 of hACRP30 as represented in SEQ ID NO 7 of WO 96/39429.

10 A preferred embodiment of the invention the polypeptide comprises the fusion polypeptide mACRP30:hFasL.

Such polypeptides and their preparation are disclosed in WO 01/49866 which content is incorporated herein by reference.

In a preferred embodiment of the present invention, amino acid sequence of the fusion polypeptide mACRP30:hFasL is the following:

15 MAIIYLILLFTAVRGHDQETTTQGGVLLPLPKGACTGWMAGIPGHPGHNGAPGR
DGRDGTGPGEKGEKGDPLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGA
KELRKVAHLTGKSNSRSMPLWEDTYGIVLLSGVKYKKGGGLVINETGLYFVYSKV
YFRGQSCNNLPLSHKVYMRNSKYPQDLVMMEGKMMSYCTTGQMWARSSYLGA
VFNLTSADHLYVNVSELSLVNFEESQTFFGLYKL

20 According to another embodiment of the invention, the hexamerization moiety comprises a Fc portion of IgG comprising amino acids 248 to 473 of gi2765420, as disclosed in WO 03/068977, which content is incorporated herein by reference.

The efficacy of multimerized forms of FasL in the prevention and treatment of GVHD is demonstrated in the following experiments, for MegaFasL

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Legend of the Figures

Figure 1: Donor allogenic HSC are isolated and cultured in presence of MFL which induces apoptosis of a fraction of cell containing alloreactive clones (1). Remaining cells, partially depleted of alloreactive T lymphocytes are transferred into irradiated host
30 (2).

Figure 2: Donor allogenic HSC are isolated and cultured (1) in presence of irradiated donor cells (2). Alloreactive T lymphocyte of donor cells get activated and proliferated (3). Addition of MFL induces cell death of alloreactive T lymphocytes (4)

while preserving the non-activated and non-alloreactive T lymphocytes. Remaining cells, depleted of alloreactive T lymphocytes are transferred into irradiated host (5).

Figure 3. Donor allogenic HSC are transferred in irradiated host (1) and because of the presence of alloreactive T cells among the donor inoculum, patient start to develop GVHD symptoms (2). Patient is treated with MegaFasL that eliminate alloreactive T lymphocytes and prevent the GVHD (3).

Figure 4: PBMC were activated with PHA (10µg/ml) for 4 days then IL-2 (50 U/ml) was added for 2 weeks. Cells were restimulated for 16 hours with various concentration of PMA/Ionomycin in presence of MFL. Cell viability was determined by forward and side scatter using flow cytometry. Presence of activated T cells was confirmed by flow cytometry where 90% of live cells express CD69 and 70% express CD25.

Figure 5: T cells were purified from PBMC, labelled with the fluorescent dye CFSE and activated with anti-CD3/anti-CD28 for 7 days. MFL was added to the culture at different days during the activation course. At the end of the activation course, cells were analyzed by flow cytometry using Propidium Iodide (PI) for apoptotic cell detection. Analysis was performed on CFSE low cells that correspond to cells that have proliferated. * Activated T cells in presence of 100ng/ml of MFL for 5, 6 and 7 days do not proliferate.

Figure 6: Expression of CD95 at the surface of naïve (CD45RA+, panel A) and memory (CD45RO+, panel B) human CD3+ T cells was analyzed by flow cytometry.

Figure 7: PBMCs were incubated for 16 hours in the presence of various concentration of MFL. Percentage of apoptotic naïve (CD3/CD45RA) and memory (CD3/CD45RO) cells was determined by annexin staining.

Figure 8: PBMC were incubated with various concentration of MFL for 5 hours. At the end of the incubation period, MFL treated cells were washed twice with PBS and stimulated with PHA (2µg/ml) for 5 days. Proliferation rate was measured by adding tritiated thymidine to the stimulated cells 16 hours before the end of the 5 days activation period.

Figure 9: PBMC were incubated with various concentration of MFL for 5 hours. At the end of the incubation period, MFL treated cells were washed twice with PBS and stimulated with irradiated PBMC from unrelated donor for 7 days. Proliferation rate was measured by adding tritiated thymidine to the stimulated cells 16 hours before the end of the 7 days activation period.

Figure 10: PBMC were activated with PHA (10µg/ml) for 4 days then IL-2 (50 U/ml) was added for 2 weeks. Cells were restimulated for 60 hours with PHA (10µg/ml) and irradiated PBMC from unrelated donor in presence of various concentrations of MFL, soluble FasL or crosslinked soluble FasL. Proliferation rate was measured by adding tritiated thymidine to the stimulated cells for the final 16 hours.

Figure 11: PBMC were activated with PHA (10µg/ml) for 4 days then IL-2 (50 U/ml) was added for 2 weeks. Cells were restimulated for 60 hours with PHA (10µg/ml) and irradiated PBMC from unrelated donor. MFL was added with tritiated thymidine to the stimulated cells for the final 16 hours.

Figure 12: ARH-77 plasma leukemia cells were incubated with various Fas agonists for 15 hours and cell viability was analysed using the PESMTS assay.

1. *In vitro* depletion of resting T lymphocytes by pre-incubation with MegaFasL as GVHD prophylaxis for allogenic HSCT.

This approach eliminates resting T cells by pre-incubation for 5 hours with MFL in absence of activation prior to the transfer into the irradiated host (figure 1).

Results: A mixed lymphocyte reaction (MLR) can be prevented when human PBMC of donor 1 were cultured for 5 hours with MegaFasL then washed twice prior being activated in presence of irradiated PBMC of the donor 2 (figure 9). The MegaFasL treatment dose is comprised between 10 and 200 ng/ml. The pre-treatment of MFL at these doses prior to the MLR eliminates or anergizes a sub-population of T lymphocyte containing alloreactive clones (figure 9) and also prevent PHA-induced activation (figure 8). Reduction of a MLR reaction corresponds to a reduction of GVHD. The prevention of GVHD is confirmed by re-infusion of treated cells in HLA-mismatched mice (for the murine model of GVHD) or reinfusion of human T lymphocytes into immunodeficient SCID mice (for the xenograft human model of GVHD).

2. *In vitro* depletion of activated T lymphocytes by incubation with MegaFasL as GVHD prophylaxis for allogenic HSCT

This approach eliminates activated alloreactive T lymphocytes (purging) *in vitro* prior to the transfer into the irradiated host (figure 2).

Results: Activated human T lymphocytes, using anti-CD3 monoclonal antibody (figure 5), PMA (phorbol 12-myristate 13-acetate)/ionomycin (figure 4), PHA (figure 4, 10,

11) or irradiated PBMC of unrelated donor (figure 10 and 11) as activating agents and memory cells (figure 6 and 7) are sensitive to MFL induced cell death in the range of 10 to 200ng/ml. In contrast naïve T cells are resistant to MFL (figure 6 and 7). In this context, alloreactive donor T lymphocytes are activated by irradiated recipient cells in a mixed lymphocyte reaction (MLR). Activated alloreactive T lymphocytes are subsequently eliminated by addition of MegaFasL to the culture in a range dose between 10 to 200 ng/ml. The remaining non-activated and MegaFasL resistant lymphocytes are compatible to the host MHC and are transferred into the irradiated host. The prevention of GVHD is confirmed experimentally by re-infusion of treated cells in HLA-mismatached mice (for the murine model of GHVD) or reinfusion of human T lymphocytes into immunodeficient SCID mice (for the xenograft human model of GVHD).

Previous art: Specific T-cell depletion of alloreactive human T lymphocytes has been used with success using antibodies recognizing activation markers. This depletion of alloreactive T lymphocytes delays the development of GVHD (2). Moreover, alloreactive T lymphocytes have been eliminated using an anti-Fas agonist antibody. Adoptive transfer of donor-derived allogenic T lymphocytes, depleted from alloreactive T cells by the anti-Fas agonist antibody prevent lethal GVHD in a mouse model (3). MFL is a more potent inducer of cell death than soluble FasL or agonist monoclonal antibody against Fas (figure 12). Therefore, sensitivity of activated alloreactive mouse and human T lymphocytes to different doses MegaFasL is tested *in vitro* in a MLR and two parameters are measured: a) the proliferation rate by ³H-thymidine incorporation and b) the apoptosis by flow cytometry using antibodies specific for T cells and activation markers in tandem with Annexin-V or Propidium Iodide (PI) staining to detect cells undergoing apoptosis.

25 3. *In vivo* depletion of activated T lymphocytes by injection MegaFasL after allogenic HSCT

The treatment protocol specifically eliminates activated alloreactive T lymphocyte *in vivo* by injection of MegaFasL, which will prevent the development of GVHD while maintaining a functional immune system (Figure 3).

30 The sensitivity of alloreactive donor T lymphocytes to MegaFasL is determined after transfer into irradiated host. Here, activated T lymphocytes are eliminated after one or several intravenous injection of MegaFasL at 0.001 to 0.020 mg/kg. The prevention of GVHD is confirmed experimentally in transfer experiments using HLA-mismatached mice

(for the murine model of GHVD) or by infusion of human T cells into immunodeficient SCID mice (for the xenograft human model of GVHD).

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2. Isabelle André-Schmutz & al., (2002). Immune reconstitution without graft-versus-host disease after haemopoietic stem-cell transplantation : a phase 1/2 study. *Lancet*, 360 : 130-37
- 10 3. Udo F., Hartwig & al., (2002). Murine acute graft-versus-host disease can be prevented by depletion of alloreactive T lymphocytes using activation-induced cell death. *Blood* 99 (8) : 3041-3049

CLAIMS

1. A method for preventing GVHD for allogenic HSCT prior transplantation, comprising the steps of culturing human PBMC from a donor in an appropriate medium
5 comprising an effective amount of a multimeric form of the soluble portion of FasL.
2. The method of claim 1, wherein human PBMC from the donor are first cultured in presence of irradiated host cells from the patient prior culture in a medium with a multimeric form of the soluble portion of FasL.
3. The method of one of claims 1 or 2, wherein concentration of multimeric
10 form of the soluble portion of FasL in the medium is comprised between 10 and 200 ng/ml.
4. The method of one of claims 1 to 3, wherein the culture in the medium comprising a multimeric form of the soluble portion of FasL is maintained for at least 1 hour, preferably between 3 to 5 hours and up to 24 hours.
5. A method for the prevention and treatment of GVHD for allogenic HSCT
15 after transplantation, wherein the patient is treated by administration of an effective amount of a multimeric form of the soluble portion of FasL.
6. The method of claim 5, wherein administration of the effective amount of the multimeric form of the soluble portion of FasL is done by any suitable method of administration known in the art, preferably by injection, more preferably by intravenous
20 injection.
7. The method of one of claims 6 or 7, wherein the multimeric form of the soluble portion of FasL is administered in a form of a pharmaceutical composition suitable for the chosen administration route, preferably in the form of a solution and/or a suspension in an acceptable carrier.
8. The method of one of claims 5 to 7, wherein the dose of multimeric form of the soluble portion of FasL is comprised between 0,001 and 0,020 mg/kg, in one or several
25 times, the dose per 24 hour being preferably below 0,020 mg/kg.
9. The method of anyone of claims 1 to 8, wherein the multimeric form of the soluble portion of FasL comprises the fusion polypeptide mACRP30:hFasL.

10. The method of claim 9, wherein the amino acid sequence of the fusion polypeptide mACRP30:hFasL is the following:

MAIIYLILLFTAVRGHDQETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGR
DGRDGTTPGEKGEKGDPLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGA
5 KELRKVAHLTGKSNSRSMPLWEDTYGIVLLSGVKYKKGGLVINETGLYFVYSKV
YFRGQSCNNLPLSHKVYMRNSKYPQDLVMMEGKMMSYCTTGQMWARSSYLGA
VFNLTSADHLYVNVSELSLVNFEESTFFGLYKL

Figure 1 :

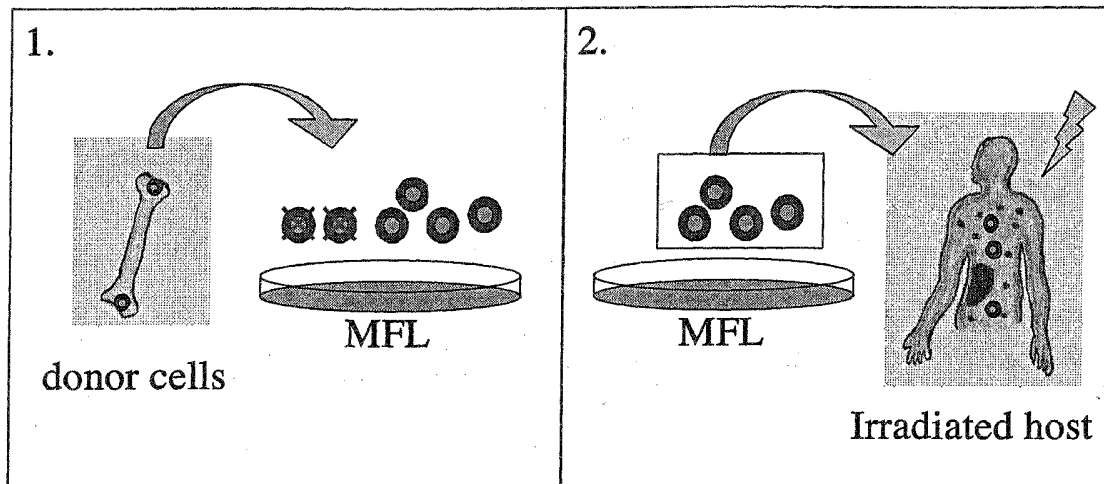
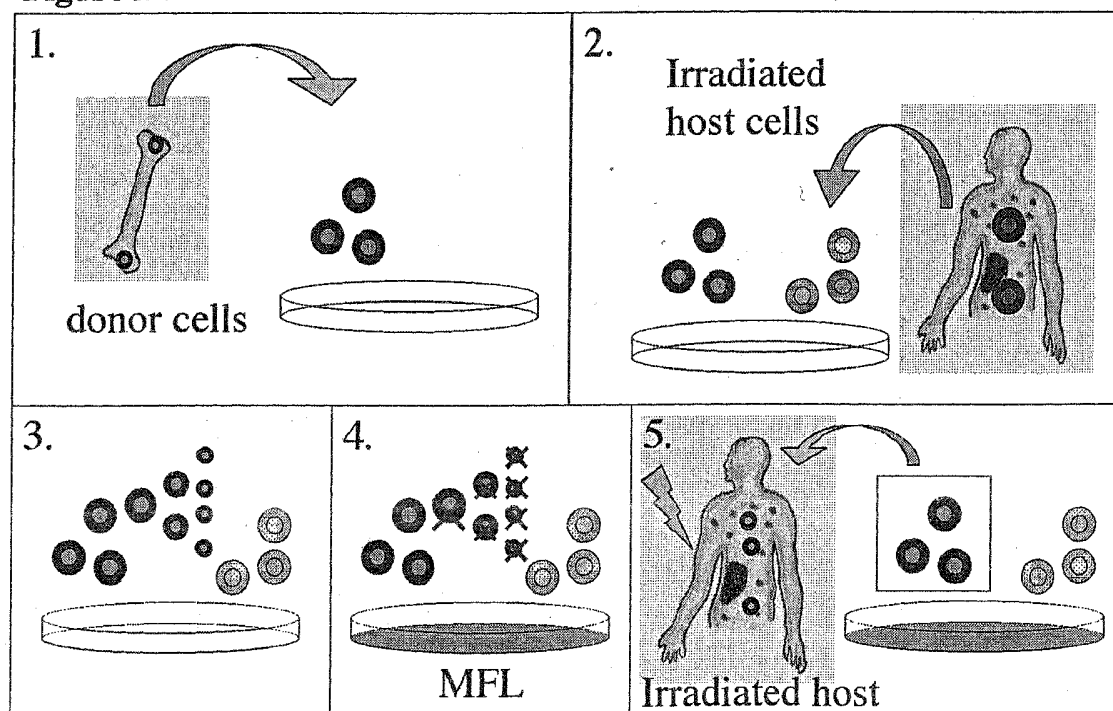


Figure 2 :



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Figure 3 :

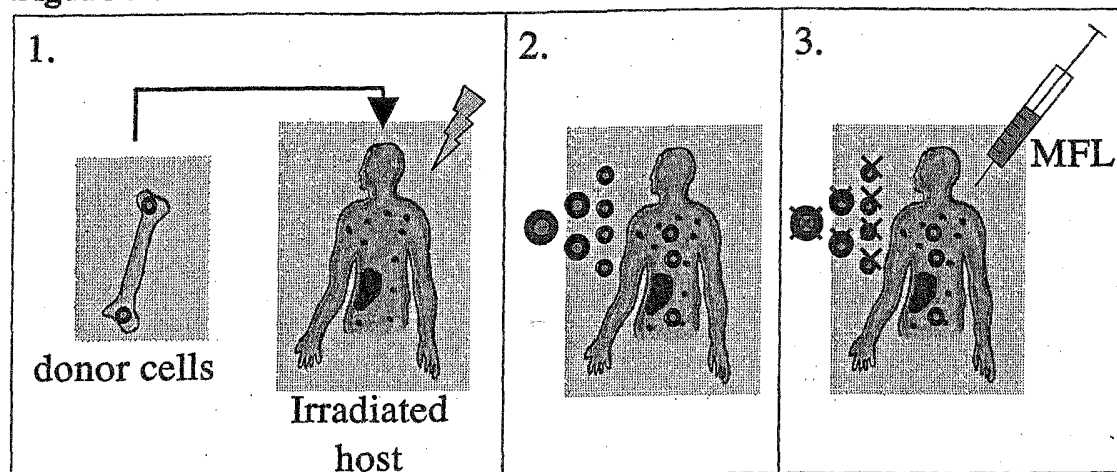
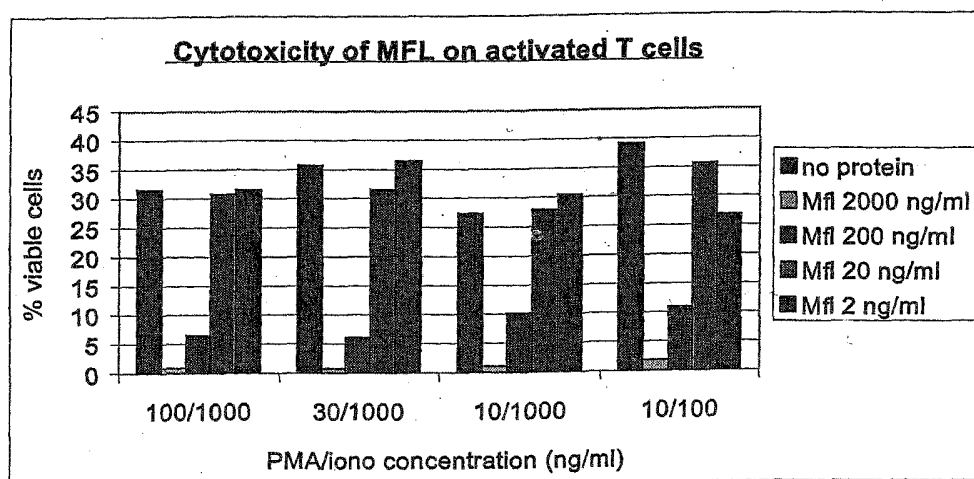


Figure 4 :



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Figure 5 :

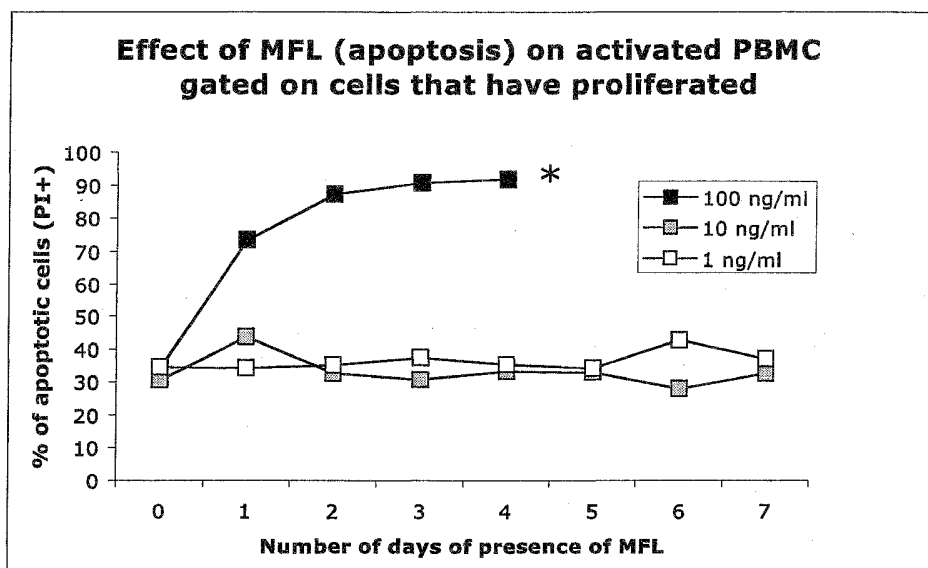
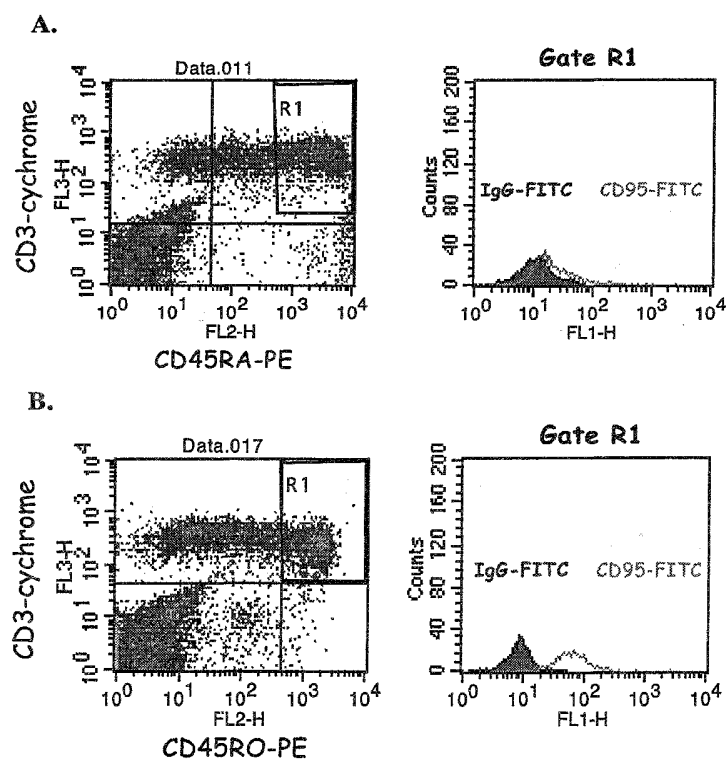


Figure 6 :



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Figure 7 :

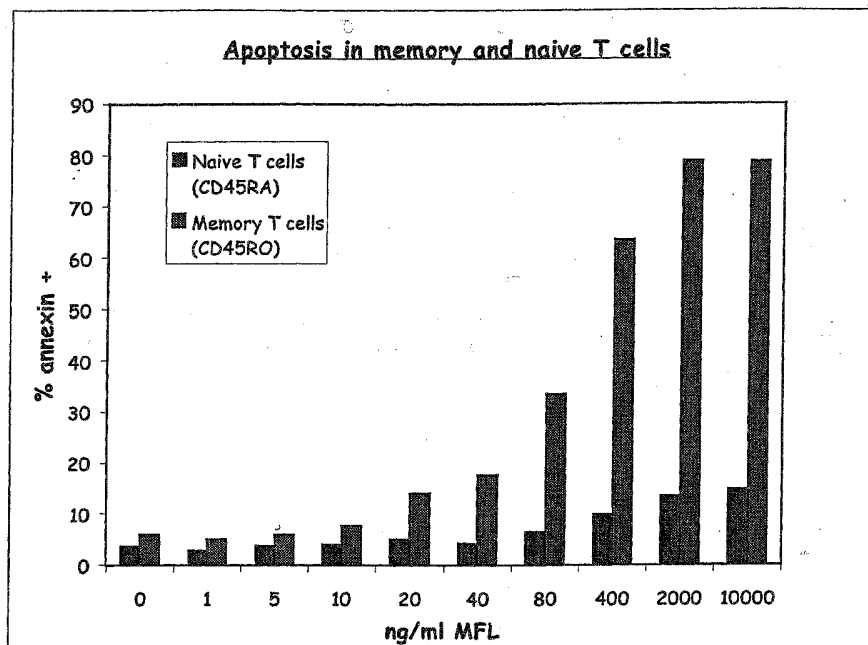


Figure 8 :

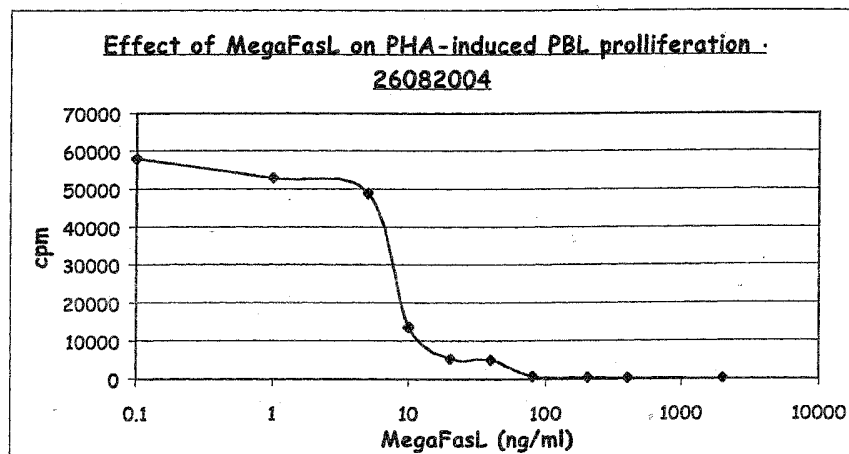


Figure 9 :

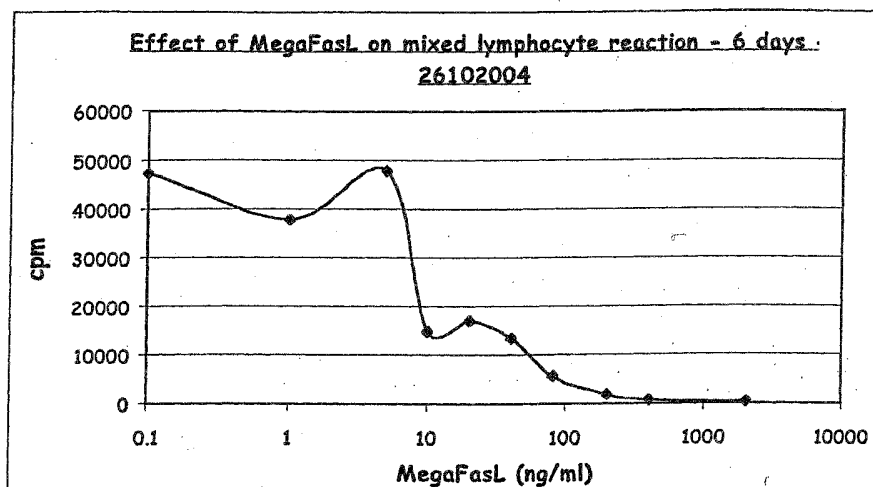
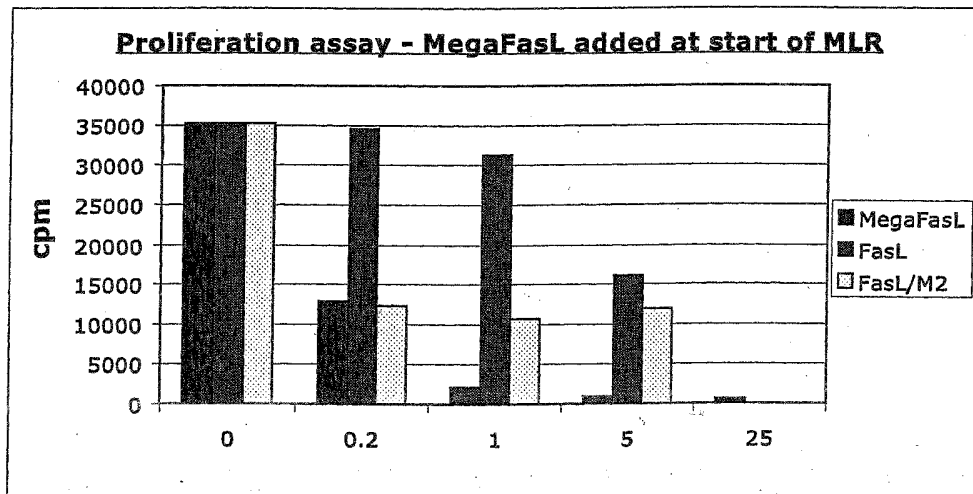


Figure 10 :



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Figure 11 :

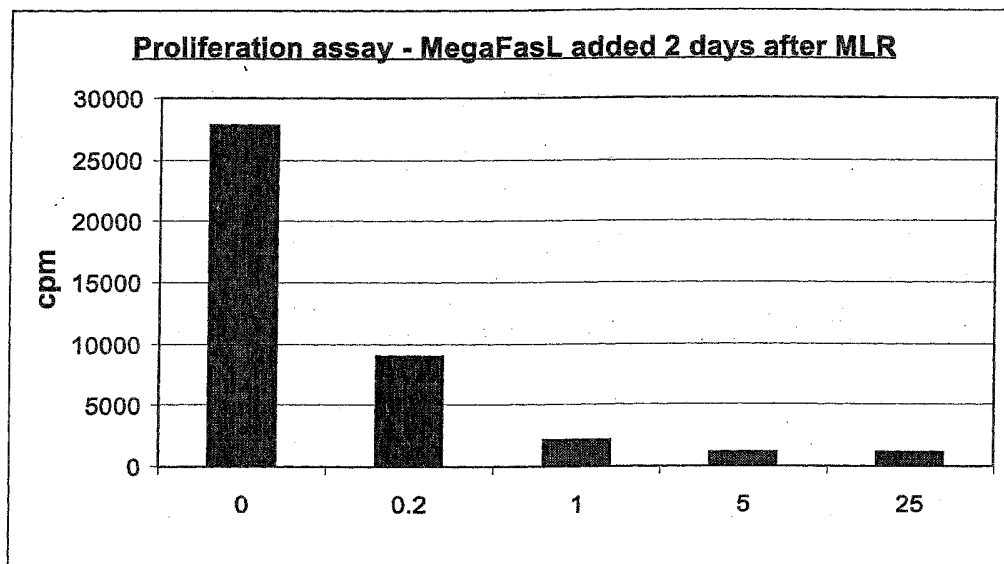


Figure 12 :

