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(54) **Title**: PEPTIDES AND USES THEREOF FOR REDUCING CD95-MEDIATED CELL MOTILITY

(57) **Abstract**: The present invention relates to peptides and uses thereof for reducing cd95 -mediated cell motility. The inventors identified peptide capable to cross the plasma membrane, bind PLCyl and disrupt the desired CD95/PLCyl interaction. In particular, the present invention relates to a peptide comprising an amino sequence of formula of X_{182}-X_{183}-RK-X_{186}-X_{187}-K (SEQ ID NO: 1) wherein X_{182} represents an amino acid selected from the group consisting of A, T, C, K, X_{183} represents an amino acid selected from the group consisting of A and C, X_{186} represents an amino acid selected from the group consisting of A, H, N, and R, and X_{187} represents an amino acid selected from the group consisting of A, K, and L.

PEPTIDES AND USES THEREOF FOR REDUCING CD95-MEDIATED CELL MOTILITY

FIELD OF THE INVENTION:

The present invention relates to peptides and uses thereof for reducing cd95-mediated cell motility.

BACKGROUND OF THE INVENTION:

CD95L is a transmembrane glycoprotein that acts locally through cell-to-cell contact (Suda et al., 1993). The extracellular domain of CD95L comprises a juxtamembrane stalk region (amino acid residues (aa) 103–136)(Orlinick et al., 1997) cleavable by metalloproteases (Fouque et al., 2014), thereby releasing CD95L into the bloodstream. CD95 (Fas/APO-1/TNFRSF6) belongs to the tumor necrosis factor receptor (TNF-R) family and is ubiquitously expressed in the body (Peter et al., 2015). When membrane-bound CD95L binds to CD95, the intracellular region of CD95 (designated the death domain - DD) orchestrates the formation of a death-inducing signaling complex (DISC) by recruitment of the adaptor molecule, Fas-associated protein with death domain (FADD), which in turn induces caspase-8 aggregation and subsequent apoptosis (Kischkel et al., 1995). Contrarily, we and others showed that metalloprotease-cleaved CD95L (cl-CD95L) promotes formation of an atypical molecular complex designated motility-inducing signaling complex (MISC) stimulating non-apoptotic signaling pathways and increasing intracellular calcium (Ca^{2+}) content (Kleber et al., 2008; Malleter et al., 2013; Tauzin et al., 2011). Recently the inventors present evidence that this CD95-mediated Ca^{2+} signaling occurs independently of the CD95-DD and exploiting this result, and they designed a novel therapeutic molecule that selectively neutralizes the CD95-mediated non-apoptotic signaling pathways (WO2015158810).

SUMMARY OF THE INVENTION:

The present invention relates to peptides and uses thereof for reducing cd95-mediated cell motility. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to a peptide comprising an amino sequence of formula of X_{182}-X_{183}-RK-X_{186}-X_{187}-K (SEQ ID NO:1) wherein X_{182} represents an amino acid selected from the group consisting of A, T, C, K, X_{183} represents an amino acid selected from the group consisting of A and C, X_{186} represents an amino acid selected from the group consisting

of A, H, N, and R, and X_{187} represents an amino acid selected from the group consisting of A, K, and L.

As used herein the term "A" or "Ala" has its general meaning in the art and refers to Alanine. As used herein the term "R" or "Arg" has its general meaning in the art and refers to Arginine. As used herein the term "N" or "Asn" has its general meaning in the art and refers to Asparagine. As used herein the term "D" or "Asp" has its general meaning in the art and refers to Aspartic acid. As used herein the term "C" or "Cys" has its general meaning in the art and refers to Cysteine. As used herein the term "E" or "Glu" has its general meaning in the art and refers to Glutamic acid. As used herein the term "Q" or "Gln" has its general meaning in the art and refers to Glutamine. As used herein the term G or "Gly" has its general meaning in the art and refers to Glycine. As used herein the term "H" or "His" has its general meaning in the art and refers to Histidine. As used herein the term "I" or "Ile" has its general meaning in the art and refers to Isoleucine. As used herein the term "L" or "Leu" has its general meaning in the art and refers to Leucine. As used herein the term "K" or "Lys" has its general meaning in the art and refers to Lysine. As used herein the term "M" or "Met" has its general meaning in the art and refers to Methionine. As used herein the term "F" or "Phe" has its general meaning in the art and refers to Phenylalanine. As used herein the term "P" or "Pro" has its general meaning in the art and refers to Proline. As used herein the term "S" or "Ser" has its general meaning in the art and refers to Serine. As used herein the term "T" or "Thr" has its general meaning in the art and refers to Threonine. As used herein the term "W" or "Trp" has its general meaning in the art and refers to Tryptophan. As used herein the term "Y" or "Tyr" has its general meaning in the art and refers to Tyrosine. As used herein the term "V" or "Val" has its general meaning in the art and refers to Valine.

In some embodiments, the peptide of the present invention comprises less than 15 amino acids, preferably less than 10 amino acids. In some embodiments, the peptide of the present invention comprises or consists of 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids.

In some embodiments, the peptide of the present invention comprise an amino acid sequence of X_{182}-X_{183}-RK-X_{186}-X_{187}-KE (SEQ ID NO:2).

In some embodiments, the peptide of the present invention comprises or consists of the amino acid sequence TCRKHRK (SEQ ID NO:3) or TCRKHRKE (SEQ ID NO:4).

In some embodiments, the peptide of the present invention is stapled. A "stapled" peptide is a peptide comprising a selected number of standard or nonstandard amino acids, further comprising at least two moieties capable of undergoing reaction to promote carbon-carbon bond formation, that has been contacted with a reagent to generate at least one cross-

linker between the at least two moieties, which modulates, for example, peptide stability. More particularly "peptide stapling" is a term coined for a synthetic methodology used to covalently join two olefin-containing side chains present in a peptide chain using an olefin metathesis reaction (J. Org. Chem. (2001) 66(16); Blackwell et al., Angew. Chem. Int. Ed. (1998) 37:3281). Stapling of a peptide using a hydrocarbon cross-linker created from an olefin metathesis reaction has been shown to help maintain a peptide's native conformation, particularly under physiological conditions (U.S. Patent Nos. 7,192,713; 7,723,469;7,786,072; U.S. Patent Application Publication Nos: 2010-0184645; 2010-0168388; 2010- 0081611; 2009-0176964; 2009-0149630; 2006-0008848; PCT Application Publication Nos: WO 2010/011313; WO 2008/121767; WO 2008/095063; WO 2008/061192; WO2005/044839; Schafmeister et al., J. Am. Chem. Soc. (2000) 122:5891-5892; Walensky et al., Science (2004) 305: 1466-1470; each of which is incorporated herein by reference in their entirety). The stapled peptide strategy in which an all-hydrocarbon cross-link is generated by olefin metathesis is an efficient approach to increase the helical character of peptides to target a-helical binding motifs. Unlike their unstapled analogues these hydrocarbon-stapled peptides have shown to be a-helical, protease-resistant, and cell permeable.

In some embodiments, the peptide of the present invention is fused to at least one heterologous peptide (i.e. a peptide which is not derived to CD95). In some embodiments, the peptide according to the invention is fused directly or via a spacer to at least one heterologous peptide. In some embodiments, the peptide according to the invention is fused either directly or via a spacer at its C-terminal end to the N-terminal end of the heterologous peptide, or at its N-terminal end to the C-terminal end of the heterologous peptide. As used herein, the term “directly” means that the (first or last) amino acid at the terminal end (N or C-terminal end) of the peptide is fused to the (first or last) amino acid at the terminal end (N or C-terminal end) of the heterologous peptide. In other words, in this embodiment, the last amino acid of the C-terminal end of said peptide is directly linked by a covalent bond to the first amino acid of the N-terminal end of said heterologous peptide, or the first amino acid of the N-terminal end of said peptide is directly linked by a covalent bond to the last amino acid of the C-terminal end of said heterologous peptide. As used herein, the term “spacer” refers to a sequence of at least one amino acid that links the peptide of the present invention to the heterologous peptide. Such a spacer may be useful to prevent steric hindrances.

In some embodiments, the heterologous peptide is a cell-penetrating peptide, a Transactivator of Transcription (TAT) cell penetrating sequence, a cell permeable peptide or a membranous penetrating sequence. The term “cell-penetrating peptides” are well known in

the art and refers to cell permeable sequence or membranous penetrating sequence such as penetratin, TAT mitochondrial penetrating sequence and compounds (Bechara and Sagan, 2013; Jones and Sayers, 2012; Khafagy el and Morishita, 2012; Malhi and Murthy, 2012).

The peptides of the present invention may be produced by any technique known per se in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination. For instance, knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said peptides, by standard techniques for production of amino acid sequences. For instance, they can be synthesized using well-known solid phase method, typically using a commercially available peptide synthesis apparatus and following the manufacturer's instructions. Alternatively, the peptides of the present invention can be synthesized by recombinant DNA techniques as is now well-known in the art. For example, these fragments can be obtained as DNA expression products after incorporation of DNA sequences encoding the desired (poly)peptide into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired peptide, from which they can be later isolated using well-known techniques. Peptides of the present invention can be used in an isolated (e.g., purified) form or contained in a vector, such as a membrane or lipid vesicle (e.g. a liposome).

A further object of the present invention relates to a nucleic acid molecule encoding for a peptide according to the invention.

These nucleic acid sequences can be obtained by conventional methods well known to those skilled in the art. Typically, said nucleic acid is a DNA or RNA molecule, which may be included in a suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or viral vector. So, a further object of the present invention relates to a vector and an expression cassette in which a nucleic acid molecule encoding for a peptide of the present invention is associated with suitable elements for controlling transcription (in particular promoter, enhancer and, optionally, terminator) and, optionally translation, and also the recombinant vectors into which a nucleic acid molecule in accordance with the invention is inserted.

The peptides of the present invention are particularly suitable of reducing CD95-mediated cell motility and thus may find various therapeutic applications.

In some embodiments, the peptide of the present invention is particularly suitable for reducing CD95-mediated cancer cell motility. In some embodiments, the peptides of the present invention are particularly suitable for the treatment of cancer in a subject in need thereof. As used herein, the term "cancer" has its general meaning in the art and includes, but

is not limited to, solid tumors and blood borne tumors. The term cancer includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term "cancer" further encompasses both primary and metastatic cancers. Examples of cancers that may be treated by methods and compositions of the present invention include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous; adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; Sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma;

mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

In some embodiments, the peptide of the present is particularly suitable for the treatment of triple negative breast cancer. As used herein the expression "Triple negative breast cancer" has its general meaning in the art and means that said breast cancer lacks receptors for the hormones estrogen (ER-negative) and progesterone (PR-negative), and for the protein HER2.

In some embodiments, the peptide of the present invention is particularly suitable for the prevention of metastases (e.g. in a subject suffering from a triple negative breast cancer).

In some embodiments, the present invention relates to the peptide of the present invention for use in enhancing therapeutic efficacy of cancer treatment in a subject in need thereof. In some embodiments, the peptide of the present invention may be administered sequentially or concomitantly with one or more therapeutic active agent such as chemotherapeutic or radiotherapeutic agents. Examples of chemotherapeutics include but are not limited to fludarabine, gemcitabine, capecitabine, methotrexate, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, platinum

complexes such as cisplatin, carboplatin and oxaliplatin, mitomycin, dacarbazine, procarbazine, epipodophyllotoxins such as etoposide and teniposide, camptothecins such as irinotecan and topotecan, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, L-asparaginase, doxorubicin, epirubicin, 5-fluorouracil and 5-fluorouracil combined with leucovorin, taxanes such as docetaxel and paclitaxel, levamisole, estramustine, nitrogen mustards, nitrosoureas such as carmustine and lomustine, vinca alkaloids such as vinblastine, vincristine, vindesine and vinorelbine, imatinib mesylate, hexamethylmelamine, kinase inhibitors, phosphatase inhibitors, ATPase inhibitors, tyrphostins, protease inhibitors, inhibitors herbimycin A, genistein, erbstatin, and lavendustin A. In some embodiments, additional therapeutic active agents may be selected from, but are not limited to, one or a combination of the following class of agents: alkylating agents, plant alkaloids, DNA topoisomerase inhibitors, anti-folates, pyrimidine analogs, purine analogs, DNA antimetabolites, taxanes, podophyllotoxins, hormonal therapies, retinoids, photosensitizers or photodynamic therapies, angiogenesis inhibitors, antimitotic agents, isoprenylation inhibitors, cell cycle inhibitors, actinomycin, bleomycin, anthracyclines, MDR inhibitors and Ca^{2+} ATPase inhibitors. The term “radiotherapeutic agent” as used herein, is intended to refer to any radiotherapeutic agent known to one of skill in the art to be effective to treat or ameliorate cancer, without limitation. For instance, the radiotherapeutic agent can be an agent such as those administered in brachytherapy or radionuclide therapy. Such methods can optionally further comprise the administration of one or more additional cancer therapies, such as, but not limited to, chemotherapies, and/or another radiotherapy.

In some embodiments, the peptide of the present invention is particularly suitable for reducing CD95-mediated lymphocyte (e.g. T cell) motility. In some embodiments, the peptide of the present invention is particularly suitable for the treatment of an auto-immune disease. As used herein, an "autoimmune disease" is a disease or disorder arising from and directed at an individual's own tissues. In some embodiments, the peptide of the present invention is particularly suitable for the treatment of an inflammatory condition.

The term "inflammatory condition" as used herein refers to acute or chronic localized or systemic responses to harmful stimuli, such as pathogens, damaged cells, physical injury or irritants, that are mediated in part by the activity of cytokines, chemokines, or inflammatory cells (e.g., neutrophils, monocytes, lymphocytes, macrophages) and is characterized in most instances by pain, redness, swelling, and impairment of tissue function. In some embodiments, the autoimmune disease or inflammatory condition is selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty

arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis (MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis,

vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA

nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome,

minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic

hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

In some embodiments, the peptide of the present invention is particularly suitable for the treatment of systemic lupus erythematosus.

In some embodiments, the peptide of the present invention is particularly suitable for preventing Th17 cell transmigration. Accordingly, the peptide of the present invention is particularly suitable for treating Th17 mediated diseases. The term "Th17-mediated disease" is used herein in the broadest sense and includes all diseases and pathological conditions the pathogenesis of which involves abnormalities of Th17 cells. As used herein, the term "Th17 cells" has its general meaning in the art and refers to a subset of T helper cells producing interleukin 17 (IL-17). "A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage". Nat. Med. 13 (2): 139-145.). The term "IL-17" has its general meaning in the art and refers to the interleukin-17A protein. Typically, Th17 cells are characterized by classical expression of Th cell markers at their cell surface such as CD4, and by the expression of IL17. Typically, as referenced herein, a Th17 cell is a IL-17+ cell. Examples of Th17 mediated diseases include but are not limited to autoimmune diseases, inflammatory diseases, osteoclasia, and transplantation rejection of cells, tissue and organs. In particular, the above-mentioned Th17-mediated diseases may be one or more selected from the group consisting of Behçet's disease, polymyositis/dermatomyositis, autoimmune cytopenias, autoimmune myocarditis, primary liver cirrhosis, Goodpasture's syndrome, autoimmune meningitis, Sjögren's syndrome, systemic lupus erythematosus, Addison's disease, alopecia greata, ankylosing spondylitis, autoimmune hepatitis, autoimmune mumps, Crohn's disease, insulin-dependent diabetes mellitus, dystrophic epidermolysis bullosa, epididymitis, glomerulonephritis, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, hemolytic anemia, multiple sclerosis, myasthenia gravis, pemphigus vulgaris, psoriasis, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroma, spondyloarthropathy, thyroiditis, vasculitis, vitiligo, myxedema, pernicious anemia and ulcerative colitis.

Typically, the peptide of the present invention is administered to the subject in a therapeutically effective amount. By a "therapeutically effective amount" is meant a sufficient amount of the peptide of the present invention for reaching a therapeutic effect (e.g. treating cancer). It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidential with the specific peptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, typically from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

Typically, the peptide of the present invention is combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Typically, the pharmaceutical compositions contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological

saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Sterile injectable solutions are prepared by incorporating the Peptide at the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. A. Each amino acid in CID sequence was replaced by alanine (alanine scanning) and all constructs were co-transfected with SH3-PLCγ1 in HEK cells. For each co-transfection, luminescence was assessed and a ratio was calculated as follows: (luminescence for mutated construct/ luminescence for wild type CID) × 100). For each condition, this value was next multiplied by the relative expression level of CID-F2 and SH3-PLCγ1-F1, evaluated by densitometric analysis of proteins as follows: ((densitometric value of mutated CID-F2 construct/ densitometric value of wild type CID-F2) × (densitometric value of co-transfected SH3-PLCγ1-F1/ densitometric value of SH3-PLCγ1-F1 co-transfected with wild type CID)). B. CID sequence of wild type CD95 (TCRKHRK) underwent double (TCAAHRK) or triple (TCAAHRA) mutations and these constructs were transiently transfected in HEK cells. Calcium signal was assessed using fluo2-AM (1 μM). Cells were stimulated with cl-CD95L (100 ng/mL; arrow), and the intracellular calcium concentration ([Ca2+]i) was monitored by measuring the F/F0 ratio (relative cytoplasmic calcium concentration ([Ca2+]CYT)). Recruitment of PLCγ1 was evaluated in HEK cells transfected with wild type, double or triple

CD95 constructs using co-immunoprecipitation experiments. Data are representative of three independently performed experiments.

Figure 2. A. CID-CD95 (aa 175–210) or DD-CD95 (aa 210-303)-F2 were co-transfected into HEK cells with the indicated domains of PLCγ1 (Ca2+ response) or FADD (apoptosis) fused to F1. Light emission indicates refolding of the luciferase and reconstitution of enzyme activity through protein/protein interactions. The inhibition of light emission for the indicated PPIs was assessed in cells incubated for the indicated times in the presence of TAT-CID (25 μM). Data represent means ± SD of three independent experiments. B. Schematic representation of the different CID constructs and the peptide control. C. Left panel: The PLCγ1-SH3 domain (aa 790–851)-F1 was co-transfected into HEK cells with the whole CID-CD95 (aa 175–210)-F2. Then, the indicated peptides were incubated for 4 hrs at the indicated concentrations and luminescence was assessed. Data represent means ± SD of three independent experiments. Right panel: DD-FADD-F1 was co-transfected into HEK cells with DD-CD95-F2. Then, the indicated peptides were incubated at the indicated concentrations for 4 hrs and luminescence was assessed. Data represent means ± the SD of three independent experiments. D. CID-CD95-F2 was co-transfected into HEK cells with SH3-PLCγ1-F1. After 24 hours, cells were incubated for 4 hrs with indicated peptides (25 μM) and light emission was assessed. Data represent means ± SD of the percentage of light emission inhibition of three independent experiments. Percentage of light emission inhibition was measured as follows: [100 - (light emission measured with peptide treatment / light emission measured without treatment (maximum)) x 100].

Figure 3: A. Th17 cells were pre-incubated for 1 h with TAT-control, TAT-minCID or minCID (1 μM) and then stimulated with cl-CD95L (100 ng/mL). [Ca2+]CYT was assessed in FuraPE3-AM (1 μM)-loaded cells. Data represent means ± the SD of 3 independent experiments. B. Th17 cells were pre-incubated for 1 h with TAT-control, TAT-CID, TAT-minCID or minCID at 1 μM and then stimulated with cl-CD95L (100 ng/mL). Endothelial T-cell transmigration was assessed by Boyden chamber. Data represent means ± the SD of three independent experiments.

EXAMPLE 1:

Some of the results presented herein after were already disclosed in the international patent application WO2015158810 and are reminded here for the comprehensive teaching of the present disclosure.

CD95 triggers a DD-independent Ca^{2+} response

We previously showed that engagement of CD95 evoked a Ca^{2+} response in activated T lymphocytes, resulting in transient inhibition of cellular apoptosis (Khadra et al., 2011) and cell migration (Malleter et al., 2013; Tauzin et al., 2011). Due to the instrumental role of the CD95-mediated apoptotic signal in anti-infectious and anti-tumor responses, we assumed that the inhibition of the CD95 non-apoptotic responses while conserving the apoptotic signal could be an attractive therapeutic option to prevent Th17 recruitment in inflamed organs without altering immune surveillance. We then wondered whether it was possible to selectively inhibit the CD95-mediated Ca^{2+} response and whether this blockade might inhibit cell migration without blocking the apoptotic signal. Human T-cells exposed to cl-CD95L rapidly formed a molecular complex containing PLCγ1 and the lack of PLCγ1 in T-cells resulted in a loss of CD95-facilitated Ca^{2+} signaling. We next investigated whether the predominant DISC components, FADD and caspase-8, might contribute to CD95-mediated Ca^{2+} signaling. Elimination of FADD and caspase-8 blocked apoptotic signaling, but not CD95-mediated Ca^{2+} signaling suggesting that PLCγ1 activation occurs independently of CD95-DISC and cell death signaling. To further investigate whether the death domain (DD) of CD95 was involved in the Ca^{2+} response, we generated CD95 constructs devoid of the entire intracellular domain of CD95 ($CD95^{1-175}$), the DD ($CD95^{1-210}$), or the last 15 aa involved in FAP-1 protein tyrosine phosphatase recruitment(Sato et al., 1995) ($CD95^{1-303}$). These constructs were expressed in the CEM-IRC T-cell line, which was selected for its low CD95 expression (Beneteau et al., 2008).

CEM-IRC cells showed minimal cell death in response to a multimeric (dodecamer) and cytotoxic CD95L (IgCD95L); however, expression of $CD95^{1-303}$ or wild-type CD95 restored cell death levels to those observed for parental CEM cells. Contrarily, introduction of $CD95^{1-175}$ or $CD95^{1-210}$ failed to induce apoptosis and, as previously observed, these constructs behaved as dominant-negative receptors (Siegel et al., 2000). Furthermore, reconstituting CEM-IRC cells with wild-type CD95 or $CD95^{1-303}$ restored CD95-mediated Ca^{2+} signaling. Of interest, while the loss of the DD from the $CD95^{1-210}$ construct prevented apoptotic signaling, CD95-DD deletion did not affect induction of Ca^{2+} signaling. Given that a CD95 construct devoid of the entire intracellular region ($CD95^{1-175}$) failed to evoke a Ca^{2+} response, we concluded that Ca^{2+} signaling is triggered by CD95 aa 175–210.

We next examined whether $CD95^{1-210}$ was capable of recruiting PLCγ1. To this end, we transiently co-transfected HEK cells with green fluorescent protein (GFP)-fused CD95 constructs and wild-type PLCγ1. Although the transfected cells expressed similar levels of CD95-GFP chimeric constructs at the cell surface, PLCγ1 was absent only from the $CD95^{1-175}$

immunoprecipitate. To demonstrate that amino acid residues 175-210 of CD95 contributes to PLCγ1 recruitment, we next generated a construct comprising $CD95^{175–210}$ (termed the calcium-inducing domain (CID)) fused to mCherry. Unlike mCherry alone, $CD95^{175–210}$/mCherry interacted with PLCγ1 and inhibited its recruitment to CD95, suggesting that interference with this juxtamembrane domain may prevent CD95-mediated Ca^{2+} signaling.

To confirm this hypothesis, we synthesized a cell-penetrating peptide, TAT-CID, by linking CID to the nine-aa human immunodeficiency virus (HIV)-TAT sequence, which serves as a carrier for protein translocation across the plasma membrane (Vives et al., 1997). Pre-incubation of activated peripheral blood lymphocytes (PBLs) with TAT-CID impaired the recruitment of PLCγ1 and abolished the induction of CD95-mediated Ca^{2+} signaling. Similarly, pre-incubation of Jurkat cells with TAT-CID inhibited PLCγ1 binding to CD95 and abrogated the CD95-facilitated Ca^{2+} response. TAT-CID also inhibited Akt phosphorylation at serine 473 (a hallmark of PI3K signaling activation) in cl-CD95L-exposed PBLs. Nevertheless, although TAT-CID impeded CD95-mediated Ca^{2+} and PI3K signaling, the peptide did not affect the apoptotic signaling pathway. Hence, we mapped a novel domain in CD95 receptor, namely CID, which recruits PLCγ1 and elicits Ca^{2+} responses.

CID interacts with the SH3 domain of PLCγ1

To address whether CD95 directly interacts with PLCγ1, we performed a Renilla luciferase-based protein fragment complementation assay (Stefan et al., 2007). The Renilla luciferase enzyme was divided into two fragments, the amino-terminal fragment (F1) and the carboxy-terminal fragment (F2). Each fragment was fused to various CD95 and PLCγ1 domains. Different domains of PLCγ1 fused to F1 were co-expressed with the whole intracellular region of CD95, CD95-CID, or CD95-DD fused to F2. Only PLCγ1-SH3 interacted with the whole CD95 intracellular region and CD95-CID, but failed to reconstitute enzyme activity when combined with CD95-DD. To strengthen this result, we evaluated whether the cell-penetrating TAT-CID peptide could inhibit the PLCγ1-SH3/CD95-CID interaction. While TAT-control peptide did not alter luciferase activity in cells co-expressing PLCγ1-SH3-F1 and CD95-CID-F2, TAT-CID efficiently blocked this activity in a dose-dependent manner, supporting the hypothesis that CD95 directly associates with PLCγ1 through the CD95-CID domain. Intriguingly, SH3 domains primary bind to peptides containing a consensus PxxP sequence that is not present in CID; however, many examples of unconventional SH3-binding peptides have previously been described (Saksela and Permi, 2012).

To understand how CD95 CID might interact with PLCγ1-SH3, we undertook two computational peptide screenings (Deng et al., 2005). First, we performed homology modeling by using the crystal structure of PLCγ1-SH3 in association with the SLP-76 heptapeptide complex (PDB:1YWO) (Deng et al., 2005). This structure served as a template, and the SLP-76 peptide was replaced by each heptapeptide combination present in CD95-CID. Second, a protein-peptide docking approach was carried out by using each aforementioned CID heptapeptide alternatively docked within the PLCγ1-SH3 domain. ***Both methods predicted that aa 182–188 (TCRKHRK) would have the highest affinity for PLCγ1-SH3, and the calculated binding energy of the PLCγ1-SH3/TCRKHRK complex was similar to that of the PLCγ1-SH3/SLP-76 complex (Table 1).*** An alanine-scanning experiment indicated that amino acid residues R184, K185, K188 and E189 were instrumental in the CID-CD95/ SH3-PLCγ1 interaction as measured by protein complementation assay (Figure 1A) and in CD95-mediated Ca^{2+} response (Figure 1B). In agreement with this cell-based assay, our computer model pointed out that R184 guanidine interacted with SH3-PLCγ1 through a hydrogen bond network involving Y845 and Q805 side-chains and K803 backbone. K185 amine function formed hydrogen bonds, in an intramolecular manner, with H186 backbone and with G826 backbone of SH3-PLCγ1. A salt bridge occurred between K188 and E825. Furthermore, the positioning of K188 in the SH3 cavity required the displacement of a water molecule (H_2O105 observed in the crystal structure), an entropically favourable process. Most importantly for this solvent exposed interface, the methylene chain of these basic residues form extensive van der Waals contacts with the SH3 hydrophobic shallow pockets. Overall, these observations indicated that even if CID sequence is not related to a typical class II ligand of SH3, it complies with its properties in terms of van der Waals interactions (Deng et al., 2005). PCA also revealed that glutamic acid 189, localized outside the minimal TAT-CID domain, participated in the CD95/PLCγ1 binding. Although *in silico* analyses indicated that E189 made few interactions with PLCγ1 SH3 domain, only forming a salt bridge with R806, this amino acid showed a role of "compass residue" evoked for typical proline-rich SH3 ligands (Musacchio, 2002). Indeed, the canonical PxxP motif-containing peptides could be docked in two opposite orientations regarding the relative positioning of a positively charged residue (+xxPxxP or xPxxPx+) interacting with a negatively charged cleft on the SH3 surface (Saksela and Permi, 2012). Similarly to this basic amino acid in PXXP motif, E189 could contribute to the positioning of the minimal CD95-CID peptide in PLCγ1 SH3 domain. Overall, these findings indicated that the CD95-CID amino-terminal region corresponded to an unconventional SH3-binding peptide.

To confirm our in silico result, we synthesized two cell-penetrating peptides consisting of the amino- and carboxyl-terminal regions of CD95 CID (CID-N, corresponding to aa 175–192, and CID-C, corresponding to aa 193–210). We then evaluated their effects on the CD95-mediated Ca^{2+} response, and showed that only CID-N abrogated CD95-mediated Ca^{2+} signaling in Jurkat cells and activated PBLs. Taken together, these findings demonstrate that the juxtamembrane region of CD95 directly interacts with PLCγ1-SH3 to evoke Ca^{2+} signaling.

Lastly, we examined whether the inhibitory actions of TAT-CID were selective for CD95-mediated Ca^{2+} signaling. Although T-cell receptor (TCR) stimulation led to a PLCγ1-dependent Ca^{2+} response in Jurkat cells, pre-treatment with the TAT-CID peptide had no such effect. Similarly, TAT-CID did not influence the PLCβ-driven Ca^{2+} response stimulated by carbachol, a cholinergic agonist that activates G-protein-coupled receptors to release calcium. Therefore, TAT-CID selectively inhibits CD95-mediated Ca^{2+} signaling.

	interaction energy (kcal/mol)	**van der Waals energy (kcal/mol)**	**Ligand Contact Surface Area (Å)**	**Receptor Contact Surface Area (Å)**	**RMSD vs 1YWO all heavy atoms (Å)**	**RMSD vs 1YWO backbone atoms (Å)**
Predicted SH3-PLCγ1 / CID-CD95 complex (TCRKHRK peptide)						
protein protein docking	-122	-26.5	203	200	na	na
homology modelling	-143	-24.4	225	222	na	na
SH3-PLCγ1 / SLP-76 complex (PPVPPQR peptide (SEQ ID NO:7))						
PDB : 1YWO	-118	-35	238	229	0	0
redocking (pose 6)	-90	-40	238	232	6.6	5.1
redocking (pose 29)	-118	-37	243	233	2.2	2.2
protein protein docking (pose 10)	-98	-36	220	218	5.5	3.6

protein protein docking (pose 20)	-138	-30	236	213	3.9	2.5

Table 1. Calculated binding energies of CID heptapeptides to PLCγ1-SH3 domain. The binding energy computed for the best complex obtained by the two molecular modelling approaches is compared to calculated binding energy of the PLCγ1 SH3 - SLP-76 complex. This control is either the co-crystallized molecules (PDB:1YWO), redocking of SLP-76 (protein-protein docking, using co-crystallized conformations), or protein-protein docking using the same protocol as for CID docking (short molecular dynamics simulation of the free peptide, then protein-protein docking).

EXAMPLE 2:

The minimum CID is a cell-penetrating peptide

We identified the minimum CID (minCID) interacting with PLCγ1, and synthesized a peptidomimetic of this domain. Two computational analyses revealed that amino acids 182 to 188 (TCRKHRK), namely minCID, of CD95 had the highest affinity for PLCγ1-SH3 and the calculated binding energy from the molecular docking of the complex consisting of PLCγ1-SH3 and TCRKHRK was similar to that of PLCγ1 with its natural ligand SLP-76 (185QPPVPPQRPM194). We then evaluated whether, like the complete 36 amino-acid sequence of CID, the heptapeptide minCID would be able to disrupt the CD95/PLCγ1 interaction. Because inhibition of CD95/PLCγ1 by TAT-CID increased in a time dependent manner (Fig.2A), we next performed the PCA experiments incubating cells with peptides for 4 hours. TAT-CID and TAT-minCID (Fig.2B) disrupted CD95/PLCγ1 interaction with similar efficacy (IC50 10.79 vs 11.47 μM, respectively) (Fig.2C) but they failed to alter CD95/FADD interaction (Fig.2C) confirming the favorable selectivity of these molecules. By contrast, amino acid residues 175 to 181 fused to TAT (Fig.2D) failed to block CD95/ PLCγ1 (Fig.2C) interaction confirming that the optimal sequence of CD95 interacting with PLCγ1 was TCRKHRK. Of note, minCID is short and positively charged, key features of a cell-penetrating peptide (CPPs). Therefore, we next evaluated whether minCID, lacking the TAT sequence, could exert inhibitory effect on the CD95/PLCγ1 interaction in non-permeabilized cells. Although to a lesser extent than TAT-CID (IC50 35.48 vs 11.47 μM, Fig.2C), minCID disrupted the CD95/PLCγ1 interaction (Fig.2C) and did not inhibit CD95/FADD interaction (Fig.2C). Overall, these findings highlighted that minCID is capable to cross the plasma membrane, bind PLCγ1 and disrupt the desired CD95/PLCγ1 interaction.

TAT-minCID and minCID inhibit endothelial transmigration of Th17 cells

Next we evaluated the effect of these CPPs on Th17 cells. Strikingly, TAT-minCID and minCID abrogated the CD95-mediated Ca2+ response in Th17 cells (Fig.3A) and thereby prevented Th17 trafficking across endothelial cells (Fig.3B).

REFERENCES:

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

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CLAIMS:

1. A peptide comprising an amino sequence of formula of X_{182}-X_{183}-RK-X_{186}-X_{187}-K (SEQ ID NO:1) wherein X_{182} represents an amino acid selected from the group consisting of A, T, C, K, X_{183} represents an amino acid selected from the group consisting of A and C, X_{186} represents an amino acid selected from the group consisting of A, H, N, and R, and X_{187} represents an amino acid selected from the group consisting of A, K, and L.

2. The peptide of claim 1 which comprises less than 15 amino acids, or preferably less than 10 amino acids.

3. The peptide of claim 1 which comprises or consists of 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids.

4. The peptide of claim 1 which comprises an amino acid sequence of X182-X183-RK-X186-X187-KE (SEQ ID NO:2).

5. The peptide of claim 1 which comprises or consists of the amino acid sequence TCRKHRK (SEQ ID NO:3) or TCRKHRKE (SEQ ID NO:4).

6. The peptide of claim 1 which is fused to at least one heterologous peptide.

7. The peptide of claim 6 wherein the heterologous peptide is a cell-penetrating peptide, a Transactivator of Transcription (TAT) cell penetrating sequence, a cell permeable peptide or a membranous penetrating sequence.

8. A nucleic acid molecule encoding for the peptide of claim 1.

9. A method of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the peptide of claim 1.

10. The method of claim 9 wherein the cancer is a triple negative breast cancer.

11. A method of treating an autoimmune disease or inflammatory condition in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the peptide of claim 1.

12. The method of claim 11 wherein the autoimmune disease or inflammatory condition is selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis (MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid,

alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or

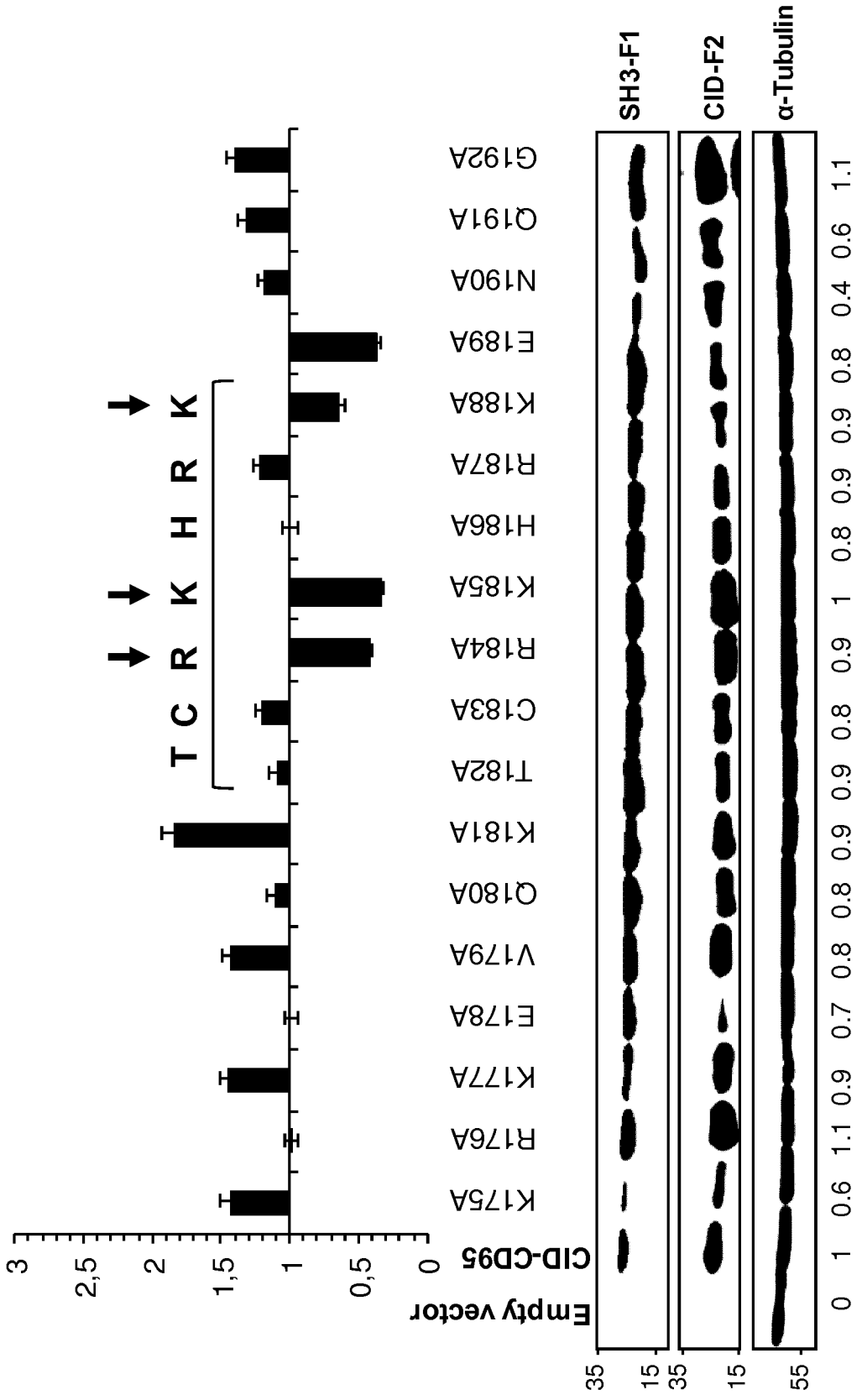
Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis,

endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral

neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

13. The method of claim 12 wherein the subject suffers from systemic lupus erythematosus.

14. A pharmaceutical composition comprising the peptide of claim 1.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K181A
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C183A
R184A
K185A
H186A
R187A
K188A
E189A
N190A
Q191A
G192A
T C R K K H R K
0 0,5 1 1,5 2 2,5 3
35 15 35 15 55
0 1 0.6 1.1 0.9 0.7 0.8 0.8 0.9 0.9 0.9 0.8 0.9 1 0.8 0.9 0.9 0.4 0.8 0.6 1.1

Figure 1A

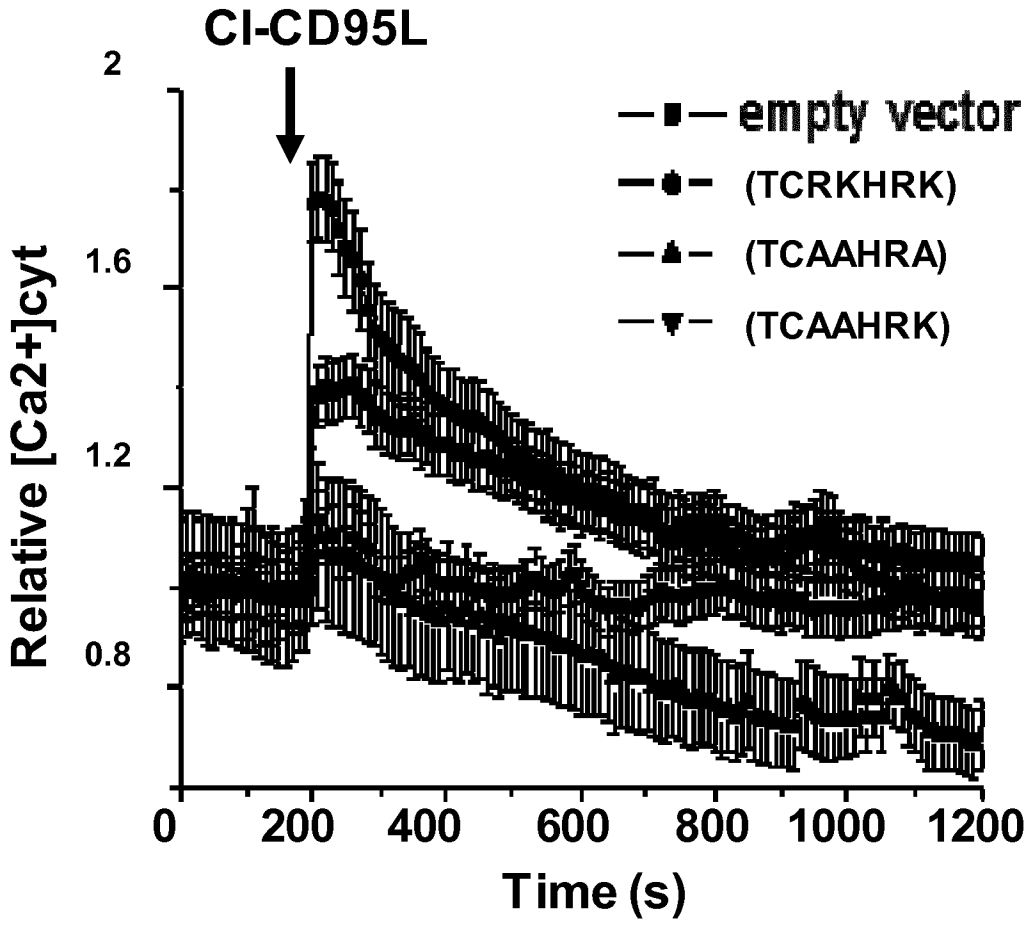
Cl-CD95L
2
1.6
1.2
0.8
Relative [Ca2+]cyt
empty vector
(TCRKHRK)
(TCAAHRA)
(TCAAHRK)
0
200
400
600
800
1000
1200
Time (s)

Figure 1B

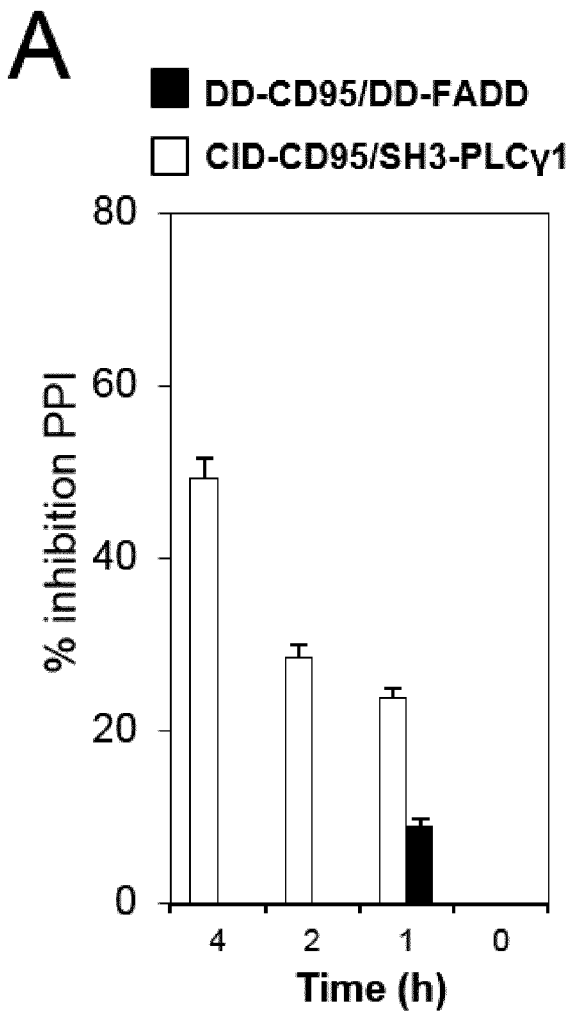

Figure 2A

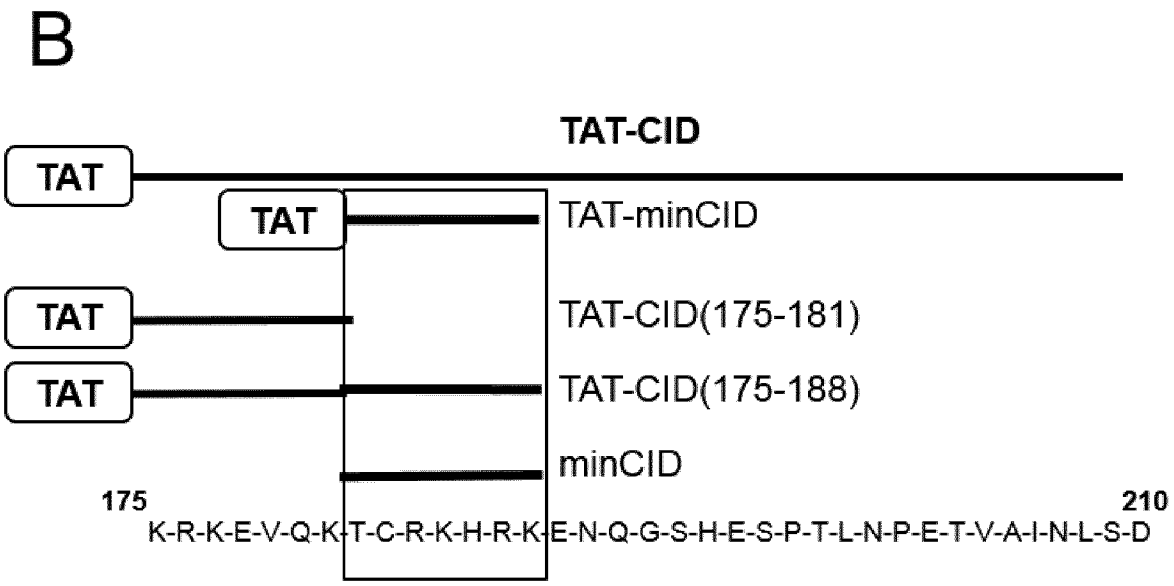

Figure 2B

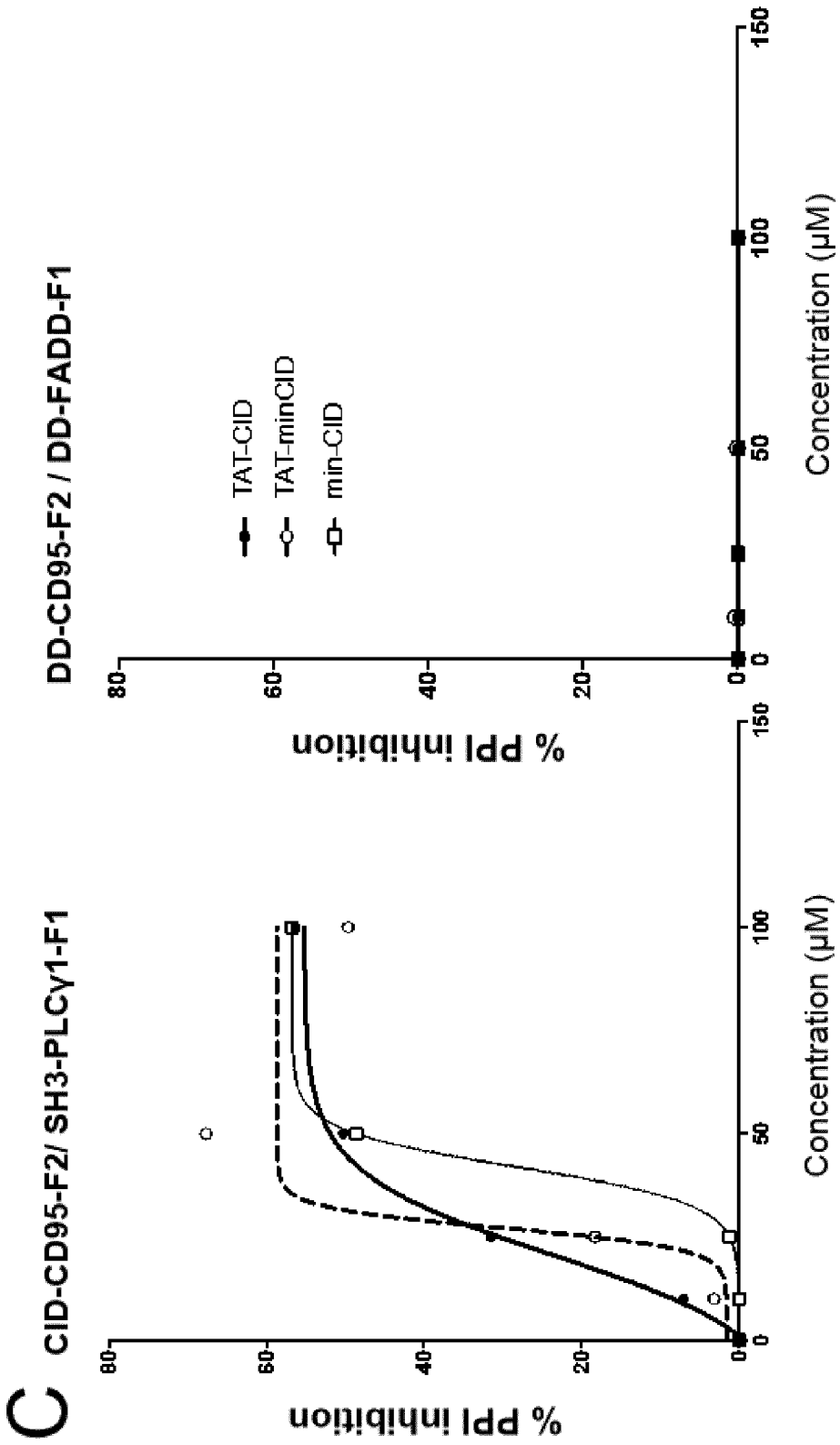
C
CID-CD95-F2/ SH3-PLCγ1-F1
% PPI inhibition
Concentration (μM)
DD-CD95-F2 / DD-FADD-F1
TAT-CID
TAT-minCID
min-CID
% PPI inhibition
Concentration (μM)

Figure 2C

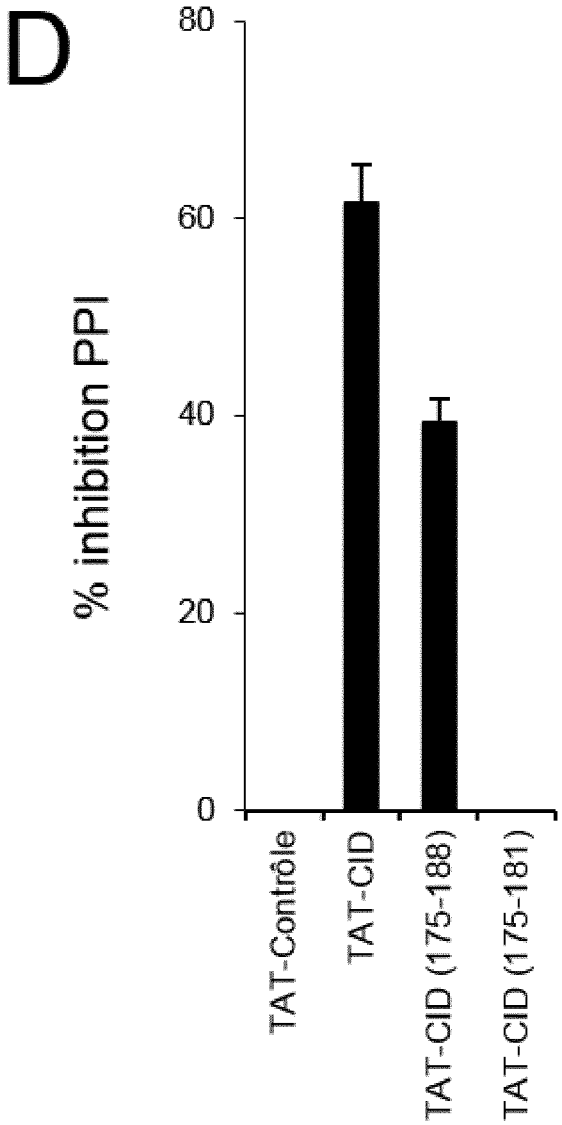
D
% inhibition PPI
80
60
40
20
0
TAT-Contrôle
TAT-CID
TAT-CID (175-188)
TAT-CID (175-181)

Figure 2D

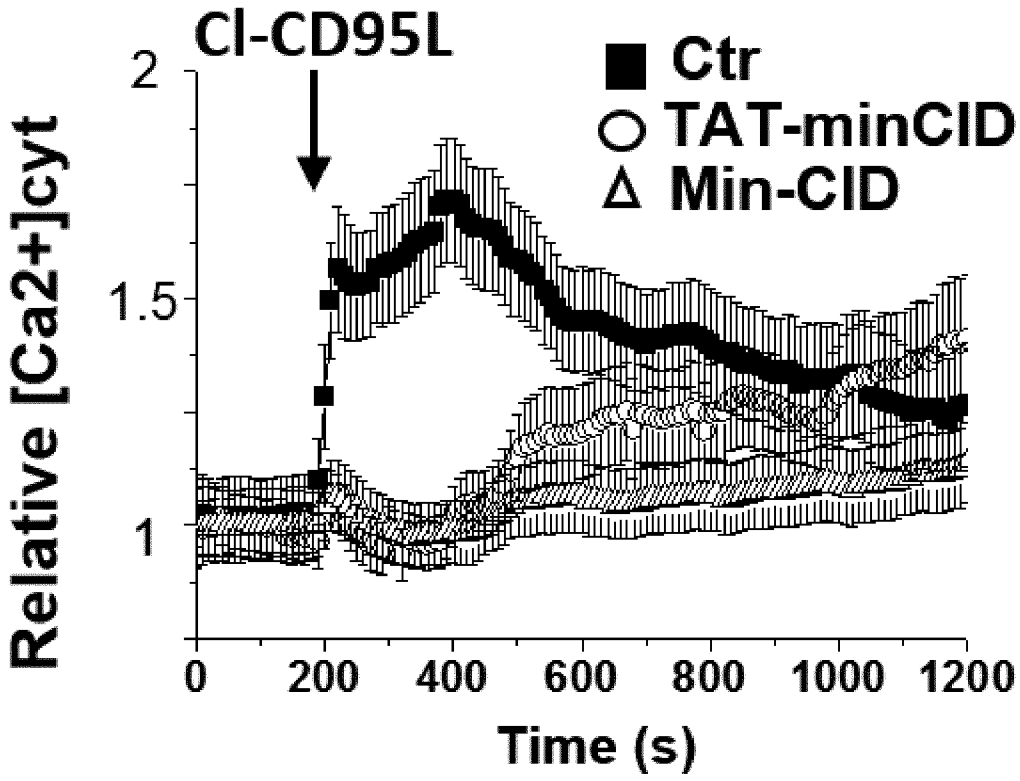
Cl-CD95L
Ctr
TAT-minCID
Min-CID
Relative [Ca2+]cyt
2
1.5
1
0
200
400
600
800
1000
1200
Time (s)

Figure 3A

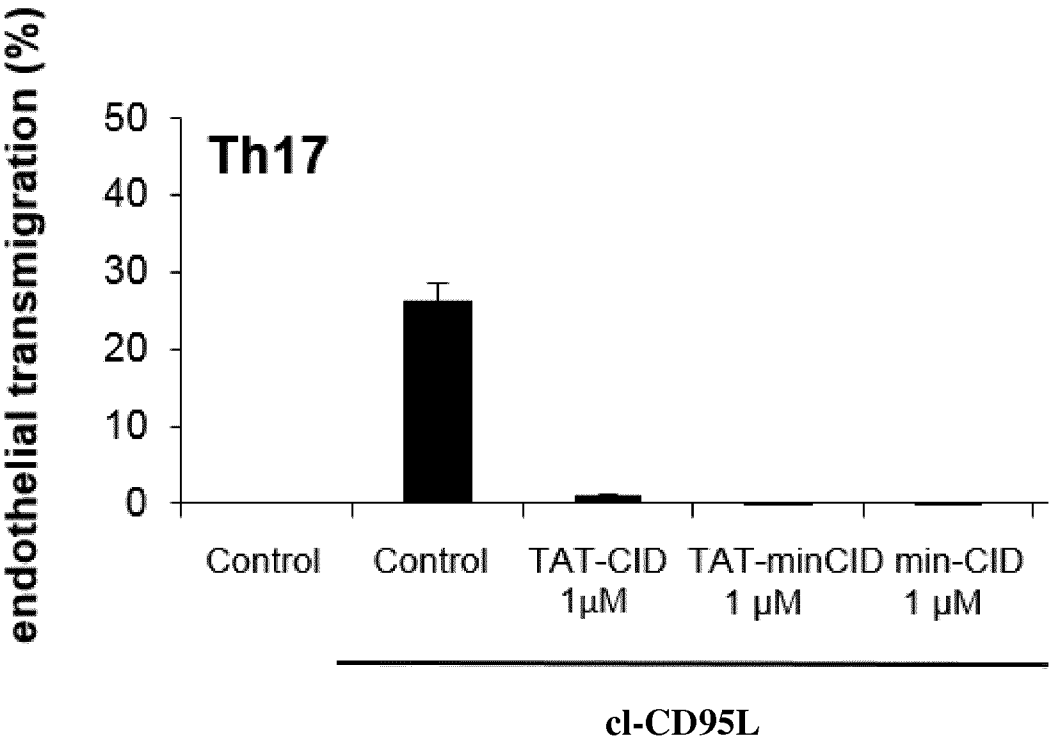
endothelial transmigration (%)
Th17
50
40
30
20
10
0
Control
Control
TAT-CID 1µM
TAT-minCID 1 µM
min-CID 1 µM
cl-CD95L

Figure 3B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/054771

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/525 A61K38/04 C07K7/06 C07K7/08 C07K14/705
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/070372 A2 (ENTREMED & AFFYMAX) 21 June 2007 (2007-06-21) sequence 121 -----	1-3,14
X	EP 1 424 343 A1 (DEUTSCHES KREBSFORSCHUNGSZENTRUM) 2 June 2004 (2004-06-02) sequence 5 -----	1-3
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April 2017	03/05/2017

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

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/054771

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S B SCHRIER ET AL.: "Prediction of calcite morphology from computational and experimental studies of mutations of a de-novo designed peptides", LANGMUIR, vol. 27, 2011, pages 11520-11527, XP002758620, American Chemical Society ISSN: 0743-7463 table 1; compound 6	1
X	Z YE ET AL.: "Structural requirements of human tissue factor pathway inhibitor (TFPI) and heparin for TFPI-heparin interaction", THROMBOSIS RESEARCH, vol. 89, 1998, pages 263-270, XP002758621, TARRYTOWN, NY ISSN: 0049-3848 table 2; compound 5	1-3
X,P	WO 2016/061133 A1 (RIPTIDE BIOSCIENCE) 21 April 2016 (2016-04-21) sequences 145, 170	1-3,14
X	WO 2015/158810 A1 (INSERM ET AL.) 22 October 2015 (2015-10-22) cited in the application the whole document	1-14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/054771

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007070372 A2	21-06-2007	US 2007203066 A1	30-08-2007
		WO 2007070372 A2	21-06-2007
EP 1424343 A1	02-06-2004	AU 2003292150 A1	23-06-2004
		EP 1424343 A1	02-06-2004
		EP 1567550 A2	31-08-2005
		US 2007123699 A1	31-05-2007
		WO 2004050698 A2	17-06-2004
WO 2016061133 A1	21-04-2016	US 2016101150 A1	14-04-2016
		US 2017020956 A1	26-01-2017
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