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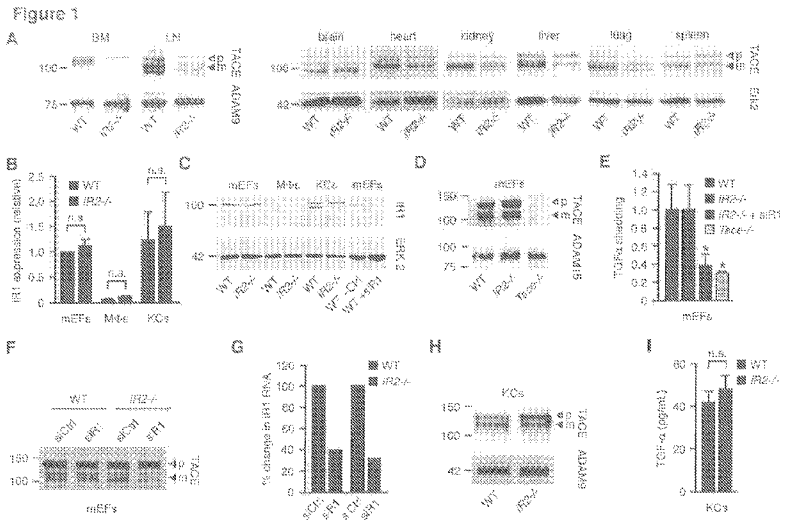
(54) **Title**: TREATMENT OF EGF-RECEPTOR DEPENDENT PATHOLOGIES



(57) **Abstract**: Disclosed are methods for treating a subject with an EGFR dependent pathology. The method comprises the step of administering to the subject an effective amount of an agent ("First Agent") that decreases the biological activity of iRhoml and an effective amount of an agent ("Second Agent") that decreases the biological activity of iRhom2. Alternatively, the method comprises the step of administering to the subject an effective amount of an agent ("First Agent") that modulates formation of a complex between iRhom 1 and TACE and an effective amount of an agent ("Second Agent") that modulates formation of a complex between TACE and iRhom2. Also disclosed are assays for identifying such agents.

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TREATMENT OF EGF-RECEPTOR DEPENDENT PATHOLOGIES

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/740,226, filed December 20, 2012, the entire contents of which are hereby incorporated by reference.

GOVERNMENT SUPPORT

The research reported herein was supported in part by grant number NIH R01 GM64750. The Government has certain rights in the invention.

BACKGROUND

EGFR (epidermal growth factor receptor) exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGFα). Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer (Yosef Yarden and Joseph Schlessinger (1987), "Epidermal Growth-Factor Induces Rapid, Reversible Aggregation of the Purified Epidermal Growth-Factor Receptor", *Biochemistry* **26** (5): 1443–1451). EGFR dimerization elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation (Oda K, Matsuoka Y, Funahashi A, Kitano H (2005), "A comprehensive pathway map of epidermal growth factor receptor signaling". *Mol. Syst. Biol.* **1** (1): 2005.0010). Such proteins modulate phenotypes such as cell migration, adhesion, and proliferation.

Mutations that lead to EGFR overexpression (known as upregulation) or overactivity have been associated with a number of cancers, including lung cancer, anal cancers (Walker F, Abramowitz L, Benabderrahmane D, Duval X, Descatoire V, Hénin D, Lehy T, Aparicio T (November 2009), "Growth factor receptor expression in anal squamous lesions: modifications associated with oncogenic human papillomavirus and human immunodeficiency virus", *Hum. Pathol.* **40** (11): 1517–27) and glioblastoma multiforme. In this latter case a more or less specific mutation of EGFR, called EGFRvIII is often observed (Kuan CT, Wikstrand CJ, Bigner DD (June 2001), (EGF mutant receptor vIII as a molecular target in cancer therapy", *Endocr. Relat. Cancer* **8** (2): 83–96). Mutations, amplifications or

misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers. Mutations involving EGFR could lead to its constant activation, which could result in uncontrolled cell division. Consequently, mutations of EGFR have been identified in several types of cancer, and it is the target of an expanding class of anticancer therapies (Zhang H, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, Greene MI (August 2007). "ErbB receptors: from oncogenes to targeted cancer therapies". *J. Clin. Invest.* **117** (8): 2051–8).

The identification of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR, including gefitinib and erlotinib for lung cancer, and cetuximab for colon cancer. Cetuximab and panitumumab are examples of monoclonal antibody inhibitors. Other monoclonals in clinical development are zalutumumab, nimotuzumab, and matuzumab. Another method is using small molecules to inhibit the EGFR tyrosine kinase, which is on the cytoplasmic side of the receptor. Without kinase activity, EGFR is unable to activate itself, which is a prerequisite for binding of downstream adaptor proteins. Ostensibly by halting the signaling cascade in cells that rely on this pathway for growth, tumor proliferation and migration is diminished. Gefitinib, erlotinib, and lapatinib (mixed EGFR and ERBB2 inhibitor) are examples of small molecule kinase inhibitors.

The membrane-anchored metalloproteinase TNFα convertase, TACE (also referred to as “ADAM17”) regulates the release of TNFα and EGFR-ligands from cells. As such, inhibiting TACE activity is another pathway by which EGFR activation can be blocked and represents a means of treating EGFR dependent pathologies.

SUMMARY OF THE INVENTION

It has now been found that iRhom1 and the related iRhom2 together support TACE (also referred to as ADAM17) maturation and shedding of the EGFR ligand TGFα. TACE is essential for activating EGFR by releasing TGFα. Based on these results, methods of treating a subject with an EGFR dependent pathology are disclosed herein.

One embodiment of the invention a method for treating a subject with an EGFR dependent pathology. The method comprises the step of administering to the subject an effective amount of an agent (“First Agent”) that decreases the biological activity of iRhom1 and an effective amount of an agent (“Second Agent”) that decreases the biological activity of iRhom2.

Another embodiment of the invention is method for treating a subject with an EGFR dependent pathology, comprising the step of administering to the subject an effective amount of an agent (“First Agent”) that modulates (increases or decreases) formation of a complex between iRhom 1 and TACE and an effective amount of an agent (“Second Agent”)

that modulates (increases or decreases) formation of a complex between TACE and iRhom2.

Another embodiment of the invention is a method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology. The method comprises the steps of

a) combining TACE, iRhom 1 and a test agent under conditions suitable for forming a complex between TACE and iRhom1; and

b) assessing the quantity of complex formation between TACE and iRhom1. A diminished or increased complex formation between TACE and iRhom1 in the presence of the test agent than in the absence is indicative that the test agent is useful for the treatment of an EGFR dependent pathology in combination with an inhibitor of a biological activity of iRhom2.

Another embodiment of the invention is a method of identifying an agent which can be used in combination with an inhibitor of a biological activity of or iRhom1 for the treatment of an EGFR dependent pathology. The method comprises the steps of

a) combining TACE, iRhom2 and a test agent under conditions suitable for forming a complex between TACE and iRhom2; and

b) assessing the quantity of complex formation between TACE and iRhom2. A diminished or increased complex formation between TACE and iRhom2 in the presence of the test agent than in the absence is indicative that the test agent is useful for the treatment of an EGFR dependent pathology in combination with an inhibitor of a biological activity of iRhom1.

Yet another embodiment of the invention is a method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology. The method comprises the following steps:

a) combining a test agent and a cell which releases an EGFR ligand under conditions suitable for stimulating release of the EGFR ligand, wherein the cell is *iRhom2-/-* (or *iRhom1-/-*) or wherein an inhibitor of a biological activity of iRhom2 is additionally combined with the cell and test agent; and

b) assessing the quantity of EGFR ligand, wherein diminished EGFR ligand release in the presence of the test agent than in the absence is indicative that the test agent is useful in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology.

Another embodiment of the invention is a method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom1 for the treatment of an EGFR dependent pathology. The method comprises the following steps:

a) combining a test agent and a cell which releases an EGFR ligand under conditions suitable for stimulating release of the EGFR ligand, wherein the cell is *iRhom1-/-* or wherein an inhibitor of a biological activity of iRhom1 is additionally combined with the cell and test agent; and

b) assessing the quantity of EGFR ligand, wherein diminished EGFR ligand release in the presence of the test agent than in the absence is indicative that the test agent is useful in combination with an inhibitor of a biological activity of iRhom1 for the treatment of an EGFR dependent pathology.

Yet another embodiment of the invention is a method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology. The method comprises the following steps:

a) combining a test agent and a cell which expresses the mature 100 kD form of TACE under conditions suitable for expression of the mature 100 kD form of TACE (e.g., on reducing SDS-PAGE), wherein the cell is either *iRhom2-/-* or an inhibitor of a biological activity of iRhom2 is additionally combined with the cell and test agent; and

b) assessing the quantity of the mature 100 kD form of TACE that is formed (e.g., on reducing SDS-PAGE), wherein diminished formation of the mature 100 kD form of TACE in the presence of the test agent than in the absence is indicative that the test agent is useful in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology.

Yet another embodiment of the invention is a method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom1 for the treatment of an EGFR dependent pathology. The method comprises the following steps:

a) combining a test agent and a cell which expresses the mature 100 kD form of TACE (e.g., on reducing SDS-PAGE) under conditions suitable for expressing the mature form of TACE, wherein the cell is either *iRhom1-/-* or an inhibitor of a biological activity of iRhom1 is additionally combined with the cell and test agent; and

b) assessing the quantity of the mature 100 kD form of TACE that is formed (e.g., on reducing SDS-PAGE), wherein diminished formation of the mature 100 kD form of TACE in the presence of the test agent than in the absence is indicative that the test agent is useful in combination with an inhibitor of a biological activity of iRhom1 for the treatment of an EGFR dependent pathology.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. iRhom2 controls TACE maturation in immune cells, but not somatic tissues. (A) Western blots of TACE in tissues and cells from *iRhom2-/-* (*iR2-/-*) and littermate controls (WT). In *iRhom2-/-* mice, mature TACE is absent in bone marrow (BM), strongly reduced in lymph nodes (LN), but present in brain, heart, kidney, liver lung and spleen (differences in mature TACE migration caused by N-linked carbohydrate modifications, blots are representative of 5). (B,C) qPCR (B, n=2) and Western blots (C, n=3) of iRhom1 in mEFs, primary macrophages (MΦs) and primary keratinocytes (KCs, i*Rhom2-/-* vs. controls , mean ± SD in B, siR1-treated WT mEFs included in C, iRhom1 runs as a doublet in KCs). (D) Representative TACE Western blot of mEFs from WT, *iRhom2-/-* or *Tace-/-* animals, n=3. (E) Shedding of TGFα from WT, *iRhom2-/-*, siR1-treated *iRhom2-/-*, or *Tace-/-* mEFs, n=4, mean ± SD, *p<0.05. (F) TACE Western blot shows reduction of mature TACE only in siR1-treated *iRhom2-/-* mEFs, but not in siR1-treated WT controls. (G) qPCR confirmed reduction of iRhom1 in siR1-treated WT or *iRhom2-/-* mEFs (representative of 3 experiments). (H, I) Western blot of TACE (H) and release of endogenous TGFα (I) from primary keratinocytes from *iRhom2-/-* or WT mice, n=2, mean ± SD. ADAM9, ADAM15 or ERK used as loading control, as indicated.

Figure 2 shows the amino acid sequence of iRohm2 (SEQ ID NO 1) and iRhom1 (SEQ ID NO 2), respectively.

Figure 3 shows the alignment of iRohm2 (top) relative to iRhom1. The sequences shown include the extracellular loop, with the most conserved sequences indicated by underlining; bold underlined sequences are the transmembrane domains that “anchor” the extracellular loop domains; shaded cysteine residues are conserved cysteine residues; and other shaded residues indicate glycosylation sites.

DETAILED DESCRIPTION

iRhom2 controls the maturation of TACE, yet *iRhom2-/-* mice are healthy (Adrain, C., Zettl, M., Christova, Y., Taylor, N., and Freeman, M. 2012. Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE. *Science* 335:225-228.McIlwain, D.R., Lang, P.A., Maretzky, T., Hamada, K., Ohishi, K., Maney, S.K., Berger, T., Murthy, A., Duncan, G., Xu, H.C., et al. 2012. iRhom2 regulation of TACE controls TNF-mediated protection against Listeria and responses to LPS. *Science* 335:229-232. Siggs, O.M., Xiao, N., Wang, Y., Shi, H., Tomisato, W., Li, X., Xia, Y., and Beutler, B. 2012. iRhom2 is required for the secretion of mouse TNFα. *Blood* 119:5769-5771), whereas *Tace-/-* mice die perinatally (Horiuchi, K., Kimura, T., Miyamoto, T., Takaishi, H., Okada, Y., Toyama, Y., and Blobel, C.P. 2007. Cutting Edge: TNF-{α}-Converting Enzyme (TACE/ADAM17)

Inactivation in Mouse Myeloid Cells Prevents Lethality from Endotoxin Shock. *J Immunol* 179:2686-2689. Peschon, J.J., Slack, J.L., Reddy, P., Stocking, K.L., Sunnarborg, S.W., Lee, D.C., Russel, W.E., Castner, B.J., Johnson, R.S., Fitzner, J.N., et al. 1998. An essential role for ectodomain shedding in mammalian development. *Science* 282:1281-1284.). To address this apparent paradox, we assessed whether iRhom2 affects TACE maturation in tissues other than macrophages. In Western blots of *iRhom2-/-* tissues, mature TACE was not detected in bone marrow (BM), was strongly reduced in lymph nodes (LN), but was clearly present in the brain, heart, kidney, liver, lung and spleen (Figure 1A), in approximate concordance with the expression of the related iRhom1 (BioGPS atlas, mu-iRhom1). We therefore tested whether it is iRhom1 that supports TACE maturation in *iRhom2-/-* mouse embryonic fibroblasts (mEFs), which express higher iRhom1 levels than macrophages (M□s, Figure 1B,C) and have normal levels of mature TACE in Western blots (Figure 1D, control: *Tace-/-* mEFs). *iRhom2-/-* mEFs shed the TACE substrate and EGFR-ligand, TGFα, at comparable levels to wild-type (WT) controls (Figure 1E). However, in *iRhom2-/-* mEFs treated with iRhom1 siRNA (siR1), TGFα shedding was strongly reduced (Figure 1E, control: *Tace-/-* mEFs). Western blots showed normal mature TACE levels in siR1-treated WT mEFs, but strongly reduced mature TACE in siR1-treated *iRhom2-/-* mEFs (Figure 1F, siR1 was effective in both WT and mutant cells, Figure 1G). Since iRhom1 is not upregulated in *iRhom2-/-* mEFs (Figure 1B,C), iRhom1 is sufficient for TACE maturation and function. In *iRhom2-/-* primary keratinocytes (KC), which expressed similar iRhom1 levels as mEFs (Figure 1B,C), mature TACE levels and the release of endogenous TGF-α were comparable to controls (Figure 1H,I). In summary, our results explain why *iRhom2-/-* mice display no obvious spontaneous pathologies: mature TACE is produced in most somatic tissues of *iRhom2-/-* mice. The related iRhom1, which is expressed in somatic tissues but not in most hematopoietic cells, appears to support TACE maturation and function in the absence of iRhom2, as shown in fibroblasts.

An “EGFR dependent pathology” is a disease or condition caused by aberrant expression (over expression or under expression) of EGFR or aberrant activity (overactivity or underactivity) of EGFR. Typically, the EGFR dependent pathology is an EGFR dependent cancer, typically a cancer which expresses (or overexpresses) EGFR. Methods of determining whether a cancer expresses or overexpresses EGFR are well known in the art and include a diagnostic immunohistochemistry assay (EGFR pharmDx) which can be used to detect EGFR expression in the tumor material. Exemplary EGFR dependent cancers (also referred to herein as “EGFR expressing cancers) include colorectal cancer, squamous cell carcinoma of the head and neck, lung cancer, anal cancer and glioblastoma multiforme. Treatment according to the disclosed invention is particularly advantageous when the cancer

(e.g., the colorectal cancer) is KRAS wild-type. KRAS mutational analysis is commercially available from a number of laboratories. Alternatively, THE EGFR expressing cancer is EGFR wild-type, or EGFR and KRAS wild-type.

Various proteins are described herein by reference to their GenBank Accession Numbers for their human proteins and coding sequences. However, the proteins are not limited to human-derived proteins having the amino acid sequences represented by the disclosed GenBank Accession numbers, but may have an amino acid sequence derived from other animals, particularly, a warm-blooded animal (e.g., rat, guinea pig, mouse, chicken, rabbit, pig, sheep, cow, monkey, etc.).

The term “iRhom1”, “Rhbdf1” or rhomboid 5 homolog 1 (Drosophila) refer to a protein having an amino acid sequence substantially identical to any of the representative iRhom1 sequences of GenBank Accession Nos. NP_071895.3 (human), AAH23469.1 or NP_034247.2 (mouse) or to the sequence shown in Figure 2.

The term “iRhom2 ”, “Rhbdf2”, or “rhomboid 5 homolog 2 (Drosophila)” refers to a protein having an amino acid sequence substantially identical to any of the representative iRhom2 sequences of GenBank Accession Nos. NP_001005498.2 or NP_078875.4 (human), NP_001161152.1 (mouse) and NP_001100537.1 (rat) or to the sequence shown in Figure 2. Suitable cDNA encoding iRhom2 are provided at GenBank Accession Nos. NM_001005498.3 or NM_024599.5 (human), BC052182.1 (mouse) and NM_001107067.1 (rat).

The term “biological activity of iRhom1” refers to any biological activity associated with the full-length native iRhom1 protein, including the biological activity resulting from its association with TACE. In suitable embodiments, the iRhom1 biological activity is equivalent to the activity of a protein having an amino acid sequence represented by GenBank Accession No. GenBank Accession Nos. NP_071895.3 (human), AAH23469.1 or NP_034247.2 (mouse) or to the sequence shown in Figure 2. Decreasing the biological activity, in one embodiment, refers to decreasing the expression of the iRhom1 mRNA or protein. Measurement of transcriptional activity can be performed using any known method, such as immunohistochemistry, reporter assay or RT-PCR, which can also be used to determine whether the biological activity of iRhom1 is decreased. In another embodiment, decreasing the biological activity refers to inhibiting or reducing maturation of TACE. TACE maturation can be detected and quantified by Western blotting. The iRhom1 referred to herein can be a mammalian iRhom1 or in a particular aspect, a human iRhom1 or a splice variant thereof.

The term “biological activity of iRhom2” refers to any biological activity associated with the full length native iRhom2 protein, including the biological activity resulting from its association with TACE. In suitable embodiments, the iRhom2 biological activity is equivalent

to the activity of a protein having an amino acid sequence represented by GenBank Accession No. NP_001005498.2, NP_078875.4, NP_001161152.1, or NP_001100537.1 or the amino acid sequence shown in Figure 2. Decreasing the biological activity, in one embodiment, refers to decreasing the expression of the iRhom2 mRNA or protein. Measurement of transcriptional activity can be performed using any known method, such as immunohistochemistry, reporter assay or RT-PCR, which can also be used to determine whether the biological activity of iRhom2 is decreased. In another embodiment, decreasing the biological activity refers to inhibiting or reducing maturation of TACE. TACE maturation can be detected and quantified by Western blotting. The iRhom2 referred to herein can be a mammalian iRhom2 or in a particular aspect, a human iRhom2, or a splice variant thereof..

The term "TACE", "ADAM17" or "ADAM metallopeptidase domain 17" refers to a protein having an amino acid sequence substantially identical to any of the representative TACE sequences of GenBank Accession Nos. NP_003174.3 (human), NP_033745.4 (mouse) and NP_064702.1 (rat). Suitable cDNA encoding TACE are provided at GenBank Accession Nos. NM_003183.4 (human), NM_009615.5 (mouse) and NM_020306.1 (rat).

Two forms of TACE are found in cells; a full-length precursor and a 100 kD mature form lacking the prodomain. Prodomain removal occurs in a late Golgi compartment, consistent with the proposed role of a furin type proprotein convertase in this process. An additional non-physiological form of TACE, lacking the pro and cytoplasmic domains, is detected when cell lysates are prepared in the presence of EDTA instead of a hydroxamate-based metalloprotease inhibitor or 1,10-phenanthroline. Mature TACE could be separated from and quantitated by Western blot, where it is the fastest migrating form of TACE McIllwein *et al., Science 335:*229 (2012) and Adrain *et al.,Science 335:*225 (2012)).

TACE and iRhom1 are believed to bind together to form a complex and to co-immunoprecipitate. The ability of an agent to modulate (increase or decrease) binding between TACE and iRhom1 is believed to correlate with the ability of the agent to modulate the activity of iRhom1, and by extension, TACE. The amount of complex formation should be measurable by methods known in the art (as described in Adrain *et al.,Science 335:*225 (2012) for iRhom2 and TACE), and include immunoprecipitation with tagged iRhom1 or tagged TACE. For example, the binding partners can be expressed in eukaryotic cell expression systems, and tested for antibodies or reagents that prevent binding, dissociate bound molecules, or stabilize the interaction, with, for example, pulldown assays, assays where one binding partner is immobilized on a plate and the second one is tagged and added. The quantity of the tagged molecule released into the supernatant can then be assessed by measuring the amount of released tagged protein by Western blot, dot blot or ELISA. An enzyme tag can be used, such as alkaline phosphatase, in which case the release can be measure by colorimetric determination of alkaline phosphatase activity in the

supernatant A fluorescent protein tag can be added, in which case the release can be measure by a fluorimeter.

TACE and iRhom2 bind together to form a complex and can immunoprecipitate (Adrain *et al.,Science 335:*225 (2012)). The ability of an agent to modulate (increase or decrease) binding between TACE and iRhom2 is disclosed herein to correlate with the ability of the agent to modulate the activity of iRhom2, and by extension, TACE. The amount of complex formation can be measured by methods known in the art (see Adrian *et al., supra*), and include immunoprecipitation with tagged iRhom2 or tagged TACE. For example, the binding partners can be expressed in eukaryotic cell expression systems, and tested for antibodies or reagents that prevent binding, dissociate bound molecules, or stabilize the interaction, with, for example, pulldown assays, assays where one binding partner is immobilized on a plate and the second one is tagged and added. The quantity of the tagged molecule released into the supernatant can then be assessed by measuring the amount of released tagged protein by Western blot, dot blot or ELISA. An enzyme tag can be used, such as alkaline phosphatase, in which case the release can be measure by colorimetric determination of alkaline phosphatase activity in the supernatant A fluorescent protein tag can be added, in which case the release can be measure by a fluorimeter.

Transforming growth factor α (TGFα) is a small 50 amino acid residue long mitogenic protein that contains three disulfide bridges. TGFα shares about 30% sequence identity with epidermal growth factor (EGF) and competes with EGF for the same membrane-bound receptor sites. High amounts of TGFα/EGF receptor complexes have been noticed in some human cancers. TGF αs are secreted by human cancer cells and retrovirus-transformed fibroblasts.

A “biological equivalent” of a protein or nucleic acid refers to a protein or nucleic acid that is substantially identical to the protein or nucleic acid. As used herein, the term “substantially identical”, when referring to a protein or polypeptide, is meant one that has at least 80%, 85%, 90%, 95%, or 99% sequence identity to a reference amino acid sequence. The length of comparison is preferably the full length of the polypeptide or protein, but is generally at least 10, 15, 20, 25, 30, 40, 50, 60, 80, or 100 or more contiguous amino acids. A “substantially identical” nucleic acid is one that has at least 80%, 85%, 90%, 95%, or 99% sequence identity to a reference nucleic acid sequence. The length of comparison is preferably the full length of the nucleic acid, but is generally at least 20 nucleotides, 30 nucleotides, 40 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 125 nucleotides, or more.

In one aspect of any of the above methods, the agent that decreases the biological activity of iRohm 1 (or iRhom2) is an antibody or antibody fragment that specifically recognizes iRhom 1 (or iRhom2) and inhibits the activity of TACE; a small molecule inhibitor

of iRhom1 (or iRhom2); a polypeptide decoy mimicking a domain necessary for the interaction of TACE and iRhom 1(or iRhom2); a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to iRhom1 (or iRhom2) DNA or mRNA; a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA; or an equivalent of each thereof. In another alternative, the agent that decreases the biological activity of iRhom1 or iRhom2 modulates (increases or decreases) formation of a complex between iRhom1 (or iRhom2) and TACE or inhibits the maturation of TACE.

In one aspect, the agent that decreases the biological activity of iRhom1 is an antibody or antibody fragment that specifically recognizes iRhom1 and inhibits the activity of TACE, or a polypeptide decoy mimicking a domain necessary for the interaction of TACE and iRhom1. In a particular aspect, the antibody or antibody fragment specifically recognizes an extracellular domain of iRhom1. For example, the antibody or antibody fragment recognizes and specifically binds to the polypeptide SAPDLAGNKRQFGSVCHQDPRVCDEPSSEDPHEWPEDITKWPICTKSSAG (SEQ ID NO 5) or an antibody binding fragment thereof containing 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acids. This polypeptide is a highly conserved fragment of the extracellular loop. Alternatively, the antibody or antibody fragment recognizes and specifically binds to a transmembrane region of iRhom2 or a region that includes both the extracellular loop and the transmembrane region. In another aspect, the agent further comprises a cell penetrating peptide. The cell penetrating peptide, in one aspect, comprises a HIV-TAT peptide.

In one aspect, the agent that decreases the biological activity of iRhom2 is an antibody or antibody fragment that specifically recognizes iRhom2 and inhibits the activity of TACE, or a polypeptide decoy mimicking a domain necessary for the interaction of TACE and iRhom2. In a particular aspect, the antibody or antibody fragment specifically recognizes an extracellular domain of iRhom2. For example, the antibody or antibody fragment recognizes and specifically binds to the polypeptide GPSDKSDLSQKQPSAVVCHQDPRTCEEPASSGAHIWPDDITKWPICTEQAQS (SEQ ID NO 6) or an antibody binding fragment thereof containing 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acids. This polypeptide is a highly conserved fragment of the extracellular loop. Alternatively, the antibody or anitbody fragment recognizes and specifically binds to a transmembrane region of iRhom2 or a region that includes both the extracellular loop and the transmembrane region. In another aspect, the agent further comprises a cell penetrating peptide. The cell penetrating peptide, in one aspect, comprises a HIV-TAT peptide.

Agents which modulate the formation of a complex between iRhom1 and TACE include compounds that increase (e.g., stabilize) or decrease (e.g., destabilize or inhibit) the

binding between the two proteins, resulting in more complex formation or less complex formation, respectively. Examples of agents that inhibit binding include an antibody or an antibody fragment that specifically recognizes the iRhom1 protein, and preferably the extracellular loop of either iRhom1 (the polypeptide SAPDLAGNKRQFGSVCHQDPRVCDEPSSEDPHEWPEDITKWPICTKSSAG (SEQ ID NO 5) or an antibody binding fragment thereof containing 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acids. Alternatively, the antibody or antibody fragment that specifically recognizes a transmembrane domain of iRohm1 or a region comprising the extracellular domain and a transmembrane domain.

Agents which modulate the formation of a complex between iRhom2 and TACE include compounds that increase (e.g., stabilize) or decrease (e.g., destabilize or inhibit) the binding between the two proteins, resulting in more complex formation or less complex formation, respectively. Examples of agents that inhibit binding include an antibody or an antibody fragment that specifically recognizes the iRhom2 protein, and preferably the extracellular loop of either iRhom2 (the polypeptide GPSDKSDLSQKQPSAVVCHQDPRTCEEPASSGAHIWPDDITKWPICTEQAQS (SEQ ID NO 6) or an antibody binding fragment thereof containing 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acids. Alternatively, the antibody or antibody fragment that specifically recognizes a transmembrane domain of iRhom1 or a region comprising the extracellular domain and a transmembrane domain.

In another alternative, the agent that inhibits binding is an antibody or an antibody fragment that specifically recognizes the extracellular domain of either TACE (the polypeptide murine TACE accession number: http://www.ncbi.nlm.nih.gov/protein/NP_033745.4 - the extracellular domain is between aa # 1 and ~670; and human TACE accession number: http://www.ncbi.nlm.nih.gov/protein/NP_003174.3 - the extracellular domain is between aa # 1 and ~670), SEQ ID NO 1) or an antibody binding fragment thereof containing 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acids. In another alternative, the inhibitor of complex formation can be a small molecule which binds either iRhom1 (or iRhom2) or TACE in the region where the two proteins bind, e.g., a fragment of either protein which binds the other or a decoy that mimics a domain necessary for the interaction of TACE and iRhom1 (or iRhom2) . This region can also include the transmembrane domain of TACE and one or more of the seven transmembrane domains of iRohm1 (or iRhom2). Agents which inhibit the formation of a complex between iRhom1 (or iRohm2) and TACE also include compounds which suppress the expression of iRohm2, e.g., iRNA can be a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to iRHom 1 (or iRhom2) DNA or mRNA, or a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA,

a vector comprising the polynucleotide. Agents that increase complex formation include antibodies or antibody fragments or small molecules that bind to and stabilize the complex. This would be identified from combinatorial chemistry inhibitor libraries by screens, and then further optimized through chemical alterations. In another aspect, the agent further comprises a cell penetrating peptide. The cell penetrating peptide, in one aspect, comprises a HIV-TAT peptide.

"Short interfering RNAs" (siRNA) refer to double-stranded RNA molecules (dsRNA), generally, from about 10 to about 30 nucleotides in length that are capable of mediating RNA interference (RNAi). "RNA interference" (RNAi) refers to sequence-specific or gene specific suppression of gene expression (protein synthesis) that is mediated by short interfering RNA (siRNA). As used herein, the term siRNA includes short hairpin RNAs (shRNAs). A siRNA directed to a gene or the mRNA of a gene may be a siRNA that recognizes the mRNA of the gene and directs a RNA-induced silencing complex (RISC) to the mRNA, leading to degradation of the mRNA. A siRNA directed to a gene or the mRNA of a gene may also be a siRNA that recognizes the mRNA and inhibits translation of the mRNA. A siRNA may be chemically modified to increase its stability and safety. See, e.g. Dykxhoorn and Lieberman (2006) Annu. Rev. Biomed. Eng. 8:377-402 and U.S. Patent Application Publication No.: 2008/0249055.

"Double stranded RNAs" (dsRNA) refer to double stranded RNA molecules that may be of any length and may be cleaved intracellularly into smaller RNA molecules, such as siRNA. In cells that have a competent interferon response, longer dsRNA, such as those longer than about 30 base pair in length, may trigger the interferon response. In other cells that do not have a competent interferon response, dsRNA may be used to trigger specific RNAi.

"MicroRNAs" (miRNA) refer to single-stranded RNA molecules of 21-23 nucleotides in length, which regulate gene expression. miRNAs are encoded by genes from whose DNA they are transcribed but miRNAs are not translated into protein (non-coding RNA); instead each primary transcript (a pri-miRNA) is processed into a short stem-loop structure called a pre-miRNA and finally into a functional miRNA. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to down-regulate gene expression.

siRNA, dsRNA, and miRNA to inhibit gene expression can be designed following procedures known in the art. See, e.g., Dykxhoorn and Lieberman (2006) Annu. Rev. Biomed. Eng. 8:377-402; Dykxhoorn et al. (2006) Gene Therapy 13:541-52; Aagaard and Rossi (2007) Adv. Drug Delivery Rev. 59:75-86; de Fougerolles et al. (2007) Nature Reviews Drug Discovery 6:443-53; Krueger et al. (2007) Oligonucleotides 17:237-250; U.S. Patent

Application Publication No.: 2008/0188430; and U.S. Patent Application Publication No.: 2008/0249055.

Delivery of siRNA, dsRNA or miRNA to a cell can be made with methods known in the art. See, e.g., Dykxhoorn and Lieberman (2006) Annu. Rev. Biomed. Eng. 8:377-402; Dykxhoorn et al. (2006) Gene Therapy 13:541-52; Aagaard and Rossi (2007) Adv. Drug Delivery Rev. 59:75-86; de Fougerolles et al. (2007) Nature Reviews Drug Discovery 6:443-53; Krueger et al. (2007) Oligonucleotides 17:237-250; U.S. Patent Application Publication No.: 2008/0188430; and U.S. Patent Application Publication No.: 2008/0249055.

"Antisense" oligonucleotides have nucleotide sequences complementary to the protein coding or "sense" sequence. Antisense RNA sequences function as regulators of gene expression by hybridizing to complementary mRNA sequences and arresting translation (Mizuno et al. (1984) PNAS 81:1966; Heywood et al. (1986) Nucleic Acids Res. 14:6771). An antisense polynucleotide comprising the entire sequence of the target transcript or any part thereof can be synthesized with methods known in the art. See e.g., Ferretti et al. (1986) PNAS 83:599. The antisense polynucleotide can be placed into vector constructs, and effectively introduced into cells to inhibit gene expression (Izant et al. (1984) Cell 36:1007). Generally, to assure specific hybridization, the antisense sequence is substantially complementary to the target sequence. In certain embodiments, the antisense sequence is exactly complementary to the target sequence. The antisense polynucleotides may also include, however, nucleotide substitutions, additions, deletions, transitions, transpositions, or modifications, or other nucleic acid sequences or non-nucleic acid moieties so long as specific binding to the relevant target sequence corresponding to the gene is retained as a functional property of the polynucleotide.

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein and known to one of skill in the art. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by de novo chemical synthesis or by cloning. For example, an antisense RNA can be made by inserting (ligating) a gene sequence in reverse orientation operably linked to a promoter in a vector (e.g., plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention.

It will be appreciated that the oligonucleotides can be made using nonstandard bases (e.g., other than adenine, cytidine, guanine, thymine, and uridine) or nonstandard backbone structures to provide desirable properties (e.g., increased nuclease-resistance, tighter-binding, stability or a desired T_m). Techniques for rendering oligonucleotides nuclease-

resistant include those described in PCT Publication WO 94/12633. A wide variety of useful modified oligonucleotides may be produced, including oligonucleotides having a peptide-nucleic acid (PNA) backbone (Nielsen et al. (1991) Science 254:1497) or incorporating 2'-O-methyl ribonucleotides, phosphorothioate nucleotides, methyl phosphonate nucleotides, phosphotriester nucleotides, phosphorothioate nucleotides, phosphoramidates. Another example of the modification is replacement of a non-bridging phosphoryl oxygen atom with a sulfur atom which increases resistance to nuclease digestion. Increased antisense polynucleotide stability can also be achieved using molecules with 2-methyoxyethyl substituted backbones. See e.g., U.S. Patent Nos. 6,451,991 and 6,900,187.

In another embodiment, ribozymes can be used (see, e.g., Cech (1995) Biotechnology 13:323; and Edgington (1992) Biotechnology 10:256 and Hu et al., PCT Publication WO 94/03596). A ribonucleic acid enzyme ("ribozymes", "RNA enzyme", or "catalytic RNA") is an RNA molecule that catalyzes a chemical reaction. Many natural ribozymes catalyze either the hydrolysis of one of their own phosphodiester bonds, or the hydrolysis of bonds in other RNAs, but they have also been found to catalyze the aminotransferase activity of the ribosome. Methods of making and using ribozymes can be found in e.g., U.S. Patent Application Publication No. 2006/0178326.

"Triplex ribozymes" configurations allow for increased target cleavage relative to conventionally expressed ribozymes. Examples of triplex ribozymes include hairpin ribozymes and hammerhead ribozymes. Methods of making and using triplex ribozymes are found in, e.g., Aguino-Jarguin et al. (2008) Oligonucleotides 18(3):213-24 and U.S. Patent Application Publication No. 2005/0260163.

Proteins have been described that have the ability to translocate desired nucleic acids across a cell membrane. Typically, such proteins have amphiphilic or hydrophobic subsequences that have the ability to act as membrane-translocating carriers. For example, homeodomain proteins have the ability to translocate across cell membranes. The shortest internalizable peptide of a homeodomain protein, Antennapedia, was found to be the third helix of the protein, from amino acid position 43 to 58 (see, e.g., Prochiantz (1996) Current Opinion in Neurobiology 6:629-634. Another subsequence, the h (hydrophobic) domain of signal peptides, was found to have similar cell membrane translocation characteristics (see, e.g., Lin et al. (1995) J. Biol. Chem. 270:14255-14258). Such subsequences can be used to translocate oligonucleotides across a cell membrane. Oligonucleotides can be conveniently derivatized with such sequences. For example, a linker can be used to link the oligonucleotides and the translocation sequence. Any suitable linker can be used, e.g., a peptide linker or any other suitable chemical linker.

The present disclosure provides, in one embodiment, a polypeptide decoy that mimics a domain necessary for the interaction of TACE and iRhom1 (or iRhom2) for

decreasing the biological activity of iRhom1 (or iRhom2). A polypeptide decoy of a protein for inhibiting the interaction between the protein and a second protein is a polypeptide that binds to the second protein but does not carry out the biological activity that such a binding would normally carry out.

In one embodiment, a polypeptide decoy is a fragment of the iRhom1 (or iRhom2) protein that includes the iRhom 1 (or iRhom2) extracellular domain responsible for binding TACE, e.g., a polypeptide with the amino sequence of SEQ ID NO 3, 4, 5 or 6 or a 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acid fragment thereof that binds TACE. In another embodiment, the polypeptide decoy does not include an iRhom1 (or iRhom2) domain that is responsible for activating TACE or contains a mutation at this domain so that the polypeptide decoy does not activate TACE. Alternatively, the polypeptide decoy also includes a portion of the transmembrane domain of iRhom1 (or iRhom2), together with or in the absence of the extracellular domain.

In another embodiment, a polypeptide decoy is a fragment of the TACE protein that includes the TACE extracellular domain responsible for binding iRhom2, e.g., or a 5 to 10, 10 to 15, 15 to 20, 20-25, 25-30, 30-40, 40-45 or more than 45 amino acid fragment thereof that binds with iRohm2. In another embodiment, the polypeptide decoy does not include a TACE domain that is responsible for its shedding activity or contains a mutation at this domain so that the polypeptide decoy does not have shedding activity. Alternatively, the polypeptide decoy also includes a portion of the transmembrane domain of TACE together with or in the absence of a portion of the extracellular domain of TACE.

“Antibody” is intended to encompass both polyclonal and monoclonal antibodies. The terms polyclonal and monoclonal refer to the degree of homogeneity of an antibody preparation, and are not intended to be limited to particular methods of production. “Antibody” also encompasses functional fragments of antibodies, including fragments of chimeric, humanized, primatized, veneered or single chain antibodies. For example, an antibody can be an IgG or antigen-binding fragment of an IgG. Antibody fragments include, but are not limited to Fv, Fab, Fab' and $F(ab')_2$ fragments. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For example, papain or pepsin cleavage can generate Fab or $F(ab')_2$ fragments, respectively. Other proteases with the requisite substrate specificity can also be used to generate Fab or $F(ab')_2$ fragments. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons has been introduced upstream of the natural stop site. For example, a chimeric gene encoding a $F(ab')_2$ heavy chain fragment can be designed to include DNA sequences encoding the CH_1 domain and hinge region of the heavy chain.

Single chain antibodies, and chimeric, humanized or primatized (CDR-grafted), or veneered antibodies, as well as chimeric, CDR-grafted or veneered single chain antibodies,

comprising fragments derived from different species, and the like are also encompassed by the term “antibody”. The various fragments of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Cabilly et al., European Patent No. 0,125,023 B1; Boss et al., U.S. Pat. No. 4,816,397; Boss et al., European Patent No. 0,120,694 B1; Neuberger, M. S. et al., WO 86/01533; Neuberger, M. S. et al., European Patent No. 0,194,276 B1; Winter, U.S. Pat. No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Queen et al., European Patent No. 0 451 216 B1; and Padlan, E. A. et al., EP 0 519 596 A1. See also, Newman, R. et al., BioTechnology, 10: 1455-1460 (1992), regarding primatized antibody, and Ladner et al., U.S. Pat. No. 4,946,778 and Bird, R. E. et al., Science, 242: 423-426 (1988)) regarding single chain antibodies.

Humanized antibodies can be produced using synthetic or recombinant DNA technology using standard methods or other suitable techniques. Nucleic acid (e.g., cDNA) sequences coding for humanized variable regions can also be constructed using PCR mutagenesis methods to alter DNA sequences encoding a human or humanized chain, such as a DNA template from a previously humanized variable region (see e.g., Kamman, M., et al., Nucl. Acids Res., 17: 5404 (1989)); Sato, K., et al., Cancer Research, 53: 851-856 (1993); Daugherty, B. L. et al., Nucleic Acids Res., 19(9): 2471-2476 (1991); and Lewis, A. P. and J. S. Crowe, Gene, 101: 297-302 (1991)). Using these or other suitable methods, variants can also be readily produced. In one embodiment, cloned variable regions can be mutated, and sequences encoding variants with the desired specificity can be selected (e.g., from a phage library; see e.g., Krebber et al., U.S. Pat. No. 5,514,548; Hoogenboom et al., WO 93/06213, published Apr. 1, 1993).

Antibodies which are specific for a mammalian (e.g., human) specific portion of iRhom1 (or iRohm2) and TACE that affect binding between the two proteins or which inhibit a biological activity of iRohm1 and iRohm2 can be raised against an appropriate immunogen, such as isolated and/or recombinant extracellular loop of iRohm 1 or iRohm2 or the extracellular domain of TACE, with or without the transmembrane domains attached, or fragments thereof (including synthetic molecules, such as synthetic peptides).

Preparation of immunizing antigen, and polyclonal and monoclonal antibody production can be performed using any suitable technique. A variety of methods have been described (see e.g., Kohler et al., Nature, 256: 495-497 (1975) and Eur. J. Immunol. 6: 511-519 (1976); Milstein et al., Nature 266: 550-552 (1977), Koprowski et al., U.S. Pat. No. 4,172,124; Harlow, E. and D. Lane, 1988, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y.); Current Protocols In Molecular Biology, Vol. 2

(Supplement 27, Summer '94), Ausubel, F. M. et al., Eds., (John Wiley & Sons: New York, N.Y.), Chapter 11, (1991)). Generally, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as SP2/0, P3X63Ag8.653 or a heteromyloma) with antibody producing cells. Antibody producing cells can be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals immunized with the antigen of interest. The fused cells (hybridomas) can be isolated using selective culture conditions, and cloned by limiting dilution. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Other suitable methods of producing or isolating antibodies of the requisite specificity (e.g., human antibodies or antigen-binding fragments) can be used, including, for example, methods which select recombinant antibody from a library (e.g., a phage display library), or which rely upon immunization of transgenic animals (e.g., mice) capable of producing a repertoire of human antibodies (see e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90: 2551-2555 (1993); Jalkobovits et al., Nature, 362: 255-258 (1993); Lonberg et al., U.S. Pat. No. 5,545,806; Surani et al., U.S. Pat. No. 5,545,807; Lonberg et al., WO97/13852).

In one embodiment, the antibody or antigen-binding fragment used in the disclosed methods binds to a fragment of the extracellular loop of iRhom1, iRohm2 or TACE. The fragment can be 5 to 10 amino acids long, 10 to 15 amino acids long, 15 to 20 amino acids long, 20-25 amino acids long, 25-30 amino acids long, 30-35 amino acids long, 35-40 amino acids long, 40-45 amino acids long or greater than 45 amino acids long.

The agent that decreases the biological activity of iRhom1 and the agent that decreases the biological activity of iRhom2 can be different compounds. Alternatively, the agent that decreases the biological activity of iRhom1 and the agent that decreases the biological activity of iRhom2 can be the same compound. For example, as shown in Figure 3, there is substantial homology between the amino acid sequence of the extracellular loop of iRhom1 and iRhom2. Therefore, it is believed that antibodies which bind both extracellular loops and that decrease a biological activity of both iRhom1 and iRhom2 can be generated. Similarly, it should be possible to generate polypeptide decoys based on the amino acid sequences of the extracellular loop of iRhom1 and iRhom2 that bind TACE in such a manner so as to inhibit a biological activity of both iRohm1 and iRhom2.

The compositions described herein for a therapeutic use may be administered with an acceptable pharmaceutical carrier. Acceptable "pharmaceutical carriers" are well known to those of skill in the art and can include, but not be limited to any of the standard pharmaceutical carriers, such as phosphate buffered saline, water and emulsions, such as oil/water emulsions and various types of wetting agents.

The term “treating” is meant administering a pharmaceutical composition for the purpose of therapeutic treatment by reducing, alleviating or reversing at least one adverse effect or symptom.

The term "administering" for in vivo and ex vivo purposes means providing the subject with an effective amount of the nucleic acid molecule or polypeptide effective to prevent or inhibit a disease or condition in the subject. Methods of administering pharmaceutical compositions are well known to those of skill in the art and include, but are not limited to, microinjection, intravenous or parenteral administration. The compositions are intended for systemic, topical, oral, or local administration as well as intravenously, subcutaneously, or intramuscularly. Administration can be effected continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the vector used for therapy, the polypeptide or protein used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. For example, the compositions can be administered prior to a subject already suffering from a disease or condition that is linked to apoptosis.

The term “effective amount” refers to a quantity of compound (e.g., an agent that decreases the biological activity of iRhom1 or iRhom2 or that modulates (increases or decreases) formation of a complex between iRhom1 (or iRhom2) and TACE) delivered with sufficient frequency to provide a medical benefit to the patient. In one embodiment, an effective amount of a protein is an amount sufficient to treat or ameliorate a symptom of an EGFR dependent pathology. Exemplary effective amounts of agent that decreases the biological activity of iRhom1 (or iRhom2) or that modulates (increases or decreases) formation of a complex between iRhom1 (or iRhom2) and TACE range from 0.1 ug/kg body weight to 100 mg/kg body weight; alternatively 1.0 ug/kg body weight to 10mg/kg body weight

An inhibitor of a biological activity of iRhom 1 and an inhibitor of a biological activity of iRhom 2 can be used alone or a combination with another anticancer agent. Anticancer agents that are commonly combined with the disclosed methods include platinum based chemotherapy. Platinum chemotherapy is the term for treatment with one of the chemotherapy drugs that contain derivatives of the metal platinum. The platinum damages the DNA of the cancer cells.

Exemplary platinum based anticancer agents include Cisplatin, carboplatin, capecitabine and oxaliplatin.

A "subject" includes mammals, e.g., humans, companion animals (e.g., dogs, cats, birds and the like), farm animals (e.g., cows, sheep, pigs, horses, fowl and the like) and

laboratory animals (e.g., rats, mice, guinea pigs and the like). In a preferred embodiment of the disclosed methods, the subject is human.

The invention also includes a method of identifying an agent to be used in combination with an agent that inhibits a biological activity of iRhom 2 (or iRhom1) for the treatment of an EGFR dependent pathology. The method assesses the ability of a test agent to modulate (increase or decrease) complex formation between iRhom1 (or iRhom2) and TACE. The method comprises the step of combining TACE, iRhom1 (or iRhom2) and a test agent under conditions suitable for forming a complex between TACE and iRhom1 (or iRhom2). This could be a pre-existing complex of iRhom1 (or iRhom2) and TACE that is immunoprecipitated from cells, such as myeloid cells to assess the interaction between iRhom2 and TACE; and keratinocytes or fibroblasts to assess the interaction between iRhom1 and TACE. It could also be a complex of recombinantly expressed extracellular loop of iRhom1 (or iRhom2) and extracellular domain of TACE, with tags added, as described above. The amount of complex formation is compared to the amount of complex formed under identical conditions in the absence of the test agent. A greater or lesser amount of complex formation in the presence of the test agent than in its absence is indicative that test agent is effective for the treatment of an EGFR mediated pathology. Methods for assessing complex formation between iRhom 2 and TACE are provided in Adrain *et al.,Science 335:*225 (2012).

The efficacy a test agent showing the ability to modulate complex formation between iRhom 1 (or iRohm2) and TACE can be further tested and/or confirmed in additional assays for assessing efficacy against any one or more disease mediated by an EGFR dependent pathology. Typically, a plurality of test agents are tested, for example as in high throughput screening, for their ability to modulate complex formation between iRhom1 (or iRhom2) TACE. Those test agents demonstrating an ability to modulate complex formation between iRhom1 (or iRhom2) and TACE are typically selected for further testing in assays for assessing efficacy against any one or more EGFR dependent pathologies.

An alternative method for identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology assesses the ability of a test agent to inhibit release an EGFR ligand. Exemplary EGFR ligands include TGFα, HB-EGF, amphiregulin, epiregulin and epigen. The method comprises combining a cell that releases an EGFR ligand (e.g., a mouse embryonic fibroblast, keratinocyte or endothelial cell) and a test agent under conditions suitable for stimulating TGFα release. The cell is either *iRhom2-/-*; or an inhibitor of iRhom 2 is additionally combined with the cell and test agent.

An alternative method for identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom1 for the treatment of an EGFR dependent

pathology assesses the ability of a test agent to inhibit release an EGFR ligand. Exemplary EGFR ligands include TGFα, HB-EGF, amphiregulin, epiregulin and epigen. The method comprises combining a cell that releases of an EGFR ligand (e.g., a mouse embryonic fibroblast, keratinocyte or endothelial cells) and a test agent under conditions suitable for stimulating TGFα release. The cell is either *iRhom1-/-*; or an inhibitor of iRhom 1 is additionally combined with the cell and test agent.

Exemplary conditions for carrying out the assay described in the previous two paragraphs and measuring the quantities of TGFα released by a cell are provided in Sahin et al., "Distinct Roles for ADAM10 and ADAM 17 in Ectodomain Shedding of six EGFR Ligands" The Journal of Cell Biology, 164:769 (2004); Sahin, et al., "Ectodomain shedding of the EGF-Receptor Ligand Epigen is Mediated by ADAM17", FEBS, 581:41 (2007); Le Gall, et al., "ADAMs 10 and 17 Represent Differentially Regulated Components of a General Shedding Machinery for Membrane Proteins Such as Transforming Growth Factor α, L-Selectin, and Tumor Necrosis Factor α", Molecular Biology of the Cell, 20:1785 (2009); Le Gall, et al., "ADAM 17 is Regulated by a Rapid and Reversible Mechanism that Controls Access to its Catalytic Site", Journal of Cell Science, 123:3913 (2010). For example, EGFR-ligand release can be measured by ELISA for TGFα, for example, or HB-EGF, or by release of tagged EGFR ligands. They can be tagged with alkaline phosphatase or any other tag that facilitates detection of the released growth factor into the supernatant. The quantity of EGFR ligand release is measured and compared with the quantity released under identical conditions in the absence of the test agent. Diminished EGFR ligand release in the presence of the test agents than in its absence is indicative of a test agent useful for the treatment of an EGFR dependent pathology. The efficacy of a test agent showing the ability to inhibit EGFR ligand release for treating EGFR dependent pathologies can be further tested and/or confirmed in additional assays for assessing efficacy against any one or more EGFR dependent pathologies. Typically, a plurality of test agents are tested, for example as in high throughput screening, for their ability to inhibit EGFR ligand release. Those test agents demonstrating an ability to inhibit EGFR ligand release are typically selected for further testing in assays for assessing efficacy against any one or more EGFR mediated pathology.

Another method for identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom1 for the treatment of an EGFR dependent pathology assesses the ability of a test agent to inhibit maturation of the 100 kD form of TACE, i.e., inhibits expression of the mature 100 kD form of TACE. The method comprises the step of combining the test agent and a cell which expresses the mature 100 kD form of TACE (e.g., on reducing SDS-PAGE) under conditions suitable for the expression of the mature form of TACE. Exemplary cells which express the mature form of TACE include Cos7 cells, mEF cells, endothelial cells, keratinocytes and many other cell types (because TACE is

ubiquitously expressed). The cell is either *iRhom1-/-*; or an inhibitor of iRhom 1 is additionally combined with the cell and test agent.

Another method for identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 for the treatment of an EGFR dependent pathology assesses the ability of a test agent to inhibit maturation of the 100 kD form of TACE, i.e., inhibits expression of the mature 100 kD form of TACE. The method comprises the step of combining the test agent and a cell which expresses the mature 100 kD (e.g., on reducing SDS-PAGE) form of TACE under conditions suitable for the expression of the mature form of TACE (e.g., on reducing SDS-PAGE). Exemplary cells which express the mature form of TACE include Cos7 cells, mEF cells, endothelial cells, keratinocytes and many other cell types (because TACE is ubiquitously expressed). The cell is either *iRhom2-/-*; or an inhibitor of iRhom2 is additionally combined with the cell and test agent.

The quantity of mature TACE that is expressed can be assessed using techniques known to one skilled in the art, e.g., Western blotting (e.g., on reducing SDS-PAGE). The quantity of mature TACE expression is measured and compared with the quantity produced under identical conditions in the absence of the test agent. Diminished expression of mature TACE in the presence of the test agents than in its absence is indicative of a test agent useful for the treatment of an EGFR dependent pathology. The efficacy of a test agent showing the ability in combination with an inhibitor of iRhom1 or iRhom2 to inhibit EGFR ligand release for treating EGFR dependent pathologies can be further tested and/or confirmed in additional assays for assessing efficacy against any one or more EGFR dependent pathologies. Typically, a plurality of test agents are tested, for example as in high throughput screening, for their ability to inhibit EGFR ligand release in combination with an inhibitor of iRhom1 or iRhom2. Those test agents demonstrating an ability to inhibit EGFR ligand release in combination with an inhibitor of iRhom1 or iRhom2 are typically selected for further testing in assays for assessing efficacy against any one or more EGFR mediated pathology.

Assays for assessing efficacy of a test agent against one or more diseases EGFR dependent pathologies are well known in the art.

WHAT IS CLAIMED IS:

1. A method for treating a subject with an EGFR dependent pathology, comprising the step of administering to the subject an effective amount of an agent ("First Agent") that decreases the biological activity of iRhom1 and an effective amount of an agent ("Second Agent") that decreases the biological activity of iRhom2.

2. A method for treating a subject with an EGFR dependent pathology, comprising the step of administering to the subject an effective amount of an agent ("First Agent") that modulates formation of a complex between iRhom 1 and TACE and an effective amount of an agent ("Second Agent") that modulates formation of a complex between TACE and iRhom2.

3. The method of Claim 1 or 2, wherein the EGFR dependent pathology is an EGFR dependent cancer.

4. The method of Claim 3, wherein the cancer is an epithelial cancer.

5. The method of Claim 3, wherein the cancer is colorectal cancer.

6. The method of Claim 3 wherein the cancer is squamous cell carcinoma of the head and neck.

7. The method of Claim 3, wherein the cancer is lung cancer, anal cancer, or glioblastoma multiforme.

8. The method of any one of Claims 3-7, wherein the colorectal cancer is KRAS wild-type.

9. The method of any one of Claims 3-8, wherein the First and Second Agents are administered in combination with an effective amount of a platinum based chemotherapy.

10. The method of Claim 9, wherein the platinum based chemotherapy is Cisplatin, carboplatin, capecitabine or oxaliplatin

11. The method of any one of Claims 1-9, wherein the cancer expresses EGFR.

12. The method of any one of Claims 1-11 wherein the First Agent and the Second Agent are the same.

13. The method of any one of Claims 1-11 wherein the First Agent and the Second agent are different.

14. The method of any one of Claims 1-13, wherein the First Agent is an antibody or antibody fragment that specifically recognizes iRhom1 and inhibits the activity of TACE; a small molecule inhibitor of iRhom1; a polypeptide decoy mimicking a domain necessary for the interaction of TACE and iRhom1 ; a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to iRhom1 DNA or mRNA; a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA for iRhom1; or a biological equivalent of each thereof.

15. The method of any one of Claims 1-13, wherein the Second Agent is an antibody or antibody fragment that specifically recognizes iRhom2 and inhibits the activity of TACE; a small molecule inhibitor of iRhom2; a polypeptide decoy mimicking a domain necessary for the interaction of TACE and iRhom2 ; a miRNA, a siRNA, a shRNA, a dsRNA or an antisense RNA directed to iRhom2 DNA or mRNA; a polynucleotide encoding the miRNA, siRNA, shRNA, dsRNA or antisense RNA for iRhom2; or a biological equivalent of each thereof.

16. The method of any one of Claims 1-14, wherein the First Agent is an antibody or antibody fragment that specifically recognizes iRhom1 and inhibits the activity of TACE.

17. The method of any one of Claims 1-16, wherein the Second Agent is an antibody or antibody fragment that specifically recognizes iRhom2 and inhibits the activity of TACE.

18. The method of any one of Claims 1-13, wherein the First Agent is an antibody or antibody fragment that specifically recognizes an extracellular domain of TACE.

19. The method of any one of Claims 1-13 or 18, wherein the Second Agent is an antibody that or antibody fragment that specifically recognizes an extracellular domain of TACE.

20. The method of Claim 1-13, wherein the First Agent is an antibody or antibody fragment that specifically recognizes an extracellular domain of iRhom1.

21. The method of Claim 1-13 or 20, wherein the Second Agent is an antibody or antibody fragment specifically recognizes an extracellular domain of iRhom2.

22. The method of any one of Claims 1-21, wherein the First and/or Second Agent further comprises a cell penetrating peptide.

23. The method of Claim 22, wherein the cell penetrating peptide comprises a HIV-TAT peptide.

24. The method of any one of Claims 1-23, wherein the iRhom1 and iRhom2 is a human iRhom1 and human iRhom2 or a splice variant thereof

25. The method of any one of Claims 1-17, wherein the First Agent is an antibody or antibody fragment that binds a portion of a transmembrane domain of iRohm1; and the Second Agent is an antibody or antibody fragment that binds a portion of a transmembrane domain of iRohm2.

26. A method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) for the treatment of an EGFR dependent pathology, comprising the steps of

a) combining TACE, iRhom 1 (or iRhom2) and a test agent under conditions suitable for forming a complex between TACE and iRhom1 (or iRhom2); and

b) assessing the quantity of complex formation between TACE and iRhom1 (or iRhom2), wherein diminished or increased complex formation between TACE and iRhom1 (or iRhom2) in the presence of the test agent than in the absence is indicative that the test agent is useful for the treatment of an EGFR dependent pathology in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1).

27. The method of Claim 26, further comprising the steps of:

c) repeating steps a) and b) one or more times with a different test agent;
d) selecting the test agents for which the amount of complex formation between TACE and iRhom1 (or iRhom2) is less in the presence of the test agent or greater in the presence of the test agent than in the absence of the test agent; and
e) assaying the test agents selected in step d) in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) in an assay for testing efficacy against an EGFR dependent pathology.

28. A method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) for the treatment of an EGFR dependent pathology, comprising the steps of

a) combining a test agent and a cell which releases an EGFR ligand under conditions suitable for stimulating release of the EGFR ligand, wherein the cell is *iRhom2-/-* (or *iRhom1-/-*) or wherein an inhibitor of a biological activity of iRhom2 (or iRhom1) is additionally combined with the cell and test agent; and
b) assessing the quantity of released EGFR ligand, wherein diminished EGFR ligand release in the presence of the test agent than in the absence is indicative that the test agent is useful in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) for the treatment of an EGFR dependent pathology.

29. The method of Claim 28, further comprising the steps of:

c) repeating steps a) and b) one or more times with a different test agent;
d) selecting the test agents for which the amount of EGFR ligand release is diminished in the presence of the test agent than in the absence of the test agent;
e) assaying the test agents selected in step d) in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) in an assay for testing efficacy against an EGFR dependent pathology.

30. The method of Claim 28 or 29, wherein the EGFR ligand is TGF-α.

31. The method of any one Claims 28-30, the cell is mouse embryonic fibroblast.

32. The method of any one of Claims 27-31, wherein the agents selected in step d) and the inhibitor of biological activity of iRhom2 (or iRhom1) are the same.

33. The method of any one of Claims 27-31, wherein the agents selected in step d) and the inhibitor of biological activity of iRhom2 (or iRhom1) are the different.

34. A method of identifying an agent which can be used in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) for the treatment of an EGFR dependent pathology,comprising the following steps:

a) combining a test agent and a cell which expresses the mature 100 kD form of TACE (e.g., on reducing SDS-PAGE) under conditions suitable for expression of the mature 100 kD form of TACE(e.g., on reducing SDS-PAGE), and wherein the cell is either *iRhom2-/-* (or *iRhom1-/-*) or an inhibitor of iRhom2 (or iRhom1) is additionally combined with the cell and test agent; and

b) assessing the quantity of the mature 100 kD form of TACE (e.g., on reducing SDS-PAGE) that is formed, wherein diminished formation of the mature 100 kD form of TACE in the presence of the test agent than in the absence is indicative that the test agent is useful in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) for the treatment of an EGFR dependent pathology.

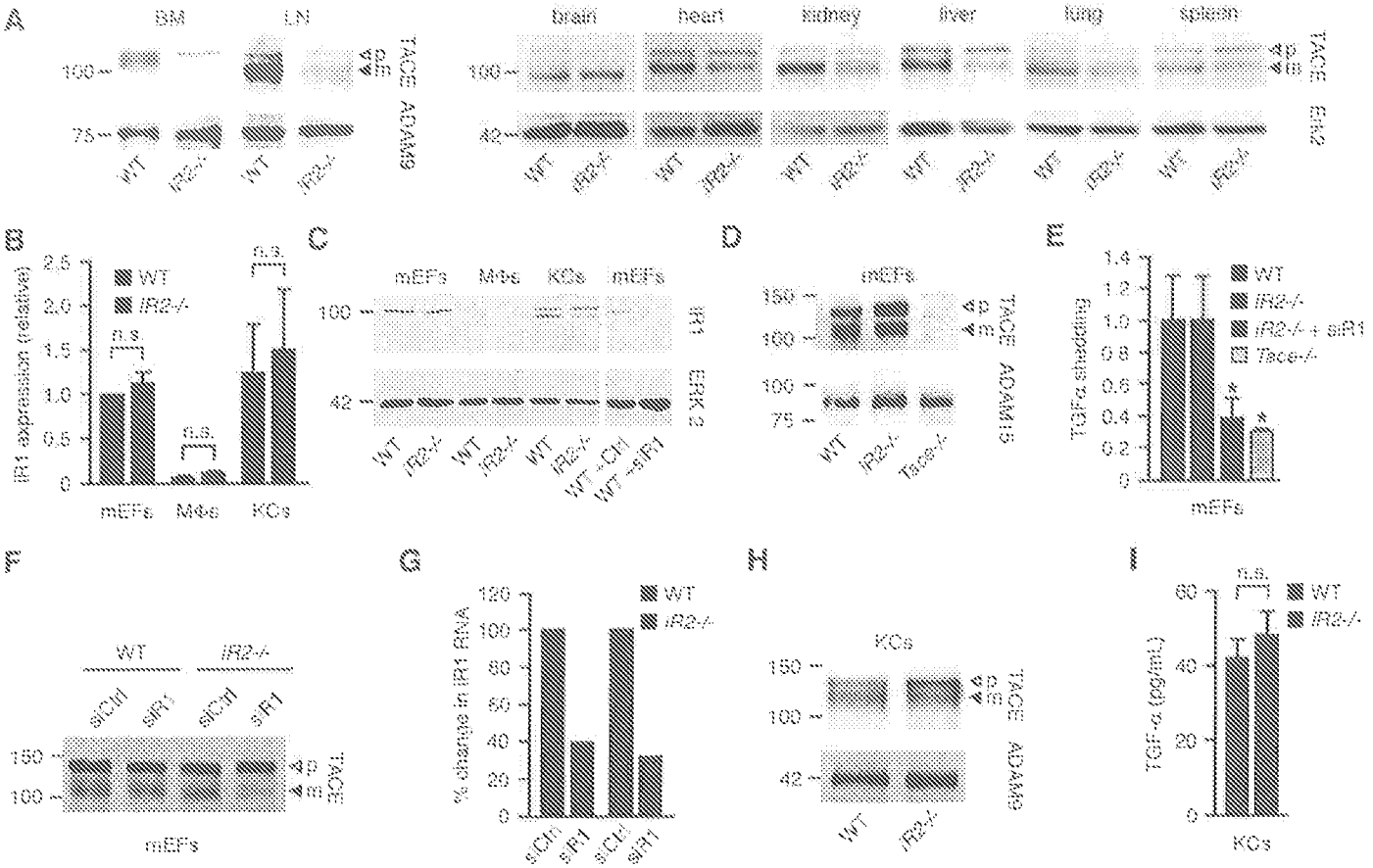
35. The method of Claim 34, further comprising the steps of:

c) repeating steps a) and b) one or more times with a different test agent;

d) selecting the test agents for which the quantity of the mature 100 kD form TACE formed (e.g., on reducing SDS-PAGE) is less in the presence of the test agent than in the absence of the test agent; and

e) assaying the test agents selected in step d) in combination with an inhibitor of a biological activity of iRhom2 (or iRhom1) in an assay for testing efficacy against an EGFR dependent pathology.

Figure 1


A
BM
LN
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heart
kidney
liver
lung
spleen
TACE
ADAM9
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n.s.
mEFs
MΦs
KCs
C
IR1
ERK 2
WT +Ctrl
WT +siR1
D
mEFs
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ADAM15
Tace-/-
E
TGF-α shedding
IR2-/- + siR1
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F
siCtrl
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I
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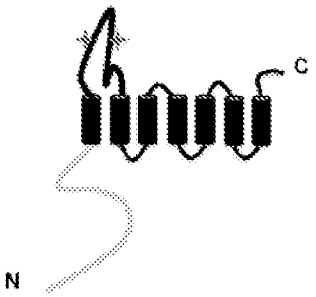

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SFQGTESPKPCKMPKIVDPLARGRAFRHPDEVDRPHAAHPPLIPGVLSLISFTSVRSGYSHLPRRKRISV
AHMSFQAAAAALLKGRSVLDATGQRCRHVKRSFAYPSFLEEDAVDGADTFDSSFFSKEEMSSMPDDVFESP
PLSASYFRGVPHSASPVSPDGVHIPLKEYSGGRALGPGTQRGKRIASKVKHFAFDRKKRHYGLGVVGNWL
NRSYRRSISSIVQRQLESFDSHRPYFI**YWLTFVHIIITLLVICTYGIA**PVGFAQHVTTQLVLKNRGVYES
VKYIQQENFWIGPSSIDLIHLGAKFSP[illegible]IRKDQQIEQLVRRERDIERISG[illegible]VQNDRSG[illegible]IQTLKKD[illegible]SE
TLATFVKWQ[illegible]GPSDKSDLSQKQPSAVV[illegible]HQDPRT[illegible]EEPASSGAHIWPDDITKWPI[illegible]TEQAQS[illegible]GLL
HID[illegible]KIKGRP[illegible]IGTKGS[illegible]EITTREY[illegible]EFMHGYFHEDATL[illegible]SQVH[illegible]LDKV[illegible]GLLPFLNPEVPDQFYRIWL
SLFLHAGIVHCLVSVVFQMTILRDLEKLAGW**HRISIIFILSGITGNLASAIF**LPYR**AEVGPAGSQFGLLA**
CLFVELFQSWQLLERPWK**AFFNLSAIVLFLFICGLLPWI**DNIA**HIFGFLSGMLLAFAFLPYITF**GTSDKY
RKRA**LILVSLLVFAGLFASLVLWLY**IYPINWPWIEYLT[illegible]FPFTSRF[illegible]EKYELDQVLH (SEQ ID NO 1)

IRHOM1

MSEARRDSTSSLQRKKPPWLKLDIPAAVPPAAEEPSFLQPLRRQAFLRSVSMPAETARVPSPHHEPRRLVLQRQT
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NVKYVQQENFWIGPSSEALIHLGAKFSP[illegible]MRQDPQVHSFILAARAREREKHSA[illegible]VRNDRSG[illegible]VQTSKEE[illegible]SSTLAV
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[illegible]IGTKGR[illegible]EITSREY[illegible]DFMRGYFHEEATL[illegible]SQVH[illegible]MDDV[illegible]GLLPFLNPEVPDQFYRLWLS**LFLHAGILHCLVSVC**
FQMTVLRDLEKLAGWHR**IAIIYLLSGITGNLASAIFL**PYRAEVG**PAGSQFGILACLFVELFQSWQIL**ARPW**RAFF**
KLLAVVLFLFAFGLLPWIDNFAHI**SGFVSGLFLSFAFLPYISF**GKFDLYRKRCQ**IIIFQVVFLGLLAGLVVLFYF**
YPVRCEWCEFLT[illegible]IPFTDKF[illegible]EKYELDAQLH (SEQ ID NO 2)

Figure 2

Alignment: iR2: top, iR1: bottom

YWLTFVHIIITLLVICTYGIAPVGFAQHVTTQLVLKNRGVYES

YWLTFVHSLVTILAVCIYGIAPVGFSQHETVDSVLRKRGVYEN

VKYIQQENFWIGPSSIDLIHLGAKFSP[redacted]IRKDQQIEQLVRRERDIERTSG[redacted]VQNDRSG[redacted]IQTLKKD[redacted]SE

VKYVQQENFWIGPSSEALIHLGAKFSP[redacted]MRQDPQVHSFILAARAREKHSA[redacted]VRNDRSG[redacted]VQTSKEE[redacted]SS

TLATFVKWQ[redacted]GPSDKSDLSQKQPSAVV[redacted]HQDPRT[redacted]EEPASSGAHIWPDDITKWPI[redacted]TEQAQS[redacted]GLL

TLAVWVKWPVHPSAPDLAGNKRQFGS--V[redacted]HQDPRV[redacted]DEPSSEDPHEWPEDITKWPI[redacted]TKSSAG[redacted]NHP

HID[redacted]KIKGRP[redacted]IGTKGS[redacted]EITTREY[redacted]EFMHGYFHEDATL[redacted]SQVH[redacted]LDKV[redacted]GLLPFLNPEVPDQFYRIWL

HMD[redacted]VITGRP[redacted]IGTKGR[redacted]EITSREY[redacted]DFMRGYFHEEATL[redacted]SQVH[redacted]MDDV[redacted]GLLPFLNPEVPDQFYRLWLS

SLFLHAGIVHCLVSVVFQMT (SEQ ID NO 3)

SLFLHAGILHCLVSVCFQMT (SEQ ID NO 4)

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/076954

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A01K67/027 C07K16/18
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)
C12N A01K C07K

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Z. YAN ET AL: "Human rhomboid family-1 gene silencing causes apoptosis or autophagy to epithelial cancer cells and inhibits xenograft tumor growth", MOLECULAR CANCER THERAPEUTICS, vol. 7, no. 6, 4 June 2008 (2008-06-04), pages 1355-1364, XP055109928, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-08-0104 whole document, especially the Abstract; page 1359, paragraph bridging columns; Figure 6B; page 1363, last paragraph ----- -/--	1-35

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

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"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
4 April 2014	10/04/2014

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	Luyten, Kattie

2

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H. ZOU ET AL: "Human rhomboid family-1 gene RHBDF1 participates in GPCR-mediated transactivation of EGFR growth signals in head and neck squamous cancer cells", THE FASEB JOURNAL, vol. 23, no. 2, 30 September 2008 (2008-09-30), pages 425-432, XP055109933, ISSN: 0892-6638, DOI: 10.1096/fj.08-112771 whole document, especially the Abstract; paragraph bridging pages 425-426	1-35
X	HE Y ET AL: "INHIBITION OF HUMAN SQUAMOUS CELL CARCINOMA GROWTH IN VIVO BY EPIDERMAL GROWTH FACTOR RECEPTOR ANTISENSE RNA TRANSCRIBED FROM THEU6 PROMOTER", JOURNAL OF THE NATIONAL CANCER INSTITUTE, OXFORD UNIVERSITY PRESS, GB, vol. 90, no. 14, 1 January 1998 (1998-01-01), pages 1080-1087, XP001009921, ISSN: 0027-8874, DOI: 10.1093/JNCI/90.14.1080 the Abstract; page 1082, second full paragraph	1-35
X	C. ADRAIN ET AL: "Tumor Necrosis Factor Signaling Requires iRhom2 to Promote Trafficking and Activation of TACE", SCIENCE, vol. 335, no. 6065, 13 January 2012 (2012-01-13), pages 225-228, XP055089578, ISSN: 0036-8075, DOI: 10.1126/science.1214400 cited in the application page 225, last paragraph; page 226; page 228; Figures 3F, 4	1-35
X,P	T. MARETZKY ET AL: "iRhom2 controls the substrate selectivity of stimulated ADAM17-dependent ectodomain shedding", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 110, no. 28, 9 July 2013 (2013-07-09) , pages 11433-11438, XP055089707, ISSN: 0027-8424, DOI: 10.1073/pnas.1302553110 the whole document	1-35

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LICHTENTHALER STEFAN F: "iRHOM2 takes control of rheumatoid arthritis", THE JOURNAL OF CLINICAL INVESTIGATION,, vol. 123, no. 2, 1 February 2013 (2013-02-01), pages 560-562, XP009174505, ISSN: 1558-8238, DOI: 10.1172/JCI67548 the whole document	1-35
X,P	PRIYA DARSHINEE A. ISSUREE ET AL: "iRHOM2 is a critical pathogenic mediator of inflammatory arthritis", JOURNAL OF CLINICAL INVESTIGATION, 1 February 2013 (2013-02-01), XP055089725, ISSN: 0021-9738, DOI: 10.1172/JCI66168 the whole document	1-35