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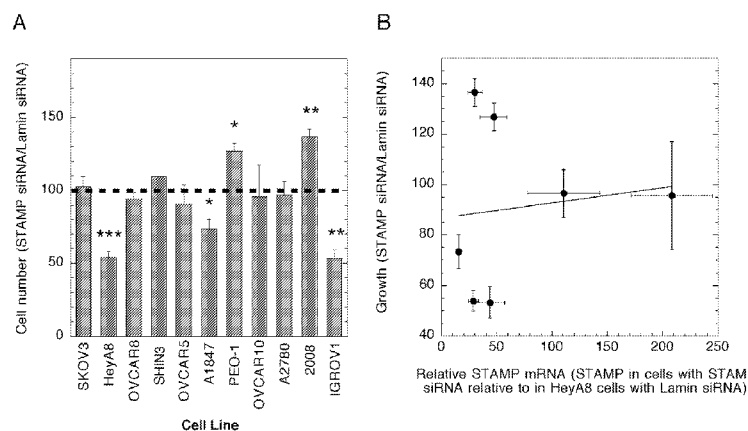


FIGURE 3

(57) Abstract: The subject invention comprises methods for diagnosing ovarian cancer using a STAMP expression product biomarker, kits for diagnosing ovarian cancer using assays for the STAMP expression producing biomarker, compositions comprising STAMP siRNA, methods for inhibiting growth of ovarian cells expressing STAMP expression product, and methods for treating patients diagnosed with ovarian cancer. The invention is premised on the unexpected discovery that STAMP affects the physiologically relevant activity of ovarian cancer cell growth apparently independent of several steroid hormones.

USE OF STAMP RELATED MOLECULES IN THE DIAGNOSIS AND INHIBITION OF OVARIAN CANCER CELLS

FEDERALLY FUNDED RESEARCH

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FIELD OF THE INVENTION

10 The subject invention relates to compositions, methods and kits for the research, diagnosis and treatment of ovarian cancer.

BACKGROUND OF THE INVENTION

15 Cell proliferation is a complicated and poorly understood process with contributions from many factors. Consequently identifying the causes for abnormal cell propagation, such as in cancers, has been an even more daunting task. Recent evidence suggests that mutant pathways (produced by a variety of mutant or dysregulated proteins) as opposed to a single mutant or dysregulated protein are responsible for at least some cancers (Jones, S. et al. (2008)). One
20 pathway that is well known for its role with normal cell growth and differentiation involves the classical steroid receptors. Androgen (ARs), estrogen (ERs), and progesterone receptors (PRs) are best recognized for their contribution in the morphogenesis of reproductive tissues (Wang et al. (2009); Hewitt et al. (2005); Ismail et al. (2003)). Conversely, preventing the actions of ARs and ERs has been the major treatment of choice for many years in combating hormone-sensitive
25 prostate and breast cancers respectively (Gradishar & Jordan (1997); Akaza (2008)). Glucocorticoids are clinically invaluable in treating lymphoid cancers and attenuating immune responses (Cole (2006)) while mineralocorticoid agonists are now thought to have significant effects on the heart and vasculature (Funder (2005); Schiffrin (2006)). A major component in the expression of steroid receptor-regulated gene expression is coactivators and corepressors,
30 which typically elevate or inhibit the action of receptor-agonist complexes (McKenna & O'Malley (2002); Nagy & Schwabe (2004)).

STAMP is a novel coregulator that enhances the ability of p160 coactivator family members TIF2 and SRC-1 to augment gene induction by AR, glucocorticoid receptor (GR), and PR. STAMP also modulates the potency (or EC₅₀) of agonists and the partial agonist activity of antisteroids (He & Simons (2007); Szapary et al. (2008)). These modulatory activities of STAMP are not limited to gene induction but are also observed for GR-mediated gene repression (Sun et al. (2008)). These actions appear to proceed via the binding of internal and C-terminal regions of STAMP to coactivators and receptors respectively in a manner that includes steroid-mediated recruitment of STAMP to the enhancer region of regulated genes. Interestingly, STAMP does not interact with ER or members of the nuclear receptor sub-family such as thyroid receptor, PPAR, RAR, or RXR (He & Simons (2007)).

The physiological role of STAMP, however, remains unclear. The absence of STAMP paralogs in the human genome suggests an important function. STAMP has been included as a member of the tyrosine tubulin ligase-like (TTLL) family (Janke et al. (2005)) on the basis of containing a tyrosine tubulin ligase (TTL) domain. However, this grouping is not applicable to several of the biological function assays of STAMP in that the coregulatory activity of STAMP with GRs does not require the TTL domain (He & Simons (2007)). Differential detection in Northern blots (He & Simons (2007)) and qPCR analysis of mouse tissues (G-S Lee and SS Simons, unpublished) is consistent with a role for STAMP in brain, ovaries, and testes. In addition, the importance of steroid receptors in normal and aberrant cell growth combined with STAMP binding to, and modulating the activity of, AR, GR, and PR suggests a possible role for STAMP in regulating the growth of some cells and cancers.

Ovarian cancer is the fifth most common cancer in the United States and is responsible for 4% of new cancer cases, and 5% of the cancer deaths, occurring in women each year (Greenlee et al. (2001); Jemal et al. (2007)). The major cause of death is from epithelial ovarian tumors, which constitute the large majority of ovarian cancers (Leung et al. (2001)). Ovarian cancer in general has a 5-year survival rate of over 90% if diagnosed and treated early, when the cancer is confined to the ovaries. Unfortunately, due to ovarian cancer's non-specific symptoms and the lack of reliable early detection methods, only about 20% of all cases are found at this beginning stage. If caught in an advanced stage, the 5-year survival rate can be as low as 29% (Jemal et al. (2002); Cvetkovic (2003)). The inability to identify and treat this cancer early underscores the necessity for better understanding its biology and unique gene and protein

expression panels. Unfortunately, the heterogeneity of epithelial ovarian cancers complicates this task. It has recently been suggested that ovarian cancer, like breast cancer, may be subdivided by differences in gene expression panels (Kobel et al. (2008); Malek et al., in press)). Therefore, it is likely that the mechanism(s) of ovarian cancer growth and metastasis will involve multiple biochemical pathways, as has been concluded for pancreatic and breast cancers (Jones et al. (2008); Easton et al. (2007)).

There is a long-felt need for a reliable diagnostic test for ovarian cancer. Further, advances in treatment are urgently needed in order to improve the 5-year survival rate, particularly for those patients who are not diagnosed until an advanced stage. The subject invention addresses these needs.

SUMMARY OF THE INVENTION

The subject invention comprises methods of diagnosing ovarian cancer in a patient, kits for diagnosing ovarian cancer, compositions comprising STAMP siRNA or variants thereof, methods of reducing ovarian cancer cell growth in ovarian cancer cells *in vitro*, and methods of reducing cancer cell growth in a patient diagnosed with ovarian cancer.

In one embodiment, the invention encompasses a method of diagnosing ovarian cancer in a patient comprising assaying ovarian tissue in said patient for expression of the STAMP gene; and determining whether the expression level of STAMP gene in the ovarian tissue corresponds to ovarian cancer. When determining whether the determined STAMP expression level corresponds to ovarian cancer, the skilled artisan can compare the determined expression level to a normal range standard for STAMP expression.

The diagnosis method expression assay can be an assay for STAMP mRNA. In one embodiment, the expression level of STAMP mRNA can be correlated to a specific stage of ovarian cancer. In the alternative, the diagnosis method expression assay is an assay for STAMP polypeptide or protein.

The subject invention also comprises a kit for diagnosing ovarian cancer. The kit comprises one or more capture reagents for capturing STAMP expression product, and means for detecting the binding of capture reagent to STAMP expression product. In one embodiment, the STAMP expression product is STAMP mRNA, and the capture reagent is a nucleic acid such as a probe. In this case, the means for detection is an appropriate label on the probe such as a

radiolabel, fluorescent label or the like. In another embodiment, the capture reagents are primers (DNA or RNA) for qRT-PCR detection of the STAMP amplicon. In yet another embodiment, the STAMP expression product is a STAMP polypeptide, and the capture reagent is an antibody. Here, the means for detection is a suitable label on the antibody such as a fluorescent tag or the like.

The invention also comprises a composition comprising a STAMP specific siRNA molecule or a variant thereof. The siRNA is selected from the group consisting of SEQ ID NOS:1-8 provided below, or variants thereof. In the following sequences, the top strand is the sense strand, and the bottom is the antisense strand. (Note that "P" indicates a 5' phosphate on the adjacent nucleotide.)

Duplex 1

5' GGUCCUACCUCGAGCAUAAUU (SEQ ID NO.:1)
 15 UUCCAGGAUGGAGCUCGUAUUP 5' (SEQ ID NO.:2)

Duplex 2

5' GUUCGUGAAUGGAAUAAUAAU (SEQ ID NO.:3)
 20 UUCAAGCACUUACCUAUUAUUAUP 5' (SEQ ID NO.:4)

Duplex 3

5' CGACGGAGUAGCAGAUUGAUU (SEQ ID NO.:5)
 25 UUGCUGCCUCAUCGUCUAACUP 5' (SEQ ID NO.:6)

Duplex 4

5' GGAUCGUGCUAUCUAAACAUAU (SEQ ID NO.:7)
 30 UUCCUAGCACGAUAGAUUUGUP 5' (SEQ ID NO.:8) .

In another embodiment, the invention comprises a method of reducing ovarian cancer cell growth *in vitro* in ovarian cancer cells responsive thereto, comprising contacting said ovarian cancer cells with an agent that reduces STAMP gene expression. The agent can be STAMP siRNA or a variant thereof, STAMP antisense molecules (DNA or RNA) or a variant thereof, or a STAMP antibody.

The invention further comprises a method of reducing cancer cell growth in a patient diagnosed with ovarian cancer comprising administering to the patient a therapeutically effective amount of a therapeutic agent that reduces STAMP gene expression, whereby ovarian cancer cell growth is substantially inhibited. The therapeutic agent can comprise STAMP siRNA, STAMP antisense molecules, and/or STAMP antibodies.

All references cited herein are incorporated in their entirety by reference.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates the effect of stably transfected STAMP on the growth rate of HEK 293 cells. Fig. 1(A) demonstrates that increased STAMP decreases cell growth; transfected cells S13 have a lower cell titre than empty vector VA cells. Figs. 1(B) and (C) provide the FACS analysis of VA and S13 cells with no steroid and G418 removed upon seeding, as described in the Examples. Fig. 1(D) sets forth the growth of VA and S13 cells after removal of G418 for more than one month as described in the Examples. Fig. 1(E) illustrates increased STAMP results in decreased cell growth with or without steroid, as described in the Examples.

Fig. 2 illustrates the levels of STAMP mRNA in different human tumor samples. Fig. 2(A) provides levels of STAMP mRNA in eight human tumor types. Fig. 2(B) provides STAMP mRNA in human ovarian cancers as described in the Examples.

Fig. 3 illustrates the effect of STAMP siRNA on growth of human ovarian cancer tissue culture cells. Fig. 3(A) provides the growth of ovarian tissue culture cells treated with STAMP siRNA relative to Lamin siRNA as described in the Examples. Fig. 3(B) provides the growth rate vs. STAMP mRNA levels of each of the cell lines in Fig. 3(A).

Fig. 4 illustrates the effect of elevated STAMP on the properties of GR induction of GREtkLUC reporter gene IN 293 CELLS. Fig. 4(A) provides the dose-response curves for GR induction of GREtkLUC reporter with varying amounts of transiently transfected GR plasmid, as described in the Examples. Fig. 4(B) illustrates modulation of A_{max} , partial agonist activity of the antiglucocorticoid Dex-Mes (% DM), and EC_{50} by elevated STAMP as described in the Examples. Fig. 4(C) provides GR mRNA levels in VA and S13 cells with or without 30 ng of transiently transfected GR plasmid, as described in the Examples. Fig. 4(D) provides Western blot with TIF2 antibody of cell lysates from cells transfected with no or 30 ng of GR plasmid as described in the Examples.

DETAILED DESCRIPTION

I. Discussion of the Results Set Forth in the Examples

5 In the subject invention, it has surprisingly been found that STAMP affects the physiologically relevant activity of cell growth apparently independent of several steroid hormones. STAMP was initially characterized as a new factor that bound to, and augmented the ability of, the coactivator TIF2 to modulate the transcriptional properties GR, AR, and PR (He et al. (2007); Szapary et al. (2008); Sun et al. (2008)). The steroid-independent effects of STAMP
10 on the growth of a variety of cells represent a new activity that differs with the cell line, even within the same cell type. As is exemplified herein, increased levels of stably transfected STAMP inhibit the growth rate of human embryonic kidney 293 cells while all possible growth responses with changing STAMP are seen in an assortment of ovarian cancer cell lines. It has also been found that a significant correlation exists between the level of STAMP mRNA and the
15 presence of ovarian cancer. Stage I-III tumor samples possess significantly higher levels of STAMP mRNA.

STAMP was increased in expression in malignant epithelial ovarian cancer compared to normal ovary of unknown epithelial component and the epithelial tumor of low malignant potential. In view of the known heterogeneity of ovarian cancers (Kobel (2008); Malek, in
20 press)), the varied responses to silencing of STAMP in the ovarian cancer cell lines suggest that there may be a select subset of ovarian cancers in which STAMP may regulate behavior. Epithelial ovarian cancer, while initiating in a steroid hormone sensitive organ and known to have functional ER and PR, has not been shown to be steroid hormone driven as in prostate and breast cancer. The lack of response to steroid hormones found in the subject Examples may be
25 an early signal as to a new role for STAMP that may ultimately explain the altered growth with modulation of STAMP in ovarian cancers.

As exemplified herein, eleven tissue culture cell lines of human ovarian cancer revealed a cell line-dependent effect of reduced STAMP mRNA, with increased or decreased growth rates seen in many lines and no changes in others. Identical cell-specific responses are seen in Fig. 4
30 regarding another activity of STAMP: its ability to modulate various parameters of GR-regulated gene induction. Thus, the effects of STAMP appear to be sensitive to unknown cell-

specific factors. These varying consequences of STAMP are reminiscent of CBP and p300, which can display gene repressive activity (Szapary et al. (1999); Fonte et al. (2005); Guidez et al. (2005); Santoso et al. (2006)) in addition to its more common coactivator properties (McKenna & O'Malley (2002); Rosenfeld et al. (2006)). For STAMP, the different responses are unrelated to the absolute gene expression level of STAMP after knockdown with STAMP siRNA (Fig. 3B). There is also no direct correlation between the growth rate of the cells and the amounts of seven common growth factors and kinases. GEO file searches uncovered several proteins, the expression of which shows some correlation with STAMP on various platforms, but no consistent connection of any protein was evident across the different platforms (data not shown). Thus we have not yet identified the molecular cause for STAMP's effects on cell growth. Nevertheless, given the heterogeneity of ovarian cancers, the statistically significant effect of decreased STAMP mRNA on the growth rates of 5 of 11 ovarian cancer tissue culture cell lines represents a medically important and useful advance in inhibition and treatment of ovarian cancer cells.

The steroid-independent effect of STAMP on the growth of stably transfected 293 cells appears to reflect a new activity in addition to its ability to alter the properties of gene induction by GR, PR, and AR (He et al. (2007); Szapary et al. (2008)). The only other characterized domain of STAMP is the tyrosine tubulin ligase-like domain in the amino-terminal third of the molecule, which overlaps the region encoding polyglutamylation activity in some TTL domain proteins including STAMP (Janke et al. (2005)). We have confirmed that STAMP is able to polyglutamylate tubulin and a few other unidentified proteins (He and Simons, data not shown). While the TTL domain, and polyglutamylation activity, of STAMP are not required for its modulation of GR induction properties (He et al. (2007)), they could be relevant for effects on cell growth. It is also possible that steroids will be found to influence the growth properties of the ovarian cancer tissue culture cell lines as their response to different levels of STAMP is often different from that of the 293 cells, where growth is unaffected by steroids. Thus, understanding the role of STAMP in ovarian cancer can lead to new insight to the deadly disease.

II. Methods, Compositions and Kits for Research and Diagnosis of Ovarian Cancer.

Compositions for Research and Diagnosis

Compositions of the subject invention that can be useful in research and/or diagnosis or
5 in any other embodiment of the invention include siRNA, antisense molecules, antibodies and probes.

RNA interference, RNAi, is a naturally occurring cellular defense in eukaryotic cells. It is an evolutionarily conserved post-transcriptional gene silencing mechanism. It is also a reverse genetic tool that can be more effective than antisense technology. Only a few molecules have
10 been found necessary to reduce the target RNA population (Fire (2004)). Small interfering RNAs, siRNAs, have been used to exploit the natural gene silencing pathway.

siRNAs are double stranded and 19-25 bps long. In the cell, siRNA form a complex with RISC protein and the siRNA then dissociates. One strand of the siRNA (“guide” strand) hybridizes to the target mRNA. Then the RISC complex cleaves the mRNA (Juliano et al
15 (2008)). The complex is capable of repeating this process multiple times, enhancing the potency of the siRNA. The silencing effect can be relatively stable and persist through several cell divisions (Tuschl et al. (2004)).

siRNA can be rationally designed from the sequence of the target mRNA using commercially available software (e.g., siGENOME™). Generally, it is important for siRNA to
20 be designed to maintain an A form (RNA-like) duplex; that the 5' position on the antisense strand be able to be phosphorylated; and that it have low thermodynamic stability in the 5' antisense region (Juliano et al. (2008)). In designing the siRNA, specificity should also be taken into consideration in order to avoid “off-target” effects. Specificity can be enhanced with the use of commercially available products (e.g., ON-TARGET™ and siSTABLE™ products). To
25 enhance siRNA stability and half-life *in vitro* or *in vivo*, siRNA can be chemically modified. . Chemical modification of the siRNA to enhance *in vivo* half-life must take into consideration maintenance of silencing capability and specificity to STAMP mRNA, and toxicity to the cell.

siRNA can be labeled (if desired) for detection purposes with radiolabels, biotin, enzymes, chemiluminescent, fluorescent labels and the like using methods known in the art.

30 Antisense molecules can also be used in diagnostic and treatment embodiments of the subject invention. Antisense molecules are designed to be complementary to STAMP mRNA.

The antisense hybridizes to the mRNA and thus blocks translation of mRNA into protein. As used herein, "antisense molecules" includes and is used interchangeable with "antisense," "antisense nucleic acids," "antisense oligonucleotides" and "antisense polynucleotides."

Antisense nucleic acids of the invention can be designed using the nucleotide sequences of the STAMP gene and constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine; 2,2-dimethylguanine; 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N-6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

Antisense can be labeled (if desired) for detection purposes with radiolabels, biotin, enzymes, chemiluminescent, fluorescent labels and the like using methods known in the art.

Probes are single-stranded DNA or RNA that hybridizes to the complementary sequence in blotting or in situ techniques. They can be as short as 14 bases long and as long as several 100 bases. They are labeled for detection purposes with radiolabels, biotin, enzymes, chemiluminescent, fluorescent, antibody labels or the like using methods known in the art.

Probes can be used in Southern blots (DNA), Northern blots (RNA), dot blots, and in situ hybridization (detection of nucleic acid in clinical specimen).

The siRNA, probes and antisense molecules of the subject invention can include variants. Generally, nucleotide sequence variants of the invention will have at least 60%, 65%, 70%, 75%,
5 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the STAMP siRNA sequence(s) disclosed herein or the STAMP gene known in the art (He & Simons (2007)).

Variants can also include sequences that hybridize under stringent conditions. As used herein, the term “hybridizes under stringent conditions” describes conditions for hybridization
10 and washing. Stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions is hybridization in 6× sodium chloride/sodium citrate (SSC, Sambrook & Russell (2001)) at about 45°C, followed by one or more washes in
15 0.2×SSC; 0.1% SDS at 50° C. Another example of stringent hybridization conditions is hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2×SSC, 0.1% SDS at 55° C. A further example of stringent hybridization conditions is hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2×SSC, 0.1% SDS at 60° C. Preferably, stringent hybridization
20 conditions are hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65° C., followed by one or more washes at
25 0.2×SSC, 1% SDS at 65° C.

An “isolated” STAMP nucleic acid is one that is separated from other nucleic acids present in the natural source of STAMP nucleic acid. Preferably, an “isolated” nucleic acid is free of sequences which naturally flank STAMP nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is
30 derived. This is particularly true for siRNA. For probes and antisense molecules, there can be some flanking 5' and 3' sequences, up to about 1-5 kB.

The isolated material can be purified to various extents. It can be part of a composition, for example, a crude extract, containing other substances, or other mixture. Or, the isolated material may be purified to essential homogeneity, for example as determined by PAGE or column chromatography such as HPLC. Preferably, an isolated nucleic acid comprises at least
5 about 50, 70, 80 or 90% (on a molar basis) of all macromolecular species present.

The invention also provides antibodies that selectively bind to the STAMP protein and/or its variants and fragments. An antibody is considered to selectively bind, even if it also binds to other proteins that are not substantially homologous with the STAMP. These other proteins share homology with a fragment or domain of STAMP. This conservation in specific regions
10 gives rise to antibodies that bind to both proteins by virtue of the homologous sequence. In this case, it would be understood that antibody binding to STAMP is still selective.

To generate antibodies, an isolated STAMP polypeptide is used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. Either the full-length protein or antigenic peptide fragment can be used. An appropriate immunogenic
15 preparation can be derived from native, recombinantly expressed, or chemically synthesized peptides.

The antigenic peptide can comprise a contiguous sequence of at least 12, 14, 15, or 30 amino acid residues. In one embodiment, fragments correspond to regions that are located on the surface of the protein, e.g., hydrophilic regions. Antibodies can be polyclonal or monoclonal.

20 An intact antibody, or a fragment thereof (e.g. Fab or F(ab')₂) can be used.

Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,
25 β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and
30 examples of suitable radioactive material include ¹²⁵I, ¹³¹I, ³⁵S or ³H.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. For an overview of this technology for producing human antibodies, see Lonberg & Huszar (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, e.g.,
5 Lonberg & Kay, U.S. Pat. Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806.

Methods for *In Vitro* Delivery into Cells and Inhibition of Expression

Oligonucleotides, including siRNA and antisense molecules, do not diffuse across intact cell membranes to any significant degree due to the hydrophobic nature of the membrane lipid
10 bilayer and the negatively charged siRNA and antisense molecules.

Antisense molecules and siRNA can be delivered into cultured cells by transfection (e.g., lipid-mediated), viral infection, electroporation or microinjection. They can also be delivered via cell target ligands (CTLs) that target specific receptors or cell penetrating peptides (CPPs). CPPs are typically polyanionic polypeptides rich in arginine and lysine that are linked to their “cargo”
15 and then bind to cell surface glycosaminoglycans; endocytotic uptake is followed by the release of cargo into the cytoplasm. CTLs include a wide variety of ligands to receptors, such as antibodies, polypeptides derived from phage display libraries and small organic molecules. Antisense molecules and siRNA can also be delivered by nanocarriers including liposomes, cationic polymer complexes and various polymeric nanoparticles (Juliano et al. (2008)).

20 Antibodies can be delivered into cultured cells by transfection (lipid or viral-mediated), electroporation or microinjection.

Silencing of gene expression can be detected by assays for mRNA, including Northern blot analysis, branched DNA and quantitative real time PCR (qRT-PCR). It can also be detected by protein assays such as immunoblotting (i.e., Western blotting), enzyme-linked
25 immunosorbent assay (ELISA), immunocytochemistry, immunohistochemistry, immunofluorescence and immunoprecipitation.

Methods and Kits for Detection of Overexpression of STAMP in Ovarian Cells

The subject invention also comprises methods for detection and diagnosis of ovarian
30 cancer in a patient comprising assaying ovarian tissue in the patient for expression of the

STAMP gene and determining whether the expression level of STAMP gene corresponds to ovarian cancer.

In one embodiment, the assay comprises a quantitative real time PCR (qRT-PCR) using DNA STAMP primers complementary to STAMP mRNA. In other embodiments, the assay can
5 use RNA primers. STAMP mRNA can also be quantitated using labeled probes or other methods known to those of skill in the art.

In another embodiment, the assay comprises an assay for a STAMP antigenic polypeptide or protein using an antibody (polyclonal or monoclonal). The antibody can be labeled for detection as described herein.

10 To make a diagnosis of ovarian cancer, the observed STAMP expression level as determined by the foregoing methods can optionally be compared to STAMP normal range levels as determined by studies of STAMP levels found in cancerous and healthy or non-cancerous tissue. In some cases, the expression level of STAMP mRNA can be correlated to a specific stage of ovarian cancer.

15 The kits of the subject invention comprise a capture reagent that captures the expression product and a detecting means that permits the detection of the complex of capture reagent and expression product.

Typically, the capture reagent is an antibody, if the expression product is a protein, or primers, a nucleic acid or probe, if the expression product is mRNA. Where the capture reagent
20 is an antibody or a probe, the detecting means can be a detectable label on the capture reagent that permits the detection of the complex using methods known in the art. Where the capture reagents are primers for use in qRT-PCR, the detecting means can be amplicon dye or label (e.g., Sybr® Green or ethidium bromide). The detected amount of expression product can optionally be compared to a standard in making a diagnosis of ovarian cancer.

25 In one embodiment, the invention encompasses kits for using antibodies to detect the presence of a STAMP protein in an *in vitro* or biological sample. The kit can comprise antibodies such as a labeled or labelable antibody for detecting STAMP in a biological sample; means for determining the amount of Ab-STAMP complex in the sample; and optionally means for comparing the amount of STAMP in the sample with a standard. The compound or agent can
30 be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect STAMP.

In another embodiment, the invention encompasses kits for detecting the presence of a STAMP nucleic acid *in vitro* or in a biological sample. For example, the kit can comprise reagents such as a labeled or labelable nucleic acid capable of detecting STAMP nucleic acid in a biological sample; means for determining the amount of labeled STAMP nucleic acid complex in the sample; and optionally means for comparing the amount of STAMP nucleic acid in the sample with a standard. The STAMP nucleic acid is typically a probe which can be designed to be complementary to STAMP mRNA. The probe can be packaged in a suitable container. The detected or quantified STAMP mRNA can then optionally be compared to a standard to determine the diagnosis. The kit can further comprise instructions for using the kit to detect STAMP mRNA.

In addition, real time PCR (qRT-PCR) now is a standard method to detect mRNA level. Therefore, kits for determining the quantity of STAMP mRNA can include any method using qRT-PCR to detect STAMP mRNA level in ovarian tissue or cells. The kit can contain specific probes or primers (DNA or RNA) against specific STAMP mRNA sequences.

Methods for determining the quantity of labeled capture reagent vary with the nature of the label. For example, a radiolabel probe comprising ^3H can be measured by liquid scintillation counting. Fluorescently labeled proteins can be measured using UV radiation.

The comparison of the amount of captured expression product with a standard requires a pre-determination of a normal range for expression product. The means for comparison can be visual, computer-assisted or any method known in the art.

The kits are useful for the detection of STAMP protein, polypeptide, mRNA or other nucleic acid in tissues and cells and their extracts. While in a preferred embodiment, the kits are used for detection and diagnosis of ovarian cancer in ovarian tissue and cells, the kits can also be used for detection of STAMP expression product in other tissues and cells.

III. Methods for Reducing Ovarian Cancer Cell Growth in a Patient

Treatment is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. "Subject," as used herein,

can refer to a mammal, e.g. a human, or to an experimental or animal or disease model. The subject can also be a non-human animal, e.g. a horse, cow, goat, or other domestic animal. A therapeutic agent includes, but is not limited to, siRNA, antibodies and antisense molecules.

siRNA and antisense molecules do not diffuse across intact cell membranes to any significant degree due to the hydrophobic nature of the membrane lipid bilayer and the negatively charged siRNA and antisense.

Antisense molecules and siRNA can be delivered *in vivo* using CPPs, CTLs and nanocarriers as discussed herein. Many examples of successful *in vivo* delivery of siRNA and antisense molecules using a variety of agents are reviewed in Juliano et al. (2008).

Pharmaceutical Compositions

The therapeutic agents of the subject invention, i.e., siRNA and antisense molecules, can be formulated into pharmaceutical compositions suitable for administration to a subject, e.g., a human. Such compositions typically comprise the nucleic acid molecule and a pharmaceutically acceptable carrier.

The term “administer” is used in its broadest sense and includes any method of introducing the compositions of the present invention into a subject. This includes altering the expression of endogenous polypeptides or polynucleotides *in vivo* by the introduction of the compositions.

As used herein, the language “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the therapeutic agent, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the

following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should 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. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the therapeutic agent (e.g., a siRNA, antisense molecule or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a 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 which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

5 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For oral administration, the agent can be contained in enteric forms to survive the stomach or further coated or mixed to be released in a particular region of the GI tract by known methods. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of
10 tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder
15 such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

20 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

 Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated
25 are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the therapeutic agents are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

EXAMPLES

The following examples are provided for illustrative purposes only and are not intended to limit the scope of the application as defined by the appended claims. All examples described herein were carried out using standard techniques which are well known and routine to those of skill in the art. Routine molecular biology techniques described in the following examples can be carried out as described in references cited therein or in standard laboratory manuals, such as Sambrook & Russell (2001).

Example 1 – Methods and Materials

The following summarizes methods and materials used in subsequent Examples (see also He et al. (2010)).

Cell lines and reagents

Various epithelial ovarian cancer cell lines were obtained as gifts: SHIN3 and OVCAR5 from Mark Levine (NIDDK), and A1847, A2780, IGROV1, OVCAR10, PEO-1, and 2008 from Charles Drescher and Beatrice Knudsen (Fred Hutchinson Cancer Research Center, Seattle WA). Renilla TS was a gift from Nasreldin M. Ibrahim, Otto Fröhlich, and S. Russ Price (Emory University School of Medicine). Predesigned STAMP SMARTpool® siRNA was purchased from Dharmacon (#M-015406), Lamin A/C siRNA from Qiagen (#1027320), STAMP TaqMan® gene expression assay probe (#Hs00367300_m1) and human GAPDH control probe (#4310884E) from ABI, and siLentFect™ siRNA transfection reagent from Bio-Rad #170-3361. GR plasmid (pSVLGR) was a gift from Keith Yamamoto (Univ. of Cal., SF). The CellTiter 96® AQueous One Solution™ cell growth assay was supplied by Promega. Mouse monoclonal anti-TIF2 antibody is from Bethyl Labs (#A300-345A). Rabbit polyclonal anti- α -tubulin antibody (used at 15 ng/ml) was from Abcam (#ab4074). The following primers were used for qRT-PCR of both human and rat GR (numbers in parentheses are positions in human GR cDNA): GTCTTCAGGCTGGAATGAACC (1443-1462), SEQ ID NO:9, and CTTTGCCCATTTCACTGCTGC (1737-1717), SEQ ID NO:10.

Stably transfected 293 cells

HEK293 cells were always grown in high glucose DMEM (Invitrogen) with 10% fetal bovine serum, sodium pyruvate (Cellgro), and MEM nonessential amino acids (Cellgro) to 70% confluency in 100 mm dishes. Cells were transfected with 10 μ g of FLAG-STAMP/pCDNA3.1neo or pCDNA3.1neo using 23 μ l Fugene® 6 (Roche Diagnostics, Branford, CT) for 24 hr. The media was changed and the cells grown for another 24 hr before being washed (PBS without Ca⁺⁺/Mg⁺⁺; Invitrogen), trypsinized, diluted 1:50-1000 in media containing 1 mg/ml G418 (Invitrogen), plated in 100 mm dishes and grown for 14 days. Colonies were selected and expanded first in 24 well plates for 10 days and then in 6 well plates until there were enough cells, all with 0.5 mg/ml G418. Positive clones were tested for Flag-

STAMP expression by Western blotting with anti-STAMP antibody prepared by Covance (3rd bleed, HL5761). All HEK293 stable cells used for experiments were grown in media free of G418 for at least 2 weeks unless otherwise noted.

5 Cell growth assay

Cells were washed (PBS without Ca⁺⁺/Mg⁺⁺), trypsinized, and then seeded at 10,000 cells/well in 96 well culture plates. Cell growth at the indicated points (day of seeding = Day 0) was determined by adding 20 µl of CellTiter Aqueous One Solution/well and measuring the OD492 three hours later in a Beckman Count DTX880® as recommended by the supplier (Promega). Medium without cells was used as the blank control.

FACS and cell counting

Cells (grown with 0.1 mg/ml G418) were washed 3 times with PBS without calcium or magnesium (Invitrogen), counted with a Beckman Coulter Vi-Cell™ XR2.02, and seeded at 50,000 viable cells/well in 6 well dishes in 3 ml of medium without G418. After 24 hours, the cells were washed once with PBS, trypsinized, resuspended in 500 µl of medium, and placed on ice. Cells were incubated at 0°C with 1 µl of 5 mg/ml of propidium iodide (Invitrogen) immediately prior to analysis on a Becton Dickinson FACS Calibur™ flow cytometry system and counting with a Beckman Coulter Vi-Cell XR2.02.

Tissue scan panels for endogenous STAMP determination

Tissue Scan panels (8 common cancers (#CSRT101), kidney cancer (#HKRT101), ovarian cancer (#HORT101 and HORT102), and breast cancer (#BCRT101)) and the accompanying pathology and tumor staging reports are available from OriGene (Rockville, MD). Each panel contains pre-normalized cDNA arrays prepared from pathologist-verified tumor tissues. Each well of the panel has same amount total cDNA. Thus, any target gene (e.g. STAMP) variation in different wells reflects real changes between tumor samples. Quantitative real-time PCR (qRT-PCR) was run once for each panel (plate) using validated STAMP primers according to manufacturer's instructions. To determine the absolute level of STAMP, 10 µl 2× Taqman PCR master, 1 µl STAMP Taqman probe, and 9 µl water (total 20 µl) was added to each

well of the 96 well plate, as recommended by OriGene. Then real-time qRT-PCR was run on an ABI HT 7900 according to the manufacturer's instructions.

Transient transfections of stably transfected cells with siRNAs or plasmids

5 For 6 well plates, cells were seeded at 5000 per well one day before transfection. One hour before transfection, 1.25 ml of fresh medium was added. siRNA (75 pM) was incubated for 20 min at r.t. with 1 µl siLentFect® reagent in Opti-mem® medium (total of 250 µl) before being added to Opti-mem medium in each well (final concentration of siRNA = 50 nM). For 96 well plates, cells were seeded at 500-2000 per well and 50 µl of fresh medium was added one
10 hour before transfection. Then 3.5 pM of siRNA was mixed with 0.05 µl siLentFect in a total of 20 µl of Opti-mem medium for 20 min before being added to each well. One day later, fresh medium was added to each well in both protocols.

Total RNA isolation, reverse transcription, real-time PCR, and luciferase assays of transiently transfected GREtkLUC were performed as previously described (He & Simons
15 (2007)). For transient transfection experiments with GREtkLUC, VA (1×10^4) or S13 (2×10^4) cells in 300 µl of media were transfected for 24 hr using 0.8 µl/well of Eugene 6 and either 0, 10 or 30 ng pSVLGR plus 100 ng GREtkLuc and 10 ng Renilla TS. Total transfected DNA was maintained at 300 ng/well with PBSK+. The cells were then induced with steroids in fresh media for 24 hr, separated from the media, and lysed for 20 min with 300 µl of passive lysis
20 buffer (Promega) at room temperature on a rotating shaker. Lysate (50 µl) was then loaded into 96 well luminometer plates and read in a Berthold luminometer.

Statistical analysis

Unless otherwise noted, the values of n independent experiments, performed in triplicate
25 for Luciferase assays, were analyzed for statistical significance by the two-tailed Student's t test using InStat® 2.03 (GraphPad® Software, San Diego, CA). In every case, each average of triplicates was treated as one value of the n experiments. When the difference between the SDs of two populations was significantly different, the Mann-Whitney or Alternate Welch t test was used. A nonparametric test was used if the distribution of values was non-Gaussian. Best-fit
30 curves (R^2 almost always ≥ 0.95) following Michaelis-Menten kinetics were obtained for the dose-response experiments with KaleidaGraph® (Synergy Software, Reading, PA).

Example 2 – STAMP Overexpression Decreases Growth of Human Embryonic Kidney
293 Cells

Two colonies of 293 cells containing stably transfected STAMP were selected for study.
5 Both colonies gave less than 50% of the increase of control cells with stably transfected empty
vector (clone VA) after 3-4 days, as determined by counting of isolated cells ($43 \pm 19\%$ [S.D., n
 $= 9$] and $33 \pm 7\%$ [S.D., $n = 2$] for clones 13 and 10 respectively vs. VA cells). One colony
(clone S13) was selected for further study. When removed from G418 selection and immediately
10 examined by "Cell Titre" assay, clone S13 cells show a 65% reduction in cell replication (Fig.
1A). Automated cell counting gave similar results (data not shown). FACS analysis showed no
increase in the proportion of dead cells in S13 vs. VA cells (Figs. 1B&C). Thus, the lower
density of S13 cells over time is due to slower growth as opposed to increased cell death.

Fig. 1 illustrates the effect of stably transfected STAMP on the growth rate of 293 cells.
Fig. 1(A) illustrates how increased STAMP decreases cell growth. The number of cells in
15 replicate plates of clone S13 (S) and VA (V) cells (no steroid, cells removed from G418 1 day
prior) after increasing days was assessed by the CellTiter Aqueous One Solution assay. The
average value ($\pm$ S.E.M.) of four independent experiments is shown. $**P < 0.005$, $***P <$
 0.0005 . (Figs. 1(B) & (C)) FACS analysis of VA and S13 cells (no steroid, cells removed from
G418 upon seeding; S = single cells, D = double cells). The number of dead cells after
20 increasing days in one of three representative experiments is plotted in Fig. 1(C) ($\pm$ SD, $n = 3$, $*P$
 < 0.05 , $**P < 0.005$). Fig. 1(D) illustrates growth of VA and S13 cells after removal from G418
for more than 1 month. The experiment was conducted and plotted ($\pm$ S.E.M., $n = 4$, $*P < 0.05$)
as in Fig. 1(A) except that $1 \mu\text{M}$ Dex was added to cells as indicated. Fig. 1(E) Increased
STAMP decreases cell growth $\pm$ steroid. The experiment was conducted as in Fig. 1(D) except
25 that four different steroids (100 nM aldosterone, $1 \mu\text{M}$ Dex, 20 nM R5020, and 10 nM R1881)
were added. Similar results were obtained in two additional independent experiments. In some
cases (e.g., S13 cells in Fig. 1A), the error bar length is smaller than the symbol.

Given the undetectable level of STAMP in VA cells by Western blotting (data not
shown), qRT-PCR was used to determine the relative amounts of STAMP. STAMP mRNA in
30 clone S13 was 32-fold higher than in VA cells but decreased by a factor of two to 14-fold after
withdrawal from G418 for at least 3 months. As expected, these reduced levels of excess

STAMP were reflected in a smaller reduction of cell growth, with or without glucocorticoid (Fig. 1D). The growth rate of clone S13 as a percent of clone VA cells was the same with EtOH ($77 \pm 0.02\%$; S.E.M, $n = 10$, $P < 0.0001$) and Dex ($76 \pm 0.03\%$; S.E.M., $n = 9$, $P < 0.0002$).

In view of the ability of STAMP to also alter the induction properties of PR and AR (He & Simons (2007)), we asked if the addition of any other steroids could influence the growth of clone S13 vs. control (clone VA) cells. As shown in Fig. 1(E), saturating levels for receptor binding of progestin (R5020), androgen (R1881), or mineralocorticoid (aldosterone) also did not change the growth rate of cells with or without overexpressed STAMP.

Example 3 – STAMP Levels in Human Tumor Samples

In view of the effect of STAMP on 293 cell growth, we asked if the abnormal growth of any cancers might be influenced by the levels of endogenous STAMP. Standardized amounts of cDNAs from each stage of 8 common human cancers were obtained from OriGene. Quantitative real time PCR analysis with STAMP TaqMan probes was performed to determine the amount of STAMP mRNA in each tumor sample.

Table 1 provides STAMP mRNA levels in a panel of 8 common human cancers. The characterization of each sample in a commercially available panel of 8 common human cancers (Origene) is listed. Also given are the Ct values from the qRT-PCR assays and the calculation of the abundance of STAMP mRNA in each sample, relative to the first sample (A1), which was arbitrarily chosen.

Fig. 2 illustrates levels of STAMP mRNA in different human tumor samples. Fig. 2(A) provides a survey of eight human tumor types. Levels of STAMP mRNA in individual samples of OriGene TissueScan® panel (each bar corresponds to a single tissue sample) were determined as described in Materials and Methods and plotted (relative to the STAMP mRNA level of the first sample in the Breast panel) vs. tumor stage. As shown in Fig. 2(A), this preliminary screen suggested that STAMP levels in kidney cancers gradually decrease as the tumor stage progresses while STAMP levels might be elevated in Stage I of ovarian cancers and several stages of breast cancer. These pilot study observations were pursued by analyzing larger panels containing 48 samples from various stages of these three cancers. This more extensive study displayed no significant differences in STAMP levels among various kidney and breast cancer samples (data not shown).

Fig. 2(B) provides STAMP mRNA in human ovarian cancers. Levels of STAMP mRNA in two different large scale OriGene TissueScan panels of only ovarian cancers was determined as in Fig. 2(A) (see also Table 2). The data from both panels were combined and plotted (box plot) as percent of the average STAMP level in normal (Stage 0) samples (n = 13) for the different stages (n for Stage IGB [low malignant potential] = 3, Stage I = 12, Stage II = 12, Stage III = 34, Stage IV = 9). P values were determined for Δ Ct values from the qRT-PCR assay before conversion to percent of STAMP in normal samples, which was plotted. Table 2 also provides the Ct values from the qRT-PCR assays, the average Ct value (and S.D.) of all samples in each Stage, and the p value of the average STAMP Ct value of each Stage vs. the Stage 0 value.

It was appreciated by the inventors that these results with ovarian tumors could be misleading as the "normal" is most likely enriched for normal ovarian stroma, which is nearly devoid of epithelial cells that are found primarily on the capsular surface of the ovary. The difference between overt Stage I-III cancers and the three low malignant potential, borderline tumors (IGB) is significant (P = 0.033 by one-tailed Student t-test) because low malignant potential ovarian neoplasm is a nonmalignant but epithelial process. These two findings led to further investigation of the role of STAMP in ovarian cancer.

Nine cultured human ovarian cancer cell lines were examined: Hey A8, SKOV3, OVCAR8, A1847, PEO-1, OVCAR10, A2780, 2008, and IGROV1. The STAMP levels in three randomly selected, untreated cell lines (Hey A8, SKOV3, OVCAR8) were determined and normalized to the amount in Hey A8 cells, which was arbitrarily selected as a reference value. STAMP mRNA levels in these ovarian cancer cell lines do not appear abnormal. They are slightly higher than that in two other transformed cell lines (U2OS.rGR osteosarcoma cells with stably transfected GR and the control 293 VA cells) and much less than in the clone S13 293 cells with stably transfected STAMP (Table 3).

Table 3 sets forth STAMP mRNA levels in the indicated cells as determined by qRT-PCR as described in Materials and Methods and then normalized to that seen in HeyA8 cells (which was arbitrarily selected as reference). Values are the averages $\pm$ S.E.M. of 3 experiments (one experiment for 293 cells).

Example 4 – Role of STAMP in Proliferation of Ovarian Cancer Cells

Because elevated STAMP decreases the growth rate of 293 cells (Fig. 1), we asked if lowering the levels of endogenous STAMP with transfected STAMP siRNA would increase the growth rate of the ovarian cancer cells. After transfecting the cells with either Lamin siRNA, as
 5 a control, or a SMARTpool (Dharmacon) of STAMP siRNAs, we again determined the STAMP levels. On average, STAMP siRNA reduced the level of endogenous STAMP mRNA in each cell line to $28 \pm 8\%$ (S.D., $n = 7$ cell lines, each being the average of three independent experiments) of that seen in the same cells treated with the Lamin siRNA control (Table 2; "percent remaining"). The "absolute" values of STAMP mRNA in each cell line following
 10 STAMP siRNA treatment, after adjusting for the different initial levels in each cell line, are also given Table 4 (values are expressed relative to Hey A8 reference cells, with STAMP levels in Hey A8 cells treated with Lamin siRNA = 100). Unexpectedly, a reduced amount of STAMP mRNA now inhibits the growth of Hey A8, A1847 and IGROV1 cells, in each case compared to the growth of the same cells treated with Lamin siRNA (Fig. 3A). However, lower STAMP
 15 mRNA levels have the opposite (and initially predicted) effect on the proliferation of PEO-1 and 2008 cells, which are significantly increased. Finally, reduced STAMP mRNA levels cause no change in the propagation of SKOV3, OVCAR8, OVCAR10, A2780 and two other ovarian cancer cell lines (SHIN3 and OVCAR5) (Fig. 3A).

Table 4 provides relative STAMP mRNA levels in ovarian cancer tissue cells following
 20 siRNA treatment. STAMP mRNA levels in the indicated cells with transfected siRNA were determined by qRT-PCR as described in Materials and Methods and then normalized to that seen in HeyA8 cells (which was arbitrarily selected as reference) after transient transfection with Lamin siRNA. The data show both the percent remaining STAMP mRNA after siRNA treatment (i.e., $100 \times \text{STAMP siRNA} / \text{Lamin siRNA}$) and the "absolute" levels relative to
 25 STAMP in HeyA8 cells treated with Lamin siRNA. For this normalization to STAMP in Lamin siRNA-treated HeyA8 cells, the ratio ($\times 100$) of (STAMP mRNA in each STAMP siRNA-treated cell)/(STAMP mRNA in STAMP siRNA-treated HeyA8 cells) is multiplied by the ratio of (STAMP mRNA in STAMP siRNA-treated HeyA8 cells)/(STAMP in Lamin siRNA treated HeyA8 cells) in the same experiment (values ranged from 0.22 to 0.38). Values are the averages
 30 $\pm$ S.E.M. of 3 experiments.

Fig. 3 illustrates the effect of STAMP siRNA on growth of ovarian cancer tissue culture cells. Fig. 3(A) provides the growth of ovarian tissue culture cells treated with STAMP siRNA relative to Lamin siRNA. The growth of cells 4 days after being transfected with STAMP siRNA or Lamin siRNA was determined as in Fig. 1. The average data $\pm$ S.E.M. for 2-6
 5 determinations ($n = 12$ for HeyA8 cells) are plotted as growth of cells treated with STAMP siRNA as percent of growth of cells treated with Lamin siRNA. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$. Fig. 3(B) provides the growth rate vs. STAMP mRNA levels. The relative growth rate (STAMP siRNA vs. Lamin siRNA) of each of the cell lines in Fig. 3(A) ($\pm$ S.E.M.) was plotted against the level of STAMP mRNA in the same cells after siRNA treatment relative to
 10 HeyA8 cells ($\pm$ S.E.M.; length of bar may be less than symbol) given in Table 2. Straight line is the best fit by Kaleidagraph ($R^2 = 0.016$).

In view of the different growth responses to lowered STAMP levels in the ovarian cancer cell lines, it seemed possible that the absolute level of STAMP, as opposed to the relative amount, might be a critical determinant for growth rates. In this case, cells with high levels of
 15 STAMP mRNA after siRNA treatment, relative to HeyA8 (see "absolute" values in Table 4), might have different growth rates than those with much lower levels. However, PEO-1 and IGROV1 cells have similar levels of STAMP mRNA after STAMP siRNA treatment ("absolute" in Table 4) but very different growth rates (Fig. 3(A)). The growth response of the cells treated with STAMP siRNA (Table 4) are plotted in Fig. 3(B). This clearly shows that there is no
 20 correlation between the amount of STAMP mRNA in STAMP siRNA-treated cells and the growth rate of STAMP siRNA-treated cells as a percent of Lamin siRNA-treated cells. These data suggest that neither absolute nor relative levels of STAMP uniquely control the growth rates of these ovarian cancer cells. In this case, the effects of decreased intracellular STAMP on cell growth would depend on additional factors in a cell-dependent manner.

25 The levels of proteins and kinases known to be associated with cancer proliferation and/or apoptosis (p21, p53, ERK, phospho-ERK, AKT, phospho-AKT and cleaved PARP) were examined in the background of STAMP or Lamin silencing to determine if they paralleled the growth rates of the ovarian cancer cells with reduced amounts of STAMP mRNA. No relationship was observed between the expression level of these proteins/kinases on Western
 30 blots and the growth rates of A1847, A2780, HeyA8, IGROV1, OVCAR10, PEO-1, and 2008 cells (data not shown). We conclude that changing the intracellular concentration or activation

status of any of these proteins does not mediate the effects of STAMP levels on the growth rates of the ovarian tissue culture cells.

Example 5 – Overexpressed STAMP In Stably Transfected 293 Cells Alters Properties of GR-Mediated Transactivation of a Cell-Specific Manner

It was theorized that the ability of higher levels of STAMP to produce opposite effects on the growth of 293 and ovarian cancer cells could be due to cell-specific factors. To test this hypothesis, we asked if similar cell-specific differences might also reverse the effects of STAMP on the parameters of GR-regulated gene expression. Previous studies revealed that transiently transfected STAMP in both CV-1 and U2OS.rGR cells not only augment the maximal amount of Dex activity ($A_{\max}$), and the partial agonist activity of antisteroids (expressed as percent of maximum Dex activity), but also shift the dose-response curve (and EC_{50}) for gene induction to lower concentrations of steroid (He & Simons (2007); Szapary et al. (2008); Sun et al. (2008)). It was therefore proposed that elevated STAMP levels in 293-derived clone S13 cells could reduce the EC_{50} of the dose-response curve for GR induction of exogenous GREtkLUC reporter relative to the same treatment of vector control VA cells. We used the identical exogenous reporter gene (GREtkLUC) as in our previously published studies (He & Simons (2007); Szapary et al. (2008); Sun et al. (2008)) was used in order to minimize the differences inherent upon comparing gene induction properties in different cell lines. Due to the relatively low level of GR in 293 cells, the effects of STAMP in the presence of two amounts (10 and 30 ng) of transiently transfected GR plasmid were the focus of the study. As expected, the magnitude of change in each parameter was proportional to the amount of transfected GR plasmid (Simons (2006)). With both amounts of transfected GR, however, the higher level of STAMP in clone S13 cells unexpectedly causes a significant right-shift in the dose-response curve to higher steroid concentrations relative to that seen with vector control VA clone cells (Figs. 4(A) & (B)). Similarly, the elevated STAMP in S13 cells decreases both the $A_{\max}$ and the partial agonist activity of the antigluocorticoid Dex-mesylate (%DM) (Fig 4B). These changes are statistically significant for each parameter in the presence of both 10 and 30 ng of transfected GR.

Fig. 4 illustrates the effect of elevated STAMP on the properties of GR induction of GREtkLUC reporter gene in 293 cells. Fig. 4(A) provides the dose-response curves for GR induction of GREtkLUC reporter with varying amounts of transiently transfected GR plasmid.

S13 (containing stably transfected STAMP) and vector control (VA) cells were cotransfected with GREtkLUC reporter and the indicated amounts of GR-encoding plasmid. Dose-response curves were conducted and plotted as described by Tao et al. (Tao et al. (2008)). Fig. 4(B) illustrates modulation of $A_{\max}$, partial agonist activity of the antiglucocorticoid Dex-Mes (%DM), and EC_{50} by elevated STAMP. Four independent experiments with clone S13 and VA cells such as in Fig. 4(A), which included assaying the activity of 1 μ M Dex-Mes, were analyzed to yield $A_{\max}$ and EC_{50} in addition to partial agonist activity of 1 μ M Dex-Mes (%DM). The average values $\pm$ S.E.M. are plotted. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ for clone S13 vs. the similarly treated clone VA sample. Fig. 4(C) provides GR mRNA levels in VA and S13 cells. The qRT-PCR was used as described in Materials and Methods to determine the level of GR mRNA in VA and S13 cells with or without 30 ng of transiently transfected GR plasmid ($\pm$ S.E.M. of triplicates). Fig. 4(D) provides Western blot with TIF2 antibody of cell lysates from cells transfected with no, or 30 ng of GR plasmid. The lower panel shows the equal levels of the loading control, α -tubulin, in each sample.

A variety of factors could be responsible for the effects of STAMP in Figs. (4)A & (B) being opposite from expected (Simons (2008)). Two well-known factors that cause increases in $A_{\max}$, and partial agonist activity, and decreases in EC_{50} are GR itself and the coactivator TIF2 (Simons (2008); Szapary et al. (1999); Chen et al. (2000)). We therefore asked whether higher levels of either GR or TIF2 in VA cells might account for the results of Figs. 4(A) & (B).

Unfortunately, the amount of GR protein is too low to detect by Western blotting, even after transient transfection with 30 ng of GR plasmid (data not shown). qRT-PCR was therefore used to determine the GR mRNA levels, which were found to be very similar in the two cell lines both with and without transfected GR plasmid (Fig. 4(C)). Likewise, Western blotting revealed equal TIF2 protein levels in clone VA and S13 cells both with and without transfected GR (Fig. 4(D)). Thus, the differences between VA and S13 cells in Figs. 4(A) & (B) cannot be explained by VA cells possessing greater amounts of GR and/or TIF2 than S13 cells. We concluded that unidentified cell-specific properties can further alter the effects of changing levels of STAMP on the expression of the same gene(s) in different cells. Therefore, just as was observed for the growth of ovarian cancer cells upon depletion of endogenous STAMP, the other known effects of STAMP are also cell-line dependent.

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
A1	Female	44	Breast / Breast	Normal	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	0	33.981	0.000	100.0
A2	Female	45	Breast / Breast	Normal	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	0	33.560	-0.421	133.9
A3	Female	32	Breast / Breast	Normal	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	0	34.441	0.460	72.7
A4	Female	47	Breast / Breast	Tumor	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	I	32.790	-1.192	228.4
A5	Female	52	Breast / Breast	Tumor	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	I	33.972	-0.010	100.7
A6	Female	57	Breast / Breast	Tumor	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	IIA	32.939	-1.042	205.9
A7	Female	42	Breast / Breast	Tumor	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	IIA	33.520	-0.461	137.7
A8	Female	63	Breast / Breast	Tumor	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	IIIA	33.092	-0.889	185.2
A9	Female	61	Breast, right lateral / Breast, right lateral	Tumor	Adenocarcinoma of breast, ductal, lobular	Adenocarcinoma of breast, ductal, lobular	IIIA	33.198	-0.784	172.1
A10	Female	55	Breast / Breast	Tumor	Adenocarcinoma of breast, ductal	Adenocarcinoma of breast, ductal	IIIC	33.016	-0.965	195.2
A11	Female	45	Breast / Lung	Tumor	Adenocarcinoma of breast, metastatic	Adenocarcinoma of breast, metastatic	IV	32.914	-1.067	209.5
A12	Female	42	Breast / Liver	Tumor	Adenocarcinoma of breast, metastatic	Adenocarcinoma of breast, metastatic	IV	32.764	-1.218	232.6

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	Δ Ct re A1	% re A1
B1	Male	61	Colon: right / Colon: right	Normal	Adenocarcinoma of colon	Within normal limits	0	33.797	-0.184	113.6
B2	Male	91	Colon: sigmoid / Colon: sigmoid	Normal	Adenocarcinoma of colon	Within normal limits	0	34.308	0.327	79.7
B3	Female	37	Colon: transverse / Colon: transverse	Normal	Adenocarcinoma of colon, mucinous, signet ring cell	Within normal limits	0	34.617	0.636	64.4
B4	Female	93	Colon: right / Colon: right	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	I	32.594	-1.387	261.6
B5	Male	65	Colon: right / Colon: right	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	IIA	33.940	-0.041	102.9
B6	Male	78	Colon: sigmoid / Colon: sigmoid	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	IIA	33.395	-0.586	150.1

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	$\Delta\text{Ct re A1}$	% re A1
B7	Male	66	Colon: right / Colon: right	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	IIA	34.193	0.211	86.4
B8	Male	86	Colon / Colon	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	III	33.971	-0.010	100.7
B9	Female	42	Cecum / Cecum	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	IIIB	34.199	0.218	86.0
B10	Male	61	Colon: sigmoid / Colon: sigmoid	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	IIIC	35.035	1.054	48.2
B11	Male	63	Colon: rectosigmoid / Colon: rectosigmoid	Tumor	Adenocarcinoma of colon	Adenocarcinoma of colon	IIIC	33.259	-0.722	165.0
B12	Female	51	Colon / Lung	Tumor	Adenocarcinoma of colon, metastatic	Adenocarcinoma of colon, metastatic	IV	33.939	-0.043	103.0
C1	Male	71	Kidney / Kidney	Normal	Carcinoma of kidney, renal cell, clear cell	Within normal limits	0	32.789	-1.192	228.5
C2	Male	66	Kidney / Kidney	Normal	Carcinoma of kidney, renal cell, clear cell	Within normal limits	0	32.925	-1.056	208.0

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
C3	Female	54	Kidney / Kidney	Normal	Schwannoma	Within normal limits	0	32.282	-1.700	324.8
C4	Male	52	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, chromophobe	Carcinoma of kidney	I	32.661	-1.320	249.7
C5	Female	55	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, clear cell	Carcinoma of kidney	I	33.203	-0.778	171.5
C6	Male	52	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, chromophobe	Carcinoma of kidney	I	33.474	-0.508	142.2
C7	Female	57	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell	Carcinoma of kidney	II	33.418	-0.563	147.7
C8	Male	59	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, clear cell	Carcinoma of kidney	III	33.553	-0.428	134.5
C9	Male	37	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, clear cell, papillary	Carcinoma of kidney	III	33.843	-0.138	110.0
C10	Male	64	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, papillary	Carcinoma of kidney	III	35.416	1.435	37.0
C11	Male	70	Kidney / Lung	Tumor	Carcinoma of kidney, renal cell, clear cell, metastatic	Carcinoma of kidney	IV	34.047	0.066	95.5

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
C12	Male	51	Kidney / Kidney	Tumor	Carcinoma of kidney, renal cell, sarcomatoid	Carcinoma of kidney	IV	34.939	0.958	51.5
D1	Male	81	Liver / Liver	Normal	Carcinoma of liver, hepatocellular	Within normal limits	0	33.289	-0.693	161.6
D2	Male	86	Liver / Liver	Normal	Carcinoma of liver, hepatocellular	Within normal limits	0	33.598	-0.383	130.4
D3	Female	33	Liver / Liver	Normal	Nodular hyperplasia of liver, focal	Within normal limits	0	32.917	-1.064	209.1
D4	Male	79	Liver: left lobe / Liver: left lobe	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	I	33.879	-0.102	107.3
D5	Female	58	Liver / Liver	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	I	33.110	-0.871	182.9
D6	Male	66	Liver / Liver	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	I	34.190	0.208	86.6
D7	Female	63	Liver / Liver	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	II	34.425	0.443	73.5
D8	Male	68	Liver / Liver	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	II	33.344	-0.638	155.6

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
D9	Female	62	Liver: right lobe / Liver: right lobe	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	II	33.760	-0.221	116.6
D10	Male	71	Liver / Liver	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	IIIA	33.532	-0.449	136.5
D11	Male	21	Liver: left lobe / Liver: left lobe	Tumor	Carcinoma of liver, hepatocellular	Carcinoma of liver, hepatocellular	IV	34.107	0.126	91.7
D12	Male	66	Bile duct / Lung	Tumor	Cholangiocarcinoma of liver, metastatic	Cholangiocarcinoma of liver, metastatic	IV	Undetermined	NMF	NMF
E1	Female	49	Lung / Lung	Normal	Carcinoma of lung, neuroendocrine	Not Reported	0	33.973	-0.008	100.6
E2	Male	79	Lung / Lung	Normal	Carcinoma of lung, large cell	Not Reported	0	33.114	-0.867	182.4
E3	Female	62	Lung / Lung	Normal	Carcinoma of lung, squamous cell	AJCC G2: Moderately differentiated	0	34.292	0.311	80.6

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
E4	Male	71	Lung: left upper lobe / Lung: left upper lobe	Tumor	Carcinoma of lung, squamous cell	AJCC G2: Moderately differentiated	IA	33.032	-0.950	193.1
E5	Male	64	Lung / Lung	Tumor	Adenocarcinoma of lung	AJCC G3: Poorly differentiated	IB	31.999	-1.982	395.0
E6	Female	85	Lung / Lung	Tumor	Carcinoma of lung, squamous cell	Not Reported	IB	33.541	-0.440	135.7
E7	Male	72	Lung / Lung	Tumor	Carcinoma of lung, squamous cell	AJCC G2: Moderately differentiated	IB	34.366	0.385	76.6
E8	Male	63	Lung / Lung	Tumor	Carcinoma of lung, non-small cell	AJCC G3: Poorly differentiated	IIB	33.103	-0.878	183.8
E9	Male	71	Lung / Lung	Tumor	Carcinoma of lung, squamous cell	AJCC G2: Moderately differentiated	IIB	32.000	-1.981	394.7
E10	Male	58	Lung / Lung	Tumor	Carcinoma of lung, squamous cell	AJCC G3: Poorly differentiated	IIIA	31.479	-2.502	566.6
E11	Male	80	Lung / Lung	Tumor	Adenocarcinoma of lung	AJCC G3: Poorly differentiated	IIIB	32.261	-1.720	329.5

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	Δ Ct re A1	% re A1
E12	Male	51	Lung: right upper lobe / Lung: left upper lobe	Tumor	Carcinoma of lung, non-small cell, metastatic	AJCC G3: Poorly differentiated	IV	Undetermined	NMF	NMF
F1	Female	70	Ovary / Ovary	Normal	Within normal limits	Not Reported	0	Undetermined	NMF	NMF
F2	Female	31	Ovary: right / Ovary: right	Normal	Within normal limits	Not Reported	0	35.680	1.699	30.8
F3	Female	42	Ovary / Ovary	Normal	Within normal limits	Not Reported	0	34.119	0.138	90.9
F4	Female	29	Ovary: right / Ovary: right	Tumor	Tumor of ovary, borderline	Not Reported	IA	32.012	-1.969	391.6
F5	Female	43	Ovary / Ovary	Tumor	Tumor of ovary, borderline	Not Reported	IB	32.173	-1.808	350.2
F6	Female	51	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, mucinous	Not Reported	IC	32.893	-1.088	212.6
F7	Female	80	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, endometrioid	Not Reported	IIB	33.519	-0.463	137.8

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
F8	Female	46	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid	Not Reported	IIIA	33.707	-0.275	121.0
F9	Female	52	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid	Not Reported	IIIB	34.759	0.778	58.3
F10	Female	74	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	Not Reported	IIIC	34.074	0.093	93.7
F11	Female	77	Ovary: left / Ovary: left	Tumor	Carcinoma of ovary	Not Reported	IIIC	33.839	-0.142	110.4
F12	Female	79	Ovary / Lymph node	Tumor	Adenocarcinoma of ovary, papillary serous, metastatic	Not Reported	IV	35.282	1.301	40.6
G1	Male	68	Prostate / Prostate	Normal	Adenocarcinoma of prostate	30% glandular epithelium, 70% fibromuscular stroma	0	34.951	0.970	51.1
G2	Male	65	Prostate / Prostate	Normal	Adenocarcinoma of prostate	20% epithelium, 80% fibromuscular stroma	0	34.635	0.654	63.6
G3	Male	76	Prostate / Prostate	Normal	Adenocarcinoma of prostate	25% epithelium, 70% fibromuscular stroma	0	32.231	-1.750	336.3

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
G4	Male	70	Prostate / Prostate	Lesion	Carcinoma of bladder, transitional cell	35% glandular epithelium, 65% stroma	I	33.203	-0.778	171.5
G5	Male	63	Prostate / Prostate	Lesion	Adenocarcinoma of prostate	25% epithelium, 75% fibromuscular stroma	II	33.920	-0.061	104.3
G6	Male	70	Prostate / Prostate	Lesion	Adenocarcinoma of prostate	45% epithelium, 55% fibromuscular stroma	II	32.233	-1.749	336.0
G7	Male	71	Prostate / Prostate	Lesion	Adenocarcinoma of prostate	55% Glandular epithelium, 60% Stroma, 5% Inflammatory cells	II	32.712	-1.270	241.1
G8	Male	56	Prostate / Prostate	Lesion	Adenocarcinoma of prostate	65% epithelium, 35% fibromuscular stroma.	II	33.811	-0.170	112.5
G9	Male	63	Prostate / Prostate	Tumor	Adenocarcinoma of prostate	Inflammation: Mild Lymphoplasmatic infiltrate	II	32.990	-0.992	198.8
G10	Male	53	Prostate / Prostate	Lesion	Adenocarcinoma of prostate	40% glandular epithelium, 60% fibromuscular stroma	III	32.607	-1.374	259.2

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	ΔCt re A1	% re A1
G11	Male	65	Prostate / Prostate	Lesion	Adenocarcinoma of prostate	55% epithelium, 45% fibromuscular stroma with chronic inflammation.	III	34.444	0.462	72.6
G12	Male	61	Prostate / Prostate	Tumor	Adenocarcinoma of prostate	Not Reported	III	33.803	-0.178	113.1
H1	Female	30	Thyroid gland / Thyroid gland	Normal	Within normal limits	Carcinoma of thyroid, papillary	0	32.970	-1.012	201.6
H2	Female	68	Thyroid gland / Thyroid gland	Normal	Within normal limits	Hamartoma of lung	0	32.928	-1.053	207.5
H3	Female	46	Thyroid gland / Thyroid gland	Normal	Within normal limits	Carcinoma of thyroid, papillary	0	33.378	-0.603	151.9
H4	Female	15	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, papillary	Carcinoma of thyroid, papillary	I	34.228	0.246	84.3
H5	Female	28	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, papillary	Carcinoma of thyroid, papillary	I	34.972	0.991	50.3

TABLE 1 –DATA FOR PANEL OF 8 COMMON HUMAN TUMORS

Well posit'n	Sex	Age	Tissue	Appear.	Diagnosis	Tumor grade	Stage	STAMP Ct	Δ Ct re A1	% re A1
H6	Female	39	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, papillary	Carcinoma of thyroid, papillary	I	35.059	1.077	47.4
H7	Male	57	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, Hurthle cell	Carcinoma of thyroid, Hurthle cell	II	33.933	-0.048	103.4
H8	Male	74	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, papillary	Carcinoma of thyroid, papillary	II	33.544	-0.437	135.4
H9	Female	76	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, papillary	Carcinoma of thyroid, papillary	III	34.110	0.129	91.5
H10	Female	52	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, follicular	Carcinoma of thyroid, follicular	III	33.058	-0.924	189.7
H11	Male	52	Thyroid gland / Neck	Tumor	Carcinoma of thyroid, medullary, metastatic	Carcinoma of thyroid, medullary	IVA	34.396	0.415	75.0
H12	Female	45	Thyroid gland / Thyroid gland	Tumor	Carcinoma of thyroid, papillary	Carcinoma of thyroid, papillary	IVA	35.104	1.123	45.9

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
C01	ovarian1	Female-70	Ovary / Ovary	Normal	Adenocarcinoma of endometrium, papillary serous		0	Unrelated to ovarian cancer	[32.977]			
C02	ovarian1	Female-37	Ovary / Ovary	Normal	Carcinoma of cervix, squamous cell		0	Unrelated to ovarian cancer	[32.083]			
C03	ovarian1	Female-23	Ovary: left / Ovary: left	Normal	Abscess of tissue		0		33.602			
C04	ovarian1	Female-42	Ovary / Ovary	Normal	Endometriosis		0		33.106			
C05	ovarian1	Female-31	Ovary: right / Ovary: right	Normal	Endometriosis		0		32.841	33.09	0.64	
C06	ovarian1	Female-31	Ovary: left / Ovary: left	Normal	Endometriosis		0		34.726			
C07	ovarian1	Female-42	Ovary / Ovary	Normal	Endometriosis		0		33.647			
C01	ovarian2	Female-46	Ovary: left / Ovary: left	Normal	Leiomyoma of myometrium	Not Applicable	0		32.576			
C02	ovarian2	Female-49	Ovary / Ovary	Normal	Leiomyoma of myometrium	Not Applicable	0		32.858			
C03	ovarian2	Female-46	Ovary: right / Ovary: right	Normal	Leiomyoma of myometrium	Not Applicable	0		32.765			
C04	ovarian2	Female-42	Ovary / Ovary	Normal	Endometrium, secretory	Not Applicable	0		32.192			
C05	ovarian2	Female-33	Ovary / Ovary	Normal	No residual malignancy	Not Applicable	0		32.568			
C06	ovarian2	Female-40	Ovary: left / Ovary: left	Normal	Cyst of ovary, follicular	Not Applicable	0		32.917			
C07	ovarian2	Female-45	Ovary / Ovary	Normal	Endometriosis	Not Applicable	0		33.047			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
C08	ovarian2	Female-35	Ovary: left / Ovary: left	Normal	Endometriosis	Not Applicable	0		33.375			
C10	ovarian1	Female-46	Ovary: right / Ovary: right	Tumor	Tumor of ovary, papillary serous, borderline	AJCC GB: Borderline malignancy	IA		34.360			
C11	ovarian1	Female-19	Ovary: right / Ovary: right	Tumor	Tumor of ovary, papillary serous, borderline	AJCC GB: Borderline malignancy	IA		32.882			
D01	ovarian1	Female-42	Ovary: left / Ovary: left	Tumor	Tumor of ovary, serous, borderline	AJCC GB: Borderline malignancy	IA	low malignant, not cancer, not normal	[33.256]	33.00	1.30	0.86
D02	ovarian1	Female-29	Ovary: right / Ovary: right	Tumor	Tumor of ovary, borderline	AJCC GB: Borderline malignancy	IA	low malignant, not cancer, not normal	[33.195]			
D03	ovarian1	Female-30	Ovary: left / Ovary: left	Tumor	Tumor of ovary, mucinous, borderline	AJCC GB: Borderline malignancy	IA		31.770			
D07	ovarian1	Female-56	Ovary: right / Ovary: right	Tumor	Tumor of ovary, mucinous, borderline	AJCC GB: Borderline malignancy	IC	low malignant, not cancer, not normal	[31.706]			
D08	ovarian1	Female-74	Ovary / Ovary	Tumor	Tumor of ovary, serous, borderline	AJCC GB: Borderline malignancy	IC	low malignant, not cancer, not normal	[32.461]			
D09	ovarian1	Female-45	Ovary / Ovary	Tumor	Tumor of ovary, serous, borderline	AJCC GB: Borderline malignancy	IC	low malignant, not cancer, not normal	[32.272]			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
C08	ovarian1	Female-34	Ovary: left / Ovary: left	Tumor	Carcinoma of ovary, endometrioid	FIGO G2: Moderately differentiated	I		32.136			
C09	ovarian2	Female-70	Ovary: left / Ovary: left	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IA		31.965			
C09	ovarian1	Female-74	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IA		32.325			
C12	ovarian1	Female-49	Ovary: right / Ovary: right	Tumor	Carcinoma of ovary, endometrioid	FIGO G1: Well differentiated	IA	low malignant, not cancer, not normal	[31.473]			
C10	ovarian2	Female-68	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IA		31.237			
C11	ovarian2	Female-48	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, clear cell	Not Reported	IA		32.649	31.98	0.47	<0.0001
D04	ovarian1	Female-63	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, mucinous	FIGO G3: Poorly differentiated	IB	low malignant, not cancer, not normal	[32.842]			
D05	ovarian1	Female-52	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IB	low malignant, not cancer, not normal	[32.360]			
D06	ovarian1	Female-43	Ovary / Ovary	Tumor	Tumor of ovary, borderline	Not Reported	IB	low malignant, not cancer, not normal	[32.820]			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
C12	ovarian2	Female-58	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G1: Well differentiated	IB		32.320			
D01	ovarian2	Female-55	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IB		32.544			
D02	ovarian2	Female-74	Ovary: bilateral / Ovary: right	Tumor	Adenocarcinoma of ovary, endometrioid, papillary serous	FIGO G3: Poorly differentiated	IB		31.502			
D10	ovarian1	Female-51	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, mucinous	FIGO G2: Moderately differentiated	IC		31.800			
D11	ovarian1	Female-58	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, endometrioid, squamous features	FIGO G2: Moderately differentiated	IC		32.299			
D03	ovarian2	Female-65	Ovary: bilateral / Ovary	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IC		31.505			
D04	ovarian2	Female-58	Ovary / Ovary	Tumor	Carcinoma of ovary, endometrioid	FIGO G2: Moderately differentiated	IC		31.454			
D05	ovarian2	Female-78	Ovary: bilateral / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIA		31.184			
D06	ovarian2	Female-44	Ovary: bilateral / Ovary: right	Tumor	Carcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIA		32.803			
D07	ovarian2	Female-73	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary	FIGO G3: Poorly differentiated	IIA		31.732			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
D12	ovarian1	Female-65	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IIB		31.972			
E01	ovarian1	Female-80	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IIB		34.024	32.05	0.84	0.0020
D08	ovarian2	Female-67	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIB		31.380			
D09	ovarian2	Female-67	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIB		32.244			
D10	ovarian2	Female-58	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IIB		32.105			
D11	ovarian2	Female-58	Ovary: bilateral / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIB		31.401			
E02	ovarian1	Female-59	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G1: Well differentiated	IIC		32.942			
D12	ovarian2	Female-59	Ovary: bilateral / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIC		31.333			
E01	ovarian2	Female-63	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIC		31.496			
E03	ovarian1	Female-69	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	III		32.504			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
E04	ovarian1	Female-56	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	III		31.203			
E02	ovarian2	Female-51	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	III		31.596			
E03	ovarian2	Female-73	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	III		32.922			
E04	ovarian2	Female-91	Ovary: bilateral / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	III		32.734			
E05	ovarian1	Female-46	Ovary / Ovary	Tumor	Carcinoma of ovary, endometrioid	FIGO G2: Moderately differentiated	IIIA		33.956			
E06	ovarian1	Female-38	Ovary: right / Ovary: right	Tumor	Tumor of ovary, serous, borderline	AJCC GB: Borderline malignancy	IIIA	low malignant, not cancer, not normal	[31.764]			
E05	ovarian2	Female-75	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IIIA		31.774			
E06	ovarian2	Female-65	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IIIA		31.568			
E07	ovarian2	Female-55	Ovary: bilateral / Ovary	Tumor	Adenocarcinoma of ovary, mucinous	FIGO G2: Moderately differentiated	IIIA		32.892			
E08	ovarian2	Female-66	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIIA		32.170			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
E07	ovarian1	Female-41	Ovary: bilateral / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB		33.661			
E08	ovarian1	Female-75	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIIB		31.165			
E09	ovarian1	Female-52	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IIIB		34.222			
E10	ovarian1	Female-60	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIIB		32.058	32.13	0.90	0.0010
E11	ovarian1	Female-52	Ovary: bilateral / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB		31.868			
E12	ovarian1	Female-77	Ovary: right / Ovary: right	Tumor	Tumor of ovary, serous, borderline	Not Reported	IIIB	low malignant, not cancer, not normal	[32.568]			
F01	ovarian1	Female-52	Ovary / Pelvic wall	Tumor	Adenocarcinoma of ovary, metastatic	Not Reported	IIIB		32.668			
E09	ovarian2	Female-80	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, serous	Not Reported	IIIB		31.937			
E10	ovarian2	Female-64	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB		30.815			
E11	ovarian2	Female-66	Ovary: bilateral / Ovary	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB		32.773			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
E12	ovarian2	Female-52	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB		30.951			
F02	ovarian1	Female-86	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G1: Well differentiated	IIIC		30.990			
F03	ovarian1	Female-74	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC		32.015			
F04	ovarian1	Female-77	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC		32.569			
F05	ovarian1	Female-44	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC		31.599			
F06	ovarian1	Female-62	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC		31.182			
F07	ovarian1	Female-77	Ovary: left / Ovary: left	Tumor	Carcinoma of ovary	FIGO G3: Poorly differentiated	IIIC		33.245			
F08	ovarian1	Female-45	Ovary / Omentum	Tumor	Adenocarcinoma of ovary, papillary serous	Not Reported	IIIC		32.996			
F09	ovarian1	Female-45	Ovary: right / Omentum	Tumor	Adenocarcinoma of ovary, serous	Not Reported	IIIC		31.588			
F01	ovarian2	Female-71	Ovary: right / Ovary: right	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC		31.629			
F02	ovarian2	Female-48	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIIC		32.223			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
F03	ovarian2	Female-50	Ovary: bilateral / Ovary: right	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC		32.383			
F04	ovarian2	Female-58	Ovary: left / Ovary: left	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIIC		32.672			
F05	ovarian2	Female-53	Ovary: right / Ovary: left	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IIIC		31.235			
F06	ovarian2	Female-66	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid, papillary serous	FIGO G3: Poorly differentiated	IIIC		30.770			
F10	ovarian1	Female-81	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IV		31.264			
F11	ovarian1	Female-77	Ovary / Colon: sigmoid	Tumor	Adenocarcinoma of ovary, metastatic	Not Reported	IV		34.783			
F12	ovarian1	Female-79	Ovary / Lymph node	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IV		34.226			
F07	ovarian2	Female-45	Ovary / Peritoneum	Tumor	Adenocarcinoma of ovary, serous, metastatic	Not Reported	IV		32.680	32.37	1.40	0.083*
F08	ovarian2	Female-80	Ovary / Ovary	Tumor	Adenocarcinoma of ovary, endometrioid, papillary serous	FIGO G3: Poorly differentiated	IV		31.830			*Mann-Whitney
F09	ovarian2	Female-68	Ovary: bilateral / Ovary: right	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IV		32.777			

TABLE 2
COMBINED DATA OF BOTH ORIGIN OVARIAN CANCER PANELS

Well posit'n	Cancer	Patient	Tissue	Appear.	Diagnosis	Tumor grade	Stage	Reason samples discarded by authors	STAMP Ct	Ave Ct of Stage	SD	P of Stage vs Stage 0
F10	ovarian2	Female-61	Ovary: bilateral / Ovary: left	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IV		30.935			
F11	ovarian2	Female-60	Ovary / Omentum	Tumor	Adenocarcinoma of ovary, papillary serous, metastatic	Not Reported	IV		30.821			
F12	ovarian2	Female-63	Ovary / Omentum	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IV		32.023			

Table 3**Relative STAMP mRNA levels in ovarian cancer tissue culture cells**

Cells:	Hey A8	SKOV 3	OVCAR 8	U2OS.rGR	293 clone VA	293 clone S13
Level:	100	269 ± 67	113 ± 8	59 ± 6	149	1864

Table 4**Relative STAMP mRNA levels in ovarian cancer tissue culture cells after siRNA treatment**

Cells:	Hey A8	A1847	PEO-1	OVCAR10	A2780	2008	IGROV1
Percent remaining:	29 ± 5	13 ± 3	33 ± 6	25 ± 5	33 ± 9	23 ± 3	38 ± 4
Absolute STAMP mRNA relative to HeyA8):	29 ± 5	16 ± 2	47 ± 12	208 ± 37	110 ± 32	30 ± 6	43 ± 14

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Lonberg & Kay, U.S. Pat. No. 5,569,825, entitled "Transgenic non-human animals capable of producing heterologous antibodies of various isotypes"

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Claims

1. A method of diagnosing ovarian cancer in a patient comprising:
assaying ovarian tissue in said patient for expression of the STAMP gene; and
determining whether expression level of STAMP gene corresponds to ovarian cancer.
5
2. The method of claim 1, wherein the assay is an assay for STAMP mRNA.
3. The method of claim 2, wherein the capture reagents are DNA primers and the
detection means is Sybr® Green label.
10
4. The method of claim 2, wherein said expression level of STAMP mRNA can be
correlated to a specific stage of ovarian cancer.
5. The method of claim 1, wherein the expression assay is an assay for STAMP
15 polypeptide or protein.
6. The method of claim 5, wherein the capture reagent is a monoclonal antibody and
the detecting means is a fluorescent label.
7. The method of claim 1, wherein said determination of whether the STAMP
20 expression level corresponds to ovarian cancer comprises comparing the determined expression
level to a STAMP expression level normal range.
8. A kit for detecting STAMP expression product in a cell or tissue comprising:
25 one or more capture reagents for capturing STAMP expression product; and
optionally means for detecting the binding of capture reagent to STAMP expression
product.
9. The kit of claim 8, wherein said STAMP expression product is STAMP mRNA,
30 and the capture reagent is a probe.

10. The kit of claim 9, wherein said detecting means is a radiolabel tag on the probe.

11. The kit of claim 8, wherein said STAMP expression product is STAMP mRNA and the capture reagents are DNA primers for performing qRT-PCR.

5

12. The kit of claim 11, wherein said detecting means is Sybr Green label.

13. The kit of claim 8, wherein said STAMP expression product is a STAMP polypeptide or protein, and the capture reagent is an antibody.

10

14. The kit of claim 13, wherein said detecting means is a fluorescent tag on the antibody.

15. The kit of claim 8, wherein said kit is for detecting or diagnosing ovarian cancer and said tissue or cell is an ovarian tissue or cell.

15

16. A method of reducing ovarian cancer cell growth in ovarian cancer cells having elevated STAMP expression product, comprising:

contacting said ovarian cancer cells with an agent that reduces the STAMP gene expression in said cancer cells.

20

17. The method of claim 16, wherein said agent is STAMP siRNA.

18. The method of claim 16, wherein said agent is a STAMP antisense molecule.

25

19. The method of claim 16, wherein said agent is STAMP antibody.

20. A method of reducing ovarian cancer cell growth in a patient diagnosed with ovarian cancer comprising:

administering to said patient, whose ovarian cancer cells exhibit elevated STAMP expression, a therapeutically effective amount of an agent that reduces STAMP gene expression, thereby substantially inhibiting ovarian cancer cell growth.

5 21. The method of claim 20, wherein said agent comprises STAMP siRNA.

 22. The method of claim 20, wherein said agent comprises a STAMP antisense molecule.

10 23. The method of claim 20, wherein said agent comprises STAMP antibodies.

 24. A composition for treatment or prevention of ovarian cancer cell growth in a patient comprising an agent capable of reducing expression of STAMP gene in ovarian cells in which STAMP gene expression is elevated, wherein the composition is administered to the
15 patient diagnosed with the ovarian cancer.

 25. The composition of claim 24, wherein the agent comprises STAMP siRNA.

 26. The composition of claim 24, wherein the agent comprises a STAMP antisense
20 molecule.

 27. The composition of claim 24, wherein the agent comprises STAMP antibodies.

25

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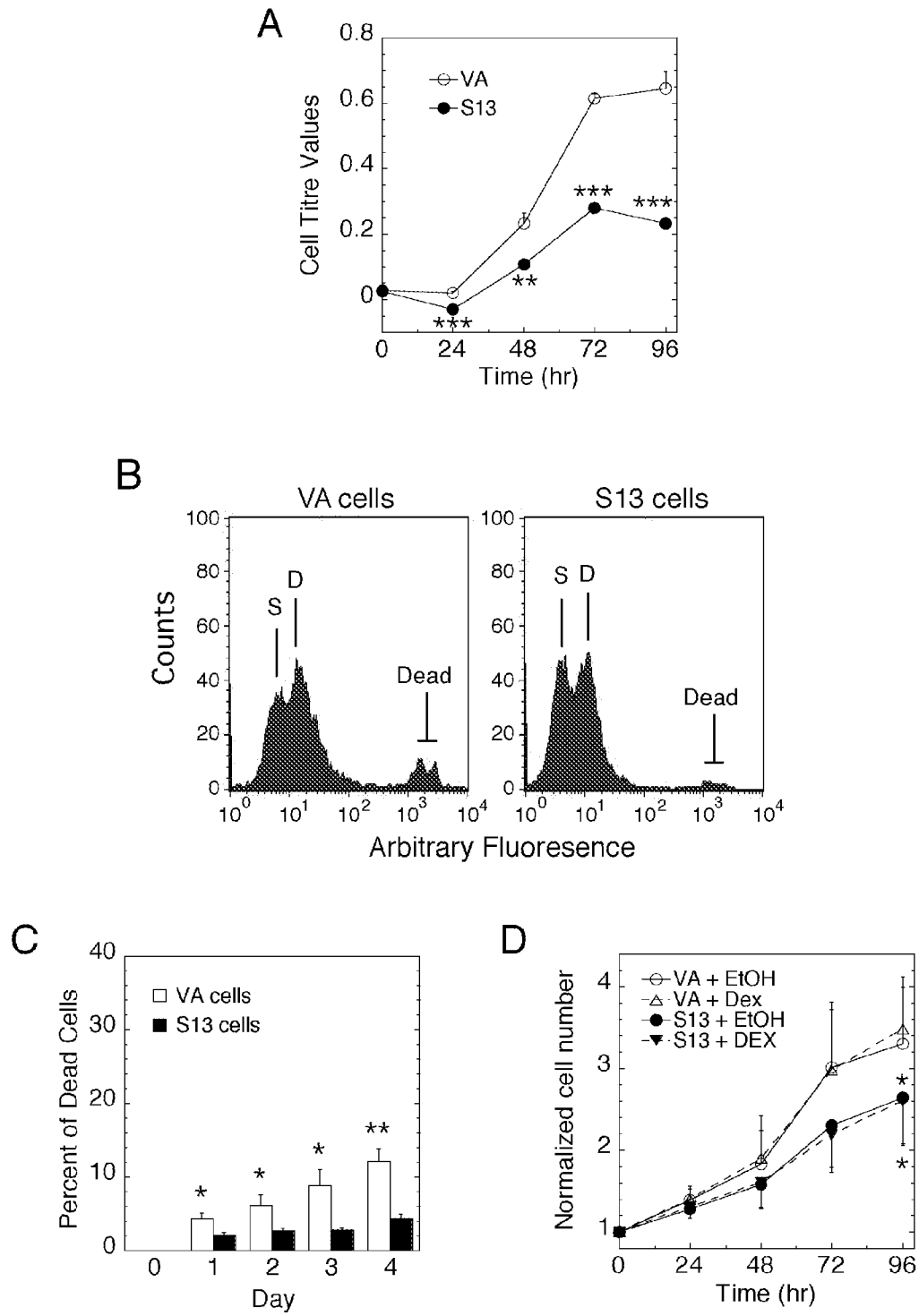


FIGURE 1

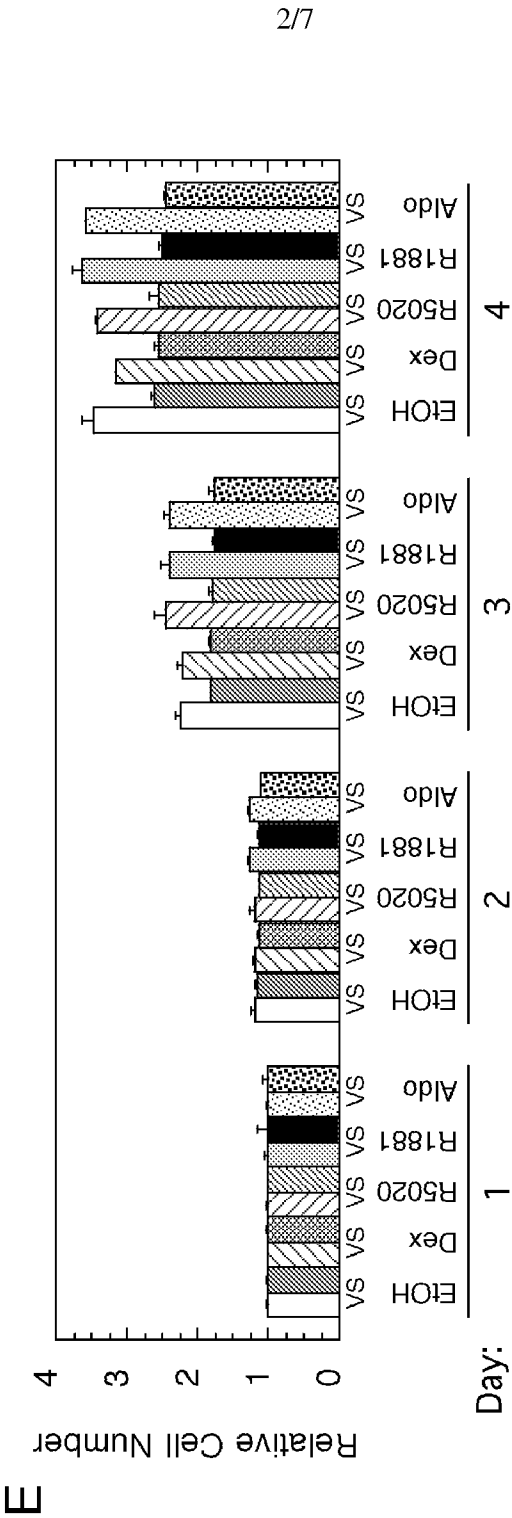
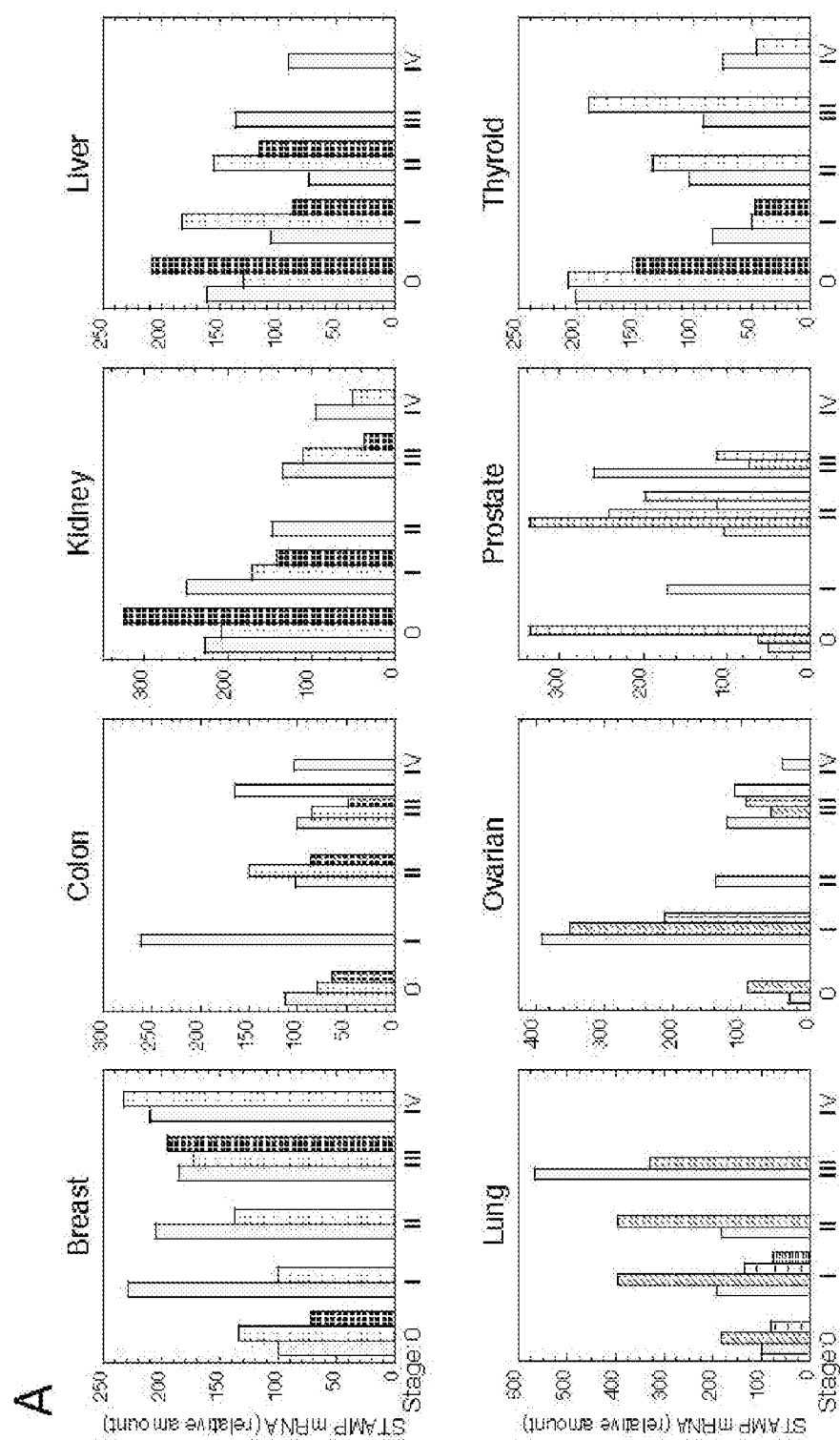


FIGURE 1 (CONT.)



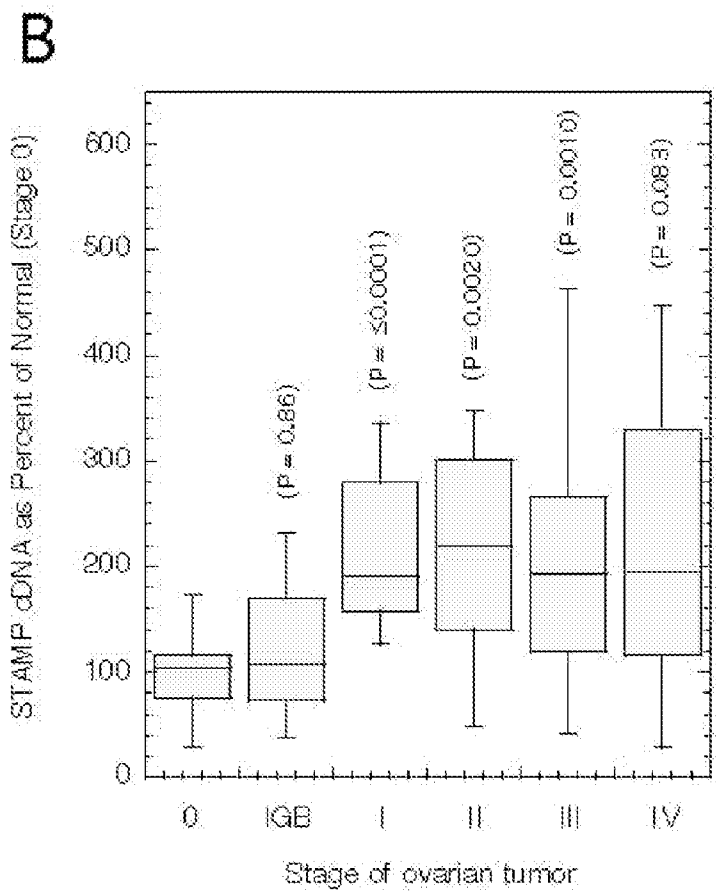


FIGURE 2 (CONT.)

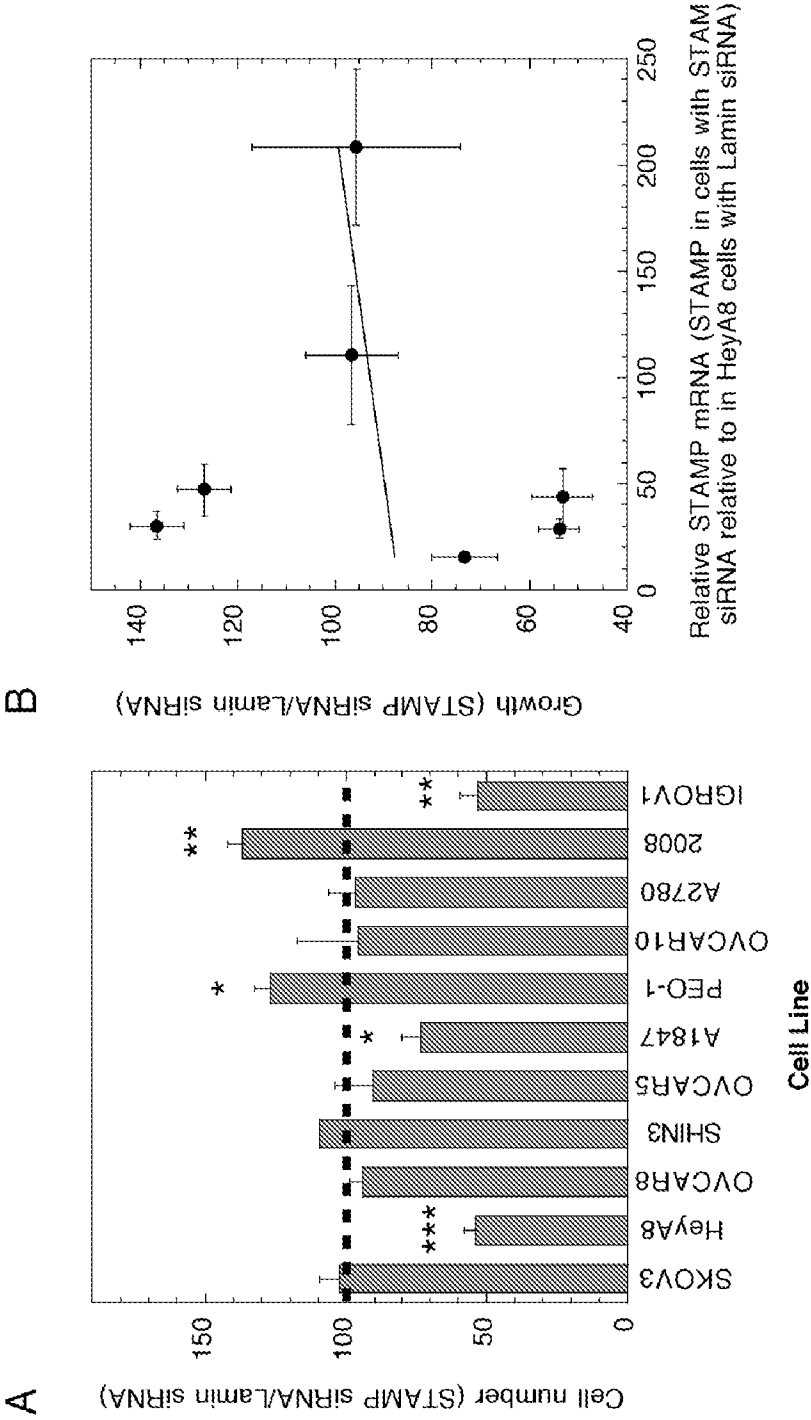


FIGURE 3

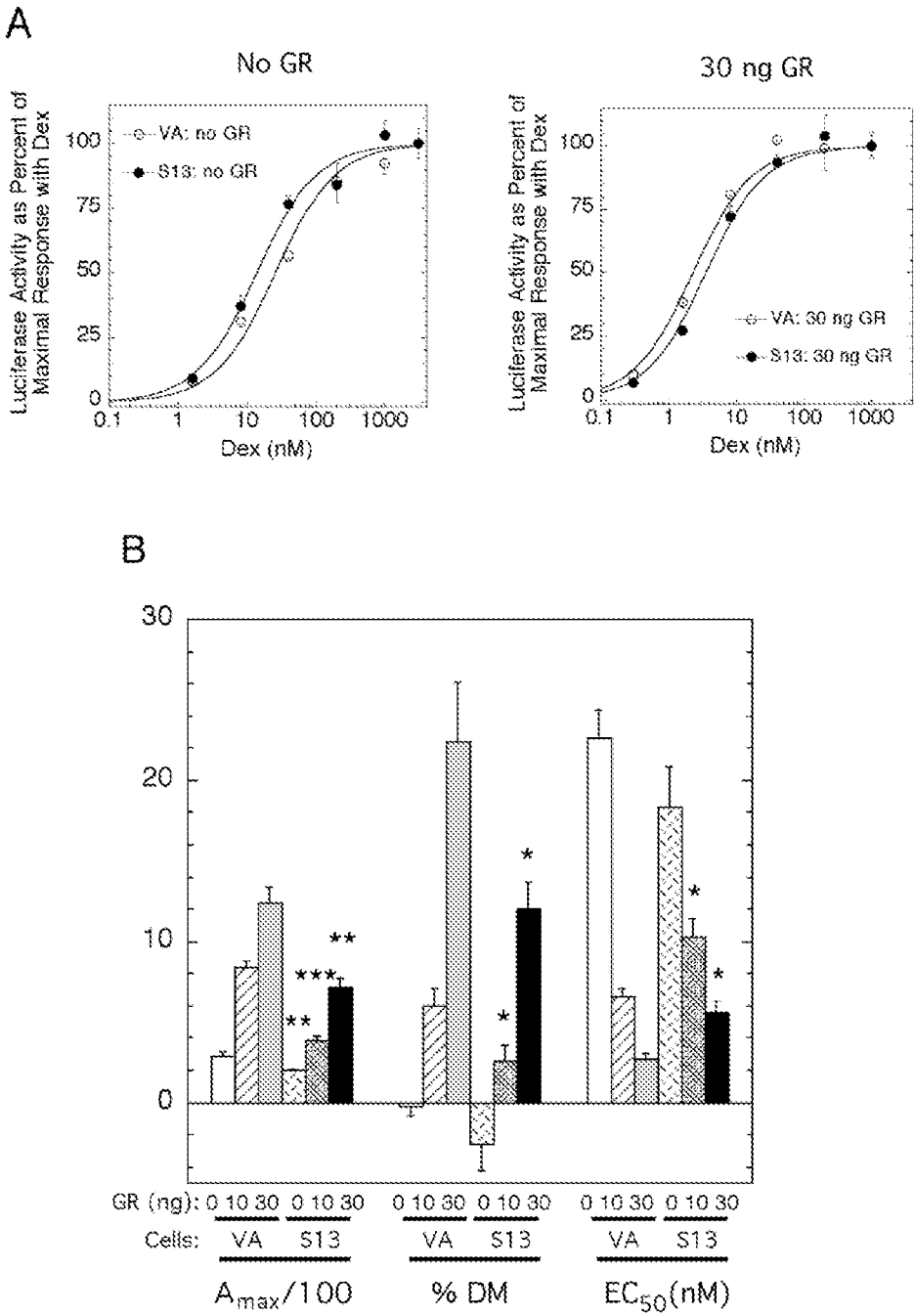
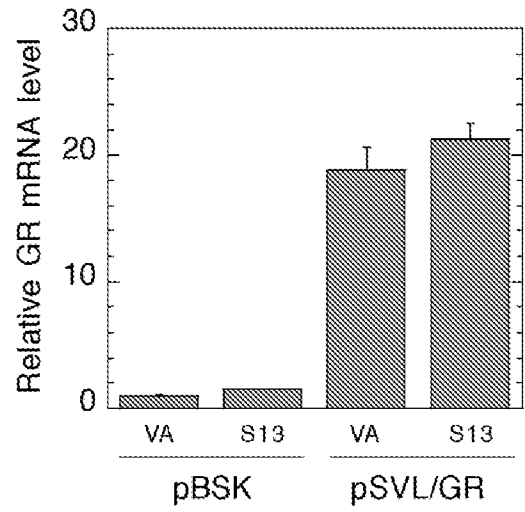


FIGURE 4

C



D

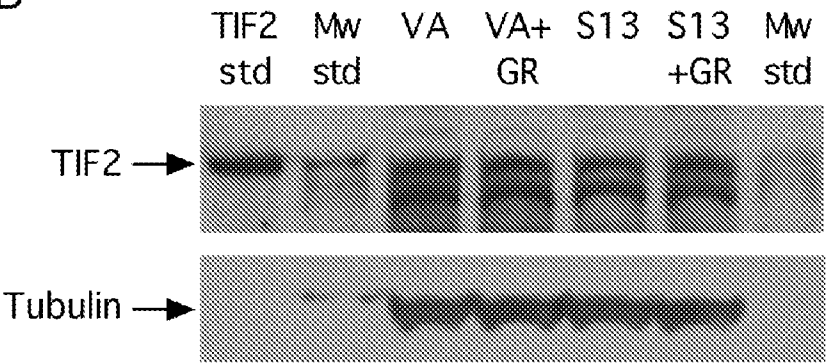


FIGURE 4 (CONT.)