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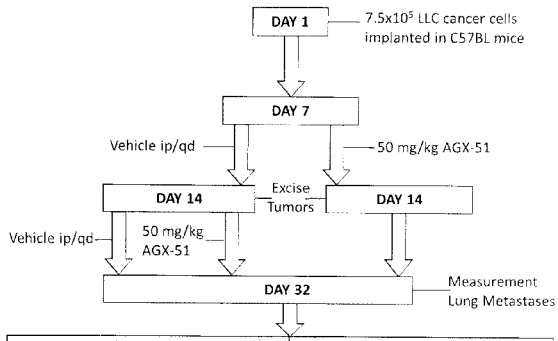
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[Continued on next page]

(54) **Title**: COMPOSITIONS AND METHODS FOR TREATING, PREVENTING AND DIAGNOSING CANCER AND OTHER PROLIFERATIVE DISORDERS

FIGURE 12



Treatment	Number with > 5 Tumor Foci
Control [Q1Dx25(7)]	7/9[a,b]
AGX-51 [Q1Dx25(7)]	3/9[b,c]
AGX-51 [Q1Dx18(14)]	3/10[d]

[a]No sample for one animal; [b]Too Numerous to Count (TNTC)=4; [c]TNTC=2 ;[d]TNTC=0

(57) **Abstract**: The present invention provides methods for impairing function or reducing or ablating levels of Id (inhibitors of differentiation) proteins to suppress metastasis of cancer cells in mammalian subjects presenting with cancer or elevated risk of metastatic disease. Exemplary anti-Id compounds include N-(3-(benzo[d] [1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N- benzylpropionamide and isolated enantiomers of N-(3-(benzo[d] [1,3]dioxol-5-yl)-3-(2- methoxyphenyl)propyl)-N-benzylpropionamide. Anti-Id compositions and methods of the invention can be combined with standard anticancer treatment modalities, such as surgery, radiation and chemotherapy. Also provided are compositions and methods to treat other hyperproliferative disorders, including pathogenic angiogenic conditions, including tumor-associated neoangiogenesis and ocular vascular pathologies including age-related macular degeneration.

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COMPOSITIONS AND METHODS FOR TREATING, PREVENTING AND DIAGNOSING CANCER AND OTHER PROLIFERATIVE DISORDERS

TECHNICAL FIELD

The present invention relates to compositions and methods for preventing, diagnosing and treating cancer and other proliferative disorders, including pathogenic angiogenesis, in mammalian subjects.

CROSS REFERENCES TO RELATED APPLICATIONS

This application claims benefit of U. S. Provisional Application No. 60/916,116, filed 13 December 2013, and U.S. Provisional Application No. 61/965,776, filed 6 February 2014, each of which is incorporated herein by reference its entirety for all purposes.

BACKGROUND

Inhibitor of Differentiation (Id) Proteins

The Id family of helix-loop-helix proteins (HLH) is implicated in the regulation of virtually all underlying cellular mechanisms and determinative events in cancer: cellular differentiation, cell cycle progression, senescence, lineage commitment and apoptosis (Perk et al., 2005; Morse, 2006; Ling et al., 2006, Nair et al, 2013). The term 'Id' itself is a characterization of the ability of these proteins to inhibit both cell differentiation and binding of important regulatory proteins to DNA (deoxyribonucleic acid). Of the four Id proteins (Id1-4), the role of Id1 in tumor invasiveness, metastasis and angiogenesis is best characterized, and findings concerning the role of Id1 in cancer provide a foundation for the discussion that follows.

Id1 is highly conserved in vertebrates and invertebrates as well as among species (Deed et al., 1994) as illustrated in the sequence alignment chart provided below that shows remarkable "homology" (sequence identity) of >90% for human Id1 (SEQ ID NO. 1) (UniProKB P41134) and mouse Id1 (SEQ ID NO. 2) (UniProKB P20067) Id1 proteins.

```
Human MKVASGSTATAAAGPSCALKAGKTASGAGEVVRCLSEQSVAISRCAGGAGARLPALLDEQ  060
Mouse MKVASGS-AAAAAGPSCSLKAGRTA---GEVVLGLSEQSVAISRCAG---TRLPALLDEQ  053
Human QVNVLLYDMNGCYSRLKELVPTLPQNRKVSKVEILQHVIDYIRDLQLELNSESEVGTPGG  120
Mouse QVNVLLYDMNGCYSRLKELVPTLPQNRKVSKVEILQHVIDYIRDLQLELNSESEVGTTGG  113
Human RGLPVRAPLSTLNGEISALTAEAACVPADDRILCR                          155
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Mouse RGLPVRAPLSTLNGEISALAAEAACVPADDRILCR 148

A comparison between human Id3 (SEQ ID NO. 3) (UniProKB Q02535) and mouse Id3 (SEQ ID NO. 4) (UniProKB P41133) Id3 proteins yields even closer sequence identity.

Human MKALSPVRGCYEAVCCLSERSLAIARGRGK**G**P**AA**EEPLSLLDDMNHCYSRLRELVPGVPR 060
Mouse MKALSPVRGCYEAVCCLSERSLAIARGRGK**S**P**ST**EEPLSLLDDMNHCYSRLRELVPGVPR 060
Human GTQLSQVEILQRVIDYILDLQVVLAEPAPGPPDGPHLPIQTAELAPELVISNDKRSFCH 119
Mouse GTQLSQVEILQRVIDYILDLQVVLAEPAPGPPDGPHLPIQTAELTPELVISKDKRSFCH 119

Human Id 2 (UniProKB Q02363) and mouse Id2 (UniProKB P41136) proteins share all but two out of 134 identical residues, while human Id4 (UniProKB P47928) and mouse Id4 (UniProKB P41139) are 100% sequence identical.

This unusually high degree of homology or sequence identity between human and murine Ids provides for heightened confidence in extrapolating experimental findings from mice, as accepted model subjects, to humans, in relation to Id structure, function and biology.

The most studied of the Ids is Id1, a regulator of transcription found in extremely low (typically non-measurable or undedtectable concentrations) in differentiated tissue of healthy adults (in contrast to elevated Id levels routinely detectable in cancer tissue or developmental (fetal) tissue). Id3 is considered a paralog of Id1, while Id2 and Id4 are distinctly different from each other and from Id1 and Id3.

The principal binding partners for Id1 and all Id helix-loop-helix (HLH) proteins are the basic helix–loop–helix (bHLH) family of transcription factors that contains over 200 members and functions broadly as regulators of developmental processes (Powell and Jarman, 2008; Massari, 2000). Particularly important members of the bHLH family are the so-called E proteins.

E proteins are bHLH proteins that are ubiquitously expressed, bind to the E-box element of DNA and are sequestered by Id proteins. E47 is a basic helix-loop-helix protein that is a splice product of the E2A gene. E-twenty six "ETS" is another family of regulatory transcription factors (approximately 20) also proposed to be inhibited by Id proteins. Both E and ETS proteins have been shown to drive cell differentiation and growth arrest in a variety of cellular contexts. Less frequent binding partners of the Id HLH proteins include retinoblastoma (RB, a protein that functions as a tumor suppressor) for Id2 (Desprez et al., 2003) and for Id1, non-bHLH proteins E-twenty-six (ETS) (a family of regulatory transcription factors implicated in the development of different tissues as well as cancer

progression through, eg, p16Ink4a, a tumor suppressor gene); Paired box (Pax); Mouse Id assocciated-1 (MIDA-1); and Sterol regulatory binding protein-1c, SREBP-1c, among others.

The functions of HLH family proteins are comparable to functions of Id proteins. Generally, all of these proteins are fundamental mediators of stasis or change in cellular differentiation, cell cycle progression, senescence, cellular commitment (to a determined lineage/fate) and/or apoptosis. However, the activities of Id proteins are typically *opposite* in effect to activities of HLH family proteins. This is attributed in part to a deactivating result from binding between the HLH and bHLH proteins, as described below.

bHLH proteins generally mediate a restrained or no growth environment in mammalian cellular systems. Consistent with this role, a reduction of E proteins in B and T-cells correlates with development of B and T-cell leukemias (Kee, 2009). In contrast, increased Id protein expression is associated with a pro-growth environment, attributable to Id's potential for neutralizing bHLH proteins.

Various reports have been published concerning the nature of interactions between bHLH proteins and other bHLH proteins (Slattery et al, 2008) and between bHLH proteins and HLH proteins such as Id proteins (Norton, 2000). All members of the bHLH family possess a highly conserved HLH region containing two amphipathic α helices separated by a shorter intervening loop. This "HLH domain" mediates homo- or hetero-dimerization that is essential for bHLH protein activity. Adjacent this HLH domain is a basic region capable of binding deoxyribonucleic acid (DNA) elements containing a canonical "E-box" sequence.

Id proteins also possess an HLH domain, but without the adjacent basic region that mediates DNA binding of bHLH proteins. As a result of this construction, Id proteins are capable of binding other HLH transcription factors and altering their activity in gene transcription. In this manner, Id1 has been reported to inhibit the activity of bHLH transcription factors like the E protein, E47, by binding and restraining the ability of the bHLH binding partner to bind DNA and mediate transcriptional changes (proposed to promote pro-differentiation and pro-apoptotic cellular changes—believed to be correlated with anti-neoplastic and anti-angiogenic activity in different cellular developmental contexts).

Id1 is present in both cytosol and nuclear compartments of cells, with shuttling from the nucleus possibly regulated by protein kinase-A (PKA) (Nishiyama et al., 2007). Intracellular levels of Id1 are also proposed to be regulated through an ubiquitin-proteasome degradation pathway (Sun et al., 2005), resulting in half-lives for Id proteins of

approximately one hour or less. This degradation process is possibly linked to TNF-α-induced apoptosis in prostate cancer cells (Ling et al., 2006). Heterodimerization with bHLH proteins reportedly affects rates of Id degradation and may be protective to extend Id protein half-life (Bounpheng et al, 1999).

Id Proteins and Angiogenesis

Id1 and Id3 have been proposed to play critical roles in the production of new blood vessels, including "pathogenic neovascularization" associated with growth and spread of tumors ("tumor-associated angiogenesis"). Transfer of the Id1 gene into HUVEC cells elevates angiopoetin-1 and confers a pro-angiogenic phenotype (Nishiyama et al., 2005). Id1 reportedly acts "downstream" of pro-angiogenic factors vascular endothelial growth factor-A (VEGF-A) Lee et al., 2006), bFGF (Ruzinova et al., 2003), HIF-1 (Kim et al., 2007), and EGF-R (Ling et al., 2004), so loss of Id1 activity may impair multiple angiogenic pathways. Such plural targeting of angiogenic pathways would be an important objective, as tumors have proven refractory to mono-directed antiangiogenic therapy (including by responding through up-regulation of alternative pro-angiogenic growth factors).

Id1 is also proposed to interact negatively with p21 (a cyclin kinase inhibitor, proposed to function via inactivation of cyclins. P21 is reported to negatively control endothelial progenitor cell formation in bone marrow neovascularization (Ciarrocchi et al., 2007), which progenitor cells are contemplated to play a pivotal role in tumor-associated angiogenesis (Seandel et al., 2008), and cancer metastasis (Gao et al., 2008; Gao et al., 2009). A direct role for endothelial Id protein expression in tumor-associated angiogenesis has been proposed based on xenograft studies in Id1 $^{-/-}$; Id3$^{+/-}$ mice. Xenograft tumors in Id1 $^{-/-}$; Id3$^{+/-}$ mice reportedly fail to grow completely, or show slower growth with extensive hemorrhage and necrosis, compared to Id wild type controls, while transplantation of wild-type bone marrow–derived endothelial precursor cells reportedly restores tumor angiogenesis and growth in Id1 $^{-/-}$; Id3 $^{+/-}$ subjects (Lyden et al., 1999; Lyden et al., 2001).

Id Proteins and Cancer

Id1 proteins have been reported to be linked to a diverse array of signaling and control elements involved in the initiation and progression of cancer (Fong et al., 2004). A

particularly intriguing link has been proposed between Id1 and cancer metastasis. Id1 has been reported to be associated in a mechanistic way with metastatic change in human breast cancer (Minn et al., 2005). Over-expression of Id1 reportedly contributes to metastasis in breast cancer cells transplanted into animals (Fong et al., 2003). In another study, over-expression of Id1 was reported to immortalize myeloid progenitor cells and lead to myeloproliferative disease in mice (Suh et al., 2008).

Id1 is highly expressed in a large number of cancers including solid tumors of the bladder (Perk et al., 2006), breast (for example, Schoppmann et al., 2003), cervix/uterus (Li et al., 2009; Schindl, et al., 2001; Maw et al., 2008), colorectal (Zhao et al., 2008), endometrium (Takai et al., 2004), gastric (Han et al., 2004; Iwatsuki et al., 2009), glia (Vandeputte et al., 2002), hepatic (Matsuda et al., 2005), ovarian (Schindl et al., 2003 and Maw et al., 2009), prostate (Forootan et al., 2007; Yu et al., 2009; Coppe et al., 2004; Ouyang et al., 2002a), renal (Li et al., 2007), squamous cell cancer head and neck (SCCHN) (Kamalian et al., 2008), thyroid (Kebebew et al., 2004; Ciarrocchi et al., 2010) and non-small cell lung cancer (NSCLC) (Bhattacharya et al., 2010) as well as liquid tumors such as acute myelogenous leukemia (AML) (Tang et al., 2009). In virtually all these cancer types, over-expression of Id1 has been reported to be associated with an aggressive phenotype and poor clinical outcome. Fundamental questions remain, however, concerning the mechanisms and pathways through which Id proteins interact and mediate negative impacts in complex developmental systems involved in cancer and other proliferative diseases and cellular pathogenic events.

The following additional observations and reports have contributed to an emerging but incomplete understanding of Id1 expression, function and biology in cancer, cancer metastasis, and pathogenic angiogenic development in mammalian subjects.

- Complex Factors Involved in Id Expression and Function Id1 gene expression is reported to be stimulated by growth factors including bone morphogenetic protein-2 (BMP-2) (Le Page et al., 2009), BMP-6 (Darby et al., 2008), growth/differentiation factor-5 (GDF5) (Chen et al., 2006), and insulin-like growth factor-1 (IGF-1) (Prisco et al., 2001; Belletti et al., 2002). Vascular-endothelial-growth-factor-A (VEGF-A) is reported to be both an upstream gene inducer (Benezra et al., 2001) and downstream mediator of Id1 (Lee et al., 2006; Lin et al., 2005). SMAD proteins are proposed to modulate activity of transforming growth factor beta ligands by forming complexes

with other SMADs that function as transcription factors (Liang et al., 2009). Early growth response protein 1 (Egr1) (Subbaramaiah et al., 2008), Sp1 (Jorga et al., 2007), a member of the Sp/KLF (specificity protein/Krüppel-like factor) family of human transcription factors involved in gene expression in the early development of an organism and activating transcription-3 (ATF3) (Li et al., 2009), are reported to positively affect promoter activity, while the tumor suppressor ATF5 is reported to suppress Id1 gene expression (Gho et al., 2008). Src, a proto-oncogene tyrosine protein kinase mediator of growth factor activity, is also reported to play a role in regulating Id1 expression (Gautschi et al., 2008). Similarly, forkhead box O-3a (FOX03a), a transcription factor reported to reverse leukemic phenotypes, may promote differentiation by transcriptional down-regulation of Id1 (Birkenkamp et al., 2007). Down-regulation of the Id1 gene has also been proposed as a mechanism for a mechanism for anti-cancer activity of non-steroidal anti-inflammatory drugs (NSAIDs) in gastric cancer (Jang et al., 2006). A reduction in pro-inflammatory prostaglandin PGE2 in breast cancer cells mediated by the NSAID celecoxib has also been correlated with reduced Id1 levels (Subbaramaiah et al., 2008)

- Complexity of Downstream Id Targets Id1 is also proposed to target various downstream oncogenic tyrosine kinases (Tam et al., 2008), such as Bcr-Abl, Tel-ABL (an aberrant fusion protein associated with chronic myelogenous leukemia), TEL-PDG-FßR (a hybrid fusion protein found in patients with myeloid neoplasms associated with eosinophilia), FLT3 (a tyrosine kinase 1 associated with one of the receptors for VEGF)-ITD, and others. Id1 is postulated to cooperate with oncogenic rat RAS (a GTPase in the mitogen-activated protein kinase (MAPK) pathway), to induce metastatic mammary carcinoma by subverting cellular senescence responses (Swarbrick et al., 2008). Id1 has been reported to stimulate a phosphatidylinositol-3-kinase (PI3K)/protein kinases B (Akt)/nuclear factor kappa beta (NFkB) signaling pathway important to cancer cell proliferation, survival and invasion (Li et al., 2007). Id1 may restrain expression of p16, which in turn positively regulates cell senescence (Zheng et al., 2004; Cummings et al., 2008). Id1 may also regulate B-cell lymphoma cell-2 (Bcl-2) and BCL-2-associated X protein (Bax), through p53 and NFkB (Kim et al., 2008), and chromosomal instability through anaphase-promoting complex-C (APC/C) during mitosis (Wang et al., 2008) to enhance survival of cancer cells.

Additionally, Id1 is proposed to activate Akt-mediated wingless type (Wnt) signaling and p27 phosphorylation through phosphatase and tensin homolog (PTEN) inhibition (Lee et al., 2009).

Id Proteins and Metastasis

Current knowledge falls even further short of practically comprehending the roles of Id proteins and other regulatory factors in cancer metastasis. Metastatic disease accounts for 90% of carcinoma-related deaths in humans. The complexity and refractory nature of metastasis present the greatest obstacles to developing new methods and tools to reduce cancer morbidity and mortality. Metastasis is a multi-step, multi-factorial process, broadly described as having two major phases: (1) physical dissemination of cancer cells from a "primary" site (i.e., initial tissue/organ where the cancer originated); and (2) colonization by cancer cells from a primary site to distant tissues/organs (Chaffer and Weinberg, 2011). Numerous studies have shown that the first phase is attended by a de-differentiation or re-activation of development, switching determined cells to a more plastic fate, commonly referred to as the "epithelial to mesenchymal transition" (EMT). EMT conversion endows primary cancer cells with a much greater metastatic potential, associated with a highly invasive phenotype (Thiery et al., 2009).

During the course of EMT, immobile epithelial cells lose their epithelial traits and acquire mesenchymal properties, coupled with an ability and propensity to migrate to other locations (secondary tumor sites) to seed new tumors. Cancer cells that have undergone EMT reportedly share key characteristics with tumor initiating cells (TICs) (Mani et al., 2008) (functionally defined by their ability to seed new tumors and restore heterogeneity of primary tumors) (Valent et al., 2012). The generation of breast cancer TICs by overexpression of EMT-inducing transcription factors, such as twist-related protein 1 (Twist1, or class A bHLH protein 38) has suggests molecular linkage of EMT-driven metastatic dissemination, and the generation of TICs (Mani et al., 2008; Morel et al., 2008). However, less is known about the biology of TICs during the second phase of metastasis (colonization of distant tissues organs). The proposal that EMT induces breast cancer TICs has been challenged based on clinical observations that most metastases present a differentiated epithelial morphology (Tarin et al., 2005). This may indicate that EMT is a transient process, and that the re-differentiation of carcinoma cells by a "mesenchymal to

epithelial transition" (MET) is a driving force in metastatic colonization, at least in some cancers (Brabletz, 2012).

While EMT may affect cancer cell dissemination (including invasion of the local parenchyma (Yook et al., 2006), intravasation into the circulatory system (Drake et al., 2009), survival during migration (Gal et al., 2008), and extravasation into the secondary site (Labelle et al., 2011; Vuoriluoto et al., 2011)), loss of mesenchymal phenotype may enhance formation of macro-metastatic colonies. This may be linked to a reversal of growth arrest associated with EMT (Brabletz et al., 2001; Mejlvang et al., 2007; Vega et al., 2004). The importance of MET in breast cancer metastasis is suggested by studies showing that after dissemination, engineered loss of the EMT transcription factor Twist1, (Tsai et al., 2012) and expression of microRNAs inhibiting the EMT transcription factor "zinc finger E-box-binding homeobox" (Zeb) (Korpal et al., 2011) enhance lung colonization by metastatic breast cancer cells. Further, the transcription factor "paired related homeobox 1" (Prrx1) (which induces EMT during dissemination but suppresses stemness traits necessary for lung colonization), must be lost prior to colonization (uncoupling in this instance EMT from the TIC phenotype) (Ocana et al., 2012). Details of how a colonizing cancer cell sheds its mesenchymal phenotype required for dissemination, while retaining TIC properties essential for founding a metastatic colony, remain unclear.

In view of the foregoing, many critical questions remain regarding the mechanisms and pathways through which Id proteins interact and mediate negative impacts in cancer and other proliferative disorders (such as tumor-associated angiogenesis). There are therefore many obstacles and hurdles to be overcome before these agents and processes can be exploited to provide new tools and methods for treating cancers in mammalian subjects. Additional needs remain unfulfilled for tools and methods to evaluate, manage and treat metastatic cancer in mammalian subjects.

SUMMARY OF EXEMPLARY EMBODIMENTS OF THE INVENTION

The present invention fulfills the foregoing needs and satisfies additional objects and advantages by providing novel tools and methods to modulate Id function in mammalian subjects suffering from a neoplasm or other cellular proliferative disorder. The invention further provides novel technical discoveries to elucidate the role and effects of Id proteins and their biochemical and molecular targets in mediating cancer and metastasis, yielding tools

and methods to manage and treat cancer and metastasis and other proliferative disorders in humans and other mammals.

In exemplary aspects the invention provides compositions and methods for treating a cellular proliferative disorder, for example inhibiting or reducing metastasis of a tumor or primary cancer cell. These methods comprise administering to a mammalian subject an effective amount of an "anti-Id compound", sufficient to reduce or prevent pathogenic cellular proliferation. angiogenesis, cancer, and/or metastatic disease in the subject. In exemplary embodiments, the compositions and methods of the invention employ an exemplary anti-Id compound of formula I, II, III, or IV as described below, or an active salt, enantiomer, polymorph, solvate, hydrate, or prodrug thereof.

In certain detailed aspects of the invention, the methods and compositions herein employ an exemplary anti-Id compound or composition comprising an anti-metastatic (or anti-proliferative, or anti-angiogenic) effective amount of racemic N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ("**AGX51**"). In distinct embodiments, the anti-Id compound is an isolated, anti-metastatically active (-)-enantiomer of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide. The (-) or "minus" enantiomer of AGX51 exhibits an extraordinary and unexpected dominance of anti-Id potency compared to the (+) or "plus" enantiomer. On this basis, novel, enantomerically-enriched preparations of (-)-AGX51 enantiomer (substantially purified to yield an increased amount or concentration of (-)-AGX51, relative to an amount or concentration of the (+)-AGX51 enantiomer (compared to conventionally-prepared racemic N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide), provide surprising advantages and clinical benefits within the compositions and methods of the invention.

In certain embodiments, the invention provides "anti-metastatic" compositions and therapeutic methods, which are effective to treat or prevent metastatic disease in mammalian subjects. These methods may employ monotherapy or coordinate or combinatorial therapy. The compounds and methods of the invention are "anti-metastatically effective", for example to reduce an incidence, size, tissue or organ distribution, or number of metastases in a subject presenting with cancer. In certain embodiments, anti-metastatic activity corresponds to an observed reduction in one or more histopathological indices of metastasis, for example quantitative reduction in occurrence, size, number or distribution of metastasized cells or

"foci" of primary tumor character observed at a secondary tissue or anatomic site. In other aspects, anti-metastatic efficacy is evinced by prevention and/or treatment of metastatic cancer, e.g., as demonstrated by an increase in a time period of disease free survival for subjects receiving anti-Id treatment according to the invention.

Other compositions and methods of the invention target distinct cellular proliferative disorders characterized by aberrant blood vessel growth, or "pathogenic angiogenesis". Examples of these disease targets include ocular disease mediated by aberrant vascular growth (e.g., macular degeneration), and tumor-associated angiogenesis. Anti-Id compounds of the invention function also as "anti-angiogenic" agents, as described below, making them useful to treat or prevent pathogenic angiogenesis, including quite powerfully tumor-associated angiogenesis (to mediate a multi-pronged, anti-metastatic and anti-angiogenic assault on secondary tumor initiation and growth.

In additional aspects of the invention, novel diagnostic assays measuring Id proteins in blood or tissues (particularly Id1 and/or Id3) are demonstrated to provide useful diagnostic tools to monitor occurrence and progression of metastatic disease, and/or evaluate effectiveness of anti-Id treatment.

In more detailed embodiments, compositions and methods of the invention may employ an effective amount of the anti-Id compound combined in a kit or formulation, or coordinately administered, along with a secondary treatment agent, treatment modality or treatment method. In exemplary embodiments, subjects are treated with the anti-Id compound simultaneously or sequentially with a secondary treatment modality or agent, for example selected from: radiation, chemotherapy, surgery, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

This patent application contains at least one figure executed in color. Copies of this patent application with color drawing(s) will be provided upon request and payment of the necessary fee.

Figure 1 provides a Western blot gel showing that the Id1 and Id3 levels are potently reduced in leukemic cells following treatment with an exemplary anti-Id compound, N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51). A purified (-)-AGX51 enantiomer exhibits surprising stereospecific effects compared to the (+)-AGX51 enantiomer.

Figure 2 provides a Western blot gel showing that the Id1 and Id3 levels are variably affected in breast cancer cells following treatment with AGX51, revealing additional surprising stereospecific effects of this anti-Id compound.

Figure 3 is an immunoblot showing rescue of p16 levels in a leukemic cell line derived from a mouse overexpressing the MML-AF9 fusion protein following treatment with AGX51.

Figure 4 is a Western blot showing rescue of p21 levels in a human bladder carcinoma line following treatment with AGX51.

Figure 5 graphically compares effects of racemic-AGX51, (+)-AGX51 ("E1") and (-)-AGX51 ("E2) on restoring cell cycle control in DU-145 human prostate cancer cells. The (-)- enantiomer of AGX51 shows pronounced stereospecific efficacy for restoring cell cycle control, whereas the (+)- enantiomer of AGX51, surprisingly, mediates no detectable effect.

Figure 6 illustrates potent anti-migration effects of an exemplary anti-Id compound of the invention, AGX51, in a model of migration-dependent metastatic disease potential. This cellular motility "Scratch" assay demonstrates that AGX51 is a potent inhibitor of metastatic activity involving migration of cancer cells.

Figure 7 is a chart illustrating strong anti-angiogenic effects of AGX51 to reduce formation of blood vessels in VEGF-165 and FGF-2 treated Matrigel plugs implanted into flanks of C57/BL mice.

Figure 8 is a chart providing data from the xenograft study performed in nude mice (n=8/treatment group) implanted with MDA 231 human breast tumors and treated iv 14 days after implantation with either vehicle (DMSO) or 50mg/kg per day of AGX51 for five days and 7.5mg/kg paclitaxel starting on days 8 and 22. Boxplots are tumor volumes 53 days post implantation (last day of study).

Figure 9 provides ion chromatograms of control (A) and low calibrant of AGX51 (B).

Figure 10 is a log-linear plot of plasma concentration data showing that AGX51 is rapidly absorbed with C_{max} estimated here at 15 minutes.

Figure 11 is a simulation of plasma concentrations following 60mg/kg, bid of AGX51.

Figure 12 shows the design and results of studies demonstrating potent anti-metastatic efficacy of AGX51, directly reducing lung metastasis of Lewis Lung Carcinoma (LLC) tumors implanted into C 57/BL mice (excised 14 days after implantation). In this

widely accepted model for predicting cancer drug efficacy in humans, AGX51 provides an unprecedented decrease in metastasis of implanted cancer cells.

Figure 13 graphically demonstrates the effects of AGX51 on lung metastasis of breast cancer (4T1) cells directly injected via tail vein into Balb/c mice. AGX51 potently reduces metastasis of injected breast cancer cells.

Figure 14 shows bioluminescence visualization of AGX51 suppression of lung metastasis in Balb/c mice following direct injection of breast cancer (4T1) cells via tail vein. The (-)-enantiomer of AGX51 potently, stereospecifically protects against metastasis in living model subjects predictive of cancer drug efficacy in humans.

Figure 15 is a graph demonstrating that a combination of paclitaxel and AGX51, even at a minimal dose, significantly decreased tumor growth in mice implanted with MDA-MB-231 tumors.

Figure 16 is a chart showing the change in final-initial tumor volume on day 19 in mice implanted with MDA-MB-231 tumors and treated with paclitaxel and varying amounts of AGX51.

Figure 17 is a graph showing that 60mg/kg, bid, AGX51 significantly increased the effectiveness of paclitaxel on reducing tumor growth.

Figure 18 is a chart showing the change in final-initial tumor volume on day 41 in mice implanted with MDA-MB-231 tumors and treated with paclitaxel and AGX51.

Figure 19 is a graph showing that the addition of AGX51 to a treatment with paclitaxel significantly increased the effectiveness of paclitaxel on decreasing tumor growth.

Figure 20 is a graph showing tumor growth in Id1 knockout mice treated with paclitaxel and AGX51.

Figure 21 is a chart showing mean tumor volume on day 20 in Id1 knockout mice treated with paclitaxel and AGX51.

Figure 22 is a chart showing a comparison of selected chemistry and hematology values in mice treated with paclitaxel and/or AGX51.

Figure 23 is a graph illustrating effects of Id3 genetic knockout on pathogenic retinal neovascularization in a mouse model.

Figure 24 is a graph demonstrating that (-)-AGX51 administered intravitreally (ivt) protects against pathogenic retinal neovascularization in a murine model of human age-related macular degeneration (AMD)

Figure 25 is a graph demonstrating that (-)-AGX51 administered intraperitoneally (ip) protects against pathogenic retinal neovascularization in a murine model of human AMD.

Figure 26 is a schematic depiction of a modified sandwich immunoassay for detection of Id protein levels/activity in biological samples for use in implementing reflexive diagnostic-treatment methods of the invention.

Figure 27 is a graph demonstrating the use of Id diagnostic tools and methods of the invention for predicting and managing cancer.

Figure 28 is a graph demonstrating the use of Id diagnostic tools and methods of the invention for predicting and managing breast cancer in human subjects.

Figure 29 is a graphic depiction of AGX51 (+)- and (-)-enantiomer elution profiles using Enantiomer Separation Method A of the invention.

Figure 30 is a graphic depiction of AGX51 (+)- and (-)-enantiomer elution profiles using Enantiomer Separation Method B of the invention.

DESCRIPTION OF EXEMPLARY EMBODIMENTS OF THE INVENTION

Before describing the present invention in detail, several terms used in the context of the present invention will be defined. In addition to these terms, others are defined elsewhere in the specification, as necessary. Unless otherwise expressly defined herein, terms of art used in this specification will have their art-recognized meanings. In addition, the following terms have the following meanings unless otherwise indicated.

Metastasis refers to the spread of a cancer from one organ or part to another non-adjacent organ or part. **Metastases** are new occurrences of cancer at secondary sites generated by metastasis.

The term **"chemotherapeutic"** drug or agent typically refers to approved anti-cancer and other anti-hyperproliferative drugs or chemical agents. "Chemotherapeutic" will generally apply to a drug or chemical active to destroy cells and tissues, typically cancer cells and alternatively or conjunctively cells of blood vessels newly formed as part of a pathologic condition attending a hyperproliferative disorder, as typically attends a neoplasm or a cancer. Chemotherapeutic agents for adjunctive use within the invention include, but are not limited to: (1) tubulin depolymerizing agents such as taxanes like paclitaxel, docetaxel, BAY 59-8862 albumin bound paclitaxel, (2) DNA damaging agents and agents that inhibit DNA

synthesis, (3) anti-metabolites, (4) anti-angiogenics and vascular disrupting agents, (5) antibodies, (6) endocrine therapy, (7) immuno-modulators, (8) histone deacetylase inhibitors, (9) inhibitors of signal transduction, (10) inhibitors of heat shock proteins, (11) retinoids such as all-trans retinoic acid, (12) inhibitors of growth factor receptors or the growth factors themselves, (13) anti-mitotic compounds, (14) anti-inflammatory agents such as COX inhibitors, and (15) cell cycle regulators, eg, check point regulators and telomerase inhibitors.

The term **"combination therapy"** refers to a therapeutic regimen that involves the provision of at least two distinct therapies to achieve an indicated therapeutic effect. For example, a combination therapy may involve the administration of two or more chemically distinct active ingredients, for example, an anti-Id compound as well as a chemotherapeutic agent. Alternatively, a combination therapy may involve the administration of an anti-Id treatment and/or one or more chemotherapeutic agents, alone or together with the delivery of another treatment, such as radiation therapy and/or surgery. In the context of the administration of two or more chemically distinct active ingredients, it is understood that the active ingredients may be administered as part of the same composition or as different compositions. When administered as separate compositions, the compositions comprising the different active ingredients may be administered at the same or different times, by the same or different routes, using the same of different dosing regimens, all as the particular context requires and as determined by the attending physician. Similarly, when one or more anti-Id treatment alone, or in conjunction, with one or more chemotherapeutic agents combined with, for example, radiation and/or surgery, the drug(s) may be delivered before or after surgery or radiation treatment. **"Monotherapy"** refers to a treatment regimen based on delivery of one therapeutically effective compound, whether administered as a single dose or in several doses over time.

"Neoplasia" refers to abnormal and uncontrolled cell growth. A **"neoplasm"**, or tumor, is an abnormal, unregulated, and disorganized proliferation of cell growth and is sometimes referred to as a cancer. A neoplasm may be benign or malignant. A neoplasm is malignant, or cancerous, if it has properties of destructive growth, invasiveness, and metastasis. Invasiveness refers to the local spread of a neoplasm by infiltration or destruction of surrounding tissue, typically including penetrating basal laminae that define boundaries of tissues (thereby often entering the body's circulatory system). Metastasis typically refers to dissemination of tumor cells to distant sites, often via lymphatics or blood vessels.

Metastasis also refers to migration of tumor cells to adjacent sites through serous cavities or subarachnoid or other spaces. Through the process of metastasis, tumor cell migration and dissemination to other compartments, tissues and areas of the body establishes "secondary" neoplasms in areas away from the "primary" site of initial cancer appearance.

A **"subject"** or **"patient"** refers to an animal in need of treatment that can be affected by methods and compositions of the invention. Subjects and patients amenable to treatment using anti-Id compounds and methods of the invention include vertebrates, particularly mammals such as bovine, canine, equine, feline, ovine, porcine, and primate (including humans and non-humans primates) mammals, presenting with, or at elevated risk of developing, cancer, metastatic disease, or any proliferative disease including pathogenic angiogenesis.The term treatment or therapy **"system"** as employed herein refers to a plurality of treatment agents or modalities, such as a combined formulation or protocol employing an anti-Id active agent, a chemotherapeutic and possibly a toxicity reducing agent—used in a coordinate treatment regimen (formulated alone or together. and administered simultaneously or sequentially). Treatment systems may also combine drug (e.g., anti-Id and/or conventional chemotherapy) treatment with another intervention or treatment modality, such as radiation therapy or surgery. Optional treatment systems employing anti-Id agents and methods of the invention can be integrated in any combinatorial "therapeutic regimen" combining complementary tools or methods, such as chemotherapeutic agents, radiation therapy, surgery, gene therapy, DNA vaccines and therapy, siRNA therapy, anti-angiogenic therapy, immunotherapy, bone marrow transplants, aptamers and other biologics such as antibodies and antibody variants, receptor decoys and other protein-based therapeutics.

The term **"treatment"** or **"treating"** means any treatment of a disease or disorder, including preventing or protecting against the disease or disorder (that is, causing the clinical symptoms not to develop); inhibiting the disease or disorder (i.e., arresting or suppressing the development of clinical symptoms; and/or relieving the disease or disorder (i.e., causing regression of clinical symptoms). The methods and compositions of the invention will often serve to prevent one or more symptoms of disease, or delay onset or recurrence/relapse of disease (**"prophylaxis"**), as well as to slow, inhibit or prevent disease progression (e.g., as marked by increased severity of disease symptoms, or onset of more advanced disease symptoms).

The novel methods and compositions of the invention flow from a surprising course of discovery. Early comparisons of gene expression data between cell lines with variable metastatic potential prompted reports that certain candidate genes are required during different steps of the metastatic disease cascade (Kang et al., 2003b; Minn et al., 2005; Yang et al., 2004). Some of these reports suggested that expression of Id proteins, including products of Id1 and Id3 genes, are involved in lung colonization of breast cancer cells (Gupta et al., 2007). Id proteins have long been reported to be dominant negative regulators of basic helix-loop-helix (bHLH) transcription factors (Perk et al., 2005).

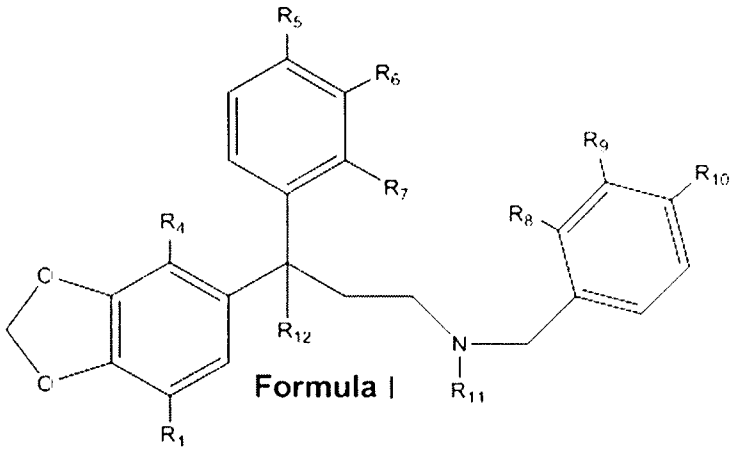
Id proteins have further been reported to play key roles in maintaining embryonic stem cell self-renewal (Romero-Lanman et al.; Ying et al., 2003), and to continue this function in adult tissue stem cells (Nam and Benezra, 2009) and hematopoietic stem cells (Jankovic et al., 2007).

Id genes have also been implicated as regulators of TIC phenotypes in certain cancers, such as glioblastoma (Anido et al., 2010; Barrett et al., 2012) and colon cancer (O'Brien et al., 2012). In human breast cancer, Id1 is predominantly expressed in the more aggressive triple negative [negative for estrogen receptor, progesterone and negative for the neu type human epidermal growth factor-2 (HER2] and metaplastic subtypes, with high Id1 expression correlating with poor clinical outcomes (Gupta et al., 2007).

The invention targets Id functionality in promoting cancer and metastasis, by employing novel suppressors of Id proteins, including suppressors of Id1 and Id3. These compounds are referred to herein as "anti-Id" compounds, and the attendant methods of the invention are collectively termed "anti-Id" methods. The anti-Id compounds of the invention are generally in an "anti-Id effective amount" by direct application or injection to a cell population, physiological compartment, tumor, or individual. These compounds and methods exhibit "anti-cancer", "anti-metastatic", "anti-proliferative", and/or "anti-angiogenic" effective activities, as described herein for different aspects and embodiments of the invention.

In exemplary aspects the invention provides compositions and methods for treating a cellular proliferative disorder, for example inhibiting or reducing metastasis of a tumor or primary cancer cell. These methods comprise administering to a mammalian subject an effective amount of an "anti-Id compound", sufficient to reduce or prevent pathogenic cellular proliferation, angiogenesis, cancer, and/or metastatic disease in the subject. In

exemplary embodiments, the compositions and methods of the invention employ an exemplary anti-Id compound of formula I, below.



Formula I

wherein R_1 may be a substituted or unsubstituted lower hydrocarbon selected from the group consisting of alkyl, alkenyl, alkanoyl, alkynyl, aryl, aroyl, aralkyl, alkylamino, aryloxy, hydrogen, carboxyl, nitro, thioalkoxy, thioaryloxy, thiol, cycloalkenyl cycloalkyl, heterocycloalkyl, heteroaryl, aralkyl, amino acid, peptide, dye, fluorophore, carbohydrate or polypeptide; R2 and R3 may independently, collectively, or in any combination that yields an active anti-Id (apoptosis inducing, cellular proliferation inhibiting, chemotherapeutic enhancing, transcription regulating, anti-inflammatory, cellular differentiation promoting, cellular transformation modulating) composition be a hydrogen, hydroxyl, sulfyhydryl, fluorine, methyl, ethyl, propyl, benzyl, 2-bromovinyl amino, hydroxymethyl, methoxy, halogen, pseudohalogen, cyano, carboxyl, nitro, thioalkoxy, thioaryloxy, thiol, substituted or unsubstituted lower hydrocarbon containing 1 to 20 carbons such as alkoxycarbonyl, allkoxycarbonylamino, amino, amino acid, aminocarbonyl, aminocarbonyloxy, aralkyl, aryloxy, carboxyl, cycloalkenyl, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, amino acid, peptide, dye, fluorophore, carbohydrate or polypeptide; R4 and R5 may be may independently, collectively, or in any combination that yields an active anti-Id (apoptosis inducing, cellular proliferation inhibiting, chemotherapeutic enhancing, transcription regulating, anti-inflammatory, cellular differentiation promoting, cellular transformation modulating) composition be an acyl or a substituted or unsubstituted lower hydrocarbon selected from the group consisting of alkyl, alkenyl, alkanoyl, aryl, aroyl, aralkyl or alkylamino; R6 may be a heteroatom such as oxygen, sulfur or nitrogen; R7 may be a heteroatom such as sulfur, nitrogen or oxygen as well as a carbon; R8, 9, 10, 11 and 12 may

independently, collectively, or in any combination that yields an active anti-Id (apoptosis inducing, cellular proliferation inhibiting, chemotherapeutic enhancing, transcription regulating, anti-inflammatory, cellular differentiation promoting, cellular transformation modulating) composition be selected from hydrogen, hydroxyl, sulfyhydryl, fluorine, methyl, ethyl, propyl, benzyl, 2-bromovinyl amino, hydroxymethyl, methoxy, halogen, pseudohalogen, cyano and a substituted or unsubstituted lower hydrocarbon containing 1 to 20 carbons.

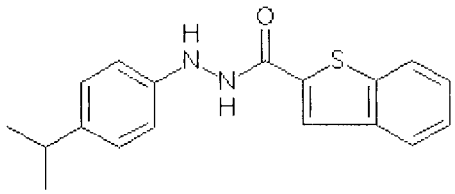
When more than one R group is present, the R group may be selected from any of the stated groups so as to be the same or different. In additional embodiments, two or more R groups may be joined together. In some embodiments, R2 and R3 may be members of a 5, or 6, member exocyclic ring structure. In other embodiments, R3 and R4 may be members of a 5, or 6, member exocyclic ring structure. In further embodiments, R5 and R6 may be members of a 5 or 6 member exocyclic ring structure. In additional embodiments, R11 and R12 may be members of a 5 or 6 member exocyclic ring structure. In some embodiments, if R7 is nitrogen, R6 and R7 may be members of a 5 or 6 member exocyclic ring structure. In other embodiments, R6 and R12 may be members of a 5 or 6 member exocyclic ring structure.

In more detailed embodiments, rationally designed anti-Id compounds of Formula I can be selected from additional candidates, wherein R_1, R_4, R_5, R_6, R_8, R_9, R_{10} are independently selected from the group consisting of hydrogen, hydroxyl, sulfyhydryl, benzyl, 2-bromovinyl amino, hydroxymethyl, methoxy, halogen, pseudohalogen, cyano, carboxyl, nitro, thioalkyl, thioaryl, thiol, substituted or unsubstituted hydrocarbons containing 1 to 20 carbons, alkoxycarbonyl, alkoxycarbonylamino, amino, amino acid, aminocarbonyl, aminocarbonyloxy, aryloxy, carboxyl, cycloalkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, peptidyl, dye, fluorophore, carbohydrate or polypeptidyl; R_8 and R_9 can also have a para configuration in addition to the ortho orientation shown in Formula I; R_7 is independently selected from hydrogen, hydroxyl, methoxy, azido, nitrile, sulfhydryl, halogen, benzoyl, substituted benzoyl or hydroxyl substituted with substituted or unsubstituted hydrocarbon containing 1 to 20 carbons, alkanoyl of a main chain of 1 to 20 carbon atoms, $CF_3(CH_2)_nCO$ where n=1 to 10, $CH_3(CF_2)_nC=O$ where n=1 to 10, $CF_3(CF_2)_n$ where n=0 to 3, aryl, alkoxy, halogen, or nitro, aryloxy,

esters of aryloxy, adamantoyl, substituted adamantoyl or aroyl of 1 to 20 carbons; R_7 cannot be methoxy if R_{11} is propionyl while R_7 and R_6 can be ortho, as shown in Formula I, or para to one another; R_{11} is independently hydrogen, propionyl, pivoyl, benzoyl, substituted benzoyl, alkanoyl of a main chain of 1 to 20 carbon atoms, $CF_3(CH_2)_nC=O$ where n=1 to 10, $CH_3(CF_2)_nC=O$ where n=1 to 10 or $CF_3(CF_2)_n$ where n=0 to 3 and R11 cannot be propionyl if R7 is methoxy; R_{12} is independently hydrogen, halogen, ^2H, $CH_3(CH_2)_n$ where n=0 to 5, $CF_3(CH_2)_nC=O$ where n=1 to 5; $CH_3(CF_2)_nC=O$ where n=1 to 5 or $CF_3(CF_2)_n$ where n=0 to 5.

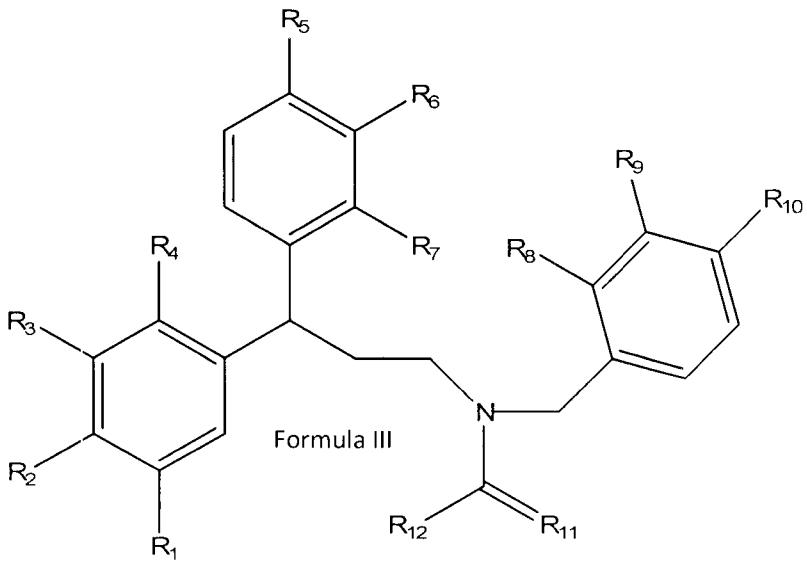
Additional description pertaining to these and other aspects of the invention can be found, for example, in United States Patent No. 8,138,356, issued 20 March 2012, and United States Patent Application Serial No. 14/341,756, filed 25 July 2014, each of which is incorporated herein by reference its entirety for all purposes.

In illustrative embodiments, the anti-Id compound selected from Formula I is N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (alternatively, "AGX51"), as shown in Figure II, below. It will be understood from the teachings herein that the various Formula I compounds, inclusive of AGX51, include pharmaceutically acceptable active salts of said compounds, as well as active enantiomers, polymorphs, metabolites, solvates, hydrates, and prodrugs of said compounds.



Formula II

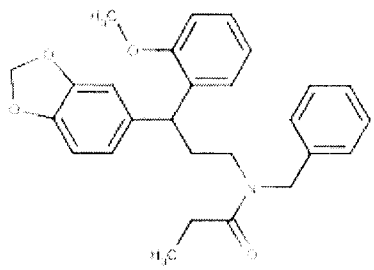
Novel and surprisingly effective methods and compositions that inhibit Id proteins may additionally comprise compounds of Formula III, below, and their active salts, enantiomers, polymorphs, metabolites, solvates, hydrates, and prodrugs.



Formula III

wherein R_1, R_2, R_3, R_4, R_5, R_6, R_8, R_9, and R_{10} may independently, collectively, or in any combination that yields an active anti-Id compound be hydrogen, hydroxyl, sulfyhydryl, fluorine, methyl, ethyl, propyl, benzyl, 2-bromovinyl amino, hydroxymethyl, methoxy, halogen, pseudohalogen, cyano, carboxyl, nitro, thioalkoxy, thioaryloxy, thiol, substituted or unsubstituted lower hydrocarbon containing 1 to 20 carbons; alkoxycarbonyl, allkoxycarbonylamino, amino, amino acid, aminocarbonyl, aminocarbonyloxy, aralkyl, aryloxy, carboxyl, cycloalkenyl, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, amino acid, peptide, dye, fluorophore, carbohydrate or polypeptide; R_7 may be selected from hydrogen, hydroxyl, benzoyl; substituted benzoyl or hydroxyl substituted with unsubstituted lower hydrocarbon containing 1 to 20 carbons; R_{11} may be a heteroatom such as oxygen, sulfur or nitrogen; R_{12} may be a lower hydrocarbon independently selected from the group consisting of alkyl, alkenyl, alkanoyl, alkynyl, aryl, aroyl, aralkyl, alkylamino, aryloxy, hydrogen, carboxyl, nitro, thioalkoxy, thioaryloxy, thiol, cycloalkenyl substituted or unsubstituted heteroatom, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, amino acid, peptide, dye, fluorophore, carbohydrate or polypeptide.

With regard to Formula III, when more than one R group is present, the R group may be selected from any of the stated groups so as to be the same or different. In additional embodiments, two or more R groups may be joined together. In some embodiments, R4 may become a member of a 5 or 6 member ring structure with neighboring rings. An exemplary compounds of Formula III for use within the invention is N-[3-(1,3-benzodioxol-5-yl)-3-(2-methoxyphenyl) propyl]-N-benzylpropanamide, as shown in Formula IV.



Formula IV

An exemplary anti-Id compound for use within the compositions and methods of the invention is N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ("AGX51"). As used herein, AGX51 refers to N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide in isolated or substantially purified form, as well all functional equivalent salts, prodrugs, metabolites, derivatives, analogs, and conjugates of AGX51. For example, those skilled in the art will that AGX51 encompasses desired prodrug forms and biotransformation products, and readily designed and tested analogs, derivatives and complexed or conjugated forms of the drug.

Thus, the invention encompasses compositions and methods employing demethylated forms of AGX15, for example N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxyphenyl)propyl)-N-benzylpropionamide; deamidated forms, for example (3-(benzo[d][1,3]dioxol-5-yl)-N-benzyl-3-(2-methoxyphenyl)propan-1-amine, or multiply-modified (e.g., demethylated and deamidated) forms, such as 2-(1-(benzo[d][1,3]dioxol-5-yl)-3-(benzylamino)propyl)phenol. In related embodiments, the anti-Id compound is a selected salt form of a racemate or purified enantiomer of a biotransformation product of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide, for example a salt of (3-(benzo[d][1,3]dioxol-5-yl)-N-benzyl-3-(2-methoxyphenyl)propan-1-amine or 2-(1-

(benzo[*d*][1,3]dioxol-5-yl)-3-(benzylamino)propyl)phenol. Exemplary salt forms of these and other anti-Id compounds herein include, but are not limited to, hydrochloride, hydrobromide, hydroiodide, phosphate, sulfate, oxalate, malate, maleate and succinate salts.

In related embodiments, the anti-Id compound is an isolated, anti-metastatically active enantiomer of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide. Surprisingly, the discoveries herein demonstrate extraordinary anti-Id potency residing in the (-)-enantiomer form of AGX51. Thus, in preferred aspects the invention employs novel, enantomerically-enriched preparations of (-)-AGX51, substantially purified to yield an increased relative amount or concentration of (-)-AGX51 (relative to an amount or concentration of the other, (+)-AGX51 enantiomer, or to an amount found in conventional, racemic preparations of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide).

In more detailed aspects, the methods and compositions of the invention employ a "substantially pure" or "essentially pure" anti-metastatic effective (-)-enantiomer of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide. In exemplary compositions, the purified (-)-AGX51 enantiomer is at least initially (i.e., pre-formulation) provided in a form that exhibits at least 80-90%, 90-95%, greater than 95%, or 98% or greater "enantiomeric enrichment" (ee) or "enantiomeric purity". As used herein, (-)-AGX51 enantiomer preparations of 90-95% ee are "substantially pure", while preparations of 98% ee or greater are or "essentially pure." In alternative aspects, the methods and compositions of the invention employ a "substantially pure" (-)-N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide preparation is defined as being "essentially free of the (+)-N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide enantiomer (i.e., having less than 5%, less than 2% or less than 1% of the total N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide present being in the form of the (+)-enantiomer with the remainder exclusively the (-)-enantiomer.

The identity and quantity of a selected AGX51 enantiomer within the compositions and methods of the invention may be determined by a variety of means. For example, these determinations and values may be demonstrated using conventional chiral chromatography and/or polarimitry. As used herein the term "purified" or "enriched" (-)-AGX51", or is intended to correspond to any composition artificially enriched to contain, in stable form, a

substantially higher concentration of (-)-AGX51 than (+)-AGX51 (or than an amount of (-)-AGX51 present in conventional (non-chiral) racemic preparations of the compound)--wherein the (-)-AGX51 occurs in an enantiomeric excess (ee) of at least about 60%, 70%, 75%, 80%, up to approximately 90% or greater, up to 95-98% or greater ee.

In certain aspects the methods and compositions of the invention employ highly purified or isolated (-)-AGX51 (at least as a starting material prior to formulation, storage or administration) in an enantiomeric excess of greater than 98% (e.g., as determined by chiral chromatography and/or optical purity assay). Preparations of (-)-AGX51 in enantiomeric excess greater than 85% or 90% are considered substantially free of the corresponding (+)-AGX51 enantiomer and are highly desired drug preparations for clinical use. Typically, pharmaceutical compositions of the invention for treating cancer, preventing or treating metastatic disease, or treating an angiogenic or other proliferative disorder, will contain no more than about 5% w/w, and in some embodiments no more than about 2% or 1% or lower w/w, of the (+)-AGX51 enantiomer (i.e., measured as a percent of total N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide mass or molar content present in the composition).

The use of substantially pure (-)-N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ((-)-AGX51), or significantly enantiomerically enriched (-)-AGX51 compositions provides enhanced therapeutic efficacy for treating cancer, preventing or treating metastatic disease, and treating angiogenic and other proliferative disorders, with reduced side effects at equivalent dosages, compared to racemic AGX51 or the (+)-AGX51 enantiomer. In alternate embodiments, enriched (-)-AGX51 yields equal or greater therapeutic efficacy with fewer side effects at lower or less frequent dosages, compared to racemic AGX51 or the (+)-AGX51 enantiomer. In certain aspects, at equivalent (by weight or molarity) dosing with racemic AGX51, the (-)-AGX51-enriched compositions and methods of the invention exhibit increased activity or therapeutic efficacy of at least 10%, 20%, 30%, or 50%, up to 75-95%, 100% or even 200% greater than an observed activity or therapeutic efficacy determined for the racemate (or for the substantially pure (+)-AGX51 enantiomer)--using any one or combination of activity measures, therapeutic indices and clinical assays employed or referenced herein.

Anti-Cancer and Anti-Metastasis Compositions and Methods

According to the discoveries and teachings herein, anti-Id compositions and methods of the invention effectively block Id binding to bHLH proteins, promote functionally significant Id degradation (i.e., therapeutically decrease Id levels in cells), and suppress Id activity when administered to cells, tissues and living subjects. These ant-Id activities in turn effectively suppress tumor metastasis, as well as tumor-associated angiogenesis and other proliferative disease states and symptoms. In this surprising manner, the compositions and methods of the invention afford effective tools to mitigate the most lethal and refractory neoplasms.

The instant invention has immense clinical promise in view that so many Americans are critically affected by cancer. The National Cancer Institute of the NIH estimates that approximately 13.7 million Americans with a history of cancer were alive in January, 2013. Some of these individuals were cancer-free, while others still had evidence of cancer and may have been undergoing treatment. In 2013, about 58,350 Americans were expected to die of cancer, or approximately 1,600 people a day. In the US, cancer accounts for 1 of every 4 deaths.

Patients typically present with cancer that is already well established, with a tumor burden that is already an existential medical problem. These patients are treated with surgery, radiation, chemotherapy (optionally including hormone therapy), sometimes with adjuvant, biologic or targeted therapy to reduce tumor burden. If this first treatment or series of treatments doesn't yield complete success, the tumor often exhibits resistance to further treatments, while in other cases the cancer metastasizes to distant organs with often fatal consequences. Death from cancer is almost universally preceded by cancer metastasis, which is therefore among the most critical, unresolved targets for cancer intervention.

Suppression of cancer metastasis is an urgent therapeutic need, in part because most existing cancer drugs inhibit only cell proliferation. The molecular biology of metastasis has proven more complex and difficult to interpret than for primary cancer cell transformation. Because the molecules that drive dissemination and colonization by malignant cells are shared among different types of cancers, drugs that inhibit these processes will be much more broadly beneficial than conventional chemotherapy drugs. Successful interventions to disrupt the metastatic cascade will focus on mechanisms and activities crucial for dissemination of cancer cells from the primary tumor, and for subsequent colonization of

secondary tumor site. As elucidated with particularity herein, Id proteins are potent inducers of these tumor initiation events, which are in turn disabled or blocked by the potent anti-Id compositions and methods of the invention. Anti-Id compounds and treatment methods provided here focus on multiple complementary anti-metastasis strategies, including: 1) prevention of cancer cell dissemination; and 2) suppression of existing metastases.

The methods and compositions of the invention find use in therapeutic applications in which anti-Id administration is indicated, as described in a clinically effective context for the first time here. Representative therapeutic applications of the invention include treatment of cellular proliferative diseases, including cancer and other diseases characterized by adverse cellular proliferation (hyperplasias).

The anti-Id compositions and treatment methods of the invention are effective to reduce or prevent cellular proliferative disorders in mammalian subjects, including clinically effective treatment of cancer in veterinary (e.g., dog, cat, large animal) and human patients.

In general terms, anti-Id effectiveness of the compositions and methods of the invention may be correlated with a by a decrease in one or more symptoms of a cellular proliferative disorders (e.g., cancer, metastatic disease, tumor-associated angiogenesis). This may be detected or quantified based on an observed decrease in cellular proliferation, vascular growth, cellular migration, secondary tumor appearance or growth, inflammation, or any other symptom associated with the targeted cellular proliferative disorder. Various assays and model systems can be readily employed to illustrate therapeutic effectiveness of the anti-Id compounds of Formula I, II, III and IV described herein. These assays are well known and include widely accepted clinical correlates for each of the subject proliferative disorders contemplated herein. Exemplary assays for ready use in this context include assays to detect or quantify cellular proliferation markers, circulating endothelial cells, circulating endothelial progenitor cells (EPCs), circulating tumor cells, various cancer/tumor markers (e.g., PSA), and histological, histochemical, and immunohistochemical markers to detect, localize, anatomically map, and/or quantify primary and metastatic cancer cells, among others. Useful, cell-specific markers for each of the foregoing cell types and functionalities (i.e., cell commitments/fates, differentiation states, developmental potentialities), are well known in the art for use within these methods and compositions of the invention. Exemplary markers for tagging, visualizing, quantifying and/or separating cells within various assays and diagnostic or clinical embodiments of the invention include: for metastatic cells bearing

markers of increased metastatic potential (e.g., markers associated with loss of epithelial character, increased migration potential, secondary site colonization potential)--Vimentin+, N-Cadherin+, E-Cadherin-; for EPCs--Lin-, GFP+, VE-Cadherin+, CD11-; for cancer stem cells (breast cancer example)--CD44(high) Cd24 (low). Many additional markers, and combinations of markers, will be routinely employed in various assays and diagnostic and clinical methods, having like or additional specificities, to distinguish Anti-Id activity, function and cellular and clinical effects, as discussed further herein.

In certain embodiments, the anti-Id compositions and methods of the invention are effective to reduce occurrence, relapse or growth of a neoplasm. Administering an anti-neoplasm effective amount of the anti-Id compound will reduce incidence, number or growth of neoplasms in treated versus control subjects by 5%, 10%, 25%, 30%, 50%, 75%, 90% or more.

In other embodiments, the invention provides "anti-metastatic" compositions and methods, which are effective to reduce an incidence or severity of tumor metastasis in mammalian subjects. These methods may employ monotherapy or coordinate or combinatorial therapy. The subject compositions and methods "anti-metastatically effective", as demonstrated by a significant reduction in incidence, number or size of metastases in treated versus control subjects presenting with, or at elevated risk for developing, cancer. In certain embodiments, anti-metastatic efficacy correlates with a reduction in one or more histopathological indices of metastasis, for example quantitative reduction in occurrence, size, number or distribution of metastasized cells or "foci" of primary tumor character observed at a secondary tissue or anatomic site. Alternatively there may be a substantial decrease in cancer cell migration or dissemination mediated by the anti-Id compound. In other embodiments, anti-metastatic efficacy is demonstrated by a significant positive increase in one or more patient therapeutic indices correlating with effective prevention and/or treatment of cancer or metastasis--for example by an increase in a time period of disease-free survival for subjects receiving the anti-Id compound compared to control subjects not receiving the anti-Id compound.

Anti-metastatic efficacy of the compounds, compositions and methods of the invention yield substantial therapeutic benefits and improved treatment outcomes in patients treated for cellular proliferative disorders and/or neoplasms. In exemplary embodiments, cancer patients treated with the anti-Id methods and compositions of the invention exhibit

improved treatment outcomes with no increase, and often a significant decrease in adverse side effects that attend conventional cancer treatments (e.g., chemotherapy and radiation therapy, which may be reduced or eliminated in patients treated with the compositions and methods herein).

Illustrative of these benefits, methods of the invention will yield at least a 20% increase in one or more positive therapeutic indices of metastatic disease prevention or arrest, for example a reduction in occurrence, size, number or distribution of metastasized cells or "foci" of primary tumor character observed at a secondary tissue or anatomic site. In certain embodiments, anti-metastatic efficacy of the anti-Id compounds and methods will yield at least a 20% increase in an accepted metastatic index, including gross indices such as disease-free survival of Id-treated patients compared to qualified control patients not treated with the anti-Id compound.

In other embodiments, anti-Id compounds, formulations and methods of the invention will yield even more significant anti-metastatic clinical benefits, for example a 20-50% increase, 50-70% increase, up to a 75%-100% reduction in a recognized metastasis indicator or marker (e.g., cancer cell migration or dissemination observed at or near a primary tumor site, metastasis observed in blood or lymphatic biological samples, or secondary tumor formation detected in a distant tissue or organ (e.g., by radiological or other imaging, biopsy, or post-surgical or post-mortum histolopathology). In many instances, effective clinical management employing anti-Id compositions and methods of the invention will yield total prevention, clearance or stable remission of primary and/or metastatic cancer symptoms, metastatic disease or other cancer symptoms enduring for 6 months to a year, 1-2 years, 2-5 years, 5-10 years or longer.

In exemplary embodiments, the anti-Id methods and compositions of the invention will be anti-metastatically effective to yield at least a 20% decrease in metastasis, a 20%-50%, a 50%-75%, up to a 90% or greater decrease in metastasis (e.g., as demonstrated by conventional, comparative histopathology, computerized tomography, positron emission tomography and/or magnetic resonance imaging to detect, locate and/or quantify metastatic cells) in anti-Id-treated versus non-treated or placebo-treated subjects.

Respective of all embodiments presented here, anti-metastatic efficacy will typically correlate with no increase, or even a decrease, in observed adverse side effects (e.g., nausea, weight loss, hair loss, immunological damage, etc.) between anti-Id treated patients and

a taxane, or an alternate cancer therapy, such as radiation therapy). In exemplary embodiments, anti-Id-treated subjects (including subjects treated with anti-Id compound monotherapy, and subjects treated with combinatorial methods, such as anti-Id plus chemotherapy, or anti-Id plus radiation therapy) will exhibit no increase in one or more generally-seen adverse cancer treatment side effects (e.g., general chemo- or radiation-therapy side effects), and will often exhibit at least a 20% reduction, a 20-50% reduction, up to a 50-90% or greater reduction in occurrence or severity of one or more adverse cancer treatment side effects (e.g., compared to positive control subjects treated with conventional chemotherapy or radiation therapy alone).

Side effects that may be associated with anti-Id therapy may include side effects associated with anti-angiogenic effects of the novel, anti-Id compounds described here. Although such prospective side effects have yet to be evaluated, various management tools can effectively limit or prevent such adverse sequelae. For example, potential impairment of wound healing due to anti-angiogenic activity of the claimed compositions and methods can be avoided by staging anti-Id treatment prior to cancer surgery when indicated, and/or post-surgery allowing for an effective healing period prior to initiation of the anti-Id therapy. Additional pro-healing agents and methods can be coordinately administered, such as coordinate administration of pro-healing cytokines or growth factors (e.g., platelet-derived growth factor (PDGF).

In more detailed aspects of the invention, the anti-Id compounds and methods are also effective to reduce a metastasis-associated increase in circulating endothelial cells. Circulating endothelial cells are generally absent in the blood of healthy individuals, but significantly elevated in individuals suffering from diseases marked by pathogenic angiogenesis, including cancer. The number (titer or hematocrit count) of circulating endothelial cells may be determined by any means applicable, such as through flow cytometry, immunobead capture, fluorescence microscopy, standard and density centrifugation, or mononuclear cell culturing on fibronectin-coated plates and immunocytochemistry. An anti-metastatic or anti-angiogenic effective amount of the anti-Id compound of the invention will decrease the number of circulating endothelial cells by 5%, 10%, 25%, 30%, 50%, 75%, 90% or more compared to levels observed in placebo-treated subjects presenting with similar pathology (e.g., an equivalent state of metastatic or

angiogenic disease prior to treatment, normally attended by tumor-associated elevation of EPCs).

In other detailed embodiments, the anti-Id compounds and methods of the invention are effective to block or reduce a metastasis-associated increase in circulating endothelial progenitor cells (EPCs) implicated in metastatic disease progression. Malignant transformation and metastasis is associated with increased numbers of circulating EPCs. Endothelial cell production is generally responsible for repair of damaged vasculature (including associated with tumors, and in other cases of pathogenic angiogenesis) through mobilization of EPCs from the bone marrow. This is followed by homing of the EPCs to target sites for repair (including sites of damaged vasculature associated with tumors) (Shaked et al., 2006; Shaked et al., 2008). This process of vascular repair often follows treatment with certain, cytotoxic "chemotherapeutic" drugs, such a paclitaxel, and vascular disrupting agents (VDAs, for example ZD6126 or AVE8062), which have attendant adverse cytoxic side effects on blood vessels. This angiogenic repair process following paclitaxel treatment is observed in normal mice, but reportedly absent in mice lacking the Id1 gene (Shaked et al, 2008).

The exact nature of the process in bone marrow responsible for tumor-associated EPC production has not been established, but the source of the EPC's is likely hematopoietic stem cells (HSCs) in the marrow. Loss of Id1 in bone marrow reportedly leads to a loss of EPCs in peripheral blood, correlated with impairment of tumor neovascularization and growth. The absence of Id1 also compromises the self-renewing capacity of HSCs in bone marrow, increasing their tendency to differentiate towards the myeloid lineage. This functional defect is associated with transcriptional changes in Id1 null HSCs, including increased expression of p21, another target of Id1 repression (Ciarrocchi et al., 2007). Id1 is required for the previously described ability of phenotypic HSCs to give rise to endothelial progeny to highlight the opposing effects of Id1 and its target gene p21 on endothelial and myeloid lineage differentiation in the HSC subset. Ablation of p21 in the Id1-/- animals restores a functional endothelial population, rescues the angiogenic defect observed in the Id1-/- mice, and reverses the premature myeloid commitment of Id1 null HSCs. Id1 appears to play a therapeutically negative, protective role for cancer cells undergoing threat with cytotoxics through a general anti-apoptotic effect (Zhang et al., 2006; Wong et al., 2004). This was reported based on studies of paclitaxel/docetaxel-induced apoptosis in prostate cancer cells,

nasopharyngeal carcinoma cells, HeLa (cervical) cancer cells and MCF7 (breast) cancer cells. Up-regulation by Id1 of Raf/MEK (Zhang et al., 2006), and/or MAPK signaling pathway (Cheung et al., 2004; Lin et al., 2005) has also been proposed to account for resistance to cytotoxics acquired by cancer cells post-treatment.

Despite the complex and indefinite pathways involved, anti-Id compounds and methods of the invention fundamentally disable Id proteins at critical intersections of these pathways. In this manner, anti -metastatic and anti-angiogenic effective anti-Id compounds of the invention also target EPC production and secondarily disable tumor-associated angiogenesis and related tumor growth. In certain embodiments, the anti-Id compositions and methods of the invention will effectively decrease the number of EPCs in a circulating blood sample of treated subjects by at least 5%, 10%, 25%, 30%, 50%, 75%, 90% or more compared to levels observed in placebo-treated subjects presenting with similar pathology (e.g., an equivalent state of metastatic or angiogenic disease prior to treatment, normally attended by tumor-associated elevation of EPCs).

Anti-Id compositions and methods of the invention further exert anti-cancer and anti-metastatic effects by shifting a cellular determination or fate of tumor cells toward senescence or apoptosis. Accordingly, in yet additional aspects of the invention, anti-Id compositions and methods of the invention will effectively increase the number of apoptotic or senescent cells in a primary or secondary tumor of anti-Id-treated patients. In certain embodiments, the anti-Id compositions and methods of the invention will increase the number of apoptotic or senescent cells in tumors (e.g., as observed through biopsy or necropsy of existing tumors) of treated subjects by at least 20%, 30%, 50%, 100%, up to 5-fold, ten-fold or higher, compared to numbers of apoptotic or senescent cells in control samples from placebo-treated subjects presenting with similar pathology (e.g., an equivalent state of metastatic or angiogenic disease prior to treatment, normally attended by tumor growth and a low incidence of apoptotic or senescent cells).

Cancer stem cells are a subopulation among bulk of tumor cells capable of initiating new tumors (with a capacity to recapitulate the lineage heterogeneity of the parental tumor). Cancer stem cells share characteristics of tissue stem cells, including self-renewal and multipotency. A positive regulatory activity of Id proteins on cancer stem cells has been reported in colon cancer and malignant glioma, although other types of cancer stem cells also appear to depend on Id proteins (Hua et al., 2006; James et al, 2010; Jankovic et al., 2007;

Perry et al., 2007; Rawlins et al, 2009; Suh et al., 2009; Lyden et al.; 1999; Anido et al., 2010; Jeon et al., 2011). In colon cancer stem cells, combined expression of Id1 and Id3 reportedly increases both self-renewal and tumor initiation (O'Brien et al, 2012). Cancer stem cells exhibit resistance to chemotherapeutic agents, and silencing of Id1 and Id3 in culture-based assays reportedly sensitizes cells to oxaliplatin (O'Brien et al, 2012). In high-grade glioma, Id proteins are co-expressed in diverse tumor cell populations, including glioma stem cells. In an orthotopic model of brain cancer driven by the *HRASV12* oncogene, deletion of conditional Id1, Id2 and Id3 alleles in the tumor cells reportedly decrease the glioma stem cell population (nestin-positive and stage-specific embryonic antigen 1 (SSEA1)-positive cells), blocks tumor growth and extends survival. Cells selected *in vitro* for self-renewal potential reportedly lose tumorigenic capacity if Id genes are deleted after implantation of the cells into mouse brains, while they retain robust tumorigenic potential if Id genes are intact (Niola et al., 2013). Embryonic neural stem cells (NSCs) reportedly lose self-renewal and multi-potency in the absence of Id1, Id2 and Id3, but these properties are sustained near normal in cells that retain one Id2 allele (suggesting that Id proteins function redundantly in NSCs) (Niola et al., 2012). In the developing mouse brain, inactivation of Id1 and Id3 (but neither gene alone) can reportedly trigger premature differentiation of NSCs (Lyden et al., 1999). Short hairpin RNA-mediated silencing of Id1 and Id3 reportedly abrogates glioma stem cell properties *in vitro* and in orthotopic transplantation experiments (Anido et al., 2010). In mouse astrocytes deficient in cyclin-dependent kinase inhibitor 2A (*Cdkn2a*$^{-/-}$), expression of Id4 induces glioma stem cell markers associated with cyclin E activation and Notch signaling (Jeon et al., 2008). Id4 has also been reported to de-repress miR-9*-mediated suppression of SRY-box 2 (*SOX2*), increasing glioma stem cell potential and chemoresistance (Jeon et al., 2011). As with somatic stem cells, cancer stem cells are anchored to a niche and derive supportive signals through cell-cell contacts with endothelial cells in blood vessels. The ability to adhere to the niche is a crucial feature of normal stem cells and cancer stem cells (Calabrese C et al., 2007; Chen S et al., 2013; Lewallen M et al., 2013; Fietz et al., 2011; Lathia JD et al., 2010; Park DM et al., 2009). Loss of Id proteins reportedly disrupts stem cell adhesion to endothelial cells in the niche in NSCs and in glioma stem cells (Niola et al., 2012; Niola et al., 2013). Id-mediated repression of bHLH transcription limits expression of RAS-related protein 1 (RAP1) GTPase-activating protein (*RAP1GAP*), a bHLH target gene that encodes an inhibitor of the RAP1 GTPase, which

controls cell adhesion via integrin signaling (Boettner et al, 2009). When Id protein levels decrease, for example, at the time of neural differentiation or in the absence of Id proteins in Id-knockout mice, de-repression of Rap1gap inhibits RAP1 and drives stem cell detachment from the niche. Thus, whereas in NSCs the Id–bHLH axis dynamically regulates cell intrinsic cues that direct stem cell interaction with the niche, continuous blockade of bHLH activity by increased Id activity in glioma stem cells may lock adhesion signals in an aberrant 'on' state, correlating with reports that RAP1GAP, Id2 and Id3 are prognostic markers in patients with high-grade glioma. In other studies high-grade glioma cells with high Id1 expression manifested high self-renewal potential *in vitro* and tumorigenic capacity when injected orthotopically, whereas cells with low Id1 expression had impaired *in vitro* self-renewal but more robust tumorigenicity (Barrett et al., 2012). These latter stem cells may be a stem cell type analogous to "transit-amplifying progenitors" in neurogenic areas of the brain. These progenitors, which express lower levels of Id proteins than NSCs (Nam et al., 2009) are capable of limited self-renewal but have high proliferative potential (Diaz-Flores et al., 2006), possibly associated with origination of certain brain tumor subtypes (Liu et al., 2011). An inverse correlation between Id1 expression and tumor progression might explain the slightly better prognosis for patients with glioblastomas having a pro-neural gene expression signature and high Id1 expression, compared patients marked by low Id1, although both subgroups fare poorly. In certain tumor types, both stem-like cells and cells with features of committed progenitors may have capacity to propagate tumors efficiently. These findings also indicate that targeting both cell populations will be important to effectively treat glioblastoma (Barrett et al., 2012).

Based on the foregoing reports, Id proteins have been postulated to play key roles as regulators of stem cell identity in both colorectal cancer and malignant glioma, essential for both self-renewal and tumor-initiating capacity of cancer stem cells. Again despite the mechanistic complexity and indefinite pathways involved in cancer stem cell development, anti-Id compounds and methods of the invention potently disable Id proteins at a critical foundation to disrupt stem cell identity and impair stem cell tumor initiation. Anti-metastatic and anti-angiogenic effective compounds of the invention specifically target tumor stem cell viability, proliferation capacity, tumor-initiation potential, and/or cell fate determination--with the result of substantially decreasing populations of new tumor induction-competent stem cells present in new or established tumors. In certain embodiments, the anti-Id

compositions and methods of the invention will effectively decrease the number of cells bearing one or more selected stem cell markers in tumors (e.g., as observed through biopsy or necropsy of existing tumors) of treated subjects, by at least 5%, 10%, 25%, 30%, 50%, 75%, 90% or more compared to levels observed in placebo-treated subjects presenting with similar pathology (e.g., an equivalent state of neoplastic or metastatic disease prior to treatment, normally attended by tumor growth, new tumor formation, and a high incidence of tumor-associated cancer stem cells). As with the determination of epithelial cells and EPCs, well known assays, markers and labeling reagents will be routinely employed to demonstrate anti-stem cell efficacy of the compositions and methods herein. Those skilled in the art will appreciate that such assays are readily designed and implemented to identify and quantify cancer stem cells, for example based on detection of positive stem cell markers, such as nestin and SSEA1, using conventional assay technologies such as cytometry, immunobead capture, and immunocytochemistry. More discrete assays will determine differences in tumor stem cell viability, proliferation capacity, tumor-initiation potential, and/or cell fate, specifically correlated in anti-Id-treated subjects with reduction of cancer disease risk, according to the teachings here and following reports of others above (all of which are incorporated herein by reference for economy of description).

Effectiveness of anti-Id treatment methods of the invention directed against cancer and metastatic disease may be monitored in terms of clinical success by any of a variety of methods, for example by tumor imaging with x-rays or MRIs (e.g., to determine if tumors have decreased in size or number in treated patients). Effectiveness will often be determined by radiographic or MRI observation of a decrease in tumor size. Effective anti-Id compositions and methods of the invention for treating cancer will routinely yield at least a 10%, 25%, 50%, 75%, 90% or greater reduction of tumor size in treated patients, or average tumor size among a group of treated patients, compared to qualified, comparable control subjects.

Effectiveness of anti-Id treatment methods of the invention directed against cancer and metastatic disease may further be determined by measuring the number of circulating tumor cells in blood samples between suitable test and control subjects. This may be accomplished by any means applicable including, but not limited to immunomagnetic selection, flow cytometry, immunobead capture, fluorescence microscopy, cytomorphologic analysis, or cell separation technology. Effective anti-Id compositions and methods of the

invention for treating cancer will routinely yield at least a 10%, 25%, 50%, 75%, 90% or greater reduction of circulating tumor cells in blood samples of treated patients, or among a group of treated patients, compared to qualified, comparable control subjects.

Effectiveness of anti-Id treatment methods of the invention directed against cancer and metastatic disease may further may also be determined by detecting or measuring primary tumor cell occurrence or number in secondary tissues or organs, including but not limited to bone, lymph nodes and lung. Effective anti-Id compositions and methods of the invention for treating cancer will routinely yield at least a 10%, 25%, 50%, 75%, 90% or greater reduction in the occurrence or number of primary tumor cells metastasized to secondary tissues or organs among treated patients compared to qualified, comparable control subjects.

In certain aspects of the invention, anti-Id compositions and methods for prevention or treatment of cancer, including for inhibiting or reducing metastasis, involve coordinate administration of an effective amount of the anti-Id compound, along with a secondary treatment agent, treatment modality or treatment method. In certain exemplary embodiments, subjects are treated with the anti-Id compound simultaneously or sequentially with a secondary treatment agent or method, selected from: a chemotherapeutic drug (i.e., using a second anti-cancer or anti-metastatic drug, compound or chemical agent), radiation, chemotherapy, surgery, or any combination of these agents/methods.

In certain "coordinate therapy" or "combinatorial treatment" embodiments, the invention employs the anti-Id compound administered simultaneously (at the same time, optionally in a combined formulation) with a secondary drug, compound or chemical agent possessing combinatorial anti-cancer or anti-metastatic activity. Secondary chemotherapy drugs in this context are contemplated to broadly include agents classified as conventional chemotherapy drugs (for example taxanes); vascular disrupting agents (VDAs); or HSP-90 inhibitors. Within these and related embodiments, the anti-Id compound and the secondary drug or treatment will be "combinatorially effective", meaning biological activity (e.g., anti-cancer or anti-metastatic activity as defined herein), side effects, patient outcomes, or other positive therapeutic indicia will be improved over results observed in relevant control subjects treated with the anti-Id compound alone, or secondary drug alone.

Anti-Id compounds and methods of the invention can be coordinately employed with any of a range of secondary anti-cancer drugs, agents or interventions, in combinatorial

formulations or coordinate treatment protocols (with anti-Id therapy administered concurrently, prior or subsequent to the secondary treatment agent or method). In exemplary coordinate treatments, an anti-Id compound such as AGX51 is administered coordinately with a chemotherapeutic drug or therapy. Chemotherapeutic drugs and therapies for use within these aspects of the invention include anti-cancer and anti-hyperproliferative agents, agents that destroy or "reprogram" cancer cells, agents that destroy blood vessels associated with neoplasms or hyperproliferative conditions, and other classes of drugs harmful to neoplastic cellular targets. In this regard, useful chemotherapeutics within the invention include, but are not limited to:

(1) tubulin depolymerizing agents like taxoids such a paclitaxel, docetaxel, BAY 59-8862 albumin bound a VDA,

(2) DNA damaging agents and agents that inhibit DNA synthesis

(3) anti-metabolites

(4) anti-angiogenics agents and vascular disrupting agents (VDAs),

(5) antibodies,

(6) endocrine therapy,

(7) immuno-modulators,

(8) histone deacetylase inhibitors,

(9) inhibitors of signal transduction,

(10) inhibitors of heat shock proteins,

(11) retinoids such as all-trans retinoic acid,

(12) inhibitors of growth factor receptors or the growth factors themselves,

(13) anti-mitotic compounds,

(14) anti-inflammatory agents such as COX inhibitors, and

(15) cell cycle regulators, eg, check point regulators and telomerase inhibitors.

In other aspects, combinatorial formulations of the invention include an anti-Id compound as described in a combined formulation with one or more conventional chemotherapeutic drugs or other anti-metastatic compound or agent, optionally including a side-effect reducing agent as known in the art (which will depend on what combinatorial therapy is being employed, e.g., chemotherapy versus radiation therapy, or both). Anti-Id compounds of the invention may be provided in the form of a pharmaceutically acceptable salt. These compounds can be routinely formulated for oral, topical, parenteral, transdermal

or intravenous (iv) administration. In certain embodiments, e.g., where multiple compounds are administered separately, multiple pharmaceutical compositions may be provided (each containing a different active agent. In other embodiments, a single formulation is provided that comprises an anti-Id active agent, a secondary chemotherapeutic agent, and optionally a side effect reducing agent.

Combinatorial efficacy observed for coordinate therapies can occur for a variety of reasons but in general is due to combined inhibition of two or more independent pathways. Individual pathways may provide "bypass" routes for targeted cells (e.g., metastatic cancer cells), requiring that multiple pathways be targeted to prevent the escape. When anti-angiogenic stress is presented, for example using known anti-angiogenic drugs, cells at the periphery of tumors may "bypass" the disruption of normal tumor-associated angiogenesis by coopting local vessels. However this is a poor escape mechanism, often subjecting the tumors to hypoxic stress. This stress in turn may be alleviated by endogenous activity of heat shock factor 90 (Hsp90). Thus, in certain embodiments of the invention an Hsp90 inhibitor is coordinately administered with an anti-Id compound to yield combinatorially effective anti-Id and anti-HSP90 activity, attended by enhanced clinical results.

Anti-Id compounds and methods of the invention can be particularly effectively employed in combination with vascular disrupting agents (VDAs), often with the attendant benefit of allowing for a lowering of VDA effective dosage and/or reduction of VDA-associated adverse side effects. VDAs are mostly chemical agents that disrupt the cytoskeletal vascular network causing cell-shape and permeability changes resulting in vascular resistance, vasoconstriction, increased vascular permeability, platelet thrombi and vascular shutdown. Vascular disruption represents a validated therapeutic strategy to deprive tumors (and, in the case of eye disease, pathologic neovascular lesions) of blood supply. In solid tumors, vascular disrupting agents rapidly disrupt the vasculature within the tumor, reduce blood flow, and deprive the tumor of oxygen and nutrients, resulting in tumor cell death. This disruption of the newly formed blood vessels contrasts with the action of anti-angiogenic therapies, which are designed to prevent new blood vessel formation. Often, because VDAs restrict tumor blood supply and drive them to hypoxic stress (ameliorated by heat shock protein (HSPs)), these combinatorial methods and formulations will often include anti-HSP 90 agents/methods to further enhance the coordinate therapeutic results.

Vascular disrupting agents target the tumor-associated, fragile and relatively newly-constructed vasculature in tumors (Tozer et al., 2005, Mita et al., 2013). The prototype VDAs are combrestatins, natural antimitotic agents isolated from the root bark of the South African tree, Combretum caffrum (Circa and Mann, 2003; Tozer et al., 2001). The most potent of these compounds is combretastatin A-4 (CA4), an antitumor drug. CA4 whose prodrug is CA4-phosphate (CA4P) binds to tubulin in endothelial cells at the same site as colchicine leading to strong inhibition of tubulin polymerization. CA4P causes shape changes, cytotoxicity, changes in cell permeability and apoptosis of proliferating endothelial cells, but not of quiescent cells. The cytoskeleton of mature cells is not sensitive to CA4P as opposed to newly formed cells, which are particularly sensitive. There is a preferential sensitivity of endothelial cells in tumor vessels to CA4P, which unlike those in normal vessels, become thrombogenic, resulting in hemorrhagic necrosis of tumors.

CA4P is currently being evaluated in multiple clinical trials as a treatment for various solid tumors as well as a treatment for age-related macular degeneration (AMD), a vision limiting condition (Nanbu et al., 2003; Eichler et al., 2006). AMD features an overgrowth of blood vessels as part of the underlying pathology (Campochiaro and Hackett, 2003). More recently, anti-angiogenic agents such as bevacizumab (Avastin™), that limit tumor growth by preventing the formation of new blood vessels, have been approved for some cancer indications and are widely used to treat solid tumors. An anti-angiogenic agent mechanistically related to bevacizumab, ranibizumab (Lucentis™), is used to treat AMD.

VDAs and anti-angiogenic agents both work in different, yet complementary ways. Anti-angiogenesis drugs attempt to keep new blood vessels from forming. Unlike VDAs, anti-angiogenic agents do not act on blood vessels that already feed existing tumors. VDAs degrade blood vessels within the tumor and cause widespread cell death in central parts of the tumor that historically have been resistant to conventional treatments, such as cytotoxic chemotherapy, radiation, and biologics. Although VDAs thus have demonstrated clinical efficacy (Hasani and Leighl, 2011; Hinnen and Eskens, 2007; McKeage and Baguley, 2010), preliminary evidence for the induction of EPCs after VDA treatment has been reported recently in Phase I clinical trials using the VDAs ZD6126, AVE8062 or CA4P (Beerepoot et al., 2006; Farace et al., 2007). A robust elevation in EPC levels has also been observed within hours of treatment with microtubule-inhibiting cytotoxic-like vascular disrupting

agents (VDAs) in mice (Shaked et al., 2006). This induction of EPCs likely diminishes effectiveness of the VDA treatment (Daenen et al., 2010).

The anti-Id compositions and methods of the invention are highly complementary to VDA treatment for reducing cancer incidence, metastasis, disease progression and tumor growth/invasiveness. As described herein, anti-Id compounds of the invention, such as ANGX51 effectively block Id-mediated EPC responses to VDA treatments. Thus, in combinatorial formulations and methods, ANGX51 provides for reduction of VDA dosing or treatment duration with comparable anti-angiogenic effects, and less angiogenic rebound involving increase of EPCs responsive to tumor vascular destruction by VDAs. Treatment of tumor-bearing mice with VDAs leads to an acute mobilization of EPCs, which home to the viable tumor rim that characteristically remains after VDA therapy. Because Id1 and Id3 promote EPC production, blockade of this EPC rebound spike by anti-Id compounds of the invention will directly reduce or prevent further tumor-associated neovascularization, blocking tumor blood flow and preventing tumor growth.

Other coordinate treatment compositions and methods herein targets cellular division as a secondary pathway for cancer and metastasis intervention. Taxanes inhibit cancer cell growth by antagonizing the machinery in cells that distribute chromosomes between mother and daughter cells. Taxane resistance has been reported to involve activation of the Notch signaling pathway, which in turn activates Id1. Combining an anti-Id1 compound with a taxane (e.g., taxol or paclitaxel) coordinately prevents this bypass. These combinatorial methods will powerfully reduce or prevent proliferation within tumors and other hyperproliferative cell populations.

In exemplary embodiments of the invention, a coordinate diagnosis and management protocol is contemplated to treat or prevent breast or ovarian cancer. Women with family histories of breast cancer or ovarian cancer may be selected for treatment, for example using a BrCA1 genetic test to establish elevated risk of breast or ovarian cancer. High risk subjects will be provided prophylactic anti-Id treatment (e.g., using (-)-AGX51) for several months, up to 1-2 years or longer, post-surgery to prevent recurrence of the disease. Periodically, systemic Id levels are determined during this time. If Id is detected at a concentration above trace or non-measureable, the anti-Id treatment is continued or increased, optionally supplemented by chemotherapy.

Other patients amenable to treatment according to the invention will be identified by routine mammography of human females positive for a breast tumor, followed by tumor excision and in some cases radiation. When no cancer cells are identified, e.g., in the lymph nodes, anti-Id compound is administered chronically daily for up to a year or more to provide protection against recurrence of the disease. Recurrence is prevented through one or more of the anti-tumor, anti-metastasis, pro-apoptosis and pro-cell cycle control effects of the drug. Periodically, systemic Id levels, optionally combined with other markers (e.g., metastatic, entothelial cell, EPC and/or cancer stem cell markers), are determined during this time. If Id is detected at a concentration above trace or non-measureable, the anti-Id treatment is continued or increased.

Additional coordinate diagnostic and management protocols are provided to treat breast cancer or prevent metastatic progression of an existing breast tumor. Patients are identified by routine mammography of human females as positive for a breast tumor, followed by tumor excision and in some cases radiation. Cancer cells are identified in the lymph nodes of the subject. The subject is administered a course of conventional paclitaxel treatment (e.g., every three weeks for 12 weeks). Over this period, anti-Id compound is administered chronically daily day to maximize the effect of the paclitaxel. The anti-Id treatment is continued for an extended period (e.g., 9-12 months) post paclitaxel treatment to provide additional protection against recurrence of the disease. Periodically, systemic Id levels and optionally other markers are determined during this time. If Id is detected at a concentration above trace or non-measureable, the anti-Id treatment is continued or increased.

In other coordinate diagnosis and management protocols, patients, previously identified by routine mammography as positive for a breast tumor and treated by tumor excision (no cancer found in the lymph nodes), are examined two years post-surgery for tumors (e.g., by computerized tomography (CT) scanning followed by Positron Emission Tomography (PET scanning). When metastasized tumors are found, and/or a lymph node test is positive for cancer cells, the subject will be administered conventional taxane therapy (e.g., paclitaxel every three weeks for 12 weeks), followed by anti-Id compound daily for 9-12 months to prevent remission of the disease, coupled with monitoring of Id levels and optionally other markers as described.

In further exemplary embodiments, subjects presenting with Stage III (metastatic) breast cancer are selected for cancer treatment and management. Subjects thus identified are

treated using an aggressive, combinatorial treatment regimen employing high doses of radiation and multiple doses of paclitaxel. After treatment, histopathology and/or bioscans [eg, computerized tomography (CT), positron emission tomography (PET) and/or magnetic resonance imaging] are used to identify patients presenting with no detectable cancer after the first line treatment above. These subjects are then treated for a prolonged period (e.g., months, six-nine months, 1-2 years or longer) with an anti-Id compound, in a prophylactic treatment regimen to block re-seeding of any remaining cancer cells to a distant organ (e.g., brain or liver). During this management phase, measurements of Id in the blood of the patient are performed periodically. The measurements continue after treatment. After another management period of several months to one year or more, Id blood levels may be observed to increase, indicating an increase in metastatic potential or even the presence of additional disease. Treatment with the anti-Id compound would then be combined with another course of treatment with a non-taxane cytotoxic such as epirubicin. Following this treatment, the anti-Id treatment would continue for a year post-treatment and longer if the Id blood levels fail to decrease to a value consistent with an acceptable (baseline or low-risk) for metastatic potential or the presence of microtumors.

Other exemplary embodiments will employ an array of combinatorial therapies. Patients identified with recurring or metastatic breast cancer may be treated first with an aggressive course of taxane therapy (e.g., 5 single doses treatment of taxane (e.g., paclitaxel, docetaxel or albumin-bound paclitaxel), every three weeks for 12 weeks), to which may be added a a vascular disrupting agent (VDA), such as combretastatin-A4 phosphate. In combination with these therapies, anti-Id compound is also administered chronically daily to maximize the effect of taxane/VDA treatment. The anti-Id treatment is continued for 9-12 months or longer to prevent recurrence or metastatic progression of the disease, with Id and other marker monitoring as described. Alternatively, these patients may be administered taxane therapy in combination with bimonthly or monthly treatment with an anti-VEGF agent (e.g., bevacizumab), or anti-VEGF receptor antagonist (e.g., sunitinib, sorafenib) reinforced by extended, prophylactic administration of anti-Id compound of the invention.

In other embodiments of the invention, coordinate diagnosis and management focuses on patients testing positive for the presence of the HER2/neu receptor. These subjects may be administered conventional taxane therapy along with bimonthly or monthly treatment with trastuzumab (Herceptin), supplemented or followed by daily anti-Id compound treatment as

described. The anti-Id treatment is continued for 6-12 months post the taxane treatment to provide additional protection against recurrence of the disease.

Clinical management methods of the invention can also be adapted to treat more specific cancers, for example estrogen and progesterone receptor-negative breast tumors. Exemplary protocols here may employ tumor excision and in some cases radiation followed by chemotherapy (e.g. with paclitaxel and doxorubicin every three weeks for 12 weeks), accompanied or followed by daily anti-Id therapy (continuing for an extended post-chemotherapy period for prevention of disease recurrence).

In other refractory cases, cancer patients will be treated coordinately with taxane chemotherapy (e.g., with paclitaxel, docetaxel or albumin-bound paclitaxel) supplemented with concurrent or sequential cisplatin treatment, and anti-Id therapy will follow the taxane therapy and extend 6-12 months or more to protect against recurrence of the disease.

Comparable coordinate diagnosis and management protocols are provided for treating other forms of cancer. For example prostate cancer patients will be selected based on PSA screening and/or biopsy to produce a Gleeson score reflecting the stage and metastatic risk of the cancer. Subjects at elevated risk are treated with radiation and chemotherapy, coupled with or followed by extended anti-Id treatment and monitoring as above. This treatment may be coordinated with conventional anti-androgen therapy.

Coordinate diagnosis and management protocols for treating melanoma *in situ* may include topical taxane treatment coupled with concurrent or subsequent, therapeutic or prophylactic anti-Id treatment according to the invention.

Coordinate diagnosis and management protocols for treating Kaposi sarcoma may include intralesional or topical taxane treatment coupled with concurrent or subsequent, therapeutic or prophylactic anti-Id treatment according to the invention. This and other coordinate treatment methods may optionally include concurrent or sequential interferon-alpha treatment.

Yet additional coordinate diagnosis and management methods are contemplated and routinely implemented according to the teachings herein, including for all types and stages of cancer and other proliferative disorders, including but not limited to bladder cancer, colon cancer, pancreatic cancer, lung cancer, brain cancer, esophageal cancer and leukemias.

In all coordinate treatment methods of the invention, the entire armamentarium of known effective chemotherapeutic agents may be combined with anti-Id therapy, likewise the

full range of vascular disrupting agents (VDAs), and HSP 90 inhibitors can be combined with anti-Id drugs such as AGX51 in combinatorial formulations, and any configuration of coordinate treatment regimens, optionally with other interventions such as radiation and surgery.

Following either surgery to excise a tumor and/or radiation treatment to shrink or destroy a tumor, metastasis from remaining tumor cells still is a risk to patients. In this regard, follow-up treatment with an anti-Id compound will decrease this risk and, in this way, increase the chance of patient survival. In addition, in brain cancer, radiation kills cells that express low levels of Id protein leaving behind cancer stem-like cells which can refuel tumor growth. Combining an anti-Id1 agent with radiation therapy is predicted to increase survival rates in this aggressive and lethal human cancer.

Other compositions and methods of the invention target distinct cellular proliferative disorders characterized by aberrant blood vessel growth, or "pathogenic angiogenesis". Examples of these disease targets include ocular disease mediated by aberrant vascular growth (e.g., macular degeneration), and tumor-associated angiogenesis. Anti-Id compounds of the invention function also as "anti-angiogenic" agents, as described below, making them useful to treat or prevent pathogenic angiogenesis, including quite powerfully tumor-associated angiogenesis to mediate a multi-pronged assault on tumor development (i.e., both anti-metastatic and anti-angiogenic). Many cancer types rely on Id protein expression in both the primary and metastatic tumor cell, as well as to support tumor-associated angiogenesis. Anti-Id compositions and methods of the invention target both tumors and their supporting blood vessels simultaneously. In these embodiments, Anti-Id compositions and methods of the invention mediate both anti-tumor (including anti-metastatic) and anti-angiogenic effects. Other anti-angiogenic agents alone have marginal efficacy for treating cancer, often requiring coordinate use of cytotoxic chemotherapies to achieve a therapeutic response.

Compositions and Methods for Treating Disease Involving Pathogenic Angiogenesis

Additional embodiments of the invention employ anti-Id compounds such as AGX51 to effectively treat or prevent any pathogenic angiogenic or neovascular condition or disease. Exemplary pathologic neovascularization phenomena are associated with the ocular disease, age related macular degeneration (AMD).

AMD is the most common cause of irreversible vision loss in the elderly (Jager et al.,

2008). This condition is mediated in large part by angiogenic changes typified by a neovascular lesion complex which can be differentiated by ocular angiography into choroidal neovascular membrane (CNV) and non-CNV components. AMD is characterized by a spectrum of clinical and pathologic findings, including drusen formation, disruption of the RPE, CNV, disciform scar formation and sub-retinal fibrosis. The sustaining event for AMD is believed to be chronic ischemia-reperfusion (I-R) injury of ocular tissue damage caused when blood supply returns to the tissue after a period of ischemia or lack of oxygen. The absence of oxygen and nutrients from blood during the ischemic period creates a condition in which the restoration of circulation results in inflammation and oxidative damage through the induction of oxidative stress rather than restoration of normal function. Tissue damage caused by cumulative insult by excess sunlight, pollution, dust and dirt is believed to be the initiating event for the ischemia that precedes the I-R.

AMD has two forms: non-exudative (dry) and exudative (neovascular or wet). Currently, the prevalence of wet AMD in the US is estimated as 1.2-1.5 million cases with approximately 200,000 new cases occurring each year. Because AMD is a disease of aging, the number of people with AMD is expected to substantially increase as people live longer and the baby-boom generation grows older. By 2030, an estimated 500,000 cases of AMD will be diagnosed each year (Seddon et al., 2004).

Recently approved anti-VEGF treatments represent a major advance in treating AMD (Rosenfeld et al., 2006; Brown et al., 2009. Useful drugs include ranibizumab, an anti-VEGF fab fragment for injection, reported to stabilize or mediate reversal of vision loss in 95% and 40% of patients, respectively. A majority of patients treated with this drug did not experience vision gain, and patients who had positive responses to treatment did not regain ability to drive or read normally. Results with bevacizumab, an anti-VEGF monoclonal antibody, for injection, appear comparable (Rosenfeld et al., 2005).

Anti-VEGF therapies appear to exert most of their beneficial effect via an anti-permeability action resulting in resolution of intra and sub-retinal edema, as the actual CNV lesion does not markedly involute (Eichler et al., 2006). However, exudative AMD- related vision loss is not due to solely choroid neovascularization (CNV) induced sub-retinal and intra-retinal edema. In addition, the long term safety of pan-VEGF inhibition in the eye has yet to be established. VEGF, produced by a number of cells in the neurosensory retina, is neuroprotective in nature and chronic inhibition could be detrimental to neuronal health

(Greenberg et al., 2005). VEGF is also constitutively expressed by RPE cells and is a survival factor for quiescent choriocapillaris endothelium (Witmer et al., 2003), suggesting that long term VEGF inhibition could be detrimental to this vital structure and the cells which rely upon it for metabolic support. With respect to the safety of chronic anti-VEGF treatment, a recent re-evaluation of patients treated for seven years with anti-VEGF treatment reported that macular atrophy was detected by fluorescein angiography in 98% of eyes, and that the area of atrophy correlated significantly with poor visual outcome (Rofagha et al, 2013). Further emphasizing the need for better treatment tools against wet AMD, the follow-up evaluations of the patients after seven years of ant-VEGF treatment found that one third of the patients had poor outcomes with vision on the Snellen chart test declining by 15 letters or more (Rofagha et al, 2013).

The three anti-VEGF agents most commonly used to treat ocular conditions are ranibizumab for injection), aflibercept for injection, and bevacizumab for injection. Besides wet AMD, these agents are also used also used to treat macular edema that results from central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO) as well as for the treatment of macular edema due to diabetic retinopathy. Besides anti-VEGF agents, a vascular disrupting agent (VDA), CA-4 phosphate, is also being evaluated in multiple clinical trials as a prospective treatment for AMD (Eichler et al., 2006).

Two surgical procedures are also occasionally used to remove the ocular lesions that are the essential pathology of AMD, particularly wet AMD: Laser photocoagulation (and photodynamic therapy (Cook et al., 2008).

The exact etiology and pathogenesis of exudative AMD is still not well understood but is thought to consist of vascular and extravascular components orchestrated by multiple factors (Tezel et al., 2004; Ambati et al., 2003). However, AMD features an overgrowth of blood vessels as part of the underlying pathology (Campchiaro et al., 2003). The vascular component of exudative AMD is comprised of vascular endothelial cells, endothelial cell precursors and pericytes. VEGF appears to be an important mediator in the pathogenesis of vascular component. However, the growth of choroidal blood vessels, involves a coordinated interaction among many other agents, not just VEGF. Targeting additional elements in the vascular process offers the opportunity to modulate the entire process. Tissue damage can result from either the vascular or extravascular component of the disease process. The extravascular component often appears to be the largest component volumetrically, and

appears, by histopathology, to be the source of the angiogenic stimuli. This extravascular component is composed primarily of inflammatory cells and less so fibroblasts and glial or RPE. Macrophages and the complement system are now known to play an important role in affecting CNV and propagating the pathogenesis of exudative AMD (Bushini et al, 2011; Gold et al., 2006; Klein et al., 2005; Hageman et al., 2005; Tsutsumi et al., 2003; Espinosa-Heidmann et al., 2005; Oh et al., 1999; Grossniklaus et al., 2002; Forrester et al., 2003). In addition, several biochemical changes in the ocular macrophages with aging are apparent, i.e., the cytokine IL-12 is lowered and the cytokine IL-10 is increased (Jager et al., 2007) which leads to an overall less robust mitigation of trauma-induced angiogenesis [IL-12 is strongly anti-angiogenic via its upregulation of IFN-γ (Dace et al., 2008) and IL-10 is pro-angiogenic (Kelly et al., 2007)]. These changes provide a compelling explanation for the pronounced age dependency of AMD.

In light of the foregoing discussion, an urgent unmet medical need exists for novel therapeutic modalities to treat exudative AMD. Given the emerging limitations of anti-VEGF therapy, these novel therapeutics should target non-VEGF-dependent steps in the development of CNV that ultimately lead to exudative AMD. Targeting Id represents such a target because of the fundamental role the Ids, particularly Id1 and Id3, in mediating the neovascularization process underlying the lesion associated with AMD.

In one aspect, the invention provides methods for inhibiting pathologic ocular neovascularization comprising administering to a subject presenting with a neoplasm an effective amount of an anti-Id compound in a monotherapy protocol employing a single drug or method of treatment. In related embodiments, treatment methods of the invention includes administering to the subject an effective amount of the anti-Id compound along with a secondary treatment agent, treatment modality or treatment method (for example by treating the subject with the anti-Id compound simultaneously or sequentially with a secondary treatment modality or agent selected from for example: An anti-VEGF agent, a VDA, interferon-γ (Naldini et al., 2005), a potent anti-angiogenic cytokine, or an agent that induces interferon-γ for example IL-12 (Del Vecchio et al., 2007 and Kleinman et al., 2008).

In certain "coordinate therapy" or "combinatorial treatment" embodiments, the invention may employ the anti-Id compound administered simultaneously (for example administered at the same time or in a combined formulation) with a secondary drug, compound or chemical agent possessing combinatorial anti-growth activity). In exemplary

embodiments, secondary chemotherapy drugs are chosen, for example an anti-VEGF agent or, a VDA, interferon-γ. Within these and related embodiments, the anti-Id compound and the secondary drug will be "combinatorially effective", meaning biological activity, eg, anti-growth or anti-angiogenesis activity as defined herein, side effects, patient outcomes, or other positive therapeutic indicia will be improved over results observed in relevant control subjects treated with the anti-Id compound or secondary drug alone.

All known treatment agents and methods for treating AMD and other pathogenic neovascular or angiogenic conditions will be useful in certain combinatorial formulations and coordinate treatment methods employing anti-Id compounds of the invention, such as AGX51. In certain embodiments, anti-Id compounds will be employed in conjuction with an anti-VEGF agent, a VDA, and/or , interferon-γ, with an attendant benefit of lowering dosage and/or side effects of these complementary treatment agents while retaining coordinate clinical benefit from their conjunctive use. For example, anti-Id therapy attended by coordinate use of an anti-VEGF agent and/or a VDA will employ lower than conventional dosages of the anti-VEGF agent and/or a VDA drug for the indicated treatment, while combinatorial efficacy will be greater than full dosage conventional anti-VEGF agent and/or a VDA, and side effects such as long term vision loss will be reduced.

In certain embodiments, anti-angiogenic compositions and methods of the invention are effective to reduce pathologic ocular neovascularization in a mammalian subject. These methods may employ monotherapy or coordinate therapy, as above. The methods (and related compounds and compositions) of the invention are "anti-angiogenic effective", for example to reduce incidence, size, or number of vascular lesions in an ocular tissue of a subject presenting with AMD. In certain embodiments, "reducing neovascularization" will correspond to an observed reduction in a histopathologic or ocular angiography index of AMD lesion size, for example a reduced occurrence, size, number or distribution of lesions or "foci" of lesions observed at a secondary ocular site. In other aspects, anti-angiogenic efficacy will be determined by a positive change in one or more patient therapeutic indices correlating with effective prevention and/or treatment of AMDs, e.g., by an increase in a time period of disease free or disease stable conditions for subjects receiving the anti-Id compound compared to suitable control subjects not receiving the anti-Id compound.

Anti-AMD lesion efficacy, i.e., efficacy diminishing or stabilizing growth of the neovascular lesion complex, of the compounds, compositions and methods of the invention

will routinely yield substantial therapeutic benefits and improved treatment outcomes in patients treated for an ocular condition (or any other pathogenic condition) with harmful angiogenesis as part of its underlying pathology. In exemplary embodiments, patients treated with the anti-Id methods and compositions of the invention will exhibit improved treatment outcomes with no increase or an observed decrease in adverse side effects. Illustrative of these benefits, methods of the invention will yield at least a 20% increase in one or more positive clinical therapeutic indices for example a beneficial change in AMD lesion index (eg, a reduction in occurrence, size, number or distribution of the lesion or "foci" of the primary lesion observed at a secondary ocular site. In illustrative embodiments, anti-AMD lesion efficacy of the anti-Id compounds and methods will be demonstrable indirectly by at least a 20% increase in a disease-free or disease stable condition for Id-treated patients compared to survival determined in suitable control patients (not treated with the anti-Id compound). In other embodiments, anti-Id compounds, formulations and methods of the invention will result in even greater anti-AMD clinical benefit, for example yielding a 20-50% increase in a positive therapeutic index, 50-90% increase, up to a 75%-100% increase, including total remission of observed primary AMD lesion enduring for 6 months to a year, 1-2 years, 2-5 years, 5 years or greater, including 10 year and longer remission. In exemplary embodiments, the anti-Id methods and compositions of the invention will be anti-AMD effective to yield at least a 20% decrease in lesion size, a 20%-50%, a 50%-75%, up to a 90% or greater decrease in lesion size, e.g., as demonstrated by comparative histopathology, ocular angiography, optical coherence tomography (OCT) or another ocular imaging technique in anti-Id-treated versus non-treated or placebo-treated subjects.

Respective of all embodiments presented here, anti-AMD efficacy will typically correlate with no increase or even a decrease in observed symptoms of AMD, e.g., loss of visual acuity between anti-Id treated patients and positive control-treated subjects. In exemplary embodiments, anti-Id-treated subjects including subjects treated with anti-Id compound monotherapy, and subjects treated with combinatorial methods such as anti-Id plus anti-VEGF therapy will exhibit no increase in Snellen chart score and will often exhibit at least a 20% increase, a 20-50% increase, up to a 50-90% or greater increase in Snellen chart score compared to positive control subjects treated with conventional (e.g., anti-VEGF) therapy.

Diagnostic Compositions and Methods, and Related Clinical Management Tools

In certain embodiments, cancer patients and other subjects identified for treatment according to the compositions and methods of the invention will be evaluated and selected for refined clinical management using novel diagnostic and managed tools discovered here.

In one embodiment, subjects are selected for treatment with anti-Id compositions and methods using novel Id diagnostic methods and kits provided here. Certain of these methods and materials provide for detection and tracking of elevated Id levels (e.g., Id1 measured in blood or a biopsied tumor sample), which will correlate with a decision to initiated, continue, lower or increase anti-Id treatment. In certain embodiments, these diagnostic methods may include diagnosing the subject for the presence of other disease indices (e.g., biochemical or histologic markers of cancer, or of metastasis, or of an angiogenic pathology) to yield coordinate diagnostic values to enhance disease assessment and management.

High Id1 levels lead to reversion of EMT to an epithelial phenotype, which is required for colonization of metastatic sites. Treatments that mitigate Id1 and Id3 (i.e., impair or inhibit their expression, activity or function) are demonstrated here as potent tools for effectuating reduction of Id levels and attendant blockade of metastatic pathways and cellular activities, along with other disease impacts in cancer patients. Dosage, duration of treatment and efficacy of the novel anti-Id compounds employed within these methods will best be determined reflexively by monitoring changing Id levels (which in these methods will often respond directly to changes in anti-Id treatment). Thus, the novel treatment methods of the invention create a need for corresponding diagnostic and reflexive (clinical managerial) monitoring of endogenous Id levels, which can be focused on any relevant test sample, such as cells (e.g., tumor cells, EPCs, cancer stem cells), tissues or organs (e.g., neoplasms, metastases, exploratory biopsy specimens, lymph nodes) or physiological fluids (e.g., blood, CNS fluid, lymphatic fluid).

Selection of subjects amenable to treatment using the anti-Id compounds of the invention is usefully guided by detection of elevated levels of Id1 and/or Id3 in a biological sample (e.g., blood, urine or saliva) taken from a patient at risk of cancer, particularly of metastatic disease. Employment of assays to detect Id levels in this manner in adjunct application with the invention yields observations of a bio-environment in certain patients with elevated Id levels, indicating a high metastatic potential and thus a critical need for effective treatment using the anti-Id compounds and methods of the invention.

Dosage and Formulation.

Generally, treatment and prophylaxis methods of the invention employ an "effective amount" of an anti-Id compound or composition as described. This may refer to an amount or dosage of AGX51 or another anti-Id compound that is effective to detectably, significantly reduce a level or concentration of a targeted Id protein (e.g., Id1 or Id3) in a cell, tissue, neoplasm, or subject. Such demonstration of efficacy is readily using standard Id protein assays, for example using labeled Id-specific antibodies or other quantitative Id detection reagents. In other embodiments, effective amounts or dosages of an anti-Id compound of the invention will be demonstrated as an amount or dosage effective to measurably inhibit or Id protein binding to a cognate binding partner (for example, Id dimerization to a bHLH protein, such as E-47. Alternate measures or assays to determine what is an effective amount or dosage of an anti-Id compound in this context, include effective amounts and dosages of the compound that mediate a reduction in any detectable and/or quantifiable Id activity or correlated biological index (e.g., a histopathological or clinical index of metastasis or tumor-associated angiogenesis), whether direct or indirect (as long as the activity is subject to modulation by reduction of Id activity or Id levels. Anti-Id compositions and methods in this context may thus be alternatively demonstrated as "effective" through a demonstrated increase in apoptosis or cellular differentiation between test and control samples, by a decrease in cellular proliferation, decrease in cell migration, decrease in secondary site colonization by primary tumor cells, decrease in tumor associated angiogenesis, decrease in EMT or MET progression, etc.

For convenience, compositions of the invention will often be referred to as comprising an "anti-cellular proliferative effective amount" or unit dosage of an anti-Id compound, for example AGX51. Alternatively, compositions will contain an "anti-metastatic dosage or amount" of the active anti-Id compound. In other references, the active compositions may comprise an "anti-angiogenic effective amount" or dosage of the anti-Id drug.

Anti-Id compounds of the invention may be formulated with one or more pharmaceutically acceptable carriers, excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects,

or provide other advantages for pharmaceutical use. Suitable effective unit dosage amounts of the active anti-Id compounds for administration to mammalian subjects, including humans, may range from 10 to 1500 mg, 20 to 1000 mg, 25 to 750 mg, 50 to 500 mg, or 150 to 500 mg. In certain embodiments, the anti-Id effective dosage may be selected within narrower ranges of, for example, 10 to 25 mg, 30-50 mg, 75 to 100 mg, 100 to 250 mg, or 250 to 500 mg. These and other effective unit dosage amounts may be administered in a single dose, or in the form of multiple daily, weekly or monthly doses, for example in a dosing regimen comprising from 1 to 5, or 2-3, doses administered per day, per week, or per month. In one exemplary embodiment, dosages of 10 to 25 mg, 30-50 mg, 75 to 100 mg, 100 to 250 mg, or 250 to 500 mg, are administered one, two, three, four, or five times per day. In more detailed embodiments, dosages of 50-75 mg, 100-200 mg, 250-400 mg, or 400-600 mg are administered once or twice daily. In alternate embodiments, dosages are calculated based on body weight, and may be administered, for example, in amounts from about 0.5mg/kg to about 100mg/kg per day, 1mg/kg to about 75mg/kg per day, 1mg/kg to about 50mg/kg per day, 2mg/kg to about 50mg/kg per day, 2mg/kg to about 30mg/kg per day or 3mg/kg to about 30mg/kg per day.

The amount, timing and mode of delivery of compositions of the invention comprising an anti-Id effective amount of a compound of Formula I and/or III will be routinely adjusted on an individual basis, depending on such factors as weight, age, gender, and condition of the individual, the acuteness of the cellular proliferative disorder and/or related symptoms, whether the administration is prophylactic or therapeutic, and on the basis of other factors known to effect drug delivery, absorption, pharmacokinetics, including half-life, and efficacy.

An effective dose or multi-dose treatment regimen for the instant anti-Id formulations will ordinarily be selected to approximate a minimal dosing regimen that is necessary and sufficient to substantially prevent or alleviate a targeted cellular proliferative disease (e.g., cancer, metastatic cancer, tumor-associated angiogenesis) in the subject, and/or to substantially prevent or alleviate one or more symptoms associated with the cellular proliferative disease in the subject. A dosage and administration protocol will often include repeated dosing therapy over a course of several days or even one or more weeks or years. An effective treatment regime may also involve prophylactic dosage administered on a day or multi-dose per day basis lasting over the course of days, weeks, months or even years.

By way of illustration, the anti-Id active agent can be admixed with conventional pharmaceutically acceptable carriers and excipients (ie, vehicles) and used in the form of aqueous solutions, tablets, capsules, elixirs, suspensions, syrups, wafers, and the like. Such pharmaceutical compositions contain, in certain embodiments, from about 0.1 to about 90% by weight of the active compound, and more generally from about 1 to about 30% by weight of the active compound. The pharmaceutical compositions may contain common carriers and excipients, such as corn starch or gelatin, lactose, dextrose, sucrose, microcrystalline cellulose, kaolin, mannitol, di-calcium phosphate, sodium chloride, and alginic acid. Disintegrators commonly used in the formulations of this invention include croscarmellose, microcrystalline cellulose, corn starch, sodium starch glycolate and alginic acid.

A liquid composition will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s), for example, ethanol, glycerine, sorbitol, non-aqueous solvent such as polyethylene glycol, oils or water, with a suspending agent, preservative, surfactant, wetting agent, flavoring or coloring agent. Alternatively, a liquid formulation can be prepared from a reconstitutable powder. For example, a powder containing active compound, suspending agent, sucrose and a sweetener can be reconstituted with water to form a suspension and a syrup can be prepared from a powder containing active ingredient, sucrose and a sweetener.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid compositions. Examples of such carriers include magnesium stearate, starch, lactose, sucrose, microcrystalline cellulose and binders, for example, polyvinylpyrrolidone. The tablet can also be provided with a color film coating, or color included as part of the carrier(s). In addition, active compound can be formulated in a controlled release dosage form as a tablet comprising a hydrophilic or hydrophobic matrix.

A composition in the form of a capsule can be prepared using routine encapsulation procedures, for example, by incorporation of active compound and excipients into a hard gelatin capsule. Alternatively, a semi-solid matrix of active compound and high molecular weight polyethylene glycol can be prepared and filled into a hard gelatin capsule, or a solution of active compound in polyethylene glycol or a suspension in edible oil, eg, liquid paraffin or fractionated coconut oil, can be prepared and filled into a soft gelatin capsule.

Tablet binders that can be included are acacia, methylcellulose, sodium carboxymethylcellulose, poly-vinylpyrrolidone (Povidone), hydroxypropyl methylcellulose, sucrose, starch and ethylcellulose. Lubricants that can be used include magnesium stearate or other metallic stearates, stearic acid, silicone fluid, talc, waxes, oils and colloidal silica.

Flavoring agents such as peppermint, oil of wintergreen, cherry flavoring or the like can also be used. Additionally, it may be desirable to add a coloring agent to make the dosage form more attractive in appearance or to help identify the product.

The compounds of the invention and their pharmaceutically acceptable salts that are active when given parenterally can be formulated for intramuscular, intrathecal or intravenous administration. A typical composition for intramuscular or intrathecal administration will be of a suspension or solution of active ingredient in an oil, for example, arachis oil or sesame oil. A typical composition for intravenous or intrathecal administration will be a sterile isotonic aqueous solution containing, for example, active ingredient and dextrose or sodium chloride, or a mixture of dextrose and sodium chloride. Other examples are lactated Ringer's injection, lactated Ringer's plus dextrose injection, Normosol-M and dextrose, Isolyte E, acylated Ringer's injection, and the like. Optionally, a co-solvent, for example, polyethylene glycol, a chelating agent, for example, ethylenediamine tetracetic acid, and an anti-oxidant, for example, sodium metabisulphite may be included in the formulation. Alternatively, the solution can be freeze dried and then reconstituted with a suitable solvent just prior to administration.

For certain applications, such as treating skin cancer lesions, the compounds of this invention are active on topical administration, and can be formulated as transdermal compositions or integrated in a transdermal delivery device (e.g., a transdermal patch). Such compositions include, for example, a backing, active compound reservoir, a control membrane, liner and contact adhesive. Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solids or granules; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, mannitol, corn starch, potato

starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are known in the art.

The anti-Id compositions of the present invention can also include aerosol formulations to be administered via pulmonary inhalation or intranasal spray. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They may also be formulated as pharmaceuticals for non-pressured preparations such as for use in a nebulizer or an atomizer.

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

Formulations suitable for topical administration may be presented as creams, gels, pastes, or foams, containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate. Suppository formulations are also provided by mixing with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous

administration may comprise the inhibitor(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a pre-determined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

Those of skill in the art will readily appreciate that dose levels can vary as a function of the specific compound, the nature of the delivery vehicle, and the like. Suitable dosages for a given compound are readily determinable by those of skill in the art by a variety of means. The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to cause a prophylactic or therapeutic response in the animal over a reasonable time frame. One skilled in the art will recognize that dosage will depend on a variety of factors including the strength of the particular compound employed, the condition of the animal, and the body weight of the animal, as well as the severity of the illness and the stage of the disease. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound. Suitable doses and dosage regimens can be determined by comparisons to anticancer or immunosuppressive agents that are known to cause the desired growth inhibitory or immunosuppressive response.

Optionally, the pharmaceutical composition may contain other pharmaceutically acceptable components such as buffers, surfactants, antioxidants, viscosity modifying agents, preservatives and the like. Each of these components is well-known in the art. See, eg, U.S. Patent No. 5,985,310, the disclosure of which is herein incorporated by reference. Other components suitable for use in the formulations of the present invention can be found in Remington, 1985. In an embodiment, the aqueous cyclodextrin solution also includes dextrose, e.g., about 5% dextrose.

Kits and Systems

Also provided within the invention are kits and systems that correlate with the foregoing description regarding combinatorial formulations, coordinate methods, diagnostic and treatment tools and systems, etc. All combinations of these primary tools disclosed for coordinate or combinatorial use are contemplated to also be provided herein in combined "kit" form. Exemplary kits and systems for practicing the subject methods may include one or more pharmaceutical formulations, which include for example an anti-Id compound packaged or referenced for use with a secondary cancer treatment agent, such as a chemotherapeutic drug, VDA, or HSP90 inhibitor. In certain embodiments the kits may include a single pharmaceutical composition, present as one or more unit dosages, where the composition includes both an anti-Id compound (e.g., (-)-AGX51 and a chemotherapeutic agent or toxicity reducing agent. In yet other embodiments, the kits may include three or more separate pharmaceutical compositions, each containing an anti-Id compound, a chemotherapeutic or possibly a toxicity reducing agent, or a combination of these elements. In addition to the above components, the subject kits may further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, e.g., as printed information or reference on a suitable medium or substrate on the packaging of the kit or on a package insert, etc.

EXAMPLES

Anti-Cancer and Anti-Metastatic Compositions and Methods

Metastasis is the greatest therapeutic challenge for prolonging survival in most cancer patients, perhaps most significantly among the most populous cancer sufferers, breast cancer patients. Identification of genes and regulatory factors involved in metastasis has proven exceedingly complex and intractable from a clinical standpoint. The inhibitor of DNA-binding (Id) proteins have been variously implicated to play diverse roles in cancer metastasis, including breast cancer metastasis. High Id1 expression is observed at metastatic sites. Id1 reportedly functions in regulating critical mesenchymal-to-epithelial transition (MET) changes which allow circulating ("disseminating") cancer cells to seed ("colonize") distant metastatic sites. Artificial genetic knockdown of Id1 is reported to disrupt MET by

tumor cells in the lung, and to block development of lung metastases in murine models of breast cancer.

The invention provides the first clinically practicable showing that a small molecule drug can effectively target and block Id function in mammalian subjects *in vivo*, to disrupt metastasis, slow or prevent cancer progression, and ultimately reduce or prevent mortality in cancer patients.

Anti-Id compounds of the invention provide a novel and profoundly effective approach for treating and/or preventing metastases in cancer patients. The Examples below illustrate development, characterization and anti-metastatic use of an exemplary small molecule anti-Id drug, N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ("AGX51"). This exemplary lead compound provides a foundation for rational design of equivalent drugs possessing anti-Id activity, based on the AGX51 platform (e.g., as described above in relation to alternate drug designs set forth in Formulae I, II, III and IV). AGX51 was discovered through exhaustive molecular screening, including a vast *in silico* small molecule screen (spanning 2 x 10^6 candidate compounds). AGX51, as herein characterized, emerged from the screen as the first small molecular agent capable of binding a hydrophobic pocket adjacent to a dimerization domain of Id1, in a manner effective to disrupt dimerization of Id1 and its cognate binding partners (including critical regulatory bHLH proteins, such as E47). This monumental discovery was surprising in many ways, not the least being the nature and complexity of HLH-bHLH dimerization rendering prior efforts to effectively disrupt these molecular interactions, using a host of candidate blocking agents, profoundly uncertain and persistently unsuccessful.

As described AGX51 and other Anti-Id compounds of the invention not only bind and impair Id dimerization function with bHLH proteins, but by this mechanism also interfere functionally, in clinically practicable ways, with downstream Id activities in critical metastatic pathways. Among these activities, AGX-51 enhances E47 binding to DNA in a dose-dependent manner (correlated with AGX-51's effective antagonism of Id1-E47 dimerization). The Examples below further show that anti-Id compounds of the invention effectively mediate reduction of Id levels in cells and living subjects (likely through "deprotection" or "unmasking" Id by disrupting Id-BHLH protein dimers, exposing Id to ubiquitin-mediated degradation, and likely other Id-degradative mechanisms). This novel

and unpredicted activity of anti-Id compounds herein, alone without other powerful anti-Id effects noted here, provides effective tools for reducing harmful Id levels in clinical subjects, to reduce or prevent cancer in these subjects by directly reducing risk of cancer development in precancerous cells, lowering cancer transformation in neoplastic cells, and reducing metastatic potential of existing neoplasms and cancers.

The Examples below demonstrate that, by treating murine breast cancer cells (4T1) with AGX51, a clinically practicable decrease of harmful Id1 protein levels results. Correlated with these surprising results, in cancer cell culture assays AGX51 decreases cell viability (as measured by alamar blue assays), and inhibits formation of mammospheres. Anti-Id compositions and methods of the invention also effect therapeutic changes in regulatory proteins p16 and p21, reducing circulating levels of Id in blood of AGX51-treated subjects, down regulate circulating endothelial cells and EPCs, directly down-regulate pathogenic neovascularization associated with metastasis and tumor growth, among many additional, distinct activities demonstrated here. These and other data, both in vitro and in vivo, are consistent with results from genetic Id knockdown experiments, which can only be construed as surprising and unexpected for a first-in-class anti-Id small molecule drug.

These exemplary data further demonstrate that anti-Id compounds and methods of the invention potently disrupt several metastatic mechanisms and pathways (as demonstrated in leukemic, lung cancer, bladder cancer, and breast cancer preclinical models, among others).

Culminating these studies, the lead candidate anti-Id drug AGX51 has now been shown to profoundly reduce actual tumor metastasis in *in vivo* murine models of multiple cancer types widely accepted as predictive of clinical drug utility in other mammalian subjects, including humans. When administered at 50mg/kg via intraperionteal injection, 24-hours after tail vein injection of 4T1 cells, AGX51 sharply reduces development of lung metastases. Significantly greater inhibition of lung metastases was observed when AGX-51 administration was increased from once to twice daily. Comparable studies and results are provided from murine breast cancer models. Additional data are provided evincing that AGX51 will block Id-dependent MET and thus prevent or curtail metastatic disease in mammalian subjects. Further demonstration of these novel activities by anti-Id compounds of the invention are ongoing studies of AGX-51 effects on the 4T1 transcriptome and EMT/MET pathway.

The Examples provided here are presented for illustrative purposes only, and will not be misconstrued by skilled artisans as limiting the scope, or practical applications, of the invention, including equivalent embodiments the teachings of this disclosure. These Examples use an exemplary small molecule inhibitor AGX57 to block and disable Id protein levels and metastasis-mediating functions, while other like compounds described herein will be constructed based on these teachings to provide additional agents effective for neutralizing Id binding and function.

Example 1

Identification and Testing of Anti-Id Compounds

The experiments disclosed here summarize the identification of 364 compounds, and concurrent design of twelve peptides useful for inhibiting Id. These experiments further provide means for identifying additional anti-Id compounds, as well as for analyzing effectiveness of candidate compounds capable of inhibiting Id. The assays described below include practicable means for testing effectiveness of candidate compounds, for example to confirm Id binding, Id dimerization blockade, and/or ability to mediate destabilization and degradation of Id in cells and living subjects.

Several million chemicals were screened using three dimensional E47-Id1 interaction mapping to identify small molecules that could potentially inhibit E47-Id1 interaction. Cognate Id1 binding structures were conceptualized and virtual screening employed a Monte Carlo simulation for a complex of Id1 and a small compound screen (helical fragments fixed) was run, including 1,000,000 steps and 100 conformations collected and analyzed. A complex conformation with the best score and total energy was selected for further analysis.

The top 3,000 compounds from virtual screening were further analyzed for ClogP < 5, tPSA < 80, Molecular Weight < 600, and chemical and biochemical stability. 364 compounds identified by the *in silico* screen are listed in Table 1.

Table 1: Chemical compounds identified by E47-Id1 interaction mapping as potentially inhibiting E47-Id1 interaction.

Compound	Formula	MW	Chemical Name
1	C21H27ClN6O3	446.94074	RCL L11, 321-2
2	C19H24O6	348.39953	2-methoxyethyl 5-(3,3-dimethyl-2-oxobutoxy)-2-methyl-1-benzofuran-3-carboxylate
3	C30H24N2O7	524.53498	di-me 3-(3,4-di-meo-ph)7-(1-naphthoyl)pyrrolo(1,2-c)pyrimidine-5,6-dicarboxylate
4	C25H30N4O6S	514.60505	RCL L18,827-1
5	C27H25FN2O6S	524.5725	RCL L19,192-2
6	C19H25N5O3	371.4428	RCL L21,483-3
7	C24H34N6O2	438.57758	RCL L34,410-9
8	C27H31BrN2O5	543.46252	RCL L34,794-9
9	C26H29N3O7	495.53693	RCL L34,836-8
10	C28H33FN2O5	496.58401	RCL L34,930-5
11	C25H26BrFN2O5	533.39877	RCL L35,342-6
12	C19H21N5O2S	383.47552	N-(4-ethoxyphenyl)-2-{(4-ET-5-(3-pyridinyl)-4H-1,2,4-triazol-3-yl)thio}acetamide
13	C19H18N4O3S2	414.50831	ME 4-((((4-allyl-5-(2-thienyl)-4h-1,2,4-triazol-3-yl)thio)acetyl)amino)benzoate
14	C25H27FN2O6	470.50214	RCL L42,816-7
15	C32H27FN2O5	538.58079	RCL L42,856-6
16	C21H17N3O4S	407.45134	RCL L43,208-3
17	C29H30N2O6	502.57225	RCL L43,214-8
18	C21H29N5O5	431.49578	RCL R13,767-7
19	C26H38O8	478.58796	3-beta,5-diacetoxy-17-alpha-ethoxycarbonyl-5-beta,14-beta-androstan-19-oic acid
20	C14H18ClN5O	307.78546	2-(4-amino-6-(pentylamino)-1,3,5-triazin-2-yl)-4-chloro-phenol
21	C18H17NO5S	359.40389	2,4-dimethoxybenzyl (2-oxo-1,3-benzothiazol-3(2h)-yl)acetate
22	C18H29NO4	323.43613	2-ethyl-4-(2-hydroxy-3-(isopropylamino)propoxy)-3,6-dimethylphenyl acetate
23	C18H21N7O3S	415.47717	RCL R60,176-4
24	C25H22BrClN2O3	513.82269	RCL R60,587-5
25	C18H22O5	318.37304	ethyl 5-(3,3-dimethyl-2-oxobutoxy)-2-methyl-1-benzofuran-3-carboxylate
26	C26H24N2O5	460.49098	ethyl 7-benzoyl-3-(3,4,5-trimethoxyphenyl)pyrrolo(1,2-c)pyrimidine-5-carboxylate
27	C17H21N7O3	371.46722	RCL R77,075-2
28	C26H23ClN2O6	494.93601	et 7-(4-cl-benzoyl)-3-(3,4,5-tri-meo-ph)pyrrolo(1,2-c)pyrimidine-5-carboxylate
29	C24H23N5O3	429.48261	RCL R82,426-7
30	C21H16FN3O4S	425.44177	RCL R84,320-2
31	C23H21N3O4S	435.50552	RCL R89,190-8
32	C23H29NO5	399.49128	4-(3,5-Dimethoxy-phenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydro-quinoline-3
33	C24H31NO6	429.51777	2,7,7-Trimethyl-5-oxo-4-(3,4,5-trimethoxy-phenyl)-1,4,5,6,7,8-hexahydro-quinolin
34	C23H20O8	424.41105	5-(acetyloxy)-3-(3,4-dihydro-2H-1,5-benzodioxepin-7-yl)-2-methyl-4-oxo-4H-chromen-7-yl acetate
35	C25H26N2O4	418.49697	ethyl 7-(4-methoxyphenyl)-2-methyl-5-oxo-4-pyridin-2-yl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate
36	C26H25N3O5S	491.57025	methyl (2E)-5-[4-(acetyloxy)phenyl]-2-[4-(dimethylamino)benzylidene]-7-methyl-3-oxo-2,3-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxylate
37	C22H32O7	408.49614	Structure contains non-supported carbohydrate derivative!
38	C16H15N7O4	369.34245	ethyl {5-[(4-methoxyphenyl)amino][1,2,5]oxadiazolo[3,4-e][1,2,4]triazolo[4,3-a]pyrazin-8-yl}acetate
39	C26H27N3O2	413.52399	N-{2-[(4-benzylpiperazin-1-yl)carbonyl]phenyl}-2-methylbenzamide
40	C25H26N2O4S	450.56097	N-(2-ethoxyphenyl)-2-{[(4-methoxyphenyl)acetyl]amino}-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxamide
41	C27H27NO5	445.51994	N-Benzo[1,3]dioxol-5-ylmethyl-N-(3-benzo[1,3]dioxol-5-yl-4-phenyl-butyl)-acetami
42	C20H28N2O3	344.45776	3-Cyclopentyl-1-[4-(3-methoxy-benzoyl)-piperazin-1-yl]-propan-1-one
43	C22H30N2O4	386.4954	1-(4-methoxybenzyl)-4-(2,4,5-trimethoxybenzyl)piperazine
44	C25H21N5O3	439.47782	N-{(1Z)-(benzoylamino)[(6-methoxy-4-methylquinazolin-2-yl)amino]methylene}benzamide
45	C17H19N5O2	325.37328	2-[(6-ethoxy-4-methylquinazolin-2-yl)amino]-5,6-dimethylpyrimidin-4(1H)-one
46	C22H24N2O6	412.44638	Acetic acid 4-(2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-
47	C15H13BrN2O4	365.18586	5-Bromo-furan-2-carboxylic acid [1-carbamoyl-2-(4-methoxy-phenyl)-vinyl]-amide
48	C24H27N3O6	453.49929	4-(2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-2-methoxyphenyl morpholine-4-carboxylate
49	C23H28N2O7S2	508.61681	ethyl 2-{[4-methoxy-3-(morpholin-4-ylsulfonyl)benzoyl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate
50	C21H23N5O3S	425.51316	N-{(1Z)-[(4,6-dimethylpyrimidin-2-yl)amino][(4-methoxyphenyl)amino]methylene}-4-methylbenzenesulfonamide

Compound	Formula	MW	Chemical Name
51	C24H24N2O5S	452.53328	ethyl 5-{[(2-methoxyphenyl)amino]carbonyl}-4-methyl-2-{[2-methylbenzoyl)amino]thiophene-3-carboxylate
52	C18H22O5	318.37304	ethyl 5-(3,3-dimethyl-2-oxobutoxy)-2-methyl-1-benzofuran-3-carboxylate
53	C17H16N4O4S2	404.46947	2-[2-(4-Methyl-6-oxo-1,6-dihydro-pyrimidin-2-ylsulfanyl)-acetylamino]-benzothiaz
54	C25H29N3O3	419.52818	N~1~-2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl-N~2~-[2-(3,4-dimethoxyphenyl)ethyl]-N~2~-methylglycinamide
55	C23H19N3O5S	449.48898	2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(4-{[(4-methylphenyl)sulfonyl]amino}phenyl)acetamide
56	C18H17F2NO5S	397.40069	ethyl 5-acetyl-2-{[(2,4-difluorophenoxy)acetyl]amino}-4-methylthiophene-3-carboxylate
57	C20H21N3O4S	399.47207	ethyl 2,5-dimethyl-3-{2-[(3-methylphenyl)amino]-2-oxoethyl}-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate
58	C19H18FN3O4S	403.43541	ethyl 4-[(4-fluorophenyl)amino]-2-(2-methoxy-2-oxoethyl)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate
59	C24H36O7	436.55032	Structure contains non-supported carbohydrate derivative!
60	C28H26ClNO5	491.97612	[9-{2-[(4-chlorobenzyl)oxy]phenyl}-1,8-dioxo-2,3,4,5,6,7,8,9-octahydroacridin-10(1H)-yl]acetic acid
61	C19H28N2O5	364.44541	ethyl 1-{3-[(2,5-dimethoxyphenyl)amino]-3-oxopropyl}piperidine-4-carboxylate
62	C18H26N2O4	334.41892	ethyl 1-{3-[(2-methoxyphenyl)amino]-3-oxopropyl}piperidine-4-carboxylate
63	C20H19N5O	345.40733	N-(4-ethoxyphenyl)-1-[4-methylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
64	C25H29N3O7	483.52578	4-(2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-2,6-dimethoxyphenyl morpholine-4-carboxylate
65	C24H19FN2O5S	466.49183	ethyl 2-[3-(4-fluorobenzoyl)-4-hydroxy-5-oxo-2-phenyl-2,5-dihydro-1H-pyrrol-1-yl]-4-methyl-1,3-thiazole-5-carboxylate
66	C21H16FN3O4S	425.44177	5-(4-Fluoro-phenyl)-3-hydroxy-4-(4-methoxy-benzoyl)-1-(5-methyl-[1,3,4]thiadiazo
67	C21H16BrN3O4S	486.34737	5-(4-bromophenyl)-3-hydroxy-4-(4-methoxybenzoyl)-1-(5-methyl-1,3,4-thiadiazol-2-yl)-1,5-dihydro-2H-pyrrol-2-one
68	C21H16FN3O4S	425.44177	5-(2-fluorophenyl)-3-hydroxy-4-(4-methoxybenzoyl)-1-(5-methyl-1,3,4-thiadiazol-2-yl)-1,5-dihydro-2H-pyrrol-2-one
69	C23H24N2O5	408.45813	ethyl 6-methoxy-4-{[4-(propoxycarbonyl)phenyl]amino}quinoline-3-carboxylate
70	C20H17ClN2O6	416.82129	(4-{(Z)-[1-(4-chlorobenzyl)-2,5-dioxoimidazolidin-4-ylidene]methyl}-2-methoxyphenoxy)acetic acid
71	C27H25FN2O4	460.5097	5-(4-fluorophenyl)-3-hydroxy-4-(3-methyl-4-propoxybenzoyl)-1-(pyridin-3-ylmethyl)-1,5-dihydro-2H-pyrrol-2-one
72	C20H20N2O5	368.3928	4-Acetyl-5-(2,4-dimethoxy-phenyl)-3-hydroxy-1-pyridin-3-ylmethyl-1,5-dihydro-pyr
73	C20H20N2O5	368.3928	4-Acetyl-5-(3,4-dimethoxy-phenyl)-3-hydroxy-1-pyridin-3-ylmethyl-1,5-dihydro-pyr
74	C24H19N3O4	413.43673	ethyl 4-[(4-isonicotinoyl-1-oxo-1,2-dihydroisoquinolin-3-yl)amino]benzoate
75	C19H23NO7	377.39766	4-(3-Hydroxy-4-methoxy-phenyl)-1-(2-methoxy-ethyl)-1,4-dihydro-pyridine-3,5-dica
76	C28H27ClN2O6	522.99019	methyl N-{[1-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinolin-3-yl]carbonyl}tyrosinate
77	C21H20N2O6	396.40335	4-[3-Hydroxy-4-(4-methoxy-benzoyl)-2-oxo-5-pyridin-3-yl-2,5-dihydro-pyrrol-1-yl]
78	C22H28N2O6	416.47826	4-{[4-(3,5-dimethoxybenzoyl)piperazin-1-yl]methyl}-2,6-dimethoxyphenol
79	C14H21N3O4S	327.40517	5-Butyl-6-hydroxy-2-(2-morpholin-4-yl-2-oxo-ethylsulfanyl)-3H-pyrimidin-4-one
80	C20H18N2O5	366.37686	3-{[3-(ethoxycarbonyl)-8-methoxyquinolin-4-yl]amino}benzoic acid
81	C23H20N6O2S	444.51885	N-(2-methoxyphenyl)-5-methyl-7-pyridin-2-yl-2-thien-2-yl-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide
82	C15H21N5O	287.36752	N~4~-(4-methoxyphenyl)-1,3,5-triazaspiro[5.5]undeca-1,3-diene-2,4-diamine
83	C20H19F3N2O4	408.38063	methyl 4-(4-(acetylamino)phenoxy]-2,3,5-trifluoro-6-pyrrolidin-1-ylbenzoate
84	C23H23BrN2O5	487.35416	ethyl 3-bromo-4-{[4-(ethoxycarbonyl)phenyl]amino}-2-methyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrrole-2-carboxylate
85	C13H19N7OS	321.40668	1-(2-{[4,6-bis(ethylamino)-1,3,5-triazin-2-yl]amino}-4-methyl-1,3-thiazol-5-yl)ethanone
86	C20H18N4O5	394.39026	2-{5-[Bis-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)-methyl]-furan-2-yl}-benzoic acid
87	C20H18N4O5	394.39026	3-{5-[Bis-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)-methyl]-furan-2-yl}-benzoic acid
88	C17H16N2O2	280.32927	2-Methoxy-6-(quinolin-3-ylaminomethyl)-phenol
89	C22H25N3O5S	443.52565	(2Z)-3-(3,4-dimethoxybenzyl)-2-[(4-methoxyphenyl)imino]-N-methyl-4-oxo-1,3-thiazinane-6-carboxamide
90	C18H21N5O2	339.40037	2-(6-Ethoxy-4-methyl-quinazolin-2-ylamino)-5-ethyl-6-methyl-1H-pyrimidin-4-one
91	C23H18ClNO4	407.85721	2-[(2-benzoyl-4-chlorophenyl)amino]-4-oxo-4-phenylbutanoic acid
92	C19H21N5O2	351.41152	2-[(6-ethoxy-4-methylquinazolin-2-yl)amino]-5,6,7,8-tetrahydroquinazolin-4(1H)-one
93	C20H29N3O2	343.47303	8-butyl-3,3-dimethyl-6-[(tetrahydrofuran-2-ylmethyl)amino]-3,4-dihydro-1H-pyrano[3,4-c]pyridine-5-carbonitrile
94	C21H23NO6	385.42056	2-Amino-4-(4-methoxycarbonyl-phenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-ch

Compound	Formula	MW	Chemical Name
95	C20H23NO4	341.41061	isobutyl 2-benzyl-1-oxo-1,2,3,6,7,7a-hexahydro-3a,6-epoxyisoindole-7-carboxylate
96	C16H17N5O5	359.34439	methyl 6-acetyl-7-(3-ethoxy-4-hydroxyphenyl)-4,7-dihydrotetrazolo[1,5-a]pyrimidine-5-carboxylate
97	C25H25N5O7	507.5073	N-{2-[(6,7-dimethoxyisoquinolin-1-yl)methyl]-4,5-dimethoxyphenyl}-1-methyl-4-nitro-1H-pyrazole-5-carboxamide
98	C17H24N2O4S	352.45583	4-{[3-(aminocarbonyl)-6-tert-butyl-4,5,6,7-tetrahydro-1-benzothien-2-yl]amino}-4-oxobutanoic acid
99	C26H31N3O3	433.55527	N-(3-morpholin-4-ylpropyl)-2-(4-propoxyphenyl)quinoline-4-carboxamide
100	C20H19NO4	337.37873	2-(2,4-diethoxyphenyl)quinoline-4-carboxylic acid
101	C20H22N4O2	350.42394	N-(2,4-dimethoxyphenyl)-2-pyrrolidin-1-ylquinazolin-4-amine
102	C20H19ClFN5O2S	447.92213	N-{(1Z)-[(3-chloro-4-fluorophenyl)amino][(4,6-dimethylpyrimidin-2-yl)amino]methylene}-1-phenylmethanesulfonamide
103	C23H23N3O5	421.45686	2-{2-[4-(1,3-benzodioxol-5-ylmethyl)piperazin-1-yl]-1-methyl-2-oxoethyl}-1H-isoindole-1,3(2H)-dione
104	C29H32N2O5	488.58879	N-(4-butoxyphenyl)-1-(4-methoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide
105	C26H25ClN2O4	464.95315	N-(4-butoxyphenyl)-1-(4-chlorophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide
106	C19H20ClN5O	369.85715	5-(4-chlorophenyl)-7-(4-ethoxyphenyl)-4,5,6,7-tetrahydro[1,2,4]triazolo[1,5-a]pyrimidin-2-amine
107	C19H20BrN5O2	430.30755	5-(4-bromophenyl)-7-(3,4-dimethoxyphenyl)-4,5,6,7-tetrahydro[1,2,4]triazolo[1,5-a]pyrimidin-2-amine
108	C23H29NO7	431.49008	ethyl 2-amino-7,7-dimethyl-5-oxo-4-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate
109	C23H22N2O6	422.44159	1',1''-propane-1,3-diylbisspiro[1,3-dioxolane-2,3'-indol]-2'(1'H)-one
110	C20H22N2O6	386.40814	2-Amino-7,7-dimethyl-4-(4-nitro-phenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate
111	C22H25N3O4S	427.52625	ethyl 10-(3-morpholin-4-ylpropanoyl)-10H-phenothiazin-3-ylcarbamate
112	C25H29N3O6	467.52638	4-(2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-2-ethoxyphenyl morpholine-4-carboxylate
113	C21H19ClN2O4	398.84958	N-{2-[3-(4-chlorobenzoyl)-4-hydroxy-5-oxo-2-phenyl-2,5-dihydro-1H-pyrrol-1-yl]ethyl}acetamide
114	C17H18N2O3	298.34461	(3aR,7aS)-2-{[(4-acetylphenyl)amino]methyl}-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione
115	C21H25N3O4	383.4511	2-Azepan-1-yl-N-(2-ethoxy-phenyl)-5-nitro-benzamide
116	C24H22N2O5S	450.51734	4-(2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-2-methoxyphenyl thiophene-2-carboxylate
117	C25H23N3O7S	509.54196	4-(2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-2-ethoxy-6-nitrophenyl thiophene-2-carboxylate
118	C23H21ClN2O3	408.88842	N'-acetyl-N'-(3-chloro-4-methylphenyl)-2-hydroxy-2,2-diphenylacetohydrazide
119	C27H22N2O7	486.48559	4-[2-amino-3-cyano-7-(2-furyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl]-2-ethoxyphenyl 2-furoate
120	C23H22FN3O5	439.44729	dimethyl 2-({(Z)-[1-(4-fluorophenyl)-5-oxo-3-propyl-1,5-dihydro-4H-pyrazol-4-ylidene]methyl}amino)terephthalate
121	C23H27N9O4	493.52954	8-[(4-amino-6-morpholin-4-yl-1,3,5-triazin-2-yl)methoxy]-1,3-dimethyl-7-(4-methylbenzyl)-3,7-dihydro-1H-purine-2,6-dione
122	C21H22N2O5	382.41989	ethyl 4-[(3,4-dimethoxyphenyl)amino]-8-methoxyquinoline-3-carboxylate
123	C31H33N3O6	543.62516	ethyl 4-{[2,3-bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-yl]carbonyl}piperazine-1-carboxylate
124	C21H23N3O6	413.43396	N~2~-acetyl-N~1~-[2-(2,3-dihydro-1,4-benzodioxin-6-ylamino)-1-(4-methoxyphenyl)-2-oxoethyl]glycinamide
125	C22H32N2O4	388.51134	ethyl 1-[2-(cyclopentylamino)-1-(4-methoxyphenyl)-2-oxoethyl]piperidine-4-carboxylate
126	C20H30N2O2	330.4743	N-cyclohexyl-2-(4-methoxyphenyl)-2-piperidin-1-ylacetamide
127	C20H29N3O7	423.47003	N-cyclohexyl-2-[(2,2-dimethoxyethyl)(methyl)amino]-2-(6-nitro-1,3-benzodioxol-5-yl)acetamide
128	C18H23N3O5	361.40111	N-cyclopentyl-2-(6-nitro-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-ylacetamide
129	C22H25N3O5	411.46165	2-[benzyl(methyl)amino]-N-cyclopentyl-2-(6-nitro-1,3-benzodioxol-5-yl)acetamide
130	C19H27N3O7	409.44294	N-cyclopentyl-2-[(2,2-dimethoxyethyl)(methyl)amino]-2-(6-nitro-1,3-benzodioxol-5-yl)acetamide
131	C23H32N4O6S2	524.66269	ethyl 1-{[6-methyl-5-[(4-methylpiperidin-1-yl)sulfonyl]-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl]acetyl}piperidine-4-carboxylate
132	C22H26N2O4	382.46352	3-(4-methoxyphenyl)-N-(3-methoxypropyl)-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide

Compound	Formula	MW	Chemical Name
133	C31H34FN3O4	531.63273	2-(4-fluorobenzyl)-3-(4-methoxyphenyl)-N-(3-morpholin-4-ylpropyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
134	C27H26N4O5	486.53207	ethyl (2Z)-2-[(2-methylbenzoyl)imino]-5-oxo-1-(tetrahydrofuran-2-ylmethyl)-1,5-dihydro-2H-dipyrido[1,2-a:2',3'-d]pyrimidine-3-carboxylate
135	C15H18N4O3	302.33571	methyl 4-[(6-tert-butyl-5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)amino]benzoate
136	C27H25F3N2O5	514.5059	6,7-dimethoxy-3-(4-methoxyphenyl)-2-methyl-1-oxo-N-[3-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
137	C26H24ClFN2O5	498.94298	N-(3-chloro-4-fluorophenyl)-6,7-dimethoxy-3-(4-methoxyphenyl)-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
138	C27H25F3N2O5	514.5059	6,7-dimethoxy-3-(4-methoxyphenyl)-2-methyl-1-oxo-N-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
139	C28H28N2O7	504.54456	N-2,3-dihydro-1,4-benzodioxin-6-yl-6,7-dimethoxy-3-(4-methoxyphenyl)-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
140	C24H30N2O5	426.5171	N-butyl-6,7-dimethoxy-3-(4-methoxyphenyl)-2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
141	C24H22ClN3O4	451.91364	N-(3-chlorophenyl)-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
142	C25H22F3N3O4	485.46699	6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-N-[3-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
143	C24H29N3O4	423.51643	N-cyclohexyl-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
144	C25H24ClN3O5	481.94013	N-(3-chloro-4-methoxyphenyl)-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
145	C26H27N3O6	477.52159	N-(2,4-dimethoxyphenyl)-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
146	C25H25N3O5	447.4951	6,7-dimethoxy-N-(3-methoxyphenyl)-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
147	C27H29N3O7	507.54808	6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-N-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
148	C25H22F3N3O4	485.46699	6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-N-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
149	C25H24BrN3O4	510.39173	N-(4-bromo-2-methylphenyl)-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
150	C26H25N3O6	475.50565	N-2,3-dihydro-1,4-benzodioxin-6-yl-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
151	C26H33N3O4	451.57061	N-(2,3-dimethylcyclohexyl)-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
152	C24H32N4O4	440.54704	N-[2-(diethylamino)ethyl]-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
153	C27H27N3O6	489.53274	ethyl 2-{[(6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinolin-4-yl)carbonyl]amino}benzoate
154	C26H26ClN3O5	495.96722	N-(4-chloro-2-methoxy-5-methylphenyl)-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
155	C28H38N4O4	494.63946	N-[3-(3,5-dimethylpiperidin-1-yl)propyl]-6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
156	C23H24N4O3	404.47273	1-{[1-[(4-methoxyphenyl)amino]isoquinolin-4-yl]carbonyl}piperidine-4-carboxamide
157	C24H31N3O4S	457.59637	N-[(1-ethylpyrrolidin-2-yl)methyl]-6,7-dimethoxy-2-methyl-1-oxo-3-thien-2-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
158	C23H29N3O5S	459.56868	6,7-dimethoxy-2-methyl-N-(2-morpholin-4-ylethyl)-1-oxo-3-thien-2-yl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
159	C22H28N2O6	416.47826	ethyl 1-[(2-ethyl-6,7-dimethoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)carbonyl]piperidine-3-carboxylate
160	C23H24N2O6	424.45753	ethyl 2-{[(2-ethyl-6,7-dimethoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)carbonyl]amino}benzoate
161	C20H16N4O3	360.37552	2-[3-(2,3-dihydro-1,4-benzodioxin-6-ylamino)imidazo[1,2-a]pyrazin-2-yl]phenol
162	C24H34N4O6S2	538.68978	ethyl 1-{[5-[(3,5-dimethylpiperidin-1-yl)sulfonyl]-6-methyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-yl]acetyl}piperidine-4-carboxylate
163	C22H24N2O5S2	460.57498	ethyl 2-({2-[(1-methyl-1H-indol-3-yl)sulfonyl]propanoyl}amino)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate
164	C24H30N2O4S	442.5817	N-butyl-6,7-dimethoxy-2-methyl-3-[4-(methylthio)phenyl]-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
165	C18H23N5O2S	373.48031	pentyl 5-methyl-7-[4-(methylthio)phenyl]-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carboxylate
166	C21H19N3O3S	393.46788	N-(4-butoxyphenyl)-4-oxo-4H-pyrido[1,2-a]thieno[2,3-d]pyrimidine-2-carboxamide

Compound	Formula	MW	Chemical Name
167	C28H31NO7	493.56177	(11Z)-11-[(2E)-1-hydroxy-3-(2,3,4-trimethoxyphenyl)prop-2-enylidene]-5-methoxy-1,2,3,4,9,9a-hexahydro-4a,9-(epiminoethano)xanthen-12-one
168	C27H28N2O4	444.53521	2-(4-ethoxy-3-methoxybenzyl)-3-oxo-N-(2-phenylethyl)isoindoline-1-carboxamide
169	C24H29N3O4S	455.58043	2-[3-(3,4-dimethylphenyl)-2,4-dioxo-3,4,6,7,8,9-hexahydro-2H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-1(5H)-yl]-N-(2-methoxyethyl)acetamide
170	C25H21N5O5	471.47662	3-[2,4-dioxo-1-[(4-oxo-4H-pyrido[1,2-a]pyrimidin-2-yl)methyl]-1,4-dihydroquinazolin-3(2H)-yl]-N-(2-furylmethyl)propanamide
171	C27H27N3O4	457.53394	N-(2-methoxybenzyl)-3-[1-(4-methylbenzyl)-2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl]propanamide
172	C29H27N5O5	525.56904	4-[2,4-dioxo-1-[(4-oxo-4H-pyrido[1,2-a]pyrimidin-2-yl)methyl]-1,4-dihydroquinazolin-3(2H)-yl]-N-(2-methoxybenzyl)butanamide
173	C30H29N5O5	539.59613	N-(2-methoxybenzyl)-4-[1-[(6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-2-yl)methyl]-2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl]butanamide
174	C24H22FN3O4	435.45904	4-[1-(2-fluorobenzyl)-2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl]-N-(2-furylmethyl)butanamide
175	C26H21N3O6	471.47377	N-(4-acetylphenyl)-2-[3-(1,3-benzodioxol-5-ylmethyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl]acetamide
176	C23H23BrN4O4	499.36816	N-(4-bromophenyl)-2-(3-(2-(cyclopentylamino)-2-oxoethyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetamide
177	C22H27NO5	385.46419	ethyl 6-(2,5-dimethoxyphenyl)-4-oxo-3-propyl-4,5,6,7-tetrahydro-1H-indole-2-carboxylate
178	C21H25NO4	355.4377	2-isopropoxyethyl 3-methyl-4-oxo-6-phenyl-4,5,6,7-tetrahydro-1H-indole-2-carboxylate
179	C30H31N3O5	513.59867	N-(2,5-dimethoxybenzyl)-3-(1H-indol-3-yl)-2-(2-methoxyethyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
180	C27H27N3O3S	473.59854	2-(2-methoxyethyl)-3-(1-methyl-1H-indol-3-yl)-1-oxo-N-(thien-2-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
181	C31H33N3O5	527.62576	N-(2,5-dimethoxybenzyl)-2-(2-methoxyethyl)-3-(1-methyl-1H-indol-3-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
182	C31H33N3O4	511.62636	N-(4-ethoxybenzyl)-2-(2-methoxyethyl)-3-(1-methyl-1H-indol-3-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
183	C28H28N2O5	472.54576	N-(4-ethoxyphenyl)-10,11-dimethoxy-8-oxo-5,8,13,13a-tetrahydro-6H-isoquino[3,2-a]isoquinoline-13-carboxamide
184	C23H21N3O4	403.44152	2-[(3,4-dimethoxyphenyl)amino]-N-(2-furylmethyl)quinoline-4-carboxamide
185	C23H27N3O3	393.48994	N-(3-isopropoxypropyl)-2-[(4-methoxyphenyl)amino]quinoline-4-carboxamide
186	C25H28N4O4	448.52631	2-[(2,4-dimethoxyphenyl)amino]-N-[3-(2-oxopyrrolidin-1-yl)propyl]quinoline-4-carboxamide
187	C24H27N3O3	405.50109	4-(azepan-1-ylcarbonyl)-N-(2,4-dimethoxyphenyl)quinolin-2-amine
188	C26H29N3O5	463.53813	ethyl 1-({2-[(2,4-dimethoxyphenyl)amino]quinolin-4-yl}carbonyl)piperidine-3-carboxylate
189	C22H25N3O4	395.46225	2-[(2,5-dimethoxyphenyl)amino]-N-(3-methoxypropyl)quinoline-4-carboxamide
190	C25H27N3O3S	449.57624	ethyl 1-[(2-{[3-(methylthio)phenyl]amino}quinolin-4-yl)carbonyl]piperidine-3-carboxylate
191	C26H32N4O2	432.57054	2-[(4-isopropylphenyl)amino]-N-(3-morpholin-4-ylpropyl)quinoline-4-carboxamide
192	C24H29N3O2	391.51763	N-(3-ethoxypropyl)-2-[(4-isopropylphenyl)amino]quinoline-4-carboxamide
193	C24H30N2O4	410.5177	N-[2-(3,4-diethoxyphenyl)ethyl]-4-methyl-3-(2-oxopyrrolidin-1-yl)benzamide
194	C17H28N2O2	292.42491	4-acetyl-N,N-dibutyl-3,5-dimethyl-1H-pyrrole-2-carboxamide
195	C18H19N3O2S	341.43503	N-(4-butylphenyl)-3-methyl-5-oxo-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxamide
196	C21H27ClN5O3	446.94074	7-(4-chlorobenzyl)-8-{[4-(2-hydroxyethyl)piperazin-1-yl]methyl}-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione
197	C18H29N5O3	363.46353	8-[(3,5-dimethylpiperidin-1-yl)methyl]-7-(3-hydroxypropyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione
198	C21H24N4O4S	428.51383	N~2~-[(3,5-dimethyl-1H-pyrazol-4-yl)sulfonyl]-N~2~-(4-methoxyphenyl)-N~1~-(4-methylphenyl)glycinamide
199	C26H29N3O5	463.53813	4-{4-[4-(1,3-benzodioxol-5-ylmethyl)piperazin-1-yl]-4-oxobutoxy}-1-methylquinolin-2(1H)-one
200	C20H22N4O5S	430.48614	methyl 2-{[2-(4-methoxyphenyl)-6,8-dimethyl-5,7-dioxo-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-4-yl]thio}butanoate
201	C21H25ClN6O4	460.9242	ethyl 4-{[7-(2-chlorobenzyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl]methyl}piperazine-1-carboxylate
202	C21H24N6O2	392.46443	8,9-dimethoxy-N-(3-methylbutyl)-2-pyridin-3-yl[1,2,4]triazolo[1,5-c]quinazolin-5-amine
203	C25H19N3O3	409.44848	2,3-di-2-furyl-N-(2-phenylethyl)quinoxaline-6-carboxamide

Compound	Formula	MW	Chemical Name
204	C22H31N3O5	417.50947	N-cycloheptyl-3-(6,7-diethoxy-2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)propanamide
205	C24H24FN3O3S2	485.60358	N-(4-fluorophenyl)-2-{[3-(3-isopropoxypropyl)-4-oxo-3,4-dihydro[1]benzothieno[3,2-d]pyrimidin-2-yl]thio}acetamide
206	C30H26N6O4S	566.64352	N-(4-methoxybenzyl)-3-(3-oxo-5-{[(4-oxo-4H-pyrido[1,2-a]pyrimidin-2-yl)methyl]thio}-2,3-dihydroimidazo[1,2-c]quinazolin-2-yl)propanamide
207	C26H23N3O5S	489.55431	2-{[3-(1,3-benzodioxol-5-ylmethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}-N-(4-ethoxyphenyl)acetamide
208	C21H19N5O3	389.41728	ethyl 4-{[1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino}benzoate
209	C21H21N5O2	375.43382	N-(2,4-dimethoxyphenyl)-1-(2,4-dimethylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine
210	C24H26N4O	386.50102	N-(3-isopropoxypropyl)-5,7-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine
211	C22H21ClN4O2	408.89127	2-(2-{[7-(4-chlorophenyl)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amino}ethoxy)ethanol
212	C23H24N4O2	388.47333	2-(2-{[7-(3-methylphenyl)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amino}ethoxy)ethanol
213	C26H27N5O	425.53799	1-(3-{[7-(3-methylphenyl)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amino}propyl)pyrrolidin-2-one
214	C28H31NO7	493.56177	2-(3,4-dimethoxybenzoyl)-6,7-dimethoxy-1-[(2-methoxyphenoxy)methyl]-1,2,3,4-tetrahydroisoquinoline
215	C26H26N2O6	462.50692	6,7-dimethoxy-1-[(4-nitrophenoxy)methyl]-2-(phenylacetyl)-1,2,3,4-tetrahydroisoquinoline
216	C28H29NO6	475.54643	methyl 4-{[6,7-dimethoxy-2-(phenylacetyl)-1,2,3,4-tetrahydroisoquinolin-1-yl]methoxy}benzoate
217	C28H31NO6	477.56237	6,7-dimethoxy-1-[(4-methoxyphenoxy)methyl]-2-(2-phenoxypropanoyl)-1,2,3,4-tetrahydroisoquinoline
218	C22H21N5O3S3	499.63637	2-({2-[5-(4-methoxyphenyl)-3-thien-2-yl-4,5-dihydro-1H-pyrazol-1-yl]-2-oxoethyl}thio)-6,7-dihydro[1,3,4]thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one
219	C31H33N3O7	559.62456	4-methoxy-N-[(Z)-1-[(8-oxo-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a][1,5]diazocin-3(4H)-yl)carbonyl]-2-(3,4,5-trimethoxyphenyl)vinyl]benzamide
220	C19H22N2O6	374.39699	ethyl 4-{[2-(3,4-dimethoxyphenyl)ethyl]amino}-3-nitrobenzoate
221	C25H27N3O2S	433.57684	2-({2-[(4-isopropylphenyl)amino]-2-oxoethyl}thio)-N-(4-methylbenzyl)nicotinamide
222	C24H34N2O4	414.54958	4-cyclopentyl-3-(3,4-dimethoxyphenyl)-1-(4-methylcyclohexyl)piperazine-2,5-dione
223	C23H30N4O3	410.52055	methyl 2-(4-butoxyphenyl)-3-(cyclohexylamino)-1H-imidazo[1,2-b]pyrazole-7-carboxylate
224	C24H28N2O3	392.50236	4-cyclopentyl-3-(4-methoxyphenyl)-1-(2-phenylethyl)piperazine-2,5-dione
225	C26H26N2O4	430.50812	2-benzyl-N-[2-(3,4-dimethoxyphenyl)ethyl]-3-oxoisoindoline-1-carboxamide
226	C19H18BrClN2O4	453.72331	N-(5-bromo-2-methoxybenzyl)-2-(6-chloro-3-oxo-2,3-dihydro-4H-1,4-benzoxazin-4-yl)propanamide
227	C19H24N2O5	360.41353	ethyl 1-[3-(3-oxo-2,3-dihydro-4H-1,4-benzoxazin-4-yl)propanoyl]piperidine-3-carboxylate
228	C20H22N2O5	370.40874	N-(4-ethoxy-3-methoxybenzyl)-2-(3-oxo-2,3-dihydro-4H-1,4-benzoxazin-4-yl)acetamide
229	C28H28N4O3S	500.62436	N-[4-(dimethylamino)phenyl]-2-[11-oxo-8-(pyrrolidin-1-ylcarbonyl)dibenzo[b,f][1,4]thiazepin-10(11H)-yl]acetamide
230	C28H27N3O4S	501.60909	N-(4-ethoxyphenyl)-2-[11-oxo-8-(pyrrolidin-1-ylcarbonyl)dibenzo[b,f][1,4]thiazepin-10(11H)-yl]acetamide
231	C28H27N3O3S	485.60969	N-(4-ethylphenyl)-2-[11-oxo-8-(pyrrolidin-1-ylcarbonyl)dibenzo[b,f][1,4]thiazepin-10(11H)-yl]acetamide
232	C30H30N4O5	526.5974	4-{[4-(1,3-benzodioxol-5-ylmethyl)piperazin-1-yl]carbonyl}-N-(2,4-dimethoxyphenyl)quinolin-2-amine
233	C26H25N3O4	443.50685	2-[(2,4-dimethoxyphenyl)amino]-N-(2-methoxybenzyl)quinoline-4-carboxamide
234	C28H27N3O6S	533.60789	N-(2,4-dimethoxyphenyl)-2-[8-(morpholin-4-ylcarbonyl)-11-oxodibenzo[b,f][1,4]thiazepin-10(11H)-yl]acetamide
235	C29H29N3O4S	515.63618	N-(4-isopropylphenyl)-2-[8-(morpholin-4-ylcarbonyl)-11-oxodibenzo[b,f][1,4]thiazepin-10(11H)-yl]acetamide
236	C19H18FN3O4S	403.43541	ethyl 4-[(2-fluorophenyl)amino]-2-(2-methoxy-2-oxoethyl)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate
237	C17H23N3O4S	365.45456	ethyl 4-(butylamino)-2-(2-methoxy-2-oxoethyl)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate
238	C20H23N5O3	381.43801	5-(4-methylphenyl)-7-(2,3,4-trimethoxyphenyl)-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidine
239	C19H20BrN5O3	446.30695	5-(4-bromophenyl)-7-(2,3,4-trimethoxyphenyl)-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidine

Compound	Formula	MW	Chemical Name
240	C21H22N4O3	378.43449	7-(2,5-dimethoxyphenyl)-5-(4-ethoxyphenyl)-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine
241	C21H24N4O2	364.45103	7-(2,5-dimethoxyphenyl)-5-(4-ethylphenyl)-4,5,6,7-tetrahydro[1,2,4]triazolo[1,5-a]pyrimidine
242	C20H23N5O2	365.43861	7-(2,5-dimethoxyphenyl)-5-(4-ethylphenyl)-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidine
243	C25H26ClNO5	455.94267	diethyl 4-(3-chlorophenyl)-1-(4-methoxybenzyl)-1,4-dihydropyridine-3,5-dicarboxylate
244	C16H15F3N2O3S	372.36875	ethyl 2-amino-4-methyl-5-({[2-(trifluoromethyl)phenyl]amino}carbonyl)thiophene-3-carboxylate
245	C20H17ClN2O4S	416.88649	1-(4-chlorophenyl)-2-([4-hydroxy-5-(4-methoxyphenoxy)-6-methylpyrimidin-2-yl]thio)ethan-1-one
246	C19H21FN2O5S	408.45202	ethyl 3-{[(4-fluorophenyl)sulfonyl]amino}-4-morpholinobenzoate
247	C28H28F6O6S2	638.65016	2,5-di(tert-butyl)-4-({[4-(trifluoromethyl)phenyl]sulfonyl}oxy)phenyl 4-(trifluoromethyl)benzene-1-sulfonate
248	C21H20N2O5S	412.46795	2-([4-hydroxy-5-(4-methoxyphenoxy)-6-methylpyrimidin-2-yl]thio)-1-(4-methoxyphenyl)ethan-1-one
249	C16H14N2O5	314.30038	Ethyl 3-hydroxy-5-methyl-6-oxo-1-phenyl-1,6-dihydropyrano[2,3-c]pyrazole-4-carboxylate
250	C19H21ClN2O4S	408.90722	1-(1,3-benzodioxol-5-ylmethyl)-4-[(4-chlorobenzyl)sulfonyl]piperazine
251	C22H23NO2	333.43411	1-benzyl-3-butyl-4-hydroxy-6-phenylpyridin-2(1H)-one
252	C18H18N2OS	310.42096	N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide
253	C16H21N3O4	319.36347	ethyl 5-[4-(2-methoxyethoxy)anilino]-3-methyl-1H-pyrazole-4-carboxylate
254	C26H22ClN3O4S	507.99994	2-({5-[({[3-(2-chlorophenyl)-5-methylisoxazol-4-yl]carbonyl}oxy)ethanimidoyl]-2-methoxybenzyl}thio)pyridine
255	C24H18N2O3S	414.48666	[2-(2,3-dihydro-1,4-benzodioxin-6-ylamino)-4-phenyl-1,3-thiazol-5-yl](phenyl)methanone
256	C21H16F3N3OS2	447.50437	2-(1,3-benzothiazol-2-ylsulfanyl)-1-[1-(trifluoromethyl)-1,3,4,9-tetrahydro-2H-beta-carbolin-2-yl]-1-ethanone
257	C24H17FN2O3S	432.47709	[2-(2,3-dihydro-1,4-benzodioxin-6-ylamino)-4-phenyl-1,3-thiazol-5-yl](4-fluorophenyl)methanone
258	C17H18F3N3O2	353.34711	ethyl 2-(4-isopropylanilino)-4-(trifluoromethyl)-5-pyrimidinecarboxylate
259	C12H11N5O4S	321.31657	ethyl 6-methyl-3-(5-nitro-2-thienyl)-1H-pyrazolo[5,1-c][1,2,4]triazole-7-carboxylate,80<90%
260	C18H18F3N3O5	413.35646	diethyl 2-({2-methyl-4-[5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl]anilino}methylidene)malonate
261	C20H18F3N3O3	405.37996	2-furylmethyl 2-(4-isopropylanilino)-4-(trifluoromethyl)pyrimidine-5-carboxylate
262	C23H29NO5	399.49128	4-(3,5-dimethoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
263	C24H25NO3	375.47175	N-[2-(3,4-dimethoxyphenyl)ethyl]-2,2-diphenylacetamide
264	C20H25NO5	359.42595	3-butyryl-4-{[2-(3,4-dimethoxyphenyl)ethyl]amino}-6-methyl-2H-pyran-2-one
265	C22H28N2O6	416.47826	4-{[4-(3,5-dimethoxybenzoyl)-1-piperazinyl]methyl}-2,6-dimethoxyphenol
266	C17H19NO5	317.34468	1,3-benzodioxol-5-yl(2,3,4-trimethoxybenzyl)amine
267	C15H19NO4	277.32298	ethyl 6-[(dimethylamino)methyl]-5-hydroxy-2-methyl-1-benzofuran-3-carboxylate
268	C24H27NO3	377.48769	(1,2-diphenylethyl)(2,3,4-trimethoxybenzyl)amine
269	C23H24N2O4S	424.52273	N-(2-methoxyethyl)-4-{[phenyl(phenylsulfonyl)amino]methyl}benzamide
270	C22H21ClN2O4S	444.94067	N^1-(4-chloro-2-methylphenyl)-N^2-(4-methoxyphenyl)-N^2-(phenylsulfonyl)glycinamide
271	C25H25ClO5	456.9274	2-[6-(2-chlorobenzoyl)-3,4-dimethoxy-2,4-cyclohexadien-1-yl]-1-(3,4-dimethoxyphenyl)ethanone
272	C21H24N2O4	368.43643	1-(4-ethoxyphenyl)-3-{[2-(4-methoxyphenyl)ethyl]amino}-2,5-pyrrolidinedione
273	C23H22N2O3	374.44339	(1-benzyl-1H-benzimidazol-2-yl)(3,4-dimethoxyphenyl)methanol
274	C18H18Cl2N2O3	381.26176	N-(4-{[2-(2,4-dichlorophenoxy)acetyl]amino}phenyl)butanamide
275	C20H24N2O3	340.42588	N-(4-{[2-(3,4-dimethylphenoxy)acetyl]amino}phenyl)butanamide
276	C20H28N2O3	344.45776	1-(3-cyclopentylpropanoyl)-4-(3-methoxybenzoyl)piperazine
277	C19H22N2O3	326.39879	N-(4-{[2-(2,4-dimethylphenoxy)acetyl]amino}phenyl)propanamide
278	C22H30N2O4	386.4954	1-(4-methoxybenzyl)-4-(2,4,5-trimethoxybenzyl)piperazine
279	C25H29N3O2	403.52878	3-benzyl-5,5-diethyl-2-[(2-hydroxyethyl)amino]-5,6-dihydrobenzo[h]quinazolin-4(3H)-one
280	C21H22BrFN2O4	465.32289	ethyl 4-[4-(3-bromo-4-methoxybenzoyl)-2-fluorophenyl]-1-piperazinecarboxylate
281	C18H30N2O2	306.452	4-{2-[(4-methoxybenzyl)amino]ethyl}-1,2,5-trimethyl-4-piperidinol
282	C27H24ClN3O3S	506.02763	4-{[(5-chloro-2-methylphenyl)(phenylsulfonyl)amino]methyl}-N-(2-pyridinylmethyl)benzamide
283	C26H27ClN2O4S	499.03309	4-{[(5-chloro-2-methylphenyl)(phenylsulfonyl)amino]methyl}-N-(tetrahydro-2-furanylmethyl)benzamide

Compound	Formula	MW	Chemical Name
284	C27H24ClN3O3S	506.02763	4-{[(5-chloro-2-methylphenyl){phenylsulfonyl)amino}methyl}-N-(3-pyridinylmethyl)benzamide
285	C23H32N2O6	432.52129	2,6-dimethoxy-4-{[4-(2,3,4-trimethoxybenzyl)-1-piperazinyl]methyl}phenol ethanedioate (salt)
286	C19H21N3O3	339.39752	3-ethyl-1-[(4-methoxyphenyl)acetyl]-5-(4-pyridinyl)-4,5-dihydro-1H-pyrazol-5-ol
287	C16H15F3N2O3S	372.36875	ethyl 2-amino-4-methyl-5-({[2-(trifluoromethyl)phenyl]amino}carbonyl)-3-*thiophenecarboxylate*
288	C18H20N2O4	328.3711	methyl 4-{[(3,5-dioxo-4-azatricyclo[5.2.1.$0^{2,6}$]dec-4-yl)methyl]amino}benzoate
289	C24H23NO3	373.45581	N-[bis(4-methoxyphenyl)methyl]-3-phenylacrylamide
290	C26H27N3O2	413.52399	N-{2-[(4-benzyl-1-piperazinyl)carbonyl]phenyl}-2-methylbenzamide
291	C25H26N2O4S	450.56097	N-(2-ethoxyphenyl)-2-{[(4-methoxyphenyl)acetyl]amino}-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxamide
292	C19H19ClN2O3	358.82788	5-(4-chlorophenyl)-1-[(4-methoxyphenyl)acetyl]-3-methyl-4,5-dihydro-1H-pyrazol-5-ol
293	C24H25ClN2O4S	472.99485	4-({(3-chlorophenyl)[(4-methylphenyl)sulfonyl]amino}methyl)-N-(2-methoxyethyl)benzamide
294	C20H19NO4	337.37873	2-(2,4-diethoxyphenyl)-4-quinolinecarboxylic acid
295	C21H20ClNO	337.85265	2-(benzylamino)-1-(4-chlorophenyl)-1-phenylethanol
296	C23H23NO4	377.44406	N-benzyl-2-hydroxy-2,2-bis(4-methoxyphenyl)acetamide
297	C21H22N2O4	366.42049	*ethyl 4-[(4-methoxyphenyl)amino]-2-methyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrrole-2-carboxylate*
298	C19H24O6	348.39953	2-methoxyethyl 5-(3,3-dimethyl-2-oxobutoxy)-2-methyl-1-benzofuran-3-carboxylate
299	C19H32ClNO4	373.92419	*1-[2-hydroxy-3-(3-methoxyphenoxy)propyl]-2,2,6,6-tetramethyl-4-piperidinol* hydrochloride
300	C20H26N2O3S	374.50582	5-{2-[(4-ethoxyphenyl)amino]-1,3-thiazol-4-yl}-3-isobutyl-5-methyldihydro-2(3H)-*furanone*
301	C23H19Cl2N3O2	440.33278	ethyl 3-{[2-(2-chlorophenyl)-4-quinazolinyl]amino}benzoate
302	C19H28N2O5	364.44541	ethyl 1-{3-[(2,5-dimethoxyphenyl)amino]-3-oxopropyl}-4-piperidinecarboxylate *hydrochloride*
303	C18H26N2O4	334.41892	ethyl 1-{3-[(2-methoxyphenyl)amino]-3-oxopropyl}-4-piperidinecarboxylate
304	C20H34N2O2	334.50618	4-{2-[(4-isopropoxybenzyl)amino]ethyl}-1,2,5-trimethyl-4-piperidinol
305	C27H37N3O3	451.61424	1-{[1-(4-ethoxybenzyl)-4-piperidinyl]carbonyl}-4-(2-ethoxyphenyl)piperazine oxalate
306	C13H16N2O3S	280.34807	ethyl 4-{[(propionylamino)carbonothioyl]amino}benzoate
307	C26H29NO5S	467.58873	2-ethoxyethyl 4-(4-methoxyphenyl)-2-methyl-5-oxo-7-(2-thienyl)-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
308	C21H24N2O5S	416.49983	*3-hydroxy-5-(5-methyl-2-furyl)-1-[3-(4-morpholinyl)propyl]-4-(2-thienylcarbonyl)*-1,5-dihydro-2H-pyrrol-2-one
309	C18H20N2O3	312.3717	N-[2-(acetylamino)phenyl]-2-(2,5-dimethylphenoxy)acetamide
310	C24H21NO5	403.43867	*4-{2-[2-(4-methoxyphenyl)-2-oxoethoxy]phenyl}-4-azatricyclo*[5.2.1.$0^{2,6}$]*dec-8-ene*-3,5-dione
311	C25H30N2O5	438.52825	1-[2-(dimethylamino)ethyl]-5-(2-ethoxyphenyl)-3-hydroxy-4-(4-methoxy-3-*methylbenzoyl*)-1,5-*dihydro*-2H-*pyrrol*-2-one
312	C27H29NO4	431.53648	N-[3-(1,3-benzodioxol-5-yl)-3-(2-methoxyphenyl)propyl]-N-benzylpropanamide
313	C22H21ClN2O3	396.87727	2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-N-cyclopropylacetamide
314	C19H20Cl2N2O	363.29005	6-chloro-2-methyl-N-(4-propoxyphenyl)-4-quinolinamine hydrochloride
315	C24H22N2O3S	418.51854	2-[(2-cyanophenyl)thio]-N-[2-(3,4-dimethoxyphenyl)ethyl]benzamide
316	C25H25N3O3	415.4963	7-[(4-ethoxy-3-methoxyphenyl)(2-pyridinylamino)methyl]-2-methyl-8-quinolinol
317	C22H22O6	382.41704	Methyl {7-[(3-methoxybenzyl)oxy]-4,8-dimethyl-2-oxo-2H-chromen-3-yl}acetate
318	C24H21NO3	371.43987	N-(3-acetylphenyl)-3-(4-methoxyphenyl)-2-phenylacrylamide
319	C22H25N3O4	395.46225	4-(2,4-dimethoxyphenyl)-N-(2,5-dimethylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide
320	C15H21N5O	287.36752	N^4-(3-methoxyphenyl)-1,3,5-triazaspiro[5.5]undeca-1,3-diene-2,4-diamine
321	C23H23N3O5	421.45686	2-{2-[4-(1,3-benzodioxol-5-ylmethyl)-1-piperazinyl]-1-methyl-2-oxoethyl}-1H-isoindole-1,3(2H)-dione
322	C26H23ClN2O5	478.93661	*N-(4-chloro-2,5-dimethoxyphenyl)-2-(2,4-dimethoxyphenyl)-4-quinolinecarboxamide*
323	C19H21ClN2O3	360.84382	N-(4-{[(4-chloro-3-methylphenoxy)acetyl]amino}phenyl)-2-methylpropanamide
324	C20H18O6	354.36286	ethyl 7-methoxy-3-(2-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylate
325	C21H21N5O3	391.43322	*1-(3-methylphenyl)-N-(3,4,5-trimethoxyphenyl)-1H-pyrazolo*[3,4-d]pyrimidin-4-amine
326	C21H25N3O3	367.4517	N-(tert-butyl)-2-{[4-(propionylamino)benzoyl]amino}benzamide
327	C19H22N2O4	342.39819	N-[2-(butyrylamino)phenyl]-2,4-dimethoxybenzamide
328	C23H23N3O4	405.45746	4-(2,5-dimethylbenzoyl)-5-(2-furyl)-3-hydroxy-1-[3-(1H-imidazol-1-yl)propyl]-1,5-dihydro-2H-pyrrol-2-one
329	C21H22N4O3S	410.49849	Ethyl 4-({[(4-ethyl-5-phenyl-4H-1,2,4-triazol-3-yl)thio]acetyl}amino)benzoate
330	C20H18ClF3N4O2S	470.90426	N-[2-chloro-5-(trifluoromethyl)phenyl]-2-{[5-(4-methoxybenzyl)-4-methyl-4H-1,2,4-

Compound	Formula	MW	Chemical Name
			triazol-3-yl)thio}acetamide
331	C22H24N2O3S	396.51218	N-{2-[(2-methoxyphenyl)amino]-2-phenylethyl}-4-methylbenzenesulfonamide
332	C23H21NO4	375.42812	benzyl 3-benzyl-4-oxo-10-oxa-3-azatricyclo[5.2.1.0^{1,5}]dec-8-ene-6-carboxylate
333	C27H32N2O5S	496.63049	N^2-[(3,4-dimethoxyphenyl)sulfonyl]-N^2-(3,5-dimethylphenyl)-N^1-mesitylglycinamide
334	C21H27NO5	389.45244	dimethyl 1-butyl-4-(2,4-dimethoxyphenyl)-1,4-dihydro-3,5-pyridinedicarboxylate
335	C24H20N2O4S	432.502	3-hydroxy-4-[(2-methyl-2,3-dihydro-1-benzofuran-5-yl)carbonyl]-1-(3-pyridinylmethyl)-5-(2-thienyl)-1,5-dihydro-2H-pyrrol-2-one
336	C21H25BrN2O4	449.3484	N-(4-{[(2-bromo-4,6-dimethylphenoxy)acetyl]amino}-2-methoxyphenyl)-2-methylpropanamide
337	C29H22ClFN2O3	500.96169	2-amino-4-{3-[(2-chloro-6-fluorobenzyl)oxy]phenyl}-5-oxo-7-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile
338	C19H25N3O3S	375.4934	ethyl {[5-cyano-3,3-dimethyl-8-(1-pyrrolidinyl)-3,4-dihydro-1H-pyrano[3,4-c]pyridin-6-yl]thio}acetate
339	C23H24F3N5O2S	491.53923	N-(2-methyl-1-{4-methyl-5-[(2-oxo-2-{[3-(trifluoromethyl)phenyl]amino}ethyl)thio]-4H-1,2,4-triazol-3-yl}propyl)benzamide
340	C20H19ClN4O3S	430.91643	ethyl 4-[({[5-(4-chlorophenyl)-4-methyl-4H-1,2,4-triazol-3-yl]thio}acetyl)amino]benzoate
341	C21H25ClN2O4	404.8974	N-(4-{[(4-chloro-3,5-dimethylphenoxy)acetyl]amino}-2-methoxyphenyl)-2-methylpropanamide
342	C15H31NO3	273.41922	1-(2-hydroxy-3-isopropoxypropyl)-2,2,6,6-tetramethyl-4-piperidinol
343	C19H19ClN4O2	370.84188	5-(4-chlorophenyl)-7-(3,4-dimethoxyphenyl)-4,5,6,7-tetrahydro[1,2,4]triazolo[1,5-a]pyrimidine
344	C18H26N6	326.44812	6-(1-azepanylmethyl)-N-(2,4-dimethylphenyl)-1,3,5-triazine-2,4-diamine
345	C23H26ClN5O2S	472.01297	N-{1-[5-({2-[(3-chloro-4-methylphenyl)amino]-2-oxoethyl}thio)-4-ethyl-4H-1,2,4-triazol-3-yl]ethyl}-2-methylbenzamide
346	C18H20N2O	280.3729	1-(2-biphenylylcarbonyl)-4-methylpiperazine
347	C27H25NO5	443.504	benzyl 7-(2-furyl)-2-methyl-4-(5-methyl-2-furyl)-5-oxo-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
348	C24H17FN2O5	432.41189	4-(1,3-benzodioxol-5-ylcarbonyl)-5-(3-fluorophenyl)-3-hydroxy-1-(3-pyridinylmethyl)-1,5-dihydro-2H-pyrrol-2-one
349	C19H20N2O3S	356.44685	N-(6-propoxy-1,3-benzothiazol-2-yl)-2,3,3a,7a-tetrahydro-1-benzofuran-2-carboxamide
350	C17H20N2O3	300.36055	2-(3-acetyl-1H-indol-1-yl)-N-(tetrahydro-2-furanylmethyl)acetamide
351	C25H25F3N2O5S	522.5476	N^2-[(3,4-dimethoxyphenyl)sulfonyl]-N^2-(3,5-dimethylphenyl)-N^1-[2-(trifluoromethyl)phenyl]glycinamide
352	C24H23ClN2O6	470.91371	4-(1,3-benzodioxol-5-ylcarbonyl)-5-(3-chlorophenyl)-3-hydroxy-1-[2-(4-morpholinyl)ethyl]-1,5-dihydro-2H-pyrrol-2-one
353	C21H18N2O4S	394.45261	methyl 4-({phenyl[(phenylsulfonyl)imino]methyl}amino)benzoate
354	C20H22Cl2N2O4	425.31534	2-(2,4-dichlorophenoxy)-N-[4-(isobutyrylamino)-3-methoxyphenyl]propanamide
355	C23H18FNO4S	423.46661	3-{3-[(4-fluorobenzyl)oxy]-4-methoxyphenyl}-2-(phenylsulfonyl)acrylonitrile
356	C21H21ClN4O3S	444.94352	ethyl 4-[({[5-(3-chlorophenyl)-4-ethyl-4H-1,2,4-triazol-3-yl]thio}acetyl)amino]benzoate
357	C23H24FN3O3	409.46443	ethyl 1-{[3-(4-fluorophenyl)-4-oxo-3,4-dihydro-2-quinazolinyl]methyl}-4-piperidinecarboxylate
358	C18H22N2O4S2	394.51504	N^2-(3-methoxyphenyl)-N^2-(methylsulfonyl)-N^1-[2-(phenylthio)ethyl]glycinamide
359	C23H17NO3	355.39684	N-(2-oxo-2H-chromen-6-yl)-2,2-diphenylacetamide
360	C24H17NO5	399.40679	4-benzoyl-2-methylphenyl (1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)acetate
361	C19H20N4O3S2	416.52425	ethyl 4-[({[4-ethyl-5-(2-thienyl)-4H-1,2,4-triazol-3-yl]thio}acetyl)amino]benzoate
362	C23H24N2O5	408.45813	2-hydroxy-2,2-bis(3-methoxyphenyl)-N'-(4-methoxyphenyl)acetohydrazide
363	C22H28N2O2	352.48066	3-[(2-ethylbutanoyl)amino]-N-(2-isopropylphenyl)benzamide
364	C21H29N3O4S	419.54698	ethyl 4-({5-cyano-3,3-dimethyl-8-(4-morpholinyl)-3,4-dihydro-1H-pyrano[3,4-c]pyridin-6-yl]thio}butanoate

Example II

Design of Synthetic Anti-Id Peptides

The E47 molecule from an X-ray of the structure of an E47-Id1 heterodimer was used as a template for peptide design. The peptides were designed to form leucine-zipper type dimers with Id1 (but not with E47), which could be stabilized by introduction of polar or charged side chains forming inter-molecular H-bonds and salt bridges with Id1. The designed peptides were deemed to have significant probability of retaining an α-helical conformation in solution. For certain peptides, the helical propensity of peptides was enhanced by introduction of polar or charged side chains to form intra-molecular H-bonds and salt bridges at positions that did not interact with Id1. Only peptides containing natural amino acids only were considered.

Multiple dynamics simulations were performed for the model of Id1-peptide complex to assess stability of proposed peptide-dimer structure. Simulations were carried out in vacuum at 300°K with the AMBER-94 force field. Positional constraints were applied to maintain backbone atoms of Id1 close to the crystal structure; side chain atoms of Id1 and all atoms of the peptide were not constrained. During the 40 picosecond equilibration and 300 picosecond acquisition simulations, dimer structure remained close to the initial model with relatively small fluctuation in potential energy. The peptide maintained an α-helical structure during entire multiple dynamics trajectory. Root mean square deviations between peptide positions in the initial model and in the final multiple dynamics structure was 0.50 Å for C^{α} atoms and 0.74 Å for all heavy atoms. The inter-molecular H-bonds anchoring the peptide to Id1 were calculated to remain stable during the multiple dynamics simulations.

Twelve peptides that met the required criteria were identified as candidate anti-Id compounds (Table 2).

Table 2: Twelve synthetic peptides created using the E47 molecule from an X-ray of the structure of E47- Id1 heterodimer as a template for peptide design.

Compound	Chemical Name
1	ELLILQRLVQVILALQ-OH (SEQ ID NO. 5)
2	ELLFLQRLVQLILALQ-OH (SEQ ID NO. 6)
3	ELLFLQALVQLILALQ-OH (SEQ ID NO. 7)
4	ELLFLQRLVQLIEALQ-OH (SEQ ID NO. 8)
5	DLLLLQRLVQLIEALQ-OH (SEQ ID NO. 9)
6	DLLFLQRLVQLIEALQ-OH (SEQ ID NO. 10)
7	ELKFLQRLVQLIEALQ-OH (SEQ ID NO. 11)
8	ELEFLQRLVQLIEALQ-OH (SEQ ID NO. 12)
9	ELEFLQRLVDLIEKLQ-OH (SEQ ID NO. 13)
10	DLEFLQRLVDLIEKLQ-OH (SEQ ID NO. 14)
11	ELKFLQRLVDLIEKLQ-OH (SEQ ID NO. 15)
12	ELKFLQRLVDLIEKLE-OH (SEQ ID NO. 16)

The identified compounds and peptides were subjected to further testing and analysis using the gel shift and cellular assays described in further detail in the examples that follow.

Example III

Gel Shift Assays of Synthetic Anti-Id Compounds

Gel shift assays were performed using the 376 small molecules identified in the *in silico* drug screen and peptide synthesis. The identified compounds were dissolved in 5% DMSO to a concentration of 100μm and reacted in binding mixtures that contained E47, Id1 and Mck. As controls, recombinant human E47 was used in a standard binding reaction that contained ^{32}P labeled MCK double stranded oligonucleotides containing consensus E-Box response element (MCK- 5' TTG ATC CCC CCA ACA CCT GCT GCC TGA AGC T (SEQ ID NO. 17)) and 100ng of Id1 was added to a standard binding reaction using E47 and p32 labeled MCK. After 30 minutes of incubation, the reaction mixtures were resolved on a 5% non-denaturing polyacrylamide gel and autoradiographed. The bound E47-MCK resulted in a shifted band whereas the unbound MCK migrated to the bottom of the gel. The reaction

mixture containing Id1 and E47 and MCK showed a failure of E47 to bind to MCK. If the identified molecules were capable of inhibiting Id1, the gel shift assay showed a shifted band of bound E47-MCK. The presence of shifted band also suggests that the small molecules have no effect on the interaction between E47 and MCK, a critical observation that demonstrates the absence of any non-specific effect of small molecules on the normal bHLH mediated transcriptional pathway.

Of the 376 small molecules identified in the *in silico* drug screen, the 17 in Table 3 were identified as demonstrating the most pronounced anti-Id activity in the gel shift assays. These molecules resulted in pronounced gel shifts in the presence of E47, Id1 and MCK.

Table 3: *Compounds showing the most pronounced anti-Id activity in gel shift assays.*

Compound	Formula	MW	Chemical Name
43	C22H30N2O4	386.4954	1-(4-methoxybenzyl)-4-(2,4,5-trimethoxybenzyl)piperazine
118	C23H21ClN2O3	408.88842	N'-acetyl-N'-(3-chloro-4-methylphenyl)-2-hydroxy-2,2-diphenylacetohydrazide
142	C25H22F3N3O4	485.46699	6,7-dimethoxy-2-methyl-1-oxo-3-pyridin-3-yl-N-[3-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydroisoquinoline-4-carboxamide
169	C24H29N3O4S	455.58043	2-[3-(3,4-dimethylphenyl)-2,4-dioxo-3,4,6,7,8,9-hexahydro-2H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-1(5H)-yl]-N-(2-methoxyethyl)acetamide
194	C17H28N2O2	292.42491	4-acetyl-N,N-dibutyl-3,5-dimethyl-1H-pyrrole-2-carboxamide
235	C29H29N3O4S	515.63618	N-(4-isopropylphenyl)-2-[8-(morpholin-4-ylcarbonyl)-11-oxodibenzo[b,f][1,4]thiazepin-10(11H)-yl]acetamide
302	C19H28N2O5	364.44541	ethyl 1-{3-[(2,5-dimethoxyphenyl)amino]-3-oxopropyl}-4-piperidinecarboxylate hydrochloride
287	C16H15F3N2O3S	372.36875	ethyl 2-amino-4-methyl-5-{{[2-(trifluoromethyl)phenyl]amino}carbonyl}-3-thiophenecarboxylate
312	C27H29NO4	431.53648	N-[3-(1,3-benzodioxol-5-yl)-3-(2-methoxyphenyl)propyl]-N-benzylpropanamide
317	C22H22O6	382.41704	Methyl (7-[(3-methoxybenzyl)oxy]-4,8-dimethyl-2-oxo-2H-chromen-3-yl)acetate
249	C16H14N2O5	314.30038	Ethyl 3-hydroxy-5-methyl-6-oxo-1-phenyl-1,6-dihydropyrano[2,3-c]pyrazole-4-carboxylate
250	C19H21ClN2O4S	408.90722	1-(1,3-benzodioxol-5-ylmethyl)-4-[(4-chlorobenzyl)sulfonyl]piperazine
252 (AGX8)	C18H18N2OS	310.42096	N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide
8	C27H31BrN2O5	543.46252	RCL L34,794-9
10	C28H33FN2O5	496.58401	RCL L34,930-5
11	C25H26BrFN2O5	533.39877	RCL L35,342-6
364	ELEFLQRLVDLIEKLQ (SEQ ID NO. 13)	1987.01	ELEFLQRLVDLIEKLQ-OH (SEQ ID NO. 13)

The molecules identified in Table 3 mediated gel shift in the presence of E47, Id1 and E box. These 17 molecules were subjected to additional gel shift assays to ensure repeatability and consistency of results. All these compounds resulted in a specific hit in the gel shift assays, indicating they are capable of disrupting Id1-E47 dimerization interactions

under conditions of the assay. Based on the intensity of the gel shift (considered equivalent to potency Id1-E47 dimerization blockade), two small molecules: N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide and N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (N-[3-(1,3-benzodioxol-5-yl)-3-(2-methoxyphenyl)propyl]-N-benzylpropanamide) were selected for further testing. However, all 376 molecules in Tables 1, 2 and specifically all 17 molecules listed in Table 3 are considered anti-Id candidate compounds for use within the methods and compositions of the invention.

N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide (HTS 03876, Maybridge) and N-[3-(1,3-benzodioxol-5-yl)-3-(2-methoxyphenyl)propyl]-N-benzylpropanamide (N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide) (6958667, Chembridge) were identified based on their ability to restore the binding between E47-MCK in the presence of Id1. The ability of these molecules to block E47-Id1 interaction was confirmed at least twice in a similar assay before analyzing their effects on cell based assays. In addition, the effect of both compounds demonstrated dose-proportionality in the gel shift assay.

Example IV

AGX51 Disruption of Id-bHLH Binding And AGX51-Mediated Id Degradation in Leukemic Cells

The instant Example demonstrates novel ability of an exemplary anti-Id compound, N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ("**AGX51**"), to disrupt Id dimerization with bHLH proteins and cause rapid degradation of unbound Id protein in cellular systems. TA leukemic cells expressing ETO chimeric protein were treated with either vehicle control (DMSO) or 20 μM, concentrations of either, racemic AGX51, purified (−)-enantiomer of AGX51 (peak "E-1" in "Enantiomer Separation A", described below), or purified (-)-enantiomer of AGX51 (peak "E-2" in Enantiomer Separation A). Cell lysates (20 μg) were separated by SDS–PAGE and transferred to nitrocellulose membranes. Immunoblots were analyzed by Western blotting and visualized by chemiluminescence (Figure 1). The primary antibody for Id1 and Id3 was obtained from BioCheck (Foster City, CA).

Figure 1 provides a Western blot gel demonstrating that Id1 and Id3 levels are potently reduced following treatment of TA leukemic cells with racemic AGX51. As in other investigations documented here, anti-Id activity of the (-)-AGX51 enantiomer (second eluting peak "E-2" from "Enantiomer Separation A" (see below)) is surprisingly much greater than the anti-Id activity of racemic AGX51, while the (+)-AGX51 (denoted as the first eluting peak "E-1" from "Enantiomer Separation A") has minimal effect on Id1 (Figure 1).

Example V

AGX51-Mediated Disruption of Id-bHLH Binding

And AGX51-Mediated Id Degradation in Breast Cancer Cells

The ability of an AGX51 to bind Id and disrupt Id dimerization with bHLH proteins and cause rapid degradation of unbound Id protein was also demonstrated in cellular models of breast cancer. 4T1 murine breast cancer cells were treated as in Example IV above, with either vehicle control (DMSO) or 20 μM, concentrations of either, racemic AGX51, purified (+)-enantiomer of AGX51 (peak "E-1 from Separation A"), or purified (-)-enantiomer of AGX51 (peak "E-2 from Separation A"). Cell lysates (20 μg) were separated by SDS–PAGE and transferred to nitrocellulose membranes. Immunoblots were analyzed by Western blotting and visualized by chemiluminescence. The same primary antibodies for Id1 and Id3 (BioCheck, Foster City, CA) were used.

Stereospecific activity of anti-Id compound AGX51 was confirmed in part in this breast cancer model (4T1 cells) (Figure 2). However, these results render the molecular basis for this stereospecificity between AGX51 enantiomers even more complex. The comparable Western blot data for the breast cancer cells (Figure 2) again showed that the (-)-AGX51 enantiomer exhibited profound dominance over the (+)-ANGX51 enantiomer in terms of anti-Id 1 binding disruption and degradation (though weak Id1 signal was still detectable in all lanes, indicating the ablation or knock-down may not have been complete in any sample).

This stereospecific (-)-ABX51-mediated destruction of Id1 protein levels in both leukemic and breast cancer cells profoundly implicates this enantiomer as a potent tool for intervention in preventing and treating metastatic disease.

In contrast to the results presented in Example IV for leukemic cells, none of the AGX51 preparations (racemate "mix", or either (+)- or (-)-enriched enantiomer prep.) appeared to effectively impair Id3 binding to promote Id3 degradation in breast cancer cells.

The unexpected stereospecificity findings for AGX51 and its enantiomers traces a predominant if not exclusive anti-Id1 activity to the (-)-AGX51 enantiomer. In comparison, anti-Id1 activity is minimal or completely lacking in the (+)-AGX51 enantiomer. The (-)-AGX51 enantiomer-specific activity is paradoxically strong for both Id1 and Id3 in leukemic cells, while comparable levels of activity were observed for the (+)-AGX51 enantiomer preparation ("E-2") in this model (Figure 1). This latter observation may be an artifact of the experiment, with possibly small amounts of the (-)-AGX51 enantiomer (5-10%) in the (+)-AGX51 preparation (fraction "E-1" from Enantiomer Separation A) being capable of mediating potent reduction of Id3 levels (as observed for all three preparations: racemate "mix"; (+)-enantiomer "E-1"; and (-)-enantiomer "E-2") (Figure 1).

The contrast of the Id-1 and Id3 stereospecificity data here, however, calls this simple "contaminant" explanation into question. Alternatively, there may be a lower concentration of Id3 in certain cancer cells, or a greater activity of all forms of AGX51 against Id3 than Id1. This latter interpretation is challenged, however, by the results in Figure 2, suggesting that neither the AGX51 racemate, nor either of the (-)-AGX51 or (+)-AGX51 enantiomers, appear to mediate significant Id3 degradation in breast cancer cells.

It must therefore be regarded as a surprising and confounding discovery that the (-)-AGX51 enantiomer mediates potent destabilization and destruction of both Id1- and Id3-bHLH complexes (corresponding to total ablation of Id1 and Id3 protein at the levels of detection afforded here) in leukemic cells, with comparable anti-Id1 stereospecific activity residing in the (-)-AGX51 enantiomer in breast cancer cells, but little or no anti-Id3 activity observed for any form of ANGX51 in breast cancer cells (while all three preparations exerted strong anti-Id3 protein ablation effects in the leukemic cells--possibly pointing to minor contamination and extraordinary anti-Id3 potency of (-)-AGX51, but exhibited only in this cell type between studies, or alternatively to an incomplete stereospecific effect (wherein (-)-AGX-51 is profoundly anti-Id1 dominant, with little or no andi-Id3 activity resides in the (+) enantiomer, yet both the (-)-AGX51 and (+)-AGX-51 enantiomer exhibit comparable anti-Id3 activity).

The surprising anti-Id potency of the (-)-AGX51 enantiomer observed here could not have been predicted based on conventional understanding of stereochemistry in biological systems. Preconceptions of stereochemical interactions in simple biochemical systems might predict a disparate activity potential between opposite polarity enantiomers. But the art clearly fails to provide a similar foundation for predicting stereochemical functional disparity among *any* compound such as ANGX51. AGX51 is a first-in-class compound shown to interact with Id and disrupt Id-dimerization with bHLH proteins and other complex binding partners. The large helix-loop-helix structures that contain homo- and hetero-dimerization domains among these complex binding pairs, have no correlates among conventional stereo-biochemistry models (e.g., simple enzyme-substrate interactions). There can be no scientifically well-founded predictions of stereospecific effects, of any kind, among *any* compound analogous to AGX51, because AGX51 is a pioneer anti-Id drug in a nascent field of discovery, first explored and charted here.

Example VI

Lethal Effects of Anti-Id Compounds on Cancer Cell Lines

Prostate cancer cell lines DU145 and PC3 (in 10% BCS) were obtained from American Type Culture Collection (Rockville, MD). The cells were cultured in Ham's F12 (Gibco, Carlsbad, CA) medium containing 10% BCS (Hyclone, Logan, UT) and appropriate antibiotics (pen/strep, fungizaone, and gentamycin (Invitrogn Inc., Carlsbad, CA). All cells were cultured at 37°C in a fully humidified atmosphere containing 5% CO_2.

At 50% confluence, the cells were treated with either 100 μM DMSO, 1000mOsmol of urea +NaCL, 1 μM N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51), 10 μM N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide, and 100 μM N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide, 1 μM N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide, 10 μM N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide, or 100 μM N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide. The cell morphology and growth was monitored daily for 1 week by microscopy for changes in morphology or cell death. Apoptosis was determined by measuring caspase 3 and caspase 7 activities using the Caspase-Glo 3/7 Assay system from Promega (Madison, WI).

N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide, at 100μm concentration led to massive cell death of PC3 cells within 3 days with no surviving cells observed after six days of treatment. While the effect of on DU145 cells was not as pronounced after three days, the cells appeared very unhealthy and were unable to proliferate as compared to the controls. Additionally, after six days, treatment of DU145 cells with N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide led to cell death. N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide was also able to induce apoptosis in DU145 at concentrations as low as 1μM (Fig. 3 (C)). Cell survival was also sensitive to N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide. N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide, at a concentration of 1 um was able to induce cell death in DU145 cells. In short, two small molecule inhibitors of E47-Id1 interaction were identified that induce massive cell death in prostate cancer cell lines.

The molecular mechanisms underlying the effects of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide on prostate cancer cells was assessed by measuring activity of primary mediators of apoptosis, caspase 3/7. Treatment with N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (1-100μm) resulted in a significant increase in caspase 3/7 in both DU145 and PC3 cells, which was higher than caspase activity in cells treated with the apoptosis inducing agent staurosporine (10μm).

Example VII

AGX51 Rescues p16 Levels in Leukemic Cells

A leukemic cell line derived from a mouse overexpressing the MML-AF9 fusion protein was treated with increasing concentrations of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51). 50% growth inhibition was observed at 10 μM of AGX51 relative to a DMSO control. Total cell lysates were collected using buffer containing 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid and 0.02% sodium azide along with freshly added complete protease inhibitors. The protein lysates (20 μg) were separated by SDS–PAGE and transferred to nitrocellulose membranes. Immunoblots were analyzed by Western blotting and visualized using a Western lightening chemiluminescence detection kit. As shown in

Figure 3, AGX51 at the IC_{50} concentration restored p16 compared to the DMSO vehicle. The primary antibodies used in this study were purchased from Epitomics, Burlingame, CA, USA.

Example VIII

AGX51 Rescues p21 Levels in Human Bladder Carcinoma Cells

A human bladder carcinoma cell line was treated with increasing concentrations of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51) and processed for p21 concentration impacts of AGX51, as described above in Example VII for AGX51-mediated rescue of p16. Total cell lysates were collected, processed, separated and transferred to a Western blot substrate as described. The Western blots were visualized using as shown in Figure 3. AGX51 mediated a pronounced dose-dependent restoration of p21. This activity, as in the case of the p16 rescue activity, indicates potent anti-Id efficacy in cellular systems modeling clinical disease. In the case of p21, Id natively impairs normal E protein regulation of p21 implicated in cell cycle control and anti-proliferative effects of p21. By virtue that the novel anti-Id compounds and methods of the invention functionally knock down or even ablate Id levels in metastatic environments, the knock down of Id here directly liberates E protein induction of p21 to restore healthy p21 levels to cancerous cells. These molecular rescue activities of AGX51 and other anti-Id compounds of the invention are shown here to mediate powerful anti-cancer and anti-metastatic impacts on cellular migration, cellular proliferation, apoptosis and other critical mechanisms for metastatic disease progression and clinical arrest.

Example IX

Anti-Id Compounds Restore Critical Cell Cycle Control in Cancer Cell Lines

DU145 prostate cancer cells were obtained from American Type Culture Collection (Rockville, MD). The cells were starved of serum for 24 h and treated 24 h with 1uM N'-(4-isopropylphenyl)-1-benzothiophene-2-carbohydrazide (AGX8) or N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51). After treatment, the cells were washed, fixed in ethanol and stained with propidium iodide before being analyzed by fluorescence-activated cell sorting (FACS) analysis (Accuri Cytometers, Inc., Ann Arbor, MI). Cell pellets were suspended and numbers of cells in each identified phases of the cell cycle were measured by flow cytometry.

AGX51 and AGX8 both exhibited distinct activity for positively restoring cell cycle control. AGX8 was less effective compared to AGX51 in this assay for rescue of cell cycle control. These data are consistent with reports that Id1 mediates cancer-promoting and metastatic induction effects in part by impairing or disabling normal cell cycle controls.

In follow on studies here, the ability of AGX51 to mediate cell cycle control was further analyzed with regard to possible stereospecific effects. Figure 5 illustrates the surprising result that one specific enantiomer, (-)-AGX51 ("E2"), is significantly more active than racemic AGX51 ("E0") in restoring cell cycle control in DU-145 prostate cancer cells, whereas the other enantiomer (+)-AGX51 ("E1") appears virtually inactive. Data are mean values of the % cells in sub-G0 (apoptotic), G1, S and G2/M phases. The error bars are SEs and an * designates $p<0.05$. The treatments concentrations were all 10 nM. Improved cell cycle control is exemplified by fewer cells in G2/m phase of cell cycle and more cells in the sub-G0 cell dormancy phase.

The foregoing findings demonstrate that anti-Id compounds of the invention mediate powerful disruption of Id-dependent cell cycle deregulation. Thus the invention provides tools and methods to directly disable cancer development through the curtailment of Id-deregulated cell cycling, correlated with uncontrolled, cancerous or metastatic cellular proliferation. In a correlated array of studies, the inventors have demonstrated direct anti-proliferative effects of anti-Id compounds described here, shown to powerfully reduce proliferation rates of many different types of cancer cells in both cellular systems and in vivo models of human disease.

Direct anti-proliferation activity of AGX51 was demonstrated in a dose-proportional fashion to limit cancer cell proliferation in human bladder cancer cells, among other models. UMUC-3 human bladder cancer cells were treated with DMSO (control) or 10μM or 20μM AGX51. After 48 hours, cell viability numbers were determined by trypan blue exclusion staining. In addition, total cell lysates were collected using a buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid and 0.02% sodium azide along with freshly added complete protease inhibitors. Three 20 μg protein lysates were separated by SDS–PAGE and transferred to nitrocellulose membranes. The immunoblots were analyzed by Western blotting for Id1, p16 or p21 and the results on the blots visualized using a Western lightening chemiluminescence detection kit. The three primary antibodies were for Id1, p16 or p21. The primary antibody for Id1 was obtained

from BioCheck, Foster City, CA. p16 and p21 antibodies were purchased from Pierce Antibodies, Rockford, IL. Actin staining was used for internal standardization.

Within these studies, ANGX51 mediated approximately a fifty percent growth inhibition (observed at 10 μM AGX51) compared to DMSO control. These studies also demonstrated that AGX51-mediated anti-proliferation activity was correlated with AGX-51-mediated decrease in Id1 protein and concomitant increase in p21 levels (with a less substantial effect on p16 levels). Acting thus as a direct modulator of p21 activity/expression, ANGX51 also functions to stabilize cell cycle control and diminish production of endothelial progenitor cells (EPCs) and EPC-dependent tumor-associated angiogenesis.

Example X

AGX51 Blocks Id-Dependent Cell Migration Essential for Metastasis

Cell migration reflects the sum of many individual biologic processes that, overall, promote the capability of a particular cell to move from one place to another. Cell migration is an integral mechanism essential for tumor metastasis, the most lethal process associated with neoplasms (Chiang and Massagué, 2008). The instant Example demonstrates how exemplary anti-Id compounds of the invention such as AGX51 can reduce or prevent metastatic disease by blocking Id-dependent metastatic cell migration.

Cellular migration potential is readily determined employing conventional "Scratch Assay" methods. The basic steps of a Scratch Assay involve creating a "scratch" in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the scratch, and comparing the images to quantify migration rates of the cells. The *in vitro* Scratch Assay is particularly suitable for studies concerning cell–matrix and cell–cell interactions in cell migration.

For this study, PC3 cells were plated and allowed to grow almost to confluence. A p200 pipet tip was used to scratch a channel in the cell layer, and the cells are washed to remove debris from the scratch area. Media containing AGX-51 dosing vehicle (DMSO) or media containing AGX-51 was then added to the cells and the cells were photographed daily.

Results are provided in the photographs presented in Figure 6. After 4 days, the scratches or channels in plated cell samples treated with media containing dosing vehicle (DMSO) have disappeared while the channels in plated cell samples treated with AGX-51

remain open. This assay, consistently repeated with comparable results, unambiguously shows that anti-Id active AGX51 potently inhibits Id-dependent cell migration. Thus the invention provides yet additional tools and methods to directly block cancer development and metastasis by reducing or disabling Id-dependent cell migration.

Example XI

AGX51 Blocks Id-Dependent Angiogenesis Essential for Cancer Growth and Metastasis

VEGF-165 and FGF-2 treated Matrigel plugs were implanted on Day 0 into C57BL/6 mice. Mice were treated with either vehicle or AGX51. The anti-Id compound was provided either in the plugs (25 μg/mg) or by daily ip treatment (30 or 100mg/kg) for 10 days. Plugs were harvested on Day 10, fixed and paraffin embedded. Three sections (5μM thickness) of each plug were stained with an anti-CD31 antibody and counterstained with hematoxylin and eosin stain. CD31-positive microvessels were counted for one entire cross-section per plug and the average microvessel density ± SD was determined. Student's t-test was used for statistical analysis.

Microvessel data from this study is provided in Figure 7. Compared to vehicle control animals, all AGX51 subjects were significantly protected against new blood vessel formation. Statistics for the study were: $p>0.05$ ($n=7$), 0.01 ($n=6$) and 0.01 ($n=7$) compared to vehicle ($n=9$) for 25 μg/plug, 30 and 100mg/kg dose groups respectively. The maximum protection was 44% at an ip dose of 100mg/kg, qd. Slices of the Matrigel plugs were histologically analyzed at the end of the study, which revealed a major decrease in the presence of complete blood vessels and therefore endothelial cells in the AGX51-treated subjects relative to control subjects.

In related studies, female nude mice were orthotopically (mammary fat pad) implanted with MDA-MB-231 human breast cancer cells. Prior to implantation, the MDA-MB-231 cells were trypsinized, centrifuged and re-suspended in a 50% DPBS/50% Matrigel solution to a concentration of 5×10^6 cells/50 μL and frozen until use. The frozen Matrigel was thawed at 4°C for 24 hours. Syringe, needle and cells were kept on ice until used. Prior to the injections, cells were gently mixed by inversion and the needed volume drawn up into a cold syringe equipped with a 25 x 5/8" gauge needle. The syringe was gently inverted to mix taking care to avoid bubbles. At four to six weeks of age, the mice were injected with 50

μL of Matrigel containing 10 x 10^6 MDA-MB-231 cells into the orthotopic site. The cells were injected orthotopically into the right, caudal mammary fat pad of the animals anesthetized by ketamine (120mg/kg)/xylazine (6mg/kg) administered *im* using a 26 x 3/8" gauge needle. Tumors were then allowed to establish to a volume of 100 mm^3, after which animals, (typically 5 animals/treatment group) were randomized and treated *ip* with vehicle (DMSO) or 60mg/kg AGX51 bid. After 41 days of treatment, the mice were sacrificed by cervical dislocation, and tumors excised and fixed in 10% buffered (neutral) formalin for histopathologic analyses. Tissue was removed from the formalin and washed with 3xPBS, soaked in 30% sucrose in PBS for 24-48 hours at 4°C to cryoprotect, and then embedded in paraffin. The paraffin blocks were stored at –20°C or –80°C until cryo-sectioning into 5μm thick sections for mounting on slides. The slides were treated with anti-CD31 and washed with PBS, and endothelial cells were detected using an avidin-biotin complex (ABC) system. The slides were counter stained with hematoxylin and eosin stain to visualize nuclei.

As consistently observed among these study specimens, blood vessels with endothelial cells were prominent in vehicle-treated histological samples, but were consistently absent in the AGX51 treated animals.

These findings directly evince that anti-Id function of AGX51 and other compounds of the invention extends to specific blockade of Id-mediated pathogenic angiogenesis. This mechanism of intervention in the metastatic cascade will provide particularly powerful tools for managing cancer and preventing and reducing metastatic disease through blockade of tumor-associated angiogenesis.

Example XII

Anti-Cancer Effects of AGX51 Mediated by Inhibition of Endothelial Progenitor Cells (EPCs)

Additional compositions and methods of the invention discretely impair tumor-associated angiogenesis by inhibiting production and reducing survival of endothelial progenitor cells (EPCs). EPCs are responsible for promoting tumor growth and metastasis by contributing to neovascularization of new tumors (and by rescuing established tumors from vascular destruction caused by tumor growth). EPC production and survival is Id-dependent in cancerous and metastatic systems, and therefore the anti-Id compositions and methods of

the invention effectively reduce or prevent metastasis through reduction or prevention of EPCs and/or by impairment of EPC function and developmental potential.

The instant Example utilizes the same test subjects as presented throughout the other Examples here, comparing anti-Id-treated animals with control animals under conditions that lead to cancer progression and metastasis in the non-treated control animals. The further object of this Example is demonstrating Id-dependent variation of EPC levels in animals treated or untreated using the compositions and methods of the invention. According to this Example, each class of treated and untreated model animal described in the foregoing Examples IV-VII will be evaluated for EPC levels (e.g., using multivariate flow sorting with antibodies directed against EPC markers (such as GFP+ and VE-cadherin+) to measure EPCs in samples from bone marrow or circulating blood). Animals treated with anti-Id compositions and methods of the invention, particularly (-)-ANGX51 will exhibit anti-Id compound-mediated, dose-dependent reduction in EPC levels (compared to control animals, and animals treated with lesser active anti-Id compounds, such as racemic ANGX51 and particularly the anti-metastatically inactive (+)-ANGX51 enantiomer.

Example XIII

Anti-Id Compounds Inhibit Tumor Growth *In Vivo*

Nude mice (n=8/treatment group) were implanted with MDA231 human cancer cells. Fourteen days after implantation, the mice were treated ip with DMSO (vehicle) or 50mg/kg AGX51 in DMSO. All mice were treated iv for 5 consecutive days with 7.5mg/kg Taxol starting on days 8 and 22. Boxplots are tumor volumes 53 days post implantation (last day of the study).

Two animals in the control group and one animal in the treated group died before day 53 (data not used). Tumor size findings for all surviving animals are provided in Figure 13. As shown in Figure 8, treatment with AGX 51 provides a significant [p=0.05 compared to vehicle (DMSO) control] negative effect of approximately 50% on tumor growth.

Example XIV

Dose optimization of AGX51

CD1 male mice (3/timepoint) were treated ip with 30mg/kg AGX51 in DMSO. Blood was collected in heparinized tubes by retro-orbital puncture pre-dose and at 0.25, 0.5, 1.0,

2.0, 4.0, 8.0 & 24 hours post dose. One μg of the internal standard for the assay (S109037 from Aldrich, St. Louis, MO) and 100 μL of pH 7.4 PBS were added to the plasma harvested from the blood. The mixture was vortexed for one minute with 1 mL methyl t-butyl ether (MTBE), and the MTBE removed after centrifugation with dry N_2 (g). The residue remaining after removal of the MTBE was reconstituted in 200 μL of ACN. Five μL of this solution was analyzed by LC/MS using a 2.0 x 250 mm C18 column coupled to an electrospray LC-MS set to monitor m/z 432 (AGX51) and m/z 468 (S109037) in the HPLC effluent. The flow rate was 200 μL/minute of ACN (85)/H_2O + 0.1% formic acid. Calibration curve samples were prepared in duplicate by fortifying 100 μL control plasma with 0, 0.03, 0.11, 0.33, 1 or 3 μg of AGX51 and analyzing the samples as described. The ratio of 432 to m/z 468 in the calibration samples (y) and the concentration of AGX51 in the sample (x) were analyzed by linear regression and the resulting slope (m) and intercept (b) used to convert the ratio in an experimental sample into a concentration of AGX51. Typical ion chromatograms from the assays are provided in Figure 9.

As shown in Figure 10, AGX51 is rapidly absorbed with C_{max} occurring at the first time point, 15 minutes. The observed profile is consistent with a two compartment model with $t_{1/2}$ for elimination from the first compartment of approximately 30 minutes and a terminal elimination $t_{1/2}$ from the second compartment of approximately 6 hours. The drug is widely distributed, Vz/F of approximately 80 liters/kg, and relatively rapidly cleared, Cl/F of >100 mL/kg/minute.

Various dosing simulations were performed using the two compartment model in WinNonlin (Pharsight, Sunnyvale, CA) and the PK parameter estimates from the fit of the plasma concentration data to the two compartment model in WinNonlin. The target criteria was to maintain the plasma concentration of AGX51 above 2 μM (0.431 μg/mL) which is the lower end of the range of IC_{50} values observed for AGX51 to inhibit proliferation in various cancer cell culture systems, e.g., DU145 (as seen in Examples IV and V, above). As shown in Figure 11, the simulations suggested that a dose of 60mg/kg, bid, would achieve the target criteria.

Example XV

AGX51 Profoundly Reduces Lung Cancer Metastasis *In Vivo* Using Accepted Murine Models Predictive of Cancer Drug Efficacy In Human Subjects

This Example demonstrates potent, *in vivo* anti-metastatic effects of the novel anti-Id compounds and methods herein. Tail vein injection of Lewis lung carcinoma was followed by measurement of lung metastases according to conventional methods. Thirty C57BL/6 mice were implanted with 7.5 x 10^5 Lewis Lung Carcinoma (LLC) cells/animal. Seven days after implantation, 5M/5F per group were treated daily intraperitoneally (ip) for 25 days with either dosing vehicle (DMSO), or 50 mg/kg racemic N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51). Fourteen days after implantation, another group of 5M/5F animals were treated daily ip for 18 days with 50 mg/kg N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide. Tumors were measured three times from Day 7 to 14. On Day 14, the tumors were excised. As illustrated in Figure 4, the animals were necropsied for the presence of lung metastases on day 32, 18 days post excision. Tumors at day 14 were approximately the same size for each treatment group, providing assurance that any difference in lung metastases found at the end of the experiment was not the result of different tumor sizes in the compared groups.

The data presented in Figure 12 demonstrate unprecedented anti-metastatic activity mediated *in vivo* through treatment of animal models of human disease using an exemplary anti-Id drug of the invention, AGX51. As the data evince, in this study racemic AGX51 mediated more than a 50% reduction in the number of treated animals presenting with greater than five metastatic tumor foci. The data here are further shown to be consistent among multiple test samples, and are further consistent with other evidence of anti-Id efficacy (AGX51-mediated Id binding disruption and ablation of Id protein in cells, and disruption of Id-dependent cellular migration crucial for metastasis). By virtue of these and other study results herein, anti-Id compounds of the invention provide potent tools for treating, reducing and preventing initiation and progression of metastatic cancer in mammalian subjects.

Example XVI

AGX51 Profoundly Reduces Breast Cancer Metastasis *In Vivo* Using a Clinically Predictive Model of Anti-Metastatic Drug Efficacy

This Example focuses on breast cancer intervention using a widely accepted murine model of clinical breast cancer treatment efficacy. Breast cancer metastasis is a leading cause of cancer mortality in humans worldwide. A common site of breast cancer metastasis is the lung. Whereas Example III above demonstrated therapeutic efficacy of AGX51 against metastasis of implanted lung tumors in mice, the instant study measures anti-metastatic effects of AGX51 against breast cancer cells directly injected into the bloodstream of test subjects.

Breast cancer cells (4T1) tagged with a luciferase gene were injected via tail vein into Balb/c mice (5.0×10^4 cells per mouse, two groups of 5 mice). Development of lung metastases was monitored by bioluminescent imaging. One day after tumor cell injection, mice were treated twice daily, i.p., for 4 weeks with either dosing vehicle (DMSO) (5 mice) or 50 mg/kg N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51) (5 mice). Bioluminescent imaging was carried out once a week. At the end of week four, the lungs were collected and analyzed for metastatic burden (Figure 13).

As shown in Figure 13, treatment with AGX51 profoundly reduced breast cancer metastasis in this direct injection model. An unprecedented 40% of the anti-Id treated animals remained completely metastasis free. In the AGX51-treated versus DMSO control subjects, there was greater than a five-fold reduction in that average number of metastases observed.

Example XVII

Anti-Metastatic Effects of AGX51 Are Stereospecific, Dominantly or Exclusively Mediated By The (-)-ANGX51 Enantiomer

Based on the pioneering studies above revealing stereospecific anti-Id binding and Id degradation (ablation) activities of AGX51 enantiomers, characterized by a profound anti-Id1 dominance of the (-)-AGX51 enantiomer, further investigations were undertaken to elucidate stereospecific effects of AGX51 affecting cellular processes within the metastatic cascade.

Following the methodology of Example V, above, anti-metastatic effects of racemic AGX51 and both the (-)-AGX51 and (+)-AGX51 enantiomers were compared side-by-side.

Metastasis was assessed according to the protocol described in Example V, above, with 5 subjects receiving DMSO or racemic AGX51, and two more groups of 5 mice each treated with either the (-)-AGX51 or (+)AGX51 enantiomer. For these studies, the AGX51 enantiomers were purified using a different separation method that yielded a higher level of enantiomeric enrichment (approximately 98% enantiomeric purity). Interestingly, the second enrichment method ("Enantiomer Separation Method B") resulted in a reversal of elution profile compared to the profile seen in the first Separation Method A (in Method B the (-)-AGX51 enantiomer eluted first ("peak 1), and the (+)-AGX51 enantiomer eluted second ("peak 2"). These distinct preparative designs and elution profiles serve to further identify and elucidate the respective physicochemical properties of the (-)-AGX51 and (+)-AGX51 enantiomers.

The instant studies revealed yet another dominant anti-Id activity exhibited by the (-)-AGX51 enantiomer; namely a profound dominance, or exclusive stereospecificity, for mediating anti-metastatic effects *in vivo*. Upon bioluminescent imaging, positive metastatic signal was observed in the lung regions of all animals receiving vehicle or the (+)-ANGX enantiomer (two of the animals in the (+)-AGX51 enantiomer group died prior to the imaging stage). In contrast, only 3 out of 5 mice receiving racemic AGX51, or the active, (-)-AGX51 enantiomer, exhibited detectable metastatic signals in the lungs.

A confirming study identical in procedure to the foregoing study has been abbreviated at a two-week preliminary visualization for inclusion in this writing. This study involves four animals per group, treated with DMSO vehicle, racemic ANGX51, (-)-ANGX51 ("peak 1"), or (+)-ANGX51 ("peak 2"). As shown in Figure 14, two of the four animals in both of the DMSO and (+)-ANGX51 ("peak 2") groups have developed extensive lung metastasis (marked by prominent bioluminescing fields in thoracic areas), whereas all animals treated with racemic ANGX51 and (-)-ANGX51 ("peak 1") remain completely clear of metastatic signal.

Example XVIII

Coordinate Anti-Cancer Drug Therapy--AGX51 and Taxanes

MDA-MB-231 cells were trypsinized, centrifuged and re-suspended in a 50% DPBS/50% Matrigel solution to a concentration of 5 x 10^6 cells/50 µL and frozen. Prior to use, the frozen Matrigel was thawed at 4°C for 24 hours. Syringe, needle and cells are kept on ice until used. Prior to injections, cells are gently mixed by inversion and the needed volume drawn up into a cold syringe equipped with a 25 x 5/8" gauge needle. The syringe is gently inverted to mix taking care to avoid bubbles.

50 µL Matrigel containing 10 x 10^6 MDA-MB 231 cells were injected orthotopically into the right, caudal mammary fat pad of four to six week old female nude mice anesthetized by ketamine (120mg/kg)/xylazine (6mg/kg) administered *im* using a 26 x 3/8" gauge needle. Tumors were allowed to establish to a volume of 100 mm^3, after which animals were randomized and treated *ip* with test compound or vehicle.

Fourteen days after implantation, the mice were treated *ip* with DMSO (vehicle), DMSO solutions of 15mg/kg paclitaxel, 15mg/kg, paclitaxel with either 6.7mg/kg, 20mg/kg, or 60mg/kg of AGX51 or 60mg/kg of AGX51 alone for five days. Tumor volumes were determined throughout the study using digital caliper and the formula: tumor volume = ½ (length × $width^2$) where the greatest longitudinal diameter is the length of the tumor and the greatest transverse diameter is the width. After 41 days of dosing, the mice were sacrificed by cervical dislocation and final tumor volumes and weights recorded and compared.

As shown in Figures 15 and 16, at day 19, paclitaxel alone decreased tumor growth by approximately 40%. AGX51 alone even at 60mg/kg, bid, did not affect tumor growth. However, all three AGX51 doses—6.7, 20 & 60mg/kg, bid significantly decreased tumor growth, $p \leq 0.01$, with the 60mg/kg dose providing the greatest effect with many tumors smaller than at the start of the study.

Additional groups of mice were treated with two other doses of paclitaxel—7.5mg/kg and 22.5mg/kg, q5d with 60mg/kg, bid, AGX51—were compared with the 15mg/kg, q5d, dose. The 7.5mg/kg dose provided no additional efficacy while the efficacy at 22.5mg/kg appeared no better than that observed with the 15mg/kg dose. Compared to the 25mg/kg dose used in the preliminary study, the animals appeared to somewhat better tolerate the 22.5mg/kg dose although the mice experienced significant hypothermia and lethargy compared to the 15mg/kg group.

In a second study, nude mice (n=5/treatment group except for the 60mg/kg, bid, AGX51 & paclitaxel doses (n=8) were again implanted with MDA231 human cancer cells. Fourteen days after implantation, the mice were treated ip with DMSO (vehicle), 15mg/kg paclitaxel, or 15mg/kg, paclitaxel with 60mg/kg of AGX51. The effect of the 15 mg/k, q5d, dose of paclitaxel on the mice in this study was considerably more pronounced than in previous studies: severe lethargy, hypothermia and poor grooming for many days. The effect of the paclitaxel treatment alone on tumor growth was slow to develop. However, ultimately paclitaxel alone did decrease tumor growth by approximately 50% and the 60mg/kg, bid in these studies. AGX51 significantly ($p \leq 0.01$) increased this effect, as shown in Figures 17 and 18.

Additional studies examined time of administration of AGX51 to maximize tumor regression. Nude mice (n=4/treatment group) were implanted with MDA231 human cancer cells. Fourteen days after implantation, the mice were treated ip with DMSO (vehicle), 40mg/kg of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide in a single dose per day or two 20mg/kg doses per day. The mean observed increase (± SD) in tumor volumes on Day 41 (final minus initial) were 242.69 ± 133.58 (qd) and 47.98 ± 86.31 mm^3 (bid) and the difference was statistically significant ($p<0.03$).

Additional studies here further evaluated combinatorial anti-cancer efficacy of AGX51 and docetaxel. MDA-MB-231 cells were trypsinized, centrifuged and re-suspended in a 50% DPBS/50% Matrigel solution to a concentration of 5 x 10^6 cells/50 µL and frozen. Prior to use, the frozen Matrigel was thawed at 4°C for 24 hours. Syringe, needle and cells were kept on ice until used. Prior to injections, cells are gently mixed by inversion and the needed volume drawn up into a cold syringe equipped with a 25 x 5/8" gauge needle. The syringe is gently inverted to mix taking care to avoid bubbles.

50 µL Matrigel containing 10 x 10^6 MDA-MB-231 cells were injected orthotopically into the right, caudal mammary fat pad of four to six week old female nude mice anesthetized by ketamine (120mg/kg)/xylazine (6mg/kg) administered *im* using a 26 x 3/8" gauge needle. Tumors are then allowed to establish to a volume of 100 mm^3, after which animals are randomized and treated *ip* with the test articles or vehicle. Fourteen days after implantation, the mice were treated ip with DMSO (vehicle), 15mg/kg of docetaxel q5d, or 15mg/kg of

docetaxel q5d and 20mg/kg of AGX51 bid. As shown in Figure 19, the mean (± SD) increase in tumor volumes on Day 41 (final-initial) were 497.47 ± 63.08 (n=5) & 57.55 ± 87.63 mm^3 (n=4), for docetaxel and docetaxel + AGX51, respectively. The difference was significant ($p<0.001$).

Example XIX

Comparison of the Effectiveness of Inhibiting Id1 Genes vs Id1 Protein

Id1 knockout mice were implanted with 10^6 LLC cells in Matrigel. The tumors were allowed to grow to approximately 350 mm^3 and then treated (5/treatment group) with DMSO vehicle control, 15mg/kg, q5d, paclitaxel or 15mg/kg, q5d, paclitaxel + 30mg/kg AGX51, bid19d. Included in the study, designated MS4, were two additional groups: C57 BL6 mice implanted as described above and treated with DMSO vehicle control or 30mg/kg AGX51, bid19d. As shown in Figures 20 and 21, AGX51 had no effect on the LLC tumors implanted into the C57BL76 mice, (mean volume ± SD of 5374.18 ± 1912.25 mm^3 for the DMSO treated animals versus 5143.44 ± 1122.17 mm^3 for the AGX51 treated animals). Contrary to reports in the literature of a modest anti-growth effect, the Id1 deletion in MS4 provided no anti-growth effect in the very rapidly growing LLC cells. Even more surprising, the paclitaxel (PAX) treatment also had only a very modest effect over the first 18 days of the study and on day 20, no effect. However, the combination of AGX51 + paclitaxel did provide a significant ($p<0.01$) anti-growth effect.

Example XX

Safety of AGX51

Anti-Id compounds of the invention appear to specifically target Id proteins and mediate their potent anti-cancer and anti-metastatic effects without disrupting fundamental cell functions or conferring substantial toxicity, contrary to observations for most conventional chemotherapeutic drugs. To evaluate the safety profile of AGX51, 8-12 week old, CD1 mice (5/treatment group) were dosed q5d with DMSO, 15mg/kg, qd, PTX, 60mg/kg AGX51, bid or 15mg/kg, qd, paclitaxel + 60mg/kg AGX51, bid (designated MS3). Heparinized blood samples were obtained by retro-orbital puncture on Day 6, 12 hours after the last N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide

(AGX51) dose. The plasma harvested from the blood was analyzed for standard clinical chemistry and hematology analytes and intact paclitaxel and AGX51.

No adverse effects were observed of AGX51 on weight or clinical chemistry (ALB, ALP, ALT, AMY, TBIL, BUN, Ca, PHOS, CRE, GLU, Na, K, TP & Glob) or hematology (WBC, Lym, Mon, Gra, RBC, Hg, Hematocrit & Platelet) values, compared to paclitaxel treatment alone. Similarly, no effect was observed for AGX51 on these same measures compared to DMSO vehicle control. The data were obtained using an Abaxis VetScan analyzer (Union City, CA). Data for a representative set of these laboratory tests are provided in Figure 22.

Example XXI

Anti-Id Compounds Protect Against Pathogenic Angiogenesis In Age-Related Macular Degeneration

The anti-Id and anti-angiogenic activity of AGX51 is demonstrated in the instant example to powerfully protect against pathogenic vascularization in the retina of mice, using a model system widely accepted as predictive of disease mechanisms and drug efficacy for treating human age related macular degeneration (AMD).

The retinas of 14 mice with the Id3 deleted (n=9 control and n=5 mice) were dilated with phenylephrine/atropine for 10 minutes and then anesthetized with ketamine/xylene (5:1) for 5 minutes prior to affecting the laser burns. A cover slip was placed on the surface of the eye (lower side) with a clear ophthalmologic media to act as a lens for the laser. A light was shone into the eye to visualize the optical nerve and the neural retina. A fine laser was then focused unto the back of the retina, set perpendicular to the back of the eye. Four burns were placed 1 optical disc (size of the optical nerve) away from the optical nerve between blood vessels (avoiding blood vessels). The settings for the laser were as follows: Duration of 100 ms, intensity of 250 mW and a diameter of 50 microns. The laser burns travel through the neural retina and focus on the retinal pigment epithelial (RPE) layer causing a rupture of Bruch's membrane. Immediately after the burn, a pocket of fluid forms around the burn and marks the spot of the burn. The pocket results from fluid expanding from the heat of the burn. The pocket eventually diminishes but a small burn spot is still observed.

Two weeks after rupture of Bruch's membrane, the animals were sacrificed and their eyes harvested and placed in paraformaldehyde overnight. The eyes were then washed in

PBS and the RPE-choroid-sclera complex isolated from the neural retina. The complex (~200microns in thickness) was then incubated overnight in PBS containing Triton X-100 and an anti-collagen IV antibody, followed by an overnight wash, then overnight incubation with AlexaFluor-conjugated secondary antibody. The complex was then washed overnight and flat mounted in anti-fade medium for evaluation. Using the Z-line imaging with confocal microscopy, 4 micron sections were imaged from the top to the bottom of the complex (~50 images). The central scar/vascularized area (~ middle of the complex) was then manually outlined and the images independently analyzed for background levels of fluorescence. Background fluorescence was subtracted from the outlined area of each image and then each area is analyzed for relative fluorescence. The total fluorescence was then calculated. Each animal, with the 4 burns, is an n of 1. Data was expressed as the mean lesion volume ± standard error of the mean (SEM) and analyzed using SPSS software. Statistical significance of the differences between the experimental groups was calculated using one-tailed Student's t-test of the volumes. As shown in Figure 23, Id3 deletion impairs neovacularization following laser induced disruption of Bruch's membrane, indicating Id3 is involved at least in part in full development of a pathogenic angiogenic response in this model system of AMD. This model is widely accepted and has been used successfully to develop numerous anti-AMD drugs.

The mean CNV area for the Id3 deleted animals was approximately half of the area of the wild type animals, and the result was highly significant statistically ($p<0.001$).

A comparable protective anti-AMD effect (as seen in the above Id genetic knockout study) has now surprisingly been achieved using a small molecule anti-Id drug of the invention, ANGX51. Even more unexpected, the anti-AMD (anti-angiogenic) effects of ANGX51 were observed in subjects treated intravitreally (directly into the affected eye), as well as intraperitoneally (requiring the drug to transit to the retinal tissue in a stable, effective state). Yet more pioneering still, the data here convincingly show that the anti-angiogenic and anti-AMD effects of AGX51 are stereospecific, with activity residing principally or exclusively with the novel (-)-AGX51 enantiomer.

In reference to Figure 24, (-)-AGX51 was administered intravitreally (ivt) using the same methods as above. C57BL Mice (5/Treatment Group) had CNV by laser treatment on day 1. The test mice were administered the (-)-enantiomer of AGX51 ("E2") (10 μg), while controls were administered vehicle (70% DMSO/30% Water). Administration was by ivt,

one hour post laser and on day 7. Sacrifice and lesion size measurements (performed using fluorescein-labeled dextran) were conducted on day 14.

In reference to Figure 25, (-)-AGX51 was administered intraperitoneally (ip) using the same methods as above. C57BL Mice (10/Treatment Group) had CNV by laser treatment on day 1. The test mice were administered the (-)-enantiomer of AGX51 ("E2") (20 mg/kg, qd) two hours post laser and then 20 mg/kg, bid, daily for 14 days, with control mice receiving vehicle (70% DMSO/30% Water). Sacrifice and lesion size measurements were completed on day 14.

The foregoing studies show powerful anti-angiogenic and anti-AMD mediated by an exemplary anti-Id compound of the invention, ANGX51. Potent anti-Id activity is again demonstrated by the (-)-AGX51 enantiomer employed in these studies. Comparing Figures 23-25, it is apparent that (-)-AGX51 mediates comparable anti-angiogenic and anti-AMD effects as is obtained by Id3 genetic knockdown. In both anti-Id drug tests (Figures 24 and 25), the extent of anti-angiogenic activity observed corresponded to a greater than 50% reduction of pathogenic angiogenic defects noted in the subjects, predicting a clinically very effective intervention provided by the invention for treating AMD and other pathogenic angiogenic diseases in humans. The nearly identical activity shown by (-)-AGX51 administered by the remote route of intraperitoneal (ip) administration (Figure 25), compared to the direct route of intravitreal (ivt) delivery, portends an unprecedented efficacy of this drug for reaching remote clinical targets in in the body, such as dense tumors, CNS targets, and other compartments that are isolated or refractory to standard drug delivery/bioavailability.

Example XXII

Cancer Treatment and Metastatic Disease Management Aided by Novel Anti-Id Diagnostic Tools

The invention also provides novel compositions, methods and kits for detecting and quantifying Id proteins, such as Id1, in biological samples for diagnosis and reflexive treatment of proliferative diseases, including cancer. These methods can be effectively integrated as well into novel therapeutic methods coordinating Id detection in patients with modifications in treatment regiments to achieve sensitive and highly successful clinical management of cancer patients and other subjects amenable to anti-Id treatment as described herein.

For clinical applications of the invention, it is important to determine anti-Id effects in treated patients on a continuing basis. Since Id proteins are degraded when Agx51 disrupts the interaction of Id with E proteins, measuring Id1 loss in patients provides useful information to modulate treatment and manage status of these patients. Id levels are elevated in the serum of both experimental mice bearing tumors, as well as in breast cancer patients. High levels of Id in these circumstances are likely associated with circulating EPCs and/or circulating tumor cells that lyse during preparations of samples, or spontaneously. Therefore, a simple blood test to determine if Id1 levels are reduced in serum of patients treated with anti-Id compounds of the invention will allow ongoing assessment of the efficacy of anti-Id compounds for reaching clinical trials. On the basis of these measurements, clinical management can be tailored to individual patient's disease status and treatment response (e.g., by lowering anti-Id drug dosing upon determination of extensive lowering of Id levels upon initial, successful drug treatment, thereby reducing potential, unnecessary treatment costs and/or side effects).

Practical implementation of reflexive diagnostic methods herein provide coordinately integrated diagnostic/treatment clinical management tools, illustrated by the following Example. Assay methods are uniquely modified for detecting of Id targets in biological samples, for example body fluids (e.g., urine, plasma, CNS fluid, expressed or extracted mammary fluid, etc.), tissues (e.g., bone marrow, blood, tumor biopsy specimens), and other diagnostically useful sample materials. A schematic representation of such a novel assay is provided in Figure 26. This assay employs modified "sandwich" techniques, whereby a highly specific monoclonal antibody against Id1 is bound to microtiter wells and then exposed to a test sample (e.g., serum) from a known or at-risk cancer screen subject, to allow binding of Id1 protein in the sample by the anti-Id monoclonal Ab. After washing the plates are exposed to E47 protein conjugated to horse radish peroxidase (E47-HRP). According to this novel assay construction, Id1 proteins from the sample bound to the monoclonal Abs bind and retain the HRP-labeled E47 in direct proportion to an amount of Id1 originally present in the sample. E47-HRP bound to the plate is then exposed to 3,3',5,5'-Tetramethylbenzidine (TMB), a chromogenic substrate of HRP for colorimetric quantitation.

The foregoing Id1-detection assay is demonstrated here with human breast cancer patients and normal subject controls to faithfully discriminate between cancer bearing and

cancer patients--thus affording sensitive and powerful clinical diagnostic, treatment and management tools.

According to the assay design illustrated in Figure 26, ELISA microtiter plates were coated with known amounts of anti-Id1-mouse monoclonal antibody (BD Pharmingen San Diego, CA). 100 µL Id1 standards (10X, 20X, 40X, 5OX, 160X, 320X and 640X (Id1 fortified mouse sera)) were dispensed into wells. The solution was then thoroughly mixed for 30 seconds and incubated at 4°C for 16 hours. After 16 hours, the incubation mixture was removed by emptying the plate contents into a waste container. The microtiter plate was rinsed 5 times with distilled water and struck sharply onto absorbent paper to remove all residual water droplets. 100 µL of E47 HRP Conjugate Reagents (1:25 or 1:50) was dispensed into each well and thoroughly mixed for 30 seconds. The plate was then incubated at room temperature for 2 hours after which the incubation was dumped out an the microtiter plate was rinsed 5 times with distilled water before being struck sharply onto absorbent paper to remove all residual water droplets. 100 µL of TMB reagent was then dispensed into each well and gently mixed for 5 seconds. The microtiter plate was then incubated in the dark at room temperature for 20 minutes. After 20 minutes, the reaction was stopped by adding 100 µL of Stop Solution to each well. The solution was gently mixed for 30 seconds and the absorbance was read at 450nm within 15 minutes. The resulting absorbance values were fit to a Michaelis-Merton hyperbolic equation using WinNonlin (Pharsight, Mountainview, CA). The correlation coefficient for the fit of the concentration data to the absorbance values was >0.99.

A total of 42 p53 knockout mice were maintained according to standard conditions. They were housed in plastic cages with hardwood chips in an air-conditioned room with a 12 h light–12 h dark cycle and were given basal diet (Oriental NMF; Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. Serum samples were obtained from the mice. 100 µL of serum from each mouse was then added to three wells on microtiter plates coated with known amounts of anti-Id1 mouse monoclonal antibody (BD Pharmingen, San Diego, CA). The solution was then thoroughly mixed for 30 seconds and incubated at 4°C for 16 hours. After 16 hours, the incubation mixture was removed by emptying the plate contents into a waste container. The microtiter plate was rinsed 5 times with distilled water and struck sharply onto absorbent paper to remove all residual water droplets. 100 µL of E47 HRP Conjugate Reagents (1:25 or 1:50) was dispensed into each well and thoroughly mixed for 30 seconds.

The plate was then incubated at room temperature for 2 hours after which the incubation was dumped out an the microtiter plate was rinsed 5 times with distilled water before being struck sharply onto absorbent paper to remove all residual water droplets. 100 µL of TMB reagent was then dispensed into each well and gently mixed for 5 seconds. The microtiter plate was then incubated in the dark at room temperature for 20 minutes. After 20 minutes, the reaction was stopped by adding 100 µL of Stop Solution to each well. The solution was gently mixed for 30 seconds and the absorbance was read at 450nm within 15 minutes.

After serum samples were obtained, the mice were sacrificed and analyzed for tumors. As shown in Figure 27, the dividing line between tumor bearing and tumor free animals was calculated using an evaluation by Receiving Operation Characteristic (ROC) curves. The ROC evaluation of the 42 concentration values predicts only one false positive and one false negative.

The powerful diagnostic utility of Id-detection assays within the invention was further demonstrated by applying the foregoing tools and methods for a breast cancer screening qualification in to populations of human female patients presenting with, and without, breast cancer. 15 serum samples were obtained from women with either normal health or advanced breast cancer (BioCheck, Inc., Foster City, CA). In a blind analysis, 15 100 µL samples were added to three wells on microtiter plates coated with known amounts of anti-Id1 mouse monoclonal antibody (BD Pharmingen, San Diego, Ca). The solution was thoroughly mixed for 30 seconds and incubated at 4°C for 16 hours. After 16 hours, the incubation mixture was removed by emptying the plate contents into a waste container. The microtiter plate was rinsed 5 times with distilled water and struck sharply onto absorbent paper to remove all residual water droplets. 100 µL of E47 HRP Conjugate Reagents (1:25 or 1:50) was dispensed into each well and thoroughly mixed for 30 seconds. The plate was then incubated at room temperature for 2 hours after which the incubation was dumped out and the plate was rinsed 5 times with distilled water before being struck sharply onto absorbent paper to remove all residual water droplets. 100 µL of TMB reagent was then dispensed into each well and gently mixed for 5 seconds. The plate was then incubated in the dark at room temperature for 20 minutes. After 20 minutes, the reaction was stopped by adding 100 µL of Stop Solution to each well. The solution was gently mixed for 30 seconds and the absorbance was read at 450nm within 15 minutes. As shown in Figure 28, there was an

extraordinary high accuracy of these assay results for distinguishing between cancer-positive and normal samples.

In more detailed diagnostic and reflexive, integrated diagnostic-treatment methods of the invention, patients may be primarily or complementarily screened using any of a number of other specific cancer markers (e.g., using prostate-specific antigen (PSA) to screen for elevated prostate cancer risk). Biopsy, optionally coupled with histopathological and/or immunohistochemical analysis may further refine primary or integrated diagnoses. In certain patients, additional tests as described herein will further refine diagnostic assessments to focus on metastatic risk (e.g., by measuring Id1 in identified tumor samples, by measuring EPCs or cancer stem cells in tumors, blood, marrow (or their markers in other samples such as urine, saliva, or CNS fluid), etc.

Illustrating reflexive diagnostic-treatment methods of the invention, subjects with organ confined cancer may be selected for treatment employing an anti-Id compound administered in a prolonged, prophylactic regimen (e.g., daily for periods extending for multiple months, six-nine months, 1-2 years, 2 years or longer), with routine follow-ups to assess maintained organ confinement of the prostate cancer. This regimen allows long-term suppression of metastasis of the cancer to avoid unnecessary trauma and quality of life costs that attend traditional surgical and chemotherapy cancer treatments. In conjunction with this novel treatment regimen, regular blood testing is conducted in the patients to test for levels of Id, as described above, particularly elevated Id1 and/or Id3, coupled with decisions based on these assay results. Reduced, static or elevated Id expression from an early compared to a successive treatment stage will often direct corresponding changes in treatment dosing, or treatment repertoire (including switching to more or fewer treatment agents or modalities, or more or less potent/aggressive interventions, generally balancing disease risk, current and projected treatment efficacy, and adverse patient impacts).

Reduced or stable Id levels may be relied upon as a future treatment and/or prognostic indicator, and may be determinative of a continuing treatment decision to maintain, or even suspend, anti-Id treatment as successful. Further elevations of Id during a treatment history might indicate a treatment decision to increase anti-Id dosing using the methods and compositions of the invention, and/or to initiate combinatorial therapy using an anti-Id compound and a secondary anti-cancer or anti-metastatic drug or alternate treatment modality (e.g., chemotherapy and/or radiation). Combined with this management paradigm, additional

methods of the invention will employ other, conventional methods and tools to diagnose and manage patients in a coordinate management regime, for example using follow-up assays for blood cancer markers, radiologic and/or histopathologic screening to continue monitoring for metastatic disease development, etc., to further refine and manage monotherapy with anti-Id compounds and combinatorial therapies as described herein.

The foregoing coordinate diagnosis and management protocols can be modified and employed to treat prostate cancer patients coordinately with both anti-Id methods and compositions and a secondary, anti-testosterone drug or therapy. Alternatively, prostate-specific radiation may be employed prior to, concurrent with, or subsequent to the anti-Id treatment.

The foregoing coordinate diagnosis and management protocols may be modified and employed to treat breast cancer or prevent metastatic progression of existing breast cancer. Patients are identified by routine mammography in human females positive for a breast tumor, followed by tumor excision where no cancer is found in the lymph nodes. Because the cancer appears localized, the subject is administered an anti-Id compound chronically to suppress metastasis of the cancer to avoid, perhaps, unnecessary trauma including greatly lowered quality of life associated with chemotherapy as well as the possibility of development of a neoplasm later in life because of the chemotherapy (Vega-Stromberg, 2003). A blood test in the patient showing elevated Id, particularly elevated Id1 and/or Id3, will support a decision to treat with an anti-Id compound, because such indicia suggest a positive environment in the body for metastasis. In related exemplary embodiments, patients within this management scheme may be coordinately treated with an anti-Id compound and chemotherapy, and/or breast-specific radiation, before, concurrent with, or subsequent to the anti-Id treatment.

Coordinate diagnosis and management protocols of the invention can also be employed to treat colon cancer or prevent metastatic progression of an existing colon cancer. Patients are selected by routine colonoscopy revealing a tumor in suitable treatment subjects, which are further selected following surgery (and/or histopathological testing) indicating that the colon cancer likely remains non-metastatic. These subjects are administered an anti-Id compound chronically to suppress metastasis of the cancer to avoid, perhaps, unnecessary trauma including lowered quality of life associated with conventional chemotherapy. A blood test in the patient showing elevated Id, particularly elevated Id1 and/or Id3, supports a

a decision to treat with an anti-Id compound, because such a measurement suggests a positive environment in the body for metastasis. In related embodiments, patients within this management scheme may be coordinately treated with an anti-Id compound and chemotherapy, and/or gastrointestinal (GI)-specific radiation, before, concurrent with, or subsequent to the anti-Id treatment.

Other coordinate diagnostic and management protocols are employed to treat melanoma. Subjects are diagnosed with melanoma in situ (the tumor remains in the epidermis, the outermost layer of skin.) The melanoma is excised by surgery and the subject is thereafter administered an anti-Id compound chronically to suppress metastasis of the cancer to avoid, perhaps, unnecessary trauma and lowered quality of life associated with chemotherapy or other conventional cancer treatments. A blood test in the patient showing elevated Id, particularly elevated Id1 and/or Id3, supports a decision to treat with an anti-Id compound, as this finding is associated with a positive environment in the body for metastasis.

In yet another exemplary coordinate diagnosis and management protocol, female human are selected presenting with Stage III (metastatic), breast cancer. Subjects thus identified are treated using an aggressive, combinatorial treatment regimen employing high doses of radiation and multiple doses of Taxol® (paclitaxel). After this primary treatment, histopathology and/or bioscans , (e.g., computerized tomography, positron emission tomography and/or magnetic resonance imaging) are used to identify patients presenting with no detectable residing cancer. These subjects are then treated for a prolonged period (e.g., six-nine months, 1-2 years or longer) with an anti-Id compound, in a prophylactic treatment regimen to block re-seeding of any remaining cancer cells to a distant organ (e.g., brain or liver). During this management phase, measurements of Id in the blood of the patient are performed periodically. After another management period of several months to one year or more, Id blood levels may be observed to increase, indicating an increase in metastatic potential. Treatment with the anti-Id compound is then increased in dosage or periodicity (or resumed). Thereafter when Id blood levels decrease to a value consistent with an acceptable (baseline or low-risk) metastatic potential the anti-Id treatment is reduced. This cycle of monitoring and reflexive intervention is maintained throughout the life of the woman.

Example XXIII

Separation of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide Enantiomers

Individual (+)- and (-)-enantiomers of AGX51 are obtained from a racemic (achiral) AGX51 composition using preparative HPLC with a chiral column. Separations on chiral stationary phases proved effective to obtaining enantiomers in acceptable purity.

Two different HPLC systems were designed and implemented here, referred to as "Enantiomer Separation Method A" and "Method B". Both methods were designed and adjusted to provide provide high mg or low gram amounts of the AGX51 enantiomers for biologic testing. Both methods employ supercritical CO_2 with an organic alcohol as the eluting solvent. However, the methods use different columns: Method A uses a Pirkle-concept stationary phase [1-(3, 5-dinitrobenzamido)-1, 2, 3, 4-tetrahydrophenanthrene] and Method B uses a polysaccharide stationary phase (cellulose with the free hydroxyl groups derivatized with 3, 5-dimethylphenylcarbamate).

The two methods are described in further detail and compared in the Tables 4 and 5 below.

Table 4. Comparison of two chiral HPLC methods used to produce the AGX 51 enantiomers.

Characteristic	Method A	Method B
Column	(S,S) Whelk-01	Chiracel-OD
Column length (mm)	250	150
Column internal diameter (mm)	30	20
Particle size column packing (μ)	5	5
Supercritical fluid	CO_2	CO_2
% CO_2	70	60
Organic solvent	Isopropanol	Methanol
% Organic solvent	30	40
Organic Modifier	None	0.5% Diethylamine
Column temperature	Room temperature	32°C
UV detection (nm)	230	260

In both methods, product isolation post fraction collection is provided using a rotovap at reduced pressure at a water bath temperature of approximately 40°C. The resulting gum can be used as is, or the enantiomer gum following isolation can be crystalized with some loss of product from methanol-hexane or ethyl acetate-hexane. Purity of the isolated enantiomers is determined by analytical HPLC using similar HPLC conditions to those used to produce the enantiomers.

Table 5. Comparison of two chiral HPLC methods used to analyze the isolated AGX 51 enantiomers.

Characteristic	Method A	Method B
Column	(S,S) Whelk-01	Chiracel-OD
Column length (mm)	250	250
Column internal diameter (mm)	4.6	4.6
Particle column packing (μ)	5	5
Pressure (bars)	128	100
Eluting Solvent(s)	100% Ethanol	CO_2 (60%) / 0.5% Triethylamine in methanol (40%)
Flow (ml/min)	1	4
UV detection (nm)	230	260

Miller, L., Orihuela, C., Froneck, R., Honda, D., Dapremont, O., Chromatographic Resolution of the Enantiomers of a Pharmaceutical Intermediate from the Milligram to the Kilogram Scale. Journal of Chromatography A. 1999; 849: 309-317. Brittain HG, Applications of chiroptical spectroscopy for the characterization of pharmaceutical compounds, Journal of Pharmaceutical and Biomedical Analysis 1998; 17: 933–940.

The enantiomers prepared by either Method A or Method B show identical LC/MS (electrospray ionization) profiles, i.e., MH^+=432, and 400 MHz proton NMR spectroscopy. i.e., 1 hydrogen triplet at 4.18 ppm (benzylic hydrogen), 12 hydrogen signals in the 6.6 to 7.4 ppm aromatic region, 3 hydrogen triplet centered at 0.96 ppm (propionyl methyl), 3 hydrogen multiplet centered at 3.73 (methoxy group), 2 hydrogen unresolved multiplet centered at 5.9 ppm (methylene in the 1,3 dioxole moiety), 2 hydrogen multiplet centered at 4.50 ppm (benzyl methylene attached to the nitrogen), 2 hydrogen multiplet centered at 3.07 ppm (second methylene attached to the nitrogen), multiple peaks corresponding to 4 hydrogens (methylene adjacent to the asymmetric carbon and methylene adjacent to the carbonyl in the propionyl moiety).

The enantiomers produced by both Method A or Method B were evaluated by polarimetry to establish the optical rotation for each enantiomer. The ability of individual enantiomers among enantiomer pairs to rotate polarized light in opposite direction to the rotation observed with the other enantiomer in the pair is universally accepted as a discrete characterization to unambiguously differentiate and quantitate enantiomers. The polarimetry evaluation repeatedly and consistently conducted here used a Jasco P-2000 with a dichrom polarizer. The concentration of each enantiomer was approximately 1% in methanol, the light source was a tungsten-halogen lamp and the wavelength of the emitted light was 589 nm. At 20°C, the enantiomers under the conditions described above rotated the polarized light either+28 degrees or -28 degrees.

HPLC profiles for analysis of racemic (achiral) AGX51 by Method A and Method B are provided in Figures 29 and and 30, respectively. The peaks in these chromatograms are labelled as to whether the individual peak rotated the polarized light in a (+) or (-) direction in

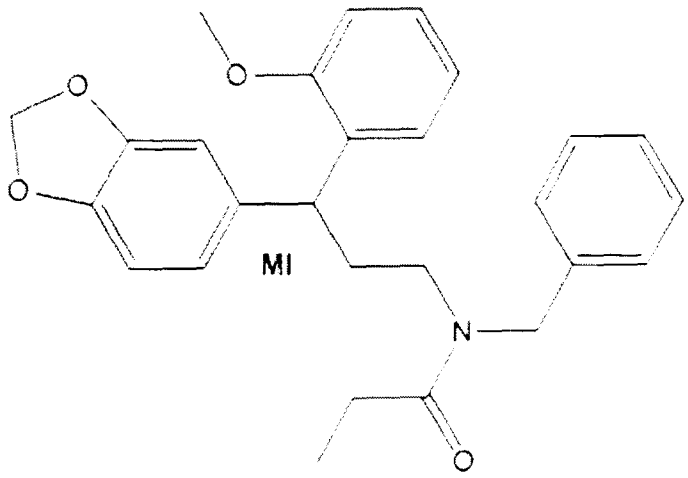
the polarimetry identification. Interestingly, the order of elution for the enantiomers differs for Method A and Method B. In Method A, the first eluting peak is the (+)-enantiomer and in Method B the first eluting peak is (-)-enantiomer.

Example XXIV

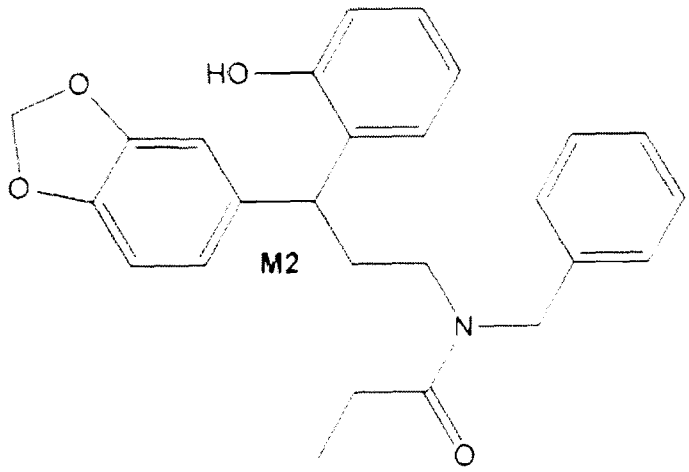
Biotransformation products of AGX51

Further illustration of the invention is presented here through description of *in vivo* biotransformation products of the exemplary anti-Id compound AGX51. CD1 male mice (3/timepoint) were treated *ip* with 30mg/kg AGX51 in DMSO. Blood was collected in heparinized tubes by retro-orbital puncture pre-dose and at 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 & 24 hours post dose. One hundred µL of pH 7.4 PBS were added to the plasma harvested from the blood by centrifugation and the samples from all time points, except pre-dose, combined. The mixture was vortexed for one minute with 1 mL methyl t-butyl ether (MTBE), and the MTBE removed after centrifugation with dry N_2 (g). The residue remaining after removal of the MTBE was reconstituted in 200 µL of ACN. Five µL of this solution was analyzed by LC/MS using a 2.0 x 250 mm C18 column coupled to an electrospray LC-MS set to monitor m/z 100-600 in the HPLC effluent. The flow rate was 200 µL/minute of ACN (85)/H_2O + 0.1% formic acid.

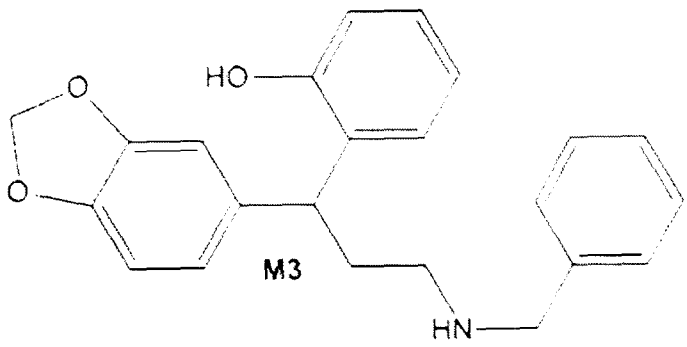
The mass spectral data was carefully screened for MH^+ ions reflecting common biotransformation routes such as dealkylation, hydroxylation, etc. Ions corresponding to demethylation, MH^+ = m/z 418 (M1), depropionylation, MH^+ = m/z 376 (M2) and demethylation + depropionylation, MH+ = m/z 348 (M3), were found and three structures, M1, M2 and M3 as shown below were identified.



N-(3-(benzo[*d*][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-*N*-benzylpropionamide



N-(3-(benzo[*d*][1,3]dioxol-5-yl)-3-(2-hydroxyphenyl)propyl)-*N*-benzylpropionamide



2-(1-(benzo[*d*][1,3]dioxol-5-yl)-3-(benzylamino)propyl)phenol

All publications and patents cited within this specification and listed below are herein incorporated by reference for economy of description, for all purposes of disclosing and describing the methods and/or materials in connection with which the publications are cited. The citation of any publication herein should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

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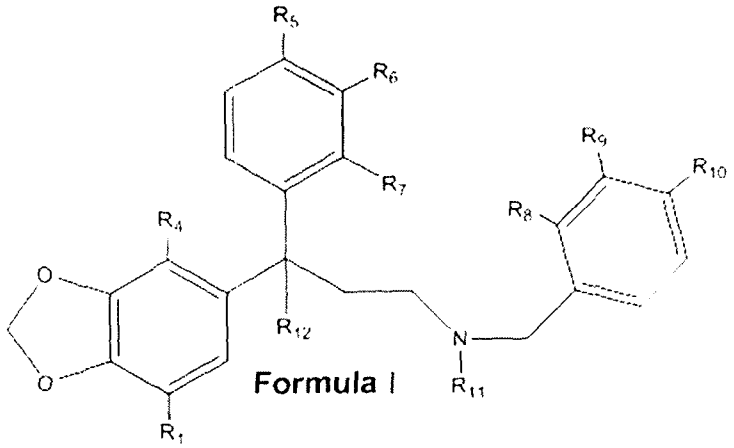
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We claim:

Claim 1. A method for reducing metastasis in a mammalian subject suffering a neoplasm comprising administering an anti-metastatic effective amount of an anti-Id (inhibitor of differentiation) compound of Formula I to said subject;



Formula I

wherein R_1, R_4, R_5, R_6, R_8, R_9, R_{10} are independently selected from the group consisting of hydrogen, hydroxyl, sulfyhydryl, benzyl, 2-bromovinyl amino, hydroxymethyl, methoxy, halogen, pseudohalogen, cyano, carboxyl, nitro, thioalkyl, thioaryl, thiol, substituted or unsubstituted hydrocarbons containing 1 to 20 carbons, alkoxycarbonyl, alkoxycarbonylamino, amino, amino acid, aminocarbonyl, aminocarbonyloxy, aryloxy, carboxyl, cycloalkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, peptidyl, dye, fluorophore, carbohydrate or polypeptidyl; R_8 and R_9 can also have a para configuration in addition to the ortho orientation shown in Formula I; R_7 is independently selected from hydrogen, hydroxyl, methoxy, azido, nitrile, sulfhydryl, halogen, benzoyl, substituted benzoyl or hydroxyl substituted with substituted or unsubstituted hydrocarbon containing 1 to 20 carbons, alkanoyl of a main chain of 1 to 20 carbon atoms, $CF_3(CH_2)_n CO$ where n=1 to 10, $CH_3(CF_2)_n C=O$ where n=1 to 10, $CF_3(CF_2)_n$ where n=0 to 3, aryl, alkoxy, halogen, or nitro, aryloxy, esters of aryloxy, adamantoyl, substituted adamantoyl or aroyl of 1 to 20 carbons; R_7 cannot be methoxy if R_{11} is propionyl while R_7 and R_6 can be ortho, as shown in Formula I, or para to one another; R_{11} is independently hydrogen, propionyl, pivoyl, benzoyl, substituted benzoyl, alkanoyl of a main

chain of 1 to 20 carbon atoms, $CF_3(CH_2)_n C=O$ where n=1 to 10, $CH_3(CF_2)_n C=O$ where n=1 to 10 or $CF_3(CF_2)_n$ where n=0 to 3 and R11 cannot be propionyl if R7 is methoxy; R_{12} is independently hydrogen, halogen, ^2H, $CH_3(CH_2)_n$ where n=0 to 5, $CF_3(CH_2)_nC=O$ where n=1 to 5; $CH_3(CF_2)_n C=O$ where n=1 to 5 or $CF_3(CF_2)_n$ where n=0 to 5.

Claim 2. The method of claim 1, wherein the anti-Id compound is racemic N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51).

Claim 3. The method of claim 1, wherein reduction of metastasis mediated by the anti-Id compound yields a greater than 20% increase in cancer free survival for anti-Id treated subjects compared to non-treated control subjects.

Claim 4. The method of claim 1, wherein reduction of metastasis mediated the anti-Id compound yields at least a 20-50% increase in cancer free survival for anti-Id treated subjects compared to non-treated control subjects.

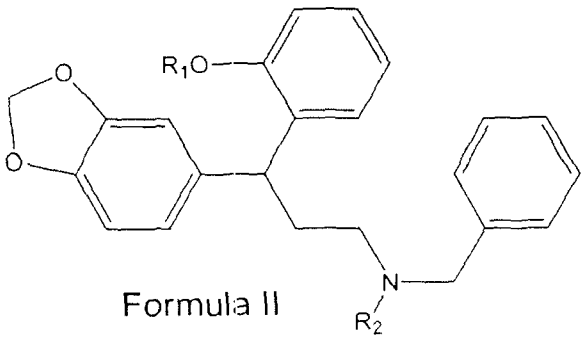
Claim 5. The method of claim 1, wherein reduction of metastasis mediated by the anti-Id compound yields at least a 20% average decrease in average size or number of organ metastases in anti-Id treated subjects compared to non-treated control subjects.

Claim 6. The method of claim 1, wherein reduction of metastasis mediated by the anti-Id compound yields a 20-50% or larger decrease in average size or number of organ metastases in anti-Id treated subjects compared to non-treated control subjects.

Claim 7. The method of claim 1, wherein reduction of metastasis mediated by the anti-Id compound yields at least a 50% decrease in average size or number of organ metastases in anti-Id treated subjects compared to non-treated control subjects.

Claim 8. The method of claim 1, wherein the anti-Id1 compound is a metabolite of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51) selected from the following (Formula II): N-(3-(benzo[*d*][1,3]dioxol-5-yl)-3-(2-hydroxyphenyl)propyl)-*N*-benzylpropionamide (Formula IIa), (3-(benzo[*d*][1,3]dioxol-5-yl)-

N-benzyl-3-(2-methoxyphenyl)propan-1-amine (Formula IIb), 2-(1-(benzo[*d*][1,3]dioxol-5-yl)-3-(benzylamino)propyl)phenol (Formula IIc) or an enantiomer of one of the aforementioned metabolites, or an isolated enantiomer of one of said metabolites.



Formula II

Compound	R_1	R_2
a	H	O=C-CH_2-CH_3
b	O=C-CH_2-CH_3	H
c	H	H

Claim 9. The method of claim 1, wherein the anti-Id compound is a salt of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51), (3-(benzo[d][1,3]dioxol-5-yl)-N-benzyl-3-(2-methoxyphenyl)propan-1-amine, or 2-(1-(benzo[d][1,3]dioxol-5-yl)-3-(benzylamino)propyl)phenol.

Claim 10. The method of claim 9, wherein the salt is a hydrochloride, hydrobromide, hydroiodide, phosphate, sulfate, oxalate, malate, maleate or succinate salt.

Claim 11. The method of claim 1, further comprising coordinately administering a secondary anti-metastatic agent selected from a chemotherapeutic agent, a vascular disrupting agent (VDA), an anti-angiogenic agent, or an HSP90 inhibitor.

Claim 12. A pharmaceutical composition comprising an enantiomerically-enriched, anti-metastatically active (-)-enantiomer of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ((-)-AGX51), in a dosage form comprising the
(-)-AGX51) enantiomer in an effective amount to reduce or prevent one or more symptom(s) of a proliferative disorder or metastatic disease in a mammalian subject.

Claim 13. The pharmaceutical composition of claim 12, wherein the composition has an enantiomeric purity of the (-)-AGX51 enantiomer of 60% enantiomeric excess (ee) or greater.

Claim 14. The pharmaceutical composition of claim 12, wherein the composition has an enantiomeric purity of the (-)-AGX51 enantiomer of 90% enantiomeric excess (ee) or greater.

Claim 15. The pharmaceutical composition of claim 12, wherein the composition has an enantiomeric purity of the (-)-AGX51 enantiomer of 98% enantiomeric excess (ee) or greater.

Claim 16. The pharmaceutical composition of claim 12, wherein the anti-metastatically active, substantially pure (-)-AGX51) enantiomer is formulated for oral, buccal, nasal, aerosol, topical, transdermal, mucosal, or injectable delivery.

Claim 17. The pharmaceutical composition of claim 12, wherein the anti-metastatically active, substantially pure (-)-AGX51) enantiomer is formulated in a sustained release mucosal delivery formulation, or a sustained release topical delivery formulation or patch device.

Claim 18. The pharmaceutical composition of claim 12, wherein the anti-metastatically active, substantially pure (-)-AGX51) enantiomer is formulated in an effective delivery form to provide sustained anti-metastatic effective plasma levels of (-)-AGX51) in human subjects over a period of approximately 8 hours or longer, wherein the sustained anti-metastatic efficacy correlates with sustained reduction of circulating plasma levels of Id1 or Id3 in treated subjects.

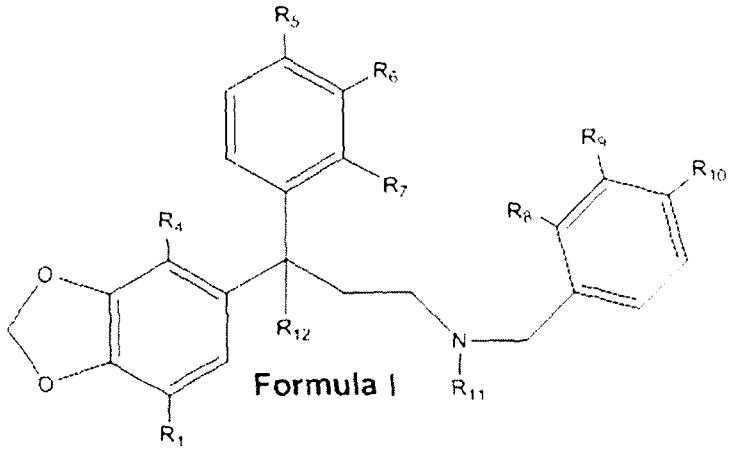
Claim 19. The pharmaceutical composition of claim 12, comprising an anti-metastatic effective daily dose of between about 100 mg to about 900 mg of the (-)-AGX51) enantiomer.

Claim 20. The pharmaceutical composition of claim xx, comprising an anti-metastatic effective dose selected from a 50-100 mg dose, a 100-200 mg dose, a 150-300 mg dose, a

300-400 mg dose, and a 400-600 mg dose, anti-metastatically effective on administration 1-3 times per day.

Claim 21. A pharmaceutical composition comprising an enantiomerically-enriched, anti-metastatically active (-)-enantiomer of N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide ((-)-AGX51), and a secondary anti-metastatic agent selected from a chemotherapeutic agent, a vascular disrupting agent (VDA), an anti-angiogenic agent, or an HSP90 inhibitor.

Claim 22. A method for treating and pathogenic vascular proliferative disease in a mammalian subject, comprising administering an anti-angiogenic effective amount of an anti-Id compound of Formula I to said subject;



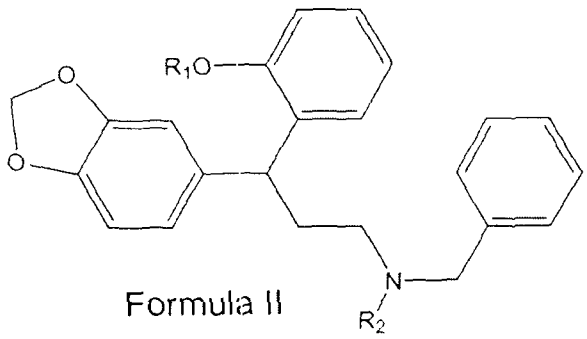
Formula I

wherein R_1, R_4, R_5, R_6, R_8, R_9, R_{10} are independently selected from the group consisting of hydrogen, hydroxyl, sulfyhydryl, benzyl, 2-bromovinyl amino, hydroxymethyl, methoxy, halogen, pseudohalogen, cyano, carboxyl, nitro, thioalkyl, thioaryl, thiol, substituted or unsubstituted hydrocarbons containing 1 to 20 carbons, alkoxycarbonyl, alkoxycarbonylamino, amino, amino acid, aminocarbonyl, aminocarbonyloxy, aryloxy, carboxyl, cycloalkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, peptidyl, dye, fluorophore, carbohydrate or polypeptidyl; R_8 and R_9 can also have a para configuration in addition to the ortho orientation shown in Formula I; R_7 is independently selected from hydrogen, hydroxyl, methoxy, azido, nitrile, sulfhydryl, halogen, benzoyl, substituted benzoyl or hydroxyl substituted with substituted or

unsubstituted hydrocarbon containing 1 to 20 carbons, alkanoyl of a main chain of 1 to 20 carbon atoms, $CF_3(CH_2)_nCO$ where n=1 to 10, $CH_3(CF_2)_nC=O$ where n=1 to 10, $CF_3(CF_2)_n$ where n=0 to 3, aryl, alkoxy, halogen, or nitro, aryloxy, esters of aryloxy, adamantoyl, substituted adamantoyl or aroyl of 1 to 20 carbons; R_7 cannot be methoxy if R_{11} is propionyl while R_7 and R_6 can be ortho, as shown in Formula I, or para to one another; R_{11} is independently hydrogen, propionyl, pivoyl, benzoyl, substituted benzoyl, alkanoyl of a main chain of 1 to 20 carbon atoms, $CF_3(CH_2)_nC=O$ where n=1 to 10, $CH_3(CF_2)_nC=O$ where n=1 to 10 or $CF_3(CF_2)_n$ where n=0 to 3 and R11 cannot be propionyl if R7 is methoxy; R_{12} is independently hydrogen, halogen, ^2H, $CH_3(CH_2)_n$ where n=0 to 5, $CF_3(CH_2)_nC=O$ where n=1 to 5; $CH_3(CF_2)_nC=O$ where n=1 to 5 or $CF_3(CF_2)_n$ where n=0 to 5.

Claim 23. The method of claim 22, wherein the anti-Id compound is racemic N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide (AGX51), or an isolated (-)-AGX51 enantiomer.

Claim 24. The method of claim 22, wherein the anti-Id compound is selected from racemic N-(3-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)propyl)-N-benzylpropionamide, N-(3-(benzo[*d*][1,3]dioxol-5-yl)-3-(2-hydroxyphenyl)propyl)-*N*-benzylpropionamide (Formula IIa), (3-(benzo[*d*][1,3]dioxol-5-yl)-*N*-benzyl-3-(2-methoxyphenyl)propan-1-amine (Formula IIb), 2-(1-(benzo[*d*][1,3]dioxol-5-yl)-3-(benzylamino)propyl)phenol (Formula IIc), or an isolated enantiomer thereof.



Formula II

Compound	R_1	R_2
a	H	O=C-CH_2-CH_3
b	O=C-CH_2-CH_3	H
c	H	H

Claim 25. The method of claim 22, wherein the anti-Id compound is in a salt form selected from a hydrochloride, hydrobromide, hydroiodide, phosphate, sulfate, oxalate, malate, maleate or succinate salt.

Claim 26. The method of claim 22, wherein the pathogenic vascular proliferative disease is an ocular disease.

Claim 27. The method of claim 25, wherein the ocular disease is selected from the group consisting of Age-Related Macular Degeneration (AMD), diabetic retinopathy, retinopathy of prematurity, sickle cell retinopathy, retinal venous occlusive disease, central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO), neovascular macular degeneration or an ocular cancer.

Claim 28. The method of claim 22, wherein the pathogenic vascular proliferative disease is Age-Related Macular Degeneration (AMD).

Claim 29. The method of claim 27, wherein the pathogenic vascular proliferative disease is wet, exudative AMD.

Claim 30. The method of claim 22, wherein the anti-Id compound is administered coordinately with a secondary drug selected from an anti-VEGF agent, an anti-VEGF receptor antagonist, a vascular disrupting agent (VDA), an HSP90 inhibitor, and an anti-inflammatory compound.

Claim 31. The method of claim 22, further comprising photodynamic therapy (PDT) or laser photocoagulation.

.

Claim 32. The method of claim 29, wherein the anti-Id compound yields a greater than 20% therapeutic reduction in occurrence (numbers of lesions among treatment groups) or size (total affected retinal surface area in a subject or among a group of subjects) of wet AMD lesions in anti-Id treated subjects compared to non-treated control subjects.

Claim 33. The method of claim 29, wherein the anti-Id compound yields a 30-50% therapeutic reduction in occurrence (numbers of lesions among treatment groups) or size (total affected retinal surface area in a subject or among a group of subjects) of wet AMD lesions in anti-Id treated subjects compared to non-treated control subjects.

Claim 34. The method of claim 29, wherein the anti-Id compound yields a 50% or greater therapeutic reduction in occurrence (numbers of lesions among treatment groups) or size (total affected retinal surface area in a subject or among a group of subjects) of the wet AMD lesion in anti-Id treated subjects compared to non-treated control subjects.

Claim 34. The method of claim 29, wherein suppression of wet AMD lesions by the anti-Id compound yields at least a 20% smaller decrease in Snellen line score over a selected treatment or monitoring period in anti-Id treated subjects compared to non-treated control subjects.

Claim 35. The method of claim 29, wherein suppression of the wet AMD lesion by the anti-Id compound yields a 20-50% smaller decrease in Snellen line score over a selected treatment or monitoring period in anti-Id treated subjects compared to non-treated control subjects.

Claim 36. The method of claim 29, wherein suppression of the wet AMD lesion by the anti-Id compound yields at least a 50% smaller decrease in Snellen line score over a selected treatment or monitoring period in anti-Id treated subjects compared to non-treated control subjects.

Claim 37. A composition or kit for use in treating subjects receiving drug treatment for a pathogenic angiogenic condition comprising: (a) an anti-Id active compound and (b) a second drug compound selected from an anti-VEGF agent, an anti-VEGF receptor antagonist, a vascular disrupting agent (VDA), an HSP90 inhibitor, or an anti-inflammatory compound.

Claim 38. A method for diagnosing a hyperproliferative disorder in a mammalian subject by determining a concentration in a biologic sample of an Id protein, comprising:

reacting a biological sample from a test subject with an Id binding protein that binds an HLH region of an Id protein;

contacting the sample with labeled antibody that binds specifically to the Id protein at an antibody binding site on the Id protein separate from the HLH region of the Id protein bound by the Id binding protein, wherein the labeled antibody binds to a different location on the Id protein than the Id binding protein; and

quantifying an amount of labeled antibody complexed with the Id binding protein.

Claim 39. The method of claim 38, wherein the Id binding protein or the anti-Id antibody is bound to a solid-phase support.

Claim 40. The method of claim 38, wherein the Id binding protein or the anti-Id antibody is labelled.

Claim 41. The method of claim 38, wherein the both the anti-Id antibody and Id binding protein are labelled.

Claim 42. The method of claim 38, wherein the Id binding protein is a bHLH protein selected from a group consisting of E12, E47, E2-2, HEB, MyoD and MRF.

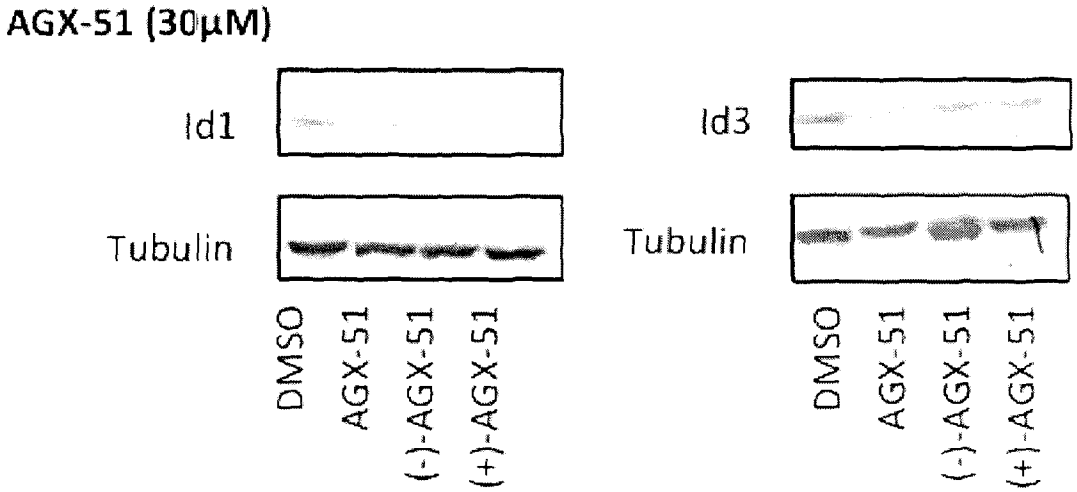
Claim 43. The method of claim 38, wherein the Id protein is Id1 or Id3.

Claim 44. The method of claim 38, wherein the biological sample is a body fluid, blood or tissue sample from a mammalian subject presenting with cancer, or with a diagnosed elevated risk of cancer or metastatic disease.

FIGURE 1



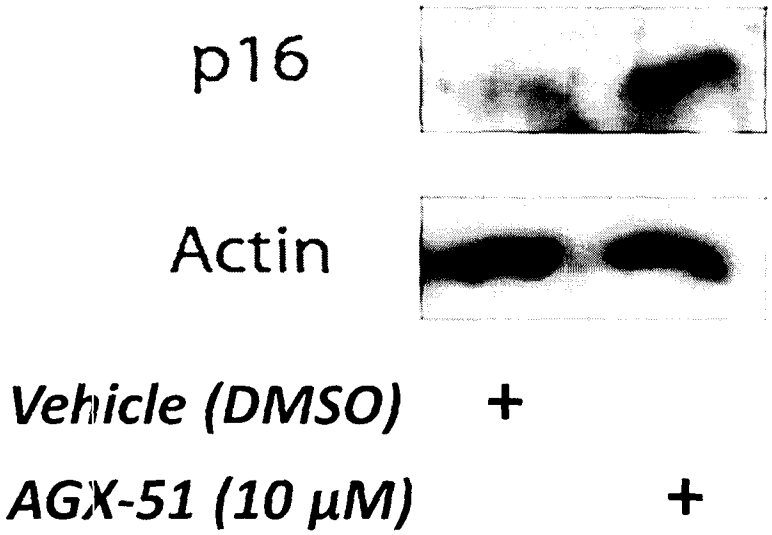
FIGURE 2


AGX-51 (30μM)
Id1
Tubulin
Id3
Tubulin
DMSO
AGX-51
(-)-AGX-51
(+)-AGX-51
DMSO
AGX-51
(-)-AGX-51
(+)-AGX-51

5

FIGURE 3

AGX51 Rescues P-16 in Leukemic Cells



5

FIGURE 4

AGX51 Rescues P-21 in UMUC-3 Human Bladder Carcinoma Cells

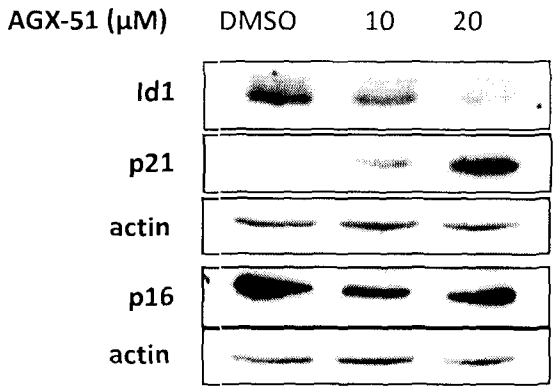
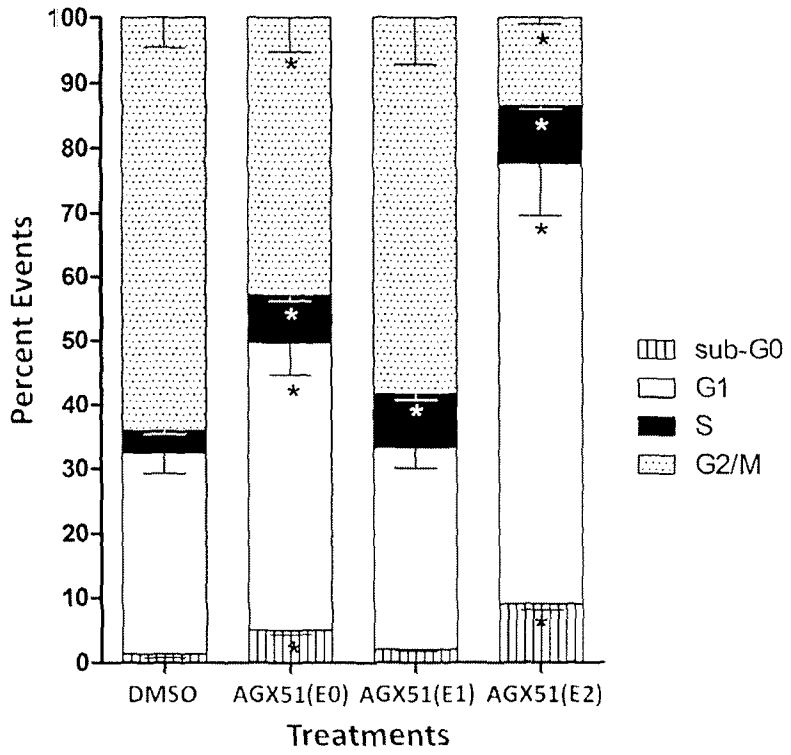


FIGURE 5

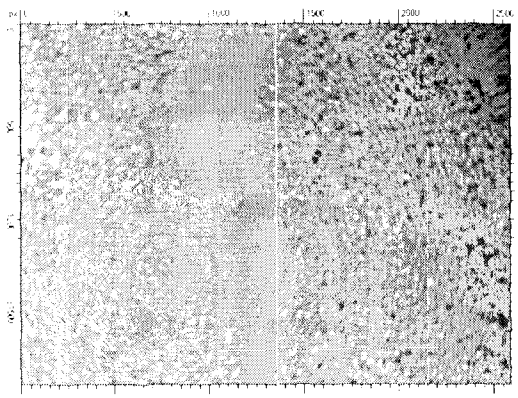
Anti-Id Compounds Restore Cell Cycle Control



5

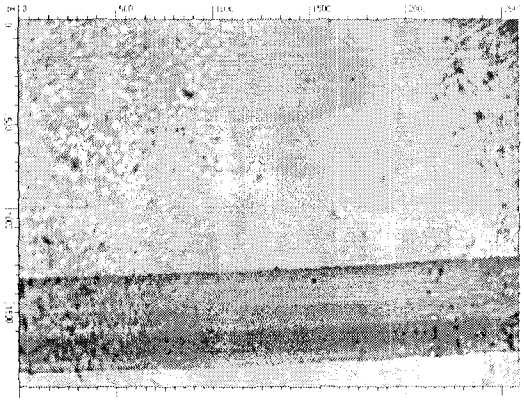
FIGURE 6

PC-3 Prostate Cancer Cells
Four Days Treatment Vehicle Control Post "Scratch"



"Scratch" Gone as Result of Migration

PC-3 Prostate Cancer Cells
Four Days Treatment 1 μM AGX-51 Post "Scr



"Scratch" Maintained-No Migration

5

FIGURE 7

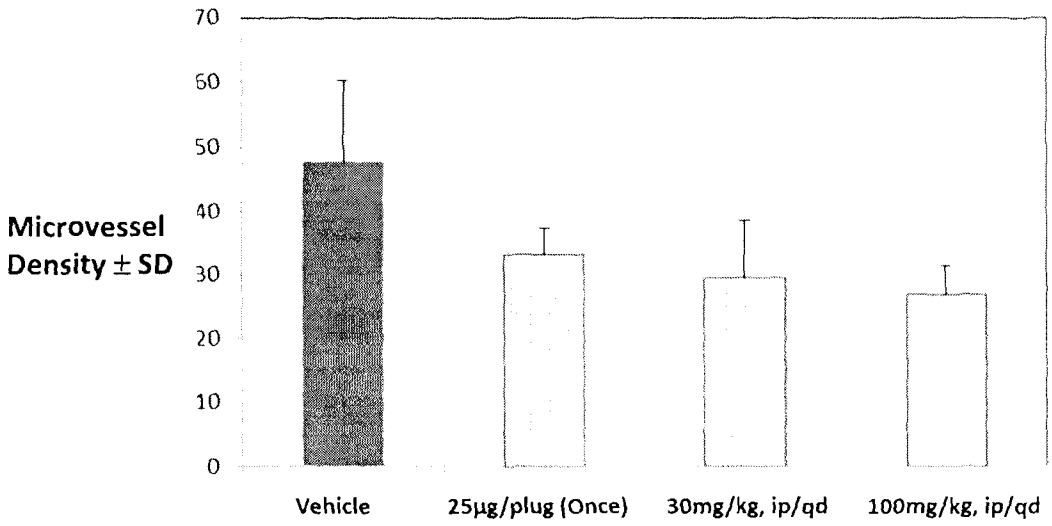
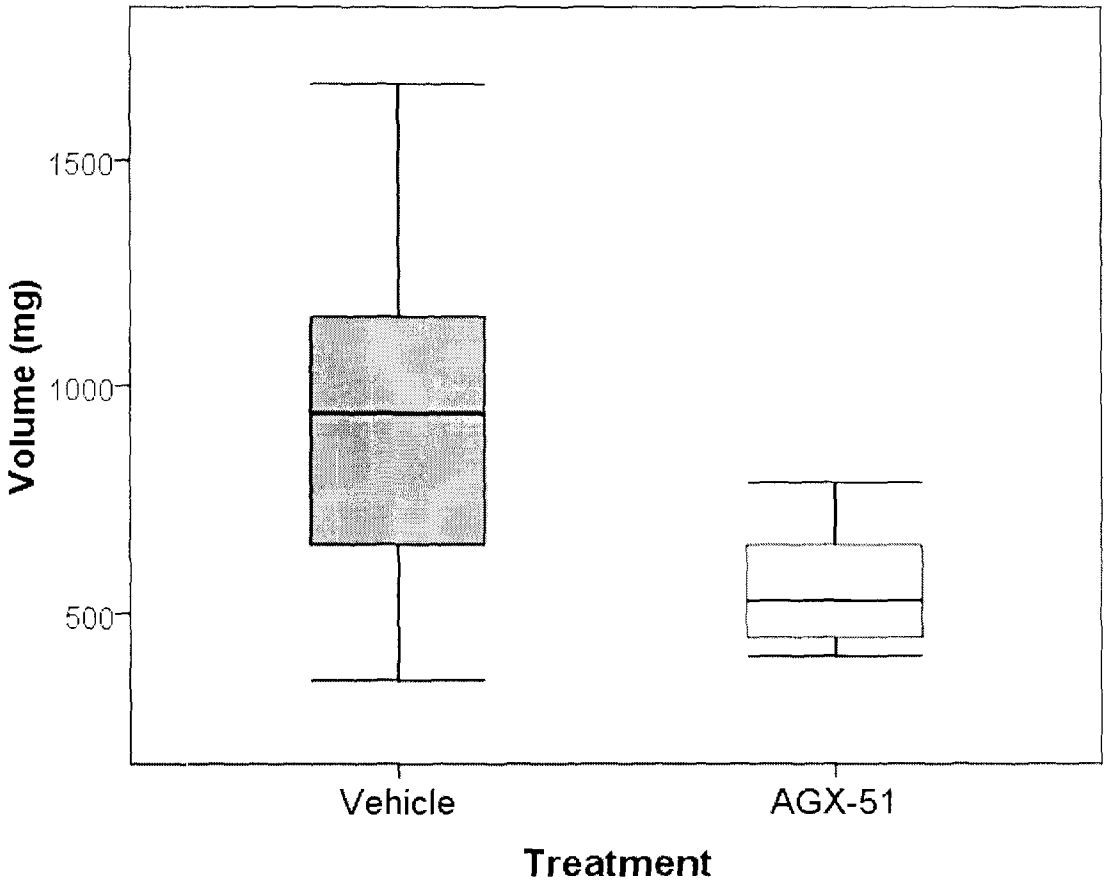

Microvessel Density ± SD
70
60
50
40
30
20
10
0
Vehicle
25μg/plug (Once)
30mg/kg, ip/qd
100mg/kg, ip/qd

FIGURE 8



5

FIGURE 9

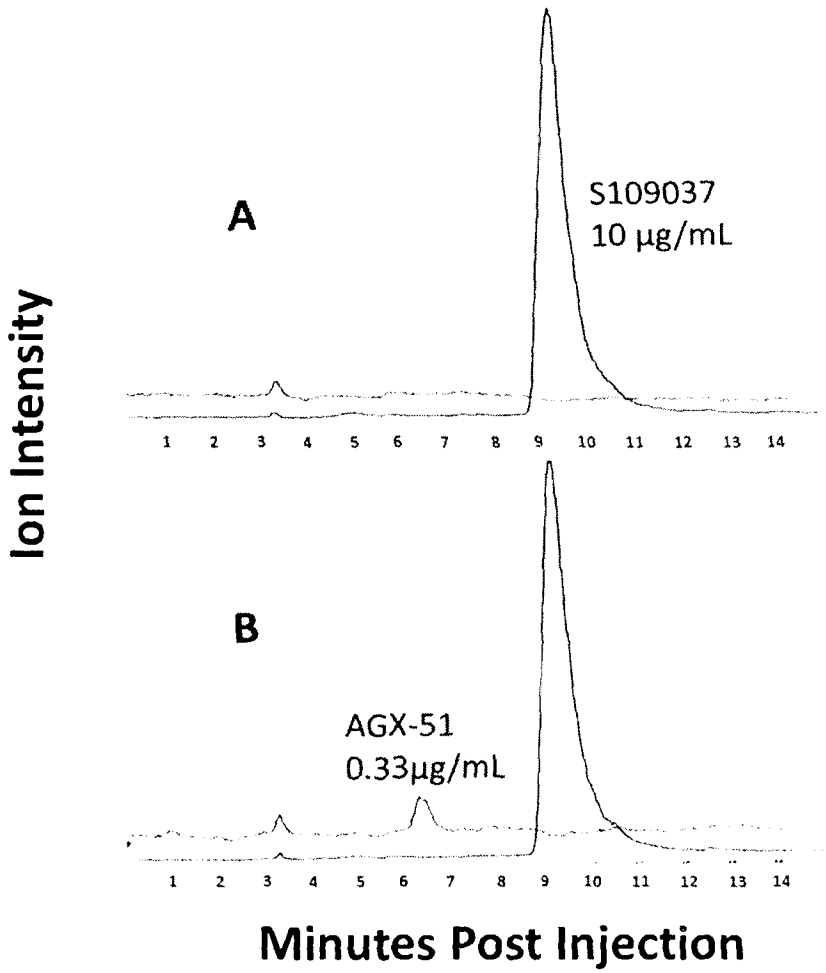

A
S109037
10 µg/mL
B
AGX-51
0.33µg/mL
Ion Intensity
1 2 3 4 5 6 7 8 9 10 11 12 13 14
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Minutes Post Injection

FIGURE 10

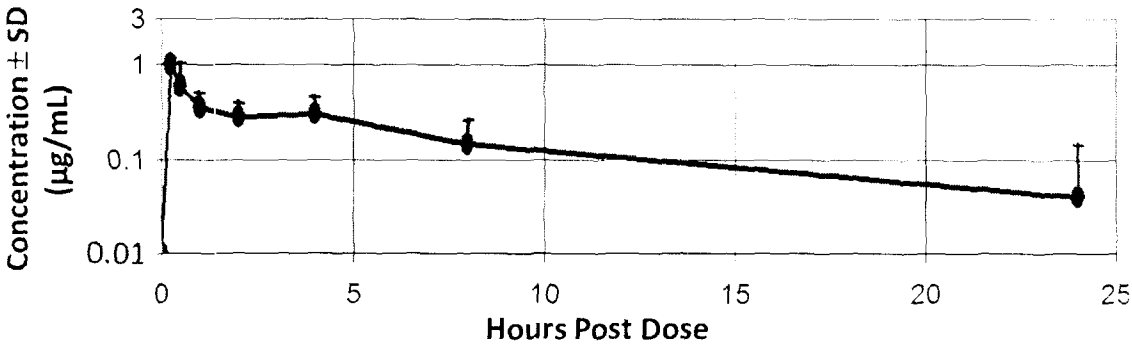
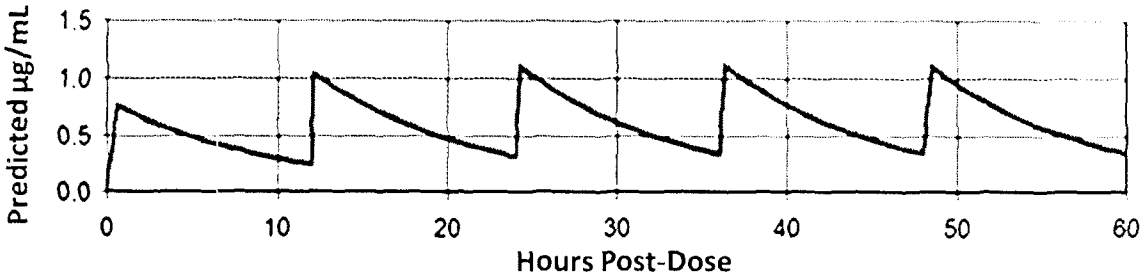
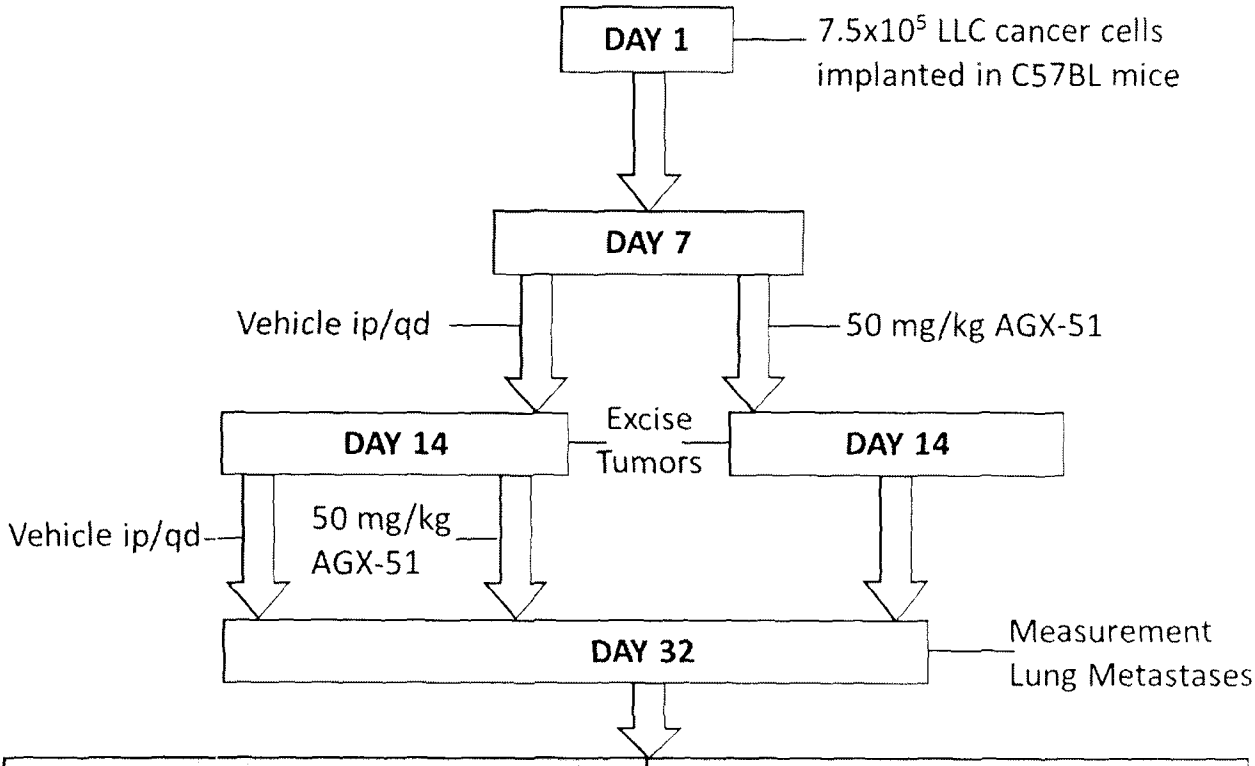


FIGURE 11



5

FIGURE 12



Treatment	Number with > 5 Tumor Foci
Control [Q1Dx25(7)]	7/9[a,b]
AGX-51 [Q1Dx25(7)]	3/9[b,c]
AGX-51 [Q1Dx18(14)]	3/10[d]

[a]No sample for one animal; [b]Too Numerous to Count (TNTC)=4; [c]TNTC=2 ;[d]TNTC=0

FIGURE 13

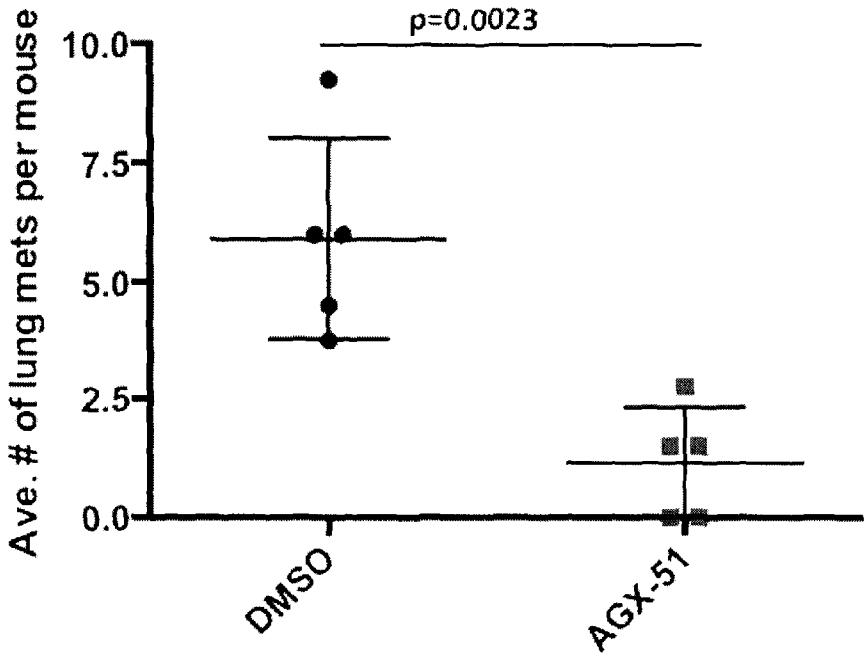


FIGURE 14

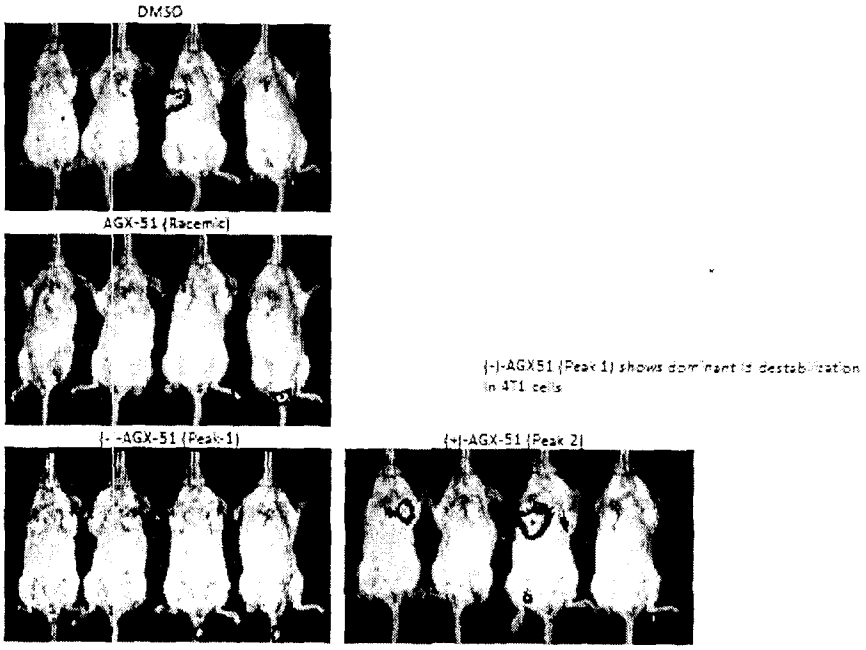

DMSO
AGX-51 (Racemic)
(-)-AGX-51 (Peak-1)
(+)-AGX-51 (Peak 2)
(+)-AGX51 (Peak 1) shows dominant destabilization in 4T1 cells

FIGURE 14

Figure 6

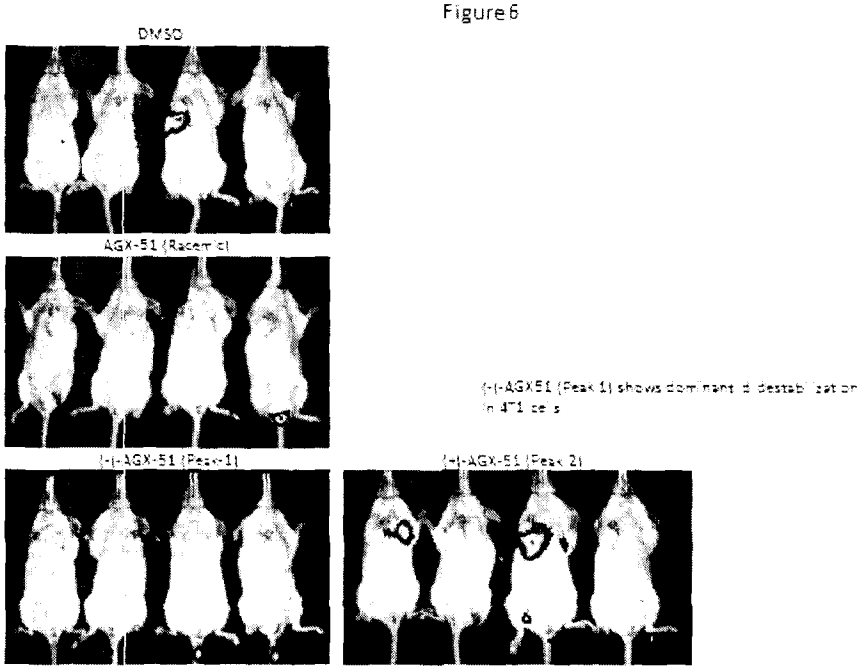


FIGURE 15

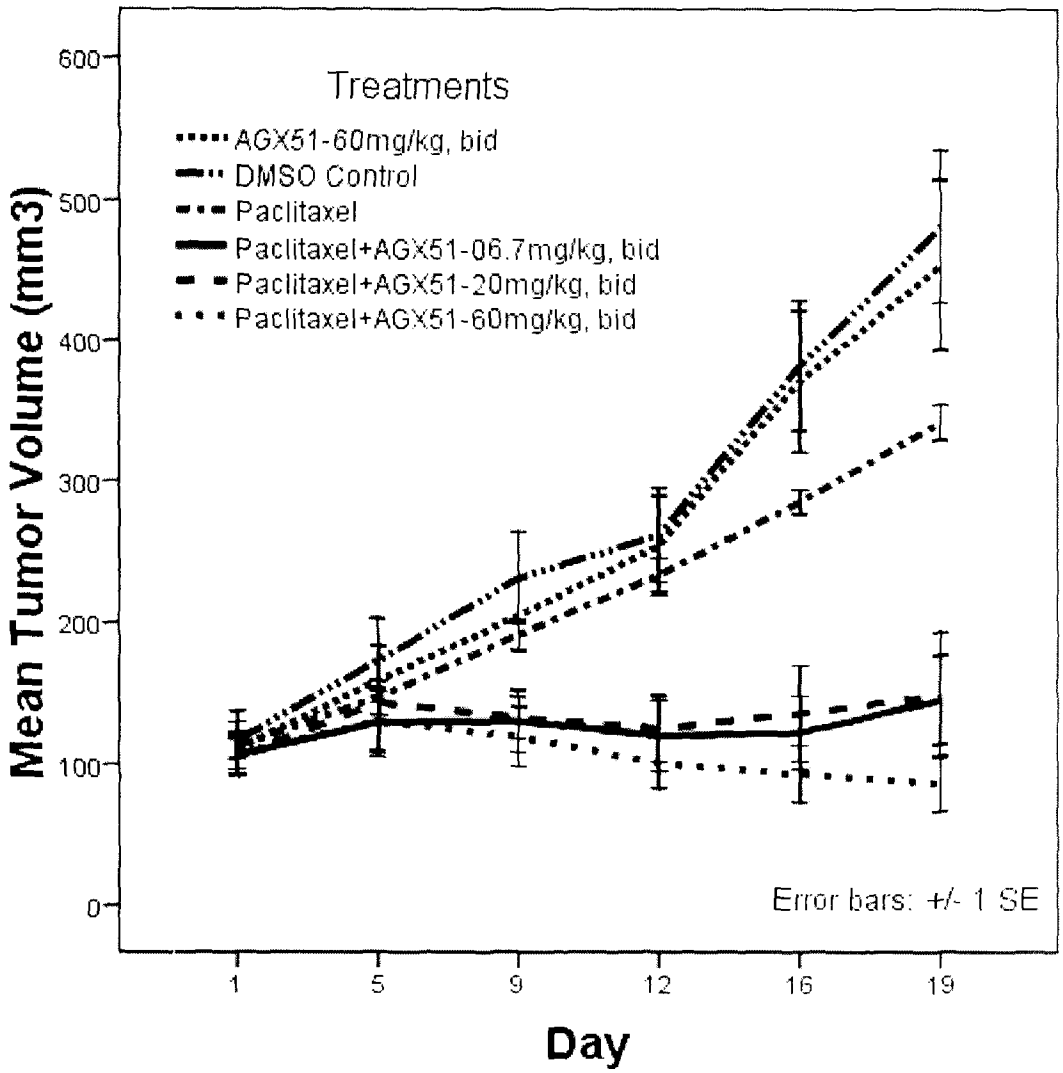

Treatments
AGX51-60mg/kg, bid
DMSO Control
Paclitaxel
Paclitaxel+AGX51-06.7mg/kg, bid
Paclitaxel+AGX51-20mg/kg, bid
Paclitaxel+AGX51-60mg/kg, bid
Mean Tumor Volume (mm3)
600
500
400
300
200
100
0
Error bars: +/- 1 SE
1
5
9
12
16
19
Day

FIGURE 16

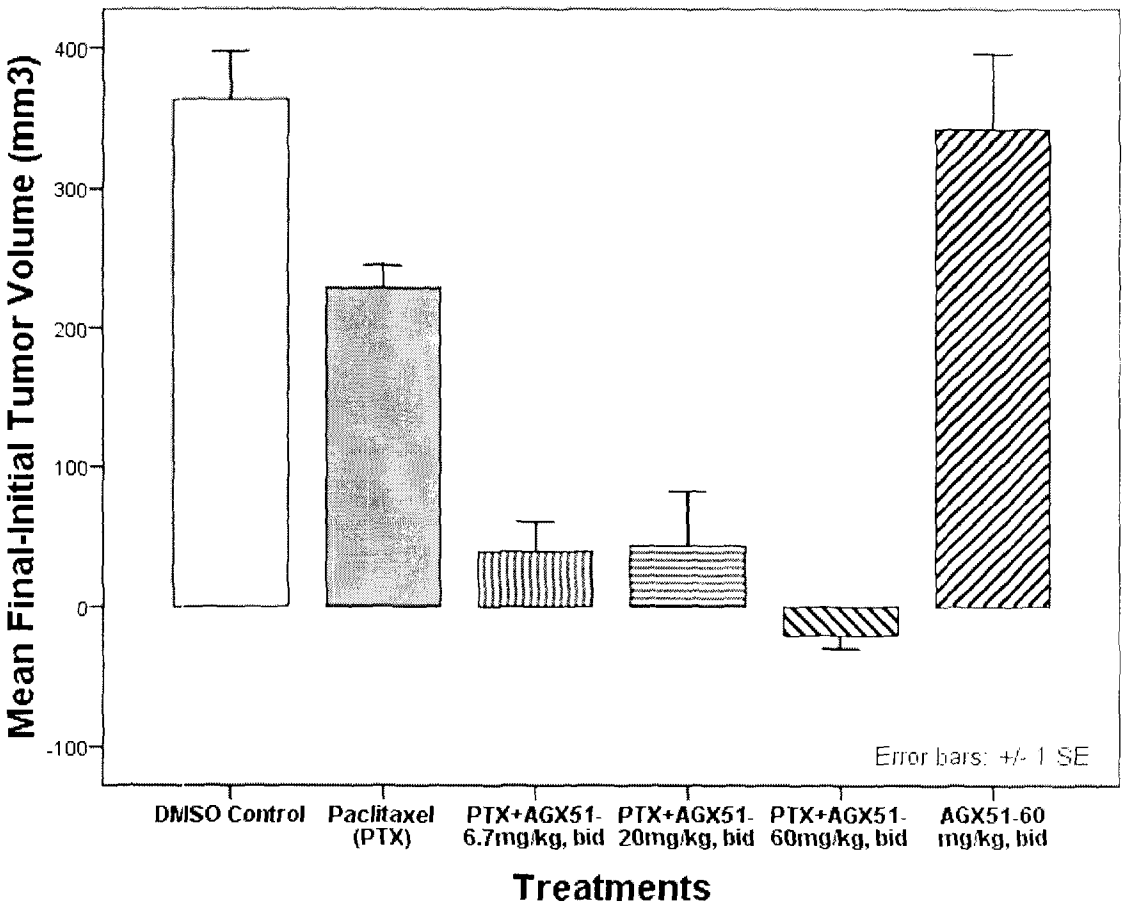

Mean Final-Initial Tumor Volume (mm3)
400
300
200
100
0
-100
Error bars: +/- 1 SE
DMSO Control
Paclitaxel (PTX)
PTX+AGX51-6.7mg/kg, bid
PTX+AGX51-20mg/kg, bid
PTX+AGX51-60mg/kg, bid
AGX51-60 mg/kg, bid
Treatments

FIGURE 17

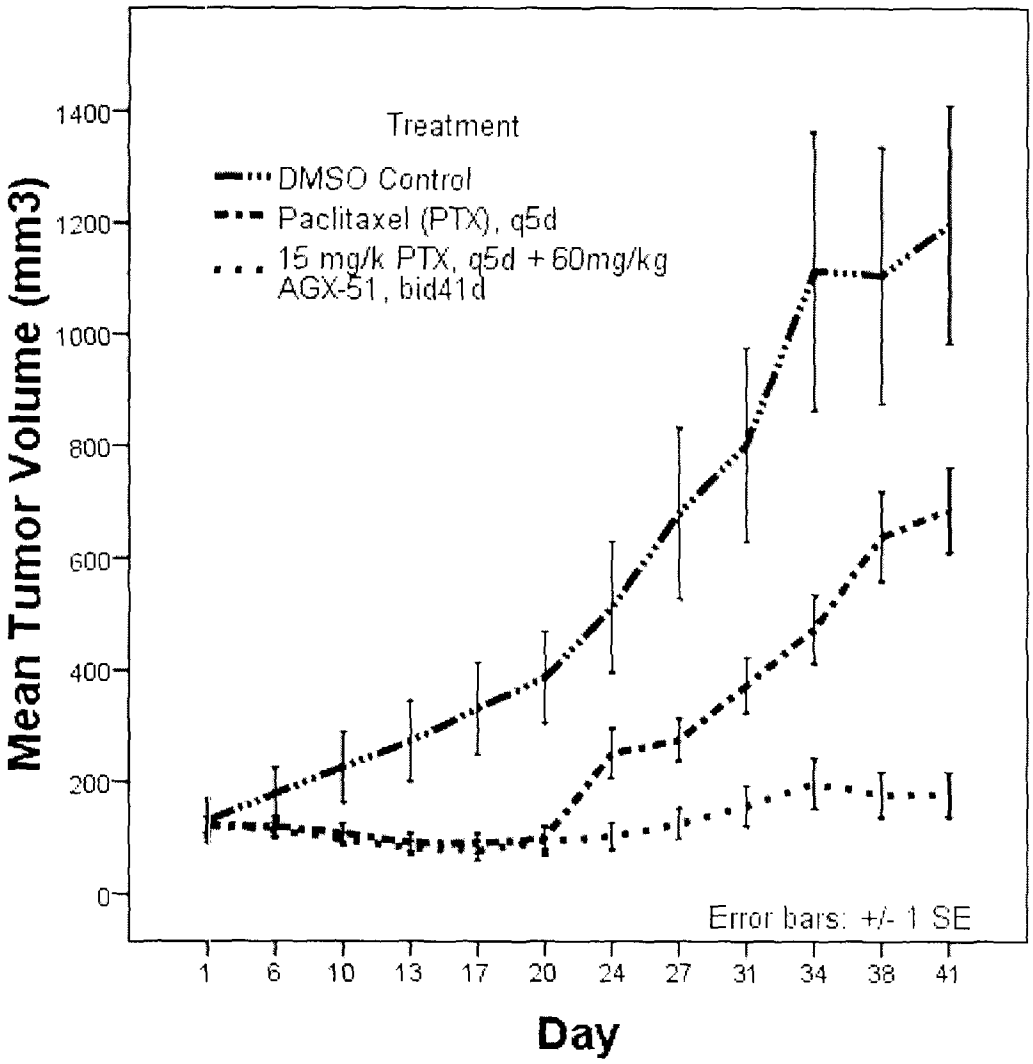
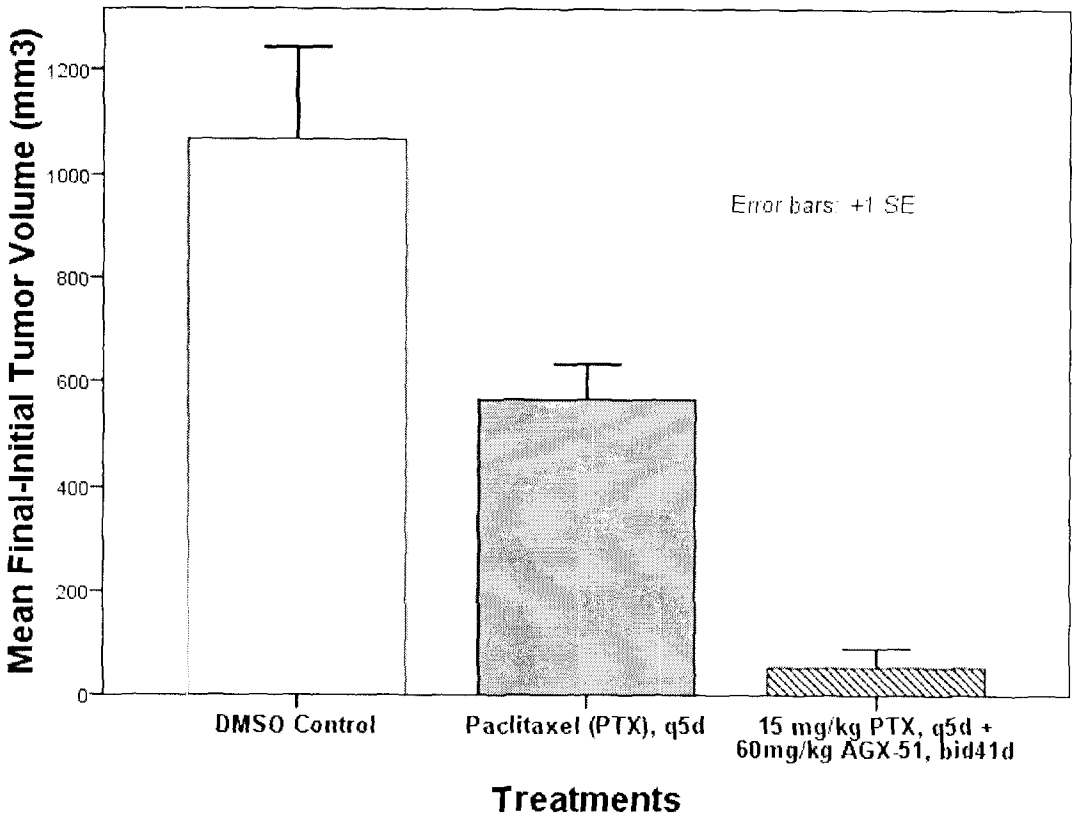
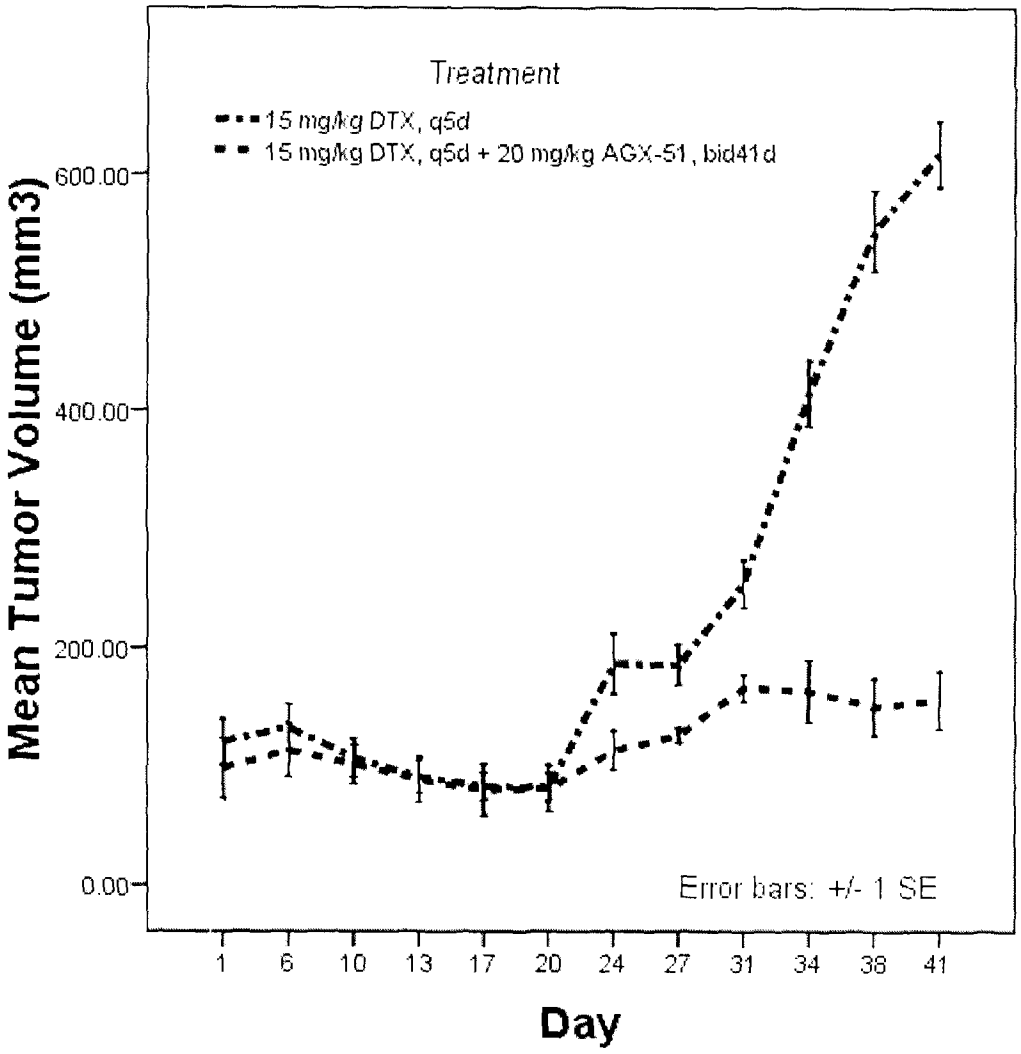

Treatment
DMSO Control
Paclitaxel (PTX), q5d
15 mg/k PTX, q5d + 60mg/kg AGX-51, bid41d
Error bars: +/- 1 SE
Mean Tumor Volume (mm3)
0
200
400
600
800
1000
1200
1400
1
6
10
13
17
20
24
27
31
34
38
41
Day

FIGURE 18


Mean Final-Initial Tumor Volume (mm3)
1200
1000
800
600
400
200
0
Error bars: +1 SE
DMSO Control
Paclitaxel (PTX), q5d
15 mg/kg PTX, q5d + 60mg/kg AGX-51, bid41d
Treatments

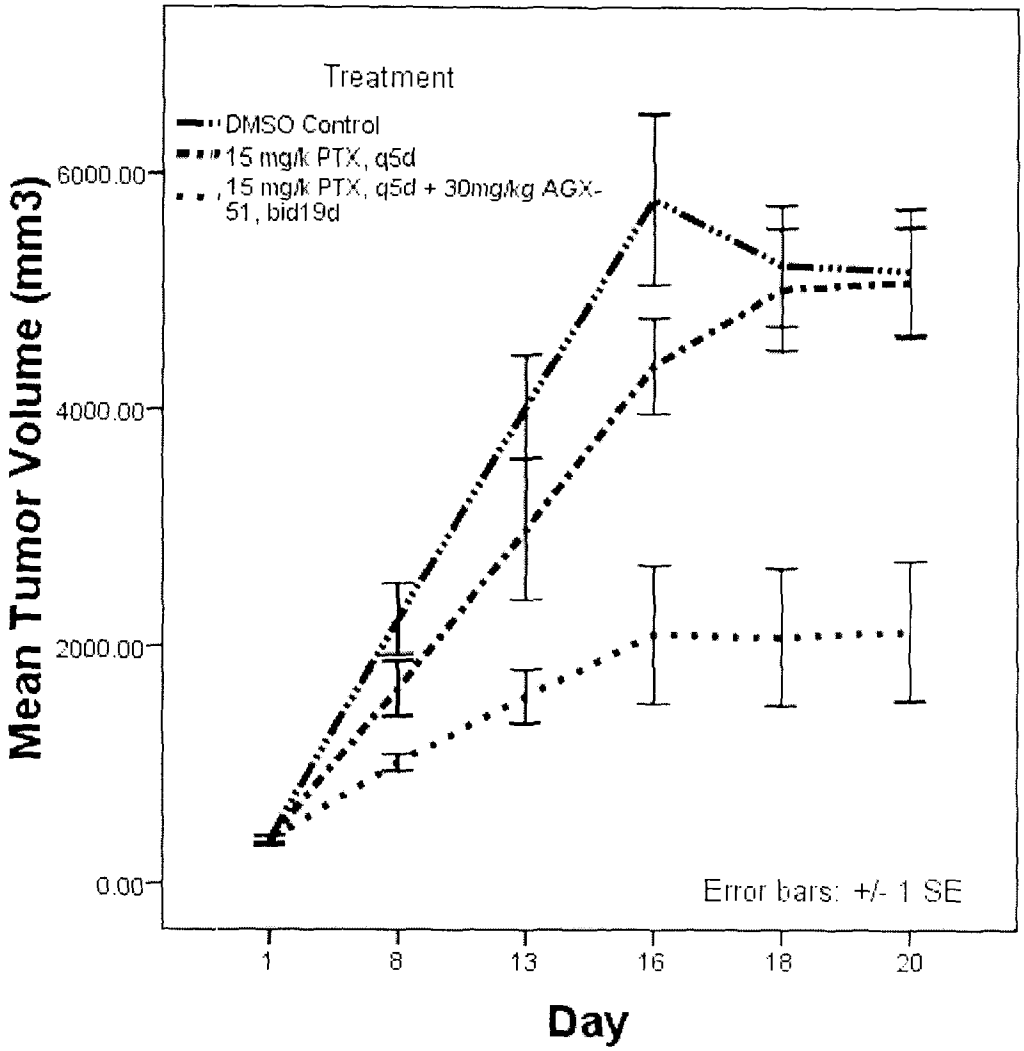
5

FIGURE 19


Treatment
15 mg/kg DTX, q5d
15 mg/kg DTX, q5d + 20 mg/kg AGX-51, bid41d
Mean Tumor Volume (mm3)
600.00
400.00
200.00
0.00
Error bars: +/- 1 SE
1
6
10
13
17
20
24
27
31
34
38
41
Day

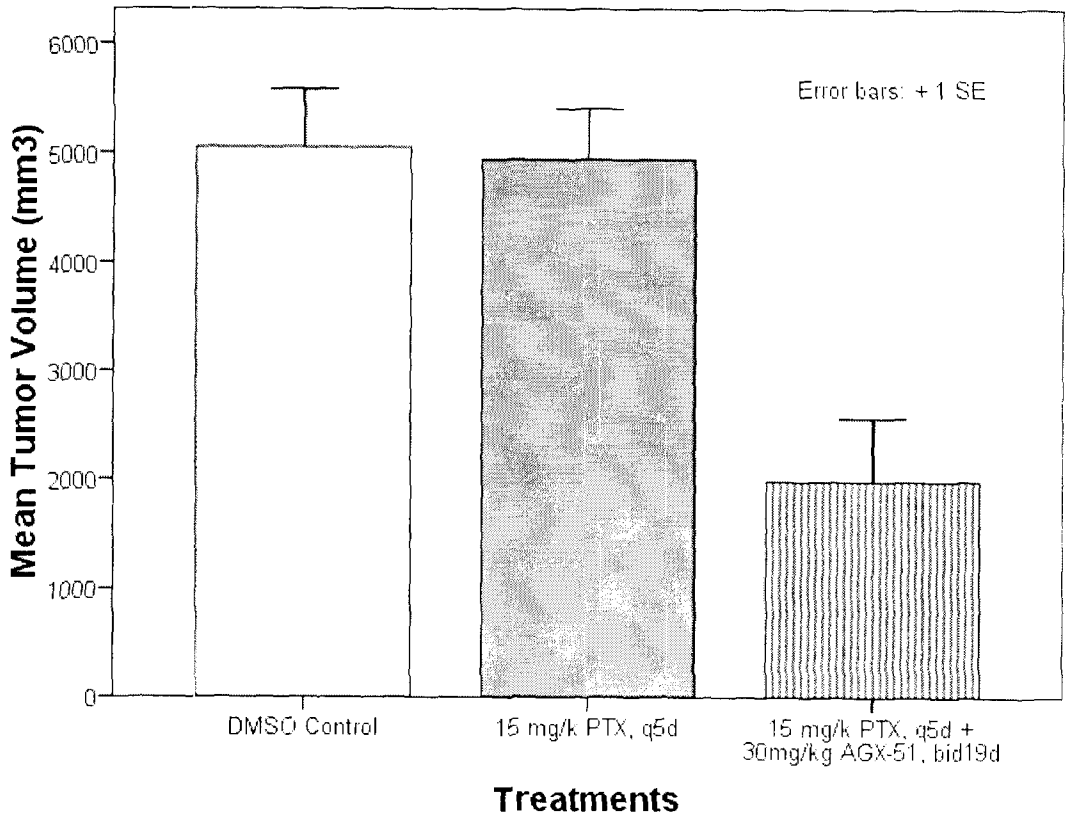
5

FIGURE 20


Treatment
DMSO Control
15 mg/k PTX, q5d
15 mg/k PTX, q5d + 30mg/kg AGX-51, bid19d
Mean Tumor Volume (mm3)
6000.00
4000.00
2000.00
0.00
Error bars: +/- 1 SE
1
8
13
16
18
20
Day

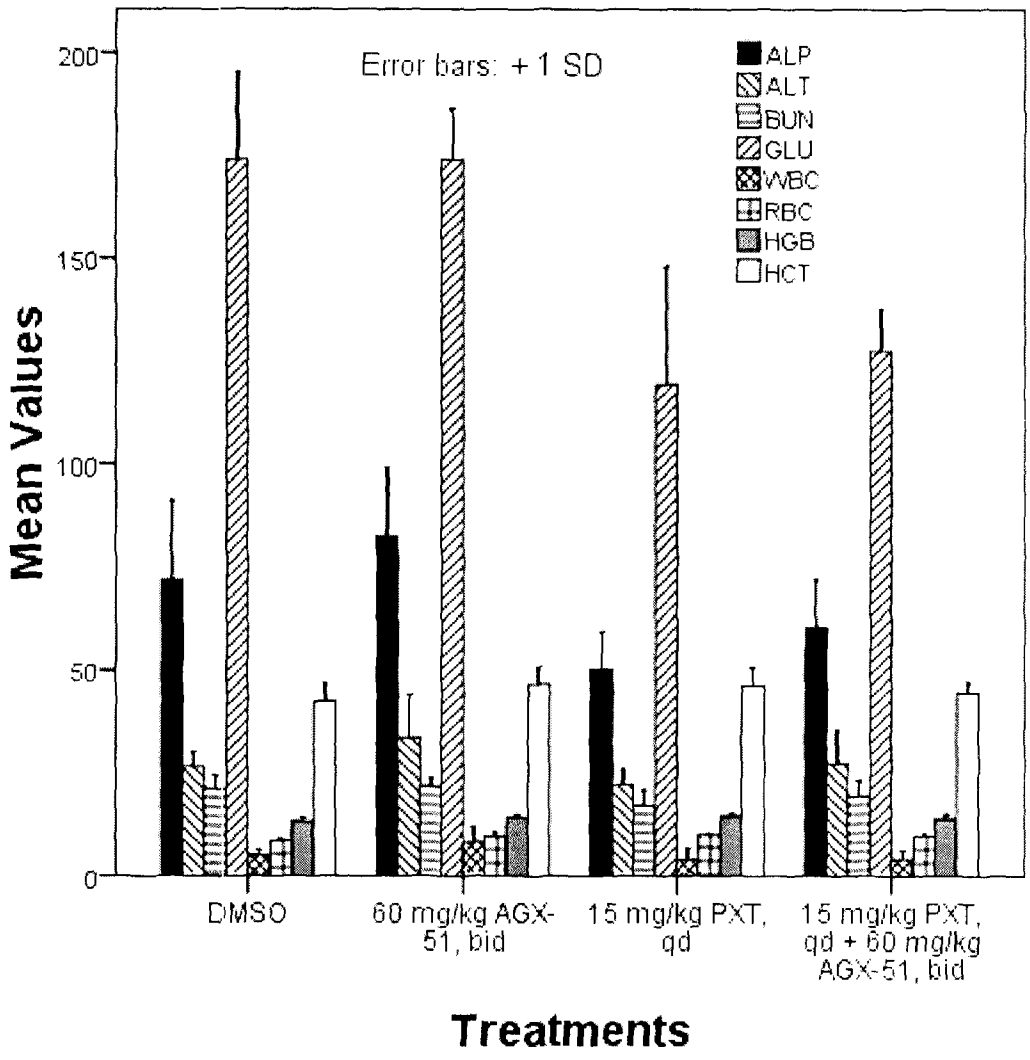
5

FIGURE 21


Mean Tumor Volume (mm3)
6000
5000
4000
3000
2000
1000
0
Error bars: + 1 SE
DMSO Control
15 mg/k PTX, q5d
15 mg/k PTX, q5d +
30mg/kg AGX-51, bid19d
Treatments

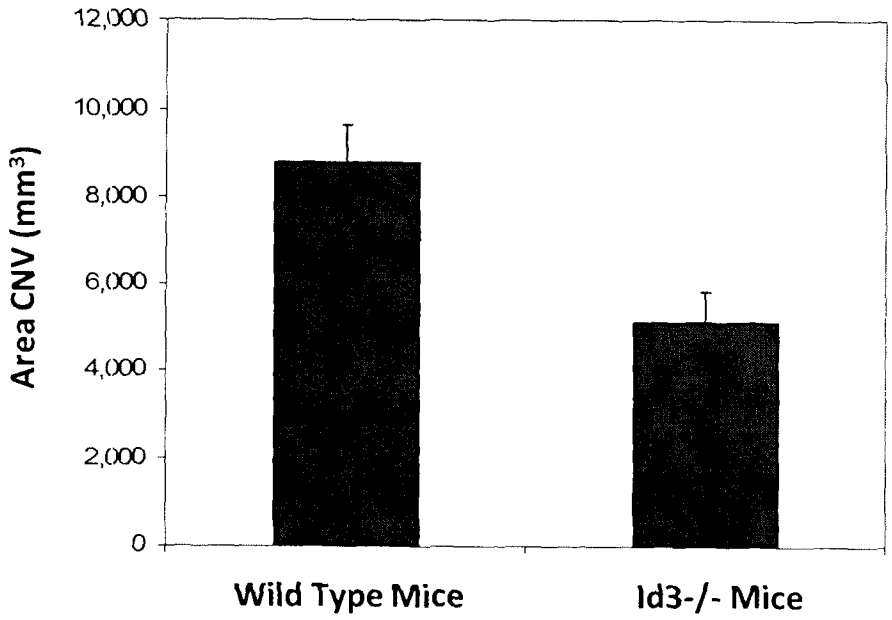
5

FIGURE 22


Error bars: + 1 SD
ALP
ALT
BUN
GLU
WBC
RBC
HGB
HCT
Mean Values
200
150
100
50
0
DMSO
60 mg/kg AGX-51, bid
15 mg/kg PXT, qd
15 mg/kg PXT, qd + 60 mg/kg AGX-51, bid
Treatments

5

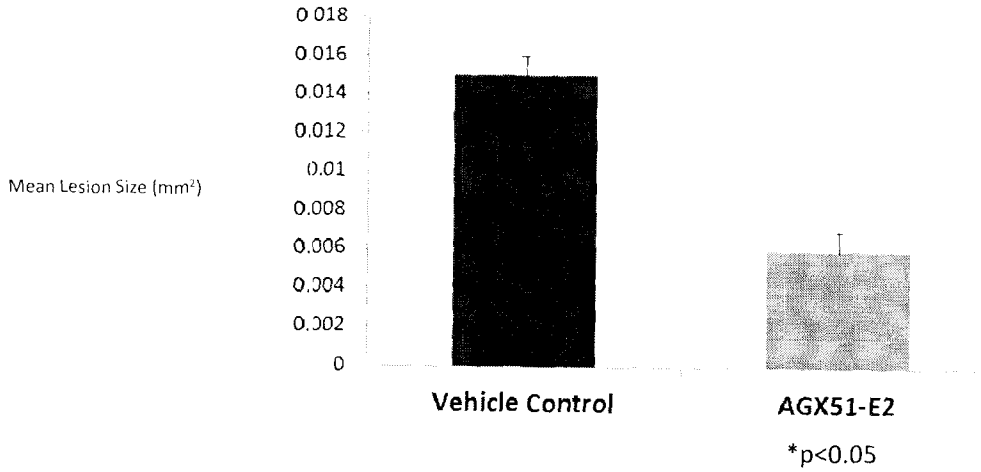
FIGURE 23


12,000
10,000
8,000
6,000
4,000
2,000
0
Area CNV (mm3)
Wild Type Mice
Id3-/- Mice

5

FIGURE 24

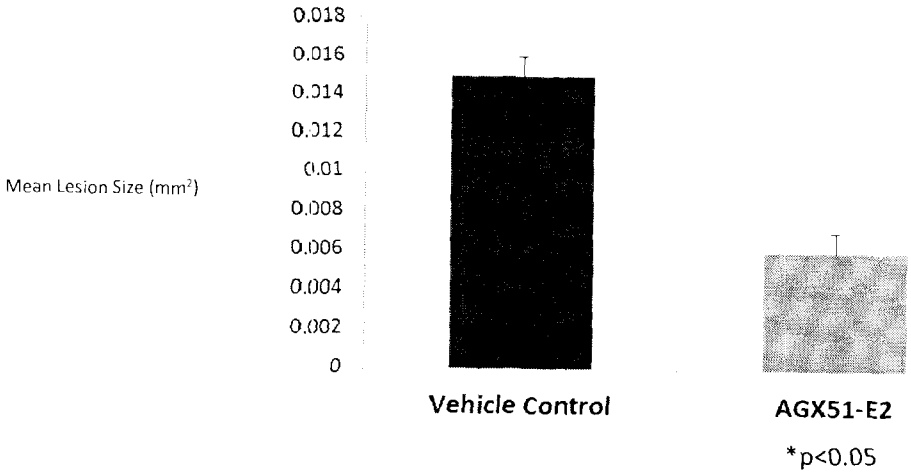
AGX51 Administered ivt Protects in Mouse Model of Human Age-Related Macular Degeneration



5

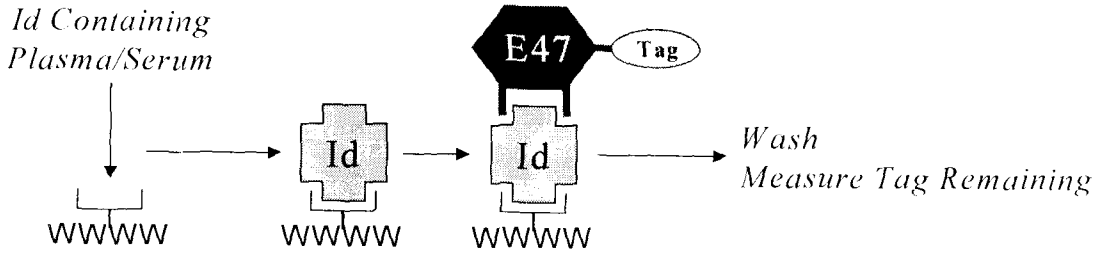
FIGURE 25

AGX51 Administered ip Protects in Mouse Model of Human Age-related Macular Degeneration



5

FIGURE 26

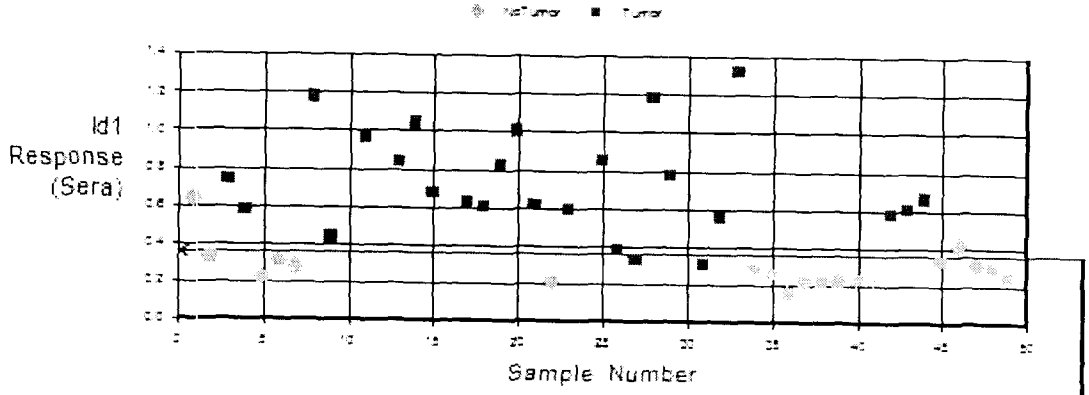

Id Containing
Plasma/Serum
E47
Tag
Id
Id
Wash
Measure Tag Remaining
Id Capture Antibody
Bound to Microtiter Plate

5

FIGURE 27

Companion Diagnostic Available to Support Clinical Studies

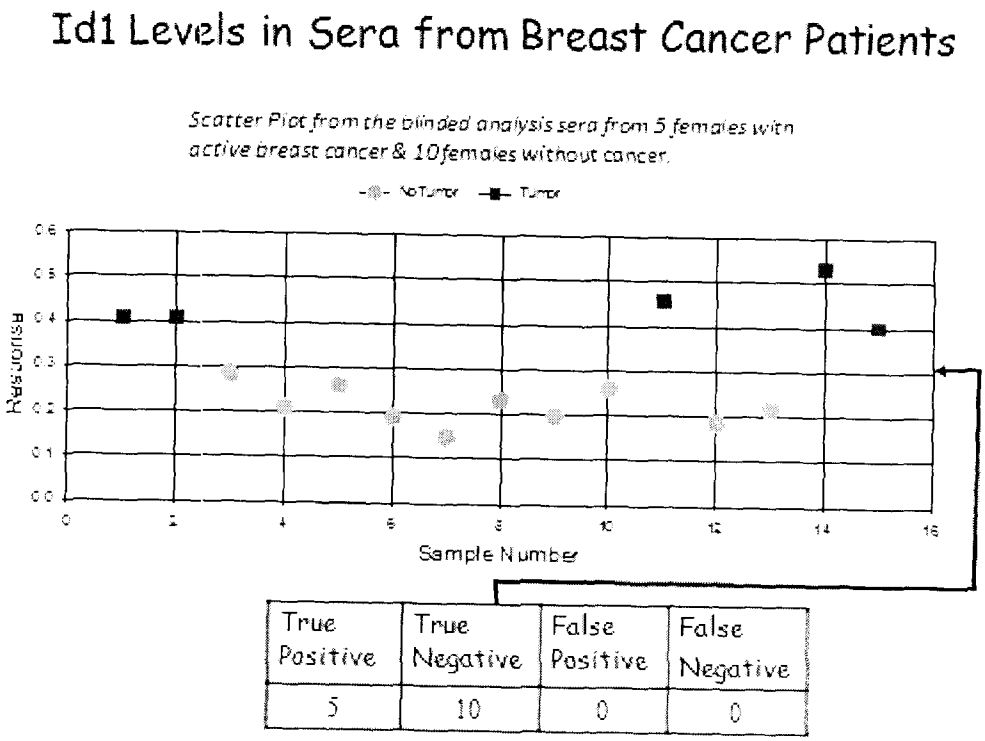
Amount of Id1 in Blood Predicts Presence of Tumors: Scattergram for analysis 44 serum samples from mice lacking p53 gene. Mice lacking p53 spontaneously develop tumors, and study explored if Id levels could predict which animals developed disease.



True Positives	True Negatives	False Positives	False Negatives
23	17	2	2

5

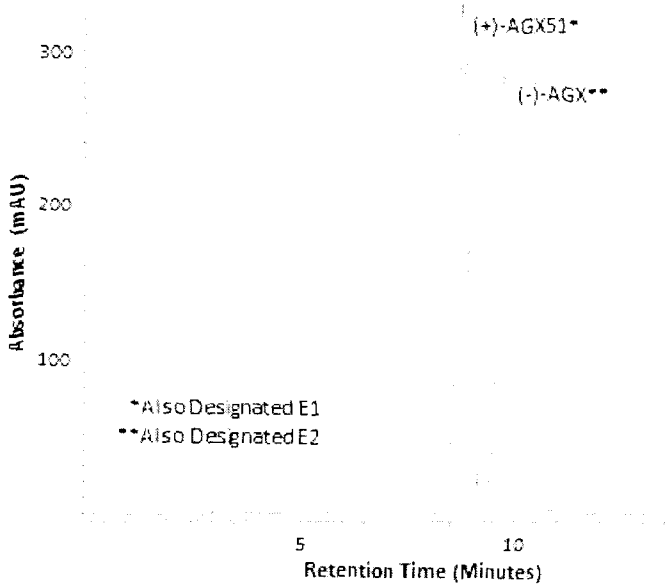
FIGURE 28



True Positive	True Negative	False Positive	False Negative
5	10	0	0

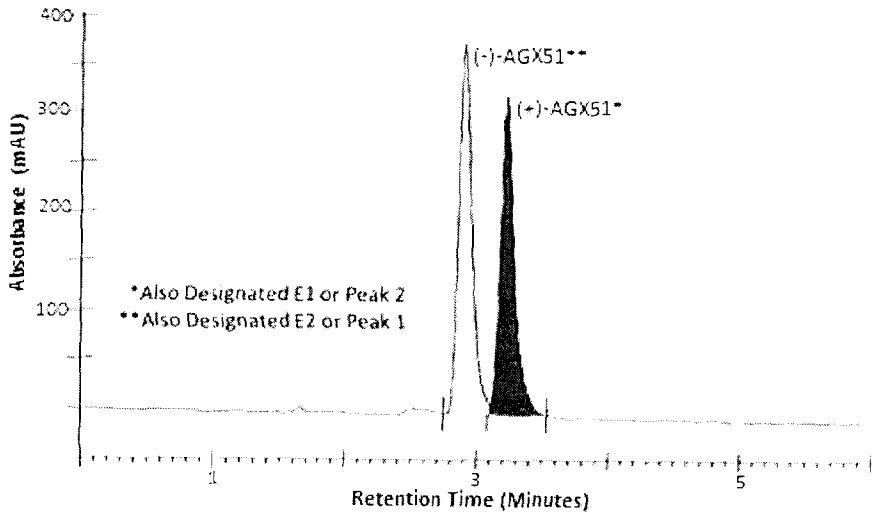
5

FIGURE 29



Typical chromatogram from separation of racemic AGX51 using chiral HPLC method A

FIGURE 30



Typical chromatogram from separation of racemic AGX51 using chiral HPLC method B