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(54) Title: ID2 AS A TARGET IN COLORECTAL CARCINOMA

(57) **Abstract:** Disclosed are compositions that include a nucleic acid component that include a nucleic acid that inhibits the expression of a gene that encodes an inhibitor of DNA binding protein (Id), and a lipid component that includes one or more phospholipids. Also disclosed are methods of treating a subject with cancer that involve administering to the subject a pharmaceutically effective amount of a composition that includes a nucleic acid component that includes a nucleic acid that inhibits the expression of a gene that encodes Id2, and a lipid component comprising one or more phospholipids.



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DESCRIPTION

ID2 AS A TARGET IN COLORECTAL CARCINOMA

BACKGROUND OF THE INVENTION

The present application claims the benefit of priority to U.S. Provisional Patent Application Serial No. 60/985,071, filed November 2, 2007, the entire contents of which is hereby specifically incorporated by reference.

1. Field of the Invention

The present invention relates generally to the fields of molecular biology and oncology. More particularly, it concerns compositions comprising an inhibitory nucleic acid, wherein the inhibitory nucleic acid is targeted to a nucleic acid encoding an inhibitor of DNA binding protein (Id). The invention also generally concerns methods of treating cancer involving administering to the subject a pharmaceutically effective amount of a composition comprising an inhibitory nucleic acid and a lipid component, wherein the inhibitory nucleic acid is targeted to a nucleic acid encoding an Id, such as Id2.

2. Description of Related Art

The inhibitor of DNA binding proteins (Ids) are a family of four homologous proteins (Id1-Id4) (Lasorella *et al.*, 2005; Lasorella *et al.*, 2001) that are helix-loop-helix-like transcription factors that lack a DNA-binding basic amino acid domain. During embryologic development, the transcriptional repressor Id2 regulates cell proliferation and differentiation. Id2 expression has also been detected in cancer cells, but its function and validity as a therapeutic target has not been elucidated. The Ids are essential for embryogenesis, and are involved in proliferation, differentiation, and cell survival (Lasorella *et al.*, 2001; Jen *et al.*, 1996). Although not typically found in most adult tissues, their reactivated expression, especially that of Id2, occurs in some human cancers (Kleef *et al.*, 1998; Lofstedt *et al.*, 2004; Rockman *et al.*, 2001; Lasorella *et al.*, 2002; Asirvatham *et al.*, 2007). Id genes have been implicated to play a role in tumor growth (Okaji *et al.*, 2006).

The Ids are believed to function primarily as dominant-negative regulators, forming non-DNA-binding heterodimers with other basic helix-loop-helix transcription factors (Benezra *et al.*, 1990). Dimerization partners of the Id family encompass members of the E protein group (E12, E47, and E2-2), whose downstream targets include the cell cycle inhibitors p21^{CIP/WAF1}, p15^{INK4B}, and p16^{INK4B} (Pagliuca *et al.*, 2000). In addition to

functioning as a transcriptional repressor, Id2 also binds to retinoblastoma (Rb) tumor suppressor proteins (Lasorella *et al.*, 2000; Iavarone *et al.*, 1994).

Rb mediates cell cycle arrest of normal cells through the sequestration of E2F transcription factors, which is mediated by the G₁ cyclin–cyclin-dependent kinase complexes (Weinberg, 1995). Developmental studies in Rb-null mice have shown that Rb can suppress Id2-mediated activities, including cell proliferation and survival (Iavarone *et al.*, 1994). Although these studies suggest that Rb acts strictly to modulate Id2 activity, additional studies reveal that Id2 can antagonize Rb-mediated repression of E2F target gene transcription. E2F targets constitute the majority of effectors required for cell cycle progression, and loss of E2F sequestration by Rb is generally thought to be a critical step in oncogenesis (Weinberg, 1995; Lasorella *et al.*, 1996).

SUMMARY OF THE INVENTION

The present invention is based on the finding that loss of Id expression in cancer can inhibit the proliferation of cancer cells. For example, the inventors have found that decreased Id2 expression, such as by shRNA targeting, results in reduction of tumor growth of colorectal cancer and fewer metastases. Further, the present invention is based on the finding that Id expression by a tumor in a subject can be reduced by administering to the subject a composition that includes an agent that reduces Id expression and a lipid, which results in reduced tumor growth. For example, the inventors have found that intraperitoneal administration of siRNA targeted to Id2 conjugated with the neutral lipid 1, 2-dioleoyl-sn-glycero-3-phosphatidylcholine significantly decreased colorectal carcinoma tumor burden in mice compared to control treatment, establishing that Id2 is a valid therapeutic target.

The present invention generally pertains to compositions that include (1) a nucleic acid component comprising a nucleic acid that inhibits the expression of a gene that encodes an inhibitor of DNA binding protein (Id); and (2) a lipid component that includes one or more neutral phospholipids. The nucleic acid may be an oligonucleotide (i.e., a nucleic acid that is 3 to about 100 nucleobases in length), or the nucleic acid may be a polynucleotide (greater than 100 nucleobases in length). The nucleic acid component may be a DNA or an RNA. In specific embodiments, the nucleic acid component is a siRNA or a nucleic acid encoding a siRNA, wherein the siRNA inhibits the expression of a gene that encodes an Id. In further specific embodiments, the nucleic acid component is a shRNA. In particular embodiments, the siRNA or shRNA comprises SEQ ID NO:10 or SEQ ID NO:11.

The Id may be any Id, including Id1, Id2, Id3, or Id4. In specific embodiments, the Id is Id2.

In certain embodiments, the lipid component forms a liposome. In some embodiments, the nucleic acid component may be encapsulated in the lipid component.

5 Any neutral phospholipid known to those of ordinary skill in the art is contemplated as a phospholipid for use in the present invention. For example, the neutral phospholipid may be a phosphatidylcholine or phosphatidylethanolamine. Specific examples of neutral phospholipids include 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), egg phosphatidylcholine ("EPC"), dilauryloylphosphatidylcholine ("DLPC"),
 10 dimyristoylphosphatidylcholine ("DMPC"), dipalmitoylphosphatidylcholine ("DPPC"), distearoylphosphatidylcholine ("DSPC"), 1-myristoyl-2-palmitoyl phosphatidylcholine ("MPPC"), 1-palmitoyl-2-myristoyl phosphatidylcholine ("PMPC"), 1-palmitoyl-2-stearoyl phosphatidylcholine ("PSPC"), 1-stearoyl-2-palmitoyl phosphatidylcholine ("SPPC"), dimyristyl phosphatidylcholine ("DMPC"), 1,2-distearoyl-sn-glycero-3-phosphocholine
 15 ("DAPC"), 1,2-diarachidoyl-sn-glycero-3-phosphocholine ("DBPC"), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine ("DEPC"), palmitoyloeyl phosphatidylcholine ("POPC"), lysophosphatidylcholine, dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine ("DSPE"), dimyristoyl phosphatidylethanolamine ("DMPE"), dipalmitoyl phosphatidylethanolamine ("DPPE"), palmitoyloeyl phosphatidylethanolamine ("POPE"),
 20 or lysophosphatidylethanolamine. In particular embodiments, the lipid component is DOPC. In some embodiments, the lipid component includes two or more neutral phospholipids.

In some embodiments, the composition that includes a lipid component and a nucleic acid component further includes a pharmaceutically acceptable carrier.

25 The lipid component may further include a positively charged lipid or a negatively charged lipid. Any charged lipid is contemplated for inclusion in the compositions of the present invention. For example, the negatively charged phospholipid may be a phosphatidylserine or phosphatidylglycerol. Specific non-limiting examples of negatively charged phospholipids include dimyristoyl phosphatidylserine ("DMPS"), dipalmitoyl phosphatidylserine ("DPPS"), brain phosphatidylserine ("BPS"),
 30 dilauryloylphosphatidylglycerol ("DLPG"), dimyristoylphosphatidylglycerol ("DMPG"), dipalmitoylphosphatidylglycerol ("DPPG"), distearoylphosphatidylglycerol ("DSPG"), or dioleoylphosphatidylglycerol ("DOPG").

In some embodiments, the composition further includes cholesterol or polyethyleneglycol (PEG).

The nucleic acid component can be of any length. For example, the nucleic acid component may be 5 to 500 nucleobases in length, 10 to 300 nucleobases in length, 18 to 100 nucleobases in length, 18 to 30 nucleobases in length. In some specific embodiments, the nucleic acid is a siRNA or shRNA that is a double stranded nucleic acid of 18 to 100
5 nucleobases in length. In more specific embodiments, the siRNA or shRNA is 18 to 30 nucleobases in length.

The compositions of the present invention may further include one or more therapeutic agents. For example, the therapeutic agent may be an anti-inflammatory agent, an antibiotic, or a chemotherapeutic agent. In specific embodiments, the therapeutic agent is a
10 chemotherapeutic agent. Any chemotherapeutic agent known to those of ordinary skill in the art is contemplated for inclusion in the compositions of the present invention. For example, the chemotherapeutic agent may be docetaxel, paclitaxel, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin,
15 plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatin, 5-fluorouracil, vincristine, vinblastine, methotrexate, oxaliplatin, or combinations thereof. In specific embodiments, the chemotherapeutic agent is 5-fluorouracil or oxaliplatin.

The present invention also generally pertains to methods of treating a subject with a
20 disease that involve administering to the subject a pharmaceutically effective amount of a composition that includes (1) a nucleic acid component that includes a nucleic acid that inhibits the expression of a gene that encodes Id2; and (2) a lipid component including one or more neutral phospholipids. In specific embodiments, the disease is cancer.

The subject may be any subject, but in particular embodiments the subject is a
25 mammal. Non-limiting examples include human, primate, horse, cow, sheep, goat, dog, chicken, cat, rat, mouse, and so forth. In specific embodiments, the subject is a human subject.

The cancer may be of any cancer type known to those of ordinary skill in the art. For example, the cancer may be breast cancer, lung cancer, prostate cancer, ovarian cancer, brain
30 cancer, liver cancer, cervical cancer, colorectal cancer, renal cancer, skin cancer, head and neck cancer, bone cancer, esophageal cancer, bladder cancer, uterine cancer, lymphatic cancer, stomach cancer, pancreatic cancer, testicular cancer, lymphoma, or leukemia. In specific embodiments, the cancer is colorectal cancer.

Some methods of the present invention are further defined as including the step of identifying a subject in need of treatment. Any method known to those of ordinary skill in the art can be used to identify a subject in need of treatment.

5 The method may further involve administering one or more additional therapies to the subject. For example, in particular embodiments, the subject has cancer, and the additional therapy is an anticancer therapy that is chemotherapy, radiation therapy, surgical therapy, immunotherapy, gene therapy, or a combination thereof. In specific embodiments, the additional anti-cancer therapy is chemotherapy. The chemotherapy may include, for example, any of those agents discussed above and elsewhere in this specification. In specific
10 embodiments, the chemotherapy is 5-fluorouracil or oxaliplatin.

The composition can be administered to the subject using any method known to those of ordinary skill in the art. Non-limiting examples include intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

15 In some embodiments of the present methods, a siRNA inhibits the expression of a gene that encodes Id2. In further embodiments, a shRNA inhibits the expression of a gene that encodes Id2. In particular embodiments, the siRNA or shRNA comprises SEQ ID NO:10 or SEQ ID NO:11.

In particular embodiments, the subject has a tumor and the method is further defined
20 as a method to reduce tumor volume in the subject. The tumor may be of any type. Non-limiting examples are set forth above and elsewhere in this specification. In specific embodiments, the tumor is a colorectal cancer.

Other aspects of the present invention concern methods of treating a subject with cancer that involve administering to the subject a pharmaceutically effective amount of a
25 composition that includes: (1) a nucleic acid component that includes a nucleic acid that inhibits the expression of a gene that encodes Id; and (2) a lipid component comprising DOPC. As set forth above, the Id may be Id1, Id2, Id3, or Id4. In particular embodiments, the Id is Id2. The cancer may be of any type. Non-limiting examples include breast cancer, lung cancer, prostate cancer, ovarian cancer, brain cancer, liver cancer, cervical cancer,
30 colorectal cancer, renal cancer, skin cancer, head and neck cancer, bone cancer, esophageal cancer, bladder cancer, uterine cancer, lymphatic cancer, stomach cancer, pancreatic cancer, testicular cancer, lymphoma, or leukemia.

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention.

Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention.

The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.”

Throughout this application, the term “about” is used to indicate that a value includes the standard deviation of error for the device and/or method being employed to determine the value.

As used herein the specification, “a” or “an” may mean one or more, unless clearly indicated otherwise. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one. As used herein “another” may mean at least a second or more.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

The following figures form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A, 1B. Assessment of Id2 expression in human colon tissues and cell lines. FIG. 1A - Representative tissue sections of nonmalignant human colonic mucosa, primary colon adenocarcinoma, colon adenocarcinoma, and liver metastasis were subjected to immunoperoxidase staining to detect expression of Id2 protein. FIG. 1B - Western blot analysis of Id2 expression in six colon adenocarcinoma cell lines. Vinculin was used as an internal loading control.

FIG. 2A, 2B, 2C. Effect of Id2 expression on proliferation and cell cycle protein expression in HCT-116 cells. FIG. 2A - Western blot analysis of Id2 levels in parental (Par) and shRNA-control (sh-Con) cells and two shRNA clones (sh-Id2C14 and sh-Id2C15). Bars

indicate standard errors of the mean. FIG. 2B - MTT analysis of growth rates of control cells (Par and sh-Con) or clones with reduced Id2 expression (sh-Id2C14, sh-Id2C15). Bars indicate standard error of the mean. FIG. 2C - Western blot analysis of cell cycle regulatory proteins from control cells (Par and sh-Con) and stable cell lines with reduced Id2 expression (sh-Id2C14 and sh-Id2C15). Vinculin was used as an internal loading control.

FIG. 3A, 3B, 3C. Effect of Id2 expression on apoptosis in HCT-116 cells.

FIG. 3A - Graphical representation of proportions of apoptotic control cells (Parental [Par] and scrambled-siRNA control [sh-Con]) and stable cell lines with reduced Id2 expression (sh-Id2C14 and sh-Id2C15) as determined by fluorescence-activated cell sorting analysis. FIG. 3B - Western analysis of Bcl-2 family members Bim/Bod, Bcl-2, Bcl-xl, Bad, and Bax from control cells (Par and sh-Con) and stable cell lines with reduced Id2 expression (sh-Id2C14 and sh-Id2C15). Vinculin levels were probed to ensure equal loading of all samples. FIG. 3C - Western analysis of caspase-7/caspase-9 and PARP from control cells (Par and sh-Con) and stable cell lines with reduced Id2 expression (sh-Id2C14 and sh-Id2C15). Vinculin levels were probed to ensure equal loading of all samples.

FIG. 4A, 4B, 4C. Effect of Id2 expression on the growth and metastasis of HCT-116

colon cancer cells *in vivo*. FIG. 4A - Mean tumor volumes after subcutaneous injection of control (shRNA-control (sh-Con)) cells or clones with reduced Id2 expression. Bars indicate standard error of the mean for 10 mice. Volumes of both siRNA clones were different from the sh-Con control ($P = 0.03$ for sh-IdC14 and $P = 0.017$ for sh-Id2C15). FIG. 4B - Average numbers of liver metastases after splenic injection of control (sh-Con) cells or clones with reduced Id2 expression. Bars indicate standard error of the mean for 10 mice. Both clones produced fewer metastases than did control cells ($P = 0.022$ for sh-Id2C14 and $P = 0.045$ for sh-Id2C15). FIG. 4C - Mean volumes of metastases from control cells (sh-Con) and clones with reduced Id2 expression. Total volume is the sum of individual metastases in each liver. Data are means $\pm$ SEM from 10 mice. Both clones produced lower-volume metastases than did control cells ($P \leq 0.02$).

FIG. 5A, 5B, 5C. Effect of *in vivo* administration of liposomal-conjugated siRNA to

Id2 on CRC tumor growth in the liver. FIG. 5A - Graphical plot of proton activity from bioluminescence imaging of mice inoculated with 1.0×10^6 HCT-116 cells and imaged at 3, 14, and 28 days after hepatic injection while being treated at 3-day intervals with either control (control siRNA-DOPC) or Id2-specific siRNA (Id2 siRNA-DOPC). FIG. 5B - Mean tumor volumes at 32 days after inoculation, with continuous intraperitoneal injections every 3 days of control (control siRNA-DOPC) or Id2-specific siRNA (Id2 siRNA-DOPC) (10 mice

per group). * $P=0.0045$ vs control siRNA. FIG. 5C – Photographs of excised livers from mice treated with either nonspecific or Id2-targeted siRNA.

FIG. 6A, 6B, 6C. Effect of *in vivo* administration of liposomal-conjugated siRNA to Id2 on CRC tumor growth in the liver. FIG. 6A – Graphical plot of immunohistochemistry (IHC) staining for proliferating cell nuclear antigen (PCNA) in tumor sections from samples in FIG. 5C. Treatment groups; control liposome (control siRNA-DOPC) and Id2-targeted liposome (Id2 siRNA-DOPC). * $P=0.02$ between treatment groups. FIG. 6B - Graphical representation of ‘fold’ change in TUNEL-positive cells (apoptotic cells) in tumor sections from control-siRNA- and Id2-siRNA-treated mice observed in five fields at x 20. Average * $P=0.002$ between treatment groups. FIG. 6C – Western analysis of tumor homogenates for Id2 expression in liposomal treatment groups from FIG. 5C; control liposome (control siRNA-DOPC) and Id2-targeted liposome (Id2 siRNA-DOPC).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

A. Id Proteins

The inhibitor of DNA binding proteins (Ids) are a family of four homologous proteins (Id1-Id4) that are helix-loop-helix-like transcription factors that lack a DNA-binding basic amino acid domain. The Ids are essential for embryogenesis, and are involved in proliferation, differentiation, and cell survival. The transcriptional repressor Id2 is a member of this family which has been shown to be indispensable for proper development in embryonic tissues. The Id proteins, including Id2, function as negative regulators by dimerizing with and blocking the DNA binding capability of other transcriptional activators.

[0001]

Table 1 lists the GenBank Accession numbers of Id protein sequences from *homo sapiens*.

Table 1

Id	GenBank Accession No.	Sequence Identifier
Id1	CAA54920	1
	NP002156	2
	NP851998	3
Id2	NP002157	4
	Q02363	5
Id3	CAA51827	6
	NP002158	7
Id4	AAA73923	8
	P47928	9

B. Therapeutic Gene Silencing

Since the discovery of RNAi by Fire and colleagues in 1981, the biochemical mechanisms have been rapidly characterized. Long double stranded RNA (dsRNA) is cleaved by Dicer, which is an RNAaseIII family ribonuclease. This process yields siRNAs of ~21 nucleotides in length. These siRNAs are incorporated into a multiprotein RNA-induced silencing complex (RISC) that is guided to target mRNA. RISC cleaves the target mRNA in the middle of the complementary region. In mammalian cells, the related microRNAs (miRNAs) are found that are short RNA fragments (~22 nucleotides). MiRNAs are generated after Dicer-mediated cleavage of longer (~70 nucleotide) precursors with imperfect hairpin RNA structures. The miRNA is incorporated into a miRNA-protein complex (miRNP), which leads to translational repression of target mRNA.

To improve the effectiveness of siRNA-mediated gene silencing, guidelines for selection of target sites on mRNA have been developed for optimal design of siRNA (Soutschek *et al.*, 2004; Wadhwa *et al.*, 2004). These strategies may allow for rational approaches for selecting siRNA sequences to achieve maximal gene knockdown. To facilitate the entry of siRNA into cells and tissues, a variety of vectors including plasmids and viral vectors such as adenovirus, lentivirus, and retrovirus have been used (Wadhwa *et al.*, 2004). While many of these approaches are successful for *in vitro* studies, *in vivo* delivery poses additional challenges based on the complexity of the tumor microenvironment.

Liposomes are a form of nanoparticles that are attractive carriers for delivering a variety of drugs into the diseased tissue. Optimal liposome size depends on the tumor target.

In tumor tissue, the vasculature is discontinuous, and pore sizes vary from 100 to 780 nm (Siwak *et al.*, 2002). By comparison, pore size in normal vascular endothelium is <2 nm in most tissues, and 6 nm in post-capillary venules. Most liposomes are 65-125 nm in diameter. Negatively charged liposomes were believed to be more rapidly removed from circulation than neutral or positively charged liposomes; however, recent studies have indicated that the type of negatively charged lipid affects the rate of liposome uptake by the reticulo-endothelial system (RES). For example, liposomes containing negatively charged lipids that are not sterically shielded (phosphatidylserine, phosphatidic acid, and phosphatidylglycerol) are cleared more rapidly than neutral liposomes. Interestingly, cationic liposomes (1,2-dioleoyl-3-trimethylammonium-propane [DOTAP]) and cationic-liposome-DNA complexes are more avidly bound and internalized by endothelial cells of angiogenic blood vessels via endocytosis than anionic, neutral, or sterically stabilized neutral liposomes (Thurston *et al.*, 1998; Krasnici *et al.*, 2003). Cationic liposomes may not be ideal delivery vehicles for tumor cells because surface interactions with the tumor cells create an electrostatically derived binding-site barrier effect, inhibiting further association of the delivery systems with tumor spheroids (Kostarelos *et al.*, 2004). However, neutral liposomes appear to have better intratumoral penetration. Toxicity with specific liposomal preparations has also been a concern. Cationic liposomes elicit dose-dependent toxicity and pulmonary inflammation by promoting release of reactive oxygen intermediates, and this effect is more pronounced with multivalent cationic liposomes than monovalent cationic liposomes such as DOTAP (Dokka *et al.*, 2000). Neutral and negative liposomes do not appear to exhibit lung toxicity (Guitierrez-Puente *et al.*, 1999). Cationic liposomes, while efficiently taking up nucleic acids, have had limited success for *in vivo* gene downregulation, perhaps because of their stable intracellular nature and resultant failure to release siRNA contents.

In vivo siRNA delivery using neutral liposomes in an orthotopic model of advanced ovarian cancer has been described (Landen *et al.*, 2005, which is incorporated herein by reference in its entirety). For example, intravenous injection of the DOPC-siRNA complex allowed a significantly greater degree of siRNA deposition into the tumor parenchyma than either delivery with cationic (positively charged) liposomes (DOTAP) or unpackaged “naked” siRNA. While the DOPC formulation delivered siRNA to over 30% of cells in the tumor parenchyma, naked siRNA was delivered only to about 3% of cells, and DOTAP delivered siRNA only to tumor cells immediately adjacent to the vasculature.

Although siRNA appears to be more stable than antisense molecules, serum nucleases can degrade siRNAs (Leung and Whittaker, 2005). Thus, several research groups have

developed modifications such as chemically stabilized siRNAs with partial phosphorothioate backbone and 2'-O-methyl sugar modifications or boranophosphate siRNAs (Leung and Whittaker, 2005). Elmen and colleagues modified siRNAs with the synthetic RNA-like high affinity nucleotide analogue, Locked Nucleic Acid (LNA), which significantly enhanced the serum half-life of siRNA and stabilized the structure without affecting the gene-silencing capability (Elmen *et al.*, 2005). Alternative approaches including chemical modification (conjugation of cholesterol to the 3' end of the sense strand of siRNA by means of a pyrrolidine linker) may also allow systemic delivery without affecting function (Soutschek *et al.*, 2004). Aspects of the present invention can use each of these modification strategies in combination with the compositions and methods described.

C. Lipid Preparations

The present invention provides methods and compositions for associating an inhibitory nucleic acid that inhibits the expression of an Id protein, such as a siNA (*e.g.*, a siRNA) or shRNA with a lipid and/or liposome. The siNA may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the polynucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. The liposome or liposome/siNA associated compositions of the present invention are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates which are not uniform in either size or shape.

Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which are well known to those of skill in the art which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes. An example is the lipid dioleoylphosphatidylcholine (DOPC).

"Liposome" is a generic term encompassing a variety of unilamellar, multilamellar, and multivesicular lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes may be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo

self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). However, the present invention also encompasses compositions that have different structures in solution than the normal vesicular structure. For example, the lipids may assume a micellar structure or merely exist as non-uniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Liposome-mediated polynucleotide delivery and expression of foreign DNA *in vitro* has been very successful. Wong *et al.* (1980) demonstrated the feasibility of liposome-mediated delivery and expression of foreign DNA in cultured chick embryo, HeLa and hepatoma cells. Nicolau *et al.* (1987) accomplished successful liposome-mediated gene transfer in rats after intravenous injection.

In certain embodiments of the invention, the lipid may be associated with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda *et al.*, 1989). In other embodiments, the lipid may be complexed or employed in conjunction with nuclear non-histone chromosomal proteins (HMG-1) (Kato *et al.*, 1991). In yet further embodiments, the lipid may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression vectors have been successfully employed in transfer of a polynucleotide *in vitro* and *in vivo*, then they are applicable for the present invention.

1. Neutral Liposomes

"Neutral liposomes or lipid composition" or "non-charged liposomes or lipid composition," as used herein, are defined as liposomes or lipid compositions having one or more lipids that yield an essentially-neutral, net charge (substantially non-charged). By "essentially neutral" or "essentially non-charged", it is meant that few, if any, lipids within a given population (*e.g.*, a population of liposomes) include a charge that is not canceled by an opposite charge of another component (*e.g.*, fewer than 10% of components include a non-canceled charge, more preferably fewer than 5%, and most preferably fewer than 1%). In certain embodiments of the present invention, a composition may be prepared wherein the lipid component of the composition is essentially neutral but is not in the form of liposomes.

In certain embodiments, neutral liposomes or lipid compositions may include mostly lipids and/or phospholipids that are themselves neutral. In certain embodiments, amphipathic lipids may be incorporated into or used to generate neutral liposomes or lipid compositions. For example, a neutral liposome may be generated by combining positively and negatively charged lipids so that those charges substantially cancel one another. For such a liposome,

few, if any, charged lipids are present whose charge is not canceled by an oppositely-charged lipid (*e.g.*, fewer than 10% of charged lipids have a charge that is not canceled, more preferably fewer than 5%, and most preferably fewer than 1%). It is also recognized that the above approach may be used to generate a neutral lipid composition wherein the lipid component of the composition is not in the form of liposomes.

In certain embodiments, a neutral liposome may be used to deliver a siRNA. The neutral liposome may contain a siRNA directed to the suppression of translation of a single gene, or the neutral liposome may contain multiple siRNA that are directed to the suppression of translation of multiple genes. Further, the neutral liposome may also contain a chemotherapeutic in addition to the siRNA; thus, in certain embodiments, chemotherapeutic and a siRNA may be delivered to a cell (*e.g.*, a cancerous cell in a human subject) in the same or separate compositions. An advantage to using neutral liposomes is that, in contrast to the toxicity that has been observed in response to cationic liposomes, little to no toxicity has yet been observed as a result of neutral liposomes.

2. Phospholipids

Lipid compositions of the present invention may comprise phospholipids. In certain embodiments, a single kind or type of phospholipid may be used in the creation of lipid compositions such as liposomes (*e.g.*, DOPC used to generate neutral liposomes). In other embodiments, more than one kind or type of phospholipid may be used.

Phospholipids include glycerophospholipids and certain sphingolipids. Phospholipids include, but are not limited to, dioleoylphosphatidylcholine ("DOPC"), egg phosphatidylcholine ("EPC"), dilauryloylphosphatidylcholine ("DLPC"), dimyristoylphosphatidylcholine ("DMPC"), dipalmitoylphosphatidylcholine ("DPPC"), distearoylphosphatidylcholine ("DSPC"), 1-myristoyl-2-palmitoyl phosphatidylcholine ("MPPC"), 1-palmitoyl-2-myristoyl phosphatidylcholine ("PMPC"), 1-palmitoyl-2-stearoyl phosphatidylcholine ("PSPC"), 1-stearoyl-2-palmitoyl phosphatidylcholine ("SPPC"), dilauryloylphosphatidylglycerol ("DLPG"), dimyristoylphosphatidylglycerol ("DMPG"), dipalmitoylphosphatidylglycerol ("DPPG"), distearoylphosphatidylglycerol ("DSPG"), distearoyl sphingomyelin ("DSSP"), distearoylphosphatidylethanolamine ("DSPE"), dioleoylphosphatidylglycerol ("DOPG"), dimyristoyl phosphatidic acid ("DMPA"), dipalmitoyl phosphatidic acid ("DPPA"), dimyristoyl phosphatidylethanolamine ("DMPE"), dipalmitoyl phosphatidylethanolamine ("DPPE"), dimyristoyl phosphatidylserine ("DMPS"), dipalmitoyl phosphatidylserine ("DPPS"), brain phosphatidylserine ("BPS"), brain sphingomyelin ("BSP"), dipalmitoyl sphingomyelin ("DPSP"), dimyristyl

phosphatidylcholine ("DMPC"), 1,2-distearoyl-sn-glycero-3-phosphocholine ("DAPC"), 1,2-diarachidoyl-sn-glycero-3-phosphocholine ("DBPC"), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine ("DEPC"), dioleoylphosphatidylethanolamine ("DOPE"), palmitoyloeoyle phosphatidylcholine ("POPC"), palmitoyloeoyle phosphatidylethanolamine ("POPE"),
5 lysophosphatidylcholine, lysophosphatidylethanolamine, and dilinoleoylphosphatidylcholine.

Phospholipids include, for example, phosphatidylcholines, phosphatidylglycerols, and phosphatidylethanolamines; because phosphatidylethanolamines and phosphatidyl cholines are non-charged under physiological conditions (*i.e.*, at about pH 7), these compounds may be particularly useful for generating neutral liposomes. In certain embodiments, the
10 phospholipid DOPC is used to produce non-charged liposomes or lipid compositions. In certain embodiments, a lipid that is not a phospholipid (*e.g.*, a cholesterol) can also be used

Phospholipids may be from natural or synthetic sources. However, phospholipids from natural sources, such as egg or soybean phosphatidylcholine, brain phosphatidic acid, brain or plant phosphatidylinositol, heart cardiolipin and plant or bacterial
15 phosphatidylethanolamine are not used in certain embodiments as the primary phosphatide (*i.e.*, constituting 50% or more of the total phosphatide composition) because this may result in instability and leakiness of the resulting liposomes.

3. Production of Liposomes

Liposomes and lipid compositions of the present invention can be made by different
20 methods. For example, a nucleotide (*e.g.*, siRNA) may be encapsulated in a neutral liposome using a method involving ethanol and calcium (Bailey and Sullivan, 2000). The size of the liposomes varies depending on the method of synthesis. A liposome suspended in an aqueous solution is generally in the shape of a spherical vesicle, and may have one or more concentric layers of lipid bilayer molecules. Each layer consists of a parallel array of molecules
25 represented by the formula XY, wherein X is a hydrophilic moiety and Y is a hydrophobic moiety. In aqueous suspension, the concentric layers are arranged such that the hydrophilic moieties tend to remain in contact with an aqueous phase and the hydrophobic regions tend to self-associate. For example, when aqueous phases are present both within and without the liposome, the lipid molecules may form a bilayer, known as a lamella, of the arrangement
30 XY-YX. Aggregates of lipids may form when the hydrophilic and hydrophobic parts of more than one lipid molecule become associated with each other. The size and shape of these aggregates will depend upon many different variables, such as the nature of the solvent and the presence of other compounds in the solution.

Lipids suitable for use according to the present invention can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma Chemical Co., dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, N.Y.); cholesterol ("Chol") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform may be used as the only solvent since it is more readily evaporated than methanol.

Liposomes within the scope of the present invention can be prepared in accordance with known laboratory techniques. In certain embodiments, liposomes are prepared by mixing liposomal lipids, in a solvent in a container (*e.g.*, a glass, pear-shaped flask). The container will typically have a volume ten-times greater than the volume of the expected suspension of liposomes. Using a rotary evaporator, the solvent may be removed at approximately 40°C under negative pressure. The solvent may be removed within about 5 minutes to 2 hours, depending on the desired volume of the liposomes. The composition can be dried further in a desiccator under vacuum. Dried lipids can be hydrated at approximately 25-50 mM phospholipid in sterile, pyrogen-free water by shaking until all the lipid film is resuspended. The aqueous liposomes can be then separated into aliquots, each placed in a vial, lyophilized and sealed under vacuum.

Liposomes can also be prepared in accordance with other known laboratory procedures: the method of Bangham *et al.* (1965), the contents of which are incorporated herein by reference; the method of Gregoriadis, as described in DRUG CARRIERS IN BIOLOGY AND MEDICINE (1979), the contents of which are incorporated herein by reference; the method of Deamer and Uster (1983), the contents of which are incorporated by reference; and the reverse-phase evaporation method as described by Szoka and Papahadjopoulos (1978). The aforementioned methods differ in their respective abilities to entrap aqueous material and their respective aqueous space-to-lipid ratios.

Dried lipids or lyophilized liposomes may be dehydrated and reconstituted in a solution of inhibitory peptide and diluted to an appropriate concentration with a suitable solvent (*e.g.*, DPBS). The mixture may then be vigorously shaken in a vortex mixer. Unencapsulated nucleic acid may be removed by centrifugation at 29,000g and the liposomal pellets washed. The washed liposomes may be resuspended at an appropriate total phospholipid concentration (*e.g.*, about 50-200 mM). The amount of nucleic acid encapsulated can be determined in accordance with standard methods. After determination of

the amount of nucleic acid encapsulated in the liposome preparation, the liposomes may be diluted to appropriate concentrations and stored at 4°C until use.

D. Inhibition of Gene Expression

siNA (*e.g.*, siRNA) are well known in the art. For example, siRNA and double-stranded RNA have been described in U.S. Patents 6,506,559 and 6,573,099, as well as in U.S. Patent Applications 2003/0051263, 2003/0055020, 2004/0265839, 2002/0168707, 2003/0159161, and 2004/0064842, all of which are herein incorporated by reference in their entirety.

Within a siNA, the components of a nucleic acid need not be of the same type or homogenous throughout (*e.g.*, a siNA may comprise a nucleotide and a nucleic acid or nucleotide analog). Typically, siNA form a double-stranded structure; the double-stranded structure may result from two separate nucleic acids that are partially or completely complementary. In certain embodiments of the present invention, the siNA may comprise only a single nucleic acid (polynucleotide) or nucleic acid analog and form a double-stranded structure by complementing with itself (*e.g.*, forming a hairpin loop). The double-stranded structure of the siNA may comprise 16, 20, 25, 30, 35, 40, 45, 50, 60, 65, 70, 75, 80, 85, 90 to 100, 150, 200, 250, 300, 350, 400, 450, 500 or more contiguous nucleobases, including all ranges therebetween. The siNA may comprise 17 to 35 contiguous nucleobases, more preferably 18 to 30 contiguous nucleobases, more preferably 19 to 25 nucleobases, more preferably 20 to 23 contiguous nucleobases, or 20 to 22 contiguous nucleobases, or 21 contiguous nucleobases that hybridize with a complementary nucleic acid (which may be another part of the same nucleic acid or a separate complementary nucleic acid) to form a double-stranded structure.

Agents of the present invention useful for practicing the methods of the present invention include, but are not limited to siRNAs. Typically, introduction of double-stranded RNA (dsRNA), which may alternatively be referred to herein as small interfering RNA (siRNA), induces potent and specific gene silencing, a phenomena called RNA interference or RNAi. This phenomenon has been extensively documented in the nematode *C. elegans* (Fire *et al.*, 1998), but is widespread in other organisms, ranging from trypanosomes to mouse. Depending on the organism being discussed, RNA interference has been referred to as “cosuppression,” “post-transcriptional gene silencing,” “sense suppression,” and “quelling.” RNAi is an attractive biotechnological tool because it provides a means for knocking out the activity of specific genes.

In designing RNAi there are several factors that need to be considered such as the nature of the siRNA, the durability of the silencing effect, and the choice of delivery system. To produce an RNAi effect, the siRNA that is introduced into the organism will typically contain exonic sequences. Furthermore, the RNAi process is homology dependent, so the sequences must be carefully selected so as to maximize gene specificity, while minimizing the possibility of cross-interference between homologous, but not gene-specific sequences. Preferably the siRNA exhibits greater than 80, 85, 90, 95, 98,% or even 100% identity between the sequence of the siRNA and the gene to be inhibited. Sequences less than about 80% identical to the target gene are substantially less effective. Thus, the greater homology between the siRNA and the STAT gene to be inhibited, the less likely expression of unrelated genes will be affected.

In addition, the size of the siRNA is an important consideration. In some embodiments, the present invention relates to siRNA molecules that include at least about 19-25 nucleotides, and are able to modulate the Id gene expression. In the context of the present invention, the siRNA is preferably less than 500, 200, 100, 50 or 25 nucleotides in length. More preferably, the siRNA is from about 19 nucleotides to about 25 nucleotides in length.

siRNA can be obtained from commercial sources, natural sources, or can be synthesized using any of a number of techniques well-known to those of ordinary skill in the art. For example, one commercial source of predesigned siRNA is Ambion®, Austin, TX. Another is Qiagen® (Valencia, CA). An inhibitory nucleic acid that can be applied in the compositions and methods of the present invention may be any nucleic acid sequence that has been found by any source to be a validated downregulator of an Id protein.

In one aspect, the invention generally features an isolated siRNA molecule of at least 19 nucleotides, having at least one strand that is substantially complementary to at least ten but no more than thirty consecutive nucleotides of a nucleic acid that encodes an Id protein, and that reduces the expression of the Id protein. In a particular embodiment of the present invention, the siRNA molecule has at least one strand that is substantially complementary to at least ten but no more than thirty consecutive nucleotides of the mRNA that encodes Id2.

In another particular embodiment, the siRNA molecule is at least 75, 80, 85, or 90% homologous, preferably 95%, 99%, or 100% homologous, to at least 10 contiguous nucleotides of any of the nucleic acid sequences encoding a full-length Id protein, such as those in Table 1. Without undue experimentation and using the disclosure of this invention, it

is understood that additional siRNAs can be designed and used to practice the methods of the invention.

The siRNA may also comprise an alteration of one or more nucleotides. Such alterations can include the addition of non-nucleotide material, such as to the end(s) of the 19 to 25 nucleotide RNA or internally (at one or more nucleotides of the RNA). In certain aspects, the RNA molecule contains a 3'-hydroxyl group. Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. The double-stranded oligonucleotide may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages. Additional modifications of siRNAs (*e.g.*, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, 5-C-methyl nucleotides, one or more phosphorothioate internucleotide linkages, and inverted deoxyabasic residue incorporation) can be found in U.S. Application Publication 20040019001 and U.S. Patent 6,673,611 (each of which is incorporated by reference in its entirety). Collectively, all such altered nucleic acids or RNAs described above are referred to as modified siRNAs.

Preferably, RNAi is capable of decreasing the expression of an Id protein, such Id2, by at least 10%, 20%, 30%, or 40%, more preferably by at least 50%, 60%, or 70%, and most preferably by at least 75%, 80%, 90%, 95% or more.

Certain embodiments of the present invention pertain to methods of inhibiting expression of a gene encoding an Id protein in a cell. In a specific embodiment, the Id protein is Id2. Introduction of siRNA into cells can be achieved by methods known in the art, including for example, microinjection, electroporation, or transfection of a vector comprising a nucleic acid from which the siRNA can be transcribed. Alternatively, a siRNA can be directly introduced into a cell in a form that is capable of binding to target mRNA transcripts. To increase durability and membrane-permeability the siRNA may be combined or modified with liposomes, poly-L-lysine, lipids, cholesterol, lipofectine or derivatives thereof. In certain aspects cholesterol-conjugated siRNA can be used (see, Song *et al.*, 2003).

The RNAi may be a small hairpin RNA (shRNA). shRNA are RNA sequences that include a hairpin turn that can be used to silence gene expression via RNA interference. The shRNA hairpin structure is cleaved in the cell to siRNA.

E. Nucleic Acids

The present invention provides methods and compositions for the delivery of siNA *via* neutral liposomes. Because a siNA is composed of a nucleic acid, methods relating to nucleic acids (*e.g.*, production of a nucleic acid, modification of a nucleic acid, *etc.*) may also be used with regard to a siNA.

The term "nucleic acid" is well known in the art. A "nucleic acid" as used herein will generally refer to a molecule (*i.e.*, a strand) of DNA, RNA or a derivative or analog thereof, comprising a nucleobase. A nucleobase includes, for example, a naturally occurring purine or pyrimidine base found in DNA (*e.g.*, an adenine "A," a guanine "G," a thymine "T" or a cytosine "C") or RNA (*e.g.*, an A, a G, an uracil "U" or a C). The term "nucleic acid" encompass the terms "oligonucleotide" and "polynucleotide," each as a subgenus of the term "nucleic acid." The term "oligonucleotide" refers to a molecule of between 3 and about 100 nucleobases in length. The term "polynucleotide" refers to at least one molecule of greater than about 100 nucleobases in length. Examples of RNA include mRNA, siRNA, and shRNA.

These definitions refer to a single-stranded or double-stranded nucleic acid molecule. Double stranded nucleic acids are formed by fully complementary binding, although in some embodiments a double stranded nucleic acid may formed by partial or substantial complementary binding. Thus, a nucleic acid may encompass a double-stranded molecule that comprises one or more complementary strand(s) or "complement(s)" of a particular sequence, typically comprising a molecule. As used herein, a single stranded nucleic acid may be denoted by the prefix "ss" and a double stranded nucleic acid by the prefix "ds".

1. Nucleobases

As used herein a "nucleobase" refers to a heterocyclic base, such as for example a naturally occurring nucleobase (*i.e.*, an A, T, G, C or U) found in at least one naturally occurring nucleic acid (*i.e.*, DNA and RNA), and naturally or non-naturally occurring derivative(s) and analogs of such a nucleobase. A nucleobase generally can form one or more hydrogen bonds ("anneal" or "hybridize") with at least one naturally occurring nucleobase in manner that may substitute for naturally occurring nucleobase pairing (*e.g.*, the hydrogen bonding between A and T, G and C, and A and U).

"Purine" and/or "pyrimidine" nucleobase(s) encompass naturally occurring purine and/or pyrimidine nucleobases and also derivative(s) and analog(s) thereof, including but not limited to, those a purine or pyrimidine substituted by one or more of an alkyl, carboxyalkyl, amino, hydroxyl, halogen (*i.e.*, fluoro, chloro, bromo, or iodo), thiol or alkylthiol moiety.

Preferred alkyl (*e.g.*, alkyl, caboxyalkyl, etc.) moieties comprise of from about 1, about 2, about 3, about 4, about 5, to about 6 carbon atoms. A nucleobase may be comprised in a nucleoside or nucleotide, using any chemical or natural synthesis method described herein or known to one of ordinary skill in the art.

2. Nucleosides

As used herein, a "nucleoside" refers to an individual chemical unit comprising a nucleobase covalently attached to a nucleobase linker moiety. A non-limiting example of a "nucleobase linker moiety" is a sugar comprising 5-carbon atoms (*i.e.*, a "5-carbon sugar"), including but not limited to a deoxyribose, a ribose, an arabinose, or a derivative or an analog of a 5-carbon sugar. Non-limiting examples of a derivative or an analog of a 5-carbon sugar include a 2'-fluoro-2'-deoxyribose or a carbocyclic sugar where a carbon is substituted for an oxygen atom in the sugar ring.

Different types of covalent attachment(s) of a nucleobase to a nucleobase linker moiety are known in the art. By way of non-limiting example, a nucleoside comprising a purine (*i.e.*, A or G) or a 7-deazapurine nucleobase typically covalently attaches the 9 position of a purine or a 7-deazapurine to the 1'-position of a 5-carbon sugar. In another non-limiting example, a nucleoside comprising a pyrimidine nucleobase (*i.e.*, C, T or U) typically covalently attaches a 1 position of a pyrimidine to a 1'-position of a 5-carbon sugar (Kornberg and Baker, 1992).

3. Nucleotides

As used herein, a "nucleotide" refers to a nucleoside further comprising a "backbone moiety". A backbone moiety generally covalently attaches a nucleotide to another molecule comprising a nucleotide, or to another nucleotide to form a nucleic acid. The "backbone moiety" in naturally occurring nucleotides typically comprises a phosphorus moiety, which is covalently attached to a 5-carbon sugar. The attachment of the backbone moiety typically occurs at either the 3'- or 5'-position of the 5-carbon sugar. However, other types of attachments are known in the art, particularly when a nucleotide comprises derivatives or analogs of a naturally occurring 5-carbon sugar or phosphorus moiety.

4. Nucleic Acid Analogs

A nucleic acid may comprise, or be composed entirely of, a derivative or analog of a nucleobase, a nucleobase linker moiety and/or backbone moiety that may be present in a naturally occurring nucleic acid. As used herein a "derivative" refers to a chemically modified or altered form of a naturally occurring molecule, while the terms "mimic" or "analog" refer to a molecule that may or may not structurally resemble a naturally occurring

molecule or moiety, but possesses similar functions. As used herein, a "moiety" generally refers to a smaller chemical or molecular component of a larger chemical or molecular structure. Nucleobase, nucleoside and nucleotide analogs or derivatives are well known in the art, and have been described (see for example, Scheit, 1980, incorporated herein by reference).

Additional non-limiting examples of nucleosides, nucleotides, or nucleic acids comprising 5-carbon sugar and/or backbone moiety derivatives or analogs, include those in U.S. Patent 5,681,947 which describes oligonucleotides comprising purine derivatives that form triple helices with and/or prevent expression of dsDNA; U.S. Patents 5,652,099 and 5,763,167 which describe nucleic acids incorporating fluorescent analogs of nucleosides found in DNA or RNA, particularly for use as fluorescent nucleic acids probes; U.S. Patent 5,614,617 which describes oligonucleotide analogs with substitutions on pyrimidine rings that possess enhanced nuclease stability; U.S. Patents 5,670,663, 5,872,232 and 5,859,221 which describe oligonucleotide analogs with modified 5-carbon sugars (*i.e.*, modified 2'-deoxyfuranosyl moieties) used in nucleic acid detection; U.S. Patent 5,446,137 which describes oligonucleotides comprising at least one 5-carbon sugar moiety substituted at the 4' position with a substituent other than hydrogen that can be used in hybridization assays; U.S. Patent 5,886,165 which describes oligonucleotides with both deoxyribonucleotides with 3'-5' internucleotide linkages and ribonucleotides with 2'-5' internucleotide linkages; U.S. Patent 5,714,606 which describes a modified internucleotide linkage wherein a 3'-position oxygen of the internucleotide linkage is replaced by a carbon to enhance the nuclease resistance of nucleic acids; U.S. Patent 5,672,697 which describes oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages that enhance nuclease resistance; U.S. Patents 5,466,786 and 5,792,847 which describe the linkage of a substituent moiety which may comprise a drug or label to the 2' carbon of an oligonucleotide to provide enhanced nuclease stability and ability to deliver drugs or detection moieties; U.S. Patent 5,223,618 which describes oligonucleotide analogs with a 2 or 3 carbon backbone linkage attaching the 4' position and 3' position of adjacent 5-carbon sugar moiety to enhanced cellular uptake, resistance to nucleases and hybridization to target RNA; U.S. Patent 5,470,967 which describes oligonucleotides comprising at least one sulfamate or sulfamide internucleotide linkage that are useful as nucleic acid hybridization probe; U.S. Patents 5,378,825, 5,777,092, 5,623,070, 5,610,289 and 5,602,240 which describe oligonucleotides with three or four atom linker moiety replacing phosphodiester backbone moiety used for improved nuclease resistance, cellular uptake and regulating RNA expression; U.S. Patent 5,858,988 which

describes hydrophobic carrier agent attached to the 2'-O position of oligonucleotides to enhanced their membrane permeability and stability; U.S. Patent 5,214,136 which describes oligonucleotides conjugated to anthraquinone at the 5' terminus that possess enhanced hybridization to DNA or RNA; enhanced stability to nucleases; U.S. Patent 5,700,922 which describes PNA-DNA-PNA chimeras wherein the DNA comprises 2'-deoxy-erythro-
5 pentofuranosyl nucleotides for enhanced nuclease resistance, binding affinity, and ability to activate RNase H; and U.S. Patent 5,708,154 which describes RNA linked to a DNA to form a DNA-RNA hybrid.

5. Polyether and Peptide Nucleic Acids

10 In certain embodiments, it is contemplated that a nucleic acid comprising a derivative or analog of a nucleoside or nucleotide may be used in the methods and compositions of the invention. A non-limiting example is a "polyether nucleic acid", described in U.S. Patent 5,908,845, incorporated herein by reference. In a polyether nucleic acid, one or more nucleobases are linked to chiral carbon atoms in a polyether backbone.

15 Another non-limiting example is a "peptide nucleic acid", also known as a "PNA", "peptide-based nucleic acid analog" or "PENAM", described in U.S. Patent 5,786,461, 5,891,625, 5,773,571, 5,766,855, 5,736,336, 5,719,262, 5,714,331, 5,539,082, and WO 92/20702, each of which is incorporated herein by reference. Peptide nucleic acids generally have enhanced sequence specificity, binding properties, and resistance to enzymatic
20 degradation in comparison to molecules such as DNA and RNA (Egholm *et al.*, 1993; PCT/EP/01219). A peptide nucleic acid generally comprises one or more nucleotides or nucleosides that comprise a nucleobase moiety, a nucleobase linker moiety that is not a 5-carbon sugar, and/or a backbone moiety that is not a phosphate backbone moiety. Examples of nucleobase linker moieties described for PNAs include aza nitrogen atoms, amido and/or
25 ureido tethers (see for example, U.S. Patent 5,539,082). Examples of backbone moieties described for PNAs include an aminoethylglycine, polyamide, polyethyl, polythioamide, polysulfonamide or polysulfonamide backbone moiety.

In certain embodiments, a nucleic acid analogue such as a peptide nucleic acid may be used to inhibit nucleic acid amplification, such as in PCR™, to reduce false positives and
30 discriminate between single base mutants, as described in U.S. Patent 5,891,625. Other modifications and uses of nucleic acid analogs are known in the art, and it is anticipated that these techniques and types of nucleic acid analogs may be used with the present invention. In a non-limiting example, U.S. Patent 5,786,461 describes PNAs with amino acid side chains attached to the PNA backbone to enhance solubility of the molecule. In another example, the

cellular uptake property of PNAs is increased by attachment of a lipophilic group. U.S. Application Ser. No. 117,363 describes several alkylamino moieties used to enhance cellular uptake of a PNA. Another example is described in U.S. Patents 5,766,855, 5,719,262, 5,714,331 and 5,736,336, which describe PNAs comprising naturally and non-naturally occurring nucleobases and alkylamine side chains that provide improvements in sequence specificity, solubility and/or binding affinity relative to a naturally occurring nucleic acid.

6. Preparation of Nucleic Acids

A nucleic acid may be made by any technique known to one of ordinary skill in the art, such as chemical synthesis, enzymatic production or biological production. Non-limiting examples of a synthetic nucleic acid (*e.g.*, a synthetic oligonucleotide), include a nucleic acid made by *in vitro* chemically synthesis using phosphotriester, phosphite or phosphoramidite chemistry and solid phase techniques such as described in EP 266,032, incorporated herein by reference, or via deoxynucleoside H-phosphonate intermediates as described by Froehler *et al.*, 1986 and U.S. Patent 5,705,629, each incorporated herein by reference. In the methods of the present invention, one or more oligonucleotide may be used. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244, each of which is incorporated herein by reference.

A non-limiting example of an enzymatically produced nucleic acid include one produced by enzymes in amplification reactions such as PCRTM (see for example, U.S. Patent 4,683,202 and U.S. Patent 4,682,195, each incorporated herein by reference), or the synthesis of an oligonucleotide described in U.S. Patent 5,645,897, incorporated herein by reference. A non-limiting example of a biologically produced nucleic acid includes a recombinant nucleic acid produced (*i.e.*, replicated) in a living cell, such as a recombinant DNA vector replicated in bacteria (see for example, Sambrook *et al.* 2001, incorporated herein by reference).

7. Purification of Nucleic Acids

A nucleic acid may be purified on polyacrylamide gels, cesium chloride centrifugation gradients, or by any other means known to one of ordinary skill in the art (see for example, Sambrook *et al.*, 2001, incorporated herein by reference).

In certain embodiments, the present invention concerns a nucleic acid that is an isolated nucleic acid. As used herein, the term "isolated nucleic acid" refers to a nucleic acid molecule (*e.g.*, an RNA or DNA molecule) that has been isolated free of, or is otherwise free of, the bulk of the total genomic and transcribed nucleic acids of one or more cells. In certain embodiments, "isolated nucleic acid" refers to a nucleic acid that has been isolated free of, or

is otherwise free of, bulk of cellular components or in vitro reaction components such as for example, macromolecules such as lipids or proteins, small biological molecules, and the like.

8. Hybridization

As used herein, "hybridization", "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or triple stranded nature. The term "anneal" as used herein is synonymous with "hybridize." The term "hybridization", "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

As used herein "stringent condition(s)" or "high stringency" are those conditions that allow hybridization between or within one or more nucleic acid strand(s) containing complementary sequence(s), but precludes hybridization of random sequences. Stringent conditions tolerate little, if any, mismatch between a nucleic acid and a target strand. Such conditions are well known to those of ordinary skill in the art, and are preferred for applications requiring high selectivity. Non-limiting applications include isolating a nucleic acid, such as a gene or a nucleic acid segment thereof, or detecting at least one specific mRNA transcript or a nucleic acid segment thereof, and the like.

Stringent conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleobase content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence or concentration of formamide, tetramethylammonium chloride or other solvent(s) in a hybridization mixture.

It is also understood that these ranges, compositions and conditions for hybridization are mentioned by way of non-limiting examples only, and that the desired stringency for a particular hybridization reaction is often determined empirically by comparison to one or more positive or negative controls. Depending on the application envisioned it is preferred to employ varying conditions of hybridization to achieve varying degrees of selectivity of a nucleic acid towards a target sequence. In a non-limiting example, identification or isolation of a related target nucleic acid that does not hybridize to a nucleic acid under stringent conditions may be achieved by hybridization at low temperature and/or high ionic strength. Such conditions are termed "low stringency" or "low stringency conditions", and non-limiting examples of low stringency include hybridization performed at about 0.15 M to about 0.9 M

NaCl at a temperature range of about 20°C to about 50°C. Of course, it is within the skill of one in the art to further modify the low or high stringency conditions to suite a particular application.

F. Treatment of Disease

1. Definitions

“Treatment” and “treating” refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For example, a treatment may include administration of a pharmaceutically effective amount of a nucleic acid that inhibits the expression of a gene that encodes an Id and a neutral lipid for the purposes of minimizing the growth or invasion of a tumor, such as a colorectal cancer.

A “subject” refers to either a human or non-human, such as primates, mammals, and vertebrates. In particular embodiments, the subject is a human.

The term “therapeutic benefit” or “therapeutically effective” as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the size of a tumor, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

A “disease” or “health-related condition” can be any pathological condition of a body part, an organ, or a system resulting from any cause, such as infection, genetic defect, and/or environmental stress. The cause may or may not be known.

In some embodiments of the invention, the methods include identifying a patient in need of treatment. A patient may be identified, for example, based on taking a patient history, based on findings on clinical examination, based on health screenings, or by self-referral.

2. Diseases

The present invention may be used to treat any disease associated with increased expression of an Id protein. For example, the disease may be a hyperproliferative disease, such as cancer.

For example, a siRNA that binds to a nucleic acid that encodes an Id protein may be administered to treat a cancer. The cancer may be a solid tumor, metastatic cancer, or non-

metastatic cancer. In certain embodiments, the cancer may originate in the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, duodenum, small intestine, large intestine, colon, rectum, anus, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In certain embodiments, the cancer is colorectal cancer (i.e., cancer involving the colon or rectum).

The cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant;

dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; 5 mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; 10 ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; hodgkin's disease; hodgkin's; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-hodgkin's lymphomas; 15 malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia. Nonetheless, it is also recognized that the present invention 20 may also be used to treat a non-cancerous disease (*e.g.*, a fungal infection, a bacterial infection, a viral infection, and/or a neurodegenerative disease). In a specific embodiment, the cancer is colorectal cancer.

G. Pharmaceutical Preparations

Where clinical application of a lipid composition containing a siNA is undertaken, it 25 will generally be beneficial to prepare the lipid complex as a pharmaceutical composition appropriate for the intended application. This will typically entail preparing a pharmaceutical composition that is essentially free of pyrogens, as well as any other impurities that could be harmful to humans or animals. One may also employ appropriate buffers to render the complex stable and allow for uptake by target cells.

30 The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one non-charged lipid component comprising a siNA or additional active ingredient will be known to those of skill in the art in

light of the present disclosure, as exemplified by Remington: The Science and Practice of Pharmacy, 21st, 2005, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

5 As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to
10 one of ordinary skill in the art. A pharmaceutically acceptable carrier is preferably formulated for administration to a human, although in certain embodiments it may be desirable to use a pharmaceutically acceptable carrier that is formulated for administration to a non-human animal but which would not be acceptable (*e.g.*, due to governmental regulations) for administration to a human. Except insofar as any conventional carrier is incompatible with
15 the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The actual dosage amount of a composition of the present invention administered to a patient or subject can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent
20 therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may
25 comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200
30 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body

weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 µg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered.

5 A gene expression inhibitor may be administered in a dose of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or more µg of nucleic acid per dose. Each dose may be in a volume of 1, 10, 50, 100, 200, 500, 1000 or more µl or ml.

Solutions of therapeutic compositions can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The therapeutic compositions of the present invention are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like.

Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to well known parameters.

Additional formulations are suitable for oral administration. Oral formulations include such typical excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders.

The therapeutic compositions of the present invention may include classic pharmaceutical preparations. Administration of therapeutic compositions according to the

present invention will be via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Topical administration may be particularly advantageous for the treatment of skin cancers, to prevent chemotherapy-induced alopecia or other dermal hyperproliferative disorder. Alternatively, administration
5 may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. For treatment of conditions of the lungs, or respiratory tract, aerosol delivery can be used. Volume of the aerosol is between about 0.01 ml and 0.5 ml.

10 An effective amount of the therapeutic composition is determined based on the intended goal. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined-quantity of the therapeutic composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be
15 administered, both according to number of treatments and unit dose, depends on the protection or effect desired.

Precise amounts of the therapeutic composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting the dose include the physical and clinical state of the patient, the route of administration, the intended goal of
20 treatment (*e.g.*, alleviation of symptoms versus cure) and the potency, stability and toxicity of the particular therapeutic substance.

H. Combination Treatments

In certain embodiments, the compositions and methods of the present invention involve an inhibitor of expression of an Id protein, or construct capable of expressing an
25 inhibitor of Id expression, in combination with a second or additional therapy. Such therapy can be applied in the treatment of any disease that is associated with increased expression or activity of an Id protein. For example, the disease may be a hyperproliferative disease, such as cancer.

The methods and compositions including combination therapies enhance the
30 therapeutic or protective effect, and/or increase the therapeutic effect of another anti-cancer or anti-hyperproliferative therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect, such as the killing of a cancer cell and/or the inhibition of cellular hyperproliferation. This process may involve contacting the cells with both an inhibitor of gene expression and a second therapy. A tissue,

tumor, or cell can be contacted with one or more compositions or pharmacological formulation(s) including one or more of the agents (*i.e.*, inhibitor of gene expression or an anti-cancer agent), or by contacting the tissue, tumor, and/or cell with two or more distinct compositions or formulations, wherein one composition provides 1) an inhibitor of gene
5 expression; 2) an anti-cancer agent, or 3) both an inhibitor of gene expression and an anti-cancer agent. Also, it is contemplated that such a combination therapy can be used in conjunction with a chemotherapy, radiotherapy, surgical therapy, or immunotherapy.

An inhibitor of gene expression may be administered before, during, after or in various combinations relative to an anti-cancer treatment. The administrations may be in intervals
10 ranging from concurrently to minutes to days to weeks. In embodiments where the inhibitor of gene expression is provided to a patient separately from an anti-cancer agent, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a
15 patient with the inhibitor of gene expression therapy and the anti-cancer therapy within about 12 to 24 or 72 h of each other and, more preferably, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between respective administrations.

In certain embodiments, a course of treatment will last 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,
20 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90 days or more. It is contemplated that one agent may be given on day 1, 2, 3, 4,
25 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and/or 90, any combination thereof, and another agent is given on day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26,
30 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and/or 90, or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment, it is contemplated that

there is a period of time at which no anti-cancer treatment is administered. This time period may last 1, 2, 3, 4, 5, 6, 7 days, and/or 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or more, depending on the condition of the patient, such as their prognosis, strength, health, etc.

5 Various combinations may be employed. For the example below an inhibitor of gene expression therapy is “A” and an anti-cancer therapy is “B”:

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A		
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A		

10 Administration of any compound or therapy of the present invention to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as
 15 well as surgical intervention, may be applied in combination with the described therapy.

In specific aspects, it is contemplated that a standard therapy will include chemotherapy, radiotherapy, immunotherapy, surgical therapy or gene therapy and may be employed in combination with the inhibitor of gene expression therapy, anticancer therapy, or both the inhibitor of gene expression therapy and the anti-cancer therapy, as described herein.

20 1. Chemotherapy

A wide variety of chemotherapeutic agents may be used in accordance with the present invention. The term “chemotherapy” refers to the use of drugs to treat cancer. A “chemotherapeutic agent” is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity
 25 within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis. Most chemotherapeutic agents fall into the following categories: alkylating agents, antimetabolites, antitumor antibiotics, mitotic inhibitors, and nitrosoureas.

30 Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin

and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; calystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a
5 sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially
10 calicheamicin gammall and calicheamicin omega11; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-
15 norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as
20 methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-
25 adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone;
30 mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol;

5 pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine;
10 novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin,
15 mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, paclitaxel, docetaxel, gemcitabien, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristin, vinblastin and methotrexate and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit
15 hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane,
20 formestane, fadrozole, vorozole, letrozole, and anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; ribozymes such as a VEGF expression
25 inhibitor and a HER2 expression inhibitor; vaccines such as gene therapy vaccines and pharmaceutically acceptable salts, acids or derivatives of any of the above.

2. Radiotherapy

Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor
30 cells. Other forms of DNA damaging factors are also contemplated such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287) and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for

prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

The terms “contacted” and “exposed,” when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with the target cell. To achieve cell killing, for example, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

3. Immunotherapy

In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Trastuzumab (Herceptin™) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells. The combination of therapeutic modalities, i.e., direct cytotoxic activity and inhibition or reduction of ErbB2 would provide therapeutic benefit in the treatment of ErbB2 overexpressing cancers.

Another immunotherapy could also be used as part of a combined therapy with gene silencing therapy discussed above. In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, i.e., is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, erb B and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines such as MIP-1, MCP-1, IL-8 and growth factors such as FLT3 ligand. Combining immune stimulating molecules, either as proteins or using gene delivery in combination with a tumor suppressor

has been shown to enhance anti-tumor effects (Ju *et al.*, 2000). Moreover, antibodies against any of these compounds can be used to target the anti-cancer agents discussed herein.

Examples of immunotherapies currently under investigation or in use are immune adjuvants *e.g.*, *Mycobacterium bovis*, *Plasmodium falciparum*, dinitrochlorobenzene and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998), cytokine therapy, *e.g.*, interferons α , β and γ ; IL-1, GM-CSF and TNF (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998) gene therapy, *e.g.*, TNF, IL-1, IL-2, p53 (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945) and monoclonal antibodies, *e.g.*, anti-ganglioside GM2, anti-HER-2, anti-p185 (Pietras *et al.*, 1998; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the gene silencing therapies described herein.

In active immunotherapy, an antigenic peptide, polypeptide or protein, or an autologous or allogenic tumor cell composition or "vaccine" is administered, generally with a distinct bacterial adjuvant (Ravindranath and Morton, 1991; Morton *et al.*, 1992; Mitchell *et al.*, 1990; Mitchell *et al.*, 1993).

In adoptive immunotherapy, the patient's circulating lymphocytes, or tumor infiltrated lymphocytes, are isolated *in vitro*, activated by lymphokines such as IL-2 or transduced with genes for tumor necrosis, and readministered (Rosenberg *et al.*, 1988; 1989).

4. Surgery

Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery is a cancer treatment that may be used in conjunction with other therapies, such as the treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy and/or alternative therapies.

Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically controlled surgery (Mohs' surgery). It is further contemplated that the present invention may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection or local application of the area with an additional anti-cancer therapy. Such treatment may be

repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

5. Other Agents

It is contemplated that other agents may be used in combination with the present invention to improve the therapeutic efficacy of treatment. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Immunomodulatory agents include tumor necrosis factor; interferon alpha, beta, and gamma; IL-2 and other cytokines; F42K and other cytokine analogs; or MIP-1, MIP-1beta, MCP-1, RANTES, and other chemokines. It is further contemplated that the upregulation of cell surface receptors or their ligands such as Fas / Fas ligand, DR4 or DR5 / TRAIL (Apo-2 ligand) would potentiate the apoptotic inducing abilities of the present invention by establishment of an autocrine or paracrine effect on hyperproliferative cells. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with the present invention to improve the treatment efficacy.

There have been many advances in the therapy of cancer following the introduction of cytotoxic chemotherapeutic drugs. However, one of the consequences of chemotherapy is the development/acquisition of drug-resistant phenotypes and the development of multiple drug resistance. The development of drug resistance remains a major obstacle in the treatment of such tumors and therefore, there is an obvious need for alternative approaches such as gene therapy.

Another form of therapy for use in conjunction with chemotherapy, radiation therapy or biological therapy includes hyperthermia, which is a procedure in which a patient's tissue is exposed to high temperatures (up to 106°F). External or internal heating devices may be involved in the application of local, regional, or whole-body hyperthermia. Local

hyperthermia involves the application of heat to a small area, such as a tumor. Heat may be generated externally with high-frequency waves targeting a tumor from a device outside the body. Internal heat may involve a sterile probe, including thin, heated wires or hollow tubes filled with warm water, implanted microwave antennae, or radiofrequency electrodes.

5 A patient's organ or a limb is heated for regional therapy, which is accomplished using devices that produce high energy, such as magnets. Alternatively, some of the patient's blood may be removed and heated before being perfused into an area that will be internally heated. Whole-body heating may also be implemented in cases where cancer has spread throughout the body. Warm-water blankets, hot wax, inductive coils, and thermal chambers
10 may be used for this purpose.

Hormonal therapy may also be used in conjunction with the present invention or in combination with any other cancer therapy previously described. The use of hormones may be employed in the treatment of certain cancers such as breast, prostate, ovarian, or cervical cancer to lower the level or block the effects of certain hormones such as testosterone or
15 estrogen. This treatment is often used in combination with at least one other cancer therapy as a treatment option or to reduce the risk of metastases.

I. Kits and Diagnostics

In various aspects of the invention, a kit is envisioned containing therapeutic agents and/or other therapeutic and delivery agents. In some embodiments, the present invention
20 contemplates a kit for preparing and/or administering a therapy of the invention. The kit may comprise one or more sealed vials containing any of the pharmaceutical compositions of the present invention. In some embodiments, the lipid is in one vial, and the nucleic acid component is in a separate vial. The kit may include at least one inhibitor of Id expression, one or more lipid component, as well as reagents to prepare, formulate, and/or
25 administer the components of the invention or perform one or more steps of the inventive methods. In some embodiments, the kit may also comprise a suitable container means, which is a container that will not react with components of the kit, such as an eppendorf tube, an assay plate, a syringe, a bottle, or a tube. The container may be made from sterilizable materials such as plastic or glass.

30 The kit may further include an instruction sheet that outlines the procedural steps of the methods, and will follow substantially the same procedures as described herein or are known to those of ordinary skill. The instruction information may be in a computer readable media containing machine-readable instructions that, when executed using a computer, cause

the display of a real or virtual procedure of delivering a pharmaceutically effective amount of a therapeutic agent.

J. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

In Vivo siRNA Targeting of Id2 Reduces Growth of Human Colorectal Cancer (CRC) in the Liver

Materials and Methods

Human tissue specimens. Formalin-fixed, paraffin-embedded specimens of colon adenocarcinoma, adjacent non-malignant colonic mucosa, and colon cancer liver metastases were obtained from an established tissue bank through a protocol approved by the institutional review board of The University of Texas M. D. Anderson Cancer Center. Histopathologic confirmation was provided by the M. D. Anderson Cancer Center's Department of Pathology. Histopathologic confirmation was provided by the M.D. Anderson Cancer Center's Department of Pathology.

Histopathologic preparation and immunohistochemical staining of tissue specimens. Patient tissue specimens were analyzed with an anti-Id2 antibody (C-20, Santa Cruz Biotechnology, Santa Cruz, CA) as described previously (Parikh *et al.*, 2004). Tumor xenografts from mice (generated as described below) were fixed in 10% neutral formalin (for paraffin embedding) or optimum cutting temperature (OCT) solution (Miles, Elkhart, IN). Immunostaining was done using standard procedures as described elsewhere (Camp *et al.*, 2006; Yang *et al.*, 2006). Staining for the endothelial cell-specific marker CD31 was performed with anti-mouse CD31 (PECAM-1) antibody (BD Biosciences, Franklin Lakes, NJ) and goat anti-mouse Alexa-594 conjugated antibody (Molecular Probes, Eugene, OR).

Apoptotic cells were detected with a TdT-mediated UTP nick-end labeling (TUNEL) Dead-End labeling kit (Promega, Madison, WI) according to the manufacturer's protocol. Image analysis of the immunohistochemical stains was done with NIH Image J 1.34 software. All analyses represent an average of a minimum of five randomly selected fields per specimen.

5 Cell lines and culture conditions. The human CRC cell lines Geo, HCT-116, HT-29, RKO, and SW-480 were obtained from the American Type Culture Collection (Manassas, VA); KM12 cells were obtained from IJ Fidler (M. D. Anderson Cancer Center). All cells were maintained in complete minimum essential medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin, and streptomycin (Life Technologies, Grand
10 Island, NY).

Preparation of siRNA expression plasmids and siRNA cell lines. siRNA plasmids were created with pSilencer 4.0 (Ambion, Austin, TX). The target sequences for Id2, 5'-gcggtgttcattgatttctt -3' (SEQ ID NO:10) and 5'- caaagcactgtgtgtgggctga -3' (SEQ ID NO:11) (Invitrogen/Life Technologies), were ligated into the expression plasmid. HCT-116 cells
15 were transfected with 0.5 ng of each siRNA plasmid and grown in selective medium containing 700 µg/mL hygromycin B (Roche Diagnostics, Mannheim, Germany). Negative controls were scrambled Id2 target sequences with no significant homology to other vertebrate genes.

Immunoprecipitation and western analysis. Cells were solubilized in 20 mM Tris-Cl
20 (pH 8.0), 137 mM NaCl, 1% Triton X-100, 1 mM Na₃VO₄, and 2 mM EDTA with protease inhibitor (Roche Diagnostics). Cell lysates were separated on 8% sodium dodecyl sulfate polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Amersham, Arlington Heights, IL). Membranes were probed with antibodies against Id2, Id1, Id3 (Santa Cruz Biotechnology), vinculin (Serotec, Raleigh, NC), cyclin D1, cyclin D2, cyclin D3,
25 cyclin A, cyclin E, p21, p27, p16, p53, Bcl-2, Bcl-xl, Bad, Bax, Bim/Bod, poly (ADP-ribose) polymerase (PARP), caspase-9, and caspase-7 (all from Cell Signaling Technology, Danvers, MA). After incubation with the appropriate antibodies, immunostained proteins were detected by chemiluminescence (New England Nuclear, Boston, MA). For western analysis of *in vivo* tumor sections, tumors were emulsified in protein lysis buffer as described above.

30 MTT analysis of cell proliferation. *In vitro* proliferation was assessed with tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described previously (Gray *et al.*, 2005). Briefly, 2,000 cells of each clone were plated per well onto 96-well microtiter plates in minimum essential medium with 10% fetal bovine serum. One

plate was developed immediately after cells had adhered (at approximately 4 h), and other plates were developed every 24 hours for 4 days. Assays were done by incubating each plate with 20 μ L of MTT substrate for 2 h followed by removal of medium and addition of 200 μ L of dimethylsulfoxide. Plates were read at a wavelength of 570 nm.

5 Flow cytometry and cell cycle analysis. Each clone was grown to 85% to 90% confluence in MEM (Eagle's minimum essential medium) supplemented with 2 mM L-glutamine, 12.5% fetal bovine serum, 100 U/mL penicillin, and 100 ng/mL streptomycin). Cells were trypsinized, washed in phosphate-buffered saline, and fixed in 70% ethanol at 4°C for 2 h. DNA was stained with propidium iodide (10 mg/mL of phosphate-buffered saline) and DNase-free RNase (Roche Diagnostics) (2.5 μ g/mL of phosphate-buffered saline) for at
10 least 30 min before cells were subjected to flow cytometry in a Coulter EPICS XL (Beckman Coulter, Fullerton, CA). Cell cycle profiles were generated by MultiCycle software (Phoenix Flow Systems, San Diego, CA).

15 In vivo tumor growth and siRNA targeting. Six- to eight-week-old male athymic nude mice were purchased from the National Cancer Institute-Frederick Cancer Research Facility (Frederick, MD) and maintained under specific pathogen-free conditions. All animal experiments met the requirements of M. D. Anderson's Animal Care Facility and the National Institutes of Health guidelines on animal care and use. Each HCT-116 clone was prepared and inoculated into 10 mice as described elsewhere (Gray *et al.*, 2005). For studies involving
20 stable cell lines, 5.0×10^5 viable Si-Control, sh-Id2C14, or sh-Id2C15 cells were injected in 0.1 mL of Hanks balanced salt solution either subcutaneously into the right rear flank, liver, or spleen (for metastasis studies) of each nude mouse. For the *in vivo* siRNA delivery experiments, 1.0×10^6 HCT-116 cells expressing the lenti-Luc reporter gene (Arumagam *et al.*, 2006) were suspended in 0.1 mL of Hanks balanced salt solution and inoculated into the
25 liver of each mouse. Four days later, control- or Id2-liposomal / siRNA (3 μ g) complexes were given as a 200- μ L intraperitoneal bolus, with identical treatment given every 3 days thereafter. After 32 days, mice were sacrificed (CO₂ inhalation) and total body weight, liver weight, and tumor volume were calculated. Tumor volume was calculated as $[(\text{length}/2) \times (\text{width}^2)] \pm \text{s.e.m.}$ For metastasis studies, tumor volume was determined as the sum of the
30 individual metastatic foci in each liver. Tissue specimens from each treatment group were snap-frozen, fixed in formalin, or frozen in optimum cutting temperature (OCT) solution.

Bioluminescence imaging. Bioluminescence imaging was done as described previously (Arumugam *et al.*, 2006) by using a cryogenically cooled IVIS 100 imaging system coupled to a data-acquisition personal computer equipped with Living Image software (Xenogen, Hopkinton, MA). For *in vivo* studies, before each imaging session, mice were anesthetized with isoflurane and injected intraperitoneally with 15 mg/mL of luciferase potassium salt (Sigma-Aldrich, St. Louis, MO) in phosphate-buffered saline at a dose of 150 mg/kg body weight. Initial images were obtained to establish baseline tumor volume/proton activity at 3 days after the hepatic inoculation of tumor cells (to allow tumor establishment), and additional images were obtained at 14 and 28 days after inoculation.

Preparation of liposomal siRNA. All Id2 and Id2 control sequences used for the *in vivo* experiments were purchased from Ambion (Landen *et al.*, 2005). Briefly, DOPC and siRNA (a control Id2-scrambled sequence or an Id2-specific sequence as described above) were mixed and lyophilized. The siRNA-liposome complexes were purified by separating the free siRNA from the liposomes by using 30,000 nominal-molecular-weight-limit filter units (Millipore, Billerica, MA). The lyophilized preparations were hydrated in 0.9% saline at a concentration of 5 µg/200 µL before being administered *in vivo*.

Quantification of autoradiographs. Autoradiographs were quantified in the linear range of the film by scanning the image using a Hewlett Packard Scanjet scanner and quantitated with Scion Image software program. Each sample measured was calculated as the ratio of the average area over the average area of actin or vinculin for immunoblots or as the ratio above the average area of basal/control levels of the protein of interest in immunoprecipitations.

Statistical analyses. Statistical analyses were performed with InStat 2.01 software (GraphPad Software, San Diego, CA), with Student's *t*-tests or Fisher's exact tests as appropriate. Significance ($P < 0.05$) was determined with 95% confidence intervals.

Results

Id2 expression in human colon mucosa, primary colon tumors, and liver metastases specimens and in human CRC cell lines. Given the paucity of published data regarding Id2 expression in human colon carcinomas and metastasis, studies were conducted to first evaluate the levels of Id2 in paraffin-embedded samples of human primary colon tumors, adjacent normal colonic mucosa, and colon cancer liver metastases by immunoperoxidase staining (FIG. 1A). No Id2 was detected in nonmalignant mucosa (0 of 40), but Id2 was expressed in 81 of 194 (42%, $P < 0.0001$) primary adenocarcinomas and in 47 of 56 liver

metastases (84%, $P < 0.0001$ vs normal mucosa, $P < 0.0001$). Id2 protein expression was also detected in all six-cell lines assayed (FIG. 1B).

Id2 expression and proliferation of CRC cells. Id2 has been reported to regulate the proliferation of human tumor cells (Lasorella *et al.*, 2002). To examine the effects of Id2 on proliferation *in vitro*, Id2 expression in HCT-116 cells was reduced by shRNA targeting. Western analysis of lysates from the siRNA-transfected clones sh-Id2C14 and sh-Id2C15 showed reductions in Id2 of more than 85% compared with controls (parental cells and cells transfected with scrambled sequence (shRNA-control (sh-Con), FIG. 1B). This targeting was specific for Id2, as levels of Id1 and Id3 did not change (Id4 protein was not detected) (FIG. 2A). Proliferation rates among controls and siRNA clones, tested by MTT assays, were similar in the two controls (doubling time for parental cells, 26.0 ± 1.2 h vs. 25.4 ± 1.0 h for sh-Con) but longer for clones with reduced Id2 expression (sh-Id2C14, 36.0 ± 2.7 h; sh-Id2C15, 33 ± 1.3 h) (FIG. 2B). Similar results showing an increase in cell doubling time occurring with a reduction of Id2 levels were also obtained with Celltiter-Glo assays (Promega, WI, USA). Next, to see if knockdown of Id2 affected cell cycle regulatory proteins in CRC cells, western blotting of cyclin molecules was used. Only cyclin D1 levels (about 75% reduction compared to control), and not levels of cyclin D2 D3, A, or E, was affected by reduction of Id2 expression (FIG. 2C). To see if the repression of Id2 levels affected the expression of cell cycle inhibitors in CRC cells, p15, p16, p21, p27, and p53 was assessed by western analysis. Levels of p21 increased twofold (as determined by densitometry) in the Id2-suppressed clones, whereas levels of the other cell cycle regulators were not altered (FIG. 2C).

Id2 expression and apoptosis of CRC cells. To determine if the impeded growth of clones with reduced Id2 expression resulted from changes in spontaneous apoptosis, fluorescence-activated cell sorting was used to examine control and shRNA-targeted cells to assess the percentage of sub-G₀ cells. Knockdown of Id2 expression in CRC cells resulted in four- to six-fold increases in the apoptotic (sub-G₀) populations (mean 12% in sh-Id2C14 cells and 8 % in sh-Id2C15 cells) compared with those of the controls (1.5% in parental cells and 2% in sh-Con cells, FIG. 3A). Western analysis was utilized to determine if the observed increase in apoptosis resulted from changes in expression of apoptotic regulatory proteins. Examination of the Bcl-2 family members (Bcl-2, Bcl-xl, Bad, Bax, and Bim/Bod) showed that only levels of the pro-apoptotic Bcl-2 family member Bim/Bod increased by twofold in response to reduction of Id2 expression in HCT-116 cells (FIG. 3B). Further examination of the apoptotic regulatory proteins caspase-7 and caspase-9 revealed that while the caspase-9

protein remained uncleaved, caspase-7 underwent a threefold increase in cleavage owing to knockdown of Id2 as did the downstream caspase-7 target poly (ADP-ribose) polymerase (PARP) (FIG. 3C).

Knockdown of Id2 expression and tumor growth *in vivo*. To understand the effect of

5 Id2 on the growth of CRC cells *in vivo*, nude mice were subcutaneously injected with sh-Con or shRNA-targeted clones with reduced Id2 levels. The resulting tumors were assessed. Tumors from cells deficient in Id2 expression were significantly smaller than those from cells with normal Id2 levels ($451 \pm 97 \text{ mm}^3$ for sh-Con vs. $161 \pm 59 \text{ mm}^3$ for sh-Id2C14 [$P = 0.03$] and $192 \pm 62 \text{ mm}^3$ for sh-Id2C15 [$P = 0.017$]) (FIG. 4A). To ascertain the effect of Id2
10 expression on hepatic metastasis of CRC cells, cells of each clone were injected into the spleen of nude mice and the resulting liver metastases were assayed. Cells with reduced Id2 expression produced fewer detectable metastases than did cells with normal Id2 levels (mean 7 ± 2 hepatic metastases for sh-Con vs 2 ± 1 for sh-Id2C14 [$P = 0.022$] or 3 ± 2 for sh-Id2C15 [$P = 0.045$]) (FIG. 4B). The mean volumes of the metastases were smaller for the reduced-
15 Id2 clones than for the siRNA control cells (mean $286 \pm 43 \text{ mm}^3$ for sh-Con vs. $57 \pm 24 \text{ mm}^3$ for sh-Id2C14 [$P = 0.015$] or $57 \pm 24 \text{ mm}^3$ for Si-Id2C15 [$P = 0.02$] (FIG. 4C).

Targeting Id2 *in vivo* and colon cancer progression. Based on the reduced tumor growth with Id2 silencing, studies were conducted to determine whether a therapeutically treatment relevant approach could be utilized. Id2 siRNA sequences incorporated into the
20 neutral liposome DOPC, which was previously shown to efficiently reduce *in vivo* gene expression (Landen *et al.*, 2005), were used. Bioluminescence imaging of tumor cell growth in nude mice showed that siRNA to Id2 impaired the growth kinetics of HCT-116 CRC cells compared with those of control cells at 30 days after hepatic inoculation (mean activity 1.8×10^6 photons for control vs. 2.8×10^5 photons for Id2 siRNA-DOPC) (FIG. 4A). At the
25 termination of the experiment, the hepatic tumor burden in the control group (control Sh-DOPC) was much greater than in the siRNA-Id2 mice (mean $932 \pm 112 \text{ mm}^3$ for control siRNA-DOPC vs. $377 \pm 100 \text{ mm}^3$ for Id2 siRNA-DOPC [$P = 0.006$, Wilcoxon rank-sum test]) (FIGS. 5B, 5C). Cellular proliferation in the excised tumor samples (assessed by immunohistochemical staining for proliferating cell nuclear antigen) was reduced in the Id2-
30 targeted group compared with those in the control group ($P=0.02$) (FIG. 6A). TdT-mediated UTP nick-end labeling staining showed that CRC cells growing in the liver of mice treated with siRNA to Id2 had four times more apoptotic cells ($P=0.002$) than did tumors treated with control siRNA (control Sh-DOPC) (FIG. 6B). Finally, Id2 expression in hepatic tumors was

shown by immunohistochemistry and western analysis to be substantially reduced in the Id2-siRNA-treated group compared with controls (FIG. 6C).

These results demonstrate that Id2 is critical for the growth of CRC and represents a therapeutic target in tumors that express it. Id2 was not expressed in normal human colonic mucosa, and was expressed infrequently in primary tumors. However, Id2 expression was upregulated in all hepatic metastases studied, suggesting either a selection of Id2 expressing cells in the process of metastasis, or environmental induction of Id2 in the liver. This observation is of particular clinical interest as the most common site of liver metastasis is the liver and hepatic tumor burden is the most common cause of death in patients with metastatic CRC. Id2 was also expressed in all six of the human CRC cell lines assayed and reduction of Id2 levels in CRC cells by siRNA decreased proliferation rates and increased spontaneous apoptosis. Further, the *in vivo* models showed that loss of Id2 expression, through either the creation of stable Id2-deficient clones or the systemic administration of siRNA to Id2, severely impaired the ability of CRC cells to grow in the liver, the major site of CRC metastasis.

Consistent with findings in other tissues, reduction of Id2 levels in CRC cells with siRNA decreased proliferation rates. These results also show that loss of Id2 increased levels of the cell cycle inhibitor p21 in the absence of changes in p53 levels. Further, the reduction of endogenous Id2 levels increased spontaneous apoptosis in CRC cells. This increase seems to be independent of p53, which remained unchanged in our Id2-deficient CRC cells.

EXAMPLE 2

Id2 Regulates Survival, Proliferation, and Chemoresistance in Colorectal Carcinomas

While the treatment of colorectal cancer (CRC) has improved with the advent of molecular targeted therapies, most patients still eventually succumb to the disease. This outcome dictates that a greater understanding of tumor growth and progression in CRC is a prerequisite to identifying new novel targets that may have validity in preclinical models. The transcriptional repressor Id2 is a member of inhibitor of differentiation (Id) family and has been shown to be indispensable for proper development in embryonic tissues. The Id proteins, including Id2, function as negative regulators by dimerizing with and blocking the DNA binding capability of other transcriptional activators. While not normally expressed in adult tissues, some human cancers show reactivated expression of Id2, where it is implicated in proliferation, and survival and angiogenesis. In view of results demonstrating that constitutive Id2 expression occurs in colon carcinoma, therefore we hypothesize that Id2

expression contributes to the aggressive nature of CRC's by enhancing proliferation, survival, and chemotherapeutic resistance. Methods. HCT-116 cells were transfected with siRNA for Id2 or scrambled sequence for Id2 (control), stable clones were selected and Id2 levels was determined by Western analysis. Proliferation rates were determined by MTT analysis and apoptosis (including chemotherapeutic studies) was determined by FACS analysis. Orthotopic xenografts were induced by injecting 0.5×10^6 cells of each clone into the right flank of nude mice, while hepatic metastasis xenografts were induced by injecting 0.5×10^6 cells of each clone into the spleen of nude mice. Results. Loss of Id2 reduces *in vitro* proliferation rates (controls; 25 h, siRNA-Id2 to 35 h) and significantly increases spontaneous apoptosis ($P < 0.05$) two fold compared cells with normal levels of Id2 expression. Treatment with the chemotherapeutic agents 5-Fluorouracil or Oxaliplatin also shows that cells with reduced Id2 expression are 2-3 fold more sensitive to increasing concentrations of each therapeutic agent than cells with normal levels of Id2. Incidence in subcutaneous xenografts was approximately the same in controls and clones with reduced Id2 expression (control; 80%, siId2 clones; 71%), however final tumor volume was significantly inhibited in clones with reduced Id2 expression (control; $54 \pm 14 \text{ mm}^3$, siRNA-Id2; $12 \pm 7 \text{ mm}^3$). Incidence of hepatic metastases was also severely reduced in cells with reduced Id2 expression (100 % in controls, 40 % in siRNA-Id2 clones), as was the average number (control; 7.4, siRNA-Id2, 2.8) and final volume of metastases (control; $31 \pm 13 \text{ mm}^3$, siRNA-Id2; $3.5 \pm 2 \text{ mm}^3$). Conclusion. These results demonstrate that Id2 promotes tumor growth, survival, and metastasis in CRC.

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All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A composition comprising:
 - (a) a nucleic acid component comprising a nucleic acid that inhibits the expression
5 of a gene that encodes an inhibitor of DNA binding protein (Id); and
 - (b) a lipid component comprising one or more neutral phospholipids.
2. The composition of claim 1, wherein the nucleic acid component comprises a siRNA
or a nucleic acid encoding a siRNA, wherein the siRNA inhibits the expression of a gene that
10 encodes an Id.
3. The composition of claim 1, wherein the nucleic acid component comprises a shRNA
or a nucleic acid encoding a shRNA.
- 15 4. The composition of claim 1, wherein the Id is Id1, Id2, Id3, or Id4.
5. The composition of claim 4, wherein the Id is Id2.
6. The composition of claim 1, wherein the lipid component forms a liposome.
- 20 7. The composition of claim 2, wherein the siRNA is encapsulated in the lipid
component.
8. The composition of claim 3, wherein the shRNA is encapsulated in the lipid
25 component.
9. The composition of claim 1, wherein the nucleic acid component comprises SEQ ID
NO:10 or SEQ ID NO:11.
- 30 10. The composition of claim 1, wherein the composition is comprised in a
pharmaceutically acceptable carrier.
11. The composition of claim 1, wherein the lipid component comprises two or more
neutral phospholipids.

12. The composition of claim 1, wherein the neutral phospholipid is a phosphatidylcholine or phosphatidylethanolamine.

5 13. The composition of claim 1, wherein the neutral phospholipid is 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), egg phosphatidylcholine ("EPC"),
 10 dilauryloylphosphatidylcholine ("DLPC"), dimyristoylphosphatidylcholine ("DMPC"), dipalmitoylphosphatidylcholine ("DPPC"), distearoylphosphatidylcholine ("DSPC"), 1-myristoyl-2-palmitoyl phosphatidylcholine ("MPPC"), 1-palmitoyl-2-myristoyl
 15 phosphatidylcholine ("PMPC"), 1-palmitoyl-2-stearoyl phosphatidylcholine ("PSPC"), 1-stearoyl-2-palmitoyl phosphatidylcholine ("SPPC"), dimyristyl phosphatidylcholine ("DMPC"), 1,2-distearoyl-sn-glycero-3-phosphocholine ("DAPC"), 1,2-diarachidoyl-sn-glycero-3-phosphocholine ("DBPC"), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine ("DEPC"), palmitoyloeyl phosphatidylcholine ("POPC"), lysophosphatidylcholine,
 20 dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine ("DSPE"), dimyristoyl phosphatidylethanolamine ("DMPE"), dipalmitoyl phosphatidylethanolamine ("DPPE"), palmitoyloeyl phosphatidylethanolamine ("POPE"), or lysophosphatidylethanolamine.

14. The composition of claim 13, wherein the neutral phospholipid is DOPC.

20

15. The composition of claim 1, wherein the lipid component further comprises a positively charged lipid or a negatively charged lipid.

16. The composition of claim 15, wherein the negatively charged phospholipid is a
 25 phosphatidylserine or phosphatidylglycerol.

17. The composition of claim 16, wherein the negatively charged phospholipid is dimyristoyl phosphatidylserine ("DMPS"), dipalmitoyl phosphatidylserine ("DPPS"), brain
 30 phosphatidylserine ("BPS"), dilauryloylphosphatidylglycerol ("DLPG"), dimyristoylphosphatidylglycerol ("DMPG"), dipalmitoylphosphatidylglycerol ("DPPG"), distearoylphosphatidylglycerol ("DSPG"), or dioleoylphosphatidylglycerol ("DOPG").

18. The composition of claim 1, wherein the composition further comprises cholesterol or polyethyleneglycol (PEG).

19. The composition of claim 2, wherein the siRNA is a double stranded nucleic acid of 18 to 100 nucleobases.
- 5 20. The composition of claim 19, wherein the siRNA is 18 to 30 nucleobases.
21. The composition of claim 1, further comprising a chemotherapeutic agent.
22. The composition of claim 21, wherein the chemotherapeutic agent is docetaxel, 10 paclitaxel, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristine, vinblastin, methotrexate, 15 oxaliplatin, or combinations thereof.
23. The composition of claim 22, wherein the chemotherapeutic agent is 5-fluorouracil or oxaliplatin.
- 20 24. A method of treating a subject with cancer comprising administering to the subject a pharmaceutically effective amount of a composition comprising
- (a) a nucleic acid component comprising a nucleic acid that inhibits the expression of a gene that encodes Id2; and
- (b) a lipid component comprising one or more neutral phospholipids.
- 25 25. The composition of claim 24, wherein the nucleic acid component comprises a siRNA or a nucleic acid encoding a siRNA, wherein the siRNA inhibits the expression of a gene that encodes an Id.
- 30 26. The composition of claim 24, wherein the nucleic acid component comprises a shRNA or a nucleic acid encoding a shRNA.
27. The method of claim 24, wherein the subject is a human subject.

28. The method of claim 24, wherein the cancer is breast cancer, lung cancer, prostate cancer, ovarian cancer, brain cancer, liver cancer, cervical cancer, colorectal cancer, renal cancer, skin cancer, head and neck cancer, bone cancer, esophageal cancer, bladder cancer, uterine cancer, lymphatic cancer, stomach cancer, pancreatic cancer, testicular cancer, lymphoma, or leukemia.
29. The method of claim 29, wherein the cancer is colorectal cancer.
30. The method of claim 24, further defined as comprising identifying a subject in need of treatment.
31. The method of claim 24, further comprising administering an additional anticancer therapy to the subject.
32. The method of claim 31, wherein the additional anticancer therapy is chemotherapy, radiation therapy, surgical therapy, immunotherapy, gene therapy, or a combination thereof.
33. The method of claim 32, wherein the additional anticancer therapy is chemotherapy.
34. The method of claim 34, wherein the chemotherapy comprises administration of docetaxel, paclitaxel, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristine, vinblastine, methotrexate, oxaliplatin, or a combination thereof.
35. The method of claim 34, wherein the chemotherapy comprises 5-fluorouracil or oxaliplatin.
36. The method of claim 24, wherein the composition is administered to the patient intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

37. The method of claim 25, wherein the siRNA inhibits the expression of a gene that encodes Id2.

38. The method of claim 24, wherein the subject has a tumor and the method is further defined as a method to reduce tumor volume in the subject.

39. The method of claim 38, wherein the tumor is colorectal cancer.

40. The method of claim 24, wherein the nucleic acid component comprises SEQ ID NO:10 or SEQ ID NO:11.

41. A method of treating a subject with cancer comprising administering to the subject a pharmaceutically effective amount of a composition comprising

(a) a nucleic acid component comprising a nucleic acid that inhibits the expression of a gene that encodes Id; and

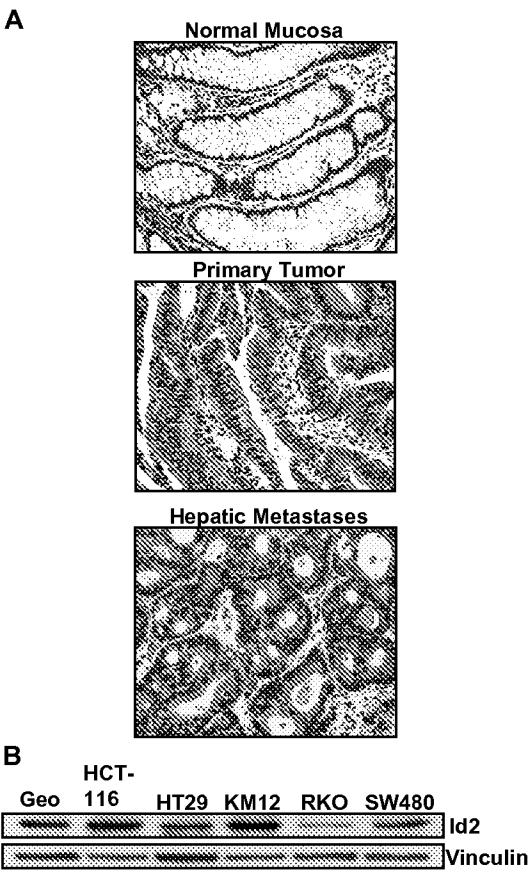
(b) a lipid component comprising DOPC.

42. The method of claim 41, wherein the Id is Id2.

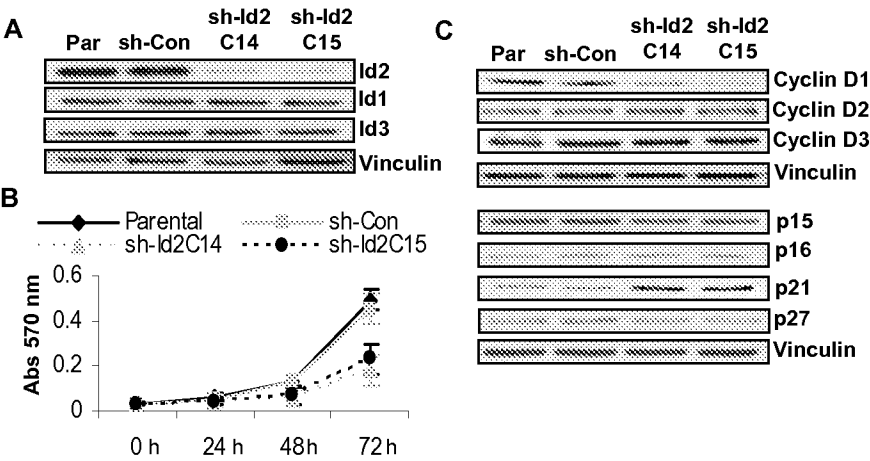
43. The method of claim 41, wherein the cancer is breast cancer, lung cancer, prostate cancer, ovarian cancer, brain cancer, liver cancer, cervical cancer, colorectal cancer, renal cancer, skin cancer, head and neck cancer, bone cancer, esophageal cancer, bladder cancer, uterine cancer, lymphatic cancer, stomach cancer, pancreatic cancer, testicular cancer, lymphoma, or leukemia.

44. The composition of claim 41, wherein the nucleic acid component comprises a siRNA or a nucleic acid encoding a siRNA, wherein the siRNA inhibits the expression of a gene that encodes an Id.

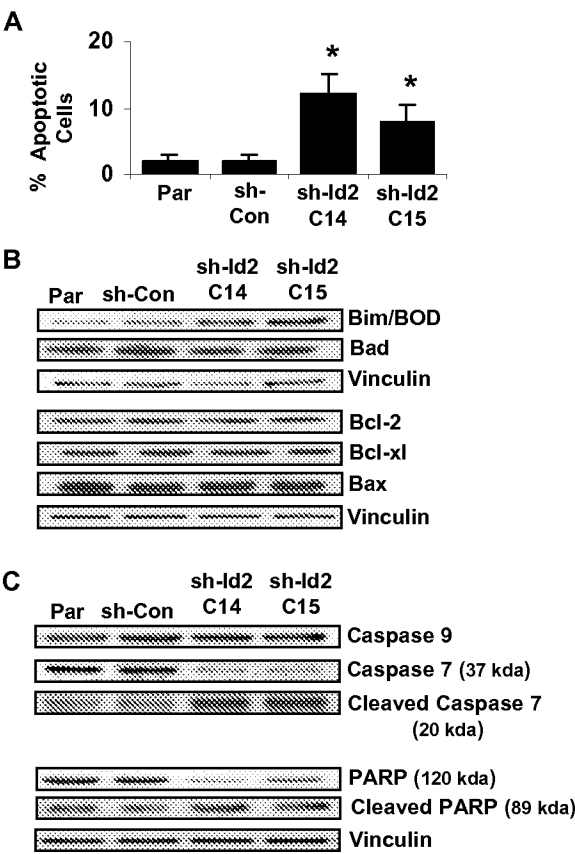
45. The composition of claim 41, wherein the nucleic acid component comprises a shRNA or a nucleic acid encoding a shRNA.



FIGs. 1A-B

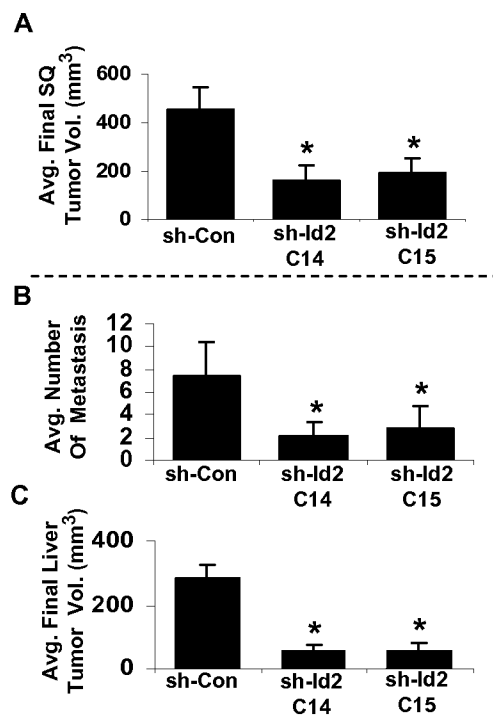


FIGs. 2A-C

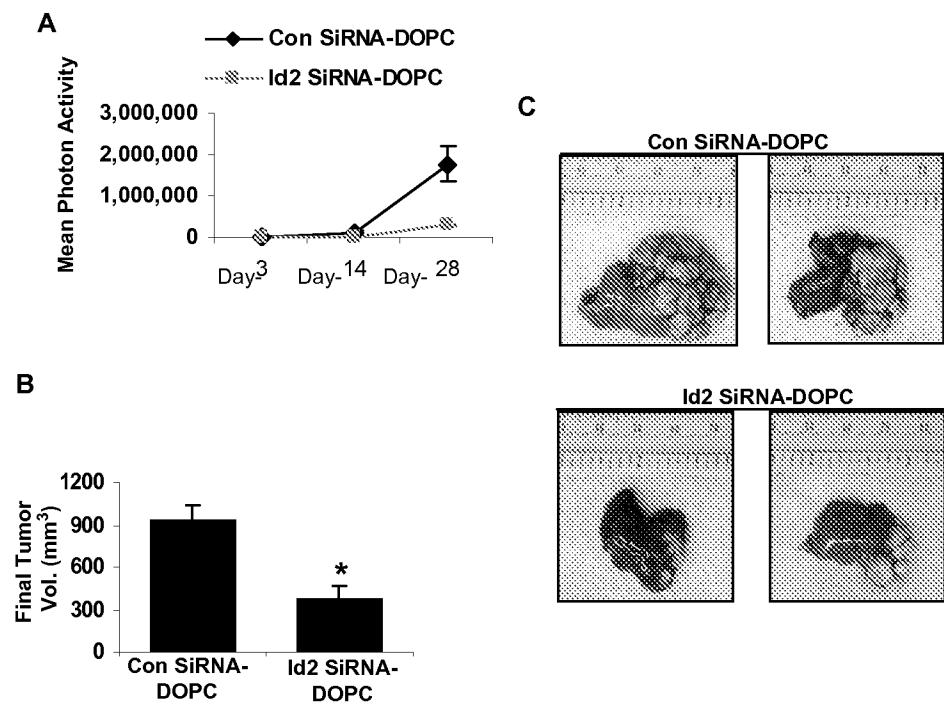


FIGs. 3A-C

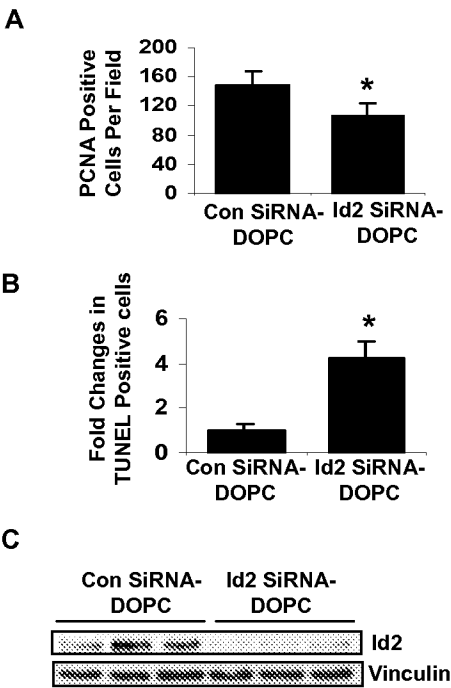
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FIGs. 4A-C



FIGs. 5A-C



FIGs. 6A-C