Abstract: IAP Antagonists are useful in the treatment of proliferative disorders associated with aberrant JAK signaling.
Title
THERAPEUTIC IAP ANTAGONISTS FOR TREATING PROLIFERATIVE DISORDERS

Field of the Invention
This invention is in the field of compositions and methods to treat proliferative disorders including cancers.

Background of the Invention
Inhibitors of Apoptosis Proteins (IAPs) are naturally occurring intra-cellular proteins that suppress caspase-dependent apoptosis. Second mitochondria-derived activator of caspases (Smac), also known as DIABLO, is an intracellular protein that functions to antagonize, i.e., inhibit, the activity of IAPs. In normal healthy cells, Smac and IAPs function together to maintain the viability of healthy cells. However, in certain disease states, e.g., cancers and other proliferative disorders, IAPs are not adequately antagonized and therefore prevent apoptosis and cause or exacerbate abnormal proliferation and survival.

Smac mimetics are synthetic small molecules that mimic the structure and IAP antagonist activity of the four N-terminal amino acids of Smac. When administered to animals suffering proliferative disorders, the Smac mimetics antagonize IAPs, causing an increase in apoptosis among abnormally proliferating cells. Various Smac mimetics are in development for use in the treatment of proliferative disorders. Smac mimetics have also been shown to promote apoptosis in chronically infected cells while sparing uninfected cells and are in development for treatment of viral and other infections.

The Janus kinase (JAK) family of cytosolic tyrosine kinases transduce cytokine-mediated signals via type I and type II cytokine receptors. Signal transduction is effected by phosphorylation and activation of transcription factors called STATs (Signal Transducer and Activator of Transcription, or Signal Transduction And Transcription). Activated STATs dissociate from the receptor and translocate from the cytoplasm to the nucleus, where they regulate transcription of selected
genes. Four members of the JAK family have been identified: JAK1, JAK2, JAK3, and Tyrosine Kinase 2 (TYK2).

Mutations in JAKs have been associated with certain cancers and other disorders. Approximately 50% of pediatric patients with acute lymphoblastic leukemia (ALL) and the Philadelphia chromosome (or similar translocations) harbor mutations in JAKs. The JAK2 V617F mutation has been associated with myeloproliferative disorders, e.g., polycythemia vera (PV), primary or essential thrombocythemia (ET), and myeloid metaplasia with myelofibrosis (MMM). JAKAFI (ruxolitinib phosphate) is an example of a JAK1/2 inhibitor approved in the U.S. for the treatment of intermediate or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera, myelofibrosis, and post-essential thrombocythemia myelofibrosis.

**Summary of the Invention**

This invention, in one aspect, relates to a method of treating a mammalian subject, e.g., a human, or a (companion) animal, a food animal, or a sporting animal, suffering from or having a proliferative disorder (e.g. associated with a Janus Kinase (JAK) mutation), such as by administering an IAP antagonist (to the subject).

Related aspects of the invention include, among others, treatment of proliferative disorders, such as those associated with aberrant (e.g. JAK) signaling, e.g., with one or more JAK mutations, for example with a combination of an IAP antagonist and a JAK inhibitor.

Related aspects of the invention include, among others, use of aberrant JAK signaling (e.g., JAK mutation) as a biomarker, e.g. for sensitivity to pro-apoptotic effects (of an IAP antagonist). For example, in an illustrative embodiment, the invention includes a method of treating (a subject presenting with one or more symptoms of) a proliferative disorder associated with aberrant JAK signaling, said method comprising:
(a) determining if the subject's disorder is associated with aberrant JAK signaling, such as but not limited to obtaining or having obtained a sample of abnormally proliferating cells from the subject and optionally determining or having determined if some or all of the sample of abnormally proliferating cells have a JAK mutation such as by genotyping/phenotyping (or having genotyped/phenotyped) the cells;

(b) if the subject manifests aberrant JAK signaling, e.g., if some or all of the cells have a JAK mutation as evidenced by presence of a JAK gene or protein mutation, then treating the patient by internally administering to the patient an effective amount of an IAP antagonist.

In certain illustrative embodiments, the IAP antagonist has the following general chemical formula:

![Chemical Structure](image)

wherein R5a and R5b are the same and are an alkyl, an alkyl substituted with hydroxyl, or an alkyl substituted with alkoxy; where R7a and R7b are the same and are alkyl; where R8a and R8b are the same and are selected from H, or alkyl; where R3a and R3b are the same and are selected from H, or hydroxy;
where $R_{12a}$, and $R_{12b}$ are both $H$; where $R_{13a}$ and $R_{13b}$ are the same and are selected from $H$ or $F$; and where, $R_{14a}$ and $R_{14b}$ are both $H$. Such compounds are described, e.g., in US7517906 and US8022230.

In a particular one of such illustrative embodiments, the IAP antagonist is birinapant, which has the chemical name: \( N\{1\,S\{-2R-(6,6'\text{-difluoro-3'}\text{-}{4S-}
\text{hydroxy-1-[2S-(2S-methylamino-propionylamino)-butyryl]-pyrrolidin-2R-ylmethyl]-}
1H,1'H-[2,2']biindolyl-3-ylmethyl\}-4S\text{-hydroxy-pyrrolidine-1 -carbonyl]-propyl\}-2S-
\text{methylamino-propionamide} \) and which has the chemical formula:

![Chemical structure of birinapant](image)

wherein $R_5$ is -CH2CH3, or a pharmaceutically acceptable salt thereof.

Birinapant is described as Compound 15 in US Patent 8603816.

Illustrative embodiments of the invention are reflected in the following brief descriptions of illustrative embodiments 2 through 5.

2. A method of treating a subject presenting with one or more symptoms of a proliferative disorder associated with a mutation in a Janus Kinase (JAK) gene, said method comprising:

(a)(i) obtaining or having obtained a sample of abnormally proliferating cells from the subject;
(a) (ii) determining or having determined if some or all of the sample of abnormally proliferating cells have a JAK mutation such as by biochemical assay, genotyping and/or phenotyping;

(b) (i) if some or all of the cells have a JAK mutation, then treating the patient by internally administering to the patient an effective amount of an IAP antagonist;

(b)(ii) if some or all of the cells do not have a JAK mutation, then treating the patient with chemotherapy other than IAP antagonist therapy.

3. A method of inhibiting the proliferation of abnormally proliferating cells, e.g. in which the abnormal proliferation is associated with aberrant JAK signaling, said method comprising treating the cells with an IAP antagonist.

4. A method for identifying a cancer in a subject (that is sensitive to treatment with an IAP antagonist), said method comprising determining or having determined if some or all of the cancerous cells suffer aberrant JAK signaling, whereby presence of a JAK mutation indicates sensitivity to treatment with an IAP antagonist.

5. A method of treating a cancer in a subject, in which subject some or all of the cancerous cells have a mutation in one or both copies of a JAK gene, said method optionally comprising (internally) administering to the subject an effective amount of an IAP antagonist.

6. Use of an IAP antagonist for the manufacture of a medicament for use in a method of treating a subject presenting with or having one or more symptoms of a proliferative disorder associated with aberrant JAK signaling, said method optionally comprising:

(a) determining if the subject’s disorder is associated with aberrant JAK signaling, such as but not limited to obtaining or having obtained a sample of abnormally proliferating cells from the subject and determining or having determined if some or all of the sample of abnormally proliferating cells have a JAK mutation such as by genotyping/phenotyping (or having genotyped/phenotyped) the cells;
(b) if the subject manifests aberrant JAK signaling, e.g., if some or all of the cells have a JAK mutation as evidenced by presence of a JAK gene or protein mutation, then treating the patient by internally administering to the patient an effective amount of an IAP antagonist and/or in any of the preceding methods of illustrative embodiments 2 through 5.

7. Use of an IAP antagonist for the treatment of a proliferative disorder, e.g. associated with aberrant JAK signaling, as described above (and as further described below).

Illustrative embodiments further include one or more of the above illustrative embodiments having one or more of the following features:
- the IAP antagonist comprises a Smac mimetic.
- the Smac mimetic comprises a bivalent Smac mimetic.
- the Smac mimetic is characterized as (i) not inhibiting XIAP E3 ubiquitin ligase activity or as only poorly inhibiting XIAP E3 ubiquitin ligase activity; (ii) not inhibiting or poorly inhibiting NOD (i.e., NOD1/2) signaling; and/or (iii) not inhibiting or poorly inhibiting NOD-mediated NF-kB activation.
- the Smac mimetic is birinapant.
- the proliferative disorder is acute lymphoblastic leukemia (ALL), a myeloproliferative disorder, breast cancer, pancreatic cancer, and/or non-small cell lung carcinoma.
- the proliferative disorder is Philadelphia chromosome-like ALL (Ph-like ALL) or a myeloproliferative disorder selected from chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) (also known as chronic idiopathic myelofibrosis or agnogenic myeloid metaplasia), chronic neutrophilic leukemia, and/or chronic eosinophilic leukemia.
- the proliferative disorder is Ph-like pediatric ALL.
- the JAK mutation is a mutation in the JAK2 gene.
- the JAK mutation is one or more of the JAK2 V617F mutation or a JAK2 exon 12, 13, 14, or 15 mutation.

- in addition to the IAP antagonist the subject is also treated by internally administering to the subject a second therapy selected from radiation, chemotherapy, immunotherapy, photodynamic therapy, and combinations thereof.

- the proliferative disorder is Ph-like ALL and the additional therapy comprises administration of one or more of a nucleoside analog such as 5-azacitidine, a tyrosine kinase inhibitor such as ruxolitinib, a TRAIL agonist such as an anti-DR4, anti-DR5, or anti-DR4/5 antibody, an NSAID such as celecoxib, or GM-CSF such as sargramostim.

- the proliferative disorder is PV, ET, or PMF and the additional therapy comprises administration of one or more of a nucleoside analog such as 5-azacitidine, a tyrosine kinase inhibitor such as ruxolitinib, a TRAIL agonist such as an anti-DR4, anti-DR5, or anti-DR4/5 antibody, an NSAID such as celecoxib, or GM-CSF such as sargramostim.

- if the abnormally proliferating cells do not have a JAK mutation, then treating the patient with an alternative therapy.

- an initial step is determining if abnormally proliferating cells have a JAK mutation such as by use of a JAK signaling biochemical assay, by genotyping or having genotyped the cells or by sequencing or having sequenced JAK protein expressed by the cells.

**Detailed Description of the Invention**

The examples, below, describe experiments leading to the discovery that IAP antagonists, as represented by birinapant, can be particularly useful in the treatment of JAK mutation-associated cancers, as represented by Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL).

In accordance with this invention, therefore, an IAP antagonist is used in the treatment of proliferative disorders that are associated with aberrant JAK
signaling, generally as a result of a mutation in one or more of JAK1, JAK2, JAK3, and TYK2. Such proliferative disorder can be, e.g., a leukemia such as but not limited to an acute lymphoblastic leukemia (ALL), a myeloproliferative disorder, a breast cancer, a pancreatic cancer, or a non-small cell lung carcinoma. Myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Other examples of such JAK mutant-associated cancers can include myelofibrosis with myeloid metaplasia (MMM), idiopathic myelofibrosis, chronic myelogenous (myelocytic) leukemia, chronic neutrophilic leukemia / hyper eosinophilic syndrome, etc. See, e.g., WO2012112847.

Some embodiments of the invention include inducing apoptosis of cells, particularly pathologically proliferating cells. The methods can be carried out in vitro or in vivo.


Many such IAP antagonists, but not all, are commonly within the genus of monovalent or bivalent Smac mimetics that have the general structure:

\[ P_{1}-P_{2}-P_{3}-P_{4} \] (Formula I)

or
wherein P1-P2-P3- and P1'-P2'-P3'- correspond to peptide replacements, i.e., peptidomimetics, of the N-terminal Ala-Val-Pro- tripeptide of mature Smac and P4 and P4' correspond to amino acid replacements of the fourth N-terminal amino acid, Phe, Tyr, Ile, or Val, and L is a linking group or bond covalently linking [P1-P2-P3-P4] to [P1'-P2'-P3'-P4'].

For example, without limitation, a Smac mimetic may reside in the following genus of compounds of Formula I or of Formula II:

P1 and P1' are NHR¹-CHR²-C(0)-;
P2 and P2' are -NH-CHR³-C(0)-;
P3 and P3' are pyrrolidine, pyrrolidine fused to a cycloalkyl, or pyrrolidine fused to a heterocycloalkyl having a -N- heteroatom, optionally substituted in each case, and wherein the pyrrolidine of P3/P3' may be bound to P2/P2' by an amide bond;
P4 and P4' are -M-Qₚ-R⁷.

The variable substituents can be, for example:

R¹: -H or -CH₃;
R²: C1-6 alkyl, C1-6 alkoxy, optionally substituted, e.g., -CH₃, -CH₂CH₃ or -CH₂OH;
R³: C1-6 alkyl, C1-6 alkoxy, C3-C7 cycloalkyl or heterocycloalkyl, or C6-C8 aryl or heteroaryl, optionally substituted in each case;
M: a covalent bond, C1-6 alkylene, substituted C1-C6 alkylene such as but not limited to -C(O)-, or C3-C7 cycloalkyl or heterocycloalkyl, optionally substituted in each case;
Q: a covalent bond, C1-6 alkylene, substituted C1-C6 alkylene, -0-, -NR₈-, or C3-C7 cycloalkyl or heterocycloalkyl, optionally substituted in each case;
P: 0 or 1;
R⁷: cycloalkyl, heterocycloalkyl, cycloalkylaryl, alkylaryl, alkylheteroaryl, aryl or heteroaryl, optionally substituted in each case;
R⁸: -H or C₁-₆ alkyl.
In compounds of Formula II, L is a linking group or bond covalently linking [P₁-P₂-P₃-P₄] to [P₁'-P₂'-P₃'-P₄].

"Alkyl" (monovalent) and "alkylene" (divalent) when alone or as part of another term (e.g., alkoxy) mean branched or unbranched, saturated aliphatic hydrocarbon group, having up to 12 carbon atoms unless otherwise specified. Examples of particular alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 2,2-dimethylbutyl, n-heptyl, 3-heptyl, 2-methylhexyl, and the like. The term, "lower," when used to modify alkyl, alkenyl, etc., means 1 to 4 carbon atoms, branched or linear so that, e.g., the terms "lower alkyl", "C₁-C₄ alkyl" and "alkyl of 1 to 4 carbon atoms" are synonymous and used interchangeably to mean methyl, ethyl, 1-propyl, isopropyl, 1-butyl, sec-butyl or t-butyl. Examples of alkylene groups include, but are not limited to, methylene, ethylene, n-propylene, n-butylene and 2-methylbutylene.

The term substituted alkyl refers to alkyl moieties having substituents replacing one or more hydrogens on one or more (often no more than four) carbon atoms of the hydrocarbon backbone. Such substituents are independently selected from the group consisting of: a halogen (e.g., i. Br, Cl, or F, particularly fluoro(F)), hydroxy, amino, cyano, mercapto, alkoxy (such as a C₁-C₆ alkoxy, or a lower (C₁-C₄) alkoxy, e.g., methoxy or ethoxy to yield an alkoxymethyl), aryloxy (such as phenoxy to yield an aryloxyalkyl), nitro, oxo (e.g., to form a carbonyl), carboxyl (which is actually the combination of an oxo and hydroxy substituent on a single carbon atom), carbamoyl (an aminocarbonyl such as NR₂C(0)-, which is the substitution of an oxo and an amino on a single carbon atom), cycloalkyl (e.g., a cycloalkylalkyl), aryl (resulting for example in aralkyls such as benzyl or phenylethyl), heterocyclylalkyl (e.g., heterocycloalkylalkyl), heteroaryl (e.g., heteroarylalkyl), alkylsulfonyl (including lower alkylsulfonyl such as methylsulfonyl), arylsulfonyl (such as phenylsulfonyl), and -OCF₃ (which is a halogen substituted alkoxy).
The invention further contemplates that several of these alkyl substituents, including specifically alkoxy, cycloalkyl, aryl, heterocyclyalkyl and heteroaryl, are optionally further substituted as defined in connection with each of their respective definitions provided below. In addition, certain alkyl substituent moieties result from a combination of such substitutions on a single carbon atom. For example, an ester moiety, e.g., an alkoxy carbonyl such as methoxycarbonyl, or tert-butoxycarbonyl (Boc) results from such substitution. In particular, methoxycarbonyl and Boc are substituted alkyls that result from the substitution on a methyl group (-CH₃) of both an oxo (=0) and an unsubstituted alkoxy, e.g., a methoxy (CH₃-O) or a tert-butoxy ((CH₃)₃C-0-), respectively replacing the three hydrogens.

Similarly, an amide moiety, e.g., an alkylaminocarbonyl, such as dimethylyaminocarbonyl or methylaminocarbonyl, is a substituted alkyl that results from the substitution on a methyl group (-CH₃) of both an oxo (=0) and a mono-unsubstitutedalkylamino or, diunsubstitutedalkylamino, e.g., dimethylamino (-N-(CH₃)₂), or methylamino (-NH-(CH₃)) replacing the three hydrogens (similarly an arylaminocarbonyl such as diphenylaminocarbonyl is a substituted alkyl that results from the substitution on a methyl group (-CH₃) of both an oxo (=0) and a mono-unsubstitutedaryl(phenyl)amino).

Exemplary substituted alkyl groups further include cyanomethyl, nitromethyl, hydroxyalkyls such as hydroxymethyl, trityloxy methyl, propionyloxymethyl, aminoalkyls such as aminomethyl, carboxylalkyls such as carboxymethyl, carboxyethyl, carboxypropyl, 2,3-dichloropentyl, 3-hydroxy-5-carboxyhexyl, acetyl (e.g., an alkanoyl, where in the case of acetyl the two hydrogen atoms on the -CH₂ portion of an ethyl group are replaced by an oxo (=0)), 2-aminopropyl, pentachlorobutyl, trifluoromethyl, methoxyethyl, 3-hydroxypentyl, 4-chlorobutyl, 1,2-dimethyl-propyl, pentafluoroethyl, alkyloxycarbonylmethyl,
allyloxycarbonylaminomethyl, carbamoyloxy methyl, methoxymethyl, ethoxymethyl, t-butoxymethyl, acetoxymethyl, chloromethyl, bromomethyl, iodomethyl, trifluoromethyl, 6-hydroxyhexyl, 2,4-dichloro (n-butyl), 2-amino (isopropyl), cycloalkylcarbonyl (e.g., cyclopropylcarbonyl) and 2-carbamoyloxyethyl. Particular substituted alkyls are substituted methyl groups.

Examples of substituted methyl group include groups such as hydroxymethyl, protected hydroxymethyl (e.g., tetrahydropyranyl-oxymethyl), acetoxymethyl, carbamoyloxymethyl, trifluoromethyl, chloromethyl, carboxymethyl, carboxyl (where the three hydrogen atoms on the methyl are replaced, two of the hydrogens are replaced by an oxo (=0) and the other hydrogen is replaced by a hydroxy (-OH)), tert-butoxycarbonyl (where the three hydrogen atoms on the methyl are replaced, two of the hydrogens are replaced by an oxo (=0) and the other hydrogen is replaced by a tert-butoxy (-0-C(CH\(_3\))\(_3\)), bromomethyl and iodomethyl.

When the specification and especially the claims refer to a particular substituent for an alkyl, that substituent can potentially occupy one or more of the substitutable positions on the alkyl. For example, reciting that an alkyl has a fluoro substituent, would embrace mono-, di-, and possibly a higher degree of substitution on the alkyl moiety.

The term substituted alkylene refers to alkylene moieties having substituents replacing one or more hydrogens on one or more (often no more than four) carbon atoms of the hydrocarbon backbone where the alkylene is similarly substituted with groups as set forth above for alkyl.

Alkoxy is -O-alkyl. A substituted alkoxy is -O-substituted alkyl, where the alkoxy is similarly substituted with groups as set forth above for alkyl. One substituted alkoxy is acetoxyl where two of the hydrogens in ethoxy (e.g., -0-CH\(_2\)-CH\(_3\)) are replaced by an oxo, (=0) to yield -0-C(0)-CH\(_2\)-CH\(_3\); another is an aralkoxy where one
of the hydrogens in the alkoxy is replaced by an aryl, such as benzyloxy, and another is a carbamate where two of the hydrogens on methoxy (e.g., -O-CH₃) are replaced by oxo (=O) and the other hydrogen is replaced by an amino (e.g., -NH₂, -NHR or -NRR) to yield, for example, -0-C(0)-NH₂. A lower alkoxy is -0-lower alkyl.

"Alkenyl" (monovalent) and "alkenylene" (divalent) when alone or as part of another term mean an unsaturated hydrocarbon group containing at least one carbon-carbon double bond, typically 1 or 2 carbon-carbon double bonds, which may be linear or branched and which have at least 2 and up to 12 carbon atoms unless otherwise specified. Representative alkenyl groups include, by way of example, vinyl, allyl, isopropenyl, but-2-enyl, n-pent-2-enyl, and n-hex-2-enyl.

The terms substituted alkenyl and substituted alkenylene refer to alkenyl and alkenylene moieties having substituents replacing one or more hydrogens on one or more (often no more than four) carbon atoms of the hydrocarbon backbone. Such substituents are independently selected from the group consisting of: halo (e.g., I, Br, Cl, F), hydroxy, amino, cyano, alkoxy (such as C₁₋₆ alkoxy), aryloxy (such as phenoxy), nitro, mercapto, carboxyl, oxo, carbamoyl, cycloalkyl, aryl, heterocyclyl, heteroaryl, alkylsulfonyl, arylsulfonyl and -OCF₃.

"Alkynyl" means a monovalent unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, typically 1 carbon-carbon triple bond, which may be linear or branched and which have at least 2 and up to 12 carbon atoms unless otherwise specified. Representative alkynyl groups include, by way of example, ethynyl, propargyl, and but-2-ynyl.

"Cycloalkyl" when alone or as part of another term means a saturated or partially unsaturated cyclic aliphatic hydrocarbon group (carbocycle group), having 3 to 8 carbon atoms unless otherwise specified, such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, and further includes polycyclic, including fused cycloalkyls such as 1,2,3,4-tetrahydonaphthalenyls (1,2,3,4-tetrahydonaphthalen-1-yl, and 1,2,3,4-tetrahydonaphthalen-2-yl), indanyls (inden-1-yl, and inden-2-yl), isoindenylns (isoinden-1-yl, isoinden-2-yl, and isoinden-3-yl) and indenyls (inden-
1-yl, inden-2-yl and inden-3-yl). A lower cycloalkyl has from 3 to 6 carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The term substituted cycloalkyl refers to cycloalkyl moieties having substituents replacing one or more hydrogens on one or more (often no more than four) carbon atoms of the hydrocarbon backbone. Such substituents are independently selected from the group consisting of: halo (e.g., i. Br, Cl, F), hydroxy, amino, cyano, alkoxy (such as C₆H₅ alkoxy), substituted alkoxy, aryloxy (such as phenoxy), nitro, mercapto, carboxyl, oxo, carbamoyl, alkyl, substituted alkyls such as trifluoromethyl, aryl, substituted aryls, heterocyclyl, heteroaryl, alkylsulfonyl, arylsulfonyl and -OCF₃. When the specification and especially the claims refer to a particular substituent for a cycloalkyl, that substituent can potentially occupy one or more of the substitutable positions on the cycloalkyl. For example, reciting that a cycloalkyl has a fluoro substituent, would embrace mono-, di-, and a higher degree of substitution on the cycloalkyl moiety. Examples of cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, tetrahydronaphthyl and indanyl.

"Aryl" when used alone or as part of another term means an aromatic carbocyclic group whether or not fused having the number of carbon atoms designated, or if no number is designated, from 6 up to 14 carbon atoms. Particular aryl groups include phenyl, naphthyl, biphenyl, phenanthrenyl, naphthacenyl, indolyl, and the like (see e.g. Lang's Handbook of Chemistry (Dean, J. A., ed) 13th ed. Table 7-2 [1985]).

The term substituted aryl refers to aryl moieties having substituents replacing one or more hydrogens on one or more (usually no more than six) carbon atoms of the aromatic hydrocarbon core. Such substituents are independently selected from the group consisting of: halo (e.g., i. Br, Cl, F), hydroxy, amino, cyano, alkoxy (such as C₆H₅ alkoxy and particularly lower alkoxy), substituted alkoxy, aryloxy (such as phenoxy), nitro, mercapto, carboxyl, carbamoyl, alkyl, substituted alkyl (such as trifluoromethyl), aryl, -OCF₃, alkylsulfonyl (including lower alkylsulfonyl), arylsulfonyl, heterocyclyl and heteroaryl. Examples of such
substituted phenyls include but are not limited to a mono-or di (halo) phenyl group such as 2-chlorophenyl, 2- bromophenyl, 4-chlorophenyl, 2,6-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 3-chlorophenyl, 3- bromophenyl, 4-bromophenyl, 3,4-dibromophenyl, 3-chloro-4-fluorophenyl, 2-fluorophenyl; 3-fluorophenyl, 4-fluorophenyl, a mono-or di (hydroxy) phenyl group such as 4-hydroxyphenyl, 3- hydroxyphenyl, 2,4-dihydroxyphenyl, the protected-hydroxy derivatives thereof; a nitrophenyl group such as 3- or 4-nitrophenyl; a cyanophenyl group, for example, 4-cyanophenyl; a mono-or di (lower alkyl) phenyl group such as 4-methylphenyl, 2,4-dimethylphenyl, 2- methylphenyl, 4- (iso-propyl) phenyl, 4-ethylphenyl, 3- (n-propyl) phenyl; a mono or di (alkoxy) phenyl group, for example, 3,4-dimethoxyphenyl, 3-methoxy-4-benzyloxy phenyl, 3- methoxy-4- (1-chloromethyl) benzyloxy-phenyl, 3-ethoxyphenyl, 4- (isopropoxy) phenyl, 4- (t-butoxy) phenyl, 3-ethoxy-4-methoxy phenyl; 3-or 4-trifluoromethylphenyl; a mono- or dicarboxyphenyl or (protected carboxy) phenyl group such 4-carboxyphenyl; a mono-or di (hydroxymethyl) phenyl or (protected hydroxymethyl) phenyl such as 3- (protected hydroxymethyl) phenyl or 3,4-di (hydroxymethyl) phenyl; a mono-or di (aminomethyl) phenyl or (protected aminomethyl) phenyl such as 2- (aminomethyl) phenyl or 2, 4- (protected aminomethyl) phenyl; or a mono-or di (N- (methylsulfonylamino)) phenyl such as 3- (N- methylsulfonylamino) phenyl.

Also, the substituents, such as in a disubstituted phenyl group, can be the same or different, for example, 3-methyl-4-hydroxyphenyl, 3- chloro-4-hydroxyphenyl, 2-methoxy-4-bromophenyl, 4-ethyl-2-hydroxyphenyl, 3-hydroxy-4- nitrophenyl, 2- hydroxy-4-chlorophenyl, as well as for trisubstituted phenyl groups where the substituents are different, as for example 3-methoxy-4-benzyloxy-6-methyl sulfonylamino, 3- methoxy-4-benzyloxy-6-phenyl sulfonylamino, and tetrasubstituted phenyl groups where the substituents are different such as 3-methoxy-4-benzyloxy-5-methyl-6-phenyl sulfonylamino. Particular substituted phenyl groups are 2-chlorophenyl, 2-aminophenyl, 2-bromophenyl, 3- methoxyphenyl, 3-ethoxy-phenyl, 4-benzyloxyphenyl, 4-methoxyphenyl, 3-
ethoxy-4- benzyloxyphenyl, 3,4-diethoxyphenyl, 3-methoxy-4-benzyloxyphenyl, 3-methoxy-4- (1-chloromethyl) benzyloxy-phenyl, 3-methoxy-4- (1-chloromethyl) benzyloxy-6-methyl sulfonyl aminophenyl groups.

When the specification and especially the claims refer to a particular substituent for an aryl, that substituent can potentially occupy one or more of the substitutable positions on the aryl. For example, reciting that an aryl has a fluoro substituent, would embrace mono-, di-, tri, tetra and a higher degree of substitution on the aryl moiety. Fused aryl rings may also be substituted with the substituents specified herein, for example with 1, 2 or 3 substituents, in the same manner as substituted alkyl groups. The terms aryl and substituted aryl do not include moieties in which an aromatic ring is fused to a saturated or partially unsaturated aliphatic ring.

"Heterocyclic group", "heterocyclic", "heterocycle", "heterocyclyl", "heterocycloalkyl" or "heterocyclo" alone and when used as a moiety in a complex group, are used interchangeably and refer to any mono-, bi-, or tricyclic, saturated or unsaturated, non-aromatic hetero-atom-containing ring system having the number of atoms designated, or if no number is specifically designated then from 5 to about 14 atoms, where the ring atoms are carbon and at least one heteroatom and usually not more than four heteroatoms (i.e., nitrogen, sulfur or oxygen). Included in the definition are any bicyclic groups where any of the above heterocyclic rings are fused to an aromatic ring (i.e., an aryl (e.g., benzene) or a heteroaryl ring). In a particular embodiment the group incorporates 1 to 4 heteroatoms.

Typically, a 5- membered ring has 0 to 1 double bonds and a 6-or 7-membered ring has 0 to 2 double bonds and the nitrogen or sulfur heteroatoms may optionally be oxidized (e.g. SO, S0₂), and any nitrogen heteroatom may optionally be quaternized. Particular unsubstituted non-aromatic heterocycles include morpholinyl (morpholino), pyrrolidinyls, oxiranyl, indolinyls, 2,3-
dihydoindolyl, isoindolyls, 2,3-dihydroisoindolyl, tetrahydroquinolinyls, tetrahydroisoquinolinyls, oxetanyl, tetrahydrofuranyls, 2,3-dihydrofuranyl, 2H-pyranly, tetrahydropyranly, aziridinyls, azetidinyls, l-methyl-2-pyrrollyl, piperazinyls and piperidinyls.

The term substituted heterocyclo refers to heterocyclo moieties having substituents replacing one or more hydrogens on one or more (usually no more than six) atoms of the heterocyclo backbone. Such substituents are independently selected from the group consisting of: halo (e.g., I, Br, Cl, F), hydroxy, amino, cyano, alkoxy (such as C_{6} alkoxy), substituted alkoxy, aryloxy (such as phenoxy), nitro, carboxyl, oxo, carbamoyl, alkyl, substituted alkyl (such as trifluoromethyl), -OCF_{3}, aryl, substituted aryl, alkylsulfonyl (including lower alkylsulfonyl), and arylsulfonyl.

When the specification and especially the claims refer to a particular substituent for a heterocycloalkyl, that substituent can potentially occupy one or more of the substitutable positions on the heterocycloalkyl. For example, reciting that a heterocycloalkyl has a fluoro substituent, would embrace mono-, di-, tri, tetra and a higher degree of substitution on the heterocycloalkyl moiety.

"Heteroaryl" alone and when used as a moiety in a complex group refers to any mono-, bi-, or tricyclic aromatic ring system having the number of atoms designated, or if no number is specifically designated then at least one ring is a 5-, 6- or 7-membered ring and the total number of atoms is from 5 to about 14 and containing from one to four heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur (Lang's Handbook of Chemistry, supra).

Included in the definition are any bicyclic groups where any of the above heteroaryl rings are fused to a benzene ring. The following ring systems are examples of the heteroaryl groups denoted by the term "heteroaryl": thienyls (alternatively called thiophenyl), furyls, imidazolyls, pyrazolyls, thiazolyls,
isothiazolyls, oxazolyls, isoxazolyls, triazolyls, thiazolyls, oxadiazolyls, tetrazolyls, thiatriazolyls, oxatriazolyls, pyridyls, pyrimidinyls (e.g., pyrimidin-2-yl), pyrazinyls, pyridazinyls, thiazinyls, oxazinyls, triazinyls, thiatriazinyls, oxatriazinyls, dithiazinyls, oxadiazinyls, dithiadiazinyls, dioxazinyls, oxathiazinyls, tetrazinyls, thiatriazinyls, oxatriazinyls, dithiadiazinyls, imidazolinyls, dihydropyrimidyls, tetrahydropyrimidyls, tetrazolo [1, 5-b] pyridazinyl and purinyls, as well as benzo-fused derivatives, for example benzoazolyls, benzofuryls, benzothienyls, benzothiazolyls, benzothiadiazolyl, benzotriazolyls, benzimidazolyls, isoindolyls, indazolyls, indolizinyls, indolyls, naphthyridines, pyridopyrimidines, phthalazinyls, quinolyls, isoquinolyls and quinazolinyls.

The term substituted heteroaryl refers to heteroaryl moieties (such as those identified above) having substituents replacing one or more hydrogens on one or more (usually no more than six) atoms of the heteroaryl backbone. Such substituents are independently selected from the group consisting of: halo (e.g., I, Br, Cl, F), hydroxy, amino, cyano, alkoxy (such as C\textsubscript{r}C\textsubscript{6} alkoxy), aryloxy (such as phenoxy), nitro, mercapto, carboxyl, carbamoyl, alky, substituted alkyl (such as trifluoromethyl), \text{OCF}_3, aryl, substituted aryl, alkylsulfonyl (including lower alkylsulfonyl), and arylsulfonyl. When the specification and especially the claims refer to a particular substituent for a heteroaryl, that substituent can potentially occupy one or more of the substitutable positions on the heteroaryl. For example, reciting that a heteroaryl has a fluoro substituent, would embrace mono-, di-, tri, tetra and a higher degree of substitution on the heteroaryl moiety.

Particular "heteroaryls" (including "substituted heteroaryls") include; 1\text{H}-pyrrolo[2,3-\text{£}]pyridine, 1, 3-thiazol-2-yl, 4- (carboxymethyl)-5-methyl-1 , 3- thiazol-2-yl, 1,2,4-thiadiazol-5-yl, 3- methyl-1 , 2,4-thiadiazol-5-yl, 1,3,4-triazol-5-yl, 2-methyl-1,3,4-triazol-5-yl, 2-hydroxy-1 , 3,4- triazol-5-yl, 2-carboxy-4-methyl-1 ,3,4- triazol-5-yl, 1, 3-oxazol-2-yl, 1,3,4-oxadiazol-5-yl, 2-methyl-1 , 3,4-oxadiazol-5-yl, 2- (hydroxymethyl)- 1, 3,4-oxadiazol-5-yl, 1, 2,4-oxadiazol-5-yl, 1, 3,4-oxadiazol-5-yl, 2-thiol-1 , 3,4-thiadiazol-5-yl, 2- (methylthio)-l , 3,4-thiadiazol-5-yl, 2-amino-1 , 3,4-thiadiazol-5-yl, 1H-tetrazol-5-yl, 1-methyl-1 H- tetrazol-5-yl, 1-(1 -
(dimethylamino) eth-2-yl)-1 H-tetrazol-5-yl, 1-(carboxymethyl)-1 H-tetrazol-5-yl, l-
(methylsulfonic acid)-IH-tetrazol-5-yl, 2-methyl-IH-tetrazol-5-yl, 1, 2,3-triazol-5-yl, 1
methyl-I , 2,3-triazol-5-yl, 2-methyl-1 , 2,3-triazol-5-yl, 4-methyl-1 , 2,3-triazol-5-
yl, pyrid-2-yl N- oxide, 6-methoxy-2- (n-oxide)-pyridaz-3-yl, 6-hydroxypyridaz-3-yl, 1-
methylpyrid-2-yl, 1- methylpyrid-4-yl, 2-hydroxypyrimid-4-yl, 1.4, 5,6-tetrahydro-
5, 6-dioxo-4-methyl-as-triazin-3-yl, 1, 4, 5, 6-tetrahydro- (formylmethyl)-5, 6-
dioxo-as-triazin-3-yl, 2,5-dihydro-5-oxo-6-hydroxy- astriazin-3-yl, 2,5-dihydro-5-
oxo-6-hydroxy-as-triazin-3-yl , 2,5-dihydro-5-oxo-6- hydroxy-2-methyl-astriazin-3-
yl , 2,5-dihydro-5-oxo-6-methoxy-2-methyl-as-triazin-3-yl, 2,5-dihydro-5-oxo-as-triazin-3-
yl , 2,5-dihydro-5-oxo-2-methyl-as-triazin-3-yl, 2,5-dihydro-5-oxo-2, 6-dimethyl-as-
triazin-3-yl, tetrazolo [1, 5-b] pyrazidin-6-yl, 8-aminotetrazolo [1, 5-b] -pyrazidin-6-yl, quinol-2-
yl, quinol-3-yl, quinol-4-yl, quinol-5-yl, quinol-6-yl, quinol-8-yl, 2-methyl-quinol-4-
yl, 6-fluoro-quinol-4-yl, 2-methyl,8-fluoro-quinol-4-yl, isoquinol-5-yl, isoquinol-8-yl, iso-
quinol-1 -yl, and quinazolin-4-yl. An alternative group of "heteroaryl" includes: 5-methyl-
2-phenyl-2H-pyrazol-3-yl, 4- (carboxymethyl)-5-methyl-1 , 3-thiazol-2-yl, 1, 3,4-
tiazol-5-yl, 2-methyl-I , 3,4-tiazol-5-yl, 1H-tetrazol-5-yl, 1-methyl-1 H-
tetrazol-5-yl, 1-(1 -(dimethylamino) eth-2-yl)-1H-tetrazol-5-yl, l-(carboxymethyl)-
1H-tetrazol-5-yl, 1- (methylsulfonic acid)-IH- tetrazol-5-yl, 1, 2,3-triazol-5-yl, 1,4, 5,6-
tetrahydro-5,6-dioxo-4-methyl-as-triazin-3-yl, 1, 4, 5, 6-tetrahydro- (2-
formylmethyl)-5, 6-dioxo- as-triazin-3-yl, 2, 5-dihydro-5-oxo-6-hydroxy-2-methyl-
as-triazin-3-yl , 2,5-dihydro-5- oxo-6-hydroxy-2-methyl-as-triazin-3-yl, tetrazolo [1,
5-b] pyrazidin-6-yl, and 8-aminotetrazolo [1, 5- b] pyrazidin-6-yl.

L is a linking group or a bond covalently linking one monomer, [P1-P2-P3-P4] to the
other monomer, [P1'P2'-P3'-P4']. Commonly, -L- links P2 to P2' position
such as at R3 or P4 to P4' such as at M, G, Q, or R7, or both P2 to P2' and P4 to
P4'. L, therefore, can be a single or double covalent bond or a contiguous chain,
branched or unbranched, substituted or unsubstituted, of 1 to about 100 atoms,
typically 1 to about 30 atoms, e.g., an optionally substituted alkylene, alkenylene,
alkylyne, cycloalkyl, alkycycloalkyl, alkylarylalkyl chain of 2 to 20 atoms
optionally with 1-4 heteroatoms selected from -O-, -NH-, and -S-. Illustrative
examples of L are a single or double covalent bond, C1-12 alkylene, substituted C1-12 alkylene, C1-12 alkenylene, substituted C1-12 alkenylene, C1-12 alkynylene, substituted C1-12 alkynylene, \( X_n \)-phenyl-Y \( n \), or \( X_n \)-(phenyl) \( 2 \)-Y \( n \), wherein X and Y are independently C1-6 alkylene, substituted C1-6 alkylene, C1-6 alkenylene, substituted C1-6 alkenylene, C1-6 alkynylene, substituted C1-6 alkynylene, or S(0) \( 2 \).

Illustrative P3/P3' groups include, without limitation:

\[
\begin{align*}
R^6 & \text{ is } -\text{H, C1-6 alkyl, substituted C1-6 alkyl, C1-6 alkoxy, substituted C1-6 alkoxy, C1-6 alkylsulfonyl, arylsulfonyl, cycloalkyi, substituted cycloalkyi, heterocycloalkyi, substituted heterocycloalkyi, aryl, substituted aryl, heteroaryl, or substituted heteroaryl;} \\
R^4, R^5, \text{ and } R^{12} & \text{ are, independently, } -\text{H, -OH, C1-6 alkyl, 0 1-6 heteroalkyl, 0 1-6 alkoxy, aryloxy, cycloalkyi, heterocycloalkyi, aryl, 0 1-6 alkyl aryl, or heteroaryl, or 0 1-6 alkyl heteroaryl, optionally substituted in each case except when } R^4 \text{ is } -\text{H or -OH.}
\end{align*}
\]

As mentioned, in certain illustrative embodiments, the Smac mimetic used in the practice of the invention is bivalent.

Compound 15, i.e., birinapant, is an example of a specific Smac mimetic. Other illustrative examples are:
In certain illustrative embodiments, a selected Smac mimetic is a small molecule that binds to a BIR domain of at least one IAP, e.g., XIAP, clAP-1, and clAP-2, leading to ubiquitination (also, referred to as ubiquitylation) and degradation of the clAPs, wherein said Smac mimetic does not inhibit NOD signaling or, if it does, then it does so only poorly. Since NOD signaling is dependent upon the E3 ubiquitin ligase activity of XIAP and mediates NF-kB activation, such Smac mimetic can be characterized as (i) not inhibiting XIAP E3 ubiquitin ligase activity or as only poorly inhibiting XIAP E3 ubiquitin ligase activity, (ii) not inhibiting or poorly inhibiting NOD (i.e., NOD1/2) signaling, and/or (iii) not inhibiting or poorly inhibiting NOD-mediated NF-kB activation. In such embodiments, the selected Smac mimetic does not inhibit XIAP E3 ligase activity by more than 50%, or even 35%, relative to untreated cells when applied in the same concentrations at which they inhibit clAP1 activity, e.g., up to 10 uM. In certain such embodiments, the Smac mimetic is selected and administered to a patient if it does not inhibit either or both of (i) XIAP-dependent NOD1/2 signaling or (ii) XIAP-dependent inhibition of pro-IL-1-beta processing by more than 50% at concentrations up to 10 uM, or even 100 nM, in the case of XIAP-NOD1/2 signaling or 1 uM, or even 500 nM, in the case of pro-IL-1-beta processing. See, e.g., US20140303090.

In certain illustrative embodiments, a selected Smac mimetic derepresses XIAP-mediated caspase-3 repression and/or degrades clAP-1 not bound to TRAF2 (non TRAF2-bound, e.g., "cytoplasmic" clAP-1 or "free" clAP-1) as well as clAP1 bound to TRAF2 and/or degrades clAP-2 bound to TRAF2 but does not degrade
clAP-2 not bound to TRAF2 or weakly degrades clAP-2 not bound to TRAF2 relative to degradation of clAP-2 bound to TRAF2. See, e.g., US20140303090.


IAP antagonists also include molecules that reduce the expression of an IAP gene, such as clAP1 or clAP2. Suitable antagonists that are capable of reducing the expression of an IAP gene would be known to persons skilled in the art. Examples include nucleic acid molecules, such as RNA or DNA molecules (including double-stranded or single-stranded), and peptides, such as antisense peptide nucleic acids, that interfere with the expression of the target gene.

Useful DNA molecules include antisense, as well as sense (e.g. coding and/or regulatory) DNA molecules. Antisense DNA molecules include short oligonucleotides. Persons skilled in the art would be able to design suitable short oligonucleotides for use in accordance with the present invention. An example is the XIAP antisense oligonucleotide, AEG35156, as described by Carter et al. [Apoptosis, 2011 Vol. 16(1):67-74]. Other examples of useful DNA molecules include those encoding interfering RNAs, such as shRNA and siRNA. Yet another example are catalytic DNA molecules known as DNAzymes.

Useful RNA molecules capable of reducing the expression of an IAP gene, also referred to herein as RNA interference molecules, include siRNA, dsRNA, stRNA, shRNA, and miRNA (e.g., short temporal RNAs and small modulatory RNAs) and ribozymes.

RNA interference (RNAi) is particularly useful for specifically inhibiting the production of a particular protein. Although not wishing to be limited by theory, Waterhouse et al. (1998) have provided a model for the mechanism by which dsRNA can be used to reduce protein production. This technology relies on the
presence of dsRNA molecules that contain a sequence that is essentially identical to the mRNA of the gene of interest or part thereof, in this case an mRNA encoding a polypeptide according to the invention. Conveniently, the dsRNA can be produced from a single promoter in a recombinant vector or host cell, where the sense and anti-sense sequences are flanked by an unrelated sequence which enables the sense and anti-sense sequences to hybridize to form the dsRNA molecule with the unrelated sequence forming a loop structure. The design and production of suitable dsRNA molecules for the present invention is well within the capacity of a person skilled in the art, particularly considering Waterhouse et al. (1998), Smith et al. (2000), WO 99/32619, WO 99/53050, WO 99/49029, and WO 01/34815.

In one example, a DNA is introduced that directs the synthesis of an at least partly double stranded RNA product(s) with homology to the target gene to be inactivated. The DNA therefore comprises both sense and antisense sequences that, when transcribed into RNA, can hybridize to form the double-stranded RNA region. In a preferred embodiment, the sense and antisense sequences are separated by a spacer region that comprises an intron which, when transcribed into RNA, is spliced out. This arrangement has been shown to result in a higher efficiency of gene silencing. The double-stranded region may comprise one or two RNA molecules, transcribed from either one DNA region or two. The presence of the double stranded molecule is thought to trigger a response from the cell that destroys both the double stranded RNA and also the homologous RNA transcript from the target gene, efficiently reducing or eliminating the activity of the target gene.

The length of the sense and antisense sequences that hybridize should each be at least 19 contiguous nucleotides, preferably at least 30 or 50 nucleotides, and more preferably at least 100, 200, 500 or 1000 nucleotides. The full-length sequence corresponding to the entire gene transcript may be used. The lengths are most preferably 100-2000 nucleotides. The degree of identity of the sense and antisense sequences to the targeted transcript should be at least 85%, preferably at least 90% and more preferably 95-100%. The RNA molecule may
of course comprise unrelated sequences which may function to stabilize the molecule. The RNA molecule may be expressed under the control of a RNA polymerase II or RNA polymerase III promoter. Examples of the latter include tRNA or snRNA promoters.

Preferred small interfering RNA (‘siRNA”) molecules comprise a nucleotide sequence that is identical to about 19-21 contiguous nucleotides of the target mRNA. Preferably, the target mRNA sequence commences with the dinucleotide AA, comprises a GC-content of about 30-70% (preferably, 30-60%, more preferably 40-60% and more preferably about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target in the genome of the cell in which it is to be introduced, e.g., as determined by standard BLAST search.

Synthesis of RNAi molecules suitable for use with present invention can be effected by first scanning the mRNA sequence of the target downstream of the AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3’ adjacent 19 nucleotides is recorded as potential siRNA target sites.

Preferably, siRNA target sites are selected from the open reading frame. Second, potential target sites are compared to an appropriate genomic database using any sequence alignment software, such as BLAST. Putative target sites which exhibit significant homology to other coding sequences are filtered out. Qualifying target sequences are selected as template for siRNA synthesis.

Preferred sequences are those including low G/C content as these have proven to be more effective in mediating gene silencing as compared to those with G/C content higher than 55%. Several target sites are preferably selected along the length of the target gene for evaluation.

MicroRNA regulation is a clearly specialized branch of the RNA silencing pathway that evolved towards gene regulation, diverging from conventional RNAi/PTGS. MicroRNAs are a specific class of small RNAs that are encoded in gene-like elements organized in a characteristic inverted repeat. When transcribed, microRNA genes give rise to stem-looped precursor RNAs from...
which the microRNAs are subsequently processed. MicroRNAs are typically about 21 nucleotides in length. The released miRNAs are incorporated into RISC-like complexes containing a particular subset of Argonaute proteins that exert sequence-specific gene repression (see, for example, Millar and Waterhouse, 2005; Pasquinelli et al., 2005; Almeida and Allshire, 2005).

DNAzymes are single-stranded polynucleotides which are capable of cleaving single and double stranded polynucleotides which are capable of cleaving both single and double stranded target sequences (Breaker, R. R. and Joyce, G. Chemistry and Biology 1995; 2:655; Santoro, S. W. & Joyce, G. F. Proc. Natl. Acad. Sci. USA 1997; 943:4262) A general model (the "10-23" model) for the DNAzyme has been proposed. "10-23" DNAzymes have a catalytic domain of 15 deoxynbonucleotides, flanked by two substrate-recognition domains of seven to nine deoxynbonucleotides each. This type of DNAzyme can effectively cleave its substrate RNA at purine:pyrimidine junctions (Santoro, S. W. & Joyce, G. F. supra; for rev of DNAzymes see Khachigian, L M. Curr Opin Mol Ther 4:119-21 (2002).

Examples of construction and amplification of synthetic, engineered DNAzymes recognizing single and double-stranded target cleavage sites have been disclosed in U.S. Pat. No. 6,326,174 to Joyce et al.

The terms "double stranded RNA" or "dsRNA" refer to RNA molecules that are comprised of two strands. Double-stranded molecules include those comprised of a single RNA molecule that doubles back on itself to form a two-stranded structure. For example, the stem loop structure of the progenitor molecules from which the single-stranded miRNA is derived, called the pre-miRNA, comprises a dsRNA molecule.

Other suitable RNA interference molecules include unmodified and modified double stranded (ds) RNA molecules including, short-temporal RNA (stRNA), small interfering RNA (siRNA), short-hairpin RNA (shRNA), microRNA (miRNA) and double-stranded RNA (dsRNA). The dsRNA molecules (e.g. siRNA) also may contain 3’ overhangs, such as 3’UU or 3TT overhangs.
In an embodiment, the siRNA molecules of the present invention have a double stranded structure. In an embodiment, the siRNA molecules of the present invention are double stranded for more than about 25%, more than about 50%, more than about 60%, more than about 70%, more than about 80%, more than about 90% of their length.

As used herein, "gene silencing" induced by RNA interference refers to a decrease in the mRNA level in a cell for a target gene (e.g., clAP1 gene and/or clAP2 gene) by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, about 100% of the mRNA level found in the cell in the absence of RNA interference.

The RNA interference molecules also include modified RNA molecules having one or more non-natural nucleotides; that is, nucleotides other than adenine "A", guanine "G", uracil "U", or cytosine "C". A modified nucleotide residue or a derivative or analog of a natural nucleotide may also be used. Any modified residue, derivative or analog may be used to the extent that it does not eliminate or substantially reduce (by at least 50%) RNAi activity of the molecule. Examples of suitable modified residues include aminoallyl UTP, pseudo-UTP, 5-l-UTP, 5-l-CTP, 5-Br-UTP, alpha-S ATP, alpha-S CTP, alpha-S GTP, alpha-S UTP, 4-thio UTP, 2-thio-CTP, 2'NH₂ UTP, 2'NH₂ CTP, and 2'F UTP. Suitable modified nucleotides also include aminoallyl uridine, pseudo-uridine, 5-l-uridine, 5-l-cytidine, 5-Br-uridine, alpha-S adenosine, alpha-S cytidine, alpha-S guanosine, alpha-S uridine, 4-thio uridine, 2-thio-cytidine, 2'NH₂ uridine, 2'NH₂ cytidine, and 2'F uridine, including the free pho (NTP) RNA molecules, as well as all other useful forms of the nucleotides.

RNA interference molecules may also contain modifications in the ribose sugars, as well as modifications in the phosphate backbone of the nucleotide chain. For example, siRNA or miRNA molecules containing α-D-arabinofuranosyl structures in place of the naturally-occurring α-D-ribonucleosides found in RNA can be used as RNA interference molecules according to the present invention. Other
examples include RNA molecules containing the o-linkage between the sugar and the heterocyclic base of the nucleoside, which confers nuclease resistance and tight complementary strand binding to the oligonucleotides molecules similar to the oligonucleotides containing 2'-O-methyl ribose, arabinose and particularly α-arabinose. Phosphorothioate linkages can also be used to stabilize the siRNA and miRNA molecules.

An "siRNA" refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the siRNA is expressed in the same cell as the gene or target gene. "siRNA" thus refers to the double stranded RNA formed by the complementary strands. The complementary portions of the siRNA that hybridize to form the double stranded molecule typically have substantial or complete identity. In an embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA. The sequence of the siRNA can correspond to the full length target gene, or a subsequence thereof.

In an embodiment, the siRNA is at least about 15-50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is about 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, preferably about 19-30 base nucleotides, preferably about 20-25 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length).

Suitable siRNAs also include small hairpin (also called stem loop) RNAs (shRNAs). In an embodiment, the shRNA comprises short, e.g. about 19 to about 25 nucleotide, antisense strand, followed by a nucleotide loop of about 5 to about 9 nucleotides, and the analogous sense strand. Alternatively, the sense strand may precede the nucleotide loop structure and the antisense strand may follow.

In an embodiment, the antagonist of IAP is siRNA, shRNA or miRNA.
Specific RNA interference molecules, such as siRNA, shRNA and miRNA molecules, can be easily designed by one skilled in the art having regard to the sequence of the target gene.


Other RNA molecules which are single stranded, or are not considered to be RNA interference molecules, may also be useful as therapeutic agents in accordance with the present invention, including messenger RNAs (and the progenitor pre-messenger RNAs), small nuclear RNAs, small nucleolar RNAs, transfer RNAs and ribosomal RNAs.

Gene therapy techniques may also be used to antagonize IAPs by introducing coding sequences for Smac and thereby amplifying expression of endogenous Smac, e.g., full or partial Smac cDNA molecules. An illustrative example of an IAP antagonist that employs gene therapy is the recombinant vaccinia virus carrying a partial Smac gene disclosed in Pan et al., "SMAC-armed vaccinia virus induced both apoptosis and necroptosis and synergizes the efficiency of vinblastine in HCC," Hum Cell. 2014 Oct; 27(4): 162-71. doi: 10.1 007/s1 3577-014-0093-z. Epub 2014 Apr 26.

Some embodiments of the invention include co-administering to the subject one or more second therapy(ies) selected from radiation, chemotherapy (which includes biological therapy), immunotherapy, photodynamic therapy, targeted therapies such as JAKAFI (ruxolitinib phosphate), and combinations thereof. The terms, "coadministering" and "coadministration," are not limited to simultaneous coadministration but more generally refer to a treatment regimen that comprises
administration of an IAP antagonist and at least a second therapy sequentially or simultaneously. Thus, e.g., the co-treatment may be initiated prior to (e.g., one day to one week prior to), concurrently with (e.g., within moments before or after to within 24 hours before or after), or after (e.g., one day to one week after) initiation of treatment with the IAP antagonist.

Biological or chemotherapeutic agents include but are not limited to the chemotherapeutic agents described in "Modern Pharmacology with Clinical Applications", Sixth Edition, Craig & Stitzel, Chpt. 56, pg 639-656 (2004). The chemotherapeutic agent can be, but is not limited to, alkylating agents, antimetabolites, anti-tumor antibiotics, plant-derived products such as taxanes, enzymes, hormonal agents, miscellaneous agents such as cisplatin, monoclonal antibodies, glucocorticoids, mitotic inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, immunomodulating agents such as interferons, cellular growth factors, cytokines, and nonsteroidal anti-inflammatory compounds (NSAID), cellular growth factors and kinase inhibitors. Other suitable classifications for chemotherapeutic agents include mitotic inhibitors, and anti-estrogenic agents.

Specific examples of suitable biological and chemotherapeutic agents include, but are not limited to, 5-azacitidine, carboplatin, cisplatin, carmustine (BCNU), 5-fluorouracil (5-FU), cytarabine (Ara-C), gemcitabine, methotrexate, daunorubicin, doxorubicin, dexamethasone, irinotecan, topotecan, etoposide, paclitaxel, docetaxel, vincristine, tamoxifen, TNFa, TRAIL and other members, i.e., other than TRAIL and TNFa, of the TNFa superfamily of molecules, other TRAIL agonists, interferon (in both its alpha and beta forms), thalidomide, thalidomide derivatives such as lenalidomide, melphalan, and PARP inhibitors.

Other specific examples of suitable biological and chemotherapeutic agents include nitrogen mustards such as cyclophosphamide, alkyl sulfonates, nitrosoureas, ethylenimines, triazenes, folate antagonists, purine analogs, pyrimidine analogs, anthracyclines, bleomycins, mitomycins, dactinomycins,
plicamycin, vinca alkaloids, epipodophyllotoxins, taxanes, glucocorticoids, L-asparaginase, estrogens, androgens, progestins, luteinizing hormones, octreotide acetate, hydroxyurea, procarbazine, mitotane, hexamethylmelamine, carboplatin, mitoxantrone, monoclonal antibodies, levamisole, interferons, interleukins, filgrastim and sargramostim.

A TRAIL receptor agonist, or TRAIL agonist, is an agent that binds to a TRAIL receptor, such as TRAIL receptor 1 (TRAIL R1, also known as "death receptor 4" or DR4), TRAIL receptor 2 (TRAIL R2, also known as "death receptor 5" or DR5), or both DR4 and DR5, and leads to apoptosis in at least one mammalian (e.g., human) cell type (such as a TRAIL-sensitive tumor cell line) when used in an amount effective to induce apoptosis under physiological conditions. TRAIL receptor agonists used in the present invention preferably do not bind to TRAIL decoy receptors.

TRAIL has received considerable attention recently because of the finding that many cancer cell types are sensitive to TRAIL-induced apoptosis, while most normal cells appear to be resistant to this action of TRAIL. TRAIL-resistant cells may arise by a variety of different mechanisms including loss of the receptor, presence of decoy receptors, or overexpression of FLIP which competes for zymogen caspase-8 binding during DISC formation. In TRAIL resistance, the compounds or compositions that are used in the method of the present invention may increase tumor cell sensitivity to TRAIL leading to enhanced cell death, the clinical correlations of which are expected to be increased apoptotic activity in TRAIL resistant tumors, improved clinical response, increased response duration, and ultimately, enhanced patient survival rate. In support of this, reduction in XIAP levels by in vitro antisense treatment has been shown to cause sensitization of resistant melanoma cells and renal carcinoma cells to TRAIL (Chawla-Sarkar, et al., 2004). The Smac mimetic compounds used in the method of the present invention bind to IAPs and inhibit their interaction with caspases, therein potentiating TRAIL-induced apoptosis.
TRAIL agonists include, for example, an antibody, such as mapatumumab or lexatumumab (Human Genome Sciences), HGS-TR2J (Human Genome Sciences), Aptomab (Genentech), CS-1008 (Daichi Sankyo), LBY135 (Novartis), APG350 (Apogenix), Conatumumab (Amgen), and TRA-8 (University of Alabama-Birmingham). The antibody can be a DR4 antibody, a DR5 antibody, or a DR4/5 antibody, i.e., an antibody that binds to both DR4 and DR5. In other embodiments the TRAIL receptor agonist is recombinant human TRAIL or a soluble TRAIL polypeptide or a Fc-TRAIL fusion peptidobody. Fc-TRAIL fusion proteins are disclosed, e.g., in US20130064838. TRAIL fusion proteins comprising a collectin trimerization domain are disclosed, e.g., in US 20130178604, US20140171622, and US8383774, among others. Other TRAIL agonists include those disclosed in, e.g., US6284236; US6998161; US7915245.

The term "antibody" includes reference to isolated forms of both glycosylated and non-glycosylated immunoglobulins of any isotype or subclass, including any combination of: 1) human (e.g., CDR-grafted), humanized, and chimeric antibodies, 2) monospecific (e.g., DR5) or multi-specific antibodies (e.g., DR4/5), and 3) monoclonal, polyclonal, or single chain (scFv) antibodies, irrespective of whether such antibodies are produced, in whole or in part, via immunization, through recombinant technology, by way of in vitro synthetic means, or otherwise. Thus, the term "antibody" is inclusive of those that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transfected to express the antibody (e.g., from a transfectoma), (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences. In some embodiments the antibodies of the present invention are monoclonal antibodies, such as humanized or fully-human monoclonal antibodies.
This also encompasses molecules with TRAIL agonist activity that may be fragments of antibodies, peptides, recombinant forms of the endogenous TRAIL, or small molecules, that effectively engage the receptor and thereby trigger TRAIL-receptor signaling. Accordingly, in addition to recombinant antibodies, new classes of therapeutic proteins are being developed and include recombinant protein scaffolds (e.g. DARPINs, anticalins, affibodies, fibronectin domains), in which binding is mediated by surface diversity that interacts with targets which include TRAIL receptors DR4 and DR5 (e.g. Veesler, D., et al., J. Biol. Chem. 284(44):30718-30726, (2009).

In an illustrative embodiment of the invention, a Smac mimetic is co-administered with GM-CSF, as disclosed, e.g., in WO2014022612. In another illustrative embodiment of the invention, a Smac mimetic is co-administered with an NSAID, as disclosed, e.g., in PCT/GB2015/053533.

JAK inhibitors, i.e., inhibitors of one or more of JAK1, JAK2, JAK3, and TYK2, include, without limitation, ruxolitinib, tofacitinib, baricitinib, CYT387, GLPG0634, GSK2586184, lestaurtinib, pacritinib, and TG101348. Fedratinib is a JAK2 inhibitor that is theoretically useful but clinical studies with this agent were terminated due to safety reasons.

In some embodiments of the invention, pharmaceutical compositions comprising an IAP antagonist, alone or in combination with one or more other active pharmaceutical ingredients, are administered to a human or veterinary subject. The pharmaceutical compositions typically comprise at least one pharmaceutically acceptable excipient, e.g., a carrier or diluent, and can be administered in the conventional manner by routes including systemic, subcutaneous, topical, or oral routes. Administration may be by intravenous injection, either as a bolus or infusion, but other routes of administration, including, among others, subcutaneous or oral administration, are not precluded. An intravenous formulation can contain, e.g., from 1 mg/mL up to and including 5 mg/mL of the IAP antagonist, such as specifically Compound 15 (birinapant), in sterile 0.05M citrate buffered PBS, pH 5.
Formulation may be by immediate release or prolonged release. Specific modes of administration and formulation will depend on the indication and other factors including the particular compound being administered. The amount of compound to be administered is that amount which is therapeutically effective, *i.e.*, the amount that ameliorates the disease symptoms, *i.e.*, that slows cancer progression or causes regression, without serious adverse effects relative to the disease being treated or causes improvement of the infectious disease or the autoimmune disease. Put another way, an effective dose is one that over the course of therapy, which may be, *e.g.*, 1 or more weeks, *e.g.*, multiple courses of 3 weeks on/1 week off, results in treatment of the proliferative disorder, *i.e.*, a decrease in the rate of disease progression, termination of disease progression, or regression or remission, or results in a decrease in infectious burden or resolution of symptoms of an infectious or autoimmune disease.

The phrase "pharmaceutical composition" refers to a composition suitable for administration in medical use.

The dosage to be administered will depend on the characteristics of the subject being treated, *e.g.*, the particular patient treated, age, weight, health, types of concurrent treatment, if any and the specific disease or disorder that is being treated. Frequency of treatments can be easily determined by one of skill in the art (*e.g.*, by the clinician).

The dose of a second or further agent, *e.g.*, a TRAIL agonist, GM-CSF, an NSAID (*e.g.*, celecoxib), a JAK inhibitor, or 5-azacitidine, when given in combination with an IAP antagonist in accordance with this invention is expected to be the same as it would be were it administered alone or with another additional chemotherapeutic agent. However in some situations the dose of the other agent(s) or the dose of the Smac mimetic required when used together or in combination, may be less than the dose of either agent when used alone.

While it is possible to combine an IAP antagonist and a second agent into a single dosage unit, *e.g.*, a sterile solution for intravenous administration, in
practice, it may be preferable to administer each agent separately, e.g., using a separate pharmaceutical dosage unit, including by administering the separate dosage units according to a different dosing regimen.

Pharmaceutical compositions to be used comprise a therapeutically effective amount of the active pharmaceutical ingredients as described above with one or more pharmaceutically acceptable excipients. The phrase "pharmaceutical composition" refers to a composition suitable for administration in medical or veterinary use. It should be appreciated that the determinations of proper dosage forms, dosage amounts, and routes of administration for a particular patient are within the level of ordinary skill in the pharmaceutical and medical arts.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active pharmaceutical ingredients, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents, emulsifying and suspending agents. Various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, and sorbic acid also may be included. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. Carrier formulation suitable for subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.
A pharmaceutical composition in intravenous unit dose form may comprise, e.g., a vial or pre-filled syringe, or an infusion bag or device, each comprising an effective amount or a convenient fraction of an effective amount such that the contents of one vial or syringe are administered at a time.

An effective dose is one that over the course of therapy, which may be, e.g., 1 or more weeks, e.g., multiple courses of 3 weeks on/1 week off, results in treatment of the disorder, e.g., a decrease in the rate of disease progression, termination of disease progression, or regression or remission.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compounds (TRAIL agonist and IAP antagonist) are admixed with at least one inert pharmaceutically acceptable excipient such as (a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (c) humectants, as for example, glycerol, (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (e) solution retarders, as for example paraffin, (f) absorption accelerators, as for example, quaternary ammonium compounds, (g) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (h) adsorbents, as for example, kaolin and bentonite, and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Solid dosage forms such as tablets, dragees, capsules, pills, and granules also can be prepared with coatings and shells, such as enteric coatings and others well known in the art. The solid dosage form also may contain opacifying agents, and can also be of such composition that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used are polymeric substances and
waxes. The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients. Such solid dosage forms may generally contain from 1% to 95% (w/w) of the active compounds. In certain embodiments, the active compounds generally range from 5% to 70% (w/w).

Since one aspect of the present invention contemplates the treatment of the disease/conditions with a combination of pharmaceutically active agents that may be administered separately, the invention further relates to combining separate pharmaceutical compositions in kit form. The kit comprises two separate pharmaceutical compositions: one composition contains the IAP antagonist used in the method of the present invention, and a second composition contains the second agent. The kit comprises a container for containing the separate compositions such as a divided bottle or a divided foil packet. Additional examples of containers include syringes, e.g., pre-filled syringes, boxes and bags. Typically, the kit comprises directions for the use of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (e.g., oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician or veterinarian.

An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process recesses are formed in the plastic foil. The recesses have the size and shape of the tablets or capsules to be packed. Next, the tablets or capsules are placed in the recesses and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are sealed in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on
the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It may be desirable to provide a memory aid on the kit, e.g., in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen which the tablets or capsules so specified should be ingested. Another example of such a memory aid is a calendar printed on the card, e.g., as follows "First Week, Monday, Tuesday, . . . etc . . . Second Week, Monday, Tuesday, . . . " etc. Other variations of memory aids will be readily apparent. A "daily dose" can be a single tablet or capsule or several pills or capsules to be taken on a given day. Also, a daily dose of a substance of the present invention can consist of one tablet or capsule, while a daily dose of the second substance can consist of several tablets or capsules and vice versa. The memory aid should reflect this variety and aid in correct administration of the active agents.

In another specific embodiment of the invention, a dispenser designed to dispense the daily doses one at a time in the order of their intended use is provided. Preferably, the dispenser is equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter which indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the compounds or composition, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil and sesame oil, glycerol, tetrahydrofurfuryl alcohol,
polyethyleneglycols and fatty acid esters of sorbitan or mixtures of these substances. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

The compounds and compositions used in the method of the present invention also may benefit from a variety of delivery systems, including time-released, delayed release or sustained release delivery systems. Such option may be particularly beneficial when the compounds and composition are used in conjunction with other treatment protocols as described in more detail below.

Many types of controlled release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di-and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the active compound is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,667,014, 4,748,034 and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,832,253, and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be desirable. Long-term release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active compounds for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those
of ordinary skill in the art and include some of the release systems described above.

The compounds used in the method of the present invention and pharmaceutical compositions comprising compounds used in the method of the present invention can be administered to a subject suffering from cancer, an autoimmune disease or another disorder where a defect in apoptosis is implicated. In connection with such treatments, the patient can be treated prophylactically, acutely, or chronically using the compounds and compositions used in connection with the method of the present invention, depending on the nature of the disease. Typically, the host or subject in each of these methods is human, although other mammals may also benefit from the present invention.

Methodologies for determining if a subject is suffering from aberrant JAK signaling are known. These include biochemical assays for analyzing JAK-mediated phosphorylation, genotyping for presence of a mutation in one or both copies of a JAK gene, e.g., in the JH2 domain, and phenotyping to identify mutant amino acid sequence(s) in a JAK gene such as by protein sequencing or antibody binding assays.

E.g., methods for detecting the presence or absence of G1849T and V617F mutations in the JAK2 gene are disclosed in US 20060288432, US20080076135, US20130189683 and US20120066776; methods for detecting the presence or absence of other JAK2 mutations, including exon 12, exon 13, exon 14, and exon 15 mutations, are disclosed in US20140057270. See also, e.g., US8841078. Mutation in a single allele is generally sufficient to determine that a proliferative disorder is associated with aberrant JAK signaling.

**Examples**

*In vitro* and *in vivo* efficacy of birinapant in preclinical models of Ph-like pediatric acute lymphoblastic leukemia

We assessed the *in vitro* sensitivity of 41 PDXs after 24 h exposure to 100 nM birinapant. Using an arbitrary cutoff of 50% of control cell viability, 9/9 Ph-like
ALL patient derived xenografts (PDXs) were sensitive to birinapant, compared with 7/13 B-Cell Precursor-ALL (BCP-ALL), 3/7 Mixed Lineage Leukemia (MLL-ALL), 1/9 T-ALL and 1/3 Early T-cell Precursor (ETP-ALL). When stratified according to B-lineage (BCP-ALL, Ph-like ALL, infant MLL-ALL) or T-lineage (T-ALL, ETP-ALL), the B subtype was significantly more sensitive than T-lineage (P < 0.001, Figure 1B). Within the B-lineage ALL PDXs the Ph-like subtype was significantly more sensitive than other high risk subtypes such as infant MLL-ALL. To verify that the sensitivity of Ph-like PDX cells was not an artifact of xenografting we next tested the birinapant sensitivity of primary ALL cells derived from 6 pediatric and 3 adult patients with Ph-like ALL, alongside 3 BCP-ALL primary samples. Using the same cutoff as for the PDX cells 9/9 primary Ph-like biopsies were sensitive to birinapant, and 2/3 non Ph-like samples were resistant. The predisposition of Ph-like ALL PDXs and primary samples to birinapant sensitivity did not appear to be due to the presence of specific kinase lesions per se, since in the BCP-ALL cohort of PDXs and primary biopsies kinase lesions were detected in 4/8 sensitive and 3/8 resistant samples. Moreover of the 4 BCR-ABL1-positive samples, 2 were sensitive (ALL-56 and #110) while 2 were resistant (ALL-4 and ALL-55).

To further investigate the mechanism by which birinapant kills Ph-like ALL cells, PDX cells were pre-incubated with the pan-caspase inhibitor QVD or the RIPK1 inhibitor Nec-1 prior to birinapant treatment. QVD significantly attenuated birinapant-induced apoptosis in 3/4 sensitive PDXs, while Nec-1 caused significant inhibition in 2/4 PDXs. Neither QVD nor Nec-1 alone significantly affected the viability of the PDX cells, nor had any effect on the cytotoxicity of birinapant in 2 resistant PDXs. These results indicate that birinapant kills Ph-like ALL cells via multiple pathways.

To confirm that birinapant resistance in BCP-ALL PDXs was not due to failure of target degradation, the levels of IAPs and other relevant proteins were assessed after birinapant treatment. Both clAP1 and clAP2 were rapidly degraded in sensitive (n=3) and resistant (n=2) PDXs within 30 min of treatment. The cleaved (activated) forms of both caspase-3 and caspase-8 were markedly upregulated in
sensitive PDXs within 6 h of birinapant treatment in contrast to the resistant PDXs, indicating that resistance occurred downstream of clAP1/2 degradation.

Since the predisposition of Ph-like ALL to birinapant sensitivity appeared to be a cell autonomous characteristic we sought confirmation of in vivo efficacy by testing 19 B-lineage PDXs. The aim of this study was to evaluate the efficacy of birinapant against patient-derived xenografts (PDXs) of pediatric ALL subtypes. Birinapant (30 mg/kg IP Q3 days × 5) significantly delayed the progression of 17/19 PDXs derived from Ph-like ALL (n=7), B-cell precursor ALL (BCP-ALL, n=8), and infant MLL-rearranged ALL (MLL-ALL, n=4) by between 2 and 77 days compared with vehicle-treated controls. Using stringent objective response criteria modeled after the clinical setting, birinapant induced objective responses in 11/19 PDXs (1 partial response, PR; 7 complete responses, CRs; 3 maintained CRs, MCRs). Similar to the in vitro data described above, the Ph-like PDXs were significantly more sensitive to birinapant in vivo compared to the BCP-ALL and MLL-ALL PDX panels, when assessed by "Treated over Controls" (T/C) or Event Free Survival (EFS) T/C values. Moreover, the in vitro birinapant sensitivity (% of control at 100 nM) of the 19 PDXs tested significantly correlated with their in vivo responses. Consistent with the in vitro results, birinapant also caused rapid (within 6 h) degradation of clAP1 in vivo regardless of the PDX response, despite clear differences in caspase-3, -7, -8, and -9 activation between a sensitive (ALL-2) and resistant (ALL-7) PDX.

Birinapant induced a CR in a Ph-like PDX at a dose 1/8th (3.8 mg/kg) of its maximum tolerated dose (30 mg/kg).

Analysis at 14 days following treatment initiation revealed >98% clearance of human leukemia cells from the bone marrow, spleen and peripheral blood of mice treated with birinapant doses that achieve drug levels attainable in humans. In vitro apoptosis assays confirmed the greater sensitivity of the Ph-like ALL PDX panel. Moreover, the clAP1 protein was rapidly degraded in PDXs upon birinapant treatment both in vitro and in vivo, regardless of their relative sensitivity.
Microarray analysis of gene expression revealed a significant correlation between baseline TNFα expression and in vivo birinapant sensitivity across 19 PDXs (P=0.002; R²=0.46). While exogenously-added TNFα did not potentiate apoptosis induced by birinapant, a TNFα blocking antibody partially reversed apoptosis in 3/4 Ph-like PDXs.

These results show that birinapant exerts profound single-agent in vivo efficacy against Ph-like pediatric ALL PDXs and indicate a role for endogenous TNFα in the birinapant mechanism of action against this high-risk pediatric ALL subtype.

We also identified a gene expression signature associated with birinapant response that was enriched in genes involved in inflammatory response, hematopoiesis, and cell death pathways.

Birinapant also significantly enhanced the anti-leukemic activity of an induction-type regimen of vincristine, dexamethasone and L-asparaginase (VXL) in vivo. These findings support further evaluation of birinapant in the treatment of Ph-like ALL.

Similar results can be obtained in experiments with non-ALL JAK-mutated tumors.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. All patent and literature references cited herein are incorporated by reference herein as though fully set forth.

The claims which follow further and specifically describe particular illustrative embodiments of the invention.
Claims

1. A method of treating a proliferative disorder associated with aberrant Janus Kinase (JAK) signaling in a subject, said method comprising (e.g. internally) administering to the subject an effective amount of an IAP antagonist.

2. The method of claim 1 wherein the proliferative disorder is associated with a mutation in a JAK gene.

3. The method of claim 1 wherein the IAP antagonist is a Smac mimetic.

4. The method of claim 3 wherein the Smac mimetic is a bivalent Smac mimetic such as birinapant.

5. The method of claim 3 wherein the Smac mimetic is characterized as (i) not inhibiting XIAP E3 ubiquitin ligase activity or as only poorly inhibiting XIAP E3 ubiquitin ligase activity, (ii) not inhibiting or poorly inhibiting NOD (i.e., NOD1/2) signaling, or (iii) not inhibiting or poorly inhibiting NOD-mediated NF-kB activation.

6. The method of any of the preceding claims wherein the proliferative disorder is acute lymphoblastic leukemia (ALL), a myeloproliferative disorder, breast cancer, pancreatic cancer, or non-small cell lung carcinoma.

7. The method of claim 6 wherein the proliferative disorder is Philadelphia chromosome-like ALL (Ph-like ALL) or a myeloproliferative disorder selected from chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocytopenia (ET), primary myelofibrosis (PMF) (also known as chronic idiopathic myelofibrosis or agnogenic myeloid metaplasia), chronic neutrophilic leukemia, or chronic eosinophilic leukemia.

8. The method of claim 7 wherein the proliferative disorder is Ph-like pediatric ALL.

9. The method of claim 6, 7, or 8 wherein the JAK mutation is a mutation in the JAK2 gene.
10. The method of claim 9 wherein the JAK mutation is the JAK2 V617F mutation or a JAK2 exon 12 mutation.

11. The method of any of the preceding claims wherein in addition to the IAP antagonist the subject is also treated by administering to the subject a second therapy selected from radiation, chemotherapy, immunotherapy, photodynamic therapy, and combinations thereof.

12. The method of claim 11 wherein the proliferative disorder is Ph-like ALL and the additional therapy comprises administration of one or more of a tyrosine kinase inhibitor such as ruxolitinib, a nucleoside analog such as but not limited to 5-azacitidine, and a TRAIL agonist such as an anti-DR4, anti-DR5, or anti-DR4/5 antibody.

13. The method of claim 11 wherein the proliferative disorder is PV, ET, or PMF and the additional therapy comprises administration of one or more of a nucleoside analog such as 5-azacitidine, a tyrosine kinase inhibitor such as ruxolitinib, and a TRAIL agonist such as an anti-DR4, anti-DR5, or anti-DR4/5 antibody.

14. The method of claim 11, 12 or 13 wherein the additional therapy comprises administration of a TRAIL agonist and an anti-inflammatory agent.

15. The method of claim 14 wherein the anti-inflammatory agent is an NSAID such as but not limited to celecoxib.

16. The method of claim 11 that comprises co-administering to the subject an IAP antagonist and a JAK inhibitor.

17. An IAP antagonist for use in a method of treating a proliferative disorder associated with aberrant JAK signaling (in a subject), said method optionally comprising internally administering to the subject an effective amount of said IAP antagonist.

18. An IAP antagonist for use in a method of treating a proliferative disorder associated with aberrant JAK signaling (in a subject), said method optionally
comprising co-administering (to the subject) an IAP antagonist and a JAK inhibitor.

19. A JAK inhibitor for use in a method of treating a proliferative disorder associated with aberrant JAK signaling (in a subject), said method optionally comprising co-administering to the subject an IAP antagonist and a JAK inhibitor.

20. A product comprising an IAP antagonist and a JAK inhibitor for simultaneous, separate or sequential use in a method of treating a proliferative disorder associated with aberrant JAK signaling.

21. A pharmaceutical composition comprising an IAP antagonist, a JAK inhibitor, and a pharmaceutically acceptable excipient.

22. Use of an IAP antagonist for the manufacture of a medicament for use in a method of treating a proliferative disorder associated with aberrant JAK signaling (in a subject), said method optionally comprising (e.g. internally) administering to the subject an effective amount of said IAP antagonist.

23. Use of an IAP antagonist for the manufacture of a medicament for use in a method of treating a proliferative disorder associated with aberrant JAK signaling (in a subject), said method optionally comprising co-administering to the subject an IAP antagonist and a JAK inhibitor.

24. Use of a JAK inhibitor for the manufacture of a medicament for use in a method of treating a proliferative disorder associated with aberrant JAK signaling (in a subject), said method optionally comprising co-administering to the subject an IAP antagonist and a JAK inhibitor.
INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/054105

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K45/06 A61K31/404 A61K31/519 A61P35/00 A61P35/02
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

X See patent family annex.

* Special categories of cited documents:

  "A" document defining the general state of the art which is not considered to be of particular relevance
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Authorized officer
Pilling, Stephen
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# INTERNATIONAL SEARCH REPORT

**International application No.**
PCT/GB2015/054105

## Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:  
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.:  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

<table>
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1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1-18, 20-23

**Remark on Protest**

- □ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

- □ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

- □ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18, 20-23

   IAP antagonists optionally in combination with a JAK inhibitor in the context of the treatment of a proliferative disorder associated with aberrant Janus Kinase (JAK) signaling.

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2. claims: 19, 24

   JAK inhibitor optionally in combination with an IAP antagonist in the context of the treatment of a proliferative disorder associated with aberrant Janus Kinase (JAK) signaling.

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

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