SYNTHETIC LETHALITY AND THE TREATMENT OF CANCER

RELATED APPLICATIONS

This application claims priority to United States Application number 61/510,686, filed July 22, 2011, the contents all of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0001] The field of the invention generally relates to compounds, compositions and methods for treatment of cancer.

BACKGROUND OF THE INVENTION

[0002] Cancer is a leading cause of death in Canada. The Canadian Cancer Society estimate there will be approximately 170000 new cases of cancer in 2011, and approximately 75000 deaths as a result of cancer.

[0003] An emerging approach for the treatment of cancer relates to the concept of synthetic lethality. Two genes (or two gene products) are synthetic lethal if mutation of either alone is compatible with viability but mutation of both leads to death. Put another way, "synthetic lethality" describe situations where a mutation and a drug (for example) together cause a cancer cell's death - either the mutation or the drug would not result in cell death. Targeting a gene (or gene product) that is synthetic lethal to a cancer-relevant mutation should kill only cancer cells and spare normal cells. Synthetic lethality therefore provides a framework for the development of anti-cancer specific agents.

[0004] The approach of synthetic lethality to the treatment of cancer is emerging, is not yet a routine approach largely due to the absence identification of synthetic lethal genes (and gene products).

[0005] *N*-myristoylation of proteins is a modification in which myristate (a 14-carbon saturated fatty acid) is covalently attached to the NH_2 terminal glycine of a variety of cellular, viral, and onco-proteins (e.g., oncogenic Src-related tyrosine kinases, heterotrimeric G alpha subunits, etc.).

[0006] Cellular myristoylated proteins have diverse biological functions in signal transduction and oncogenesis. Modification of proteins by myristoylation is required for the subcellular targeting, protein conformation and biological activity of many important proteins in eukaryotic cells, including those required for signal transduction and regulatory functions important in cell growth. Tyrosine kinases of the Src family (proto-oncogenes) are among the most extensively studied myristoylated proteins.

[0007] Myristoylation of proteins is catalyzed *N*-myristoyltransferase (NMT). NMT is responsible for this activity in eukaryotic cells and works by modifying its polypeptide substrate after the removal of the initiator methionine residue by methionyl aminopeptidase. This modification occurs primarily as a cotranslational process, although myristoylation can also occur post-translationally after proteolytic cleavage of proteins, typically during apoptosis. Two isozymes of the mammalian NMT enzymes have been cloned and are designated NMT1 and NMT2. NMTs play a pro-survival role in cells. The two NMTs are present in all normal cells.

[0008] There remains a need for compounds, composition and method for the treatment of cancer.

[0009] This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should it be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY

[0010] In accordance with one example of the present disclosure there is provided compounds and compositions for the treatment of a subject with cancer. There are also provided methods for identifying subject with cancer that are suitable for treatment with the compounds, composition and methods are described herein.

[0011] In accordance with one example of the present disclosure, there is provided a method of treating a subject having a cancer deficient in NMT2, comprising: administering to said subject an NMT inhibitor. In a specific example, said NMT inhibitor is a NMT1 inhibitor.

[0012] In a specific example, said cancer is a lymphoma. In an example, said lymphoma is a B cell lymphoma. In a more specific example, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt's lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[0013] In a specific example, said NMT inhibitor is a small molecule, an antibody, a peptide fragment, or a nucleic acid.

[0014] In a specific example, said small molecule is Tris-DBA, HMA, or DDD85646, or a derivative thereof. In a specific example, said antibody is a monoclonal antibody or a polyclonal antibody. In a specific example, said nucleic acid comprises a dsRNA molecule, a RNAi molecule, miRNA molecule, a ribozyme, a shRNA molecule, or a siRNA molecule.

[0015] In a specific example, said subject is a human subject.

[0016] In another example of the present disclosure, the method further comprises administering a chemotherapeutic agent. In a specific example, said chemotherapeutic agent is CHOP, GAP-BOP, m-BACOD, ProMACE-MOPP, ProMACE-CytaBOM, MACOP-B, IMVP-16, MIME, DHAP, ESHAP, CEFF(B), CAMP, VABCD, ABDIC, CBVD, PCVP, CEP, EVA, MOPLACE, MIME, MINE, MTX-CHOP, CEM, CEVD, CAVP, EVAP, or EPOCH.

[0017] In another example of the present disclosure, there is provided a method of treating a subject having cancer, comprising: measuring a sample from said subject to determine whether said sample is deficient in NMT2; and administering an inhibitor of NMT to said subject when said sample is deficient in NMT2. In a specific example, said NMT inhibitor is a NMT1 inhibitor.

[0017a] In one aspect, the present disclosure refers to a method of detecting a subject having cancer being a B cell lymphoma, comprising: measuring a sample from said subject to determine whether said sample is deficient in NMT2 by determining epigenetic inactivation a NMT2 gene or loss of protein function or having a level of NMT2 protein that is low or absent compared to a control being normal B cell; wherein the B cell lymphoma is Burkett's lymphoma.

[0018] In a specific example, said NMT inhibitor is a NMT1 inhibitor.

[0019] In a specific example, said cancer is a lymphoma. In a specific example, said lymphoma is a B cell lymphoma. In a more specific example, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt's lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[0020] In a specific example, said NMT inhibitor is a small molecule, an antibody, a peptide fragment, or a nucleic acid.

[0021] In a specific example, said small molecule is Tris-DBA, HMA, or DDD85646, or a derivative thereof. In a specific example, said antibody is a monoclonal antibody or a polyclonal antibody. In a specific example, said nucleic acid comprises a dsRNA molecule, a RNAi molecule, miRNA molecule, a ribozyme, a shRNA molecule, or a siRNA molecule.

[0022] In a specific example, said subject is a human subject.

[0023] In another example of the present disclosure, the method further comprises administering a chemotherapeutic agent. In a specific example, said chemotherapeutic agent is CHOP, GAP-BOP, m-BACOD, ProMACE-MOPP, ProMACE-CytaBOM, MACOP-B, IMVP-16, MIME, DHAP, ESHAP, CEFF(B), CAMP, VABCD, ABDIC, CBVD, PCVP, CEP, EVA, MOPLACE, MIME, MINE, MTX-CHOP, CEM, CEVD, CAVP, EVAP, or EPOCH.

[0024] In another example, measuring of said sample is carried out using quantitative fluorescence activated cell sorting, enzyme linked immunosorbent assay, immunohistochemistry, quantitative immunohistochemistry, fluorescence resonance energy transfer, Forster resonance energy transfer, biomolecular fluorescence complementation, mass spectrometry, immunoblot assay or coimmunoprecipitation assay.

[0025] In another example of the present disclosure, there is provided a kit for treating cancer in of treating a subject having a cancer deficient in NMT2, comprising: an NMT inhibitor; and instructions for the use thereof. In one example, said NMT inhibitor is a NMT1 inhibitor.

[0026] In a specific example, said cancer is a lymphoma. In a specific example, said lymphoma is a B cell lymphoma. In a more specific example, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt's lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[0027] In a specific example, said NMT inhibitor is a small molecule, an antibody, a peptide fragment, or a nucleic acid.

[0028] In a specific example, said small molecule is Tris-DBA, HMA, or DDD85646, or a derivative thereof. In a specific example, said antibody is a monoclonal antibody or a polyclonal antibody. In a specific example, said nucleic acid comprises a dsRNA molecule, a RNAi molecule, miRNA molecule, a ribozyme, a shRNA molecule, or a siRNA molecule.

[0029] In a specific example, said subject is a human subject.

[0030] In another example of the present disclosure, the method further comprises administering a chemotherapeutic agent. In a specific example, said chemotherapeutic agent is CHOP, GAP-BOP, m-BACOD, ProMACE-MOPP, ProMACE-CytaBOM, MACOP-B, IMVP-16, MIME, DHAP, ESHAP, CEFF(B), CAMP, VABCD, ABDIC, CBVD, PCVP, CEP, EVA, MOPLACE, MIME, MINE, MTX-CHOP, CEM, CEVD, CAVP, EVAP, or EPOCH.

[0031] In another example of the present disclosure, there is provided a use of an inhibitor NMT for treating a subject having a cancer deficient in NMT2. In a specific example, said NMT inhibitor is a NMT1 inhibitor.

[0031a] In another aspect, the present disclosure refers to use of an NMT1 inhibitor for the preparation of a medicament for treating a subject having a cancer being a B cell lymphoma deficient in NMT2 by determining epigenetic inactivation a NMT2 gene or loss of protein function or having a level of NMT2 protein that is low or absent compared to a control being normal B cell, wherein the B cell lymphoma is Burkett's lymphoma.

[0032] In a specific example, said cancer is a lymphoma. In a specific example, said lymphoma is a B cell lymphoma. In a more specific example, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt’s lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[0033] In a specific example, said NMT inhibitor is a small molecule, an antibody, a peptide fragment, or a nucleic acid.

[0034] In a specific example, said small molecule is Tris-DBA, HMA, or DDD85646, or a derivative thereof. In a specific example, said antibody is a monoclonal antibody or a polyclonal antibody. In a specific example, said nucleic acid comprises a dsRNA molecule, a RNAi molecule, miRNA molecule, a ribozyme, a shRNA molecule, or a siRNA molecule.

[0035] In a specific example, said subject is a human subject.

[0036] In another example of the present disclosure, the method further comprises administering a chemotherapeutic agent. In a specific example, said chemotherapeutic agent is CHOP, GAP-BOP, m-BACOD, ProMACE-MOPP, ProMACE-CytaBOM, MACOP-B, IMVP-16, MIME, DHAP, ESHAP, CEFF(B),

CAMP, VABCD, ABDIC, CBVD, PCVP, CEP, EVA, MOPLACE, MIME, MINE, MTX-CHOP, CEM, CEVD, CAVP, EVAP, or EPOCH.

[0037] In another example of the present disclosure, there is provided a use of an inhibitor NMT for the preparation of a medicament for treating a subject having a cancer deficient in NMT2.

[0038] In a specific example, said NMT inhibitor is a NMT1 inhibitor.

[0039] In a specific example, said cancer is a lymphoma. In a specific example, said lymphoma is a B cell lymphoma. In a more specific example, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt's lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[0040] In a specific example, said NMT inhibitor is a small molecule, an antibody, a peptide fragment, or a nucleic acid.

[0041] In a specific example, said small molecule is Tris-DBA, HMA, or DDD85646, or a derivative thereof. In a specific example, said antibody is a monoclonal antibody or a polyclonal antibody. In a specific example, said nucleic acid comprises a dsRNA molecule, a RNAi molecule, miRNA molecule, a ribozyme, a shRNA molecule, or a siRNA molecule.

[0042] In a specific example, said subject is a human subject.

[0043] In another example of the present disclosure, the method further comprises administering a chemotherapeutic agent. In a specific example, said chemotherapeutic agent is CHOP, GAP-BOP, m-BACOD, ProMACE-MOPP, ProMACE-CytaBOM, MACOP-B, IMVP-16, MIME, DHAP, ESHAP, CEFF(B), CAMP, VABCD, ABDIC, CBVD, PCVP, CEP, EVA, MOPLACE, MIME, MINE, MTX-CHOP, CEM, CEVD, CAVP, EVAP, or EPOCH.

[0044] In another example of the present disclosure, there is provided a use of NMT2 as a marker for one or more of diagnosis, prognosis, classifying, or monitoring of cancer in a subject.

[0044a] In another aspect, the present disclosure refers to use of NMT2 as a marker by determining epigenetic inactivation a NMT2 gene or loss of protein function or having a level of NMT2 protein that is low or absent compared to a control being normal B cell for one or more of *in vitro* diagnosis, prognosis, classifying, or monitoring of cancer being B cell lymphoma in a subject, wherein the B cell lymphoma is Burkitt's lymphoma.

[0045] In another example of the present disclosure, there is provided a use of protein myristoylation as a marker for one or more of diagnosis, prognosis, classifying or monitoring cancer in a subject.

[0045a] In another aspect, the present disclosure refers to use of protein myristoylation as a marker for one or more of *in vitro* diagnosis, prognosis, classifying or monitoring cancer being a B cell lymphoma in a subject, wherein the B cell lymphoma is Burkitt's lymphoma.

[0046] In another example of the present disclosure, there is provided a use of protein acylation as a marker for one or more of diagnosis, prognosis, classifying or monitoring cancer in a subject.

[0047] In a specific example, said cancer is lymphoma.

[0048] In a specific example, said lymphoma is B cell lymphoma.

[0049] In a specific example, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt's lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[0050] In a specific example, said marker is measured using an assay selected from immunoassays or nucleic acid detection, or protein activity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] Embodiments of the present invention will now be described, by way of example only, with reference to the attached Figures, wherein:

[0052] Figure 1 depicts immunoblot analysis of NMT1 and NMT2 expression in one type of normal B cells (L0) and various B cell lymphomas and T cell leukemias;

[0053] Figure 2 is a graph illustrating sensitivity of various normal cells and various B cell lymphomas and T cell leukemias to the NMT inhibitors tris-dibenzylideneacetone-dipalladium (Tris-DBA);

[0054] Figure 3 is a bar graph illustrating inhibition of N-myristoyltransferase (NMT) by tris-dibenzylideneacetone-dipalladium (Tris-DBA); and

[0055] Figure 4 are immunoblotts depicting lymphoma cell lines probed with antibodies against NMT1 and NMT2.

[0056] Figure 5 is a line graph showing the sensitivity of NMT inhibitors on a Burkitt's Lymphoma cell line in comparison to an immortalized normal B lymphocytic cell line; and

[0057] Figure 6 depicts the results of transfection of Ramos B lymphoma cells with pcDNA3.1-V5-NMT2 showing increased survival to TrisDBA (5 ug/ml) 2.5 fold vs control cells transfected with empty plasmid vector (Panel A) showing cell viability, and (Panel B) an immunoblott.

[0058] In the Detailed Description that follows, the numbers in bold face type serve to identify the component parts that are described and referred to in relation to the drawings depicting various embodiments of the invention. It should be noted that in describing various embodiments of the present invention, the same reference numerals have been used to identify the same of similar elements. Moreover, for the sake of simplicity, parts have been omitted from some figures of the drawings.

DETAILED DESCRIPTION

[0059] As will be described in more detail below, there is described herein compounds, composition and methods for the treatment of a subject with cancer. There are also described here methods for identifying subject with cancer that are suitable for treatment with the compounds, composition and methods are described herein. There are also described here methods for identifying subject with cancer.

[0060] The present application provides methods and compositions for the treatment of NMT deficient cancers. NMT-deficient cancers include cancers deficient in NMT2 or NMT1. In a specific example, the NMT deficient cancer is a NMT2 deficient cancer.

[0061] The term "cancer", as used herein, refers to a variety of conditions caused by the abnormal, uncontrolled growth of cells. Cells capable of causing cancer, referred to as "cancer cells", possess characteristic properties such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and/or certain typical morphological features. Cancer cells may be in the form of a tumour, but such cells may also exist alone within a subject, or may be a non-tumorigenic cancer cell. A cancer can be detected in any of a number of ways, including, but not limited to, detecting the presence of a tumor or tumors (e.g., by clinical or radiological means), examining cells within a tumor or from another biological sample (e.g., from a tissue biopsy), measuring blood markers indicative of cancer, and detecting a genotype indicative of a cancer. However, a negative result in one or more of the above detection methods does not necessarily indicate the absence of cancer, e.g., a patient who has exhibited a complete response to a cancer treatment may still have a cancer, as evidenced by a subsequent relapse.

[0062] In a specific example of the present disclosure, the cancer is lymphoma.

[0063] The term "lymphoma" as used herein refers to a malignant growth of B or T cells in the lymphatic system. "Lymphoma" includes numerous types of malignant growths, including Hodgkin's Lymphoma and non-Hodgkin's lymphoma. The term "non-Hodgkin's Lymphoma" as used herein, refers to a malignant growth of B or T cells in the lymphatic system that is not a Hodgkin's Lymphoma (which is characterized, e.g., by the presence of Reed-Sternberg cells in the cancerous area). Non-Hodgkin's lymphomas encompass over 29 types of lymphoma, the distinctions between which are based on the type of cancer cells.

[0064] In a more specific example of the present disclosure, the cancer is a B-lymphoma.

[0065] Thus, in one embodiment, the compounds, compositions and methods of the disclosure are suitable for the treatment of a subject with B cell lymphoma.

[0066] Examples of B-cell lymphomas include, but are not limited to, for example, follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, and MALT-type/monocytoid B cell lymphoma. Also contemplated are the treatment of pediatric lymphomas such as Burkitt's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, precursor B-LBL, precursor T-LBL, and anaplastic large cell lymphoma.

[0067] The term "subject", as used herein, refers to an animal, and can include, for example, domesticated animals, such as cats, dogs, etc., livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.), mammals, non-human mammals, primates, non-human primates, rodents, birds, reptiles, amphibians, fish, and any other animal. In a specific example, the subject is a human.

[0068] The term "treatment" or "treat" as used herein, refers to obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable

or undetectable. "Treating" and "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treating" and "treatment" as used herein also include prophylactic treatment. For example, a subject with early cancer, for example an early stage lymphoma, can be treated to prevent progression or alternatively a subject in remission can be treated with a compound or composition described herein to prevent recurrence.

[0069] It is shown herein that B cell lymphoma cells express NMT1, but not NMT2. This is in contrast to the leukemic and other cells tested which express both NMT1 and NMT2. (As shown in Figures 1 and 4)

[0070] It is further shown herein that B lymphoma cells are sensitive to inhibition of cell viability by NMT inhibitors.

[0071] In one example, the NMT inhibitor is tris-dibenzylideneacetone-dipalladium (Tris-DBA) (Figure 2)

[0072] In other examples, the NMT inhibitor 2-hydroxymyristae (HMA) is used to inhibit B lymphoma cells.

[0073] In yet another example, the pyrazole sulphonamide inhibitor of *T. brucie* NMT [J.A.Frearson et al (2010) Nature. 464.728-723)] (DDD85646) is used to inhibit B lymphoma cells. (Figure 5).

[0074] In a specific example, treatment of a subject with B lymphoma comprises administering said subject with an NMT inhibitor.

[0075] NMT inhibitor compounds or derivatives may be used in the present invention for the treatment of NMT2 deficient cancer.

[0076] There term "deficient" as used herein refers broadly to inhibition, reduction or elimination of (as compared to wild type or control samples), for example, NMT synthesis, levels, activity, or function, as well as inhibition of the induction or stimulation of synthesis, levels, activity, or function of the protein of NMT (for example NMT 1 or NMT2). The term also refers to any metabolic or regulatory pathway which can regulate the synthesis, levels, activity, or function of NMT. The term includes also includes inhibition, reduction or elimination resulting form binding with other molecules and complex formation. Therefore, the term "NMT deficient" refers to that which results in the inhibition, reduction, or elimination of protein function or protein pathway function. However, the term does

not imply that each and every one of these functions must be inhibited at the same time.

[0077] In some examples, a cancer may be identified as being deficient in NMT by determining the presence of a mutation in a NMT gene. Such methods of nucleic acid detection and amplification are well known to the skilled worker.

[0078] For example the nucleic acid to be amplified may be from a biological sample. Various methods (such as phenol and chloroform extraction) of extraction are suitable for isolating the DNA or RNA. Nucleic acid extracted from a sample can be amplified using nucleic acid amplification techniques well known in the art. Non limiting examples include chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), nested PCR, ligase chain reaction, amplifiable RNA reporters, Q-beta replication, transcription-based amplification, boomerang DNA amplification, strand displacement activation, cycling probe technology, isothermal nucleic acid sequence based amplification (NASBA), or other sequence replication assays or signal amplification assays may also be used.

[0079] Methods of amplification are well-known in the art. Some methods employ reverse transcription of RNA to cDNA.

[0080] In one example, PCR is used to amplify a target sequence of interest, e.g., a NMT2 sequence.

[0081] Nucleic acids may be amplified prior to detection or may be detected directly during an amplification step, e.g., "real-time" methods. In some embodiments, the target sequence is amplified using a labeled primer such that the resulting amplicon is detectably labeled. In some embodiments, the primer is fluorescently labeled. In some embodiments, the target sequence is amplified and the resulting amplicon is detected by electrophoresis.

[0082] The level of gene expression can be determined by assessing the amount of NMT2 mRNA in a sample. Methods of measuring mRNA in samples are known in the art. To measure mRNA levels, the cells in the samples can be lysed and the levels of mRNA in the lysates or in RNA purified or semi-purified from lysates can be measured by any variety of methods familiar to those in the art. Such methods include, without limitation, hybridization assays using detectably labeled DNA or RNA probes, e.g., northern blotting, or quantitative or semi-quantitative RT-PCR methodologies using appropriate oligonucleotide primers. Alternatively, quantitative

or semi-quantitative in situ hybridization assays can be carried out using, for example, tissue sections, or unlysed cell suspensions, and detectably labeled, e.g., fluorescent, or enzyme-labeled, DNA or RNA probes. Additional methods for quantifying mRNA include RNA protection assay ("RPA"), cDNA and oligonucleotide microarrays, representation difference analysis ("RDA"), differential display, EST sequence analysis, serial analysis of gene expression ("SAGE"), and multiplex ligation-mediated amplification with the Luminex FlexMAP ("LMF").

[0083] Amplification can also be monitored using "real-time" methods. Real time PCR allows for the detection and quantitation of a nucleic acid target. Typically, this approach to quantitative PCR utilizes a fluorescent dye, which may be a double-strand specific dye, such as SYBR Green.RTM. I. Alternatively, other fluorescent dyes, e.g., FAM or HEX, may be conjugated to an oligonucleotide probe or a primer. Various instruments capable of performing real time PCR are known in the art. The fluorescent signal generated at each cycle of PCR is proportional to the amount of PCR product. A plot of fluorescence versus cycle number is used to describe the kinetics of amplification and a fluorescence threshold level is used to define a fractional cycle number related to initial template concentration. When amplification is performed and detected on an instrument capable of reading fluorescence during thermal cycling, the intended PCR product from non-specific PCR products can be differentiated using melting analysis. By measuring the change in fluorescence while gradually increasing the temperature of the reaction subsequent to amplification and signal generation it may be possible to determine the (Δct) of the intended product(s) as well as that of the nonspecific product.

[0084] The methods may include amplifying multiple nucleic acids in sample, also known as "multiplex detection" or "multiplexing." As used herein, the term "multiplex PCR" refers to PCR, which involves adding more than one set of PCR primers to the reaction in order to detect and quantify multiple nucleic acids, including nucleic acids from one or more target gene markers. Furthermore, multiplexing with an internal control, e.g., 18s rRNA, GADPH, or .beta.-actin) provides a control for the PCR without reaction.

[0085] In some examples, a cancer may be identified as being deficient in NMT by determining epigenetic inactivation a NMT gene, or loss of the loss of protein function.

[0086] In some examples, a cancer may be identified as being deficient in NMT by determining the activity of NMT (including NMT1 or NMT2) in a sample of cells from a subject. Activity may be determined relative to a control, for example in the case of defects in cancer cells, relative to non-cancerous cells, preferably from the same tissue. Thus, a cancer deficient in NMT may have reduced or eliminated NMT activity and/or expression. The activity of NMT may be determined by using techniques well known in the art. In these examples, a cancer deficient in NMT has a reduced or eliminated activity.

[0087] In some examples, a cancer may be identified as NMT deficient by determining the amount, concentration and/or levels of NMT protein.

[0088] In some examples, a cancer may be identified as NMT deficient by determining the amount of myristoylated proteins in a biological sample from a subject with cancer, or suspected of having cancer. In this example, the presence, absence or amount of myristoylated protein can be determined, for example, using click chemistry using appropriate fatty acid analogs. Non-limiting methods are described herein, in the Materials and Method. Alternate methods of determining the presence, absence, or amount of myristoylated proteins will be known to the skilled worker. A sample which has a reduced amount myristoylated protein in a sample (optionally as compared to a control) is indicative of an NMT deficient sample, or NMT deficient cancer.

[0089] In some examples, a cancer may be identified as NMT deficient by determining the amount of the amount of acylation of proteins in a biological sample from a subject with cancer, or suspect of having cancer. In this example, the presence, absence or amount of acylation of proteins can be determined. Such methods would be know to the skilled worker. A sample which as a reduced amount of acylation of proteins in a sample (optionally as compared to a control) is indicative of an NMT deficient sample, or NMT deficient cancer.

[0090] In some examples, a cancer may be identified as a NMT deficient by determining the presence of one or more sequence variations such as mutations and polymorphisms may include a deletion, insertion or substitution of one or more nucleotides, relative to the wild-type nucleotide sequence. The one or more variations may be in a coding or non-coding region of the nucleic acid sequence and, may reduce or abolish the expression or function of NMT. Thus, the variant nucleic acid

may encode a variant polypeptide which has reduced or abolished activity or may encode a wild-type polypeptide which has little or no expression within the cell, for example through the altered activity of a regulatory element.

[0091] A variety of methods may be used for determining the presence or absence of a particular nucleic acid sequence in a sample obtained from a subject.

[0092] In some examples, a cancer may be identified as NMT-deficient by assessing the level of expression or activity of a positive or negative regulator of NMT of a component of the NMT pathway. Expression levels may be determined, for example, by immunoassays, such as immoblotts and ELISA, and nucleic acid detection methods, such as RT-PCR, nanostring technology, RNA-seq, nucleic acid hybridisation or karyotypic analysis.

[0093] In some examples, a cancer may be identified as being deficient in NMT by determining the presence in a cell sample from the individual of one or more variations, for example, polymorphisms or mutations in NMT.

[0094] Mutations and polymorphisms associated with cancer may also be detected at the protein level by detecting the presence of a variant (i.e. a mutant or allelic variant) polypeptide.

[0095] In another example, there is provided a method a treating a subject with cancer, wherein said cancer comprises cancer cells which are deficient in NMT2, comprising administering to said subject an NMT inhibitor and/or an NMT1 inhibitor.

[0096] The term "inhibit" or "inhibitor" as used herein, refers to any method or technique which inhibits protein synthesis, levels, activity, or function, as well as methods of inhibiting the induction or stimulation of synthesis, levels, activity, or function of the protein of interest, for example NMT2. The term also refers to any metabolic or regulatory pathway which can regulate the synthesis, levels, activity, or function of the protein of interest. The term includes binding with other molecules and complex formation. Therefore, the term "inhibitor" refers to any agent or compound, the application of which results in the inhibition of protein function or protein pathway function. However, the term does not imply that each and every one of these functions must be inhibited at the same time.

[0097] In another example, there is provided a method of treating a subject with cancer, wherein said cancer comprises cancer cells deficient in NMT1, comprising administering to said subject an NMT inhibitor and/or an NMT2 inhibitor.

[0098] In some examples, treatment methods comprise administering to a subject a therapeutically effective amount of a compound described herein and optionally consists of a single administration, or alternatively comprises a series of applications. In a specific example, said compound is a NMT inhibitor, an NMT1 inhibitor and/or an NMT2 inhibitor.

[0099] In a more specific example, the NMT inhibitor is Tris-DBA, HMA, DDD85646, or derivatives thereof.

[00100] In other examples, the compounds and/or compositions are provided in a pharmaceutically effect amount suitable for administration to a subject.

[00101] The term "pharmaceutically effective amount" as used herein refers to the amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by a researcher or clinician. This amount can be a therapeutically effective amount.

[00102] The compounds and compositions are provided in a pharmaceutically acceptable form.

[00103] The term "pharmaceutically acceptable" as used herein includes compounds, materials, compositions, and/ or dosage forms which are suitable for use in contact with the tissues of a subject without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. is also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

[00104] The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

[00105] A compound or composition may be administered alone or in combination with other treatments, either simultaneously or sequentially, dependent upon the condition to be treated.

[00106] The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing the active compound into association with a

carrier, which may constitute one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[00107] The compounds and compositions may be administered to a subject by any convenient route of administration, whether systemically/peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot / for example, subcutaneously or intramuscularly.

[00108] Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in- water liquid emulsion or a water- in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

[00109] Formulations suitable for parenteral administration (e.g., by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non- aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection.

[00110] The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection

solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs .

[00111] Compositions comprising compounds disclosed herein may be used in the methods described herein in combination with standard chemotherapeutic regimes or in conjunction with radiotherapy.

[00112] In the case of lymphoma in a patient, know treatments are dependent upon the subject being treated, the type of disease, and its stage. Existing treatment modalities for lymphoma are known to the skilled worker. Accordingly, there know treatments may be used together with the NMT inhibitors disclosed herein.

[00113] Common drug combinations for use in treating lymphomas include, but are not limited, to CHOP (i.e., cyclophosphamide, doxorubicin, vincristine, and prednisone), GAP-BOP (i.e., cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone), m-BACOD (i.e., methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and leucovorin), ProMACE-MOPP (i.e., prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin with standard MOPP), ProMACE-CytaBOM (prednisone, doxorubicin, cyclophosphamide, etoposide, cytarabine, bleomycin, vincristine, methotrexate, and leucovorin), and MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin, and leucovorin). For relapsed aggressive non-Hodgkin's lymphoma the following chemotherapy drug combinations may be used with the compounds and compositions described herein: IMVP-16 (i.e., ifosfamide, methotrexate, and etoposide), MIME (i.e., methyl-gag, ifosfamide, methotrexate, and etoposide), DHAP (i.e., dexamethasone, - 16 high dose cytarabine, and cisplatin), ESHAP (i. e., etoposide, methylprednisone, high dosage cytarabine, and cisplatin), CEFF(B) (i.e., cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin), and CAMP (i.e., lomustine, mitoxantrone, cytarabine, and prednisone).

[00114] Treatment for salvage chemotherapy used for certain lymphomas such as for relapsed, resistant Hodgkin's Disease include but are not limited to VABCD (i.e., vinblastine, doxorubicin, dacarbazine, lomustine and bleomycin), ABDIC (i.e., doxorubicin, bleomycin, dacarbazine, lomustine, and prednisone), CBVD (i.e.,

lomustine, bleomycin, vinblastine, dexamethasone), PCVP (i.e., vinblastine, procarbazine, cyclophosphamide, and prednisone), CEP (i.e., lomustine, etoposide, and prednimustine), EVA (i.e., etoposide, vinblastine, and doxorubicin), MOPLACE (i.e., cyclophosphamide, etoposide, prednisone, methotrexate, cytaravine, and vincristine), MIME (i.e., methyl-gag, ifosfamide, methotrexate, and etoposide), MINE (i.e., mitoquazone, ifosfamide, vinorelbine, and etoposide), MTX-CHOP (i.e., methotrexate and CHOP), CEM (i.e., lomustine, etoposide, and methotrexate), CEVD (i.e., lomustine, etoposide, vindesine, and dexamethasone), CAVP (i.e., lomustine, melphalan, etoposide, and prednisone), EVAP (i.e., etoposide, vinblastine, cytarabine, and cisplatin), and EPOCH (i.e., etoposide, vincristine, ; doxorubicin, cyclophosphamide, and prednisone).

[00115] It will be appreciated that alternate methods to inhibit NMT1 or NMT2 may be used in a synthetic lethal strategy for the treatment of cancer, and in particular the treatment of B cell lymphoma. For example, expression of NMT1 or NMT2 may be inhibited using anti-sense or RNAi technology. The use of these approaches to down-regulate gene expression and/or protein activity is known to the skilled worker.

[00116] In another embodiment of the present disclosure there is provided a method for determining the benefit of NMT2-inhibitor and/or NMT1-inhibitor treatment of a patient.

[00117] In one example, a method of the present disclosure comprises qualitatively or quantitatively determining, analyzing or measuring a sample from a subject with cancer, or suspected of having cancer, for the presence or absence, or amount or concentration, of NMT1 and/or NMT2.

[00118] In another example, a method of the present disclosure comprises qualitatively or quantitatively determining, analyzing or measuring a sample from a subject with cancer, or suspected of having cancer, for the presence or absence, or amount or concentration, of myristolayted proteins.

[00119] In another example, a method of the present disclosure comprises qualitatively or quantitatively determining, analyzing or measuring a sample from a subject with cancer, or suspect of having cancer, for the presence or absence, or amount of concentration of acylated proteins.

[00120] The term "sample" as used herein refers to any sample from a subject, including but not limited to a fluid, cell or tissue sample that comprises cancer cells,

or which is suspected of containing cancer cells, which can be assayed for gene expression levels, proteins levels, enzymatic activity levels, and the like. The sample may include, for example, a blood sample, a fractionated blood sample, a bone marrow sample, a biopsy, a frozen tissue sample, a fresh tissue specimen, a cell sample, and/or a paraffin embedded section, material from which RNA can be extracted in sufficient quantities and with adequate quality to permit measurement of relative mRNA levels, or material from which polypeptides can be extracted in sufficient quantities and with adequate quality to permit measurement of relative polypeptide levels.

[00121] The determination, analysis or measurement of NMT1 or NMT2, or the presence or absence of NMT1 and/or NMT2 can be correlated with the benefit of NMT1-inhibtor or NMT2-inhibitor treatment of cancer in the patient.

[00122] The determination, analysis or measurement of myristolyated proteins, or the presence or absence of myristolyated proteins can be correlated with the benefit of NMT1-inhibtor or NMT2-inhibitor treatment of cancer in the patient.

[00123] In a specific example, antibodies of the present invention are immunoreactive or immunospecific for, and therefore specifically and selectively bind to a protein of interest, for example the protein NMT1 or NMT2. In one example, antibodies which are immunoreactive and immunospecific for human NMT1 or NMT2 can be used. Antibodies for human NMT1 or NMT2 are preferably immunospecific. The term "antibody" and "antibodies" includes, but is not limited to, monoclonal and polyclonal antibodies.

[00124] In another example, antibodies of the present invention are immunoreactive or immunospecific for, and therefore specifically and selectively bind to both NMT1 and NMT2 protein. In this example, antibodies which are immunoreactive and immunospecific for both human NMT1 and NMT2 can be used. Antibodies for human NMT1 and NMT2 are preferably immunospecific. In this example, and owing to the different molecular mass of NMT1 and NMT2, it is possible identify the presence or absence of both proteins using a single antibody, using, for example SDS-PAGE and immunoblotting. The term "antibody" and "antibodies" includes, but is not limited to, monoclonal and polyclonal antibodies.

[00125] The term "binds specifically" refers to high avidity and/or high affinity binding of an antibody to a specific polypeptide e.g., an epitope of NMT1 or NMT2.

Antibody binding to its epitope on this specific polypeptide is stronger than binding of the same antibody to any other epitope, particularly those which may be present in molecules in association with, or in the same sample, as the specific polypeptide of interest. Antibodies which bind specifically to a polypeptide of interest may be capable of binding other polypeptides at weak, yet detectable, level. Such weak binding, or background binding, is readily discernable from the specific antibody binding to the compound or polypeptide of interest, e.g., by use of appropriate controls, as would be known to the worker skilled in the art.

[00126] In one example, a sample containing cancerous cells or suspected as containing cancerous cells is obtained from a subject with cancer. Collection of such a sample is well known to the skilled worker. In a specific example, the sample is a blood sample. Methods of obtaining a sample sample, processing and/or storage of such a sample are also well known to the skilled worker.

[00127] In a specific example, the detection, analysis or measurement of NMT1 or NMT2 protein within a sample is carried out using immunohistochemistry. It will be clear to the skilled worker that other immuno assays, both qualitative or quantitative, may be used in the present invention.

[00128] Other examples that may be used in the detection, analysis or measurement of NMT1 or NMT2 include, but are not limited to, immunoblotting, ELISA, indirect immuno-fluorescence, multiplexing bead technology, immunoprecipitation and mass spectrometry from sample obtain from the subject. In practice, in the example in which a patient sample is determined to have low or absent NMT2 staining, the subject is considered a good candidate for NMT-inhibitor therapy.

[00129] In another example, a method of the present disclosure comprises qualitatively or quantitatively determining, analyzing or measuring the activity of NMT1 and/or NMT2 protein activity in biological sample from a subject with cancer patient for the presence or absence or amount of NMT1 and/or NMT2 activity. In this example, the uses of substrates (natural or synthetic) of NMT1 or NMT2 are used to identify a sample in which NMT1 or NMT2 activity is present, absent, or the amount thereof.

[00130] In practice, in the example in which a subject sample is determined to be NMT2 deficient, , the subject is considered a good candidate for administration on an NMT inhibitor.

[00131] In practice, in the example in which a patient sample is determined to have low or absent NMT2 activity, the subject is considered a good candidate for administration on an NMT inhibitor.

[00132] In practice, in the example in which a patient sample is determined to have low or absent amount of myristolated protein, the subject is considered a good candidate for administration on an NMT inhibitor.

[00133] In practice, in the example in which a subject sample is determined to have a low or absent amount of acylated protein, the subject is considered a good candidate for administration on an NMT inhibitor.

[00134] In another example, a method of the present disclosure comprises identifying a mutation, deletion, or the like, in the NMT1 or NMT2 gene in a sample from a subject with cancer or suspect of having cancer. Wherein, said mutation, deletion, or the like, in NMT1 or NMT2 gene results in a loss of diminishment of NMT1 or NMT2 protein activity in cancer cells within said sample. Methods of identifying such mutations, deletions, or the like, in NMT1 or NMT2 are known to the skilled worker, and include, but are not limited to, RFLP, RT-PCT, microarray analysis, and/or any suitable type of DNA sequencing. In practice, in the example in which a patient sample is determined to have a mutation, deletion, or the like, in NMT2 which results in a low or absent NMT2 protein activity, the subject is considered a good candidate for NMT-inhibitor therapy.

[00135] In another example, a method of the present disclosure comprises identifying a mutation, deletion, or the like, in the NMT1 or NMT2 mRNA in a sample from a subject with cancer or suspect of having cancer. Wherein, said mutation, deletion, or the like, in NMT1 or NMT2 mRNA results in a loss of diminishment of NMT1 or NMT2 protein activity in cancer cells within said sample. Methods of identifying such mutations, deletions, or the like, in NMT1 or NMT2 mRNA are known to the skilled worker, and include, but are not limited to, Northern blotting, RT-PCR, microarray analysis, and/or any suitable type of mRNA sequencing. In practice, in the example in which a patient sample is determined to have a mutation, deletion, or the like, in NMT2 mRNA which results in a low or

absent NMT2 protein activity, the subject is considered a good candidate for NMT-inhibitor therapy.

[00136] In another example, a method of the present disclosure, there is provided a method for the treatment of a subject suffering from cancer, associated with a defect in NMT1 or NMT2, comprising administering to said subject an inhibitor of NMT.

[00137] Examples of inhibitors include, but are not limited to, small molecules, antibodies, peptide fragments, and/or nucleic acid molecules.

[00138] Specific examples of small molecules include Tris-DBA, HMA, DDD85646, and their derivatives. The term "derivatives" as used herein includes, but is not limited to, salts, coordination complexes, esters such as in vivo hydrolysable esters, free acids or bases, hydrates, prodrugs or lipids, coupling partners.

[00139] Peptide fragments may be prepared wholly or partly by chemical synthesis that active site of NMT1. Peptide fragments can be prepared according to established, standard liquid or solid-phase peptide synthesis methods, which will be known to the skilled worker.

[00140] Nucleic acid inhibitors, or the complements thereof, inhibit activity or function by down-regulating production of active polypeptide. This can be monitored using conventional methods well known in the art, for example by screening using real time PCR as described in the examples.

[00141] Examples of nucleic acid inhibitors include anti-sense or RNAi technology, the use of which is to down-regulate gene expression is well-established in the art. Anti-sense oligonucleotides may be designed to hybridise to the complementary sequence of nucleic acid, pre-mRNA or mature mRNA, interfering with the production of the base excision repair pathway component so that its expression is reduced or completely or substantially completely prevented. In addition to targeting coding sequence, anti- sense techniques may be used to target control sequences of a gene, e.g. in the 5' flanking sequence, whereby the anti-sense oligonucleotides can interfere with expression control sequences.

[00142] An alternative to anti-sense is to use a copy of all or part of the target gene inserted in sense, that is the same, orientation as the target gene, to achieve reduction in expression of the target gene by co-suppression.

[00143] Additionally, double stranded RNA (dsRNA) silencing may be used. dsRNA mediated silencing is gene specific and is often termed RNA interference (RNAi).

[00144] In another example, nucleic acid is used which on transcription produces a ribozyme, able to cut nucleic acid at a specific site and therefore also useful in influencing NMT.

[00145] In yet another example, small RNA molecules may b e employed to regulate gene expression. These include targeted degradation o f mRNAs by small interfering RNAs (siRNAs), post transcriptional gene silencing (PTGs), developmentally regulated sequence-specific translational repression of mRNA by micro-RNAs (miRNAs) and targeted transcriptional gene silencing.

[00146] In yet another example, the expression of a short hairpin RNA molecule (shRNA) in the cell may be used. A shRNA consists of short inverted repeats separated by a small loop sequence. One inverted repeat is complimentary to the gene target. In the cell the shRNA is processed by DICER into a siRNA which degrades the target NMT gene mRNA and suppresses expression. In a preferred embodiment the shRNA is produced endogenously (within a cell) by transcription from a vector.

[00147] A defect in NMT1 or NMT2 is a NMT1 or NMT2 deficient phenotype which may be deficient in a component of a NMT1 or NMT2 mediated pathway i.e., expression of activity of a component of the pathway may be reduced or abolished in the cancer cell relative to control cells. In some embodiments, the cancer cell may be deficient in NMT1 or NMT2 i.e., expression of activity of NMT1 or NMT2 may be reduced or abolished in the cancer cell relative to control cells.

[00148] Accordingly, there is provided the use of NMT2 as a marker for one or more of diagnosis, prognosis, classifying, or monitoring of cancer in a subject. In some examples, NMT2 is said measured using an assay selected from immunoassays or nucleic acid detection, or protein activity.

[00149] There is also provided the use of protein myristoylation as a marker for one or more of diagnosis, prognosis, classifying or monitoring cancer in a subject.

[00150] There is also provided the use of protein acylation as a marker for one or more of diagnosis, prognosis, classifying or monitoring cancer in a subject.

[00151] In some example, said cancer is lymphoma. In more specific examples, said lymphoma is B cell lymphoma. In more specific examples, said B cell lymphoma is follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, B-CLL/SLL, immunocytoma/Waldenstrom's, MALT-type/monocytoid B cell lymphoma, Burkitt's lymphoma, a pediatric lymphoma, or anaplastic large cell lymphoma.

[00152] Methods of the invention are conveniently practiced by providing the compounds and/or compositions used in such method in the form of a kit. Such a kit preferably contains the composition. Such a kit preferably contains instructions for the use thereof.

[00153] To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these example are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

[00154] **EXAMPLES**

[00155] In the following examples, standard methodologies were employed, as would be appreciated by the skilled worker.

[00156] **MATERIALS AND METHODS**

[00157] **Antibodies and reagents.**

[00158] Tris dibutylbenzinylidene acetone paladium (TrisDBA) was a kind gift of Dr. Arbiser (U. Alabama). DDD85646 was synthesised as described [J.A.Frearson et al (2010) Nature. 464.728-723)] and was obtained from Dr. David Gray and Paul Wyatt, Dundee University)

[00159] Mouse anti-NMT1 (clone 14; 1:1000) and mouse anti-NMT2 (clone 30; 1:2000) antibodies were from BD Biosciences, San Jose, CA, USA. Rabbit anti-NMT1 (polyclonal, 1:3000) was purchased from Proteintech, Chicago, IL, USA. Rabbit anti-GFP (1:20,000), anti-PARP-1 (1:5000), anti-GAPDH (1:5000) and anti-c-terminal PAK2 (1:2000) antibodies were from Eusera (www.eusera.com), Edmonton, AB, Canada. Mouse anti-α-tubulin (1:15,000) and rabbit-anti-V5 (1:10,000) antibodies were purchased from Sigma Aldrich, St. Louis, MO, USA. Mouse anti-His (1:2000) was from Qiagen, Germany. Rabbit anti-cleaved caspase-8 (1:1000) and anti-cleaved caspase-3 (1:1000) were both from Cell Signaling, Danvers,

MA, USA. Enhanced chemiluminesce (ECL) Plus and ECL Prime western blotting detection kits were purchased from GE Healthcare, Pittsburgh, PA, USA. Unless stated otherwise, all chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of the highest purity available.

[00160] DNA constructs. Engineering of V5- and His-tagged NMT1 and NMT2 constructs. NMT1 and NMT2 entry vectors which are compatible with the Gateway cloning system (Life Technologies, Grand Island, N.Y., USA) were purchased from Genecopoeia (Rockville, MD, USA). The NMT1 and NMT2 genes were incorporated into the destination vector pcDNA3.1/nV5 DEST (Life Technologies) using the LR clonase enzyme (Life Technologies) according to the manufacturer's instructions to generate the plasmids N-terminally-tagged NMTs (His-NMT1, His-NMT2, V5-NMT1 and V5-NMT2). V5-tagged NMT constructs were used for mammalian cell expression, whereas His-NMT constructs were used for bacterial expression. The cloning products were confirmed by DNA sequencing (Eurofins MWG Operon, Huntsville, AL, USA).

[00161] Cell culture. Origin of the B cells were a gift from Dr. Jim Stone or were obtained from ATCC. All reagents from cell culture were purchased from Invitrogen. B cells were cultured at 37°C and 5% CO_2 in a humidified incubator and maintained in RPMI media supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin.

[00162] Cell lysis. Cells were washed in cold PBS, lysed in 0.1% SDS-RIPA buffer [50 mM Tris, 150 mM NaCl, 1% Igepal CA-630, 0.5% NaDC, 2 mM $MgCl_2$, and 1x complete protease inhibitor (Roche Diagnostics); pH 8.0] and rocked for 15 min at 4°C. Cell lysates supernatant were obtained after a 16,000g centrifugation for 15 min at 4°C.

[00163] Induction of apoptosis. Unless mentioned otherwise, apoptosis was induced using 2.5 μM staurosporine (STS) (Sigma Aldrich, St. Louise, MO, USA) and 5 μg/mL cycloheximide (ICN Biochemicals Inc. Aurora, OH, USA) in order to inhibit protein translation and enhance apoptosis induction.

[00164] Incubation with NMT inhibitors. Tris dibutylbenzinylidene acetone paladium (TrisDBA) was a kind gift of Dr. Arbiser. Cells were incubated at increasing concentrations for 24 hours with TrisDBA (or DMSO for control) or for 24 and 48 hours with DDD85646.

[00165] **B cell transfection.** B cells were transfected using the Neon® transfection system (Life technologies) following manufacturer's instructions and optimized protocol for Ramos B cells transfection (pulse voltage 1,300V; pulse width 20 ms, 2 pulses and $7.7.10^6$ cells/mL) adapted for 100 µL tips. Classically, two transfections were pulled to obtained enough living cells to perform a viability assay.

[00166] **Cell viability assay.** B and T cell viability was measured using the trypan blue exclusion method. Cells were grown in confluency conditions (2×10^6 cells/mL maximum) assuring the minimum basal apoptosis. After incubation with NMT inhibitors, about 20 000 cells (10 µL) were incubated with 10 µL of TC10™ Trypan Blue Dye (Biorad) for 15 min. Cell viability was quantified using the TC10™ automated cell counter (Biorad).

[00167] ***In vitro*** **NMT activity assay.** N-myristoyltransferase activity assay protocol was adapted from Raju, R. V., and Sharma, R. K. (1999) Preparation and assay of myristoyl-CoA:protein N-myristoyltransferase. *Methods Mol Biol* **116**, 193-211. [^{3}H] myristoyl-CoA was freshly synthesized for each experiment, as previously described by Towler, D., and Glaser, L. (1986) Protein fatty acid acylation: enzymatic synthesis of an N-myristoylglycyl peptide. *Proc Natl Acad Sci U S A* **83**, 2812-2816. Briefly, cells were resuspended in 0.25 M sucrose buffer (50 mM NaH_2PO_4, pH 7.4) and subjected to 2 rounds of sonication at level 6.0 on a Branson Sonicator. Reaction mixture is composed of 10 µL of cell extract (about 20 µg of proteins) incubated in NMT activity buffer (0.26M Tris-HCl, 3.25 mM EGTA, 2.92 mM EDTA and 29.25 mM 2-mercaptoethanol, 1% Triton X-100, pH 7.4) and myristoylable or non-myristoylable decapeptide corresponding to the N-terminal sequence of truncated-Bid (0.1 mM dissolved in water). Reaction was started by the addition of 7.4 µL (≈ 10pMol) of freshly synthesized [^{3}H] myristoyl-CoA (final mixture volume = 25 µL) and incubated for 15 min at 30°C. The reaction is stopped by spotting 15 µL of the reaction mixture on a P81 phosphocellulose paper disc (Whatman, Kent, UK) and dried for 30 seconds. Discs were washed (washing buffer: 25 mM Tris buffer, pH 7.4) to remove the residual radioactivity ([^{3}H]-myristate and [^{3}H]-myristoyl-CoA) while [^{3}H]-myristoyl-peptide is retained on the phosphocellulose paper. Radioactivity was quantified by liquid scintillation counting and converted into pMol of myristoylated peptide (Raju, R. V., and Sharma, R. K. (1999) Preparation and assay of myristoyl-CoA:protein N-myristoyltransferase. *Methods Mol Biol* **116**, 193-211

[00168] **RT-PCR.** qRT-PCR was performed with Taqman NMT1 and NMT2 probes using an 18S probe as an internal control. The difference in the number of cycle times (Δct) was calculated by subtracting the cycle time (ct) at which we see an exponential increase in the expression of the 18S internal control for each cell type from the NMT cycle time, again at a point where exponential increase of the signal is seen.

[00169] **Example 1**

[00170] Figure 1 depicts the analysis of NMT1 and NMT2 expression in normal cells and various B cell lymphomas and T cell leukemias. This figure shows the near complete absence of expression of NMT2 in B Lymphoma cell lines (BL-2, Ramos), which express only NMT1 in comparison to normal B cells (EBV transformed human B lymphocytes, L0) and human leukemic T cell lines (Jurkat, MOLT-4, CEM).

[00171] While not wishing to be bound by theory, those cells which express only one NMT isozyme, for example Burkitt's lymphoma cells which shows the near complete absence of NMT2, are likely to have altered myristoylated protein profiles.

[00172] A sample which has a reduced amount myristoylated protein in a sample (optionally as compared to a control) is indicative of an NMT deficient sample, or NMT deficient cancer. Such an NMT deficient cancer is suitable to treatment with an inhibitor or NMT1.

[00173] **Example 2**

[00174] Figure 2 depicts the sensitivity of various B cell lymphomas and T cell leukemias to the NMT inhibitors tris-dibenzylideneacetone-dipalladium (Tris-DBA). Various B and T cells were incubated for 24h with increasing concentration of Tris DBA. Cell viability was measured using trypan blue exclusion method and adjusted to 100% for control. Cell survival curves measured by trypan blue exclusion show that B cell lymphomas are more sensitive to the NMT inhibitor tris-dibenzylideneacetone-dipalladium (Tris-DBA).

[00175] **Example 3**

[00176] Figure 3 depicts the inhibition of N-myristoyltransferase (NMT) by tris-dibenzylideneacetone-dipalladium (Tris-DBA).

[00177] NMT activity was assayed using a peptide myristoylation assay with purified recombinant NMT1 and NMT2. NMT activity was calculated from the

amount of radiolabeled myristoylpeptide produced and detected on phosphocellulose paper (adapted from King et al. 1991, Anal Biochem.).

[00178] This figure shows that tris-dibenzylideneacetone-dipalladium (Tris-DBA) inhibits NMT *in vitro* using purified recombinant NMTs enzymes.

[00179] **Example 4**

[00180] Figure 4 depicts the results of immunoblotts in which lymphoma cell lines were probed with antibodies against NMT 1 (Panel A) and NMT 2 (Panel B). The legend of Figure 4 corresponds as follows: IM9: B lymphoblast; BL2: Burkitt's lymphoma; CEM: T cell leukemia; Karpas 299: T cell lymphoma; Sup-M2: ALCL; UCONN: ALCL (ALCL: Anaplastic large-cell lymphoma); DAUDI: Burkitt's lymphoma; Ramos: Burkitt's lymphoma BJAB: Burkitt's lymphoma; HD-MYZ: Hodgkin lymphoma; KM-H2: Hodgkin lymphoma; L428: Hodgkin lymphoma; Jurkat: T cell leukemia.

[00181] **Example 5**

[00182] Figure 5 depicts the effectiveness of NMT inhibitors on Burkitt's Lymphoma cell line Ramos in comparison to immortalized normal B lymphocytic cell line (IM9) after 48 hours, at different concentrations.

[00183] **Example 6**

[00184] In this example, transfection of Ramos B lymphoma cells (which, as shown herein, expresses NMT1) with pcDNA3.1-V5-NMT2 increased survival to TrisDBA (5 ug/ml) 2.5 fold vs control cells transfected with empty plasmid vector. In Figure 6, 20 X 10^6 Ramos B lymphoma cells were transfected with 32μg of DNA (pcDNA3.1-V5-empty or pcDNA3.1-V5-NMT2) using the Neon Transfection System (Invitrogen) following the recommended protocol for Ramos cell line (1,350 Volt, 30 ms). Transfected cells were centrifuged 5 minutes at 1200rpm to remove dead cells and cellular debris. Cells in the supernatant were allowed to recover for 6 hours in complete RPMI. After a PBS wash, cells were resuspended and grown in RPMI containing TrisDBA (5ug/ml) for 24hours then counted using the trypan blue exclusion method (Panel A). Cells were lysed and western blotting (ECL) was performed to confirm expression NMT2 with antibodies against NMT2, and GAPDH for loading control (Panel B).

[00185] **Example 7**

[00186] In this example, qRT-PCR was performed with Taqman NMT1 and NMT2 probes using an 18S probe as an internal control. The difference in the number of cycle times (Δct) was calculated by subtracting the cycle time (ct) at which we see an exponential increase in the expression of the 18S internal control for each cell type from the NMT cycle time, again at a point where exponential increase of the signal is seen. As shown in Table 1, below, the ratio of NMT2 to NMT1 expression is decreased (up to 60 fold) in B lymphoma cell lines. While not wishing to be bound by theory, these results may suggest that that a reduction in mRNA encoding for NMT2 may be responsible for the reduction of NMT 2 protein levels assessed by Western blotting.

[00187] Table 1

Analysis of NMT mRNA expression by qRT-PCR

		mRNA sequence	Δct (ctNMT-ct18S)	NMT mRNA expression normalized to 18S	mRNA fold decrease of NMT2 vs NMT1
Immortalized Normal B cell line	IM9	NMT1	1.25	0.42	0.27
		NMT2	3.12	0.12	
	L0	NMT1	4.02	0.06	1.95
		NMT2	3.06	0.12	
B cell lymphoma cell line	Ramos	NMT1	-1.21	2.31	24.68
		NMT2	3.42	0.09	
	BL2	NMT1	-0.088	1.06	60.41
		NMT2	5.83	0.02	

[00188] All publications, patents and patent applications mentioned in this Specification are indicative of the level of skill those skilled in the art to which this invention pertains and are herein incorporated by reference to the same extent as if each individual publication patent, or patent application was specifically and individually indicated to be incorporated by reference.

[00189] The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modification as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method of detecting a subject having cancer being a B cell lymphoma, comprising:

measuring a sample from said subject to determine whether said sample is deficient in NMT2 by determining epigenetic inactivation a NMT2 gene or loss of protein function or having a level of NMT2 protein that is low or absent compared to a control being normal B cell; wherein the B cell lymphoma is Burkett's lymphoma.

2. The method of claim 1, wherein said subject is a human subject.

3. The method of claim 1 or claim 2, wherein measuring of said sample is carried out using quantitative fluorescence activated cell sorting, enzyme linked immunosorbent assay, immunohistochemistry, quantitative immunohistochemistry, fluorescence resonance energy transfer, Forster resonance energy transfer, biomolecular fluorescence complementation, mass spectrometry, immunoblot assay or coimmunoprecipitation assay.

4. Use of an NMT1 inhibitor for the preparation of a medicament for treating a subject having a cancer being a B cell lymphoma deficient in NMT2 by determining epigenetic inactivation a NMT2 gene or loss of protein function or having a level of NMT2 protein that is low or absent compared to a control being normal B cell, wherein the B cell lymphoma is Burkett's lymphoma.

5. The use of claim 4, wherein said NMT1 inhibitor is a small molecule, an antibody, a peptide fragment, or a nucleic acid.

6. The use of claim 5, wherein said small molecule is DDD85646, or a derivative thereof.

7. The use of claim 4, wherein said small molecule is Tris-DBA, HMA, or DDD85646, or a derivative thereof.

8. The use of claims 5, wherein said antibody is a monoclonal antibody or a polyclonal antibody.

9. The use of claim 5, wherein said nucleic acid comprises a dsRNA molecule, a RNAi molecule, miRNA molecule, a ribozyme, a shRNA molecule, or a siRNA molecule.

10. The use of any one of claims 4-9, wherein said subject is a human subject.

11. The use of any one of claims 4-10, wherein a chemotherapeutic agent is to be further administered, optionally wherein said chemotherapeutic agent is CHOP, GAPBOP, m-BACOD, ProMACE-MOPP, ProMACE-CytaBOM, MACOP-B, IMVP-16, MIME, DHAP, ESHAP, CEFF(B), CAMP, VABCD, ABDIC, CBVD, PCVP, CEP, EVA, MOPLACE, MIME.

12. Use of NMT2 as a marker by determining epigenetic inactivation a NMT2 gene or loss of protein function or having a level of NMT2 protein that is low or absent compared to a control being normal B cell for one or more of *in vitro* diagnosis, prognosis, classifying, or monitoring of cancer being B cell lymphoma in a subject, wherein the B cell lymphoma is Burkitt's lymphoma.

13. Use of protein myristoylation as a marker for one or more of *in vitro* diagnosis, prognosis, classifying or monitoring cancer being a B cell lymphoma in a subject, wherein the B cell lymphoma is Burkitt's lymphoma.

14. The use of claim 13, wherein said marker is measured using an assay selected from immunoassays or nucleic acid detection, or protein activity.