The use of canagliflozin and derivatives thereof in the treatment and prevention of cancer is disclosed. Said compounds have been previously determined to be selective sodium glucose cotransporter 2 (SGLT2) inhibitors useful in the treatment of diabetes with effects that are similar to metformin. Canagliflozin has now been determined to activate AMP-activated protein kinase (AMPK) and inhibit the growth of a range of cancers. Further to this, use of canagliflozin in combination with other chemotherapeutics has now been determined to give rise to increased anti-cancer activity.
CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority from pending U.S. provisional application nos. 62/121,539 filed on February 27, 2015 and 62/169,156 filed on June 1, 2015, the contents of both of which are incorporated herein by reference in their entirety.

FIELD

[0002] The present application relates to methods, uses, compositions and kits for treating cancer, including cancer prevention. In particular, the present application relates to the use of Canagliflozin, optionally in combination with an adjunct cancer treatment, for the treatment of cancer.

BACKGROUND

[0003] Cancer is the leading cause of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012.\(^1\) Deaths from cancer are projected to continue rising, with an estimated 13.1 million deaths in 2030. An age-related increase in cancer incidence exists since somatic mutations occur at a constant rate over time. The prospect of targeting cancerous cells between the time intervals in which these mutations appear provides an opportunity to prevent cancers from transitioning to successive stages of malignancy.\(^2\) Early interventions have therefore been associated with improved patient outcomes. However, the apparent failure to control cancer related deaths highlights the need for discovering biological agents that will better protect against neoplastic events.

[0004] Given the increase in global cancer incidence with its associated mortality, there is increasing interest in strategies for disease prevention. One approach is chemoprevention, which can be defined as the use of natural, synthetic or biological agents to protect against the progression of premalignant cells to invasive disease or the use of chemicals, bioactive plant compounds or dietary components to block or reverse cancer progression.

[0005] 5′-Adenosine monophosphate activated protein kinase (AMPK) is a cellular energy sensor conserved throughout eukaryotes. This
heterotrimeric enzyme is composed of a catalytic subunit and regulatory β and Y subunits. AMPK is activated >100-fold by phosphorylation of Thr172 in the a subunit by upstream kinases liver kinase B1 (LKB1) or the Ca²⁺-dependent kinase CaMKK. 3,4

[0006] The activation of AMPK switches off ATP consuming pathways (fatty acid and protein synthesis) and switches on ATP-generating pathways (glucose uptake, fatty acid oxidation and stimulating appetite) to regulate energy homeostasis in response to nutrients, hormones, and high-density lipoprotein (HDL) cholesterol (for review see Steinberg & Kemp).

[0007] An important means to conserve energy under metabolic stress is to inhibit protein translation and cellular growth. AMPK suppresses protein synthesis through inhibition of the mammalian target of rapamycin (mTOR), a protein kinase that regulates cell growth, proliferation, protein synthesis, and has been implicated in malignant transformation and cancer. AMPK can target and block the mammalian target of rapamycin complex 1 (mTORC1) complex through multiple mechanisms to conserve energy expenditure. For example, AMPK can inhibit mTOR through phosphorylation of the tuberous sclerosis complex (TSC) proteins (TSC1 :TSC2). TSC1 :TSC2 have GTPase activity towards the small G-protein Rheb, which activates mTORC1 when it is GTP-bound. AMPK triggers TSC2 activity via direct phosphorylation on its Thr1227 and Ser1345 residues, which in turn inactivates Rheb by converting it to a GDP-bound confirmation. AMPK also inhibits mTORC1 activity through phosphorylation and inhibition of its binding partner Raptor.

[0008] In addition to inhibiting mTOR, AMPK also regulates cell survival and growth by phosphorylating a wide range of transcription factors, their co-activators, and histones. In response to metabolic stress AMPK can phosphorylate the transcription factor p53 on its Ser15 residue and cause G1-S phase cell cycle arrest. Alternatively, AMPK was also shown to phosphorylate the cyclin-dependent kinase inhibitor (CDKI) p27 on Thr198 to sequester it in the cytoplasm and promote survival in response to nutrient or growth-factor withdrawal. Furthermore, AMPK can provoke transcriptional regulation of
genes in response to bioenergenic strain through direct phosphorylation of histone H2B on Ser36.\textsuperscript{16} Lastly, AMPK induces a metabolic switch that inhibits pathways that are important for cell growth and proliferation.\textsuperscript{17} Thus, AMPK can target multiple signaling pathways by acute phosphorylation or alterations in gene transcription to attenuate stress and modulate cell survival; effects that may limit cancer cell proliferation and survival.

[0009] The homeostatic mechanisms regulating blood glucose involve an intricate balance between multiple organ systems. The inhibition of sodium glucose transporter 2 (SGLT2) prevents reabsorption of glucose in the distal kidney; thus resulting in the excretion of glucose into the urine.\textsuperscript{18}

[0010] Phlorizin is a phenolic-glucoside that was first isolated from the bark of apple trees in 1835. Phlorizin is known to induce glucosuria in animals and humans as is it able to non-selectively inhibit the sodium-dependent glucose transporters (SGLT1-2) at low nanomolar concentrations.\textsuperscript{19} However, it is not orally bioavailable and causes gastrointestinal distress because it inhibits SGLT1 (the glucose transporter found mainly in the small intestine) as well as SGLT2 (which is expressed almost exclusively in the kidney).\textsuperscript{18} These features unfortunately limit the compound’s therapeutic use.\textsuperscript{20}

[0011] Selective SGLT2 inhibitors have therefore been developed to avoid such impediments. C-glucosides have been used as an alternative to β-glucosides as they are resistant to degradation by β-glucosidase enzymes.\textsuperscript{21} Furthermore, they increase glucosuria and reduce hyperglycemia in an insulin-independent manner. Two such drugs include Canagliflozin (trade name Invokana\textsuperscript{TM}) and Dapagliflozin (trade name Forxiga\textsuperscript{TM}) which have recently received approval by the FDA, Health Canada and the European Commissions for the treatment of type 2 diabetes.\textsuperscript{22} Both Canagliflozin and Dapagliflozin display similar half-maximal inhibitory concentrations (IC\textsubscript{50}) to the SGLT2 transporter (approximately 1-2 nM). They exhibit a 400 and 1200-fold selectively towards SGLT2 respectively, which to date, is their only described target.\textsuperscript{23}

[0012] Canagliflozin has been shown to lower blood sugar in patients with type 2 diabetes. Its primary mechanism of action is reported to involve
the inhibition of the sodium glucose transporter 2 (SGLT2) protein which in turn blocks glucose resorption in the distal kidney resulting in increased glucose excretion. Studies have established that these drugs do not induce carcinogenesis or increase the risk of bladder cancer. However, whether or not Canagliflozin inhibits cancer cell proliferation and cancer cell clonogenic survival has not previously been reported.

Type 2 diabetics on SGLT2 inhibitors have increased insulin-sensitivity, reduced body weight and increased lipid metabolism, effects which are similar to other anti-diabetic agents such as the biguanide metformin. Metformin has been shown to inhibit cancer cell growth and survival and is currently the subject of over 600 cancer clinical trials.

One of the molecular targets of metformin is AMPK (discussed above) that is activated during cellular energetic challenges and apart from inhibition of mTOR also it inhibits lipogenesis through a number of mechanisms including suppression of Acetyl-CoA carboxylase (ACC); a rate limiting step in de novo fatty acid synthesis.

Cytotoxic therapy with chemotherapy or radiotherapy is used to treat inoperable cancers including metastatic disease or to prevent tumor recurrence. Cytotoxic therapy combinations are frequently used for tumors showing resistance to chemotherapy or radiation, which develops secondary to activation of cell survival mechanisms and due to genomic instability that leads to development of more aggressive tumor cell sub-populations. Single treatments often will not provide identical response in tumour cells.

In order to prevent broad-spectrum drug resistance, combination therapies will employ drugs that are effective as single agents and have different mechanisms of action. For example, Docetaxel, an inhibitor of microtubule disassembly, is commonly used as first line chemotherapy in combination with prednisone in patients who have developed castrate-resistant prostate cancer (CRPC). However, the prognosis for CRPC remains poor. It is designated as a spectrum disorder where patients can present as asymptomatic or have incurred significant metastases and
morbidities while on androgen deprivation therapies. In addition, few second-line chemotherapies have been shown to improve survival beyond docetaxel treatments. It is therefore desirable to discover novel treatments that can potentiate the effects of current therapies with minimal toxicity.

[0017] Similarly, cisplatin, a drug that produces cross-links within DNA, is used for the treatment of patients with stage IB-IV non-small cell lung cancer (NSCLC). Based on the stage presented, cisplatin is used in combination with either a non-platinum based drug or radiation. Lung cancer is the most prevalent malignancy worldwide and NSCLC accounts for approximately 80-85% of cases. Despite the improvements in overall survival with the current treatment routines, approximately 1.6 million lung cancer related deaths were reported in 2012.

[0018] Radiation is also a key cancer treatment. However, while it is used as a curative therapy for unresected lung and prostate cancers both of these diseases show high degree of radio-resistance, leading to poor outcomes and increased toxicity due to dose escalation. In this setting it is desirable to develop radiation sensitizers to enhance cancer killing.

SUMMARY

[0019] Canagliflozin is a medication that lowers blood sugar in type 2 diabetics. It is a sodium glucose transporter 2 (SGLT2) inhibitor; a medication that lowers blood sugar in type 2 diabetes by increasing glucose excretion from the kidney. It is disclosed herein that clinical concentrations of Canagliflozin (10 μM) activate AMP-activated protein kinase (AMPK) and inhibits the clonogenic survival and proliferation of lung (A549, H1299), prostate (22RV1, PC3), colon (MC38, HCT116), liver (HepG2), breast (MCF7) and ovarian (SKOV-3) cancer cells. This effect was also observed with Dapagliflozin but only at concentrations outside the clinically effective range. Canagliflozin is also disclosed herein to enhance the effectiveness of radiation and commonly used chemotherapeutics such as Cisplatin and Docetaxel. These data indicate that Canagliflozin enhances the effectiveness of radiation and anti-neoplastic therapies for the treatment of cancer.
Accordingly, the present application includes a method for the treatment or prevention of cancer progression in a subject, said method comprising administering to the subject an effective amount of Canagliflozin, or an active analog thereof. In an embodiment of the present application, the Canagliflozin, or the active analog thereof, is administered in combination with an adjunct cancer treatment.

The present application also includes a use of Canagliflozin, or an active analog thereof, for treating cancer in a subject in need thereof. In an embodiment, the Canagliflozin, or the active analog thereof, is for use in combination with an adjunct cancer treatment.

The present application also includes a method of treating cancer comprising administering an effective amount of Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment to a subject in need thereof. The present application further includes a use of Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment for treating cancer in a subject.

The present application also includes a method of improving the efficacy of an adjunct cancer treatment comprising administering an effective amount of Canagliflozin, or an active analog thereof, in combination with the adjunct cancer treatment to a subject in need thereof. The present application further includes a use of Canagliflozin, or an active analog thereof, for improving the efficacy of an adjunct cancer treatment.

The present application also includes a pharmaceutical composition comprising Canagliflozin, or an active analog thereof, in combination with one or more other anti-cancer agents.

The present application also includes a kit for the treatment of cancer, the kit comprising:

- Canagliflozin, or an active analog thereof;
- one or more other anti-cancer agents; and
optionally instructions for administration of the Canagliflozin, or
the active analog thereof, and the one or more other anti-cancer agents
to a subject in need thereof.

[0026] The present application also includes a kit for improving the
efficacy of an anti-cancer agent for the treatment of cancer, the kit comprising:
Canagliflozin, or an active analog thereof;
the anti-cancer agent; and
optionally instructions for administration of the Canagliflozin, or
the active analog thereof, and the anti-cancer agent to a subject in
need thereof.

In an embodiment, the adjunct cancer treatment is one or more other anti-cancer
agents and/or radiation therapy.

[0027] In another embodiment, the other anti-cancer agent is a biguanide
derivative, such as Metformin and/or Phenformin. In another embodiment, the
other anticancer agent is a salsalate/salicylate derivative. In another
embodiment, the other anti-cancer agent is selected from Metformin,
Phenformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin, Paclitaxel,
Cyclophosphamide, Methotrexate, Cisplatin, 5-Fluorouracil, Etoposide,
carboptatin, Gemcitabine, Vinorelbine and combinations thereof. In another
embodiment, the other anti-cancer agent is Cisplatin or Docetaxel. In a further
embodiment, the other anti-cancer agent is Metformin. In an alternative
embodiment, the other anti-cancer agent is a biological agent.

[0028] In an embodiment, the cancer is selected from prostate cancer,
pancreatic cancer, ovarian cancer, lung cancer, breast cancer, bladder
cancer, colon cancer, brain cancer, head and neck cancer, endometrial
cancer, leukemia, lymphoma and sarcoma. In another embodiment, the
cancer is of the lung, prostate, colon, breast or ovary.

[0029] The present application also includes a method for treating or
preventing cancer progression comprising administration to the subject a
combination of therapeutically effective amounts of Canagliflozin, or an active analog thereof, and an additional anti-neoplastic agent.

[0030] In the present application, a method for treating or preventing cancer progression by administering to a subject a therapeutically effective combination treatment is disclosed.

[0031] The present application also includes a method for treating or preventing cancer progression comprising administration to the subject therapeutically effective amounts of Canagliflozin or an active analog thereof, in combination with an anti-neoplastic agent or radiation.

[0032] In an embodiment, the active analog of Canagliflozin is a compound of the Formula I:

![Chemical Structure]

wherein $R^A$ is selected from halo and $C_i^4$alkyl; and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, $C_i^4$alkyl, halo-substituted $C_i^4$alkyl, $OC_i^4$alkyl, halo-substituted $OC_i^4$alkyl, methylenedioxy, ethyleneoxy, mono-$C_i^4$alkylamino, di-$C_i^4$alkylamino, carbamoyl, mono-$C_i^4$alkylcarbamoyl and di-$C_i^4$alkylcarbamoyl.

[0033] Other features and advantages of the present application will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating embodiments of the application, are given by way of illustration only and the scope of the claims should not be limited by these embodiments, but should be given the broadest interpretation consistent with the description as a whole.
The embodiments of the application will now be described in greater detail with reference to the attached drawings in which:

Figure 1 shows plots demonstrating the proliferation of cancers of the lung (A549 and H1299; Figures 1A and B, respectively) prostate (PC3 and 22RV-1; Figures 1C and D, respectively) colon (HCT116; Figure 1E), liver (HepG2; Figure 1F), breast (MCF7; Figure 1G) and ovarian (SKOV-3; Figure 1H) cancer cells treated with Canagliflozin, or Dapagliflozin at the indicated concentrations relative to vehicle treated controls for 72 h. The results are expressed as the mean and standard error of the mean (SEM) over at least three independent experiments where * = p <0.05, ** = p<0.01 , *** = p<0.001 and **** = p<0.0001 as calculated by one-way ANOVA.

Figure 2 shows the clonogenic survival of cancers of the lung (A549: Figure 2A, left panel; Figure 2B, top panel; and H1299: Figure 2A, right panel; Figure 2B, bottom panel), prostate (PC3: Figure 2C, left panel; Figure 2D, top panel; and 22RV-1: Figure 2C, right panel, Figure 2D: bottom panel) and colon (MC38; Figure 2E) treated with Canagliflozin or Dapagliflozin at the indicated concentrations relative to the vehicle treated controls for 5-10 days. The results are expressed as the mean and standard error of the mean (SEM) over at least three independent experiments where * = p <0.05, ** = p<0.01 , *** = p<0.001 and **** = p<0.0001 as calculated by one-way ANOVA.

Figure 3 shows that Canagliflozin rapidly induces the activation of AMPK in cancer cells. Canagliflozin quickly increases the phosphorylation of AMPK and ACC while potently inhibiting mTORC1 activity in PC3 and H1299 cells. Canagliflozin (30 uM) increased the expression of phosphorylated AMPK and ACC within 0.5 hours in PC3 (A, B) and H1299 (C, D) cells, which was sustained up to 24 h over three independent experiments. The phosphorylation of S6 kinase and its substrate, the ribosomal protein S6, decreased time dependently in PC3 and H1299 cells. Dapagliflozin treatment did not influence the phosphorylation status of AMPK or ACC in these cells. The results are expressed as the mean and standard error of the mean (SEM).
of at least three independent experiments where * = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001 as calculated by one-way ANOVA.

[0038] Figure 4 shows that Canagliflozin dose-dependently activates AMPK in cancer cells. Canagliflozin also dose dependently increases the phosphorylation of AMPK and ACC in PC3 and H1299 cells at clinically effective concentrations. PC3 (A, B) and H1299 (C, D) cancer cells treated with the indicated concentrations of drug relative to the vehicle treated controls at 0.5 and 1 hour, respectively. Canagliflozin and Dapagliflozin significantly increased the expression of phosphorylated AMPK and ACC at doses >10 µM and >100 µM respectively in PC3 cells. A similar response was observed in H1299 cells at doses >30 µM and >100 µM respectively. The results are expressed as the mean and standard error of the mean (SEM) of at least three independent experiments where * = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001 as calculated by one-way ANOVA.

[0039] Figure 5 shows that Dapagliflozin is a weak AMPK activator. Dapagliflozin dose response is shown for PC3 (A) and H1299 (B) cells for the numeric data presented in Figures 4B and 4D.

[0040] Figure 6 shows the ¹⁴C-acetate synthesis into PC3 cancer cells treated with indicated concentrations of vehicle or Canagliflozin for 2 hours.

[0041] Figure 7 shows Oxygen consumption of H1299 cancer cells treated with indicated concentrations of vehicle or Canagliflozin for 2 hours.

[0042] Figure 8 shows that Canagliflozin (30 µM) decreased complex-I supported respiration in PC3 and H1299 cancer cells (A). Percent inhibition of complex-I facilitated respiration in PC3 and H1299 cells is shown in (B). Cell proliferation as a function of Canagliflozin dose in the presence of 11 mM glucose, no galactose or in the presence of 10 mM galactose, no glucose is shown for PC3 (left panel) and H1299 (right panel) cells (C). The results are expressed as the mean and standard error of the mean (SEM) of at least three independent experiments where * = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001 as calculated by one or two-way ANOVA.
Figure 9 shows that Canagliflozin inhibits the activity of Akt in PC3 cells. Canagliflozin (30 µM) decreases the phosphorylation of Akt at residues Thr308 and Ser473 within 0.5 h in PC3 cells (A-B). Under these same conditions, the phosphorylation status of Akt in H1299 cells remained generally unchanged (C-D). The results are expressed as the mean and standard error of the mean (SEM) of three independent experiments where * = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001 as calculated by a t-test.

Figure 10 shows that Canagliflozin but not Dapagliflozin inhibits H3-2DG uptake in PC3 (A) and H1299 (B) cells treated at the indicated concentrations relative to the vehicle treated controls. The results are expressed as the mean and standard error of the mean (SEM) of three independent experiments where * = p<0.05, ** = p<0.01, *** = p<0.001 and **** = p<0.0001 as calculated by one-way ANOVA.

Figure 11 demonstrates graphically that Canagliflozin potentiates Docetaxel's anti-clonogenic (A) and anti-proliferative (B) effects in PC3 cells. Figure 11A shows clonogenic survival as a function of Canagliflozin dose for PC3 cells treated either with Canagliflozin or Canagliflozin and 0.5 nM Docetaxel. Figure 11B shows cell proliferation as a function of Canagliflozin dose for PC3 cells treated either with Canagliflozin or Canagliflozin and 2 nM Docetaxel.

Figure 12 shows graphically that Canagliflozin potentiates Cisplatin's anti-proliferative effects in H1299 cells. Figure 12A shows cell proliferation as a function of Canagliflozin dose for H1299 cells treated either with Canagliflozin or Canagliflozin and 5 µM Cisplatin. Figure 12B shows cell proliferation as a function of Cisplatin dose for H1299 cells treated either with Cisplatin or Cisplatin and 30 µM Canagliflozin.

Figure 13 shows graphically that Canagliflozin potentiates the anti-clonogenic effects of radiation in PC3 cells. Clonogenic survival is shown as a function of Canagliflozin dose for PC3 cells treated with 0, 2 or 4 Gy radiation.
DETAILED DESCRIPTION

1. Definitions

[0048] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present application herein described for which they are suitable as would be understood by a person skilled in the art.

[0049] In understanding the scope of the present application, the term "comprising" and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, "including", "having" and their derivatives. The term "consisting" and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The term "consisting essentially of", as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps.

[0050] Terms of degree such as "substantially", "about" and "approximately" as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least ±5% of the modified term if this deviation would not negate the meaning of the word it modifies.

[0051] As used in this application, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise. For example, an embodiment including "an anti-cancer agent" should be understood to present certain aspects with one anti-cancer agent or two or
more additional anti-cancer agents. In embodiments comprising an "additional" or "second" component, such as an additional or second anti-cancer agent, the second component as used herein is chemically different from the other components or first component. A "third" component is different from the other, first, and second components, and further enumerated or "additional" components are similarly different.

[0052] The term "and/or" as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that "at least one of" or "one or more" of the listed items is used or present.

[0053] In embodiments of the application, the compounds described herein have at least one asymmetric center. Where compounds possess more than one asymmetric center, they may exist as diastereomers. It is to be understood that all such isomers and mixtures thereof in any proportion are encompassed within the scope of the present application. It is to be further understood that while the stereochemistry of the compounds may be as shown in any given compound listed herein, such compounds may also contain certain amounts (e.g. less than 20%, suitably less than 10%, more suitably less than 5%) of compounds of the application having alternate stereochemistry.

[0054] The term "radiation therapy" as used herein includes any form of ionizing radiation therapy for cancer, including external beam radiotherapy such as conventional fraction radiotherapy, hypofractionated radiotherapy (treatment delivered in a shorter period of time), 3D-conformal, intensity modulated and volumetric arc therapy radiation, stereotactic radiotherapy and radiosurgery; brachytherapy and injectable radio-active therapies.

[0055] The term "subject" as used herein includes all members of the animal kingdom including mammals, and suitably refers to humans.

[0056] The term "pharmaceutical composition" as used herein refers to a composition of matter for pharmaceutical use.

[0057] The term "pharmaceutically acceptable" means compatible with the treatment of subjects, for example, mammals such as humans.
As used herein, the terms "effective amount" or "therapeutically effective amount" and the like means an amount effective, at dosages and for periods of time necessary to achieve a desired result. For example, in the context of treating cancer, an effective amount of the Canagliflozin, or the active analog thereof, is an amount that, for example, reduces the cancer compared to the cancer without administration or use of the Canagliflozin, or the active analog thereof. For example, reducing the cancer can refer to reducing the tumor burden compared to the tumor burden without administration or use of the Canagliflozin, or the active analog thereof. Furthermore, reducing the cancer also includes preventing tumor progression or reducing the rate of progression of cancer compared to tumor progression or rate of progression of cancer without administration or use of the Canagliflozin, or the active analog thereof. Effective amounts may vary according to factors such as the disease state, age, sex, weight and/or species of the subject. The amount of Canagliflozin, the active analog thereof, or another anti-cancer agent that will correspond to such an amount will also vary depending upon various factors, such as the given anti-cancer agent, the pharmaceutical formulation, the route of administration or use, the type of cancer being treated, the identity of the subject being treated, and the like, but can nevertheless be routinely determined by one skilled in the art.

The terms "to treat", "treating" and "treatment" as used herein and as is well understood in the art, mean an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms, 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 a disease, and remission (whether partial or total, whether detectable or undetectable). In an embodiment, "to treat", "treating" and "treatment" mean prolonging survival as compared to expected survival if not receiving treatment. The terms "to treat", "treating" and "treatment" as used herein include prophylactic treatment. For example, in an embodiment, a
subject with early cancer is treated to prevent progression. In another embodiment, a subject in remission is treated to prevent recurrence.

[0060] Treatment methods comprise administering to a subject or use of a therapeutically effective amount of Canagliflozin, or the active analog thereof, and optionally consist of a single administration or use, or alternatively comprise a series of administrations or uses. For example, in an embodiment, the Canagliflozin, or the active analog thereof, is administered or used at least once a week. However, in another embodiment, the Canagliflozin, or the active analog thereof, is administered to the subject or used from about one time per 2, 3 or 4 weeks, or less, or about one time per week to about once daily for a given treatment. In another embodiment, the Canagliflozin, or the active analog thereof, is administered or used 2, 3, 4, 5 or 6 times daily. The length of the treatment period depends on a variety of factors, such as the severity of the cancer, the age of the subject, the concentration and/or the activity of a Canagliflozin, or an active analog thereof formulation, and/or a combination thereof. It will also be appreciated that the effective dosage of the Canagliflozin, or the active analog thereof, used for the treatment may increase or decrease over the course of a particular treatment regime. Changes in dosage result and become apparent by standard diagnostic assays known in the art. In some embodiments, chronic administration or use is required. For example, in an embodiment, the Canagliflozin, or the active analog thereof, is administered to the subject or used in an amount and for duration sufficient to treat the subject.

[0061] "Palliating" a cancer means that the extent and/or undesirable clinical manifestations of a cancer are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the cancer.

[0062] The term "prevention" or "prophylaxis", or synonym thereto, as used herein refers to a reduction in the risk or probability of a subject becoming afflicted with cancer or manifesting a symptom associated with cancer.

[0063] The term "administered" as used herein means administration of a therapeutically effective amount of the Canagliflozin, or the active analog
thereof, optionally in combination with another anti-cancer agent to a cell either in cell culture or in a subject.

[0064] The term “Canagliflozin” as used herein refers to a compound having the following structure:

![Structure of Canagliflozin](image)

and includes all crystalline forms and/or solvates thereof. Canagliflozin is available commercially. Its synthesis is described, for example, in U.S. Patent No. 7,943,788. A crystalline form of the hemihydrate of Canagliflozin is described in U.S. Patent No. 7,943,582 and is within the scope of the present application.

[0065] The term "active analog of Canagliflozin" refers to compounds having a structure that is analogous to Canagliflozin, with structural variations that do not substantially impact the compound's activity as an activator of AMPK and/or inhibitor of the proliferation of cancer cells. Whether an analog of Canagliflozin is an activator of AMPK and/or inhibitor of the proliferation of cancer cells can be determined by a person skilled in the art using standard assays, for example as described in the Examples herein.

[0066] The term "Dapagliflozin" as used herein refers to a compound having the following structure:

![Structure of Dapagliflozin](image)
II. Methods and Uses

[0067] The selective sodium-dependent glucose transporter 2 (SGLT2) inhibitors are a new class of anti-diabetic medications that lower blood glucose. It was tested whether the SGLT2 inhibitors Canagliflozin and Dapagliflozin inhibit the growth and survival of cancer cells. It was found that Canagliflozin inhibited lung, prostate, colon, liver, breast and ovarian cancer cell proliferation at concentrations that can be clinically achieved (20-65 µM). Low dose treatment also inhibited colony formation of lung and prostate cancer cells (IC50s of 8-13 µM). These effects were potentiated when combined, for example, with gamma radiation or the cytotoxic drugs Cisplatin and Docetaxel. In contrast, Dapagliflozin was 5-10-fold less effective in all of these same assays. Reductions in growth and survival with Canagliflozin were accompanied by activation of the AMP-activated protein kinase (AMPK) and reductions in Akt and the mTORC1 substrate S6 kinase. Consistent with the activation of AMPK, Canagliflozin reduced mitochondrial respiration through the inhibition of complex-I. These data suggest that in addition to being a medication for the treatment of diabetes, Canagliflozin is also useful in limiting, for example, the growth and survival of adenocarcinomas.

[0068] Accordingly, the present application includes a method of treating cancer comprising administering Canagliflozin, or an active analog thereof, to a subject in need thereof. The present application also includes a use of Canagliflozin, or an active analog thereof, for treating cancer in a subject; a use of Canagliflozin, or an active analog thereof, for preparation of a medicament for treating cancer in a subject; and Canagliflozin, or an active analog thereof, for use to treat cancer in a subject.

[0069] The present application further includes a method for the treatment or prevention of cancer progression in a subject, said method comprising administering to the subject an effective amount of Canagliflozin, or an active analog thereof. The present application also includes a use of Canagliflozin, or an active analog thereof, for treatment or prevention of cancer progression in a subject; a use of Canagliflozin, or an active analog
thereof, for preparation of a medicament for treating or preventing cancer progression in a subject; and Canagliflozin, or an active analog thereof, for use to treat or prevent cancer progression in a subject.

[0070] In an embodiment, the cancer is selected from the group consisting of prostate cancer, pancreatic cancer, ovarian cancer, lung cancer, breast cancer, bladder cancer, colon cancer, brain cancer, head and neck cancer, endometrial cancer, leukemia, lymphoma and sarcoma. In another embodiment, the cancer is of the lung, prostate, colon, liver, breast or ovary. In a further embodiment, the cancer is of the lung, prostate, colon, breast or ovary. It is an embodiment that the cancer is of the lung, prostate or colon.

[0071] In an embodiment, the subject is a human.

[0072] In an embodiment, the Canagliflozin, or the active analog thereof, is useful as an adjunct therapy with other cancer treatments such as radiation and/or other anti-cancer agents. Accordingly, the present application also includes a method of treating cancer comprising administering, to a subject in need thereof, Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment. The present application also includes a use of Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment for treating cancer in a subject; a use of Canagliflozin, or an active analog thereof, in combination with one or more other anti-cancer agents for preparation of a medicament for treating cancer in a subject; and Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment for use to treat cancer in a subject.

[0073] In an embodiment, the adjunct cancer treatment is radiation therapy. In another embodiment, the adjunct cancer treatment is one or more other anti-cancer agents. It is an embodiment that the adjunct cancer treatment is a combination of radiation and one or more other anti-cancer agents.

[0074] In an embodiment, the administration or use of Canagliflozin, or the active analog thereof, in combination with the adjunct cancer treatment enhances the effectiveness of the adjunct cancer treatment in the treatment of cancer.
Accordingly, the present application also includes a method of improving the efficacy of an adjunct cancer treatment for treating cancer comprising administering Canagliflozin, or an active analog thereof, in combination with the adjunct cancer treatment, to a subject in need thereof. The present application also includes a use of Canagliflozin, or an active analog thereof, for improving the efficacy of an adjunct cancer treatment; a use of Canagliflozin, or an active analog thereof, for preparation of a medicament for improving the efficacy of an adjunct cancer treatment; and Canagliflozin, or an active analog thereof, for use to improve the efficacy of an adjunct cancer treatment.

The other anti-cancer agent can be any suitable other anti-cancer agent, the selection of which can be made by a person skilled in the art. In an embodiment, the anti-cancer agent is a drug targeting cancer mitochondrial metabolism, glucose uptake, isocitrate dehydrogenase or lipid metabolism. In another embodiment, the anti-cancer agent is a direct small molecule activator of AMPK.

In another embodiment, the other anti-cancer agent is a biguanide derivative, such as Metformin and/or Phenformin.

In another embodiment, the other anticancer agent is a salsalate/salicylate derivative.

In another embodiment, the one or more other anti-cancer agents are selected from the group consisting of Phenformin, Metformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin, Paclitaxel, Cyclophosphamide, Methotrexate, Cisplatin, 5-Fluorouracil, Etoposide, carboplatin, Gemcitabine, Vinorelbine and combinations thereof. In a further embodiment, the other anti-cancer agent is Cisplatin or Docetaxel. It is an embodiment that the other anti-cancer agent is Cisplatin. In another embodiment of the present application, the other anti-cancer agent is Docetaxel. In a further embodiment, the other anti-cancer agent is Metformin. It is an embodiment that the other anti-cancer agent is a chemotherapeutic agent
used for the treatment of lung cancer such as Etoposide, carboplatin, Gemcitabine or Vinorelbine.

[0080] In an embodiment, the other anti-cancer agent is a biological agent. The biological agent can be any suitable biological agent, the selection of which can be made by a person skilled in the art. In another embodiment, the biological agent targets tumor growth mechanisms such as epidermal growth factor receptor (EGFR) and associated pathway-related, anaplastic lymphoma kinase (Alk)-related, K-Ras-related, p53-related therapy (for lung cancer), androgen receptor-related therapy (for prostate cancer) and immune-system modulating therapi es (for all cancers). In a further embodiment, the biological agent is selected from the group consisting of: monoclonal antibodies, cytokines, vaccines, oncolytic viruses and combinations thereof.

[0081] In an embodiment, the active analog of Canagliflozin is a compound of the Formula I:

\[
\begin{align*}
\text{R}^\text{A} & \text{ is selected from halo and } \text{C}_{1-4}\text{ alkyl;} \text{ and } \text{Ring } \text{C} \text{ is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, } \text{C}_{1-4}\text{ alkyl, halo-substituted } \text{C}_{1-4}\text{ alkyl, } \text{OCl}_{4}\text{ alkyl, halo-substituted } \text{OCl}_{4}\text{ alkyl, methylenedioxy, ethylenedioxy, mono-} \text{C}_{1-4}\text{ alky lamino, di-} \text{C}_{1-4}\text{ alky lamino, carbamoyl, mono-} \text{C}_{1-4}\text{ alky carbamoyl and di-} \text{C}_{1-4}\text{ alky carbamoyl.}
\end{align*}
\]

[0082] In an embodiment, Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, \( \text{C}_{1-4}\text{ alkyl, halo-substituted } \text{C}_{1-4}\text{ alkyl, OCl}_{4}\text{ alkyl, halo-substituted } \text{OCl}_{4}\text{ alkyl, mono-} \text{C}_{1-4}\text{ alky lamino and di-} \text{C}_{1-4}\text{ alky lamino. In a further embodiment, Ring C is a phenyl group substituted with 1 substituent selected from F, Cl, CH}_3, \text{OCH}_3, \text{cyano, CF}_3, \text{OCF}_3 \text{ and }
In an embodiment, the substituent on Ring C is located at the para position. In an embodiment, Ring C is para-fluorophenyl.

[0083] In a further embodiment, \( R^A \) is selected from F, Cl and \( \text{CH}_3 \). In a further embodiment, \( R^A \) is \( \text{CH}_3 \).

[0084] In an embodiment, the methods and uses of the application comprise the use or administration of Canagliflozin, a hydrate, solvate and/or crystalline polymorph thereof, including a crystalline hemihydrate form of Canagliflozin.

[0085] The Canagliflozin, or the active analog thereof, and optionally the one or more other anti-cancer agents can be administered to a subject or used in a variety of forms depending on the selected route of administration or use, as will be understood by those skilled in the art. In an embodiment, the Canagliflozin, or the active analog thereof, and/or optionally the one or more other anti-cancer agents are administered to the subject, or used, by oral (including sublingual and buccal) or parenteral (including intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, topical, patch, pump and transdermal) administration or use and the Canagliflozin, or the active analog thereof, and/or optionally the other anti-cancer agent(s) formulated accordingly. For example, the Canagliflozin, the active analog thereof, and/or optionally the other anti-cancer agent(s) are administered or used by injection, in a spray, in a tablet/caplet, in a powder, topically, in a gel, in drops, by a patch, by an implant, by a slow release pump or by any other suitable method of administration or use, the selection of which can be made by a person skilled in the art.

[0086] In an embodiment, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are orally administered or used, for example, with an inert diluent or with an assimilable edible carrier, or enclosed in hard or soft shell gelatin capsules, or compressed into tablets, or incorporated directly with the food of the diet. In an embodiment, for oral therapeutic administration or use, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are
incorporated with excipient and administered or used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Oral dosage forms also include modified release, for example immediate release and timed-release, formulations. Examples of modified-release formulations include, for example, sustained-release (SR), extended-release (ER, XR, or XL), time-release or timed-release, controlled-release (CR), or continuous-release (CR or Contin), employed, for example, in the form of a coated tablet, an osmotic delivery device, a coated capsule, a microencapsulated microsphere, an agglomerated particle, e.g., as of molecular sieving type particles, or, a fine hollow permeable fiber bundle, or chopped hollow permeable fibers, agglomerated or held in a fibrous packet. In an embodiment of the present application, timed-release compositions are formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable coatings, such as by microencapsulation, multiple coatings, etc. Liposome delivery systems include, for example, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. In an embodiment, liposomes are formed from a variety of phospholipids, such as cholesterol, stearylamine and/or phosphatidylcholines.

[0087] In another embodiment, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are freeze dried and the lyophilizates obtained, are used for example, for the preparation of products for injection.

[0088] In another embodiment of the present application, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are administered or used parenterally. In an embodiment, solutions of the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. In a further embodiment, dispersions of the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and administration or use, these
preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations.

[0089] In an embodiment, the pharmaceutical form is suitable for injectable administration or use and includes sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists.

[0090] In an embodiment, compositions for nasal administration or use are formulated as aerosols, drops, gels or powders. In an embodiment, the aerosol formulation comprises a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and is presented in single or multidose quantities in sterile form in a sealed container, which, for example, take the form of a cartridge or refill for use with an atomising device. In another embodiment, the sealed container is a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after administration or use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which is, for example, a compressed gas, such as compressed air or an organic propellant such as a fluorochlorohydrocarbon. In another embodiment, the aerosol dosage forms take the form of a pump-atomizer.

[0091] In an embodiment, the composition is suitable for buccal or sublingual administration or use such as in tablets, lozenges, and pastilles, wherein the active ingredient is formulated with a carrier such as sugar, acacia, tragacanth, gelatin and/or glycerine. In another embodiment, the composition is suitable for rectal administration or use such as in suppositories containing a conventional suppository base such as cocoa butter.

[0092] In another embodiment, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are coupled with soluble polymers as targetable drug carriers. Such polymers include, for example, polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxy-ethylaspartamide-
phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. In another embodiment, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

[0093] The Canagliflozin, or active analog thereof, is administered or used alone or, as noted above, in combination with other known anti-cancer agents. When administered or used in combination with other known anti-cancer agents, it is an embodiment that the Canagliflozin, or active analog thereof, is administered or used contemporaneously with those anti-cancer agents. As used herein, “contemporaneous” administration or use of two substances to a subject means providing each of the two substances so that they are both biologically active in the individual at the same time. The exact details of the administration or use will depend on the pharmacokinetics of the two substances in the presence of each other, and include, for example, administering or using the two substances at the same time, within a few hours of each other, or administering or using one substance within 24 hours of administration or use of the other, if the pharmacokinetics are suitable. Design of suitable dosing regimens is routine for one skilled in the art. In particular embodiments, two substances will be administered or used substantially simultaneously, i.e., within minutes of each other, or in a single composition that contains both substances. It is a further embodiment of the present application that Canagliflozin, or active analog thereof, and the other anti-cancer agent(s) are administered to a subject or used in a non-contemporaneous fashion.

[0094] The dosage of the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents can vary depending on many factors such as the pharmacodynamic properties of the compound, the mode of administration or use, the age, health and weight of
the recipient, the nature and extent of the symptoms of the cancer, the frequency of the treatment and the type of concurrent treatment, if any, and the clearance rate of the compound in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. In an embodiment, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are administered or used initially in a suitable dosage that is optionally adjusted as required, depending on the clinical response. As a representative example, oral dosages of the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents will range between about 1 mg per day to about 1000 mg per day for a human adult. In an embodiment of the present application, the pharmaceutical compositions are formulated for oral administration or use and the Canagliflozin, the active analog thereof, and/or optionally the one or more other anti-cancer agents are, for example in the form of tablets containing 0.25, 0.5, 0.75, 1.0, 5.0, 10.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 75.0, 80.0, 90.0, 100.0, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 mg of active ingredient per tablet. In an embodiment, the Canagliflozin, the active analog thereof and/or optionally the one or more other anti-cancer agents are administered or used in a single daily dose. In another embodiment, the total daily dose is divided into two, three or four, or more, daily doses.

[0095] In an embodiment wherein the Canagliflozin, or active analog thereof, is administered or used in combination with one or more other anti-cancer agents, the dosage of the Canagliflozin, or active analog thereof, is less than the dosage of the Canagliflozin, or active analog thereof, when administered or used alone. In another embodiment wherein the Canagliflozin, or active analog thereof, is administered or used in combination with one or more other anti-cancer agents, the dosage of the other anti-cancer agent is less than the dosage of the other anti-cancer agent when administered or used alone.
III. Compositions

[0096] The present application also includes a composition comprising Canagliflozin, or an active analog thereof, one or more other anti-cancer agents and optionally a carrier.

[0097] The Canagliflozin, or active analog thereof, and the one or more other anti-cancer agents are suitably formulated into pharmaceutical compositions for administration to subjects or use in a biologically compatible form suitable for administration or use in vivo. Accordingly, the present application further includes a pharmaceutical composition comprising Canagliflozin, or active analog thereof, one or more other anti-cancer agents and optionally a pharmaceutically acceptable carrier.

[0098] In another embodiment, the one or more other anti-cancer agents are selected from the group consisting of Phenformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin, Paclitaxel, Cyclophosphamide, Methotrexate, Cisplatin, 5-Fluorouracil, Etoposide, carboplatin, Gemcitabine, Vinorelbine and combinations thereof. In a further embodiment, the other anti-cancer agent is Cisplatin or Docetaxel. In an embodiment that the other anti-cancer agent is Cisplatin. In another embodiment of the present application, the other anti-cancer agent is Docetaxel. It is an embodiment that the other anti-cancer agent is selected from Etoposide, carboplatin, Gemcitabine and Vinorelbine.

[0099] In an embodiment, the other anti-cancer agent is a biological agent. The biological agent can be any suitable biological agent, the selection of which can be made by a person skilled in the art. In another embodiment, the biological agent targets tumor growth mechanisms such as epidermal growth factor receptor (EGFR) and associated pathway-related, anaplastic lymphoma kinase (Alk)-related, K-Ras-related, p53-related therapy (for lung cancer), androgen receptor-related therapy (for prostate cancer) and immune-system modulating therapies (for all cancers). In a further embodiment, the biological agent is selected from the group consisting of: monoclonal antibodies, cytokines, vaccines, oncolytic viruses and combinations thereof.
It will be appreciated by a person skilled in the art that embodiments relating to the Canagliflozin, or active analog thereof can be varied as detailed herein for the methods and uses of the present application.

In an embodiment, the active analog of Canagliflozin is a compound of the Formula I:

wherein $R^A$ is selected from halo and $\text{Cl}_4\text{alkyl}$; and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, $\text{Cl}_4\text{alkyl}$, halo-substituted $\text{Cl}_4\text{alkyl}$, $\text{OCI}_4\text{alkyl}$, halo-substituted $\text{OC-I}_4\text{alkyl}$, methylenedioxy, ethylenedioxy, mono-$\text{Cl}_4\text{alkylamino}$, di-$\text{Cl}_4\text{alkylamino}$, carbamoyl, mono-$\text{Cl}_4\text{alkylcarbamoyl}$ and di-$\text{Cl}_4\text{alkylcarbamoyl}$.

In an embodiment, Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, $\text{C_1}_4\text{alkyl}$, halo-substituted $\text{C}_1\text{alkyl}$, $\text{OCI}_4\text{alkyl}$, halo-substituted $\text{OCI}_4\text{alkyl}$, mono-$\text{Cl}_4\text{alkylamino}$ and di-$\text{Cl}_4\text{alkylamino}$. In a further embodiment, Ring C is a phenyl group substituted with 1 substituent selected from $F$, $Cl$, $CH_3$, $OCH_3$, cyano, $CF_3$, $OCF_3$ and $N(CH_3)_2$. In an embodiment, the substituent on Ring C is located at the para position. In an embodiment, Ring C is para-fluorophenyl.

In a further embodiment, $R^A$ is selected from $F$, $Cl$ and $CH_3$. In a further embodiment, $R^A$ is $CH_3$.

In an embodiment, the compositions of the application comprise Canagliflozin, a hydrate, solvate and/or crystalline polymorph thereof, including a crystalline hemihydrate form of Canagliflozin.
IV. Kits

[00105] The present application also includes a kit for the treatment of cancer, the kit comprising:

Canagliflozin, or an active analog thereof;

one or more other anti-cancer agents; and

optionally instructions for administration of the Canagliflozin, or the active analog thereof, and the one or more other anti-cancer agents to a subject in need thereof.

[00106] The present application also includes a kit for the treatment of cancer, the kit comprising:

Canagliflozin, or an active analog thereof; and

instructions for administration of the Canagliflozin, or the active analog thereof, to a subject being administered one or more other anti-cancer agents for the treatment of cancer.

[00107] The present application also includes a kit for improving the efficacy of an anti-cancer agent for the treatment of cancer, the kit comprising:

Canagliflozin, or an active analog thereof;

the anti-cancer agent; and

optionally instructions for administration of the Canagliflozin, or the active analog thereof, and the anti-cancer agent to a subject in need thereof.

[00108] The present application also includes a kit for improving the efficacy of an anti-cancer agent for the treatment of cancer, the kit comprising:

Canagliflozin, or an active analog thereof; and

instructions for administration of the Canagliflozin, or the active analog thereof, to a subject being administered the anti-cancer agent for the treatment of cancer.
[00109] It will be appreciated by a person skilled in the art that embodiments relating to the kits of the present application can be varied as discussed herein for the methods and uses of the present application.

[00110] In an embodiment, the active analog of Canagliflozin is a compound of the Formula I:

\[
\text{R}^A \quad \text{S} \quad \text{C}
\]

wherein $R^A$ is selected from halo and $\text{Cl}_4$-alkyl; and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, $\text{Cl}_4$-alkyl, halo-substituted $\text{Cl}_4$-alkyl, $\text{OCI}_4$-alkyl, halo-substituted $\text{OC}-\text{I}_4$-alkyl, methylenedioxy, ethyleneoxy, mono-$\text{Cl}_4$-alkylamino, di-$\text{Cl}_4$-alkylamino, carbamoyl, mono-$\text{Cl}_4$-alkylcarbamoyl and di-$\text{Cl}_4$-alkylcarbamoyl.

[00111] In an embodiment, Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, $\text{C}_1$-$\text{C}_2$-alkyl, halo-substituted $\text{C}_1$-$\text{C}_2$-alkyl, $\text{OCI}_1$-$\text{C}_4$-alkyl, halo-substituted $\text{OCI}_1$-$\text{C}_4$-alkyl, mono-$\text{Cl}_4$-alkylamino and di-$\text{Cl}_4$-alkylamino. In a further embodiment, Ring C is a phenyl group substituted with 1 substituent selected from F, Cl, CH$_3$, OCH$_3$, cyano, CF$_3$, OCF$_3$ and N(CH$_3$)$_2$. In an embodiment, the substituent on Ring C is located at the para position. In an embodiment, Ring C is para-fluorophenyl.

[00112] In a further embodiment, $R^A$ is selected from F, Cl and CH$_3$. In a further embodiment, $R^A$ is CH$_3$.

[00113] In an embodiment, the kits of the application comprise Canagliflozin, a hydrate, solvate and/or crystalline polymorph thereof, including a crystalline hemihydrate form of Canagliflozin.
The following non-limiting examples are illustrative of the present application:

EXAMPLES

Example 1: The SGLT2 inhibitor Canagliflozin activates AMPK and Inhibits the Growth and Survival of Cancer Cells

i. Materials and Methods

Cell Lines and Treatments: Human lung (A549, H1299), prostate (PC3, 22RV-1), breast (MCF-7), colon (HCT16), liver (HepG2) and ovarian (SKOV-3) cancer cells were purchased from the American Type Culture Collection (ATCC: Manassa, VA). The MC38 cells were derived from a murine colon adenocarcinoma. The class, origin and driving mutation(s) of each cell line are described in Table 1. A549, H1299, PC3, 22RV-1, HCT16 and SKOV-3 cells were cultured in RPMI 1640 media (Gibco: Mississauga, ON) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) antibiotic-antimycotic (100x) solution (Gibco: Mississauga, ON). HepG2 and MCF-7 cells were cultured in MEM media (Gibco: Mississauga, ON) supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic-antimycotic (100x). MCF-7 media was also supplemented with 1% (v/v) non-essential amino acids (100X) solution (Gibco: Mississauga, ON) and 1% sodium-pyruvate (100X) solution (Gibco: Mississauga, ON). All cells were maintained at 37°C in 5% CO₂ and were treated with the concentrations of Canagliflozin and Dapagliflozin (Selleck Chemicals LLC), Phenformin, Metformin, Galactose (Sigma: Toronto, ON), Cisplatin and Docetaxel (Cayman: Ann Arbor, MI) indicated herein. Working solutions were prepared so that the vehicle comprised less than 0.1% of the media.

Clonogenic Survival Assay: A549, H1299, PC3 and 22RV-1 cells were seeded at a density of 500-1000 cells per well. The following day, cells were treated in triplicate with the indicated drug for 5-10 days. Cells were fixed with 0.5% crystal violet DNA stain (1 g crystal violet (Sigma: Toronto, ON) in 50% methanol) and viable colonies (≥ 50 cells) were counted.
[00117] **Proliferation Assay:** A549, H1299, PC3, 22RV-1, HepG2, HCT116, MCF-7 and SKOV-3 cells were seeded at a density of 1000-2000 cells per well into 96-well plates. The following day, cells were treated in quadruplicate with the indicated drug for 72 hours. Cells were fixed with 10% formalin and stained with 0.5% crystal violet DNA stain (1 g of crystal violet in 20% methanol). Once dry, the intracellular stain was solubilized with 0.05 M NaH₂PO₄ and the absorbance at 570 nm was measured.

[00118] **Lipogenesis Assay:** PC3 cells were treated in triplicate with a Canagliflozin solution prepared with 0.5 mM Na-acetate and 10 nCi [H³]-Na-acetate (Perkin Elmer) in supplemented media. After 4 hours, plates were placed on ice, washed with ice cold phosphate buffered saline (PBS) and snap frozen to physically disrupt cell membranes. Cells were scraped from plates and collected at room temperature. A methanol:chloroform (2:1) solvent was used for lipid extraction as previously described. 34

[00119] **2-Deoxyglucose Uptake Assay:** PC3 and H1299 were treated in triplicate with a Canagliflozin solution prepared with 2nCi [H³]-2-deoxy-D-glucose (2-DG; Perkins Elmer) in HEPES buffer (140 mM NaCl, 20 mM HEPES-Na, 5 mM KCl, 2.5 mM MgSO₄, 1 mM CaCl₂, pH 7.4). After 10 minutes, plates were placed on ice, washed with ice cold PBS and snap frozen in lysis buffer (1M DTT, 200 mM Na₃VO₄, 20% triton-X, protease inhibitor cocktail tablet (Roche), 50 mM HEPES, 150 mM NaCl, 100 mM NaF, 10 mM Na pyrophosphate, 5 mM EDTA, 250 mM Sucrose). Cells were scraped and measured for radioactive counts.

[00120] **Mitochondrial Respiration Assay:** Cells were treated in duplicate with the indicated treatment for 30-90 minutes. Cells were subsequently washed and collected in MiR05 buffer (1/10 mM sucrose, 60 mM potassium lactobionate, 20 mM HEPES, 10 mM KH₂PO₄, 3 mM MgCl₂, 0.5 mM EGTA, 1 g/L BSA, pH 7.1) supplemented with an equivalent dose of the indicated treatment. Respiratory measurements were conducted using the Oxygraph-2K OROBOROS® apparatus at 30 °C with stirring. Cells were permeabilized with digitonin (4-8 µM) and then treated with glutamate (5 mM), malate (2 mM)
and ADP (5 mM) to induce state III respiration. This rate represented mitochondrial complex-I supported respiration, which was subsequently confirmed through blockade with the complex-I specific inhibitor rotenone (0.5 μM). Cells were dosed with Cytochrome C (5 μM) to check for mitochondrial membrane integrity. Succinate (10 mM) was then used to examine complex-II supported respiration, which was later inhibited by malanate (5 mM), a complex II inhibitor. Oxygen flux was calculated over time using the Datl_ab4 software (OROBOROS®, Austria).

[00121] Cellular ATP Assay: PC3 and H1299 cells were seeded at a density of 20,000 cells per well into white-walled 96-well plates. The following day, cells were treated in duplicate with the indicated treatment for 30 minutes. The Abeam Luminescent ATP Detection Assay Kit (ab1 13849) was used to detect levels of cellular ATP according to the manufacturer’s protocol.

[00122] Immunoblotting and Densitometry: Cells were washed in PBS and collected in ice-cold lysis buffer. Samples were snap frozen, thawed and manually collected on ice. Samples were prepared with 4x SDS sample buffer (40% glycerol, 240 mM Tris-HCl pH 6.8, 8% SDS, 0.04% bromophenol blue, 5% β-mercaptoethanol). 30 μg of boiled protein sample was separated using SDS-PAGE. Proteins were electrically transferred at 4°C onto a nitrocellulose membranes using 10% (v/v) MeOH transfer buffer. Membranes were blocked (5% BSA solution in TBST (50 mM Tris, 150 mM NaCl, 1M HCl, pH 7.4, 0.1 % Tween-20)) and incubated with the indicated primary and HRP-conjugated secondary antibodies. Densitometry values were quantified using Image J software and are expressed as percent of control.

[00123] Statistical Analysis: All results are expressed as a mean with standard error of the mean (SEM). All statistics were calculated using GraphPad Prism 6 software (La Jolla, CA). A p value <0.05 was considered significant and was indicated by a (*). Statistical analyses were performed using two-tailed t-tests or one-way analysis of variance (ANOVA) along with the Fisher-Least Significant Difference post-hoc test. Two-way ANOVAs were used to evaluate the significance between treatment types (single agents
versus combination). Clonogenic and proliferative IC50 values were calculated using a non-linear regression model (with normalized slope).

II. Results

**Canagliflozin blocks the cellular proliferation and clonogenic survival of cancer cells.**

[00124] The proliferation of lung (Figure 1 A-B) prostate (Figure 1 C-D), colon (Figure 1E), liver (Figure 1F), breast (Figure 1G) and ovarian (Figure 1H) cancer cells was significantly inhibited by doses of Canagliflozin within the therapeutic window of exposure (10-50 µM) observed in type 2 diabetic patients.\(^\text{18}\) Prostate (PC3 and 22RV-1) and breast (MCF-7) cancer cells were observed to be the most sensitive to Canagliflozin treatment. The half maximal inhibitory concentration (IC50) for the effects of Canagliflozin to inhibit cancer cell proliferation for each cell line is reported in Table 2. The (IC50 of Canagliflozin in each cell line was calculated to be <65 µM. Lung cancer cells appeared to be wholly resistant to the same concentrations of Dapagliflozin, while prostate, colon, breast and ovarian cancer cell proliferation was only significantly reduced at concentrations of 100 µM. Additionally, the estimated IC50s for Dapagliflozin are well above the tolerable clinical exposure level. Liver cancer cells (HepG2) were the only cell line whose proliferation was inhibited by Dapagliflozin at lower doses (IC50 <55 µM).

[00125] The ability of Canagliflozin and Dapagliflozin to inhibit colony formation was also tested. Clonogenic survival of lung (Figure 2A-B) and prostate (Figure 2C-D) cancer cells was significantly inhibited at doses of Canagliflozin as low as 5 µM (H1299) or 10 µM (A549, PC3, 22RV-1). These inhibitory effects were found to be significant by 10 µM. Canagliflozin also inhibited the clonogenic survival of cancers of colon (MC38; Figure 2E). The IC50 for the effects of Canagliflozin to inhibit cancer cell clonogenic survival ranged from -8-23 µM and is reported in Table 3. Dapagliflozin was approximately 2-10 fold less effective (Figure 2, Table 3).
Canagliflozin activates the AMPK-ACC and mTORCI signalling axes in lung and prostate cancer cells

[00126] Immunoblotting was conducted on the lung and prostate cell lines that were observed to be most sensitive to Canagliflozin to investigate the potential mechanisms mediating these effects.

[00127] Activating phosphorylation of AMPK at T172 and the phosphorylation of its downstream substrate ACC at Ser 79/221 was measured following treatment of prostate, lung and colon cancer cells with 30 μM Canagliflozin or Dapagliflozin for varying times. Within 30 minutes of treatment, Canagliflozin increased the phosphorylation of AMPK (Thr172) and ACC (Ser79) by 7 and 4-fold respectively in PC3 prostate cancer cells, a response that was sustained for up to 24 hours (Figure 3A-B). A smaller response was detected in the H1299 cells (2-fold increase, Figure 3C-D).

[00128] Canagliflozin also decreased the phosphorylation of S6K (Thr389) and its substrate S6 (Ser 240/244) by approximately 50% at the 0.5 and 1 hour time point respectively, in the PC3 cells. A similar response by S6 was not observed until the 24 hour time point in the H1299 cells. Dapagliflozin did not significantly alter the phosphorylation status of any of the markers measured in the PC3 or H1299 cells over time.

[00129] Given this robust activation after an acute exposure, dose responses were then conducted with both SGLT2 inhibitors at 0.5 h and 1 h in the PC3 and H1299 cells. Dose dependency, Canagliflozin significantly increased the phosphorylation of AMPK and ACC in PC3 and H1299 cells at concentrations >10 μM and >30 μM respectively (Figure 4A-D). In contrast, Dapagliflozin increased the phosphorylation of these same markers by only 2-fold once a 100 μM dose was applied to the PC3 cells (Figure 4B, Figure 5A). This same dose was observed to increase the phosphorylation of ACC by 20% in the H1299 cells (Figure 4D, Figure 5B).

Canagliflozin inhibits cell growth and proliferation through AMPK regulation of protein and lipid synthesis.
While not wishing to be limited by theory, AMPK may prevent cancer cell growth and proliferation due to the inhibition of protein synthesis and lipogenesis; effects mediated through mTOR and ACC respectively. mTOR is a useful pathway controlling cancer cell growth and proliferation whose activity can be inferred through the phosphorylation of downstream effectors such as p70S6. Canagliflozin (30 μM) totally eliminated the phosphorylation of p70S6 within 1 hr of treatment without altering total protein expression indicating the potent ability of this compound to suppress mTOR activity (Figure 3). In contrast, Dapagliflozin had no effect on the phosphorylation of p70S6 (Figure 3). Canagliflozin inhibits de novo lipogenesis in PC3 prostate cancer cells

Consistent with phosphorylation of ACC Canagliflozin also dose dependently suppressed de novo lipogenesis (Figure 6). Canagliflozin dose-dependently decreased the incorporation of [H³]-acetate into fatty acids in the PC3 cells at concentrations >15 μM. Over 50% inhibition was observed at concentrations of 30 μM (Figure 6).

Canagliflozin reduces oxygen consumption; SGLT2 inhibitors block mitochondrial respiration through the inhibition of complex-I.

AMPK can be activated by reductions in cellular energy charge, increases in cellular calcium or through a direct allosteric mechanism involving Ser108 of the AMPK betal subunit. Most xenobiotics and drugs that activate AMPK reduce mitochondrial oxidative phosphorylation leading to increases in cellular AMP/ADP therefore the effects of Canagliflozin on oxygen consumption were measured.

It was found that Canagliflozin dose dependently reduced oxygen consumption in H1299 cancer cells (Figure 7). While not wishing to be limited by theory, these data suggest that Canagliflozin activates AMPK by inhibiting oxidative phosphorylation and the adenylate charge of the cell.

A 30 minute treatment of Canagliflozin (30 μM) reduced the rate of oxygen consumption through complex-I by 35 and 80% in PC3 and H1299 cells, respectively (Figure 8A-B). It was then tested whether the cancer cells
would be more sensitive to Canagliflozin when grown in galactose; a method used to enhance cellular reliance on oxidative phosphorylation for ATP production. Under these conditions, the proliferation of these cancer cells was further inhibited (Figure 8C). The proliferative IC50 for Canagliflozin dropped from 23.41 to 12.64 \( \mu \text{M} \), and from 35.96 to 19.55 \( \mu \text{M} \) in PC3 and H1299 cells, respectively. In addition, cancer cells, which were cultured in growth media containing galactose, were subjected to a greater drop in cellular ATP with a 30 minute treatment of Canagliflozin, in comparison to cells cultured in growth media containing glucose (Table 4). This response was also observed when the cells were treated with the known complex-I inhibitor phenformin.

**Canagliflozin inhibits Akt activity in PC3 prostate cancer cells**

[00135] A 30-minute treatment with Canagliflozin (30 \( \mu \text{M} \)) reduced phosphorylation of Akt at residues Thr308 and Ser473 in PC3 cells by 60% and 70% respectively (Figure 9A-B).

[00136] In contrast, Akt phosphorylation at these same sites was only reduced by 10 and 20 % in H1299 cells (Figure 9C-D), respectively. This was observed using an equivalent dose at the 1-hour time point.

**Canagliflozin inhibits glucose uptake independently of SGLT2**

[00137] Canagliflozin but not Dapagliflozin decreased \( ^3 \text{H}-2\text{DG} \) uptake in PC3 and H1299 cells (Figure 10A-B). In PC3 cells, a 10 and 30 \( \mu \text{M} \) dose of Canagliflozin reduced 2-DG uptake by over 30 and 60%, respectively. These same doses reduced 2-DG uptake by 50 and 60% in H1299 cells.

**Canagliflozin potentiates Docetaxel’s anti-proliferative and anticlonogenic effects in PC3 cells.**

[00138] Figure 11A shows the clonogenic survival of prostate cancer cells treated with Canagliflozin at the indicated concentrations relative to the vehicle treated control as a single agent (grey) or in combination with 0.5 \( \text{nM} \) Docetaxel (black). 5 \( \mu \text{M} \) and 10 \( \mu \text{M} \) doses of Canagliflozin combined with 0.5 \( \text{nM} \) docetaxel inhibited PC3 clonogenic survival by an additional 50% and 90%, respectively, compared to docetaxel treatment alone.
[00139] The effect of a similar combination regimen towards these cells' proliferative ability was also tested. Figure 11B shows the proliferation of prostate cancer cells treated with Canagliflozin at the indicated concentrations relative to the vehicle treated control as a single agent (grey) or in combination with 2 nM Docetaxel (black). Consistently, a 30 μM dose of Canagliflozin combined with a 2 nM dose of Docetaxel inhibited PC3 cell proliferation by an additional 50% compared to docetaxel treatment alone.

Canagliflozin potentiates Cisplatin's anti-proliferative effects in H1299 cells.

[00140] Figure 12A shows the proliferation of lung cancer cells treated with Canagliflozin at the indicated concentrations relative to the vehicle treated control as a single agent (grey) or in combination with 5 μM Cisplatin (black). As can be seen from the data in Figure 12A, a 30 μM dose of Canagliflozin combined with a 5 μM dose of Cisplatin inhibited H1299 cell proliferation by an additional 12% compared to Cisplatin treatment alone.

[00141] Figure 12B shows the proliferation of lung cancer cells treated with Cisplatin at the indicated concentrations relative to the vehicle treated control as a single agent (grey) or in combination with 30 μM Canagliflozin (black). As can be seen from the data in Figure 12B, a 30 μM dose of Canagliflozin combined with a 10 μM dose of Cisplatin inhibited H1299 cell proliferation by an additional 25% compared to Canagliflozin treatment alone.

Canagliflozin potentiates radiation's anti-clonogenic effects in PC3 cells.

[00142] Canagliflozin's ability to sensitize cancer cells to radiation therapy was also tested. Figure 13 shows the clonogenic survival of prostate cancer cells treated with a 5 or 10 μM dose of Canagliflozin combined with different doses of radiation (0, 2 or 4 Gy). As can be seen from the data in Figure 13, when combined with 2 Gy of radiation, a 5 μM and 10 μM dose of Canagliflozin inhibited PC3 clonogenic survival by an additional 60 and 80% respectively when compared to radiation treatment alone.
III. Discussion

[00143] Cancers of the lung and prostate are among the 5 most common sites of cancer diagnoses across genders. In view of these striking cancer risks, it is an object to discover novel chemotherapeutic agents that will improve the clinical outcomes of patients currently living with cancer.

[00144] It was found that Canagliflozin, at concentrations within the therapeutic window of exposure used for treating type 2 diabetes, displays significant anti-cancer effects across a variety of cell lines; effects not observed with the other approved SGLT2 inhibitor Dapagliflozin. Furthermore, it has been observed that suppression of cancer growth and survival are associated with the activation of AMPK, and the inhibition of de novo lipogenesis, glucose uptake and AKT/mTOR signalling.

[00145] Inhibition of Cancer Cell Growth: The proliferation of lung (A549, H1299), prostate (PC3, 22RV-1), colon (HCT116), liver (HepG2), breast (MCF-7) and ovarian (SKOV-3) cancer cells was significantly inhibited by concentrations of Canagliflozin as low as 10 µM over a 72-hour period (Figure 1, IC50 <65 µM, Table 2). The clonogenic survival of lung and prostate cells was also significantly inhibited at doses of Canagliflozin as low as 10 µM (Figure 2). High doses of Dapagliflozin inhibited the clonogenic survival of lung and prostate cancer cells, but had little effect on cellular proliferation. In prostate, colon, breast and ovarian cancer cells, Dapagliflozin only reduced proliferation at concentrations of 100 µM. However, liver cancer cells demonstrated the least resistance to this drug.

[00146] Canagliflozin is an AMPK Activator: Consistently, a 10-30 µM dose of Canagliflozin increased the phosphorylation of AMPK (Thr172) and its downstream substrate ACC (Ser79) within 30 minutes in PC3 and H1299 cells (Figure 3). Other AMPK activators such as metformin, salicylate and AICAR have only been able to induce similar patterns of activation at millimolar concentrations in these same cell lines.

[00147] Furthermore, it has been demonstrated herein for the first time that Canagliflozin treatment also leads to the inhibition of mTORCI signalling. A
decrease in the phosphorylation of mTORd's effector protein S6K (Thr389) and its substrate S6 (Ser 240/S244) was observed within 1 hour in PC3 cells and within 24 hours in H1299 cells. These timely responses are similar to those of rapamycin on mTORC1 signalling in these same cell lines.\textsuperscript{35} Prostate carcinoma appeared to be the most sensitive, as a significant increase in the phosphorylation of ACC was detected at doses as low as 10 \( \mu \text{M} \) (Figure 2). In contrast, Dapagliflozin was a weak activator of AMPK in these cancer cells.

[00148] To determine how Canagliflozin induces AMPK activation, the rate of oxygen consumption (OCR) was examined. It was discovered that Canagliflozin inhibits oxygen consumption (Figure 7).

[00149] It was found that a 30 \( \mu \text{M} \) dose was able to decrease complex-I facilitated respiration in the H1299 and PC3 cells by 80\% and 35\%, respectively (Figure 8A-B). It was surprising to see that the OCR in PC3 cells, which have the greatest activation of AMPK following Canagliflozin treatment, had small reductions in complex-I activity compared to the H1299 cancer cells. However, the resting rate of respiration in the PC3 cells was approximately 75\% less than that of the H1299 cells, suggesting, while not wishing to be limited by theory, that the PC3 cells have a reduced mitochondrial content and thus less mitochondrial reserve capacity (Figure 9A). As many complex-I inhibitors, such as metformin\textsuperscript{36} and berberine, indirectly activate AMPK\textsuperscript{37} while not wishing to be limited by theory, this may be the mechanism by which Canagliflozin increases AMPK activity. Therefore, while not wishing to be limited by theory, even small reductions in complex-I activity would be expected to compromise the ability of these cells to maintain energy homeostasis.

[00150] To provide further evidence that Canagliflozin is toxic to mitochondrial function, the cancer cells were cultured in growth media containing galactose. The oxidation of galactose to pyruvate generates no net ATP. The cells are therefore compelled to oxidize pyruvate in order to sustain their energy demands.\textsuperscript{38} Under these conditions, the PC3 and H1299 cells proved to be more sensitive to the anti-proliferative effects of Canagliflozin (Figure 8C).
Canagliflozin's effects on mitogen signaling were also investigated. Canagliflozin was observed to suppress the activating phosphorylation of Akt by more than 50% in PTEN negative PC3 cells but had minimal inhibitory activity in H1299 cells which express the PTEN protein\(^{39}\) (Figure 9). Typically, PTEN suppresses tumour cell growth by inhibiting PI3K-Akt-mTOR pathway by dephosphorylating phosphatidylinositol-(3,4,5)-triphosphate (PIP\(_3\)), an activator of 3-phosphoinositide-dependent kinase (PDK) and Akt.\(^{40}\) While not wishing to be limited by theory, PC3 cells may have elevated Akt activity to which Canagliflozin can suppress, as opposed to the H1299 cells whose Akt activity is within the normal homeostatic range. While not wishing to be limited by theory, Canagliflozin's ability to suppress this pathway suggests that it may be targeting proteins that reside upstream. In line with the findings in the PC3 cells, Akt has recently been discovered to directly phosphorylate the a1-subunit of AMPK at residue Ser487 and prevent the activating phosphorylation at Thr172 in HEK293 cells.\(^{41}\) Canagliflozin's ability to inhibit Akt activity, in combination with complex-I inhibition and a subsequent reduction in the cellular adenylate charge, may therefore further promote AMPK activation in the PC3 cancer cells.

Investigating Canagliflozin's Mechanism of Action: Canagliflozin dose dependently decreased 2-DG uptake). While not wishing to be limited by theory, it is possible that Canagliflozin inhibits other glucose transporters expressed on cancer cell surfaces. However, thus far, it has only been noted that Canagliflozin is a weak inhibitor of GLUT1.\(^{42}\) This is noted because acute glucose deprivation induces an energetic challenge within the cell to which AMPK will respond.\(^{43}\) However, this activity is likely not sustainable for 24 hours suggesting, while not limited by theory, that there is an alternative mechanism of AMPK activation. Still, by inhibiting glucose uptake, a considerable source of energy on which cancer cell growth may depend is reduced. This effect may become useful when, as discussed above, mitochondrial function is also impaired.

Reductions in lipid synthesis: Not only is ACC considered a sensitive measure of cellular AMPK activity; it is also the rate-limiting enzyme in
the fatty acid biosynthetic pathway. Since an increase in the phosphorylation of ACC (Ser79) in response to Canagliflozin treatment was detected, while not wishing to be limited by theory, a proportional decrease in de novo lipogenesis may be observed. Furthermore, it has previously been demonstrated that the inhibition of lipogenesis is strongly correlated with salicylate activated AMPK and inhibition of clonogenic survival in lung and prostate carcinomas\textsuperscript{44}, a result which has been replicated by other groups using other AMPK activators.\textsuperscript{45} Consistently, a dose-dependent decrease in fatty acid synthesis by Canagliflozin was noted at concentrations >15 µM in PC3 cells (Figure 6).

[00154] Canagliflozin’s use in concurrent chemotherapy and radiation therapy: Canagliflozin is an effective single agent in terms of its ability to inhibit PC3 and H1299 cell viability. It was therefore of interest to determine whether this drug could also be useful in combination with the chemotherapy drugs Docetaxel and Cisplatin as well as radiation; well established therapies for the treatment of castrate resistant prostate cancer (CRPC) and non-small cell lung cancer (NSCLC) respectively. Docetaxel is the first-line therapy for the treatment of metastatic CRPC as it increases the overall survival of patients when used in combination with prednisone.\textsuperscript{46} It is reported herein that low dose Canagliflozin (5-30 µM) in combination with clinically relevant concentrations of Docetaxel inhibited PC3 colony formation and cell proliferation by 50-90% more in comparison to Canagliflozin treatment alone.

[00155] When combined with radiation therapy, a low dose of Canagliflozin inhibited PC3 clonogenic survival by at least 60% more when compared to radiation treatment alone (Figure 13).

IV. Summary

[00156] This study is the first to investigate the effects of sodium-dependent glucose transporter inhibitors on AMPK activity in cancer models. It was found that Canagliflozin, at concentrations within the therapeutic levels used for treating type 2 diabetes, inhibits the growth and survival of a variety of different cancer cell lines. These effects were not observed with the other approved SGLT2 inhibitor Dapagliflozin. It was demonstrated that clinically
relevant concentrations of Canagliflozin activate AMPK; while not wishing to be limited by theory, potentially through a mechanism involving the inhibition of the mitochondrial complex I. Furthermore, it was shown that suppression of growth and survival in PC3 cells is associated with the inhibition of glucose uptake, de novo lipogenesis and AKT/mTORC1 signaling. Further, the usefulness of combining low dose Canagliflozin with Docetaxel, Cisplatin and radiation therapies to further reduce the growth of lung and prostate cancer was shown.

[00157] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.
FULL CITATIONS FOR DOCUMENTS REFERRED TO IN THE APPLICATION


Scheen, A.J. Pharmacodynamics, Efficacy and Safety of Sodium-Glucose Co-Transporter Type 2 (SGLT2) Inhibitors for the Treatment of Type 2 Diabetes Mellitus. Drugs 75, 33-59 (2015).


Table 1. Cancerous cell lines - listed class/disease, origin and relevant mutation(s).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Line</th>
<th>Class/Disease</th>
<th>Origin</th>
<th>Mutations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>A549</td>
<td>Non-Small Cell Lung Cancer</td>
<td>Lung Carcinoma</td>
<td>LKB1/ KRAS (G12V)</td>
<td>Giard et al. 1973</td>
</tr>
<tr>
<td></td>
<td>H1299</td>
<td>Non-Small Cell Lung Cancer</td>
<td>Lung Carcinoma – Metastatic tumours in the lymph node</td>
<td>p53/ NRAS (Q61K)</td>
<td>Phelps et al. 1996</td>
</tr>
<tr>
<td>Prostate</td>
<td>PC3</td>
<td>Castrate-Resistant Androgen-Insensitive</td>
<td>Prostate Grade IV Adenocarcinoma – Metastatic tumours in the bone</td>
<td>p53/ PTEN/</td>
<td>Kaignh et al. 1979, Fraser et al. 2012</td>
</tr>
<tr>
<td></td>
<td>22RV-1</td>
<td>Androgen-Sensitive</td>
<td>Prostate Carcinoma – CWR22 xenograft</td>
<td>PI3K (Q546R) p53 (Q331R) AR (H874Y)</td>
<td>Sramkoski et al. 1999, Attardi et al. 2004, van Bokhoven et al. 2003</td>
</tr>
<tr>
<td>Colon</td>
<td>HCT116</td>
<td>Colorectal Carcinoma</td>
<td>Colon Carcinoma</td>
<td>PI3K (H1047R) KRAS (G13D)</td>
<td>Jhawer et al. 2008</td>
</tr>
<tr>
<td>Liver</td>
<td>HepG2</td>
<td>Hepatocellular Carcinoma</td>
<td>Liver Carcinoma</td>
<td>NRAS (Q61L)</td>
<td>Vollmer et al. 1995, Omerovic et al. 2005</td>
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<tr>
<td>Breast</td>
<td>MCF-7</td>
<td>ER+/PgR+ Mammary Carcinoma</td>
<td>Mammary Carcinoma – Metastatic tumour in the pleural effusion</td>
<td>PI3K (E545K)</td>
<td>Ikediobi et al. 2006</td>
</tr>
<tr>
<td>Ovarian</td>
<td>SKOV-3</td>
<td>Ovarian Carcinoma</td>
<td>Ovarian Carcinoma</td>
<td>PI3K (H1047R) p53 (S90)</td>
<td>Ikediobi et al. 2006</td>
</tr>
</tbody>
</table>
Table 2. Half maximal inhibitory concentrations (IC50) of Canagliflozin and Dapagliflozin on the proliferation of lung (A549, H1299), prostate (22RV1, PC3) colon (MC38, HCT116), liver (HepG2), breast (MCF7) and ovarian (SKOV-3) cells.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Cell Line</th>
<th>Cell Proliferation IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Canagliflozin</td>
</tr>
<tr>
<td>Lung</td>
<td>A549</td>
<td>55.13</td>
</tr>
<tr>
<td></td>
<td>H1299</td>
<td>34.21</td>
</tr>
<tr>
<td>Prostate</td>
<td>22RV1</td>
<td>28.88</td>
</tr>
<tr>
<td></td>
<td>PC3</td>
<td>27.52</td>
</tr>
<tr>
<td>Colon</td>
<td>MC38</td>
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</tr>
<tr>
<td>Colon</td>
<td>HCT116</td>
<td>45.81</td>
</tr>
<tr>
<td>Liver</td>
<td>HepG2</td>
<td>35.12</td>
</tr>
<tr>
<td>Breast</td>
<td>MCF7</td>
<td>19.73</td>
</tr>
<tr>
<td>Ovarian</td>
<td>SKOV-3</td>
<td>63.50</td>
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</tbody>
</table>
Table 3. Half maximal inhibitory concentrations (IC50) of Canagliflozin and Dapagliflozin on the clonogenic survival of lung (A549, H1299) and prostate (22RV1, PC3) cancer cells.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Cell Line</th>
<th>Clonogenic Survival IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Canagliflozin</td>
</tr>
<tr>
<td>Lung</td>
<td>A549</td>
<td>11.70</td>
</tr>
<tr>
<td></td>
<td>H1299</td>
<td>10.32</td>
</tr>
<tr>
<td>Prostate</td>
<td>22RV-1</td>
<td>13.19</td>
</tr>
<tr>
<td></td>
<td>PC3</td>
<td>8.78</td>
</tr>
<tr>
<td>Colon</td>
<td>MC38</td>
<td>23.40</td>
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Table 4. Cellular ATP content in PC3 and H1299 cells\(^1\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th></th>
<th>Glucose</th>
<th></th>
<th>Galactose</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>PC3</td>
<td></td>
<td>H1299</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% ATP Decrease</td>
<td>Standard Error</td>
<td>% ATP Decrease</td>
<td>Standard Error</td>
<td>Statistical Significance(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (\mu)M CAN</td>
<td>4.75</td>
<td>± 4.87</td>
<td>14.00</td>
<td>± 1.87</td>
<td>p=0.08</td>
<td></td>
</tr>
<tr>
<td>30 (\mu)M CAN</td>
<td>23.90</td>
<td>± 2.81</td>
<td>34.04</td>
<td>± 1.00</td>
<td>p=0.06</td>
<td></td>
</tr>
<tr>
<td>100 (\mu)M CAN</td>
<td>88.37</td>
<td>± 5.79</td>
<td>98.25</td>
<td>± 4.25</td>
<td>p=0.06</td>
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<tr>
<td>3 mM PHEN</td>
<td>18.75</td>
<td>± 6.34</td>
<td>33.66</td>
<td>± 4.27</td>
<td>p=0.007</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Cells were maintained in either 11 mM glucose or 10 mM galactose for 24 hours, then treated with Canagliflozin or Phenformin (positive control) at the indicated concentrations relative to the vehicle treated controls for 30 minutes.

\(^2\) The results are expressed as the mean and standard error of the mean (SEM) of four independent experiments where \(p\) was calculated by one ANOVA.
Claims:

1. Method for the treatment or prevention of cancer progression in a subject, said method comprising administering to the subject an effective amount of Canagliflozin, or an active analog thereof.

2. The method according to claim 1, wherein the Canagliflozin, or the active analog thereof, is administered in combination with an adjunct cancer treatment.

3. A method of treating cancer comprising administering an effective amount of Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment to a subject in need thereof.

4. A method of improving the efficacy of an adjunct cancer treatment comprising administering an effective amount of Canagliflozin, or an active analog thereof, in combination with the adjunct cancer treatment to a subject in need thereof.

5. The method according to any one of claims 2 to 4, wherein the adjunct cancer treatment is one or more other anti-cancer agents and/or radiation therapy.

6. The method according to claim 5, wherein the other anti-cancer agent is biguanide derivative, salsalate or salicylate derivative, or a biological agent.

7. The method according to claim 5, wherein the other anti-cancer agent is Cisplatin or Docetaxel.

8. The method according to claim 5, wherein the other anti-cancer agent is selected from Phenformin, Metformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin, Paclitaxel, Cyclophosphamide, Methotrexate, Cisplatin, 5-Fluorouracil, Etoposide, carboplatin, Gemcitabine, Vinorelbine and combinations thereof.
9. The method according to claim 5, wherein the other anti-cancer agent is a biological agent.

10. The method according to any one of claims 1 to 9, wherein the cancer is selected from prostate cancer, pancreatic cancer, ovarian cancer, lung cancer, breast cancer, bladder cancer, colon cancer, brain cancer, head and neck cancer, endometrial cancer, leukemia, lymphoma and sarcoma.

11. The method according to claim 10, wherein the cancer is of the lung, prostate, colon, breast or ovary.

12. The method according to any one of claims 1 to 11, wherein the active analog of Canagliflozin is a compound of the formula (I):

\[
\begin{align*}
R^A & \quad \text{Ring C} \\
\text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

wherein \( R^A \) is selected from halo and \( \text{Cl}_4\text{alkyl} \); and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, \( \text{C}_1\text{alkyl} \), halo-substituted \( \text{C}_4\text{alkyl} \), \( \text{OC}_4\text{alkyl} \), halo-substituted \( \text{OC}_1\text{alkyl} \), methylenedioxy, ethylenedioxy, mono-\( \text{Cl}_4\text{alkylamino} \), di-\( \text{Cl}_4\text{alkylamino} \), carbamoyl, mono-\( \text{Cl}_4\text{alkylcarbamoyl} \) and di-\( \text{Cl}_4\text{alkylcarbamoyl} \).

13. The method according to claim 12, wherein Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, \( \text{Cl}_4\text{alkyl} \), halo-substituted \( \text{C}_4\text{alkyl} \), \( \text{OC}_4\text{alkyl} \), halo-substituted \( \text{OC}_4\text{alkyl} \), mono-\( \text{d}_4\text{alkylamino} \) and di-\( \text{Cl}_4\text{alkylamino} \).

14. The method according to claim 12 or 13, wherein \( R^A \) is selected from \( \text{F} \), \( \text{Cl} \) and \( \text{CH}_3 \).
15. The method according to any one of claims 1 to 11, comprising administration of Canagliflozin, or a hydrate, solvate and/or crystalline polymorph thereof.

16. The method according to any one of claims 1 to 15, wherein the subject is a human.

17. A pharmaceutical composition comprising Canagliflozin, or an active analog thereof, in combination with one or more other anti-cancer agents.

18. The pharmaceutical composition according to claim 17, wherein the other anti-cancer agent is a biguanide derivative, salsalate or salicylate derivative, or a biological agent.

19. The pharmaceutical composition according to claim 17, wherein the other anti-cancer agent is selected from Phenformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin, Paclitaxel, Cyclophosphamide, Methotrexate, Cisplatin, 5-Fluorouracil, Etoposide, carboplatin, Gemcitabine, Vinorelbine and combinations thereof.

20. The pharmaceutical composition according to claim 17, wherein the other anti-cancer agent is a biological agent.

21. The pharmaceutical composition according to any one of claims 17 to 20, wherein the active analog of Canagliflozin is a compound of the formula (I):

![Chemical Structure Image]

wherein $R^A$ is selected from halo and $\text{C}_i$-$\text{alkyl}$; and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, $\text{C}_i$-$\text{alkyl}$, halo-substituted $\text{C}_i$-$\text{alkyl}$, $\text{OC}_i$-$\text{alkyl}$, halo-substituted $\text{OC}_i$-$\text{alkyl}$, halo-substituted $\text{OC}_i$. 
alkyl, methylenedioxy, ethyleneoxy, mono-\(\text{Cl}_4\)alkylamino, di-\(\text{Cl}_4\)alkylamino, carbamoyl, mono-\(\text{Cl}_4\)alkylcarbamoyl and di-\(\text{Cl}_4\)alkylcarbamoyl.

22. The pharmaceutical composition according to claim 21, wherein Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, \(\text{Cl}_4\)alkyl, halo-substituted \(\text{Cl}_4\)alkyl, \(\text{OCl}_4\)alkyl, halo-substituted \(\text{OCl}_4\)alkyl, mono-\(\text{C}_1-4\)alkylamino and di-\(\text{C}_1-4\)alkylamino.

23. The pharmaceutical composition according to claim 21 or 22, wherein \(R^A\) is selected from F, Cl and CH₃.

24. The pharmaceutical composition according to any one of claims 17 to 20, comprising Canagliflozin, or a hydrate, solvate and/or crystalline polymorph thereof.

25. A use of Canagliflozin, or an active analog thereof, for treating cancer in a subject in need thereof.

26. The use according to claim 25, wherein the Canagliflozin, or the active analog thereof, is for use in combination with an adjunct cancer treatment.

27. A use of Canagliflozin, or an active analog thereof, in combination with an adjunct cancer treatment for treating cancer in a subject.

28. A use of Canagliflozin, or an active analog thereof, for improving the efficacy of an adjunct cancer treatment.

29. The use according to any one of claims 26 to 28, wherein the adjunct cancer treatment is one or more other anti-cancer agents and/or radiation therapy.

30. The use according to claim 29, wherein the other anti-cancer agent is a biguanide derivative, salsalate or salicylate derivative, or a biological agent.

31. The use according to claim 29, wherein the other anti-cancer agent is selected from Phenformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin,
Paclitaxel, Cyclophosphamide, Methotrexate, Cisplatin, 5-Fluorouracil, Etoposide, carboplatin, Gemcitabine, Vinorelbine and combinations thereof.

32. The use according to claim 29, wherein the other anti-cancer agent is Metformin.

33. The use according to claim 29, wherein the other anti-cancer agent is a biological agent.

34. The use according to any one of claims 25 to 33, wherein the cancer is selected from prostate cancer, pancreatic cancer, ovarian cancer, lung cancer, breast cancer, bladder cancer, colon cancer, brain cancer, head and neck cancer, endometrial cancer, leukemia, lymphoma and sarcoma.

35. The use according to claim 34, wherein the cancer is of the lung, prostate, colon, breast or ovary.

36. The use according to any one of claims 25 to 35, wherein the active analog of Canagliflozin is a compound of the formula (I):

\[
\text{(I)}
\]

wherein \( R^A \) is selected from halo and \( \text{Ci}_4\text{alkyl} \); and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, \( \text{Ci}_4\text{alkyl} \), halo-substituted \( \text{Ci}_4\text{alkyl} \), OCi\(_4\text{alkyl} \), halo-substituted OC-i\(_4\text{alkyl} \), methylenedioxy, ethylenedioxy, mono-Ci\(_4\text{alkylamino} \), di-Ci\(_4\text{alkylamino} \), carbamoyl, mono-Ci\(_4\text{alkylcarbamoyl} \) and di-Ci\(_4\text{alkylcarbamoyl} \).

37. The use according to claim 36, wherein Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, Ci\(_4\text{alkyl} \), halo-
substituted \( \text{Cl}_4 \text{alkyl}, \text{OCI}_4 \text{alkyl}, \) halo-substituted \( \text{OCI}_4 \text{alkyl}, \) mono-\( \text{C}_1-4 \text{alkylamino} \) and di-\( \text{Cl}_4 \text{alkylamino}. \)

38. The use according to claim 36 or 37, wherein \( R^k \) is selected from \( \text{F}, \text{Cl} \) and \( \text{CH}_3 \).

39. The use according to any one of claims 25 to 35 comprising use of Canagliflozin, or a hydrate, solvate and/or crystalline polymorph thereof.

40. The use according to any one of claims 25 to 39, wherein the subject is a human.

41. A kit for the treatment of cancer, the kit comprising:
   - Canagliflozin, or an active analog thereof;
   - one or more other anti-cancer agents; and
   - optionally instructions for administration of the Canagliflozin, or the active analog thereof, and the one or more other anti-cancer agents to a subject in need thereof.

42. A kit for improving the efficacy of an anti-cancer agent for the treatment of cancer, the kit comprising:
   - Canagliflozin, or an active analog thereof;
   - the anti-cancer agent; and
   - optionally instructions for administration of the Canagliflozin, or the active analog thereof, and the anti-cancer agent to a subject in need thereof.

43. The kit according to claim 41 or 42, wherein the other anti-cancer agent is a biguanide derivative, salsalate or salicylate derivative, or a biological agent.

44. The kit according to claim 41 or 42, wherein the other anti-cancer agent is selected from Phenformin, Doxorubicin, Docetaxel, Duanorubicin, Epirubicin, Paclitaxel, Cyclophosphamide, Methotrexate, Cisplatin, 5-
Fluorouracil, Etoposide, carboplatin, Gemcitabine, Vinorelbine and combinations thereof.

45. The kit according to claim 43, wherein the other anti-cancer agent is Metformin.

46. The kit according to claim 41 or 42, wherein the other anti-cancer agent is a biological agent.

47. The kit according to any one of claims 41 to 46, wherein the cancer is selected from prostate cancer, pancreatic cancer, ovarian cancer, lung cancer, breast cancer, bladder cancer, colon cancer, brain cancer, head and neck cancer, endometrial cancer, leukemia, lymphoma and sarcoma.

48. The kit according to claim 47, wherein the cancer is of the lung, prostate, colon, breast or ovary.

49. The kit according to any one of claims 41 to 48, wherein the active analog of Canagliflozin is a compound of the formula (I):

```
\[ \text{Formula Image} \]
```

wherein \( R^A \) is selected from halo and \( \text{C}_4\text{-alkyl} \); and Ring C is phenyl unsubstituted or substituted with 1 to 3 substituents selected from halo, cyano, \( \text{C}_4\text{-alkyl} \), halo-substituted \( \text{C}_4\text{-alkyl} \), \( \text{OC}_4\text{-alkyl} \), halo-substituted \( \text{OC}-i-4\text{-alkyl} \), methylenedioxy, ethylenedioxy, mono-\( \text{C}_4\text{-alkylamino} \), di-\( \text{C}_4\text{-alkylamino} \), carbamoyl, mono-\( \text{C}_4\text{-alkylcarbamoyl} \) and di-\( \text{C}_4\text{-alkylcarbamoyl} \).

50. The kit according to claim 49, wherein Ring C is a phenyl group substituted with 1-2 substituents selected from halo, cyano, \( \text{C}_4\text{-alkyl} \), halo-
substituted $\text{Cl}_4\text{alkyl}$, $\text{OCl}_4\text{alkyl}$, halo-substituted $\text{OCl}_4\text{alkyl}$, mono-$\text{C}_1\text{alkylamino}$ and di-$\text{Cl}_4\text{alkylamino}$. 

51. The kit according to claim 49 or 50, wherein $R^A$ is selected from $\text{F}$, $\text{Cl}$ and $\text{CH}_3$.

52. The kit according to any one of claims 41 to 48, comprising Canagliflozin, or a hydrate, solvate and/or crystalline polymorph thereof.

53. The kit according to any one of claims 41 to 52, wherein the subject is a human.
**FIG. 1**

**A**

**A549**

- Black squares: Dapagliflozin
- Grey circles: Canagliflozin

**Cell Proliferation** vs **Dose (μM)**

**B**

**H1299**

- Black squares: Dapagliflozin
- Grey circles: Canagliflozin

**Cell Proliferation** vs **Dose (μM)**
FIG. 1 (cont.)
FIG. 1 (cont.)
FIG. 2D
MC38-Colon Cancer

Percent Inhibition (% of Control)

Concentration (µM)

- Dapagliflozin
- Canagliflozin

FIG. 2E
FIG. 3A
FIG. 3C
FIG. 4A
FIG. 6
FIG. 7
FIG. 8
FIG. 8 (cont.)
FIG. 9
FIG. 9 (cont.)
FIG. 11
H1299

**A**

- Canagliflozin
- Canagliflozin + 5 µM Cisplatin

**B**

- Cisplatin
- Cisplatin + 30 µM Canagliflozin

**FIG. 12**
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2006.01): A61K 31/381, A61P 35/00, C07D 409/10

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)
IPC (2006.01): A61K31, A61P35, C07D 409

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: Canadian Patent Database, Orbit (FAMPAT), PubMed, Scopus
Keywords: canagliflozin, invokana, dapagliflozin, fortiga, cancer, neoplastic, neoplasms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>P, X, Y</td>
<td>SAITO, et al. ENDOCRINE JOURNAL, Vol. 62, epub 31 October 2015 (31-10-2015), pages 1133-1137. See abstract; Figure 4; and page 1137.</td>
<td>X: 1, 10, 11, 15, 16, 25, 34, 35, 39, and 40</td>
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* Further documents are listed in the continuation of Box C. See patent family annex.

| “T” | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| “X” | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| “Y” | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |

Date of the actual completion of the international search
27 May 2016 (27-05-2016)

Date of mailing of the international search report
10 June 2016 (10-06-2016)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, CI 14 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer
Philip O. Brown, Ph.D. (819) 994-1622
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **Claim Nos.: 1-16**
   because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-6 are directed to methods for treatment of the human or animal body by surgery or therapy which, under Article 17(2)(b) and Rule 39.1(iv), the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the products appearing in said claims.

2. **Claim Nos.: 1-4, 6, 9, 17, 18, 20, 25-28, 30, 33, 41-43, and 46**
   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:

The above claims do not clearly and concisely define the subject matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. By the same token, the subject matter encompassed by said claims has not been clearly and completely disclosed in the description, such that the application does not comply with Article 5 of the PCT. **See extra sheet for further details.**

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

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

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 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 claim 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 claim Nos.:

**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.
<table>
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Y: 12-14, 21-23, 36-38, and 49-51 |
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Continuation of Box II:

Claims 1-4, 5, 9, 17, 18, 20, 25-28, 30, 33, 41-43, and 46 do not clearly define the matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. The expressions "an active analog", "biguanide derivative" (emphasis added), "salsalate or salicylate derivative" (emphasis added), and "biological agent" attempt to define various compounds in terms of qualitative similarity or generic origin. As there is no clear definition as to what constitutes an analog, derivative, or "biological agent", such expressions therefore fail to clearly define the subject matter for which protection is sought. Furthermore, the disclosure relating to such genera is so incomplete with regards to the meaning of Article 5 such that said claims appear to lack support with regards to the meaning of Article 6. This renders a search across the entire breadth of these claims impossible. As a result, said claims have only been searched with regards to those compounds clearly defined in the application (i.e., canagliflozin, dapagliflozin, and the derivatives of such compounds that are structurally defined in the claims), optionally in combination with anti-cancer agents in general.

Claims 4, 28, and 42 do not clearly define the matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. The expression "improving the efficacy of an adjunct cancer treatment" attempts to define the result to be achieved in terms of a incompletely defined qualitative comparison with another treatment. It therefore fails to clearly define what applications are actually encompassed by such a claim. In particular, it is unclear how such claims differ from the claims to combination chemotherapy appearing in the application (e.g., claim 3). As a result, for search purposes, said claims have been interpreted as being simply directed to combination chemotherapy involving canagliflozin and a secondary agent.