(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 October 2006 (19.10.2006)

PCT

(10) International Publication Number
WO 2006/108276 A1

(51) International Patent Classification:
A61K 31/19 (2006.01)   A61P 35/00 (2006.01)
C12Q 1/00 (2006.01)

(21) International Application Number:
PCT/CA2006/000548

(22) International Filing Date: 11 April 2006 (11.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/669,884   11 April 2005 (11.04.2005)   US

(71) Applicant (for all designated States except US): THE
GOVERNORS OF THE UNIVERSITY OF ALBERTA
[CA/CA]; #4000 Research Transition Facility, 8308 - 114
Street, Edmonton, Alberta T6G 2E1 (CA).

(72) Inventors; and
(75) Inventors/Applicants (for US only): MICHELAKIS,
Evangelos [CA/CA]; 8728-103 Street, Edmonton, Alberta
T6E 6K3 (CA). ARCHER, Stephen [CA/CA]; 450 Olsen
Close, Edmonton, Alberta T6R 1L1 (CA).

(74) Agents: NADOR, Anita et al.; McCarthy Tétrault LLP,
Box 48, Suite 4700, Toronto Dominion Bank Tower,
Toronto, Ontario M5K 1E6 (CA).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI,
NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,
SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US,
UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
—   with international search report

[Continued on next page]

(54) Title: A METHOD OF TREATING CANCER USING DICHLOROACETATE
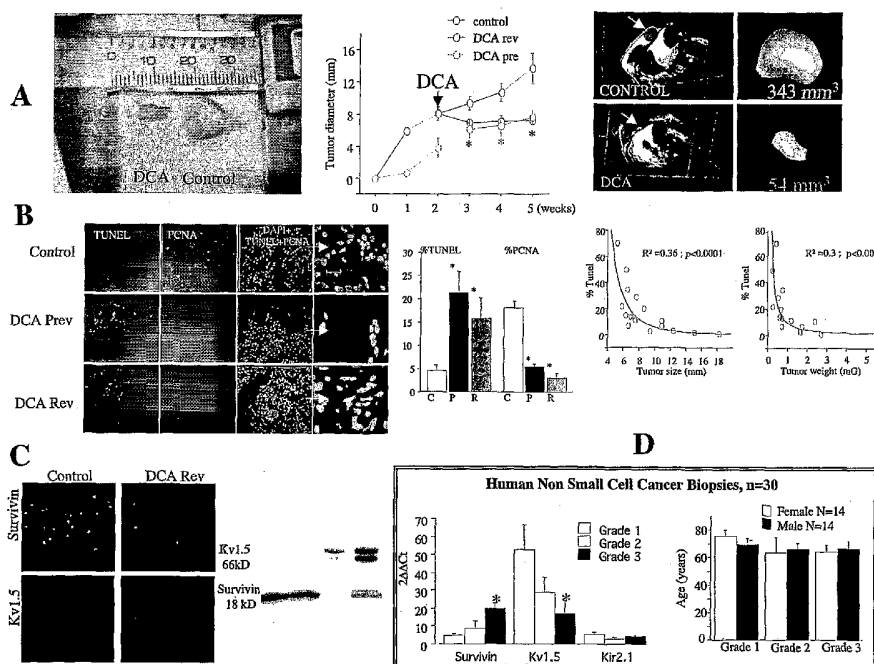
(57) Abstract: The invention relates to the use of dichloroacetate and chemical equivalents thereof for the treatment of cancer by
inducing apoptosis or reversing apoptosis-resistance in a cell Preferably, the dosage is 10-100 mg/kg Preferably, sodium dichloroac-
etate is used. The dichloroacetate may optionally be given in combination with a pro-apoptotic agent and/or a chemotherapeutic
agent Preferably, the cancers treated are non-small cell lung cancer, glioblastoma and breast carcinoma.

TITLE: A Method of Treating Cancer Using Dichloroacetate

This patent application claims priority from United States Provisional Patent Application No. 60/669,884 filed April 11, 2005, the content of which is hereby incorporated by reference herein.

5   **FIELD OF THE INVENTION**

The invention relates to the use of dichloroacetate and obvious chemical equivalents thereof in the treatment of cancer. Related uses and diagnostic and screening methods are also included in one aspect of the present invention.

BACKGROUND OF THE INVENTION

10   Most cancers are characterized by a resistance to apoptosis that makes them prone to proliferation and resistant to most cancer therapies. Most of the available cancer treatments aim to induce apoptosis but are highly toxic. There are two main categories of apoptosis: the receptor–mediated and the mitochondria–dependent apoptosis. Mitochondria-dependent apoptosis is not very well studied and only

15   recently have the mitochondria been viewed as anything more than an organelle that produces energy. As such there is a need for a cancer therapy that can overcome apoptosis resistance in cancer cells.

SUMMARY OF THE INVENTION

A cell can become resistant to apoptosis in a variety of ways one of which is

20   altering its metabolism and having hyperpolarized mitochondria. Since apoptosis is initiated by depolarization of mitochondria, the more hyperpolarized a mitochondrion is, the further it is from the depolarization threshold and the more resistant it is to the initiation of apoptosis.

In one embodiment the present inventors have surprisingly found that one

25   can modulate mitochondrial function to treat cancer. In one embodiment, the present invention provides a method for inducing apoptosis in cancer. In another embodiment, the inventors provide a method for inducting apoptosis in cancer but normal cells. In another embodiment, the invention provides a method of reversing apoptosis resistance in cancer cells, such as cancer cells with hyperpolarized

30   mitochondria. In one embodiment, the method comprises administering to cancer cells, in one embodiment cells having or suspected of having hyperpolarized mitochondria, an effective amount of dichloroacetate or salts thereof or obvious

chemical equivalents thereof.

In one embodiment, the dichloroacetate or obvious chemical equivalent thereof is administered in combination with another pro-apoptotic agent and/or chemotherapeutic agent, and/or other cancer therapy.

5   In one embodiment, the invention provides a method for inducing apoptosis and/or reversing apoptosis resistance in a cancer cell, comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the invention provides a method for inhibiting proliferation of cancer cells, comprising administering to the cells an effective

10   amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the invention provides a method of decreasing survivin in a cancer cell, comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the invention provides a method of increasing Kv1.5 protein in a cancer cell comprising

15   administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the invention provides a method of increasing AIF in a cancer cell comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the invention provides a method of increasing H_2O_2 in a cancer cell

20   comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the methods of the invention cancer cells, but not normal or non-cancerous cells are affected by the treatment with dichloroacetate or obvious chemical equivalent thereof.

In one embodiment, the present invention provides a method for treating a

25   cancer. In another embodiment, the invention provides a method of treating a cancer associated with hyperpolarized mitochondria. In another embodiment the invention provides a method of treating cancer by restoring mitochondrial membrane potential ($\Delta\Psi m$) (essentially depolarizing the hyperpolarized cancer cell mitochondria). This molecular metabolic therapy is accomplished by administering

30   to a patient in need thereof a therapeutically effective amount of dichloroacetate or obvious chemical equivalent thereof. In another embodiment, the invention provides a use of dichloroacetate or obvious chemical equivalent thereof in the

treatment of cancer.

In one embodiment, the dichloroacetate is a salt of dichloroacetic acid. In another embodiment, the dichloroacetic acid is a sodium salt of dichloroacetic acid.

In one embodiment, the cancer to be treated using the DCA or obvious chemical equivalent thereof is selected from the group consisting of: non-small cell lung cancer, glioblastoma and breast carcinoma.

In another embodiment, the dichloroacetate, or obvious chemical equivalent thereof, is administered in the form of a pharmaceutical composition comprising dichloroacetate or obvious chemical equivalent thereof and a pharmaceutically acceptable carrier. In yet another embodiment the invention provides a use of dichloroacetic acid or dichloroacetate or obvious chemical equivalent thereof in the preparation of a medicament or pharmaceutical composition for the treatment of cancer, such as a cancer associated with hyperpolarized mitochondria. In yet another embodiment, the dichloroacetate, or obvious chemical equivalent thereof, is administered orally.

In yet another embodiment, the dichloroacetate is administered in a water-based formulation. In one embodiment the water-based formulation of DCA comprises 0.0075g of DCA/l to 7.5 g of DCA/l). In another embodiment the dichloroacetate or obvious chemical equivalent thereof is administered at a total daily dose of ~25-50mg/kg bid of dichloroacetate. In another embodiment the dose is 10-100 mg/kg given twice a day is administered to the patient. In one embodiment the dose is 25-50 mg bid.

In another embodiment, the invention constitutes a method for determining whether a cancer is associated with hyperpolarized mitochondria, which would predict its therapeutic response to dichloroacetate or obvious chemical equivalents thereof or similar compounds. In one embodiment such method comprises administering an effective amount of dichloroacetate, or chemical equivalent thereof to a cancer tissue sample from a patient and measuring its apoptosis sensitivity and mitochondrial membrane potential using confocal microscopy or flow cytometry. This diagnostic test would determine whether the individual patient could benefit from dichloroacetate or other therapies that cause apoptosis through similar mechanism.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various

5   changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 shows that dichloroacetate depolarizes mitochondria, releases

10   **cytochrome-c and AIF from the mitochondria and enhances H_2O_2 production.**
Figures 1A, B and C are confocal images of cancer and normal cells stained with dyes for mitochondrial membrane potential or antibodies to cytochrome c and apoptosis inducing factor (AIF) as explained in Example 1.

Figure 1D shows the results of an assay measuring production of hydrogen peroxide

15   (H_2O_2) in cancer cells treated with dichloroacetate, as explained in Example 1.

Figure 2 shows that dichloroacetate increases K^+ current and repolarizes cancer cells without affecting normal cells.
Figure 2A and B show patch clamping data in cancer and normal cells in response to dichloroacetate and a variety of inhibitors, as explained in Example 2.

20   Figure 2C shows the results of quantitative reverse tanscriptase polymerase chain reaction (qRT-PCR) and immunobloting, studying the expression of Kv1.5 in cancer cells treated with dichloroacetate, as explained in Example 2.

Figure 3 shows that DCA increases Kv1.5 expression via the Ca++/calcineurin-dependent transcription factor NFAT.

25   Figure 3A are confocal images of cancer cells loaded with the calcium-sensitive dye Fluo-3 and mean data for calcium levels, as explained in Example 3.

Figure 3B are confocal images of cancer cells treated with antibodies against Kv1.5 and NFAT, as explained in Example 3.

Figure 4 shows that dichloroacetate increases apoptosis and decreases

30   **proliferation of cancer cells in vitro.**
Figure 4A are confocal images of cancer cells assayed for Annexin, TUNEL, PCNA, BrdU and surviving, as explained in Example 4.

Figure 4B are immunoblots in cancer cells for expression and activity of caspase 3 and 9, as explained in Example 4.

Figure 5 shows that dichloroacetate prevents and reverses tumor growth in vivo in rats and a similar correlation between apoptosis-K+ channels and malignancy grade in human cancers.

Figure 5A are representative pictures of explanted tumors and MRI images form the tumors in vivo, as well as a plot of tumor size over time in response to dichloroacetate, as explained in Example 5.

Figure 5B are confocal images of tumors stained for TUNEL and PCNA as well as mean data, as explained in Example 5.

Figure 5C are confocal pictures of tumors stained with surviving and Kv1.5 as well as an immunoblot for surviving and Kv1.5, as explained in Example 5.

Figure 5D illustrates mean data from qRT-PCR analysis for survivin and Kv1.5, plotted over histological grade score, from non small cancer of the lung specimens from 30 patients, as explained in Example 5.

Figure 6 is a schematic drawing illustrating dichloroacetate's proposed mechanism of action.

Figure 7 is a heat map of genes modified by dichloroacetate in cancer cells, using a Affymetrix gene microarray, as explained at the bottom of the results a discussion section.

DETAILED DESCRIPTION OF THE INVENTION

"Dichloroacetate (DCA) or obvious chemical equivalent thereof" means dichloroacetate acid or salt thereof or other analog, derivative of dichloroacetate that has the same desired therapeutic effect in the treatment of cancer.

For example, salts of dichloroacetic acid are well known and commercially available. Generally, such salts of dichloroacetic acid will have the following formula:
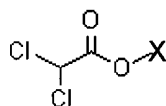
X=Na, K+, CH3 or OH

Specific salts include those formed by the alkali metal and alkaline earth metal ions such as sodium, potassium, calcium, and magnesium, ammonium, and substituted ammonium where the substituent is a mono-ordi-lower alkyl radical of 1-4 carbon atoms and ethylene diammonium. Pharmaceutically acceptable salts,

5   with minimum cell cytotoxicity, such as sodium, are preferred.

Specific pharmaceutical salts useful in this invention include sodium dichloroacetate, potassium dichloroacetate, and diisoproyl ammonium dichloroacetate. The sodium dichloroacetate and free base forms are highly preferred. Generally, salt and free base forms of dichloroacetate are particularly

10   preferred for use in the invention because of their ready availability and economical price. In one embodiment, salts of dichloroacetic acid can be used at a concentration between about 0.5 mM to about 100mM, preferably about 50 mM. Preferably such compounds are used at a concentration of at concentrations of 0.1-10mM, more preferably at least about 0.5 mM (estimated drug concentration at tumor based on

15   cell culture studies).

"Administering to the cell" as used herein, means any mode wherein a substance is administered to the cell (in vivo or in vitro) and has an effect on said cell, such methods are known to those skilled in the art. By way of example, in vitro, it can be administered to the cell media, or cell culture media. In vivo, by way

20   of example, it can be administered through known forms of pharmaceutical administration.

A "patient in need thereof" as used herein is a patient that has or is suspected of having a cancer, such as lung cancer, glioblastoma or breast cancer, which is characterized by apoptosis resistance and/or hyperpolarized mitochondria.

25   Preferably said patient is a mammal, and in one embodiment a human. It is acknowledged that because of the difficulty obtaining human tissue to allow direct measurement of hyperpolarized mitochondria that one could measure survivin (as a surrogate of mitochondria dependent apoptosis) and Kv1.5 mRNA. As shown in Figure 5 this index of survivin/Kv1.5 is high in all tumor cell lines tested and in a

30   cohort of 30 patients with non-small cell lung cancer. Furthermore DCA reversed this index in vitro and in vivo. Therefore in one embodiment of the invention this index can be used clinically to select the patients most likely to benefit from DCA

therapy. However, the method of the invention is not necessarily restricted to such patients as clinical trials using DCA therapy could be used to identify patients who may benefit from the therapy in whom the index is unknown, unavailable or low.

As such, in one embodiment, the invention provides a method for determining whether a cell, or patient comprising cells suspected of being cancer cells can benefit from dichloroacetate or obvious chemical equivalent treatment.

In one embodiment, the invention provides a method for identifying cells or a patient that may benefit from treatment with dichloroacetate or obvious chemical equivalent thereof to induce or reverse resistance to apoptosis and/or to treat cancer, comprising:

(i) obtaining a cell sample, tissue sample, or sample comprising cancerous cells or cells suspected of being cancerous;

(ii) administering to said sample dichloroacetate or obvious chemical equivalent thereof;

(iii) determining whether said apoptosis is induced and/or apoptosis resistance is reversed in said sample,

wherein when said apoptosis is induced and/or apoptosis resistance is reversed in said sample, this is indicative that the cells and/or patient from where the sample was obtained may benefit from dichloroacetate or obvious chemical equivalent treatment.

In one embodiment, the method of determining whether said apoptosis is induced and/or reversed or whether a patient or cell may benefit from dichloroacetate or obvious chemical equivalent treatment is by monitoring the samples survivin/Kv1.5 index. This can be monitored with respect to an external or internal control. For instance, in one embodiment, the index compared to a pre-determined level or range that is known to benefit from treatment, and/or the index is obtained from the sample prior to treatment and if it is lowered or reversed, then the cell or patient may benefit from treatment.

The dichloroacetate and obvious chemical equivalents of the invention may be formulated into pharmaceutical compositions for administration to humans in a biologically compatible form suitable for administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the

substance to be administered in which any toxic effects are outweighed by the therapeutic effects.

Administration of a therapeutically effective amount or effective amount of the pharmaceutical compositions or DCA or obvious chemical equivalents thereof

5   of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of dichloroacetate or obvious chemical equivalent thereof or formulation comprising

10   thereof to elicit a desired response in the individual. The dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

Accordingly, the present invention provides a pharmaceutical composition

15   for treating a patient in need thereof, such as a human, comprising dichloroacetate or obvious chemical equivalent thereof of the invention and a pharmaceutically acceptable carrier, diluents or excipients.

The term "treating a human" as used herein means administering the pharmaceutical composition of the invention to a human to prevent, alleviate or

20   cure a cancer.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle.

25   In one embodiment, the pharmaceutical compositions may be used in methods for treating humans. The dosage and type of dichloroacetate of the invention to be administered will depend on a variety of factors, which may be readily monitored in human subjects. Such factors include the type and severity of the disease.

30   A further aspect of the present invention relates to methods for therapeutic treatment of humans using dichloroacetate compositions or obvious chemical equivalents thereof.

In a further embodiment of the invention, the dichloracetate or obvious chemical equivalent thereof can be administered in combination with other pro-apoptotic or chemotherapeutic agents. "In combination with" as used in this context means that the agents can be administered at the same time or at different times, but

5   in a combination treatment regimen, such as combination regimens know to those skilled in the art. In one embodiment, the agents can be formulated into a single or different pharmaceutical compositions.

It is further understood that the therapeutic compositions of the invention may be used in conjunction with pharmaceutically acceptable excipient or carriers.

10   The pharmaceutical compositions according to the present invention are prepared conventionally, comprising substances that are customarily used in pharmaceuticals, [e.g. Remington's Pharmaceutical Sciences, (Alfonso R. Gennaro ed. 18th edition 1990) Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985 or Remington's The Sciences

15   and Practice of Pharmacy, 21st Edition", (University of the Sciences in Philadelphia, 2005) or Handbook of Pharmaceutical Additives (compiled by Michael and Irene Ash, Gower Publishing Limited, Aldershot, England (1995))], including excipients, carriers adjuvants and buffers. The compositions can be administered, e.g. orally, parentally, enterally, intramuscularly, subcutaneously,

20   intravenously or other routes useful to achieve an effect. For example, in one embodiment, the active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, intramuscular, etc.), oral administration or inhalation. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and

25   other natural conditions which may inactivate the compound. Conventional excipients include pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral, oral and other routes of administration that do not deleteriously react with the agents. For parental application, particularly suitable are injectable sterile solutions, preferably oil or aqueous solutions, as well

30   as suspensions, emulsions or implants, including suppositories. Ampules are convenient unit dosages. The pharmaceutical preparations can be sterilized and, if desired, mixed with stabilizers, wetting agents, emulsifiers, salts for influencing

osmotic pressure, buffers or other substances that do not react deleteriously with the active compounds. In one embodiment, the pharmaceutical compositions of the invention are compositions that can be administered orally.

The invention will now be described by the following non-limiting examples.

EXAMPLES

METHODS

Cell Culture: The non-small cell lung cancer cell line A549, the glioblastoma cell line MO59K, and the mammary carcinoma cell line, MCF 7, were purchased from ATCC (VA USA). Normal small airway epithelial cells (NSAEC) were purchased from Cambrex Bio-science. A549 were maintained on F12K medium, glioblastoma on DMEM/F12, (Gibco/Invitrogen ON Canada), MCF-7 cells on DMEM (Sigma-Aldrich, ON Canada) and NSAEC on special small airway epithelial cell basal medium provided by the company. All media were supplemented with 10% FBS (Sigma-Aldrich) and 5% PSF (Gibco) as antibiotic.

Tumorigenicity Assays in Nude rats. Cells from A549 cell lines were harvested and resuspended in PBS. The cell suspension (3.106 cells per injection) was injected s.c. in the back (below the scapula level) of athymic nude rats. Rats were randomly divided into 3 groups: control (received only the cells at day 0), DCA prevention group (cells + DCA at day 0) and DCA reversal (cells at day 0+ DCA 2 weeks post injection). The DCA 0.075 g/L was added to the drinking water and the rats had free access to water. At this dose the DCA solution is colourless and odorless. Rats were observed weekly for the visual appearance of tumors at injection sites, and tumor sizes were measured every week in the 3 groups during one month. At the end of the month, rats were killed, and the tumors were excised and fixed for apoptosis and proliferation measurements. Some tumors were imaged in anesthetized rats with A SIEMENS 1.5T MRI systems, using standard sequences, allowing for 3D reconstruction and calculation of the tumor volume in vivo.

Immunoblotting : Antibodies to K^+ channels were purchased from Alomone (Jerusalem, Israel). Cells or tumor were collected and immunoblotting was performed on pooled samples from 4 T-25 dishes or 4 rats in each of the 2 groups (25 μg protein in pooled sample/lane), as previously described. The films were

digitized and quantitated using 1D Image Analysis Software (Kodak, Rochester, NY). Expression was normalized to both Ponceau-S and smooth muscle actin signal to correct for loading differences.

qRT-PCR: Samples were added to a microwell plate with TaqMan probes and RT-
5   PCR reagents (Applied Biosystems, Foster City, CA). qRT-PCR was performed with an ABI PRISM 7700 Sequence Detector (Applied Biosystems) and primers for human Kv1.5, survivin, 18s and Beta 2 microglobulin as described.

Electrophysiology: Whole cell electrophysiology was performed on cultured cells. Cells were voltage-clamped at a holding potential of -70 mV. Currents were evoked
10   by 200-ms test pulses from -70 to +70 mV filtered at 1kHz and sampled at 2-4kHz.

Confocal Microscopy: Imaging was performed using a Zeiss LSM 510 confocal microscope as described. Apoptag apoptosis detection kit (TUNEL stain, Serologicals, Norcross, GA) and the proliferating cell nuclear antigen (PCNA) antibody (DAKO, Carpinteria, CA) were used as per manufacturer's instructions on
15   both formaldehyde fixed cells and paraffin-embedded tissue sections after antigen retrieval. Nuclear staining was made using 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI, 300nM; Molecular Probes) in fixed tissue or cells as previously described Apoalert Annexin V kit (Clontech, Palo Alto, CA), cytochrome c antibody (Pharmingen, San Diego, CA), Apoptosis inducing factor
20   (Santa Cruz California USA) NFAT and Kv1.5 (Sigma) and mitotracker red (500 nM, Molecular Probes) were used as described by the company instructions. Mitochondrial membrane potential ($\Delta\Psi$m) was studied in live cancer cultured cells, using tetramethylrhodamine methyl-ester perchlorate (TMRM) (20 nmol/L) for 30 minutes (37°C) and Hoechst (1.0 umol/L) nuclear staining for 10 minutes
25   (Molecular Probes, Canada).

H_2O_2 measurements. Cancer cells were propagated on LabTek multiwell slides (Nalge/Nunc, VWR, ON Canada) until confluent. Monolayers were pre-incubated with 500μM DCA (Sigma-Aldrich) in the presence or absence of 5μM rotenone (Sigma-Aldrich) for one hour. Production of H_2O_2 was measured by Amplex Red
30   (Molecular Probes, Eugene OR) following the manufacturer's recommendations over a one hour period in the presence of the relevant drug(s). Fluoresence was

measured at 590nm with excitation at 530nm and H_2O_2 levels determined by reference to a standard curve. Each assay was done four times.

DNA microarrays: Total RNA from A549 and MO59K treated (DCA 500 μM for 48 H) and untreated cells were extracted using Quiagen RNeasy kit (Ontario, Canada). Differences in genes expression between control A549, MO59K and DCA-A549, DCA-MO59K cells was assessed using human DNA chip set U133A (Affymetrix, California , USA). Analysis was performed using Affymetrix software. Changes in gene expression were considered significant only if the expression was altered by ≥ 1.75 fold. Special intention was made on genes that were change in both A549 and MO59K. Then a pathway analysis (GO analysis) was performed.

Statistics: Values are expressed as the mean±SEM. Inter-group differences were assessed by Kruskal Wallis or One-way ANOVA as appropriate with post hoc analysis using Fisher's Exact Test (Statview 4.02, SAS Institute, Cary, NC).

Example 1: DCA depolarizes mitochondria, releases cytochrome-c and AIF from the mitochondria and enhances H_2O_2 production (Figure 1).

A: 48 H of DCA (500 μM) significantly depolarized the mitochondria in A549, MO59K, MCF-7cancer cells, but had no effect on normal epithelial cells (EC).

B: In the upper panel, cytochrome-c in green is co-localized with the mitotracker red staining in control, whereas after 48H DCA cytochrome-c leaked out the mitochondria and is localized into the cytosol.

C: In control (upper panel) AIF in red is not localized in nucleus, whereas after 48H DCA AIF is mainly localized into the nucleus.

D: DCA increases H_2O_2 production in a rotenone and dose-dependent manner.

Example 2: DCA increases K+ current and repolarizes cancer cells without affecting normal cells (Figure 2).

A: 48H DCA (500 μM) increases K+ current density in all cancer cells lines (A549, MO59K and MCF-7) but does not affect normal cells such as the normal epithelial cells. On the right, original traces representing the K+ current in both control (untreated cells) and DCA treated cells. Increase in K+ current density was mainly due to an increase in Kv current, as shown by the 4-AP-sensitive current in the legend. Increased K+ current density results in a significant decrease in membrane

capacitance, (which suggests a decrease in cell volume) and repolarization of the resting membrane potential.

B: The mechanism by which DCA increases K+ current was assessed by cancer cells acutely exposed to DCA (10 min, 500 μM). The effects of DCA on K+ current were blocked by 4-AP and blocked by both catalase and rotenone (50 μM). TTFA, a specific blocker of complex II did not prevent the effects of DCA.

C: DCA treated cells (48hrs) had increased Kv1.5 protein and mRNA, suggesting that DCA is able to increase Kv channel expression.

Example 3: DCA increases Kv1.5 via the Ca++/calcineurin-dependent transcription factor NFAT (Figure 3).

A: Free cytosolic calcium concentration was measured using fluo-3. Control cells had more calcium than both DCA and H_2O_2 treated cells. Acute DCA decreased the calcium concentration through a 4-AP and rotenone sensitive pathway confirming the electrophysiology data.

B: Confocal imaging of A549 cells showed that NFAT (green) is activated as it is mainly localized in the nucleus of most of the untreated cells. Interestingly, these cells had a very low level of Kv1.5 expression (red). Both DCA and H_2O_2 blocked the activation of NFAT as shown by its localization out of the nucleus in the cytoplasm, and had increased Kv1.5 expression.

Example 4: DCA increases apoptosis and decreases proliferation of cancer cells in vitro (Figure 4).

A: DCA significantly increases apoptosis as a higher number of DCA treated cells are Annexin V positive and TUNEL positive. DCA also significantly decreases proliferation as less DCA treated cells were positive to PCNA, BrDU or survivin.

B: Increased apoptosis is also shown by the DCA-induced activation of both caspase3 and 9 as immunoblots reveal an active band for both caspases.

Example 5: DCA prevents and reverses tumor growth in vivo (Figure 5).

A: Injection of A549 cells into the flank of nude rats induced the development of tumor within a week. DCA treated rats in both prevention and reversal protocols had smaller tumors. The size of tumors were assessed by caliper as shown on the picture, by weight and by using MRI in vivo.

B: DCA treated rats had tumors with an increase in apoptosis as shown by TUNEL. Interestingly a significant negative correlation was observed between %TUNEL and both tumor size and weight.

C: As in the in vitro experiments, DCA increased Kv1.5 expression and decreased survivin expression in vivo. Representative confocal immunohistochemistry is shown on the left and immunobloting on the right. The 2 lanes on the left are from non treated rats while the right 2 lanes are from DCA-treated rats.

D: Human non-small cell lung cancers show a positive correlation between histological grade and the survivin/Kv1.5 index (the higher the tumor grade the more aggressive the tumor).

RESULTS AND DISCUSSION

Human A549 (non small cell lung cancer), MO59K (glioblastoma) and MCF-7 (breast cancer) cancer cells have hyperpolarized mitochondria; DCA treatment induces $\Delta\psi$m depolarization, mitochondrial release of both AIF and cytochrome C and increases mitochondrial H_2O_2 production (Figure 1). TMRM (tetramethylrhodamine methyl-ester) is a positively charged dye that accumulates in the negatively charged mitochondria in a mitochondria membrane potential-dependent manner, i.e. the more the red signal of TMRM the more hyperpolarized the mitochondria. All cancer cell types have more red fluorescence, i.e. they were more hyperpolarized than the normal epithelial cells (EC) at the bottom. Hyperpolarized mitochondria reflect an apoptosis resistance state, since it is mitochondrial depolarization that initiates apoptosis. DCA depolarizes mitochondria in all the cancer cell types. DCA did not alter the mitochondrial membrane potential in normal human lung epithelial cells (Figure 1). Interestingly, DCA brought the mitochondria membrane potential to the level of the normal epithelial cells. Together these findings likely explain the relative lack of toxicity of DCA on normal tissue and should confer a wide therapeutic window to this cancer treatment strategy. The mitochondrial depolarization was associated with the release from the mitochondria of both apoptosis inducers, cytochrome-c and apoptosis inducing factor (AIF). Cytochrome c leaks to the mitochondria and activates caspases, the effectors of apoptosis. AIF is translocated to the nucleus where it initiates caspase-independent apoptosis. DCA treatment of all A549, MCF-7 and MO59K causes

leakage of cytochrome c to the cytoplasm and translocation of AIF to the nucleus, as shown by the multiple immunostaining and confocal imaging in Figure 1B. The cells were stained green with a monoclonal antibody to cytochrome c and red with mitotracker red, a mitochondrial marker. The green staining in control was confined

5   to discrete areas (mitochondria) whereas in the DCA-treated cells it diffusely stains the cytoplasm. The cells were stained red with AIF antibody and blue for DAPI a nuclear marker. The red staining in control was confined in the cytosol whereas in the DCA-treated cells it stains in the nucleus. An opening of the mitochondrial transition pore (MTP) is required for both cytochrome c and AIF release and this is

10   associated with mitochondrial depolarization in mitochondria-dependent apoptosis.

A major stimulus of the redox-sensitive MTP is an increase in the production of activated oxygen species (AOS) produced in the electron transport chain of the mitochondria. AOS production is increased as the mitochondrial depolarize. Due to the presence of MnSOD in the mitochondria, superoxide is

15   dismutated to H_2O_2, a radical that is relatively stable and can leak in the cytoplasm and the plasma membrane, where it can affect redox-sensitive mechanism, including the opening of K^+ channels.

Exposure to DCA (50, 500 and 5000μM) for 4 hours increased H_2O_2 production in A549 cells in a dose-dependent manner. The effect of DCA was

20   totally inhibited by rotenone, a complex I inhibitor, suggesting that most of free radical used to produce H_2O_2 was produced in the complex I (Figure 1C).

Chronic DCA treatment increases Kv current density and hyperpolarizes the plasma membrane (Figure 2).

As illustrated in Figure 2A, Kv1.5 has been implicated in the mechanism of

25   apoptosis, at least in pulmonary artery smooth muscle cells. Increase in both Kv1.5 expression and current density was responsible of decreased $[K+]_i$ leading to shrinkage of the cells and a decrease in the tonic inhibition that K^+ exerts on caspases, thus promoting apoptosis. In the cancer cells of the present examples, there was a significant increase in K^+ current in DCA treated cells, compared to the

30   cancer untreated cells. Most of the increased K^+ current in the DCA treated cells is 4-aminopyridine sensitive (i.e. Kv current).

The DCA-induced activation of Kv channels is due to the increase in the H_2O_2 production (Figure 2C).

In order to determine the mechanism of the increase in the Kv current, pharmacology experiments were performed on the acute effect of DCA on cancer cells. DCA (500 µM) superfused over A549 cells for 5 to 10 minutes also caused an increased K^+ current, similar to that caused by chronic exposure to DCA. The effect of DCA on K^+ current was blocked by both catalase and rotenone (10 µM) but not by TTFA (50 µM, an inhibitor of complex II of the electron transport chain), suggesting that H_2O_2 derived from complex I, but not complex II, was responsible for the Kv current activation (as it was fully blocked by 4-AP).

DCA upregulates the Kv1.5 expression via a [Ca++]i-NFAT pathway (Figure 3).

In addition to the acute activation of existing Kv channels, DCA might increase the outward K^+ current by upregulating the expression of the Kv1.5 protein. In fact, downregulation of Kv1.5 might be a feature of the cancer cells, contributing to the resistance to apoptosis of cancer cells. Both mRNA and protein levels of Kv1.5 channels were increased in DCA treated cells compared to the controls in agreement with the electrophysiology data (Figure 2C). The transcription factor NFAT is regulated by intracellular calcium and calcineurin. Increase in the intracellular Ca^{++} activates calcineurin, which de-phosphorylates NFAT, allowing its translocation to the nucleus, where it decreases Kv1.5 expression. Since the DCA hyperpolarized the plasmalemmal membrane, it is very likely that the influx of Ca^{++} via voltage gated Ca^{++} channels will decrease. Cells were studied using confocal microscopy and Fluo-3. DCA treated cells had a significant decrease of the [Ca++] compared to the untreated cells (Figure 3A). Decreased [Ca++] induced by DCA was blocked by both 4-AP and rotenone, reinforcing the hypothesis that complex I-produced H_2O_2 activated Kv channels and thus repolarized the membrane potential. Furthermore, exogenous H_2O_2 mimics the effect of DCA and decreases the intracellular Ca++ levels (Figure 3A). As expected, in the untreated cancer cells, NFAT was localized in the nucleus whereas in both DCA and H_2O_2 treated cells NFAT was localized in the cytosol (Figure 3B). Interestingly, when cells had a nuclear localization of NFAT they expressed very

- 17 -

little Kv1.5, studied by co-staining with an antibody to NFAT and Kv1.5 and DAPI staining for imaging of the nucleus (Figure 3B).

The data showed that DCA depolarizes mitochondria and causes the release of pro-apoptotic mediators. Furthermore, the increase in Kv current (acutely via the production of H_2O_2 and chronically via the NFAT mediated increase in Kv1.5 expression) would further potentiate apoptosis more distally in the pathway, via the decrease in the K+-induced caspase inhibition. Thus, with a "double hit" mechanism, DCA would be a powerful inducer of apoptosis and reverse both causes of potential resistance to apoptosis, i.e. mitochondrial hyperpolarization and Kv inhibition. Apoptosis was then measured with a number of assays. The apoptosis-resistance cells would actually be the ones proliferating the most. Therefore, by eliminating these cells, DCA will likely decrease indices of cell proliferation in the cancer cells; for this reason indices of cell proliferation was also measured.

The DCA-induced apoptosis is mitochondria-dependent, and occurs early (Figure 4). DCA treatment (500μM) of A549 cells causes early induction of apoptosis as evidenced by the annexin V staining, which stains phosphatidylserine expressed early on the surface of apoptotic cells (Figure 4A). Non-treated cells showed almost no apoptosis (measured by TUNEL) and significant cell proliferation (measured by PCNA, BrDu and DAPI counting) (Figure 4A). In contrast, DCA-treated cells showed a significant increase in apoptosis and a decrease in cell proliferation (Figure 4A). Shrunk and pyknotic nuclei, a feature of late apoptosis, were seen in the apoptotic cells. Both the caspase-3 and 9 activity was as well enhanced in DCA treated cells (Figure 4B). In addition, DCA treatment decreased the expression of survivin, an inhibitor of apoptosis protein that is selectively expressed in cancer (Figure 4A). Survivin expression is considered an index of poor prognosis in a large number of cancer types.

Since DCA decreases proliferation and increases apoptosis without affecting normal cells, it would be beneficial in the treatment of cancer in vivo.

DCA blocks tumor growth in nude rats (Figure 5)

Nude rats were injected with 3.10^6 A549 cells. The rats developed tumors within a week. 20 animals were divided into 3 groups: controls did not receive DCA, DCA-prevention rats received DCA just after cell injection for 5 weeks, and

- 18 -

DCA-reversal rats received DCA 2 weeks post cell injection for 3 more weeks. The control rats rapidly develop tumor with a constant and exponential tumor growth (Figure 5A). Both DCA-treated groups had a significant decrease in the tumor size, measured by tumor weight at the time of rat sacrifice, and tumor maximal diameter

5   measured weekly in alive rats using calipers as well as using MRI in alive rats (Figure 5A). The decrease in the tumor growth in the DCA treated group was associated with an increase in apoptosis (TUNEL) and a decrease in proliferation (PCNA and survivin) (Figure 5B), confirming the in vitro results. In fact, there was a negative correlation between apoptosis and tumor size (the higher the apoptosis

10   the smaller the size) suggesting that the induction of apoptosis is in fact the mechanism by which the tumor size was decreased (Figure 5B). In addition, Kv1.5 was upregulated and survivin was downregulated in the treated rats (Figure 5C), again confirming the in vitro data.

Since survivin is regulating mitochondria-dependent apoptosis, via the

15   mechanism that was described above, the depolarization in mitochondria might eventually cause an up regulation of Kv1.5. Thus survivin and Kv1.5 might be regulated in parallel and together serve as an index of apoptosis resistance in tumors. In order to validate this hypothesis and increase the clinical relevance of the animal data tissues from 30 patients with non small cell lung cancer were analyzed

20   from the archives of the University of Alberta pathology department. Since all tissues were archived functional studies (i.e. measure mitochondrial membrane potential) could not be performed, but survivin and Kv1.5 expression could be measured.   The survivin/Kv1.5 ratio index correlated positively with the aggressiveness of the tumor (i.e. the higher the index the higher the tumor

25   histological grade as assessed by blinded pathologists) (Figure 5D). The age and the sex of the patients were similar among the 3 standard histological grades. These results validate the relevance of the animal work and for the first time link the mitochondria-dependent apoptosis with the expression of Kv channels, under a comprehensive mechanism offering cancer cells resistance to apoptosis.

30   **DCA's effects are restricted to mitochondrial pathways (Figures 6 and 7)**

DCA is a prototype inhibitor of the mitochondrial enzyme pyruvate dehydrogenase kinase and thus DCA activates pyruvate dehydrogenase and

promotes glucose oxidation. Consequently, DCA increases the delivery of NADH in the complex one of the electron transport change. This results in an increase in the AOS production within the mitochondrial complex I and the depolarization of mitochondria, initiating apoptosis, as described in the proposed mechanism of

5   action of Figure 6. In order to confirm that the effects of DCA are not nonspecific but are indeed metabolic and regulate apoptosis pathways, a gene chip and GO analysis of treated and non-treated cells was performed. We used a "subtraction" strategy to reveal relevant changes in tumor gene expression that were solely due to DCA. Studying both the A549 and glioblastoma cell line (i.e. a very different tumor

10   than the lung cancer, epithelial versus glial cells) and focusing on the changes that occurred in a similar pattern in response to DCA therapy revealed changes in gene expression due to DCA, rather than idiosyncratic tumor-specific gene changes.

The genes that were modified by DCA in parallel inA549 (lung) and MO59K (glioblastoma) are listed and their expression levels were plotted in a heat

15   map (Figure 7). LC=lung cancer cells control, LD=lung cancer cells treated with DCA, GC=glioblastoma cells controls, GD=glioblastoma cells treated with DCA. Most of these genes were related to mitochondria and complex I and interestingly, among all the voltage-gated potassium channels only Kv1.5 was significantly upregulated. This gene chip analysis further supports the model described in Figure

20   6.

CONCLUSION

The present study concludes that DCA is an attractive treatment for cancer, such as human cancer. The present invention shows the interplay of mitochondria membrane Kv channels and apoptosis.

25   In one embodiment, not being bound by a particular mechanism, the positive effects of DCA can potentially be explained by the fact that mitochondrial hyperpolarization and downregulation of K+ channels contribute to the apoptosis resistance state that characterizes cancer. In one embodiment, DCA increases the delivery of NADH in the mitochondrial ETC complex I, resulting in an increase in

30   the production of H2O2 and mitochondrial depolarization. The mitochondrial depolarization initiates apoptosis by causing a leak of proapoptotic mediators in the cytoplasm. At the same time, the H2O2 can activate the plasmalemal Kv channels

and can activate redox-sensitive transcription factors in the nucleus (our preliminary findings suggest activation of NFAT) and also upregulate Kv1.5 expression. There is strong evidence that shows that DCA only depolarizes mitochondria that are abnormally hyperpolarized. DCA does not change the mitochondrial membrane

5   potential and the K+ current in normal vascular and epithelial cells. This provides the basis for the selectivity of the DCA, i.e DCA will not affect non-cancerous cells, a highly desirable feature of all candidate cancer therapies. DCA by itslelf blocks tumor prevention, but by reversing the apoptosis resistance state, DCA would make tumors more sensitive to proapoptotic chemotherapies, decreasig the required doses

10   and toxicities. Metabolic modulators might be a new class of orally available anticancer drugs. The present invention illustrates for the first time that targeting a mitochondria-K+ channel axis is proposed and shown to be effective in cancer.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the

15   invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent

20   or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

We Claim:

1.   A method for treating cancer associated with hyperpolarized mitochondria comprising administering to a patient in need thereof a therapeutically effective amount of dichloroacetate or obvious chemical equivalent thereof.

2.   The method of claim 1 wherein the dichloroacetate is a salt of dichloroacetic acid.

3.   The method of claim 2 wherein the dichloroacetate is a sodium dichloroacetate.

4.   The method of claim 1 wherein the cancer associated with hyperpolarized mitochondria is selected from the group consisting of: non-small cell lung cancer, glioblastoma and breast carcinoma.

5.   The method of claim 1 wherein the dichloroacetate or obvious chemical equivalent thereof is administered in the form of a pharmaceutical composition comprising dichloroacetate or obvious chemical equivalent thereof and a pharmaceutically acceptable carrier.

6.   The method of claim 1 wherein the dichloroacetate or obvious chemical equivalent thereof is administered orally.

7.   The method of claim 6 wherein 10-100 mg/kg of DCA or obvious chemical equivalent is administered per day.

8.   The method of claim 7 wherein 10-100 mg/kg of DCA or obvious chemical equivalent is administered twice per day.

9.   The method of claim 7 wherein the dose is 25-50 mg/kg.

10.   The method of claim 8 wherein the dose is 25-50 mg/kg.

11.   A method of any one of claims 1 – 10 wherein the dichloroacetate or obvious chemical equivalent thereof is administered in combination with another pro-apoptotic agent and/or chemotherapeutic agent, and/or other cancer therapy.

12.   A method for inducing apoptosis and/or reversing apoptosis resistance in a cancer cell, comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof.

13.   A method for inhibiting proliferation of cancer cells, comprising administering to the cells an effective amount of dichloroacetate or obvious chemical equivalent thereof.

14.   A method of decreasing survivin in a cancer cell, comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof.

15   A method of increasing Kv1.5 protein in a cancer cell comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof.

16.   A method of increasing AIF in a cancer cell comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof.

17.   A method of increasing H_2O_2 in a cancer cell comprising administering to the cell an effective amount of dichloroacetate or obvious chemical equivalent thereof.

18.   A method of anyone of claims 1 – 17, wherein cancer cells, but not normal or non-cancerous cells are affected by the treatment with dichloroacetate or obvious chemical equivalent thereof.

19. A method for identifying cells or a patient that may benefit from treatment with dichloroacetate or obvious chemical equivalent thereof to induce or reverse resistance to apoptosis, comprising:

  (i) obtaining a cell sample, tissue sample, or sample comprising cancerous cells or cells suspected of being cancerous;

  (ii) administering to said sample dichloroacetate or obvious chemical equivalent thereof;

  (iii) determining whether said apoptosis is induced and/or apoptosis resistance is reversed in said sample,

wherein when said apoptosis is induced and/or apoptosis resistance is reversed in said sample, this is indicative that the cells and/or patient from where the sample was obtained may benefit from dichloroacetate or obvious chemical equivalent treatment.

20. In one embodiment, the method of determining whether said apoptosis is induced and/or reversed or whether a patient or cell may benefit from dichloroactate or obvious chemical equivalent treatment is by monitoring the samples survivin/Kv1.5 index, wherein the index compared to a pre-determined level or range that is known to benefit from treatment, and/or the index is obtained from the sample prior to treatment and if it is lowered or reversed, then the cell or patient may benefit from treatment.
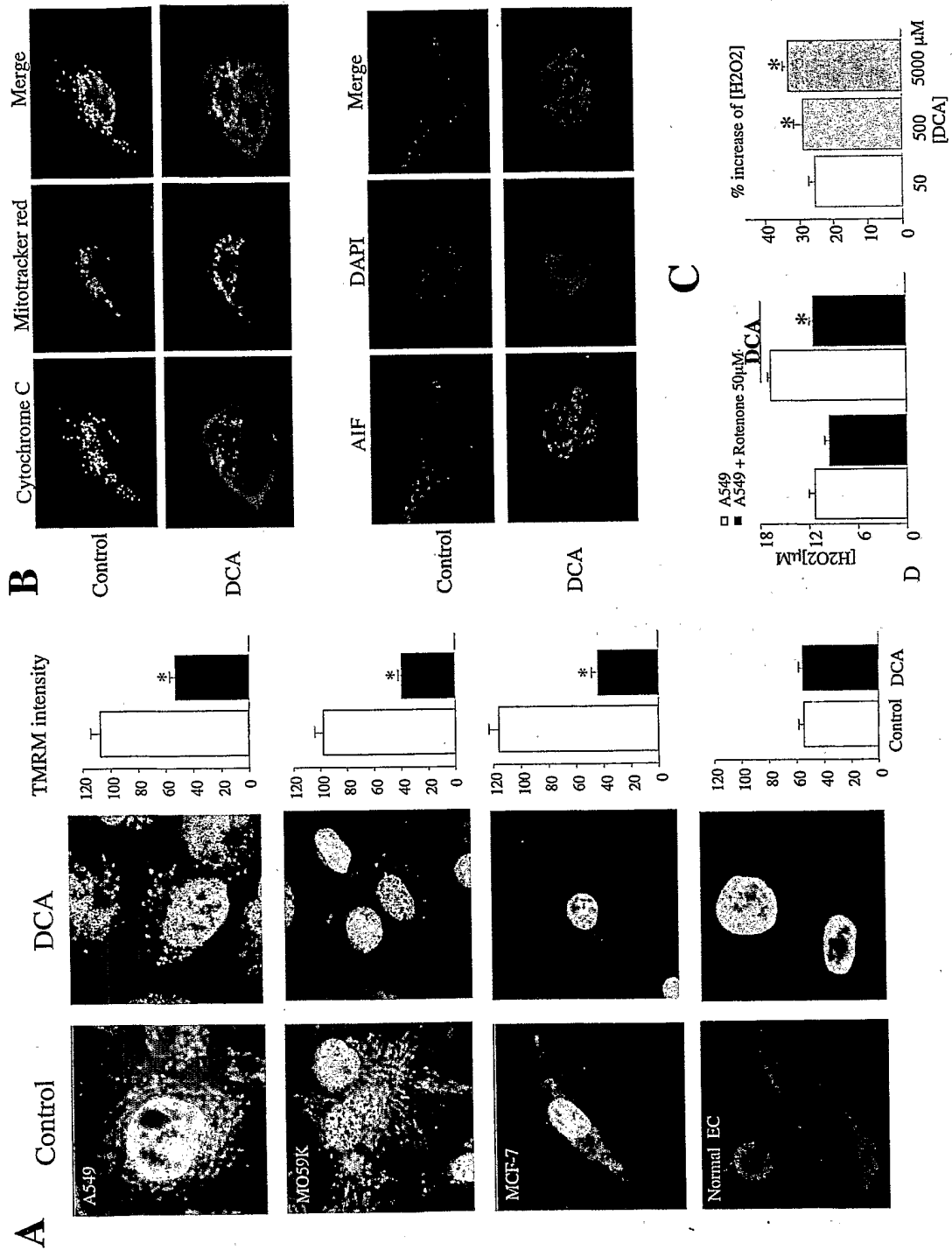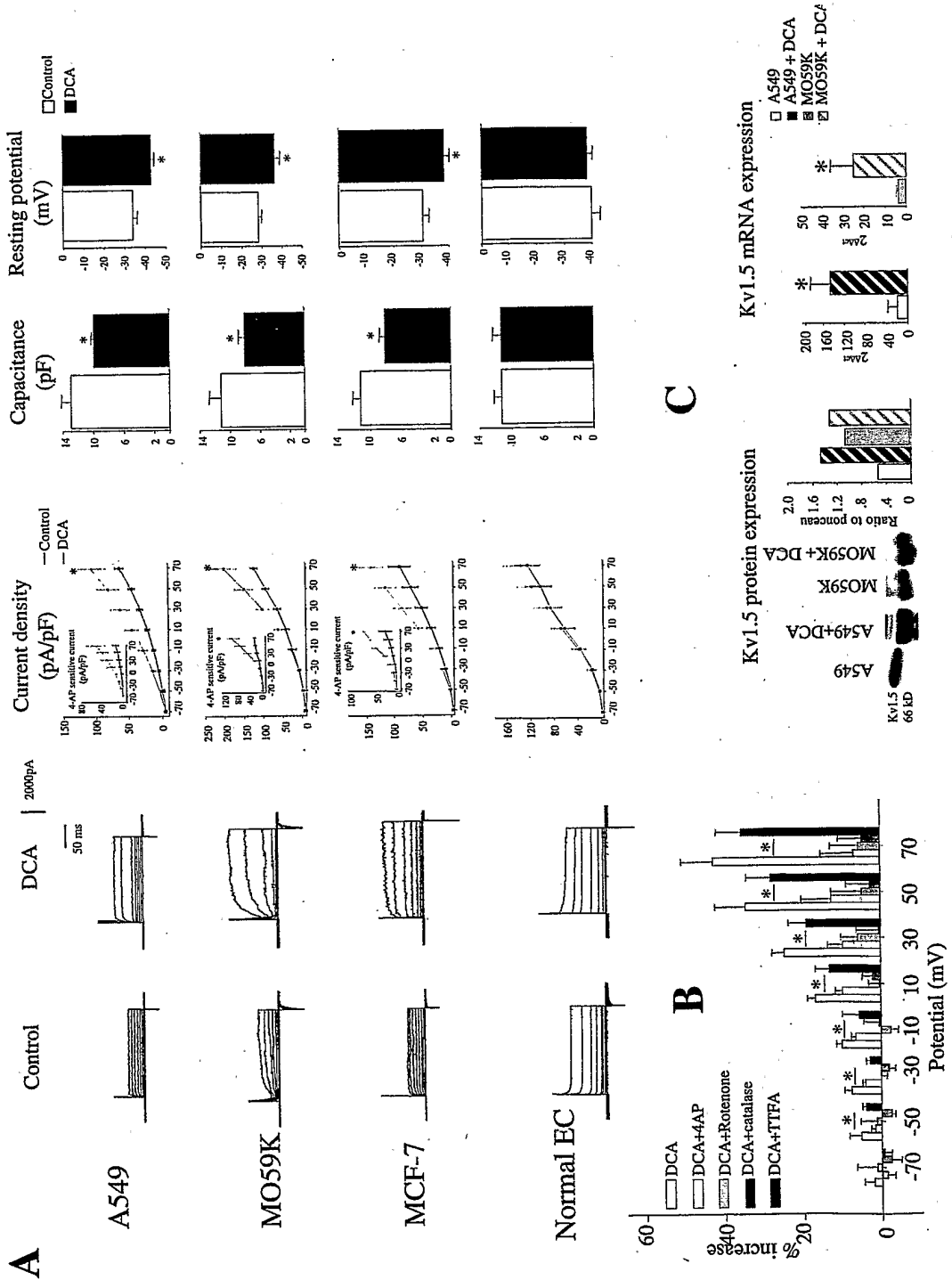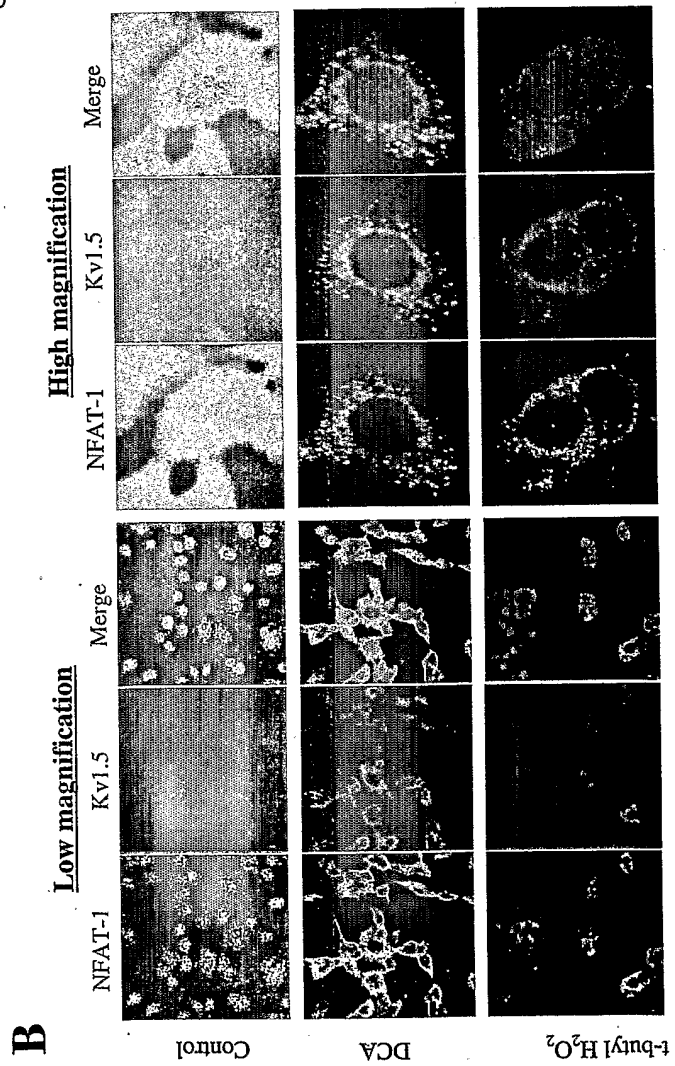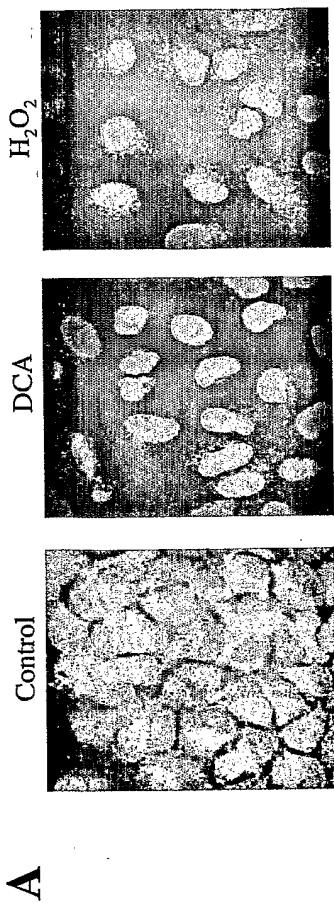
1/7

FIGURE 1

FIGURE 2

3/7

FIGURE 3

FIGURE 4

FIGURE 5

6/7

FIGURE 6

FIGURE 7

Decrease

Increase

Mitochondria related genes

HPD: 4-hydroxyphenylpyruvate dioxygenase (Up 5.8)
SOD2: Superoxide dismutase 2 mitochondrial (Up 4.2)
SOD1: Superoxide dismutase 1, soluble (ALS 1 (adult)) (Up 1.8)
NQO1: NAD(P)H dehydrogenase, quinone 1 (Up 1.6)
NDUFS5: NADH dehydrogenase, (ubiquinone) Fe-S protein 5 (Up 1.5)
NDUFV2: NADH dehydrogenase, (ubiquinone) flavoprotein 2 (Up 1.5)
PDK2: Pyruvate dehydrogenase kinase, isoenzyme (Down 3)
ATP5C1: ATP syntahse, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1 (Down 2.5)
TK2: Thymidine kinase 2, mitochondrial (Down 2)
VDAC3: Voltage-dependent anion channel 3 (Down 1.8)
NDUFA6: NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (Down 1.8)
NDUFA7: NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7 (Down 1.5)
NDUFB7: NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7 (Down 1.5)
SLC25A17: Solute carrier family 25 (mitochondria carrier; peroxisomal membrane protein (Down 2)
UcP2: Uncoupling protein 2 (mitochondrial, proton carrier) (Down 2)
ETFDH: Uncoupling protein 2 (mitochondrial, proton carrier) (Down 2)

Apoptosis/Proliferation related genes

PAK1: p21/Cdc42/rac1-activated kinase 1 (STE20 homolog, yeast) (Up 2.5)
TACR1:tachykinkin receptor 1 (Up 2)
NOL8: Nucleolar protein 8 (Down 7)
PDCD6: Programmed cell death 6 (Down 3)
SNX1: Sorting nexin 1 (Down 3)
API5: Apoptosis inhibitor 5 (Down 2)

Ion channels related genes

KCNA5: Potassium voltage-gated channel, shaker-related subfamily, member 5 (Up 5)
CACNAII: Calcium channel, voltage-dependent, alpha 1I subunit (Up 4)
KCTD12: Potassium channel tetramerisation domain containing 12 (Up 3)
KCNK1: Potassium channel, subfamily K, member 1 (Up 2)
KCTD3: Potassium channel tetramerisation domain containing 3 (Down 3)

Others

NFATc1: Nuclear factor of activated T-cells, cytoplasmic, calcineurin dependent 1 (Down 3)
COAS2: Cyclophilin LC (Up 2)

LC   LD   GC   GD

A.   CLASSIFICATION OF SUBJECT MATTER
       IPC: *A61K 31/19* (2006.01) , *C12Q 1/00* (2006.01) , *A61P 35/00* (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
       A61K 31/19 (2006.01) , *C12Q 1/00* (2006.01) , *A61P 35/00* (2006.01)

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)

Canadian Patent Database, Delphion, Scopus, PubMed, Google™ (dichloroacetate, dichloroacetic acid, cancer, apoptosis, mitochondria, mitochondrion, membrane potential hyperpolarized mitochondria, hyperpolarized membrane,Kv1.5, survivin,, hydrogen peroxide, apoptosis inducing factor, tumour, glioblastoma, non-small cell lung cancer, breast carcinoma, breast cancer, lung cancer)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2006/042062 A2 (THE JOHNS HOPKINS UNIVERSITY) 20 April 2006 see abstract; pages 4, 19-22, 64-67; claims 1-3	1-20
Y	COSTANTINI, P. ET AL.: "Mitochondrion as a novel target of anticancer chemotherapy" JOURNAL OF THE NATIONAL CANCER INSTITUTE vol. 92 no. 13, 5 July 2000, Oxford University Press pages 1042-53 see whole document	1-20
Y	FANTIN, V.R. ET AL.: "A novel mitochondriotoxic small molecule that selectively inhibits tumor cell growth" CANCER CELL vol. 2, July 2002, Cell Press pages 29-42 see whole document	1-20

[X] Further documents are listed in the continuation of Box C.   [X]   See patent family annex.

*	Special categories of cited documents :	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent but published on or after the international filing date	"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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2006 (16-05-2006)	1 August 2006 (01-08-2006)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Tania Nish (819) 934-3592

C (Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MCMURTRY M.S. ET AL.: "Dichloroacetate prevents and reverses pulmonary hypertension by inducing pulmonary artery smooth muscle cell apoptosis" CIRCULATION RESEARCH vol. 95, 2004, American Heart Association Inc. pages 830-840 see whole document	1-20
A	YANG, J. & SMITH, R.A.: "The effect of dichloroacetate on the phosphorylation of mitochondria proteins" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS vol. 111 no. 3, 1983, Academic Press Inc. pages 1054-1058	1-20
A	NAGY, G. ET AL.: "T cell activation-induced mitochondrial hyperpolarization is mediated by Ca^{+2} amd redox-dependent production of nitric oxide" JOURNAL OF IMMUNOLOGY vol. 171, 2003, The Am. Assoc. of Immunologists Inc. pages 5188-5197 see whole document	1-20
A	PAL, S. ET AL.: "Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels" THE JOURNAL OF NEUROSCIENCE vol. 23 no. 12, 15 June 2003, Society for Neuroscience pages 4798-4802 see whole document	1-20

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2006042062	20-04-2006	NONE	

Box No. II	**Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

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

1. [X] Claim Nos. :   1-18
    because they relate to subject matter not required to be searched by this Authority, namely :

    Claims 1-18 are methods of medical treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search (Rule 39.1(iv), PCT). Regardless, this Authority has carried out a search based on the alleged effects of dichloroacetate for the treatment of cancer.

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

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

Box No. III	**Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

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

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.