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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 April 2011 (21.04.2011)

PCT

(10) International Publication Number
WO 2011/045575 A1

(51) International Patent Classification:
A61N 1/32 (2006.01) *A61N 1/30* (2006.01)
A61N 1/05 (2006.01)

(21) International Application Number:
PCT/GB2010/001919

(22) International Filing Date:
15 October 2010 (15.10.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0918066.2 15 October 2009 (15.10.2009) GB

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF YORK** [GB/GB]; Heslington, York YO10 5DD (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **ROBINSON, Martin Paul** [GB/GB]; Department of Electronics, University of York, Heslington, York YO10 5DD (GB).

(74) Agent: **GILHOLM, Stephen Philip**; IPheions Intellectual Property, Buzzard Office, The Hawk Creative Busines Park, Easingwold YO61 3FE (GB).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2011/045575 A1

(54) Title: IMPLANT FOR INDUCING ELECTROPORATION USING ELECTROMAGNETIC INDUCTION

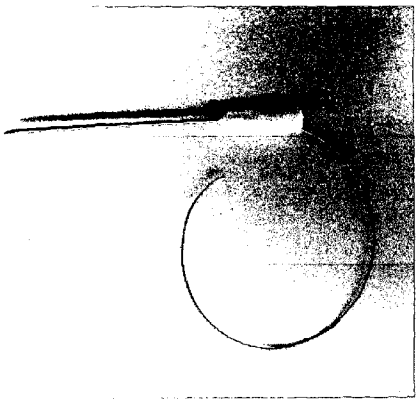

Figure 1

(57) Abstract: There is described a method of electroporation which comprises wirelessly applying an induced time varying voltage pulse across a gap between a pair of spaced apart terminals wherein the terminals comprise a portion of an implant. There is also described an electrically conducting annular implant which comprises a plurality of electrical terminals.

IMPLANT FOR INDUCING ELECTROPORATION USING ELECTROMAGNETIC INDUCTION

Field of the invention

The present invention relates to a novel device, especially a medical device and to methods of delivery of therapeutic agents related thereto.

More particularly, the invention relates a novel method of electroporation *in vivo* electroporation and to a medical device for use in such a method.

Background of the invention

Electroporation comprises applying an external electrical field across a cell plasma membrane which induces a significant increase in the electrical conductivity of the membrane. This increase in electrical conductivity causes a corresponding increase in permeability of the membrane. Therefore, the technique of electroporation is considered to involve producing temporary pores in cell membranes which may enable matter to pass through. Electroporation is used in molecular biology as a way of introducing a substance, such as DNA, a therapeutically active agent, a diagnostic agent or a molecular probe. Although well established as a laboratory tool, there is much topical interest in developing clinical *in-vivo* applications e.g. for enhancing DNA cancer vaccines.

Electroporation is conventionally performed *in-vitro* by placing cell suspensions between two electrodes, for example, in a cuvette, and applying a high voltage pulse across the medium.

More recently, there have been developments in electroporation *in vivo*. Thus it has been found that *in vivo* electroporation can be combined with, for example, DNA therapy, such as a DNA vaccine, to boost the body's immune response against cancer cells and the like. A recent review in Nature explains the benefits of electroporation for vaccine delivery and states that 'multiple trials are in progress in infectious diseases and cancer'.

For example, in cancer therapy the currently used method for *in vivo* electroporation comprises inserting electrodes into a tumour in order to apply high electric fields direct to the tumour to induce pore formation. However, this procedure is disadvantageous in that, *inter alia*, the high electric field must be applied multiple times, which is generally uncomfortable for the patient and it is necessary to re-insert the needles multiple times which can be painful for the patient and requires a great deal of accuracy if the needles are to be positioned in exactly the same place each time to ensure the same tissue is treated accurately and uniformly.

With the increase in the need for targeted therapies in disease like cancer there is therefore a need for an improved *in vivo* electroporation technique.

Summary of the Invention

We have now found a novel method of electroporation and a novel system of inducing electroporation.

Thus, we have found that by applying a time varying magnetic field, rather than an electric field, across a substantially annular electrically conducting implant,

electromagnetic induction occurs and electroporation occurs in the surrounding cells. Desirably the implant comprises a split annulus, e.g. with a gap between either end of the substantially annular implant, such that when the magnetic flux passes through the ring the time varying magnetic flux induces a high voltage across the gap.

Furthermore, we have found that this novel technique generates an alternating current (AC) rather than a DC electric field, but that the generated AC pulses can efficiently achieve electroporation.

Thus, according to a first aspect of the invention we provide a method of electroporation which comprises wirelessly applying an induced time varying voltage pulse across a gap between a pair of spaced apart terminals wherein the terminals comprise a portion of an implant.

Desirably the method of the invention comprises *in vivo* electroporation and therefore the method comprises applying a generated time varying voltage pulse across a gap between a pair of implant terminals.

The implant terminals may comprise a plurality of individual terminals, however in an especially preferred aspect of the invention the pair of terminals consists of the ends of an annulus. According to this aspect of the invention the pair of terminals consists of either end of the same annulus. Thus, the annulus may comprise a simple, essentially two dimensional, ring provided it is an incomplete loop. Alternatively, the ring may comprise multiple turns, e.g. a helical implant in the form of a coil, provided that the turns in the coil have the same orientation to enable an EMF to be induced.

Such a coil may be a flat coil or a cylindrical coil, e.g. in the form of a solenoid, However, it is essential that the ends of the ring or coil are provided with a gap between the ends so that the induced electrical field across the gap causes electroporation in the tissue in which the implant is placed. In its simplest form the implant may be a split ring such that the ends of the split ring can act as the terminals of the implant.

Thus, an implant comprising a plurality of terminals as hereinbefore described is novel *per se*. Therefore, according to a yet further aspect of the invention we provide an electrically conducting implant which comprises a plurality of electrical terminals.

As hereinbefore described the electrically conducting annular implant may comprise a split or incomplete annulus or a coil provided with at least a pair of terminals.

The implant comprising a split or incomplete annulus may comprise any electrically conducting material, e.g. metal. However, in order to facilitate the insertion of the split or incomplete annulus implant, the implant desirably comprises a flexible material. In a preferred aspect of the invention the implant comprises a memory material, such as, a shape memory metal, such as a shape memory alloy.

A variety of shape memory alloys may be used in the manufacture of the implant of the present invention. In one aspect of the invention the shape memory alloy will exhibit a one way shape memory effect. It is desirable that the whole of the implant comprises a shape memory alloy as hereinbefore described. However, it will be understood that an implant suitable for *in vivo* electroporation may consist of an

implant a portion of which comprises a shape memory alloy and the remainder may comprise other conventional implant material. Furthermore, it is within the scope of the present invention that one or more shape memory alloys may be used in the implant of the invention imparting differing shape memory properties in the implant as desired.

Examples of shape memory alloys include, but shall not be limited to, cadmium alloys, such as, Ag-Cd, e.g. Ag-Cd 44/49 or Au-Cd, e.g. Au-Cd 46.5/50; copper alloys, such as, Cu-Al-Ni, e.g. Cu-Al-Ni 14/14.5 wt.% Al and 3/4.5 wt.% Ni or Cu-Sn approx. 15 wt.% Sn or Cu-Zn 38.5/41.5 wt.% Zn or Cu-Zn-X (X = Si, Al, Sn); iron alloys, such as, Fe-Pt, e.g. Fe-Pt approx. 25 at.% Pt; manganese alloys, such as, Mn-Cu, e.g. Mn-Cu 5/35 at.% Cu or Fe-Mn-Si; platinum alloys; nickel alloys, such as, cobalt-nickel alloys, e.g. Co-Ni-Al or Co-Ni-Ga, or nickel-iron alloys, such as, Ni-Fe-Ga; titanium alloys, such as, Ti-Pd or Ni-Ti. An especially desirable material is Nitinol™ is an example of shape memory alloy that is finding many applications in medicine and has excellent biocompatibility, very high corrosion resistance, and excellent cytocompatibility.

A flexible split annular split-ring is especially advantageous in that the implant may comprise a wire implant. Such a wire implant may be inserted into a patient using minimally invasive surgery, such as therapeutic endoscopy. Furthermore, a wire implant is advantageous in that, *inter alia*, it may remain *in situ*, even when the patient is not undergoing treatment, which reduces the invasiveness of the technique further.

It is this aspect of the invention which makes a 'memory metal' especially desirable, since the memory metal may be formed into a straight wire before insertion, but wire will conform to an annular implant once inserted to the desired site, e.g. into a tumour, in a patient.

Thus, a preferred material has a relatively high yield strength so that, for example, a coiled implant may be surgically inserted and the material will then regain its three dimensional form once it is *in situ*. Although the yield strength may vary it is desirably >300MPa.

Furthermore a material which has a relatively high Young's modulus, i.e. a material that is substantially stiff, is preferred. A material desirably has a Young's modulus of >80 GPa.

In addition the electroconductive material may optionally be provided with a biocompatible coating and the biocompatibility of the implant of the invention may be improved by applying or grafting on a more biocompatible material.

It is well known to coat the surface of medical devices such as needles with siloxanes, such as, polydimethylsiloxanes, for lubrication.

US Patent No. 3,574,673 discloses the use of organosiloxane and aminosiloxane polymers which can be cured on various surfaces such as needles to provide a lubricating film. US Patent No. 4,720,521 discloses coating devices such as needles or catheters with a curable silicone composition to form a crosslinked, adherent

coating which serves as a matrix for a non-reactive lubricating silicone polymer. Other biocompatible coatings may include a coating of a biocompatible metal, such as gold or titanium and alloys thereof. Other biocompatible coatings which may be mentioned include, but shall not be limited to, low friction coatings, such as Teflon®, clotting agents, such as fibrin, or endothelial cells, The advantage of a biocompatible coating is that, *inter alia*, any electroconductive material with a suitable yield strength and optionally Young's modulus may be used as an implant according to the invention.

An especially preferred material for use as an implant is an optionally coated tempered high or medium carbon steel or spring steel, such as "piano wire". The diameter of the spring steel may vary but may be similar to those that are commercially available, i.e. from 0.15 to 2mm although it will be understood that for most implants a smaller diameter will be preferred due to its ease of implantation.

The method of electroporation described herein provides a novel form of delivery of one or more therapeutic agents. Such a method is advantageous in that it provides specific localised delivery of a therapy.

Thus, according to this aspect of the invention we provide a method of delivery of a therapeutic agent which comprises administering the therapeutic agent simultaneously, sequentially or separately whilst "administering" electroporation.

Although it will be understood by the person skilled in the art that the method of electroporation may be applied to delivery of a variety of active agents to a variety of

sites, the method is especially advantageous in the delivery of anticancer agents to a tumour. The term "therapy" or "therapeutic agent" used herein shall include prophylactic agents, such as vaccines, e.g. DNA vaccines.

Suitable proliferative diseases, such as cancer, which may be treated shall include, but shall not be limited to, cancers, such as, melanoma, colorectal cancer, ovarian cancer, prostate, renal, gliomas, adenocarcinomas, sarcomas, breast cancer and liver cancer .

Suitable anticancer agents which may be administered include, but shall not be limited to, one or more anti-proliferative, gyrostatic or cytotoxic compounds, e.g. one or more chemotherapeutic agents selected from the group comprising an inhibitor of polyamine biosynthesis; an inhibitor of a different protein kinase, especially protein kinase C or of a tyrosine protein kinase, such as epidermal growth factor receptor protein tyrosine kinase; an inhibitor of a growth factor, such as vascular endothelial growth factor; a cytokine; a negative growth regulator; hormones or hormone analogues; and a conventional cytostatic agent. Alternatively or in addition the anticancer agent may comprise one or more of the following:

An adrenal cortex antagonist, i.e. a compound which targets, decreases or inhibits the activity of the adrenal cortex and changes the peripheral metabolism of corticosteroids, resulting in a decrease in 17- hydroxycorticosteroids. An example of an adrenal cortex antagonist includes, but is not limited to, Mitotane.

An AKT pathway inhibitor, i.e. a compound which targets, decreases or inhibits cell proliferation. Akt, also known as protein kinase B (PKB), a serine/threonine kinase, is

a critical enzyme in several signal transduction pathways involved in diabetes. The principal role of Akt in the cell is to facilitate growth factor-mediated cell survival and to block apoptotic cell death. A target of the AKT pathway inhibitor includes, but is not limited to, Pi3K/AKT. Examples of an AKT pathway inhibitor, include, but are not limited to, Deguelin and Triciribine.

An alkylating agent, i.e. a compound which causes alkylation of DNA and results in breaks in the DNA molecules as well as cross-linking of the twin strands, thus interfering with DNA replication and transcription of RNA. Examples of an alkylating agent include, but are not limited to, Chlorambucil, cyclophosphamide, Dacarbazine, Lomustine, Procarbazine, Thiotepa, Melphalan, Temozolomide, Carmustine, Ifosfamide, Mitomycin, Altretamine, Busulfan, Machlorethamine hydrochloride, nitrosourea, Streptozocin, and estramustine.

An angiogenesis inhibitor, i.e. a compound which targets, decreases or inhibits the production of new blood vessels. Examples of an angiogenesis inhibitor include, but are not limited to, Fumagillin; Shikonin, Tranilast; ursolic acid; suramin; and thalidomide.

An anti-androgen, i.e. a compound which blocks the action of androgens of adrenal and testicular origin which stimulate the growth of normal and malignant prostatic tissue. Examples of an anti-androgen include, but are not limited to, Nilutamide; bicalutamide.

An anti-oestrogen, i.e. a compound which antagonizes the effect of estrogens at the oestrogen receptor level. Examples of an anti-oestrogen include, but are not limited to, Toremifene; Letrozole; Testolactone; Anastrozole; Bicalutamide; Flutamide; Tamoxifen Citrate; Exemestane; Fulestrant; tamoxifen; fulvestrant; raloxifene and raloxifene hydrochloride.

An anti-hypercalcemia agent, i.e. a compound which is used to treat hypercalcemia. Examples of an anti-hypercalcemia agent include, but are not limited to, gallium (III) nitrate hydrate; and pamidronate disodium.

An antimetabolite, i.e. a compound which inhibits or disrupts the synthesis of DNA resulting in cell death. Examples of an antimetabolite include, but are not limited to, 6-mercaptopurine; Cytarabine; Fludarabine; Flexuridine; Fluorouracil; Capecitabine; Raltitrexed; Methotrexate; Cladribine; Gemcitabine; Gemcitabine hydrochloride; Thioguanine; Hydroxyurea; DNA demethylating agents, such as 5-azacytidine and decitabine; edatrexate; and folic acid antagonists such as, but not limited to, pemetrexed.

An apoptosis inducer, i.e. a compound which induces the normal series of events in a cell that leads to its death. Examples of an apoptosis inducer include, but are not limited to, ethanol, gambogic acid; Embelin and Arsenic Trioxide.

An aurora kinase inhibitor, i.e. a compound which targets, decreases or inhibits later stages of the cell cycle. An example of an aurora kinase inhibitor includes, but is not limited to Binucleine 2.

A Bruton's Tyrosine Kinase (BTK) inhibitor, i.e. a compound which targets, decreases or inhibits human and murine B cell development. An example of a BTK inhibitor includes, but is not limited to terreic acid.

A calcineurin inhibitor, i.e. a compound which targets, decreases or inhibits the T cell activation pathway. Examples of a calcineurin inhibitor include, but are not limited to Cypermethrin; Deltamethrin; Fenvalerate, and Tyrphostin 8.

A CaM kinase II inhibitor, i.e. a compound which targets, decreases or inhibits CaM Kinases. CaM Kinases constitute a family of structurally related enzymes that include phosphorylase kinase, myosin light chain kinase, and CaM kinases I-IV. Examples of a CaM kinase II inhibitor include, but are not limited to, 5-Isoquinolinesulfonic acid, 4-[(2S)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4- phenyl-1-piperazinyl) propyl]phenyl ester (9Cl); and benzenesulfonamide, N-[2-[[[3-(4-chlorophenyl)-2-propenyl]methyl]amino] methyl]phenyl]-N-(2-hydroxyethyl)-4-methoxy-(9CI).

A CD45 tyrosine phosphatase inhibitor, i.e. a compound which targets, decreases or inhibits dephosphorylating regulatory pTyr residues on Src-family protein-tyrosine kinases. An example of a CD45 tyrosine phosphatase inhibitor includes, but is not limited to, Phosphonic acid, [[2-(4-bromophenoxy)-5-nitrophenyl]hydroxymethyl]-(9CI).

A CDC25 phosphatase inhibitor, i.e. a compound which targets, decreases or inhibits overexpressed dephosphorylate cyclin-dependent kinases in tumours. An example of

a CDC25 phosphatase inhibitor includes 1,4-naphthalenedione, 2,3-bis[(2-hydroyethyl)thio]-(9CI).

A CHK kinase inhibitor, i.e. a compound which targets, decreases or inhibits overexpression of the antiapoptotic protein Bcl-2. An example of a CHK kinase inhibitor includes, but is not limited to, Debromohymenialdisine.

An agent for regulating genistein, olomucine and/or tyrphostins. Examples of such regulating agents include, but are not limited to, Daidzein, which is also known as 4H-1- benzopyran-4-one, 7-hydroxy-3-(4-hydroxyphenyl)-(9CI); Iso-Olomoucine, and Tyrphostin 1.

A cyclooxygenase inhibitor e.g. Cox-2 inhibitors. Examples of a COX-2 inhibitor, include but are not limited to, 1 H-indole-3-acetamide, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-N-(2-phenylethyl)-(9CI); 5-alkyl substituted 2-arylamino phenylacetic acid and derivatives, such as celecoxib (CELEBREX), rofecoxib (VfOXX), etoricoxib, valdecoxib; or a 5-alkyl-2-arylaminophenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid, lumiracoxib; and celecoxib.

A cRAF kinase inhibitor, which targets, decreases or inhibits the up-regulation of E-selectin and vascular adhesion molecule-1 induced by TNF. Examples of a cRAF kinase inhibitor include, but are not limited to, 3-(3,5-dibromo-4-hydroxybenzylidene)-5-iodo-1 ,3- dihydroindol-2-one; and benzamide, 3-(dimethylamino)-N-[3-[(4-hydroxybenzoyl)amino]-4-methylphenyl]-(9CI).

A cyclin dependent kinase inhibitor, which targets, decreases or inhibits cyclin dependent kinase which play a role in the regulation of the mammalian cell cycle. Examples of targets of a cyclin dependent kinase inhibitor include, but are not limited to, CDK, AHR, CDK1 , CDK2, CDK5, CDK4/6, GSK3beta, and ERK. Examples of a cyclin dependent kinase inhibitor include, but are not limited to, N9-lsopropyl-Olomoucine; Olomoucine; Purvalanol B, which is also known as Benzoic acid, 2-chloro-4-[[2-[[(1 R)-1-(hydroxymethyl)-2-methylpropyl]amino]-9-(1-methylethyl)-9H-purin-6-yl]amino]-(9Cl); Roascovitine; Indirubin, which is also known as 2H-lndol-2-one, 3-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,3-dihydro-(9Cl); Kenpaullone, which is also known as lndolo[3,2-d][1]benzazepin-6(5H)-one, 9-bromo-7,12-dihydro- (9Cl); purvalanol A, which is also known as 1-Butanol, 2-[[6-[(3-chlorophenyl)amino]-9-(1-methylethyl)-9H-purin-2-yl]amino]-3-methyl-, (2R)-(9Cl); and lndirubin-3'-monooxime.

A cysteine protease inhibitor, i.e. a compound which targets, decreases or inhibits cysteine protease which plays a vital role in mammalian cellular turnover and apoptosis. An example of a cysteine protease inhibitor includes, but is not limited to, 4-morpholinecarboxamide,N-[(1S)-3-fluoro-2-oxo-1-(2-phenylethyl)propyl] amino]-2-oxo-1-(phenylmethyl)ethyl]-(9CI).

A DNA intercalator, i.e. a compound which binds to DNA and inhibits DNA, RNA, and protein synthesis. Examples of a DNA intercalator include, but are not limited to, Plicamycin and Dactinomycin.

A DNA strand breaker, i.e. a compound which causes DNA strand scission and results in inhibition of DNA synthesis, inhibition of RNA and protein synthesis. An example of a DNA strand breaker includes, but is not limited to, Bleomycin.

An E3 Ligase inhibitor, i.e. a compound which targets, decreases or inhibits the E3 ligase. An example of an E3 ligase inhibitor includes, but is not limited to, N-(3,3,3-trifluoro-2-trifluoromethyl)propionyl)sulphanilamide.

An endocrine hormone, i.e. a compound which by acting mainly on the pituitary gland causes the suppression of hormones in males and females. An example of an endocrine hormone includes, but is not limited to, Leuprolide and megestrol acetate.

Compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family, i.e. a compound which targets, decreases or inhibits the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers Examples of such compounds include but shall not be limited to erlotinib, gefitinib, guanylyl cyclase (GC-C), HER2, tyrphostin, 2-propenamide, 2-cyano-3-(3,4-dihydroxyphenyl)-N-phenyl-,(2E)-(9CI); Tyrphostin Ag 1478; Lavendustin A; and 3-pyridineacetonitrile.

A farnesyltransferase inhibitor, i.e. a compound which targets, decreases or inhibits the Ras protein Examples of a farnesyltransferase inhibitor include, but are not limited to, a- hydroxyfarnesylphosphonic acid; butanoic acid, 2-[[(2S)-2-[[(2S,3S)-2-[[(2R)-2-amino-3-mercaptopropyl]amino]-3-methylpentyl]oxy]-1-oxo-3-phenyl propyl]amino]-4-(methylsulfonyl)-1-methylethyl ester, (2S)-(9cl); and Manumycin A.

An Flk-1 kinase inhibitor, i.e. a compound which targets, decreases or inhibits Flk-1 tyrosine kinase activity. An example of an Flk-1 kinase inhibitor includes, but is not limited to, 2-propenamide, 2-cyano-3-[4-hydroxy-3,5-bis(1-methylethyl)phenyl]-N-(3-phenylpropyl)-,(2E)-(9CI).

A Glycogen synthase kinase-3 (GSK3) inhibitor, i.e. a compound which targets, decreases or inhibits glycogen synthase kinase-3 (GSK3). An example of a GSK3 inhibitor includes, but is not limited to, indirubin-3'-monooxime.

A histone deacetylase (HDAC) inhibitor, i.e. a compound which inhibits the histone deacetylase and which possess anti-proliferative activity. Examples include but is not limited to compounds such as, Suberoylanilide hydroxamic acid (SAHA); [4-(2-amino-phenylcarbamoyl)-benzyl]-carbamic acid pyridine-3-ylmethyl ester and derivatives thereof; butyric acid, pyroxamide, trichostatin A, Oxamflatin, apicidin, Depsipeptide; depudecin and trapoxin. Other examples include depudecin; HC Toxin, which is also known as Cyclo[L-alanyl-D-atanyl-([alpha]S,2S)-D-amino-G-oxooxiraneoctanoyl-D-prolyl] (9Cl); sodium phenylbutyrate, suberoyl bis-hydroxamic acid; and Trichostatin A.

An HSP90 inhibitor, i.e. a compound which targets, decreases or inhibits the intrinsic ATPase activity of HSP90. Examples of an HSP90 inhibitor include geldanamycin, 17-demethoxy-17-(2-propenylamino)-(9CI); and Geldanamycin.

An l-kappa B-alpha kinase inhibitor (IKK), i.e. a compound which targets, decreases or inhibits NF-kappaB. An example of an IKK inhibitor includes, but is not limited to, 2-propenenitrile, 3-[(4-methylphenyl)sulphonyl]-(2E)-(9CI).

An insulin receptor tyrosine kinase inhibitor, i.e. a compound which modulates the activities of phosphatidylinositol 3-kinase, microtubule- associated protein, and S6 kinases. An example of an insulin receptor tyrosine kinase inhibitor includes, but is not limited to, hydroxyl-2-naphthalenylmethylphosphonic acid.

A c-Jun N-terminal kinase (JNK) kinase inhibitor, i.e. a compound which targets, decreases or inhibits Jun N-terminal kinase. Examples of a JNK kinase inhibitor include, but are not limited to, pyrazoleanthrone and/or epigallocatechin gallate.

A Mitogen-activated protein (MAP) kinase-inhibitor, i.e. a compound which targets, decreases or inhibits Mitogen-activated protein. An example of a MAP kinase inhibitor includes, but is not limited to, benzenesulfonamide, N-[2-[[[3-(4-chlorophenyl)-2- propenyl]methyl]amino]methyl]phenyl]-N-(2-hydroxyethyl)-4-methoxy-(9CI).

An MDM2 inhibitor, i.e. a compound which targets, decreases or inhibits the interaction of MDM2 and the p53 tumour suppressor. An example of a MDM2 inhibitor includes, but is not limited to, trans-4-iodo, 4'-boranyl-chalcone.

An MEK inhibitor, i.e. a compound which targets, decreases or inhibits the kinase activity of MAP kinase, MEK. An example of a MEK inhibitor includes, but is not limited to, butanedinitrile, bis[amino[2-aminophenyl)thio]methylene]-(9CI).

An MMP inhibitor, i.e. a compound which targets, decreases or inhibits a class of protease enzyme that selectively catalyze the hydrolysis of polypeptide bonds including the enzymes MMP-2 and MMP-9 that are involved in promoting the loss of tissue structure around tumours and facilitating tumour growth, angiogenesis, and metastasis. Examples of an MMP inhibitor include, but are not limited to, Actinonin, which is also known as Butanediamide, N4-hydroxy-N1-[(1S)-1-[[(2S)-2-(hydroxymethyl)-1-pyrrolidinyl]carbonyl]-2-methylpropyl]-2-pentyh(2R)-(9Cl); epigallocatechin gallate; collagen peptidomimetic and non-peptidomimetic inhibitors; tetracycline derivatives, e.g., hydroxamate peptidomimetic inhibitor batimastat; and its orally-bioavailable analogue marimastat, prinomastat, metastat, Neovastat and Tanomastat.

An NGFR tyrosine-kinase-inhibitor, i.e. a compound which targets, decreases or inhibits nerve growth factor dependent tyrosine phosphorylation. An example of a NGFR tyrosine-kinase-inhibitor includes, but is not limited to, Tyrphostin AG 879.

A phosphatidylinositol 3-kinase inhibitor, i.e. a compound which targets, decreases or inhibits PI 3-kinase. Examples of a phosphatidylinositol 3-kinase inhibitor include, but are not limited to, Wortmannin, which is also known as 3H-Furo[4,3,2-de]indeno[4,5-h]-2-benzopyran-3,6,9-thone, 11 -(acetyl oxy)- 1 ,6b,7,8,9a, 10, 11 , 11

b-octahydro-1-(methoxymethyl)-9a,11-b-dimethyl-(1S,6bR,9aS,11R,11bR)-(9Cl); 8-phenyl-2-(morpholin-4-yl)-chromen-4-one; and/or Quercetin Dihydrate.

A platinum agent, i.e. a compound which contains Platinum and inhibits DNA synthesis by forming interstrand and intrastrand cross-linking of DNA molecules. Examples of a platinum agent include, but are not limited to, Carboplatin; Cisplatin; Oxaliplatin; cisplatinum and Satraplatin.

The implant and method of the invention may also be advantageous for the delivery of prophylactic therapeutic agents, such as vaccines, especially DNA vaccines. Therefore, in a further aspect of the invention we provide a method of introducing a DNA vaccine formulation into a cell of a tissue which comprises the steps of:

(i) placing an electrically conducting implant comprising at least a pair of terminals, in contact with the selected tissue;

(ii) delivering the DNA vaccine formulation into the tissue, and

(iii) simultaneously, sequentially or separately wirelessly maintaining an electrical voltage across the pair of terminals so that the nucleic acid expression construct is introduced into the cell.

The DNA plasmid may be present in the vaccine formulation at a concentration of at least 1 mg/ml, and the poly-L-glutamate may be present in the amount of weight that is 1% of the amount of DNA plasmid.

According to this aspect of the invention we provide a method of gene delivery to cells. Thus, the gene delivery may, for example, be the DNA encoding an immunogen of interest.

Thus, the invention further provides, a method of enhancing an immune response generated in a patient which comprising the steps of

(i) inserting an implant of the invention as hereinbefore described;

(ii) administering DNA encoding one or more immunogens to the patient; and

(iii) applying a time varying magnetic field adjacent to the site of the implant.

Exemplary immunogens which may be encoded according to the present invention may be a DNA vaccine which may comprise a protein or peptide of a pathogen. Such a pathogen may be a bacteria e. g, E. coli strains, Salmonella, Clostridria, Vibrio, Corynebacteria, Listera, Nocardia, Legionella, Bacilli, such as, B. anthracis, Staphylococcus, Streptococci, such as, S. pneumoniae, Borrelia, Mycobacterium, such as, M. tuberculosi, Neisseria, such as, N. gonorrhoeae, Trepanoma, etc.; viruses, such as, parvoviruses, orthomyxoviruses, such as, those causing influenza, paramyxoviruses, picornaviruses, such as, rhinoviruses or polioviruses, papoviruses, herpesviruses, togaviruses, retroviruses, such as, HIV, rhabdoviruses, adenoviruses, etc.; and lower eukaryotes e. g. fungi, protozoa, yeast, helminths, nematodes, etc., such as, Dermatophytes, Pneumocystis, Trypanosoma, Plasmodium, Candida, Cryptococcus, Histoplasma, Coccidioides, amoeba, schistosomes, etc.

Alternatively, the immunogens administered as a part of the present invention may encode antigens that are produced by aberrant or diseased cells of the recipient e. g., cancer cells, etc., such that the recipient animal will form appropriate antibodies.

The DNA vaccine of the present invention may comprise more than one species of DNA.

According to a yet further aspect of the invention we provide a method of treatment of a patient suffering from a disorder, such as cancer, which comprises the simultaneous, sequential or separate steps of applying a voltage across a conductive implant; and administering one or more agents therapeutically active against the disorder to be treated.

Desirably the voltage applied across the conductive implant is achieved as hereinbefore described, by, for example, applying time varying magnetic field across a substantially annular electromagnetically conductive implant

The method according to this aspect of the invention may include the step of first inserting the implant in a patient to be treated. However, it will be understood by the person skilled in the art that once the implant is inserted, it may remain *in situ* and be used for multiple drug therapies.

The method of this aspect of the invention may suitably be used for treatment of a variety of disorders, however, it is especially suitable for use in administering one or more therapeutically active agents which should be "targeted". One example of such

a disorder is proliferative diseases, such as, cancer. Therefore, a particular aspect of the present invention comprises a method of treating or alleviating a proliferative disease, such as, cancer which comprises the steps as hereinbefore described.

Thus, we especially provide a method of treating a cancer patient.

According to a further aspect of the invention we provide a kit comprising;
an electrically conducting annular implant provided with a plurality of electrical terminals, e.g. a split annulus;
means, such as a syringe, for insertion of the implant; and
optionally one or more therapeutic agents as hereinbefore defined.

Referring to the examples that follow, compounds of the preferred embodiments are synthesized using the methods described herein, or other methods, which are known in the art.

It is understood that the invention is not limited to the embodiments set forth herein for illustration, but embraces all such forms thereof as come within the scope of the above disclosure.

EXAMPLES

Figures 1 to 5 illustrate the principle of implanting a split-ring device via hypodermic applicator needle. The sequence of pictures shows the implant wire, e.g. a spring steel implant is illustrated, inserted into the applicator needle from the right. The wire implant is then fed through the hollow applicator needle, emerging from the tip at the

left. As the wire implant comes out of the applicator, its own springiness (yield strength) forces it to curve. In the final picture (Figure 5) the wire implant has formed the required incomplete ring shape.

Method of treatment

Preliminary tests with a transcutaneous magnetic stimulator (TMS) from Magstim Ltd. show that sufficiently high field strengths can be generated for electroporation.

The novel technique generates AC rather than DC fields, but our previous research has shown that AC pulses can efficiently achieve electroporation. The technique requires the implantation of the split ring which could be done via a fine needle. However this is less invasive than the current method which requires electrodes to be inserted multiple times. Invasive procedures are also an accepted risk in the taking of biopsies.

Claims

1. A method of electroporation which comprises wirelessly applying an induced time varying voltage pulse across a gap between a pair of spaced apart terminals wherein the terminals comprise a portion of an implant.

2. A method according to claim 1 which comprises *in vivo* electroporation by applying a generated time varying voltage pulse across a gap between a pair of implanted terminals.

3. A method according to claim 1 wherein the implanted terminals comprise a plurality of individual terminals.

4. A method according to claim 1 wherein the implanted terminals comprise a pair of terminals consisting of the ends of an annulus.

5. A method according to claim 1 wherein the annulus comprises one or more of an essentially two dimensional ring or a ring comprising multiple turns.

6. A method according to claim 1 wherein the two dimensional ring is a split ring.

7. A method according to claim 1 wherein the ring comprising multiple turns is in the form of a coil.

8. A method according to claim 1 wherein the pair of terminals consists of either end of the same split annulus.

9. An electrically conducting annular implant which comprises a plurality of electrical terminals.

10. An implant according to claim 6 which comprises a split annulus.

11. An implant according to claim 6 which comprises a coil provided with at least a pair of terminals.

12. An implant according to claim 6 wherein the implant material is a flexible material.

13. An implant according to claim 6 wherein the implant material is a memory material.

14. An implant according to claim 8 wherein the implant material is a shape memory metal.

15. An implant according to claim 9 wherein the implant material is a shape memory alloy.

16. An implant according to claim 1 wherein the implant material is Nitinol™.

17. An implant according to claim 9 wherein the implant material has a relatively high yield strength.

18. An implant according to claim 9 wherein the implant material has a Young's modulus of >80 GPa.

19. An implant according to claim 9 wherein the implant material is provided with a biocompatible coating.

20. An implant according to claim 9 wherein the implant is an optionally coated tempered high carbon steel or spring steel.

21. An implant according to claim 9 wherein the diameter of the spring steel is from 0.15 to 2mm.

22. A method of delivery of a therapeutic agent which comprises administering the therapeutic agent simultaneously, sequentially or separately whilst "administering" electroporation.

23. A method according to claim 12 wherein the therapeutic agent is an anticancer agent.

24. A method of treatment of a patient suffering from a disorder, such as cancer, which comprises the simultaneous, sequential or separate steps of applying a voltage across a conductive implant; and

administering one or more agents therapeutically active against the disorder to be treated.

25. A method of introducing a DNA vaccine formulation into a cell of a tissue which comprises the steps of:

(i) placing an electrically conducting implant comprising at least a pair of terminals, in contact with the selected tissue;

(ii) delivering the DNA vaccine formulation into the tissue, and

(iii) simultaneously, sequentially or separately wirelessly maintaining an electrical voltage across the pair of terminals so that the nucleic acid expression construct is introduced into the cell.

26. A method of enhancing an immune response generated in a patient which comprises the steps of

(i) inserting an implant of the invention as hereinbefore described;

(ii) administering DNA encoding one or more immunogens to the patient; and

(iii) applying a time varying magnetic field adjacent to the site of the implant.

27. A method according to any one of claims 14, 15 or 16 wherein the immunogen is a DNA vaccine comprising a protein or peptide of a pathogen wherein the pathogen is a bacteria e. g, E. coli strains, Salmonella, Clostridria, Vibrio, Corynebacteria, Listera, Nocardia, Legionella, Bacilli, such as, B. anthracis, Staphylococcus, Streptococci, such as, S. pneumoniae, Borrelia, Mycobacterium, such as, M. tuberculosi, Neisseria, such as, N. gonorrhoeae, Trepanoma, etc.; viruses, such as, parvoviruses, orthomyxoviruses, such as, those causing influenza, paramyxoviruses,

picornaviruses, such as, rhinoviruses or polioviruses, papoviruses, herpesviruses, togaviruses, retroviruses, such as, HIV, rhabdoviruses, adenoviruses, etc.; and lower eukaryotes e. g, fungi, protozoa, yeast, helminths, nematodes, etc., such as, Dermatophytes, Pneumocystis, Trypanosoma, Plasmodium, Candida, Cryptococcus, Histoplasma, Coccidioides, amoeba, schistosomes, etc.

28. A method according to claim 14 wherein the conductive implant is a substantially annular electromagnetically conductive implant

29. A method according to claim 15 wherein the patient is a cancer patient.

30. A method according to claim 14 wherein the method comprises a first step of inserting the implant in a patient to be treated.

31. A method according to claims 1 or 14 wherein the method comprises targeting a therapeutically active agent.

32. A kit comprising;

an electrically conducting annular implant provided with a plurality of electrical terminals;

means for insertion of the implant; and

optionally one or more therapeutic agents.

33. A method, implant or kit as hereinbefore described with reference to the accompanying examples.

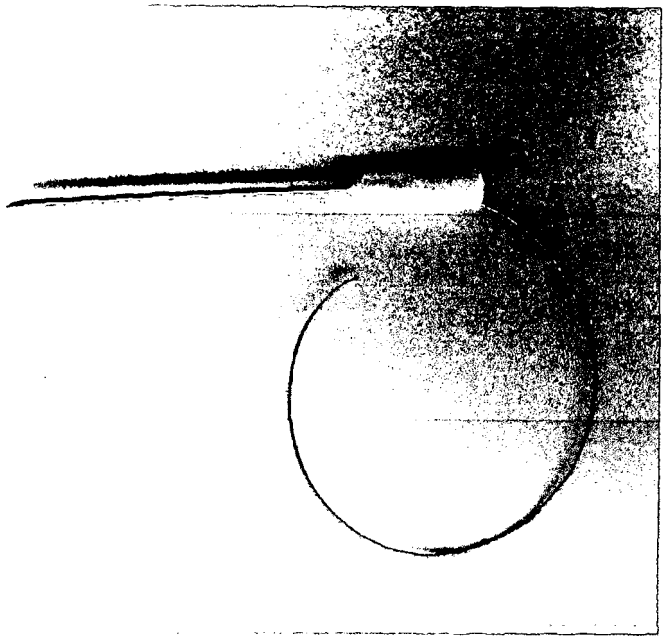

Figure 1

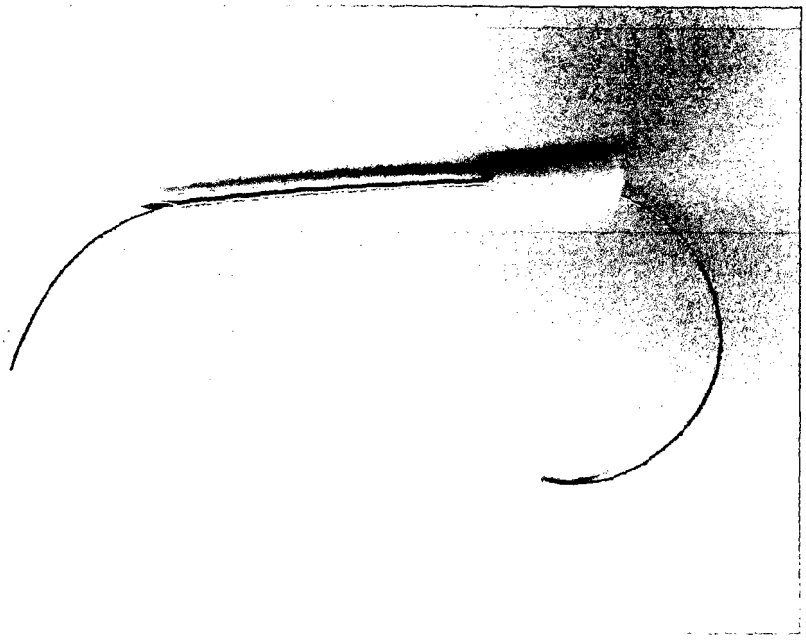

Figure 2

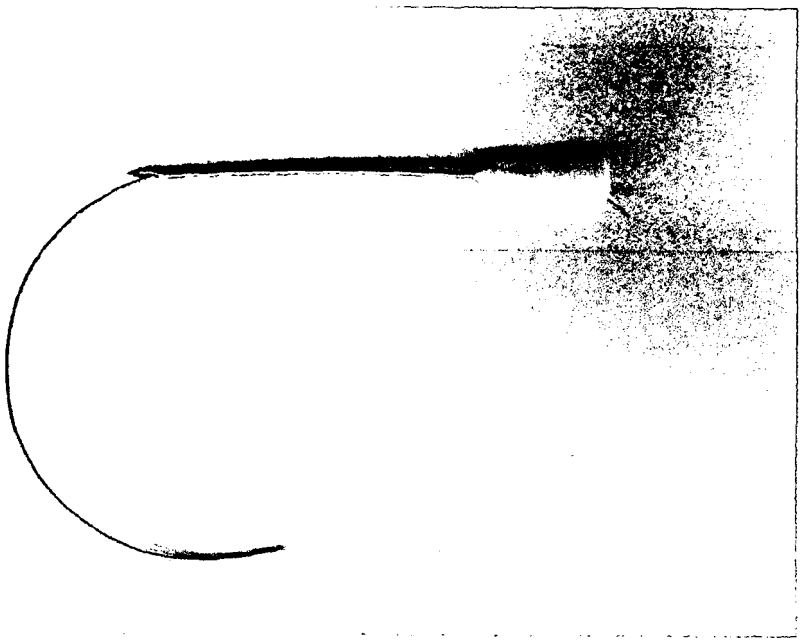

Figure 3

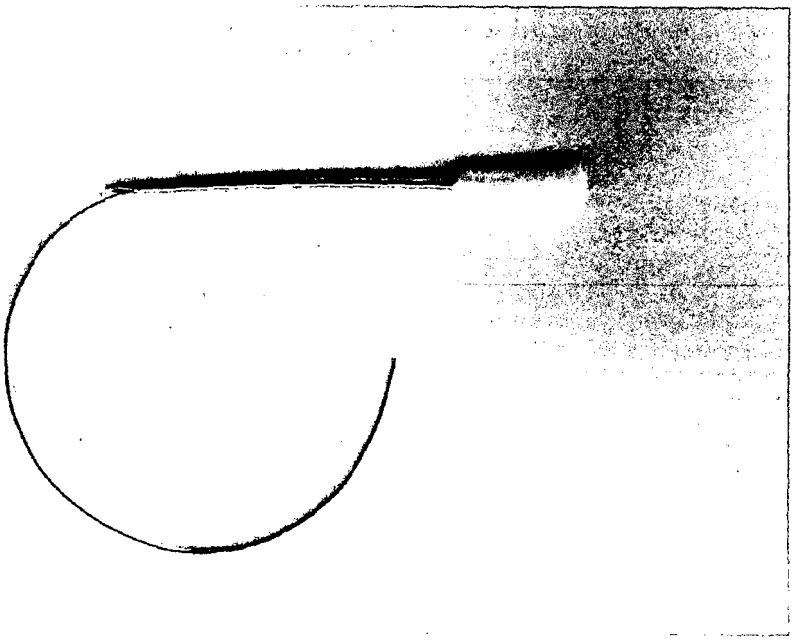

Figure 4

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001919

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61N1/32 A61N1/05
ADD. A61N1/30

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)
A61N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 501 662 A (HOFMANN GUNTER A [US]) 26 March 1996 (1996-03-26) figures 5-6 column 2, lines 9-36 column 5, lines 4-38	9-12, 19-21
X	US 2009/149918 A1 (KRULEVITCH PETER [US] ET AL) 11 June 2009 (2009-06-11) figure 1a paragraphs [0013], [0024]	9-18,21, 32

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"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)

"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

"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.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 March 2011	23/03/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gentil, Cédric

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2010/001919

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

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

1. [X] Claims Nos.: 1-8, 22-31, 33
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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

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

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 an additional fees, this Authority did not invite payment of additional fees.

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

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

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.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001919

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	CHEN C ET AL: "Electroporation of cells using EM induction of ac fields by a magnetic stimulator", PHYSICS IN MEDICINE AND BIOLOGY IOP PUBLISHING LTD. UK, vol. 55, no. 4, 21 February 2010 (2010-02-21), pages 1219-1229, XP002626120, ISSN: 0031-9155 the whole document -----	9-21,32

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2010/001919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5501662 A	26-03-1996	NONE	
US 2009149918 A1	11-06-2009	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 1-8, 22-31, 33

Rule 39.1(iv) PCT - Method of treatment of the human or animal being by therapy. The electroporation methods as defined in claims 1-8, 22-31 and 33 are directed towards delivery of active therapeutic agents, such as vaccines, to a patient and therefore relate to the curing of diseases or malfunctions of the body in order to restore or maintain health; reference is also made to the description, page 7, line 16 - page 8, line 4 and claims 22-26 and 33. It is therefore considered that claims 1-8, 22-31 and 33 define different methods of treatment of the human or animal body by therapy, for which no international search needs to be carried out (Rule 39.1(iv) PCT).