

(19) AUSTRALIAN PATENT OFFICE

- (54) Title
Oligonucleotide-containing microspheres, their use for the manufacture of a medicament for treating diabetes type 1
- (51)⁶ International Patent Classification(s)
A61K 9/16 (2006.01) 9/00
A61K 9/00 (2006.01) 20060101ALI2007022
C12N 15/11 (2006.01) ^{4BMEP} **C12N**
A61K 9/16 15/11
20060101AFI2006072 20060101ALI2007072
^{2BMEP} **A61K** ^{1BMEP}
PCT/US2005/016689
- (21) Application No: 2005244851 (22) Application Date: 2005.05.12
- (87) WIPO No: W005/112885
- (30) Priority Data
- | (31) Number | (32) Date | (33) Country |
|-------------|------------|--------------|
| 60/570,273 | 2004.05.12 | US |
| 60/625,483 | 2004.11.05 | US |
- (43) Publication Date : 2005.12.01
- (71) Applicant(s)
Baxter Healthcare S.A., Baxter International Inc., Children's Hospital of Pittsburgh
- (72) Inventor(s)
Scott, Terrence L., Giannoukakis, Nick, Lafreniere, Debra, Machen, Jennifer, Bisker-Leib, Vered, Brown, Larry L.
- (74) Agent/Attorney
Peter Maxwell & Associates, Level 6 60 Pitt Street, Sydney, NSW, 2000
- (56) Related Art
US 6475995 B1
WO 2000/041679 A1
US 2003/0059474 A1
US 6458387 B1
US 2002/0146459 A1
US 6268053 B1

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 December 2005 (01.12.2005)

PCT

(10) International Publication Number
WO 2005/112885 A3

(51) International Patent Classification: ⁷ A61K 9/16,
38/00, 39/00

(21) International Application Number:
PCT/US2005/016689

(22) International Filing Date: 12 May 2005 (12.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/570,273 12 May 2004 (12.05.2004) US
60/625,483 5 November 2004 (05.11.2004) US

(71) Applicant (for all designated States except US): **BAXTER INTERNATIONAL INC.** [US/US]; One Baxter Parkway, Mail Stop DF3-2E, Deerfield, IL 60015 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SCOTT, Terrence, L.** [US/US] (US). **LAFRENIERE, Deborah** [US/US] (US). **GIANNOUKAKIS, Nick** [US/US] (US). **BISKER-LIEB, Vered** [US/US] (US). **BROWN, Larry, L.** [US/US] (US).

(74) Agent: **MEHLER, Raymond, M.**; Cook, Alex, McFar-
ron, Manzo, Cummings & Mehler, Ltd., Suite 2850, 200
West Adams Street, Chicago, IL 60606 (US).

(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, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SH, 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, 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
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
9 February 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: OLIGONUCLEOTIDE-CONTAINING MICROSPHERES, THEIR USE FOR THE MANUFACTURE OF A MEDICAMENT FOR TREATING DIABETES TYPE 1

(57) Abstract: This application refers to microspheres comprising oligonucleotides for treatment of type 1 diabetes, said oligonucleotides comprise between about 30 weight percent and about 100 weight percent of the microspheres, based on the total weight of the microspheres, said microspheres having an average particle size of not greater than about 50 microns. The oligonucleotides are targeted to bind to primary transcripts selected from the group consisting of CD40, CD80 and CD86 primary transcripts, and combinations thereof.

WO 2005/112885 A3

DELIVERY OF AS-OLIGONUCLEOTIDE MICROSPHERES
TO INDUCE DENDRITIC CELL TOLERANCE FOR THE TREATMENT
OF AUTOIMMUNE TYPE 1 DIABETES

Description

Cross References to Related Application

[0001] Provisional Patent Application Serial No. 60/570,273, filed May 12, 2004 and Provisional Patent Application Serial No. 60/625,483 filed November 5, 2004.

Background of the Invention

Field of the Invention

[0002] The present invention generally relates to microsphere delivery of AS-oligonucleotides in order to induce dendritic cell tolerance, particularly in the non-obese-diabetic (NOD) mouse model. More particularly, the invention relates to drug delivery technology by way of microspheres that are fabricated using totally aqueous conditions, which microspheres incorporate antisense (AS) oligonucleotides. These microspheres are used for an antisense approach to prevent an autoimmune diabetes condition in NOD mice *in vivo* and *in situ*.

Background of the Invention

[0003] Microparticles, microspheres, and microcapsules are solid or semi-solid particles having a diameter of less than one millimeter, more preferably less than 100 microns, which can be formed of a variety of materials, including synthetic polymers, proteins, and polysaccharides. Microspheres have

been used in many different applications, primarily separations, diagnostics, and drug delivery.

[0004] A number of different techniques can be used to make these microspheres from synthetic polymers, natural polymers, proteins and polysaccharides, including phase separation, solvent evaporation, emulsification, and spray drying. Generally the polymers form the supporting structure of these microspheres, and the drug of interest is incorporated into the polymer structure. Exemplary polymers used for the formation of microspheres include homopolymers and copolymers of lactic acid and glycolic acid (PLGA) as described in U.S. Pat. No. 5,213,812 to Ruiz, U.S. Pat. No. 5,417,986 to Reid et al., U.S. Pat. No. 4,530,840 to Tice et al., U.S. Pat. No. 4,897,268 to Tice et al., U.S. Pat. No. 5,075,109 to Tice et al., U.S. Pat. No. 5,102,872 to Singh et al., U.S. Pat. No. 5,384,133 to Boyes et al., U.S. Pat. No. 5,360,610 to Tice et al., and European Patent Application Publication Number 248,531 to Southern Research Institute; block copolymers such as tetronic 908 and poloxamer 407 as described in U.S. Pat. No. 4,904,479 to Illum; and polyphosphazenes as described in U.S. Pat. No. 5,149,543 to Cohen et al. Microspheres produced using polymers such as these exhibit a poor loading efficiency and are often only able to incorporate a small percentage of the drug of interest into the polymer structure. Therefore, substantial quantities of microspheres often must be administered to achieve a therapeutic effect.

[0005] Spherical beads or particles have been commercially available as a tool for biochemists for many years. For example, antibodies conjugated to beads create relatively large particles specific for particular ligands. The large antibody-coated particles are routinely used to crosslink receptors on the surface of a cell for cellular activation, are bound to a solid phase for immunoaffinity purification,

and may be used to deliver a therapeutic agent that is slowly released over time, using tissue or tumor-specific antibodies conjugated to the particles to target the agent to the desired site.

[0006] One disadvantage of the microparticles or beads currently available is that they are difficult and expensive to produce. Microparticles produced by these known methods have a wide particle size distribution, often lack uniformity, and fail to exhibit long term release kinetics when the concentration of active ingredients is high. Furthermore, the polymers used in these known methods are dissolved in organic solvents in order to form the microparticles. They must therefore be produced in special facilities designed to handle organic solvents. These organic solvents could denature proteins or peptides contained in the microparticles. Residual organic solvents could be toxic when administered to humans or animals.

[0007] In addition, the available microparticles are rarely of a size sufficiently small to fit through the aperture of the size of needle commonly used to administer therapeutics or to be useful for administration by inhalation. For example, microparticles prepared using polylactic glycolic acid (PLGA) are large and have a tendency to aggregate. A size selection step, resulting in product loss, is necessary to remove particles too large for injection. PLGA particles that are of a suitable size for injection must be administered through a large gauge needle to accommodate the large particle size, often causing discomfort for the patient.

[0008] Generally, many currently available microparticles are activated to release their contents in aqueous media and therefore must be lyophilized to prevent premature release. In addition, particles such as those prepared using the PLGA system exhibit release kinetics based on both erosion and

diffusion. In this type of system, an initial burst or rapid release of drug is observed. This burst effect can result in unwanted side effects in patients to whom the particles have been administered.

[0009] Microparticles prepared using lipids to encapsulate target drugs are known. For example, lipids arranged in bilayer membranes surrounding multiple aqueous compartments to form particles may be used to encapsulate water soluble drugs for subsequent delivery as described in U.S. Pat. No. 5,422,120 to Sinil Kim. These particles are generally greater than 10 microns in size and are designed for intra articular, intrathecal, subcutaneous and epidural administration. Alternatively, liposomes have been used for intravenous delivery of small molecules. Liposomes are spherical particles composed of a single or multiple phospholipid and cholesterol bilayers. Liposomes are 30 microns or greater in size and may carry a variety of water-soluble or lipid-soluble drugs. Liposome technology has been hindered by problems including purity of lipid components, possible toxicity, vesicle heterogeneity and stability, excessive uptake and manufacturing or shelf-life difficulties.

[00010] An objective for the medical community is the delivery of nucleic acids to the cells in an animal for diabetes treatment. For example, nucleic acids can be delivered to cells in culture (*in vitro*) relatively efficiently, but nucleases result in a high rate of nucleic acid degradation when nucleic acid is delivered to animals (*in vivo*).

[00011] In addition to protecting nucleic acid from nuclease digestion, a nucleic acid delivery vehicle must exhibit low toxicity, must be efficiently taken up by cells and have a well-defined, readily manufactured formulation. As shown in clinical trials, viral vectors for delivery can result in a

severely adverse, even fatal, immune response *in vivo*. In addition, this method has the potential to have mutagenic effects *in vivo*. Delivery by enclosing nucleic acid in lipid complexes of different formulations (such as liposomes or cationic lipid complexes) has been generally ineffective *in vivo* and can have toxic effects. Complexes of nucleic acids with various polymers or with peptides have shown inconsistent results and the toxicity of these formulations has not yet been resolved. Nucleic acids also have been encapsulated in polymer matrices for delivery, but in these cases the particles have a wide size range and the effectiveness for therapeutic applications has not yet been demonstrated.

[00012] Therefore, there is a need for addressing nucleic acids delivery issues, and there is an on-going need for development of microspheres and to new methods for making microspheres. Details regarding microspheres are found in US Patents No. 6,458,387 to Scott et al., No. 6,268,053, No. 6,090,925, No. 5,981,719 and No. 5,599,719 to Woiszwilllo et al., and No. 5,578,709 to Woiszwilllo. These and all references identified herein are incorporated by reference hereinto.

Summary of the Invention

[00013] In accordance with the present invention, DNA to be delivered to dendritic cells is delivered as microspheres. It is believed that such a delivery approach prevents access of the nucleases to the nucleic acids within the microsphere. Microsphere delivery of AS-oligonucleotides is carried out in order to induce dendritic cell tolerance, particularly in the NOD mouse model. The microspheres are fabricated using aqueous conditions, which microspheres incorporate antisense (AS) oligonucleotides. These microspheres are used to inhibit

gene expression and to prevent an autoimmune diabetes condition in NOD mice *in vivo* and *in situ*.

[00014] In a preferred aspect of the invention, three AS-oligonucleotides targeted to the CD40, CD80 and CD86 primary transcripts are synthesized, and an aqueous solution of the oligonucleotide mixture is prepared and combined with a polymer solution. After processing, microspheres containing the oligonucleotides are provided, and these are delivered to the NOD mice.

[00015] These and other aspects, objects, features and advantages of the present invention, including the various combinations, will be apparent from and clearly understood through a consideration of the following detailed description.

Brief Description of the Drawings

[00016] In the course of this description, reference will be made to the attached drawings, wherein:

[00017] Fig. 1 is a schematic illustration of the role of dendritic cells in the autoimmune destruction of pancreatic insulin-producing beta-cells in Type 1 diabetes;

[00018] Fig. 2 is a diagram of the Beta-Galactosidase gene-containing plasmid vector;

[00019] Fig. 3 shows photomicrographs providing evidence for transfection of NIH 3T3 fibroblast cells with the plasmid DNA microspheres;

[00020] Fig. 4 is a photomicrograph of agarose electrophoresis gel of naked plasmid DNA and of two plasmid DNA microsphere formulations according to the invention, each after exposure to DNAase;

[00021] Fig. 5 is a bar graph of Beta-Galactosidase activity in four different plasmid DNA applications.

[00022] Fig. 6 is a scanning electron micrograph of microspheres of AS-oligonucleotides and poly-L-lysine polycation;

[00023] Fig. 7 is a scanning electron micrograph of microspheres of AS-oligonucleotides and poly-L-ornithine polycation; and

[00024] Fig. 8 is a plot summarizing diabetes incidence in three groups of NOD mice treated with the microspheres and according to other procedures for delivery of the three primary transcripts.

Description of the Preferred Embodiments

[00025] As required, detailed embodiments of the present invention are disclosed herein; however, it is to be understood that the disclosed embodiments are merely exemplary of the invention, which may be embodied in various forms. Therefore, specific details disclosed herein are not to be interpreted as limiting, but merely as a basis for the claims and as a representative basis for teaching one skilled in the art to variously employ the present invention in virtually any appropriate manner.

[00026] The preferred embodiment prevents autoimmune insulin-dependent diabetes by formulating and injecting antisense (AS)-oligonucleotide microspheres described herein targeting the primary transcripts of CD40, CD80 and CD86. These oligonucleotides are designed to induce immune tolerance in an attempt to prevent destruction of the insulin producing beta cells in the NOD mouse model. The events leading to the destruction of these beta cells is illustrated in Fig. 1. This illustrates how Type 1 diabetes is manifested by the autoimmune destruction of the pancreatic insulin-producing beta cells in the NOD mouse, as well as in humans. At the time of clinical onset, humans have 10-20% residual beta cell

mass. Sparing of this residual mass can result in remaining insulin levels which are adequate to regulate glucose levels. The microparticles of the invention are provided to interfere with the autoimmune destruction of the beta cells which is illustrated in Fig. 1.

[00027] It will be appreciated that dendritic cells (DC) can be activated to be potent antigen presenting cells found in all tissues and which are highly concentrated under the skin. These antigen presenting dendritic cells function as triggers of the immune response through the activation of T-cells, particularly in lymph nodes.

[00028] Fig. 2 is a drawing of a plasmid vector containing the Beta-galactosidase gene that can be used to transfect NIH 3T3 fibroblast cells. *In vitro* evidence for the transfection of NIH 3T3 fibroblast cells with the plasmid DNA microspheres is shown in Fig. 3 by the cells which stain blue in color in response to the addition of the Beta-Galactosidase x-gal (5-bromo-4-chloro-3-indolyl-beta-galactopyranoside) substrate.

[00029] Fig. 4 illustrates the ability of microspheres to protect DNA in solution. This is an agarose electrophoresis gel showing nuclease protection imparted by microspheres of plasmid DNA produced generally as noted herein. In the Plasmid samples 1, 2 and 3, naked plasmid DNA was exposed to DNAse, with the smears indicating plasmid DNA degradation at each of the three levels of DNAase application. In the Particle 1 and Particle 2 samples, plasmid DNA microsphere formulations were exposed to DNAase. The lack of smearing indicates the microsphere formulations show shielding of the plasmid DNA from degradation.

[00030] Fig. 5 reports on Beta-Galactosidase activity in four different plasmid DNA applications. The naked plasmid DNA application showed very low levels. Somewhat greater levels are indicated for plasmid DNA cationic lipid complex

application using lipofectamine, a commercial cationic lipid, as the delivery vehicle. Substantially greater activity is shown for two pDNA microspheres, with Microspheres 1 corresponding to Particle 1 of Fig. 4, and Microspheres 2 corresponding to Particle 2 of Fig. 4.

[00031] In making the microspheres that are used for autoimmune treatment of diabetes in mice, three AS-oligonucleotides are dissolved in aqueous solution and combined with water soluble polymer(s) and a polycation. The solution typically is incubated at about 60-70°C, cooled to about 23°C, and the excess polymer is removed. Microspheres are formed which are believed to contain the three AS-oligonucleotides having the following sequences, wherein an asterisk indicates thioation:

Seq ID 1: CD 40-AS: 5'-C*AC* AG*C C*GA* GG*C* AA*A
GA*C* AC*C A*T*G C*AG* GG*C* A-3'

Seq ID 2: CD80-AS: 5'-G*GG* AA*A G*CC* AG*G A*AT* CT*A
G*AG* CC*A A*TG G*A-3'

Seq ID 3: CD86-AS: 5'-T*GG* GT*G C*TT* CC*G T*AA*
GT*T C*TG* GA*A C*AC* G*T*C-3'

[00032] More particularly, the nucleic acids typically comprise between about 30 and about 100 weight percent of the microspheres and have an average particle size of not greater than about 50 microns. Typically, they are prepared as follows. An aqueous solution of the oligonucleotide mixture is prepared by combining aliquots from three oligonucleotide solutions, each solution containing one of these three types. A solution containing the three types of oligonucleotides is prepared. The solutions preferably contain about 10 mg/ml oligonucleotide. These are combined with aliquots of a 10

mg/ml stock solution of polycation solution at volumetric ratios of polycation:oligonucleotide of from about 1:1 to about 4:1. Polymer solutions of polyvinyl pyrrolidone and/or of polyethylene glycol are prepared and combined with the other solutions. Heating, cooling, centrifuging and washing multiple times provide an aqueous suspension which typically is frozen and lyophilized to form a dry powder of microspheres comprising oligonucleotide and polycation.

[00033] Microspheres according to the invention are a viable non-viral delivery tool for plasmid DNA and antisense oligonucleotides and other nucleic acids. They allow for *in vitro* delivery of Beta-Galactosidase plasmid DNA in 3T3 fibroblast cells. The microspheres protect plasmid DNA from nuclease activity. High levels of Beta-Galactosidase activity are expressed following transfection with the microsphere formulations.

[00034] Microspheres containing the antisense oligonucleotides of interest down-regulate surface cell antigens CD40, CD80 and CD86, known to be critical in the activation of the autoimmune reaction that results in destruction of insulin-producing beta cells of the pancreas. This can be accomplished by subcutaneous injection to dendritic cells located under the skin. NOD mice studies demonstrate effective prevention of the autoimmune destruction of beta cells. The DNA and oligonucleotide microspheres are effective transfection vehicles *in vitro* and *in vivo*. Dendritic cells appear to take up the oligonucleotide microspheres and suppress the expression of surface cell antigens CD40, CD80 and CD86. The antisense oligonucleotide microspheres effectively prevent diabetes development in the NOD mouse.

[00035] The following Examples illustrate certain features and advantages of the invention in order to further illustrate

the invention. The Examples are not to be considered limiting or otherwise restrictive of the invention.

EXAMPLE 1

[00036] Three AS-oligonucleotides targeted to the CD40, CD80 and CD86 primary transcripts were synthesized by the DNA synthesis facility at University of Pittsburgh (Pittsburgh, PA). The AS-oligonucleotides sequences are:

Seq ID 1: CD 40-AS: 5'C*AC* AG*C C*GA* GG*C* AA*A
GA*C* AC*C A*T*G C*AG* GG*C* A-3'

Seq ID 2: CD80-AS: 5'-G*GG* AA*A G*CC* AG*G A*AT* CT*A
G*AG* CC*A A*TG G*A-3'

Seq ID 3: CD86-AS: 5'-T*GG* GT*G C*TT* CC*G T*AA*
GT*T C*TG* GA*A C*AC* G*T*C-3'

[00037] An aqueous solution of the oligonucleotide mixture was prepared by combining aliquots of three oligonucleotide solutions, each of which contained one type of oligonucleotide, to form a 10[mg/ml] solution of the three types of oligonucleotides. 10 [mg/ml] poly-L-lysine·HBr in diH₂O (poly-L-lysine·HBr up to 50,000 by Bachem, King of Prussia, PA) was prepared. Poly-L-lysine·HBr was added to the oligonucleotides solution at a volumetric ratio of 1:1. The mixture was vortexed gently. A 25% polymer solution containing 12.5% PVP (polyvinyl pyrrolidone, 40,000 Daltons, Spectrum Chemicals, Gardena, CA) and 12.5% PEG (polyethylene glycol, 3,350 Daltons, Spectrum Chemicals, Gardena, CA) in 1M Sodium Acetate (Spectrum, Gardena, CA) at pH=5.5 was made. The polymer solution was added in a 2:1 volumetric ratio as follows: 750 µl of AS-oligonucleotides, 0.75 ml of poly-L-lysine·HBr, 3.0 ml of PEG/PVP, and a total volume of 4.50 ml.

[00038] The batch was incubated for 30 minutes at 70°C and then cooled to 23°C. Upon cooling, the solution became turbid and precipitation occurred. The suspension was then centrifuged, and the excess PEG/PVP was removed. The resulting pellet was washed by resuspending the pellet in deionized water, followed by centrifugation and removal of the supernatant. The washing process was repeated three times. The aqueous suspension was frozen and lyophilized to form a dry powder of microspheres comprising oligonucleotide and poly-L-lysine.

[00039] Fig. 6 presents a scanning electron micrograph (SEM) of the 1:1 poly-L-lysine:oligonucleotide ratio material. Microspheres, 0.5-4 μm in size, with an average particle size of approximately 2.5 μm were fabricated. Precipitation of an unknown material was also observed. Additional studies by HPLC determined that the precipitation was comprised of residual PEG/PVP, mostly PVP.

EXAMPLE 2

[00040] AS-oligonucleotides targeted to the CD40, CD80 and CD86 primary transcripts were the AS-oligonucleotides sequences of Example 1. An aqueous solution of the oligonucleotide mixture was prepared by combining aliquots of the three oligonucleotide solutions, each of which contained one type of oligonucleotide, to form a 10[mg/ml] solution of the three types of oligonucleotides. A solution of oligonucleotide mixture was prepared. 5 [mg/ml] poly-L-ornithine·HBr in diH₂O (poly-L-ornithine·HBr 11,900 (vis) by Sigma) was prepared. Poly-L-ornithine·HBr was added to the oligonucleotides solution. The mixtures were vortexed gently. A 25% polymer solution containing 12.5% PVP (40,000 Daltons, Spectrum Chemicals, Gardena, CA) and 12.5% PEG (3,350 Daltons, Spectrum Chemicals, Gardena, CA) in 0.1M Sodium Acetate (Spectrum Chemicals, Gardena, CA) at pH=5.5 was made. The

polymer solutions were added. Incubation and rinses followed as described in Example 1. 1.5 ml of the AS-oligonucleotides, 1.5 ml of the poly-L-ornithine·HBr, 3 ml of the PEG/PVP, and a total volume of 6.0 ml was prepared.

[00041] Fig. 7 presents an SEM of this 1:1 poly-L-ornithine:oligonucleotide ratio material. Microspheres, 0.2-8 μm in size, with an average particle size of approximately 2 μm were fabricated. Precipitation of an unknown material was also observed. Additional HPLC studies were able to prove that this precipitation was comprised of residual PEG/PVP, mostly PVP.

EXAMPLE 3

[00042] *In vivo* studies were conducted using the NOD mouse model of Type 1 diabetes mellitus. Type 1 diabetes is manifested by the autoimmune destruction of the pancreatic insulin-producing beta cells as illustrated in Fig. 1. AS-oligonucleotides were used in three applications in an attempt to interfere with the autoimmune destruction of beta cells. The goal was to interfere with the dendritic cell function by targeting the primary transcripts of CD40, CD80 and CD86, which encode dendritic cell surface proteins required for T-cell activation. Dendritic cells with low levels of CD40, CD80 and CD86 are known to promote suppressive immune cell networks *in vivo*. These cascades can result in T-cell hyporesponsiveness to beta cells *in vivo*.

[00043] In the first group of test animals, dendritic cells were propagated *ex vivo* from bone marrow progenitors of NOD mice. Combinations of the three AS-oligonucleotides targeting the primary transcripts of CD40, CD80 and CD86 were added to the cells in tissue culture. After incubation, the AS-oligonucleotide transfected dendritic cells were injected into syngeneic recipients of 5 to 8 weeks of age (not yet diabetic). This is a known *ex-vivo* delivery approach.

[00044] In parallel, AS-oligonucleotide microspheres were injected directly into other NOD mice of the same age. A single injection was carried out on each thus-treated mouse. Another group of these NOD mice was not treated and served as a control.

[00045] Fig. 8 shows that the control, untreated NOD mice all developed diabetes by age 23 weeks. The *ex vivo* AS-oligonucleotide transfected and re-infused dendritic cells group (AS-ODN DC) showed delayed development of diabetes, with 20% remaining "Diabetes Free", indicating glucose levels are maintained within a non-diabetic range. Of the microspheres *in vivo*-injected NOD mice, 71% remained "Diabetes Free" at 43 weeks.

[00046] It will be understood that the embodiments of the present invention which have been described are illustrative of some of the applications of the principles of the present invention. Numerous modifications may be made by those skilled in the art without departing from the true spirit and scope of the invention. Various features which are described herein can be used in any combination and are not limited to precise combinations which are specifically outlined herein.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition comprising microspheres, wherein said microspheres contain a first antisense sequence that targets CD40 primary transcript, a second antisense sequence that targets CD80 primary transcript, and a third antisense sequence that targets CD86 primary transcript, wherein each of said first, second and third oligonucleotides reduces or suppresses in vivo expression of CD40, CD80 and CD86 respectively, and wherein said oligonucleotides comprise between about 30 weight percent and about 100 weight percent of the microspheres, based on the total weight of the microspheres, said microspheres having an average particle size of not greater than about 50 microns.
2. A composition comprising microspheres wherein the microspheres comprise oligonucleotides targeted to bind primary transcripts selected from the group consisting of CD40, CD80, and CD86 primary transcripts and combinations thereof, wherein the oligonucleotides reduce or suppress the expression of CD40, CD80 and/or CD86, and wherein the oligonucleotides comprise greater than about 30 weight percent of the microspheres based on total weight of the microspheres.
3. The composition of claim 2, wherein the microspheres comprise a mixture of three different antisense oligonucleotides targeted to bind CD40, CD80, and CD86 primary transcripts.
4. The composition of any one of claims 1 to 3, wherein the microspheres further comprise a polycation.

03/08/10

5. The composition of claim 4, wherein the microspheres consist essentially of antisense oligonucleotides and the polycation.
6. The composition of any one of claims 1 to 5, wherein the microspheres are capable of being taken up by dendritic cells.
7. The composition of any one of claims 1 to 6, wherein the composition is an injectable composition suitable for in vivo delivery.
8. The composition of any one of claims 1 to 7, wherein the composition is suitable for subcutaneous administration.
9. An injectable composition for the treatment of type 1 diabetes comprising microspheres, said microspheres comprising a first antisense oligonucleotide sequence that targets CD40 primary transcript, a second antisense oligonucleotide sequence that targets CD80 primary transcript, and a third antisense oligonucleotide sequence that targets CD86 primary transcript, wherein each of said first, second and third antisense oligonucleotides reduces or suppresses the expression of CD40, CD80 and CD86 respectively, wherein said first, second and third antisense oligonucleotides comprise greater than about 30% by weight of said microspheres.
10. The composition of any one of claims 2 to 9, wherein the microspheres have an average particle size less than about 50 microns.

03/08/10

11. The composition of any of claims 1 to 9, wherein the microspheres have a particle size of 0.2 microns to 8 microns.
12. The composition of any of claims 1 to 9, wherein the microspheres have a particle size of 0.5 microns to 4 microns.
13. The composition of any of claims 1 to 9, wherein the microspheres have an average particle size of about 2 microns.
14. The composition of any of claims 1 to 9, wherein the microspheres have an average particle size of about 2.5 microns.
15. The composition of any one of claims 1 to 14, wherein the antisense oligonucleotides comprise greater than 60% by weight of the microspheres.
16. A process for delivering nucleic acids in the form of a microsphere composition in accordance with any one of claims 1 to 15 to an individual with type 1 diabetes, wherein the process comprises administering said composition using a route of administration selected from the group consisting of intravenous, intramuscular, subcutaneous, topical, intradermal, intraperitoneal, oral, pulmonary, ocular, nasal or rectal delivery.
17. A process for protecting beta cells of the pancreas of non-obese diabetic mice from autoimmune destruction, comprising subcutaneously injecting the composition in accordance with any one of claims 1 to 15.

03/08/10

18. A process for protecting beta cells of the pancreas of individuals from autoimmune destruction, comprising subcutaneously injecting the composition in accordance with any one of claims 1 to 15.

19. A process for protecting beta cells of the pancreas of humans from autoimmune destruction, comprising subcutaneously injecting the composition in accordance with any one of claims 1 to 15.

20. A process for protecting beta cells of the pancreas of individuals from autoimmune destruction and onset of type 1 diabetes, comprising subcutaneously injecting the composition in accordance with any one of claims 1 to 15.

Dated this 3rd day of August 2010

Baxter International Inc.

Baxter Healthcare S.A., and

Children's Hospital of Pittsburgh

Patent Attorneys for the Applicant

PETER MAXWELL AND ASSOCIATES

03/08/10

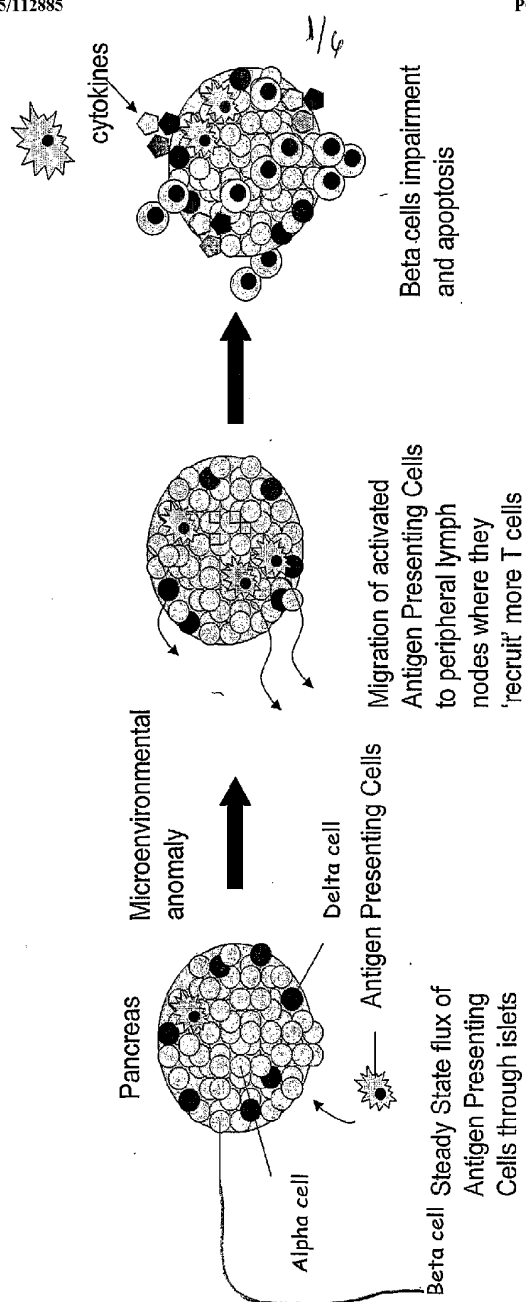


Figure 1 Role of dendritic cells or antigen presenting cells in the autoimmune destruction of the pancreatic insulin-producing beta-cells in Type 1 Diabetes.

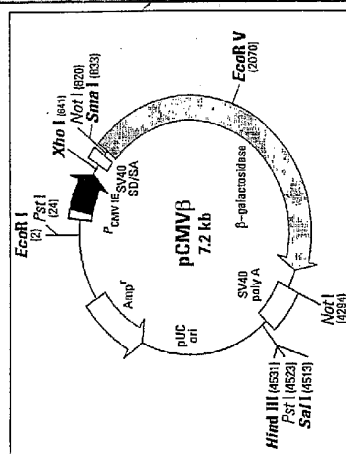
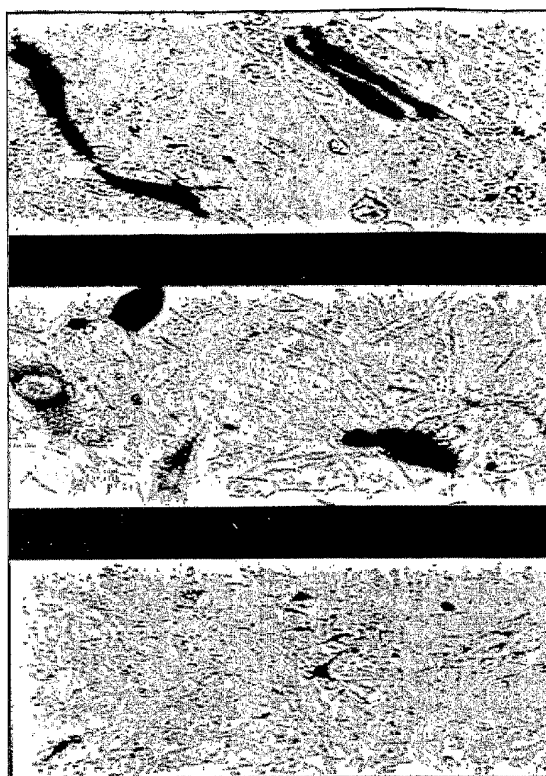


FIGURE 2

Figure 3: PROMAXX plasmid DNA and transfected cells: 5-bromo-4-chloro-3-indolyl-β-galactopyranoside addition to active β-galactosidase turns the NIH 3T3 fibroblasts dark blue in color.

3/6

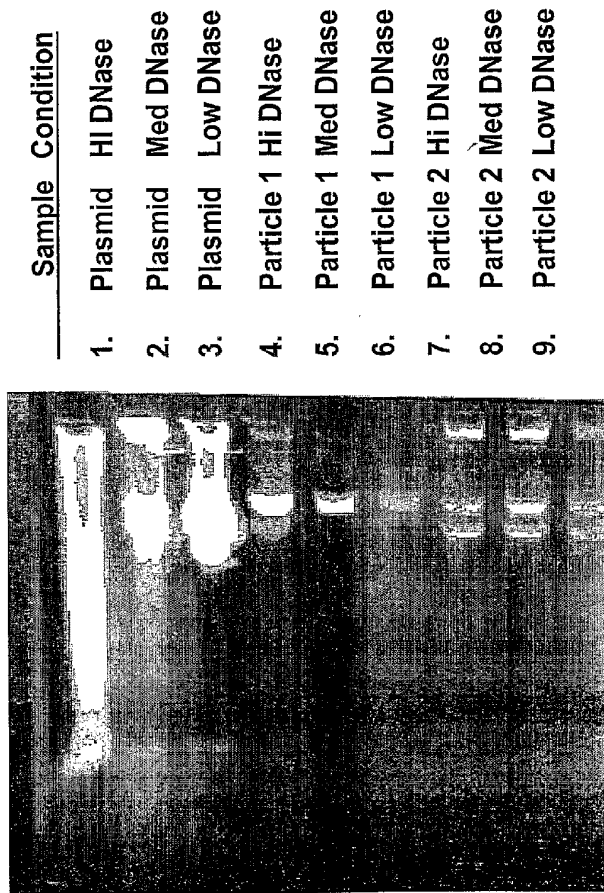


Figure 4: Agarose electrophoresis gel of naked pDNA and two PROMAXX pDNA microspheres formulations exposed to DNase. PROMAXX microspheres apparently shielded the pDNA from degradation. PROMAXX pDNA formulation 1 shows enhanced protection over formulation 2.

4/6

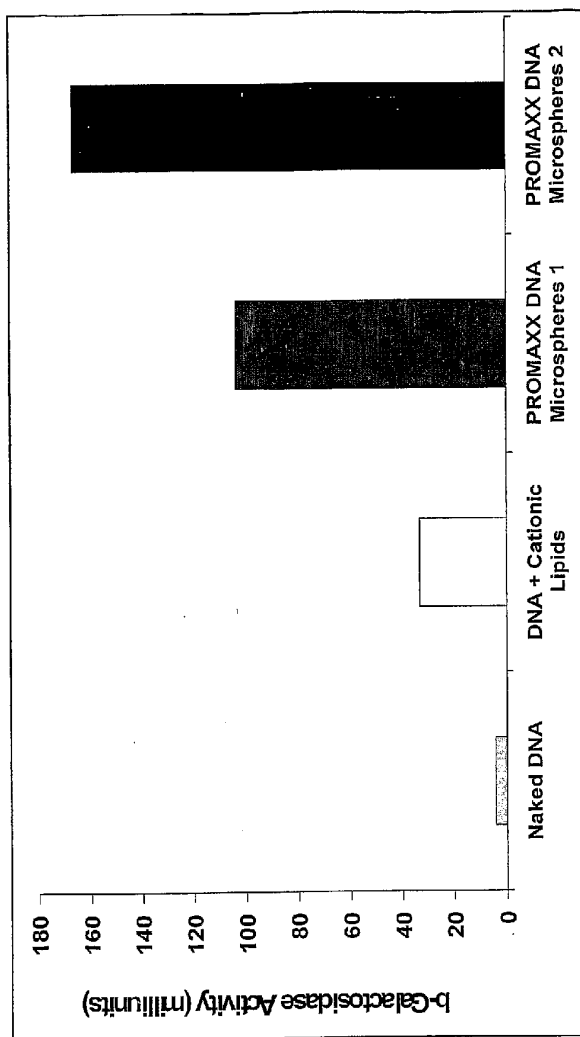


Figure 5: β -Galactosidase activity in PROMAXX pDNA microspheres transfected cells compared with β -Galactosidase activity in pDNA cationic lipid complex and naked pDNA.

5/6

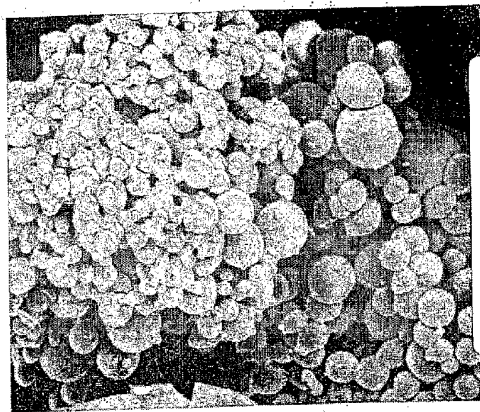


FIG. 7

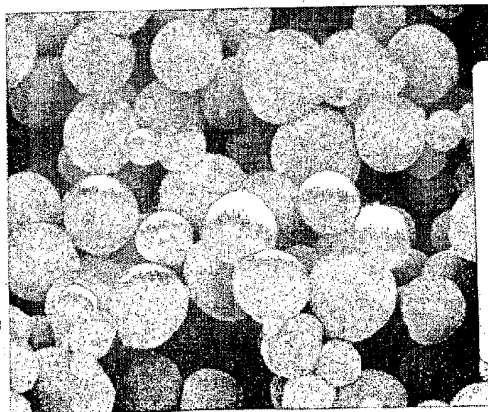


FIG. 6

Scanning electron micrographs of PROMAXX Microspheres comprised of AS-oligonucleotides and poly-L-lysine (left) or AS-oligonucleotides and poly-L-ornithine (right).

6/6

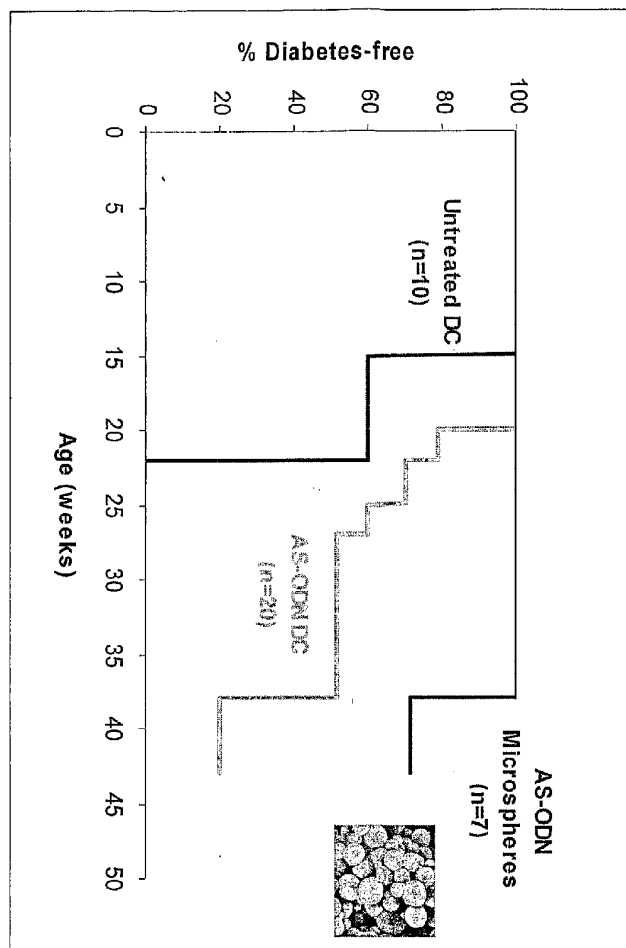


Figure 8: Summary of Diabetes incidence in the three groups of treated NOD mice.

SEQUENCE LISTING

<110> Brown, Larry R.; Bisker-Leib, Vered; Scott, Terrence L.;
Lafreniere, Deborah; Machen, Jennifer; Giannoukakis,
Nick.

<120> Delivery of AS-oligonucleotide Microspheres to Induce
Dendritic Cell Tolerance for the Treatment of Autoimmune
Type I Diabetes

<130> 6143PCT-1 (4362-0002)

<141> 2005-05-12

<160> 3

<210> 1

<211> 31

<212> DNA

<213> Mus musculus

<400> 1

cacagccgag gcaaagacac catgcagggc a 31

<210> 2

<211> 29

<212> DNA

<213> Mus musculus

<400> 2

gggaaagcca ggaatctaga gccaatgga 29

<210> 3

<211> 30

<212> DNA

WO 2005/112885

PCT/US2005/016689

<213> Mus Musculus

<400> 3

tgggtgcttc cgtaagttct ggaacacgtc 30