

APPLICATION DATA SHEET

Publication Filing Type: New Provisional
Application Type: Provisional
Title of Invention: ORAL FORMULATIONS OF CYTIDINE ANALOGS

Customer Number Attorney and Correspondence Address: 25871

INVENTORS:

ETTER, Jeffrey B.
1318 Deer Trail Rd.
Boulder, CO 80302
(US Citizen)

ASSIGNEE:

Pharmion Corporation
2525 28th Street
Boulder, Colorado 80301
(Delaware Corporation)

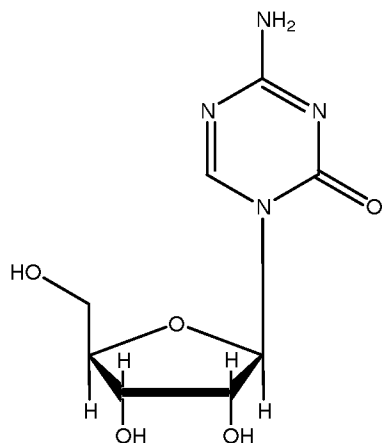
ORAL FORMULATIONS OF CYTIDINE ANALOGS

BACKGROUND OF THE INVENTION

[0001] Cellular proliferative disorders are responsible for numerous diseases resulting in major morbidity and mortality and have been intensively investigated for decades. Cancer now is the second leading cause of death in the United States, and over 500,000 people die annually from this proliferative disorder.

[0002] Nucleoside analogs have been used clinically for the treatment of viral infections and proliferative disorders for decades. Most of the nucleoside analog drugs are classified as antimetabolites. After they enter cells, nucleoside analogs are successively phosphorylated to nucleoside 5'-monophosphates, 5'-diphosphates, and 5'-triphosphates. In most cases, nucleoside triphosphates are the chemical entities that inhibit DNA or RNA synthesis, either through a competitive inhibition of polymerases or through incorporation of modified nucleotides into DNA or RNA sequences. Nucleosides may act also as their diphosphates.

[0003] 5-Azacytidine (also known as azacitidine and 4-amino-1- β -D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one; Nation Service Center designation NSC-102816; CAS Registry Number 320-67-2) has undergone NCI-sponsored trials for the treatment of myelodysplastic syndromes (MDS). See Kornblith et al., J. Clin. Oncol. 20(10): 2441-2452 (2002) and Silverman et al., J. Clin. Oncol. 20(10): 2429-2440 (2002). 5-Azacytidine may be defined as having a molecular formula of $C_8H_{12}N_4O_5$, a relative molecular weight of 244.21 and a structure of:



[0004]

[0005] Azacitidine (also referred to herein as 5-azacytidine herein) is a nucleoside analog, more specifically a cytidine analog. Azacitidine is an antagonist of its related natural

nucleoside, cytidine. Azacitidine, as well as decitabine, i.e., 5-aza-2'-deoxycytidine, are antagonists of decitabine's related natural nucleoside, deoxycytidine. The only structural difference between the analogs and their related natural nucleosides is the presence of nitrogen at position 5 of the cytosine ring in place of oxygen.

[0006] Other members of the class of deoxycytidine and cytidine analogs include arabinosylcytosine (Cytarabine), 2'-deoxy-2',2'-difluorocytidine (Gemcitabine), 5-aza-2'-deoxycytidine (Decitabine), 2(1H) pyrimidine riboside (Zebularine), 2',3'-dideoxy-5-fluoro-3'thiacytidine (Emtriva), N⁴-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine (Capecitabine), 2'-cyclocytidine, arabinofuanosyl-5-azacytidine, dihydro-5-azacytidine, N⁴-octadecyl-cytarabine, elaidic acid cytarabine, and cytosine 1-β-D-arabinofuranoside (ara-C).

[0007] In general, oral delivery of members of this class of compounds has proven difficult due to combinations of chemical instability, enzymatic instability, and/or poor tissue permeability. For example, these compounds are known to be acid labile and thus unstable in the acidic gastric environment. In the case of azacitidine, ara-C, decitabine and gemcitabine, an enzyme thought to be responsible for a significant portion of drug metabolism is cytidine deaminase. Strategies to improve the oral bioavailability of this drug class have included the use of prodrugs to modify chemical and enzymatic instability, and/or the use of enzymatic inhibitors.

[0008] For example, DeSimone et al describe the ability of azacitidine to induce fetal hemoglobin production in baboons when administered via the intravenous (IV), subcutaneous (SC), or perioral (PO) route. In the case of PO administration the author states that co-administration of THU (tetrahydrouridine) was necessary to achieve fetal hemoglobin induction, however no specific data is provided on the doses or responses observed without THU. Azacitidine doses ranged from 0.25 mg/kg/d to 8 mg/kg/d with co-administration of 20 mg/kg/d THU. Administration of THU alone was shown to result in a significant decrease in peripheral cytidine deaminase activity.

[0009] Neil, et al describe the effect of THU on the pharmacokinetics and pharmacodynamics of inter peritoneal (I.P.) and peri oral (P.O.) azacitidine when administered to leukemic mice. Pharmacokinetic parameters were determined using a bioassay that did not discriminate between azacitidine and its degradation and metabolism products. Inclusion of THU with IP administration had little effect on the clearance or degradation of azacitidine. Inclusion of THU with PO administration significantly increased both C_{max} and t_{1/2}. In both

acute and chronic IP dosing the inclusion of THU did not influence the pharmacodynamic effects of azacitidine except at the highest chronic dose which was toxic. Conversely, co-administration of THU with PO azacitidine resulted in increased efficacy at all doses except the highest chronic dose which was again toxic.

[0010] Dunbar, et al describe the administration of azacitidine via IV and PO routes for increased production of total hemoglobin in a β^0 -thalassemic patient. Doses of 2 mg/kg/d IV resulted in a measurable increase to hemoglobin levels. Administration of 2 mg/d tid (three times daily) PO with co-administration of THU did not result in increased hemoglobin levels.

[0011] Dover, et al describe administration of azacitidine via the SC and PO routes for increased production of total hemoglobin, fetal hemoglobin and F cells in sickle cell patients. Azacitidine oral bioavailability was assessed by clinical response only. Dover reports that oral doses of azacitidine (2 mg/kg/d) alone or THU (200 mg/d) alone did not result in increased F reticulocyte production. However oral doses of 200 mg/d of THU were observed to result in a significant suppression of peripheral cytidine deaminase activity for several days post administration. When azacitidine was co-administered with THU good clinical response was observed as determined by total hemoglobin, fetal hemoglobin and F cell levels. In fact comparable clinical response was observed with doses of 2 mg/kg/d SC without THU versus 0.2 mg/kg/d PO with co-administration of 200 mg/d THU. Oral doses of azacitidine and THU were prepared by encapsulation at the clinical site. No information was provided with respect to excipients.

[0012] Efforts to increase bioavailability of this class of compounds have also been described in, for example, U.S. Patent Application Publication No. 2004/0162263 (Sands, et al.) In this publication, delivery of azacitidine in an enteric-coated formulation are disclosed such that the drugs are preferably absorbed in the upper regions of the small intestine, such as the jejunum.

[0013] Despite these efforts, a need remains for more effective methods and compositions which increase oral bioavailability of this class of compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 represents a graph showing Absolute Mucosal to Serosal Permeability of Azacitidine in Human Intestinal Tissue with and without Enzymatic Inhibition.

[0015] Figure 2 represents a graph showing Relative Mucosal to Serosal Permeability of Azacitidine in Human Intestinal Tissue with and without Enzymatic Inhibition with Respect to Atenolol.

[0016] Figure 3 represents a graph showing Absolute Mucosal to Serosal Permeability of Azacitidine in Human Colonic Tissue with Various Concentrations of TPGS or Labrafil without Enzymatic Inhibition.

[0017] Figure 4 represents a graph showing Relative Mucosal to Serosal Permeability of Azacitidine in Human Colonic Tissue with Various Concentrations of TPGS or Labrafil without Enzymatic Inhibition.

SUMMARY OF THE INVENTION

[0018] In a first embodiment, the present invention comprises a controlled release pharmaceutical composition for oral administration for enhanced systemic delivery of a cytidine analog comprising a therapeutically effective amount of a cytidine analog and a drug release controlling component which is capable of providing release of the cytidine analog primarily in the large intestine. After ingestion by a patient, the cytidine analog is released primarily in the large intestine.

[0019] In another embodiment, the present invention includes a method for treating a patient having a disease associated with abnormal cell proliferation. The method includes orally administering to the patient a controlled release pharmaceutical composition, comprising a therapeutically effective amount of a cytidine analog and a drug release controlling component which is capable of providing release of the cytidine analog primarily in the large intestine. After ingestion by a patient the cytidine analog is released primarily in the large intestine.

[0020] In another embodiment, the present invention includes a method of increasing the bioavailability of a cytidine analog upon administration to a patient, comprising the following steps. First, provided is a controlled release pharmaceutical composition, comprising a therapeutically effective amount of a cytidine analog and a drug release controlling component capable of providing release of the cytidine analog primarily in the large intestine. Second, the patient ingests the composition, whereupon the composition contacts the biological fluids of the patient's body and increases the bioavailability of the cytidine analog.

[0021] In one embodiment, a condition to treat using the present invention is a myelodysplastic syndrome. In one embodiment, the cytidine analog is azacitidine. In one embodiment, the drug release controlling component is an enteric coating.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention is based on the surprising discovery that azacitidine and related compounds are best absorbed in the lower gastrointestinal tract, i.e., the large intestine (colon). Conventionally, it is expected that the upper gastrointestinal tract is the more desirable location for absorption, due to greater surface area, relatively greater liquidity, and the fact that typically the greater part of absorption of nutrients takes place therein. However, the inventors have found that in the case for cytidine analogs, absorption is greatest and most consistent between patients in colonic tissue. Accordingly, the present invention demonstrates the preparation of a solid oral dosage form of a cytidine analog, such as azacitidine, using common pharmaceutical excipients designed for delivering pharmaceutical compositions to the large intestine and colon. The term "absorb", "absorption", "absorbed" and the like are used to indicate transfer of a cytidine analog across a relevant tissue, such as, for example, intestinal tissue. In some embodiments, absorbed cytidine analogs are taken up by the blood stream making the cytidine analog available at least partially systemically. In some embodiments, absorption occurs without substantive degradation (i.e., undesirable chemical modification of) of the cytidine analog.

[0023] Furthermore, the inventors have demonstrated that inclusion of THU (taught by others as a requirement to facilitate bioavailability of this drug class) is not necessary to achieve useful oral bioavailability of cytidine analogs via delivery in the large intestine and colon. Accordingly, formulations of the present invention obviate the need to utilize enzymatic inhibitors such as THU in formulations to increase bioavailability of cytidine analogs. Avoidance of enzymatic inhibitors is a desirable attribute for a therapeutic dosage form since such inclusion increases the formulation cost and complexity, and may result in instability, or undesirable, pharmacological, toxicological or other effects. Accordingly, oral delivery of azacitidine without inclusion of an enzymatic inhibitor is possible when the target tissue to which the drug is delivered is the colon. In the case of PO delivery of azacitidine to humans, data suggests that delivery to the upper GI tract may well benefit from enzymatic inhibition, however

delivery to the colon does not require the inclusion of such an inhibitor. Targeting to the colon may be achieved with commercially available and pharmaceutically acceptable coatings such as, for example, enteric coatings.

[0024] Furthermore, the inventors have demonstrated the preparation of solid oral dosage forms containing excipients and coatings which possess acceptable production and stability characteristics for use as a pharmaceutical dosage form.

[0025] In one embodiment, the present invention includes a controlled release pharmaceutical composition for oral administration comprising a) a therapeutically effective amount of a cytidine analog and b) a drug release controlling component for providing the release of the cytidine analog primarily in the large intestine. The controlled release pharmaceutical compositions of the present invention will in one embodiment lack THU.

[0026] In one embodiment, the cytidine analog useful in the present invention includes any moiety which is structurally related to cytidine or deoxycytidine and functionally mimics and/or antagonizes the action of cytidine or deoxycytidine. These analogs may also be called cytidine derivatives herein. In one embodiment, cytidine analogs to use with the present invention include 5-aza-2'-deoxycytidine (decitabine), 5-azacytidine, 5-aza-2'-deoxy-2',2'-difluorocytidine, 5-aza-2'-deoxy-2'-fluorocytidine, 2'-deoxy-2',2'-difluorocytidine (also called gemcitabine), or cytosine 1- β -D-arabinofuranoside (also called ara-C), 2(1H) pyrimidine riboside (also called zebularine), 2'-cyclocytidine, arabinofuanosyl-5-azacytidine, dihydro-5-azacytidine, N⁴-octadecyl-cytarabine, and elaidic acid cytarabine. In one embodiment, is 5-azacytidine and 5-aza-2'-deoxycytidine The definition of cytidine analog used herein also includes mixtures of cytidine analogs.

[0027] Cytidine analogs useful in the present invention may be manufactured by any methods known in the art. In one embodiment, methods to manufacture include methods as disclosed in U.S. Serial No. 10/390,526 (U.S. Patent No. 7,038,038); U.S. Serial No. 10/390,578 (U.S. Patent No. 6,887,855); U.S. Serial No. 11/052615 (U.S. Patent No. 7,078,518); U.S. Serial No. 10390530 (U.S. Patent No. 6,943,249); and U.S. Serial No. 10/823,394.

[0028] In one embodiment, the amounts of a cytidine analog to use in methods of the present invention and in the oral formulations of the present invention include a therapeutically effective amount. Therapeutic indications are discussed more fully herein below. Precise amounts for therapeutically effective amounts of the cytidine analog in the pharmaceutical

compositions of the present invention will vary depending on the age, weight, disease and condition of the patient. For example, pharmaceutical compositions may contain sufficient quantities of a cytidine analog to provide a daily dosage of about 150 mg/m²(based on patient body surface area) or about 4 mg/kg (based on patient body weight) as single or divided (2-3) daily doses.

[0029] The controlled release pharmaceutical compositions of the present invention include a drug release controlling component. The drug release controlling component is adjusted such that the release of the cytidine analog occurs primarily in the large intestine. In one embodiment, at least about 95% of the cytidine analog is released in the large intestine, or at least about 90% of the cytidine analog is released in the large intestine. In other embodiments, at least about 80% of the cytidine analog is released in the large intestine, at least about 70% of the cytidine analog is released in the large intestine, at least about 60% of the cytidine analog is released in the large intestine, or at least about 50% of the cytidine analog is released in the large intestine. In other embodiments, the amount released in the intestines is at least about 40%, at least about 30%, or at least about 20% of the cytidine analog. The term "release" refers to the process whereby the cytidine analog is made available for uptake by or transport across the epithelial cells that line the large intestine and is made available to the body.

[0030] The pharmaceutical compositions of the present invention are intended for oral delivery. Oral delivery includes formats such as tablets, capsules, caplets, solutions, suspensions and/or syrups, and may also comprise a plurality of granules, beads, powders or pellets that may or may not be encapsulated. Such formats may also be referred to as the "drug core" which contains the cytidine analog. Such dosage forms are prepared using conventional methods known to those in the field of pharmaceutical formulation and are described in the pertinent texts, e.g., in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 20th Edition, Lippincott Williams & Wilkins, 2000). Tablets and capsules represent the most convenient oral dosage forms, in which case solid pharmaceutical carriers are employed. Tablets are used in one embodiment. Tablets may be manufactured using standard tablet processing procedures and equipment. One method for forming tablets is by direct compression of a powdered, crystalline or granular composition containing the cytidine analog, alone or in combination with one or more carriers, additives, or the like. As an alternative to direct compression, tablets can be prepared using wet-granulation or dry-granulation processes. Tablets may also be molded rather

than compressed, starting with a moist or otherwise tractable material; particularly, compression and granulation techniques are used in one embodiment.

[0031] In another embodiment, capsules may be used. Soft gelatin capsules may be prepared in which capsules contain a mixture of the active ingredient and vegetable oil or non-aqueous, water miscible materials such as, for example, polyethylene glycol and the like. Hard gelatin capsules may contain granules of the active ingredient in combination with a solid, pulverulent carrier, such as, for example, lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives, or gelatin. A hard gelatin capsule shell can be prepared from a capsule composition comprising gelatin and a small amount of plasticizer such as glycerol. As an alternative to gelatin, the capsule shell may be made of a carbohydrate material. The capsule composition may additionally include colorings, flavorings and opacifiers as required.

[0032] The cytidine analog in one embodiment is prepared as a tablet or capsule which represents a drug core comprising the pharmaceutical composition and the pharmaceutical composition is a controlled release pharmaceutical composition, and as such, comprises at least one additional component comprising a drug release controlling component. Accordingly, the formulations of the present invention are formulated with a drug release controlling component in order to provide for release of the cytidine analog primarily in the large intestine. In one embodiment, minimal release of the cytidine analog occurs in the upper reaches of the gastrointestinal tract, e.g., the stomach and small intestine.

[0033] The small intestine extends from the pylorus to the colic valve where it ends in the large intestine. The small intestine is about 6 meters long and is divisible into three portions: the duodenum, the jejunum, and the ileum. The small intestine is especially adapted for transport and absorption of nutrients and other molecules from ingested material, passing through the lining of the small intestine into the blood. The surface cells of the small intestine are highly specialized for digestion and absorption of nutrients. Almost all the body's nutrient absorption occurs in the small intestine, along its three sub-divisions: the duodenum, jejunum, and ileum. Sites for absorption of specific nutrients (eg: iron, vitamin.B12) are located in these divisions, but most absorption occurs in the jejunum (middle section). Specialized cells contain digestive enzymes, carrier proteins and other secretions. Blood vessels transport nutrients away from the intestine to the liver in the first instance.

[0034] Indigestible food passes into the large intestine. By the time ingested material leaves the small intestine, virtually all nutrient absorption will have occurred. The large intestine extends from the end of the ileum (distal ileum) to the anus. The large intestine is divided into the cecum, colon, rectum, and anal canal. The colon is divided into four parts: the ascending, transverse, descending, and sigmoid. The substantial release of the cytidine compound of the present invention may occur in any portion of the large intestine. In one embodiment, release primarily occurs at the upper regions of the large intestine, such as, for example, at the distal ileum, cecum, and/or the ascending colon.

[0035] It is known that there are major variations in acidity in the gastrointestinal tract. The stomach is a region of high acidity (about pH 1 to 3). Specific glands and organs emptying into the small intestine raise the pH of the material leaving the stomach to approximately pH 6.0 to 6.5. The large intestine and the colon are about pH 6.4 to 7.0. The transit time through the small intestine is approximately three hours. In contrast, the transit time through the large intestine is approximately 35 hours.

[0036] Methods by which to formulate compositions to target specific regions of the gastrointestinal tract are known in the art, described in numerous publications, and all references specifically cited within the present document are incorporated by reference herein. For example, release of drug in the gastrointestinal tract may be accomplished by choosing a drug release controlling component to work together with some physical, chemical or biochemical process in the gastrointestinal tract. A drug release controlling component may take advantage of processes and/or conditions within the gastrointestinal tract and in specific regions of the gastrointestinal tract such as, for example, osmotic pressure, hydrodynamic pressure, vapor pressure, mechanical action, hydration status, pH, bacterial flora, and enzymes. Specific U.S. Patents incorporated by reference herein include, among others, U.S. Pat. No. 3,952,741, U.S. Pat. No. 5,464,633, U.S. Pat. No. 5,474,784, U.S. Pat. No. 5,112,621.

[0037] Optionally, pharmaceutical compositions of the present invention may further comprise a seal coating material that seals the drug to prevent decomposition due to exposure to moisture, such as hydroxylpropylmethylcellulose. Accordingly, the core of the pharmaceutical composition (containing the cytidine analog) may first be sealed with the seal coating material and then coating with the drug release controlling component to prevent decomposition of the cytidine analog by exposure to moisture. Seal coating materials include, in one embodiment,

acetyltributyl citrate, acetyltriethyl citrate, calcium carbonate, carauaba wax, cellulose acetate, cellulose acetate phthalate, cetyl alcohol, chitosan, ethylcellulose, fructose, gelatin, glycerin, glyceryl behenate, glyceryl palmitostearate, hydroxyethyl cellulose, hydroxyethylmethyl cellulose, hydroxypropyl cellulose, hypromellose, hypromellose phthalate, isomalt, latex particles, maltitol, maltodextrin, methylcellulose, microcrystalline wax, paraffin, poloxamer, polydextrose, polyethylene glycol, polyvinyl acetate phthalate, polyvinyl alcohol, povidone, shellac, shellac with stearic acid, sodium carboxymethyl cellulose, sucrose, titanium oxide, tributyl citrate, triethyl citrate, vanillin, white wax, xylitol, yellow wax, and zein. Compositions of the present invention may also include film forming agents, which include, for example, ammonium alginate, calcium carbonate, chitosan, chlorpheniramine maleate, copovidone, dibutyl phthalate, dibutyl sebacate, diethyl phthalate, dimethyl phthalate, ethyl lactate, ethylcellulose, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, hypromellose, hypromellose acetate succinate, maltodextrin, polydextrose, polyethylene glycol, polyethylene oxide, polymethylacrylates, poly(methylvinyl ether/maleic anhydride), polyvinylacetate phthalate, triethyl citrate, and vanillin.

[0038] In one embodiment, drug release controlling components include, for example, coatings, matrices, or physical changes. Coatings are used in one embodiment. Coatings include, for example, enteric coatings, time delay coatings, bacterially degradable coatings, and mixtures thereof. The pharmaceutical composition may comprise multiple coatings of either the same or different types of coatings. In choosing an appropriate coating or mixture thereof, the formulations practitioner may consider a number of variables influencing the location in which a drug will become available in the gastrointestinal tract, e.g., the pH at which coatings dissolve; the time of dissolution (which is influenced by thickness of the coatings and/or additional components in the coatings); time of transit through the gastrointestinal tract, and whether the coatings can be degraded by the patient's digestive enzymes or require enzymes present only in bacteria residing in the lower intestine. As an example of a combination drug release controlling component is, for example, an inner core with two polymeric layers. The outer layer, an enteric coating, may be chosen to dissolve at a pH level above 5. The inner layer, may be made up of hydroxypropylmethylcellulose to act as a time delay component to delay drug release for a predetermined period. The thickness of the inner layer can be adjusted to determine the lag time.

[0039] Methods by which skilled practitioners can assess where a drug is released in the gastrointestinal tract of either animal models or human volunteers are known in the art, and include scintigraphic studies, testing in biorelevant medium which simulates the fluid in relevant portions of the gastrointestinal tract, among others.

[0040] In one embodiment, a drug release controlling component may include an enteric coating. The term "enteric coating" refers to a coating that allows a cytidine analog formulation to pass through the stomach substantially intact and subsequently disintegrate substantially in the intestines. In one embodiment, the disintegration occurs in the large intestine.

[0041] The coating of pH-sensitive (enteric) polymers to tablets, capsules and other oral formulations of the present invention provided delayed release and protect the active drug from gastric fluid. In general, enteric coatings should be able to withstand the lower pH values of the stomach and small intestine and be able to disintegrate at the neutral or slightly alkaline pH of the large intestine. Enteric coatings are a well known class of compounds. Coating pharmaceutically active compositions with enteric coatings is well known in the art to enable pharmaceutical compositions to bypass the stomach and its low acidity. Enteric coatings generally refer to a class of compounds that dissolve at or above a particular pH and include a number of pH-sensitive polymers. The pH dependent coating polymer may be selected from those enteric coatings known to those skilled in the art. Such polymers may be one or more of the group comprising hydroxypropylmethylcellulose phthalate, polyvinyl acetate phthalate (PVAP), hydroxypropylmethylcellulose acetate succinate (HPMCAS), alginate, carbomer, carboxymethyl cellulose, methacrylic acid copolymer (such as, for example, a cationic copolymer of dimethyl aminoethyl methacrylate and neutral methacrylic esters), polyvinyl acetate phthalate, cellulose acetate trimellitate, shellac, cellulose acetate phthalate (CAP), starch glycolate, polacrylin, methyl cellulose acetate phthalate, hydroxymethylcellulose phthalate, hydroxymethylmethylcellulose acetate succinate, hydroxypropylcellulose acetate phthalate, cellulose acetate terephthalate, cellulose acetate isophthalate, and includes the various grades of each polymer such as HPMCAS-LF, HPMCAS-MF and HPMCAS-HG, or mixtures thereof. Other enteric coatings suitable for the present invention include acetyltributyl citrate, carbomers, guar gum, hypromellose acetate succinate, hypromellose phthalate, polymethacrylates, tributyl citrate, triethyl citrate, white wax, and zein.

[0042] In one embodiment, the pH dependent coating is selected from the group consisting of methacrylic acid copolymers of varying threshold pH (such as, but not limited to EUDRAGIT S 100 (a cationic copolymer of dimethyl aminoethyl methacrylate and neutral methacrylic acid esters manufactured by Rohm Pharma GmbH of Darmstadt, Germany)).

[0043] Multiple coatings of enteric polymers may be utilized. In one embodiment, the first coating (closest to the core) is an enteric coating that will survive until the dosage form arrives at the large intestine/colon. To target the large intestine, in one embodiment an enteric coating comprises a series of methacrylic acid anionic copolymers known as EUDRAGIT S. The EUDRAGIT S films are colorless, transparent and brittle. In one embodiment, the enteric coating comprises EUDRAGIT S100. The EUDRAGIT S coatings are insoluble in pure water, in buffer solutions below a pH of 6.0 and also in natural and artificial gastric juices. They are slowly soluble in the region of the digestive tract where the juices are neutral to weakly alkaline (i.e., the large intestine and the colon) and in buffer solutions above a pH of 7.0. Mixtures of these various enteric polymers recited above, can be used in the present invention. Further, the use of plasticizers is included in one embodiment with the enteric polymer coatings useful herein.

[0044] As known in the art and discussed in sources such as Patel et al. "Colon Specific Delivery" *Drug Delivery Technology* (2006) Vol. 6 62-71, and Khan et al., *J. Controlled Release* 1999; 58:215-222, the disintegration rates of enteric coated tablets are dependent on the polymer combination used to coat the tablets, the pH of the disintegration media, and the coating level of the tablets (i.e., thickness of the coating). The presence of plasticizer and the nature of the salts in the dissolution medium also influence the dissolution rate. A number of specific formulations effective for release in the colon in human volunteers, using *in vivo* scintigraphic studies, is disclosed in Patel et al., and are incorporated by reference herein.

[0045] The enteric coating may also be modified through the inclusion of an edible acid to retard or slow the dissolution of the coating in the intestines. Any edible acid may be used. Representative edible acids include acetic acid, benzoic acid, fumaric acid, sorbic acid, propionic acid, hydrochloric acid, citric acid, malic acid, tartaric acid, isocitric acid, oxalic acid, lactic acid, the phosphoric acids and mixtures thereof. One embodiment includes fumaric acid and malic acids. The weight percent of the edible acid in the enteric coating solution (polymer, plasticizer, anti-tack agents, water and the like) can range from about 5 to about 40%, with 10 to 30%

present in one embodiment and 10 to 25% in another embodiment. Those skilled in the art will readily be able to determine the exact amount of edible acid to include in the coating solution, depending upon the pKa of the particular edible acid and the desired delay in dissolution of the enteric coating. After application of the enteric coating solution, as further described below, the percent of edible acid in the coating will range from about 10 to about 80 weight % of the coating; 20 to 60% in one embodiment; and 25-50% in another.

[0046] Enteric coatings can be obtained from a number of manufacturers, such as, for example, Rohm Pharma GmbH of Darmstadt, Germany (EUDRAGIT). Particular blends of pH sensitive polymers and types can be selected by one of skill in the art. As an example, the manufacturer of EUDRAGIT polymers teaches that the EUDRAGIT grades for sustained release formulations are based on copolymers of acrylate and methacrylates with quaternary ammonium groups as functional groups as well as ethylacrylate methylmethacrylate copolymers with a neutral ester group. EUDRAGIT polymers are available insoluble and/or permeable. For example, the EUDRAGIT RL-types are highly permeable, the EUDRAGIT RS-types are poorly permeable, the EUDRAGIT NE-types are swellable and permeable. The release profiles and locations of release can be determined by varying mixing ratios of the polymers and/or film thickness of the coatings and such profiles can be adjusted by those of skill in the art.

[0047] In some embodiments, coatings include those that selectively dissolve at a pH at or above the pH generally prevailing in the large intestine, for example, above about pH 6, above about pH 6.2, above about pH 6.4, above about pH 6.6, above about pH 6.8, or above about pH 7. In one embodiment, the enteric coating will selectively dissolve in the pH range of about 6.0 to about 7.5, in the pH range of about 6.2 to about 7.5, in the pH range of about 6.4 to about 7.2, in the pH range of about 6.5 to about 7, in the pH range of about 6.5 to 6.8. As an example of coatings and their "threshold" pH (the pH at which the coating will dissolve) which the skilled practitioner may consider include, but are not limited to, cellulose phthalates (e.g, hydropropylmethylcellulose phthalates (HPMCPs)) that selectively dissolve at pH above 5.6, the EUDRAGIT family of polymers which are anionic polymer based on methacrylic acid and methacrylates with carboxyl functional groups (e.g., EUDRAGIT L30D with threshold pH of 5.6, EUDRAGIT L with threshold pH of 6.0, and EUDRAGIT S with threshold pH of 6.8), AQUATERIC with threshold pH of 5.8, polyvinylacetate phthalate (PVAP) that releases drug at pH values above about 5.0, shellac that is obtained from a gummy exudation produced by female

insects, *Laccifer lacca kerr*, and releases drug at about pH 7.0, and cellulose acetate phthalate (CAP) with threshold pH of 6.0. In a one embodiment, the drug is enteric-coated with EUDRAGIT S100 with threshold pH of 7.0, which will degrade measurably at slightly lower pH such as pH 6.8.

[0048] In one embodiment, prior to application to the tablets, capsules, or drug core of the present invention, the drug release controlling component, such as, for example, the enteric coatings useful in the present invention, will be dissolved in a non-aqueous solution in order to create the solid oral formulation of the present invention. Examples of such non aqueous solutions include any known in the art suitable for pharmaceutical formulation procedures, including, for example, acetone-isopropanol solvent mixtures, methylene chloride-ethanol solvent mixtures, acetone-ethanol solvent mixtures, benzene-methanol solvent mixtures, acetate-ethanol solvent mixtures, among others. Proportions of each solvent to use and conditions will be readily determined by those of skill in the art. The solid dispersion of the composition of the present invention, in one embodiment, can be formed by spray drying techniques, although it will be understood that suitable solid dispersions may be formed by a skilled addressee utilizing other conventional techniques, such as co-grinding, melt extrusion, freeze drying, rotary evaporation or any solvent removal process. In one embodiment, spray drying is utilized. The enteric coating may be applied over the entire surface area or portions thereof. In one embodiment, the entire surface area is coated.

[0049] According to the invention, the controlled release pharmaceutical composition comprising a cytidine analog and an enteric coating, in one embodiment, will not substantially disintegrate in an acidic, aqueous medium at about pH 1-3 for at least one hour, at least two hours, or at least three hours. The composition is considered to be substantially disintegrated if at least 50% of the composition disintegrates, e.g., undergoes rupture and release. In some embodiments, the controlled release pharmaceutical composition comprising a cytidine analog and an enteric coating disintegrates substantially in an aqueous medium at about pH 7 or above within about ten hours, within about eight hours, within about six hours, within about four hours, within about two hours, within about one hour, within about 45 minutes, within about 30 minutes, and within about 15 minutes. In some embodiments, the controlled release pharmaceutical composition comprising a cytidine analog and an enteric coating disintegrates substantially in an aqueous medium at about pH 6.8 or above within about ten hours, within

about eight hours, within about six hours, within about four hours, within about two hours, within about one hour, within about 45 minutes, within about 30 minutes, and within about 15 minutes. In some embodiments, times are within about two hours or less. In some embodiments, the controlled release pharmaceutical composition comprising a cytidine analog and an enteric coating does not disintegrate substantially in an aqueous medium at about pH 5 to about pH 6.5 for at least one hour, for at least about 1.5 hours, for at least about two hours, for at least about 2.5 hours, for at least about 3 hours, for at least about 3.5 hours, or for at least about four hours.

[0050] According to the invention, the controlled release pharmaceutical composition comprising a cytidine analog and an enteric coating, in one embodiment, will not substantially disintegrate after ingestion by a patient, on average, for at least about one hour, at least two hours, at least about three hours, at least about four hours, at least about five hours, at least about six hours, at least about seven hours, at least about eight hours, at least about nine hours, at least about ten hours, at least about twelve hours, at least about fourteen hours, at least about sixteen hours, at least about eighteen hours, at least about twenty hours, at least about twenty four hours, at least about twenty eight hours, or at least about thirty two hours. According to the invention, the controlled release pharmaceutical composition comprising a cytidine analog and an enteric coating, in some embodiments, will substantially disintegrate after ingestion by a patient within about three hours, within about four hours, within about five hours, within about six hours, within about seven hours, within about eight hours, within about nine hours, within about ten hours, within about twelve hours, within about fourteen hours, within about sixteen hours, within about eighteen hours, within about twenty hours, within about twenty two hours, within about twenty four hours, within about twenty six hours, within about twenty eight hours, or within about thirty hours. The composition is considered to be substantially disintegrated if at least 50% of the composition disintegrates, e.g., undergoes rupture and release.

[0051] The amount of the enteric-coating material, when the coating material is EUDRAGIT S100, in one embodiment is about 1-10% w/w in the composition, about 3-8% w/w in the composition, or 4-7% w/w in the composition. Appropriate amounts of coating to use will vary depending on the type of coating used, tablet size, surface preparation, target dissolution time at a given pH, etc. All things being equal, to determine the amount and/or thickness of the enteric coating, as the pH threshold of the enteric coating material increases, the relative amount

and thickness of the coating can be decreased to achieve dissolution of the tablet in a specified time frame at a particular pH. Routine empirical studies to determine the optimum conditions for targeting the large intestine may be carried out by the skilled person.

[0052] As examples of more specific types of controlled release pharmaceutical compositions, the following is described. For example, a pharmaceutical composition comprising a solid oral dosage form may be coated with a coating of a 60 to 150 micrometer thick layer of an anionic polymer which is insoluble in gastric juice and in intestinal fluid below pH 7 but soluble in colonic intestinal juice, so that the dosage form remains intact until the colon. For example, a pharmaceutical composition may be coated with a material which dissolves in the intestine and contained within a capsule which is also coated with a material which dissolves in the intestine. The composition is for selectively administering the drug to the intestine. The granules are in one embodiment contained within an enterically coated capsule which releases the granules in the small intestine and that the granules are coated with a coating which remains substantially intact until they reach at least the ileum and in one embodiment, thereafter provide a sustained or immediate release of the drug through the colon. Also there may be a non-disintegratable solid enteric pharmaceutical composition comprising a cytidine analog having relatively rapid dissolution at pH 6.5 from an excipient matrix, and dosage forms containing pellets of the composition. The rapid dissolution is increased by the presence of a rheological modifying super-disintegrant in an amount of at least 5% by weight, but insufficient to cause disintegration of the composition. It is stated that the composition may comprise a plurality of such pellets, which may be coated in an enteric coating such as cellulose acetate phthalate or, partly methyl esterified methacrylic acid polymers having a ratio of free acid groups to ester groups of about 1:2, contained in a capsule that is enterically coated with a suitable coating material. The coating material on the pellets is in one embodiment, one that is insoluble in gastric juices and intestinal fluid below pH 7, but is soluble in the lower intestine. The enteric coating material of the capsule is chosen to protect the capsule during passage through the stomach. In another embodiment, the pharmaceutical compositions of the present invention may include time delay coatings to delay release of the cytidine analog until reaching the large intestine. In accordance with this embodiment, solid dosage forms, whether tablets, capsules, caplets, or particulates, may, if desired, be coated so as to provide for delayed release. Sustained release dosage forms provide for drug release over an extended time period, and may or may not be

delayed release. Generally, as will be appreciated by those of ordinary skill in the art, sustained release dosage forms are formulated by either dispersing a drug within a matrix of, a gradually bioerodible (hydrolyzable) material such as an insoluble plastic, a hydrophilic polymer, or a fatty compound, or by coating a solid, drug-containing dosage form with such a material. Insoluble plastic matrices may be comprised of, for example, polyvinyl chloride or polyethylene, vinyl polymers and copolymers such as polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers, zein, and shellac, ammoniated shellac, shellac-acetyl alcohol, and shellac n-butyl stearate. Fatty compounds for use as a sustained release matrix material include, but are not limited to, waxes generally (e.g., carnauba wax) and glyceryl tristearate. In particular, time release coatings useful in the present invention may include the following: acetyltributyl citrate, acetyltriethyl citrate, aliphatic polyesters, bentonite, carbomers, carrageenan, cellulose acetate, cellulose acetate phthalate, ceratonia, cetyl alcohol, cetyl esters wax, chitosan, dibutyl sebacate, ethylcellulose, glycerin monostearate, glyceryl behenate, glyceryl monooleate, glyceryl monostearate, glyceryl palmitostearate, guar gum, hydroxypropyl cellulose, hypromellose acetate succinate, isopropyl palmitate, magnesium aluminum silicate, magnesium oxide, methylcellulose, microcrystalline wax, paraffin, peanut oil, potassium polacrilin, polycarbophil, polyethylene oxide, polymethacrylates, povidone, stearic acid, stearyl alcohol, talc, tributyl citrate, triethyl citrate, white wax, xanthan gum, yellow wax, and zein.

[0053] The type, amount and/or thickness of the matrix or coating can be readily adjusted by one of skill in the art to obtain the desired release profiles and timing.

[0054] In one embodiment, the controlled release formulation comprising a time delay component does not allow substantial release of the cytidine analog for at least about three hours after oral ingestion by a patient, for at least about four hours after oral ingestion by a patient, for at least about five hours after oral ingestion by a patient, for at least about six hours after ingestion by a patient, for at least about eight hours after ingestion by a patient, or for at least about ten hours after ingestion by a patient. In one embodiment, a dissolution time period is, in one embodiment, between about three hours and about ten hours, between about four hours and about six hours. A nominal lag time of five hours is usually considered sufficient, since small intestinal transit has been considered relatively constant at about 3 to 4 hours. Multiple coatings

of either the same or different types of a time delay release component may be used by one of skill in the art to target the large intestine and/or colon.

[0055] Examples of time delay methods are known in the art, and include PULSINCAP device, which consists of a non-disintegrating half capsule shell sealed at the open end with a hydrogel plug. The plug hydrates on contact with gastrointestinal fluid, and swells to an extent that it is expelled from the capsule body, thus releasing the drug. Usually, the time it takes the hydrogel plug to hydrate and eject from the capsule shell defined the lag time prior to drug release, and hence, by altering the composition and size of the hydrogel plug, it is possible to achieve drug release after varying lag times. As another example, a pulsed system, called the TIME CLOCK SYSTEM, comprises a solid dosage form coating with a hydrophobic surfactant layer to which a water-soluble polymer is attached to improve adhesion to the core. The thickness of the outer layer determines the time required to disperse in an aqueous environment. After dispersion of the outer layer, the core becomes available for dispersion.

[0056] In another embodiment of the present invention, the pharmaceutical compositions may include a bacterially degradable component, such as a coating, in order to delay release of the cytidine analog until reaching the large intestine. For example, the digestive excretions in the human gastrointestinal tract lack specific enzymes that can degrade certain types of oligosaccharides, such as, for example, cellulose. In contrast, bacteria existing at the level of the large intestine have the ability to digest many of these types of polysaccharides. Accordingly, a coating or matrix which undergoes bacterial degradation in the colonic surroundings and dissolves/degrades causing release of its drug content is compatible with the present invention. A number of flora are typically found in the human gastrointestinal tract. The flora may change depending upon the physiological condition of the person or animal being treated. Drug delivery may be designed to specifically target a type of flora known to be in abundance in a patient or a population of patients.

[0057] A partial list of oligosaccharides suitable for incorporation into the controlled release pharmaceutical compositions of the present invention, in one embodiment, includes those which can be digested by colonic bacteria but not by the enzymes present at the level of a patient's stomach or small intestine. Examples of such oligosaccharides are cellobiose, lactulose, the trisaccharide raffinose and stachyose and polymers thereof, such as cellulose. Oligosaccharides also include amylose, arabinogalactan, chitosan, chondroitin, cyclodextrin,

dextran, guar gum, inulin, pectin, and xylan. Natural polymers such as mucopolysaccharides can also be the basis of bacterially degradable coatings. Most of these natural polymers are, in their unmodified form, soluble in water and gastric fluid. Accordingly, in one embodiment, natural polymers are cross linked to reduce the hydrophilicity of these polymers and thus allow their utility in the compositions and methods of the invention as colonic drug carriers which pass the small intestine and degrade in the colon. Accordingly, bacterially degradable components are, in one embodiment, covalently bonded to a polymer, in one embodiment an acrylic polymer, including, a methacrylic polymer. An non-limiting example of one cross-linking method is amide protection by the reaction of diamine with the polymer. Diamines that can be used include: 1,4 butanediamine, 1,6 hexanediamine, 1,7 heptanediamine and 1,12 dodecanediamine. A number of U.S. patents and patent application publications discuss methods by which to control release of drugs via bacterially degradable coatings and/or matrices, such as, for example, U.S. 5,525,634, U.S. 5,849,327, U.S. 4,432,966, U.S. 5,112,621, and U.S. 5,536,507, all of which are incorporated by reference herein in their entireties.

[0058] The presence of microbial anaerobic organisms is known to provide reducing conditions in the large intestine and colon. Thus, the coating may also suitably comprise a material that is redox sensitive. Such coatings typically consist of azo-polymers, which can, for example, be composed of a random co-polymer of styrene and hydroxyethyl methacrylate cross-linked with divinyl azo-benzene synthesised by free radical polymerization. The azo-polymer is broken down enzymatically and specifically in the colon delivery system. When drugs are coated with these polymers, they are protected against gastric and intestinal enzymes. The drugs are subsequently released in the large intestine where enzyme activity is low and the azo bond is broken by only microbial enzymes in the colon. In vitro evaluation of azo-containing polysaccharide gels, more specifically azo-inulin and azo-dextran gels, for colonic delivery has been shown that *in vitro* azo-polysaccharide gels can be degraded through both reduction of the azo group in the cross-links as well as enzymatic breakdown of the polysaccharide backbone. The azo-polysaccharide gels were synthesized by radical cross-linking of a mixture of methacrylated inulin or methacrylated dextran and NN-bis-(methacryloylamino) azo-benzene (B(MA)AB), and were characterised by dynamic mechanical analysis and swelling measurements. Azo-dextran gels could be obtained from methacrylated dextran having low degrees of substitution but not from minimally substituted methacrylated inulin. Increasing the

amount of B(MA)AB resulted in denser azo-inulin and azo-dextran networks. Compared with their swelling in dimethyl formamide, all azo-dextran gels became more swollen in water while azo-inulin gels shrank upon exposure to water, indicating a more hydrophobic character of the azo-inulin gels. Breakdown of the inulin and dextran chains by inulinase and dextranase, respectively, were observed.

[0059] Below are discussed additional examples of art-known methods of colonic delivery, which incorporate one or more of the methods discussed hereinabove and are suitable for formulating the cytidine compounds. As discussed above, as a general rule, those skilled in the art consider the small intestine to be the preferred target for delivery of acid-labile compounds. Controlled release methods and compositions for targeting or controlling the release of an active compound in the large intestine and colon have been disclosed, including the following. A formulation for site specific release of a cytidine analog in the colon comprises a cytidine analog and amorphous amylose with an outer coating of cellulose or an acrylic polymer material. The active compound is in one embodiment coated with glassy amylose, which tends not to degrade until it reaches the colon where it is attacked by amylose cleaving enzymes provided by microbial flora normally present in the colon. The composition may optionally be further coated with a cellulose or acrylic polymer material, which enhances the delayed release property of the amylose coated composition. The rate of release of the active compound from the composition once it reaches the colon may be controlled by varying the thickness of inner amylose coating provided. It is also possible to vary the release in the colon by coating different particles of the active compound with amylose of different thicknesses. Release characteristics can be further varied by drying, which affects pore size and permeability or by adding a fatty or waxy substance to retard penetration of water. In one embodiment, the cellulose or acrylic polymer outer coating material displays pH independent degradation. Another controlled release composition suitable for delivery of cytidine analog to the colon includes cytidine analog containing spheres also containing microcrystalline cellulose and having diameters in the range 1.00 to 1.40 mm, which spheres are coated with a mixed solvent (water and an organic water miscible solvent) amylose/ethyl cellulose composition, although the latter may instead be an acrylic polymer or hydrophobic coating. Where higher amylose concentrations are present in the coatings, a thicker coating is applied such that release of the cytidine analog should not take place before the colon. Another sustained release pharmaceutical formulation includes coating

particles with different thicknesses of a material (such as cellulose acetophthalate) in order to release the drug compound at different rates so as to provide sustained release over a predetermined time period.

[0060] Generally, methods for sustained release and/or delivery to the large intestine and/or colon include the following methods disclosed in the following U.S. issued patents, all of which are incorporated herein by reference in their entireties. U.S. Pat. No. 5,529,790 discloses pharmaceutical formulations which provide delayed and sustained release of a drug from the formulation by means of a hydratable diffusion barrier coating. The delay is a consequence of the rate of hydration and the thickness of the coating and the sustained release results from the permeability and thickness of the coating. The diffusion barrier, in one embodiment, consists of a film-forming material that is insoluble in intestinal conditions and at least one further additive which controls the rate of hydration and permeability of the diffusion barrier. One embodiment includes an embodiment where film-forming polymers are non-aqueous or aqueous dispersions of fully esterified acrylic resins (e.g. EUDRAGIT NE30D), fully esterified acrylic resins containing quaternary amine side chains (e.g. EUDRAGIT RS30D) or aqueous dispersions of ethyl cellulose. In one embodiment, an additive for controlling the rate of hydration and the permeability is magnesium stearate. The drug (e.g. diltiazem hydrochloride) may be formulated as spherical microparticles having a diameter in the range 500-1500 micrometers and is, in one embodiment, formulated in two batches of particles, a long delay batch having a low rate of hydration and low permeability and a short delay batch having a relatively high rate of hydration and a high permeability, so that sustained release of the drug can be effected over an extended period of time. Dissolution studies were carried out on particles having different coating thicknesses. U.S. Pat. No. 5,260,069 and U.S. Pat. No. 5,834,024 disclose pharmaceutical compositions comprising at least two pluralities of particles. The pluralities may be coated with different thicknesses of a coating material comprising a polymer blend. The blend comprises, as a major component, at least one water insoluble polymer and, as a minor component, a polymer whose solubility is dependent on pH. U.S. Pat. No. 5,260,069 exemplifies compositions in which nifedipine and zidovudine are active components and U.S. Pat. No. 5,834,024 exemplifies the use of diltiazem as the active component. U.S. Pat. No. 6,267,990 discloses a pharmaceutical composition comprising three pluralities of particles, one of which is uncoated and the other two are coated with different thicknesses of a pH dependent release coating material. U.S. Pat. No.

6,267,990 exemplifies the use of the ACE inhibitor, captopril, as the active component. U.S. Pat. No. 5,834,021 exemplifies a pharmaceutical composition comprising a plurality of pellets comprising prednisolone metasulphobenzoate. The pellets are coated with a first pH dependent release coating material and then filled into a capsule which is then itself coated with a second pH dependent release coating material.

[0061] In addition to the cytidine and drug release controlling component, pharmaceutical compositions of the present invention will generally contain other excipients such as diluents (bulking agents), lubricants, disintegrants, fillers, stabilizers, surfactants, preservatives, coloring agents, flavoring agents, binding agents, excipient supports, glidants, permeability enhancement excipients, plasticizers and the like, all of which are known in the art. It will be understood by those in the art that some substances serve more than one purpose in a pharmaceutical composition. For instance, some substances are binders that help hold a tablet together after compression, yet are disintegrants that help break the tablet apart once it reaches the target delivery site. Selection of excipients and the amounts to use may be readily determined by the formulation scientist based upon experience and consideration of standard procedures and reference works in the field.

[0062] Binders are used to impart cohesive qualities to a tablet, and thus ensure that the tablet remains intact after compression. Suitable binder materials include, but are not limited to, starch (including corn starch and pregelatinized starch), gelatin, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, propylene glycol, waxes, and natural and synthetic gums, e.g., acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose, hydroxypropylmethylcellulose, methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose and the like), veegum, carbomer (e.g. carbopol), sodium, dextrin, guar gum, hydrogenated vegetable oil, magnesium aluminum silicate, maltodextrin, polymethacrylates, povidone (e.g. KOLLIDON, PLASDONE), microcrystalline cellulose, among others. Binding agents also include acacia, agar, alginic acid, cabomers, carrageenan, cellulose acetate phthalate, ceratonia, chitosan, confectioner's sugar, copovidone, dextrates, dextrin, dextrose, ethylcellulose, gelatin, glyceryl behenate, guar gum, hydroxyethyl cellulose, hydroxyethylmethyl cellulose, hydroxypropyl cellulose, hydroxypropyl starch, hypromellose, inulin, lactose, magnesium aluminum silicate, maltodextrin, maltose, methylcellulose, poloxamer, polycarbophil, polydextrose, polyethylene oxide,

polymethylacrylates, povidone, sodium alginate, sodium carboxymethylcellulose, starch, pregelatinized starch, stearic acid, sucrose, and zein.

[0063] Diluents are typically necessary to increase bulk so that a practical size tablet is ultimately provided. Suitable diluents include dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch, microcrystalline cellulose (e.g. AVICEL), microfine cellulose, pregelatinized starch, calcium carbonate, calcium sulfate, sugar, dextrates, dextrin, dextrose, dibasic calcium phosphate dihydrate, tribasic calcium phosphate, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, polymethacrylates (e.g. EUDRAGIT), potassium chloride, sodium chloride, sorbitol and talc, among others. Diluents also include ammonium alginate, calcium carbonate, calcium phosphate, calcium sulfate, cellulose acetate, compressible sugar, confectioner's sugar, dextrates, dextrin, dextrose, erythritol, ethylcellulose, fructose, fumaric acid, glyceryl palmitostearate, isomalt, kaolin, lactitol, lactose, mannitol, magnesium carbonate, magnesium oxide, maltodextrin, maltose, medium-chain triglycerides, microcrystalline cellulose, microcrystalline silicified cellulose, powdered cellulose, polydextrose, polymethylacrylates, simethicone, sodium alginate, sodium chloride, sorbitol, starch, pregelatinized starch, sucrose, sulfobutylether- β -cyclodextrin, talc, tragacanth, trehalose, and xylitol.

[0064] Lubricants are used to facilitate tablet manufacture; examples of suitable lubricants include, for example, vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, and oil of theobroma, glycerin, magnesium stearate, calcium stearate, and stearic acid. Stearates, if present, in one embodiment represent at no more than approximately 2 wt. % of the drug-containing core. Further examples of lubricants include calcium stearate, glycerin monostearate, glyceryl behenate, glyceryl palmitostearate, magnesium lauryl sulfate, magnesium stearate, myristic acid, palmitic acid, poloxamer, polyethylene glycol, potassium benzoate, sodium benzoate, sodium chloride, sodium lauryl sulfate, sodium stearyl fumarate, stearic acid, talc, and zinc stearate.

[0065] Disintegrants are used to facilitate disintegration of the tablet, and are generally starches, clays, celluloses, algin, gums or crosslinked polymers. Disintegrants also include alginic acid, carboxymethylcellulose calcium, carboxymethylcellulose sodium (e.g. AC-DI-SOL, PRIMELLOSE), colloidal silicon dioxide, croscarmellose sodium, crospovidone (e.g. KOLLIDON, POLYPLASDONE), guar gum, magnesium aluminum silicate, methyl cellulose,

microcrystalline cellulose, polacrillin potassium, powdered cellulose, pregelatinized starch, sodium alginate, sodium starch glycolate (e.g. EXPLOTAB) and starch. Additional disintegrants include alginic acid, calcium alginate, calcium carboxymethylcellulose, chitosan, colloidal silicon dioxide, sodium croscarmellose, crospovidone, sodium docusate, guar gum, hydroxypropyl cellulose, magnesium aluminum silicate, methylcellulose, microcrystalline cellulose, potassium polacrillin, povidone, powdered cellulose, sodium alginate, sodium carboxymethyl cellulose, sodium starch glycolate, starch, and pregelatinized starch.

[0066] Stabilizers are used to inhibit or retard drug decomposition reactions that include, by way of example, oxidative reactions. Surfactants may be anionic, cationic, amphoteric or nonionic surface active agents. Additional stabilizing agents include acacia, albumin, alginic acid, aluminum stearate, ammonium alginate, ascorbic acid, ascorbyl palmitate, bentonite, butylated hydroxytoluene, calcium alginate, calcium stearate, calcium carboxymethylcellulose, carrageenan, ceratonia, colloidal silicon dioxide, cyclodextrins, diethanolamine, edetates, ethylcellulose, ethyleneglycol palmitostearate, glycerin monostearate, guar gum, hydroxypropyl cellulose, hypromellose, invert sugar, lecithin, magnesium aluminum silicate, monoethanolamine, pectin, poloxamer, polyvinyl alcohol, potassium alginate, potassium polacrillin, povidone, propyl gallate, propylene glycol, propylene glycol alginate, raffinose, sodium acetate, sodium alginate, sodium borate, sodium carboxymethyl cellulose, sodium stearyl fumarate, sorbitol, stearyl alcohol, sufobutyl-b-cyclodextrin, trehalose, white wax, xanthan gum, xylitol, yellow wax, and zinc acetate.

[0067] Glidants can be added to improve the flow properties of a powder composition or granulate and improve the accuracy of dosing. Excipients that may function as glidants include colloidal silicon dioxide, magnesium trisilicate, powdered cellulose, starch, tribasic calcium phosphate, calcium silicate, powdered cellulose, colloidal silicon dioxide, magnesium silicate, magnesium trisilicate, silicon dioxide, starch, tribasic calcium phosphate, and talc.

[0068] Permeation enhancers are an included excipient in one embodiment. Permeation enhancers act to enhance uptake of a substance through the intestinal wall and deliver more of a substance to the bloodstream. Movement through the intestinal wall may occur by passive diffusion, the movement of drug across a membrane in a manner driven solely by the concentration gradient; by carrier-mediated diffusion, movement of drug across a cell membrane via a specialized transport system embedded in the cell membrane; paracellular diffusion, the

movement of drug across a membrane by going between, rather than through, two cells; and transcellular diffusion, the movement of a drug across the cell. Additionally, there are numerous cellular proteins capable of preventing intracellular accumulation of drugs by pumping drug that enters the cell back out. These are sometimes called efflux pumps. One of the most important is p-glycoprotein, which is present in many different tissues in the body (e.g., intestine, placental membrane, blood-brain barrier). Permeation enhancers can work by facilitating any of the processes mentioned above (such as by increasing fluidity of membranes, opening "tight junctions" between cells, and/or inhibiting efflux.)

[0069] Examples of suitable permeation inhibitors include, for example, but are not limited to, surfactants. Suitable examples for the present invention include are known and commercially available, e.g. from the BASF company under the trade mark SOLUTOL. An example is SOLUTOL HS15 which is known, e.g. from the BASF technical leaflet MEF 151E (1986), to comprise of about 70% polyethoxylated 12-hydroxystearate by weight and about 30% by weight unesterified polyethylene glycol component. SOLUTOL HS 15 has a hydrogenation value of 90 to 110, a saponification value of 53 to 63, an acid number of maximum 1, and a maximum water content of 0.5% by weight. Polyoxyethylene-polyoxypropylene co-polymers and block co-polymers are included in one embodiment, for example of the type known and commercially available under the trade names PLURONIC, EMKALYX and POLOXAMER. A further example of this class is POLOXAMER F127. Propylene glycol mono- and di-fatty acid esters such as propylene glycol dicaprylate (also known and commercially available under the trade name MIGLYOL 840), propylene glycol dilaurate, propylene glycol hydroxystearate, propylene glycol isostearate, propylene glycol laurate, propylene glycol ricinoleate, propylene glycol stearate and so forth are also included in some embodiments. Other examples include propylene glycol mono C:8 esters include SEFSOL 218 (Nikko Chemicals) and CAPRYOL 90 (Gattefosse) and tocopherol esters, e.g. tocopheryl acetate and tocopheryl acid succinate (HLB of about 16), transesterified ethoxylated vegetable oils are known and are commercially available under the trade name LABRAFIL. Examples are LABRAFIL M 2125 CS (obtained from corn oil and having an acid number of less than about 2, a saponification number of 155 to 175, an HLB value of 3 to 4, and an iodine number of 90 to 110), and LABRAFIL M 1944 CS (obtained from kernel oil and having an acid number of about 2, a saponification number of 145 to 175 and an iodine number of 60 to 90). LABRAFIL M 2130 CS (which is a transesterification product of

a C.sub.12-18 glyceride and polyethylene glycol and which has a melting point of about 35 to 40.degree. C., an acid number of less than about 2, a saponification number of 185 to 200 and an iodine number of less than about 3). In one embodiment, the transesterified ethoxylated vegetable oil is LABRAFIL M 2125 CS which can be obtained, for example, from Gattefosse, Saint-Priest Cedex, France. In one embodiment, a permeation enhancer includes water soluble tocopheryl polyethylene glycol succinic acid esters (TPGS), e.g. with a polymerisation number ca 1000, e.g. available from Eastman Fine Chemicals Kingsport, Tenn., USA. Other embodiments include POLOXAMER compounds, particularly F127, chitosan, carboxymethylcellulose, SOLUTOL compounds, sodium laurate, and LABRAFIL compounds. Other permeation enhancers include alcohols, dimethyl sulfoxide, glyceryl monooleate, glycofurol, isopropyl myristate, isopropyl palmitate, lanolin, linoleic acid, myristic acid, oleic acid, oleyl alcohol, palmitic acid, polyoxyethylene alkyl ethers, 2-pyrrolidone, sodium lauryl sulfate, and thymol.

[0070] In some embodiments, the present invention consists or consists essentially of a controlled release pharmaceutical composition for oral administration for enhanced systemic delivery of a cytidine analog comprising a therapeutically effective amount of a cytidine analog and a drug release controlling component which is capable of providing release of the cytidine analog primarily in the large intestine. The present invention in some embodiments includes a controlled release pharmaceutical composition for oral administration for enhanced systemic delivery of azacitidine consisting essentially of or consisting of a therapeutically effective amount of azacitidine and a drug release controlling component which is capable of providing release of the cytidine analog primarily in the large intestine. In other embodiments, the present invention includes a controlled release pharmaceutical composition for oral administration of a cytidine analog for enhanced systemic delivery of the cytidine analog consisting essentially or consisting of a therapeutically effective amount of a cytidine analog and an enteric coating which is capable of providing release of the cytidine analog primarily in the large intestine. In other embodiments, the present invention includes a controlled release pharmaceutical composition for oral administration for enhanced systemic delivery of a cytidine analog consisting essentially of (or consisting of) a therapeutically effective amount of azacitidine and an enteric coating which is capable of providing release of the cytidine analog primarily in the large intestine and at least one excipient which improves the cohesive qualities of the, and/or increases the bulk of, and/or

improves the manufacture of, or facilitates disintegration of, and/or retards the drug decomposition reactions occurring in, or enhances uptake through the intestinal wall of, the controlled release pharmaceutical compositions of the present invention.

[0071] A tablet can be made by compressing a powder composition granulate between a punch and dye. Some excipients and active ingredients have a tendency to adhere to the surfaces of the punch and dye, which can cause the tablet to have pitting and other surface irregularities. A lubricant may be added to the composition to reduce adhesion and ease release of the product from the dye. Lubricants include magnesium stearate, calcium stearate, glyceryl monostearate, glyceryl palmitostearate, hydrogenated castor oil, hydrogenated vegetable oil, mineral oil, polyethylene glycol, sodium benzoate, sodium lauryl sulfate, sodium stearyl fumarate, stearic acid, talc and zinc stearate.

[0072] Suitable patients to treat include humans; birds such as chickens, ostriches, quail, and turkeys; mammals such as companion animals (including dogs, cats, and rodents) and economic food and/or fur or other product animals, such as horses, cattle, llamas, chinchillas, ferrets, goats, sheep, rodents, minks, rabbits, raccoons, and swine.

[0073] In another embodiment, the present invention includes a method of increasing the bioavailability of a cytidine analog comprising administering the controlled release pharmaceutical compositions of the present invention to a patient. Specifically, a controlled release pharmaceutical composition of the present invention is provided to a patient, and ingested by the patient, where the composition contacts the biological fluids of the patient's body and increases the bioavailability of the cytidine analog. Oral bioavailability of a cytidine analog in the compositions of the present invention can be more than 5%, more than 10%, more than 15%, more than 20%, more than 25%, more than 30% or more than 50% greater than the oral bioavailability of prior art formulations of a cytidine analog. Average maximum plasma concentration achieved relative to the dose administered may be more than 2 fold higher, 3 fold higher, 5 fold higher, about 10 fold higher than the oral bioavailability of prior art formulations of a cytidine analog when a cytidine analog is administered orally in the controlled release formulations of the present invention.

[0074] In another embodiment, the present invention includes methods for treating a patient having a disease associated with abnormal cell proliferation, comprising administering the controlled release pharmaceutical compositions of the present invention. In one embodiment,

the controlled release pharmaceutical compositions of the present invention allow for enhanced bioavailability of the cytidine analog to the patient.

[0075] In some embodiments, indications that may be treated using the pharmaceutical compositions of the present invention include those involving undesirable or uncontrolled cell proliferations. Such indications include benign tumors, various types of cancers such as primary tumors and tumor metastasis, hematological disorders (e.g. leukemia, myelodysplastic syndrome and sickle cell anemia), restenosis (e.g. coronary, carotid, and cerebral lesions), abnormal stimulation of endothelial cells (arteriosclerosis), insults to body tissue due to surgery, abnormal wound healing, abnormal angiogenesis, diseases that produce fibrosis of tissue, repetitive motion disorders, disorders of tissues that are not highly vascularized, and proliferative responses associated with organ transplants.

[0076] Generally, cells in a benign tumor retain their differentiated features and do not divide in a completely uncontrolled manner. A benign tumor is usually localized and nonmetastatic. Specific types of benign tumors that can be treated using the present invention include hemangiomas, hepatocellular adenoma, cavernous haemangioma, focal nodular hyperplasia, acoustic neuromas, neurofibroma, bile duct adenoma, bile duct cystanoma, fibroma, lipomas, leiomyomas, mesotheliomas, teratomas, myxomas, nodular regenerative hyperplasia, trachomas and pyogenic granulomas.

[0077] In a malignant tumor cells that become undifferentiated, do not respond to the body's growth control signals, and multiply in an uncontrolled manner. The malignant tumor is invasive and capable of spreading to distant sites (metastasizing). Malignant tumors are generally divided into two categories: primary and secondary. Primary tumors arise directly from the tissue in which they are found. A secondary tumor, or metastasis, is a tumor which is originated elsewhere in the body but has now spread to a distant organ. The common routes for metastasis are direct growth into adjacent structures, spread through the vascular or lymphatic systems, and tracking along tissue planes and body spaces (peritoneal fluid, cerebrospinal fluid, etc.)

[0078] Specific types of cancers or malignant tumors, either primary or secondary, that can be treated using this invention include leukemia, breast cancer, skin cancer, bone cancer, prostate cancer, liver cancer, lung cancer, brain cancer, cancer of the larynx, gall bladder, pancreas, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary

type, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, gallstones, islet cell tumor, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuronmas, intestinal ganglioneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyoma tumor, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, medulloblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoides, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythemia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, epidermoid carcinomas, and other carcinomas and sarcomas.

[0079] Hematologic disorders include abnormal growth of blood cells which can lead to dysplastic changes in blood cells and hematologic malignancies such as various leukemias. Examples of hematologic disorders include but are not limited to acute myeloid leukemia, acute promyelocytic leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, the myelodysplastic syndromes, and sickle cell anemia.

[0080] Acute myeloid leukemia (AML) is the most common type of acute leukemia that occurs in adults. Several inherited genetic disorders and immunodeficiency states are associated with an increased risk of AML. These include disorders with defects in DNA stability, leading to random chromosomal breakage, such as Bloom's syndrome, Fanconi's anemia, Li-Fraumeni kindreds, ataxia-telangiectasia, and X-linked agammaglobulinemia.

[0081] Acute promyelocytic leukemia (APML) represents a distinct subgroup of AML. This subtype is characterized by promyelocytic blasts containing the 15; 17 chromosomal translocation. This translocation leads to the generation of the fusion transcript comprised of the retinoic acid receptor and a sequence PML.

[0082] Acute lymphoblastic leukemia (ALL) is a heterogeneous disease with distinct clinical features displayed by various subtypes. Reoccurring cytogenetic abnormalities have been demonstrated in ALL. The most common cytogenetic abnormality is the 9;22 translocation. The resultant Philadelphia chromosome represents poor prognosis of the patient.

[0083] Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder of a pluripotent stem cell. CML is characterized by a specific chromosomal abnormality involving

the translocation of chromosomes 9 and 22, creating the Philadelphia chromosome. Ionizing radiation is associated with the development of CML.

[0084] The myelodysplastic syndromes (MDS) are heterogeneous clonal hematopoietic stem cell disorders grouped together because of the presence of dysplastic changes in one or more of the hematopoietic lineages including dysplastic changes in the myeloid, erythroid, and megakaryocytic series. These changes result in cytopenias in one or more of the three lineages. Patients afflicted with MDS typically develop complications related to anemia, neutropenia (infections), or thrombocytopenia (bleeding). Generally, from about 10% to about 70% of patients with MDS develop acute leukemia. In one embodiment, MDS is a condition to treat with the present invention, and includes the following myelodysplastic syndrome subtypes: refractory anemia, refractory anemia with ringed sideroblasts (if accompanied by neutropenia or thrombocytopenia or requiring transfusions), refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia.

[0085] Treatment of abnormal cell proliferation due to insults to body tissue during surgery may be possible for a variety of surgical procedures, including joint surgery, bowel surgery, and cheloid scarring. Diseases that produce fibrotic tissue include emphysema. Repetitive motion disorders that may be treated using the present invention include carpal tunnel syndrome. An example of cell proliferative disorders that may be treated using the invention is a bone tumor.

[0086] The proliferative responses associated with organ transplantation that may be treated using this invention include those proliferative responses contributing to potential organ rejections or associated complications. Specifically, these proliferative responses may occur during transplantation of the heart, lung, liver, kidney, and other body organs or organ systems.

[0087] Abnormal angiogenesis that may be may be treated using this invention include those abnormal angiogenesis accompanying rheumatoid arthritis, ischemic-reperfusion related brain edema and injury, cortical ischemia, ovarian hyperplasia and hypervascularity, (polycystic ovary syndrom), endometriosis, psoriasis, diabetic retinopathy, and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplastic), macular degeneration, corneal graft rejection, neurovascular glaucoma and Oster Webber syndrome.

[0088] Diseases associated with abnormal angiogenesis require or induce vascular growth. For example, corneal angiogenesis involves three phases: a pre-vascular latent period,

active neovascularization, and vascular maturation and regression. The identity and mechanism of various angiogenic factors, including elements of the inflammatory response, such as leukocytes, platelets, cytokines, and eicosanoids, or unidentified plasma constituents have yet to be revealed. The pharmaceutical composition of the present invention may also be used for treating diseases associated with undesired or abnormal angiogenesis alone or in conjunction with an anti-angiogenesis agent.

[0089] The particular dosage of these agents required to inhibit angiogenesis and/or angiogenic diseases may depend on the severity of the condition, the route of administration, and related factors that can be decided by the attending physician. Generally, accepted and effective daily doses are the amount sufficient to effectively inhibit angiogenesis and/or angiogenic diseases. According to this embodiment, the pharmaceutical composition of the present invention may be used to treat a variety of diseases associated with undesirable angiogenesis such as retinal/choroidal neovascularization and corneal neovascularization. Examples of retinal/choroidal neovascularization include, but are not limited to, Best's diseases, myopia, optic pits, Stargardt's diseases, Paget's disease, vein occlusion, artery occlusion, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, carotid obstructive diseases, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales disease, diabetic retinopathy, macular degeneration, Behçet's diseases, infections causing a retinitis or chorioiditis, presumed ocular histoplasmosis, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy. Examples of corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium, keratitis sicca, Sjögren's, acne rosacea, phlyctenulosis, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, polyarteritis, Wegener's sarcoidosis, Scleritis, peripheral radial keratotomy, neovascular glaucoma and retrolental fibroplasia, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections and Kaposi's sarcoma.

[0090] The pharmaceutical composition of the present invention may be used for treating chronic inflammatory diseases associated with abnormal angiogenesis. The chronic inflammation depends on continuous formation of capillary sprouts to maintain an influx of inflammatory cells. The influx and presence of the inflammatory cells produce granulomas and thus, maintains the chronic inflammatory state. Inhibition of angiogenesis using the composition of the present invention may prevent the formation of the granulomas, thereby alleviating the disease. Examples of chronic inflammatory disease include, but are not limited to, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis, and rheumatoid arthritis.

[0091] Inflammatory bowel diseases such as Crohn's disease and ulcerative colitis are characterized by chronic inflammation and angiogenesis at various sites in the gastrointestinal tract. For example, Crohn's disease occurs as a chronic transmural inflammatory disease that most commonly affects the distal ileum and colon but may also occur in any part of the gastrointestinal tract from the mouth to the anus and perianal area. Patients with Crohn's disease generally have chronic diarrhea associated with abdominal pain, fever, anorexia, weight loss and abdominal swelling. Ulcerative colitis is also a chronic, nonspecific, inflammatory and ulcerative disease arising in the colonic mucosa and is characterized by the presence of bloody diarrhea. These inflammatory bowel diseases are generally caused by chronic granulomatous inflammation throughout the gastrointestinal tract, involving new capillary sprouts surrounded by a cylinder of inflammatory cells. Inhibition of angiogenesis by the composition of the present invention should inhibit the formation of the sprouts and prevent the formation of granulomas. The inflammatory bowel diseases also exhibit extra intestinal manifestations, such as skin lesions. Such lesions are characterized by inflammation and angiogenesis and can occur at many sites other the gastrointestinal tract. Inhibition of angiogenesis by the composition of the present invention should reduce the influx of inflammatory cells and prevent the lesion formation.

[0092] Sarcoidosis, another chronic inflammatory disease, is characterized as a multisystem granulomatous disorder. The granulomas of this disease can form anywhere in the body and, thus, the symptoms depend on the site of the granulomas and whether the disease is active. The granulomas are created by the angiogenic capillary sprouts providing a constant supply of inflammatory cells. By using the composition of the present invention to inhibit angiogenesis, such granulomas formation can be inhibited. Psoriasis, also a chronic and recurrent

inflammatory disease, is characterized by papules and plaques of various sizes. Treatment using the composition of the present invention should prevent the formation of new blood vessels necessary to maintain the characteristic lesions and provide the patient relief from the symptoms.

[0093] Rheumatoid arthritis (RA) is also a chronic inflammatory disease characterized by non-specific inflammation of the peripheral joints. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis. Treatment using the composition of the present invention alone or in conjunction with other anti-RA agents should prevent the formation of new blood vessels necessary to maintain the chronic inflammation and provide the RA patient relief from the symptoms.

[0094] The pharmaceutical composition of the present invention may also be used to treat autoimmune diseases. Autoimmune diseases refer to a wide range of degenerative diseases caused by the immune system attacking a person's own cells. Autoimmune diseases are usually classified clinically in a variety of ways. In light of affected parts by the diseases, there are, for example, degenerative diseases of supporting tissues and connective tissues; autoimmune degenerative diseases of salivary glands, particularly Sjogren's disease; autoimmune degenerative diseases of kidneys, particularly systemic lupus erythematoses (SLE) and glomerulonephritis; autoimmune degenerative diseases of joints, particularly rheumatoid arthritis; and autoimmune degenerative diseases of blood vessels such as generalized necrotizing angitis and granulomatous angitis; and multiple sclerosis. Alternatively, autoimmune diseases can be classified in one of the two different categories: cell-mediated disease (i.e. T-cell) or antibody mediated disorders. Examples of cell-mediated autoimmune diseases include multiple sclerosis, rheumatoid arthritis, autoimmune thyroiditis, and diabetes mellitus. Antibody-mediated autoimmune disorders include myasthenia gravis and SLE.

[0095] Dosing schedules for the compositions and methods of the present invention, for example, can be adjusted to account for the patient's characteristics and disease status. Appropriate dose will depend on the disease state being treated. Appropriate biomarkers may be used to evaluate the drug's effects on the disease state and provide guidance to the dosing schedule. In some cases, daily doses, and in others, selected days of a week, month or other time

interval. In one embodiment, the drug will not be given more than once per day. In one embodiment, dosing schedules for administration of pharmaceutical compositions of the present invention including the daily administration to a patient in need thereof of. Dosing schedules may mimic those that are used for non-oral formulations of a cytidine analog, adjusted to maintain, for example, substantially equivalent therapeutic concentration in the patient's body.

[0096] The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

[0097] Absorption Potential Assessment of Azacitidine using Caco 2 Monolayers.

[0098] The permeability of azacitidine was determined in a Caco 2 monolayer model system using phosphate buffered saline as the system medium. The Caco-2 cells are an intestinal epithelial cell line (human colon adenocarcinoma established from the primary colon tumor (adenocarcinoma)) Monolayers of Caco-2 cells are used to classify the intestinal absorption potential of a drug candidate molecule. The assay was carried out in accordance with P. Artursson and J. Karlsson, "Correlation between Oral Drug Absorption in Humans and Apparent Drug Permeability Coefficients in Human Intestinal Epithelial (Caco-2) Cells", *Biochem. Biophys. Res. Commun.* 175, 880 (1991).

[0099] Briefly, Caco-2 cells were grown to confluence on collagen-coated, microporous polycarbonate in 12-well plates. For the assay buffer, Dulbecco's Phosphate Buffered Saline at pH 7.4 was used. The chamber on the apical side of the cells was filled with DPBS containing 1000 micromolar azacitidine with or without additional excipients. The test compound was then dosed on either the apical or basolateral side of the Caco-2 monolayer, and flux across the monolayer was determined both one and two hours after dosing. Results were compared to control high permeability compounds metoprolol and antipyrine and low permeability compounds atenolol and ranitidine, and were expressed as Papp (apparent permeability) compared to reference compound standards. Integrity of the monolayers was determine both before and after testing by measuring the transendothelial electrical resistance (TEER) value. Apparent permeability is calculated as $(dC_r/dt) \times V_r / (A \times C_0)$ where dC_r/dt is the slope of the cumulative concentration in the receiver compartment versus time in micromolar per second, V_r

is the volume of the receiver compartment in cubic centimeters, A is the area of the cell monolayer, and C_0 is the measured initial donor concentration in micromolar.

[00100] In the absence of additional excipients the measured apical to basolateral azacitidine permeability for azacitidine was $0.15 \pm 0.02 \times 10^{-6}$ cm/sec.

[00101] Additionally, a series of pharmaceutically acceptable excipients were screened for their ability to increase the apparent permeability of azacitidine in this model system. The excipients evaluated and their effects on azacitidine permeability in this model system are presented in Table 1.

[00102] Table 1. Sodium laurate was obtained from Sigma Chemical (available from St. Louis, MO); Vitamin E TPGS (TPGS-TPGS –d-alpha tocopherol polyethylene glycol 1000 succinate (available from Eastman, Kingsport, TN); LABRAFIL M 1944 CS(2) (Oleyl macrogolglycerides) (available from Gattefosse, France).

Table 1: Effect of Azacitidine Permeability in Caco 2 Monolayer Model with Various Excipients

Excipient	Excipient Concentration (%)	P_{app} ($\times 10^{-6}$ cm/sec)
Control	NA	0.15 ± 0.02
Poloxamer F127	0.1	0.13 ± 0.02
Chitosan	0.1	0.15 ± 0.01
Carboxymethyl Cellulose	0.1	0.26 ± 0.09
Solutol	0.1	0.26 ± 0.04
Sodium Laurate	0.1	$15.77 \pm 1.77^*$
	0.05	0.17 ± 0.03
	0.01	0.19 ± 0.02
	0.001	0.19 ± 0.04
Labrafil	0.1	0.34 ± 0.002
	0.05	0.38 ± 0.13
	0.01	0.23 ± 0.04
	0.001	0.27 ± 0.02
TPGS	0.1	0.38 ± 0.09
	0.05	0.45 ± 0.18
	0.01	0.30 ± 0.05
	0.001	0.33 ± 0.08

* High permeability due to toxic effects of sodium laurate on Caco 2 cells

[00103] Conclusion

[00104] Azacitidine was found to permeate through Caco-2 monolayers, indicating that colonic epithelial cells are a good candidate for delivery of azacitidine for enhanced bioavailability. Permeation of azacitidine was found to be enhanced by TPGS and Labrafil, having a significant effect with respect to increased azacitidine permeability. For both excipients, there appeared to be a shallow dose response relationship between the amount of excipient and the observed permeability. Inclusion of appropriate amounts of TPGS and/or Labrafil results in an increase in the apparent permeability of azacitidine without adverse effects on Caco 2 monolayers. Inclusion of one or both of these excipients should improve oral bioavailability of azacitidine through enhanced GI absorption.

Example 2

[00105] Permeability in Human Intestinal Strips

[00106] The permeability of azacitidine has also been assessed in viable human intestinal strips derived from specific sections of the GI tract. This model allows evaluation of the absorption potential of drugs and differences in drug absorption across the human jejunum, ileum, and colon and is based on Ungell et al. "Membrane Transport of Drugs in Different Regions of the Intestinal Tract of the Rat", *J. Pharm. Sci.* 87:360-366, (1998) and Nejdfors et al. "Mucosal *in vitro* Permeability in the Intestinal Tract of the Pig, the Rat, and Man: Species and Region Related Differences", *Scand. J. Gastroenterol.* 35:501-507, (2000). Permeation experiments were performed for azacitidine in the jejunal, ileal, and colonic tissues originating from the same human donor. Post-mortem human whole intestine was obtained from the International Institute for the Advancement of Medicine (IIAM). Tissues were used within 24 hours of the organ's removal. Tissues were maintained in cold transport media before being stripped. The segments were cut along the mesenteric border, the underlying musculature was stripped off, and the epithelium was rinsed with ice cold normal saline. The stripped tissues were mounted within the vertical USSING chambers (Harvard Apparatus, Holliston, MA). In these studies, azacitidine permeability was measured in both absolute rate and relative to atenolol (internal control). In all cases the net apparent permeability was assessed in the mucosal to serosal direction only. Evaluation conditions included the use of azacitidine alone and for some samples azacitidine in the presence of ketoconazole and THU as enzymatic inhibitors of CYP3A4 and cytidine deaminase, respectively.

[00107] Absolute permeability values for multiple donors at different intestinal sites with and without enzymatic inhibition are presented in graphical form in FIG. 1. Figure 1 shows absolute mucosal to serosal permeability of azacitidine in human intestinal tissue with and without enzymatic inhibition. In general permeability appeared greatest in the colon relative to the jejunum and ileum. Inclusion of enzymatic inhibitors increased the absolute permeability in all GI tract regions, however the size of the increase was maximal in the jejunum and ileum and was relatively small in the colon.

[00108] The permeability of azacitidine in the same human intestinal strips relative to the internal control (atenolol) is shown graphically in FIG. 2. Figure 2 shows relative mucosal to serosal permeability of azacitidine in human intestinal tissue with and without enzymatic inhibition with respect to atenolol. The use of internal controls ensures that small variations due to tissue viability and processing are normalized. Qualitatively, the same conclusions can be drawn from the azacitidine permeability data relative to atenolol. Absorption was greatest and most consistent between donors in colonic tissue, and the effects of enzymatic inhibition were minimized in tissues derived from the colon.

[00109] Permeation Enhancement in Human Tissues

[00110] Evaluation of the effects of azacitidine permeability in human colonic strips with various levels of the permeation enhancers identified in the Caco 2 model system has also been performed. Absolute and relative permeability of azacitidine with the two excipients at various levels are presented in FIGS. 3 and 4, respectively. Figure 3 shows absolute mucosal to serosal permeability of azacitidine in human colonic tissue with various concentrations of TPGS or LABRAFIL without enzymatic inhibition, and Figure 3 shows relative mucosal to serosal permeability of azacitidine in human colonic tissue with various concentrations of TPGS or LABRAFIL without enzymatic inhibition.

[00111] There was a trend toward greater permeability with increasing levels of TPGS, albeit to a lesser extent than observed in the model Caco2 system. Conversely, under the same conditions Labrafil did not appear to affect azacitidine permeability in human colonic tissue.

[00112] Conclusions

[00113] The available data from viable human intestinal tissue shows that enzymatic degradation of azacitidine was more prevalent in upper GI segments. Effective administration of azacitidine with delivery to the jejunum and/or ileum may require the use of an enzymatic

inhibitor such as THU. By contrast, both the absolute and relative permeability of azacitidine appeared maximal in human colonic tissue. Furthermore, enzymatic inhibition in colonic tissue did not provide a dramatic improvement in azacitidine permeability. Therefore, an oral azacitidine dosage form that targets the colon for delivery has been shown to maximize bioavailability without the need to include an enzymatic inhibitor.

Example 3

[00114] Solid Oral Dosage Form

Solid oral dosage forms of azacitidine were prepared using standard pharmaceutical excipients and techniques. TPGS was first adsorbed onto either microcrystalline cellulose or calcium silicate in an independent step. Dry ingredients were then dry blended and tablets prepared by direct compression. Tablets were then enteric coated with EUDRAGIT S100 from an acetone - isopropanol solvent mixture or with AQUAT AS-HG from a methylene chloride – ethanol solvent mixture.

Clinical Trial Material cores were composed of the following materials in these ratios:

Ingredient	mg/tablet	% w/w
Azacitidine	20.0	20.0
Mannitol, USP	58.2	58.2
Microcrystalline Cellulose, NF	15.0	15.0
Crospovidone, NF	3.0	3.0
Magnesium Stearate, NF	1.8	1.8
Vitamin E TPGS, NF	2.0	2.0

Cores were then be coated to approximately 7% w/w with the following mixture:

Ingredient	mg/tablet	% w/w
EUDRAGIT S100, NF	5.0	71.5
Triethyl citrate, NF	0.5	7.0
Talc, USP	1.5	21.5

Excipient compatibility studies have demonstrated that azacitidine is compatible with each excipient. Stability studies have demonstrated excellent stability of both cores and coated tablets

under long term (25°C, 60% RH) and accelerated (40°, 70% relative humidity) storage conditions. The formulation composition that has been manufactured is presented in Table 2.

Table 2: Oral Azacitidine Tablet Composition

Material	Trade Name	Purpose	Quality Standard
Azacitidine	NA	Active	In-House
Mannitol	PARTECK M200	Bulking Agent	USP
Microcrystalline Cellulose	PROSOLV 90HD	Binding Agent	USP
Crospovidone	POLYPLASON E XL	Disintegrant	USP
Magnesium Stearate	NA	Lubricant	USP
Vitamin E TPGS	NA	Absorption Enhancement	NF
Methacrylic acid copolymer ¹	EUDRAGIT S100 ²	Enteric Coating	USP
Triethyl Citrate	MORFLEX	Plasticizer	USP
Talc,		Anticaking Agent	USP

¹ Hypromellose Acetate Succinate, NF alternate material

² AQUAT AS-HG, alternate trade name

Example 4

[00115] A clinical study using the oral formulation described in Example 3 is performed to assess the safety and bioavailability of single oral doses of azacitidine in patients with myelodysplastic syndromes, acute myelogenous leukemia, or solid tumors. The study is a multicenter, open-label, single treatment study. One patient receives an oral dose of the study drug starting at 60 mg. Subsequent patients are treated with escalating doses, in 20 mg increments, up to 200 mg. The study assesses the safety and tolerability of escalating doses, provides pilot information on the oral bioavailability of the study drug, and provides information on the single dose pharmacokinetics of the study drug after oral administration.

[00116] The outcome from the clinical trial is shown to be that patients dosed with the current oral azacitidine formulation show measurable levels of azacitidine in plasma samples. The amount of azacitidine measured in the plasma is proportional to the administered dose, and the apparent oral bioavailability is acceptable for therapeutic treatment. The conclusions drawn from such results are that the current formulation is delivering azacitidine to the colonic region, and that site is capable of efficiently absorbing azacitidine without the degradation associated

with cytidine deaminase. Ultimately, such observations lead to broader clinical applications of azacitidine.

[00117] While the invention has been particularly shown and described with reference to a number of embodiments, it would be understood by those skilled in the art that changes in the form and details may be made to the various embodiments disclosed herein without departing from the spirit and scope of the invention and that the various embodiments disclosed herein are not intended to act as limitations on the scope of the claims.

CLAIMS

What is claimed is:

1. A controlled release pharmaceutical composition for oral administration for enhanced systemic delivery of a cytidine analog comprising a) a therapeutically effective amount of a cytidine analog and b) a drug release controlling component capable of providing release of the cytidine analog primarily in the large intestine, wherein after ingestion by a patient the cytidine analog is released primarily in the large intestine.
2. The pharmaceutical composition of claim 1, wherein at least about 90% of the cytidine analog is released in the large intestine.
3. The pharmaceutical composition of claim 1, wherein at least about 80% of the cytidine analog is released in the large intestine.
4. The pharmaceutical composition of claim 1, wherein at least about 70% of the cytidine analog is released in the large intestine.
5. The pharmaceutical composition of claim 1, wherein the drug release controlling component is selected from the group consisting of an enteric component, a time delay component, a bacterially degradable component, and mixtures thereof, wherein the patient lacks the digestive enzymes required to degrade the bacterially degradable component.
6. The pharmaceutical composition of claim 5, wherein the enteric component comprises an enteric coating.
7. The pharmaceutical composition of claim 6, wherein the coating primarily dissolves at a pH at above about pH 6.4.

8. The pharmaceutical composition of claim 6, wherein the enteric coating primarily dissolves in the pH range of about pH 6.5 to 6.8.

9. The pharmaceutical composition of claim 6, wherein the enteric coating primarily dissolves in the pH of above about pH 7.

10. The pharmaceutical composition of claim 6, wherein the enteric coating does not substantially dissolve in aqueous solution at a pH of above about pH 6.4 for at least about 30 minutes.

11. The pharmaceutical composition of claim 6, wherein the enteric coating does not substantially dissolve in aqueous solution at a pH of above about pH 6.4 for at least about one hour.

12. The pharmaceutical composition of claim 6, wherein the enteric coating does not substantially dissolve in aqueous solution at a pH of above about pH 6.4 for at least about two hours.

13. The pharmaceutical composition of claim 1, wherein the enteric coating material comprises an agent selected from the group consisting of any grade of hydroxypropylmethylcellulose phthalate, polyvinyl acetate phthalate (PVAP), hydroxypropylmethylcellulose acetate succinate (HPMCAS), alginate, carbomer, carboxymethyl cellulose, methacrylic acid copolymer, shellac, cellulose acetate phthalate (CAP), starch glycolate, polacrylin, methyl cellulose acetate phthalate, hydroxymethylcellulose phthalate, hydroxymethylmethylcellulose acetate succinate, hydroxypropylcellulose acetate phthalate, cellulose acetate terephthalate, cellulose acetate isophthalate, cellulose acetate trimellitate, and mixtures thereof.

14. The pharmaceutical composition of claim 13, wherein the enteric coating is a methacrylic acid copolymer or mixtures thereof.

15. The pharmaceutical composition of claim 14, wherein the methacrylic acid copolymer primarily dissolves at a pH at above about pH 6.4.

16. The pharmaceutical composition of claim 14, wherein the methacrylic acid copolymer primarily dissolves at a pH range of about pH 6.5 to 6.8.

17. The pharmaceutical composition of claim 14, wherein the methacrylic acid copolymer primarily dissolves at a pH at above about pH 7.

18. The pharmaceutical composition of claim 13, wherein the methacrylic acid copolymer is selected from the group consisting of a cationic copolymer of dimethyl aminoethyl methacrylate and neutral methacrylic esters, trimethylaminoethylmethacrylate and neutral methacrylic esters.

19. The pharmaceutical composition of claim 13, wherein the enteric coating comprises between about 1 and about 15 wt % of the total weight of the composition.

20. The pharmaceutical composition of claim 13, wherein the enteric coating comprises between about 3 and about 11 wt % of the total weight of the composition.

21. The pharmaceutical composition of claim 13, wherein the enteric coating comprises a methacrylic acid copolymer in the amount of between about 6 and about 8 wt % of the total weight of the composition.

22. The pharmaceutical composition of claim 5, wherein the cytidine analog and one or more excipients are blended together to form a drug core which is then enteric-coated with the enteric coating.

23. The pharmaceutical composition of claim 22, wherein the excipient is selected from the group consisting of calcium carbonate, carboxymethyl cellulose, cellulose acetate, colloidal silica, crospovidone, dextrose, hydroxypropylmethylcellulose, lactose, magnesium

stearate, mannitol, methylcellulose, microcrystalline cellulose, microcrystalline silicified cellulose, powdered cellulose, povidone, pregelatinized starch, sodium alginate, sodium carboxymethyl cellulose, sodium croscarmellose, sodium starch glycolate, sodium stearyl fumarate, sorbitol, starch, and stearic acid.

24. The pharmaceutical composition of claim 22, wherein the excipient is selected from the group consisting of mannitol, microcrystalline cellulose, crospovidone, magnesium stearate, tocopheryl polyethylene glycol succinic acid esters, and talc.

25. The controlled release pharmaceutical composition of claim 5, wherein the drug release controlling component comprises a time delay component.

26. The controlled release pharmaceutical composition of claim 25, wherein the time delay component is a matrix or coating and is selected from the group consisting an insoluble plastic selected from the group consisting of polyvinyl chloride, polyethylene, vinyl polymers and copolymers selected from the group consisting of polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymer; hydroxypropyl methyl cellulose, shellac, ammoniated shellac, shellac-acetyl alcohol, and shellac n-butyl stearate; and fatty compounds selected from the group consisting of wax and glyceryl tristearate.

27. The controlled release pharmaceutical composition of claim 25, wherein the time delay component does not allow substantial release of the cytidine analog for at least about three hours after oral ingestion by a patient.

28. The controlled release pharmaceutical composition of claim 25, wherein the time delay component does not allow substantial release of the cytidine analog for at least about four hours after oral ingestion by a patient.

29. The controlled release pharmaceutical composition of claim 25, wherein the time delay component does not allow substantial release of the cytidine analog for at least about six hours after oral ingestion by a patient.

30. The controlled release pharmaceutical composition of claim 5, wherein the drug release controlling component comprises a bacterially degradable component, wherein patients lack the digestive enzymes required to degrade the component.

31. The controlled release pharmaceutical component of claim 30, wherein the bacterially degradable component is selected from the group consisting of an azo polymer, an oligosaccharide susceptible to bacterial degradation, mucopolysaccharides and combinations thereof.

32. The controlled release pharmaceutical component of claim 31, wherein the oligosaccharide is selected from the group consisting of cellobiose, lactulose, raffinose and stachyose, and polymers thereof.

33. The controlled release pharmaceutical component of claim 31, wherein the bacterially degradable component is covalently bonded to an acrylic polymer.

34. The pharmaceutical composition of claim 1, further comprising one or more pharmaceutically acceptable excipients selected from the group consisting of bulking agent, binding agent, excipient support, diluent, lubricant, disintegrant, glidant, permeability enhancement excipient, plasticizer, and combinations thereof.

35. The pharmaceutical composition of claim 34, wherein the excipient comprises a permeability enhancement excipient.

36. The pharmaceutical composition of claim 34, wherein the permeability enhancement excipient is selected from the group consisting of polyoxyethylene-polyoxypropylene copolymers, chitosan, carboxymethylcellulose, polyethoxylated

hydroxystearate/unesterified polyethylene glycol, sodium laurate, transesterified ethoxylated vegetable oil, and tocopherol polyethylene glycol succinate acid esters.

37. The pharmaceutical composition of claim 34, further comprising mannitol, calcium silicate, microcrystalline cellulose, crospovidone, magnesium stearate, Vitamin E TPGS, and triethyl citrate.

38. The pharmaceutical composition of claim 1, wherein the cytidine analog is selected from the group consisting of 5-aza-2'-deoxycytidine (decitabine), 5-azacytidine, 5-aza-2'-deoxy-2',2'-difluorocytidine, 5-aza-2'-deoxy-2'-fluorocytidine, 2'-deoxy-2',2'-difluorocytidine (also called gemcitabine), or cytosine 1- β -D-arabinofuranoside (also called ara-C), 2(1H) pyrimidine riboside (also called zebularine), 2'-cyclocytidine, arabinofuanosyl-5-azacytidine, dihydro-5-azacytidine, N⁴-octadecyl-cytarabine, and elaidic acid cytarabine.

39. The pharmaceutical composition of claim 1, wherein the cytidine analog is 5-azacytidine.

40. The pharmaceutical composition of claim 1, wherein the controlled release pharmaceutical composition allows for enhanced bioavailability of the cytidine analog.

41. A method for treating a patient having a disease associated with abnormal cell proliferation, comprising: orally administering to the patient a pharmaceutical composition in accordance with claim 1.

42. The method of claim 41, wherein the disease associated with abnormal cell proliferation is a myelodysplastic syndrome.

43. A method of increasing the bioavailability of a cytidine analog upon administration to a patient, comprising orally administering to the patient a pharmaceutical composition in accordance with claim 1.

44. A method for treating a patient having a disease associated with abnormal cell proliferation, comprising: orally administering to the patient a controlled release pharmaceutical composition, comprising a) a therapeutically effective amount of a cytidine analog and b) a drug release controlling component capable of providing release of the cytidine analog primarily in the large intestine, wherein after ingestion by a patient the cytidine analog is released primarily in the large intestine.

45. The method of claim 44, wherein the disease associated with abnormal cell proliferation is a myelodysplastic syndrome.

46. A method of increasing the bioavailability of a cytidine analog upon administration to a patient, comprising: (I) providing a controlled release pharmaceutical composition to a patient, comprising a) a therapeutically effective amount of a cytidine analog and b) a drug release controlling component capable of providing release of the cytidine analog primarily in the large intestine, wherein after ingestion by a patient the cytidine analog is released primarily in the large intestine; and (II) ingesting of said composition by the patient, whereby said composition contacts the biological fluids of the patient's body and increases the bioavailability of the cytidine analog.

ABSTRACT

[00118] The present invention provides an oral formulation of a cytidine analog, including, azacitidine, for delivery to the lower gastrointestinal tract, including, the large intestine; methods to treat diseases associated with abnormal cell proliferation by treatment with the oral formulations of the present invention; and methods to increase the bioavailability of a cytidine analog upon administration to a patient by providing an oral formulation of the present invention.

S:\ClientFolders\Pharmion\PHAR05\PR\Application.doc

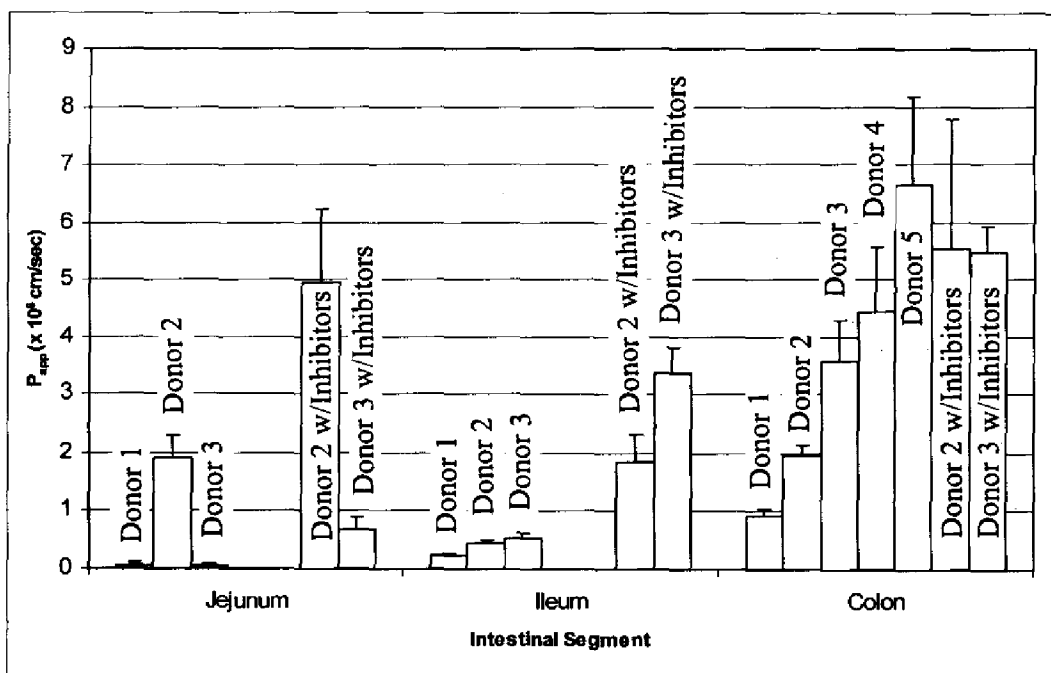


Fig. 1

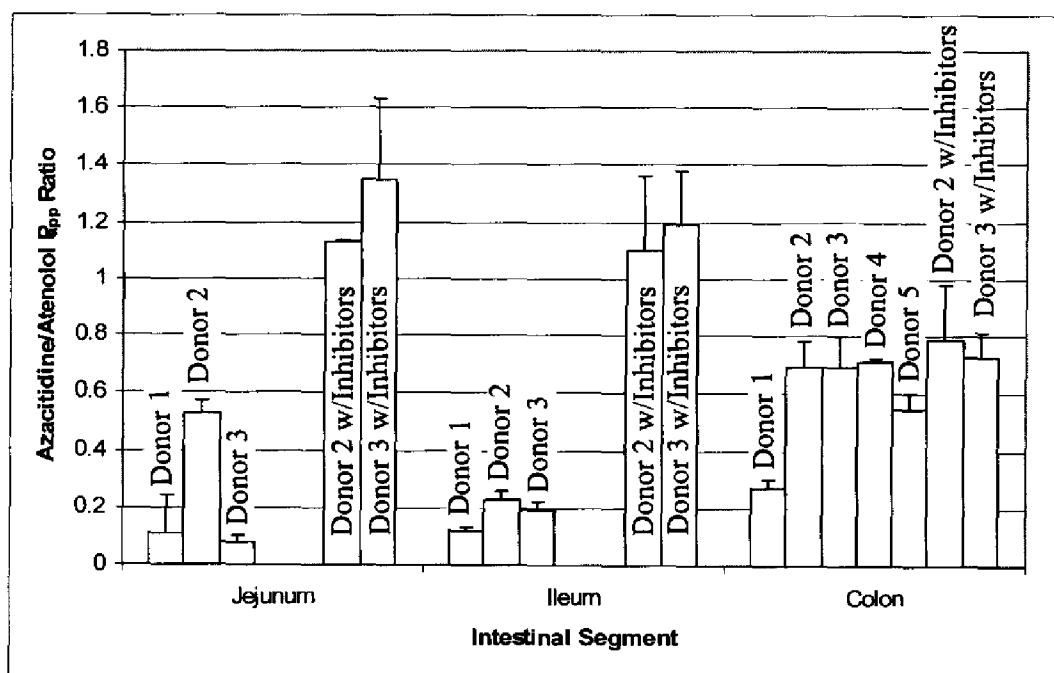


Fig. 2

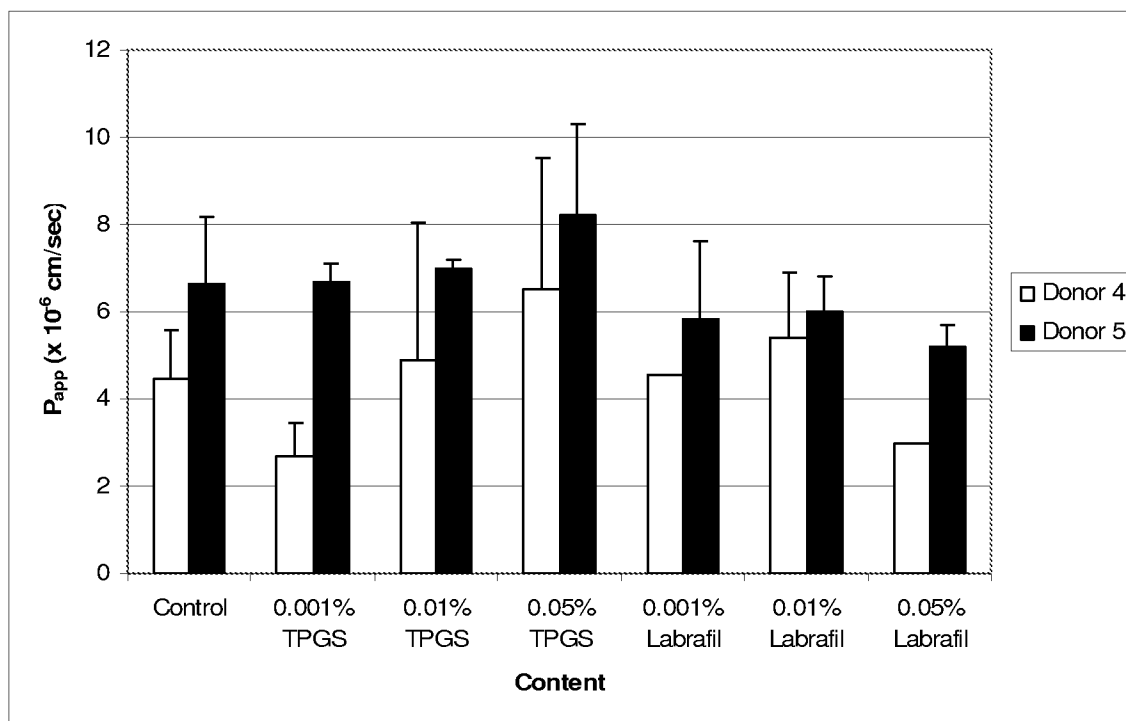


Fig. 3

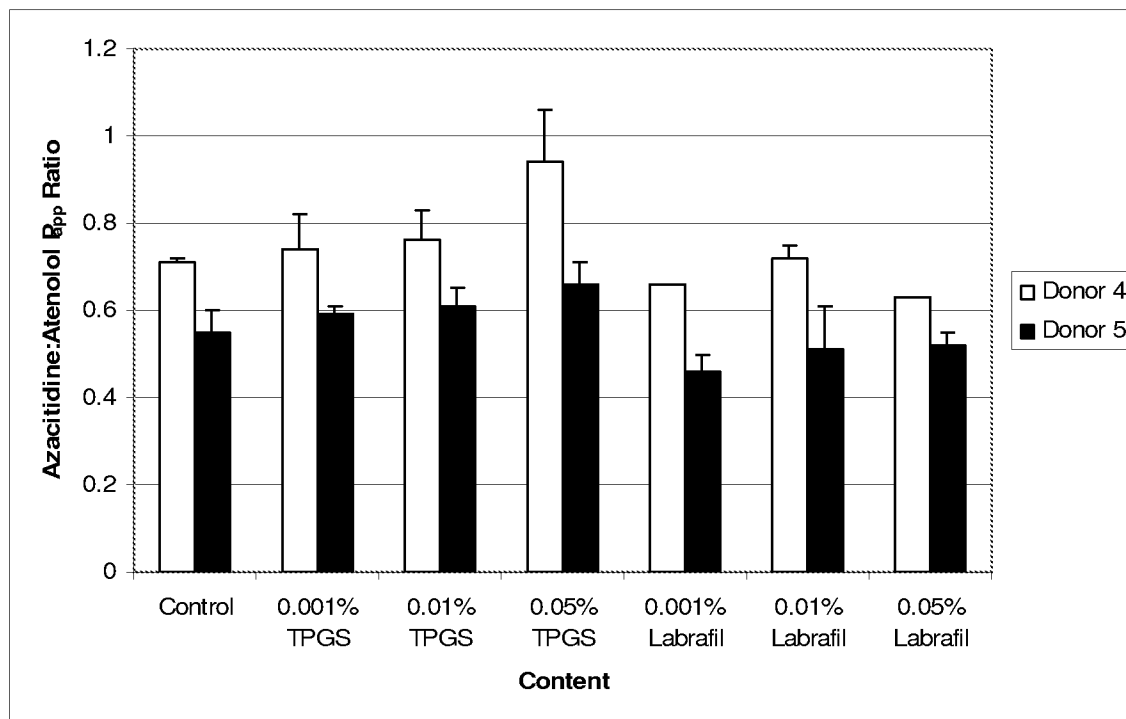


Fig. 4

Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		ORAL FORMULATIONS OF CYTIDINE ANALOGS		
First Named Inventor:		Jeffrey B Etter		
Filer:		Mary Breen Smith.		
Attorney Docket Number:		PHAR.05/PR		
Filed as Small Entity				
Provisional Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Provisional Application filing fee	2005	1	100	100
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				100

Electronic Acknowledgement Receipt

EFS ID:	1182787
Application Number:	60824320
Confirmation Number:	6680
Title of Invention:	ORAL FORMULATIONS OF CYTIDINE ANALOGS
First Named Inventor:	Jeffrey B Etter
Customer Number:	25871
Filer:	Mary Breen Smith.
Filer Authorized By:	
Attorney Docket Number:	PHAR.05/PR
Receipt Date:	01-SEP-2006
Filing Date:	
Time Stamp:	13:39:20
Application Type:	Provisional
International Application Number:	

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	\$ 100
RAM confirmation Number	1210
Deposit Account	195117
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 and 1.17	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part	Pages
1	Application Data Sheet	ADS.pdf	16684	no	1
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
2		Application.pdf	226031	yes	48
	Multipart Description				
	Doc Desc		Start	End	
	Specification		1	40	
	Claims		41	47	
	Abstract		48	48	
Warnings:					
Information:					
3	Drawings	Figures.pdf	32147	no	2
Warnings:					
Information:					
4	Fee Worksheet (PTO-875)	fee-info.pdf	8101	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			282963		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.					