The present invention relates to stabilized nanodispersions of rapamycin micelles, nanoparticles or microcapsules and to their compositions and use. Rapamycin is a macrolide antibiotic produced by the soil fungus Streptomyces hygroscopicus. It is used for the prevention of organ transplant rejection and in the treatment of lymphoproliferative disorders. Rapamycin binds to the mammalian target of rapamycin (mTOR) complex, leading to inhibition of cell growth and proliferation, and is used in cancer therapy.

The invention provides stabilized rapamycin nanodispersions, which are stable preparations of rapamycin in aqueous media or in water-miscible solvents. These nanodispersions can be used in various fields such as medicine, pharmacy, and cosmetics. The stabilized nanodispersions can improve the bioavailability and therapeutic efficacy of rapamycin, and are less affected by factors such as pH and temperature, compared to the free drug form. The invention also provides compositions comprising these nanodispersions and their use in medical and pharmaceutical fields.

**Abstract**

Oral preparations of microcapsules and nanoparticles including an inhibitor of the mammalian target of rapamycin. The preparations are intended to assist with the treatment and prevention of cancer, neurocognitive dysfunction, genetically predisposed disorders, and age-related disorders. The embodiments discussed address the present need for alternative preparations or manufacturing processes that ensure efficacy while improving other performance characteristics such as storage stability, biodistribution, dosage cost, etc.

**Title:** Oral Rapamycin Nanoparticle Preparations and Use

**Rapamycin Nanoparticles**

![Nanoparticles Diagram](image)

**Figure 1**

**Abstract:** Oral preparations of microcapsules and nanoparticles including an inhibitor of the mammalian target of rapamycin. The preparations are intended to assist with the treatment and prevention of cancer, neurocognitive dysfunction, genetically predisposed disorders, and age-related disorders. The embodiments discussed address the present need for alternative preparations or manufacturing processes that ensure efficacy while improving other performance characteristics such as storage stability, biodistribution, dosage cost, etc.
ORAL RAPAMYCIN NANOPARTICLE PREPARATIONS AND USE

Inventors: Dana Vaughn, PhD & Neal K. Vail, PhD

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application relates to the subject matter of US Patent Application Serial No. 13/128,800, filed November 11, 2009, published under Publication No. 2012/0064143, entitled "Inhibition of Mammalian Target of Rapamycin," which has original priority dating to November 11, 2008 (for reference, the "Related UT Application"). Subject matter disclosed or claimed in this patent application has been developed in cooperation with representatives of the Board of Regents of the University of Texas System, which is assignee of record for the Related UT Application.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not applicable for this patent application, although certain rights in the subject matter of the Related UT Application may be owned by the United States government.

BACKGROUND

1. Field of the invention.

[0003] The present invention relates generally to manufacture and use of mTOR inhibitors for oral administration in the prevention and treatment of medical maladies in humans and other animals. More particularly, the invention relates to manufacture and use of preparations for oral administration that include an mTOR and/or mTOR complex 1 (mTORC1) inhibitor together with protective polymers and stabilizers, for prevention and treatment of medical maladies, most especially
in the fields of oncology, neurology and autoimmunities, as well as healthy lifespan extension in humans and other animals.

2. Description of Related Art

Rapamycin (also known as sirolimus) is a well-known pharmaceutical agent that has long been used to minimize organ transplant rejection. Rapamycin and its numerous analogs and derivatives (collectively known as "rapalogs") famously act to inhibit its namesake metabolic pathway in mammals -- the mammalian target of rapamycin ("mTOR"). The critical metabolic roles of the mTOR pathway have long led to broad speculation about possible medical uses for rapamycin outside of organ transplant rejection. However, despite the success with prevention of transplant rejection, and despite the many long-felt needs and corresponding tremendous efforts in developing rapamycins for other indications, effective use of rapamycin for treating or preventing other disorders has not been widely successful and has been very limited at best. The reader should refer to the Related UT Application, which has been incorporated by reference, for additional technical descriptions and a detailed description of the related art.

Particular formulations taught in the Related UT Application (the "2008 Discoveries") provided particles or "cores" containing the active rapamycin ingredient, and those cores were microencapsulated within a protective polymer matrix, for oral administration of the rapamycin. The rapamycin cores were preferably microencapsulated using a spinning disk atomization coating process with a protective polymer matrix known under the "EUDRAGIT® S 100" name. The EUDRAGIT® S 100 polymer matrix principally consists of a particular methacrylate polymer that is generally stable at pH levels below 7 and was used to protect the rapamycin from degrading in the acidic conditions of the stomach.
Then, once the microencapsulated rapamycin entered basic conditions (i.e., pH greater than 7) within the intestines, the protective matrix would dissolve and, theoretically, the undegraded rapamycin would be absorbed through the intestinal walls and become bioavailable for its intended medical purposes.

[0006] Unfortunately, theory and practice do not always match perfectly. Despite tremendous hope for broad efficacy of the orally administered use of such microencapsulated rapamycin preparations, and despite widespread national and international attention to the 2008 Discoveries, significant concerns remained about whether effective levels of rapamycin could be reliably delivered to the body in this form. For reasons that long remained uncertain in practice, stability of the basic rapamycin molecule within such formulations has been less reliable than desired, and uncertainties have mounted with respect to whether enteric absorption levels can be reliable enough for adequate market acceptance of the 2008 Discoveries.

[0007] Other challenges exist. It is counterintuitive to even consider the use of rapamycin and other mTOR inhibitors for prevention or treatment of conditions such as feline gingivitis or canine hemolytic anemia. Particularly because one of the contraindications or precautions commonly associated with rapamycin relates to mouth ulcers. For a variety of reasons, rapamycin tends to cause mucous membrane breakdown in oral cavities in some subjects, particularly in certain doses. That alone would sufficiently deter someone from using rapamycin for these applications.

[0008] Consequently, there is a need for improved encapsulated rapamycin preparations - preparations that still capitalize on the 2008 discoveries but that
improve various performance characteristics, such as storage stability, biodistribution, dosage cost, etc.

[0009] In addition, because the potential applications are so wide and varied and yet relatively unproven for an oral form of rapamycin, that wide variety itself presents an impediment to realizing publically available use of such a preparation. Given the market dynamics and regulatory requirements of pharmaceutical industries, a successful effort to actually make embodiments of the 2008 Discoveries available for use by the public would require much more than minimizing uncertainties about the preparation itself. A successful effort to do so must identify and validate a particular, highly-impactful indication for which the benefits of using a microencapsulated rapamycin would be relatively irrefutable, and the effort must likewise develop corresponding methods and strategies for effectively and reliably addressing as much.

**SUMMARY OF THE INVENTION**

[0010] While the present invention is multifaceted, it can be embodied in numerous improved forms of encapsulated rapamycins and in methods for reliably producing and using these improved forms. The improved forms of encapsulated rapamycins preferably provide nanoparticles containing mTOR inhibitors within a protective polymer matrix for oral administration of rapamycin. The result is not only more durable and stable, but is also more bioavailable and efficacious for treatment and prevention of medical maladies, particularly of genetically-predisposed disorders and age-related disorders, especially in the fields of oncology, neurology and auto-immune disorders in humans and other animals.

[0011] The various embodiments improve on the related art, including by optimizing stability, manufacturability, bioabsorption, biodistribution, dosage cost,
efficacy and the like. Although the embodiments addressed below do not compose an exhaustive list, this specification describes embodiments comprising controlled release encapsulated rapamycin; rapamycin nanoparticle inclusions; rapamycin nanoparticle morphology; free radical scavengers and oxidative stabilizers; and an albumin-rapamycin nanoparticle.

[0012] In the disclosed methods, the composition comprising rapamycin or an analog of rapamycin may be delivered in any suitable manner. In a preferred embodiment, the composition comprising rapamycin or an analog of rapamycin is orally administered to the subject.

[0013] Compositions comprising rapamycin or an analog of rapamycin may include a nanoparticle construct combined with a carrier material preferably an enteric composition for purposes of minimizing degradation of the composition until it passes the pylorus to the intestines of the subject. Compositions comprising rapamycin or an analog of rapamycin may also include a hydrophilic, swellable, hydrogel forming material. Such compositions may be encased in a coating that includes a water insoluble polymer and a hydrophilic water permeable agent. In some embodiments, the water insoluble polymer is a methyl methacrylate-methacrylic acid copolymer. Compositions comprising rapamycin or an analog of rapamycin may further include a thermoplastic polymer for purposes of gradual or controlled release of the rapamycin or an analog of rapamycin. Examples of the thermoplastic polymer include EUDRAGIT® Acrylic Drug Delivery Polymers (Evonik Industries AG, Germany).

[0014] In some embodiments, rapamycin particles or particles of rapamycin analogs or other mTOR inhibitors or analogs thereof, are encapsulated or coated, or the composition comprising the rapamycin or other mTOR inhibitor or analog
thereof is encapsulated or coated. For reference purposes in these descriptions, "microencapsulation" (and its grammatical variations) should be interpreted to refer to protection of microparticle or nanoparticle forms of rapamycins (preferably in the nanoparticle forms according to the descriptions herein) by combining such particles with an enteric coating material or the like that is formulated to resist degradation in acidic conditions. Further, the designations "microencapsulated rapamycin" and "enteric-coated rapamycin" are used interchangeably to refer generically to each and every variation of microencapsulated rapamycins, especially to those variations that are described or particularly suggested in these descriptions, and equivalents thereof. Exceptions in particular contexts should be understood, nonetheless, to the extent that the context makes more specific or contrary clarifications for that context. In some embodiments, the encapsulant or coating used for and incorporated in enteric-coated rapamycin preparations may be an enteric coating. In another aspect of these descriptions, general references to "prevention and treatment" (or the like) of a malady should be interpreted to include reference not only to prevention and treatment of the actual malady, but also to delay or reduction in the progression of that malady as well as prevention and treatment of its precursors and sequelae.

[0015] In many embodiments involving enteric-coated rapamycin preparations, the rapamycins, rapamycin analogs, or related compositions within the enteric-coated rapamycin preparation are provided in the form of nanoparticles that include the rapamycin or other mTOR inhibitor, in which cases the designation "nanoRapa" is generically used for reference purposes in these descriptions, while the form of rapamycin used may preferably include, but not be limited to, an encapsulated form in the form of nanoparticles designated as
"enteric-coated rapamycinNP2g." After preparing the nanoRapa preparations through any of various approaches that may be understood and/or described herein, the nanoRapa preparation may then be coated with an enteric coating to provide an enteric-coated rapamycin preparation formed from nanoRapa particles. For reference purposes in these descriptions, the designation "e-nanoRapa" is generically used to refer to each and every enteric-coated rapamycin variation formed from nanoRapa particles.

[0016] Many other objects, features and advantages of the present invention will become apparent to those of ordinary skill in the art, particularly after a thorough review of the public literature in the field, and all the more from the following detailed descriptions and accompanying illustrations and claims. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from these detailed descriptions.
The accompanying drawings form part of the present specification and are included to further demonstrate and illustrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Fig. 1 is a graphic illustration of microscopic aspects of a preferred process for producing a dispersion of preferred forms of rapamycin nanoparticles according to the teachings of the present invention.

Fig. 2 is a graphic illustration of two basic steps in a preferred process for producing a dispersion of preferred forms of rapamycin nanoparticles according to the teachings of the present invention.

Fig. 3 provides a photograph of a dispersion of rapamycin nanoparticles produced as a result of Step 2 in the preferred process illustrated in Fig. 2, together with a graph of nanoparticle size distribution for the dispersion shown in the photograph.

Figs. 4A-4B are flowcharts illustrating detailed steps of a more comprehensive preferred process for producing preferred forms of enteric-coated rapamycin nanoparticles, which includes the process for producing a nanoparticle dispersion as illustrated in Fig. 2, together with additional steps for microencapsulating the rapamycin nanoparticles.

Fig. 5 presents summary data to illustrate how extended regular use of microencapsulated rapamycin nanoparticles was effective at reducing FCGS disease scores in 100% of sixteen feline subjects.
Figs. 6A-6B illustrate data to support that encapsulated rapamycin increases life span and health in Apc\textsuperscript{Min/+} mice.

Figs. 7A-7C illustrate data to support that encapsulated rapamycin has positive efficacy in Apc\textsuperscript{Min/+} mice. (A) Polyp count in Apc\textsuperscript{Min/+} mice at time of death in mice treated with no enteric-coated rapamycin (left cluster, solid circles), the medium dose of enteric-coated rapamycin as described above (middle cluster, solid squares), or the high dose of enteric-coated rapamycin as described above (right cluster, solid triangles). (B) Encapsulated Rapa improves physical activity in Apc\textsuperscript{Min/+} mice, as shown by measuring the average number of beam breaks (activity) for two time periods of the day, light and dark. Mice were treated with Eudragit control diet (left column, hatched), the medium dose of enteric-coated rapamycin as described above (middle column, white with black dots), or the high dose of enteric-coated rapamycin as described above (right column, black with white dots). The food area of the cage was excluded. (C) Encapsulated Rapa maintains normal hematocrits in Apc\textsuperscript{Min/+} mice. Mice were treated with no enteric-coated rapamycin (solid black circles), the medium dose of enteric-coated rapamycin (solid black squares), or the high dose of enteric-coated rapamycin (solid black triangles). Age at the time of hematocrits is indicated on the X-axis.

Figs. 8A-8C illustrate data to support that encapsulated rapamycin inhibits mTOR complex 1 (mTORC1) downstream effector, ribosomal protein subunit S6 (rpS6) phosphorylation by S6 kinase 1 (S6K1) in the distal segment of small intestine. enteric-coated rapamycin was fed to C57BL/6 mice, the same genetic background for the Apc\textsuperscript{Min/+}, and intestines were collected and prepared for immunoassay. (A) Immunoblot showing detection of total rpS6 (bottom panel), Ser240/244 phosphorylated rpS6 (middle panel) and pan actin as a
loading control. (B) Signal intensities for each band in (A) were quantified and the ratio of phosphorylated rpS6 to total rpS6 was calculated and graphed as a scatter plot using Prism Software. Statistical significance of the reduction in this ratio was determined using an un-paired t-test (Prism). These data show that enteric-coated rapamycin effectively inhibited mTORCI and its effector rpS6, which is known to play a vital role in biogenesis of ribosomes used in protein synthesis needed for cell growth and proliferation. This is likely the effect of enteric-coated rapamycin that inhibits polyp development and growth in ApcMin/+ mice and extends longevity. (C) Blood levels of rapamycin at 217 days (174 days treatment with enteric-coated rapamycin) in mice treated with 14 ppm enteric-coated rapamycin (the medium dose of enteric-coated rapamycin as described above, see bottom cluster, shown in solid black squares) and 42 ppm enteric-coated rapamycin (the high dose of enteric-coated rapamycin as described above, see top cluster, shown in solid black circles).

[F0026] Figs. 9A-9E illustrate data to support that microencapsulated increases life and health span for Ape Min/+ mice. (A) Enteric-coated rapamycin increases life span for Ape Min/+ mice. The grey box represents a range of median life spans for C57BL/6J female mice taken from previous studies (Winjnhoven, et al., 2005), which is also representative of syngeneic colonies at the Barshop Institute animal facility used in the present study. Note that 60% of the 42 ppm enteric-coated rapamycin fed Ape Min/+ mice have lived beyond this range. (B) Polyp count for Ape Min/+ mice at the time of death. Note the first and third mouse that died from the 42 ppm cohort had no polyps. (C) Enteric-coated rapamycin improves physical activity in Ape Min/+ mice. Graphed is the mean number of beam breaks (activity) for light (inactive) and dark (=active) phases of the day. The food
area of the cage is excluded. (D) enteric-coated rapamycin maintains normal hematocrits in Ape Mn/s mice. Note the dose response. Also note the normal hematocrit as compared to wild type C57BL/6 mice (~40%) in the high dose group even at late time points when many wild type C57BL/6 mice would die from natural causes. (E) enteric-coated rapamycin compared to RAD001 (everolimus). Survival curves taken from Fujishita, et al., 2008, using RAD001 delivered by gavage at the doses indicated below the graph. Wild type survival is shown as in (A). Note the lower dose of enteric-coated rapamycin extends survival better than the highest dose of RAD001 and the higher dose far exceeds RAD001 in life span extension.

[0027] Figs. 10A-1QH illustrate pharmacodynamic data for C57BL/6 mice fed enteric-coated rapamycin. (A) Western blots developed with indicated antibodies. C57BL/6 mice (607-627 days old) were fed enteric-coated rapamycin for 42 days, intestine dissected and immunoassayed. Segments of the small intestine (proximal and distal) were assayed as indicated. Graphs show the quantitation of the immunoblot data as measured by the ratio of intensity values for the phosphorylation state-dependent signal (P(240/244)rpS6) to phosphorylation state-independent (rpS6) signal (B) and rpS6 to actin antibody signal (C). (D) Rapamycin blood levels of C57BL/6 mice at 607-627 days of age (42 days on enteric-coated rapamycin diets, which averaged 37 ng/mL and 170 ng/mL for the 14 and 42 ppm groups, respectively). (E) Rapamycin tissue levels in proximal and distal small intestine of C57BL/6 mice (607-627 days of age and 42 days on diet). Mean levels (µg/g) are: proximal 14 ppm 91.62 ± 14.91, 42 ppm 266.7 ± 26.35; distal 14 ppm 627.3 ± 135.5, 42 ppm 1488 ± 141.6. P values are: 0.001 (proximal 14 ppm compared to 42 ppm); 0.035 (distal 14 ppm compared to
42 ppm); 0.0077 (proximal 14 ppm compared to 42 ppm); 0.0001 (proximal 42
ppm compared to distal 42 ppm). (F) Rapamycin blood levels in Apc\(^{Min}\) mice at
217 days (174 days on enteric-coated rapamycin diets, which averaged 14ng/mL
or 26.6 ng/mL on 14 ppm diets, respectively) or at 308 days of age (265 days on
enteric-coated rapamycin diet, which averaged 10.2 ng/mL or 28.1 ng/mL on 14
ppm or 42 ppm diets, respectively). (G&H) Graphs showing quantification of
phosphorylation state-dependent signal P(Ser473)Akt to phosphorylation state-
independent signal (D) and Akt to actin antibody signal (E). Note that chronic
treatment with enteric-coated rapamycin does not lead to rebound activation of
Akt.

[0028] Fig. 11 illustrates data to support that enteric-coated rapamycin
represses mTORC1 in colon. Immunoblots developed with the antibodies shown
indicate that a robust reduction in Ser240/244 phosphorylation of rpS6 relative to
untreated colon. Note that levels of rpS6 do not appear to change. Rapamycin
blood levels are indicated below each lane, which represents a colon from one
C57BL/6 mouse consuming chow with or without enteric-coated rapamycin as
shown in the lanes. Note also that the lowest mobility, least phosphorylated (a)
band of 4E-BP1 increases with enteric-coated rapamycin treatment. The 4E-BP1
antibody is phosphorylation state-independent and therefore detects all three of
the mobility forms of 4E-BP1 indicated. These data suggest that both major
outputs (4E-BP1 and S6K1 rpS6) from mTORC1 are repressed in the colon by
chronic treatment.

[0029] Fig. 12 illustrates data to support that encapsulated rapamycin
improves memory and restores cerebral blood flow (CBF) in AD mice treated with
rapamycin after the onset of disease, a, Spatial learning. While learning in AD
mice was impaired, P<0.01, Bonferroni's post hoc test applied to a significant effect of genotype and treatment, F(3,188)=6.04, P=0.0014, repeated measures (RM) 2-way ANOVA], performance of rapamycin-fed AD mice was indistinguishable from non-Tg littermates' and from control-fed AD mice. No significant interaction was observed between day number and genotype (P=0.96), thus genotype and treatment had the same effect at all times during training. Overall learning was effective in all groups [F(4,188)=3.36, P=0.01, RM two-way ANOVA].

b, Spatial memory is restored by rapamycin treatment. While memory in control-fed AD mice was impaired [P values as indicated, Tukey's test applied to a significant effect of genotype and treatment (P<0.0001), one-way ANOVA], memory in rapamycin-fed AD mice was indistinguishable from non-Tg groups and was significantly improved compared to control-fed AD mice (P=0.03).

c-g, Rapamycin restores CBF in AD mice. c, CBF maps and regional CBF maps (e) of representative control-and rapamycin-treated non Tg and AD mice obtained by MRI. d, Decreases in CBF in AD mice are abrogated by rapamycin treatment (P as indicated, Bonferroni's test on a significant effect of genotype and treatment on CBF, F(1,16)=14.54, P=0.0015, two-way ANOVA). f and g, Decreased hippocampal (f) but not thalamic (g) CBF in AD mice is restored by rapamycin treatment (P as indicated, Bonferroni's test on a significant effect of treatment on CBF, F(1,16)=13.62, P=0.0020, two-way ANOVA). Data are means ± SEM. Panels a-b, n=10-17 per group. Panels c-g, n = 6 per group.

[0030] Figs. 13A-13D illustrates data indicating increased vascular density without changes in glucose metabolism in rapamycin-treated AD mice. A, Cerebral metabolic rate of glucose (CMRglc) maps of representative control-and rapamycin-treated non Tg and AD Tg mice obtained by positron emission
tomography. b, CMRolc as standardized uptake values (SUV) for the region of interest were not different among experimental groups (F(1,20)=0.77, P=0.39 for the effect of genotype and F(1,20)=3.63, P=0.071 for the effect of treatment, two-way ANOVA). c, Magnetic resonance angiography images of brains of rapamycin-treated non Tg and AD mice. Representative regions showing loss of vasculature in control-treated AD mice and its restoration in rapamycin-treated animals are denoted by arrows. d, Decreased cerebral vessel density in control-treated AD mice is abrogated by rapamycin treatment (P as indicated, Bonferroni's post hoc test applied to a significant effect of treatment on vascular density, F(1,16)=24.47, P=0.0001, two-way ANOVA). Data are means ± SEM. n = 6 per group.

[0031] Fig. 14 illustrates rapamycin levels in different brain regions of AD mice chronically fed with rapamycin-supplemented chow.
DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0032] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in these examples are thought to represent techniques that function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, in light of the present disclosure, those of skill in the art should appreciate that many changes can be made in the specific embodiments which are disclosed while still obtaining a like or similar result without departing from the spirit and scope of the invention.

[0033] For purposes of these descriptions, a few wording simplifications should also be understood as universal, except to the extent otherwise clarified in a particular context either in the specification or in any claims. The use of the term "or" in the specification is used to mean "and/or" unless explicitly indicated to refer to alternatives only, or unless the alternatives are inherently mutually exclusive. When referencing values, the term "about" is used to indicate an approximate value, generally one that includes the standard deviation of error for any particular embodiments that are disclosed or that are commonly used for determining such value. "A" or "an" may mean one or more, unless clearly indicated otherwise. Such "one or more" meanings are most especially intended when references are made in conjunction with open-ended words such as "having," "comprising" or "including." Likewise, "another" may mean at least a second or more.
GENERAL EMBODIMENTS

[0034] Any inhibitor of mTOR is contemplated for inclusion in the present compositions and methods. In particular embodiments, the inhibitor of mTOR is rapamycin or an analog of rapamycin, preferably administered orally in the form of an enteric-coated rapamycin and/or e-nanoRapa preparation.

[0035] Rapamycin binds to a member of the FK binding protein (FKBP) family, FKBP 12. The rapamycin/FKBP 12 complex binds to the protein kinase mTOR to block the activity of signal transduction pathways. Because the mTOR signaling network includes multiple tumor suppressor genes, including PTEN, LKB1, TSC1, and TSC2, and multiple proto-oncogenes including P13K, Akt, and eEF4E, mTOR signaling plays a central role in cell survival and proliferation. Binding of the rapamycin/FKBP complex to mTOR causes arrest of the cell cycle in the G1 phase (Janus 2005).

[0036] mTORC1 inhibitors also include rapamycin analogs. Many rapamycin analogs are known in the art. Non-limiting examples of analogs of rapamycin include, but are not limited to, everolimus, tacrolimus, CC1-779, ABT-578, AP-23675, AP-23573, AP-23841, 7-epi-rapamycin, 7-thiomethyl-rapamycin, 7-epi-trimethoxyphenyl-rapamycin, 7-epi-thiomethyl-rapamycin, 7-demethoxy-rapamycin, 32-emethoxy-rapamycin, 2-desmethyl-rapamycin, and 42-0-(2-hydroxy)ethyl rapamycin.

[0037] Other analogs of rapamycin include: rapamycin oximes (U.S. Pat. No. 5,446,048); rapamycin aminoesters (U.S. Pat. No. 5,130,307); rapamycin dialdehydes (U.S. Pat. No. 6,680,330); rapamycin 29-enols (U.S. Pat. No. 6,677,357); O-alkylated rapamycin derivatives (U.S. Pat. No. 6,440,990); water soluble rapamycin esters (U.S. Pat. No. 5,955,457); alkylated rapamycin
derivatives (U.S. Pat. No. 5,922,730); rapamycin amidino carbamates (U.S. Pat. No. 5,637,590); biotin esters of rapamycin (U.S. Pat. No. 5,504,091); carbamates of rapamycin (U.S. Pat. No. 5,567,709); rapamycin hydroxyesters (U.S. Pat. No. 5,362,718); rapamycin 42-sulfonates and 42-(N-carbalkoxy)sulfamates (U.S. Pat. No. 5,346,893); rapamycin oxepane isomers (U.S. Pat. No. 5,344,833); imidazolidyl rapamycin derivatives (U.S. Pat. No. 5,310,903); rapamycin alkoxyesters (U.S. Pat. No. 5,233,036); rapamycin pyrazoles (U.S. Pat. No. 5,164,399); acyl derivatives of rapamycin (U.S. Pat. No. 4,316,885); reduction products of rapamycin (U.S. Pat. Nos. 5,102,876 and 5,138,051); rapamycin amide esters (U.S. Pat. No. 5,118,677); rapamycin fluorinated esters (U.S. Pat. No. 5,100,883); rapamycin acetals (U.S. Pat. No. 5,151,413); oxorapamycins (U.S. Pat. No. 6,399,625); and rapamycin silyl ethers (U.S. Pat. No. 5,120,842).

[0038] Other analogs of rapamycin include those described in U.S. Pat. Nos. 6,015,809; 6,004,973; 5,985,890; 5,955,457; 5,922,730; 5,912,253; 5,780,462; 5,665,772; 5,637,590; 5,567,709; 5,563,145; 5,559,122; 5,559,120; 5,559,119; 5,559,112; 5,550,133; 5,541,192; 5,541,191; 5,532,355; 5,530,121; 5,530,007; 5,525,610; 5,521,194; 5,519,031; 5,516,780; 5,508,399; 5,508,290; 5,508,286; 5,508,285; 5,504,291; 5,504,204; 5,491,231; 5,489,680; 5,489,595; 5,488,054; 5,486,524; 5,486,523; 5,486,522; 5,484,791; 5,484,790; 5,480,989; 5,480,988; 5,463,048; 5,446,048; 5,434,260; 5,434,260; 5,411,967; 5,391,730; 5,389,639; 5,385,910; 5,385,909; 5,385,908; 5,378,836; 5,378,696; 5,373,014; 5,362,718; 5,358,944; 5,346,893; 5,344,833; 5,302,584; 5,262,424; 5,262,423; 5,260,300; 5,260,299; 5,233,036; 5,221,740; 5,221,670; 5,202,332; 5,194,447; 5,177,203; 5,169,851; 5,164,399; 5,162,333; 5,151,413; 5,138,051; 5,130,307; 5,120,842; 5,120,727; 5,120,726; 5,120,725; 5,118,678; 5,118,677; 5,100,883; 5,023,264;
5,023,263; 5,023,262; all of which are incorporated herein by reference. Additional rapamycin analogs and derivatives can be found in the following U.S. Patent Application Pub. Nos., all of which are herein specifically incorporated by reference: 20080249123, 20080188511; 20080182867; 20080091008; 20080085880; 20080069797; 20070280992; 20070225313; 20070203172; 20070203171; 20070203170; 20070203169; 20070203168; 20070142423; 20060264453; and 20040010002.

[0039] Rapamycin or a rapamycin analog can be obtained from any source known to those of ordinary skill in the art. The source may be a commercial source or a natural source. Rapamycin or a rapamycin analog may be chemically synthesized using any technique known to those of ordinary skill in the art. Non-limiting examples of information concerning rapamycin synthesis can be found in Schwecke et al., 1995; Gregory et al., 2004; Gregory et al., 2006; Graziani, 2009.

[0040] Preferred embodiments of the present invention provide an improved form of encapsulated rapamycin - an encapsulated rapamycin nanoparticle that is more durable, stable and bioavailable, which enhances efficacy and predictability and ensures better biodistribution while also allowing improved patient compliance relative to raw rapamycin, as well as being produced at a reasonable cost. The improved form of encapsulated rapamycin preferably provides the rapamycin nanoparticles within a polymer matrix, forming an encapsulated rapamycin nanoparticle in a single drug delivery structure for oral administration of rapamycin. The polymer matrix, more particularly, is a controlled release matrix, as described elsewhere in these descriptions. This encapsulated rapamycin nanoparticle may also be referred to as an enteric-coated rapamycin nanoparticle. In addition, many of the preferred embodiments also include a stabilizing
compound (for our purposes, a "stabilizer") within the controlled release matrix either to improve compatibility of the rapamycin with the controlled release matrix, to stabilize the crystalline morphology of the rapamycin, or to help further prevent degradation of the rapamycin, particularly when the encapsulated rapamycin nanoparticle is exposed to air, atmospheric moisture, or room temperature or warmer conditions. Particularly preferred embodiments incorporate the stabilizers within each rapamycin nanoparticle, although certain aspects of the invention may be embodied with stabilizers on the surface of the encapsulated rapamycin nanoparticles or otherwise dispersed in the controlled release matrix. To different levels depending on the particular approach used for producing the nanoparticles, with or without other additives, the result is more efficacious for treatment and prevention of genetically-predisposed disorders and age-related disorders, especially in the fields of oncology and neurology in humans and other animals.

Rapid anti-solvent precipitation, or controlled precipitation, is a preferred method of preparing the rapamycin nanoparticles as it provides for minimal manipulation of the rapamycin and exquisite control over nanoparticle size and distribution, and the crystallinity of the rapamycin. Several controlled precipitation methods are known in the art, including rapid solvent exchange and rapid expansion of supercritical solutions, both of which can be implemented in batch or continuous modes, are scalable, and suitable for handling pharmaceutical compounds. Preferred embodiments use an anionic approach, producing micelles (illustrated in Fig. 1) or other molecular aggregations of amphipathic compounds (e.g. sodium cholate or similar surfactants with amphipathic tendencies) in concentrations greater than their critical micelle concentrations.
As part of a preferred process for producing microencapsulated rapamycin nanoparticles, Figs. 1 & 2 illustrate basic preferred steps for producing a dispersion of preferred rapamycin nanoparticles through controlled precipitation. Rapamycin itself (sometimes referred to "raw" or "neat" rapamycin) is available in powder forms from multiple sources readily identifiable to those in the field. Although rapamycin is not readily soluble in water, solubility can be achieved in some aqueous miscible solvents.

Step 1 in Fig. 2 illustrates a first basic step in the preferred process of producing preferred rapamycin nanoparticles, whereby raw rapamycin is mixed and dissolved into an aqueous miscible solvent 160 (the mixture represented by 140 in Fig.1). As illustrated by Step 2 in Fig. 2, the resultant solvent mixture is injected into rapidly stirred water containing an appropriate aqueous soluble dispersant, preferably sodium cholate, which is a polar amphipathic molecule that tends to form micelles from solution.

After mixing the solvent mixture with the micelle-producing aqueous dispersant in Step 2, the effects of solubility cause the rapamycin to partition to the hydrophobic micelle cores 130. Appropriate solvents 160 and dispersants 110 are discussed in greater detail below. Although the core of the micelles is relatively hydrophobic, which tends to attract the rapamycin from the solvent mixture, the results create a nanoparticle 150 having an outer surface decorated with hydrophilic ends of sodium cholate, which tend to keep the resulting nanoparticles in suspension within the final mixture.

A sample of a rapamycin nanoparticle dispersion 310 resulting from Step 2 is shown in the photograph in Fig. 3. Fig. 3 also shows a representative graph 320 of the resultant rapamycin nanoparticle size distribution, as indicated by
the intensity of light scattered by corresponding particle sizes within the sample dispersion. The sodium cholate results in a hydrophilic surface, stabilizing the nanoparticles in the aqueous media and thereby preventing aggregation and particle growth. This product ensures enhanced and prolonged rapamycin stability - i.e., improved resistance to moisture degradation and/or oxidation for the final product - as well as good intestinal bioabsorption characteristics for the rapamycin protected in this manner.

[0046] Rapamycin nanoparticles prepared by controlled precipitation methods can be stabilized against irreversible aggregation, Ostwald ripening, and/or reduced dispersibility, by control of colloid chemistry, particle surface chemistry and particle morphology. For example, nanoparticles prepared by antisolvent solidification can be stabilized by ionic and non-ionic surfactants that adsorb to nanoparticle surfaces and promote particle colloid stability through either charge repulsion or steric hindrance, respectively. Moreover, stabilizers can affect nanoparticle crystallinity, which may be preferred to promote different biodistribution and bioavailability in certain indications.

[0047] Rapamycin nanoparticles can consist of molecular rapamycin bound by suitable methods to other nanoparticles. Suitable methods of attaching rapamycin to a nanoparticle carrier or substrate may include physical adsorption through hydrogen van der Waals forces or chemisorption through covalent or ionic bonding. Nanoparticle substrates may be either natural or synthetic, and modified to promote specific interactions with rapamycin. Natural nanoparticles include albumin and other proteins, and DNA. Synthetic nanoparticles include organic and inorganic particulates, micelles, liposomes, dendrimers, hyperbranched polymers, and other compounds.
The rapamycin nanoparticles can be processed by any suitable method, such as by milling, high pressure atomization, or rapid anti-solvent precipitation. Milling is suitable provided care is taken to minimize both rapamycin degradation and particle agglomeration. Rapamycin degradation can be reduced with the aid of cooling or cryogenic processes. Agglomeration due to the increased surface area and concomitant adhesive forces can be reduced by the use of dispersants during the milling process.

The individual rapamycin nanoparticles are preferably sized in the range between about 1 nanometer and about 1 micron. Smaller sized rapamycin nanoparticles are preferred, preferably at less than 1 micron diameter, for various reasons, including better control of final particle size, improved stability within the particles, and the ability to tune bioavailability by controlling the crystallinity and composition of the rapamycin nanoparticles.

Manufacturing approaches for the encapsulated rapamycin nanoparticle drug delivery structure embodiments of the present invention include creating a solution of the controlled release matrix, with the rapamycin nanoparticles dispersed therein, in appropriate proportion and producing a heterogeneous mixture. The solvent for such mixtures can be a suitable volatile solvent for the controlled release matrix, although it is preferred the solvent be either a poor solvent or non-solvent for the rapamycin nanoparticles so that when the rapamycin nanoparticles are dispersed into the controlled release matrix solution they remain as discrete nanoparticles. The resulting dispersion of rapamycin nanoparticles in the controlled release matrix solution can then be reduced to a dry particulate powder by a suitable process, thereby resulting in microparticles of a heterogeneous nature comprised of rapamycin nanoparticles.
randomly distributed in the controlled release matrix. The particulate powder may also be tailored by a suitable process to achieve a preferred particle size for subsequent preparation, which may be from about 20 to about 70 microns in diameter.

[0051] The rapamycin nanoparticles are microencapsulated with the controlled release matrix using a suitable particle-forming process to form the encapsulated rapamycin nanoparticle. An example of a particle-forming process is spinning disk atomization and drying. For a detailed discussion of the apparatus and method concerning the aforementioned spin disk coating process, this application incorporates by references US Patent Applications 2011/221337 and 2011/220430, respectively. Alternatively, for example, the encapsulated rapamycin nanoparticles can be prepared by spray drying.

[0052] In some embodiments, not all of the rapamycin nanoparticles will be encapsulated within the controlled release matrix. Instead the rapamycin nanoparticles may be enmeshed with the controlled release matrix, with some of the rapamycin nanoparticles wholly contained within the controlled release matrix while another other rapamycin nanoparticles apparent on the surface of the drug delivery structure, constructed in appearance similar to a chocolate chip cookie.

[0053] Depending on the size of the rapamycin nanoparticles, the encapsulated rapamycin nanoparticles are preferably of diameter between 10 and 50 microns, although diameters as large as 75 microns may be suitable for alternatives with corresponding compromises due to the larger size.

[0054] The controlled release matrix of the encapsulated rapamycin nanoparticles can be selected to provide preferred release characteristics of the encapsulated rapamycin nanoparticles. For example, the matrix may be pH
sensitive to provide either gastric release, or preferably, enteric release of the rapamycin. Enteric release of the rapamycin is preferred to achieve improved absorption and bioavailability of the rapamycin. Many materials suitable for enteric release are known in the art, including fatty acids, waxes, natural and synthetic polymers, shellac, and other materials. Polymers are a preferred enteric coating and may include copolymers of methacrylic acid and methyl methacrylate, copolymers of methyl acrylate and methacrylic acid, sodium alginate, polyvinyl acetate phthalate, and various succinate or phthalate derivatives of cellulose and hydroxypropyl methylcellulose. Synthetic polymers, such as copolymers of methacrylic acid and either methyl acrylate or methyl methacrylate, are preferred enteric release polymers due the ability to tune the dissolution pH range of these synthetic polymers by adjusting their comonomer compositions. Examples of such pH sensitive polymers are EUDRAGIT® polymers (Evonik Industries, Essen, Germany). Specifically, EUDRAGIT® S 100, a methyl methacrylate and methacrylic acid copolymer with comonomer ratio of 2:1, respectively, has a dissolution pH of about 7.0, thereby making it suitable for enteric release of rapamycin.

[0055] The encapsulated rapamycin nanoparticles may be delivered in various physical entities including a pill, tablet, or capsule. The encapsulated rapamycin nanoparticles may be pressed or formed into a pellet-like shape and further encapsulated with a coating, for instance, an enteric coating. In another embodiment, the encapsulated rapamycin nanoparticles may be loaded into a capsule, also further enterically coated.

[0056] Various performance enhancing additives can be added to the encapsulated rapamycin nanoparticles. For example, additives that function as
free radical scavengers or stabilizers can be added to improve oxidative and storage stability of the encapsulated rapamycin nanoparticles. Free radical scavengers are preferably chosen from the group that consists of glycerol, propylene glycol, and other lower alcohols. Additives alternatively incorporate antioxidants, such as a-tocopherol (vitamin E), citric acid, EDTA, a-lipoic acid, or the like.

[0057] Methacrylic acid copolymers with methyl acrylate or methyl methacrylate are moderate oxygen barriers. Furthermore, these polymers will exhibit an equilibrium moisture content. Oxygen transport due to residual solvent, moisture or other causes, can lead to degradation of the encapsulated rapamycin nanoparticles. Oxygen barrier materials can be added to the encapsulated rapamycin nanoparticles formulation to improve oxygen barrier properties. Preferred oxygen barrier polymers compatible with the preferred polymers are polyvinyl alcohol (PVA) and gelatin.

PREFERRED MICROPARTICLE AND NANOPARTICLE EMBODIMENTS

[0058] Preferred embodiments with rapamycin nanoparticle inclusions comprise discrete nanoparticles of rapamycin heterogeneously dispersed in a controlled release matrix. As illustrated in accompanying drawings, the rapamycin nanoparticles are prepared by a suitable method and may contain additives to promote nanoparticle stability, modify rapamycin crystallinity, or promote compatibility of the rapamycin nanoparticles with the controlled release matrix. The controlled release matrix is formulated to promote release of rapamycin to specific parts of the body, such as the intestine, to enhance oxidative and storage stability of the encapsulated rapamycin nanoparticles, and to maintain the discrete, heterogeneously distributed nature of the rapamycin nanoparticles.
Rapamycin nanoparticles are preferably prepared by anti-solvent precipitation or solidification, also sometimes referred to as controlled precipitation or solidification. Antisolvent solidification is a preferred approach as it provides exquisite control of particle size and distribution, particle morphology, and rapamycin crystallinity. For example, it is possible to prepare nanoparticles with narrow particle size distribution that are amorphous, crystalline, or combinations thereof. Such properties may exhibit additional benefits, by further controlling the biodistribution and bioavailability of rapamycin in specific indications.

Rapamycin is dissolved in a suitable water-miscible solvent and then rapidly injected into rapidly stirred water containing an appropriate aqueous soluble dispersant. Water-miscible solvents for rapamycin include methanol, ethanol, isopropyl alcohol, acetone, dimethylsulfoxide, dimethylacetamide, n-methylpyrrolidone, tetrahydrofuran, and other solvents. Low boiling point, high vapor pressure water-miscible solvents are preferred to facilitate their removal during subsequent microparticle formation. Some preferred water-miscible solvents are methanol, acetone, and isopropyl alcohol. A preferred water-miscible solvent is methanol. Some aqueous soluble dispersants include ionic surfactants such as sodium dodecyl sulfate and sodium cholate, non-ionic surfactants such as Pluronics, Poloxomers, Tweens, and polymers, such as polyvinyl alcohol and polyvinylpyrrolidone. Some preferred aqueous-soluble dispersants are sodium cholate, Pluronic F-68, and Pluronic F-127. A preferred aqueous-soluble dispersant is sodium cholate, which provides surprisingly beneficial properties in the present application.

Not only is sodium cholate a surfactant and a dispersant, it serves to produce multimolecular structures which tend to cause aggregation of rapamycin.
within those structures, particularly when the pH and other condition of the aqueous solution are controlled to allow aggregation of the rapamycin from that aqueous solution. The resulting process allows for rapamycin nanoparticle production that not only tends to produce nanoparticles in highly predictable size ranges, but also provides a resulting nanoparticle with surprisingly desirable levels of colloidal stability. Moreover, while sodium cholate tends to be a polar molecule as well as an amphoteric surfactant, it induces an ionic charge in each hydrophilic nanoparticle when it is enmeshed in the EUDRAGIT® matrix. It is believed that when the nanoparticle is released from the EUDRAGIT® matrix within the animal subject's enteric passages where conditions are basic, the same properties cause the nanoparticle to be more readily received and absorbed through the intestinal walls.

[R0062] Rapamycin is dissolved in the water-miscible solvent 160 at a concentration of about 0.01 % w/v to about 10.0 % w/v preferably about 0.1 % w/v to about 1.0 % w/v. The aqueous-soluble dispersant 110 is dissolved in water at a concentration above its critical micelle concentration, or CMC, typically at about 1 to about 10 times the CMC. The rapamycin solution is injected into the aqueous-soluble dispersant solution with agitation at a volumetric ratio of about 1:10 to about 1:1, preferably about 1:5 to about 1:1.

[R0063] The controlled release matrix is prepared from a water-soluble polymer, preferably a copolymer of methacrylic acid with either methyl acrylate or methyl methacrylate, such as those marketed under the trade name of EUDRAGIT® and having pH-dependent dissolution properties. More preferably the controlled release matrix is comprised of EUDRAGIT® S 100, although other water-soluble enteric controlled release would be suitable. Water-soluble
controlled release matrices are selected so as either not to compromise the integrity of rapamycin nanoparticles or to provide a medium in which rapamycin nanoparticles may be prepared by the controlled precipitation methodology described previously.

[0064] In preparing the water-soluble polymer it is preferable to maintain conditions that do not compromise the integrity of the rapamycin nanoparticles. Firstly, since the rapamycin nanoparticles are susceptible to solubilization by certain co-solvents, it is important to maintain a suitable quantity of certain co-solvents to achieve controlled release matrix solubility while not deleteriously affecting the morphology of the rapamycin nanoparticles. Secondly, rapamycin nanoparticles will be susceptible to chemical degradation by high pH; therefore, it is important to modulate the controlled release matrix solution pH so that rapamycin is not chemically altered. It is preferable the controlled release matrix solution pH be maintained below about pH 8. Lastly, it is preferable to achieve near to complete solubilization of the controlled release matrix in solution so that microencapsulation of the rapamycin nanoparticles by the controlled release matrix in subsequent processing steps may proceed with high efficiency. When using the EUDRAGIT® S 100 as the controlled release matrix, it is preferable to achieve a controlled release matrix solution by using a combination of co-solvents and solution pH modulation. It is preferable the co-solvents be about 40% or less by volume. Similarly, it is preferable that the pH of the controlled release matrix solution be about 8 or less, such that the EUDRAGIT® S 100 is not completely neutralized and is preferably only about 80% or less neutralized. These preferred conditions achieve nearly complete to complete solubilization of the EUDRAGIT® S 100 in a medium that is mostly aqueous and that maintains the integrity of the
rapamycin nanoparticles, therefore leading to their microencapsulation by the controlled-release matrix in subsequent processing steps.

[0065] The rapamycin nanoparticles prepared by the preferred controlled precipitation method are added to the aqueous solution of the controlled released matrix, resulting in a nanoparticle dispersion in the solubilized controlled release matrix. Alternatively, the rapamycin solubilized in a suitable or preferred co-solvent can be dispersed into the aqueous solution of controlled release matrix leading to controlled precipitation of rapamycin particles, thereby leading to a rapamycin nanoparticle dispersion in fewer processing steps, but of appropriate composition to permit subsequent microencapsulation processing.

[0066] As an alternative embodiment, the encapsulated rapamycin nanoparticles are created using pre-existing nanoparticle substrates, such as albumin, to create, in the case of albumin, "albumin-rapamycin nanoparticles." Within this general class of alternatives, preferred approaches for creating the albumin-rapamycin nanoparticles involve encapsulating rapamycin within albumin nanoparticles or preferentially associating rapamycin with albumin nanoparticles through physical or chemical adsorption. The albumin nanoparticles themselves are preferably formed from human serum albumin, a plasma protein derived from human serum.

[0067] More particularly, this embodiment preferably involves use of a therapeutic peptide or protein that is covalently or physically bound to albumin, to enhance its stability and half-life. With the albumin stabilized, the rapamycin is mixed with the stabilized albumin in an aqueous solvent and passed under high pressure to form rapamycin-albumin nanoparticles in the size range of 100-200 nm (comparable to the size of small liposomes).
Preferred embodiments also address degradation risks and other limits imposed by the related art by preparing encapsulated rapamycin nanoparticles as a heterogeneous mixture of rapamycin nanoparticles in a polymer matrix. Distributed nanoparticles are morphologically different than homogeneous rapamycin and are less susceptible to degradation because of the bulk nature of the nanoparticles compared to the smaller size of molecular rapamycin.

Another alternative embodiment involves biodegradable polymers loaded with rapamycin. Biodegradable polymers loaded with drugs can be microparticles. "Microparticle" refers to particles between about 0.1 and 300 pm in size. Drug-loaded biodegradable polymers release drug in a time-dependent manner.

As used herein, "biodegradable" refers to any natural means by which a polymer can be disposed of in a patient's body. This includes such phenomena as, without limitation, biological decomposition, bioerosion, absorption, resorption, etc. Biodegradation of a polymer in vivo results from the action of one or more endogenous biological agents and/or conditions such as, without limitation, enzymes, microbes, cellular components, physiological pH, temperature and the like.

In some aspects, the biodegradable polymers can be poly-ε-caprolactone (PCL) microparticles. PCL is a biodegradable, biocompatible, and semicrystalline polymer. PCL is useful for drug delivery because it is highly permeable to many drugs and is non-toxic. Sinha et al. 2004. Rapamycin can also be loaded onto microparticles of other biodegradable polymers, including but not limited to aliphatic polyester, polylactide, polyglycolide, poly(lactide-co-
glycolide), mixtures thereof, and their copolymers. Such biodegradable polymers are known in the art.

[0072] Rapamycin may be loaded onto microspheres of PCL alone or of PCL copolymers or blends to obtain the desired drug release characteristics. Copolymers of PCL can be formed using many different monomers, including, but not limited to, ethylene oxide, polyvinyl chloride, chloroprene, polyethylene glycol, polystyrene, diisocyanates (urethanes), tetrahydrofuran (THF), diglycolide, dilactide, δ-valeractone, substituted caprolactones, 4-vinyl anisole, styrene, methyl methacrylate, and vinyl acetate.

[0073] Drug-loaded PCL microspheres can be prepared by several different methods known by persons of skill in the art, including, but not limited to, and o/w emulsion solvent extraction/evaporation method, a w/o/w emulsion solvent evaporation technique, a spray drying technique, a solution-enhanced dispersion method, and a hot melt technique. These methods are described in more detail in Sinha et al., 2004, which is hereby incorporated by reference. Briefly, as a non-limiting example, the o/w emulsion solvent extraction evaporation method can be performed by dissolving the required amount of polymer and drug in an organic phase, emulsifying under stirring with polyvinyl alcohol to form an o/w emulsion, stirring for 3 hours at about 500 rpm to evaporate the organic phase, and filtering and drying the formed microspheres.

[0074] Drug-loaded microspheres of aliphatic polyesters, polylactide, polyglycolide, and poly(lactide-co-glycolide) can be prepared by several different methods known by persons of skill in the art. Non-limiting examples can be found in the following references, all of which are hereby incorporated by reference: Kemala et al., 2012; Ghassemi et al., 2009; Corrigan & Heelan, 2001; Cleland et
[0075] In some aspects of this alternative embodiment, the microparticles loaded with rapamycin are encased, encapsulated, or coated to provide for release in the intestinal tract, including the colon.

[0076] In some aspects, the microparticles are coated with an enteric coating, which is a coating that prevents release and absorption of active ingredients until they reach the intestine. Some enteric coatings facilitate delivery of agents to the colon. In some embodiments, the enteric coating is a EUDRAGIT® coating. EUDRAGIT® coatings include EUDRAGIT® L 100-55, Poly(methacrylic acid-co-ethyl acrylate) 1:1; EUDRAGIT® L 30 D-55, Poly(methacrylic acid-co-ethyl acrylate) 1:1; EUDRAGIT® L-100, Poly(methacrylic acid-co-methyl methacrylate) 1:1; EUDRAGIT® S 100, Poly(methacrylic acid-co-methyl methacrylate) 1:2; EUDRAGIT® FS 30 D, Poly(methyl acrylate-co-methyl methacrylate-co-methacrylic acid) 7:3:1; EUDRAGIT® RL, Poly(ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride) 1:2:0.2; EUDRAGIT® RS, Poly(ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride) 1:2:0.1; and EUDRAGIT® E, Poly(butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate) 1:2:1. Other coatings include EUDRAGIT® RS, EUDRAGIT® RL, ethylcellulose, and polyvinyl acetate. Benefits include pH-dependent drug release, protection of active agents sensitive to gastric fluid, protection of gastric mucosa from active agents, increase in drug effectiveness, good storage stability, and GI and colon targeting, which minimizes risks associated with negative systemic effects and maintains effective dosing.
In some aspects, colon targeting of rapamycin can be achieved by creating PCL microparticles loaded with rapamycin or rapamycin analog and subsequently coating the microparticles with EUDRAGIT® S 100. Methods of making such coated microparticles can be found in Ghorab et al., 2011, which is hereby incorporated by reference. Briefly, drug-loaded PCL microparticles are suspended in a solution containing an appropriate amount of EUDRAGIT® S 100 dissolved in ethyl alcohol. The suspension is poured into distilled water. The resulting mixture is homogenized for five minutes and then mechanically stirred until the organic solvent is completely evaporated. Microparticles are collected, washed with cyclohexane twice, and dried overnight in a dessicator.

Some other examples of enteric coating components include cellulose acetate phthalate, methyl acrylate-methacrylic acid copolymers, cellulose acetate succinate, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose acetate succinate, polyvinyl acetate phthalate, methyl methacrylate-methacrylic acid copolymers, sodium alginate, and stearic acid. The coating may include suitable hydrophilic gelling polymers including, but not limited to, cellulosic polymers, such as methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, and the like; vinyl polymers, such as polyvinylpyrrolidone, polyvinyl alcohol, and the like; acrylic polymers and copolymers, such as acrylic acid polymer, methacrylic acid copolymers, ethyl acrylate-methyl methacrylate copolymers, natural and synthetic gums, such as guar gum, arabic gum, xanthan gum, gelatin, collagen, proteins, polysaccharides, such as pectin, pectic acid, alginic acid, sodium alginate, polyaminoacids, polyalcohols, polyglycols, and the like; and mixtures
thereof. Any other coating agent known to those of ordinary skill in the art is contemplated for inclusion in the coatings of the microcapsules set forth herein.

[0079] The coating may optionally comprise a plasticizer, such as dibutyl sebacate, polyethylene glycol and polypropylene glycol, dibutyl phthalate, diethyl phthalate, triethyl citrate, tributyl citrate, acetylated monoglyceride, acetyl tributyl citrate, triacetin, dimethyl phthalate, benzyl benzoate, butyl and/or glycol esters of fatty acids, refined mineral oils, oleic acid, castor oil, corn oil, camphor, glycerol and sorbitol or a combination thereof. The coating may optionally include a gum. Non-limiting examples of gums include homopolysaccharides such as locust bean gum, galactans, mannans, vegetable gums such as alginates, gum karaya, pectin, agar, tragacanth, acacia, carrageenan, chitosan, alginic acid, other polysaccharide gums (e.g., hydrocolloids), acacia catechu, salai guggal, indian bodellum, sopaiba gum asafetida, cambi gum, Enterolobium cyclocarpum, mastic gum, benzoin gum, sandarac, gambier gum, butea frondosa (Flame of Forest Gum); myrrh, konjac mannan, guar gum, welan gum, gellan gum, tara gum, locust bean gum, carrageenan gum, glucomannan, galactan gum, sodium alginate, xanthan gum deacetylated xanthan gum, pectin, sodium polypectate, gluten, karaya gum, tamarind gum, ghatti gum, Acaroid/Yacca/Red gum, dammar gum, juniper gum, ester gum, ipil-ipil seed gum, gum talha (acacia seyal), and cultured plant cell gums including those of the plants of the genera: acacia, actinidia, aperenia, carbobrotus, chickorium, cucumis, glycine, hibiscus, hordeum, letuca, lycopersicon, malus, medicago, mesembryanthemum, oryza, panicum, phalaris, phleum, poliathus, polycarbophil, sida, solanum, trifolium, trigonella, Afzelia africana seed gum, Treculia africana gum, detarium gum, cassia gum, carob gum,
Prosopis africana gum, Colocassia esulenta gum, Hakea gibbosa gum, khaya gum, scleroglucan, zea, mixtures of any of the foregoing, and the like.

[0080] A variety of other encasing materials and systems for delivering rapamycin-loaded biodegradable microspheres to the colon can be used alone or in combination with a pH-dependent coating like EUDRAGIT® S 100. Non-limiting examples follow.

[0081] Hydrophilic gelling polymers or copolymers can be included in a material encasing one or more microspheres to provide a time-dependent release of drug-loaded microspheres. Non-limiting examples of hydrophilic gelling copolymers include methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carbomers, polyvinyl alcohols, polyoxyethylene glycols, polyvinylpyrrolidones, poloxamers, or natural or synthetic rubbers. An intermediate layer of these polymers can be included to delay release of active ingredient for a desired amount of time, as described in Poli et al., (EP0572942). Another example of a time-dependent encasing material is a wax matrix including, for example, behenic acid, as described in Otuska & Matsuda, 1994.

[0082] Polysaccharides that are resistant to digestive enzymes but are enzymatically broken down by bacteria in the colon can be included in an encasing material. Non-limiting examples include chitosan and pectin as described in Coulter (EP2380564), and azopolymers, disulfide polymers, amylose, calcium pectinate, and chondroitin sulfate as described in Watts (EP0810857).

[0083] A starch capsule coated with an enteric coating such as EUDRAGIT® S 100 or EUDRAGIT® L 100 may be used, as described in Watts (EP0180857). A variety of starches, including modified starches and starch
derivatives may be used. Non-limiting examples include hydroxyethyl starch, hydroxypropyl starch, carboxymethyl starch, cationic starch, acetylated starch, phosphorylated starch, succinate derivatives, or grafted starches.

[0084] A layer of insoluble or relatively insoluble rupturable polymer can be used as part of a strategy to provide for abrupt release of drug-loaded microspheres in the colon. The rupturable polymer can comprise one or more of a variety of suitable polymers known by those of skill in the art, including, but not limited to, cellulose acetate, cellulose acetate propionate, or ethyl cellulose. A variety of strategies for causing rupture of the polymer in the colon can be employed. As a non-limiting example, the rupturable polymer can be designed to rupture upon encountering increased pressure due to intestinal peristalsis, as described in Muraoka et al., 1998. As another example, the rupturable polymer can be semi-permeable, and an effervescent solid can be included in a core containing the drug-loaded microparticles, as described in Krogel & Bodmeier, 1999. As another example, a layer of swellable material, including, but not limited to, croscarmellose sodium or hydroxypropylmethyl cellulose, can be disposed within the rupturable polymer layer, as described in Bussemer et al., 2001. Controlled entry of water past the rupturable polymer layer can be provided by embedded hydrophilic particulate material, as described in Lerner et al., (WIPO Pub. No. WO 1999/018938).

[0085] A two-piece encasing system, as described in McNeill et al., (WIPO Pub. No. WO 1990/009168) can be used to provide for release of drug-loaded microspheres in the colon. One of the pieces is a relatively water insoluble capsule with an open orifice, which is covered by a second piece that swells as it
takes up water. The swelling causes displacement from the orifice and release of the capsule contents.

EXAMPLES OF PREFERRED MTOR INHIBITING PREPARATIONS

Example 1 - Development of methods to produce rapamycin nanoparticles. Rapid solvent exchange was used to examine the formation of rapamycin nanoparticles. Three water-miscible solvents 160 and three water-soluble surfactants were selected to study their respective effects on the formation and morphology of rapamycin nanoparticles. The water-miscible solvents 160 were isopropyl alcohol (Solvent 1), acetone (Solvent 2), and methanol (Solvent 3). The water-soluble surfactants were Pluronic F-68 (Dispersant 1, a non-ionic PEO-PPO-PEO block copolymer), Pluronic F-127 (Dispersant 2, a non-ionic PEO-PPO-PEO block copolymer), and sodium cholate (Dispersant 3, an anionic surfactant). Rapamycin was dissolved in each of the water-miscible solvents 160 at a concentration of 0.25% w/v. The water-soluble surfactants were dissolved in deionized water at concentrations of 0.5% w/v, 0.5% w/v, and 1.0% w/v, respectively, for each of the dispersants. Each experimental combination (e.g. NP-1 to NP-9 in following table) consisted of 5mL of rapamycin solution and 25ml_ of surfactant solution, resulting in a dilution factor of 1:5 solventwater. 25ml_ of surfactant solution was transferred to a 50ml_ beaker and stirred with the aid of magnetic micro stir bar. Rapamycin solution was rapidly injected at 500µL increments with the aid of a micropipette with the pipette tip placed below the surface of the rapidly stirred surfactant solution. The visual appearance of the resulting nanoparticles and their colloidal stability after 24-hours were qualitatively assessed. The following table summarizes the qualities of the rapamycin nanoparticle dispersions. Qualitatively, rapamycin nanoparticle dispersions
having a colorless to blue, opalescent appearance will have particle sizes on the order of less than about 300nm as evidenced by their interaction with the ultraviolet wavelengths of visible light. Whereas, dispersions having a more white appearance will have particle sizes larger than about 300nm due to their interaction with the broader spectrum of visible light. Rapamycin nanoparticle formulations NP-7 and NP-9 were selected as preferred methods of nanoparticle preparation.

<table>
<thead>
<tr>
<th>Solvent 1</th>
<th>Dispersant 1</th>
<th>Dispersant 2</th>
<th>Dispersant 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent 1</td>
<td>NP-1 : White, settled, resdispensible</td>
<td>NP-2 : Blue, opalescent, settled, redispensible</td>
<td>NP-3 : Clear, aggregated, redispensible</td>
</tr>
<tr>
<td>Solvent 2</td>
<td>NP-4 : Blue, opalescent, some settling</td>
<td>NP-5 : White, settled, redispensible</td>
<td>NP-6 : Blue, opalescent, settled, redispensible</td>
</tr>
<tr>
<td>Solvent 3</td>
<td>NP-7 : Blue, opalescent, stable</td>
<td>NP-8 : Blue to white, settled, redispensible</td>
<td>NP-9 : Blue, opalescent, stable</td>
</tr>
</tbody>
</table>

[0087] Example 2 - Preparation of a high concentration rapamycin nanoparticle dispersion. The water-miscible solvent 160 and water-soluble dispersant 110 of NP-9 from Example 1 was used to prepare rapamycin nanoparticles. 656mg of rapamycin were dissolved in 6.56mL of Solvent 3 to yield a 1.0% w/v solution. This volume of rapamycin solution was injected into 26.25mL of 1.0% w/v Dispersant 1 in deionized water. The resulting rapamycin nanoparticle dispersion had a final rapamycin content of 2.4% w/w. The particle
size of the dispersion was determined by dynamic light scattering to be 230nm ± 30nm with a single peak.

[0088] Example 3 - Preparation of a water-soluble enteric coating. 3.5g of EUDRAGIT® S 100 were added to 70mL of deionized water with light stirring, resulting in a white dispersion. 1.4g of sodium hydroxide were added to the dispersion with continued stirring. The resulting dispersion gradually turned clear and colorless indicating an aqueous solution of S-100. The estimated concentration of sodium hydroxide was 0.5N.

[0089] Example 4 - Preparation of a feedstock containing rapamycin nanoparticles and a water-soluble enteric coating. Rapamycin nanoparticles were prepared as described in Example 2 and then slowly added to an aqueous solution of EUDRAGIT® S 100 prepared as in Example 3. The ratio of rapamycin to EUDRAGIT® S 100 was 1:9, or 10% wt. rapamycin payload. The resulting dispersion was allowed to stir for several minutes to observe stability. After one hour, the dispersion had transformed to a clear yellow, indicating destruction of the rapamycin nanoparticles and a change in the rapamycin. Addition of a small amount of acetic acid to reduce the solution pH to below neutral resulted in a clear, colorless solution.

[0090] Example 5 - Preparation of water-soluble enteric coating with a water-miscible co-solvent. 3.5g of EUDRAGIT® S 100 were added to 30/70 v/v methanol/deionized water, resulting in a white dispersion. The dispersion was stirred continuously until a clear solution was formed.

[0091] Example 6 - Preparation of a feedstock containing rapamycin nanoparticles and a water-soluble enteric coating. Rapamycin nanoparticles were prepared as described in Example 2 and then slowly added to an aqueous
solution of EUDRAGIT® S 100 prepared as in Example 5. The ratio of rapamycin to S 100 was 1:9, or 10% wt. rapamycin payload. The white dispersion was allowed to stir for several minutes after which the dispersion was transformed into a clear solution indicating the rapamycin nanoparticles had been destroyed.

Example 7 - Preparation of a partially-neutralized, water-soluble enteric coating with a water-miscible co-solvent. 3.5g of EUDRAGIT® S 100 were added to 10/90 v/v methanol/deionized water, resulting in a white dispersion. The dispersion was titrated to clarity with 2.000mL of 4.8M sodium hydroxide. The estimated neutralization of the S-100 was 78%.

Example 8 - Preparation of a feedstock containing rapamycin nanoparticles and a water-soluble enteric coating. Rapamycin nanoparticles were prepared as described in Example 2 then slowly added to an aqueous solution of EUDRAGIT® S 100 as prepared in Example 7. The ratio of rapamycin to EUDRAGIT® S 100 was 1:9, or 10% wt. rapamycin payload. The resulting white dispersion remained stable for several hours as indicated by no change in color or change in optical clarity. The final pH was 7.5. The particle size of the final dispersion was determined by dynamic light scattering to be 756nm ± 52nm with a single peak and indicating possible clustering of the rapamycin nanoparticles in the resulting feedstock.

Example 9 - Preparation of a feedstock containing rapamycin nanoparticles and a water-soluble enteric coating. The rapamycin solution used in Example 2 was injected, with stirring, into the aqueous solution of EUDRAGIT® S 100 prepared in Example 7. The ratio of rapamycin to EUDRAGIT® S 100 was 1:9, or 10% wt. rapamycin payload. A blue, opalescent colloid was formed and it remained stable for several hours as indicated by no change in color or change in
optical clarity. The final pH was 7.5. The particle size of the final dispersion was determined by dynamic light scattering to be 305nm ± 60nm with a single peak.

[0095] Example 10 - Spray drying of feedstock containing rapamycin nanoparticles and a water-soluble enteric coating. The feedstocks prepared in Examples 8 and 9 were spray dried and analyzed for rapamycin content. Particles prepared from Example 8 had a rapamycin content of 9.5% wt. (87% rapamycin yield). Particles prepared from Example 9 had a rapamycin content of 7.9% wt. (80% rapamycin yield).

[0096] Example 11 - Storage stability of enteric-coated encapsulated rapamycin nanoparticles. Microparticles prepared by spray drying in Example 10 were stored under controlled conditions at room temperature and 50% relative humidity. Samples were analyzed weekly for rapamycin content. All samples maintained at least 95% of their original rapamycin content at all time points for at least three weeks.

[0097] Example 12 - Preparation of nanoparticles in EUDRAGIT® S 100 as illustrated in Figs. 4A & 4B. A rapamycin solution was prepared by combining rapamycin with methanol (at Steps 402 & 404) in a 10% w/v ration as 3.03g rapamycin and 30.25ml methanol. A 1% w/w sodium cholate solution was prepared by combining 1.2g sodium cholate with 120ml deionized water as shown in Step 424. Nanoparticle formation was achieved by transferring the rapamycin solution with a 60ml plastic syringe equipped with a 20ga needle, injecting the rapamycin solution below the surface of the sodium cholate solution in a 250 ml beaker (Steps 406 & 408). Mixing was accomplished with a paddle mixer operating at 300rpm yielding a rapamycin nanoparticle suspension. At Step 410, a 10% w/w EUDRAGIT® S 100 solution was prepared by combining 20g
EUDRAGIT® S 100 in a 9.7% w/v mixture with 180mL deionized water, 25.72ml methanol in a 12.5% v/v mixture, and 1.8g sodium cholate in a 0.875% w/v mixture. This 10% w/w EUDRAGIT® S 100 solution was titrated with 4M sodium hydroxide to achieve a pH of between about 7.5 and about 7.6. Encapsulated rapamycin particles were then fabricated by combining the EUDRAGIT® S 100 solution with the rapamycin nanoparticle suspension at Step 412. The EUDRAGIT® S 100 solution and the rapamycin nanoparticle suspension were combined in a 500ml bottle, adding 2.13g of glycerol and mixing with a magnetic stir bar. The combined EUDRAGIT® S 100 solution and rapamycin nanoparticle suspension were then spray dried and collected. The spray drying parameters (shown at Step 418) included a 0.4mm nozzle, nozzle air pressure of 3bar, input air temperature of 110°C, a sample pump rate of 5mL/min and an air speed of 0.30 m3/min. After the preferred nanoparticle microencapsulation process is complete, the nanoparticles may then be graded and sorted according to the desired size range at Step 414. Alternatively, the resulting dispersion of rapamycin nanoparticles in the controlled release matrix solution can be reduced to a dry particulate powder by a suitable process, thereby resulting in microparticles of a heterogeneous nature comprised of rapamycin nanoparticles randomly distributed in the controlled release matrix. This dry particulate powder can then be combined with excipients and pressed into tablet form as indicated at Step 420.

METHODS OF USING RAPAMYCIN COMPOSITIONS

"Treatment" and "treating" refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit for a disease or health-
related condition. For example, the rapamycin compositions of the present invention may be administered to a subject for the purpose of treating or preventing intestinal adenomas or polyps and cancer in a patient who has been identified as being at risk for developing intestinal polyps or intestinal cancer.

[0099] The terms "therapeutic benefit," "therapeutically effective," or "effective amount" refer to the promotion or enhancement of the well-being of a subject. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease.

[0100] "Prevention" and "preventing" are used according to their ordinary and plain meaning. In the context of a particular disease or health-related condition, those terms refer to administration or application of an agent, drug, or remedy to a subject or performance of a procedure or modality on a subject for the purpose of preventing or delaying the onset of a disease or health-related condition. For example, one embodiment includes administering the rapamycin compositions of the present invention to a subject at risk of developing intestinal polyps and cancer (e.g., a patient who has been diagnosed with FAP) for the purpose of preventing intestinal polyps and cancer.

[0101] Rapamycin compositions, as disclosed herein, including preferably encapsulated rapamycin nanoparticles, may be used to prevent, treat, delay or reduce any disease or condition (or its precursors or sequelae) for which an inhibitor of mTOR is contemplated as effective for treatment, prevention, or delaying or reducing its progression. For example, methods are disclosed herein of using rapamycin compositions to treat or prevent diseases or conditions which a patient has been identified as being at risk for developing, including: intestinal polyps or intestinal cancer, such as colorectal cancer or FAP; vascular cognitive
impairment; canine hemolytic anemia; and feline chronic gingivostomatitis (FCGS) and other gum and gingival diseases.

[0102] Other uses of rapamycin compositions, as disclosed herein, including preferably encapsulated rapamycin nanoparticles, are also contemplated. For example, U.S. Pat. No. 5,100,899 discloses inhibition of transplant rejection by rapamycin; U.S. Pat. No. 3,993,749 discloses rapamycin antifungal properties; U.S. Pat. No. 4,885,171 discloses antitumor activity of rapamycin against lymphatic leukemia, colon and mammary cancers, melanocarcinoma and ependymoblastoma; U.S. Pat. No. 5,206,018 discloses rapamycin treatment of malignant mammary and skin carcinomas, and central nervous system neoplasms; U.S. Pat. No. 4,401,653 discloses the use of rapamycin in combination with other agents in the treatment of tumors; U.S. Pat. No. 5,078,999 discloses a method of treating systemic lupus erythematosus with rapamycin; U.S. Pat. No. 5,080,899 discloses a method of treating pulmonary inflammation with rapamycin that is useful in the symptomatic relief of diseases in which pulmonary inflammation is a component, i.e., asthma, chronic obstructive pulmonary disease, emphysema, bronchitis, and acute respiratory distress syndrome; U.S. Pat. No. 6,670,355 discloses the use of rapamycin in treating cardiovascular, cerebral vascular, or peripheral vascular disease; U.S. Pat. No. 5,561,138 discloses the use of rapamycin in treating immune related anemia; U.S. Pat. No. 5,288,711 discloses a method of preventing or treating hyperproliferative vascular disease including intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion with rapamycin; and U.S. Pat. No. 5,321,009 discloses the use of rapamycin in treating insulin dependent diabetes mellitus.

PHARMACEUTICAL PREPARATIONS
Certain methods and compositions set forth herein are directed to administration of an effective amount of a composition comprising the rapamycin compositions of the present invention.

1. Compositions

A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (Remington's, 1990). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated. The compositions used in the present invention may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it needs to be sterile for such routes of administration as injection.

The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions, and these are discussed in greater detail below. For human administration, preparations should meet sterility, pyrogenicity, and general safety and purity standards as required by FDA Office of Biologies standards.
The formulation of the composition may vary depending upon the route of administration. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure.

In addition to the compounds formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g., tablets or solids for oral administration; liposomal and nanoparticle formulations; enteric coating formulations; time release capsules; formulations for administration via an implantable drug delivery device; and any other form. One may also use nasal solutions or sprays, aerosols or inhalants in the present invention.

The capsules may be, for example, hard-shell capsules or soft-shell capsules. The capsules may optionally include one or more additional components that provide for sustained release.

In certain embodiments, pharmaceutical composition includes at least about 0.1% by weight of the active compound. In other embodiments, the pharmaceutical composition includes about 2% to about 75% of the weight of the composition, or between about 25% to about 60% by weight of the composition, for example, and any range derivable therein.

The compositions may comprise various antioxidants to retard oxidation of one or more components. Additionally, the prevention of the action of microorganisms can be accomplished by preservatives such as various antibacterial and antifungal agents, including, but not limited to, parabens (e.g.,
methylparabens, propylparabens), chlorobutanol, phenol, scorbic acid, thimerosal or combinations thereof. The composition should be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0111] In certain preferred embodiments, an oral composition may comprise one or more binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, EUDRAGIT® Acrylic Drug Deliver Polymers, or any combination thereof.

[0112] In particular embodiments, prolonged absorption can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin, EUDRAGIT® Acrylic Drug Deliver Polymers or combinations thereof.

2. Routes of Administration

[0113] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

[0114] The composition can be administered to the subject using any method known to those of ordinary skill in the art. For example, a pharmaceutically effective amount of the composition may be administered intravenously, intracerebrally, intracranially, intraventricularly, intrathecally, into the cortex, thalamus, hypothalamus, hippocampus, basal ganglia, substantia nigra or
the region of the substantia nigra, cerebellum, intradermal, intraarterially, intraperitoneally, intralesionally, anally, subcutaneously, orally, topically, locally, by inhalation (e.g., aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in creams, in lipid compositions (e.g., liposomes), or by other method or any combination of the foregoing as would be known to one of ordinary skill in the art (Remington's, 1990).

[0115] In particular embodiments, the composition is administered to a subject using a drug delivery device. Any drug delivery device is contemplated for use in delivering an effective amount of the inhibitor of mTOR or mTOR Complex 1 (mTORC1).

3. Dosage

[0116] A pharmaceutically effective amount of an inhibitor of mTORC1 is determined based on the intended goal. The quantity to be administered, both according to number of treatments and dose, depends on the subject to be treated, the state of the subject, the protection desired, and the route of administration. Precise amounts of the therapeutic agent also depend on the judgment of the practitioner and are peculiar to each individual.

[0117] The amount of rapamycin or rapamycin analog or derivative to be administered will depend upon the disease to be treated, the length of duration desired and the bioavailability profile of the implant, and site of administration. Generally, the effective amount will be within the discretion and wisdom of the
patient’s physician. Guidelines for administration include dose ranges of from about 0.01 mg to about 500 mg of rapamycin or rapamycin analog.

For example, a dose of the inhibitor of mTORCl may be about 0.0001 milligrams to about 1.0 milligrams, or about 0.001 milligrams to about 0.1 milligrams, or about 0.1 milligrams to about 1.0 milligrams, or even about 10 milligrams per dose or so. Multiple doses can also be administered. In some embodiments, a dose is at least about 0.0001 milligrams. In further embodiments, a dose is at least about 0.001 milligrams. In still further embodiments, a dose is at least 0.01 milligrams. In still further embodiments, a dose is at least about 0.1 milligrams. In more particular embodiments, a dose may be at least 1.0 milligrams. In even more particular embodiments, a dose may be at least 10 milligrams. In further embodiments, a dose is at least 100 milligrams or higher.

In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5
microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

[0120] The dose can be repeated as needed as determined by those of ordinary skill in the art. Thus, in some embodiments of the methods set forth herein, a single dose is contemplated. In other embodiments, two or more doses are contemplated. In some embodiments, the two or more doses are the same dosage. In some embodiments, the two or more doses are different dosages. Where more than one dose is administered to a subject, the time interval between doses can be any time interval as determined by those of ordinary skill in the art. For example, the time interval between doses may be about 1 hour to about 2 hours; about 2 hours to about 6 hours; about 6 hours to about 10 hours; about 10 hours to about 24 hours; about 1 day to about 2 days; about 1 week to about 2 weeks, or longer, or any time interval derivable within any of these recited ranges. In specific embodiments, the composition may be administered daily, weekly, monthly, annually, or any range therein.

[0121] Doses for encapsulated rapamycin (enteric-coated rapamycin) and for encapsulated rapamycin nanoparticles may be different. According to preferred embodiments of the present invention, doses are contemplated in a range of more than 50 micrograms and up to (or even exceeding) 200 micrograms per kilogram for daily administration, or the equivalent for other frequencies of administration. Although dosing may vary based on particular needs and preferred treatment protocols according to physician preference, maximum tolerable daily bioavailable dosings (trough levels) for a 28-day duration are about 200 micrograms of rapamycin (or equivalent) per subject kilogram, for both human and canine subjects, although those of ordinary skill would understand that greater
dose amounts ranges would be tolerable and suitable when administered less often than once per day, and lesser ranges would be tolerable when administered more often than once per day.

[0122] In certain embodiments, it may be desirable to provide a continuous supply of a pharmaceutical composition to the patient. This could be accomplished by catheterization, followed by continuous administration of the therapeutic agent. The administration could be intra-operative or post-operative.

4. Secondary and Combination Treatments

[0123] Certain embodiments provide for the administration or application of one or more secondary or additional forms of therapies. The type of therapy is dependent upon the type of disease that is being treated or prevented. The secondary form of therapy may be administration of one or more secondary pharmacological agents that can be applied in the treatment or prevention of, for example, intestinal polyps or cancer or a disease, disorder, or condition associated with intestinal polyps and cancer in a patient who has been identified as being at risk for developing intestinal polyps or intestinal cancer. Other secondary forms of therapy may be administration of one or more secondary pharmacological agents that can be applied in the treatment or prevention of vascular cognitive impairment or a disease, disorder, or condition associated with vascular pathology or vascular cognitive impairment. For example, the secondary or additional form of therapy may be directed to treating high blood pressure, high cholesterol, high blood sugar (or diabetes), an autoimmune disease, an inflammatory disease, a cardiovascular condition, or a peripheral vascular condition.
If the secondary or additional therapy is a pharmacological agent, it may be administered prior to, concurrently, or following administration of the inhibitor or mTORC1.

The interval between administration of the inhibitor of mTORC1 and the secondary or additional therapy may be any interval as determined by those of ordinary skill in the art. For example, the inhibitor of mTORC1 and the secondary or additional therapy may be administered simultaneously, or the interval between treatments may be minutes to weeks. In embodiments where the agents are separately administered, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that each therapeutic agent would still be able to exert an advantageously combined effect on the subject. For example, the interval between therapeutic agents may be about 12 hours to about 24 hours of each other and, more preferably, within about 6 hours to about 12 hours of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations. In some embodiments, the timing of administration of a secondary therapeutic agent is determined based on the response of the subject to the inhibitor of mTORC1.

5. Kits

Kits are also contemplated as being used in certain aspects of the present invention. For instance, a rapamycin composition of the present invention can be included in a kit. A kit can include a container. Containers can include a bottle, a metal tube, a laminate tube, a plastic tube, a dispenser, a pressurized container, a barrier container, a package, a compartment, or other types of
containers such as injection- or blow-molded plastic containers into which the hydrogels are retained. The kit can include indicia on its surface. The indicia, for example, can be a word, a phrase, an abbreviation, a picture, or a symbol.

[0127] Further, the rapamycin compositions of the present invention may also be sterile, and the kits containing such compositions can be used to preserve the sterility. The compositions may be sterilized via an aseptic manufacturing process or sterilized after packaging by methods known in the art.

GENERAL USES OF THE ORAL MTOR PREPARATIONS

[0128] When orally administered daily, or at other regular frequencies, in correspondingly effective doses, pharmaceutical preparations prepared according to the foregoing descriptions, and their equivalents, are effective for preventing and treating various maladies in humans and other animals, and for reducing the progression of those maladies and their sequelae. For example, such oral administration enables a human subject or his/her caregiver to prevent or treat various cancer conditions and neurological conditions, and precursors and sequelae thereof in humans.

[0129] Preferably, preparations according to the preferred embodiments are administered at a regular frequency, preferably at frequencies varying from three times per week (either on three consecutive days, or on three regularly distributed days of the week).

[0130] Although dosing may vary based on particular needs and preferred treatment protocols according to physician preference, maximum tolerable daily bioavailable dosings (trough levels) for a 28-day duration are about 200 micrograms of rapamycin (or equivalent) per subject kilogram, for both human and canine subjects, although those of ordinary skill would understand that greater
dose amount ranges would be tolerable and suitable when administered less often than once per day, and lesser ranges would be tolerable when administered more often than once per day.

[0131] Whereas prior art uses of rapamycin may have involved recommended daily dosings of roughly 13 micrograms per kilogram in human subjects, oncology protocols according to preferred embodiments of the present invention use higher dosings than the prior art, preferably in a range of more than 50 micrograms and up to (or even exceeding) 200 micrograms per kilogram for daily administration, or the equivalent for other frequencies of administration. Other conditions addressed by oral administration protocols of the present invention include preventing and treating gingival diseases in humans, dogs and cats, whether through the preferred preparations of rapamycin (or the equivalent) or through combination therapies with stem cell therapy and/or other active pharmaceutical or botanical treatment protocols.

[0132] In contrast to oncology-related dosings, preferred protocols for oral administration of the preparations taught herein when used for prevention and treatment of targeted neurological conditions, and reducing the progression thereof, use lower dosings than the prior art. Such lower dosings are preferably about 5 micrograms of bioavailable rapamycin (or the equivalent) per daily oral dose, and such dosings otherwise more generally fall in the preferred range of between 1 and 7 micrograms per kilogram for once-daily administration, or the equivalent for other frequencies of administration.

[0133] Although various neurological indications are targeted in alternative embodiments, preferred embodiments of oral administration protocols according to the present invention are used for preventing and treating, and reducing the
progression of Alzheimer’s disease, pre-Alzheimer’s disease, vascular dementia and other variations of cognitive impairment in general, in humans, canines, felines and other animal subject types. Other neurologic conditions for which embodiments of the present invention are thought to be effective also include the treatment and therapy for traumatic brain injury and traumatic spinal cord injury as well as the prevention and delay of their advancement and sequelae. Such embodiments include preventing and treating anxiety disorders in canines and felines, as well as reducing the progression of neurological impairment in human subjects exhibiting indications related to Alzheimer’s disease, vascular dementia, or precursors to onset of Alzheimer’s disease.

SPECIFIC USES OF ORAL MTOR PREPARATIONS

[0134] The following disclosures describe uses of oral mTOR preparations for specific maladies, and the teachings of the present invention contemplate use of microencapsulated rapamycin nanoparticle preparations for these same purposes. In combination with background information regarding these maladies are specific example descriptions as observed by the inventors and their collaborators.

A. Intestinal Cancer and Familial Adenomatous Polyposis (FAP)

[0135] Particularly beneficial results are appreciated through oral administration in the prevention and treatment of familial adenomatous polyposis (FAP), as well as colon cancer and other sequelae of FAP, particularly in human subjects who are identified as being genetically predisposed to develop FAP. Particular benefits are also appreciated in reducing and preventing the progression of FAP and in preventing or delaying the need for colonic resection which is often required before the age of 25 years in humans with FAP.
1. Intestinal Cancer

Intestinal cancer encompasses a variety of cancers, including cancer of the small intestine, gastric cancer, and colorectal cancer. Symptoms of intestinal cancer often include, but are not limited to, pain throughout the body, unexplained weight loss, pain or cramping in the middle of the abdomen, a lump in the abdomen, blood in the stool, nausea, bloating, iron-deficient anemia, and jaundice. Historically, the most common treatment of intestinal cancer is surgery and radiation therapy.

Intestinal cancer is more likely to occur in some patients than others. For example, intestinal cancer is more likely to occur in a patient that has been diagnosed with an inflammatory bowel disease, an intestinal polyp or an adenoma, familial adenomatous polyposis (FAP), or as having a mutation which is known to cause increased WNT signaling. In other embodiments, the patient has a family history of intestinal polyps or intestinal cancer.

Small intestine cancer can be further divided into a variety of subtypes, including cancer of the jejunum and ileum, duodenal cancer, adenocarcinoma, gastrointestinal stromal tumors, lymphoma, and ileal carcinoid tumors. Adenocarcinoma is a type of cancer that begins in the lining of the small intestine, and makes up 40-50% of all small intestinal cancers. This type of intestinal cancer occurs most often later in life. People with Crohn's Disease and certain other inherited conditions such as familial adenomatous polyposis (FAP) and Peutz-Jeghers Syndrome are at higher risk of developing adenocarcinomas. Carcinoid tumors occur when neuroendocrine cells grow abnormally, and may also be referred to as neuroendocrine tumors or neuroendocrine cancer. People with a family history of multiple endocrine neoplasia or a family history of
neurofibromatosis are more likely to get carcinoid tumors. Carcinoid tumors are also more common among women, African Americans, and people with certain diseases that damage the stomach and reduce the amount of stomach acid. Gastrointestinal stromal tumors start in the interstitial cells of Cajal (ICCs) in the walls of the GI tract. It is believed that a family history of neurofibromatosis or familial gastrointestinal stromal tumor syndrome will increase a patient's risk of getting stromal tumors. Gastrointestinal lymphomas are a cancer of the lymphatic system that begins in the lymphoid tissue. It is believed that old age, genetic risk factors that cause abnormal function of the immune system, a diet high in animal fat and low in fruits and vegetables, exposure to radiation and certain chemicals, immune deficiencies, and some infections increase the likelihood of a lymphoma developing.

Colorectal cancer, commonly also known as colon cancer or bowel cancer, is a cancer from uncontrolled cell growth in the colon, rectum, or appendix. The majority of colorectal cancers are due to lifestyle and increasing age, but some are associated with an underlying genetic disorder. For example, people with inflammatory bowel disease (ulcerative colitis and Crohn's Disease) are at increased risk for developing colon cancer. Those with a family history of colorectal cancer in two or more first-degree relatives have a two to threefold greater risk of disease, and a number of genetic syndromes are also associated with higher rates of colorectal cancer. The most common of these is hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome) which is present in about 3% of people with colorectal cancer. Other syndromes that are strongly associated include: Gardner syndrome and FAP.
Gastric cancer refers to cancer arising from any part of the stomach, and is often either asymptomatic or causes only nonspecific symptoms in its early stages. Infection by Helicobacter pylori is believed to be the cause of most stomach cancer while autoimmune atrophic gastritis, intestinal metaplasia, and various genetic factors are associated with increased risk levels. A very important but preventable cause of gastric cancer is tobacco smoking. Gastric cancers due to smoking mostly occur in the upper part of the stomach near the esophagus.

2. Familial Adenomatous Polyposis (FAP)

Familial adenomatous polyposis (FAP) is an autosomal dominant disease caused by mutation of the adenomatous polyposis coli (APC) gene, located on chromosome 5 (Kinzler, 1991). This germ line defect accelerates the initiation of the adenoma—carcinoma, resulting in the development of numerous adenomatous colorectal polyps at a young age. Polyposis inevitably progresses to colorectal cancer if left untreated. Given the predictable development of colorectal cancer in patients with FAP, the safest preventative strategy is surgical resection of the colon when polyposis develops. The two main prophylactic surgeries are colectomy with ileorectal anastomosis (IRA) and proctocolectomy with ileal pouch-anal anastomosis (IPAA) (Vasen, 2008). Genetic screening and endoscopy in concert with prophylactic surgery significantly improved the overall survival of FAP patients. A pharmacological prophylactic approach to prevent these outcomes for this population of patients is obviously in great need.

However, less well appreciated is the second leading cause of death in FAP, duodenal adenocarcinoma. Nearly 90% of patients with FAP develop duodenal polyps, the precursor lesions of duodenal adenocarcinoma and 4.5% will develop duodenal adenocarcinoma in their lifetime (Wallace, 1998; Bulow, 2004).
In contrast to the colon, prophylactic surgical resection of the ampulla and/or duodenum is accompanied by significant morbidity. Duodenal surgery is currently indicated for patients with severe duodenal polyposis or duodenal carcinoma. This patient population has a strong need for adjuvant therapies to surgery to prevent or reduce the polyp formation and carcinogenesis in the gastrointestinal tract.

3. WNT Signaling Pathway

WNTs comprise a family of 19 secreted glycoproteins which function in diverse biological processes such as cell proliferation, survival and segment polarity during development (Anastas, 2013). WNTs signal via transmembrane receptors included in 10 member of the frizzled (FZD) family of G-protein coupled receptors and receptor tyrosine kinases. The first WNT gene was identified in cancer arising in mouse models of mammary cancer and in mouse and human colon cancer. WNTs promote stabilization of a transcription factor called β-catenin (also known as CTNNB1). WNTs control both the canonical β-catenin-dependent and non-canonical β-catenin-independent pathways. Studies point to a vital role for hyper-activated WNT-β-catenin signaling in colorectal cancer (Korinek, 1997; Morin, 1997). Inherited inactivating mutations of the adenomatous polyposis coli (APC) gene, the product of which is a negative controller of β-catenin stability, are found in patients with FAP. Polyps of FAP patients progress to colorectal carcinomas upon inactivation of the tumor suppressor p53 and activating mutations of KRAS. Both APC and CTNNB1 are commonly mutated in colorectal cancers of non-FAP patients.

The high prevalence of WNT pathway mutations in many types of cancer is evidence for the importance of the WNT-β-catenin pathway in
carcinogenesis. Mutations in other members of the WNT signal pathway implicated in carcinogenesis include: TCF7L2 (transcription factor 7-like), CTNNB1, WTX (Wilms tumor gene on the X chromosome), and AXIN (See Table 1 of Anastas, 2013).

[0145] The risk for developing intestinal polyps or intestinal cancer may be determined by genetic analysis. The treatment or prevention of the disease may be instituted before or after any related surgical intervention such as polypectomy or any form of a full or partial colectomy or colon resection. Dosing regimens may include multiple doses per day, one dose per day, or regular doses one or more days apart.

[0146] In some aspects, colon-targeted, rapamycin-loaded microspheres can be used to treat or prevent some intestinal cancers, particularly colorectal cancer.

EXAMPLES

[0147] The following examples are included to demonstrate certain non-limiting aspects of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques that are thought to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, would also appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

[0148] Encapsulated rapamycin in EUDRAGIT® S 100, sometimes referred to as enteric-coated rapamycin, increases life span in a mouse model of colon
cancer, referred to as $\text{Apc}^{\text{Min}^+}$. This mouse model carries a germ line mutation in one copy of the mouse tumor suppressor gene encoding adenomatous polyposis coli (Ape). Min in $\text{Apc}^{\text{Min}^+}$ refers to a condition called multiple intestinal neoplasms, which in this mouse model develop very early in life, resulting in a short life span of about 180 days. The cause of death of $\text{Apc}^{\text{Min}^+}$ mice is usually severe anemia due to bleeding from the multiple neoplastic polyps in the intestine. $\text{Apc}^{\text{Min}^+}$ mice model impart an inherited condition in humans called familial adenomatous polyposis (FAP).

[0149] Since FAP patients develop polyps that eventually progress to colon cancer, and since $\text{Apc}^{\text{Min}^+}$ mice develop similar neoplasms, this mouse model (and other containing similar mutations in Ape) are widely used by intestinal cancer researchers.

[0150] Showing that enteric-coated rapamycin treatment beginning early in life in $\text{Apc}^{\text{Min}^+}$ mice prevents polyps from developing and progressing to the bleeding stage thereby resulting in a life span equal to and perhaps greater than wild type, normal mice (See Figs. 6A-6B for comparison to life span of normal, wild type) strongly suggests a similar approach in FAP (and other types of GI cancers) in human patients will be of great benefit.

[0151] $\text{Apc}^{\text{Min}^+}$ mice were fed Eudragit control chow (0 ppm rapamycin), a medium dose of 14 ppm encapsulated rapamycin (2.24 mg/kg/day), or a high dose of 42 ppm encapsulated rapamycin (6.72 mg/kg/day) chow beginning at 6 weeks of age (Fig. 6A, arrow). All mice consuming 0 ppm chow died by 181 days of age, while the rapamycin-treated mice survived to between 570 and 685 days (median 668 days) for the mice dosed at 14 ppm and to between 559 days to 1,093 days (median 937 days). This extension of life span was statistically
significant (Logrank Test; p<0.0025) for each dose (See Fig. 6B). In Fig. 6B, life span of enteric-coated rapamycin-treated Apc<sup>Min</sup><sup>/+</sup> mice was compared to wild type C57BL6 mice or mice treated with RAD001 (everolimus), as reported by Fujishita T, Aoki K, Lane HA, Aoki M, Taketo MM in "Inhibition of the mTORC1 pathway suppresses intestinal polyp formation and reduces mortality in Apc<sup>Δ716</sup> mice" (published in Proc. Natl. Acad. Sci. USA, 2008 Sep. 9;105(36):13544-9). This experiment reveals that enteric-coated rapamycin is more effective than the RAD001 treatment because the lower dose of enteric-coated rapamycin (2.24 mg/kg) results in a longer life span than the highest dose of RAD001 (10 mg/kg). In addition, 60% of the Apc<sup>Min</sup><sup>/+</sup> mice receiving 42 ppm enteric-coated rapamycin diets lived beyond mice treated with the highest dose of RAD001 and wild type, normal mice.

[0152] Intestinal polyp counts of Apc<sup>Min</sup><sup>/+</sup> mice at necropsy show a reduction, especially the mice treated with high dose. The first mouse to die in the high dose treatment group showed no visible signs of intestinal neoplasms. The second mouse that died had three polyps. This is evidence of prevention of neoplastic disease in a highly prone mouse model.

[0153] Encapsulated rapamycin also improves the general health and healthy lifespan of animals to which preparations according to the present invention are administered. For instance, with reference to the treated Apc<sup>Min</sup><sup>/+</sup> mice as described above, Figs. 6A-6B illustrate data to support that encapsulated rapamycin increases life span and health in such mice. Fig. 6A, more particularly, shows how encapsulated rapamycin increased life span in Apc<sup>Min</sup><sup>/+</sup> mice, and Fig. 6B illustrates how the life span of rapamycin-treated Apc<sup>Min</sup><sup>/+</sup> mice compared to wild type C57B6 mice or mice treated with RAD001 (everolimus).
The health of the mice was tested by monitoring their activity. Older or sick mice move less than younger, healthy mice. The Nathan Shock Healthspan and Functional Assessment Core of the Barshop Institute for Longevity and Aging Studies documented the activity of the rapamycin-treated and control mice. The data shown in Fig. 7B reveal the decline in movement by the 0 ppm fed group (labeled control in the graphs), which has been prevented by both the medium (2.24 mg/kg/day) and high (6.72 mg/kg/day) doses of rapamycin. Both the mid and high doses are equally effective in maintaining this aspect of health. The data shown in Fig. 7B also show a difference between movement between light and dark phases of the day cycle for the medium and high doses of rapamycin, the difference being absent in the control 0 ppm dose mice. These data indicate the maintenance of a diurnal rhythm and activity levels similar to wild-type C57BL/6 mice, suggesting better health versus \(Apc^{Min/+}\) mice on control chow.

Fig. 7A demonstrates that polyp count at the time of death was lower in \(Apc^{Min/+}\) mice that were treated with enteric-coated rapamycin. The first mouse that died after treatment with 42 ppm enteric-coated rapamycin had no polyps, and the second one had only 3 polyps. Fig. 7C demonstrates that encapsulated Rapa maintains normal hematocrits in \(Apc^{Min/+}\) mice. The hematocrit in enteric-coated rapamycin-treated mice (in the high dose group) was normal as compared to wild type C57BL/6 mice (44%) even at 550 days, a time when about 5% of wild type C57BL/6 mice were reported to die from natural causes. It is clear that the high dose enteric-coated rapamycin is more effective in maintaining normal hematocrits, which is indicative of the repression of mTORCI (shown in Fig. 8) and inhibition of polyp development and growth leading to extended longevity in this tumor-prone model.
Figs. 8A & 8B shows a dose-dependent depression of the phosphorylation of rpS6 by chronic enteric-coated rapamycin treatment. rpS6 was recently shown to have a vital role in ribosome biogenesis needed for protein synthesis, development and growth of intestinal neoplasms. Chauvin C, Koka V, Nouschi A, Mieulet V, Hoareau-Aveilla C, Dreazen A, et al., Oncogene. 2013. Both mid and high doses are equally effective in repressing this part of mTORC1 downstream signaling.

Also shown are blood levels of rapamycin by the 2.24 mg/kg and 6.72 mg/kg enteric-coated rapamycin doses (Fig. 8C). These blood concentrations are higher than the therapeutic range used for organ transplant recipients. Trepanier D, Gallant H, Legatt D, Yatscoff R. Clin Biochem 1998, 31:345-351. A dose response was observed in proximal and distal small intestine tissue levels of rapamycin, the increase in the distal intestine compared with the proximal, which is consistent with the pH gradient approaching neutrality thereby resulting in an increase drug release by Eudragit delivery. This implies that enteric-coated rapamycin may be an effective and convenient method to deliver rapamycin to both the small intestine and blood, indicating that enteric-coated rapamycin may have both local and systemic effects.

These data of increased lifespan, increased activity levels, increased hematocrit, dose-dependent depression of the phosphorylation of rpS6, decreased polyp production, and other health indicators is not due exclusively to the dose of rapamycin delivered in the chow. The low dose of 2.24 mg/kg/day is lower than other reported doses of rapamycin such as 3 mg/kg/day and 10 mg/kg/day by oral gavage (Fujishita, et ai, Proc Natl Acad Sci USA. 105(36): 13544-9, 2008) and 40 mg/kg food pellet (Koehl, et ai, Oncogene, 29:1553-60, 2010). However, and
surprisingly, the rapamycin encapsulated in the Eudragit provided a superior therapeutic benefit than rapamycin delivered alone.

[0159] The studies described above demonstrate that encapsulated rapamycin in the disclosed formulation, which is enterically delivered, prevents, delays the development of, or slows the growth and progression of intestinal polyps (and subsequent cancer) in this mouse model.

EXAMPLE 2
[0160] enteric-coated rapamycin increases survival in mouse FAP models more than uncoated rapamycin or everolimus. Chronic rapamycin improved survival for \textit{Apc}\textsuperscript{Min/+} mice (n=5 females/group) in a dose-dependent manner (Fig. 9A, \(p<0.005\), logrank test). The \textit{Apc}\textsuperscript{Min/+} cohort fed control chow lived 164-182 days (median 174) versus the 14 ppm enteric-coated rapamycin cohort that lived

EXAMPLE 3
[0161] Enteric-coated rapamycin directly affects the intestines of mice. In the \textit{Apc}\textsuperscript{Min/+} model, polyps rarely form in the colon (Tankak \textit{et al.}, 2006), and since enteric-coated rapamycin’s effects on polyp development and/or growth are likely due to its direct action in the intestine, mTORCI status has been assayed in various segments of the intestine of mice. For this purpose, immunoassays of mTORCI activity were used. In this assay, protein lysates are prepared from intestinal tissue dissected form treated and untreated mice. The protein lysates are separated on SDS-polyacrylamide gels and transferred to membranes. The membranes are then probed with phosphorylation state-dependent or phosphorylation state-independent antibodies (indicated in Fig. 10A). In addition, the membrane is also probed with an antibody specific for a protein encoded by a Cap-independent (not regulated by mTORC1) mRNA (Actin, Fig. 10A).
Antibodies are detected with an Odyssey Infrared Imaging System. Ratios for each antibody against the pan-actin loading control using I.I. K counts were calculated. The respective antibody to pan-actin ratio was then used to calculate phosphorylated protein to total protein ratio. Prism 5 (GraphPad Software, Inc.) was used to analyze and graph the data. An unpaired two-tailed f-test or Mann Whitney test (nonparametric for rpS6) was used to obtain p values. P values below 0.05 were considered significant.

The mTORCI substrate S6 kinase 1 phosphorylates the small ribosomal subunit protein S6 (rpS6) in response to nutrient and growth factor stimuli (Magnuson B, Ekim B, Fingar DC). Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signaling networks (Biochem J. 2012 Jan 1; 441 (1):1-21). Therefore, rpS6 phosphorylation in 607-627 day old female C57BL/6 mice fed enteric-coated rapamycin for 42 days using the same doses as the NIA Intervention Testing Program (14 and 42 ppm) (Wilkinson et al., 2012; Miller et al., 2011; Harrison et al., 2009) was measured. Chronic enteric-coated rapamycin treatment inhibited rpS6 Ser 240/244 phosphorylation in the proximal and distal segments of the small intestine, demonstrating mTORCl inhibition (Fig. 10A). These results were reproduced in the distal intestine from multiple mice (Fig. 10A, right panel, Fig. 10B). Because rpS6 is encoded by a 5' TOP-containing mRNA (Meyhaus et al., 2009) and since mTORCI regulated translation of 5' TOP-containing mRNAs (Thorren et al., 2012), rpS6 content in lysates was also assayed. There were no differences in the levels of rpS6 relative to actin, encoded by a non-5' TOP mRNA (Fig. 10C). Blood levels from treated mice averaged 37 ng/mL and 170 ng/mL for the 14 and 42 ppm groups, respectively (a 4.6-fold increase in response to 3 fold higher concentration in the food) (Fig. 10D).
These blood concentrations are higher than the therapeutic range used for organ transplant recipients (Trepanier et al., 1998). A dose response in proximal and distal small intestine tissue levels of rapamycin was also observed (Fig. 10E). Importantly, there is an increase in rapamycin levels in distal intestine compared to proximal (Fig. 10F), which is consistent with the pH gradient approaching neutrality, thereby resulting in an increase drug release by EUDRAGIT®. Since phosphorylation of rpS6 by S6 kinase plays a role in ribosome biogenesis (Chauvin et al., 2013), enteric-coated rapamycin-mediated depression of rpS6 phosphorylation indicated decreased ribosome biogenesis, which would inhibit cell growth needed for polyp formation. Thus, enteric-coated rapamycin is an effective and convenient method to deliver rapamycin to both the small intestine and blood indicating enteric-coated rapamycin could have both local and systemic effects.

The status of the other major arm of mTORC1 effectors, the Cap-binding translation initiation factor, eIF4E and its binding repressor, 4E-BP1, which are implicate in cancer growth (Alain, et al., 2012) were also examined. No significant effects on this arm of the pathway were detected, including no alterations in the ratio of eIF4E to its repressor 4E-BP1 (data not shown). Thus, the major effect of chronic rapamycin treatment directed to the small intestine appears to be a reduction of S6K1 activity manifested by decreased rpS6 phosphorylation.

As observed by the Fujishita et al. study of Ape716 mice (Fujishita, et al., 2008), ApcMin/- mice treated with both low and high dose enteric-coated rapamycin also developed adenocarcinoma in the intestine, albeit in lower numbers in the high dose group (Table 1). Finally, treatment with rapamycin or rapamycin analogs results in the activation of a negative feedback loop resulting in
activation of mTORC2 that phosphorylates and activates Akt (Sarbassov, et al., 2006; O'Reilly, et al., 2006), which is a pro-growth, pro-survival pathway suggested to cause rapamycin resistance (Efeyan, et al., 2010). In our analysis of tissues, we observe no significant effects on Akt Ser473 phosphorylation and a small decrease in Akt levels in distal small intestine (Figs. 10G & 10H). This suggests that rebound activation of this pro-growth signaling pathway is less likely to be a factor in resistant tumors.

Since FAP patients develop colonic neoplasia, the effects of enteric-coated rapamycin on colons of C57BL/6 mice were also assessed. Fig. 11 shows that enteric-coated rapamycin treatment resulted in significant reduction in phosphorylation of Ser240/244 in the colon compared to control, consistent with colonic release of rapamycin.

Showing that enteric-coated rapamycin targeted to the intestine of Apc\[^{Min/+}\] mice prevents polyps from developing and progressing to the bleeding stage, thereby increasing life span, strongly suggests a similar approach—targeting rapamycin to the colon—will be of great benefit in treating and preventing FAP, colorectal cancer, inflammatory bowel diseases, and age-related intestinal diseases.

B. Vascular Cognitive Impairment

Vascular cognitive impairment is a cognitive impairment that results from underlying vascular pathology. Current approaches to treating and preventing vascular cognitive impairment focus on controlling risk factors for the vascular pathologies that underlie vascular cognitive impairment, such as high blood pressure, high cholesterol, high blood sugar or diabetes, or an autoimmune or inflammatory disease. While others have proposed treatments for some types
of dementia, there is no known cure for vascular cognitive impairment, and no drug has been approved by the FDA for the treatment of vascular cognitive impairment. Thus, there is a need for methods and compositions that can treat and prevent vascular cognitive impairment.

[0168] The inventors and/or their collaborators have discovered an effective treatment for vascular cognitive impairment comprising rapamycin, an analog of rapamycin, or another inhibitor of mTOR. Alzheimer's disease (AD) in mice was found to exhibit underlying vascular pathology, which was improved by enteric-coated rapamycin treatment. The rapamycin treatment also improved the cognitive defects (e.g., learning and memory) that are characteristic of AD mice. Thus, rapamycin and other inhibitors of TOR (e.g., rapamycin analogs) can be used to treat or prevent vascular cognitive impairment.

[0169] The term "vascular cognitive impairment" refers to various defects caused by an underlying vascular pathology, disease, disorder, or condition that affects the brain. For example, strokes, conditions that damage or block blood vessels, or disorders such as hypertension or small vessel disease may cause vascular cognitive impairment. As used herein, the term "vascular cognitive impairment" includes mild defects, such as the milder cognitive symptoms that may occur in the earliest stages in the development of dementia, as well as the more severe cognitive symptoms that characterize later stages in the development of dementia.

[0170] The various defects that may manifest as vascular cognitive impairment include mental and emotional symptoms (slowed thinking, memory problems, general forgetfulness, unusual mood changes such as depression or irritability, hallucinations, delusions, confusion, personality changes, loss of social
skills, and other cognitive defects); physical symptoms (dizziness, leg or arm weakness, tremors, moving with rapid/shuffling steps, balance problems, loss of bladder or bowel control); or behavioral symptoms (slurred speech, language problems such as difficulty finding the right words for things, getting lost in familiar surroundings, laughing or crying inappropriately, difficulty planning, organizing, or following instructions, difficulty doing things that used to come easily, reduced ability to function in daily life).

EXAMPLES

[0171] The following examples are included to demonstrate certain non-limiting aspects of the invention as relates to rapamycin preparations made and used according to teachings of the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques for which preferred preparations function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1 (In Vivo Effects of Rapamycin)

[0172] Applicants' collaborators have used magnetic resonance imaging (MRI) arterial spin labeling (ASL) techniques in vivo, as well as other functional imaging, in vivo optical imaging, and behavioral and biochemical tools to determine whether rapamycin treatment affects the progression of established deficits in the transgenic PDAPP mouse model of Alzheimer's Disease ("AD mice"). AD mice and unaffected littermates were treated with rapamycin after the onset of AD-like impairments at 7 months of age for a total of 16 weeks.
Rapamycin levels in brain regions of AD mice chronically fed with rapamycin ranged from 0.98 to 2.40 pg/mg. Levels in hippocampus were 1.55 pg/mg (see Fig. 14).

Control-fed symptomatic AD mice showed significant deficits during spatial training in the Morris water maze, as previously described (Fig. 12a). Learning deficits of AD mice, however, were partially abrogated by rapamycin treatment. Rapamycin-induced amelioration of learning deficits was most pronounced as an inversion in the rate of acquisition early during spatial training (Fig. 12a). Control-fed AD mice showed worsening performance as training progressed, a behavioral pattern associated with increased anxiety levels in animals that do not learn well. In contrast, acquisition of the spatial task for the rapamycin-treated AD group improved during the first 3 days of training in a manner indistinguishable from non-transgenic littermates, but in contrast to this group, reached a plateau at day 4 (Fig. 12a). Memory of the trained location for the escape platform was significantly impaired in control-fed AD mice (Fig. 12b), as previously described. Memory in rapamycin-treated mice, however, was indistinguishable from that of non-transgenic littermates and was significantly improved compared to that of control-fed AD mice (Fig. 12b). Thus, chronic administration of rapamycin, started after the onset of AD-like cognitive deficits, improved spatial learning and restored spatial memory in symptomatic AD mice.

Applicants’ collaborators have also examined the effects of chronic rapamycin treatment on hemodynamic function in brains of AD mice using high-field MRI. Control-fed AD animals had significantly lower global cerebral blood flow (CBF) compared to non-transgenic littermates, (Figs. 12e-d), which indicated that the AD mice had vascular abnormalities. Global CBF in rapamycin-treated
mice, in contrast, was indistinguishable from that of non-transgenic groups (Figs. 12e-d). At its earliest stages, AD is associated with synaptic dysfunction in entorhinal cortex and hippocampus while other brain regions such as thalamus are largely spared. Applicants' collaborators have observed that hippocampal, but not thalamic CBF was reduced in control-treated AD mice (Figs. 12e-g). Hippocampal CBF, however, was restored to levels indistinguishable from those of non-transgenic littermates by rapamycin treatment (Figs. 12e-g).

[0175] Applicants' collaborators have also determined cerebral glucose uptake in control-and rapamycin-fed AD mice using positron emission tomography (PET). In spite of the observed differences in CBF, cerebral metabolic rate of glucose (CMRGlc) was not significantly different between control-and rapamycin-treated groups (Figs. 13a-b). To test whether changes in CBF were caused by changes in cerebral vascularization, Applicants' collaborators measured vascular density in control-and rapamycin-fed AD mouse brains using high-resolution magnetic resonance angiography (MRA). Control-treated AD mice showed a pronounced reduction in cerebral vessel density with respect to non-transgenic littermates, further demonstrating that the AD mice exhibited vascular pathology. The reduction in brain vascularity observed in the AD mice was abrogated by rapamycin treatment (Figs. 13c-d). Thus, decreases in CBF in AD mice likely arise from cerebrovascular damage, and restored CBF reflects the preservation of vascular density as a result of rapamycin treatment.

[0176] The resulting data indicates that vascular deterioration can be reversed by chronic rapamycin treatment through a mechanism that involves NO-dependent vasodilation. Rapamycin-mediated maintenance of vascular integrity led to decreased Aβ deposition in brain vessels, significantly lower Aβ plaque
load, and reduced incidence of microhemorrhages in AD brains, suggesting that decreasing Aβ deposition in vasculature preserves its functionality and integrity, enabling the continuing clearance of Aβ from brain, thus resulting in decreased plaque load. Because memory deficits were ameliorated in rapamycin-treated AD mice, data suggests that continuous Aβ clearance through preserved vasculature may be sufficient to improve cognitive outcomes in AD mice. Alternatively, a role of increased autophagy and the chaperone response may play a role.

[0177] Many of the studies described above provide evidence for an effective role of enteric-coated rapamycin as well as microencapsulated rapamycin nanoparticles as taught herein. Some studies particularly evidence use in the inhibition of NO release in brain vascular endothelium during the progression of disease in AD mice, suggesting that mTOR-dependent vascular deterioration may be a critical feature of brain aging that enables AD. Moreover, data further indicates that chronic inhibition of mTOR by enteric-coated rapamycin, an intervention that extends lifespan in mice, negates vascular breakdown through the activation of eNOS in brain vascular endothelium, and improves cognitive function after the onset of AD-like deficits in transgenic mice modeling the disease. Rapamycin, already used in clinical settings, is expected to be an effective therapy for the vascular pathologies in AD humans and AD mice. By protecting against vascular pathologies that may cause vascular cognitive impairment, rapamycin is thus expected to be an effective therapy to prevent and treat vascular cognitive impairment as well as a variety of neurological disorders such as pre-Alzheimers and canine cognitive dysfunction.

EXAMPLE 2 (Materials and Methods)
Mice. The derivation and characterization of AD [AD(J20)] mice has been described elsewhere. AD mice were maintained by heterozygous crosses with C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME). Even though the human (h)APP transgene is driven by a neuron-specific promoter that is activated at ~el4, heterozygous crosses were set up such that the transgenic animal in was the dam or the sire in approximately 50% of the breeding pairs to avoid confounds related to potential effects of trans gene expression during gametogenesis, or imprinting effects. AD mice were heterozygous with respect to the transgene. Non-transgenic littermates were used as controls. Experimental groups were: control-fed non-Tg, n=17; rapamycin-fed non-Tg, n=18; control-fed Tg, n=10; rapamycin-fed Tg, n=10; all animals were males and 11 month-old at the time of testing. Rapamycin was administered for 16 weeks starting at 7 months of age. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Texas Health Science Center at San Antonio (Animal Welfare Assurance Number: A3345-01).

Rapamycin Treatment. Mice were fed chow containing either microencapsulated enteric-coated rapamycin at 2.24 mg/kg or a control diet as described by Harrison et al. (9). Rapamycin was used at 14 mg per kg food (verified by HPLC). On the assumption that the average mouse weighs 30 gm and consumes 5 gm of food/day, this dose supplied 2.24 mg rapamycin per kg body weight/day. All mice were given ad libitum access to rapamycin or control food and water for the duration of the experiment. Body weights and food intake were measured weekly. Food consumption remained constant and was comparable for control-and rapamycin-fed groups. Littermates (transgenic and non-transgenic mice) were housed together, thus we could not distinguish effects of genotype on
food consumption. Even though there were no differences in food consumption, body weights of rapamycin-fed nontransgenic, but not transgenic, females increased moderately during treatment, (6.8% increase for rapamycin-fed vs control-fed non-transgenic females). The higher increase in body weight for non-transgenic animals is not unexpected, since non-transgenic animals of both genders tend to be slightly (1-3 g) heavier than AD transgenic.

Animal Preparation for Functional Neuroimaging. Mice were anesthetized with 4.0% isoflurane for induction, and then maintained in a 1.2% isoflurane and air mixture using a face mask. Respiration rate (90-130 bpm) and rectal temperature (37±0.5°C) were continuously monitored. Heart rate and blood oxygen saturation level (SaO2) were recorded using a MouseOx system (STARR Life Science Corp., Oakmont, PA) and maintained within normal physiological ranges.

Cerebral Metabolic Rate of Glucose (CMR_Glc). 0.5 mCi of ^18FDG dissolved in 1 ml of physiologic saline solution was injected through the tail vein. 40 min were allowed for ^18FDG uptake before scanning. The animal was then moved to the scanner bed (Focus 220 MicroPET, Siemens, Nashville, USA) and placed in the prone position. Emission data was acquired for 20 min in a three-dimensional (3D) list mode with intrinsic resolution of 1.5 mm. For image reconstruction, 3D PET data was rebinned into multiple frames of 1s duration using a Fourier algorithm. After rebinning the data, a 3D image was reconstructed for each frame using a 2D filtered back projection algorithm. Decay and dead time corrections were applied to the reconstruction process. CMR_Glc was determined using the mean standardized uptake value (SUV) equation: SUV = (AxW)/A_int, where A is the activity of the region of interest (ROI; i.e., brain region in the study),
W is the body weight of the mice, and $A_{inj}$ is the injection dose of the $^{18}$FDG.

[0182]  Cerebral Blood Flow. Quantitative CBF (with unit of ml/g/min) was measured using the MRI based continuous arterial spin labeling (CASL) techniques on a horizontal 7T/30cm magnet and a 40G/cm BGA12S gradient insert (Bruker, Billerica, MA). A small circular surface coil (ID = 1.1 cm) was placed on top of the head and a circular labeling coil (ID = 0.8 cm), built into the cradle, was placed at the heart position for CASL. The two coils will be positioned parallel to each other, separated by 2 cm from center to center, and were actively decoupled. Paired images were acquired in an interleaved fashion with field of view (FOV) = 12.8 x 12.8 mm$^2$, matrix = 128 x 128, slice thickness = 1 mm, 9 slices, labeling duration = 2100 ms, TR = 3000 ms, and TE = 20 ms. CASL image analysis employed codes written in Matlab and STIMULATE software (University of Minnesota) to obtain CBF.

[0183]  In Vivo Imaging Experiments. Details of experimental procedures were identical to protocols previously published by Applicant's collaborators with the University of Texas. Briefly, mice were anesthetized with volatile isoflurane through a nosecone (3% induction, 1.5% maintenance). The depth of anesthesia was monitored by regular checking of whisker movement and the pinch withdrawal reflex of the hind limb and tail. Also, during surgery and imaging, three main vital signs including heart rate, respiratory rate, and oxygen saturation were periodically assessed by use of the MouseOx system (STARR Life Sciences). Body temperature was maintained at 37°C by use of feedback-controlled heating pad (Gaymar T/Pump). Initially, the scalp was shaved, incised along the midline and retracted to expose the dorsal skull. Then removal of periosteum by forceps and cleaning of skull by a sterile cotton swab were performed. A stainless steel
head plate was glutted (VetBond, 3M, St. Paul, MN) to dorsal skull and screwed to a custom-made stereotaxic frame. To create a thin-skull cranial window over the somatosensory cortex, skull was initially thinned by high-speed electric drill (Fine Science Tools, Foster City, CA) and subsequently thinned to approximate 50 μm using a surgical blade under a dissecting microscope (Nikon SMZ800). The optimal thinness was indicated by high transparency and flexibility of skull.

Artificial cerebrospinal fluid (aCSF) was used to wash the thinned area and enable pial vasculature clearly visible through the window. In vivo imaging of cortical vasculature was performed by using an Olympus FV1000 MPE with a 40X 0.8 NA water-immersion objective (Nikon). To illuminate vasculature, FITC-dextran or Rhodamine-dextran dissolved in sterilized PBS (300 μl, 10 mg/ml) was injected through tail vein at the beginning of the experiments. To observe nitric oxide (NO) derived from blood vessels, the NO indicator dye DAF-FM (Molecular Probes) was dissolved in DMSO, diluted in Rhodamine-dextran solution (250 μl), and induced into blood vessels through tail-vein injection. High-resolution z stacks of cortical layer I vasculature were sequentially acquired at different times. The NIH image J plugins stackreg and turboreg were used to align the z stacks or maximal intensity z-projections of z stacks to facilitate identification and comparison of the same blood vessels. The diameter of blood vessels was analyzed by Image J plugin vessel diameter. For the drug application, rapamycin (250 III, 10 mg/kg solution in PBS) or a NO synthase inhibitor L-NAME (250 μl, 30 mg/kg solution in PBS) was injected intraperitoneally. Acetylcholine (300J.II, 7.5 μl/ml solution in PBS), as a positive control for vasodilation, together with Rhodamine-dextran and DAF-FM were injected intravenously via tail vein.
Behavioral Testing. The Morris water maze (MWM) (54) was used to test spatial memory. All animals showed no deficiencies in swimming abilities, directional swimming or climbing onto a cued platform during pre-training and had no sensorimotor deficits as determined with a battery of neurobehavioral tasks performed prior to testing. All groups were assessed for swimming ability 2 days before testing. The procedure described by Morris et al. was followed as described. Experimenters were blind with respect to genotype and treatment. Briefly, mice were given a series of 6 trials, 1 hour apart in a light-colored tank filled with opaque water whitened by the addition of non-toxic paint at a temperature of 24.0±1.0°C. In the visible portion of the protocol, mice were trained to find a 12x12-cm submerged platform (1 cm below water surface) marked with a colored pole that served as a landmark placed in different quadrants of the pool. The animals were released at different locations in each 60' trial. If mice did not find the platform in 60 seconds, they were gently guided to it. After remaining on the platform for 20 seconds, the animals were removed and placed in a dry cage under a warm heating lamp. Twenty minutes later, each animal was given a second trial using a different release position. This process was repeated a total of 6 times for each mouse, with each trial ~20 minutes apart. In the non-cued part of the protocol, the water tank was surrounded by opaque dark panels with geometric designs at approximately 30 cm from the edge of the pool, to serve as distal cues. The animals were trained to find the platform with 6 swims/day for 5 days following the same procedure described above. At the end of training, a 45-second probe trial was administered in which the platform was removed from the pool. The number of times that each animal crossed the previous platform location was determined as a measure of platform location retention. During the course of
testing, animals were monitored daily, and their weights were recorded weekly. Performance in all tasks was recorded by a computer-based video tracking system (Water2020, HVS Image, U.K). Animals that spent more than 70% of trial time in thigmotactic swim were removed from the study. Data were analyzed offline by using HVS Image and processed with Microsoft Excel before statistical analyses.

Western blotting and $\beta$-determinations. Mice were euthanized by isoflurane overdose followed by cervical dislocation. Hemibrains were flash frozen. One hemibrain was homogenized in liquid N2 while the other was used in immunohistochemical determinations (57 per group). For Western blot analyses, proteins from soluble fractions of brain LN2 homogenates were resolved by SDS/PAGE (Invitrogen, Temecula, CA) under reducing conditions and transferred to a PVDF membrane, which was incubated in a 5% solution of nonfat milk or in 5% BSA for 1 hour at 20°C. After overnight incubation at 4°C with anti-APP (CTI5 or anti-GFAP) the blots were washed in TBS-Tween 20 (TBS-T) (0.02% Tween 20, 100mM Tris pH 7.5; 150 nM NaCl) for 20 minutes and incubated at room temperature with appropriate secondary antibodies. The blots were then washed 3 times for 20 minutes each in TBS-T and then incubated for 5 min with Super Signal (Pierce, Rockford, IL), washed again and exposed to film or imaged with a Typhoon 9200 variable mode imager (GE Healthcare, NJ). Human $\beta_{40}$ and $\beta_{42}$ levels, as well as endogenous mouse $\beta_{40}$ levels were measured in guanidine brain homogenates using specific sandwich ELISA assays (Invitrogen, Carlsbad, CA) as described.

Immunohistochemistry and confocal imaging of fixed tissues. Ten-micrometer coronal cryosections from snap-frozen brains were post-fixed in 4%
paraformaldehyde and stained with Αβ-specific antibodies (6E10, 10 pg/ml) followed by AlexaFluor594-conjugated donkey anti-rabbit IgG (1:500, Molecular Probes, Invitrogen, CA), and with Biotinylated Lycopersicon Esculentum (Tomato) Lectin (1:4000, Vector Laboratories, Burlingame, CA) followed by strepavidin-AlexaFluor488, conjugate (1:500, Molecular Probes, Invitrogen, CA) and imaged with a laser scanning confocal microscope (Nikon Eclipse TE2000-U) using a 488 Argon laser and a 515/30nm filter for the AlexaFluor488 fluorophore and a 543.5 Helium-neon laser and a 590/50nm filter for the AlexaFluor594 fluorophore. Stacks of confocal images for each channel were obtained separately at z=0.15 μm using a 60X objective. Z-stacks of confocal images were processed using Volocity software (Perkin Elmer). Images were collected in the hilus of the dentate gyrus (and/or the stratum radiatum of the hippocampus immediately beneath the CA1 layer) at Bregma ~2.18. The MBL Mouse Brain Atlas was used for reference.

[0187] Microhemorrhages. Ten-micrometer coronal cryosections from snap-frozen brains post-fixed in 4% paraformaldehyde were washed 3 X in Tris-buffered Saline (TBS) (Fisher BioReagents, NJ) and immersed in 1% Thioflavin-S (Sigma Life Sciences, St. Louis, MO). Sections were then washed 3 X in distilled water and immersed in 2% potassium hexacyanoferrate(III) trihydrate (Santa Cruz Biotechnology, CA) and 2% hydrochloric acid (Sigma Life Sciences). After three washes in TBS, sections were coverslipped with ProLong® Gold antifade reagent with DAPI (Life Technologies, CA). The number of microhemorrhages per section was counted at Bregma ~2.18 using a 40X objective on a Zeiss Axiovert 200M microscope (Carl Zeiss AG, Germany) using 4 sections per animal, and numbers of microhemorrhages were averaged for each animal.
Statistical analyses. Statistical analyses were performed using GraphPad Prism (GraphPad, San Diego, CA) and StatView. In two-variable experiments, two-way ANOVA followed by Bonferroni's post-hoc tests were used to evaluate the significance of differences between group means. When analyzing one-variable experiments with more than 2 groups, significance of differences among means was evaluated using one-way ANOVA followed by Tukey's post-hoc test. Evaluation of differences between two groups was evaluated using Student's t test. Values of POD.05 were considered significant.

EXAMPLE 3 (Other Animal Models of Vascular cognitive impairment)

Other animal models of vascular cognitive impairment (including rodent models) may be used to further characterize the beneficial effects of rapamycin treatment that were observed in the studies described above. See Nishio K et al., Stroke 41(6):1278-84, 2010; Ihara M & Tomimoto H, J. of Aging Research. Article ID 978761 , 2011; Tomimoto H et al., J. of Cerebral Blood Flow & Metabolism 25:S263 (2005). Such rodent models may be tested as described above in Examples 1 and 2. For example, rodent subjects may be administered rapamycin or a negative control and subsequently evaluated using the behavioral, imaging, biochemical, and metabolic and blood flow protocols described in Examples 1 and 2.

C. FCGS, AIHA, Lichen Planus, Lupus & Other Autoimmune Disorders

When orally administered daily, or at other regular frequencies (such as three times per week), in correspondingly effective doses, pharmaceutical preparations prepared according to the foregoing descriptions, and their equivalents, are thought to be effective for preventing and treating various autoimmune maladies in humans, canines, felines and other animals, and for
delaying or reducing the progression of those maladies and their sequelae. For example, such oral administration in a canine subject enables prevention or treatment of canine hemolytic anemia and other autoimmune hemolytic anemias (AIHA), and such oral administration in humans or other animals enables prevention and treatment of lichen planus and lupus.

[0191] As another example, when orally administered daily or three times per week, or at other regular frequencies, in correspondingly effective doses, pharmaceutical preparations prepared according to the foregoing descriptions, and their equivalents, are effective for preventing and treating and reducing the progression of various gingival diseases. Preferably, preparations according to the preferred embodiments are administered at a regular frequency, preferably in periods in excess of one year on a daily or three times per week regiment. Note that dosing may occur more frequently or less frequently. Particularly identified gingival diseases include gingivitis stomatitis (a/k/a Gingivostomatitis), which includes diseases known as Lymphocytic or Plasmacytic Gingivostomatitis.

[0192] For instance, positive efficacy was observed in felines with chronic gingiva-stomatitis, when microencapsulated rapamycin nanoparticle preparations according to the present invention were administered three times a week orally, in capsules containing doses at 200, 400 and 600 micrograms/kilogram, variously for two, four, six and eight week durations. Particularly, in controlled studies following a protocol that confirmed the initial presence of medium to severe Feline Chronic Gingivostomatitis (FCGS), an autoimmune gingival disease, microencapsulated nanoparticle preparations produced according to the process illustrated in Figs. 1-4B not only stopped progression of FCGS in all subjects tested, but also significantly reduced the severity of FCGS in most if not all of the tested subjects.
More particularly, as illustrated in Fig. 5, a protocol of treating medium to severe FCGS with microencapsulated nanoparticle preparations produced according to the process illustrated in Figs. 1-4B three times a week over 8 consecutive weeks showed reduced severity of FCGS in all sixteen of the feline subjects that participated in the study.

 Particularly beneficial results are also appreciable through regular multi-week oral administration in the prevention and treatment of canine hemolytic anemia as well as gingival diseases. "Regular" oral administration may include oral administration of capsules, tablets or other oral dosing forms of microencapsulated rapamycin nanoparticles (or their equivalents) at least twice weekly, and preferably at least three times weekly, while alternative treatment protocols may be achieved through multiple dosings per day as well.

 In any particular treatment protocol, it should be appreciated that dosing may vary based on particular needs and preferred treatment protocols according to preference, and depending on weight of the particular subject. Those of ordinary skill would understand that greater dose amount ranges would be tolerable and suitable when administered less often than once per day, and lesser ranges would be tolerable when administered more often than once per day.

 Whereas prior art uses of rapamycin may have involved recommended daily dosings of roughly 13 micrograms per kilogram in human subjects, oncology protocols according to preferred embodiments of the present invention use higher dosings than the prior art, preferably in a range of more than 50 micrograms and up to (or even exceeding) 200 micrograms per kilogram for daily administration, or the equivalent for other frequencies of administration. Other conditions addressed by oral administration protocols of the present
invention include preventing and treating hemolytic anemia in canines, felines, humans, and other animals, whether through the preferred preparations of rapamycin (or the equivalent) or through combination therapies with stem cell therapy and/or other active pharmaceutical or nutraceutical treatment protocols.

[0196] In contrast to oncology-related dosings, preferred protocols for oral administration of the preparations taught herein when used for prevention and treatment of targeted neurological conditions, and reducing the progression thereof, use lower dosings than the prior art. Such lower dosings are preferably about 5 micrograms of bioavailable rapamycin (or the equivalent) per daily oral dose, and such dosings otherwise more generally fall in the preferred range of between 1 and 7 micrograms per kilogram for once-daily administration, or the equivalent for other frequencies of administration.

[0197] Although various indications are targeted in alternative embodiments, preferred embodiments of oral administration protocols according to the present invention are, more stable, more bioavailable and efficacious, and finds better biodistribution, for treatment and prevention and reducing the progression of genetically-predisposed disorders and age-related disorders, with surprising benefits especially in the fields of the prevention and treatment of hemolytic anemia in canines and gingivitis in felines and is thought to have analogous benefits in humans and other animals.

ALTERNATIVE EMBODIMENTS WITH OTHER RAPAMYCINS

[0198] Although many aspects of the present invention relate directly to rapamycin itself, possible broader aspects of the invention relate also to analogs and derivatives of rapamycin, and to producing a more stable and effective oral
preparation for delivering an agent to bind, interact with or otherwise regulate activity of the mTOR pathway.

[0199] Accordingly, as alternatives that benefit from many but not necessarily all of the teachings of the present invention, any of the particular embodiments described above may be modified by substituting one or more other rapamycins in place of (or in addition to) rapamycin. For corresponding purposes of these descriptions, rapalogs and all mTOR pathway inhibitors should be considered as "rapamycins" (i.e., the plural of rapamycin). Also, in this context and wherever else a context relates to any of the rapamycins rather than just rapamycin, any related references to "encapsulated rapamycin" should be read as teaching not only about discrete particles that include rapamycin, but also about discrete particles that include any one or more rapamycins.

ADMINISTRATION IN COMBINATION WITH OTHER THERAPIES

[0200] It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention. Alternative embodiments may involve administration of rapamycin in combination with other therapies. Such therapies include but are not limited to dental scaling; long term use of antibacterial dental hygiene products; professional scaling and long-term tooth brushing with 0.2% chlorohexidine; corticosteroids, gold salts; antibiotics; chlorohexidine gluconate gel; radical dental extraction techniques of premolars, molars or other teeth; radiation therapy; cryotherapy; antibiotics with activity against gram-negative and anaerobic organisms (including amoxicillin-clavulanic acid combination, enrofloxacin, lincomycin, clindamycin, spiramycin, metrodinazole, and tetracyclines); corticosteroids; subgingival injection of up 10 milligrams triamcinolone; long-term prednisolone, methylprednisolone, or
triamcinolone; methylprednisolone; sodium aurothimalate; aurothioglucose; azathioprine; cyclophosphamide; chlorambucil; immunostimulatory; PIND-ORF; megoestrol acetate; lactoferrin; sodium salicylate; meloxicam; interferon; thalidomide; anti-viral agents; AZT; PMEA; soft-tissue lasers; multivitamin; antioxidant supplementation; and chemical cautery.

GENERAL ALTERNATIVES

[0201] It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all substitutions, modifications or alternatives equivalent thereto should be presumed to fall within the spirit and scope of the invention. While reference is made in many respects to incorporation of various rapamycin nanoparticle embodiments, it should also be recognized that the spirit and scope of the invention may not be limited to nanoparticles as such, nor to the other particular compounds or the like referenced herein.

[0202] In all respects, it should also be understood that the drawings and detailed description herein are to be regarded in an illustrative rather than a restrictive manner, and are not intended to limit the invention to the particular forms and examples disclosed. Rather, the invention includes all embodiments and methods within the scope and spirit of the invention as claimed, as the claims may be amended, replaced or otherwise modified during the course of related prosecution. Any current, amended, or added claims should be interpreted to embrace all further modifications, changes, rearrangements, substitutions, alternatives, design choices, and embodiments that may be evident to those of skill in the art, whether now known or later discovered. In any case, all substantially equivalent systems, articles, and methods should be considered
within the scope of the invention and, absent express indication otherwise, all structural or functional equivalents are anticipated to remain within the spirit and scope of the present inventive system and method.

[0203] It is also specifically contemplated that any of the particular encapsulated rapamycin embodiments described herein may be provided in daily oral doses (once or twice daily) for any of the medical or veterinary applications referenced throughout this specification or that may be referenced in US Patent Application 2012/0064143 and any other publications describing possible uses for encapsulated rapamycin. It should also be understood that the dosing regimens described herein with regard to specific indications may also be used with any or all of the other indications discussed. Dosing regimen would include both the concentration of rapamycin administered as well as the frequency of administration.

[0204] Alternative embodiments of the present invention include administering rapamycin locally to the oral cavity and at least one polymer, such that said system is attached to a surface in the oral cavity and remains attached thereto for at least 1 hour. Administration may also include a sustained release and a liquid precursor varnish composition to this system. This process is discussed in detail in Friedman et al., (US 2013/0018069), which is incorporated by reference.

[0205] For other alternatives, it should be understood that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Moreover, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Any embodiment of
the present invention may consist of or consist essentially of — rather than comprise/include/ contain/have — the described features and/or steps.

[0206] Accordingly and otherwise, many other alternatives will be evident to those of skill in the art. Rather than being limited by the embodiment descriptions as set forth above, the invention itself should ultimately be contemplated based on any claims that may be appended hereto or added in the course of prosecuting this patent application or other patent applications that claim direct or indirect priority to this patent application. All descriptive materials referenced herein are incorporated by reference in their entirety, for all purposes. These descriptive materials include: *Feline Gingivostomatitis*, Ross Harley PhD; *Feline Chronic Gingivitis Stomatitis*, Dentalvets 2013, [http://www.dentalvets.co.uk/files/Docs/Common%20Case%20Types/FCGS/FCGS_May2013.pdf](http://www.dentalvets.co.uk/files/Docs/Common%20Case%20Types/FCGS/FCGS_May2013.pdf); *The Disease Formerly Known as Lymphocytic/Plasmacytic Gingivostomatitis*, Fraser A. Hale, [www.toothvet.com](http://www.toothvet.com) (December 2010).
CLAIMS

1. A method of orally administering a rapamycin to an animal subject (which may be a human animal subject), comprising:
   a. preparing or otherwise obtaining a microparticle pharmaceutical preparation, comprising:
      i. a solid excipient matrix comprising a polymer composition that remains intact when exposed to acidic conditions of the alimentary canal of an animal of the type of said subject animal and that disintegrates when exposed to basic conditions of targeted intestinal portions of said alimentary canal; and
      ii. rapamycin nanoparticles dispersed within said solid excipient matrix, said rapamycin nanoparticles comprising a micelle-inducing compound, such as sodium cholate, and a pharmaceutically active core;
      iii. said pharmaceutically active core comprising rapamycin;
      iv. said micelle-inducing compound naturally inducing formation of micelles within a solution of said micelle-inducing compound, said micelles having properties that promote stability of said rapamycin when said rapamycin nanoparticles are dispersed within said matrix; and
   b. orally administering said preparation to said animal subject multiple times per week over a multi-week duration, in efficacious amounts, wherein said animal subject is in need of prevention or treatment, or
delayed progression, of one or more oncologic, neurologic or autoimmune maladies:

2. The method of claim 1, wherein said orally administering step comprises orally administering said preparation at a frequency of three or more times per week, in a dosage that is therapeutically effective for preventing or treating a gingival disease or disorder in the animal subject when administered at said frequency.

3. The method of claim 1, wherein said one or more oncologic, neurologic or autoimmune maladies include one or more of the following: gingival disease; cognitive impairment in canines; neurological impairment in human subjects exhibiting indications related to Alzheimer's disease, vascular dementia, or precursors to onset of Alzheimer's disease; familial adenomatous polyposis (FAP), colon cancer, or other sequelae of FAP; and anxiety disorders.
Rapamycin Nanoparticles

Dispersant Molecule

Dispersant Micelle (20 – 30 nm)

Rapamycin in Water-miscible Solvent

Dispersant Stabilized Rapamycin Nanoparticle (70 – 300 nm)

FYI: Approx. Number of Rapamycin Molecules in a Nanoparticle can be Estimated as Follows:

\[ N_R = \frac{1.18 \times 602 \times \pi \times D^3}{914.2 \times 6} \]

e.g. D = 230nm, then \( N_R = 4.9 \times 10^6 \), or about 5 million molecules

Fig. 1
RAW RAPAMYCIN
(or other suitable mTor inhibitor)

+ MeOH
(or other suitable aqueous solvent)

+ Sodium Cholate
(stabilizes & aggregates rapamycin molecules from aqueous solution into nanoparticles, & creates hydrophobic charge w/ dispersant effect around each nanoparticle)

RAPAMYCIN NANOPARTICLES

Prepare Polymer Matrix
Prepare solution or other liquid form of Eudragit S-100 (or other suitable controlled-release polymer) w/ solvent characteristics that won't dissolve nanoparticles.

Microencapsulate
Disperse nanoparticles within the liquid matrix & quickly form & solidify microparticles of same, such as through spray drying.

Size
Grade & sort microparticles to desired size range.

MICROENCAPSULATED RAPAMYCIN NANOPARTICLES

FIG. 4A
Flow Chart of Rapamycin Nanoparticles in Eudragit S-100 Procedure (20 g scale)

Eudragit S-100 Stock Solution
10% w/w Eudragit S-100 in water
20g Eud S-100 (9.7% w/v)
180 mL DI water
25.72mL methanol (12.5% w/v)
1.8g NaChol (0.875% w/v)
Titrate with 4M NaOH to pH 7.5-7.6
(~14 mL, 2.24g, 1% w/v)
Total solids: 24.04g

Combine S-100 Solution and Nanoparticle Suspension and Spray Dry
In a 500mL bottle
Combine rapa nanoparticle suspension and S100 solution. Add 2.13 g of glycerol and mix with mag stir bar.
Spray dry and collect

NaChol Stock Solution
1% w/w NaChol in water
1.2g NaChol (1% w/v)
120 mL DI water
Total solids: 1.2g

Rapamycin Stock Solution
10% w/v Rapamycin in MeOH
3.03g Rapamycin (10% w/v)
30.325 mL MeOH
Total solids: 3.03g

Nanoparticle Formation
Transfer rapa solution to 60mL plastic syringe, 20ga needle
Inject below surface at 4mL/min into NaChol solution mixing with paddle mixer at 300 rpm
In a 250mL beaker
Total solids: 1.2g NaChol
3.03g Rapamycin

Spray Drying Parameters
Bi-fluid nozzle 0.4mm (no cap)
Nozzle air pressure: 3 bar
Air in temp: 110 C
Sample pump rate: 5mL/min
Air speed: 0.30 m/s per min

Total Solids, Theo Yield and Payload
Solids from S-100 Stock: 24.04g
Solids from NaChol Stock: 1.2g
Solids from rapamycin stock: 3.03g
Glycerol: 2.13g
Total solids/Theo yield: 30.4g
Rapamycin: 3.03/30.4 = 9.97%
Eud S-100: 66%
NaChol: 9.9%
NaOH: 7.9%
Glycerol: 7.0%

Tablet Formation
Dry Powder Product will then be combined with four excipients and pressed into tablet form, the excipients being:
Lactose monohydrate
Macrogol
Magnesium stearate
Talc

Fig. 4B
FIG. 5
A. $\text{Apc}^{\text{Min/+}} \text{ Females}$

- 6.72 mg/kg (n = 5)
- 2.24 mg/kg (n = 5)
- $P = 0.0025$

B. 

- eRapa (6.72)
- RAD (10)
- RAD (0)
- C57BL6 WT
- eRapa (2.24)

Figs. 6A – 6B
Figures 7A – 7C
FIGS. 8A – 8C
FIGS. 9A – 9E
FIGS. 10A – 10C
**Fig. 11**

- eRapa Diet (ppm rapamycin)
- Pan Actin
- P-(Ser240/244)rpS6
- rpS6
- \( \gamma \)
- \( \beta \) 4E-BP1
- \( \alpha \)

[Rapamycin] in blood (ppm)

- 0
- 42
- <1.5
- 156.0
- 97.0
- 92.0
- 143.0
- 77.0
FIGS. 12A – 12G
FIG. 14
### INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/US2014/073097

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**A. CLASSIFICATION OF SUBJECT MATTER**

- **IPC(8)**: A61K 9/14
  - **CPC**: A61K 9/14

According to International Patent Classification (IPC) or to both national classification and IPC

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**B. FIELDS SEARCHED**

<table>
<thead>
<tr>
<th>IPC(8)</th>
<th>CPC</th>
<th>USPC</th>
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</table>

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**Orbit, Google Patents, Google Scholar.**

Search terms used: Rapamycin, sirolimus, matrix, micelle

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C.

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Special categories of cited documents:

- **"A"** document defining the general state of the art which is not considered to be of particular relevance
- **"E"** earlier application or patent but published on or after the international filing date
- **"L"** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- **"O"** document referring to an oral disclosure, use, exhibition or other means
- **"P"** document published prior to the international filing date but later than the priority date claimed
- **"T"** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- **"X"** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- **"Y"** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- **"&"** document member of the same patent family

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Date of the actual completion of the international search: **02 March 2015**

Date of mailing of the international search report: **28 APR 2015**

Name and mailing address of the ISA/US:

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