

LONG-ACTING COLLOIDAL PHARMACEUTICAL COMPOSITIONS
OF INTEGRASE STRAND TRANSFER INHIBITORS AND RELATED METHODS

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of U.S. Patent Application No. 63/498461, filed April 26, 2023, expressly incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

 This invention was made with government support under Grant No. R61/33 AI149665
10 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

 Advances in the successful development of many effective drug substances have enabled the introduction of single or multiple oral drug products to maximally inhibit HIV
15 virus replications. However, all the available oral drug products require at least daily or more frequent dosing to suppress virus in HIV⁺ patients. People living with HIV can expect virus free survival for extended periods as long as pills are taken chronically and daily. HIV daily pills are referred to as highly active antiretroviral therapies (or HAART) are composed of combinations anti-retroviral therapeutics, which is often referred to as cART. The cART's are
20 intended to prevent HIV replication with drug substances intended to inhibit multiple replication checkpoints and maximally suppress virus replication. HAART or cART typically compose of two to three drug substances intended to inhibit viral reverse transcriptase (RT), protease, and/or integrase (often referred to as integrase strand transfer inhibitor or INSTI). With worldwide implementation of HAART and worldwide effort to identify, treat and benefit
25 viral suppression of cART, people living with HIV can now live to their old age. However, chronic daily oral pills pose pill fatigues. Stoppage of cART leads to viral rebound, disease progression to AIDS and premature death. Treatment interruption and non-adherence to

prescribed daily dosing increase the likelihood of drug resistance virus in patients that could also lead to viral rebound and progression to AIDS. While modern one-pill-a-day dosing have demonstrated to save lives, WHO reported that in 2021 about 1.5 million are newly infected with HIV; and more than half-a-million (about 650,000) people died from HIV.

5 To overcome the pill fatigue, avoid preventable death and address above mentioned challenges, HIV drugs with long-acting (LA) pharmacokinetic properties are developed to increase dosing intervals with intent to increase treatment adherence and reduce pill burden. Typical approach is to produce long-acting HIV medicinal product involves selecting water insoluble derivatives that exhibit longer terminal half-life in the blood to produce injectable
10 dosage form that will sustain drug levels for weeks-to-months. For example, recently FDA-licensed long-acting cabotegravir (LA-CAB, an INSTI) product assembled in CABENUVA [composed of two drug products- LA cabotegravir injection, and LA rilpivirine (LA-RPV, an RT inhibitor) injection- which are given intramuscularly in two separate sites]. Both LA-CAB and LA-RPV are manufactured with excipients such as, PEG400, and poloxamer (polymeric
15 compounds) to form small drug crystals amenable for injection. These products retain native crystalline states. Nanocrystalline or nanocrystals are indented to improve solubility of water insoluble drugs. As a result, nano-drug crystal platforms, by reduction the size of natural state of large particulate drug crystalline, are typically used to increase drug dissolution rate to enhance oral absorption. However, nanocrystalline are metastable state and tend to reverse
20 to more stable larger structure of drug in polymorphic crystalline form. Metastable nanocrystalline drugs are known to be unstable to enhance water solubility. Thus, formation of stable drug particles that provide sustained plasma drug levels for extended time, or a long-acting drug product based on new and non-obvious composition and method is urgently needed. The goal is to make it stable, scalable, easy to resuspend for providing
25 pharmaceutically acceptable injectable product, yet provide long and extended plasma drug concentrations to overcome the requirement of daily oral dosing. Even with a long-acting or sustained drug formulation packaged in oral dosage form, the drug is expelled from the gut

(intestine) within about 24 hr in humans thus daily pill uptake is still required to provide drug levels that effective to suppress virus replication.

In some aspect, approaches and technologies have been described to produce long-acting drug products. Some of the technologies include (1) conjugation of drug molecule to erodible bio-polymers that release drug molecules as it detached from polymer over time; (2) encapsulation into polymeric particles that are biodegradable (e.g. PLGA or PLA); or hydrogel for slowly releasing encapsulates drug molecules; (3) encapsulating into lipid vesicles, silica, clay and other carriers; and (4) grinding of large drug crystals in insoluble suspension with polymeric excipients to form small, nano-crystalline drug product that slowly dissolve upon injection

In one aspect, current approaches to produce long acting and sustained release of drug require cumbersome and time-consuming chemical modification of drug substances. Some encapsulation processes may require removal of unencapsulated materials, or involve mechanical milling processes which could introduce contamination, heat-induced-degradation, increasing cost and production risks.

According to the WHO, there are 38.4 million people living with HIV in 2021, and this number continues to grow by about 1 million from the previous year. Thus, there is an urgent need to develop a simple, scalable long-acting HIV product that is easy to make, distribute and use by HIV patients and people at risk of exposure.

The disclosure described herein provides a novel composition and simple process that enable production of colloidal drug product that are stable and appropriate for making injectable, pharmaceutical suspension product for achieving long-acting drug presence in the body.

SUMMARY

In one aspect, the present disclosure provides a colloidal integrase strand transfer inhibitor particle comprising an integrase strand transfer inhibitor and an amino acid. Representative integrase strand transfer inhibitors useful in the particle include dolutegravir,

bictegravir, cabotegravir, raltegravir, and elvitegravir. Representative amino acids useful in the particle include glutamine and tryptophan.

In another aspect, the present disclosure provides an injectable pharmaceutical composition, comprising a suspension of the colloidal integrase strand transfer inhibitor
5 particles described herein and an aqueous carrier.

In a further aspect, the present disclosure provides a method for treating or preventing HIV in a subject, comprising administering to subject in need thereof a therapeutically effective amount of the integrase strand transfer inhibitor particles described herein or the injectable pharmaceutical composition described herein.

10 In another aspect, the present disclosure provides a method for treating a disease or condition preventable or treatable by administering an integrase strand transfer inhibitor, comprising administering to a subject in need thereof a therapeutically effective amount of the integrase strand transfer inhibitor particles described herein or the injectable pharmaceutical composition described herein.

15 DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings.

FIG. 1 is a schematic illustration of the binding of an integrase strand transfer inhibitor
20 (INSTI) to the integrase catalytic site (e.g., E152, D64, and D116) of HIV integrase enzyme by a representative triad of coplanar oxygen atoms in the inhibitor via bridging Mg^{2+} ions.

FIG. 2 compares the chemical structures of representative integrase strand transfer inhibitors, each with a triad of coplanar oxygen atoms (circled). Halogenated phenyl ring substituents also circled.

25 FIG. 3A is an image of representative colloidal integrase strand transfer inhibitor particles comprising dolutegravir (DTG) and glutamine (Gln). This product is referred to as DTG-CS product and has a final molar ratio of glutamine (Gln)-to-dolutegravir (DTG) of 9:2.

The particles are spherical in shape and have a diameter of about 354 nm. The size of particles can be tuned to produce a range of size based by the ratio of Gln to DTG and to rate of Gln addition.

FIG. 3B is an image of dolutegravir nanocrystals having a diameter of about 10 μ m.

5 FIG. 3C compares the Small Angle X-ray diffraction analysis of DTG-CS particles (DTG-CS, Gln:DTG = 9.2) (top curve) to an admix of DTG and Gln (Gln:DTG = 9.2) (middle curve) and the differential (bottom curve).

FIGS. 4A and 4B are images comparing representative colloidal integrase strand transfer inhibitor particles: dolutegravir (DTG) and glutamine (Gln) (FIG. 4A); and
10 dolutegravir (DTG) and tryptophan (Trp) (FIG. 4B).

FIG. 5 is a time-course pharmacokinetics of dolutegravir concentration (ng/mL) over time (weeks) in three in non-human primates (NHPs) after a single subcutaneous 5 mg/kg injection of DTG-CS particles (DTG-CS, Gln:DTG = 9.2 m/m). The single-dose injection of DTG in CS formulated product produced long-acting plasma drug concentrations time-course
15 of dolutegravir. Data presented were obtained with 2 non-human primates *M. Nemestrina*. No notable untoward effects in NHP observed over 15 weeks. For comparison plasma time-course of 5mg/kg soluble DTG in NHP after a single subcutaneous dosing is presented.

FIG. 6 is an image of representative colloidal integrase strand transfer inhibitor particles: bictegravir (BIC) and glutamine (Gln). The BIC-CS particles (BIC-CS, Gln:BIC =
20 9:2 m/m) have diameters from about 2 to about 300 nm.

FIG. 7 compares DTG-CS particle size (nm) as a function of glutamine concentration (% wt/v). Varying the ratio of DTG:Gln showed a direct relationship between Gln concentration (% wt/v) and particle size. Increasing the Gln concentration, increased the DTG-Gln CS particle size over the range of Gln % tested.

25 FIG. 8 shows bictegravir particle (BIC-CS) formation as a function of glutamine concentration. Increasing concentrations of glutamine were added to a fixed concentration of 2.23 mM of bictegravir in solution. The increase in bictegravir particle formation was apparent

immediately and completed within 1-2 min. The resulting particle in apparently turbid suspension was quantified with a spectrophotometer set to measure absorbance at 700 nm and expressed as A 700. The higher the absorbance means higher degree of bicitegravir particle formed in suspension. The data were expressed as glutamine-to-bicitegravir mole ratio.

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DETAILED DESCRIPTION

The present disclosure provides compositions comprising stable colloids of HIV integrase inhibitors for making long-acting pharmaceutical injectable products for the treatment and prevention of HIV infection. The compositions provide extended plasma time-course of the HIV integrase inhibitors for 15 weeks or more when injected into a subject.

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In one aspect, the present disclosure provides a colloidal integrase strand transfer inhibitor particle comprising an integrase strand transfer inhibitor and an amino acid.

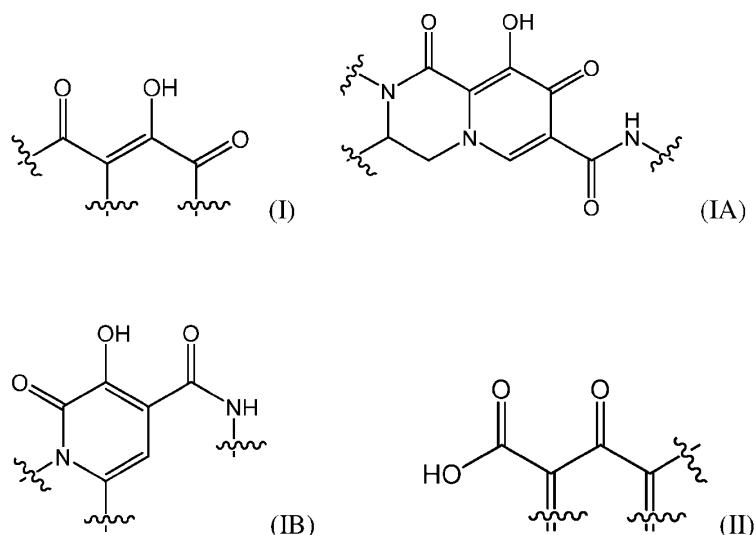
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As used herein, the term “colloidal integrase strand transfer inhibitor particle” refers to a particle that includes a mixture of small particles of one substance (i.e., the INSTI inhibitor) distributed substantially uniformly throughout another substance (i.e., the amino acid). The colloid particles are generally greater in size than those of a drug substance fully dissolved in a solution and lesser in size than those in a drug suspension.

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As used herein, the term “integrase strand transfer inhibitor” (INSTI) refers to a compound that inhibits the HIV integrase enzyme by binding to the integrase catalytic site (e.g., E152, D64, and/or D116) by a triad of coplanar oxygen atoms in the inhibitor via bridging Mg^{2+} ions as shown schematically in FIG. 1. The integrase strand transfer inhibitors described herein have a triad of coplanar oxygen atoms as shown in FIGS. 1 and 2. FIG. 2 illustrates the chemical structures of representative integrase strand transfer inhibitors with the triad of coplanar oxygen atoms, circled in each. Representative triads of coplanar oxygen atoms include the following moieties of Formulae (I), (IA), (IB), and (II):

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Representative integrase strand transfer inhibitors described herein include dolutegravir, bictegravir, cabotegravir, raltegravir, and elvitegravir. Dolutegravir, bictegravir, and cabotegravir include the moiety of Formulae (I) and (IA), raltegravir includes the moiety of Formula (IB), and elvitegravir includes the moiety of Formula (II).

10 In addition to the integrase strand transfer inhibitor, the colloidal integrase strand transfer inhibitor particles described herein include an amino acid. The amino acid stabilizes the colloidal particle. As described herein and without being bound by theory, the colloidal particles described herein appear to be held together by hydrogen-bonding (H-bonding) interactions, not by ionic bonding interactions, between the integrase strand transfer inhibitor
 15 (e.g., DTG) and the amino acid acids (e.g., glutamine and tryptophan) and maintained in the colloidal particle structure by van der Waals forces. It is believed that the H-bonding interaction between the amino acid and the integrase strand transfer inhibitor is H-bonding between the amino acid and the integrase strand transfer inhibitors' triad of coplanar oxygen atoms (see FIG. 2 and the moieties of Formulae (I), (IA), (IB), and (II), which include triad of

coplanar oxygen atoms). The halogenated phenyl ring of the INSTIs may also interact with phenyl ring of tryptophan potentially through pi-pi electron interactions.

The colloidal integrase strand transfer inhibitor particles described herein advantageously carry, transport, and ultimately deliver the integrase strand transfer inhibitor
5 contained therein, have advantageous pharmacokinetic properties and profiles compared to their respective integrase strand transfer inhibitors alone, and are effective for the prolonged delivery of the integrase strand transfer inhibitor, which are releasable over time from the particles. By virtue of their stable colloidal form, the integrase strand transfer inhibitor particles described herein are long-acting particles for the delivery of their integrase strand
10 transfer inhibitor contained therein.

In certain embodiments, the integrase strand transfer inhibitor is selected from the group consisting of dolutegravir, bictegravir, cabotegravir, raltegravir, and elvitegravir. In other embodiments, the integrase strand transfer inhibitor is selected from the group consisting of dolutegravir, bictegravir, and cabotegravir. In further embodiments, the integrase strand
15 transfer inhibitor is selected from the group consisting of raltegravir and elvitegravir. In one embodiment, the integrase strand transfer inhibitor is dolutegravir.

As noted above, in addition to the integrase strand transfer inhibitor, the colloidal integrase strand transfer inhibitor particles described herein include an amino acid. In certain
20 embodiments, the amino acid is glutamine. In other embodiments, the amino acid is tryptophan. Suitable amino acids include L-isomers, D-isomers, and racemic amino acids.

In certain embodiments of the colloidal particles, the integrase strand transfer inhibitor is dolutegravir (DTG) and the stabilizing amino acid is glutamine (Gln). In certain of these
25 embodiments, the molar ratio of dolutegravir to glutamine (DTG:Gln) is 1: 0.5-12 m/m. In other of these embodiments, the molar ratio of dolutegravir to glutamine (DTG:Gln) is 1:4.9 m/m.

In other embodiments of the colloidal particles, the integrase strand transfer inhibitor is dolutegravir (DTG) and the stabilizing amino acid is tryptophan (Trp). In certain of these

embodiments, the molar ratio of dolutegravir to tryptophan (DTG:Trp) is 1: 0.5-12 m/m. In other of these embodiments, the molar ratio of dolutegravir to glutamine (DTG:Trp) is 1:4.9 m/m.

5 In further embodiments of the colloidal particles, the integrase strand transfer inhibitor is bictegravir (BIC) and the stabilizing amino acid is glutamine (Gln). In certain of these embodiments, the molar ratio of bictegravir to glutamine (BIC:Gln) is 1: 0.5-12 m/m. In other of these embodiments, the molar ratio of bictegravir to glutamine (BIC:Gln) is 1:5 m/m.

In other embodiments of the colloidal particles, the integrase strand transfer inhibitor is bictegravir (BIC) and the stabilizing amino acid is tryptophan (Trp). In certain of these
10 embodiments, the molar ratio of bictegravir to tryptophan (DTG:Trp) is 1 : 0.5-12 m/m.

The preparation of representative integrase strand transfer inhibitor particles (DTG:Gln) and corresponding injectable composition is described in Example 1. Other representative integrase strand transfer inhibitor particles (e.g., DTG:Trp and BIC:Gln) and corresponding injectable composition were prepared according to the procedure described in
15 Example 1. Integrase strand transfer inhibitor particles that include cabotegravir, raltegravir, and elvitegravir and corresponding injectable composition are prepared according to the procedure described in Example 1.

In another aspect, the present disclosure provides an injectable pharmaceutical composition, comprising a suspension of the colloidal integrase strand transfer inhibitor
20 particles described herein in an aqueous solvent.

In certain embodiments, the injectable pharmaceutical composition (or formulation) includes from about 1 to about 2 mg integrase strand transfer inhibitor/mL.

In certain embodiments, the injectable pharmaceutical composition (or formulation) includes from about 10 to about 15 mg amino acid /mL.

25 In certain embodiments, the injectable pharmaceutical composition (or formulation) further includes a biocompatible pharmaceutical excipient. Suitable excipients include histidine, parabens, sucrose, lactose, Tween 20, and Tween 80.

In certain embodiments, the injectable pharmaceutical composition (or formulation) further includes a pegylated modifier. Suitable pegylated modifiers include lipid-conjugated PEGs with varying PEG MW (range: 500-5000) and acylated PEGs with varying PEG MW (range: 500-5000).

5 In a further aspect, the present disclosure provides methods for using the colloidal particles and injectable pharmaceutical compositions.

 In one aspect, the present disclosure provides a method for treating or preventing HIV in a subject. In certain embodiments, the method comprises administering a therapeutically effective amount of the integrase strand transfer inhibitor particles described herein or the
10 injectable composition described herein to subject in need thereof.

 It will be appreciated that certain integrase strand transfer inhibitors have been approved for both HIV prevention and HIV treatment. Some have been approved only for HIV treatment.

 In another aspect, the present disclosure provides a method for treating or preventing a
15 disease or condition treatable by administering an integrase strand transfer inhibitor in a subject. In certain embodiments, the method comprises administering a therapeutically effective amount of the integrase strand transfer inhibitor particles described herein or the injectable composition described herein to subject in need thereof. Other conditions or diseases known to be treatable by integrase strand transfer inhibitor include certain leukemias
20 and cancers related to retroviral induced oncogene activation.

 In the above methods, the integrase strand transfer inhibitor particles or the injectable compositions are administered by injection. Such as intravenous, subcutaneous, or intramuscular injection. In certain embodiments, 0.5 – 6 mL of the injectable compositions (formulations) described herein are administered in one to four injections subcutaneously or
25 intramuscularly. The administration may be at any interval between 7 and 365 days.

Subjects that benefit from the above methods include subjects at risk of HIV exposure, those living with HIV, those recently exposed to HIV, and individual living with HIV that cannot swallow pills.

The following is a description of the preparation, evaluations, and use of representative
5 colloidal integrase strand transfer inhibitor particles and their injectable compositions (formulations).

In one aspect, the present disclosure provides a long-acting HIV therapeutic product comprising an HIV integrase (INSTI) inhibitor (e.g., dolutegravir or DTG, a current HIV drug substance). The therapeutic product described herein is a colloidal pharmaceutical injectable
10 product (e.g., colloidal DTG product). The colloidal pharmaceutical product includes a colloid (e.g., DTG colloid), which is distinct from current drug delivery technologies, including liposome, nano-crystals, polymer conjugation or encapsulation, encasing in clay, carbon, silicon and other structure or materials used to produce a long-acting drug product. The DTG colloid disclosed herein is stable in suspension and suitable for use as an injectable
15 pharmaceutical product that exhibit long-acting pharmacokinetics in mammals. Dolutegravir is a current first-line HIV drug that effectively inhibits HIV viral sequences to integrate into host human genomic DNA to product HIV progeny.

The DTG colloid disclosed herein includes an amino acid that stabilizes the colloid. To determine whether glutamine (Gln), one of the essential amino acids for protein building
20 block for animals and humans, was effective for stabilizing the colloid, dolutegravir sodium (DTG) was dissolved in water at 20 g/L at 25°C. Glutamine (Gln) is also dissolved in water 15 g/L at 25°C. To 8 parts of DTG [20g/L], 2 parts of Gln (15g/L) were added in a glass container and mixed at 25°C. Immediately after mixing, the mixture form colloid exhibiting cloudy appearance. These spherical particles, detectable and observable under a high-
25 resolution polarized microscope (see FIG. 3A and 4A). The particles exhibited a diameter of 354 nm. These particles are stable at room temperature or 4°C storage and not subject to

destabilization upon dilution (after DTG-CS is formed). The resulting DTG-Gln particle is also referred to herein as DTG-CS as they are verified to be colloid and stable product.

Additional dose response studies with varying concentrations of Gln in the mixture with fixed concentration of DTG-sodium in acidic solution (pH 5-6) confirmed that the above
5 stated composition and mixing method produce optimal results (which was verified to have all DTG in solution are found in DTG-CS; and no excess free DTG remaining in the solution containing DTG-CS). The range of Gln:DTG tested was 0-10 mole ratio. Excess DTG or Gln leads to either agent remaining in the solution and requires removal of soluble DTG or Gln as by product. Having defined molar ratio of DTG:Gln to form DTG-CS product provides a
10 distinctive advantage in making DTG-CS product as no-free drug removal is required. Thus, greatly reducing the time, cost and resources in manufacturing of DTG-CS as a long-acting drug product.

With respective molecular weight consideration in the mixture of 8:2 v/v 20g/L DTG: 15g/L Gln; the mole ratio of DTG to Gln is 2:9 (m/m). Thus, the DTG-Gln product or DTG-
15 CS composed of 2:9 m/m DTG-to-Gln was used as representative as described, unless otherwise noted.

To analyze physical characteristics of DTG-CS particles, X-ray diffraction techniques were employed. These techniques enable probing of new structural organizational distinction of DTG and Gln molecular arrangements. X-ray diffraction enables elucidation of structural
20 distinction of native DTG crystalline, DTG and Gln admixture or either DTG or Gln alone. As shown in FIG. 3C, the DTG and Gln admixture (middle curve) was determined to be different from DTG-CS (top curve).

However, it was surprising that when the X-ray diffraction results were compared for the admixture (FIG. 3C, middle curve) to that of DTG-Gln in colloid form (FIG. 3C, top curve),
25 it was found that an X-ray diffraction pattern that did not exist and was distinct from that of native profile in the DTG-Gln admixture powder. Based subtractive analysis of 2θ angle versus paraf intensity analysis (FIG. 3C bottom curve) indicates notable increases in the

intensity at 2θ angle 13.4, 16.3, 22.4, 25.9, 34.4 and 46.7; and notable decreases at 20.32, 24.6 and 29.6. Thus, DTG-CS is physically and crystallographically distinct from that of DTG drug crystal and admixture of DTG-Gln. The DTG-CS is a novel structure previously unknown until now.

5 The stability of DTG-CS product made with either glutamine (Gln) or tryptophan (Trp) at the same ratio (2:9 (m/m)) was evaluated. These DTG-CS products are stable in storage for extended periods. These DTG-CS products cannot be destabilized when challenged with ionic amino acids, such as lysine or arginine in 10- to 100-fold excess relative to glutamine or tryptophan in solution. Furthermore, the addition of positively charged lysine (in ionic form)
10 or negatively charged glutamic acid (in ionic form) did not destabilize the DTG-CS product. Thus, these data suggest that the interaction of DTG with Trp or with Gln are not ionic in nature.

To probe whether the H-bonding proposed as a key and important attribute of INSTI, and its interaction with non-ionic amino-acid, glutamine or tryptophan, DTG-CS products
15 were tested with urea, a well-understood H-bond breaker and generally referred to as chaotropic agent. Urea has been known to protein scientist as chaotropic agent to denature the structure of protein composed of specific amino acid polymer sequence that folding and held together by hydrogen bonds and van der Waal's forces to provide 3-dimensional structure for specific functions. Urea is able to break these bonds and unfold or denature proteins and break
20 inter-amino acid H-bond (non-covalent) interactions as well as van der Waal's interactions. It was surprising that adding increasing concentrations of urea to the DTG-CS products resulted in destabilize the DTG-CS products. The DTG-CS products were destabilized and reverted the DTG-CS products (opaque in appearance) to soluble DTG (soluble and clear appearance) only at very high urea concentration (15-20 % w/v). While there are some differences in
25 sensitivity to urea between DTG-CS products made with Gln and Trp becoming unstable, the DTG-CS products were resistance up to 10-15 w/v % of urea chaotropic agent challenge. The DTG-CS products appear to be held together not by ionic bonding but likely through H-

bonding between DTG and stabilizers such as amino acid Trp and Gln and maintained in the DTG-CS structure by van der Waals forces.

In preparation of the pharmaceutically acceptable particle product, excipients to reduce self-aggregations or injectable colloid drug products could interfere with formation or ability of product to resuspend before use. After testing a range of common excipients and appropriate surfactants, three pharmaceutical excipients, dextrose, sucrose, and less common excipient, polyethylene-glycol polymer (MW=2000; or mPEG₂₀₀₀) conjugated to distearylphosphatidylethanolamine (DSPE) generally referred to as mPEG₂₀₀₀-DSPE were determined not to interfere with DTG-CS formation and resultant particle suspensibility.

The DTG-CS suspensions were made at 2 mg/ml DTG with Gln stabilizer to determine ability to form a DTG-CS suspension. In preparation of the DTG-CS formulation for injection, 0.1% sucrose, 0.1% dextrose, or 0.014% pegylated lipid (mPEG₂₀₀₀-DSPE) were added to determine formation of DTG-CS. After collecting DTG-CS particles by centrifugation at 500g x 10 min, they were resuspended in water at 1/10 of original volume. The results indicate that while the noted pharmaceutical excipients did not interfere in formation of DTG-CS, the presence of 0.014 w/v% of mPEG₂₀₀₀-DSPE in 2 mg/mL DTG-CS has improved re-suspendability of DTG-CS for an injectable DTG-CS pharmaceutical preparation.

Colloidal bicittegravir particles (BIC-CS) particles were also prepared. FIG. 6 is an image of BIC-CS particles prepared using glutamine as the amino acid. As shown in FIG. 6, the BIC-CS particles (BIC-CS, Gln:BIC = 9:2) have diameters from about 2 to about 300 nm.

Bicittegravir particle (BIC-CS) formation as a function of glutamine concentration is shown in FIG. 8. Referring to FIG. 8, increasing concentrations of glutamine were added to a fixed concentration of 2.23 mM of bicittegravir in solution. The increase in bicittegravir particle formation was apparent immediately and completed within 1-2 min. The resulting particle in apparently turbid suspension was quantified with a spectrophotometer set to measure absorbance at 700 nm and expressed as A₇₀₀. The higher the absorbance means higher degree

of bictegravir particle formed in suspension. The data were expressed as glutamine-to-bictegravir mole ratio.

As noted above, the present disclosure provides an injectable, long-acting HIV therapeutic product comprising an HIV integrase (INSTI) inhibitor (e.g., dolutegravir or DTG, a current HIV drug substance). The DTG colloid disclosed herein is stable in suspension and suitable for use as an injectable pharmaceutical product that exhibit long-acting pharmacokinetics in mammals. FIG. 5 is a time-course pharmacokinetics of dolutegravir concentration (ng/mL) over time (weeks) in three in non-human primates (NHPs) after a single subcutaneous 5 mg/kg injection of DTG-CS particles (DTG-CS, Gln:DTG = 9.2 m/m). The single-dose injection of DTG in CS formulated product produced long-acting plasma drug concentrations time-course of dolutegravir. Data presented were obtained with 2 non-human primates *M. Nemestrina*. No notable untoward effects in NHP were observed over 15 weeks. For comparison plasma time-course of 5mg/kg soluble DTG in NHP after a single subcutaneous dosing is presented. The free drug (DTG) is prepared as a 1.6 mg/mL solution and given at equivalent dose 5mg/kg in NHP via subcutaneous route. The plasma drug concentrations were measured with LC-MS/MS assay (the same as that for plasma NHP samples collected from NHP dose with equivalent 5 mg/kg DTG subcutaneous injection in DTG-CS dosage form. Data expressed were the mean value of 2 NHP. The plasma drug level is below detection limit of the LC-MS/MS assay by day 2.

As used herein, the term “about” refers to $\pm 5\%$ of the specified value.

The following examples are provided to illustrate, not limit, the present disclosure.

EXAMPLE

Example 1

Preparation of a Representative Colloidal Integrase Strand Transfer Inhibitor Particle

In this example, the preparation of a representative colloidal integrase strand transfer inhibitor particle, dolutegravir:glutamine (DTG-Gln CS) particles, and their injectable formulations are described.

In this example, the terms “DTG-Gln CS” and “DTG-CS” are used interchangeably.

The following materials, compositions, and methods were used to prepare DTG-CS initial DTG-Gln particles suspension with about 1.4 mL DTG in DTG-CS dosage form.

Reagents

- 5 dolutegravir sodium salt (DTG) CAS # 1051375-19-9, MW: 419.38 (Cipla)
 glutamine (Gln): CAS #56-85-9, MW: 146.1 (Sigma)
 mPEG₂₀₀₀-DSPE Sodium Salt: CAS #247925-28-6, MW: 2805.5 (Lipoid)
 sterile water for injection

Preparation of stock solutions

- 10 2 mg/mL stock solution of DTG was prepared by adding 2 mg DTG into each mL of water.

21 mg/mL stock solution of glutamine was prepared by adding 21 mg of glutamine powder into each mL of water.

- 15 7 mg/mL stock solution of mPEG₂₀₀₀-DSPE was prepared by adding 7 mg of mPEG₂₀₀₀-DSPE into each mL of water.

For the sterile injectable product, all the 3 items were sterile filtered and the formulation process was carried out in a sterile hood.

Formulation of DTG-CS in suspension at 25°C

- 20 In 600 mL DTG (2 mg/mL) solution, 120 mL of glutamine (21 mg/mL) was added and mixed with a stirrer at 60 rpm.

Subsequently and within 1-2 min, 12 mL mPEG₂₀₀₀-DSPE (7mg/mL) was added with continued mixing.

The resultant mixture was allowed to mix for 5 min at low speed (about 60 rpm).

Final DTG concentration was about 1.64 mg/mL in the final DTG-CS suspension.

- 25 DTG-CS particle size and concentration

DTG-CS particle size and DTG concentration are adjustable with varying ratio of DTG: Gln in the preparation step.

To increase DTG concentration, the following steps were performed.

Allow the above DTG-CS suspension (about 1.64 mg/mL) to rest overnight resulting in the DTG-CS particles settled to the bottom of the flask.

Remove about 92-93% of the liquid volume without particles.

5 Resuspend the particles in 60 mL to provide a final concentration of DTG in DTG-CS of about 20 mg/mL.

The final product is stable at 25°C or 4°C with respect to particles size and drug concentration over the 6-8 months study period.

10 The method and formulation process are readily scalable and intentionally designed in simple and scalable steps for making injectable sterile drug product. No heating or cooling is required. The size of the particles was determined by dynamic light scattering (DLS) (hydrodynamic size of particles determined by optically measuring particles undergoing Brownian motion in a small scattering volume). Depending on DTG:Gln ratio, the particles had an initial size of from about 200 to about 700 nm (preferred nanoparticle size) or a large
15 size in the range of about 1 to about 2 microns.

As described above, DTG-CS (dolutegravir:glutamine) particles were prepared by controlled addition of glutamine to dolutegravir solution. DSPE-mPEG2000 was included in the formation to avoid particle aggregation. DTG-CS (dolutegravir:glutamine) particles can be concentrated 10-100X by settling or centrifugation (low speed).

20 Effect of glutamine on DTG-Gln CS particle size

Varying the concentration of glutamine in the formulation step affects DTG-Gln CS particle size. To reduce the particle size of the DTG-Gln CS, the ratio of DTG:Gln was adjusted. A direct relationship between Gln concentration (% w/v) and particle size was observed. Increasing the Gln concentration, increased the DTG-Gln CS particle size.

25 FIG. 7 illustrates the effects of varying concentration of Gln on DTG-Gln CS particle size.

DTG-Gln CS particle stability

DTG-Gln CS particles prepared as described herein are stable at 25°C or 4°C with respect to particles size and drug concentration over a period of at least 6-8 months and are not subject to reversal by magnesium ion (Mg^{2+}). DTG-Gln CS particles can be reversed by
5 addition of urea at high concentration.

While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A colloidal integrase strand transfer inhibitor particle comprising an integrase strand transfer inhibitor and an amino acid.
2. The particle of Claim 1, wherein the integrase strand transfer inhibitor is selected from the group consisting of dolutegravir, bictegravir, cabotegravir, raltegravir, and elvitegravir.
3. The particle of Claim 1, wherein the integrase strand transfer inhibitor is selected from the group consisting of dolutegravir, bictegravir, and cabotegravir.
4. The particle of Claim 1, wherein the integrase strand transfer inhibitor is dolutegravir.
5. The particle of Claim 1, wherein the integrase strand transfer inhibitor is selected from the group consisting of raltegravir and elvitegravir.
6. The particle of any one of Claims 1-5, wherein the amino acid is glutamine or tryptophan.
7. The particle of Claim 1, wherein the integrase strand transfer inhibitor is dolutegravir (DTG) and the stabilizing amino acid is glutamine (Gln).
8. The particle of Claim 1, wherein the integrase strand transfer inhibitor is dolutegravir (DTG) and the stabilizing amino acid is tryptophan (Trp).
9. The particle of Claim 1, wherein the integrase strand transfer inhibitor is bictegravir (BIC) and the stabilizing amino acid is glutamine (Gln).

10. The particle of Claim 1, wherein the integrase strand transfer inhibitor is bictegravir (BIC) and the stabilizing amino acid is tryptophan (Trp).

11. An injectable pharmaceutical composition, comprising a suspension of the colloidal integrase strand transfer inhibitor particles of any of Claims 1-10 in an aqueous carrier.

12. The composition of Claim 11 further comprising a biocompatible pharmaceutical excipient.

13. The composition of Claims 11 or 12 further comprising a pegylated modifier.

14. A method for treating or preventing HIV in a subject, comprising administering a therapeutically effective amount of the integrase strand transfer inhibitor particles of any one of Claims 1-10 or the composition of any one of Claims 11-13 to subject in need thereof.

15. A method for treating a disease or condition preventable or treatable by administering an integrase strand transfer inhibitor, comprising administering a therapeutically effective amount of the integrase strand transfer inhibitor particles of any one of Claims 1-10 or the composition of any one of Claims 11-13 to a subject in need thereof.

16. The methods of Claims 14 or 15, wherein the integrase strand transfer inhibitor particles or the composition are administered by subcutaneous or intramuscular injection.

17. The method of Claim 16, wherein the administration is at any interval between 30 and 365 days.

18. The integrase strand transfer inhibitor particles of any one of Claims 1-10 or the composition of any one of Claims 11-13 for use in the treatment or prevention of an HIV infection in a human.

19. The integrase strand transfer inhibitor particles of any one of Claims 1-10 or the composition of any one of Claims 11-13 for use in the treatment or prevention of a disease or condition treatable by administering an integrase strand transfer inhibitor.