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Enhancement of oral bioavailability of an HIV-attachment inhibitor by nanosizing and amorphous formulation approaches

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ABSTRACT

BMS-488043 is an HIV-attachment inhibitor that exhibited suboptimal oral bioavailability upon using conventional dosage forms prepared utilizing micronized crystalline drug substance. BMS-488043 is classified as a Biopharmaceutics Classification System (BCS) Class-II compound with a poor aqueous solubility of 0.04 mg/mL and an acceptable permeability of 178 nm/s in the Caco2 cell-line model. Two strategies were evaluated to potentially enhance the oral bioavailability of BMS-488043. The first strategy targeted particle size reduction through nanosizing the crystalline drug substance. The second strategy aimed at altering the drug's physical form by producing an amorphous drug. Both strategies provided an enhancement in oral bioavailability in dogs as compared to a conventional formulation containing the micronized crystalline drug substance. BMS-488043 oral bioavailability enhancement was ~5- and 9-folds for nanosizing and amorphous formulation approaches, respectively. The stability of the amorphous coprecipitated drug prepared at different compositions of BMS-488043/polyvinylpyrrolidone (PVP) was evaluated upon exposure to stressed stability conditions of temperature and humidity. The drastic effect of exposure to humidity on conversion of the amorphous drug to crystalline form was observed. Additionally, the dissolution behavior of coprecipitated drug was evaluated under discriminatory conditions of different pH values to optimize the BMS-488043/PVP composition and produce a stabilized, amorphous BMS-488043/PVP (40/60, w/w) spray-dried intermediate (SDI), which was formulated into an oral dosage form for further development and evaluation.

1. Introduction

BMS-488043, Fig. 1, is a novel, first-in-class, HIV-attachment inhibitor (antiretroviral) that targets the viral envelope (Ho et al., 2006). It is a small molecule which blocks viral entry by preventing the binding of gp120 to cellular CD4 receptor (Castagna et al., 2005; Colonno et al., 2004; De Clercq, 2005a,b). BMS-488043 is classified as a Biopharmaceutics Classification System (BCS) Class-II compound having an acceptable permeability but poor aqueous solubility with dissolution-rate-dependant absorption. The free base neat form (m.p. = 245 °C) has two pK_a values of 2.6 (weak base) and 9.3 (weak acid) and exhibits low solubility (0.04 mg/mL) in the pH range of 4–8. The aqueous solubility increases modestly at pH values <4 and >8 providing minimal advantage for bioavailability enhancement with a change in solution pH. Based on *in vivo* studies using crystalline drug, BMS-488043 was found to exhibit suboptimal bioavailability due to dissolution-rate/solubility-limited absorption. Formulation or particle engineering approaches have been proposed as attractive alternatives to modify bioavailability of a poorly water-soluble molecule (Chen et al., 2004; Hu et al., 2004; Keck and Muller, 2006; Law et al., 2004; Leuner and Dressman, 2000; Liversidge and Cundy, 1995; Serajuddin, 1999). Two commonly used successful techniques, albeit drug substance dependent, include particle size-reduction through nanosizing and other nanoparticle engineering processes (Kesisoglu et al., 2007; Hu et al., 2004), and formation of a high-energy, stabilized amorphous form (Sethia and Squillante, 2003; Kaushal et al., 2004).

Particle size reduction through nanosizing of crystalline drug has been lauded as a generally acceptable approach to enhance the bioavailability of poorly water-soluble compounds that exhibit dissolution-rate-limited absorption (Liversidge and Cundy, 1995; Merisko-Liversidge et al., 2003). In such cases, the dissolution rate of the drug is a function of its intrinsic solubility and its particle

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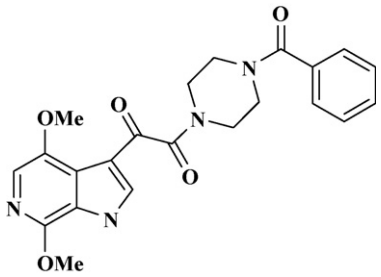

Fig. 1. Chemical structure of BMS-488043.

size. Nanosizing greatly increases the surface area of drug particles, improves its dissolution rate, and thus, enhances the potential of its bioavailability. Nanomilling is an attrition process where drug crystals are media milled in a water-based stabilizer solution. The process generates physically stable dispersions consisting of nanometer or sub-micron sized drug crystals that are suitable delivery systems for all commonly used routes of administration. Size reduction of naproxen was associated with an increase in its rate of absorption attributed to an increase in surface area available for dissolution from the nanocrystal formulation (Liversidge and Conzentino, 1995).

Improving dissolution rate and bioavailability, by producing a high-energy amorphous form, is another very attractive concept for drugs with poor aqueous solubility (Sethia and Squillante, 2003) provided that the generated metastable form is maintained (Yoshioka et al., 1994; Kaushal et al., 2004). If not properly stabilized, the metastable amorphous material generally tends to convert back to a more stable crystalline form. The use of polymers and other glass forming solids to form stabilized amorphous pharmaceutical coprecipitates, with excipients acting as antiplasticizers, has been widely reported (Law et al., 2004; Leuner and Dressman, 2000; Serajuddin, 1999; Yoshioka et al., 1995). Studies with indomethacin-polyvinylpyrrolidone (PVP) coprecipitates have shown that PVP, used as a stabilizer, does act as a crystallization inhibitor (Yoshioka et al., 1994). In addition to PVP, other polymers such as Eudragits®, polyvinylpyrrolidone-*co*-vinyl acetate (PVP-VA), hydroxypropylcellulose (HPC), and hydroxypropylmethylcellulose (HPMC), are also widely used (Sethia and Squillante, 2003; Kaushal et al., 2004). The higher internal energy of the amorphous state reflects enhanced thermodynamic properties relative to the lower energy crystalline state (e.g. solubility, vapor pressure) leading to enhanced dissolution and bioavailability. Such a state provides greater molecular mobility with the expectation that amorphous systems would exhibit greater chemical reactivity and show some tendency to spontaneously crystallize (Yoshioka et al., 1994). The crystallization could also possibly occur at different rates below and above the glass transition temperature (T_g) of the material (Hancock and Zografi, 1997; Fukuoka et al., 1989). In cases where amorphous character is desirable in a pharmaceutical formulation, the amorphous material may be stabilized using strategies based on understanding of the thermodynamic and kinetic properties of the amorphous systems. As in the current case with BMS-488043, the stabilization and formulation development of the amorphous material (spray-dried intermediate, SDI) was critical to its dosing and *in vivo* evaluation of its pharmacokinetic properties.

Based on *in vivo* studies using crystalline drug, BMS-488043 was found to exhibit suboptimal bioavailability due to dissolution-rate/solubility-limited absorption. The goal of the current study was to explore alternate formulation strategies to enhance the drug's oral exposure. We assessed particle size reduction (nanosizing), and conversion of the drug to a high-energy stabilized amorphous form as potential bioavailability enhancing techniques. Prototype nanosuspension formulations and capsule/tablet formulations containing stabilized amorphous drug substance were prepared and characterized to confirm their physical form. The *in vitro* performance was evaluated with dissolution and stability studies while the *in vivo* performance was tested by conducting relative bioavailability studies in dogs.

2. Materials and methods

2.1. Materials

BMS-488043 drug substance was synthesized at Bristol-Myers Squibb.

Microcrystalline cellulose (Avicel® PH101), lactose (Fast Flo), polyvinylpyrrolidone (Povidone, PVP K-29/30, K-90), polyvinylpyrrolidone-vinyl acetate (PVP-VA), sodium starch glycolate, docusate sodium (DOSS), magnesium stearate, sodium lauryl sulfate and silicon dioxide were compendial grade and were obtained through Bristol-Myers Squibb's Material Management Group. Hydroxypropyl cellulose (HPC-SL) was purchased from Nisso (Nippon Chemical Co., Japan). Common solvents such as dichloromethane and acetonitrile were analytical grade and were used as received through Bristol-Myers Squibb's laboratory services/stockrooms.

2.2. Methods

2.2.1. HPLC method

A reversed-phase gradient HPLC method was developed to quantify BMS-488043 using a Waters® 2690 Separation Module attached to a Waters® 996 Photo Diode Array Detector (Waters®, Milford, MA, USA). The column, an YMC ODS-AM, S-5 μm particle size, [4.6 mm × 250 mm; YMC, Inc.; Wilmington, NC, USA] maintained at 35 °C was used to effect separation and analysis. Two mobile phase systems [A: 0.05% (v/v) trifluoroacetic acid in water] and [B: 0.05% (v/v) trifluoroacetic acid in acetonitrile] were delivered at a flow rate of 1.0 mL/min according to a gradient table that provided detection of BMS-488043 at a typical retention time of 32.9–33.3 min using a detection wavelength (λ) of 230 nm. The HPLC method was used to estimate the drug's loading in the different formulations and to evaluate the drug's relative stability in its formulations.

2.2.2. Dissolution method

A USP II (paddle) dissolution method at paddle speeds of 50–100 rpm was used for dissolution testing of crystalline active pharmaceutical ingredient (API), amorphous spray-dried intermediates and formulated prototype capsules and tablets. The closed loop dissolution arrangement consisted of a DISTEK Dissolution System 2100B (DISTEK Inc., North Brunswick, NJ, USA) and a Hewlett Packard 89092A solvent transfer system (Hewlett Packard Co., Palo Alto, CA, USA) that is in-line connected to a Hewlett Packard 8453 UV/Vis detector/analyzer (Hewlett Packard Co., Palo Alto, CA, USA). Each vessel contained 750 or 1000 mL of the desired medium (water, 0.02N HCl, 0.1N HCl) maintained at 37 °C. Three-prong sinkers were used to hold the capsules at the bottom of the vessels. All samples were filtered through the recommended in-line 10-μm filters (DISTEK Inc., North Brunswick, NJ, USA) that were designed to eliminate any back pressures, and analyzed by the Hewlett Packard 8453 UV/Vis spectrophotometer at an absorbance wavelength of 230 nm. Standard calibration curves in the linear BMS-488043 concentration ranges of 0.06–0.25 or 0.1–0.23 mg/mL were prepared in 20% acetonitrile/pH 2 aqueous systems and were used to quantify the relative amounts of dissolved drug

using the system's internal calculation program (calculated %dissolved).

2.2.3. Preparation and stability of nanosuspensions

High-density polystyrene beads (500 μm) were used as the milling media to prepare aqueous nanosuspensions of the drug substance at concentrations of 10% (w/w) using nanomills [Nanomill™ 10–100 mL, Nanosystems™ Elan Group, King of Prussia, PA, USA]. Hydroxypropyl cellulose (HPC-SL, 1.25%, w/w and 2.1%, w/w) was used as the suspending agent (stabilizer) along with a surfactant [e.g. sodium lauryl sulfate (SLS), docusate sodium (DOSS) and polyvinylpyrrolidone (Povidone, PVP K-29/30)]. The nanosuspensions were prepared at 4 °C using speeds of 1800–5500 rpm for 45–60 min. The harvested samples were diluted with water and analyzed for particle size distribution using a laser light scattering analyzer (Horiba LA-910 Laser Scattering Particle Size Distribution Analyzer; Horiba Instruments, Inc., Irvine, CA, USA). Nanosuspension samples were stored at 4 °C and room temperature for up to 4 weeks to monitor its physical stability (particle growth, agglomeration and resuspendability).

2.2.4. Preparation and stability of amorphous drug

2.2.4.1. Spray drying. A Buchi B-191 Mini Spray Dryer (Brinkmann Instruments, Westbury, NY, USA), with inlet/outlet temperatures of 100/70, or 100/45 °C at a solution pumping rate of ~3.5 mL/min, with an atomizing N_2 flow rate of 700 NL/h at 100% aspiration, was used to prepare the stabilized amorphous drug. In the current investigation, polymers including PVP-K30, PVP-K90, PVP-VA, and HPC-EF were considered. Isopropyl alcohol/water (IPA/H_2O) mixtures at compositions of 70/30–90/10 (v/v) were used to solubilize the drug at total solid concentrations of 4.0–5.0% (w/v) at 60 °C. Amorphous spray-dried intermediates of BMS-488043/PVP at compositions of 100/0, 90/10, 80/20, 20/80 and 40/60 (w/w) were prepared in house using 70/30 or 75/25 (v/v) IPA/water mixtures. The BMS-488043/PVP 40/60 (w/w) SDI was scaled up to provide supplies for formulation development activities.

The amorphous SDIs were stored at 5 °C, 25 °C/60% RH (open and close), 40 °C/75% RH (open and close), and 50 °C for evaluation of physical and chemical stability. A 6-month comprehensive physical and chemical stability study was conducted to identify stability boundaries in support of storage recommendations. The material was characterized by the general thermal analysis methods of Differential Scanning Calorimetry (DSC 2910, TA Instruments, New Castle, DE), Modulated Differential Scanning Calorimetry (2920 Modulated DSC, TA Instruments, New Castle, DE), thermogravimetric (TGA) analysis (Thermogravimetric Analyzer, TGA 2050, TA Instruments, New Castle, DE) and by powder X-ray diffraction (PXRD, Rigaku Miniflex XRD, Rigaku MSC, the Woodlands, TX) to confirm its amorphous character.

2.2.4.2. Flash evaporation. A flash-evaporation method using a Brinkmann/Buchi Rotovap (Buchi Rotovap RE 1200, Buchi Laboratories, Switzerland) was employed to prepare "as is" amorphous material and stabilized amorphous drug containing 10% and 20% (w/w) of polyvinylpyrrolidone (PVP, K-30). The drug, or the drug-stabilizer physical mixture, was initially dissolved in acetonitrile with stirring at elevated temperatures. The hot solution was filtered through a 0.45 μm PTFE membrane filter (Whatman Inc., Clifton, NJ), into an appropriate round-bottomed glass container. The solvent was flash evaporated at reduced pressure in a water bath heated at 40–50 °C. A stabilized amorphous BMS-488043 containing 10% (w/w) PVP, prepared by this procedure was mixed with Lactose Fast Flo and used for dosing dogs in a relative bioavailability (BA) evaluation study.

2.2.5. Quantitation of crystalline content in amorphous SDI

PXRD was evaluated as a sensitive method to detect and ultimately quantify the crystalline drug content in its amorphous compositions initially during the development phase, and/or later during storage.

To investigate PXRD as a technique to quantify the crystalline drug content in the SDI amorphous phase, amorphous BMS-488043/PVP 40/60 (w/w) SDI was prepared by spray drying. Physical mixtures of crystalline API/amorphous SDI were then prepared at 5/95, 10/90 and 20/80 (w/w) equivalents and PXRD data was obtained on these mixtures.

2.2.6. Preparation of a prototype micronized tablet formulation

A prototype 200 mg tablet formulation was prepared for a relative BA dog study using micronized API of particle size of 95% <7 μm (Malvern Mastersizer 2000; Malvern Instruments, Southborough, MA, USA) and a surface area of 3.7 m^2/g (Gemini 2360 Surface Area Analyzer, Micromeritics, Norcross, GA, USA). The formulation was wet-granulated and contained a 70% (w/w) drug loading, 13.3% (w/w) microcrystalline cellulose, 5.7% (w/w) lactose, 2.5% (w/w) PVP-K30, 8% (w/w) sodium starch glycolate and 0.5% (w/w) magnesium stearate.

2.2.7. Preparation of prototype capsule formulations

Prototype capsules were prepared using amorphous BMS-488043/PVP coprecipitates for evaluation of stability, dissolution behavior and relative bioavailability in dogs. Based on HPLC analysis of the spray dried material (SDI), an amount equivalent to 200 mg BMS-488043 potency was mixed with ~500 mg of lactose and filled into size #000 hard gelatin capsules and dosed in dog BA studies. *In vitro* dissolution of the formulated amorphous material was also examined for evaluation of the dissolution-rate differences prior to initiating dog bioavailability studies.

2.2.8. In vivo pharmacokinetic studies in dogs

BMS-488043 was administered orally as a single 200 mg dose/animal in a crossover study to female beagle dogs. The dose was comparable to the first human dose and was well tolerated in exploratory pharmacokinetic studies in dogs with no gender differences. Thus, the 200 mg/animal dose was used as the standard dose for evaluating relative bioavailability from prototype formulations. The animals (n = 3 or 4) were fasted overnight prior to dosing. Each treatment was administered as a single dose followed by a washout period of 1 week before administering the next formulation. The nanosuspension formulation was administered via a standard gavage tube or by using size #10 or #12 Torpac "Lock Ring" gelatin capsules (Torpac Inc., Fairfield, NJ). Blood samples were collected at pre-dose, 0.5, 1, 2, 4, 8 and 24 h post dose into dipotassium EDTA vacutainers and centrifuged at 2000 × g for 5 min to harvest plasma for LC/MS/MS analysis. Plasma samples were treated with an appropriate volume of acetonitrile containing an internal standard, centrifuged and a portion of the clear supernatant was injected onto an HPLC system (YMC ProC18 column, 2.0 mm × 50 mm, 3 μm particles, Waters Co., Milford, MA). The HPLC was interfaced to a tandem mass spectrometer (Micromass Quattro LC, Beverly, MA) equipped with an electrospray interface. An eleven point standard curve was constructed for quantifying the compound in the desired range. The BMS-488043 pharmacokinetic parameters were estimated from plasma concentration-time data by noncompartmental analysis using the Kinetica™ software (v.3.0, InnaPhase Co., Philadelphia, PA). All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and Bristol-Myers Squibb Animal Care and Use Committee guidelines.

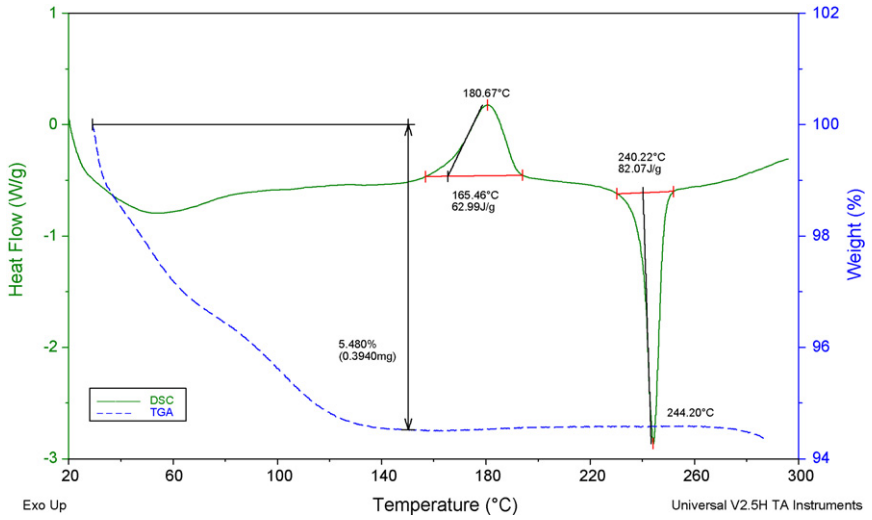

Fig. 2. DSC and TGA scans of amorphous BMS-488043/PVP (90/10, w/w) prepared by flash evaporation (FE).

3. Results and discussion

Based on initial oral *in vivo* data using crystalline drug formulated as a capsule using a wet-granulation process, it was recognized that for a poorly water-soluble drug such as BMS-488043, alternate formulations would be required to optimize its *in vivo* exposure. Since the drug's high Caco2 permeability usually translates into a high absorption rate, it was recognized that low exposure obtained in initial dog bioavailability studies, carried out with crystalline drug, was dissolution-rate or solubility-rate limited. Particle size reduction of crystalline drug and formation of stabilized amorphous drug (physical form change) were evaluated as potential particle engineering strategies to improve the drug's *in vitro* and *in vivo* performance, and boost its exposure to achieve the desired therapeutic levels.

Stabilized nanosuspensions and amorphous SDIs were successfully prepared. The particles were initially characterized to confirm their desired physical state (nanocrystalline or amorphous) and to assess their stability upon storage.

3.1. Preparation and stability assessment of nanosuspensions

Prototype BMS-488043 nanosuspension formulations were prepared at 10% (w/w) drug concentrations. Nanosuspensions prepared using 2% (w/w) HPC-SL as the stabilizer and 0.1% (w/w) SLS as the surfactant were physically stable (no caking, agglomeration or changes in particle size) when stored at both 4 °C and room temperature for up to 4 weeks. A nanosuspension formulation of BMS-488043 prepared at 100 mg/g (10%, w/w, 0.1%, w/w, SLS, mean cumulative particle size = 0.120 μm) was dosed at 200 mg BMS-488043 equivalents, for assessment in a relative bioavailability study in dogs. No further development activities were performed on the nanosuspensions due to the relatively moderate bioavailability enhancement obtained with this approach and for formulation developability issues.

3.2. Preparation and stability assessment of amorphous drug

The formation of amorphous material from neat crystalline drug by flash evaporation (Buchi Rotovap RE 1200, acetonitrile) proved to be a practical process to initially prepare small quantities of stabilized amorphous coprecipitates using PVP. The DSC thermogram produced by non-isothermal heating of the amorphous coprecipitate prepared at a composition of BMS-488043/PVP 90/10 (w/w), is shown in Fig. 2. The exotherm at ~181 °C is indicative of crystallization (T_c) to form the crystalline drug that eventually melts at ~244 °C. Fig. 3 represents the PXRD pattern of the same amorphous coprecipitate in comparison to a physical mixture of crystalline BMS-488043/PVP 90/10 (w/w). It can be clearly seen that the PXRD pattern of the amorphous coprecipitate appears as

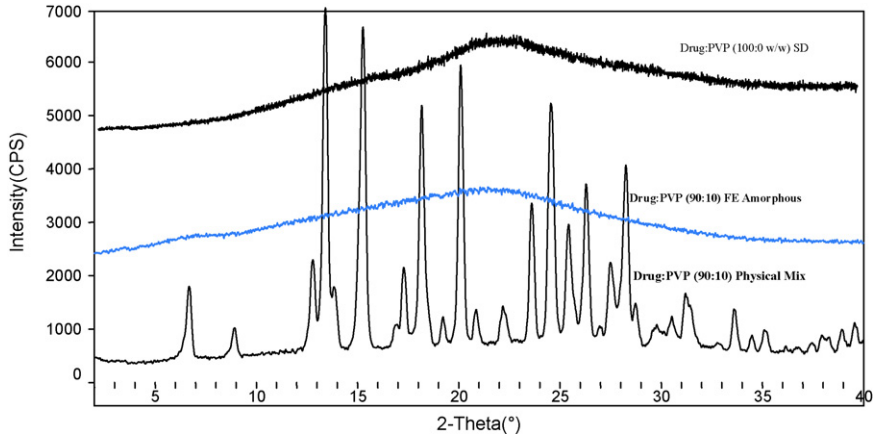

Fig. 3. PXRD diffraction patterns of amorphous BMS-488043/PVP (90/10, w/w) prepared by flash evaporation (broad halo) and its corresponding physical mixture (crystalline peaks), along with BMS-488043/PVP (100/0, w/w) prepared by spray drying (broad halo).

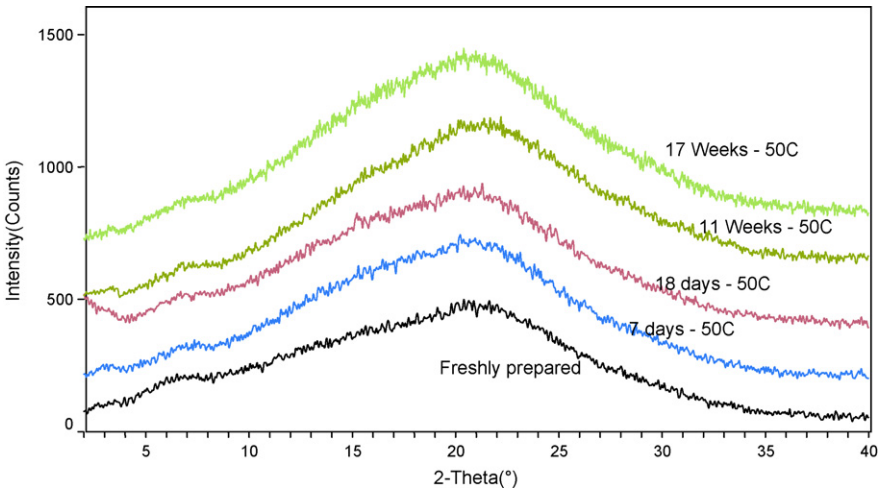

Fig. 4. Solid state stability (50 °C) of BMS-488043/PVP (80/20, w/w) amorphous coprecipitate prepared by flash evaporation.

a broad halo and lacks the peaks characteristic of crystalline BMS-488043.

Stability was monitored (by PXRD) for coprecipitates prepared at BMS-488043/PVP compositions of 100/0, 90/10 and 80/20 (w/w) by flash evaporation. Conversion to the crystalline form was observed within a week for the 100/0 BMS-488043/PVP amorphous drug sample stored at 50 °C. Similarly, conversion to the crystalline form was observed for the 90/10 BMS-488043/PVP amorphous coprecipitate sample stored open at 40 °C/75% RH for 5 days. However, no conversion was detected for the same sample stored at 50 °C for up to 18 days. Similarly, no conversion was observed for the 80/20 BMS-488043/PVP amorphous coprecipitate sample stored at 50 °C for 17 weeks (Fig. 4). These observations confirmed the need to include PVP as a crystallization inhibitor in such amorphous coprecipitates and to identify its optimal composition (%PVP load). The role of PVP as a crystallization inhibitor is generally not well understood, although well documented in the literature (Yoshioka et al., 1995; Crowley and Zografi, 2001). The mechanism of inhibition could be related to PVP acting as an anti-plasticizer to raise the drug's T_g, or via adsorption on the crystal surfaces thus suppressing further crystal growth, or by a dilution effect to inhibit nucleation. The deleterious effect of moisture, as an inducer of crystallization, on the stability of the amorphous drug was also evident.

Spray drying is a commonly used method to transform a crystalline drug substance into its high energy, thermodynamically metastable, amorphous state. The 100/0 BMS-488043/PVP amorphous drug was initially obtained by spray drying a 4% (w/v) API solution prepared in a 70/30 (v/v) IPA:water mixture. As shown in Fig. 3, the PXRD pattern for this spray-dried material lacks the crystalline peaks and shows a halo reflecting the amorphous character of the material produced. Similarly, the DSC thermogram obtained on this amorphous drug material, Fig. 5, reflects an exothermic recrystallization event followed by an endothermic melting event that corresponds to the melting point of the crystalline drug. A BMS-488043/PVP (40:60, w/w) SDI was initially prepared and its short-term stability was assessed when stored at room temperature/room light (RT/RL), 40 °C and open at 40 °C/75% RH (Fig. 6).

A long-term physical and chemical stability study was initiated on amorphous BMS-488043/PVP-K30 (40/60, w/w) SDI to identify its stability boundaries in support of its storage recommendation. The stability data supported the physical and chemical stabilization of the amorphous drug in presence of PVP when stored under low relative humidity at ambient room temperature or under refrigeration. Hence, this BMS-488043/PVP (40/60, w/w) SDI was chosen for further development based on its physical stability and processability. The spray-drying process was optimized to generate large-scale batches. An amorphous powder was obtained with no drug degradation during processing being detected.

3.2.1. Quantitation of crystalline content in amorphous SDI

Two commonly used analytical techniques namely, dissolution and PXRD, were evaluated as sensitive methods to detect and quantify the crystalline drug content in its amorphous compositions initially or during storage. The PXRD method was chosen as the more versatile and less time consuming method.

To investigate PXRD as a technique to quantify the crystalline drug content in the SDI amorphous phase, amorphous BMS-488043/PVP 40/60 (w/w) SDI was prepared by spray drying. Physical mixtures of crystalline API/amorphous SDI were then prepared at 5/95, 10/90 and 20/80 (w/w) equivalents. PXRD data obtained on these mixtures, as presented in Fig. 7, clearly show that 5% (w/w) crystalline drug was a lower detection limit achieved by the PXRD method employed in the study. The PXRD method was optimized, validated and used as an official analytical method to monitor stability samples (Sarsfield et al., 2006).

3.2.2. Dissolution performance of amorphous drug

The dissolution behavior (paddles, 150 rpm) of 25-mg BMS-488043 equivalents derived from two compositions of amorphous powders relative to crystalline "as is" drug was compared in water.

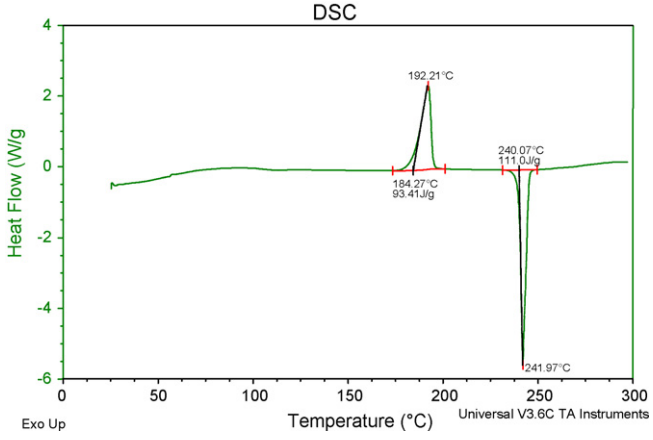

Fig. 5. DSC thermograph of BMS-488043/PVP (100/0, w/w) amorphous drug prepared by spray drying. The exothermic event at 190 °C is indicative of recrystallization.

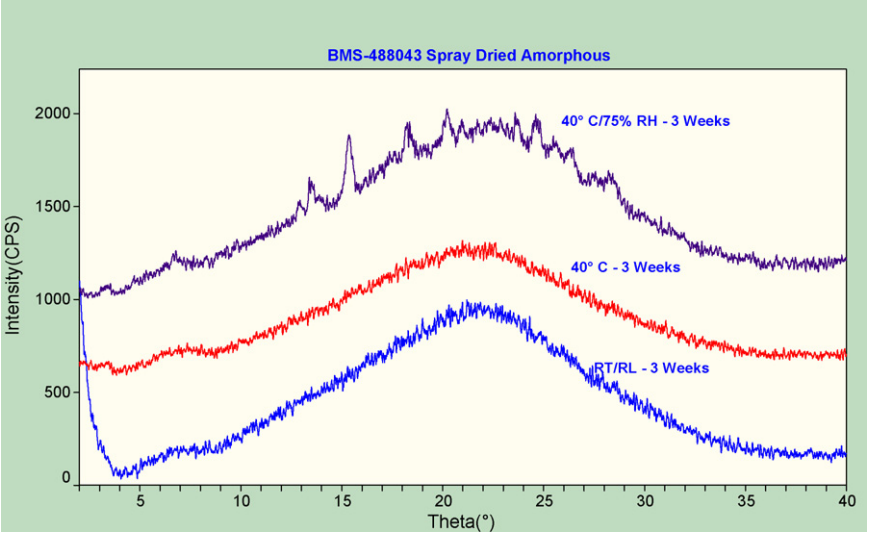

Fig. 6. Solid state stability of BMS-488043/PVP (40/60, w/w) amorphous coprecipitate prepared by spray drying and stored for 3 weeks at room temperature/room light (RT/RL), 40 °C and 40 °C/75% RH (open).

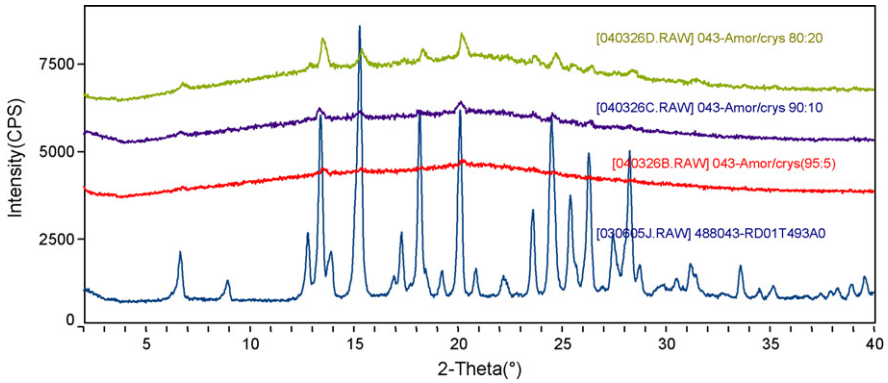

Fig. 7. PXRD patterns of physical mixtures of crystalline BMS-488043/amorphous SDI prepared at 5/95, 10/90 and 20/80 (w/w) equivalents.

The data as depicted in Fig. 8, clearly attest to the fact that both amorphous compositions of low (90/10, w/w) and high (100/0, w/w) drug loading dissolved faster than the crystalline drug. This observation supports the premise of using amorphous material to enhance the initial dissolution rate of the drug.

Dissolution behavior of capsule formulations, prepared at 100-mg BMS-488043 potencies using amorphous material, was also evaluated. The experiment was conducted using USP II dissolution method (paddles) in 1000 mL of 0.1N HCl (pH ~ 1.2) maintained at 37 °C and at paddle speeds of 100 rpm. Although dissolution was conducted using a less discriminatory medium than water (higher solubility at pH < 4), the dissolution profiles as depicted in Fig. 9 clearly show a slow down in the dissolution rates for the amorphous material relative to the crystalline drug. The two capsule formulations of the amorphous drug were prepared in a microcrystalline cellulose (MCC) blend. The slow down in dissolution upon exposure to the aqueous dissolution medium may be attributed to the initial rapid conversion of the amorphous material to crystalline BMS-488043. The deposition of the crystalline drug on the insoluble polymer (MCC) at the surface of the capsules formed a hard plug that inhibited dissolution. Thus, it was proposed to maintain an initial dissolution/disintegration rate that is faster than the rate of conversion of the amorphous drug to its crystalline form. This became apparent in the improved dissolution rate obtained when the amorphous material was blended with lactose, a fast dissolving or readily dispersible filler, as shown in Fig. 9.

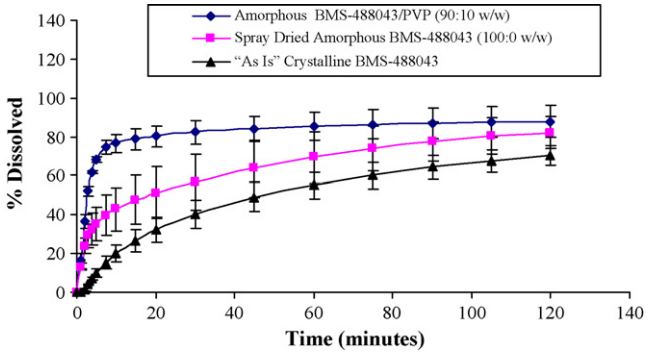

Fig. 8. Dissolution of BMS-488043 powder, 25 mg, in water at 37 °C (paddle speed = 150 rpm).

This observation highlighted the need to identify and employ fast dissolving or readily dispersible fillers in the formulation. In such a case, no crystalline material formed or got deposited on the insoluble matrix and the dissolution profile became comparable to that of the neat crystalline material. Although the formulated amorphous drug formulation might have exhibited slower *in vitro*

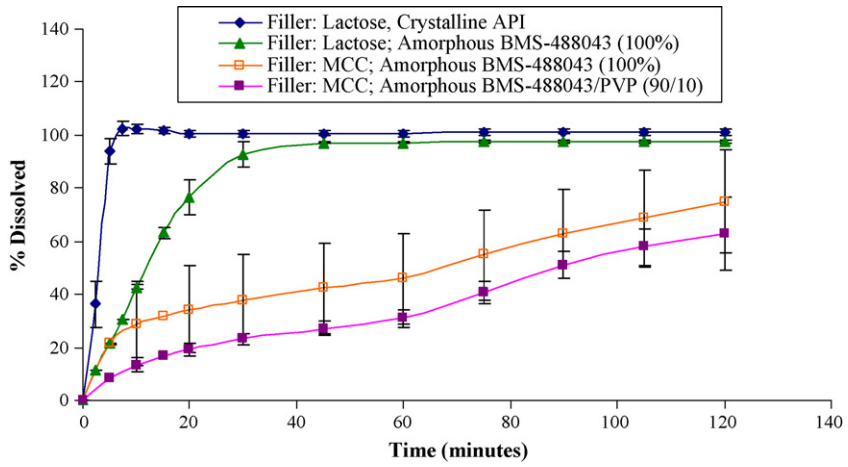

Fig. 9. Dissolution of BMS-488043 capsules, 100 mg, in 0.1N HCl (pH 1.2) at 37 °C (paddle speed = 100 rpm).

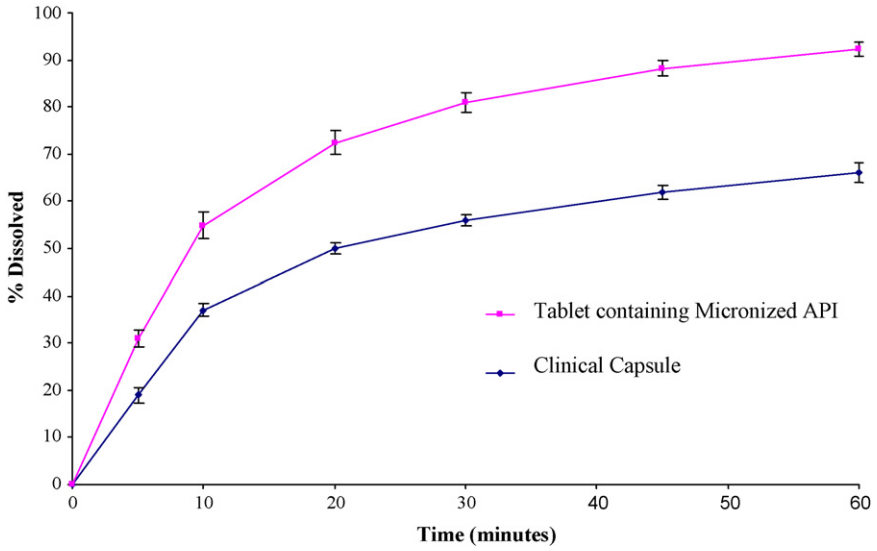

Fig. 10. Dissolution of BMS-488043 tablets containing micronized API, 200 mg and BMS-488043 capsules, 200 mg, in 0.02N HCl (pH 1.4) at 37 °C (paddle speed = 50 rpm).

dissolution, such behavior did not adversely impact its *in vivo* performance (no *in vitro–in vivo* correlation). This was reflected in the improved *in vivo* exposure obtained from the amorphous formulations as seen in Table 2.

3.2.3. Dissolution performance of tablets containing micronized API

The dissolution profile of a tablet formulation containing micronized API is shown in Fig. 10. For comparison, the dissolution profile of the capsule formulation used in early clinical studies is included. The dissolution results reflect modest enhanced dissolution for the tablet formulation (drug SA = 3.7 m^2/g) relative to the capsule formulation (drug SA = 0.8 m^2/g).

3.3. Evaluation of in vivo exposure

Dosing a 200 mg equivalent of micronized crystalline BMS-488043 in dogs as a tablet formulation produced modest increases in C_{max} and AUC relative to dosing a capsule formulation prepared with wet-milled drug (Table 1). This observation demonstrates the

Table 1
Mean and standard deviation values of pharmacokinetic parameters obtained upon dosing 200 mg equivalents of single dose oral BMS-488043 in female dogs in the "fasted" state (n = 3) as a tablet formulation and as a nanosuspension. Relative[a] exposure ratios are also tabulated for clarity.

Crossover group/formulation	Treatment	C_{max} (ng/mL)	AUC (0–24) (ng h/mL)
Capsule[a]	Wet-milled	1,401 (175)	9,424 (2,539)
Tablet	Micronized	2,518 (1,702)	17,411 (12,512)
Suspension	Nanosuspension	6,609 (745)	43,159 (6,403)
Relative exposure ratios			
Capsule[a]	Wet-milled	1.0	1.0
Tablet	Micronized	1.8	1.8
Suspension	Nanosuspension	4.7	4.6

[a] Reference material as prepared from wet-milled crystalline API (95% < 23 μm, SA = 0.8 m^2/g).

Table 2
Mean and standard deviation values of pharmacokinetic parameters obtained upon dosing of 200 mg equivalents of single dose oral BMS-488043 in female dogs ($n = 4$) in the "fasted" state as capsules containing amorphous stabilized material relative to the clinical capsule. Relative[a] exposure ratios are also tabulated for clarity.

Treatment/dosage form	C_{max} (ng/mL)	AUC (0–24) (ng h/mL)
Capsule[a]	2,179 (1,789)	25,657 (22,316)
20% (w/w) SDI (amorphous)	39,549 (12,332)	177,979 (39,777)
40% (w/w) SDI (amorphous)	34,312 (7,353)	223,646 (37,980)
Relative exposure ratios		
Capsule[a]	1.0	1.0
20% (w/w) SDI (amorphous)	18.2	7.0
40% (w/w) SDI (amorphous)	15.7	8.7

[a] Reference material as prepared from wet-milled crystalline API (95% < 23 μm, SA = 0.8 m^2/g).

modest *in vitro–in vivo* correlation exhibited by this BCS Class-II compound upon particle size reduction (Table 1).

Dosing a nanosuspension formulation resulted in significantly greater C_{max}, and exposure (AUC), compared to the capsule formulation (as shown in Table 1). The C_{max} increased 4.7-fold and AUC by 4.6-fold indicating bioavailability enhancement by direct particle size reduction. Comparatively, the relative increase in exposure obtained from dosing nanosized drug was lower than that observed upon dosing the amorphous material. As shown in Table 2, an increase in C_{max} and exposure (AUC) was observed with stabilized amorphous drug prepared at both 20% (w/w) and 40% (w/w) drug loading relative to the capsule formulation prepared with wet-milled crystalline drug. The amorphous SDI provided ~15–18-fold enhancement in C_{max}, and 7–9-fold enhancement in AUC relative to the capsule formulation. The relative increase in exposure (C_{max}) from SDI appears to be somewhat dependent on the drug loading (Table 2), being higher at the lower drug load (20%, w/w). However, the relative increase in AUC value at drug load of 40% (w/w) could be attributed to partial crystallization of the drug. These *in vivo* observations attest to the superior performance of the amorphous coprecipitates and their important role in optimizing BMS-488043 exposure for future development.

4. Conclusions

Formulation strategies of using nanosized crystalline drug dosage form and stabilized amorphous drug were investigated as ways to enhance bioavailability of BMS-488043 and optimize its *in vivo* performance. Tablets prepared from micronized crystalline drug, with controlled particle size reduction, produced modest increase in exposure relative to capsules prepared from wet-milled crystalline drug in the dog model. However, significantly higher BMS-488043 exposures were obtained in the dog model using a nanosuspension formulation and stabilized amorphous material relative to the capsule formulation. Dissolution studies with amorphous BMS-488043 prototypes demonstrated a rapid dissolution profile which could account for improved absorption observed *in vivo*. Physical and chemical stability studies supported the consideration of these formulation strategies for further development. Nanosuspension and stabilized amorphous dosage forms were both viable options to consider for clinical development of the poorly water-soluble BMS-488043, with amorphization being the superior option.

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References

Chen, X., Vaughn, J.M., Yacaman, M.J., Williams, R., Johnston, K., 2004. Rapid dissolution of high-potency danazol particles produced by evaporative precipitation into aqueous solution. J. Pharm. Sci. 93, 1867–1878.
Castagna, A., Biswas, P., Beretta, A., Lazzarin, A., 2005. The appealing story of HIV entry inhibitors: from discovery of biological mechanisms to drug development. Drugs 65, 879–904.
Colonno, R., Ho, H., Zhou, N., Masterson, T., Hanna, G., Kadow, J., Lin, P.-F., 2004. BMS-488043: a novel small molecule HIV-1 attachment inhibitor. In: 13th International Symposium on HIV & Emerging Infectious Diseases, Toulon, France.
Crowley, K.J., Zografi, G., 2001. The use of thermal methods for predicting glass-former fragility. Thermochim. Acta 380, 79–93.
De Clercq, E., 2005a. New approaches toward anti-HIV chemotherapy. J. Med. Chem. 48, 1297–1313.
De Clercq, E., 2005b. Anti-infectives: emerging anti-HIV drugs. Exp. Opin. Emerg. Drugs 10, 241–273.
Fukuoka, E., Makita, M., Yammamura, S., 1989. Glassy state of pharmaceuticals. III. Thermal properties and stability of glassy pharmaceuticals and their binary glass systems. Chem. Pharm. Bull. 37, 1047–1050.
Hancock, B.C., Zografi, G., 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. J. Pharm. Sci. 86, 1–12.
Ho, H.-T., Fan, L., Nowicka-Sans, B., McAuliffe, B., Li, C.-B., Yamanaka, G., Zhou, N., Hua, F., Dicker, I., Dalterio, R., Gong, Y.-F., Wang, T., Yin, Z., Ueda, Y., Matiskella, J., Kadow, J., Clapham, P., Robinson, J., Colonno, R., Lin, P.-F., 2006. Envelope conformational changes induced by human immunodeficiency virus type I attachment inhibitors prevent CD4 binding and downstream entry events. J. Virol. 80, 4017–4025.
Hu, J., Johnston, K.P., Williams III, R.O., 2004. Nanoparticle engineering Processes for enhancing the dissolution rates of poorly water soluble drugs. Drug Dev. Ind. Pharm. 30, 233–245.
Kaushal, A.M., Gupta, P., Bansal, A.K., 2004. Amorphous drug delivery systems: molecular aspects, design, and performance. Crit. Rev. Ther. Drug Carrier Syst. 21, 133–193.
Keck, C.M., Muller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur. J. Pharm. Biopharm. 62, 3–16.
Kesisoglu, F., Panmai, S., Wu, Y., 2007. Nanosizing-oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 59, 631–644.
Law, D., Schmitt, E.A., March, K.C., Everitt, E.A., Wang, W., Fort, J., Krill, S.L., Qiu, Y., 2004. Ritonavir-PEG 8000 amorphous solid dispersions: *in vitro* and *in vivo* evaluation. J. Pharm. Sci. 93, 563–570.
Leuner, C., Dressman, J., 2000. Improving drug solubility for oral delivery using solid dispersions. Eur. J. Pharm. Biopharm. 50, 47–60.
Liversidge, G.G., Conzentino, P., 1995. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int. J. Pharm. 125, 309–313.
Liversidge, G.G., Cundy, C.C., 1995. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs. I. Absolute oral bioavailability of nanocrystalline danazol in Beagle Dogs. Int. J. Pharm. 125, 91–97.
Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. 18, 113–120.
Sarsfield, B.A., Davidovich, M., Desikan, S., Fakes, M., Futernik, S., Hilden, J.L., Tan, J.S., Yin, S., Young, G., Vakkalagadda, B., Volk, K., 2006. Proceedings for 2005 Denver X-Ray Conference, published by International Centre for Data Diffraction. Powder X-ray diffraction detection of crystalline phases in amorphous pharmaceuticals, 322–327.
Serajuddin, A.T.M., 1999. Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. J. Pharm. Sci. 88, 1058–1066.
Sethia, S., Squillante, E., 2003. Solid dispersions: revival with greater possibilities and applications in oral drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 20, 215–247.
Yoshioka, M., Hancock, B.C., Zografi, G., 1994. Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. J. Pharm. Sci. 83, 1700–1705.
Yoshioka, M., Hancock, B.C., Zografi, G., 1995. Inhibition of indomethacin crystallization in poly(vinylpyrrolidone) coprecipitates. J. Pharm. Sci. 84, 983–986.