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Systemic in vitro and in vivo evaluation for determining the feasibility of making an amorphous solid dispersion of a B-Raf (rapidly accelerated fibrosarcoma) inhibitor

Yong Cui [a], Po-Chang Chiang [a], Edna F. Choo [b], Jason Boggs [b], Joachim Rudolph [c], Jonas Grina [d], Steve Wenglowsky [d], Yingqing Ran [a,*]

[a] Department of Small Molecule Pharmaceutics, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[b] Department of Drug Metabolism and Pharmacokinetics, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[c] Department of Discovery Chemistry, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[d] Department of Chemistry, Array BioPharma, Boulder, CO 80301, USA

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ABSTRACT

It is well acknowledged that oral bioavailability of a drug candidate is often influenced by factors such as the permeability, physico-chemical properties, and metabolism of the drug. Among the physico-chemical properties, solubility and dissolution rate are considered the most critical factors affecting the oral bioavailability of a compound G–F is a potent and selective B-Raf inhibitor with poor solubility and adsorption is limited by solubility at high doses. In order to overcome this issue using a spray-dried amorphous dispersion (SDD) formulation was evaluated. A combination of theoretical solubility prediction and in vitro dissolution, were used to predict the in vivo exposure of G–F. The predicted value was found to have good agreement with the in vivo exposure from dosing the crystalline and amorphous form of G–F.

In general, this combined approach demonstrated that the amorphous form of G–F offers an advantage over the crystalline form of G–F in terms of solubility; in vitro dissolution and in vivo absorption were predictable and consistent with the literature. This systemic approach provides a great value for compound development.

1. Introduction

It has been a common challenge for pharmaceutical industry to develop poorly soluble compounds, in particular due to their lack of oral bioavailability, resulting in low/limited exposure. At the discovery stage, in order to evaluate the efficacy and the safety profiles of lead candidates, there is a need to provide exposure-enhancing formulations with conventional excipients. This can be highly challenging with poorly soluble compounds, especially for toxicology studies that require compound exposures multiple times over the efficacious exposure (Li and Zhao, 2007). Numerous approaches have been widely used to improve solubility and dissolution including pH adjustment or salt formation (if the compound has a p*K*a within physiological range), solubilization by adding cosolvents, surfactants and complexation agents such as cyclodextrins, nano-suspension by wet milling, lipid-based formulation, and amorphous solid dispersion (Al-Obaidi et al., 2009; Chiang et al., 2012; Hauss, 2007; Leuner and Dressman, 2000; Li and Zhao, 2007; Loftsson and Brewster, 1996; Pouton, 2000, 2006; Rajewski and Stella, 1996; Ran et al., 2005; Serajuddin, 1999; Shanbhag et al., 2008; Strickley, 2004; Yalkowsky, 1999). Among all those solubility enhancement strategies, spray-dried solid dispersion (SDD) has drawn lots of attention lately in both discovery and development stages. Compared with other approaches, SDD provides advantages such as low toxicity concern, capability of scale up and commercialization (Al-Obaidi et al., 2009; Chiang et al., 2012; Hauss, 2007; Leuner and Dressman, 2000; Serajuddin, 1999; Shanbhag et al., 2008).

Conventionally, the feasibility of SDD is evaluated through a series in vitro and in vivo testing. It is a typical industrial approach

Abbreviations: AUC, area under the concentration-timer profile; CL, clearance; C_{max}, maximum concentration; C_p, isobaric heat capacity; DSC, differential scanning calorimetry; F, bioavailability; HPLC, High Performance Liquid Chromatography; T_g, glass transition temperature; HPMC-E4, hydroxypropyl methylcellulose (grade E4); HPMCAS-MF, hydroxypropyl methylcellulose acetate succinate (grade-MF); *K*, first-order terminal elimination rate constant; K_a, absorption constant; K12, intercompartment rate constant from central to peripheral compartment; K21, intercompartment rate constant from peripheral to central compartment; MP, melting point; Raf, rapid accelerated fibrosarcoma; SEM, scanning electron microscopy; SDD, spray-dried amorphous dispersion; XRPD, powder X-ray diffraction.

* Corresponding author. Tel.: +1 650 467 1423; fax: +1 650 225 6238.
E-mail address: yran@gene.com (Y. Ran).

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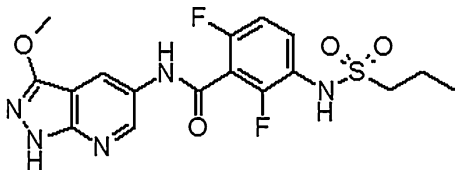

Fig. 1. Chemical structure of G–F (2,6-difluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(propylsulfonamido)benzamide).

for limited compounds with abundant supplies. In discovery stage, however, this is less likely to be the case where compounds are often made in small amount (i.e. 1–100 mg). The conventional approach of SDD cannot be applied due to large compound demand (i.e. several grams).

In order to solve this issue, several approaches have been proposed to evaluate the feasibility of SDD. A thermal based approach has been developed to help this issue (Hancock et al., 1995; Stillinger, 1998). Compared with other methods, the key advantages of this method are the less compound requirement and minimized risks of SDD failure, which, in most cases are the rate limiting factors in drug discovery. In this study, the potential of solubility enhancement was evaluated by measuring the heat capacity difference of amorphous and crystalline forms, by using the calculation of the free energy difference to predict the potential of solubility enhancement (Hancock et al., 1995; Stillinger, 1998).

After obtaining the favorable predicted value (i.e. 10–100 fold increase) of solubility of amorphous over the crystalline form, the SDD was then considered to be a suitable approach to boost solubility of poorly soluble compound. In order to prove the hypothesis, SDD was made, in vitro and in vivo tests were conducted on both SDD and crystalline form. In vitro dissolution and supersaturation profile of SDD and crystalline form were compared. One or two preclinical species were selected based on efficacy and/or safety models to evaluate in vivo performance of SDD and crystalline form. The ultimate goal of using SDD is to increase in vivo exposure at the discovery stage in order to enable efficacy and safety evaluation of the lead compounds. In addition, the in vivo data provides confirmation of the solubility enhancement prediction of the amorphous form based on free energy differences.

G–F (2,6-difluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)-3(propylsulfonamido)benzamide) Fig. 1, a potent (B-Raf^{V600E} IC_{50}: 4.8 nM; phospho-ERK (Malme-3 M) EC_{50}: 19 nM) and selective B-Raf kinase inhibitor is a highly crystalline compound with a melting point (MP) ranging from 190 °C to 228 °C depending on the polymorphs (Wenglowsky et al., 2011). It has poor intrinsic aqueous solubility and solubility limited absorption in rats at high dose with the most stable crystalline form of MP ~228 °C (Choo et al., 2011; Wenglowsky et al., 2011). Several approaches like jet-milling, adding cosolvents and salt formation have been attempted in order to improve solubility and exposure of this highly crystalline compound. Unfortunately those approaches have not been successful especially for efficacy and safety study with high exposure requirements (Ran, 2012).

SDD is one of the options to ensure preclinical studies after the failure of the other conventional approaches. However, depending on the physico-chemical properties of a compound, the solubility improvement of SDD compared with crystalline form is not predictable, Friesen (Friesen et al., 2008) observed around 2–10 fold increase of SDD solubility, which in some cases, may not be sufficient to enable efficacy or safety studies. A thermal based prediction was performed with G–F on solubility enhancement between crystalline and amorphous form. The target is to achieve at least 10 fold solubility enhancement of amorphous form over crystalline state of G–F from thermal prediction in order to ensure success of SDD. Based on the favorable prediction, G–F with HPMCAS-MF SDD was made and followed by in vitro and in vivo testing. A good correlation was obtained for G–F between solubility prediction and in vitro dissolution and in vivo exposure measurements

2. Materials

G–F was synthesized by a team of chemists from Array Biopharma (Boulder, CO). HPMCAS-MF (AQOAT) was purchased from Shin-Etsu Co. Ltd. (Tokyo, Japan). HPLC (High Performance Liquid Chromatography) grade acetonitrile and water were purchased from Brudick and Jackson (Muskegon, MI). All other chemicals and solvents used for solubility and dissolution measurement, as well as spray-drying were purchased from Sigma–Aldrich Co. (St. Louis, MO). The pH buffers were prepared based on the procedure by Chiang (Chiang and Hu, 2009). This is a universal buffer with pH range from 1.54 to 12.05. The in vitro dissolution medium used is a phosphate buffer of pH 6.5 (Friesen et al., 2008).

3. Experimental

3.1. Solubility measurement

Samples were prepared by weighing excess G–F powder into 4-mL glass vials, which contained about 1–2 mL solution of buffers with pH range from 1 ± 0.2 to 12 ± 0.2. Duplicate vials were prepared for each pH value and were put on an end-to-end Labquake® rotator (Barnstead Thermolyne, Sparks, NV) at 5 rpm at ambient temperature (25 ± 3 °C) for about 7 days. Samples were inspected every day to make sure that there were excess solid drug in the vials. After 7 days, the samples were filtered through a 0.22-μm PTFE filter and diluted with 50% acetonitrile and 50% water before being injected into a HPLC system (Agilent 1100; Agilent Technologies, Waldbronn, Germany).

3.2. HPLC (High Performance Liquid Chromatography) assay

An Agilent 1100 HPLC system with a G1315B DAD detector was used for all analyses. An Xterra® RP 18 (3.5 μm, 4.6 mm × 50 mm; Waters, Milford, MA) column was used with a mobile phase composed of 0.05% trifluoroacetic acid in water (mobile phase A) and 0.05% trifluoroacetic aid in acetonitrile (mobile phase B). The method was a gradient method starting with 10% mobile phase B and reached 65% mobile phase B at 3 min in a 6.5 min total run time. A wavelength of 320 nm nm was selected to detect G–F, and the flow rate was controlled at 1.25 mL/min and the injection volume was 10 μL. The temperature of the column also was controlled at 30 °C. The retention time of G–F was 3.2 ± 0.2 min. The standard concentration of G–F was in the range of 1–100 μg/mL. The average of duplicate values was used for the experimental solubility data. The deviation of the duplicates in this study is less than 5%.

3.3. Dissolution testing

To evaluate the solubility enhancement of the amorphous form of G–F relative to the crystalline form of this compound, an in vitro dissolution test in PBS buffer with pH 6.5 was performed using a pION μ dissolution system (PION Inc., Woburn, MA) equipped with a six channel fiber-optic probe. The media temperature of 37 °C in the sample vial was controlled by connecting it to a circulating water-bath, 150 rpm was chosen as the stir speed to ensure good mixing. About 10 mg drug was added into each vial containing 20 mL PBS buffer, the concentration was obtained via a UV spectrometer equipped with a single fiber-optic probe. In order