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CMC development of [^{14}C]-labeled sotorasib for the conduct of microtracer human ADME study

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Abstract

Human absorption, distribution, metabolism, and excretion (hADME) studies of new drugs are required for global regulatory filings. Recent advances in high sensitivity analytical technologies have enabled microtracer hADME studies wherein very low radioactive doses can be administered to healthy volunteers to study drug pharmacokinetic profile. Microtracer hADME studies are advantageous to accelerate study timelines during drug development. However, there are limited examples in peer-reviewed journals that highlight the key chemistry, manufacturing, and control (CMC) development challenges and requirements for enabling these clinical microtracer hADME studies. The current manuscript summarizes the CMC activities, risk assessment, and mitigation strategies that were put in place and executed to enable and accelerate the microtracer hADME study of [^{14}C]-labeled sotorasib (AMG 510, compound 1). Sotorasib is a first in class KRAS^{G12C} inhibitor used to treat non-small cell lung cancer (NSCLC) in patients with a KRAS^{G12C}mutation (Canon et al., Nature 575:217–223, 2019). The key CMC activities included the synthesis of low nanocurie [^{14}C]-labeled AMG 510 drug substance, development of a drug-in-bottle (DIB) formulation, use of simulation software to predict absorption profiles, associated drug substance and drug product analytical control strategies development, and the utilization of accelerator mass spectrometry (AMS) as a CMC tool enabling low radioactive strength formulation analysis.

Keywords Microtracer, Human ADME, CMC, Isotopic dilution, Radiolabeling, DIB, AMS

Introduction

Human absorption, distribution, metabolism, and excretion (hADME) studies are important to understand the fate of investigational small molecule drugs upon oral administration at their active pharmacological dose level. These studies involve the use of radiolabeled drug substance and are required by regulatory agencies for new drug registration (International 2010; European 2012; International 2013). This study is typically conducted before initiating phase III clinical trials to establish an understanding of the drug disposition and consists of a single dosing event in a small cohort of patients or healthy volunteers. The mass balance studies have been regulatory area of concern during marketing authorization dossier review (Coppola et al. 2019). Amgen has earlier published a review paper on chemistry, manufacturing, and controls (CMC) for macrotracer hADME study with a radiolabeled dose of ~ 100 µCi (Roberts et al. 2016). However, there are limited peer-reviewed journals that highlight the key CMC requirements for microtracer studies with low radiolabeled dose (~ 1.0 µCi). Main highlights of our current publication are the microtracer ADME study of [^{14}C]-labeled sotorasib conducted with

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the synthesis of low nanocurie radiolabeled drug substance (DS), development of an extemporaneous drug-in-bottle (DIB) suspension, use of simulation software to predict absorption profiles, and the application of accelerator mass spectrometer (AMS) technology as a CMC tool for formulation analysis (Fig.1). Sotorasib is a first in class $KRAS^{G12C}$ inhibitor used to treat non-small cell lung cancer (NSCLC) in patients with a $KRAS^{G12C}$ mutation (Canon et al. 2019).

Advances in highly sensitive AMS's have enabled microtracer hADME studies in which very low levels of radioactivity (~ 1.0 µCi total dose) are dosed at or below pharmacologically active dose levels. The AMS technique has been extensively used to fully characterize low levels of radioactivity in body fluids allowing generation the pharmacokinetic profile of a small molecule (Ings 2009; Spracklin et al. 2020). Microtracer studies provide four major advantages over conventional studies: (i) patients or healthy volunteers are not exposed to high levels of radioactivity; (ii) accelerated study timelines as the FDA (Food and Drug Administration) does not require supporting rodent dosimetry data for microtracer studies, consistent with Amgen's commitment to use the absolute minimum number of research animals necessary (Lozac'h et al. 2018; Amgen 2022); (iii) opportunity to evaluate ADME profile early in drug development process to validate the other species; and (iv) enables the possibility of administering a low radioactive dose in vulnerable populations such as pediatrics where a conventional radioactive hADME study is prohibited (van Groen et al. 2020).

A major challenge with microtracer hADME studies is establishing a robust CMC package for very low radioactive strength clinical material. Although the radioactive dose utilized in a microtracer study is low enough to fall within range of normal background variation, administration of radioactive drugs at pharmacological cold dose levels still requires an appropriate CMC dataset. Clinical studies involving radioactive dose administration require approval by a special ethics committee as well as submission and approval of Investigational New Drug Application (IND, US) or Investigational Medicinal Product Dossier (IMPD, EU) (Atzrodt et al. 2017). Regulatory requirements related to Good Manufacturing Practices (GMP) for radiolabeled drugs are not clearly defined. However, ICH Q7A Chap. 19 guidance for the manufacture of APIs used in clinical trials is generally followed (US 2016). Regulatory ADME CMC submissions generally outline the manufacturing process, clinical dose preparation procedure and process qualifications, analytical methods, and release and stability details per local requirements. There are limited examples in peer-reviewed journals that highlight the key chemistry, manufacturing and control (CMC) development challenges and requirements for enabling these clinical microtracer hADME studies.

Radiolabeled drug substance (DS) synthesis is usually based on the counterpart non-radiolabeled synthetic scheme. Most of the [^{14}C] radiolabeled DS syntheses use labeled barium carbonate as a precursor (Abdel-Magid and Caron 2006). While it is ideal to introduce the radiolabel as late in the synthesis as possible, this may not always be feasible due to the integral label position

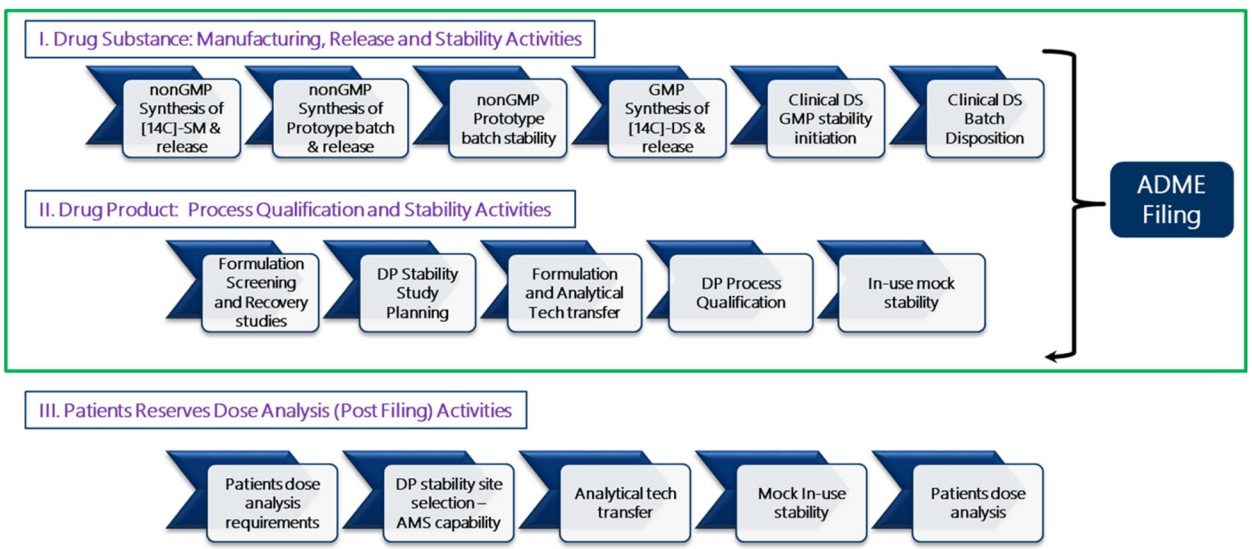

Fig. 1 Accelerated microtracer ADME CMC activities overview

required for metabolic stability and early label introduction, prior to even established non-labeled starting materials may be required, leading to significantly longer synthesis time (Roberts 2009). The isotopic dilution approach, wherein a few milligrams of high specific activity (HSA) material is synthesized first followed by HSA material dilution with unlabeled GMP-manufactured DS, is generally preferred to obtain blended radiolabeled DS with desired radioactive concentration. Synthesis of HSA material is also advantageous from a CMC standpoint to allow positive radiolabel position confirmation in the molecule which otherwise may be difficult to detect in blended material of very low radioactive strength (~ nanoCurie) used in microtracer studies.

^{14}C-labeled compounds frequently show higher decomposition rate than their unlabeled counterparts. The primary degradation pathway for these compounds is through transmutation of radioactive atoms emitting beta-radiations, resulting in structural damage to the compound. Subsequently, these high-energy beta-radiations can be self-absorbed by the molecule leading to the generation of reactive intermediates and chain decomposition reactions (Roberts 2009). These decomposition rates are influenced by storage temperatures, specific activity, and molecular structure. To mitigate inherent instability issues, radiolabeled compounds are generally stored at low temperatures (≤ -20 °C or ≤ -70 °C) or as a solution in a solvent such as ethanol (Atzrodt et al. 2020). Nevertheless, re-purification may be inevitable in instances where there is longer duration between radiolabeled compound synthesis and clinical study dosing. Ideally, stability studies should be designed to enable sufficient shelf life on clinical material allowing clinical study to be completed obviating the need for material re-purification.

Liquid preparations for oral delivery have a long history in drug development and are the preferred formulation for hADME studies as compared to solid dosage forms. These preparations include solutions, suspensions, or emulsions containing one or more active ingredients in a suitable vehicle, or simply a liquid active ingredient (The 2016). Powders for oral solution or oral suspension are often differentiated depending on the nature of the powder. If the powder is pure active pharmaceutical ingredient (API), it is commonly referred to as API-in-Bottle or Drug-in-Bottle (DIB). If the powder is a blend of API and excipients, it is commonly known as Powder-in-Bottle (PIB) (Skultety 2015). It is preferred that radioactive clinical compounds are released under cGMP at the manufacturing site post isotopic dilution and shipped in bulk to the clinical site for extemporaneous DP compounding due to stability and logistical considerations (Roberts et al. 2016).

Several decision elements, including biopharmaceutical properties of the molecule, chemical and physical stability of the molecule, project timeline and logistics, etc., should be evaluated when considering a DIB approach for a clinical study. DIB formulations must meet the essential characteristics of drug performance: bioavailability, manufacturability, and stability (Pudipeddi and Serajuddin 2005; Center 1995). Achieving these requirements in the DIB formulation space requires an understanding of critical formulation attributes. Critical formulation attributes for a suspension DIB formulation include solubility, particle size, dissolution, and redispersibility of the drug substance. For hADME studies, additional critical attributes include the target radioactive dose and in-use stability determination. Non-labeled representative drug substance is utilized to assist in formulation development and to monitor critical formulation attributes. Once formulation is finalized, a prototype radiolabeled drug substance is used to perform drug product qualification along with establishing proper stability. This data is required to support regulatory CMC filing.

Results and discussion

[^{14}C]-AMG 510 drug substance manufacturing under Current Good Manufacturing Practice (cGMP)

In determining where to position the [^{14}C] label into AMG 510 (1), we first considered incorporating the

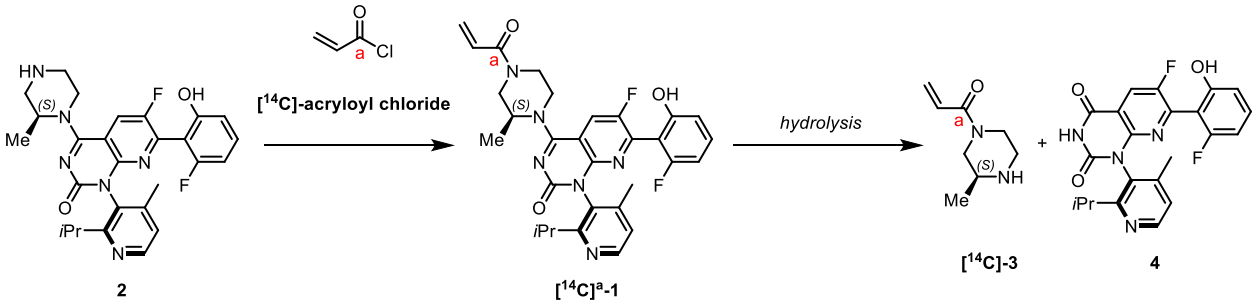

Fig. 2 Options for inclusion of [^{14}C] in compound 1

radiolabel in the carbonyl of the acrylamide (position *a*, Fig. 2) since it presented the most efficient approach from a chemistry perspective (one step from intermediate 2). However, forced degradation studies of 1 showed that hydrolysis of the piperazine functional group is a common chemical decomposition pathway under both acidic and basic conditions, forming functionalized piperazine 3 and pyrimidione 4. Due to the propensity for hydrolysis, it was considered undesired to include the [^{14}C] label into the piperazine of 1.

Next, the team sought to develop a synthesis that would introduce a radiolabel in pyrimidione moiety 4. Inclusion of a radiolabel in this group would require introduction of the [^{14}C] at an early stage of the synthesis, resulting in a long synthetic sequence after radiolabel introduction. Functionalized benzamide [^{14}C]-5 bearing a [^{14}C] label at the amide carbonyl (position *b*, Fig. 3) was chosen as a raw material due to the high degree of confidence that this molecule could be prepared and that the downstream steps for conversion to 1 were well established. This synthesis involved 6 chemical transformations and a chiral chromatographic separation.

The initial synthetic strategy sought to access [^{14}C-5] through lithium halogen exchange with bromopyridine 6 followed by quenching with [^{14}C-CO_2]. Subsequent amidation with ammonia would afford the desired [^{14}C-5]. Initial attempts at the lithiation/carboxylation step resulted in functionalization at the 4-position of the pyridine, which occurred with or without protodebromination to form compounds 7 and 8 (characterized by mass spectrometry and comparison to reference standard HPLC retention time) (Fig. 4).

Due to the challenges encountered with the lithiation approach to prepare [^{14}C-5], an alternative three-step strategy was pursued focusing on a copper-mediated cyanation to introduce the [^{14}C] label. The first step of this sequence involves a Sandmeyer reaction of aniline 9 to afford the corresponding iodopyridine. Subsequent copper-mediated cyanation and hydrolysis would provide the desired compound [^{14}C-5].

Proof-of-principle for the cyanation approach was first achieved using non-radiolabeled potassium cyanide, with the Sandmeyer iodination, cyanation, and hydrolysis reactions performing well under standard literature procedures. The initial step of the radiosynthesis was performed by treating 9 with sodium nitrite followed by potassium iodide to afford iodopyridine 10. Subsequently, a copper-mediated cyanation using potassium [^{14}C]-cyanide generated benzonitrile [^{14}C]-11, which was hydrolyzed using sulfuric acid to complete the synthesis of [^{14}C]-5 (Fig. 5).

With the key radiolabeled compound [^{14}C]-5 in hand, the remaining synthesis was completed as shown in Fig. 6 to provide the desired HSA [^{14}C]-1 with 98.1% radiochemical purity. The approach to synthesize [^{14}C]-1 from [^{14}C]-5 uses the same fundamental chemical transformations as the synthesis of unlabeled 1 (Zhang et al. 2022) and therefore provided material with an equivalent

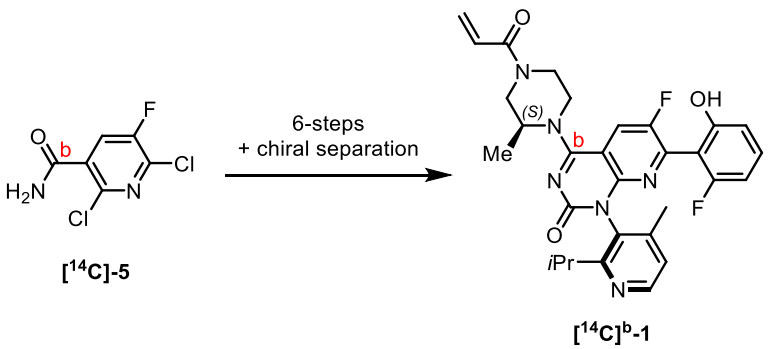

Fig. 3 Proposed synthesis of [^{14}C]-1

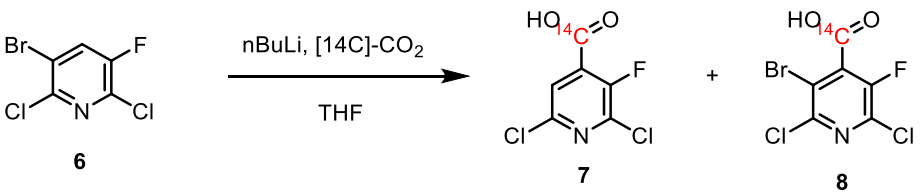

Fig. 4 Lithiation/carboxylation of 6

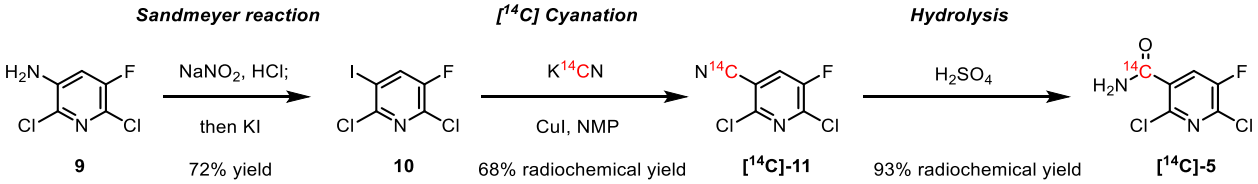

Fig. 5 Synthesis of [^{14}C-5]

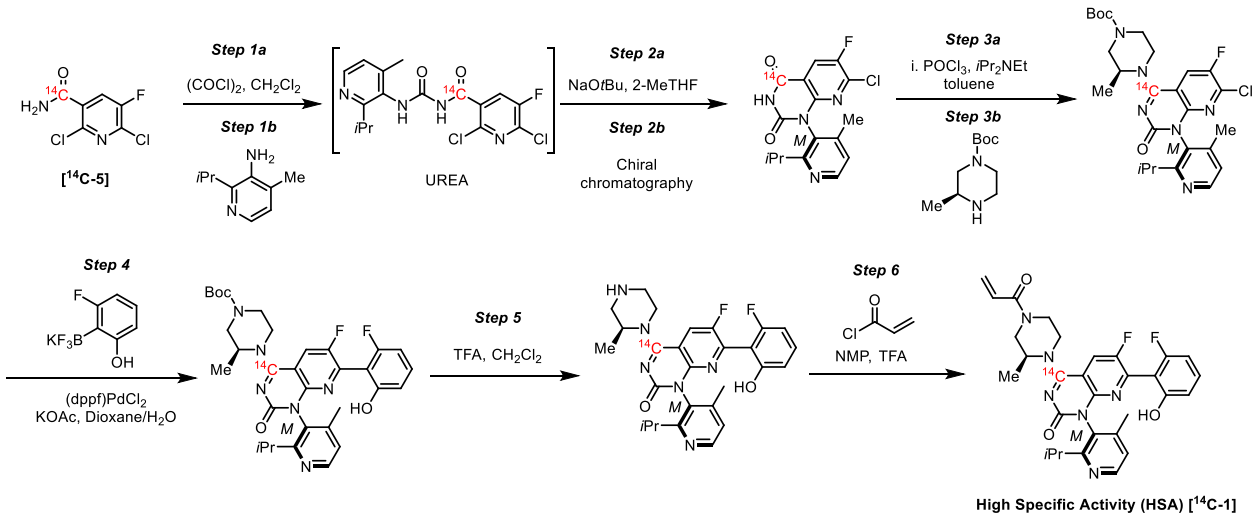

Fig. 6 Synthesis of [^{14}C-1] high specific activity

purity profile as cGMP-manufactured unlabeled drug substance.

The HSA [^{14}C]-1 was produced with a specific activity of 99.3 µCi/mg. With the target clinical dose of 720 mg and a maximum allowed radiolabeled dose of 1 µCi for hADME microtracer study, the target specific activity level of [^{14}C]-1 clinical DS was 1.4 nCi/mg, requiring a dilution of HSA with unlabeled 1 at a ratio of approximately 1:70,000. Given the high dilution ratio, the synthesis of unlabeled 1 and the final blending step with HSA [^{14}C]-1 were executed under cGMPs; however, the synthesis of HSA [^{14}C]-1 was conducted as development lot. This approach was justified due to the high dilution ratio, ensuring any residual impurities in HSA [^{14}C]-1 would not pose a risk to subjects dosed in the study (Table 1).

[^{14}C]-1 clinical DS was released under cGMP using qualified methods, and all the specification acceptance criteria were met. Release testing for HSA [^{14}C]-1 and [^{14}C]-1 clinical DS included description, identification (ID) by mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR, ^{1} H and ^{13} C), specific activity, radiochemical purity/impurities, chemical purity/impurities, chemical assay (%w/w), residual solvents, and chiral impurities tests, with ID by MS,

Table 1 HSA [^{14}C]-1 (high specific activity) results

Test	Criteria	Result
Radiochemical purity by HPLC-beta ram detector	≥ 96.0% area Any single impurity ≤ 2.5% area	98.1% area All single impurities ≤ 2.5% area
Assay (as is) by HPLC-UV[a]	Report (%w/w)	89.8% w/w
Specific activity by LSC	Report (µCi/mg)	99.3 µCi/mg
Chemical purity by HPLC-UV[a]	≥ 96.0% area	99.2% area

[a] HPLC-UV analysis was performed at 256-nm wavelength

specific activity, and radiochemical purity/impurities tests being specific to [^{14}C] part of DS. High dilution played critical role in release control strategy development. For HSA [^{14}C]-1, ID was performed by MS as specific test for [^{14}C]-label while for [^{14}C]-1 clinical DS, ID test was performed using 1 H-NMR and 13 C-NMR due to low [^{14}C] mass resulting from the high dilution. Additionally, radiochemical purity and impurities testing was not performed as part of [^{14}C]-1 DS release (Table 2) since levels of any potential radiochemical impurities from HSA [^{14}C]-1 were well below the detection limit of the radiochemical purity method after such a high dilution. Instead, radiochemical purity/

Table 2 [^{14}C]-1 clinical AMG 510 drug substance results

Test	Criteria	Result
Chemical purity by HPLC-UV[a]	≥ 96.0% area	99.7% area
Total organic impurities	≤ 4.0% area	0.32% area
Assay (as is) by HPLC-UV[a]	95.0–105.0% w/w	98.9% w/w
Specific Activity by LSC	Report (nCi/mg)	1.36 nCi/mg

[a] HPLC-UV analysis was performed at 256-nm wavelength

impurities were tested and appropriately controlled in the HSA [^{14}C]-1 (Table 1).

The stability studies were conducted consistent with ICH Q1A guidelines (Guidance 2003) at ≤ −70 °C (long-term storage) and −20 °C (accelerated condition) in amber vials with black phenolic caps lined with PTFE/14B (polytetrafluoroethylene) rubber. The overall stability studies' design and purposes are outlined in Fig.7. The storage conditions were representative of the actual storage conditions used in product storage. We first performed a lead lot stability study using a prototype batch of higher radioactivity (~3 µCi/mg, Table 2) as a worst-case scenario for preliminary stability assessment and to support clinical batch disposition. This material was tested for appearance, chemical assay/purity/impurities, and radiochemical purity. Subsequently, when [^{14}C]-1 clinical DS (~1.4 nCi/mg radioactive strength) was synthesized, cGMP stability study was initiated which encompassed complete hADME clinical study up to clinical study closure. This material was tested for appearance and chemical assay/purity/impurities and a 6-month expiry was established at ≤−70 °C based on the stability data per regulatory guidance. Both materials (prototype and clinical DS) showed no degradation at ≤−70 °C and −20 °C temperatures for 6 months. Considering the criticality to maintain radiolabeled DS stability to ensure successful hADME study completion, it is recommended to have not more than 3 months difference between radiolabeled DS manufacturing and clinical dosing. No formal stability study was conducted on HSA [^{14}C]-1 material considering the expected complete utilization of HSA material in the isotopic dilutions and the possibility of performing material re-purification, should any significant change in HSA [^{14}C]-1 material quality was observed during testing before performing isotopic dilutions. The HSA [^{14}C]-1 material was also stored at ≤−70 °C post synthesis. Any impact on the final blended material from the HSA instability, which may have occurred from isotopic blending process, was considered insignificant due to the high dilutions performed.

Additionally, in-use radiolabeled DS stability data for the prototype batch in amber glass vials with black phenolic caps lined with PTFE/14B rubber was generated. Results showed that appearance, chemical assay, chemical purity, organic impurities, and radiochemical purity remained within specification for up to 48 h at room temperature after removal from the ≤ −70 °C storage condition. This data supported the [^{14}C]-1 clinical drug substance handling at room temperature for up to 48 h for the formulation preparation at the clinical site.

Oral formulation: drug-in-bottle (DIB) suspension

Formulation development and in silico biopharmaceutical modeling

AMG 510 is an amphoteric molecule with low solubility (0.06 mg/mL) in water. With a target clinical dose of 720 mg, a suspension formulation had to be developed to meet the clinical dosing requirements. A 1% HPMC and 0.5% polysorbate 80 formulation was nominated based on viscosity of the vehicle and thorough qualitative observations such as redispersibility of the drug substance during the compounding process and storage. Redispersibility was evaluated by recording the number of 180° inversions it took to form a homogenous suspension. Additional observations were recorded to ensure robust vehicle selection for the hADME study such as foaming of the vehicle and drug substance caking at the bottom of the formulation vial during compounding/storage.

Milling DS presents challenge for cleanup and containment of radiolabeled materials. As a result, the radiolabeled DS manufacturing site could not mill blended clinical DS and hence particle size was identified as a critical quality attribute for the formulation. The impact of particle size on dissolution and absorption profiles was

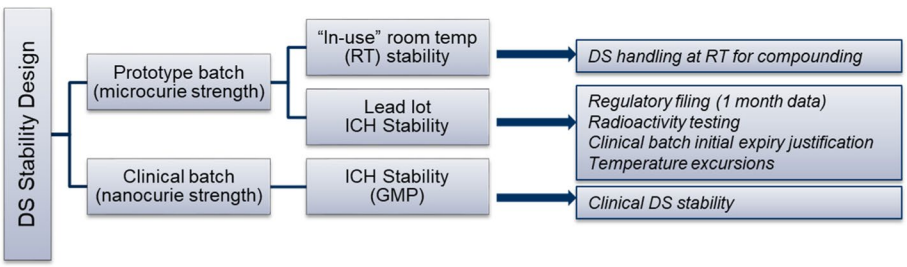

Fig. 7 [^{14}C]-labeled drug substance stability design and purpose

evaluated. Non-radiolabeled DS material was used to determine excipient compatibility of the drug substance and to define the formulation protocol during the compounding processes.

The two-step formulation compounding procedure for AMG 510 DIB formulation is described in Fig. 8. During formulation development, the particle size of the drug was monitored during the formulation process, before and after the sonication step (Fig. 9). Impact of particle size on absorption was evaluated through in vitro dissolution experiments. Almost immediately upon the addition of the suspension to the dissolution vessel, the drug showed a particle size dependent dissolution and release (Fig. 10).

The particle size (D90) of unmilled AMG 510 DS was about ~ 200 µm, while the particle size (D90) of milled AMG 510 DS used for tablet preparation was < 50 µm. At the higher particle size range, there is a potential for longer dissolution time that could have negative impact on dissolution and thereby absorption and exposure. This was confirmed by GastroPlus modeling and simulation. GastroPlus is a mechanistic physiologically based pharmacokinetic simulation software package that simulates oral absorption, biopharmaceutics, and pharmacodynamics in humans and animals. Physiologically based pharmacokinetic (PBPK) models represent the body as compartments parameterized based on physiology of tissues and organs including composition, volumes, and blood flows. Physiologically based pharmacokinetic models integrate this physiological description with compound-specific data to predict the pharmacokinetics of drugs, allowing simulation of the time course of drug concentrations in plasma and tissues. A PBPK model was built in GastroPlus™ V9.0 (SimulationsPlus) using the physiochemical properties, dissolution data in Fig. 10, and the available Human PK data. A parameter sensitivity analysis was conducted which allowed to determine the effect of an input parameter, e.g., solubility, dissolution rate, and particle size on a pharmacokinetic property like fraction

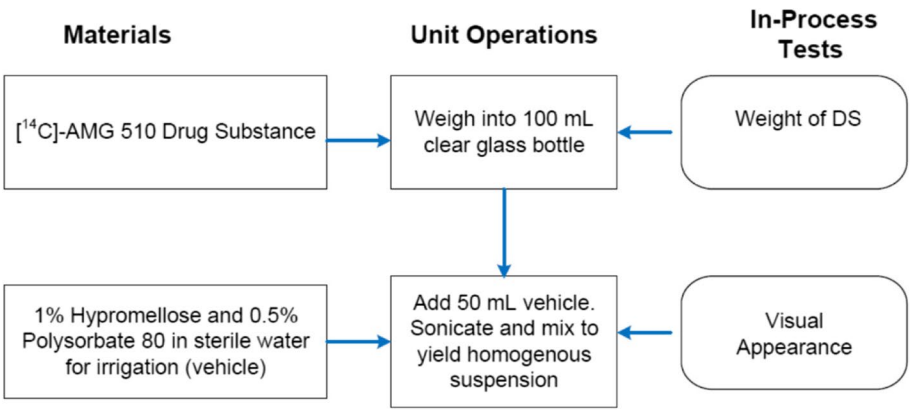

Fig. 8 Flow diagram of the compounding process of [^{14}C] AMG 510 oral suspension

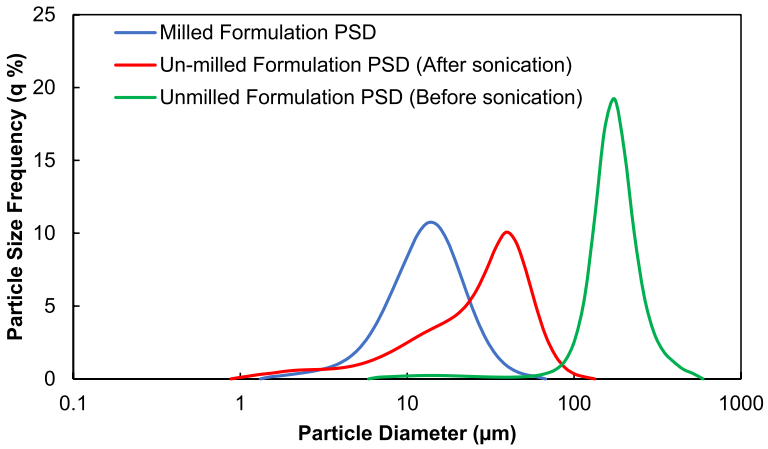

Fig. 9 Summary of particle size distribution of milled and unmilled (before and after sonication) AMG 510 during DIB formulation development

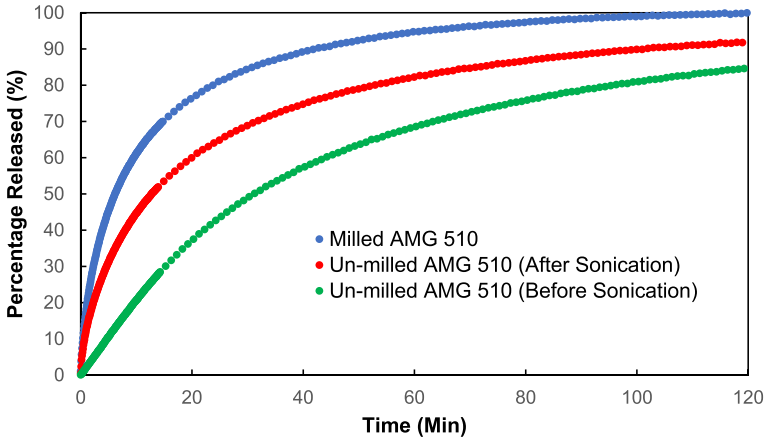

Fig. 10 Summary of dissolution profiles of formulated milled and unmilled (before and after sonication) AMG 510 during DIB formulation development

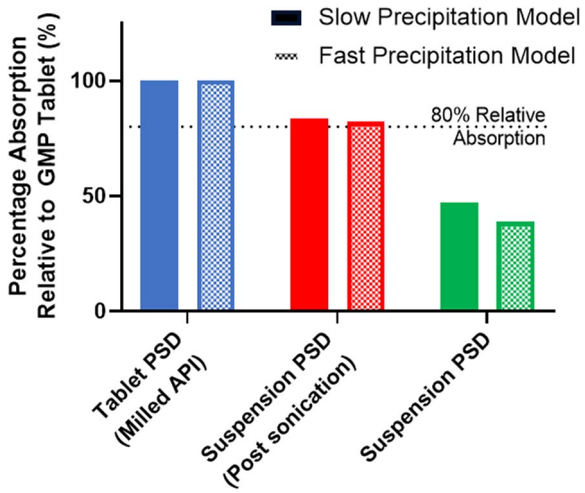

Fig. 11 Parameter sensitivity analysis of AMG 510 showing effect of particle size and precipitation rate on absorption

absorbed. Figure 11 captures the parameter sensitivity analysis of particle size distribution and precipitation rate on the absorption of AMG 510. Detailed description of the model parameters is not disclosed due to its confidential nature.

AMG 510 final dose preparation as a suspension included a 90-min sonication step which was modified to ensure that the particle size (D90) of radiolabeled AMG 510 in the suspension was reduced from ~ 200 to ~ 50 µm; this reduction in particle size is not predicted to have a significant impact on the absorption of AMG 510, irrespective of the precipitation rate, based on the GastroPlus™ modeling data presented above. This sonication step ensured proper drug solubilization, homogenization of suspension, and break down of any drug substance agglomerates into primary particles. The particle size (D90) was confirmed to be < 50 µm for the mock preparation (using unlabeled drug substance) at the clinical site.

Dosing regimen

For suspension administration, it is critical to confirm through analytical recovery studies that the dosing instructions ensure accurate dose delivery to the subjects. During development, the dosing instructions were evaluated by preparing analytical samples following exact dosing instructions, i.e., suspension bottle was shaken well, and the content was transferred into analytical flask followed by rinsing the bottle three times with approximately 50-mL water and transferring the rinses into the same flask. Another 50-mL water was transferred to the same flask. Subsequently, samples are dissolved using acetonitrile (~ 250 mL volume) and brought to the nominal method concentration (0.3 mg/mL) by diluting 10mL stock to 50 mL in a flask using method diluent (1:1 acetonitrile and water mixture) for %recovery determination against DS standards. Dosing instructions were demonstrated to be suitable, with nearly 100% recovery values. Thus, the following dosing regime was followed during clinical study:

> *"The entire content (~ 50 ml) of each subject's dose was directly administered from the dosing bottle after shaken well. To ensure the complete dose was given to the subject, the empty bottle was rinsed thrice with water (approx. 50 mL each rinse) and each rinse solution was administered to the subject. After oral administration of rinse solutions (approximately 150 mL total in volume), the subjects were given additional 50 mL of sterile water for injection to drink. In total, each sub-*

ject received 720 mg of AMG 510 dose containing 1μCi of ^{14}C-AMG510 in 50 mL suspension vehicle and with nearly 200 mL of total rinses/water."

Process qualification and stability

DP process qualification was executed at the clinical site using [^{14}C]-prototype DS batch (~3 μCi/mg) to prepare individual DIB suspensions at 14.4 mg/mL concentration using 100 mL clear Pyrex glass bottles with an unlined polypropylene cap (representative of clinical dosing bottles). The 14.4 mg/mL oral suspension was prepared by mixing 720-mg radiolabeled AMG 510 drug substance in 50-mL aqueous vehicle containing 1% hypromellose and 0.5% polysorbate 80, resulting in a homogenous well-dispersed suspension. The pH of the resulting AMG 510 suspension was within acceptable range for oral administration. The resulting mixture is sonicated for about 90 min to obtain a well-dispersed, homogenous suspension.

The specifications were set based on the use of various chemical and physical tests that provide information on the characteristics of the drug product. All the testing criteria, including appearance, chemical identity, radiochemical identity, assay (%label claim), chemical purity and impurities, radiochemical purity, and radioactivity concentration, were met (Table 3). Process qualification data indicated that the %label claim met the acceptance criterion of 90.0–110.0% (i.e., 648 mg/dose to 792 mg/dose) of the target 720 mg/dose. The drug product compounding pharmacy released excipients based on manufacturer's certificate of analysis which provided sufficient control for this phase I clinical study. The qualification DP batch was placed on stability at room temperature for 48 h and samples showed no change in appearance, assay, chemical purity, organic impurities, radiochemical purity and radioactive concentration after 48 h. This data supported chemical and radiochemical stability of the [^{14}C]-drug substance 14.4 mg/mL clinical oral suspension for up to 48 h at room temperature.

Clinical dose control strategy

Based on successful process qualification using the prototype batch, the in-process tests during clinical compounding of [^{14}C]-AMG 510 14.4 mg/mL oral suspension included a DS weight check and a visual appearance test to ensure a uniform suspension was performed (Fig. 9). The radioactivity concentration in each dose was determined from the actual weight of the [^{14}C]-AMG 510 DS recorded during the oral suspension compounding and the specific activity determined at the [^{14}C]-clinical DS release. Prior to dose administration, the dosing suspensions of [^{14}C]-DS 14.4 mg/mL were stored at temperatures below 30 °C protected from light and were administered within 48 h stability window obviating the need for post-administration testing.

AMS-based formulation stability study and patients dose reserves analysis

AMS is highly sensitive mass spectrometry technique used for the detection of long-lived radioisotopes such as ^{14}C. While the AMS has been extensively utilized in bioanalysis of drug metabolites in pharmacokinetic (PK) studies, this technique has been utilized in this study as a CMC tool to characterize formulation stability and patient analytical reserves due to the low specific activity (~1 μCi) of clinical dose suspensions.

During clinical dose preparations, it is customary to preserve a few clinical dose suspensions as analytical reserves that are intended to be analyzed in the event of an unexpected finding during the clinical study. Since the clinical study target completion date was far beyond dose stability window and the impact of deep freezing the suspension samples was unknown, it was decided to analyze these analytical reserves within 48 h post preparation for radiochemical purity/impurities and radioactive concentration.

A mock 14.4 mg/mL DIB suspension in-use stability study was conducted at the AMS site using clinical DS (1.4 nCi/mg radioactive strength) as outlined in the Table 4. Since clinical dose analytical reserves had to be shipped from clinical site to the AMS testing site for analysis post preparation, this stability study was designed to assess the shipping impact as well. These stability samples

Table 3 DP process qualification results

Test	Criteria	Result
Radiochemical purity by HPLC-beta ram detector	NLT 96.0% area	100.0% area
Assay (% label claim) by HPLC-UV[a]	90.0–110.0% w/w	98.5% w/w
Chemical Purity by HPLC-UV[a]	NLT 96.0% area (Total impurities NMT 4.0% area)	99.7% area (Total impurities 0.31% area)
Radioactive concentration by LSC (Target 1.0 μCi/dose)	80.0–120% target	99.1%

[a] HPLC-UV analysis was performed at 256 nm wavelength

Table 4 Mock clinical formulation stability design using HPLC-AMS

Mock dose preparation	Testing site	Test purpose	Storage temperature	Time points		
				T0	T24h	T48h
AMS site	AMS site	In-use stability	Below 30 °C, protected from light	x	x	x
Clinical site	AMS site	Shipping mitigation		-	x	-

"x" denotes appearance and radiochemical purity and impurities tests were performed

"-" denotes no tests were performed

Table 5 AMS-based stability study results

Test	Criteria	T0	T24h	T48h	Clinical site T24h
Appearance	White, opaque, well dispersed suspension	Conforms	Conforms	Conforms	Conforms
Radiochemical Purity	Radiochemical purity: ≥ 96.0% area Any single unspecified impurity ≤ 2.5% area	98.7% All impurities <2.5%	99.1% All impurities <2.5%	98.4% All impurities <2.5%	99.1% All impurities <2.5%
Radioactivity Concentration	Target 1.0 µCi/dose	1.0 µCi	1.0 µCi	1.0 µCi	1.0 µCi

were tested and passed criteria for appearance, radiochemical purity and impurities (HPLC-AMS), and radioactive concentration (LSC) as shown in Table 5. Thus, clinical dose suspension was demonstrated to be stable for 48 h with no impact from shipping.

Post-clinical patients dosing, actual clinical dose analytical reserve samples ($n=2$) were analyzed at the AMS site and met the specification criteria with radiochemical purity of 98.7–99.0%; any single impurity was observed at less than 0.5% area and radioactive concentration of 0.95–1.0 µCi/dose.

Conclusion

The CMC activities related to accelerated microtracer hADME study for [^{14}C]-labeled Sotorasib have been outlined here. Several contract manufacturing sites were utilized to accomplish end-to-end campaign activities such as manufacturing, release, and stability of GMP [^{14}C]-labeled drug substance, DP process qualification, and finally AMS-based patients reserves analysis. A longer synthetic route was executed due to the integral radiolabel position required to achieve desired metabolic stability. Due to solubility limitations, a DIB suspension formulation was developed. The risk of particle size dependent absorption was assessed and mitigated by designing a compounding procedure that included an additional sonication step and in silico biopharmaceutical modeling. A phase-appropriate approach was implemented during analytical technology transfers and control strategies development. The first-person utilization (to the best of our knowledge) of AMS technology is demonstrated as a CMC tool enabling the stability and testing of low radioactive strength formulations.

Methods

Analytical characterizations were performed using standard reversed-phase high-performance liquid chromatography (HPLC) instruments equipped with different detectors, such as an ultraviolet detector (UV, 256 nm) utilized for chemical purity/impurities/potency determinations, a beta ram detector utilized for radiochemical purity/impurities determinations and an AMS (accelerator mass spectrometry) used for clinical dose analysis. A standard Liquid Scintillation Counting (LSC) was used for specific activity determinations.

Particle size was assessed using a light scattering analyzer (Malvern Mastersizer 3000). The in vitro dissolution was conducted using a µDISS Profiler (Pion Inc., Billerica, MA) equipped with in situ fiber optic UV probes and a mini-bath platform for temperature and agitation control. The fiber optic probe assembly with 1-mm pathlength tip was used to build a standard curve. The dissolution experiment was performed with fasted state simulated gastric fluid (FaSSGF) at pH 1.6 and at the characteristic wavelength of AMG 510 (256 nm), where 2 mL of AMG 510 suspension was added to 8 mL of dissolution media. Accuracy of the fiber optic UV results was verified by manual sampling and analyzed by HPLC. The impact of particle size on dissolution were confirmed by

GastroPlus modeling and simulation using GastroPlus™ V9.0 (SimulationsPlus).

Abbreviations

hADME	Human absorption, distribution, metabolism, elimination
CMC	Chemistry, manufacturing, and controls
AMS	Accelerator mass spectrometer
DIB	Drug in bottle
DS	Drug substance
FDA	Food and Drug Administration
IND	Investigational New Drug Application
IMPD	Investigational Medicinal Product Dossier
cGMP	Current Good Manufacturing Practices
GMP	Good manufacturing practices
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
NSCLC	Non-small cell lung cancer
HSA	High specific activity
PIB	Powder in bottle
API	Active pharmaceutical ingredient
ID	Identification
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
PTFE	Polytetrafluoroethylene
HPLC	High-performance liquid chromatography
UV	Ultra-violet
LSC	Liquid Scintillation Counting
PBPK	Physiologically based pharmacokinetic
PSD	Particle size distribution
PK	Pharmacokinetic

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Authors' contributions
SS contributed to the analytical specifications and testing strategies development and execution for drug substance, intermediates/starting materials, and drug product. PA and JEH contributed to the oral formulation development, in silico biopharmaceutical modeling, and drug product process qualification. ATP contributed to the radiolabeled drug substance process development and manufacturing controls. TLC provided subject matter expertise during ADME study planning. SS, PA, ATP, and JEH wrote the manuscript with TLC being the reviewer. All authors read and approved the final manuscript.

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Availability of data and materials
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Declarations

Competing interests
The authors declare the following competing financial interest(s): All authors are current Amgen employees and may own Amgen stocks/shares.

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