Expanding the Repertoire for "Large Small Molecules": Prodrug ABBV-167 Efficiently Converts to Venetoclax with Reduced Food Effect in Healthy Volunteers

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ABSTRACT

Since gaining approval for the treatment of chronic lymphocytic leukemia (CLL), the BCL-2 inhibitor venetoclax has transformed the treatment of this and other blood-related cancers. Reflecting the large and hydrophobic BH3-binding groove within BCL-2, venetoclax has significantly higher molecular weight and lipophilicity than most orally administered drugs, along with negligible water solubility. Although a technology-enabled formulation successfully achieves oral absorption in humans, venetoclax tablets have limited drug loading and therefore can present a substantial pill burden for patients in high-dose indications. We therefore generated a phosphate prodrug (**3**, ABBV-167) that confers significantly increased water solubility to venetoclax and, upon oral administration to healthy volunteers either as a solution or high drug-load immediate release tablet, extensively converts to the parent drug. Additionally, ABBV-167 demonstrated a lower food effect with respect to venetoclax tablets. These data indicate that beyond-rule-of-5 molecules can be successfully delivered to humans via a solubility-enhancing prodrug moiety to afford robust exposures of the parent drug following oral dosing.

Introduction

Apoptosis is a highly regulated form of cell death that is critical for multiple processes including embryonic development, tissue homeostasis, and regulation of the immune system (1). The process of and commitment to intrinsic apoptosis is governed by a dynamic equilibrium of binding interactions between the proapoptotic and antiapoptotic BCL-2 family members (2, 3). Defects in apoptotic signaling are a common requirement for oncogenesis (4) that are often driven by an overabundance of antiapoptotic factors. One such factor is BCL-2, which plays a dominant role in the survival of lymphoid malignancies as well as in some solid tumors, where its overexpression is regulated by a variety of mechanisms. Venetoclax (**1**, **Fig. 1**) is a first-in-class selective inhibitor of BCL-2 approved for the treatment of chronic lymphocytic leukemia (CLL; ref. 5) and acute myelogenous leukemia (AML; ref. 6). Venetoclax-based therapy has also shown encouraging activity in other malignancies, such as multiple myeloma (MM; ref. 7) and estrogen receptor–positive breast cancer (8). The clinical maintenance dose of venetoclax in CLL is 400 milligrams (mg) per day, which is achieved following a ramp-up over five weeks to manage tumor lysis syndrome. In some indications, doses as high as 1,200 mg have been explored (9).

Given the multitude of diseases where BCL-2 inhibition could yield therapeutic benefit (10), the continued clinical evaluation of venetoclax is critical. Moreover, as the magnitude of BCL-2 expression and dependency varies across diseases, the ability to achieve a wide range of drug exposures could increase the chances of identifying the most optimal dose for patient benefit in distinct clinical settings. Although venetoclax can be safely administered up to 1,200 mg (9), it is classified as a Biopharmaceutical Classification System (BCS class IV) drug (11), reflecting its low aqueous solubility and permeability. To overcome the challenging physicochemical properties (**Table 1**) and enhance the oral bioavailability of venetoclax, an amorphous solid dispersion (ASD) formulation was developed (12, 13). Although the resultant tablets have been effective to date, the drug loading by weight within each tablet is limited to ensure robust stability and clinical performance (13); this confers a pill size, and hence total pill burden, that can present challenges for patients in high-dose indications. Additionally, venetoclax has a significant food effect in humans, with a 3–5-fold increase in exposure depending on the fat content of the meal (14). As venetoclax is prescribed to be taken with a meal, this food effect can result in pharmacokinetic variability as noted in population pharmacokinetic analyses (15, 16).

Given the potential of BCL-2 inhibition in human diseases, we set out to explore alternatives to the venetoclax tablet that could potentially offer patients a lower pill burden option, and one with decreased food effect. To this end, we envisioned the incorporation of a labile and ionizable pro-moiety into venetoclax that could confer sufficient water solubility yet allow for efficient transformation to and absorption of the

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Table 1. Geometric mean (mean, %CV) pharmacokinetic parameters of venetoclax following administration of venetoclax tablet or ABBV-167 as a solution or tablet.

Pharmacokinetic parameters (units)	Venetoclax tablet fasting ($N = 12$)	ABBV-167 solution fasting ($N = 11$)	ABBV-167 tablet fasting ($N = 11$)	ABBV-167 tablet high-fat ($N = 12$)
C_{max} (μg/mL)	0.137 (0.149–47)	0.460 (0.496–35)	0.297 (0.311–37)	0.505 (0.543–37)
T_{max}[a] (h)	4.2 (4.0–6.0)	4.0 (4.0–6.0)	4.0 (2.0–6.0)	6.0 (4.0–10.0)
$t_{1/2}$[b] (h)	13.6 (5.11)	15.3 (2.98)	14.6 (4.38)	13.3 (3.52)
AUC_t (μg•h/mL)	1.70 (1.85–45)	6.03 (6.51–34)	3.43 (3.67–43)	6.87 (7.35–35)
AUC_∞ (μg•h/mL)	1.78 (1.93–45)	6.22 (6.74–35)	3.55 (3.81–44)	7.09 (7.60–36)

[a]Median (minimum through maximum).
[b]Harmonic mean (pseudo-standard deviation).

active drug in the gastrointestinal (GI) tract upon oral dosing (17). This water-soluble prodrug approach, in turn, would provide the opportunity to develop a higher drug-load tablet formulation using conventional technologies and achieve a reduction in pill burden. Finally, we hypothesized that increased aqueous solubility of the prodrug accompanied by rapid conversion to parent in the GI tract could alleviate the impact of food on drug exposure.

The approach of adding labile phosphate or other charged groups to increase water solubility has been successfully applied to advanced clinical-stage molecules (17–19). However, venetoclax is a complex "beyond-rule-of-5" (BRO5) molecule (20) with physicochemical properties that fall well outside those of typical orally bioavailable drugs. Although prodrugs of poorly soluble compounds in the BRO5 space have been generated (17), none have reported suitable conversion to the parent molecule in humans following oral administration (21, 22). Likewise, the ability of a water-soluble prodrug to meaningfully reduce food effect in humans has not been previously described to our knowledge. This lack of precedent indicated that successful oral delivery of a venetoclax-based prodrug in humans could represent a considerable advancement for patients, as well as drug delivery.

Starting with venetoclax, we designed a prodrug ABBV-167 (**3**, **Fig. 1**) that achieves a significant increase in solubility in aqueous media at neutral pH. This molecule is rapidly converted to venetoclax upon intravenous (i.v.) or oral dosing in preclinical species, with minimal prodrug remaining in circulation. The noteworthy property transformation facilitated the generation of a high drug-load immediate release (IR) tablet with significantly lower mass than the venetoclax tablet at the same dose strength. Oral administration of ABBV-167 as either a solid dosage form or an aqueous solution to healthy volunteers rendered higher venetoclax exposure in the fasted state compared with venetoclax tablets, while also showing robust exposure and a reduced food effect compared with these tablets in the presence of a high-fat meal. These results suggest that ABBV-167 could serve as a lower pill burden alternative for patients with BCL-2–mediated diseases. Additionally, this study indicates that a prodrug strategy aimed at oral pill burden reduction may be amenable to other molecules within the BRO5 space.

Materials and Methods

Initial preparation of ABBV-167

To a solution of venetoclax (**1**) (1.2 g, 1.4 mmol/L) in acetonitrile (20 mL) was added di-tert-butyl chloromethyl phosphate (1.1 g, 4.15 mmol/L) and *N, N*-diisopropylethylamine (1.2 mL, 6.9 mmol/L). The mixture was heated in a Biotage microwave synthesizer at 80°C for 1.5 hours and concentrated. The residue was dissolved in dichloromethane (5 mL), treated with trifluoroacetic acid (5 mL) for 1 hour and concentrated. The residue was purified by reverse phase chromatography, eluting with 40%–65% acetonitrile in 0.1% trifluoroacetic acid water to give compound **3** as a trifluoroacetic acid salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.73 (d, 1 H), 8.60 (t, 1 H), 8.53 (d, 1 H), 8.46 (d, 1 H), 7.98 (d, 1 H), 7.81 (dd, 1 H), 7.57 (d, 1 H), 7.40 (d, 2 H), 7.15 (d, 1 H), 7.09 (d, 2 H), 6.85 (d, 1 H), 6.79 (dd, 1 H), 6.46 (d, 1 H), 6.25 (d, 2 H), 3.28 (m, 4 H), 3.22 (s, 3 H), 3.03 (m, 3 H), 2.25 (m, 3 H), 2.01 (m, 5 H), 1.78 (m, 3 H), 1.61 (m, 2 H), 1.46 (m, 4 H), 1.03 (m, 6 H), 0.95 (s, 6 H). MS (ESI) *m/z* 978.3 $(M + H)^+$.

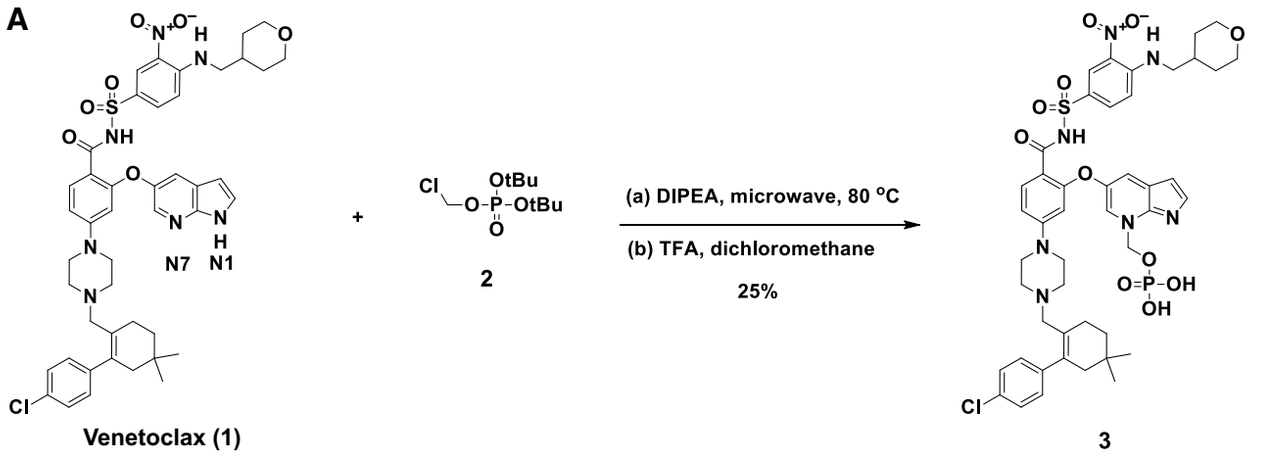
Solubility assessments

Reagents used included 0.1N HCl, 50 mmol/L acetate buffers μ = 0.155 with NaCl, and phosphate-buffered saline. Simulated intestinal fluid prepared using powder from Biorelevant.com. Equipment/Instruments used included balance Mettler Toledo, UMX2; water bath Vankel Rotating Apparatus, set at 37°C and 25 RPM; HPLC Agilent 1100.

Samples were tested in aqueous media at 37°C. Excess amount of the bulk drug was weighed out and mixed with an aliquot of target media in a clear glass vial. The vial was sealed with a cap and wrapped with aluminum foil, then tumbled in a 37°C water until equilibrated. When equilibration was completed, the samples were removed from the water bath and the final pH values were measured. The suspensions were filtered through 4-mm, 0.2-μm Millex-LG syringe filters (Millipore). Each filter was only used for one sample and the first three droplets were discarded. The filtrate was assayed after appropriate dilution with the same solvent as used for stock solution. Three replicates were prepared. The concentration of the sample was determined by injecting in HPLC and calculated against a calibration curve for the compound.

Pharmacokinetic assays

Studies across species were conducted to evaluate the pharmacokinetic profile of **3** after a single i.v. or oral dose. CD-1 mice (male) were obtained from Charles River Laboratories. Beagle dogs (male/female) were obtained from Marshall Farms. Doses were administered as the free base. **3** was prepared as a solution in D5W with 2.1 equivalents of sodium hydroxide for the intravenous dosing or as a solution or suspension in 0.2% hydroxypropyl methyl cellulose (HPMC) with 2.1 equivalents of sodium hydroxide in mouse (solution) and dog (suspension). Mice were permitted food and water ad libitum. Dogs were fasted overnight prior to dosing, with free access to water. Food was returned to dogs 4 (i.v.) or 12 (p.o.) hours after drug administration. The intravenous dose in mouse was administered as a slow bolus to the penile vein under



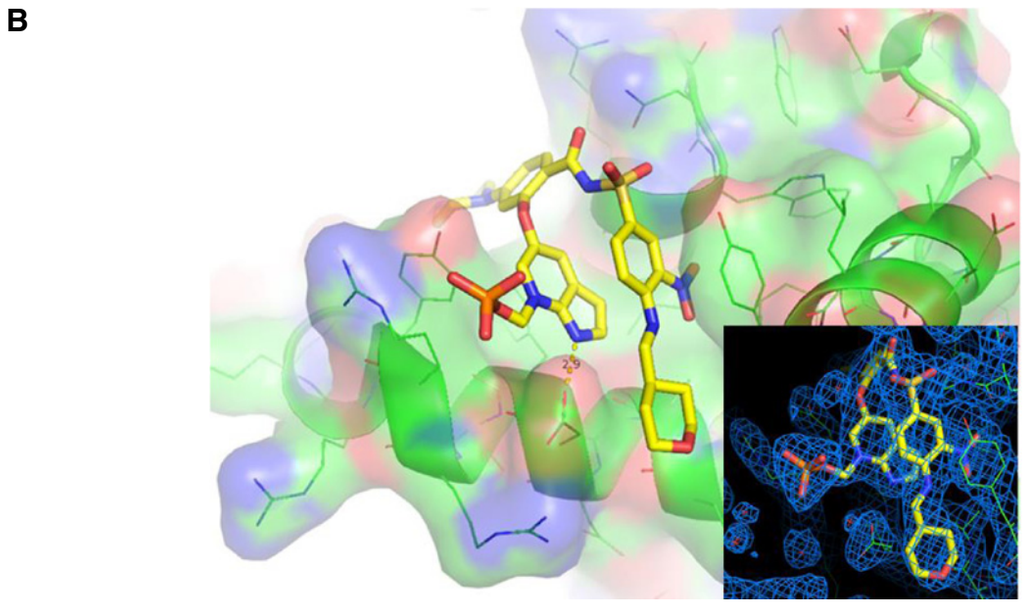


Figure 1.

Initial synthesis route to prodrug **3** and X-ray structure of **3** complexed to BCL-2. **A,** Structure of venetoclax (1) and initial synthesis route of prodrug **3**. **B,** X-ray structure of **3** complexed with BCL-2 (PDB code 7LHB), 2.07Å with insert showing the electron density for the compound (mesh 1σ). Crystallography methods and diffraction statistics are found within the Supplementary Information section.

anesthesia at a dose volume of 10 mL/kg. The intravenous dose in dog was administrated as a slow bolus to the cephalic vein at 0.5 mL/kg. Oral doses were administered via gavage at 10 and 5 mL/kg in mouse and dog, respectively. Blood samples collected into EDTA anticoagulant for plasma concentration analysis were obtained from each animal after dosing. Nine to 12 serial samples were obtained over a period of 24 to 72 hours for mice and dogs, respectively, after i.v. dosing and for 72 hours in both species following oral dosing. Plasma was separated by centrifugation (3,000 g × 10 minutes, ∼4°C) and stored frozen (< 15°C) until analysis. All animal studies were conducted in accordance with the guidelines and protocols established and approved by the internal Institutional Animal Care and Use Committee (IUCUC) of AbbVie.

Preparation of clinical formulations of ABBV-167

The oral solution was prepared at the clinical site via extemporaneous dose preparation by dissolving the API into a neutral-pH buffered solution immediately prior to administration. Dibasic sodium phosphate was dissolved in water and mixed with dilute HCl solution to a target pH of approximately 7.5. The API was then added to this media at 1.13 mg/mL on a free basis and stirred until dissolution. 100 mL was administered to each subject.

The IR tablets were prepared by wet granulating the API with a binder, filler, and glidant in the presence of water in a high shear mixer (Gral 25L, GEA). The granulation was tray dried, delumped, and blended with lubricant and a disintegrant. This tablet blend was then compressed in a high-speed tablet press (XL100, Korsch AG) with

7 mm round tooling. The tablet target weight was 172.5 mg with an active dose of 113 mg ABBV-167 (free base).

Clinical study

Twelve adult healthy female subjects were enrolled in an open-label, single-dose, 4-period crossover study conducted in the United States. Subjects were randomly assigned in equal numbers to one of four sequences of regimens A, B, C, and D as shown in **Fig. 4A**. On the morning of day 1 of each period, subjects received either a 100-mg single dose of venetoclax tablet (regimen A), 100 mg equivalent of ABBV-167 solution (regimen B), or 100 mg equivalent of ABBV-167 tablet (regimens C and D). Each dose of the study drug was taken orally with approximately 240 mL of water under fasting conditions except regimen D, which was given 30 minutes after starting after a high-fat breakfast. More details on the study design, pharmacokinetic, statistical, and safety assessments are provided in the Supplementary Information. The clinical study was conducted in accordance with Good Clinical Practice guidelines and ethical principles that have their origin in the Declaration of Helsinki. The protocol was approved by the institutional review board at Vista Health System, and written informed consent was obtained from each patient before any study-related procedures were performed.

Determination of sample size in the clinical study

Complete data from 12 subjects would provide at least 81.5% power to detect a 55% increase in the venetoclax C_{max} central value between ABBV-167 formulations and the venetoclax formulation under fasting condition. The power calculations were performed using logarithmic transformation. The calculation assumed the crossover model error term variance of 0.1321 for the natural logarithm of C_{max}. This value is selected based upon the within-subject variance for log-transformed venetoclax C_{max} observed in a previous venetoclax bioavailability and food effect study (14).

Sample collection and bioanalytical methods in the clinical study

Blood samples for venetoclax and ABBV-167 assay were collected in 3 mL K_2EDTA containing plastic tubes by venipuncture prior to dosing (0 hour) and at 1, 2, 4, 6, 8, 10, 12, 24, 48, and 72 hours after dosing in each study period. Plasma concentrations were determined using liquid–liquid extraction and liquid chromatography with tandem mass spectrometric detection. The lower limits of quantitation for venetoclax and ABBV-167 were 2.14 ng/mL and 0.103 ng/mL, respectively.

Data sharing statement

AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications.

These clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement. Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

Results

Design and generation of high-molecular-weight venetoclax prodrug 3

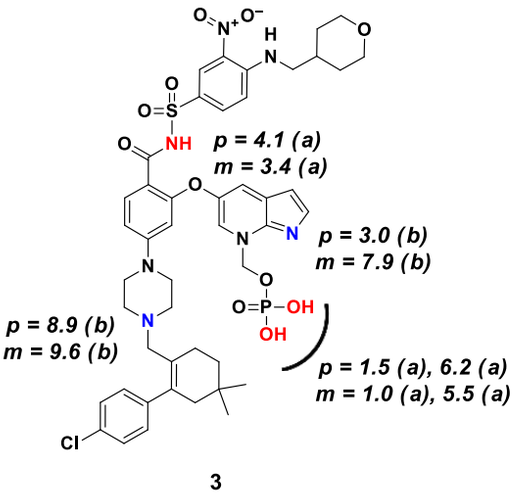
Our aspirations for a venetoclax prodrug intended for daily oral delivery included solubility sufficient to enable high drug-loading formulations via conventional technologies (23). Minimal systemic exposure of the prodrug was also desired to simplify toxicological evaluation (24). Both attributes pointed to a highly charged pro-moiety that could confer elevated solubility while exhibiting limited passive permeability within the intestinal lumen. We therefore focused on phosphate-based pro-moieties that have been shown to confer enhanced solubility to organic molecules along with the ability to be cleaved *in vivo* via alkaline phosphatases (23). However, this approach lacked precedent for molecules with the atypical physicochemical properties of venetoclax, suggesting potential challenges during preclinical development.

To explore modification of the pharmacophore with solubilizing pro-moieties, we first evaluated the various potential attachment points on the venetoclax structure. As robust stability of the final product would be required throughout manufacturing, formulation, and storage, bond strength was deemed a critical factor. The 5-substituted azaindole group of venetoclax offered a mildly reactive aromatic system that could potentially allow for installation of the collapsible pro-moiety methyleneoxyphosphate (23) at one of two sp2-hybridized endocyclic nitrogen atoms (*N1, N7*; **Fig. 1A**). To effect this transformation, a mixture of venetoclax (**1**) and three equivalents of di-tert-butyl chloromethyl phosphate in acetonitrile was heated in a microwave reactor. Upon cooling, the mixture was treated with trifluoroacetic acid to provide a single isomer. Subsequent 2-D NMR indicated that alkylation had occurred at *N7*, thus rendering product **3**. This structural assignment was subsequently and unambiguously confirmed via X-ray cocrystal structure of **3** in complex with BCL-2 (**Fig. 1B**).

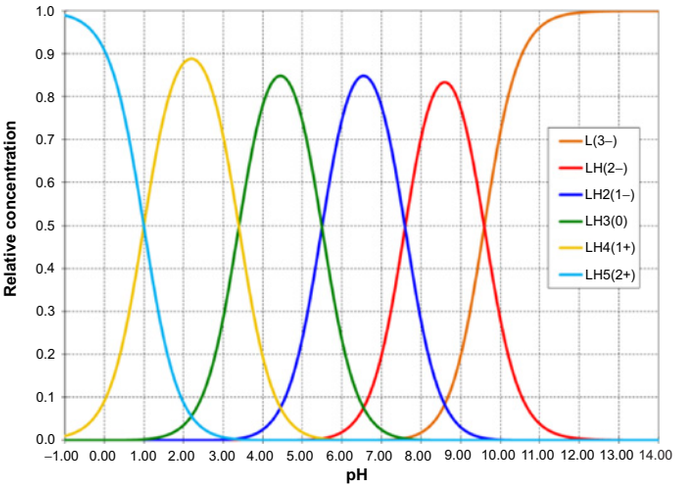
With a small amount of material in hand, we evaluated the preliminary solubility of **3** in a neutral (pH = 7.4) aqueous buffer; gratifyingly, solubility in excess of 3.5 mg/mL was observed, a more than 1,000-fold increase relative to venetoclax. Although this result was highly encouraging, the initial synthesis protocol proved inefficient for larger scale production of material with suitable purity.

To supply sufficient quantity and quality of material to support further evaluation, the initial prodrug synthesis protocol was optimized to afford highly enriched **3** as an HCl salt (see Supplementary Information). With high-purity material in hand, we then assessed the properties of **3** both experimentally and *in silico*. The venetoclax prodrug has high molecular weight, high polar surface area (PSA), and a measured logD that is substantially lower than venetoclax (**Fig. 2C**). The complexity of the molecule is reflected by the presence of five measurable dissociation constants (pK_a) over a physiologically relevant pH range (**Fig. 2A**; Supplementary Table S1). The existence of multiple pK_a values inadvertently led to a highly complex speciation landscape for prodrug **3** in solution (**Fig. 2B**), with major implications for stability and isolation. Solution stability as a function of pH became a key design factor as did the importance of tight control of pH range during isolation to ensure control of stoichiometry. Despite the resulting constraints and negligible solubility of the parent molecule, we ultimately crystallized a hydrate of the HCl salt of **3** and

A



B



C

Property	Venetoclax (1)	3
Molecular weight	868	978
Calculated PSA (Å^2)	183.09	246.58
log$D_{pH7.4}$ (shake flask)	5.5	0.96
Solubility at pH 7, 37°C	<0.004 μg/mL	>3.5 mg/mL

Figure 2.
Prodrug **3** has a complex speciation profile resulting from multiple dissociation constants. **A,** Predicted (p) and measured (m) pK_a values of **3**. **B,** pH-Speciation diagram of venetoclax prodrug **3** showing the relative concentrations of its all related species (2+, 1+, 0, 1−, 2−, 3−) over pH range from −1 to 14. The pH-speciation diagram was constructed by solving the mass-balance equations using measured pK_a values (25). **C,** Summary of physical and chemical properties of venetoclax and **3**.

demonstrated aqueous solubility of >3.5 mg/mL at neutral pH (**Fig. 2C**). To prevent chemical degradation to venetoclax and loss of crystallinity due to dehydration, the drug substance was stored at 2°C–8°C between 20% and 80% relative humidity.

Prodrug 3 is rapidly cleaved to afford venetoclax upon intravenous dosing and affords robust venetoclax exposure upon oral dosing in animals

Desired aspects of the prodrug included rapid conversion to venetoclax within the GI tract upon oral dosing and minimal circulating exposure of the prodrug such that toxicological evaluation would be straightforward. The pro-moiety was designed such that alkaline phosphatases located within the intestinal brush border would first hydrolyze the phosphate group, affording the transient aminal intermediate **4** (**Fig. 3**). Rapid decomposition of **4** would then afford venetoclax and formaldehyde, the latter of which would be oxidized *in vivo* to afford formic acid (26). The amount of formaldehyde produced even at doses commensurate with 1,200 mg venetoclax would be well below the estimated daily intake from food, thus presenting a negligible risk for toxicity (26).

Although oral administration was the primary intended goal of the venetoclax prodrug program, we first administered a single i.v. dose to both mice and dogs to understand the clearance of **3** in the two species that would ultimately be used for toxicological evaluation. As shown in **Fig. 4**, the preclinical pharmacokinetic profiles of **3** were characterized by high clearance and rapid conversion to venetoclax in both species. Less than 1% of the original prodrug concentrations remained in circulation 1 hour after administration (Supplementary Table S2).

Oral dosing of **3** in an aqueous solution was conducted at multiple doses to determine the prodrug and parent exposures in both mouse and dog. Each dose was adjusted for molecular weight, thus affording the molar equivalents of 5, 30, 100, and 150 mg/kg. The total concentration of **3** in dogs was consistently <0.1% of the quantified venetoclax levels on an AUC basis, and <1.5% on a C_{max} basis, for all doses. Plasma levels in mice were below the limits of detection (Supplementary Table S3). These data indicated that **3** was rapidly converted to venetoclax upon either i.v. or oral administration.

The exposure profiles of venetoclax following dosing of **3** were very similar to those observed when dosing the parent itself (27). Nonlinearity was seen in the exposures with increasing doses for both C_{max} and AUC (Supplementary Table S4). Plasma levels of venetoclax in mice continued to increase through all dose levels, whereas the 100 and 150 mg/kg doses provided similar exposures in dog, indicating the former dose to be near saturation. Overall, the ability to

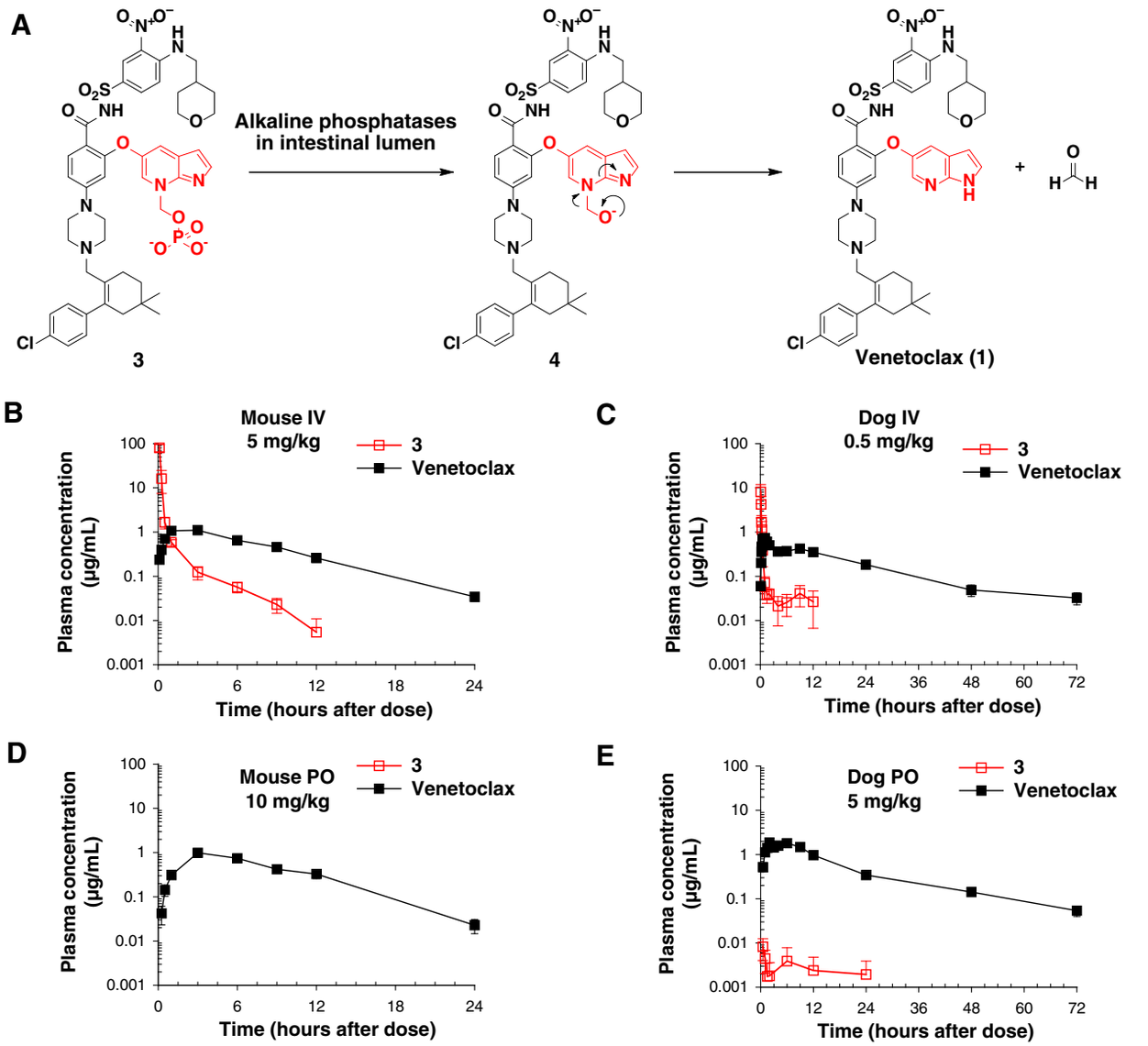


Figure 3.

A, Proposed bioconversion of **3** via alkaline phosphatases in the intestinal lumen to furnish venetoclax plus formaldehyde, and single-dose pharmacokinetics of prodrug **3** and parent venetoclax following an i.v. dose of prodrug **3** in mouse (**B**) and dog (**C**), or after an oral dose in mouse (**D**) and dog (**E**). Prodrug **3** was formulated in (w/v) 0.2% HPMC and dose at 10 mL/kg in mice, and D5W (5% dextrose in water) with 2.1 eq NaOH and dosed at 0.5 mL/kg in dog.

realize significant multiples of clinically relevant concentrations of venetoclax (14, 15) indicated that **3** was efficiently converting to the desired parent molecule. Additionally, the extremely low level of circulating prodrug, even at high doses, was deemed highly beneficial for simplifying toxicological evaluation.

Given the encouraging pharmaceutics and pharmacokinetic properties of **3**, this molecule was nominated as clinical candidate ABBV-167 and further evaluated in IND-enabling toxicology studies. Repeat dosing of mice and dogs afforded the expected on-target pharmacology based on previous data with venetoclax (28). These data indicated that ABBV-167 was suitable for clinical evaluation in human volunteers.

Clinical evaluation of ABBV-167 (3) shows robust conversion to venetoclax and reduced food effect in healthy human volunteers

An open-label, randomized, four-sequence crossover study in 12 healthy adult female subjects was conducted to characterize the pharmacokinetics of ABBV-167 and evaluate the bioavailability of venetoclax from two prodrug formulations under fasting and fed conditions relative to the venetoclax tablet (**Fig. 4A**). One formulation consisted of ABBV-167 dissolved in an aqueous solution, which served to remove the dissolution step and hence provide the most fundamental assessment of prodrug conversion in the GI tract. The solid dosage formulation consisted of an IR tablet containing 65.5% ABBV-

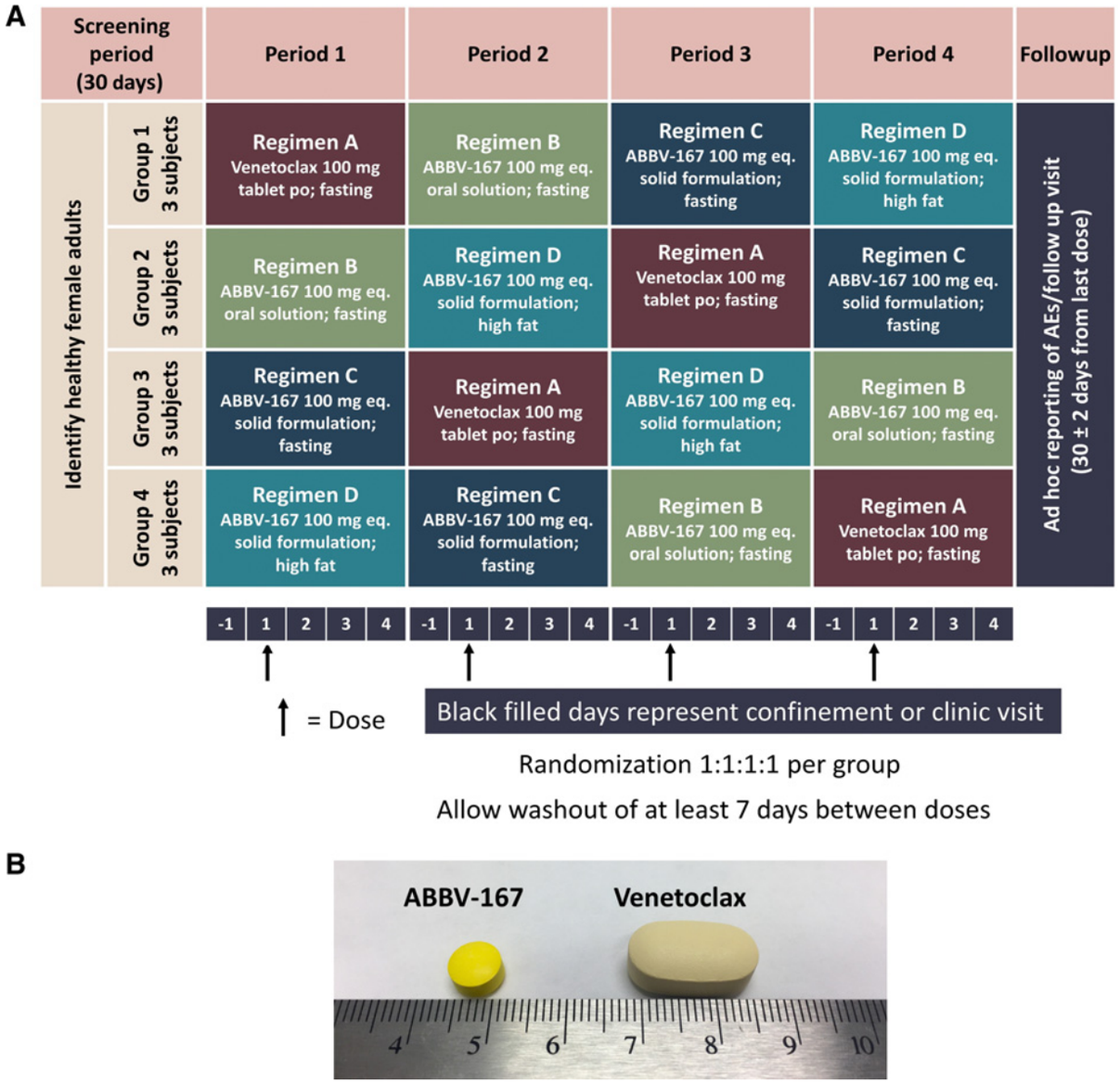


Figure 4.

Study design for clinical evaluation of ABBV-167 and venetoclax in healthy volunteers, and pictorial comparison of solid dosage forms of ABBV-167 and venetoclax tablet (100 mg strength). **A,** Schematic of the clinical bioavailability study. **B,** IR tablet of ABBV-167 and 100 mg strength venetoclax tablet.

167 by weight, at the molar equivalent of a 100-mg venetoclax tablet. This level of API loading was significantly higher than that utilized in the venetoclax tablet, contributing to a substantially reduced overall size (**Fig. 4B**). The total weight of the ABBV-167 prototype IR tablet (172.5 mg) is less than one fifth of the venetoclax tablet (>1 g).

Ten healthy female subjects completed the clinical bioavailability study. Eleven healthy female subjects completed the PK evaluation in the clinical bioavailability study and one subject discontinued due to an adverse event, possibly related to ABBV-167. The mean (SD) age of subjects was 38 (11) years, and the mean (SD) weight was 72 (5) kg. The mean (+SD) plasma concentration–time profiles and pharmacokinetic parameters for venetoclax are shown in **Fig. 5A** and **Table 1**, respectively. No new adverse events were identified from this study compared with the known venetoclax safety profile (see Supplementary Information).

The impact of formulation and food effect are presented in **Fig. 5** and Supplementary Table S5. Venetoclax C_{max} and AUC_{∞} from ABBV-167 tablets were 2.1- and 1.9-fold higher, respectively, than the venetoclax tablet under fasting conditions. Venetoclax C_{max} and AUC_{∞} from ABBV-167 solution were 1.6- and 1.8-fold higher, respectively, than the ABBV-167 tablets under fasting conditions. A food effect was also observed as a high-fat meal increased the venetoclax C_{max} and AUC_{∞} from the ABBV-167 tablet formulation by 1.7- and 2.1-fold, respectively, compared with a fasted state.

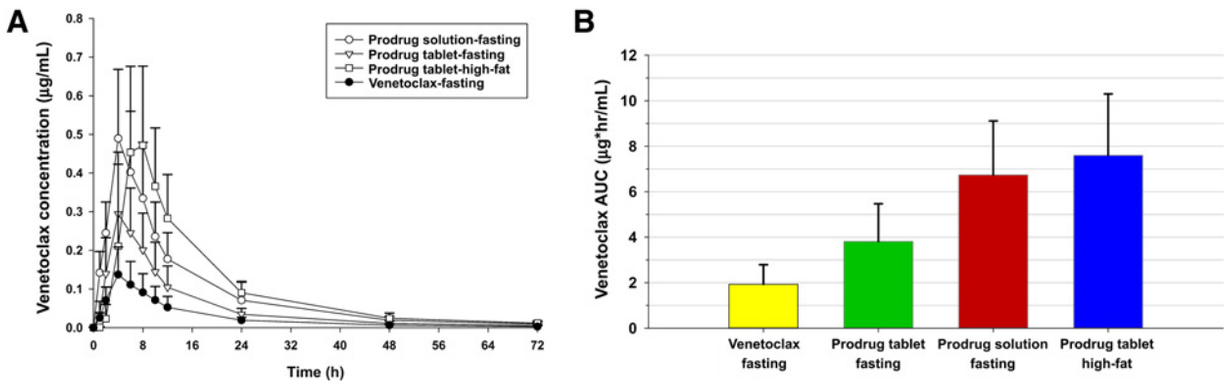


Figure 5.

Exposure of venetoclax delivered as ABBV-167 via solution or solid dosage form, and under fed and fasted conditions. **A,** Mean venetoclax plasma concentration-time profiles in clinical study. **B,** Comparison of venetoclax exposures from the tablets and the prodrug formulations under both fasting and high-fat meal conditions.

Systemic ABBV-167 exposures were < 0.01% of plasma venetoclax exposures, suggesting almost complete conversion of ABBV-167 to venetoclax. The mean ABBV-167 terminal half-life was 2 hours. Median ABBV-167 T_{max} for the solution and tablet formulations under fasting conditions were 2 and 4 hours, respectively. Median ABBV-167 T_{max} was delayed by 2 hours when administered with food.

Discussion

BCL-2 is a prosurvival BCL-2 family protein that can protect malignant and other cells by sequestering and neutralizing the action of prodeath factors such as BAD (BCL-2–associated death protein), BIM (BCL-2–interacting mediator of cell death), and BAX (BCL-2–associated X protein), thus conferring a distinct survival advantage. Interactions among BCL-2 family proteins are characterized by extremely high-affinity binding events that occur within the large, shallow, and hydrophobic BH3-binding grooves of the prosurvival proteins (2). Because of this, successful inhibition of these protein–protein interactions (PPI) at the cellular level has been quite challenging, and for some time these targets were deemed to be "undruggable" (29). Although we utilized a variety of techniques to generate the first marketed drug within this family of proteins (30), the physicochemical properties of molecules like venetoclax reflect the challenges associated with competitively inhibiting PPIs. These properties include extremely low aqueous solubility and pose a significant challenge for drug development (23).

Poorly soluble molecules dissolve sparingly in the GI tract, a feature that contributes to low bioavailability. Given the properties of venetoclax, we explored various technology-enabled formulations ahead of FIH studies. ASD technology is one such approach that has garnered attention within the pharmaceutical industry. ASD formulations can generate physically stable and processable amorphous forms of APIs that, relative to crystalline APIs, often show improved bioavailability (12). The application of this technology to venetoclax culminated in robust oral absorption in patients and healthy volunteers in clinical studies. The efficacy and safety features of this drug that have been established since, along with the widespread expression of BCL-2 across various cancer indications (10), have prompted further evaluation in multiple hematologic cancer indications as well as solid tumor settings such as breast (8) and non–small cell lung cancer (31). In several of these indications, doses of 800–1,200 mg have been or are currently being explored (8, 32).

In order to maximize the formulation stability and performance of the venetoclax tablet, less than 15% drug (by weight) per tablet was utilized, resulting in tablet size in excess of 1 g for the highest marketed dose strength of 100 mg. Patients with diseases such as cancer often present with comorbidities and require multiple concomitant medications to treat and manage their underlying disease and symptoms (33, 34). Given the comedications that are typical of patients with cancer, we anticipated that reducing the pill burden of venetoclax could alleviate some of these complications and contribute to an improved quality of life.

Our effort to pursue a higher drug loading, and hence lower pill burden, alternative to the venetoclax tablet began with the design and generation of novel prodrug ABBV-167. Despite high molecular weight and complexity, ABBV-167 demonstrates dramatically improved water solubility relative to the parent molecule and robust conversion to venetoclax upon oral or intravenous administration to preclinical species. Characterization of ABBV-167 in a crossover study in healthy volunteers demonstrated the bioavailability of venetoclax from the prodrug tablet to be superior compared with the venetoclax tablet under fasting conditions. Moreover, the food effect on the prodrug tablet bioavailability was lower than venetoclax tablets reported in previous studies (14, 15). Because venetoclax is prescribed to be taken with food, these data suggest that the PK variability due to differences in the composition of the meal consumed could be lower for the prodrug. The reduced food effect needs to be further confirmed with a population size that affords statistical evaluation.

Venetoclax has received five Breakthrough Therapy Designations and several marketing approvals (35) and may achieve broader adoption in the coming years. Reflecting the nature of the PPIs it is designed to inhibit, venetoclax is a BRO5 compound that presents substantial challenges for drug development. Although soluble prodrugs of BRO5 compounds have been reported, none have demonstrated robust conversion in humans following oral dosing, nor the potential to mitigate a positive food effect observed with the parent formulation. We report herein the first successful demonstration of

clinical performance with ABBV-167, an orally administered BRO5 prodrug. This approach may therefore encourage the additional generation of soluble prodrugs that can reduce pill burden of BRO5 drugs and improve quality of life for patients.

Authors' Disclosures

A.J. Souers reports personal fees from AbbVie outside the submitted work; in addition, A.J. Souers has a patent for U.S. Patent 9,006,247 issued. A.H. Salem reports other from AbbVie during the conduct of the study; other from AbbVie outside the submitted work. Z. Tao reports a patent for U.S. Patent 9,006,247 issued. O.F. Bueno reports employment with AbbVie; may hold stock or other options. S.W. Elmore reports being an employee of and a stock holder in AbbVie. J.C. Kalvass reports personal fees and other from AbbVie outside the submitted work. J.D. Leverson reports being an employee of and shareholder in AbbVie, Inc. R.M. Menon reports personal fees and other from AbbVie outside the submitted work. D.C. Phillips reports personal fees from AbbVie Inc. and other from stock holder in AbbVie Inc. during the conduct of the study; personal fees and other from employee of AbbVie Inc. and other from stock holder in AbbVie Inc. outside the submitted work. S.H. Rosenberg reports personal fees from AbbVie outside the submitted work. A.A. Suleiman reports other from AbbVie outside the submitted work. X. Wang reports a patent for U.S. Patent 9,006,247 issued. N.D. Catron reports a patent 9,006,247 issued. No disclosures were reported by the other authors.

Authors' Contributions

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References

1. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007;35:495–516.
2. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol 2008;9:47–59.
3. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007;26:1324–37.
4. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
5. Deeks D. Venetoclax: first global approval. Drugs 2016;76:979–87.
6. Strati P, Konopleva MY. Venetoclax for AML: changing the treatment paradigm. Blood Adv 2019;3:4326–35.
7. Vaxman L, Sidiqi H, Gertz M. Venetoclax for the treatment of multiple myeloma. Review Expert Rev Hematol 2018;11:915–20.
8. Lok SW, Whittle JR, Vaillant F, Teh CE, Lo LL, Policheni AN, et al. A phase Ib dose-escalation and expansion study of the BCL2 inhibitor venetoclax combined with tamoxifen in ER and BCL2–positive metastatic breast cancer. Cancer Discov 2019;9:354–69.
9. Juárez-Salcedo LM, Desai V, Dalia S. Venetoclax: evidence to date and clinical potential. Drugs Context 2019;8:212574.
10. Leverson JD, Sampath D, Souers AJ, Rosenberg SH, Fairbrother WJ, Amiot M, et al. Found in translation: how preclinical research is guiding the clinical development of the BCL2-selective inhibitor venetoclax. Cancer Discov 2017;7:1376–93.
11. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications. Int J Pharm 2011;420:1–10.
12. Schittny A, Huwyler J, Puchkov M. Mechanisms of increased bioavailability through amorphous solid dispersions: a review. Drug Deliv 2020;27:110–27.
13. Saboo S, Moseson DE, Kestur US, Taylor LS. Patterns of drug release as a function of drug loading from amorphous solid dispersions: a comparison of five different polymers. Eur J Pharm Sci 2020;155:105514.
14. Salem AH, Agarwal SK, Dunbar M, Nuthalapati S, Chien D, Freise KJ, et al. Effect of low- and high-fat meals on the pharmacokinetics of venetoclax, a selective first-in-class BCL-2 inhibitor. J Clin Pharmacol 2016;56:1355–61.
15. Jones AK, Freise KJ, Agarwal SK, Humerickhouse RA, Wong SL, Salem AH. Clinical predictors of venetoclax pharmacokinetics in chronic lymphocytic leukemia and non-Hodgkin's lymphoma patients: a pooled population pharmacokinetic analysis. AAPS J 2016;18:1192–202.
16. Freise KJ, Jones AK, Verdugo ME, Menon RM, Maciag PC, Salem AH. Moving beyond maximum tolerated dose for targeted oncology drugs: use of clinical utility index to optimize venetoclax dosage in multiple myeloma patients. Clin Pharmacol Ther 2017;102:970–76.
17. Rautio J, Meanwell NA, Di L, Hageman MJ. The expanding role of prodrugs in contemporary drug design and development. Nat Rev Drug Discov 2018;17:559–87.
18. Liu C, Lin J, Hynes J, Wu H, Wrobleski ST, Lin S, et al. Discovery of ((4-(5-(Cyclopropylcarbamoyl)-2-methylphenylamino)-5-methylpyrrolo[1,2-f][1,2,4]triazine-6-carbonyl)(propyl)carbamoyloxy)methyl-2-(4-(phosphonooxy)phenyl)acetate (BMS-751324), a Clinical Prodrug of p38α MAP Kinase Inhibitor. J Med Chem 2015;58:7775–84.
19. Baluom M, Grossbard EB, Mant T, Lau DTW. Pharmacokinetics of fostamatinib, a spleen tyrosine kinase (SYK) inhibitor, in healthy human subjects following single and multiple oral dosing in three phase I studies. Br J Clin Pharmacol 2013;76:78–88.
20. DeGoey DA, Chen HJ, Cox PB, Wendt MD. Beyond the rule of 5: lessons learned from AbbVie's drugs and compound collection. J Med Chem 2018;61:2636–51.
21. DeGoey DA, Grampovnik DJ, Flosi WJ, Marsh KC, Wang XC, Klein LL, et al. Water-soluble prodrugs of the human immunodeficiency virus protease inhibitors lopinavir and ritonavir. J Med Chem 2009;52:2964–70.

22. Lasseter KC, Gambale J, Jin B, Bergman A, Constanzer M, Dru J, et al. Tolerability of fosaprepitant and bioequivalency to aprepitant in healthy subjects. J Clin Pharmacol 2007;47:834–40.
23. Jornada DH, dos Santos Fernandes GF, Chiba DE, de Melo TR, dos Santos JL, Chung MC. The prodrug approach: a successful tool for improving drug solubility. Molecules 2016;21:42.
24. U.S. Department of Health and Human Services Food and Drug Administration. M3(R2) nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals (2012). https://www.fda.gov/media/82666/download.
25. Alderighi L, Gans P, Ienco A, Peters D, Sabatini A, Vacca A, et al. Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species. Coord Chem Rev 1999;184:311–18.
26. European Health Authority. Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. EFSA J 2014;12:3550.
27. Choo EF, Boggs J, Zhu C, Lubach JW, Catron ND, Jenkins G, et al. The role of lymphatic transport on the systemic bioavailability of the Bcl-2 protein family inhibitors navitoclax (ABT-263) and ABT-199. Drug Metab Dispos 2014;42:207–12.
28. Center for Drug Evaluation and Research. Application number: 208573Orig1s000 (2015). Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208573Orig1s000MedR.pdf. Cited 2020 Nov 23.
29. Dang C, Reddy E, Shokat K, Soucek L. Drugging the 'undruggable' cancer targets. Nat Rev Cancer 2017;17:502–8.
30. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013;19:202–8.
31. AbbVie Inc. A Study of Budigalimab (ABBV-181) in Participants With Advanced Solid Tumors. ClinicalTrials.gov identifier: NCT03000257. Updated Jan 5, 2021. https://clinicaltrials.gov/ct2/show/NCT03000257. Cited 2021 Apr 23.
32. Harrison S, Cavo M, De La Rubia J, Popat R, Gasparetto C, Hungria V, et al. T(11;14) and high BCL2 expression are predictive biomarkers of response to venetoclax in combination with bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma: biomarker analyses from the phase 3 Bellini study. Blood 2019;134:142.
33. Given BA, Given CW, Sikorskii A, Vachon E, Banik A. Medication burden of treatment using oral cancer medications. Asia Pac J Oncol Nurs 2017;4:275–82.
34. Lees J, Chan A. Polypharmacy in elderly patients with cancer: clinical implications and management. Lancet Oncol 2011;12:1249–57.
35. Fowler M. Venetoclax with azacytidine improved overall survival for patients with AML. Cancer Network March 23, 2020. Available from:https://www.cancernetwork.com/view/venetoclax-azacytidine-improved-overall-survival-patients-aml. Cited 2020 Nov 23.