The role of lymphatic transport on the systemic bioavailability of the Bcl-2 protein family inhibitors navitoclax (ABT-263) and ABT-199

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Running Title

Lymphatic transport of navitoclax and ABT-199

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Abbreviations: Navitoclax, (ABT-263; (Benzamide, 4-[4-[(2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl)methyl]-1-piperazinyl]-N-[4-[[1R]-3-(4-morpholiny1)-1-[(phenylthio)methyl] propyl]amino]-3-[(trifluoromethyl)sulfonyl]phenyl] sulfonyl]-; ABT-199, (2-(1H-pyrrolo[2,3-b]pyridin-5-yl oxy)-4-(4-(2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)-N-(3-nitro-4-((tetrahydro-2H-pyran-4-yl)methylamino)phenyl)sulfonyl)benzamide); TDC, thoracic lymph duct cannulated, CL, clearance, F, bioavailability; AUC, area under the curve; LCT, long chain triglyceride
Abstract

Navitoclax (ABT-263), a Bcl-2 family inhibitor and ABT-199, a Bcl-2 selective inhibitor, are high molecular weight, high logP molecules that show low solubility in aqueous media. While these properties are associated with low oral bioavailability (F), both navitoclax and ABT-199 showed moderate F in preclinical species. The objective of the described study was to determine if lymphatic transport contributes to the systemic availability of navitoclax and ABT-199 in dogs. The IV pharmacokinetics of navitoclax and ABT-199 were determined in intact (non-cannulated) dogs. In oral studies, tablets (100 mg) of navitoclax and ABT-199 were administered to both intact and thoracic lymph duct cannulated (TDC) dogs. The clearance of navitoclax and ABT-199 was low; 0.673 and 0.779 mL/min/kg, respectively. The volume of distribution of both compounds was low (0.5-0.7 L/kg). The half-life of navitoclax and ABT-199 were 22.2 and 12.9 h, respectively. The F of navitoclax and ABT-199 were 56.5 and 38.8%, respectively in fed intact dogs. In fed TDC dogs, 13.5 and 4.67% of the total navitoclax and ABT-199 dose were observed in lymph with the %F of navitoclax and ABT-199 of 21.7 and 20.2%, respectively. The lower lymphatic transport of ABT-199 corresponds to the lower overall %F of ABT-199 vs navitoclax despite similar systemic availability via the portal vein (similar % F in TDC animals). This is consistent with the higher long chain triglyceride solubility of navitoclax (9.2 mg/mL) vs. ABT-199 (2.2 mg/mL). In fasted TDC animals, lymph transport of navitoclax and ABT-199 decreased by 1.8-fold and 10-fold, respectively.
Introduction

Navitoclax (ABT-263; (Benzamide, 4-[[2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl]-N-[[4-[(1R)-3-(4-morpholinyl)-3-[(trifluoromethyl)sulfonyl]phenyl] sulfonyl] is a small molecule Bcl-2 family protein inhibitor that binds with high affinity (Ki ≤ 1 nM) to multiple antiapoptotic Bcl-2 family proteins including Bcl-X<sub>L</sub>, Bcl-2, and Bcl-w, while showing lower affinity for Mcl-1 (Park et al., 2006; Tse et al., 2008). ABT-199 (2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)-4-(4-((2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)-N-(3-nitro-4-((tetrahydro-2H-pyran-4-yl)methylamino)phenylsulfonyl)benzamide) is a first-in-class orally bioavailable small molecule Bcl-2 family protein inhibitor that binds with high affinity (Ki < 0.10 nM) to Bcl-2 and with lower affinity to other Bcl-2 family proteins Bcl-X<sub>L</sub>, Bcl-w, and Mcl-1 (> 480-fold and > 2,000-fold lower affinity than to Bcl-2, respectively) (Souers et al., 2013).

Over expression of antiapoptotic Bcl-2 family proteins is associated with tumor maintenance, progression and increased resistance to chemotherapy, and are thus compelling targets for anticancer therapy (Strasser et al., 2000; Cory et al., 2003; Adams et al., 2005). Navitoclax is a first-in-class orally bioavailable inhibitor of Bcl-2 and Bcl-xL that is currently in Phase II clinical trials. To date, navitoclax has shown a reduction in tumor burden in patients with hematological malignancies believed to be dependent on Bcl-2 for survival (Tse et al., 2008; Wilson et al., 2010; Roberts et al., 2012). ABT-199 is a first-in-class Bcl-2 selective inhibitor that has recently entered clinical trials in patients with chronic lymphocytic leukemia (CLL), where it has shown clinical activity upon oral administration (Davids et al., 2012; Seymour et al., 2012).
The deep and large hydrophobic BH3-binding groove of the Bcl-2 family members necessitates that small molecules that occupy the space are hydrophobic and have high molecular weight (Park et al., 2006; Petros et al., 2006; Petros et al., 2010). Therefore, the physicochemical properties of both the BH3 mimetics navitoclax and ABT-199 (Table 1) do not conform to the “Lipinski rule of 5” (Lipinski et al., 2001) and it would be predicted that both compounds have low bioavailability after oral dosing. However, both compounds have reasonable exposure pre-clinically and clinically (Wilson et al., 2010; Davids et al., 2012; Roberts et al., 2012; Seymour et al., 2012). One possible explanation for the relatively high oral exposure observed in preclinical species as well as human patients is the high lipophilicity of these compounds, a physicochemical attribute that could contribute to oral absorption via lymphatic transport (Porter and Charman, 2001; Trevaskis et al., 2008).

While in vitro measurements such as long chain fatty acid solubility, logD/P (Trevaskis et al., 2008) or association with plasma chylomicrons (Gershkovich and Hoffman, 2005) may provide some indication of whether a compound will undergo lymphatic transport, in vivo animal studies provide the only means of directly measuring the amount of compound in lymph. The added advantage of the conscious dog model is the ability to administer human dose forms/tablets. Therefore the objective of this study was to determine if lymphatic transport contributes to the systemic availability of navitoclax and ABT-199 using the conscious thoracic lymph cannulated dog model.
Materials and Methods

Navitoclax and ABT-199 were synthesized and tablets were manufactured by Abbott Laboratories (Abbott Park, IL). All other reagents or material used in these studies were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Determination of Physicochemical Properties

The thermodynamic solubility of navitoclax and ABT-199 in aqueous or oil-based media were determined by equilibrating navitoclax or ABT-199 in the presence of excess crystalline active pharmaceutical ingredient (API). For aqueous solubility, samples were equilibrated for 48 h at 25°C (buffers) or 37°C in Fasted- and Fed-State Simulated Intestinal Fluid; FaSSIF or FeSSIF) followed by centrifugation at 3000 g for 10 minutes and filtration through a syringe filter. Sample concentration in the supernatant was measured by HPLC. ABT-199 HPLC analysis was conducted on an Agilent 1100 HPLC using 0.1% trifluoroacetic acid (TFA) in water and 50:50 acetonitrile:methanol as mobile phases on a 150x4.6mm 3.5µm Xorbax XDB-C18 (Agilent) column with gradient from 60 to 95% B over 7 minutes with UV monitoring at 314 nm. Navitoclax HPLC analysis was conducted on an Agilent 1100 HPLC using 10mM Ammonium bicarbonate pH 9.5 and 85:15 acetonitrile:methanol as mobile phases on a 100x3.0mm, 2.5µm Luna C-18(2)-HST (Phenomenex) with an isocratic hold at 60% B for 20 minutes followed by a gradient to 85% B to 45 minutes with UV monitoring at 280 nm.

For long chain triglyceride (LCT) solubility, excess crystalline API in soybean oil were agitated at 37°C for 4 days, then centrifuged at 13000 rpm for 10 min. Supernatants were diluted in acetone and concentrations were measured using HPLC (Agilent 1100, Santa Clara, CA) with UV detection at 254 nm. The column was a Waters X Terra RP18 3.5...
μm, 50 x 4.6 mm (Waters, Milford, MA), mobile phase A was 0.05% TFA in water, and mobile phase B was 0.05% TFA in acetonitrile.

LogP and topological polar surface area (TPSA) were calculated using ACDLabs Version 11 software suite (Ontario, Canada). LogD<sub>pH7.4</sub> was determined by RT-HPLC method modified from the EPA method (EPA Product Properties Test Guidelines OPPTS 830.7570) for very lipophilic compounds. Briefly, the method is carried out by injecting the test compounds in a reversed phase HPLC system against a test mixture of compounds with known logD values. The logD of the test compounds are calculated from their retention factor and the calibration curve.

Studies in Beagle Dogs

This study was conducted at WuXi AppTec Co., Ltd., in accordance with the IACUC guidelines that are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

For each compound, the same 3 male Beagle dogs (Marshall Bioresources, Beijing, China; 10-12 kg) were used in each phase of the study. A 7-day wash-out period was inserted between each phase of the study with the exception of the final phase, where a 3-day washout period was utilized to avoid the loss of patency in the thoracic lymph cannulas. All animals were fed (50g of a human FDA high fat meal) ~10 min to 1 h prior to dosing and again at ~5 h post dose and water was available ad libitum throughout the study in all but the final phase of the experiment, where animals were fasted overnight until 10 h post-dose. In the first phase of the study, animals were administered IV 1 mg/kg of navitoclax or ABT-199 formulated as a solution in 10% dimethylsulfoxide,
90% polyethylene glycol 400. In phase 2 (fed animals), dogs were dosed with a 100 mg tablet (clinical formulation) of either navitoclax or ABT-199 followed by 60 mL of water. Before phase 3 (fasted animals), the thoracic lymph duct (TDC) was cannulated under surgical anesthesia according to the previously described methods (Khoo et al., 2001). Following surgery animals were allowed to recover unrestrained overnight and returned to normal ambulatory movement before dosing a 100 mg tablet of navitoclax or ABT-199. To maintain hydration, animals were administered intravenous saline (25 mL) hourly for the first 12 h post dose.

For all studies, blood samples were collected pre-dose and at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h postdose. In TDC animals, lymph was collected into drainage packs (containing 1% v/v; 60% Tween 20, 20% methanol and 20% water) predose, and at hourly intervals until 12 h post-dose after which a 12-24 hr interval collection was made, the amount of lymph collected at each interval was measured gravimetrically. All plasma and lymph samples were collected with potassium (K₂) EDTA and heparin sodium as anticoagulant, respectively. Blood samples were processed for plasma within 1 h of sample collection, plasma was stored at -70°C or lower until analyzed by LC/MS/MS. All animal studies were conducted by WuXi AppTec Co. Ltd (Shanghai, China).

**LC-MS/MS Analysis**

Navitoclax and ABT-199 concentrations were determined by LC-MS/MS following protein precipitation with acetonitrile, and injection of the supernatant onto the column. The column used for analysis of navitoclax was an ACQUITY UPLC® BEH-C18, (50 x 2.1 mm, 1.7 μm particle size) and a Phenomenex Kinetex C-18 (50 x 2.1 mm, 12.6 μm particle size) column was used for analysis of ABT-199. An Acuity ultra performance
liquid chromatography system (Waters, Milford, MA) coupled with a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) were used for the LC-MS/MS assay. The aqueous mobile phase was water with 0.1% formic acid and the organic mobile phase was acetonitrile with 0.1% formic acid for analysis of navitoclax in plasma. For all lymph samples and plasma samples with ABT-199, the aqueous mobile phase was water with 2mM ammonium acetate and the organic mobile phase was acetonitrile with 0.1% formic acid. The total run time was ~1.6 min and the ionization was conducted in the positive ion mode using the transition \( m/z \ 487.9 \rightarrow 233.3 \) for navitoclax and \( m/z \ 868.6 \rightarrow 233.4 \) for ABT-199. The lower and upper limits of quantitation of the assay for lymph and plasma were 0.005 µM and 10 µM, respectively.

**Pharmacokinetic Analysis**

Pharmacokinetic parameters were calculated by non-compartmental methods as described in Gibaldi and Perrier (Gibaldi and Perrier, 1982) using WinNonlin version 5.1.1 (Pharsight Corporation, Mountain View, CA). Parameters are presented as mean ± standard deviation (SD). The % of dose recovered in lymph was calculated as the cumulative mass of navitoclax or ABT-199 in lymph divided by the oral dose. Bioavailability (%F) in both intact and TDC animals were determined by dividing the dose-normalized AUC\(_{0-\infty}\) of each animal dosed PO by the respective dose-normalized AUC\(_{0-\infty}\) determined from the animals dosed IV. The proportion of dose (as a %) transported through the lymph was determined as the difference in AUC observed between intact and cannulated animals; \((\text{AUC}_{\text{intact}} - \text{AUC}_{\text{TDC}})/\text{AUC}_{\text{intact}})*100.\)
Results

Physicochemical Properties

The physicochemical properties of both navitoclax and ABT-199 are shown in Table 1. These are characterized by high molecular weight and logD_{pH7.4} values, which contribute to very low solubility in aqueous media. Despite the high logD_{7.4} of both compounds, the LCT solubility of both navitoclax and ABT-199 were relatively low (~9 and 2 mg/mL, respectively).

Pharmacokinetics in Intact Dogs

Following IV administration of both navitoclax and ABT-199, the plasma CL of both compounds was low (<3% of liver blood flow; Figure 1A and Table 2) which contributed to the long observed half-lives (13-22 h; Table 2). The volume of distribution of both compounds was low, consistent with high protein binding (>99%; data not shown). After oral administration of 100 mg of navitoclax or ABT-199 to fed intact dogs, the absolute bioavailability (%F), representing the collective portal and lymphatic transport was ~1.5-fold higher for navitoclax than ABT-199, 56.5% vs. 38.8%, respectively (Figure 1B, 1C and Table 2).

Pharmacokinetics in Thoracic Duct Cannulated Dogs

As shown in figures 1B and C, in TDC dogs, systemic exposure of both navitoclax and ABT-199 were lower than exposure in intact animals. This is evident in the initial absorptive (from enterocyte into systemic circulation)/distribution phase where for navitoclax in particular, there was a ~4-fold difference in C_{max} between intact and TDC animals. In TDC dogs, the lower initial plasma exposure of navitoclax and ABT-199 is accompanied by increasing concentrations of compound in lymph (Figure 2A).
Cumulative lymph levels of both navitoclax and ABT-199 increased linearly up to 8 hr then plateaued (Figure 2A and B). The amount of navitoclax and ABT-199 in lymph represented 13.5% and 4.68% of the 100 mg dose administered, respectively (Figure 2B). The %F in TDC dogs, representing compound absorbed via the portal vein only, was similar between navitoclax and ABT-199 (~20%). However, taking into account the difference in AUC between intact and TDC dogs (Table 2), the contribution of lymphatic transport to overall F (in intact dogs) of navitoclax and ABT-199 ((AUC\text{intact} - AUC\text{TDC})/AUC\text{intact})*100) was 59 ± 5% vs. 43 ± 8%, respectively (Figure 2B), i.e., roughly half of systemically available navitoclax and ABT-199 is available by lymphatic transport.

**Effect of Food on Lymphatic Transport**

The effect of food on lymphatic transport of navitoclax and ABT-199 was tested, however because the patency of the lymph duct cannulas could not be maintained for a number of the animals, only n=1 and n=2 data in fasted animals were obtained for navitoclax and ABT-199, respectively. In the one animal with a patent cannula, the plasma AUC\text{0-t\text{last}}, C\text{max} and F of navitoclax were 47.9 μM.h, 5.47 μM and 16.8%, respectively. In TDC fasted animals (n=2) mean plasma AUC\text{0-∞}, C\text{max} and F of ABT-199 were 57.2 μM.h, 1.91 μM and 22.0%, respectively. In TDC animals, without the contribution from lymphatic transport, the plasma exposure (and corresponding %F) of navitoclax and ABT-199 in fasted animals was similar to exposures in fed TDC animals. However, the % dose of navitoclax recovered from lymph in fasted animals was lower, 7.58 % vs. 13.5% in fed animals, and for ABT-199, 0.454% in fasted animals vs. 4.68%
in fed animals (Figure 2A). Thus, in intact animals, the %F in fasted animals would be predicted to be lower, particularly for ABT-199.

**Discussion**

It has been proposed that the prerequisite for substantial intestinal lymphatic drug transport is a logP >4.7 and a long chain triglyceride (LCT) solubility >50 mg/mL (Trevaskis et al., 2008). However, recently it has been reported that compounds such as the CETP inhibitor CP532,623 with LCT solubility <50 mg/mL are transported by the intestinal lymphatics (Trevaskis et al., 2010b; Trevaskis et al., 2010c). Therefore, it is conceivable that transport by the intestinal lymphatics could still play a role in the absorption of the Bcl-2 family inhibitors with LCT solubility <10 mg/mL.

In intact dogs, the CL of both navitoclax and ABT-199 were low, resulting in relatively long half-lives in dogs. Moderate bioavailability and relatively high oral exposures of navitoclax and ABT-199 were observed despite their low aqueous solubility. Of note, the half-life of both compounds after oral dosing to intact animals was similar to IV dosing, suggesting that absorption was not rate limiting (lack of flip-flop kinetics) (Table 1).

The clearest indication that lymphatic transport is playing a role in the disposition of both navitoclax and ABT-199 is in the observed ~2-fold decrease in plasma exposure in TDC animals compared to intact animal (Table 2). This is accompanied by a gradual and linear increase in lymph exposures over 8 hours (Figure 2A). Interestingly, while the absorptive profile of navitoclax and ABT-199 were different in TDC animals compared to intact animals, the elimination half-lives of navitoclax and ABT-199 were similar. This
suggests that lymphatic transport does not affect CL. Based on the similar %F (~20%) of navitoclax and ABT-199 in TDC dogs, portal availability of both compounds appear to be similar. Thus, the overall difference in F in intact animals, (56.6 % and 38.8%, for navitoclax and ABT-199, respectively, vs. F of ~20% in TDC animals) reflects a difference in lymphatic transport (Figure 2B). If this was not the case, the bioavailability of navitoclax and ABT-199 would be ~20%. The higher F of navitoclax (in intact animals) is consistent with the 4-fold higher LCT solubility of navitoclax (9.2 mg/mL) compared to ABT-199 (2.2 mg/mL).

Food may impact absorption/bioavailability by increasing the solubilization capacity due to the presence of lipids, stimulation of bile acid production, and/or slowing the GI transit time thereby increasing the absorption window. In addition, lipids in food may also promote drug access into the intestinal lymphatic system, where fat in food stimulates the formation of triglyceride-rich lipoprotein in the enterocyte and provides a lipoprotein rich environment whereby a drug/compound can associate with lipid transport pathways (Trevaskis et al., 2008). The latter appears to apply to navitoclax and ABT-199, since similar solubilities were observed in FaSSIF or FeSSIF, which differ in their bile salt content (Table 1). The positive effect of food in dog on lymphatic transport has been reported, for example with halofrantrine, CP524,515 and CP532,623, where food enhanced lymphatic transport by 67- , 2.2-, and 1.4-fold, respectively (Khoo et al., 2001; Khoo et al., 2003; Trevaskis et al., 2010b)

In fasted TDC dogs, lymphatic transport of navitoclax and ABT-199 were decreased by
1.8- and 10-fold, respectively (Figure 2). Thus, fasting did not greatly alter the availability of either compound via the portal vein (no change in plasma exposure) but altered lymphatic transport. It is worth noting that in the animals where the cannulas were patent, 660 mL (n = 1; navitoclax) and 1110 mL of lymph (ABT-199; mean from 2 animals) were collected over 24 hours. This was similar to the lymph volume collected in fed animals, suggesting that fasting did not appear to affect the flow of lymph. While in this set of studies we did not conduct an arm with fasted intact dogs, the resulting decrease in lymph transport in fasted animals for ABT-199, would predict a % F of ~20% (assuming little/no contribution from lymphatic transport) and ~40% for navitoclax (with a ~50% decrease in contribution from lymphatic transport). This in keeping with previous data showing lower exposure in fasted vs. fed (intact) animals (Data on file Abbott Laboratories). In dogs it appears that ABT-199 lymphatic transport was much more sensitive to the fed/fasted state perhaps due to its lower lipid solubility and consequently higher reliance on an exogenous source of lipid to drive lymphatic transport. Interestingly, this observation is consistent with observations in patients where under fed conditions, a greater food effect was observed with ABT-199 (Davids et al., 2012) compared to navitoclax (Wilson et al., 2010). Exposures (AUC) of navitoclax and ABT-199 were increased by 1.2-fold (Wilson et al., 2010) and 3-4-fold (Davids et al., 2012), respectively.

Apart from increasing absorption, lymphatic transport may enhance systemic exposure by avoiding hepatic first pass since the lymphatic system bypasses the liver and empties directly into the systemic circulation. This has been reported in both dogs and rats, where
post-prandial administration of halofantrine resulted in a decreased metabolite to parent ratio (Porter et al., 1996; Khoo et al., 2001). In addition, it has also been suggested that enteric metabolism may be reduced by lymphatic transport (Trevaskis et al., 2006). It is unlikely however, for navitoclax or ABT-199 that avoidance of enteric metabolism/hepatic first pass lymphatic transport is playing a role in increasing systemic oral exposure of either navitoclax or ABT-199, as $^{14}$C preclinical data suggest that the fraction absorbed is limiting $F$ rather than $CL$ (data not shown). In addition to enhancing systemic exposure, it has been suggested that lymphatic transport may enhance the therapeutic effect of immune-modulatory or anti-cancer compounds since drug levels are enhanced at the target site of action, e.g, lymphocytes (Trevaskis et al., 2010a; Trevaskis et al., 2011). For example, the administration of the immunosuppressant JWH015 in a high-lipid formulation (40 mg of oleic acid) to rats elicited a significant increase in ex-vivo mitogen stimulated release of “anti-inflammatory” cytokines (IL-4 and IL-10), relative to a low lipid formulation, despite similar systemic plasma levels observed from both formulations (Trevaskis et al., 2010a; Trevaskis et al., 2011). Further focused studies are needed to determine if clinically therapeutic advantage can be gained by targeting lymphatic transport for drugs that target lymphocytes and lymphoid compartments. In addition, it is possible that increasing lipophilicity or lipid solubility may increase metabolism as well as introduce complexities such as sensitivity to changes in formulation or fed/fasted conditions. This may result in variable pharmacokinetics or potentially pharmacodynamics depending on the site of action.
In conclusion, we have shown that lymphatic transport plays a role in the overall systemic availability of lipophilic compounds such as navitoclax and ABT-199. These studies further indicate that lymphatic transport could be leveraged in the drug discovery process to increase the exposure of compounds with low aqueous solubility by “dialing in” lipophilicity/lipid solubility.
DMD #55053

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Authorship Contributions:

Participated in research design: Boggs, Catron, Choo, Jenkins, Lubach, Voorman, Zhu

Conducted experiments: Catron, Lubach, Zhu

Contributed new reagents or analytic tools: Catron, Lubach, Souers

Performed data analysis: Boggs, Choo

Wrote or contributed to the writing of the manuscript: Choo, Catron, Lubach, Souers
DMD #55053

References


DMD #55053


Trevaskis NL, Shanker RM, Charman WN and Porter CJ (2010c) The mechanism of lymphatic access of two cholesteryl ester transfer protein inhibitors (CP524,515
and CP532,623) and evaluation of their impact on lymph lipoprotein profiles.


Footnotes:

The authors are employees and stockholders of their respective affiliations.

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**Figure 1.** Mean ± SD (n=3) systemic plasma concentration-time profiles (A) after intravenous administration of 1 mg/kg of navitoclax or ABT-199 to fed dogs, (B) after oral dosing of 100 mg tablet of navitoclax to fed thoracic lymph cannulated dogs (TDC) and intact dogs and (C) after oral dosing of 100 mg tablet of ABT-199 to fed TDC and intact dogs.

**Figure 2.** (A) Cumulative lymphatic transport of drug (as a % of dose) in thoracic lymph-cannulated dogs (mean ± SD; n=3) following oral administration of 100 mg tablet of navitoclax or ABT-199 to fed or fasted dogs; (B) Mean ± SD (%) of the absorbed dose of navitoclax and ABT-199 recovered in lymph; the plasma bioavailability in lymph cannulated and intact dogs and the proportion (as a %) of the systemic availability from lymphatic transport.
Tables:

Table 1: Physical and Chemical Properties of navitoclax and ABT-199

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<th>Navitoclax</th>
<th>ABT-199</th>
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<tr>
<td>Molecular Weight (Da)</td>
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<td>868</td>
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<td>172</td>
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<td>2.24</td>
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<td>Aqueous Solubility; pH</td>
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<tr>
<td>1, 7.4, Fasted SSIF, Fed</td>
<td></td>
<td></td>
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<td>SSIF (mg/mL)</td>
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<sup>a</sup> – Topological polar surface area (TPSA) and <sup>b</sup> – clogP calculated using ACDLabs Ver 11 software; <sup>c</sup> – experimentally determined using HPLC retention technique modified from EPA method (EPA Product Properties Test Guidelines OPPTS 830.7570); <sup>d</sup> – LCT = long chain triglyceride
Table 2: Pharmacokinetics of navitoclax and ABT-199 after IV (1 mg/kg) and oral administration (100 mg/dog; ~10 mg/kg) to intact (non-cannulated) and thoracic lymph cannulated fed dogs. Data are presented as mean ± SD (n=3; for each compound the same animals were dosed after a washout period)

<table>
<thead>
<tr>
<th></th>
<th>Navitoclax</th>
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<th>ABT-199</th>
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<tbody>
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<td>IV</td>
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<td>IV</td>
<td>PO</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>Intact</td>
<td>Thoracic lymph</td>
<td>Intact</td>
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<tr>
<td><strong>AUC&lt;sub&gt;0-tlast&lt;/sub&gt; (µM.h)</strong></td>
<td>26.9 ± 0.2</td>
<td>158 ± 24</td>
<td>61.2 ± 2.6</td>
<td>24.6 ± 4.1</td>
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<tr>
<td><strong>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µM.h)</strong></td>
<td>26.9 ± 0.2</td>
<td>164 ± 24</td>
<td>67.3 ± 3.8</td>
<td>25.1 ± 4.1</td>
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<td><strong>CL (mL/min/kg)</strong></td>
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<td>NA</td>
<td>0.774 ± 0.134</td>
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<td><strong>Vss (L/kg)</strong></td>
<td>0.481 ±0.031</td>
<td>NA</td>
<td>NA</td>
<td>0.729 ± 0.143</td>
</tr>
<tr>
<td><strong>T&lt;sub&gt;1/2&lt;/sub&gt; (hr)</strong></td>
<td>22.2 ±1.0</td>
<td>17.4 ± 1.9</td>
<td>22.9 ± 8.1</td>
<td>12.9 ±0.9</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt; (µM)</strong></td>
<td>NA</td>
<td>22.5 ± 3.2</td>
<td>5.18 ±1.58</td>
<td>NA</td>
</tr>
<tr>
<td><strong>T&lt;sub&gt;max&lt;/sub&gt; (hr)</strong></td>
<td>NA</td>
<td>2.0 ± 0.0</td>
<td>4.0 ± 2.0</td>
<td>NA</td>
</tr>
<tr>
<td><strong>F (%)</strong></td>
<td>NA</td>
<td>56.5 ± 3.8</td>
<td>21.7 ± 2.4</td>
<td>NA</td>
</tr>
</tbody>
</table>
Figure 2

A. Cumulative % of Dose

B. Dose in Lymph

- Navitoclax - Fed (n=3)
- ABT-199 - Fed (n=3)
- Navitoclax - Fasted (n=1)
- ABT-199 - Fasted (n=2)