

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

217202Orig1s000

NON-CLINICAL REVIEW(S)



Memorandum

Division of Pharmacology/Toxicology
Office of Cardiology, Hematology, Endocrinology, & Nephrology
Center for Drug Evaluation and Research

Date:	10/11/2024
Application #	NDA 217202
Applicant:	AOP Orphan Pharmaceuticals
Drug:	Landiolol
Primary Reviewer:	Paul Grimm
Subject:	Nonclinical Review

Change in Nonclinical Review Team: The application NDA 217202 for landiolol HCl, was transferred to DCN pharm/tox team during the current application cycle. We reference the previous review completed by Feleke Eshete, Ph.D., that was previously submitted into DARRTs dated 4/7/2023. The review that follows provides a brief overview and addresses pharm/tox concerns that contributed to the issuing of a Complete Response (CR). Previously raised concerns regarding labeling will also be addressed.

Introduction: Landiolol HCl (LAN) is a beta-1-adrenergic blocker indicated for the short-term reduction of ventricular rate in patients with supraventricular tachycardia, including atrial fibrillation and atrial flutter. It is presumed to inhibit the positive chronotropic effects of catecholamines on the heart. AOP Orphan Pharmaceuticals AG (AOP pharma) has submitted a 505(b)(2) marketing application for LAN 300 mg injection formulation relying on published nonclinical data for Onoact®, a landiolol HCl product (ONO-1101) developed in Japan, and additional nonclinical studies conducted by the applicant. The applicant conducted studies included GLP-compliant genotoxicity, local tolerance, and impurity qualification studies. At the Divisions request, the applicant also completed 1) receptor binding profiles of parent and the major metabolite; 2) effects on hERG & other cardiac ion channels; 3) confirmation of the presence of human metabolites in animal species used for toxicology studies; and 4) qualifications of impurities and degradants in the drug product. In addition to Onoact, clinical pharmacology data for the U.S. listed drug Brevibloc® (esmolol), which is also a beta-adrenergic blocker and contains the same active salt as Onoact, was used in bridging studies and to guide label language.

The applicant has not conducted carcinogenicity study because of short-term clinical use (consistent with the reference drug) nor juvenile studies. Potential developmental and reproductive toxicities have been evaluated based on published literature with the Division's concurrence.

(b) (4) **Concern:** A potential safety concern was identified during the previous review and contributed to the Agency issuing a CR letter. (b) (4)

Resolution of (b) (4) **Concern:** In the intervening period between the Agency issuing the CR (May 31, 2023) and the Applicant resubmitting (May 29, 2024), the (b) (4)

Guidance for Industry was published (b) (4). Based on this updated guidance and through a consult with CTCS, the (b) (4) was applied (b) (4) to determine its (b) (4) and associated AI limit. (b) (4), which has an associated AI of (b) (4) ng/day. Drug product lots stored for 18 months contain (b) (4) at (b) (4) ppb, which provides an AI of (b) (4) ng/day. For this reason, this safety issue is considered resolved and the nonclinical team has no additional safety concerns. The consult review by CTCS on the determination of carcinogenic PC of (b) (4) using (b) (4) is attached to this review.

Recommendation: Landiolol HCl 300 mg injection formulation is approvable from a nonclinical perspective at the proposed dose for the short-term reduction of ventricular rate in patients with supraventricular tachycardia.

Labeling: The previous nonclinical team raised several questions regarding animal exposure levels listed on the LDL label. The sponsor provided the requested information, and the current nonclinical team considers the label acceptable.

To: Feleke Eshete (CDER/OND/OCHEN/DPTCHEN)
cc: David Carlson (CDER/OND/OCHEN/DPTCHEN)
From: CDER/OTS/OCP/DARS: Computational Toxicology Consultation Service
Re: NDA 217202 – (b) (4) Calculation for (b) (4)
Date: September 12, 2023

Summary

The CDER/OTS/OCP/DARS Computational Toxicology Consultation Service (CTCS) was consulted to apply the (b) (4) and associated acceptable intake (AI) limit of the (b) (4) (b) (4)-related impurity (b) (4). CTCS concludes that (b) (4) has an associated AI of (b) (4) ng/day.



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/s/

PAUL R GRIMM
10/14/2024 02:36:45 PM

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10/14/2024 02:44:56 PM
Concur.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 217202
Supporting document/s: 001
Applicant's letter date: 31-May-2022
CDER stamp date: 31-May-2022
Product: Landiolol HCl 300 mg for Injection
Indication: Short-term reduction of ventricular rate in patients with supraventricular tachycardia including atrial fibrillation and atrial flutter.
Applicant: AOP Orphan Pharmaceuticals GmbH
Clinical Review Division: Division of Cardiology and Nephrology
Pharm/Tox Division: Division of Pharm/Tox for Cardiology, Hematology, Endocrinology, and Nephrology (DPT-CHEN)
Reviewer: Feleke Eshete, Ph.D.
Supervisor/Team Leader: David Carlson, Ph.D.
Project Manager: Maryam Kord Bacheh Changi

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 217202 are owned by AOP Orphan Pharmaceuticals or are from published literature cited by the applicant on a landiolol product marketed outside of the United States. The publications were translated from Japanese, and this review provides abbreviated citations to the references that are provided in the application; the complete citation is available in module 4.3 of the marketing application. Note that the terms ONO-1101, Onact, and landiolol HCl (LAN) are interchangeably used in this review and represent the same active pharmaceutical.

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1 Executive Summary

1.1 Introduction

Landirolol HCl (LAN) is a beta-1-adrenergic blocker indicated for the short-term reduction of ventricular rate in patients with supraventricular tachycardia, including atrial fibrillation and atrial flutter. It is presumed to inhibit the positive chronotropic effects of catecholamines on the heart. AOP Orphan Pharmaceuticals AG (AOP pharma) has submitted a 505(b)(2) marketing application for LAN 300 mg injection formulation relying on published nonclinical information from a landiolol HCl product (ONO-1101/Onoact®), and additional nonclinical data from studies conducted by the applicant that are listed under **Section 3 - Studies Newly Submitted**.

It is concluded in this review that the reliance on ONO-1101 literature for nonclinical support is justifiable since both products share the same active salt and appear to be pharmaceutically equivalent. Both products are administered intravenously for the same medical condition and comparable PK parameters were obtained for the LAN and Onact in healthy volunteers at the same doses.

1.2 Safety evaluation

Repeat i.v. bolus daily dose of ONO-1101 at ≥ 100 mg/kg/day were overtly toxic and caused death of rats and rabbits. These animals presented clonic convulsions, apnea/bradypnea, hypoactivity and reddish lacrimation. Death also occurred within 12 hrs when ONO-1101 was continuously infused at 5 mg/kg/min (~61-times the AUC at the maximum recommended human dose (MRHD)). However, a clinical trial conducted by the applicant that simulated the proposed dosing regimen showed a very high safety margin to the NOAEL exposure in a 4-week 24-hr infusion study in dogs suggesting that the high exposure scenarios noted during bolus daily administration are unlikely in the clinic with the recommended dosing regimen.

Table 1 below presents the safety margins to the clinical dose based on the NOAEL in rats and dogs. Most of the toxicity studies conducted with ONO-1101 that the applicant cited were conducted with daily intravenous bolus doses, while intravenous infusion is recommended for the clinical use. Exposure information is available from a single dose rat study in which LAN was continuously infused for 24 hrs (details in section **6.1 Single-Dose Toxicity**) and a 4-week infusion study conducted in dogs for which exposure information is available for the parent drug and the two metabolites (details under **6.2 Repeat-Dose Toxicity**). A maximum clinical dose of 40 $\mu\text{g/kg/min}$ LAN (37.3 $\mu\text{g/kg/min}$ as base) has been proposed for marketing, which is equivalent to 57.6 mg/kg/day. This daily dose in mg/m^2 has been used for safety margin calculation relative to the NOAEL proposed in the reports cited by the applicant from the Japanese product (ONO-1101). The calculated margins based on body surface area (BSA) approximated the exposure at the MRHD.

However, comparison based on daily dosing is unreasonable since the $t_{1/2}$ is very short in animals as well as humans; theoretically, LAN would be expected to completely clear

within 15 minutes based on the $t_{1/2}$ of about 5 minutes in all species. Clearance of LAN in rats, dogs and humans was also comparable and is equivalent to hepatic blood flow for each species.

Thus, the safety evaluation in this review is based on the rat single dose study after 24-hrs continuous i.v. infusion of doses of 250, 500 & 1,000 mg/kg, and a 4-week dog study after 24 hr continuous infusion at 0.04 (57.6 mg/day), 0.2 (288 mg/day), and 1 mg/kg/min (1,440 mg/day) (PMDA 2006). The animal exposures at the NOAEL doses have been compared with the exposure in patients with tachycardic atrial fibrillation or atrial flutter [protocol# LDLL600.201] that simulated the recommended clinical dosing regimen.

Rats tolerated a continuous i.v. infusion of up to 1,000 mg/kg for 24 hrs. The exposure (AUC) multiple to the clinical exposure at this dose was 2-times the exposure at 40 $\mu\text{g/kg/min}$. In a 4-week dog study, LAN was administered by continuous infusion for 24 hrs at 0.04, 0.2, and 1 mg/kg/min. The steady state plasma concentrations of LAN, M-1 and M-2 were similar in both sexes and exhibited approximate dose proportionality. The level of M-1 was 5- to 12-fold the parent compound, with highest ratio at the high dose. The level of M-2 was 2- to 8-fold with the highest ratio at the low dose.

In patients with tachycardic atrial fibrillation or atrial flutter, LAN was infused either continuously at 40 $\mu\text{g/kg/min}$ for a mean duration of 130.7 min or given as a 100 $\mu\text{g/kg/min}$ bolus and continuous infusion of 40 $\mu\text{g/kg/min}$ for a mean duration of 102.1 min. An average dose of LAN infused over the course of the study averaged 48.5 ± 18.9 $\mu\text{g/kg/min}$. Mean C_{max} of 1.717 $\mu\text{g/mL}$ and AUC of 1.926 $\mu\text{g}\cdot\text{h/mL}$ were achieved in the study. The C_{max} and AUC of LAN corrected to 40 $\mu\text{g/kg/min}$ were 1.385 $\mu\text{g/mL}$ and 2.076 $\mu\text{g}\cdot\text{h/mL}$. The NOAEL exposure in the 4-week 24-hr infusion dog study was 136-times the exposure in the MRHD. Moreover, the exposures achieved for the M-1 and M-2 metabolites in the clinical study are less than the exposure in the single dose rat study and the 4-week, 24 hr infusion dog study, and the kinetic parameters (T_{max} , $t_{1/2}$, clearance) for the metabolites showed similar trends in all species. Thus, it is concluded in this review that the potential risk from administration of LAN at the recommended dose and duration is minimal for the proposed infusion rate of up to 40 $\mu\text{g/kg/min}$ LAN in patients with normal cardiac function or for a maximum infusion rate of (b) (4) $\mu\text{g/kg/min}$ for patients with impaired cardiac function to achieve desired reductions in ventricular rate.

(b) (4), a potential carcinogenic impurity, was identified in drug product batches tested at the request of the Agency's quality review team. A review by the Agency's computational toxicology experts recommended an "acceptable intake" of (b) (4) ng/day (b) (4), equivalent to the level recommended for (b) (4) impurities which lack robust carcinogenicity data, but which would confer negligible excess carcinogenic risk after lifetime exposure. Available data are considered inadequate to assess carcinogenic risk of (b) (4) exposure exceeding (b) (4) ng/day.

Overall, the nonclinical data submitted by AOP pharma along with the publicly available literature on ONO-110 support the safety of the proposed clinical dosing regimen for the LAN 300 mg injection formulation.

Table 1: Safety Margins to the Maximum Recommended Clinical Dose Based on Animal Exposure

Study	Toxicity	Species	NOAEL (mg/kg) M/F	Based on AUC*	Based on BSA
<i>General toxicity Studies</i>					
Single dose 24-hr continuous infusion (250, 500, 1,000 mg/kg)	None	rat	1000	2	-
IV bolus 4-wks (12.5, 25, 50, 100 mg/kg/d (Yamaguchi, 1997c))	Clonic convulsions and death @100 mg/kg	Rat	50	-	<1
IV. bolus 4-wks(12.5, 25, 50 mg/kg/d (Yamaguchi, 1997b))	GI and effects on erythrocyte indices	Dog	12.5		<1
Continuous iv infusion (24 hr/day), 4-wks (0.04, 0.2, 1, 5 mg/kg/min (PMDA 2006))	Death @ 5 mg/kg/min	Dog	1 mg/kg/min	136	-

*Exposure in humans: 2.07 µg.hr/mL and 1.385 µg/mL after continuous infusion of 40 µg/kg/min (57.6 mg/kg/day) for 3.5 hrs (source: Study name: LDLL600.201 (study # 2014-001905-42).

1.3 Recommendations

1.3.1 Approvability

Landiolol HCl 300 mg injection formulation is approvable from a nonclinical perspective at the proposed dose for the short-term reduction of ventricular rate in patients with supraventricular tachycardia contingent upon controlling the (b) (4) impurity to the acceptable intake level recommended by the Agency.

1.3.2 Additional Non-Clinical Recommendations

No additional post-approval nonclinical studies are required.

1.3.3 Labeling

Labeling recommendations have been provided to the applicant and the label language communicated to the applicant is summarized below. There are numerous listed drugs in the 'beta adrenergic blocker' class and the sponsor referred to the U.S. listed drug Brevibloc® (esmolol) to guide label language since Onoact (landiolol) is not approved in the United States. Published study reports were reviewed to confirm exposure margins and body surface area comparisons have been recommended in the review when plasma exposure information was not available. Toxicokinetic data were not available in the publication cited in support of the reproductive studies. The applicant has used

(b) (4)

(b) (4)

to

estimate the exposures at doses of 25, 50, or 100 mg/kg/day of study. This extrapolation is not considered appropriate for the referenced studies and has not been accepted in this review. Thus, the clinical exposure margins have been calculated based on body surface area.

8 USE IN SPECIFIC POPULATIONS

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

1.3 Summary of Nonclinical Data and Information Submitted in the Application

Primary Pharmacology

Landiolol is a β_1 -blocker that inhibits the positive chronotropic effects of the catecholamines adrenaline and noradrenaline on the heart.

In Vitro and Ex Vivo studies

In vitro studies conducted with ONO-1101 using membrane fractions obtained from cells expressing the different β -adrenergic receptors show that LAN is a potent and selective inhibitor of the β_1 -receptor. The relative potency for the β_2 - and β_3 -adrenergic receptors based on $K_i\beta_2/K_i\beta_1$ and $K_i\beta_3/K_i\beta_1$ were 30.4 and 312, respectively. A study sponsored by AOP pharmaceuticals with LAN, the M-1 metabolite, esmolol (another selective β_1 -adrenergic receptor blocker) and isoproterenol on the human β_3 -adrenergic receptor had K_i values of 21.3 μ M, >300 μ M, 10.1 μ M and 3.3 μ M for LAN, M-1, esmolol and isoproterenol, respectively. The K_i value for the β_1 -adrenergic receptor was 90 nM for LAN and 194 nM for esmolol and 3.8 μ M for the M-1 metabolite. The M-1 metabolite that has been shown to represent the major metabolite after LAN administration in all species is not expected to activate the β_1 -adrenergic receptor at the clinical exposure. The C_{max} of M-1 is 3-times and its $t_{1/2}$ is 16-times longer than that of LAN in humans, but its β_1 -adrenergic receptor binding potency is ~42-times lower.

The binding affinity of ONO-1101 to various receptors and ion channels was assessed in membrane fractions obtained from Beagle dogs and Wistar rats. Membrane fractions from dog papillary muscle, left atrial muscle (β_1) and lung (β_2) as well as fractions from rat forebrain regions to examine binding to other receptors and ion channels were

tested. The study with the dog membrane fractions showed cardioselectivity of LAN, and the rat forebrain studies indicated a very low potency of ONO-1101 at α_1 , α_2 , 5-HT₁, 5-HT₂, M-1, M-2, M3, nicotinic, D₁ and D₂ receptors as well as calcium and sodium channels. The study corroborated the selectivity LAN for the β_1 -adrenergic receptor and suggested the absence of secondary pharmacological effects on receptors of major neurotransmitters in the brain.

In a different study, atrial rate and smooth muscle tension were measured ex-vivo in guinea pig right atrium and tracheal tissue with ONO-1101, esmolol and propranolol. ONO-1101 was more cardio selective (~8-fold) than esmolol and propranolol (375-fold); the β_1/β_2 -receptor activity ratios were 255, 33, and 0.68 for ONO-1101, esmolol and propranolol, respectively. The intrinsic sympathomimetic activity of ONO-1101, esmolol and propranolol were also assessed in the guinea pig right atrium and tracheal tissue by measuring the blocking effect against isoproterenol-induced spontaneous heart rate (HR) and tracheal muscle relaxation. All tested compounds were devoid of intrinsic sympathomimetic activity.

Effects on cardiac function and hemodynamics were assessed after direct application of ONO-1101 and esmolol in isolated, Langendorff perfused guinea pig heart system and patch clamping of isolated myocytes. ONO-1101 had minimal cardiac effects relative to esmolol that inhibited cardiac performance (see section **4.1 Primary Pharmacology** for details). The potential chronotropic and inotropic effects of ONO-1101 and esmolol were evaluated after isoproterenol-induced tachycardia in rabbit heart. Both ONO-1101 and esmolol produced dose-dependent decrease in HR, left ventricular developed pressure, maximal rate of left ventricular force development, and myocardial O₂ consumption, indicating that LAN affects the rate and contractility of the heart. This has been corroborated by a study in anesthetized dogs that evaluated electrophysiological, and cardiovascular hemodynamics after ONO-1101 infusion.

In a related study, the time courses of blocking norepinephrine-induced effects of ONO-1101 were compared to those of esmolol and propranolol using isolated, blood-perfused sinoatrial node and papillary muscle preparations of mongrel dogs. Norepinephrine-induced increases in sinoatrial rate (SAR) and tension on papillary muscle (PMDT) were maximally inhibited 3 to 6 min after intravenous injections of ONO-1101. The test-articles decreased SAR and PMDT in a dose-related manner starting at 10 $\mu\text{g/kg}$. Recovery from inhibition was also dose-dependent, 50% recovery was noted 12 min for SAR and 13 min for PMDT at 10 $\mu\text{g/kg}$.

In vivo pharmacology studies

Cardioselectivity of ONO-1101 was tested in anesthetized Beagle dogs by examining its inhibitory effects on isoproterenol-induced increase in heart rate (β_1), decrease in diastolic blood pressure (β_2), and inhibition of histamine-induced increase in tracheal lateral pressure (β_2). Landiolol selectively blocked the β_1 -receptors present in the heart without blocking β_2 -receptors that play an important role for the relaxation of peripheral vessels and trachea. When the effects of bolus intravenous administration of LAN and

esmolol were evaluated on HR, mean arterial pressure (MAP) and renal sympathetic nerve activity of anesthetized rabbits, dose-dependent negative chronotropic effects were noted that appeared to occur at faster rate for ONO-1101. MAP was dose-dependently decreased with esmolol, but not with LAN. Renal sympathetic activity was increased transiently for esmolol, but no effect was noted with ONO-1101.

The onset and recovery time from the effects of ONO-1101, esmolol and propranolol were assessed in isoproterenol-induced tachycardia in anesthetized dogs after intravenous infusion for 3 hrs. ONO-1101 exerted inhibition of the HR increase within 20 minutes and a 50% disappearance rate of 11-20 minutes. Similar study in dogs suggested that the inhibitory effects of ONO-1101 and esmolol on isoproterenol-induced tachycardia disappears within 10 minutes (50%) and completely recovered after 30 minutes.

Additional studies were performed to evaluate the effects of ONO-1101 on several tachycardia and arrhythmia dog models. The results in halothane-epinephrine, electrical stimulation, and aconitine-induced-arrhythmia and tachycardia models indicated that ONO-1101 has a potential to improve experimental tachycardic atrial flutter and fibrillation and increased sympathetic nerve activity. The halothane-epinephrine and aconitine models emulate clinical tachyarrhythmia and clinical pathophysiology.

In dogs, 10 µg/kg/min of epinephrine was infused to decrease cardiac output, stroke volume, and increase average blood pressure. ONO-1101 improved the cardiac output by increasing ventricular filling time because of decreased heart rate and increased stroke volume. In another study, electrophysiological, and cardiovascular hemodynamics (LVdp/dt, left ventricular pressure, cardiac output) were continuously monitored in anesthetized dogs after infusion of ONO-1101 and esmolol for 30 min. The test-articles were administered with concomitant infusion of isoproterenol to produce ~50% increase in contractility (LVdp/dt). Both compounds decreased HR, LVdp/dt, left ventricular pressure and cardiac output without significantly affecting mean blood pressure. LVdp/dt that signifies changes in contractility was dose-dependently decreased by both compounds.

Cardiac electrical activity, including QTc were not significantly different from control mean. However, both sinus node recovery time and the sinus cycle duration were prolonged by ONO-1101 and esmolol in a dose-related manner. Both functional and effective recovery periods of the AV node were increased by both compounds. However, conduction time from atria to bundle of His were increased at sinus rhythm at ≥ 3 µg/kg/min for LAN and ≥ 30 µg/kg/min for esmolol. Conduction time from bundle of His to myocardium was not affected. Both compounds increased refractory period of the right ventricle at ≥ 0.3 µg/kg/min. The monophasic action potential duration at 90% recovery level (MAP₉₀) was increased during sinus rhythm at ≥ 30 µg/kg/min in the right ventricle but was not affected at pacing cycle length of 400 ms (MAP₉₀ (CL400)). The increase in the refractory period with maintained MAP₉₀ (CL400) is suggestive of the antiarrhythmic potential of ONO-1101.

Although ONO-1101 improved HR above 3 µg/kg/min (HED of 1.62 µg/kg/min) and restored atrioventricular conduction at a dose of >1,000 µg/kg/min (HED of 540.5 µg/kg/min) in aconitine-induced atrial tachyarrhythmia, higher doses decreased blood pressure and caused bradycardia and resulted in death at 3,000 µg/kg/min of ONO-1101 administered to dogs. Thus, it is prudent to monitor hemodynamic parameters when higher doses of landiolol HCl are administered in the clinic.

Safety Pharmacology

Transient decrease in spontaneous motor activity and bradypnea were noted at 100 mg/kg of ONO-1101 in mice, and a 300 mg/kg dose was lethal. The NOEL in Irwin test was 30 mg/kg (2.4 mg/kg HED). The test-article did not affect the autonomic function in isolated ileum from Guinea pigs and rabbits at concentrations up to 300 µM (~73-times the C_{max} at MRHD).

The potential effects of LAN and the M-1 & M-2 metabolites on hERG and other cardiac ion channels were investigated by patch clamping of cells expressing the channels in a study sponsored by the applicant. IC₅₀ of >300 µM (>21µM free) was predicted for LAN, representing 5-fold the C_{max} at MRHD. IC₅₀ of >1,000 µM was predicted for the metabolites M-1 and M-2.

Multiple studies were conducted to evaluate the potential effects of ONO-1101 on HR & mean blood pressure (MBP) in conscious rats and ECG parameters in anesthetized/conscious dogs. A 300 mg/kg (162.2 mg/kg/day HED) dose decreased HR and MBP in rats and the animals died within 5 minutes. The NOEL for MBP after infusion was 30 mg/kg/min (HED of 4.7 mg/kg/min), while the NOEL for the bolus dose was 100 mg/kg (HED 16.21 mg/kg). Significant prolongation of the PR interval was reported at ≥0.1 mg/kg with no significant changes in QTc in anesthetized dogs. Transient effects were noted in respiratory rate at the high dose. No effect was observed on HR, ECG parameters and respiratory rate in conscious dogs. Additionally, ONO-1011 (40 µg/kg/min) did not have effect on acetylcholine-induced ova-sensitized Guinea pigs on anesthesia when increase in total pulmonary resistance and decrease in lung compliance were assessed as endpoints.

The effects of ONO-1011 on GI motility was evaluated in mice, and gastric secretion volume, acidity, and pH effects were assessed in rats. Bolus i.v. injections of up to 100 mg/kg to mice had no effect on gastric motility. In rats with ligated pyloric region of the stomach, neither ONO-1011 administered by i.v. bolus injection (30 mg/kg) nor by continuous infusion (up to 10 mg/kg/min) affected gastric secretion volume, acidity, and pH.

The effect of ONO-1011 on urine volume, and urine Na⁺, K⁺, Cl⁻ and pH was evaluated after i.v. bolus injection of 30 mg/kg, or a continuous infusion of up to 10 mg/kg/min for 5 hrs. Bolus injection had no effect, but ONO-1011 caused a significant increase in urine volume and Na⁺, K⁺ and Cl⁻ after infusion. Urine pH decreased significantly at all doses and there was positive occult blood reaction that was caused by hemolysis at 10 mg/kg/min. The potential hemolytic effects of LAN were further investigated (see section

11.3 Hemolytic Effect of Landiolol) that showed that 10 mM concentration was hemolytic in vitro; the C_{max} at the MRHD was 4 μ M.

Pharmacodynamic Drug Interactions

The applicant cited (ONO, 2006; PMDA, 2002) and reported that class I antiarrhythmic agents (Na^+ channel blockers), calcium blockers, and anesthetics potentiates one or more of the effects of LAN, i.e., decreased HR, prolonged the PR interval, and decreased the MBP. The information in Onoact®, states that sedatives, psychotropic drugs, muscle relaxants, anticonvulsants, as well as general anesthetics used for induction and maintenance of anesthesia can possibly influence the circulatory system and result in pharmacodynamic drug interactions with LAN. However, many clinical studies were conducted under anesthesiology for ONO-1011 and have been referenced in the application.

Pharmacokinetics and Toxicokinetics

Absorption

Exposure in terms of C_{max} and AUC_{inf} was dose-proportional in rats after single intravenous bolus and continuous infusion of ONO-1101. Dose-proportionality appears to also occur in dogs after bolus dose. The $t_{1/2}$ in rat (aver $t_{1/2}$ =5 min; $t_{1/2terminal}$ = 30 min) and dogs (aver $t_{1/2}$ = 1.6 min; $t_{1/2terminal}$ = 6 min) increased upon continuous infusion. The $t_{1/2}$ in rats was comparable after single and repeat dosing. In rats, the T_{max} increased with dose after 24-hr infusion. Exposure (AUC_{inf}) and $t_{1/2}$ didn't change after repeat dosing in rats (**Table 6**). After continuous infusion of the LAN to the target population, the median $t_{1/2}$ in humans was 5 min, while that of metabolites M-1 and M-2 were ~80 min and 22 min, respectively (protocol LDLL600.201)¹.

In a 4-week dog study, ONO-1101 was administered intravenously by 24 hr continuous i.v. infusion up to 1 mg/kg/min. The steady state plasma concentrations of LAN, M-1 and M-2 were similar in both sexes and exhibited approximate dose proportionality. The level of M-1 was 5- to 12-fold higher than the parent compound, with the highest ratio at the high dose. The level of M-2 was 2 to 8-fold with the highest ratio at the low dose. After continuous infusion of LAN for 24 hrs in female rats, exposure was more than dose-proportional between the mid and low dose (see **6.1 Single-Dose Toxicity**). The M-1 metabolite exposure on average was ~270-times the parent compound, while the average exposure of the M-2 metabolite is ~2-times. Both metabolites had a longer rat $t_{1/2}$ than that of the parent drug with a mean $t_{1/2}$ of ~30 min after continuous infusion. The AUC_{inf} of M-1 metabolite was ~6-fold higher than that of LAN in healthy Japanese volunteers after 1 min bolus and an hr of infusion. The M-1 metabolite also showed very long $t_{1/2}$ (~20-fold) in these volunteers relative to that of LAN that was between 3-5 minutes.

¹ Study no. 2014-001905-42: Open-label PD/PK study in patients with tachycardic atrial fibrillation or atrial flutter

Distribution

ONO-1101 has low binding to plasma proteins in all species. After a single i.v. bolus dose of 1 mg/kg ^{14}C -ONO-1101 to rats, high concentrations of test article related radioactivity was found in the kidney, liver, lungs, trachea, skin, and carcass within 15 min and declined to about or below detection limit in all tissues at 24 hrs. Radioactivity levels in the brain and fetuses were much lower than that of plasma; drug-related radioactivity was not observed in the brain after repeat dosing. When the same bolus dose was administered to lactating rats, the radioactivity in milk reached the highest levels at 1 hr and then declined, reaching negligible level after 24 hrs.

Metabolism and PK interactions

Landiolol is metabolized in all species by hydrolysis of the ester moiety that produces M-1, which undergoes β -oxidation to the M-2 metabolite. LAN is metabolized by carboxylesterase in rat and dog plasma and liver, and pseudocholinesterase in human plasma, and carboxylesterase in human liver.

The stability of LAN was assessed in plasma obtained from rats, dogs, and humans, and in liver homogenates from rats and dogs. The $t_{1/2}$ of LAN in plasma was 2.0, 70.4 and 4.1 minutes, for rats, dogs, and humans, respectively. The $t_{1/2}$ in liver homogenate was 3.3 minutes in rats and 16.8 minutes in dogs. The hydrolysis of LAN is expected to generate metabolite M-1, which has a longer half-life than the parent compound in humans. Hydrolysis was inhibited by esterase inhibitors. The estimated mean $t_{1/2}$ of M-1 was 96 min and that of M-2, 216 min after 60 min infusion of 10 $\mu\text{g/kg/min}$ LAN.

Inhibition of CYP enzymes in pooled human liver microsomes indicated that LAN and metabolite M-1 may be inhibiting CYP2C19. Greater than 50% inhibition of CYP2C19 was noted at concentrations of 0.1 mM LAN and 1.3 mM M-1. The C_{max} at the MRHD is 4.11 μM suggesting these inhibitions may not occur in the clinic. The applicant has not conducted further drug transporters effects of LAN and its metabolites citing the Agency guidance about the need for $\geq 25\%$ hepatic uptake or renal clearance for evaluation.

Excretion

ONO-1101 is mainly excreted in the urine, fecal excretion accounting for $<11\%$ ONO-1101 related radioactivity in rats. In lactating rats, $\sim 70\%$ of the radioactivity in plasma was measured in milk after 1 hr, but negligible level was measured after 24-hrs of dosing. There was no significant difference in clearance and the volume of distribution between conscious and anesthetized dogs. ONO-1101 clears at about the dog hepatic blood flow but has low distribution in dogs after continuous infusion.

In humans, the cumulative urinary excretion rate in 24 hrs was 7-8% for the parent drug, 63-84% for M-1 and 15-20% for M-2, which confirmed that most of the administered dose is excreted in urine in multiple species.

Toxicology

In addition to a single dose 24-hr infusion study conducted by AOP Health, a 4-week i.v. bolus study in rats and dogs and a continuous infusion study of the same duration in dogs with ONO-1101 were cited. The high doses evaluated by the applicant in the one-day continuous infusion study in rats were without effect except body weight loss at ≥ 250 mg/kg dose.

In a 4-week i.v. bolus study in rats with ONO-1101, animals receiving 100 mg/kg/day (HED of 16.1 mg/kg) experienced clonic convulsions and died within 2-minutes of test-article administration. The cause of death was unclear but have been suggested to be related to the pharmacology of the drug. Transient bradypnea, dyspnea, tremor, chromodacryorrhea and loss of righting reflex were observed in the 100 mg/kg groups of both sexes shortly after the administration but not in animals receiving ≤ 50 mg/kg. These effects occurred shortly after dosing and were reversible in animals that survived. The conditions were attributed to the β -blocking effects of ONO-1101 and death is thought to result from disproportionate adrenergic receptor blocking effect.

Macroscopic changes were noted in the liver, kidney, bladder, uterus, testes, epididymis, and seminal vesicles at the 100 mg/kg dose but were not associated with microscopic findings. A NOAEL of 50 mg/kg/day was identified in the study. This dose is lower than the MRHD based on allometric scaling. However, comparison at this scale with a bolus dose wouldn't be appropriate since the $t_{1/2}$ is very short in rats and humans are expected to completely clear within 3-hrs assuming an upper $t_{1/2}$ of 30 minutes.

A high daily dose of 50 mg/kg was administered as an i.v. bolus dose in the 4-week dog study. Sinus arrhythmia, bradycardia and ECG wave-pattern changes were observed at this dose and decreased erythrocytes and hemoglobin values were reported at ≥ 25 mg/kg. The investigators identified a NOAEL of 12.5 mg/kg/day for both sexes.

Two 4-weeks studies were conducted in dogs by continuously administering the test article intravenously for 24 hrs (PMDA 2006). Doses of 0.2, 1 & 5 mg/kg/min (rate of 0.1 mL/kg/min) were evaluated with blood sampling for the measurement of the levels of the parent drug and the two metabolites, M-1, and M-2 up to 72 hrs. All dogs in the 5 mg/kg/min group died within 9 to 11 hrs (92-fold the clinical exposure at MRHD based on concentration- $\mu\text{g/mL}$). The animals showed signs of $\downarrow$ motor activity, decumbence & cyanosis and microscopic findings of cardiac & renal discoloration and pleural effusion. The plasma concentration at 5 mg/kg/min was 61.99 $\mu\text{g/mL}$ 1 hr after the start of administration and 127.30 $\mu\text{g/mL}$ at 6 hrs.

Steady state concentration of the parent drug was reached within half an hr at the 0.2 and 1 mg/kg/min dose and its level was below the limit of quantification after 1 hr. The 24-hr plasma concentration prior to the termination of administration was 2.13 and 11.81 $\mu\text{g/mL}$ at 0.2 and 1.0 mg/kg/min, respectively, while $\text{AUC}_{0-96\text{h}}$ was 52.25 and 281.19 $\mu\text{g}\cdot\text{h/mL}$, respectively. The plasma concentration of M-1 and M-2 reached

steady state in 6 hrs and showed nearly constant changes in concentration over time till the end of administration. The terminal concentration of M-1 was 6.2x and 8.4x that of the parent drug at 0.2 and 1.0 mg/kg/min, respectively, while the corresponding AUC_{0-96h} values for M-1 were 7.5x and 9.3x. The concentration of the M-2 metabolite at the end of dosing was 4.1x and 2.1x at 0.2 and 1.0 mg/kg/min, respectively, while the corresponding AUC_{0-96h} values for M-2 were 4.8x and 2.7x, respectively.

The second 4-week dog study evaluated doses of 0.04, 0.2 or 1.0 mg/kg/min administered at the rate of 0.05 mL/kg/min by continuously administering the test article intravenously for 24 hrs at the rate of 0.1 mL/kg/min with concomitant monitoring of blood levels of the parent drug and metabolites (PMDA 2006). Shortening of APTT was observed in females at the mid dose and both sexes at the high dose. There were no other findings that indicated activation of the coagulation cascade. The PR interval was increased, which is related to the pharmacological effect. Exposure was dose-proportional and there were no sex differences in exposure. Steady state was reached within 1 hr and constant changes occurred over time until 4-weeks. The NOAEL was 1 mg/kg/min, with the average AUC_{0-96h} of 281.2 µg.h/mL providing ~136-fold margin to the MRHD based on AUC. The concentration of M-1 was ~11-times the parent while M-2 was 2-fold, and steady state for M-1 was reached within 6-hrs.

Genotoxicity

AOP Pharmaceuticals sponsored a battery of genotoxicity assays that have been validated during this review. LAN did not show genotoxic potential under the conditions of the assays (details in section **7 Genetic Toxicology**).

Reproductive toxicity

The applicant has cited a fertility and early embryonic development study that was conducted in male and female rats at i.v. doses of 25, 50 or 100 mg/kg/day ONO-1101 administered to the male 64 days prior to mating and during mating and to females 15-days before mating and during mating (Nishimura et al, 1997a). Parental death occurred at the high dose and hypoactivity, clonic convulsions, bradypnea/apnea and reddish lacrimation were observed in both sexes at the same dose. Body weight and food consumption were decreased in females during premating and mating periods at the high dose. Treatment did not affect the estrous cycle, mating, and other fertility indices; skeletal or visceral changes were absent in the fetuses. A NOAEL of 50 mg/kg/day for general toxicity in parents, and 100 mg/kg/day for the reproductive performance in parents and for the development of fetuses were identified.

The same doses of ONO-1101 were administered intravenously to pregnant rats and rabbits during the time of organogenesis (Nishimura et al, 1997b). The effect on lactation was also assessed from the day of delivery to Day 21 in rats. Two rats and three rabbits receiving the 100 mg/kg dose, and another rabbit at 50 mg/kg died during the study. Maternal rats and rabbits presented clinical signs of bradypnea, clonic convulsions, and hypoactivity; chromodacryorrhea was reported in rats.

Viability was low for rat pups in the 50 or 100 mg/kg/day groups, and body weight of male fetuses were decreased in the 100 mg/kg/day group, but there were no malformations. Weaned animals were tested for behavior, sensory and reproductive functions. F-1 males in the 25 and 100 mg/kg dose groups had a slightly lowered copulation rate, but there were no histopathology findings in reproductive tissues of animals that failed to copulate and animals that failed to conceive didn't reveal any adverse findings. A NOAEL of 50 mg/kg/day for dams, and 25 mg/kg/day for their offspring were identified.

Pregnant rabbits were administered intravenous doses of 25, 50 or 100 mg/kg/day on gestation days 6 to 18. Animals in the 50 mg/kg dose group had decreased number of implantations, number of male fetuses and increased percentage of dead fetuses, and an increase in female fetal weight compared to the control group. Visceral and skeletal variations were reported in both the control and treated animals, but teratogenic effects were absent. A maternal and fetal NOAEL of 25 mg/kg and 100 mg/kg were identified, respectively in the study (Nishimura et al, 1997c).

The same doses that were tested in rat fertility and EFT studies were tested in the rat pre/postnatal developmental study (Nishimura, 1997d). Five maternal animals in the 100 mg/kg group died immediately after administration; death occurred both during gestation and lactation. Hypoactivity, reddish lacrimation, clonic convulsions and bradypnea/apnea were observed after administration of the high dose, and reddish lacrimation was also reported at 50 mg/kg. Body weight and food consumption were low in maternal animals at weaning. Total resorptions occurred in one animal each in the 25 and 50 mg/kg dose groups. Fetal viability decreased on day 4 after birth in the 100 mg/kg group, but external malformations were absent. Potential effects of ONO-Behavioral, learning, pregnancy, delivery, and lactation of the F1 generation were not affected by ONO-1101. NOAEL of 50 mg/kg was identified for both dams and offspring when administered in the perinatal and lactation periods.

In **Table 2** below, the maternal and fetal NOAEL doses were compared based on BSA with the proposed infusion dose converted to a daily dose. The margins to the human dose based on bolus daily dosing in animals doesn't represent the actual exposure to be expected after infusion as previously discussed (e.g., section **1.2 Safety evaluation**). Moreover, maximum tolerated doses were evaluated in the reproductive toxicity studies. The clinical relevance of daily bolus dosing is also questionable since the applicant is seeking licensure for acute infusion for maximum of (b) (4). Thus, it is concluded in this review that the clinical reproductive toxicity risks at the recommended infusion dose are minimal.

Table 2: Safety Margins to the Clinical Dose for the Reproductive toxicity Studies

Study	Toxicity	Species	NOAEL (mg/kg) M/F	Based on BSA [‡]
Seg 1 (25, 50, 100 mg/kg/day i.v. bolus)	Clonic convulsions, bradypnea and death @ 100 mg/kg	Rat	Maternal: 50	<1
	No effect on fertility parameters		Fetal: 100	<1
Seg 2 (25, 50, 100 mg/kg/day i.v. bolus)	Convulsions, bradypnea and death @ 100 mg/kg	Rat	Maternal: 50	<1
	Decreased fetal viability at > 50 mg/kg		Fetal: 25	<1
Seg 2 (25, 50, 100 mg/kg/day i.v. bolus)	Death at ≥50 mg/kg/d	Rabbit	Maternal: 25	<1
	None		Fetal:100	<1
Seg 3 (25, 50, 100 mg/kg/day i.v. bolus)	Death @ 100 mg/kg	Rat	Maternal: 50	<1
	Effects on reproductive parameters of F1 animals @100 mg/kg		Fetal: 50	<1

[‡]Ratio of the NOAEL (mg/kg/day) and continuous infusion of 40 µg/kg/min converted to 57.6 mg/kg/day.

Special toxicology studies

Local tolerance and irritation potential tests were conducted in rabbits with the 300 mg injection landiolol HCl formulation, Onact®, Rapibloc 300 mg injection formulation (approved in EU) and formulations containing 5-fold concentrations of excipients present in LAN were tested. Parenteral routes other than i.v. were also used for testing. No local reactions or systemic toxicities were noted, except administration related findings.

The hemolytic potential and the effects of LAN on coagulation parameters and platelet function were evaluated in vitro. LAN exerted a slight aPTT- and PT-prolongation at very high concentration (>200-times the MRHD). Hemolysis was observed in rat, human and dog blood samples treated with 10 mM LAN (>2,000-times MRHD) compared to control (saline treated) samples.

The impurities reported in the drug product were (b) (4)

All impurities are expected to be above the qualification threshold at product target shelf-life. In silico structure activity analyses and toxicology studies with LAN and a mixture of synthesized impurities were conducted for qualification. These studies support the conclusion that the impurities are qualified at the limits specified by the applicant (see section 11.4 Impurities/Degradants for details).

(b) (4) Impurity

The CMC review team requested assessment for the risk of (b) (4) in the LAN product due to the reported presence of (b) (4). The applicant developed a new validated analytical method and identified (b) (4) in the US registration drug product batches. The average level of (b) (4) measured in samples stored for about 15-months was ~ (b) (4) ng/day for MRHD of 2,688 mg/day free base) that exceeded the default acceptable intake (AI) of (b) (4) ng/day.

Thus, the applicant used the ICH M7(R1) approach and proposed a surrogate (b) (4) molecule and calculated an AI limit per CDER's (b) (4) impurities guidance (details under **11.4 Impurities/Degradants**).

Figure 1: (b) (4)



(b) (4) was proposed as a surrogate with an AI of (b) (4) ng/day, which is equivalent to (b) (4) ppm for a dose of 2,688 mg/day landiolol for a 50 kg person.

The nonclinical review team consulted with the CDER (b) (4) Safety Team (b) (4) and the computational toxicology consultation service (CTCS) to perform (Q)SAR analysis and surrogate evaluation. The (b) (4) safety team indicated that (b) (4) has not been accepted for (b) (4) due to the potential formation of (b) (4) in the pathway and recommended a default AI of (b) (4) ng/day for (b) (4), corresponding to ~ (b) (4) ppb at the maximum recommended dose of 2,688 mg/day.

The applicant was informed in the late-cycle meeting communication that the surrogate selected for AI determination may not adequately characterize the risks of (b) (4), and a default AI of (b) (4) ng/day may be recommended (DARRTs, 03-Mar-2023). A virtual late-cycle meeting was held with the applicant on March 14, 2023. The applicant presented a case for the selection (b) (4) and discussed an alternative surrogate, (b) (4) with stipulated AI of (b) (4) ng/day ((b) (4) ppm for daily dose of 2,688 mg for a 50-kg person). The CTCS also evaluated the proposed (b) (4) surrogate.

The CTCS (Q)SAR analyses predicted (b) (4) to be positive for bacterial mutagenicity due to the (b) (4) structure. In addition to the surrogates proposed by the applicant, CTCS identified (b) (4) as a potential surrogate. However, none of the three compounds were appropriate for (b) (4) and none could

be used to support an AI calculation for (b) (4) (details under **11.4 Impurities/Degradants**).

(b) (4) Risk evaluation and recommendation

The structural information and analyses by CTCS have suggested that (b) (4) has a carcinogenic potential through a DNA-reactive genotoxic mechanism. However, based on the structure, (b) (4) the applicant and the Agency reviewers have judged that (b) (4) is expected to have a lower potential to interact with DNA and is a less potent carcinogen compared to the potential impurities identified in the CDER guidance. However, based on the absence of an acceptable surrogate (b) (4) an AI of (b) (4) ng/day has been proposed for the impurity in the drug product by the CTCS and the (b) (4).

The nonclinical review team considered whether an acceptable intake could be calculated using a less-than-lifetime approach to (b) (4) exposure since the use of the drug is less than 3-month total exposure (discussed in ICH M7(R1)²). However, the Agency (b) (4) team has used a more conservative lifetime cancer risk estimate, consistent with some other regulatory agencies and a concern that DNA repair mechanisms could be saturated during acute high exposure periods.

It should be also noted that a lifetime AI of (b) (4) ng/day was proposed by the Agency for (b) (4) and (b) (4) impurities in (b) (4) as (b) (4) (Figure 2). (b) (4) like (b) (4) contains a (b) (4) group and it was shown to be positive for mutagenicity in the Ames assay.

² CDER/CBER, (b) (4), Guidance for Industry (b) (4).

Figure 2: (b) (4)

Thus, in the absence of an acceptable surrogate and lack of support for an acceptable risk level for LTL exposure, and the precedent with other (b) (4), the default AI value of (b) (4) ng/day, corresponding to ~ (b) (4) ppb at the maximum recommended dose of 2,688 mg/day has been accepted in this review. This level is about 5-fold less than the average (b) (4) level measured in the registration batches.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

144481-98-1

INN Name

Landirolol Hydrochloride

Code Name

LDLL E103/P00065

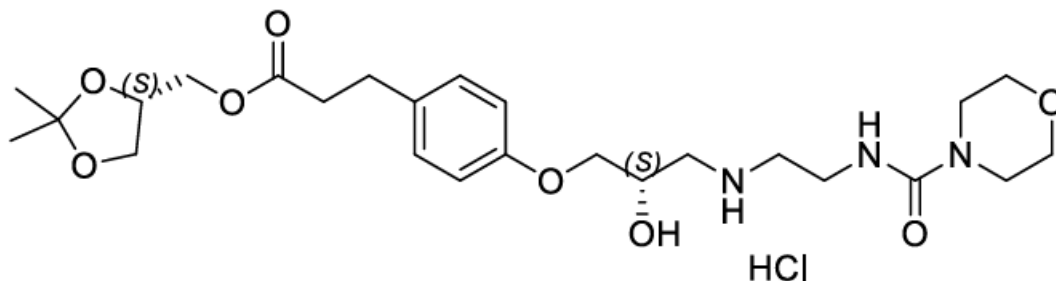
Chemical Name

((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl-3-(4-((S)-2-hydroxy-3-((2-(morpholine-4-carboxamido)ethyl)amino)propoxy)phenyl)propanoate hydrochloride

Molecular Formula/Molecular Weight

C₂₅H₃₉N₃O₈.HCl /546.06 g/mol

Structure



Pharmacologic Class

Beta-adrenergic receptor blocker (β 1-adrenergic receptor inhibitor)

2.2 Relevant INDs

IND 131330

2.3 Drug Formulation

Table 3: Landiolol HCL 300 mg for Injection Formulation

Name of ingredient	Quantity (per vial)	Function	Reference to Standards	% (w/v) Reconstituted solution	Concentration per mL Reconstituted solution
Landiolol hydrochloride (Landiolol)	300 mg (280 mg)	Active Pharmaceutical Ingredient	In-house specification	0.6	6 mg/mL
Mannitol (b) (4)	300 mg	(b) (4)	USP	0.6	6 mg/mL
Sodium hydroxide*	(b) (4)	pH adjustment	NF	(b) (4)	
Water for injection**	(b) (4)	(b) (4)	USP		

* (b) (4)

** Removed during lyophilization process

2.4 Comments on Novel Excipients

Sodium hydroxide is used for pH adjustment and the maximum daily intake (MDE) of mannitol is under the IID MDE value.

2.5 Comments on Impurities/Degradants of Concern

Process-related and potential genotoxic impurities above the qualification threshold have been qualified (more detailed review in section 11.4 Impurities/Degradants).

2.6 Proposed Clinical Population and Dosing Regimen

LAN is indicated for short-term reduction of ventricular rate in patients with supraventricular tachycardia including atrial fibrillation and atrial flutter in patients with normal and impaired cardiac function, (b) (4).

Table 4: Proposed Dosage and use

<i>Stepwise Dosing with normal cardiac function</i>	
Step	Action
1	Titrate the using ventricular rate at 10-min interval
2	Start at 10 µg/kg/min landiolol HCl; adjust dose as needed up to 40 µg/kg/min in 10 µg/kg/min increments to achieve desired reduction in ventricular rate.
3	If necessary, increase the dose up to 40 mcg/kg/min in 10 mcg/kg/min increments no sooner than 10 min after the last dose increase on an individual patient basis based on patient heart rate and if the cardiovascular status of the patient requires and allows such an increase of the dose.
<i>Stepwise Dosing in impaired cardiac function</i>	
Step	Action
1	Titrate using ventricular rate at 15-min intervals
2	Start at 1 µg/kg/min, adjust dose as needed up to (b) (4) µg/kg/min in 1 µg/kg/min increments to achieve desired reduction in ventricular rate.

2.7 Regulatory Background

A type B pre-IND meeting background document with questions was submitted on September 6, 2016, to the Division of Cardio-renal products. The applicant was seeking to develop a 300 mg (b) (4) lyophilized LAN powder for i.v. infusion (b) (4) through the 505(b)(2) pathway based on published nonclinical data with a LAN product (ONOact®) marketed in Japan with limited bridging studies conducted by the applicant. The current marketing application is intended for a 300 mg injection of landiolol HCl; (b) (4).

In an open-label PD/PK study in patients with tachycardic atrial fibrillation or atrial flutter [protocol# LDLL600.201], LAN was either infused continuously at 40 µg/kg/min for mean duration of 130.7 min or given as a 100 µg/kg/min bolus and then by continuous infusion of 40 µg/kg/min for mean duration of 102.1 min. An average dose of LAN infused over the course of the study ranged from 19 to 93 µg/kg/min (average 48.5±18.9 µg/kg/min). A dose of landiolol between 40 to 100 µg/kg/min (average 61.0±22.0 µg/kg/min) was evaluated in the study. A C_{max} of 1.717 and the AUC of 1.926 µg.h/mL was achieved in the study. Means of C_{max} and AUC of LAN corrected to 40 µg/kg/min were 1.385 µg/mL and 2.076 µg*h/mL. The time to reach maximum blood concentration corrected to 40 µg/kg/min (T_{max}) was 1.135 h. The mean t_{1/2} for LAN was 5 minutes, compared to 92 min for metabolite M-1 and 22 min for metabolite M-2. Total body clearance was lowest for M-1 while the parent drug and M-2 showed much higher CL:

2,431.5 mL/(kg*h) for LAN, 271.1 mL/(kg*h) for M-1 and greater than 17,000 mL/(kg*h) for M-2.

The nonclinical team issued comments during the pre-IND meeting and additionally in a type C guidance meeting on July 14, 2020, about the need for receptor binding data for the parent and major human metabolites, and testing for effects on hERG, potassium, sodium, and calcium ion cardiac channel activity. The applicant has responded to these comments (summary below).

Table 5: Summary of Nonclinical Issues and Status from Prior FDA Meetings (reproduced from the submission)

Issue/FDA Comment	Current Status	Hyperlink to Relevant NDA Module
Provide data addressing receptor binding profiles for parent and major human metabolites.	The sponsor performed the required studies (Study No. AOP-(b) (4)2015 and 2020). The data is presented in the pharmacology written and tabulated summaries.	2.6.2.2.1
Provide data on any effects on hERG, potassium, sodium, and calcium cardiac ion channel activity.	The sponsor performed the following GLP in vitro studies: hERG with Landiolol (Study No. 161108.CPQ) hERG with Metabolite M-1 (Study No. 161109.CPQ) hERG, nCav1.2, hKvLQT1/hminK, hNav1.5 and torsadogenic risk for Landiolol, Metabolite M-1 and M-2 (Study No. 160927.CPQ). The results are presented in the pharmacology written and tabulated summaries.	2.6.2.4.1
Demonstrate that human metabolites are present in the animal species evaluated in the toxicology studies	The sponsor has included information regarding the presence of human metabolites M-1 and M-2 in rat and/or dog.	2.6.6.2.4
Impurities and degradation products that exceed qualification threshold should be qualified per ICH Q3A(R2) and Q3B(R2).	Information regarding this issue has been provided for the landiolol batches used in the nonclinical studies included in this NDA.	2.6.6.5.1 3.2.P.5.1
Provide tabulated (particularly for toxicology and safety pharmacology studies) as well as written summaries for all nonclinical studies that support nonclinical evaluation of your drug. FDA acknowledged that for publications, not all the data needed for populating detailed tabulated summaries would be available and it was agreed that the sponsor would indicate appropriately in those instances.	Provided.	2.6
Provide study reports and literature articles addressing nonclinical evaluation.	Provided	4
Both tabulated and written nonclinical summaries should provide links to the full study reports and literature articles.	Links included in Module 2.6 written and tabulated summaries.	2.6.4
Safety factors based on human equivalent doses and plasma drug exposures relative to human exposure at the maximum recommended dose should be provided for all pivotal toxicology studies and presented with a summary table.	Provided.	2.4.5 (Table 2)
For the reproductive toxicology studies without toxicokinetic data, you may extrapolate the exposures from other appropriate toxicology studies in the same species and estimate the safety factors based on the exposures. Provide a clear explanation of how exposures from other animal studies were extrapolated/derived to cover exposure conclusions for reproductive toxicity studies.	Provided.	2.4.5 (Table 2)
FDA suggested that the proposed overview tables (see example in appendix to these meeting minutes that was originally provided in the above clarification communication) planned for all nonclinical subsections including primary/secondary pharmacology as well as pharmacokinetics should contain key findings for each referenced study	Key findings for each referenced study or literature references have been included.	2.6.2 2.6.4 2.6.5

3 Studies Newly Submitted

Study #. 161108.CPQ: Effect of Landiolol Hydrochloride on hERG, hKvLQT1/hminK hCav1.2 and hNav1.5 Channels Expressed in Mammalian Cells.

Study #. 161109.CPQ Effect of Metabolite M-1 on hERG, hKvLQT1/hminK hCav1.2 and hNav1.5 Channels Expressed in Mammalian Cells.

Study #. 160927.CPQ: Effects of Test Articles on Ion Channels Expressed in Mammalian Cells

Study#. US034-0012488: ADME-TOX - Study of Landiolol HCl (Compound B) and Landiolol Metabolite M-1 (Compound A).

Study #. US034-0014432: ADME-TOX - Study of Landiolol HCl (Compound B) and Landiolol Metabolite M-1 (Compound A).

Study #. US034-0013986: CYP2C19 inhibition (HLM, omeprazole substrate) - Study of Landiolol HCl (Compound B) and Landiolol Metabolite M-1 (Compound A).

Study #. 610774: Landiolol Hydrochloride: Single Dose Intravenous (24 h) Infusion Toxicity Study with a 2 Day Observation Period in the Rat.

Study #. 787325: Landiolol HCl: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA.

Study #. 787330 :Landiolol HCl Mouse Lymphoma Mutation Study.

Study #. 790584: Landiolol HCl: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow: 0 h and 24 h Intravenous Dosing and 48 h Sampling with Toxicokinetic Blood Sampling

Study #. 787346: Landiolol HCl: Micronucleus Test in Bone Marrow Cells of CD-1 Mice: 0 h + 24 h Oral Dosing and 48 h Sampling.

Study #. 19199/ Local Tolerance Study in Rabbits After a Single Injection of Landiolol by the Intravenous Route

Study 524235 #. A Single Dose Study to Investigate the Local Tolerance of LDLLE103/P00065 after Injection in Rabbits.

Study #. 522109: A Single Dose Study to Investigate the Local Tolerance of LDLE101/P00071 after Injection in Rabbits

Study #. 312069 Evaluation of the Hemolytic Potential of Landiolol Hydrochloride in Human and Rat Whole Blood In Vitro.

Study # 510638: 2-Week Study of (b) (4) Impurity (b) (4) by Intravenous (Bolus) Injection in Rats.

Study #. 504598/ A Single Dose Toxicity Study of Rapibloc 600 mg (600 mg powder for solution for injection and infusion) by Intravenous (24 hr) Infusion in Rats with a 3 Day Observation Period.

Study #. 795100/Rapibloc Conc (Landiolol HCl 20 mg/2 mL solution for injection): Bacterial Reverse Mutation Test in Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 and Escherichia coli WP2uvrA.

APN001drkls/Toxicological analysis of twelve potential impurities using Derek Nexus and Leadscope.

3.1 Studies Reviewed

3.2 Studies Not Reviewed

All newly submitted studies have been reviewed.

3.3 Previous Reviews Referenced

None

4 Pharmacology

Landiolol HCl 300 mg for injection is supplied in glass vials as lyophilized powder for reconstitution in saline or 5% glucose solution to a final concentration of 6 mg/mL. The product contains equal amounts of the drug substance and mannitol as an excipient. The intended maximum recommended daily dose of landiolol HCl is 57.6 mg/kg/day with the proposed infusion rate of up to 40 µg/kg/minute in patients with normal cardiac function or a maximum infusion rate of (b) (4) µg/kg/minute for patients with impaired cardiac function.

4.1 Primary Pharmacology

In Vitro and Ex Vivo studies

The inhibitory potency based on K_i values for LAN and M-1 metabolite were determined using membrane fractions from CHO-K1 or HEK293 cells stably expressing the β_1 -, β_2 - and β_3 -adrenergic receptors. The β_1 -selectivity ($K_{i\beta_2}/K_{i\beta_1}$ or $K_{i\beta_3}/K_{i\beta_1}$) for LAN/Onoct was 30.4 against β_2 and 312 for β_3 . The K_i value for β_1 -adrenergic receptor was 90 nM for LAN and 3.8 µM for the metabolite M-1 indicating the high affinity and selective binding of LAN on the β_1 -adrenergic receptor compared to the major metabolite. A study sponsored by AOP pharmaceuticals with LAN, M-1 metabolite, and isoproterenol on the human β_3 -adrenergic receptor had K_i values of 21.3 µM, >300 µM, 10.1 µM and 3.3 µM for LAN, M-1, esmolol and isoproterenol, respectively.

The binding affinity of LAN to various receptors and ion channels was assessed in membrane fractions obtained from Beagle dogs and Wistar rats. Membrane fractions from dog papillary muscle, left atrial muscle (β_1 -AR) and lung (β_2 -AR) were used. Fractions from rat forebrain regions were used to examine binding to additional receptors and ion channels in the brain; striatal (muscarinic M-1 receptors, dopamine D1 and D2 receptors), frontal cerebral cortices (β_2 -AR, serotonin 5-HT₁ and 5-HT₂ receptors, nicotinic receptors), calcium channels (mixed T- and L-type), sodium channels (site 1), heart (muscarinic M-2 receptors) as well as submandibular glands (muscarinic M3 receptors).

The results shown in the tables reproduced from Shiroya, et al, 1997a below show that ONO-1101 has greater selectivity to the β_1 -AR than propranolol and esmolol. The K_i values of ONO-1101 for α_1 , α_2 , 5-HT₁, 5-HT₂, M-1, M-2, M3, nicotinic, D1 and D2

receptors as well as calcium and sodium channels were 82, 180, >1000, 113, 43, 41, >1,000, >1,000, >1,000, 429, >1,000 and >1,000 μM , respectively; these affinities are less than 1/40 of that for β_1 -receptors.

Table 6: Cardioselectivity and Receptor and Ion Channel Binding in Dog and Membrane Fractions

Table 6: K_i values and cardioselectivity for β -receptors in canine tissues (myocardium and lung)

Drug	K_i Value [nM]			Cardioselectivity	
	β_1		β_2	$K_i \beta_2/K_i \beta_1$	
	Left atrial muscle	Papillary muscle	Lung	Left atrial muscle	Papillary muscle
Landiolol	993.2 $\pm$ 39.8	834.0 $\pm$ 157.9	12,416 $\pm$ 1,034.2	12.5 $\pm$ 1.1 (73.5)	17.8 $\pm$ 4.6 (118.7)
Esmolol	1,054.1 $\pm$ 218.6	1,226 $\pm$ 405.7	5,900.0 $\pm$ 340.2	6.59 $\pm$ 1.3 (38.8)	7.85 $\pm$ 2.7 (52.3)
Propranolol	9.2 $\pm$ 2.5	10.1 $\pm$ 2.1	1.3 $\pm$ 0.2	0.17 $\pm$ 0.03 (1)	0.15 $\pm$ 0.03 (1)

Calculation formula: $= K_i$ value β_1/K_i value β_2 .

Values in parentheses show the selectivity relative to propranolol (arbitrarily set to 1).

Data are presented as mean $\pm$ standard deviation ($n = 5$).

Table 7: K_i values for respective receptors and ion channels

Receptor/Channel	K_i (μM)
α_1	81.5 $\pm$ 11.9
α_2	180.1 $\pm$ 37.2
5-HT ₁	>1,000
5-HT ₂	112.9 $\pm$ 31.4
M ₁	42.7 $\pm$ 12.9
M ₂	40.7 $\pm$ 6.1
M ₃	>1,000
Nicotine	>1,000
D ₁	>1,000
D ₂	429.0 $\pm$ 20.3
Ca Channel (T, L)	>1,000
Na Channel (site 1)	>1,000

Data are presented as mean $\pm$ standard deviation ($n = 3$).

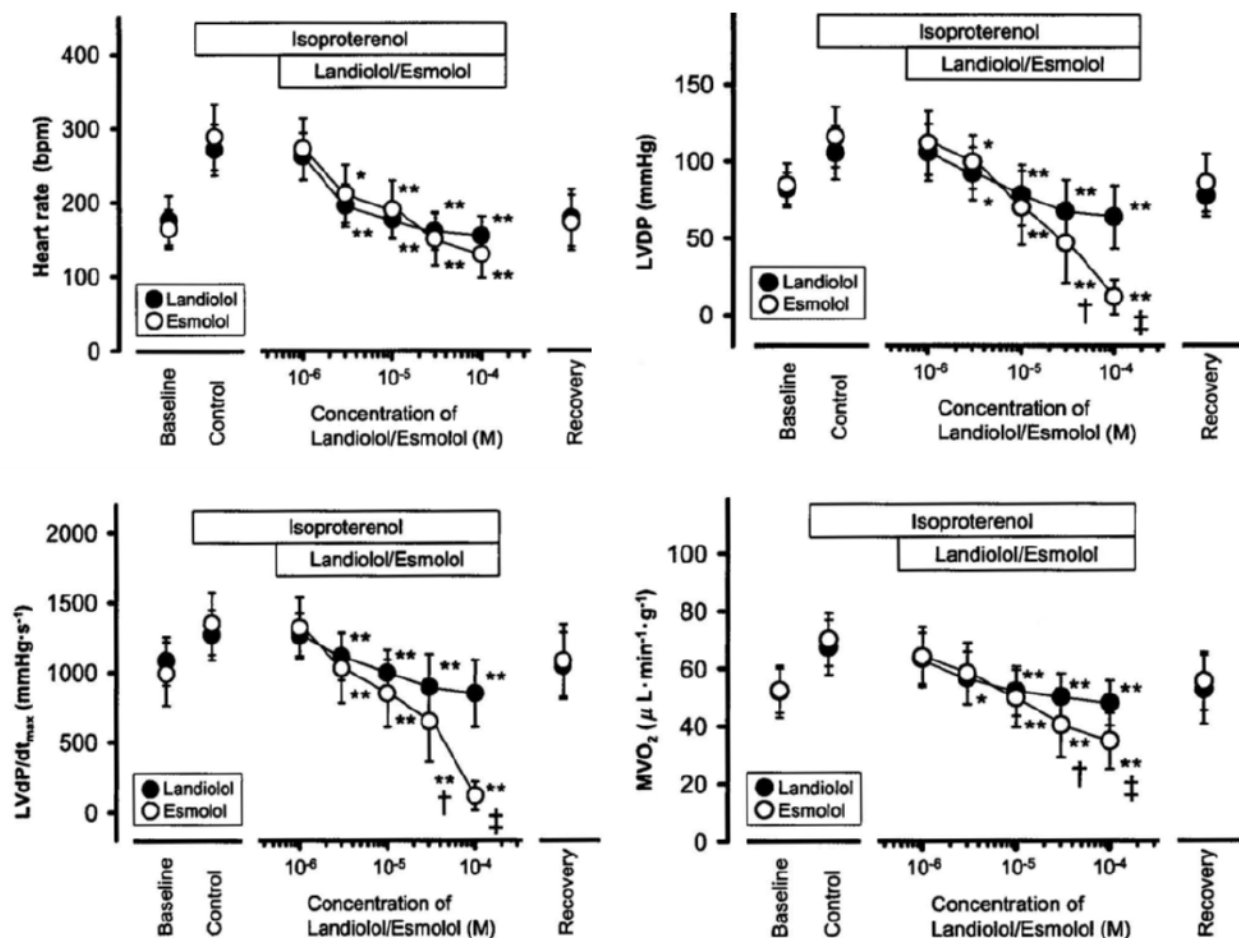
Another study referenced from ONO-1101 evaluated the cardioselectivity of LAN relative to that of esmolol and propranolol conducted with isolated guinea pig right atrium and tracheal tissue. Maximum changes in atrial rate and smooth muscle tension were measured with and without the test-articles and compared as pA₂ values. The cardioselectivity of landiolol was shown to be ~8-fold greater than that of esmolol, and 375-fold greater than that of propranolol; the β_1/β_2 -receptor activity ratio was 255, 33, and 0.68 for ONO-1101, esmolol and propranolol, respectively. The intrinsic sympathomimetic activity of ONO-1101, esmolol and propranolol were also assessed in the guinea pig right atrium and tracheal tissue by measuring the blocking effect against isoproterenol-induced effect on spontaneous hear rate and tracheal muscle relaxant action. All tested compounds were devoid of intrinsic sympathomimetic activity. At

therapeutic dosages, LAN does not exhibit any membrane stabilizing activity or intrinsic sympathomimetic activity in vitro.

Effects on cardiac function and hemodynamics were assessed after direct application of ONO-1101 and esmolol in isolated, Langendorff perfused guinea pig heart system. Electrophysiological parameters were measured on isolated ventricular myocytes with patch clamping. Landiolol had minimal cardiac effects relative to esmolol that inhibited cardiac performance ($\downarrow$ systolic left ventricular pressure and HR, increased coronary perfusion pressure and decreased APD) at $>10\mu\text{M}$ (4-times the C_{max} at MRHD). Shortening of action potential duration (APD) with esmolol was maintained even when the delayed rectifier K^+ current or ATP sensitive K^+ current was blocked. Moreover, esmolol inhibited both the inward rectifier K^+ current (IK_1) and the L-type Ca^{2+} current (ICaL) and dose-dependently increased the outward current.

The potential chronotropic and inotropic effects of LAN and esmolol were evaluated after isoproterenol-induced tachycardia with isolated whole rabbit heart. Both LAN and esmolol produced dose-dependent decrease in HR, left ventricular developed pressure (LVDP), maximal rate of left ventricular force development ($\text{LVdP/dt}_{\text{max}}$), and myocardial O_2 consumption (MVO_2). The results reproduced from Ikeshita, et al., 2008 below indicated that both compounds dose-dependently decreased HR, LVDP, $\text{LVdP/dt}_{\text{max}}$, and MVO_2 . Thus, both agents showed equipotent chronotropic effects and effects on contractility. However, at concentration $>30\mu\text{M}$ esmolol produced lower LVDP, $\text{LVdP/dt}_{\text{max}}$ (contractility), and MVO_2 .

In a related study, the time courses of blocking norepinephrine-induced effects of LAN were compared to those of esmolol and propranolol using isolated, blood-perfused sinoatrial node and papillary muscle preparations from mongrel dogs. Norepinephrine-induced increases in sinoatrial rate (SAR) and tension on papillary muscle (PMDT) were maximally inhibited 3 to 6 min after intravenous injections of LAN. The test-articles decreased SAR and PMDT in a dose-related manner starting at $10\mu\text{g/kg}$ i.v. dose. Maximum inhibitions by LAN of NE-induced increases in SAR on average were 54, 78, and 96% at 0.01, 0.1, and 1 mg/kg of the drug, respectively. Maximum inhibitions by LAN of NE-induced increases in PMDT were 50, 93, and 100%, respectively. Recovery from inhibition was dose-dependent, and 50% recovery was noted 12 min for SAN and 13 min for PMDT at $10\mu\text{g/kg}$ i.v. dose of landiolol.

Figure 3: Effects of Landiolol and Esmolol on Isoproterenol-Induced Changes in Isolated Heart

In vivo studies

Cardioselectivity of ONO-1101 was tested in anesthetized beagle dogs by examining its inhibitory effects on isoproterenol-induced increase in HR (β_1), decrease in diastolic blood pressure (β_2), and inhibition of histamine-induced increase in tracheal lateral pressure (β_2). LAN selectively blocked the β_1 -receptors present in the heart without blocking β_2 -receptors that plays an important role for the relaxation of peripheral vessels and the trachea. The β_1/β_2 ratio of ONO-1101 was 799 when heart rate and diastolic blood pressure were used as indicators, and 277 when HR and respiratory resistance were used as indicators. The β_1 -selectivity of ONO-1101 was 11- to 14-times higher than that of esmolol and 460 to 670-times higher than that of propranolol.

Another study compared the effects of bolus intravenous administration of LAN and esmolol on HR, mean arterial pressure (MAP) and renal sympathetic nerve activity in anesthetized rabbits. LAN and esmolol exerted dose-dependent negative chronotropic effects that appeared to occur at faster rate for LAN. Landiolol also exhibited relatively greater potency for negative chronotropic effects than esmolol. LAN (3.0 mg/kg) and

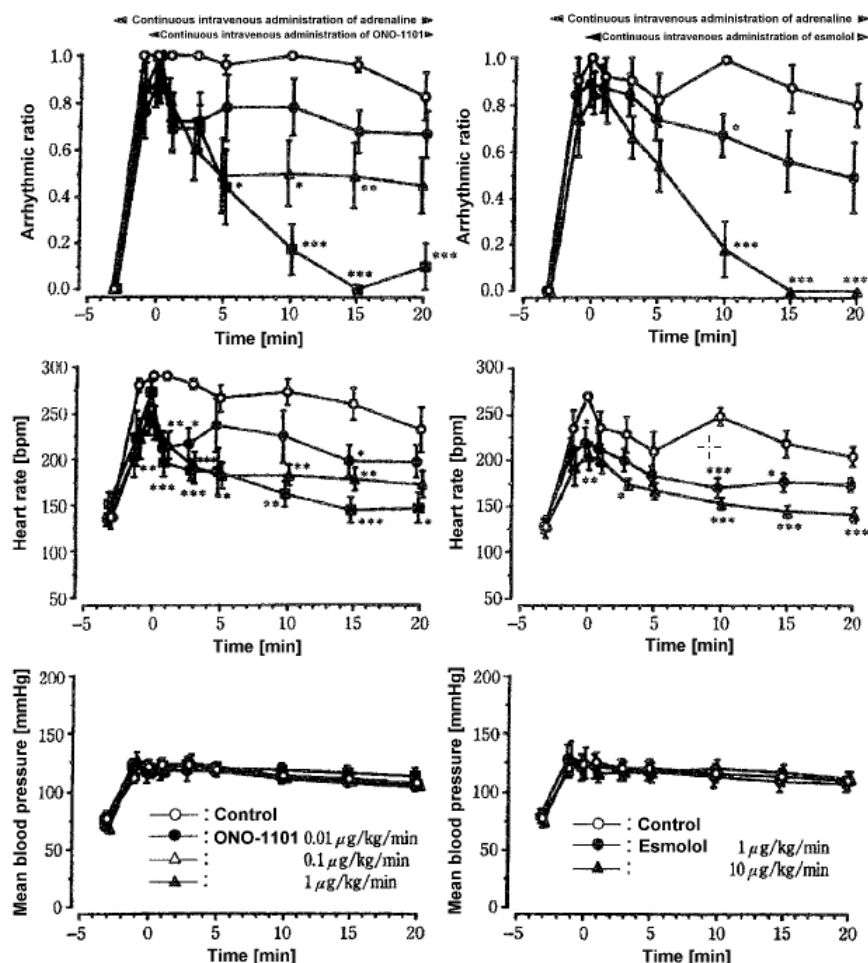
esmolol (5 .0 mg/kg) produced approximately the same (~14%) maximal HR reduction. MAP was dose-dependently decreased with esmolol, but not with LAN. Renal sympathetic activity increased transiently for esmolol, but no effect was noted with LAN.

The onset and disappearance of LAN, esmolol and propranolol was assessed in isoproterenol-induced tachycardia in anesthetized dogs after intravenous infusion for 3 hrs. LAN exerted increased inhibition of HR increase compared to esmolol with the onset of ~ 20 minutes and disappearance rate of 11-20 minutes for both compounds. Similar study in dogs suggested that the inhibitory effects of LAN and esmolol on isoproterenol-induced tachycardia disappears within 10 minutes (50%) and completely recovered after 30 minutes.

Additional studies were performed to evaluate the effects of LAN on several tachycardia and arrhythmia models in dogs. In the halothane-epinephrine model, saline, LAN, esmolol were administered by infusion for 20 minutes in the background of intravenous epinephrine. Propranolol, verapamil & lidocaine were administered as bolus doses as shown below:

Drug	Dose (i.v., bolus) [µg/kg]	Dose (i.v., continuously for 20 min) [µg/kg/min]
Landiolol	10	0.01
	100	0.1
	-	1
Esmolol	100	1
	1000	10
Propranolol	3	-
	30	-
Verapamil	30	-
	300	-
Lidocaine	3000	-

Antiarrhythmic effect was evaluated from the percentage of arrhythmic beats in heartbeat per unit of time (arrhythmic ratio). As shown in the figure by Shiroya, 1997b below, ONO-1101 and esmolol significantly suppressed arrhythmia induced by halothane and epinephrine in a dose-dependent manner when administered by continuous i.v. infusion at 0.1 and 1 µg/kg/min. Hypotensive effect was observed for both agents at a very high dose.

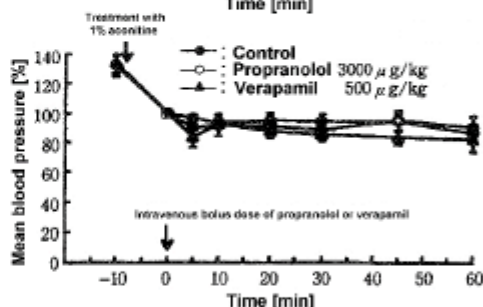
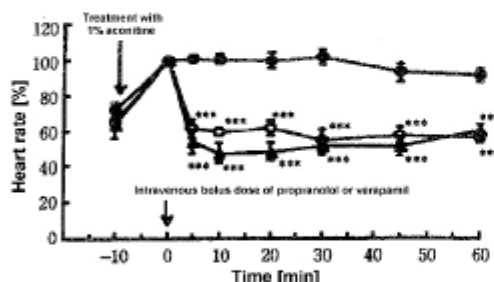
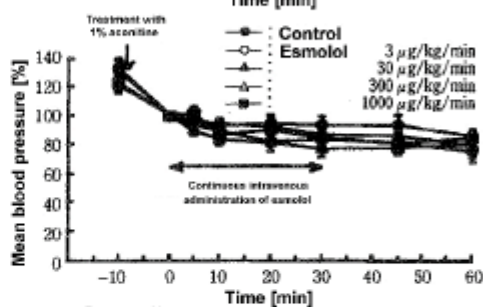
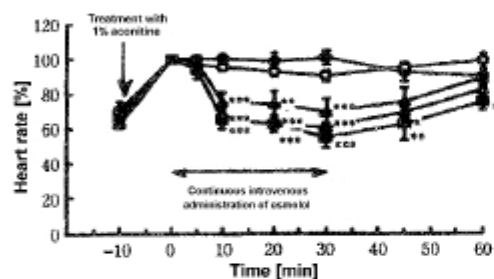
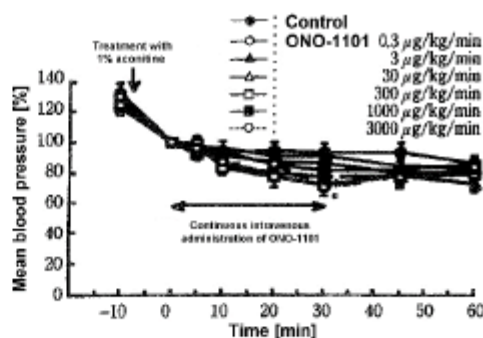
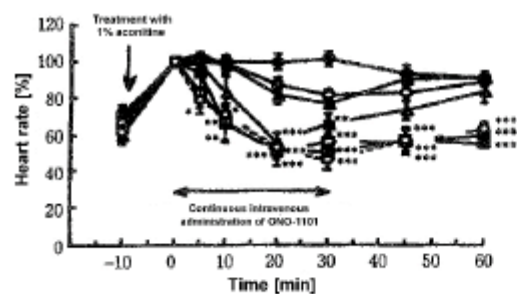


Effect of continuous intravenous administration of ONO-1101 and esmolol on halothane-adrenaline-induced arrhythmia (arrhythmic ratio, heart rate and blood pressure)

Data are shown as mean $\pm$ standard error ($n = 5$)

*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Dunnett's test vs. control)

When aconitine-induced atrial tachyarrhythmia was used, ONO-1101 significantly increased the number of cases with improved heart rate (subjects with a heart rate decrease of at least 20%) at a dose of $>3 \mu\text{g/kg/min}$ and the number of cases with improved electrocardiogram (subjects in whom atrioventricular conduction was restored) at a dose of $>1,000 \mu\text{g/kg/min}$. Esmolol also significantly increased the number of cases with improved heart rate at a dose of $>30 \mu\text{g/kg/min}$ and the number of cases with improved electrocardiogram at a dose of $>1,000 \mu\text{g/kg/min}$. The report also stated that "significant hypotensive effect was noted 30 minutes after the start of treatment for ONO-1101, but not other drugs at $>1,000 \mu\text{g/kg/min}$ ".



Effect on changes in heart rate and blood pressure in aconitine-induced atrial arrhythmia model

ONO-1101 0.3 µg/kg/min group ($n = 5$), 3 µg/kg/min group ($n = 5$), 30 µg/kg/min group ($n = 5$), 300 µg/kg/min group ($n = 8$), 1,000 µg/kg/min group ($n = 8$), 3,000 µg/kg/min group ($n = 7$)

Esmolol 3 µg/kg/min group ($n = 5$), 30 µg/kg/min group ($n = 5$), 300 µg/kg/min group ($n = 8$), 1,000 µg/kg/min group ($n = 6$)

Propranolol 3,000 µg/kg group ($n = 7$)

Verapamil 500 µg/kg group ($n = 5$)

Control group ($n = 8$)

Data are shown as mean $\pm$ standard error

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ (Dunnett's test vs. control)

Table 7: Effect of ONO-1101, Esmolol, Propranolol and Verapamil in Aconitine-Induced Atrial Arrhythmia Model (reproduced from Shiroya 1997b)

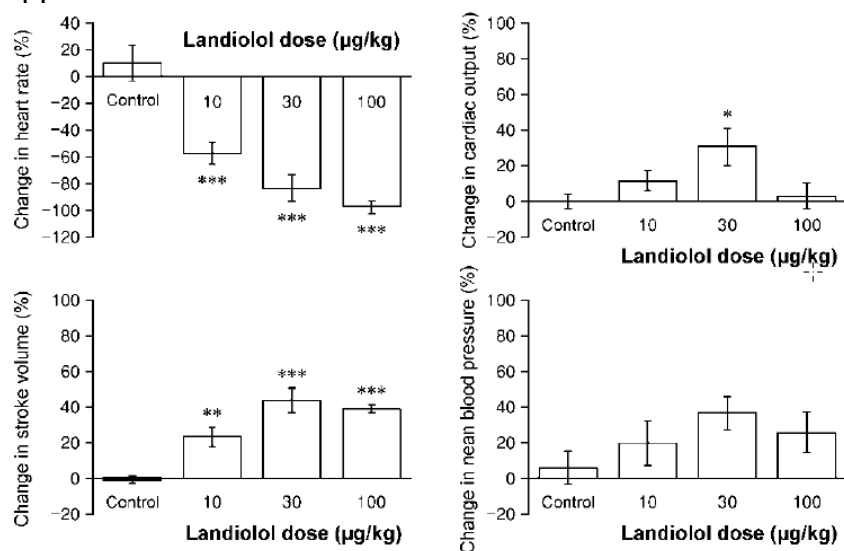
Treatment	Cases with improved heart rate	Cases with improved electrocardiogram	Deaths
Control	1/8	0/8	0/8
ONO-1101 (continuous i.v. infusion)			
0.3 µg/kg/min	2/5	0/5	0/5
3 µg/kg/min	4/5*	1/5	0/5
30 µg/kg/min	5/5**	1/5	0/5
300 µg/kg/min	8/8***	1/8	0/8
1,000 µg/kg/min	8/8***	4/8*	0/8
3,000 µg/kg/min	7/7**	6/7*	1/8
Esmolol (continuous i.v. infusion)			
3 µg/kg/min	1/5	0/5	0/5
30 µg/kg/min	4/5*	1/5	0/5
300 µg/kg/min	8/8***	1/8	0/8
1,000 µg/kg/min	6/8**	4/8*	2/8
Propranolol (i.v. bolus)			
3,000 µg/kg	7/7**	2/7	1/8
Verapamil (i.v. bolus)			
500 µg/kg	5/5**	0/5	3/8

Animals whose atrioventricular conduction was restored to 1:1 were counted as cases with an improved electrocardiogram, and animals with improved heart rate and improved electrocardiogram were counted as cases showing efficacy/number of surviving animals

Deaths are shown as number of deaths/number of experimental animals

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ (Fisher's exact test vs. control)

In male dogs, 10 µg/kg/min of epinephrine was infused to decrease cardiac output, stroke volume, and increase average blood pressure. LAN improved the cardiac output by decreasing heart rate and increased stroke volume by increasing ventricular filling time as shown in the figure reproduced from the study summary provided by the applicant.

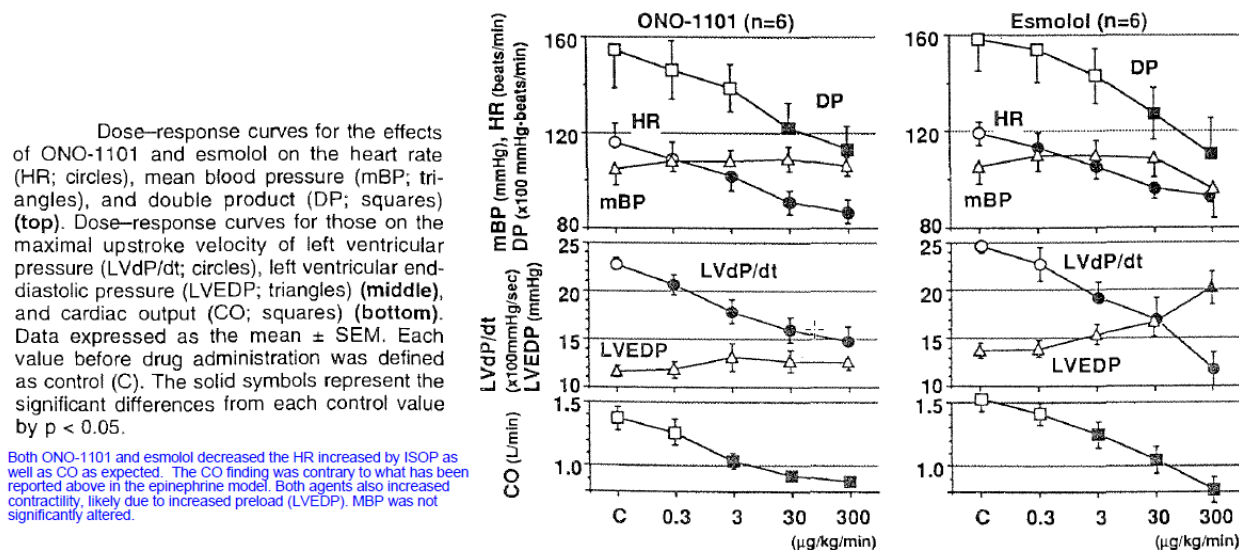


Effect of Landiolol on Epinephrine-Induced Decreased Cardiac Output

Data shown represent means $\pm$ SD (n = 10). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ (Dunnett's test versus control).

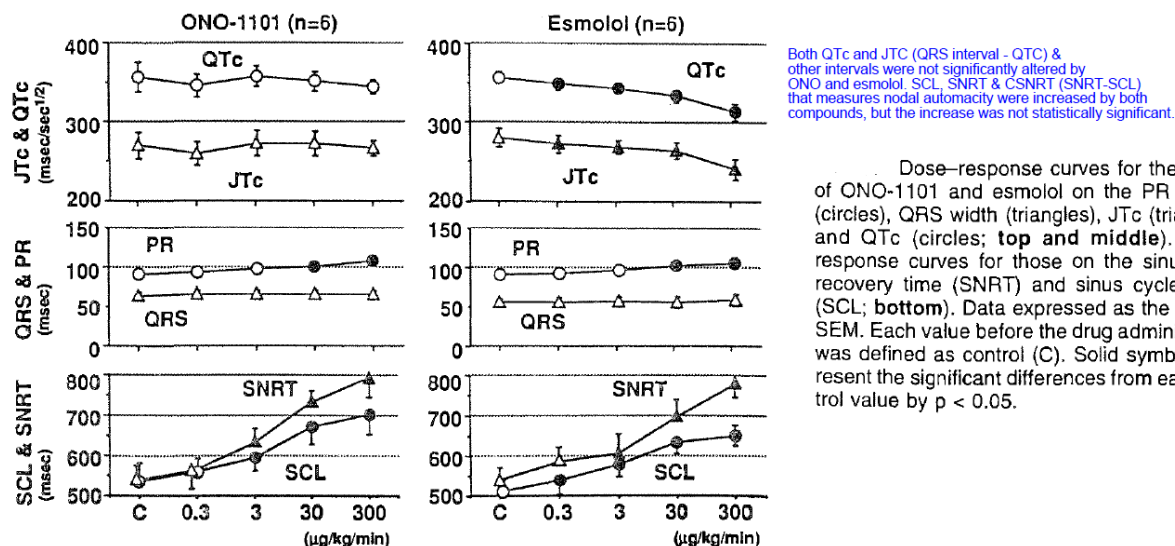
In another study cited by the applicant (Sugiyama, et al, 1999), electrophysiological, and cardiovascular hemodynamics parameters (LVdp/dt, left ventricular pressure, cardiac output) were continuously monitored in anesthetized dogs after infusion of ONO-1101 and esmolol for 30 min. The test-articles were administered with concomitant infusion of isoproterenol to produce ~50% increase in contractility (LVdp/dt). As shown in the two figures reproduced from Sugiyama, et al, 1999, both compounds decreased HR, LVdp/dt, LVEDP and cardiac output without significantly affecting mBP. The left ventricular end diastolic pressure that could be affected by the HR, preload, afterload, and contractility of the ventricles remained unchanged with LAN but appears to increase with esmolol. Cardiac output LVdp/dt that signifies changes in contractility were dose-dependently decreased by both compounds.

Figure 4: The Effects of Landiolol and Esmolol on Hemodynamic Parameters in Dogs (reproduced from Sugiyama, et al, 1999)



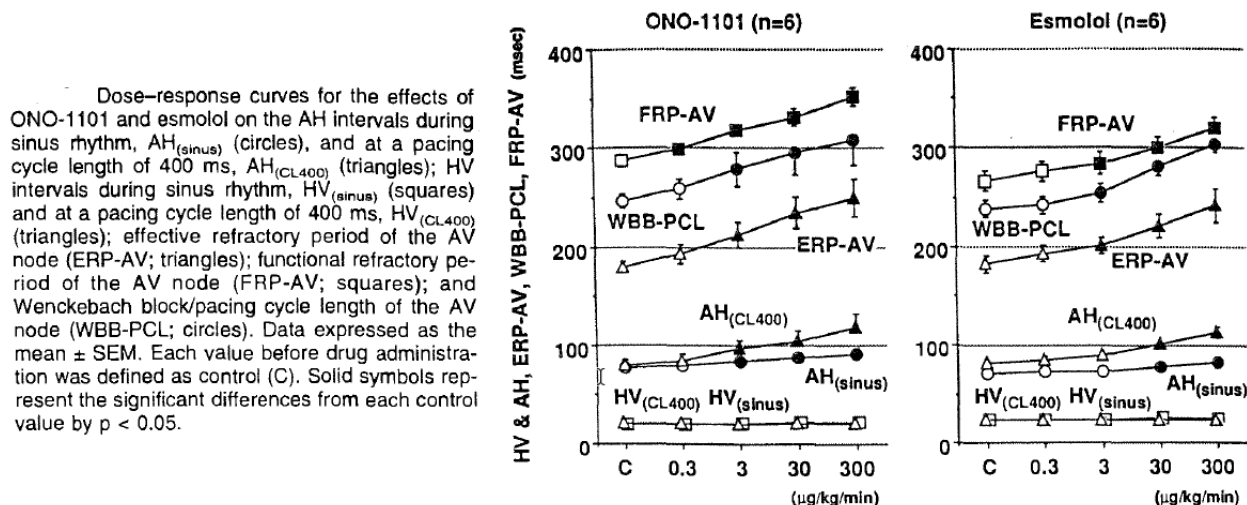
Cardiac electrical activity, including QTc were not significantly different from control mean. However, both sinus node recovery time (SNRT) and the sinus cycle duration were prolonged by LAN and esmolol in a dose-related manner. However, CSNRT, a surrogate for intrinsic automaticity was not affected significantly.

Figure 5: Effects of Landiolol and Esmolol on ECG Parameters and Nodal Function (reproduced from Sugiyama, et al 1999)



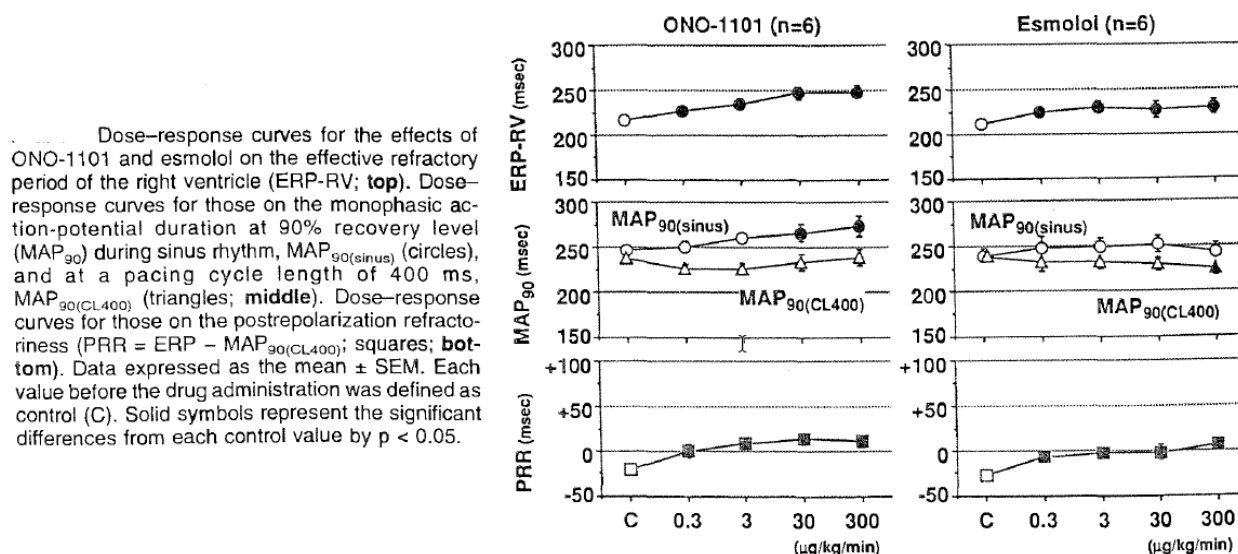
Both functional and effective recovery periods of the AV node were increased by both compounds. However, AH (conduction time from atria to bundle of His) were increased at sinus rhythm at ≥ 3 $\mu\text{g/kg/min}$ for LAN and ≥ 30 $\mu\text{g/kg/min}$ for esmolol. The HV (time from bundle of His to myocardium) was not affected as shown in Figure 4 below.

Figure 6: The Effects of Landiolol and Esmolol on the Conduction Velocity and Refractory Periods of the AV Node and Right Ventricle (reproduced from Sugiyama, et al 1999)

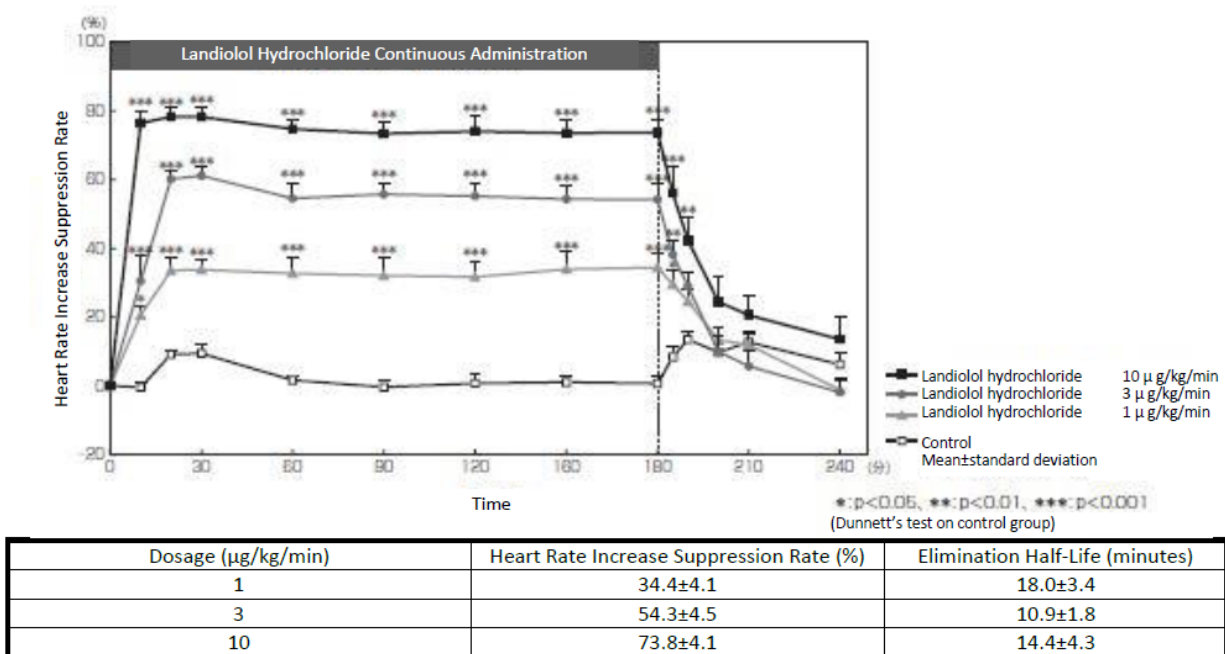


The $MAP_{90(sinus)}$ was increased at ≥ 30 $\mu\text{g/kg/min}$ in the right ventricle, but not $MAP_{90(CL400)}$ suggesting the effect of HR. Both compounds affected the refractory period of the right ventricle as shown in the figure 5 below. The increase in the refractory period with maintained MAP_{90} is suggestive of the antiarrhythmic potential of both compounds.

Figure 7: The Effects of Landiolol and Esmolol on Action Potential Duration and Effective Refractory Period of the Right Ventricle (reproduced from Sugiyama, et al, 1999)



The inhibitory effect of LAN on HR increases lasted 11 to 60 minutes after discontinuation of administration in a dog model. Result from one study is shown below.



Suppression rate is the value 180 minutes after the start of landiolol hydrochloride administration.

4.2 Secondary Pharmacology

The potential membrane stabilizing activity of LAN was evaluated in single ventricular myocytes from guinea pigs and atrial cells from rabbits by measuring I_{Ca2+} , I_K and

inward commutative potassium current (I_{K1}), Na^+ and A-type transient outward potassium current (I_t). No membrane stabilizing effects were noted at concentrations of 10, 30 and 100 μM of LAN.

Landiolol was profiled in membrane fractions from rat forebrain (alpha-1 adrenergic receptors), striatum (M-1 muscarinic and D_1 & D_2 dopamine receptors), anterior cerebral cortex (alpha-2 adrenergic receptors, 5-HT₁ & 5-HT₂ receptors), nicotine receptors, calcium channel (mixed T, L), and sodium channel (site 1), heart (M-2 muscarinic receptor, and submandibular gland (M3 muscarinic receptor). The K_i values are summarized in the table below (reproduced from ONO, et al., 2020).

K _i values for various receptors (in vitro)			
Receptor	K _i Value (μM)	Receptor	K _i Value (μM)
Adrenergic alpha-1 receptor	81.5±11.9	Muscarinic M3 receptor	>1000
Adrenergic alpha-2 receptor	180.1±37.2	Nicotine receptor	>1000
Serotonin 5-HT ₁ receptor	>1000	Dopamine D1 receptor	>1000
Serotonin 5-HT ₂ receptor	112.9±31.4	Dopamine D2 receptor	429.0±20.3
Muscarinic M1 receptor	42.7±12.9	Calcium channel (T, L mixed)	>1000
Muscarinic M2 receptor	40.7±6.1	Sodium channel (site 1)	>1000

Mean±standard deviation (n=3)

Isoproterenol-induced metabolic changes and renin release were assessed in anesthetized dogs with LAN, esmolol and propranolol. LAN and esmolol had no effect on this increase, whereas propranolol at high dose suppressed the isoproterenol-induced increase in glucose, lactic acid and serum insulin and renin activity. Free fatty acid concentration was decreased at $\geq 30 \mu g/kg/min$ for all the adrenergic blockers.

4.3 Safety Pharmacology

Nervous system

Published studies were summarized by the sponsor on the potential effects of LAN administration on the function of the nervous system (Akimoto, 1997). Irwin test was conducted at single doses ranging from 3 to 300 mg/kg. Transient decrease in spontaneous motor activity and bradypnea were reported at 100 mg/kg, and the 300 mg/kg dose was lethal. The NOEL was 30 mg/kg. Administration of up to 100 mg/kg single i.v. doses of LAN to mice didn't affect spontaneous motor activity. Nor did it have any effect on hexobarbital induced sleep time & writhing response to pain induced by acetic acid or tail pinching.

Targeting of the autonomic nervous system was assessed at concentrations of 10, 30, 100, 300 μM , 1 mM in isolated ileum from Guinea pigs or rabbits alone or on the contractive responses of histamine (0.1 to 0.3 μM), acetyl choline (0.03 to 0.3 μM) and $BaCl_2$ (1 mM). LAN was without effect alone, but 1 mM LAN inhibited histamine-acetylcholine and $BaCl_2$ -induced contractile responses on average by 99%, 61%, 52%, respectively.

Cardiovascular system

Based on the recommendation of the Division, the sponsor has investigated the potential effects of LAN and the M-1 metabolite on hERG and other cardiac ion channels. The results of these studies are summarized below:

Study/ Study No.	Findings
Effect of Landiolol Hydrochloride on hERG, hKvLQT1/hminK hCav1.2 and hNav1.5 Channels Expressed in Mammalian Cells/161108.CPQ	The cardiac hERG (I _{Kr}), and, hKvLQT1/hminK potassium channels and hNav1.5 sodium were expressed in HEK293 cells, and hCav1.2, L-type cardiac channel was expressed in CHO cells. Currents were measured by patch clamping. Terfenadine (hERG), chromanol 293B (hKvLQT1/hminK), Nifedipine (hCav1.2) and lidocaine (hNav1.5) were used as positive controls. At 300 μ M, LAN inhibited hERG by 21.4%, hKvLQT1/hminK by 7.7%, hCav1.2 by 18.3% and hNav1.5 3.9% relative to control mean. The IC ₅₀ was not calculated but was predicted at $\geq$ 300 μ M (21 μ M free drug). This is about 118-times higher than the C _{max} at the MRHD.
Effect of metabolite M-1 on hERG, hKvLQT1/hminK hCav1.2 and hNav1.5 Channels Expressed in Mammalian Cells/161108.CPQ /161109.CPQ	Currents were measured in channels expressed in HEK293 and CHO cells by patch clamping as described above. At 300 μ M, M-1 inhibited hERG by -0.5%, hKvLQT1/hminK by 1.9%, hCav1.2 by -1.3% and hNav1.5 -2.3% relative to control mean. The IC ₅₀ was not calculated but was predicted $\geq$ 300 μ M (21 μ M free drug). The C _{max} of M-1 is 3-times and its t _{1/2} is 18-times longer than that of LAN, but its β 1-adrenergic receptor binding potency is ~42-times lower in humans.
Effects of Test Articles on Ion Channels Expressed in Mammalian Cells/160927.CPQ	This study examined the effects of LAN, its two metabolites (M-1 and M-2), esmolol and its metabolite (M-1) on hERG expressed in HEK293, hKvLQT1/hminK co-expressed with I _{Ks} (slow delayed rectifier potassium current) and hNav1.5 sodium expressed in CHO cells, and hCav1.2, channel co-expressed with I _{CaL} (high threshold calcium current) in CHO cells. Currents were measured with automatic parallel patch clamp system. The IC ₅₀ values are given below.

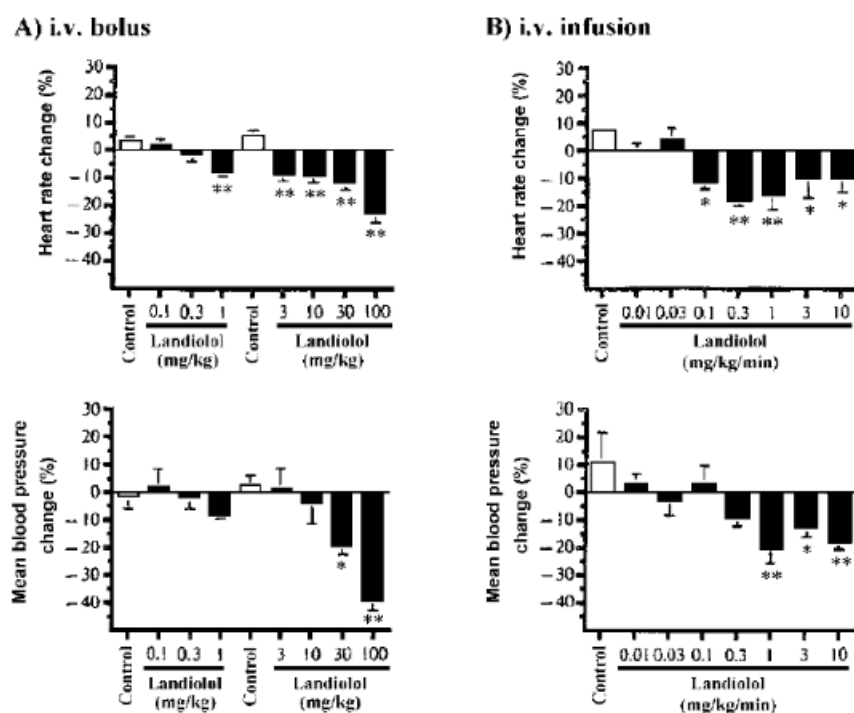
Test Article	IC ₅₀ (μ M)				
	hERG	Cav1.2	Nav1.5 (Tonic)	Nav1.5 (Phasic)	KvLQT1/minK
Esmolol Hydrochloride	84.688	637.395	264.124	154.632	422.425
Esmolol M1	>1000	>1000	>1000	>1000	>1000
Landiolol Hydrochloride	998.65*	3003*	4405.71*	>1000	>1000
Landiolol Metabolite 1	>1000	>1000	>1000	>1000	>1000
Landiolol Metabolite 2	>1000	>1000	>1000	>1000	>1000

The sponsor has used a statistical model to compare the torsadogenic risk for LAN and Esmolol (Kramer et al., 2013). LAN has a low torsadogenic risk.

ECG & Hemodynamic Parameters

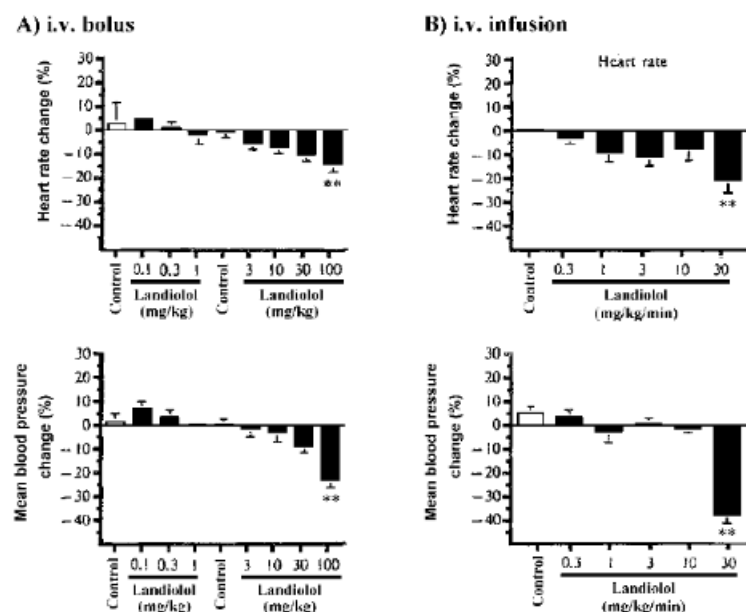
The potential effects of LAN on Mean blood pressure (MBP) and HR were evaluated in anesthetized and conscious rats after bolus and continuous infusion (Akimoto, 1997). Bolus i.v. doses of 0.1, 0.3, 1, 3, 10, 30, 100 and 300 mg/kg, and doses of 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 mg/kg/min were continuously infused for 60 min. The results shown in **Figure 7** and **Figure 8** below indicate that significant decrease in HR and MBP occurred at lower doses when infused than given as a bolus dose in both anesthetized and conscious rats, and the effects are prominent in anesthetized rats. The 300 mg/kg dose markedly decreased HR and MBP and the animals died within 5 minutes. The i.v. infusion NOEL for MBP was 30 mg/kg/min (HED of 4.7 mg/kg/min), while the NOEL for the bolus dose was 100 mg/kg (HED 16.21 mg/kg).

Figure 8: Effects of Landiolol on Heart Rate and Mean Blood Pressure in Anesthetized Rats (*reproduced from Akimoto 1997*)



Data shown represent means $\pm$ SE (n = 3-6). A) i.v. bolus; B) Continuous i.v. infusion. *: $p < 0.05$; **: $p < 0.01$ (Dunnett's test versus control).

Figure 9: Effects of Landiolol on Heart Rate and Mean Blood Pressure in Conscious Rats (*reproduced from Akimoto 1997*)



Anesthetized dogs were treated with i.v. bolus doses of 0.001 to 30 mg/kg and ECG parameters, blood pressure, blood flow and respiration were assessed (Akimoto, 1997). ECG parameters were measured prior to administration and 1, 5, 10, 20, 30 and 60 minutes after administration of LAN. Blood pressure was decreased at ≥ 1 mg/kg with mean reduction after 1 minute and recovery within 30 min at the high dose. HR was decreased moderately starting 0.01 mg/kg and recovery took a longer time compared to blood pressure. Blood flow volume in femoral artery was decreased significantly at 10 mg/kg without dose-dependence. Significant prolongation of the PR interval was reported at ≥ 0.1 mg/kg with no significant changes in QTc. Transient effects were noted on respiratory rate at the high dose.

Another study was conducted in conscious dogs (Yamaguchi, 1997b), after daily bolus i.v. infusion of 12.5, 25 and 50 mg/kg for 4-weeks with a 4-week recovery period. No effect was observed on HR, ECG parameters and respiratory rate.

Isometric contractions of isolated atria and HR were measured from naïve Guinea pigs, and pigs pretreated with 5 mg/kg reserpine intraperitoneally to assess the effect of LAN (0.1 μ M to 1 mM) alone, and after pretreatment with 0.1 μ M epinephrine or 0.1 μ M or 0.3 μ M acetylcholine. When used alone, clear negative inotropic and chronotropic effects of LAN were shown at concentrations of ≥ 0.3 mM (>118 -fold the C_{max} at MRHD) or higher irrespective of reserpine treatment of animals. In specimens without pretreatment, mean contractile force and heart rate at a LAN concentration of 1 mM were 63% and 14%, respectively compared to the values prior to administration, which were 71% and 11%, respectively. Upon propranolol treatment, both groups of specimens (with and without reserpine pretreatment) showed negative inotropic and chronotropic effects.

Effects on Guinea Pig Respiratory System

The effect of 40 µg/kg/min LAN and 150 µg/kg/min esmolol were evaluated on ova-sensitized, anesthetized Guinea pigs after i.v. injections of acetylcholine to measure the effects on total pulmonary resistance and dynamic lung compliance. Neither of the β₁-adrenergic blockers had effect on acetylcholine-induced increase in total pulmonary resistance and decreased lung compliance.

Effects on Gastrointestinal and Urinary System of Mice and Rats

Overnight fasted mice receiving i.v. bolus dose of LAN (3-100 mg/kg) were orally administered activated charcoal. Atropine was given intravenously as a positive control. LAN did not affect GI motility based on charcoal movement relative to the control group. In rats with ligated pyloric region of the stomach, neither LAN administered by i.v. bolus injection (30 mg/kg) nor by continuous infusion (1-10 mg/kg/min) affected gastric secretion volume, acidity, and pH.

The effects on urine volume, Na⁺, K⁺, Cl⁻ and pH were evaluated after i.v. bolus injection of LAN at 30 mg/kg, or after continuous infusion of 1, 3, 10 mg/kg/min for 5 hrs. Bolus injection had no effect, but LAN caused a significant increase in urine volume and the concentration of all excreted electrolytes (Na⁺, K⁺ and Cl⁻) when infused. Urine pH levels decreased significantly at all doses and there was positive occult blood reaction that was caused by hemolysis at 10 mg/kg/min. The potential hemolytic effects of LAN were further investigated (section 10.3) that showed that 10 mM concentration is hemolytic in vitro.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The applicant summarized published non-clinical data of the landiolol-containing product ONO-1101.

Absorption

As summarized in the sponsor provided table below, exposure in terms of C_{max} and AUC_{inf} was dose-proportional in rats after single intravenous bolus administration and continuous infusion. Dose-proportionality appears to also occur in dogs after bolus dose. The test article showed biphasic elimination with similar t_{1/2} values in rat and dogs after bolus injection. Steady state concentration was achieved in rats after 2 hrs and in dogs after 30 minutes of infusion. In rats, the T_{max} increased with dose after 24-hr infusion. Exposure (AUC_{inf}) and t_{1/2} didn't change after repeat dosing in rats.

In a TK measurement of a 4-week dog study, LAN was administered intravenously at 0.04 (57.6 mg/day), 0.2 (288 mg/day), and 1 mg/kg/min (1,440 mg/day) by 24hr continuous i.v. administration (PMDA 2006). The steady state plasma concentrations of

LAN, M-1 and M-2 were similar in both sexes and exhibited approximate dose proportionality. The level of M-1 was 5 to 12-fold higher than the parent compound, with highest ratio at the high dose. The level of M-2 was 2 to 8-fold higher than parent with the highest ratio at the low dose.

Table 8: PK Parameters after Single and Repeat dosing in Rats and Dogs
(reproduced from the sponsor summary)

Type of Study	Test System	Method of Administration	Dose	Key Findings	Study No./ Reference	eCTD
<i>Absorption</i>						
Single dose (Single dose toxicity)*	Rat/Sprague-Dawley (SD) strain	infusion (24-hours); 173.6, 347.2, 694.4 µg/kg/min	250, 500, 1000 mg/kg/day	- dose-proportional increase in C_{max} and $AUC_{0-\infty}$ - T_{max} : 0.53, 1, 4 h, respectively - C_{max} : 47.4, 141, and 252 ng/mL, respectively - $AUC_{0-\infty}$: 305, 1,779, 4,795 ng*h/mL, respectively	Study No. 610774	4.2.2.2
Single dose	Rat/SD strain	i.v. (bolus); 0.3, 1, 3, 10 mg/kg	0.3, 1, 3 or 10 mg/kg/day	- dose proportional increase in C_{max} and $AUC_{0-\infty}$ - $T_{1/2}$: ~5 min; $T_{1/2\text{ terminal}}$ ~30 min - no sex differences	Tsunekawa (1997a)	4.3
	Rat/SD strain	infusion (3-hours); 18 mg/kg/h	54 mg/kg/day	- C_{ss} (approx. 2 hours): 27.0 ± 4.3 µg eq/mL - $AUC_{0-\infty}$: 98.1 ± 13.4 µg eq*h/mL - $T_{1/2}$ (180-300 min): 34 ± 3 min		4.3
Single dose	Dog/Beagle	i.v. (bolus); 0.3 and 3 mg/kg	0.3, 3 mg/kg/day	- $AUC_{0-\infty}$: 4.4 ± 0.4 , 38.1 ± 9.3 µg*min/mL - $T_{1/2}$ (1 - 5 min): 1.6 ± 0.1 min - $T_{1/2}$ (10 - 30 min): 6.0 ± 0.6 min	Tsunekawa (1997d)	4.3
	Dog/Beagle	infusion (30-min); 0.3 mg/kg/min	9 mg/kg/day	- C_{ss} (approx. 30 min): 4.8 ± 1.1 µg/mL - $AUC_{0-\infty}$: 147.6 ± 26.8 µg*min/mL - $T_{1/2}$ (31 - 60 min): 4.9 ± 1.9 min		4.3
Repeated dose (7 days)	Rat/SD strain	i.v. (bolus); 7 consecutive days	1 mg/kg/day	<u>Single Dose:</u> - $AUC_{0-\infty}$: 1.97 ± 0.2 (M), 2.1 ± 0.27 (F) µg*h/mL - $T_{1/2}$ (1 - 5 min): 6.0 ± 1.5 (M), 5.0 ± 0.7 (F) min - $T_{1/2}$ (15 - 240 min): 32 ± 2 (M), 33 ± 4 (F) min <u>Repeated Dose:</u> - $AUC_{0-\infty}$: 1.6 ± 0.18 (M), 1.75 ± 0.33 (F) µg*h/mL - $T_{1/2}$ (1 - 5 min): 4.4 ± 0.8 (M), 5.2 ± 0.8 (F) min - $T_{1/2}$ (15 - 240 min): 30 ± 2 (M), 28 ± 3 (F) min	Tsunekawa (1997b)	4.3

PK parameters were determined after single intravenous bolus or continuous infusion of [^{14}C]-ONO-1101 to male and female rats (Tsunekawa, 1997a). Bolus doses of 0.3, 1, 3, and 10 mg/kg were administered to both male and females, and 18 mg/kg/hr was administered for 3 hr (144 mg/kg/day) to male rats. LAN concentration was measured in lysed whole blood. The results are summarized in the table below reproduced from the publication. Exposure was dose-proportional and plasma radioactivity showed a bi-phasic elimination pattern, with an initial half-life of 4.5 - 6.0 minutes and a terminal half-life of 27 - 33 minutes in rats after i.v. bolus dosing. Clearance was similar in both sexes of rats and was similar at all doses. There was no difference in clearance when LAN was administered either as a bolus or infusion.

PK Parameters of Landiolol After i.v. Administration of ^{14}C -Landiolol to Male and Female Rats

Dose [mg/kg]	Sex	AUC _{0-∞} [μg eq·h/mL]	CL _{tot} [mL/h/kg]	t _{1/2} (1-5 min) [min]	t _{1/2} (15-240 min) [min]
0.3	f	0.72 ± 0.04	419 ± 25	4.9 ± 0.6	27 ± 2
	m	0.68 ± 0.07	444 ± 53	4.9 ± 0.6	30 ± 2
1	f	1.97 ± 0.20	512 ± 57	6.0 ± 1.5	32 ± 2
	m	2.11 ± 0.27	478 ± 55	5.0 ± 0.7	33 ± 4
3	f	6.16 ± 0.82	493 ± 58	4.8 ± 0.6	31 ± 1
	m	5.73 ± 1.12	542 ± 126	4.5 ± 0.7	30 ± 4
10	f	19.8 ± 1.87	508 ± 48	4.7 ± 0.7	31 ± 2
	m	18.8 ± 1.32	535 ± 40	4.8 ± 0.8	31 ± 3

Results are expressed as the mean ± SD of 4 animals. AUC - Area under the Curve, CL - Clearance, eq - Radioactive Equivalent, f - Females, m - Males, T_{1/2} - Half-Life.

PK Parameters After i.v. Infusion of ^{14}C -Landiolol (18 mg/kg/h for 3 h) to Male Rats

Dose [mg/kg/h]	AUC _{0-∞} [μg eq·h/mL]	CL _{tot} [mL/h/kg]	T _{1/2} (180-300 min) [min]	C _{ss} (2 h) [μg eq/mL]
18	98.1 ± 13.4	558 ± 71	34 ± 3	27.0 ± 4.3

Results are expressed as the mean ± SD of 4 animals. AUC - Area under the Curve, CL - Clearance, C_{ss} - Plasma Concentration at Steady State, eq - Radioactive Equivalent, T_{1/2} - Half-Life.

After continuous infusion of LAN for 24 hrs in female rats, exposure was more than dose-proportional between the mid and low dose (see **6.1 Single-Dose Toxicity**). The M-1 metabolite exposure on average was ~270x the parent compound, while the average exposure of the M-2 metabolite was ~2x. Both metabolites appear to have t_{1/2} longer than that of the parent drug with a mean t_{1/2} of ~30 min after continuous infusion. The AUC_{inf} of M-1 metabolite was ~6-fold higher than that of LAN in healthy Japanese volunteers after 1 min bolus and after an hr of infusion. The M-1 metabolite also showed very long t_{1/2} (>21-fold) relative to that of LAN that was between 3-5 minutes (Nakashima and Kanamaru, 2000).

Table 9: PK Parameters After Continuous i.v. Infusion of Landiolol for 24 Hrs to Female rats (reproduced from the application)

Dose [mg/kg/day]	C _{max} [ng/mL]	C _{max} /Dose [ng/mL]/ [mg/kg/day]	T _{max} [h]	AUC _(0-t) [ng*h/mL]	AUC _(0-t) /Dose [ng*h/mL]/ [mg/kg/day]	AUC _(0-∞) [ng*h/mL]	AUC _(0-∞) /Dose [ng*h/mL]/ [mg/kg/day]	T _{1/2} [h]
Landiolol								
250	47.4	0.127	0.53	304.9	n.c.	n.c.	n.c.	n.c.
500	141	0.282	1.0	1,779	n.c.	n.c.	n.c.	n.c.
1,000	252	0.252	4.0	4,795	n.c.	n.c.	n.c.	n.c.
Metabolite M-1								
250	8,180	32.7	4.0	137,100 ^{450x}	548.4	137,400	549.5	0.68
500	17,600	35.1	24.0	375,500 ^{211x}	751.0	376,800	753.6	0.71
1,000	38,600	38.6	24.1	772,500 ^{161x}	772.5	774,000	774.0	0.51
Metabolite M-2								
250	80.4	0.321	24.5	718.1 ^{2.4x}	2.873	n.c.	n.c.	n.c.
500	215	0.429	12.0	3,254 ^{1.8x}	6.507	3,336	6.672	1.36
1,000	285	0.285	12.0	4,960 ^{1x}	4.960	5,075	5.075	1.07

AUC - Area under the Curve, C_{max} - Maximum Plasma Concentration, n.c. - not calculated, T_{1/2} - Half-Life, T_{max} - Time to Maximum Plasma Concentration.

The plasma concentration-time profile of [¹⁴C]ONO-1101 was assessed in male dogs after bolus dose (0.3 and 3.0 mg/kg) and continuous i.v. infusion (0.3 mg/kg/min for 30 min). The effects of anesthesia on the PK of ONO-1101 and metabolites were also evaluated after continuous i.v. infusion (0.3 mg/kg/min for 30 min) of [¹⁴C]ONO-1101 (Tsunekawa, 1997d). The results shown below indicate the absence of effects due to anesthesia. There was no effect on clearance after bolus and i.v. infusion, while t_{1/2} and AUC values were higher for infusion as may be expected based on the half-life.

Table 10: PK parameters of ONO-1101 after Intravenous Bolus and Continuous Infusion of ONO-1101 in Dogs (reproduced from Tsunekawa, 1997d)

i.v. bolus dose		Dose (mg/kg)	
parameters		0.3	3.0
$T_{1/2}$ (1~5 min)	(min)	1.6 ± 0.1	1.6 ± 0.1
$T_{1/2}$ (10~30 min)	(min)		6.0 ± 0.6
$AUC_{0-\infty}$	($\mu\text{g} \cdot \text{min}/\text{ml}$)	4.4 ± 0.4	38.1 ± 9.3
CL_{tot}	($\text{ml}/\text{min}/\text{kg}$)	69.1 ± 6.9	82.5 ± 23.1
MRT	(min)		4.4 ± 0.7
V_{ss}	(ml/kg)		370.2 ± 163.1

i.v. infusion	Parameters	Conscious	Anesthetized
$T_{1/2}$ (31~60 min)	(min)	4.9 ± 1.9	4.6 ± 1.1
$AUC_{0-\infty}$	($\mu\text{g} \cdot \text{min}/\text{ml}$)	147.6 ± 26.8	211.5 ± 79.3
C_{ss} (30 min)	($\mu\text{g}/\text{ml}$)	4.8 ± 1.1	7.4 ± 2.6
CL_{tot}	($\text{ml}/\text{min}/\text{kg}$)	62.2 ± 10.8	47.4 ± 19.9
MRT	(min)	20.1 ± 1.1	19.6 ± 0.6
V_{ss}	(ml/kg)	324.5 ± 124.9	212.6 ± 61.8

i.v. infusion		M-1		M-2	
		Conscious	Anesthetized	Conscious	Anesthetized
$T_{1/2}$	(min)	174.1 ± 4.9	163.6 ± 7.2	144.2 ± 36.4	94.2 ± 16.3
$AUC_{0-\infty}$	($\mu\text{g} \cdot \text{min}/\text{ml}$)	774.9 ± 26.6	876.4 ± 194.5	676.4 ± 108.4	745.2 ± 231.6
T_{max}	(min)	32.7 ± 2.1	31.3 ± 0.6	110.0 ± 34.6	110.0 ± 34.6
C_{max}	($\mu\text{g}/\text{ml}$)	4.5 ± 0.4	6.1 ± 1.4	2.4 ± 0.4	3.3 ± 0.7

Results are expressed as the mean $\pm$ SD of three animals

Distribution

As summarized by the sponsor below, LAN has low binding to plasma proteins in all species. After 1 mg/kg single i.v. bolus dose of ^{14}C -ONO-1101 to rats, high concentrations of the test article related radioactivity was found in the kidney, liver, lung, trachea, skin, and carcass within 15 min and declined to about or below detection limit in all tissues at 24 hr. Radioactivity levels in the brain and fetus were much lower than in the plasma; drug-related radioactivity was not observed in the brain after repeat dosing.

When the same bolus dose was administered to lactating rats, the radioactivity in milk reached the highest levels at 1 hr and then began to decline, reaching a negligible level after 24 hrs. Within 72 hr after intravenous administration of 1 mg/kg ^{14}C -ONO-1101, 85.6% and 9.9% of administered radioactivity were excreted into urine and feces in male animals and 84.2% and 12.0% in female rats, respectively. Within 24 hr of intravenous administration of ^{14}C -ONO-1101 at the dose of 1 mg/kg, 6.1% of administered radioactivity were excreted into bile in male and 9.5% in female rats.

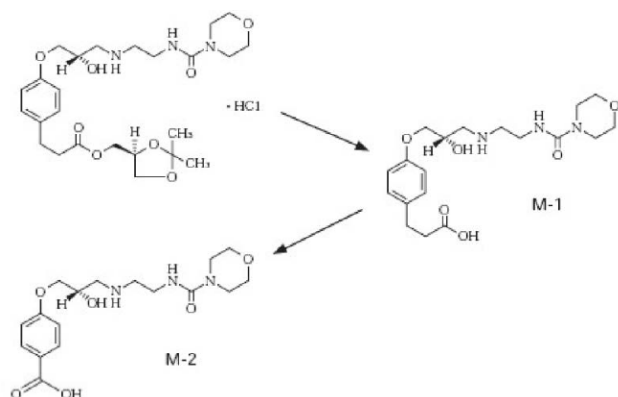
Table 11: Tissue distribution of Radioactive Landiolol

Type of Study	Test System	Method of Administration	Dose	Key Findings	Study No./ Reference	eCTD
<i>Distribution</i>						
Plasma Protein Binding	Serum rat, dog, human	<i>in vitro</i>	0.1, 1, 10 mg/kg/day or 50 µg/mL	- low serum protein binding in all species - rat: range 2.7 - 5.3% - dog: range 14.7 - 21.4% - human: range 1.5 - 7.0%	Tsunekawa (1997c)	4.3
Single Dose Distribution	Rat/SD strain	i.v. (bolus); single dose	1 mg/kg/day	- 5 and 15 minutes after administration: high concentration in kidneys and liver, followed by lungs, bronchi, bladder; further localized in skin and carcasses - 1 and 4 hours: concentrations in kidneys, liver, and lungs decreased, concentration in gut contents increased - 24 hours: tissue concentrations very low/not detectable, little as a 1% recorded for intestines - low CNS distribution at all times	Tsunekawa (1997a)	4.3
Repeat Dose Distribution (7 days)	Rat/SD strain	i.v. (bolus); 7 consecutive days	1 mg/kg/day	- high drug concentrations in liver, kidneys, skin, bladder, and lungs 15 minutes after the 7 th administration - 1 hour post 7 th administration: high levels still recorded in liver, bladder, kidneys, and small intestine - 4 hours post 7 th application: high levels only in intestinal contents - 72 hours post 7 th application: almost not detectable - not detected in any sections of the CNS at any timepoint	Tsunekawa (1997b)	4.3

Metabolism

The stability of LAN was assessed in plasma obtained from rats, dogs and humans, and liver homogenates from rats and dogs. The $t_{1/2}$ of LAN in plasma was 2.0, 70.4 and 4.1 minutes, respectively for rats, dogs, and humans. The half-life in liver homogenate was 3.3 min in rats and 16.8 min in dogs. The hydrolysis of LAN is expected to generate metabolite M-1, which has a longer half-life than the parent compound in humans. Hydrolysis in both samples was inhibited by esterase inhibitors. The estimated mean $t_{1/2}$ of M-1 was 96 min and M-2 was 216 min for a 60 min infusion of 10 µg/kg/min LAN (Study CPA368-10).

Landiolol is metabolized in all species by hydrolysis of the ester moiety that produces M-1, which undergoes β -oxidation to the M-2 metabolite. LAN is metabolized by carboxylesterase in rat plasma and liver as well as in dog liver, and pseudocholinesterase in human plasma, and carboxylesterase in human liver.

Figure 10: The Metabolic Fate of Landiolol

Excretion

The excretion of ^{14}C -landiolol in urine, feces, bile, and milk was assessed after a single intravenous bolus injection to rats. Most of the radioactivity was in the urine, fecal excretion accounting for <11%. In lactating rats, ~70% of the radioactivity in plasma was measured in milk after 1 hr, but negligible level was measured after 24-hrs of dosing.

Table 12: Excretion in Urine and Feces After i.v. Administration of ^{14}C -Landiolol to Rats (reproduced from the submission)

Animal		Excretion [% of dose]		
		0-24 h	0-48 h	0-72 h
Male	Urine	85.3 ± 3.7	85.4 ± 3.6	85.6 ± 3.4
	Feces	8.6 ± 2.5	9.8 ± 2.4	9.9 ± 2.4
	Total	93.8 ± 4.3	95.2 ± 3.7	95.5 ± 3.5
Female	Urine	83.5 ± 3.8	84.0 ± 4.0	84.2 ± 4.1
	Feces	11.1 ± 0.7	11.8 ± 0.7	12.0 ± 0.6
	Total	94.6 ± 2.9	95.8 ± 3.5	96.2 ± 3.7

Each value represents the mean ± SD of 4 animals.

After daily administration of 1 mg/kg of ^{14}C -landiolol to male and female rats for seven consecutive days by i.v. bolus injection, excretion rates into urine and feces plateaued after the fourth consecutive day of administration in both sexes, and the cumulative excretion rates in urine and feces were 89.7% and 14.9%, respectively, in male rats and 86.5% and 17.0%, respectively, in female rats.

There was no significant difference in LAN clearance and the volume of distribution between conscious and anesthetized dogs. LAN clears at about dog hepatic blood flow but has low distribution after continuous infusion.

Pharmacokinetic drug interactions

The sponsor has conducted additional studies using human liver microsomal preparations and CYP-specific reference compounds to determine the potential for LAN and its metabolite M-1 to inhibit CYP enzymes *in vitro*. The studies corroborated findings from the published studies as shown in **Table 13** below.

Study# US034-0012488: ADME-TOX - Study of Landiolol HCl (Compound B) and Landiolol Metabolite M-1 (Compound A).

Study # US034-0014432: ADME-TOX - Study of Landiolol HCl (Compound B) and Landiolol Metabolite M-1 (Compound A).

Study # US034-0013986: CYP2C19 inhibition (HLM, omeprazole substrate) - Study of Landiolol HCl (Compound B) and Landiolol Metabolite M-1 (Compound A).

CYP 1A, 2C8, 2C9, 2C19, 2D6, and 3A4 activities were tested *in vitro* with pooled human liver microsomes at 0.1 mM LAN and 1.3 mM M-1 concentrations. Inhibition of >50% was noted for CYP2C19 with omeprazole as substrate for LAN and M-1 and CYP2D6 with dextromethorphan for LAN. CYP2D6 with dextromethorphan inhibition for both LAN and M-1 were >50%, but not that of CYP2C19 in a repeated experiment. Another study was conducted for CYP2C19 at concentrations ranging from 1 µM to 0.1 mM LAN and 0.03 mM to 1.3 mM M-1 that showed >50% inhibition at 0.1 mM LAN and 1.3 mM M-1. The C_{max} at the MRHD is 2.54 µM suggesting these inhibitions may not occur in the clinic.

Table 13: Potential Cytochrome P450 Enzyme Inhibition by Landiolol and M-1 Metabolite (reporduced from the submission)

Pharmacokinetic Drug Interaction						
CYP inhibition	Human, liver microsomes	<i>in vitro</i>	Landiolol 100 µM; M-1: 1300 µM	- Landiolol and M-1, at a screening concentration 50-fold the clinical plasma concentration, no relevant inhibition of CYP1A, CYP2C8, CYP2C9, CYP3A, CYP2D6. - potential for CYP2C19 inhibition, IC ₅₀ determination	Study No. 100058422	4.2.2.6
	Human, liver microsomes	<i>in vitro</i>	Landiolol: 1 – 100 µM M-1: 30 – 1500 µM	- CYP2C19 IC ₅₀ : landiolol 69 µM, M-1 1300 µM	Study No. 100059009	4.2.2.6
	Human, liver microsomes	<i>in vitro</i>	Landiolol 100 µM; M-1: 1300 µM	Landiolol and M-1 tested at concentrations 50-fold the clinical plasma concentration: - no TDI inhibition of CYP3A, CYP2C9, CYP2C19 - TDI of CYP1A: 21.0% with landiolol and 14.5% with M-1, TDI of CYP2D6: 54.9% with landiolol and 51.5% with M-1	Study No. 100059780	4.2.2.6
	Human, liver microsomes	<i>in vitro</i>	landiolol: 3×10^{-3} M (16.4 µg/mL); M-1 and M-2 1×10^{-4} M	- Neither landiolol, M-1, and M-2 showed relevant inhibitory effects on CYP450 enzymes (CYP 1A2, 2C9, 2C19, 2D6, and 3A4)	ONO (2020)	4.3

The applicant has not conducted the potential effects of LAN and its metabolites on drug transporters citing the Agency's guidance about the need for ≥25% hepatic uptake or renal clearance for evaluation and based on the clinical experience with Onact and subsequently with Rapibloc® that has been approved many European countries.

6 General Toxicology

6.1 Single-Dose Toxicity

Study Number/ Title: 610774/ Landiolol Hydrochloride: Single Dose Intravenous (24 h) Infusion Toxicity Study with a 2 Day Observation Period in the Rat

Key Study Findings

- The mean weight of all males at 250 and 500 mg/kg was lower than controls (~20%) and 2 animals at the 1,000 mg/kg lost weight by the end of treatment period. The BW loss was not correlated with food intake for the low and high dose animals.
- Glucose was increased in animals receiving LAN and lactate dehydrogenase activity was decreased. The toxicological significance of these findings is unclear. The NOAEL of 1,000 mg/kg/day proposed by the sponsor is upheld in this review.

Sprague-Dawley rats were administered single dose of LAN at 250, 500 and 1000 mg/kg/day continuously over 24 hr by intravenous infusion. The doses were justified based on the NOAEL of 50 mg/kg/day in a 4-week daily intravenous bolus injection study (Yamaguchi et al., 1997c) and the clinical use at concentrations of 5 µg/kg/min over 2 days and 40 µg/kg/min for 10 hrs that showed no toxicity or local intolerance in humans (Ito et al., 2006, Yoshida et al., 2008).

Table 14: Study Information 24-Hr Bolus Infusion Toxicity Study in Rats

Study Features and Methods	Details
GLP Compliance:	Yes
Dose and frequency of dosing:	250, 500, 1,000 mg/kg
Route of administration:	24-hr intravenous infusion
Formulation/vehicle:	water
Species/strain:	Rats/Sprague Dawley
Number/sex/group:	5
Age:	8-9 weeks (309-387 g (♂), 203-270 g (♀))
Satellite groups/unique design:	9 females/group (samples collected 5 min, 15min, 30 min, 1, 4, 12, 24 hr after dosing and 5 min, 15 min, 30 min and 2 hr after ending the infusion),

Table 15: Observations and Results of 24-Hr Bolus Infusion Toxicity Study in Rats

Parameters	Major findings
Mortality	None
Clinical signs	Not remarkable
Body weights	All males at 250 and 500 mg/kg (~20%) and 2 animals 1,000 mg/kg lost weight by the end of treatment period. This effect was less pronounced at the high dose based on the change in weight on Day 3 compared to Day 1.
Food consumption	Males dosed with LAN consumed less food compared to control males and predose values; ~20% at 500 mg/kg.
Hematology	Total and differential leukocyte counts were increased at 500 mg/kg in both sexes.
Clinical chemistry	Glucose concentration was increased in males at ≥500 mg/kg and females at the high dose. LDH was decreased in a dose-unrelated manner.
Urinalysis	No finding
Gross pathology	No test-article related finding
Organ weights	Not evaluated
Histopathology	Not evaluated

Exposure to LAN was colinear with dose. The AUC_{0-t} at the high dose for M-1 was 161-times higher, while M-2 metabolite was like that of the parent compound. The T_{max} and t_{1/2} values for the metabolites were higher.

Table 16: TK Parameters in Rats Receiving Landiolol Infusion for 24-Hrs**Landiolol**

Analyte	Group	C _{max} (ug/mL)	C _{max} /D (ug h/mL)/ (mg/kg/day)	T _{max} (h)	AUC(0-t) (ug h/mL)	AUC(0-t)/D (ug h/mL)/ (mg/kg/day)	T _{1/2} (h)	AUC(0-inf) (ug h/mL)	AUC(0-inf)/D (ug h/mL)/ (mg/kg/day)	CL (mL/h/kg)	V _d (mL/kg)	Rsq	Flags*
Landiolol	2F	0.0316	0.000127	0.53	0.2046	0.0008185	NC	NC	NC	NC	NC	NC	h
	(Excluding Animal 33F)	0.0474	0.0001898	0.53	0.3049	0.001220	NC	NC	NC	NC	NC	NC	h
	3F	0.141	0.000282	1.00	1.779	0.003557	NC	NC	NC	NC	NC	NC	h
	4F	0.252	0.000252	4.00	4.795	0.004795	NC	NC	NC	NC	NC	NC	h

*Flags applicable to slope-dependent (T_{1/2}, AUC(0-inf), AUC(0-inf)/D, CL and V_d) parameters only.**Landiolol M1**

Analyte	Group	C _{max} (ug/mL)	C _{max} /D (ug h/mL)/ (mg/kg/day)	T _{max} (h)	AUC(0-t) (ug h/mL)	AUC(0-t)/D (ug h/mL)/ (mg/kg/day)	T _{1/2} (h)	AUC(0-inf) (ug h/mL)	AUC(0-inf)/D (ug h/mL)/ (mg/kg/day)	Rsq	Flags*
Landiolol M1	2F	8.18	0.0327	4.00	107.0	0.4281	0.67	107.3	0.4292	0.55	g
	2F (Excluding Animal 33F)	8.18	0.0327	4.00	137.1	0.5484	0.68	137.4	0.5495	0.71	g
	3F	17.6	0.0351	24.08	375.5	0.7510	0.71	376.8	0.7536	0.98	a
	4F	38.6	0.0386	24.00	772.5	0.7725	0.51	774.0	0.7740	1.00	a

*Flags applicable to slope-dependent (T_{1/2}, AUC(0-inf), AUC(0-inf)/D) parameters only.**Landiolol M2**

Analyte	Group	C _{max} (ug/mL)	C _{max} /D (ug h/mL)/ (mg/kg/day)	T _{max} (h)	AUC(0-t) (ug h/mL)	AUC(0-t)/D (ug h/mL)/ (mg/kg/day)	T _{1/2} (h)	AUC(0-inf) (ug h/mL)	AUC(0-inf)/D (ug h/mL)/ (mg/kg/day)	Rsq	Flags*
Landiolol M2	2F	0.0804	0.000321	24.50	0.5256	0.002102	NC	NC	NC	NC	h
	2F (Excluding Animal 33F)	0.0804	0.000321	24.50	0.7181	0.002873	NC	NC	NC	NC	h
	3F	0.215	0.000429	12.00	3.254	0.006507	1.36	3.336	0.006672	0.82	b
	4F	0.285	0.000285	12.00	4.960	0.004960	1.07	5.075	0.005075	0.98	ab

*Flags applicable to slope-dependent (T_{1/2}, AUC(0-inf), AUC(0-inf)/D) parameters only.**6.2 Repeat-Dose Toxicity**

The toxicology studies conducted for a LAN product, ONO-1101 listed below have been referenced by the sponsor. Reports of intravenous bolus and continuous infusion studies in rats and dogs up to 4-week duration were available.

Repeated Dose Toxicity									
Repeated dose toxicity	Rat/SD	i.v. bolus	28 days	0, 12.5, 25, 50, 100	NOAEL = 50 mg/kg	n.d.	Ono Pharm.	Yamaguchi (1997b)	4.3
Repeated dose toxicity	Dog/Beagle	i.v. bolus	28 days	0, 12.5, 25, 50	NOAEL = 12.5 mg/kg	n.d.	Ono Pharm.	Yamaguchi (1997c)	4.3
Repeated dose toxicity	Dog/Beagle	i.v. continuous inf. (60 min/day)	14 days	120 (2 mg/kg/min)	No findings.	n.d.	Ono Pharm.	PMDA (2002)	4.3
Repeated dose toxicity	Dog/Beagle	i.v. continuous inf. (24 h/day)	28 days	57, 288, 1140 (0.04, 0.2, 1.0 mg/kg/min)	NOAEL = 1140 mg/kg	n.d.	Ono Pharm.	PMDA (2006)	4.3

Four-week i.v. bolus study in rats with 4-week recovery period ([Yamaguchi 1997c](#))

Daily doses of 12.5, 25, 50 and 100 mg/kg/day (69.4 µg/kg/min) LAN were administered in a 4-week intravenous rat study with a 4-week recovery group. Mortality, clinical observations, ophthalmology, body weight, food consumption, clinical pathology, organ weights, macroscopic and microscopic examination, and electron microscopy of liver and kidney were evaluated.

- Three animals receiving the 100 mg/kg dose (HED of 16.2 mg/kg) with clonic convulsions died within two minutes of test-article administration between Days 1

and 17. Histopathological evaluation did not reveal the cause of death, although dark reddening of the lungs was noted in 3 females.

- Bradypnea and dyspnea, tremor, chromodacryorrhea and loss of righting reflex were observed in the 100 mg/kg groups of both sexes shortly after the administration. Most of these symptoms disappeared within one minute after administration in animals that survived and were absent in animals receiving ≤ 50 mg/kg.
- There were no effects on body weight, food consumption, and ophthalmic endpoints.
- Erythrocyte indices were decreased in a dose-unrelated manner and were present after drug withdrawal. Total protein and triglyceride levels were decreased in the study in one or both sexes.
- Macroscopic changes were noted in the liver, kidney, bladder, uterus, testes, epididymis, and seminal vesicles, but were not associated with microscopic findings.
- There were no electron microscopic findings in the liver and renal cortex and medulla of the kidneys.
- Moderate thickening of the vessel wall was noted at the administration site in the 100 mg/kg/day dose group, and fibrosis of perivascular tissue was seen in females, and slight thickening of the vessel wall was noted in one female after drug withdrawal. Slight thickening of the vessel wall was present in one male in the 50 mg/kg/day dose group and one female in the control group.
- The investigators identified a NOAEL of 50 mg/kg for the study.

Four-week iv bolus study in dogs with 4-week recovery period (Yamaguchi 1997b)

Beagle dogs were administered 12.5, 25, 50 mg/kg/day LAN for 4-weeks, with 4-week recovery period. Mortality, clinical observations, ophthalmology, cardiovascular examinations, respiratory rate, body temperature, local tolerance, body weight, food consumption, hematology, clinical chemistry, urinalysis, cystoscopy, organ weights, macroscopic and microscopic examinations, and electron microscopy of selected organs. Animals were also subjected to a kidney function test by administration of 1 mg of phenolsulfonphthalein (PSP) into the cephalic vein, and liver function test with 0.5 mg of indocyanine green (ICG) via the cephalic vein.

- There was no mortality in the study.
- Nausea and vomiting were noted in both sexes within 10 minutes of test-article administration at ≥ 25 mg/kg. Dose-related increase in soft feces and diarrhea were noted at ≥ 25 mg/kg.
- There was no effect on body weight and food intake.
- Sinus arrhythmia, bradycardia and ECG wave-pattern changes were observed.
- Decreased erythrocytes and hemoglobin reported at ≥ 25 mg/kg.
- No significant change in plasma concentration of PSP and ICG was recorded in LAN-treated groups compared with the respective control groups. No evidence for changes in kidney function was observed.
- The sponsor identified a NOAEL of 12.5 mg/kg/day for both sexes.

Four Week continuous i.v. infusion study in dogs (PMDA 2006)

Male Beagle dogs were administered 0.2, 1 and 5 mg/kg/min continuously for 24-hrs at a rate of 0.1 mL/kg/min. Blood was collected at 0.5, 1, 6, 12 and 24 hrs after start of administration as well as 1, 2, 6, 24, 48 and 72 hrs after the end of administration to measure plasma concentration of the test-article and metabolites, M-1, and M-2.

All animals in the 5 mg/kg/min died 9-11 hrs after the start of dosing. Cardiac and renal discoloration and pleural effusion were observed upon autopsy. Dogs vomited, and exhibited decreased spontaneous motor activity, decumbence, and cyanosis after start of administration. The plasma concentration of LAN after the administration of 5 mg/kg/min group was 61.99 µg/mL and 127.30 µg/mL after 1 hr and 6 hrs of the administration, respectively. Thus, animals died at 61-fold AUC relative to the exposure at the MRHD.

Steady state concentration was reached within 0.5 hr and the level was below LLOQ after 1 hr. Exposure to LAN after 24 hrs of the start of administration and immediately prior to the end of administration at 0.2 and 1.0 mg/kg/min was 2.13 and 11.81 µg/mL, and AUC_{0-96h} was 52.25 and 281.19 µg.h/mL, respectively. The plasma concentration of M-1 at the same period was 13.33 and 98.67 µg/mL, respectively and AUC_{0-96h} was 390.43 and 2,606.35 µg.h/mL. The concentration of M-2 was 8.71 and 24.21 µg/mL and AUC_{0-96h} for M-2 was 252.45 and 745.93 µg.h/mL, at 0.2 and 1.0 mg/kg/min, respectively.

Four Week continuous iv infusion study in dogs (PMDA 2006)

Both male and female dogs were administered doses of 0.04, 0.2 and 1 mg/kg/min LAN for 4-weeks and 24 hrs a day using saline as control at a rate of 0.05 mL/kg/min. Blood was collected to measure plasma concentration of the test-article and metabolites, M-1, and M-2, 1, 6, 24 hrs, 14 days and 4 weeks after start of administration, as well as 1, 6 & 24 hrs after the end of administration.

- No mortality in the study.
- Shortening of APTT was observed in females at the mid dose and both sexes at the high dose. There were no other findings that indicated the activation of the coagulation cascade.
- Decreases in total cholesterol and phospholipid were observed in females of the 0.2 mg/kg/min females and both sexes at 1 mg/kg/min.
- There was an increase in PR interval, which is related to the pharmacological effect. There were no other significant findings.
- Exposure was dose-proportional and there were no sex differences. Steady state was reached with 24 hrs for the lower doses, but later at the high dose.
- The NOAEL was 1 mg/kg/min, with the average concentration of 11.2 µg/mL providing 8-fold margin to the MRHD.
- The level of M-1 was 10.6-times to that of the parent while M-2 was 2-fold higher than parent.

7 Genetic Toxicology

The applicant sponsored a battery of GLP-compliant tests to evaluate the genotoxicity potential of LAN. All studies conducted by (b) (4). The in vitro studies were conducted with and without S9 fractions at concentrations selected based on range finding studies. The studies were appropriately controlled and validated. LAN did not show genotoxic potential under the conditions of the assays.

Bacterial reverse mutagenicity studies (study #. 787325 and 795100) were conducted with LAN up to the limit concentrations (5,000 µg/plate). Study 795100 was conducted for qualification of product-related impurities and is reviewed in 11.4

Impurities/Degradants

Study Number/ Title: 787325/Landiolol HCl: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 100, TA 1537 and TA 98 and *Escherichia coli* \VP2uvrA

This study evaluated LAN at concentrations ranging from 17 to 5,000 µg/plate in *S. typhimurium* and *E. coli* strains with and without S9-mix by the plate incorporation and pre-incubation methods.

Landiolol HCl did not exhibit genotoxicity potential in strains of *Salmonella typhimurium* and *Escherichia coli*, when tested up to a maximum concentration of 5,000 µg per plate in the absence and presence of metabolic activation.

Study Number/ Title: 787330/ Landiolol HCl Mouse Lymphoma Mutation Study

Landiolol HCl concentrations that resulted in reduction of cell growth by 28.3% and 43.8% after 4 hr and 24 hr incubations with S9 mix were used in the study. In the presence of S9 mix a concentration of 5,000 µg/mL reduced relative cell growth to 2.4%. Four independent assays were conducted with duplicate cultures as shown below:

Assay No.	Presence or absence of S9	Treatment time (hours)	Final concentrations [†] (µg/mL)
1	Absence	4	200, 400, 700, 1100, 1600
2	Presence	4	1600, 2200, 2900, 3700, 4600
3	Absence	24	600, 1000, 1400, 1800, 2200
4	Presence	4	3000, 3400, 3800, 4200, 4600

Landiolol HCl did not induce genetic toxicity in mouse lymphoma L5178Y cells, in either the absence or the presence of S9 mix, when tested at cytotoxic concentrations.

Study Number/ Title: 790584/ Landiolol HCl: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow: 0 h and 24 h Intravenous Dosing and 48 h Sampling with Toxicokinetic Blood Sampling

The study was conducted in CD rats with intravenous doses of 37.5, 75 and 150 mg/kg/day. The study design, including blood sampling for TK analyses is shown below:

Dose Group	Daily Test Dose	Treatment and Number of Rats		
		Dosing (h)	Sampling (h)	No. of Rats
Vehicle Control	Sodium chloride, (0.9% (w/v); 12.5 mL/kg)	0 + 24	48	5M + 5F
Low Dose	Landiolol HCl (37.5 mg/kg)			5M
Mid Dose	Landiolol HCl (75 mg/kg)			5M
High Dose	Landiolol HCl (150 mg/kg)			8M + 8F
Positive Control	Cyclophosphamide (50 mg/kg)			3M

Dose Group	Sex	Blood Sampling Times and Animal Numbers	
		0h + 5 min	24h + 5 min
High Dose	M	201-203	201-203
	F	204-206	204-206

The highest dose tested was the maximum tolerable dose based on a prior toxicity study. Bone marrow was sampled after 48 hrs of initial dosing (after the second dose) and at least 2,000 polychromatic erythrocytes (PCE) per animal were scored for the frequency of micronucleated cells (MN-PCE). Acceptance and evaluation criteria, including historical frequency of MN-PCE in negative and positive control data were provided. Landiolol HCl did not induce micronuclei in bone marrow cells under the testing conditions.

The concentrations of LAN, M-1 and M-2 metabolites were determined at the high dose. The highest concentration of LAN was 1.88 µg/mL in the female after 5 minutes. The M-1 metabolite ranged from 205 to 593 µg/mL, and M-2 was below the LLOQ. There was no difference in exposure between the male and female and no accumulation was noted.

Study Number/ Title:787346/ Landiolol HCl: Micronucleus Test in Bone Marrow Cells of CD-1 Mice: 0 h + 24 h Oral Dosing and 48 h Sampling

The applicant sponsored another study in CD-1 mice evaluating 2,000 mg/kg/day (limit dose per OECD 474) administered twice intravenously every 24-hrs.

Dose Group	Daily Test Dose	Treatment and Number of Mice		
		Dosing (h)	Sampling (h)	No. of Mice
Vehicle Control	10 mL water/kg	0 + 24	48	5M + 5F
High Dose	2000 mg Landiolol HCl/kg	0 + 24	48	7M + 7F
Positive Control	50 mg Cyclophosphamide/kg	0 + 24	48	5M

Bone marrow samples were smeared on a slide dried and stained in 15% Giemsa. At least 2,000 PCE per animal were scored for micronuclei and the frequency of MN-PCE were determined. Landiolol HCl did not induce an increase in micronucleated polychromatic cells in male and female CD-1 mice when administered orally at the limit dose.

8 Carcinogenicity

The sponsor has not conducted carcinogenicity studies citing ICH M3 (R2) since the product is intended for short-term use. ICH S1A may be also relevant, and the issue of carcinogenicity study was not raised by the Division during the Pre-NDA meeting.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

The applicant didn't conduct fertility and early embryonic developmental studies. A published rat study with a LAN product (ONO-1101) was cited (Nishimura, 1997a). The test article in the published study was administered intravenously at 25, 50 or 100 mg/kg/day to males 64 days prior to mating and during mating until necropsy. Females were dosed from Day 15th before mating until Day 7 of gestation.

Three males and 2 females receiving the high dose died. Hypoactivity, clonic convulsion, bradypnea/apnea and reddish lacrimation were observed after administration in both sexes. Red lacrimation was noted in male animals at 50 mg/kg/day. Absolute and relative thymus weight was decreased at the high dose. Body weight and food consumption were decreased in females during premating and mating periods at the high dose. Treatment did not affect the estrous cycle, mating, and other fertility indices, and no skeletal or visceral changes were present in the fetuses. Thus, a NOAEL of 50 mg/kg/day was identified for general toxicity in parents, and 100 mg/kg/day for the reproductive performance in parents and for the development of fetuses.

9.2 Embryonic Fetal Development

Rat study

The potential effects ONO-1101 on embryofetal development were assessed after bolus daily i.v. administration of 25, 50, and 100 mg/kg to pregnant SD rats from GD7 to GD17 (Nishimura, 1997b). Female animals with successful copulation were divided into groups and necropsied on GD20. Another group was allowed to give birth naturally since the study included investigations of reproductive ability of the F1 generation including macroscopic examination of fetuses at GD20. The effect on lactation was also assessed from the day of delivery to Day 21.

Two animals died immediately after administration of 100 mg/kg. Bradypnea, clonic convulsions, loss of righting reflex, hypoactivity and chromodacryorrhea were observed at high frequency at the same dose. Food consumption was decreased in this group during the treatment period, and placental weight was decreased but there were no effects on fetal weight. There were no effects on pregnancy & delivery indices, and gestation length was comparable in treated and control animals.

On day 4 after birth, viability of the offspring was decreased in the 50 or 100 mg/kg/day groups, and body weight of male fetuses were decreased in the 100 mg/kg/day group. The Day 4 survival rate was low and increased incidence of unossified talus was seen in the 50 or 100 mg/kg/day group of fetuses culled on day 4 after birth. However, there were no-treatment related malformations of fetuses.

Weaned animals were used for general Irwin, sensory function, and reproductive competency tests. Sensory function tests (tail suspension, pain response, and righting, pupillary, Preyer, corneal & ear twitch reflexes) were performed on postpartum day 28.

Reproductive ability of the F1 generation was undertaken by mating 1 male and 1 female per dam from the same treatment group avoiding sibling mating. A 14-day mating period and an additional 7-day mating period for unsuccessful mating was performed. Females with confirmed copulation (Day 0) were checked for general condition, body weight & food consumption. Animals were necropsied on GD20 and the number of corpora lutea and implantation sites were counted, and the intrauterine distribution of fetuses, number of fetal deaths and resorptions and number of live fetuses were determined.

Males in the 25 and 100 mg/kg dose groups had a slightly lowered copulation rate in the reproductive ability test, so an additional test was conducted using untreated females for 14-days and subsequently for 7-days again. Necropsy was performed on animals of both sexes with no confirmed copulation and that did not successfully inseminate or conceive. Microscopic examination of sections of the testes, epididymis, seminal vesicle and prostate in males and the uterus and ovaries in females were performed.

The results overall indicated absence of significant differences in copulation and insemination rates between treated and control F1 animals. There were no abnormal findings in necropsy and microscopic examination of animals that failed to

copulate and animals that failed to conceive. A NOAEL of 50 mg/kg/day for dams, and 25 mg/kg/day for their offspring were identified in the study.

Rabbit study

The potential effects ONO-1101 on embryofetal development were assessed after bolus daily i.v. administration of 0 (normal saline) 25, 50, and 100 mg/kg dose to pregnant KAR:NZW rabbits from GD6 to GD18 (Nishimura, 1997c). Daily observations were made during dosing and body weight was measured on GD0, GD6 to GD19, and on GD24 and GD29. Food consumption was measured every day. Does were necropsied on GD29 and the number of corpora lutea, intrauterine distribution of fetuses, number of resorptions and number of live fetuses were recorded. Fetal and placental weights were measured, and live kits were examined for external anomalies. Maternal brain, heart, lung, kidney, spleen, adrenal gland, pituitary gland, and ovaries were weighed. Fetuses were examined for macroscopic anomalies and examined for potential skeletal malformations.

One doe in the 50 mg/kg and three in the 100 mg/kg dose group died immediately after test-article administration. Reddish pleural fluid, ascitic fluid and bloody contents were noted in the female receiving 50 mg/kg and died. This was not observed in the 100 mg/kg group that died. However, animals in the 100 mg/kg dose group presented transient bradypnea/apnea, clonic convulsion and hypoactivity immediately after each dose. There was no effect on body weight and food consumption. Necropsy of dams on gestation day 29 revealed splenomegaly in 5/15, 6/12 and 6/11 animals in the 25, 50 and 100 mg/kg dose groups, respectively, but there were no histopathological correlates for the finding. Whether the effect on the spleen is related to the effects of LAN on the erythrocyte parameters is not known.

Females in the 50 mg/kg dose group had decreased number of implantations, implantation rate, number of male fetuses and percentage of dead fetuses, and an increase in female fetal weight compared to the control group. Visceral examination revealed thymic remnants in the neck in one animal each in the control group and the 50 and 100 mg/kg dose groups, and anomaly of the subclavian arteries and hypertrophy of the left ventricle in one animal each in the 100 mg/kg dose group.

Skeletal examination revealed poorly ossified hyoid bone in the 25 and 50 mg/kg dose groups, but not at 100 mg/kg. Sternebral asymmetry, split and excess lumbar ribs, hypoplasia of 12th ribs and presence of 8 lumbar vertebrae were noted as skeletal variations, but the incidences were not different between the control group and the ONO-1101 treatment groups.

The authors of the study concluded that the maternal NOAEL is 25 mg/kg, and 100 mg/kg for fetuses. The proposed label indicates that this is 3-fold higher than the human exposure at the MRHD, (b) (4). This discrepancy should be addressed during the labeling discussion

9.3 Prenatal and Postnatal Development

In a study summarized by the applicant (Nishimura, 1997d), intravenous doses of 25, 50 and 100 mg/kg/day ONO-1101 were evaluated from GD17 to PND20 of parturition to examine the effects on pregnancy, delivery, lactation and the effects on postnatal growth and development of the offspring. Pregnant animals in each group delivered naturally and gestation length, birth rate, delivery rate, live birth rate (# live-born pups/implantation sites x 100) were calculated.

Lactation status was inspected from delivery (Day 0) until lactation Day21 after which dams were necropsied. The weaning rate was calculated after culling of pups on postpartum Day4. In addition, the presence of milk spots visible through the abdominal wall of the offspring was taken as an index of the milk secretion ability. External development was examined by checking for separation of auricles on postpartum Day4, eruption of incisors and appearance of abdominal hair on postpartum day 14, separation of eyelids on postpartum Day17, descent of testes on postpartum Day28, and opening of vagina on postpartum Day42. General behavior of animals was evaluated in Irwin test and the test animals were necropsied and organ weights were measured, and the skeletons examined for malformations and ossification. Sensory function and open field tests were assessed on postpartum Day28.

Five animals in the 100 mg/kg group died immediately after administration (2 animals on GD17 and GD21, and 3 animals on lactation day 0 to 5). At necropsy, lungs of animals that died in the 100 mg/kg dose group during the gestation or lactation period were dark red. Hypoactivity, reddish lacrimation, clonic convulsion and bradypnea/apnea were observed after administration of the high dose. Body weight and food consumption at weaning were also lower than the control group. Reddish lacrimation was noted in animals receiving the 50 mg/kg.

One animal each in the 25 and 50 mg/kg dose groups had not given birth by GD24. In both dams, implantation sites were present and total resorption had occurred. Relative kidney weight was increased in the 100 mg/kg dose group and increased relative lung weight and decreased absolute liver weight in the 25 mg/kg dose group.

Viability of offspring on day 4 after birth decreased in the 100 mg/kg/day group, and body weight gain was low during suckling but recovered after weaning. The day 4 survival rate decreased in the 100 mg/kg dose group compared to the control group. The weaning rate and growth rate did not differ between the control group and treatment groups.

Up on the skeletal examination of weaned offsprings culled on postpartum Day4, decreased mean number of ossified phalanges of the hind paw and increased unossified talus bone were seen in the 100 mg/kg/day group, however, no delay of ossification was found. The test-article did not affect external differentiation, functional, behavioral, or learning abilities or reproductive performance of the offspring. From the

above results, it was estimated that the NOAEL of ONO-1101 under these experimental conditions was 50 mg/kg/day for dam and offspring.

Reproductive ability of the F1 generation was evaluated beginning week 12 postpartum as described under section 9.2 above. Decreased implantation rates were noted in the 25 and 100 mg/kg dose groups compared to the control group on GD20. Copulation rates and insemination or conception rates in F1 males and females did not differ between the control and treatment groups. In general, pregnancy, delivery, lactation and necropsy findings or organ weights of the dams was not affected by the test-article at ≤50 mg/kg. Thus, a NOAEL of 50 mg/kg was proposed both for dams and offspring when administered in the perinatal and lactation periods. The proposed label indicates that this is 5-fold higher than the human exposure at the MRHD. There is no TK information in the published study with ONO-1101, thus clarification of the source of the rat exposure data would be needed during the labeling discussion.

10 Juvenile Toxicity Studies

Juvenile toxicity study has not been conducted with LAN.

11 Special Toxicology Studies

11.1 Local Tolerance

The studies evaluating local tolerance and the irritation potential of LAN formulations in rabbits were conducted by (b) (4). The three studies below indicated that different LAN formulations administered via i.v. and other routes were tolerated.

Study Number/ Title: 519199/ Local Tolerance Study in Rabbits After a Single Injection of Landiolol by the Intravenous Route

In this GLP study, the local tolerability of LAN as a single i.v. injection of either Rapibloc Conc or Onoact® were investigated in NZW rabbits. Each animal received a single injection into the left ear of either landiolol hydrochloride (AOP) or Onoact®, at a concentration of 10 mg/mL, and a single injection of the corresponding vehicle into the right ear. There were no signs of systemic toxicity nor local reactions at the injection sites in any of the groups. There were sporadic inflammatory changes at the injection sites that were judged to be unrelated to the test-articles.

Study Number/ Title: 524235/ A Single Dose Study to Investigate the Local Tolerance of LDL E103/P00065 after Injection in Rabbits

The objective of this GLP complaint study was to assess the local tolerance and irritation potential of landiolol (Rapibloc 300 mg = landiolol HCl 300 mg for injection) and its vehicle when given to rabbits at unintended injection sites. Vehicle and the test-article were administered via the intermuscular (thigh), perivenous (marginal ear vein), intra-arterial (ear artery) and subcutaneous routes (flank). Animals were checked for viability; body weight and tissues samples were collected from euthanized animals from the injection sites and microscopically examined.

Minor dermal reactions were noted in the marginal veins in both vehicle and LAN injections. There were no microscopic findings.

Study Number/ Title: 522109/ A Single Dose Study to Investigate the Local Tolerance of LDLA E101/P00071 after Injection in Rabbits

The objective of this GLP study was to assess the local tolerance and irritation potential of landiolol (Rapibloc Conc) and its vehicle when given to rabbits at unintended injection sites (intramuscular, perivenous, intra-arterial, subcutaneous). The concentration of landiolol hydrochloride (4 mg/mL) and excipients were 2-fold higher in the test item formulation than the intended clinical concentration. The concentrations of the excipients in the control item formulation were 5-fold higher than the maximal concentrations used in a clinical setting. In addition, the study evaluated the potential reversibility of any findings. Dermal reactions, clinical signs, macroscopic abnormalities, and histological changes were evaluated.

Injection site reactions were noted in animals that received the vehicle and the test-article in comparable routes. However, intramuscular injection of control formulation with increased excipient concentration resulted in myofiber degeneration and perivenous and intra-atrial injection resulted in inflammation. Thus, it was concluded that injections of formulations of LDLA E101/P00071 elicited only those findings at the injection sites which are typical of administration procedure-related findings in rabbits.

11.2 Effect on Coagulation and platelet functions

Blood was collected from rats and centrifuged at 1,000 RPM for 10 minutes to get platelet rich plasma that was further centrifuged at 3,000 RPM for 10 minutes to get platelet-poor plasma (PPP). Aliquots of this PPP were added to landiolol solution to get a final concentration of 0.01 μ M to 1 mM. Plasma recalcification time, activated partial thromboplastin time (aPTT), prothrombin time (PT), total plasmin activity, clot contraction and platelet aggregation activity were measured. LAN exerted only a slight effect on aPTT- and PT-prolongation.

11.3 Hemolytic Effect of Landiolol

Heparinized blood was collected from rats, dogs, and humans for purification of erythrocytes in saline. LAN was added to 0.05 μ M to 10 mM and incubated at 37°C for 30 min and centrifuged at 3,000 RPM for 2 min. The supernatant was diluted and the

O.D. measured at 540 nM. Hemolysis was observed in rat, human and dog blood samples treated with 10 mM landiolol compared to control (saline treated) samples.

11.4 Impurities/Degradants

Background

Potential process impurities, (b) (4) and any other unknown impurities were controlled at “not more than (b) (4) %” for the maximum daily dose >2g/day. The limit of “not more than (b) (4) %” for (b) (4) was qualified in the rat studies. Total impurities were controlled with the limit of “not more than (b) (4) %”.

The impurities reported in the drug product were (b) (4)

(see table below). All impurities are expected to be above the qualification threshold at product target shelf-life. In silico structure activity analyses and a toxicology study with LAN and a mixture of synthesized impurities were conducted for qualification.

Table 17: Impurities in the Landiolol Product

Impurity	Occurrence	Formation
(b) (4)	LDLL600 / LDLL300 LDLA	(b) (4)
	LDLL600 / LDLL300 LDLA	
	LDLA	
	LDLL600 / LDLL300	
	LDLL600 / LDLL300	
	LDLL600 / LDLL300	
	LDLL600 / LDLL300	
	LDLL600 / LDLL300	
	LDLA	
	LDLA	
	LDLA	
	LDLA	

Study Number/ Title: 510638/GLP 2 Week Study of

(b) (4)

by

Intravenous (Bolus) Injection in Rats

The impurities were synthesized, except possibly impurity (b) (4)³ and administered as a mixture at doses listed in the table below.

Table 18: Methods of 2-Week Rat Intravenous Impurity Study in Rats

GLP Compliance: Yes

Dose and frequency of dosing:

Twice daily for 2-weeks at doses shown below:

Group No.	Treatment	Total Dose (mg/kg/day)	Total Volume ^a (mL/kg/day)	Volume Per Application (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
						Males	Females
1	Control	0			0	1001-1008	1501-1508
2 ^b	(b) (4)		2.5	1.25	(b) (4)	2001-2008	2501-2508

Control (vehicle) = Saline (0.9%) solution

^a Dose was based on the most recent body weight measurement.

^b All impurities were pooled together and given twice a day by intravenous bolus injection.

Route of administration: Intravenous bolus injection

Formulation/vehicle: Saline

Species/strain: Rat/Sprague Dawley

Number/sex/group: 8

Age: 6-7 weeks [214 to 264 g (♂); 156 to 200 g (♀)]

Satellite groups/unique design: Not included

³ Impurity could not have been synthesized due to technical limitations (source module 3.2.P.5.5.3.2)

Table 19: Observations and Results 2-Week Impurity Study in Rats	
Parameters	Major findings
Mortality	Absent
Clinical signs	No treatment related clinical signs
Body weights	BW and food consumption was not different from control mean
Ophthalmoscopy	No treatment related finding
ECG	Not reported
Hematology	Total and differential counts of leucocytes were increased in animals receiving the impurities. The values were judged to be within historical control data. No findings in coagulation parameters.
Clinical chemistry	Mean calcium level was higher in males receiving the impurities, in comparison to controls.
Urinalysis	Not remarkable
Gross pathology	No finding
Organ weights	Increased absolute spleen weight (+16%) in males and decreased absolute uterus & thyroid weight (-16%) in females in impurity group relative to control mean. No microscopic correlates to the findings.
Histopathology	Minimal lung emboli were noted at greater frequency in animals treated with the impurity. This finding is considered test-article related and may be related to i.v. dosing based on the hemorrhage, cellular infiltration, and mild fibrosis at the injection site of 5 out of 6 animals with the emboli.
Adequate battery:	
Yes	Cellular infiltration in both sexes and minimal to mild perivascular fibrosis in females receiving impurities.

Conclusion:

Minimal lung emboli were noted at greater frequency in female animals treated with the pooled impurities. It is not known whether the emboli were due to any specific impurity or whether it was injection related. Increased total and differential leukocyte count associated with microscopic finding of hemorrhage, cellular infiltration, and mild fibrosis were also noted at the injection site of 5 animals out of 6 animals with lung emboli. The applicant has concluded that the lung finding is not treatment related because it only occurred in female animals. The lung finding is probably treatment related considering the targeting of the lungs by LAN at high doses that probably resulted in death in rats and other species at 100 mg/kg/day.

The proposed specification for total impurities is (b) (4) % which is equivalent to (b) (4) mg/kg in the maximum clinical dose of 57.6 mg/kg. The estimated impurity level in the 100 mg/kg dose in rat studies is equivalent to (b) (4) mg/kg HED.

Moreover, this study tested (b) (4) mg/kg of pooled impurities, which corresponded to (b) (4) mg/kg HED, indicating the existence 8-fold margin to the level of impurity at the lethal dose in rats and the total impurity level qualified in this study.

Study Number/ Title: 504598/ A Single Dose Toxicity Study of Rapibloc 600 mg (600 mg powder for solution for injection and infusion) by Intravenous (24 hr) Infusion in Rats with a 3 Day Observation Period

Key Study Findings

- There were no treatment related findings in this study.

A 600 mg formulation of LAN was continuously infused at 1,000 mg/kg/day (41.7 mg/kg/day). The toxicity batch (lot 0000241593) was aged to represent levels of stressed (long-term stability at 25 °C) with spiked impurity (b) (4) and (b) (4). The final concentration of (b) (4), impurity (b) (4), impurity (b) (4), impurity (b) (4), impurity (b) (4), impurity (b) (4), impurity (b) (4), impurity (b) (4) and impurity (b) (4).

Table 20: Infusion study of LAN in Rats with 3-Day Observation

Study Features and Methods	Details
GLP Compliance:	Yes
Dose and frequency of dosing:	1,000 mg/kg
Route of administration:	24 hr intravenous infusion
Formulation/vehicle:	Saline
Species/strain:	Rats/Sprague Dawley
Number/sex/group:	5
Age:	8-9 weeks (309-387 g (♂), 203-270 g (♀))
Satellite groups/unique design:	None
Deviation from study protocol affecting interpretation of results:	None

Study Number/ Title: 795100/Rapibloc Conc (Landiolol HCl 20 mg/2 mL solution for injection): Bacterial Reverse Mutation Test in Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98, and Escherichia coli WP2uvrA

This study conducted by (b) (4), evaluated Rapibloc at concentrations ranging from 5 to 5,000 µg/plate in *S. typhimurium* and *E. coli* with and without S9-mix by plate incorporation method. (b) (4) (batch # H101/P00071 is from the 40°C stress study with concentration of (b) (4), impurity (b) (4), impurity (b) (4), impurity (b) (4), and others as a total impurity level of (b) (4)%. The study was a GLP-compliant study that has been validated in this review.

The test was negative for mutagenic potential.

Study Number/ Title: APN001drklis/Toxicological analysis of twelve potential impurities using Derek Nexus and Leadscape

Key Study Findings

All structures analyzed triggered an EQUIVOCAL alert in humans for carcinogenicity due to the presence of an (b) (4) moiety. The (b) (4) structure is associated with carcinogenicity in rodents with unlikely relevance to humans. No alerts for chromosome damage, genotoxicity or mutagenicity were triggered by any structure.

Study Information

A structure-activity relationship (analysis was conducted on 12 impurities (see **Table 15**) using Derek Nexus and Leadscape in silico systems. These 12 impurities are expected to be above qualification threshold at target shelf life (7 out of 12 impurities in each formulation).

The finding from Derek Nexus and Leadscape genotoxicity suite suggested that the impurities have no genotoxic and mutagenic potential. Potential genotoxic impurities of LAN were identified in the drug substance per ICH M7 guidance. The levels of (b) (4)

(class 1 mutagen), (b) (4) (class 1), (b) (4) (class 3), (b) (4) (class 3) and (b) (4) (class 2) were specified as acceptable for a less than life-time exposure of ≤ 1 -month (120 $\mu\text{g/day}$). (b) (4) (same as (b) (4) impurity in the DP) is considered qualified. (b) (4) is the main (b) (4). The potential genotoxic impurities are also qualified in silico consistent with ICH M7 guidance.

The studies reviewed above in totality support the applicant's conclusion that the impurities are qualified at the specified limits.

The table below provided by the sponsor presents the specification limits, the level of a specific impurity at the maximum proposed dose of 57.6 mg/kg. The dose multiples calculated for the impurities in the rat studies as HED relative to the specified impurity levels in the MRHD are consistent with the reviewer's calculations and support the proposed specifications.

Table 21: Impurities and Dose Multiples (*reproduced from the application*)

Impurity	Proposed impurity specification limit	Impurity level administered	Impurity dose level in rats ^b	Clinical impurity dose acc to limit ^c	Rat impurity HED ^d	Safety margin rat/clinical dose ^e
	(%)	(%)	(mg/kg)	(mg/kg)	(mg/kg)	(x-fold)
Study No. 504598						
(b) (4)						2.1
(b) (4)						2.8
(b) (4)						1.9
(b) (4)						1.2
(b) (4)						2.4
(b) (4)						1.6
(b) (4)						1.5
Study No. 612546						
(b) (4)						1.9
(b) (4)						0.8
(b) (4)						1.7
(b) (4)						1.3
(b) (4)						1.7
(b) (4)						1.6
(b) (4)						1.1
Study No. 510638						
(b) (4)						1.5
(b) (4)						1.5
(b) (4)						1.5
(b) (4)						1.5
(b) (4)						1.4

a No Landiolol product was used in Study No. 510638; instead, the impurities were pooled for the study

b Actual impurity dose on a mg/kg as calculated from impurity levels of test item at 1000 mg/kg Landiolol HCl

c Maximum clinical impurity dose in mg/kg, based on the proposed DP impurity specification limits for a dose of 57.6 mg/kg/day Landiolol HCl

d Human equivalent dose (HED) for the rat toxicity dose applying body weight to surface conversion using allometric scaling.

e Safety margin for the clinical impurity dose (mg/kg) in comparison to the rat toxicity study impurity dose (mg/kg).

f Impurity considered qualified as metabolite in accordance to ICH Q3B(R2).

(b) (4) **Impurity**

The CMC review team requested assessment for the risk of (b) (4) in the LAN product due the reported presence of (b) (4). The sponsor provided the requested information on February 15, 2023. The applicant developed a new validated analytical method targeting a default limit of (b) (4) ng/day with an analytical limit of (b) (4) ppb (b) (4). The applicant then measured (b) (4) in US registration batches of the drug product (two of the batches after 15- and 16-months) and determined the maximum daily intake (AI) for a daily dose of 2,688 mg landiolol free base at maximum infusion rate of 37.3 µg/kg/min free base for a 50-kg body weight. The results are shown in the sponsor provided table below; the (b) (4) impurity exposures from the three batches would exceed the default acceptance intake of (b) (4) ng/day.

Batch	Concentration in drug product ng /g (ppb)	Concentration in landiolol free base ng /g (ppb)	Maximum daily intake, 50 kg body weight ng / day
L32102	(b) (4)		
L32103			
L32104			

Since there was no published AI limit for (b) (4), the applicant used the ICH M7(R1) approach and calculated the AI limit per CDER's (b) (4) impurities guidance.⁴

Two approaches were used:

Approach 1: Used literature based read-across related surrogate (b) (4) derivation of AI for (b) (4)

4

(b) (4)

5

(b) (4)

Figure 11: Structures of

(b) (4)



(b) (4)					
Structural group	Structure	CAS no.	TD ₅₀ [mg/kg/day]	AI [ng/day]	AI for landiolol base for MDD 2688 mg [ppb]
(b) (4)					

A surrogate AI of (b) (4) ng/day was determined by this approach, which is equivalent to (b) (4) ppm for a dose of 2,687.5 mg/day of landiolol for a 50 kg person.

Approach 2: This approach was used for confirmation of the AI determination. Multicase QSAR Flex software including its underlying (b) (4) library for structural read-across was used. Five surrogates were identified by the software as potential read-across surrogates, and only two had (b) (4). One of these compounds was (b) (4) with a TD₅₀ value of (b) (4) mg/kg/day based on a two-dose carcinogenicity study. For a landiolol free base daily dose, the (b) (4) specification limit is ~ (b) (4) ppm ((b) (4) ppm with molecular weight adjusted AI of (b) (4) ng/day).



The sponsor also discussed the carcinogenicity risk of (b) (4) based on the mechanism of (b) (4) compound formation considering the presence of (b) (4) for metabolic conversion, which is the key step in the formation of (b) (4), and the potential

steric hinderance in DNA alkylation (b) (4)

(b) (4). They present lower potential concern as carcinogens with a TD₅₀ below (b) (4) mg/kg/day. Substitutions at the (b) (4) might partially block the (b) (4) metabolically and limit its DNA reactivity, thereby contributing to the reduction of the carcinogenic potency of the molecule. (b) (4) also contains a (b) (4) substituent, which is expected to contribute negatively to the carcinogenic potency.

The applicant also discussed the relevance of (b) (4). Six (b) (4) were found to induce in vitro DNA breaks and repair synthesis in primary cultures of both rat and human hepatocytes (b) (4). When tested for genotoxicity in vivo at a high dose level of (b) (4) mg/kg all produced a modest, but statistically significant increase in micronucleated cells in rat hepatocytes with no evidence of clastogenicity in bone marrow and the spleen. The applicant has thus judged that the genotoxic potential of (b) (4) would be lower than that of volatile, small (b) (4) compounds like (b) (4), justifying a higher individual limit.

The nonclinical review team consulted with the CDER (b) (4) safety team and the computational toxicology consultation service (CTCS). The (b) (4) safety team indicated that (b) (4) previously has not been accepted for (b) (4) due to the potential formation of (b) (4) in the pathway and a default AI of (b) (4) ng/day was used for the (b) (4). For (b) (4), a (b) (4) ng/day AI would correspond to ~ (b) (4) ppb at the maximum recommended dose of 2,687.5 mg/day. This level is 5-fold less than the average level measured in the registration batches.

The applicant was informed in the late-cycle meeting communication that the surrogate selected for AI determination may not adequately characterize the risks of (b) (4), and a default AI of (b) (4) ng/day may be recommended (DARRTs, 03-Mar-2023). A virtual late-cycle meeting was held with the applicant on March 14, 2023. The applicant presented a case for the selection was (b) (4) and discussed an alternative surrogate, (b) (4) with a stipulated AI of (b) (4) ng/day (b) (4) ppm for 2,688 mg/day dose for a 50-kg person). This surrogate, like the earlier ones, was not accepted by CTCS due to the potential formation of (b) (4) in the metabolic pathway.

The potential for acceptability of a risk level for less-than-lifetime (LTL) exposure to (b) (4) was considered in the review since the drug product will be administered for less than 3-months. The LTL approach discussed in the ICH M7(R1) guidance was considered since the guidance doesn't explicitly exclude the cohort of concern carcinogenic chemicals from the LTL approach. However, Agency (b) (4) recommendations have incorporated a

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more conservative lifetime cancer risk estimate, consistent with other regulatory agencies and based on a concern that DNA repair mechanisms could be saturated during acute high exposure periods.

Although relevant guidances describe such approaches to allow higher levels of mutagenic impurities for LTL clinical exposures, the Agency, as communicated by the (b) (4) safety team currently lacks policy mechanism to calculate an AI based on LTL for (b) (4) impurities. Moreover, other regulatory authorities, such as the Committee for Medicinal Products for Human Use ((b) (4) do not recommend setting higher (b) (4) limits in drug products for LTL assumptions. It is reasoned that cellular mechanisms repairing the alkylated DNA might become saturated during short-term/high exposure scenarios.

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/s/

FELEKE ESHETE
04/07/2023 12:06:54 PM

DAVID B CARLSON
04/07/2023 01:55:19 PM

I agree with the primary reviewer's approval recommendation contingent on control of the identified (b) (4) impurity