CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/10722883

https://www.iajps.com/volumes/volume11-february-2024/13-issue-02-february-24/

Available online at: http://www.iajps.com Review Article

# A REVIEW ARTICLE ON BEMPEDOIC ACID – ANTI HYPERLIPIDEMIC DRUG

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**Abstract:**

*Bempedoic acid is a prodrug that, once activated, decreases LDL-C levels by the inhibition ofadenosine triphosphate citrate lyase in the liver. Five clinical trials have demonstrated the safety and efficacy of bempedoic acid and the bempedoic acid/ezetimibe combination in lowering LDL-C in patients with atherosclerotic CVD and heterozygous familial hypercholesterolemia and also in high-risk primary prevention, and statin-intolerant patients. Bempedoic acid has been demonstrated to lower LDL-C levels by 15-25% in clinical trials and up to 38% when combined with ezetimibe. In 2020, the FDA approved bempedoic acid. Furthermore, the combination of bempedoic acid with ezetimibe is FDA approved for the treatment of adults with heterozygous familial hypercholesterolemia or established atherosclerotic CVD who require additional LDL-C lowering after maximally tolerated statin therapy. The ongoing CLEAR OUTCOMES trial aims to evaluate whether bempedoic acid can reduce cardiovascular events in patients with statin intolerance and results will be available in the next 3 years. This outcomes trial will be pivotal for determining the role of bempedoic acid in the non-statin lipid-lowering armamentarium.*

***Keywords:*** *bempedoic acid; cardiovascular events; dyslipidemia; hypercholesterolemia; low-density lipoprotein-cholesterol.*

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*Please cite this article in press Nikhath Kousar et al.,* ***A Review Article On Bempedoic Acid – Anti Hyperlipidemic Drug****, Indo Am. J. P. Sci, 2024; 11 (02).*

**NTRODUCTION:**

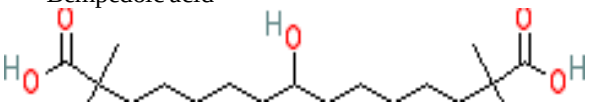
Bempedoic acid is a first in class ,oral inhibitor of cholesterol biosynthesis that is approve in patients with atherosclerotic cardiovascular disease (ASCVD) and for primary prevention in individuals with Heterozygous familial hypercholesterolemia (HEFH) by the united states food and drug administration .pooled data from the phase 3 clinical trials, clear wisdom have demonstrated the safety and efficacy of Bempedoic acid with regard to loweringof low density lipoprotein cholesterol (LDL-C) in patients with HEFH as an adjunct or alternative to currently existing lipid lowering therapies.

Clear Outcomes is a cardio vascular outcomes trial that is currently underway that will provide additional insight as to where Bempedoic acid will fit into treatment regimens among the non-statin lipid lowering therapy options patients who might particularly benefit from Bempedoic acid are those with [HEFH] and those unable to take adequate doses of stains or take any statin therapy altogether who need additional LDL-C lowering

**DRUG DISCOVERY:**

Brand Name : Nexletol

Generic Name : Bempedoic acid



Dosage form : Tablets

FDA Approved: First Approved [FEB 212020]
COMPANY: Esperion therapeutics, Inc. TREATMENT: High cholesterol, familial heterozygous

Nexletol [Bempedoic acid] is a first in class, adenosine triphosphate-citrate lyase inhibitor forthe treatment of adults with heterozygous cardiovascular disease who require additionallowering of LDL cholesterol.

**DISCOVERY TIMELINE FOR BEMPEDIOC ACID**

| DATE | ARTICLE |
|---|---|
| Feb 21,2020 | FDA approves Nexletol (Bempedoicacid) to lower LDL-cholesterol |
| May 5,2019 | Esperion announces of new drug applications (NDAs)for both Bempedioc acid and the Bempedioc acid/ezetimibe combination tablet for fillingand regulatory review |
| Mar 13,2019 | Esperion announces publication ofBempedioc acid study 1 resultsin the new England journal of medicine |

**HISTORICAL APPROACHES IN DRUG DISCOVERY**

There were two clinical trials that evaluated the benefits and side effects of Bempedoicacid. The trial designs were similar. All enrolled subjects were on a lipid-lowering diet and taking the highest dose of a statin (drug commonly used to lower cholesterol) for high cholesterol. Inboth trials, subjects were randomly assigned to receive Bempedoic acid or placebo tablets everyday for 52-weeks. Neither the subjects nor the health care providers knewwhich treatment wasbeing given in The trials measured percent change in LDL cholesterol (LDL-C) blood levels from baseline to week twelve and compared Bempedoic acidto placebo. In one clinical trial, Bempedoic acid reduced LDL-C by about 20 mg/dl compared to placebo and had a similar frequency of side effects to placebo, although a higher percentageof drug-receiving subjects dropped out of the study because of side effects (11% vs. 7% under placebo). In one randomized controlled trials patients who could not tolerate therapy with statins had a reduced risk of major adverse cardiovascular events after being treated with Bempedoic acid.


In January 2020, the committee for medicinal products for human use (CHMP) in the European Union recommended granting of a marketing authorization for Bempedoic acid as both a standalone drug (brand name Nilemdo) and as a fixed dose combination medication with ezitimibe (brand name Nustendi). Bempedoic acid was approved for use in the European Union in April
2020, and the combination Bempedoic acid/ezitimibe was approved in March 2020.

In February 2020, Bempedoic acid was approved for use in the United States both as a standalone drug (brand name Nexletol) and in a fixed-dose combination with ezetimibe (brand name Nexlizet). The U.S. food and drug administration (FDA) granted the approval of Nexletolto Esperion Therapeutics

The FDA approved Bempedoic acid based on evidence from two clinical trials (Trial 1/ NCT02666664 and Trial 2/NCT02991118) of 3009 subjects with high LDL cholesterol and known atherosclerotic cardiovascular disease or HEFH. The trials were conducted in United States, Canada, and Europe.

CHARACTERAZATION OF BEMPEDOIC ACID
PHYSICOCHEMICAL PROPERTIES
The intrinsic physico chemical properties of Bempedoic acid confer its low aqueous solubilityand high permeability. However Bempedoic acid is a weak with high solubility

MELTING POINT : 87-92 ˚c

BOILING POINT : 506.5±35.0˚C (Predicted)
APPEARANCE : Solid
COLOUR : White

**SIDE EFFECTS :**

In allergic reactions such as hives ; difficulty in breathing; swelling of your face; lips ,tongue ,throat .A less common but more serious adverse effects was tendon rupture in the rotator cuff of the shoulder.

**USES:**

Bempedoic acid is used to lower cholesterol and reduce the risk of heart disease.

It is not known if Bempedoic acid can lower the risk of complications related to high cholesterolsuch as heart attack, stroke, or death.

**FORMULATION:**

Bempedoic acid (180mg) constitutes of the total weight of the tablet formulation. In addition
,the tablet formulation contains colloidal silicon dioxide ,hydroxyl propyl cellulose lactose monohydrate ,magnesium stearate micro crystalline cellulose ,and sodium starch glycolate. Thetablets are coated with film compromising partially hydrolysed polyvinyl alcohol, polyethylene glycol, talc, and titanium dioxide. All excipients present in the drug product are USP/NF grade. The components of coating agent conformed to USP grade excipients.

**DOSAGE**:

180mg taken orally once daily with or without food.

**PHARMACOKINETIC ASPECT OF BEMPEDOIC ACID:**

Bempedoic acid pharmacokinetic parameters are presented as the mean [standarddeviation±(SD)] unless otherwise specified.

Absorption :

Bempedoic acid is absorbed with a median time to maximum concentration of 3.5 hour. When administered as NEXLETOL 180mg tablets .

Effect of food:

Concomitant food administration had no effect on the oral bioavailability of Bempedoic acid.Distribution:
The Bempedoic acid apparent volume of distribution was 18L. plasma protein binding of Bempedoic acid ,its glucuronide and its metabolite were 99.3%&98.8% .

It does not partition into blood cells.Metabolism:
The primary route of elimination for Bempedoic L[ is through metabolism of the acyl glucuronide. Bempedoic acid is also reversibly converted to an active metabolite. Based on aldo-keto reductase activity observed invitro from human liver .

Elimination:

The study state clearance of Bempedoic acid was 11.2ml/min after once daily dosing; renal clearance of unchanged Bempedoic acid represented <2% of total clearance.

Half-life:21±11hours at steady state.

**DRUG DISPOSITION:**

Bempedoic acid disposition in human subjects:

A single 240 mg dose of Bempedoic acid was administered as an oral solution to six healthy male subjects and Asian subjects (age•33 to 59 year of age) had a median body weight of 79.3kg and body mass index of 25.2kg/m².one subject experienced mild headache, which was possibly related to the study drug.no serious AEs, deaths , or clinically important changes in laboratory assessments ,vital signs ,electrocardiograms ,or physical examinations were reported during the study. Total recovery of radioactivity was monitored in excreta for 240 hours after dosing. The cumulative recovery of radioactivity in urine and faeces combined was87.4% of dose ,with less than 5%of dose being excreted unchanged. excretion of radioactivity in urine was the dominant route of elimination, accounting of 62.1% of dose recovery on average. Approximately 25.4% of dose recovery in faeces. Approximately 50% of total recovery was achieved within 72 hours of radioactive dose administration.

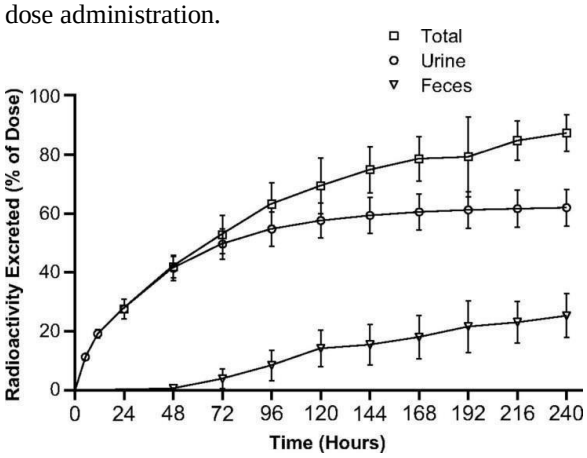



**Fig1:** Mean (± SD) cumulative percent of radioactivity excreted in urine and faeces and in combined excreta after single oral dose administration of 240 mg [14C] Bempedoic acid. Mean cumulative recovery of radioactivity was 87.4% of dose, with approximately 62.1% of dose excreted in urine and 25.4% in faeces.

The distribution of radioactivity between whole blood and plasma was determined after [14C] Bempedoic acid dosing. Geometric mean estimates of the ratio of blood to plasma radioactivityranged from 0.518 to 0.572 over the 0- to 48-hour sampling time course, with corresponding geometric mean (CV%) blood:plasma ratios of 0.489 (6.7%) for Cmax and 0.498 (14.8%) for AUCinf parameters.

Mean (± SD) plasma concentration–time profiles of total radioactivity, Bempedoic acid, and ESP15228 after administration of a single oral solution dose of 240 mg [14C] Bempedoic acid are shown in Fig. 2. +The time course of total radioactivity and Bempedoic acid concentrations in plasma were similar, rapidly attaining peak levels at 1 hour after oral dosing and characterized by similar multiexponential profiles. Geometric mean (CV%) estimates of elimination half-life were 26.0 (15.9%) hours for total radioactivity and 22.4 (24.0%) hours forBempedoic acid (Table 1).

The corresponding ESP15228 concentration–time profile indicated peak concentrations were attained at 11.0 hours with a similar elimination half-life of 31.1 (26.7%) hours as observed for Bempedoic acid. A comparison of AUCinf estimates indicates Bempedoic acid accounted for approximately 59.3% of total radioactivity in plasma and approximately 90.4% of peak radioactivity concentration. Based on AUCinf exposure, the metabolites ESP15228, Bempedoic acid-glucuronide, and ESP15228-glucuronide accounted for approximately 12.1%, 28.9%, and 10.4% of total radioactivity in plasma, respectively (Table 1).

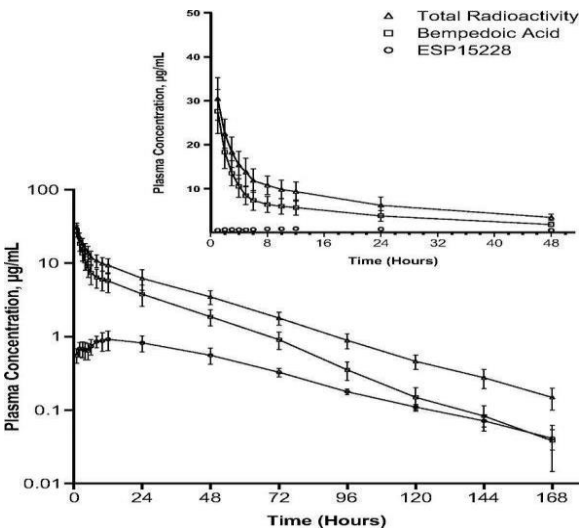



Fig2: Mean (± SD) plasma concentrations of total radioactivity, Bempedoic acid, and ESP15228 after single oral dose administration of 240 mg [14C] Bempedoic acid. Inset: Linear concentration–time profiles plotted from time 0 to 48 hours.

**PRECLINICAL TOXICITY STUDIES:**

In the event of an novel dose , the regional or local poison control center should be promptly notified .Currently a no specific antidote for Bempedoic acid overdose has been identified .

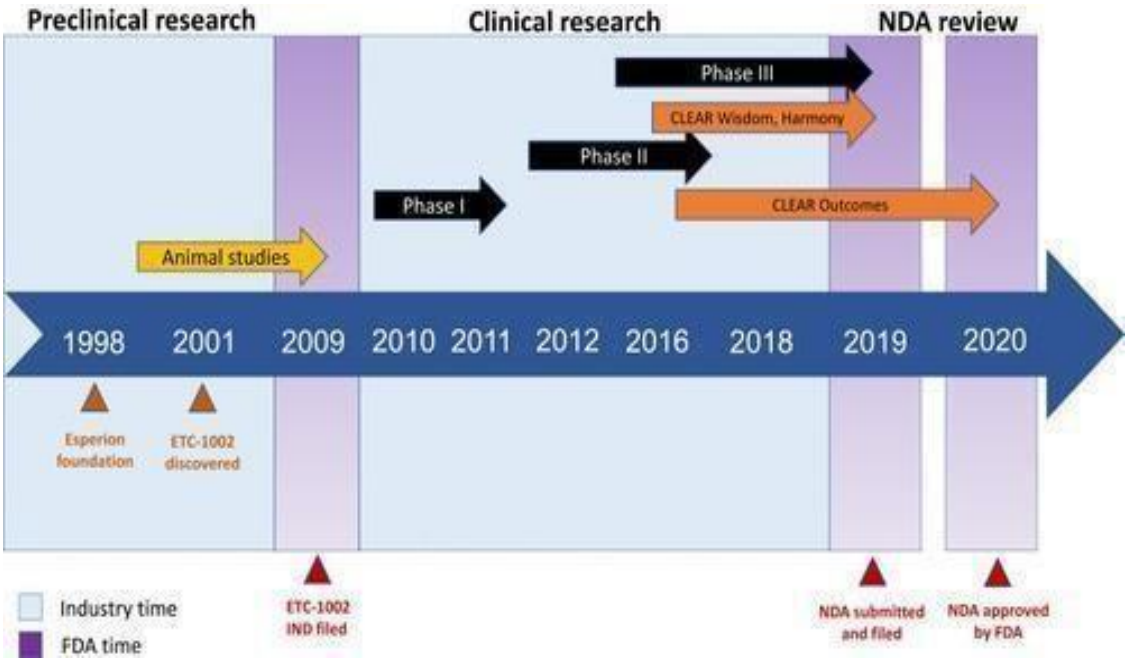
**INVESTIGATIONAL NEW DRUG APPLICATIONS OF BEMPEDIOC ACID**

An Investigational New Drug (IND) application was submitted by Esperion® in October 2009. Bempedoic acid has been evaluated in Phase 1 and Phase 2 clinical studies, and most recently in Phase 3 trials. Two New Drug Applications (NDAs) were submitted for Bempedoic acid andthe Bempedoic acid/ezetimibe combination to the US FDA on February 20, 2019 and February 26, 2019, respectively. Simultaneously, Esperion® submitted two Marketing Authorization Applications (MAAs) to the European Medicines Agency (EMA) for Bempedoic acid and Bempedoic acid/ezetimibe combination on February 11, 2019.

Both NDAs received their first approval in the US (as monotherapy on February 21, 2020 andas the fixed-dose combination with ezetimibe on February 26, 2020) as an adjunct to diet and maximally tolerated statin therapy in adults with HeFH, or established ASCVD. In the European Union, Bempedoic acid was approved for use in adults withprimary hypercholesterolemia or mixed dyslipidemia in combination with ezetimibe (March 2020) and as monotherapy (April 2020). A summary of the timeline for this drug developmentis shown in Figure 2. On April 6, 2020, the European Commission approved the use of Bempedoic acid for use in adults with primary hypercholesterolemia, as an adjunct to statin therapy, or as an alternative to statin therapy in those who are statin intolerant or in who statintherapy is contraindicated.

Figure 2 Timeline for the development of Bempedoic acid

NOTE : Industry time is the time during which the company or industry was developing thedrug and FDA refers to all the steps that were performed to get FDA approval



**BIOANALYTICAL TESTING AND CLINICAL TRIALS**

This report details new bioanalytical methods for Bempedoic acid and ESP15228 in human plasma and urine based on mixed mode solid phase extraction and UHPLC with negative ion electrospray tandem mass spectrometry. The methods were validated to regulatory standards and have been successfully applied to 26 clinical studies in the past 7 years. The concurrent development and validations of similar methods supporting toxicological studies have also been completed and will be reported separately.

**Reference standards**

ETC-1002, its phase I metabolite ESP15228, and their respective tetra-deuterated internal standards were synthesized and fully characterized in certificates of analysis by Ricerca Biosciences, Concord, OH, and supplied by Esperion Therapeutics. All were stored at room temperature. Structures are presented in Fig. 1, Fig. 2.

For stability testing and method development purposes, ESP15228-acyl-glucuronide was synthesized by Ricerca but not fully characterized. ETC-1002 acyl glucuronide was **Extraction from plasma**

A prior unpublished method employed positive ion electrospray LC-MS/MS and Oasis HLB solid phase extraction based on hydrophobicity. The extraction wash solutions were acidic and highly aqueous to facilitate retention. The method was sometimes inaccurate in lipemic plasma samples due to ion suppression effects, and the extraction and chromatographic separation modes were similar rather than complementary. The mixed mode extraction presented here used both aqueous and organic wash solutions and Robust methods for the measurement of ETC-1002 and its oxidized metabolite ESP15228 were developed to support clinical trials designed to studythe safetyand efficacy of Bempedoic acid for the treatment of hyperlipidemia. Strong anion exchange solid phase extraction and negative ion electrospray LC-MS/MS analysis were the basis for selective methods in human plasma and urine .The methods require 125–150 µL of sample and the entire procedure is executed in 96-well plates.

**CLINICAL TRIALS**

PHASE I AND II:

Fifteen phase I clinical trials evaluated the safety and tolerability of single and multiple doses of Bempedoic acid in healthy individuals as well as those with mild dyslipidemia.16–18 The results from phase I trials demonstrated on-treatment reductions in LDL-C levels ranging between 17% and 36%. No dose-related side effects were noted in phase I clinical trials.16–18 Ten Phase II clinical trials investigated the safety and efficacy of Bempedoic acid in patients with hypercholesterolemia. Specific subpopulations explored in the phase II trials include patients with type 2 diabetes, statin intolerance, hypertension, on a background of either low- or high- intensity statin therapy, on a background of ezetimibe with and without statin therapy, and patients on a PCSK9 inhibitor (evolocumab).19–27 Bempedoic acid was generally well tolerated across these trials and resulted in LDL-C lowering that ranged from 13% to 43%.19–27.

PHASE III TRIALS

The efficacy and safety of Bempedoic acid among patients with HEFH was assessed in two placebo-controlled Phase 3 studies: Cholesterol Lowering via Bempedoic Acid, an ACL- Inhibiting Regimen (CLEAR) Wisdom and CLEAR Harmony.

CLEAR Harmony randomized patients with ASCVD with or without the presence of HEFH. Atotal of 2230 patients with LDL-C levels ≥70 mg/dL taking maximally tolerated statin therapy were randomized to receive either Bempedoic acid at a dose of 180 mg daily (n = 1487) vs placebo (n = 742) and followed for 52 weeks with a primary endpoint of safety and a secondary endpoint of percent change in LDL-C at week 12. It should be noted in this trial, that nearly 100% of the patients were taking statins and approximately 50% were on a high-intensity statin. At week 12, Bempedoic acid demonstrated a 16.5% reduction in LDL-C compared to baseline and an 18.1% reduction compared to the placebo arm ($p<0.001$). Furthermore, there was an 11.9%reduction in apoB and a 21.5% reduction in high-sensitivity C-reactive protein (HSCRP) at week 12 compared to baseline ($p<0.001$ for both). These data confirmed the safety and efficacy of Bempedoic acid as an add-on to statin therapy regardless of background statin intensity.

CLEAR Wisdom enrolled adults with ASCVD with or without HEFH and LDL-C level ≥70 mg/dL on maximally tolerated lipid-lowering therapy. The study randomized 779 patients to either 180 mg Bempedoic acid or placebo once daily for a total of 52 weeks. The primary endpoint was percent change

in LDL-C from baseline at week 12 of the study. Baseline LDL-
C levels were 120 mg/dL, and treatment with Bempedoic acid

resulted in a 15.1% decrease in LDL-C levels at week 12 compared to baseline levels. Reductions were also noted in total cholesterol(TC) (9.9%), ApoB (9.3%), and HSCRP (median reduction of 18.7%). Patients receiving Bempedoic acid vs placebo experienced a reduction of 15.1% vs a 2.4% increase, respectively (difference of 17.4%, p <0.001).

Pooled analyses from CLEAR Harmony and CLEAR Wisdom included a total of 3009 participants with ASCVD, of which 112 (3.7%) had a diagnosis of HEFH. The primary efficacy endpoint was mean percent change in LDL-C compared with baseline at week 12. Characteristics of patients from the pooled analysis with and without HEFH are shown in Table 1. Baseline LDL-C levels were higher in individuals with HEFH (n = 112; Bempedoic acid, 144.1 [45.1] mg/dL; placebo, 156.5 [69.2] mg/dL) compared to those without HEFH (n = 2897; Bempedoic acid, 106.3 [30.9] mg/dL; placebo, 105.6 [30.0] mg/dL). At week 12, patients who received Bempedoic acid exhibited a significant reduction in LDL-C levels with placebo- corrected reduction of −22.3% (95% CI −33.3, −11.4) vs −18.3% (95% CI −18.3, −16.6) among individuals with and without HEFH, respectively (P <0.001).

| CHARACTERISTICS | BEMPEDIOC ACID | PLACEBO |
|---|---|---|
| **N** | **76** | **36** |
| AGE (mean ± SD)Female, n(%) White race, n(%) ASCVD, n(%) Hypertension,n(%) | 58.3±10.8 | 56.1±10.6 |
| Diabetes ,n(%) | 41(54) | 22(61) |
| Baseline LDL- C(mg/dl)(mean ±SD) | 73(96) | 34(94) |
| Baseline statin use, n(%)Baseline ezetimibeuse, n(%) | 27(36) | 14(39) |
| | 37(49) | 21(58) |
| | 7(9) | 4(11) |
| | 144.1±45.1<br>71(93)<br>35(46) | 156.5±59.2<br>31(86)<br>16(44) |

**Table 2** Secondary Efficacy Endpoints in Patients with and without Heterozygous Familial Hypercholesterolemia in Pooled Phase III Analysis

| | HeFH | | | No HeFH | | |
|---|---|---|---|---|---|---|
| | Bempedoic Acid | Placebo | P value | Bempedoic Acid | Placebo | P value |
| Non-HDL-C | −15.8 (2.3) | 2.1 (4.5) | **<0.001** | −11.9 (0.4) | 1.7 (0.7) | **<0.001** |
| Total Cholesterol | −14.2 (1.8) | 1.8 (3.8) | **<0.001** | −10.4 (0.3) | 0.9 (0.5) | **<0.001** |
| ApoB | −11.7 (2.4) | 3.8 (3.7) | **<0.001** | −9.1 (0.4) | 3.5 (0.6) | **<0.001** |
| HsCRP mean % change Q1,Q3 | −18.8 (−47.6, 20.0) | 4.3 (−14.4, 42.1) | **0.03** | −21.1 (−49.4, 23.3) | −1.9 (−34.8, 53.6) | **<0.001** |
| HDL-C mean % change (SD) | −8.3 (17.3) | 0.8 (10.1) | NS | −6.0 (14.2) | −0.2 (12.1) | NS |

Secondary efficacy endpoints included percent change at week 12 in non–high-density lipoprotein cholesterol (non-HDL-C), TC, ApoB, HSCRP, triglycerides (TGs), and HDL-C levels. Significant reductions were noted in non-HDL-C, TC, ApoB, and HSCRP in patients with HEFH treated with Bempedoic acid vs placebo (Table 2). Additionally, no differences were observed in the safety profile among individuals from CLEAR Harmony and CLEAR Wisdom with and without HEFH with regard to adverse events, serious adverse events, and adverse events leading to discontinuation of the drug.

The CLEAR Serenity trial examined Bempedoic acid in patients with statin intolerance. This trial enrolled 345 patients with hypercholesterolemia and a history of statin intolerance; 93% with a history of SAMS. This trial included 2% (7 out of 345) of patients with HEFH. At 12 weeks, Bempedoic acid compared to placebo reduced LDL-C by 21% and HSCRP by 24%. Myalgias occurred less commonly with Bempedoic acid than placebo (4.7% vs 7.2%). There was no interaction between the primary outcome of change in LDL-C from baseline to week 12 by patient subgroups identified as primary prevention or secondary prevention/HEFH. Thesedata support safety and efficacy of Bempedoic acid among patients who cannot tolerate statins.

Bempedoic acid has also been evaluated in combination with ezetimibe in patients with hypercholesterolemia. Out of 301 patients in the primary analysis (108 of which were randomized to the Bempedoic acid ezetimibe fixed-dose combination), use of the fixed-dose combination of Bempedoic acid 180 mg and ezetimibe 10 mg daily reduced LDL-C by 36.2%compared with Bempedoic acid alone (−17.2%), ezetimibe alone (−23.2%) or placebo (+1.8%). Thus, the placebo-corrected difference for this fixed-dose combination was −38.0%; P <0.001. Similar LDL-C reductions were seen across varying intensity of statins. The fixed-dose combination regimen also reduced HSCR by 35.1%, which was greater than ezetimibe alone(−8.2%) or placebo (+21.6%). These data suggest that Bempedoic acid and ezetimibe are moreeffective together in an additive manner, and the fixed-dose combination may be an attractiveoption for patients to reduce their overall pill burden.

While LDL-C reduction is the primary target of therapy, there has been a consistently noted reduction in HSCRP with Bempedoic acid. The Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) showed that a therapy (canakinumab) that lowered inflammation (HSCRP) without influencing lipid levels also lowered ASCVD events. Although canakinumabwas ultimately not given FDA label approval for cardiovascular event reduction, CANTOS was a landmark study in proving the concept that targeting inflammation can reduce cardiovascular events in high-risk patients.

## NEW APPROACHES IN DRUG DISCOVERY

### Introduction

For years, the mainstay of lipid-lowering therapy has been medications in the HMG-CoA reductase (statin) drug class. Known to significantly decrease low-density lipoprotein cholesterol (LDL-C), raise high-density lipoprotein (HDL-C), and lower triglycerides (Tg), alongside their pleiotropic effects, statins are highly recommended in diseases related to atherosclerotic cardiovascular disease (ASCVD) or familial hypercholesterolemia (FH).[1]

The term *ASCVD* refers to CVD caused by plaque buildup on the arterial walls and linked to hyperlipidemia and hypertriglyceridemia.[1] It typically encompasses cardiovascular events suchas myocardial infarction, sudden cardiac death, and ischemic stroke, as well as peripheral artery disease, coronary artery disease, or a need for arterial revascularization. FH is an autosomal dominant trait that causes a decreased binding of LDL-C to LDL receptors. The binding of LDL-C to LDL receptors results in internalization and degradation of LDL-C, thus resulting ina reduction in its circulating levels.[2]

The 2018 multiorganization guidelines on cholesterol management directly addressed the secondary prevention of ASCVD (ie, in patients with established, or “clinical,” ASCVD), while indirectly addressing heterozygous FH (HEFH) via patients with severe primary hypercholesterolemia (LDL-C ≥190 mg/dL).[1] Statins are considered to be first-line treatment agents for both disease states. However, up to 40% of patients may not succeed in reaching their lipid goals on statins alone.[3] The current guidelines recommend 2 agents as potential add- to maximum tolerated–dose statins: ezetimibe and proprotein convertase subtilisin/kexin type 9 (PCSK-9) inhibitors.[1] Ezetimibe offers lipid-lowering benefits similar to those of moderate- intensity statins and is a preferred alternative/add-on therapy due to a lower cost andoral formulation. Even though PCSK-9 inhibitors are significantly efficacious in lowering LDL-C, they are less often used due to cost and the burden from their injection method of administration.[1] Therefore, there is still a need for additional oral options for lipid-lowering therapy.

In February 2020, the use of Bempedoic acid∗ was approved as an adjunct to diet and maximum tolerated–dose statin therapy along with diet and exercise for reducing LDL-C in the treatment of HEFH or established ASCVD.[4] Along with this approval, the use of combination Bempedoic acid/ezetimibe† was also approved.[5] The present article discusses the pharmacology of Bempedoic acid; the trials that led to the Food and Drug Administration (FDA) approvals of these therapies; the tolerability and efficacy of these therapies; and the implications for the treatment of hypercholesterolemia, ASCVD, and HEFH.

## MATERIALS AND METHODS:

Trials from the CLEAR series were selected, as they played a pivotal role in the establishment of FDA approval, along with additional trials published after FDA approval, which provided novel evidence on the use of Bempedoic acid in the treatment of hypercholesterolemia. Publications that were not randomized, controlled trials were not included in this review. Only randomized controlled trials in which ezetimibe was used in conjunction with Bempedoicacid were included in this review as they were relevant to the new FDA approval of Bempedoicacid.

## FINDINGS

The findings of the present review show that Bempedoic acid is both an effective and well-tolerated option for the treatment of hypercholesterolemia when used without ezetimibe in addition to standard therapy. It also appears that the combination with ezetimibe increases the cholesterol-lowering effect more than either agent alone when added to standard therapy.

## IMPLICATIONS

Hypercholesteremia continues to be a major contributing factor leading to ASCVD. Bempedoic acid is an additional treatment option, along with both statins and diet and exercise, for reducing cholesterol levels and ASCVD events. With the new FDA approval, Bempedoic acidmay offer an effective therapy for reducing low-density lipoprotein cholesterol in patients at high risk for cardiovascular events due to established ASCVD or heterozygous familial hypercholesterolemia.

## DISCUSSION

The CLEAR trials9, 10, 11 showed a net benefit in adding Bempedoic acid to current cholesterol-lowering therapy to further lower LDL-C in patients at high risk for CVD. Ezetimibe may lower LDL-C by ~25% compared to ~16% with Bempedoic acid when used as add-on therapy with statins.[8,15] Ballantyne et al[16] reported a −17.2% reduction in LDL in the Bempedoic acid group, whereas the ezetimibe group saw a −23.2% reduction. This finding is in contrast to those in the monotherapy arms of a Phase III

## CONCLUSSION

In patients at high risk for cardiovascular events due to established ASCVD or HEFH, Bempedoic acid alone or in combination with ezetimibe can be considered as add-on therapy with statins along with diet and exercise for a reduction in LDL-C. It is likely that Bempedoic acid will be considered ahead of the PCSK9 inhibitors due to cost, but behind ezetimibe alone due in equal parts to lesser efficacy and fewer overall efficacy data. This being said, Bempedoicacid/ezetimibe combination therapy.

THE CPCSEA (THE COMMITTEE FOR THE PURPOSE OF CONTROL ANDSUPERVISION OF EXPERIMENTS ON ANIMALS ) GUIDELINES FOR THE CAREAND USE OF LABORATORY ANIMALS :

Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and safety of animals used in laboratory studies while conducting biomedical and behavioral research and testing of products.

## GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioral research and testing with the basic objective of providing specifications that wil enhance animal wellbeing, quality in the pursuit of advancement of biological knowledge thatis relevant to humans and animals.

## VETERINARY CARE

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour, and well-being is conveyed to the attending veterinarian. The veterinarian can also contribute to the establishment of appropriatepolicies and procedures for ancillary aspects of veterinary care, such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

## ANIMAL PROCUREMENT

All animals must be acquired lawfully as per the

CPCSEA guidelines. A health surveillance program for screening incoming animals should be carried out to assess animal quality. Methods of transportation should also be taken into account (Annexure – 4). Each consignmentof animals should be inspected for compliance with procurement specifications, and the animals should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

**QUARANTINE, STABILIZATION AND SEPARATION**

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. A minimum duration of quarantine for small lab animals is one week and larger animals is 6 weeks (cat, dog and monkey) Effective quarantine procedures should be used for non-human primates to help limit exposure of humans to zoonotic infections. Regardless of the duration of quarantine, newly received animals should be given a period forphysiologic, psychologic and nutritional stabilization before their use and the intended use of the animals. Physical separation of animals by species is recommended to prevent interspecies disease.

**SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE**

All animals should be observed for signs of illness, injury, or abnormal behavior by animal house staff. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis (Annexure 1 and 2). Unexpected deaths and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of acontagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (e.g. Mycobacterium tuberculosis in non-human primates), the group should be kept intact and isolated during the process of diagnosis, treatment, and control. Diagnostic clinical laboratory may be made available.

**ANIMAL CARE AND TECHNICAL PERSONNEL**

Animal care programs require technical and husbandry support. Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training to ensure effective implementation of the program .

**PERSONAL HYGIENE**

It is essential that the animal care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided e.g. showers, change of uniforms, footwears etc. Clothing suitable for use in the animal facility should be supplied andlaundered by the institution. A commercial laundering service is acceptable in many situations; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. In some circumstances, it is acceptable to use disposable wear such as gloves, masks, head covers, coats, coveralls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility. Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics in animal rooms. A separate area or room should be made available for these purposes.

**ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS**

Institutions should have policies governing experimentation with hazardous agents. Institutional Biosafety Committee whose members are knowledgeable about hazardous agentsare in place in most of the higher level education, research institutes and in many pharmaceutical industries for safety issues. This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions . Since the use of animals in such studies requires special consideration, the procedures and the facilities to be used must be reviewed by both the Institutional Biosafety Committee and Institutional Animal Ethics Committee (IAEC).

**MULTIPLE SURGICAL PROCEDURES ON SINGLE A SINGLE ANIMAL :**

Multiple surgical procedures on a single animal for any testing or experiment are not to be practiced unless specified in a protocol only approved by the IAEC. DURATIONS OF EXPERIMENTS No animal should be used for experimentation for more than 3 years unless adequate justification is provided

**PHYSICAL FACILITIES**

**(a)** Building materials should be selected to facilitate efficient andhygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

**(b)** Corridor(s) should be wide enough to facilitate the movement of personnel as well as equipment's and should be kept clean.

**(c)** Utilities such as water lines, drain pipes and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

**(d)** Animal room doors Doors should be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities. **(e)** Exterior windows Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate source of light and ventilation. In primate rooms, windows can be provided.

**(f)** Floors should be smooth, moisture proof, nonabsorbent, skid-proof, resistant to wear,acid, solvents, adverse effects of detergents and disinfectants. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints. A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passageof equipment.

**(g)** Drains Floor drains are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces.

**(h)** Walls and ceilings Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners. Surface materials should be capable of withstanding scrubbing with detergents and disinfectants and the impact of water under high pressure.

**(i)** Storage areas Separate storage areas should be designed for feed, bedding, cages and materials not in use. Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste.

**(j)** Facilities for sanitizing equipment and supplies An area for sanitizing cages and ancillary equipment is essential with adequate water supply Experimental area All experimental procedures in small animals should be carried out in aseparate area away from the place where animals are housed. For larger animal functional areas for aseptic surgery should include a separate surgical support area, a preparation area, the operating room or rooms, and an area for intensive care and supportive treatment of animals

**ENVIRONMENT**

(a) Temperature and humidity control Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions or differences in the number and kind of room occupants. Ideally, capability should be provided to allow variations within the range of approximately 18 to 29°C (64.4 to 84.2øF), which includes the temperature ranges usually recommended for common laboratory animals. The relative humidity should be controllable within the range of 30% to 70% throughout the year. For larger animals a comfortable zone (18 to 37°C) should be maintained during extreme summer by appropriate methods for cooling.

(b) Ventilation In renovating existing or in building new animal facilities, consideration shouldbe given to the ventilation of the animals' primary enclosures. Heating, ventilating, and air- conditioning systems should be designed so that operation can be continued with a standby system. The animal facility and human occupancy areas should be ventilated separately.

(c) Power and lighting The electrical system should be safe and provide appropriate lighting and a sufficient number of power outlets. It is suggested that a lighting system be installed that provides adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals. Fluorescent lights are efficient and available in a variety of acceptable fixtures. A time-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required. Emergency power should be available in the event of power failure.

(d) Noise control The facility should be provided

with noise free environment. Noise control is an important consideration in designing an animal facility. Concrete walls are more effective than metal or plaster walls in containing noise because their density reduces sound transmission.

**Anesthesia**

Unless contrary to the achievement of the results of study, sedatives, analgesics and anesthetics should be used to control pain or distress under experiment. Anesthetic agents generally affect cardiovascular, respiratory and thermo-regulatory mechanism in addition to central nervous system. Before using actual anesthetics the animal is prepared for anesthesiaby overnight fasting and using pre-anesthetics, which block parasympathetic stimulation of cardio- pulmonary system and reduce salivary secretion. Atropine is the most commonly used anticholinergic agent. This should be carried out under expert supervision for regional infiltration of surgical site, nerve blocks and for epidural and spinal.

anesthesia. A number of general anesthetic agents are used in the form of inhalants. General anesthetics are also used in the form of intravenous or intramuscular injections such as barbiturates. Species characteristics and variation must be kept in mind while using an anesthetic. Side effects such as excessive salivation, convulsions, excitement and disorientation should be suitably prevented and controlled. The animal should remain under veterinary care till it completely recovers from anesthesia and postoperative stress.

**Euthanasia**

Euthanasia is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting an euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species ofanimal to be killed . The method should in all cases meet the following requirements:

**(a)** Death, without causing anxiety, pain or distress with minimum time lag phase.

**(b)** Minimum physiological and psychological disturbances.

**(c)** Compatibility with the purpose of study and minimum emotional effect on the operator.

**(d)** Location should be separate from animal rooms and free from environmental contaminants. Tranquilizers have to be administered to larger species such as monkeys, dogs and cats beforean euthanasia procedure

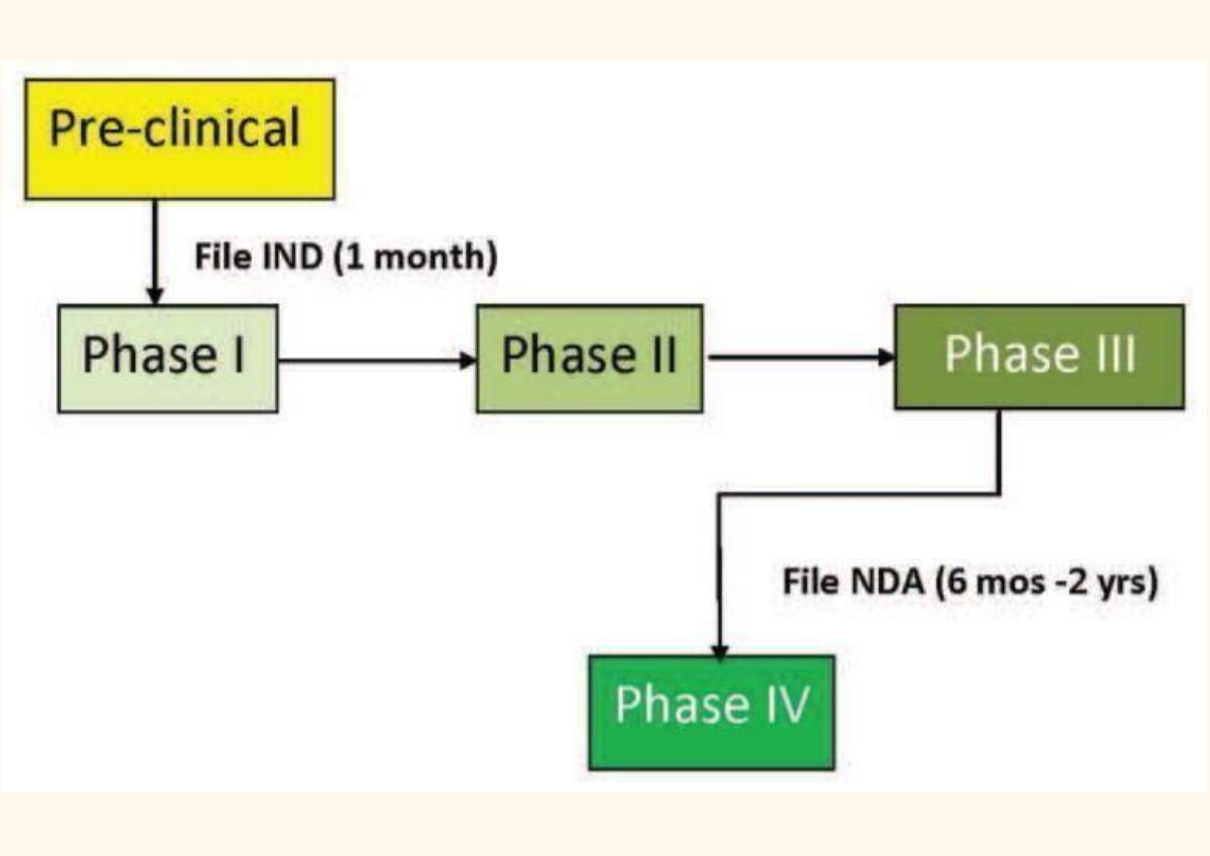
**PRE-CLINICAL STUDY**

Bempedoic acid (initially known as ESP-55016 and later as ETC-1002) was initially identified after chemical synthesis as ω-hydroxy-alkane dicarboxylic acid (ESP-55016) and found to haveremarkable lipid-lowering activity in female Zucker (fa/fa rats), a model of diabetic dyslipidemia.12 Further investigations on ETC-1002 showed that this molecule acts as an inhibitor of ACL, now known to be its major mode of action.13 In view of its complex mechanism, specific investigations on its properties were performed in primary human monocyte macrophages and in vivo models of inflammation, which showed that Bempedoic acid drives reduction in proinflammatory cytokines and chemokines.14 More recent investigations were specifically targeted to animal models with hypercholesterolemia. For instance, Bempedoic acid was shown to significantly reduce cholesterol and triglyceride levels as well as improve glucose tolerance in LDLR-/- mice, in addition to a clear suppression of cholesterol ester accumulation in the aortas from treated animals.15Finally, the most definitive evidence of ACL inhibition as the mechanism of action was provided by pinkosky et al.11 Bempedoic acid is activated by very-long chain acyl-CoA synthetase-1 (ACSVL1) to produce its downstream effects of inhibiting ACL, thereby reducing visceral adiposity, proinflammatory cytokines and chemokines, and atherosclerotic plaque size in mouse models.

**BIO STATISTICS IN PRE- CLINICAL STUDIES**

The development of new therapy for a particular disease from concept to market is an extensiveprocess that is costly in terms of time, effort and finances. The process starts with preclinical studies involving in vitro (e.g., tissue culture studies) and in vivo (animal studies) experiments in a laboratory. When the required information and results are obtained from preclinical studies, an Investigational New Drug (IND) application is submitted to the Food and Drug Administration (FDA) accompanied by the results of the preclinical studies. Researcher are allowed to conduct studies in humans only after receiving an approved IND.

Human studies start at Phase I where human volunteers are recruited with the goal of obtaining information about the side effects of the drug, and in some cases, determining the maximum tolerated dose. Phase II begins after Phase I study shows no issues with toxicity. The goal of a Phase II study is to obtain preliminary information that will show some indication of effectiveness and safety of the drug applied to the population with the disease targeted by the new therapy. After successful completion of the Phase II study, a large-scale Phase III clinical trial is conducted with the goal of establishing evidence of effectiveness in a broader and larger population as well as collecting additional information about safety. Upon successful completion of Phase III, a New Drug Application (NDA) is filed to the FDA to obtain approval to market the drug. In the NDA, results from the animal studies and human studies (phases I– III) are reviewed by FDA before giving the final stamp of approval. The last phase of the drug development (Phase IV) is post-marketing surveillance. Figure 1 summarizes the different stages in drug development.




Stages of Drug Development

Clearly, preclinical studies being the first stage in the process play a crucial role in drug development. Unfortunately, a high proportion of these preclinical studies conducted on animals that indicated some therapeutic effect do not translate to similar results in studies in humans. This issue is mostly attributed to poor planning, conduct and reporting of most preclinical studies (see for instance Perin, 2014; Warner et al., 2014; Henderson et al., 2013; Landis et al., 2012; and Kilkenny et al., 2009) Consequently, the National Institute of Neurological Diseases calls for more rigorous reporting of these studies to raise awareness on the proper design and conduct of future preclinical studies as well as the proper interpretation of the results of completed studies (Landis et al., 2012). In line with this goal, this paper aims to review some of the basic statistical elements of clinical trials which will help researchers understand and appreciate the relevance of these concepts in the context of preclinical studies.

## STUDY PLANNING AND CONDUCT

The details of how the study will be conducted relies

heavily on the question. Without a well- defined question or hypothesis, the study will most likely result in a "fishing exploration". Given the question of interest, primary outcome can be defined and appropriate study design can be chosen. The number of primary outcomes should be kept at a minimum; the ideal case would be to have only one primary outcome. However, this may not be possible in some cases. For instance in myasthenia gravis (MG) animal studies, therapeutic effect may be reflected in different aspects such as change in strength, weight, disease severity, serum cytotoxicity and acetylcholine receptor (ACHR) antibody concentration to name a few. Having multiple outcomes as contrasted to single outcome will have consequences in the sample size calculationand data analysis.

**DESIGN AND SAMPLE SIZE**

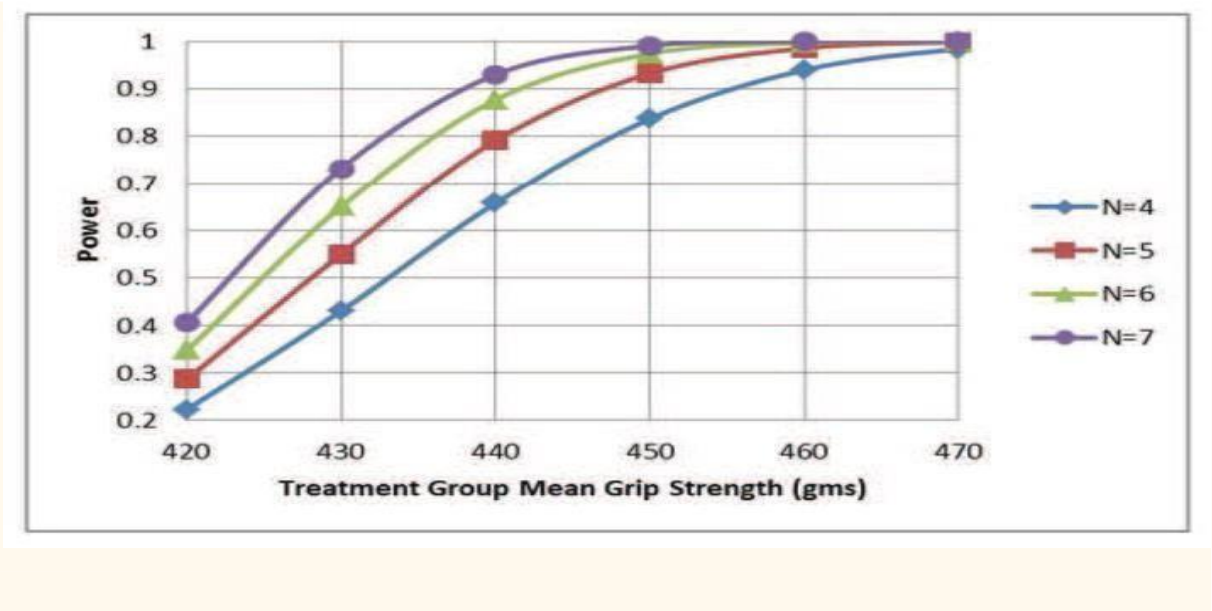
Design of the study, characteristics of the outcome, and the number of outcomes are some factors that affect the determination of the appropriate sample size. Researchers must carefully consider the choice of study design based on the question of interest. They must aim to use thesimplest appropriate design as study design dictates the method of data analysis and interpretation, and the method of data analysis dictates the method of calculating sample size. The most popular design used is the parallel group design where different animals are used in each of the *M* treatment group. The simplest of this design is the case where there are only twogroups, i.e, $M = 2$, for example comparing the outcome of untreated group to the outcome of the group treated with a new drug. The analysis associated with this design is typically a t-testfor two independent samples when the outcome follows a normal distribution or a Fisher's exact test (or chi-squared test for large samples) to compare two proportions when the outcome is a binary variable (e.g., with improvement or no improvement). Increasing the number of groups to compare, say from 2 to 3, will increase the required sample size. Designs such as cross-over design, where each animal serves as their own control, will require smaller sample size than parallel design but it has other requirements that may not be feasible for some experiments (for instance, cases where animals are euthanized to obtain the outcome of interest). Having multiple primary outcomes which then result in multiple statistical testing in the data analysis stage will require larger sample size relative to a single outcome due to the required adjustments necessary to avoid inflation of the false positive error rates. When the outcome is binary (e.g., compare the proportion showing improvement between the treated and untreated group), a larger sample will be required compared to the case where the outcome is continuous (e.g., measuring actual weight or strength). Also, the case where one of the two binary outcomes is rare in both groups will require a larger sample size than a case where both possibilities are common. When the outcome of interest is the time to occurrence of an event where methods of data analyses are based on survival analyses, power is highly dependent on the expected number of events for a given period of time in addition to the overall sample size, and the number of events that will be observed is highly dependent on the length of follow-up time. Censored observations, i.e., outcomes of subjects who did not experience the event due to drop-out or end of follow-up, are not uncommon in survival analysis studies. However, the higher percent of censoring the less amount of information is available resulting in lower power to detect a given effect size. Therefore, power can be increased while keeping the sample size and effect size constant by increasing the follow up time that will result in an increase in the expected number of observed events in that period. Note that although it may be of clinical interest to model a continuous outcome as the time for it to reach a certain cutoff point and use survival analysis methods, doing so sacrifices a large amount of statistical efficiency (e.g., loss of power) and thus should be avoided .

We illustrate the process of sample size determination for a single continuous outcome based on a two-tailed t-test for comparing two independent sample (e.g., untreated versus treated groups). For an MG study, one may be interested in the grip strength as the outcome. Assuming grip strength (in grams) follows a normal distribution, we examine how the power changes across different scenarios. We set the significance level at 5%, the mean grip strength of the placebo group at 400 and standard deviation of 20 (common between the treatment groups). The effect size is defined as the difference between the mean grip strength of the untreated andtreated group. Figure 2 shows the power for the different values of the mean of the treatment group for a given group sample size. [Calculations done using PASS 11 software . As the meanof the treated group increases relative to the mean of the untreated group, the effect size also increases, and the sample size necessary to achieve a fixed desired power level decreases. Another way of looking at this is that for a fixed sample size, the power increases as the effect size increases. These observations are intuitive because a larger difference is easier to detect than a smaller difference.

Figure 2 may also be used to conclude that 5 rats in each group is sufficient to detect an effectsize of 40

grams (i.e., an increase in expected grip strength from 400 to 440 grams) or more with power of about 80%. Although not shown, increasing the value of the assumed common standard deviation (fixing all other parameters) will require larger sample size because a larger standard deviation implies more noise in the outcome which makes it more challenging to detect a given treatment effect. Increasing the sample size will compensate for the increase in noise and achieve the desired power.

We highly recommend that researchers investigate different scenarios in a manner similar to this illustration when determining the sample size so that they are aware of what to expect from the study if an assumption about a parameter is not correct.




Power Analysis for comparing two independent groups: two-tailed 5% significance level t-test assuming a common standard deviation of 20 grams and a mean of 400 grams for untreated group

Using the above illustration, suppose that we categorize the actual grip strength as at or above a certain cut-off. Thus, the primary outcome is changed from the actual grip strength to a binary "success" or "failure" based on a target grip strength. Assuming the same normal distribution for actual grip strengths in each group as described in the preceding illustration (i.e., mean of 400 grams in placebo group and 440 grams in treated group with common standard deviation of 20 grams), the sample size required to test the proportion of animals demonstrating the target grip strength depends on the cut off. If the target grip strength is defined as 430 grams, then it is expected that 6.68% of the placebo and 30.85% of the treated group will demonstrate a gripstrength of at least 430 grams.

To detect a difference of 24.17% (=30.85–6.68) or higher in proportion of animals demonstrating this target grip strength, using a two-tailed large-sample z-test for two independent proportions with 5% significance level and power of at least 80%, the number of animals in each group assuming equal allocation is calculated to be 37 requiring a total of 74 animals (using PASS 11). This is a case in point of binary outcomes requiring larger sample size than a continuous outcomes – compare 74 animals required for this binary outcome example to the 10 animals that we previously calculated when the primary outcome is the actual grip strength. If the target is decreased to 425 grams, the proportions of animals reaching this target change to 10.56% in the placebo and 22.33% in the treated groups. The sample size required in each group increases to 148

(a total of 296 animals) because the effect size to be detected is down to 11.77%.

Although sample size is usually associated with power, there may be preclinical studies for which the goal is to estimate the treatment effect on a particular outcome (for instance, AChR concentration level) as preliminary data for subsequent preclinical studies. In this case, sample size calculation will be based on a confidence interval around the treatment effect. Instead of power, the goal is to minimize the margin of error of the confidence interval for a fixed confidence level (typically at 95% confidence level).

As a final note for this section, researchers must avoid using rules of thumb for sample sizes. As an example, in regression modeling, a commonly used rule of thumb is to have 10 observations per predictor variable so that if one is interested in testing 5 predictor variables, 50 observations must be obtained. The problem with rules of thumb is that, in most cases, there is no theoretical justification for the rule. Referring back to the regression model example, one should define the main goal to determine the appropriate sample size. For instance, is the goal to find predictors, or is the goal to accurately predict the outcome? Sample size for regression depends on the variability of the outcome, the number of predictor variables of interest, the number of covariates to be adjusted for, and the degree of correlation among the variables (outcom0e, predictors and covariates) to name a few. A researcher must work with a biostatistician in determining the best approach to determine and justify the sample size based onthe specific aim of the study.

**RANDOMIZATION AND BINDING**

Another important aspect of study design for a study with the goal of comparing different groups is the process of treatment assignment. To avoid selection bias and minimize confounding of treatment effect with covariates (measured or unmeasured), treatment assignment must be done in a random manner. Consider a study that compares two treatment groups. Figure 3 displays 3 different schemes (S1, S2 and S3) to assign the treatments (new drug=T, control=C). Schemes S1 and S2 are not acceptable because they follow a predictable pattern, and hence may still result in selection bias. Scheme S1 also has the problem that treatment effects will be confounded with the conditions in the laboratory during the early/later part of the study. Scheme S3 follows no predictable pattern and the treatment and control are distributed across the study period, and hence, a good treatment assignment scheme produced through randomization.

| Scheme | SUBJECT NUMBER | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | |
| S1 | T | T | T | T | T | T | T | T | T | T | C | C | C | C | C | C | C | C | C | C | NOT GOOD |
| S2 | C | T | C | T | C | T | C | T | C | T | C | T | C | T | C | T | C | T | C | T | NOT GOOD |
| S3 | T | T | T | C | T | C | C | T | T | T | T | C | C | C | T | C | C | C | C | T | GOOD |

Sample treatment assignment schemes

Randomization is a process of assigning treatment to subjects as they are enrolled in the study in a non-subjective and unpredictable manner. Randomization scheme must be determined ahead of time and must only be known to the staff who would not be involved in the laboratory. Laboratorystaff in charge of giving the treatment will only be aware of the treatment assignment(s) at the time that the animals are ready to be treated. There are variations in randomization schemes to accommodate availability of animals. One of these variations is stratified randomization. For instance, if animals arrive in batches, researchers need to make sure that treatment types are well represented in each batch or group.

Stratified randomization creates a different randomization scheme for each batch (i.e., stratum) as if each batch is a different study and will be more appropriate than the

In addition to avoiding or minimizing selection bias with randomization of treatment, observer bias must also be minimized.

Observer bias typically occurs when the outcome of interest is measured in a more subjective manner. In MG preclinical studies, an observer bias may influence the assessment of strength or disease severity. To minimize observer bias, the person evaluating the outcome and providing dose (if not fixed ahead of time) must be blinded to the treatment group.

**REFERENCES:**


1. Khera AV, Hegele RA. What is familial hypercholesterolemia, and why does it matter? Circulation. 2020;141(22):1760–1763. doi:10.1161/CIRCULATIONAHA.120.046961

2. Michos ED, McEvoy JW, Blumenthal RS. Lipid management for the prevention of atherosclerotic cardiovascular disease. N Engl J Med. 2019;381(16):1557–1567. doi:10.1056/NEJMra1806939

3. Cannon CP, Blazing MA, Giugliano RP, et al. Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med. 2015;372(25):2387–2397. doi:10.1056/NEJMoa1410489

4. Robinson JG, Farnier M, Krempf M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med. 2015;372(16):1489–1499. doi:10.1056/NEJMoa1501031

5. S.M. Grundyetal.**2018**AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APHA/ASPC/ NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American heart association task force on clinical practice guidelines

6. J Am Coll Cardiol (**2019)** S.M. Boekholdt et al. Very low levels of atherogenic lipoproteins and the risk for cardiovascular events: a meta analysis of statin trials

7. J Am Coll Cardiol **(2014 )**C. Ballantyne et al. Efficacy and safety of Bempedoic acid added to ezetimibe in statin-intolerant patients with hypercholesterolemia: a randomized, placebo-controlled study Atherosclerosis

8. C. Gagné et al. Efficacy and safety of ezetimibe added to ongoing statin therapy for treatment of patients with primary hypercholesterolemia

9. Am J Cardiol **(2002)**P. Thompson et al. Treatment with ETC-1002 alone and in combination with ezetimibe lowers LDL cholesterol in hypercholesterolemic patients with or without statin intolerance

10. J Clin Lipidol (**2016)**A.A.A. Asbeutah et al. A meta-analysis of cardiovascular outcomes in patients with hypercholesterolemia treated with Bempedoic acid.

11. Am J Cardiol **(2020)** B. Sjouke et al. Homozygous autosomal dominant hyper cholesterolaemia in The Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome

12. Amore BM, Cramer CT, MacDougall DE, Sasiela WJ, and Emery MG (2021a) Absence ofeffect of steady state Bempedoic acid on cardiac repolarization: Results of a thorough QT/QTcstudy in healthy volunteers. Clin Transl Sci 14:2487–2496.OpenUrlGoogle Scholar

13. Amore BM, Sasiela WJ, and Emery MG (2021b) The effects of impaired hepatic function on pharmacokinetics of Bempedoic acid,

14,A first-in-class adenosine triphosphate (ATP) citrate lyase inhibitor, evaluated in an open-label, single-dose, parallel-group study (Abstract PIII-030). Clin Pharmacol Ther 109:S53.

15. Amore BM, Sasiela WJ, Ries DK, Tresh P, and Emery MG (2022) Pharmacokinetics of Bempedoic acid in patients with renal impairment. Clin Transl Sci 15:789–798.OpenUrlGoogleScholar

16. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J et al. (2010) Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet 376:1670–1681.OpenUrlCrossRefPubMedGoogle Scholar

17. Ballantyne CM, Banach M, Mancini GBJ, Lepor NE, Hanselman JC, Zhao X, and Leiter LA (2018) Efficacy and safety of Bempedoic acid added to ezetimibe in statin-intolerant patients with

hypercholesterolemia: A randomized, placebo-controlled study. Atherosclerosis 277:195–203.OpenUrlCrossRefPubMedGoogle Scholar

18. Bays HE, Banach M, Catapano AL, Duell PB, Gotto Jr AM, Laufs U, Leiter LA, Mancini GBJ, Ray KK, Bloedon LT, et al. (2020) Bempedoic acid safety analysis: Pooled data from four phase 3 clinical trials. J Clin Lipidol 1

19. Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, et al. Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. *PLoS ONE*. 2009;4(11):e7824. doi: 10.1371/journal.pone.0007824. 19.Landis SC, Amara SG.

20. Asadullah K, et al. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature.* 2012;490(7419):187–191.

21. Little RJA, Rubin DB. Statistical analysis with missing data. 2. New York: Wiley & Sons;2002.

22. Piddlesden SJ, Jiang S, Levin JL, et al. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. *J Neuroimmunol.* 1996;71:173177. [PubMed ] [Google Scholor ]

23. Soltys J, Kusner L, Young A, Richmonds C, Hatala D, Gong B, Shanmugavel V, Kaminski H. Novel Complement Inhibitor Limits Severity of Experimentally Myasthenia Gravis. Ann Neurol. 2009;65(1):6775.

24. Warner DS, James ML, Laskowitz DT, Wijdicks EF. Translational research in acute central nervous system injury: lessons learned and the future. *JAMA Neurol.* (71) 2014 Oct 1;(10):1311–8. [Pub med ] [ Google Scholor ]

25. PinkoskySL, FilippovS, SrivastavaRA, et al. AMP-activated protein kinase and ATP-citrate lyase are two distinct molecular targets for ETC-1002, a novel small molecule regulator of lipid and carbohydrate metabolism. *J Lipid Res*. 2013 34–151. doi:10.1194/jlr.M03052823118444

26. JeongHW, HsuKC, LeeJW, et al. Berberine suppresses proinflammatory responses throughAMPK activation in macrophages. *Am J Physiol Endocrinol Metab*. 2009; E955–964. doi:10.1152/ajpendo.90599.200819208854

27. SamsoondarJP, BurkeAC, Sutherl and BG, et al. Prevention of diet-induced metabolic dysregulation, inflammation, and atherosclerosis in LDLR(-/-) mice by treatment with the ATP-citrate lyase inhibitor Bempedoic acid. *Arterioscler Thromb Vasc Biol*. 2017:647–656. doi:10.1161/ATVBAHA.116.30896328153881

28. A Multiple Ascending Dose Study of ETC-1002 in healthy subjects; 2019. Available from Accessed 79, 2019.