Prospective evaluation of the efficacy, safety, and optimal biomarker enrichment strategy for nangibotide, a TREM-1 inhibitor, in patients with septic shock (ASTONISH): a double-blind, randomised, controlled, phase 2b trial

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Summary

Background Activation of the triggering receptor expressed on myeloid cells-1 (TREM-1) pathway is associated with septic shock outcomes. Data suggest that modulation of this pathway in patients with activated TREM-1 might improve survival. Soluble TREM-1 (sTREM-1), a potential mechanism-based biomarker, might facilitate enrichment of patient selection in clinical trials of nangibotide, a TREM-1 modulator. In this phase 2b trial, we aimed to confirm the hypothesis that TREM1 inhibition might improve outcomes in patients with septic shock.

Methods This double-blind, randomised, placebo-controlled, phase 2b trial assessed the efficacy and safety of two different doses of nangibotide compared with placebo, and aimed to identify the optimum treatment population, in patients across 42 hospitals with medical, surgical, or mixed intensive care units (ICUs) in seven countries. Non-COVID-19 patients (18–85 years) meeting the standard definition of septic shock, with documented or suspected infection (lung, abdominal, or urinary [in patients ≥65 years]), were eligible within 24 h of vasopressor initiation for the treatment of septic shock. Patients were randomly assigned in a 1:1:1 ratio to intravenous nangibotide 0·3 mg/kg per h (low-dose group), nangibotide 1·0 mg/kg per h (high-dose group), or matched placebo, using a computer-generated block randomisation scheme (block size 3). Patients and investigators were masked to treatment allocation. Patients were grouped according to sTREM-1 concentrations at baseline (established from sepsis observational studies and from phase 2a change to data) into high sTREM-1 (≥ 400 pg/mL). The primary outcome was the mean difference in total Sequential Organ Failure Assessment (SOFA) score from baseline to day 5 in the low-dose and high-dose groups compared with placebo, measured in the predefined high sTREM-1 (≥ 400 pg/mL) population and in the overall modified intention-to-treat population. Secondary endpoints included all-cause 28-day mortality, safety, pharmacokinetics, and evaluation of the relationship between TREM-1 activation and treatment response. This study is registered with EudraCT, 2018-004827-36, and Clinicaltrials.gov, NCT04055909.

Findings Between Nov 14, 2019, and April 11, 2022, of 402 patients screened, 355 were included in the main analysis (116 in the placebo group, 118 in the low-dose group, and 121 in the high-dose group). In the preliminary high sTREM-1 population (total 253 [71%] of 355; placebo 75 [65%] of 116; low-dose 90 [76%] of 118; high-dose 88 [73%] of 121), the mean difference in SOFA score from baseline to day 5 was 0·21 (95% CI –1·45 to 1·87, p=0·80) in the low-dose group and 1·39 (–0·28 to 3·06, p=0·104) in the high-dose group versus placebo. In the overall population, the difference in SOFA score from baseline to day 5 between the placebo group and low-dose group was 0·20 (–1·09 to 1·50; p=0·76),and between the placebo group and the high-dose group was 1·06 (–0·23 to 2·35, p=0·108). In the predefined high sTREM-1 cutoff population, 23 (31%) patients in the placebo group, 35 (39%) in the low-dose group, and 25 (28%) in the high-dose group had died by day 28. In the overall population, 29 (25%) patients in the placebo, 38 (32%) in the low-dose, and 30 (25%) in the high-dose group had died by day 28. The number of treatment-emergent adverse events (111 [96%] patients in the placebo group, 113 [96%] in the low-dose group, and 115 [95%] in the high-dose group) and serious treatment-emergent adverse events (28 [24%], 26 [22%], and 31 [26%]) was similar between all three groups. High-dose nangibotide led to a clinically relevant improvement in SOFA score (of two points or more) from baseline to day 5 over placebo in those with higher cutoff concentrations (≥532 pg/mL) of sTREM-1 at baseline. Low dose nangibotide displayed a similar pattern with lower magnitude of effect across all cutoff values.

Interpretation This trial did not achieve the primary outcome of improvement in SOFA score at the predefined sTREM-1 value. Future studies are needed to confirm the benefit of nangibotide at higher concentrations of TREM-1 activation.

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See Online for appendix

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Introduction

Septic shock is defined as a subgroup of patients with sepsis, persistent hypotension requiring vasopressor support, and an elevated serum lactate concentration despite adequate fluid resuscitation.[1,2] The recommendations for treatment of septic shock remain largely supportive[3] in spite of extensive efforts to develop new therapies.[4,5] Almost all novel therapeutic approaches that have shown promise in preclinical development have failed in clinical trials.[6] This has led to the recognition that conventional randomised controlled trial designs in sepsis might be inadequate for the development of new therapies, and that new enhanced or precision-based trial designs targeting specific subpopulations of patients with septic shock are required.[7]

The triggering receptor expressed on myeloid cells-1 (TREM-1) is an immunomodulatory receptor expressed on innate immune cells, endothelial cells, and platelets.[8–10] The biological function of TREM-1 is the amplification of the inflammatory response following the initial activation of toll-like receptors. In sepsis, this amplification might contribute to the dysregulated immune response,[11] which plays a key role in the development and progression of septic shock. Exaggerated activation of this pathway in septic shock can be measured by an elevated circulating level of expression of the cleaved portion of the TREM-1 receptor: soluble TREM-1 (sTREM-1),[12] which is associated with increased mortality.[13]

Nangibotide is a 12 amino-acid peptidic fragment derived from TREM-like transcript-1 (TLT-1), a receptor protein belonging to the TREM-1 family. Nangibotide binds the TREM-1 agonist ligand and thereby modulates the amplification of the immune response caused by the activation of the TREM-1 pathway in sepsis.[10]

Extensive preclinical modelling of TREM-1 modulation in rodent, porcine, and primate septic shock revealed a protective effect of nangibotide in terms of organ function, cardiovascular status, and survival.[10,14,15] Following a phase 1 study that showed no adverse safety signals,[16] a phase 2a clinical trial investigated three doses of nangibotide therapy for up to 5 days in 49 patients with septic shock and the investigational intervention was found to be safe and well tolerated.[17] Although the phase 2a trial was not designed to prove efficacy, patients treated with nangibotide showed numerical improvements in organ function evaluated with Sequential Organ Failure Assessment (SOFA) score. This signal was larger in the subgroup of patients with high circulating concentrations of sTREM-1. Following this phase 2a trial, we did the phase 2b ASTONISH trial to confirm the hypothesis that TREM1 inhibition might improve outcomes in patients with septic shock.

Methods

Study design

ASTONISH (Efficacy, Safety and Tolerability of Nangibotide in Patients with Septic Shock) was a double-blind, randomised, placebo-controlled, multicentre, dose finding phase 2b trial in patients with septic shock. This exploratory study had three parallel objectives: the first two related to the assessment of the safety and tolerability of nangibotide, and the assessment of efficacy of the two doses of nangibotide on organ dysfunction and 28-day mortality in a predefined high sTREM-1 population and in all participants. The third objective was evaluation of the relationship between the degree of TREM-1 activation as measured by sTREM-1, and the efficacy of the two doses of nangibotide on organ dysfunction and mortality.

The trial was done in 42 hospitals with medical, surgical, or mixed intensive care units (ICUs) in seven countries: France, Belgium, Spain, Denmark, Finland, Ireland, and the USA. Hospitals included in the trial

Research in context

Evidence before this study

Observational data support an association between the degree of triggering receptor expressed on myeloid cells-1 (TREM-1) activation, defined by the concentration of soluble TREM-1 (sTREM-1) and outcome in patients with septic shock. A phase 2a trial in 49 patients with septic shock suggested that TREM-1 modulation with nangibotide was well tolerated and could be effective in modifying clinically relevant outcomes in patients with septic shock and elevated sTREM-1 concentrations.

Added value of this study

Despite the fact that it didn't reach significance for the primary endpoint, this study shows in a large phase 2b study, designed to assess the effect of nangibotide on acute morbidity in septic shock, that treatment with a higher dose of nangibotide (1·0 mg/kg per h) for 5 days could result in greater improvements in SOFA score in patients with sTREM-1 concentrations higher than 532 pg/mL; a finding which remains to be confirmed in phase 3. Nangibotide therapy was well tolerated.

Implications of all the available evidence

This study builds on existing evidence to support progression to definitive evaluation of the efficacy of nangibotide in septic shock. A future phase 3 trial will test the efficacy of nangibotide treatment at a dose of 1·0 mg/kg per h for up to 5 days in patients with an elevated sTREM-1 concentration, higher than that anticipated in the ongoing phase 2b, within 24 h of the onset of septic shock.

were university teaching (n=38), public (n=3), and private (n=1) hospitals. Details of the planned trial design have been reported previously.[18]

The trial procedures and the informed consent form process were approved by the respective independent ethics committees following international standards and the national requirements of each participating country. The study was registered in the EU Clinical Trials Register (EudraCT number 2018-004827-36) and with Clinicaltrials.gov, NCT04055909.

Participants

Patients (aged 18–85 years inclusive) were eligible for enrolment within 24 h of the initiation of vasopressor for the treatment of septic shock if they met all inclusion and no exclusion criteria. The main inclusion criteria were the presence of septic shock based on consensus definitions[1] and related to a documented or suspected infection in the lung, in the abdominal cavity, or in the urinary tract (appropriate routine microbiological cultures, including blood, had to be obtained before starting antimicrobial therapy). Patients with urinary tract infection were only eligible if they were aged 65 years or older at inclusion. The main exclusion criteria related to the presence of severe life limiting comorbidity and profound immunosuppression. A full list of inclusion and exclusion criteria is included in the appendix (p 17). Patients with infection or septic shock solely due to SARS-CoV-2 were not eligible. After written and informed consent was provided by the patient, his or her legal representative, or, in relevant countries, an independent physician, a clinical coordinating centre composed of sepsis experts confirmed patient eligibility for enrolment in the trial.

Randomisation and masking

A computer-generated block randomisation scheme (block size 3) was developed by an independent statistician who was not part of the study team. Randomisation assignment was implemented by means of an interactive response platform. Eligible patients were randomly assigned in a 1:1:1 ratio to one of three treatment groups (low-dose nangibotide, high-dose nangibotide, or placebo) with stratification according to site only. Patients, study investigators, and treating clinicians were masked to treatment allocation.

Procedures

Patients received a loading dose of nangibotide over 15 min followed by infusion of one of two doses (low dose 6·66 mg/kg for loading plus 0·3 mg/kg per h or high dose 20 mg/kg for loading plus 1·0 mg/kg per h) or a matched placebo. Study drug was issued as a lyophilised white powder in 50 mL glass vials containing either nangibotide or placebo. The powder was solubilised with water for injection at the study site and infused at the prescribed rate on the basis of actual bodyweight as a continuous infusion via a central vein.

Treatment was initiated as early as possible, but no later than 24 h after the onset of septic shock, defined by the start of vasopressor therapy. Patients were treated with study drug until 24 h (±2 h) after vasopressor withdrawal, with a minimum duration of 3 days (72 h [±2 h]) and up to a maximal duration of 5 days (120 h [±2 h]), even if not weaned from vasopressors. The rationale for administering nangibotide was that this is the period of sustained TREM-1 activation observed in both preclinical models and observational human data sets and is consistent with the primary inflammatory period in patients.[11]

Blood samples for pharmacokinetic and exploratory pharmacodynamic analyses, including the evaluation of sTREM-1 before the initiation of study drug, were collected before, during, and after the treatment period. The sampling schedule is provided in the appendix (pp 25–27). sTREM-1 concentration was measured centrally; a preliminary cutoff value of 400 pg/mL was chosen to define the high sTREM-1 population on the basis of the analysis of observational data derived from the AdrenOSS-1 study,[19] and exploratory data from the phase 2a MOT-C-201 study in 49 patients with septic shock treated with nangibotide.[17]

Outcomes

The primary endpoint was the difference in total SOFA score from baseline to day 5 between the low-dose and high-dose nangibotide groups and the placebo group. The difference was assessed in the subgroup of patients with high sTREM-1 baseline concentrations (≥400 pg/mL) and in the overall population. The quality and consistency of data collection for each SOFA subscore was controlled in various ways. In the initiation phase of the trial, each site underwent specific SOFA score training on the basis of a series of guidelines that were provided to the sites to standardise the collection of SOFA scores (appendix pp 48–61), and that were in part published before initiation of the study.[20] Regular refresher training was also provided. Furthermore, internal validation of the consistency and quality of the SOFA data was done on an ongoing basis throughout the trial. Comparison, in a fully masked fashion, of the reported SOFA subscore values was made with data on organ function and support received by patients in other sections of the case report form (all data that was validated during site visits). The cross-comparison of these data with more than 12 000 individual subscores identified a possible discrepancy between the data sources in approximately 6% of cases. Of these, approximately one-third resulted in a change of the reported SOFA subscore, one-third resulted in a revision to another aspect of the case report form, and for the remainder, following discussion with the site, no changes were considered necessary. All score revisions were conducted in a masked fashion and no changes were made after database lock. At the time when the data were unmasked, one patient was missing a single

baseline SOFA subscore (hepatic). All other baseline data were available for analysis.

The key secondary endpoints were all-cause mortality at day 28, analysed by means of a logistic regression model including baseline SOFA score and treatment group as covariates, and the evaluation of the relationship between baseline sTREM-1 and treatment response. The goal of the evaluation was to establish the optimal sTREM-1 threshold value in a larger sample size than that used in the previous phase 2a study.

Other secondary endpoints included the daily change of total SOFA score and subscores, the outcome of death, length of ICU stay, and length of hospital stay. The proportion of patients who were classified as dead or receiving organ support on day 28 was also explored following a post-hoc revision to the initial analysis plan to include data from follow-up visits imputing missing data from 35 patients. Descriptive listings of the handling of the missing data in this group of patients are provided in the appendix (pp 22–23). The end-of-study visit was on day 28. A complete list of evaluations is provided in the appendix (pp 25–27).

Safety and pharmacokinetic data collection is described in the appendix (p 24). Planned long-term follow-up of mortality and functional status at 1 year will be done as part of a separate analysis after all patients have completed 12-month follow-up.

Statistical analysis

The planned sample size of 225 patients in the predefined high sTREM-1 group was based on a treatment effect of a 2-point difference in total SOFA score between the placebo group and for one dose and 1·15-point difference between the placebo group and for the other dose at day 5, with an SD of 3·3 for each group and a 1-sided alpha level of 0·025. On the basis of evaluation of phase 2a data and observational datasets, it was expected that around 50% of the study population would have a sTREM-1 higher than the preliminary cutoff value in the ASTONISH trial (ie, >400 pg/mL). The sample size was established considering the missing data and the proposed approach to impute missing data (ie, assuming a missing-not-at-random mechanism). Because of the penalty applied to the SOFA score in the event of death (see below), we anticipated a larger treatment effect considering that there would be more deaths in the placebo group compared with the nangibotide group. We also anticipated, because of death occurrence, and the method for penalising missing data due to death, that the SD of the change in SOFA score would be higher than that observed in the phase 2a study as a consequence. However, we assumed that the standardised effect size would remain the same as that which would have been observed without the penalty for death.

Two preplanned interim analyses were done by an independent unmasked data monitoring committee after the first 128 patients and after 223 patients had been randomly assigned. The first interim analysis reviewed safety and the second interim analysis assessed both safety and futility of both doses independently. The data monitoring committee charter and statistical analysis plan (SAP) for the futility analysis are provided in the appendix (pp 62–107).

Detailed summaries of the methods used in the analysis of safety and efficacy of nangibotide are provided in the SAPs (appendix pp 69–161).

Efficacy endpoints were assessed in all patients who were randomly assigned to a treatment group and received at least one dose of study drug and are presented in the modified intention to treat (mITT) set. Safety and tolerability outcomes are presented in the safety set of patients who received at least one dose of study drug as treated. The per-protocol analysis included all patients who, in addition, received the trial medication according to the protocol with minor deviations only and satisfied all major entry criteria. Demographic and medical background data, safety variables, and secondary endpoints were analysed by means of descriptive statistics. Individual vasopressor dose was converted into norepinephrine-equivalent dose; a conversion table is provided in the data monitoring committee SAP (p 84). Continuous data were analysed on the basis of mean (SD) or median (IQR) depending on the distribution of the data. Categorical variables are summarised by means of counts and frequencies for contingency tables. The change in SOFA score was assessed by means of an ANCOVA model adjusting for randomised treatment and the baseline SOFA score as independent variables. The primary outcome of the difference in least-squares mean (95% CI) SOFA score from baseline to day 5 was calculated by use of the estimated mean difference between placebo and nangibotide at day 5 with a positive value showing an additional benefit of nangibotide therapy over placebo. In brief, the primary method for handling the SOFA score included the replacement of missing SOFA values not due to death by the last available post-randomisation value of the relevant SOFA subscore (ie, last observation carried forward method). Missing values due to death were replaced by the last available post-randomisation value of the total SOFA score increased by an additional penalty of four points. The primary analysis did not assume a missing at random mechanism, instead, missing data due to death, discharge alive from the ICU before day 5, or missing individual subscores for other reasons were imputed by means of the last observation carried forward, a missing-not-at-random method.

Sensitivity analyses, which used different penalty scores and alternative methods for handling missing data, such as multiple imputations, were done and are described in the SAP (pp 135–140). The analysis of other endpoints is described in the SAP. A post-hoc correction exploring the effect of variation in the baseline characteristics between groups was applied by means of

an established method.[21] In brief, an adjusted (primary) analysis was done by means of an ANCOVA model including treatment (nangibotide studied dose *vs* placebo), baseline SOFA score, and baseline covariates (Acute Physiology and Chronic Health Evaluation [APACHE II], sTREM-1, interleukin-6 [IL-6], age, gender, BMI, and site of infection), while considering the primary method for imputing missing data. Additional exploratory analyses included evaluation of the relationship between treatment response and clinical characteristics including the presence of confirmed infection and the use of glucocorticoids for the treatment of septic shock. Analytical methods were the same as those used in the primary analysis.

The relationship between the degree of TREM-1 activation (defined by sTREM-1 concentration at baseline) and treatment effect was explored with regard to selected clinically relevant outcomes. The treatment effect of each dose of nangibotide versus placebo was evaluated for potential sTREM-1 cutoff values between each percentile up to the 90th percentiles with a step length of 1. Exploratory statistical analysis of the effect size at each sTREM-1 threshold was done by means of the same methodology used in assessment of the relevant primary and secondary outcomes.

Adjustment for multiplicity to control the type 1 error at the usual two-sided 0·05 alpha level was only planned for the primary analysis among patients with high

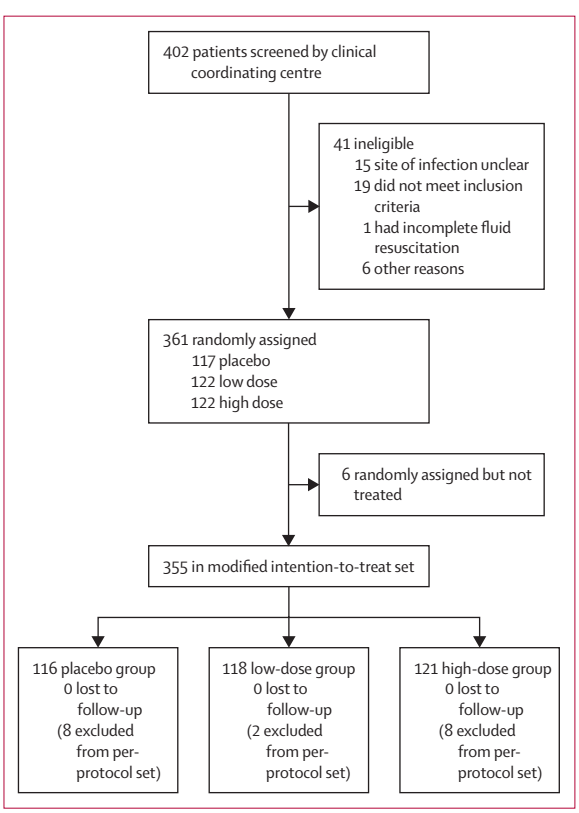

Figure 1: **Trial profile**

	Placebo group (n=116)	Low-dose nangibotide group (n=118)	High-dose nangibotide group (n=121)
Sex			
Female	37 (32%)	41 (35%)	43 (36%)
Male	79 (68%)	77 (65%)	78 (64%)
Age, years	66·7 (12·9)	67·4 (12·7)	69·2 (10·7)
BMI, kg/m²	27·4 (5·0)	26·2 (4·9)	27·2 (5·3)
Ethnicity			
White	109 (94%)	114 (97%)	117 (97%)
Black or African American	2 (2%)	1 (1%)	1 (1%)
Asian	2 (2%)	1 (1%)	0
Other	2 (2%)	0	3 (3%)
Parameters at inclusion			
Sequential Organ Failure Assessment score	9·0 (8–11)	10·0 (8–12)	10·0 (9–12)
Acute Physiology And Chronic Health Evaluation II, points	22·0 (19·3–26·0)	23·0 (18·0–27·0)	24·0 (19·0–28·0)
Lactate*, mmol/L	4·0 (2·9–6·4)	4·0 (3·2–7·0)	4·5 (3·2–6·1)
Microbiology†			
Any positive culture	100 (86%)	97 (82%)	99 (82%)
Positive blood culture	67 (58%)	71 (60%)	64 (53%)
Site of infection			
Abdominal cavity	65 (56%)	62 (53%)	60 (50%)
Lung	35 (30%)	36 (31%)	36 (30%)
Urinary tract	16 (14%)	20 (17%)	25 (21%)
Infection characteristics			
Nosocomial	17 (16%)	19 (16%)	12 (11%)
Community acquired	84 (78%)	82 (71%)	88 (78%)
Other	7 (6%)	15 (13%)	13 (11%)
Source of admission			
Medical	64 (55%)	73 (62%)	78 (65%)
Emergency surgery	49 (42%)	39 (33%)	40 (33%)
Elective surgery	3 (3%)	6 (5%)	3 (3%)
Chronic comorbidities			
Charlson Comorbidity Index	5·0 (0–20)	4·0 (0–18)	4·0 (0–18)
Biomarkers			
sTREM-1 (min–max)	530·5 (68–2030)	626·0 (121–2510)	631·0 (146–2560)
sTREM-1 ≥400 pg/mL	75 (65%)	90 (76%)	88 (73%)
IL-6, pg/mL	1171 (172–7831)	1414 (252–6517)	1637 (337–10 365)
Treatment at inclusion			
Vasopressor*	116 (100%)	117 (99%)	120 (99%)
Invasive ventilation	78 (67%)	78 (66%)	82 (68%)
Renal replacement therapy	9 (8%)	10 (9%)	14 (12%)
Time from start of vasopressor to initiation of intervention, h	16·3 (10·4–20·3)	13·9 (7·7–19·5)	14·2 (9·3–19·8)
Therapy			
Systemic antibiotic therapy	116 (100%)	118 (100%)	121 (100%)
Glucocorticoid therapy†	79 (68%)	78 (66%)	92 (76%)

Data are median (IQR), mean (SD), or n (%). *Lactate is value at screening. †Any positive culture result reporting a result at screening or within 3 days of randomisation was considered a positive microbiology result. *Two patients (one in the low-dose group and one in the high-dose group) completed their first episode of vasopressor therapy in the period between randomisation and the start of intervention. †Glucocorticoid therapy administered at an equivalent dose of 200 mg/day hydrocortisone for the treatment of septic shock was established by an independent masked data adjudication committee.

Table 1: **Baseline characteristics for the modified intention-to-treat set**

sTREM-1 baseline concentrations (≥400 pg/mL) and in the overall population and is described in the SAP. Hence, p values provided for all other efficacy endpoints, key secondary, and others, are exploratory only. Statistical analyses were done by means of SAS version 9.3, and R version 3.4.3. Preparation of the mITT, safety set, and per-protocol analyses was done on the basis of preplanned SAP by the biostatistics team of the contract research organisation. The exploratory evaluation of the relationship between the degree of TREM-1 activation and treatment response was done by the study statistician, JMG.

Role of the funding source

The funder of the study contributed to study design, design of the statistical analysis plan, post-hoc exploratory data analyses, writing of the report, and the decision to submit for publication. The funder played no role in patient screening, patient recruitment, data collection, or planned data analysis.

Results

The first patient was enrolled on Nov 14, 2019, and the last on April 11, 2022. Masked analysis of sTREM-1 levels in the overall population provided by the contract research organisation revealed that 71% of the patients included in the trial were in the a priori defined preliminary high sTREM-1 population (>400 pg/mL)—a higher proportion than the 50% anticipated before initiation of the study (n=225). As the sample size goal for the high sTREM-1 population was reached, the study was terminated with 361 patients recruited in total and 253 in the preliminary high sTREM-1 population. 402 patients were formally assessed for eligibility by the clinical coordinating centre, with 41 considered not suitable for inclusion owing to inadequate confidence regarding the presence of proven suspected infection at a relevant site (n=15), inclusion or exclusion criteria issues (n=19), fluid resuscitation being considered incomplete (n=1), or other reasons (n=6). Six patients (one in the placebo group, four in the low-dose group, one in the high-dose group) were randomly assigned but died before the initiation of study drug and were excluded from the mITT and safety set analysis (n=355, figure 1). In total, 355 patients received either placebo (n=116), low-dose (n=118), or high-dose nangibotide (n=121). 18 (5%) patients were excluded from the per-protocol analysis, five owing to deviation from protocolised drug administration and 13 owing to deviation from inclusion or exclusion criteria (details are listed in the appendix p 35).

Baseline characteristics for the overall population are shown in table 1 and for the high sTREM-1 population in the appendix (p 28). The population displayed typical features of patients with septic shock; 249 (70%) of patients received glucocorticoids for the treatment of septic shock. The placebo group displayed a consistent pattern of lower disease severity than the low-dose and high-dose groups in terms of total SOFA and APACHE II scores. The distribution of site of infection was not significantly different across study groups. Duration of shock and drug exposure are provided in the appendix (p 21). Patient characteristics for the per-protocol population were similar to those of the safety set.

The primary outcome did not reach significance: in the preliminary high sTREM-1 population, the difference in SOFA score from baseline to day 5 was 0·21 (95% CI –1·45 to 1·87; p=0·80) in the low-dose group and 1·39 (–0·28 to 3·06; p=0·104) in the high-dose group (figure 2A, appendix p 29) versus placebo. In the overall population, the difference between the placebo group and low-dose group was 0·20 (–1·09 to 1·50; p=0·76), and between the placebo group and the high-dose group was 1·06 (–0·23 to 2·35, p=0·108; figure 2B, appendix p 29). Total SOFA score at baseline and day 5 in each group is provided in the appendix p 30. No relevant difference in the primary outcome was observed in the per-protocol population compared with the mITT set (appendix p 36).

Planned analysis of the change in SOFA score by means of a range of analytical approaches to control for missing data showed a consistent pattern of effect associated with nangibotide therapy (appendix pp 30–31). In a post-hoc exploratory analysis of the effect of nangibotide on patients with microbiologically confirmed infection (296 [83%] of 355 patients), the pattern observed in the mITT set was similar to the primary outcome results: the change in SOFA score was 0·86 (–0·95 to 2·67) when comparing the high-dose group with the placebo group in the preliminary high sTREM-1 population and was 0·66 (–0·78 to 2·09) in the overall population. A consistent pattern was observed in patients who received glucocorticoid therapy for the treatment of septic shock

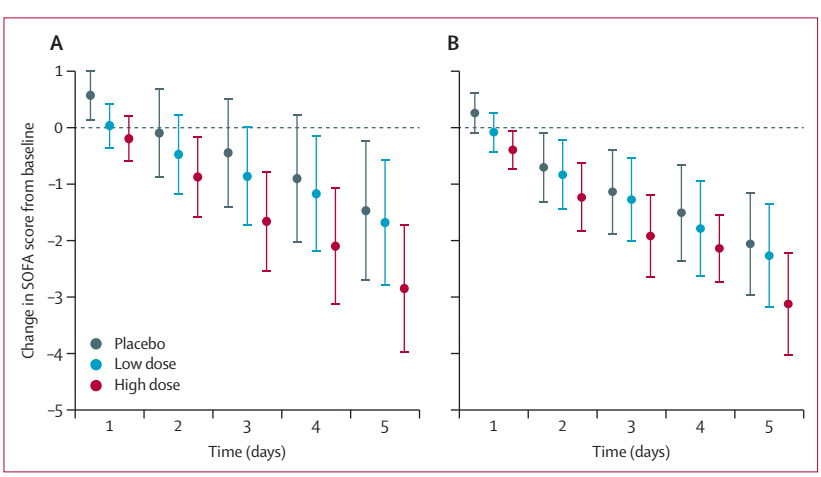

Figure 2: **Change in SOFA score from baseline to day 5**
Least squares mean and 95% CIs on days 1–5 in the group of patients with a sTREM-1 concentration of ≥400 pg/mL at baseline (A) and the overall population (B). SOFA score was assessed at each study day and the difference from baseline estimated. Missing data due to death were replaced with the LOCF plus a penalty of four points. Missing data at random were replaced using the LOCF method. SOFA=Sequential Organ Failure Assessment. LOCF=last observation carried forward.

(249 [70%] of 355): the change in SOFA score was 2·09 (0·27 to 3·90) when comparing the high-dose group to the placebo group in the preliminary high sTREM-1 population and was 1·84 (0·37 to 3·32) in the overall population.

The key secondary outcome was all-cause mortality at day 28. In the preliminary high sTREM-1 cutoff population, 23 (31%) patients in the placebo group, 35 (39%) in the low-dose, and 25 (28%) in the high-dose group had died by day 28. In the overall population, 29 (25%) patients in the placebo, 38 (32%) in the low-dose, and 30 (25%) in the high-dose group had died by day 28 (table 2). Post-hoc adjustment correcting for imbalances in the baseline characteristics of the population resulted in a risk difference of –4·2% (95% CI 10·0 to –18·5) in the low-dose group and 3·0% (16·1 to –10·2) in the high-dose group, in the high sTREM-1 population compared with the placebo group. In the overall population, the risk difference was –1·8% (9·0 to –12·6) for the low-dose group and 2·4% (12·8 to –7·9) for the high-dose group.

Mortality related to septic shock was evaluated by an independent masked data adjudication committee. No difference in this outcome was observed in the overall or preliminary high sTREM-1 cutoff population (appendix p 31). The time to liberation from any organ support, the proportion of patients alive and free of organ support, the rate of secondary infection, and exploratory analysis of the duration of hospitalisation also displayed no significant difference in response to either nangibotide dose in the overall or preliminary high sTREM-1 populations (appendix pp 32–34).

Results of the analysis of safety and tolerability are summarised in table 3. The number of treatment-emergent adverse events and serious treatment-emergent adverse events was similar between all three study groups. 339 (95%) of 355 patients had at least one treatment-emergent adverse event (classification of treatment-emergent adverse event by system organ class is provided in the appendix pp 162–190). Eight (2%) patients had a serious treatment-emergent adverse event considered possibly related to study drug with two patients each in the placebo group and high-dose group and four in the low-dose group; no pattern in these events was observed and summaries of the cases are provided in the appendix (p 37). No study drug-related treatment-emergent adverse event led to the premature cessation of study drug. The independent unmasked data monitoring committee raised no safety concerns at either interim analysis or at any other time during the study and recommended that both doses continue to be assessed without modification to the protocol.

Nangibotide treatment did not trigger specific anti-drug immune responses in this study, and no clinical signs related to immunogenicity were detected. Pharmacokinetics were similar to those observed in the previous phase 2a trial and are reported in the appendix (pp 38–39).

	n	28-day mortality	Between-group comparison	
			Estimate for odds ratio (95% CI)	p value
High sTREM-1 concentration (≥400 pg/mL) population				
Placebo	75	23 (31%)	..	..
Low-dose nangibotide group	90	35 (39%)	1·42 (0·74–2·73)	0·85
High-dose nangibotide group	88	25 (28%)	0·86 (0·44–1·71)	0·34
Overall population				
Placebo	116	29 (25%)	..	..
Low-dose nangibotide group	118	38 (32%)	1·37 (0·77–2·45)	0·86
High-dose nangibotide group	120	30 (25%)	0·91 (0·50–1·66)	0·38

Data are n (%), unless stated otherwise. Estimates for odds ratio analysed in a logistic regression model adjusting for treatment group and baseline SOFA score, the covariates used for the primary endpoint analysis. One patient with a missing baseline SOFA subscore was excluded from the analysis. SOFA=Sequential Organ Failure Assessment.

Table 2: **All-cause mortality at day 28 in the overall population and the high sTREM-1 concentration population**

	Placebo group (n=116)	Low-dose nangibotide group (n=118)	High-dose nangibotide group (n=121)
Any treatment-emergent adverse event	111 (96%)	113 (96%)	115 (95%)
Any serious treatment-emergent adverse event	28 (24%)	26 (22%)	31 (26%)
Any serious treatment-emergent adverse event possibly related to study drug	2 (2%)	4 (3%)	2 (2%)
Any adverse event leading to death	34 (29%)	40 (34%)	35 (29%)

Table 3: **Treatment-emergent adverse events**

The change in SOFA score from baseline to day 5, all-cause mortality, and the proportion of patients that were dead or receiving organ support at day 28 were assessed in a preplanned analysis at a range of potential cutoff values for sTREM-1 by means of the safety set to explore the optimal population for progression to phase 3 trial. The evaluation of the change in SOFA score revealed that at higher cutoff values than the preliminary 400 pg/mL threshold, nangibotide therapy consistently delivered a greater effect size, with the high dose displaying greater effect size than the low dose (figure 3A, appendix pp 40–41). From cutoff values of at least 532 pg/mL representing the 45th percentile of sTREM-1 values in the study population, the mean difference in total SOFA score between the placebo group and the high-dose group was 2·3 (95% CI 4·1 to 0·4; p=0·018) in favour of nangibotide. After post-hoc adjustment for various covariates to account for imbalances in the baseline characteristics between

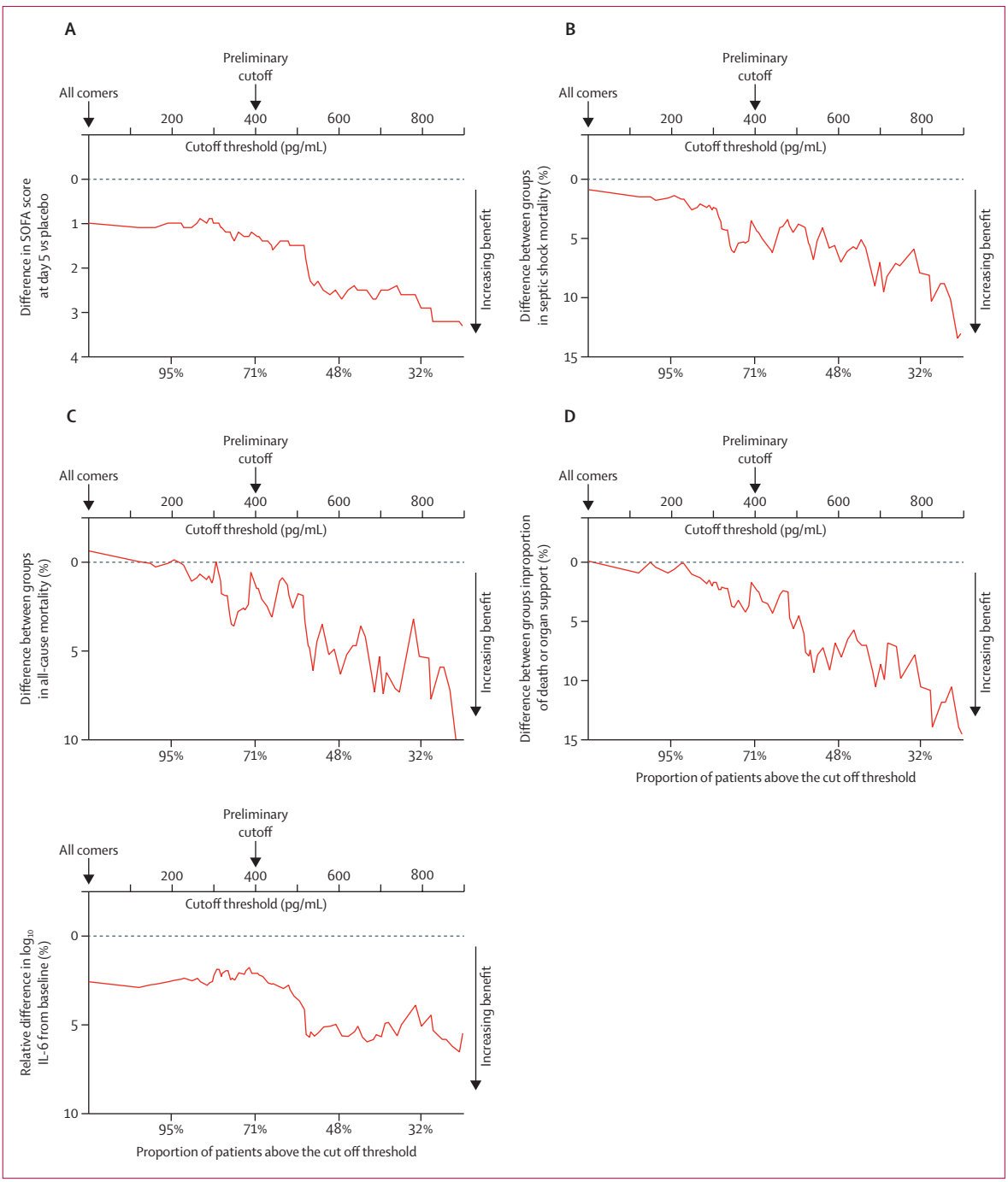

Figure 3: **The effect of different cutoff values for sTREM-1 on treatment response to high-dose nangibotide therapy compared with placebo**
The difference between the high-dose group and the placebo group in change in SOFA score from baseline to day 5 (A), septic shock-related mortality at day 28 (B), all-cause mortality at day 28 (C), and proportion of patients either dead or receiving organ support at day 28 (D), and exploratory analysis of mean change in IL-6 from baseline to day 2 (E). Representative performance of nangibotide for all comers and at increasing cutoff thresholds for each percentile of sTREM-1 in increments up to 900 pg/mL are presented. SOFA=Sequential Organ Failure Assessment.

treatment groups, the effect of the high dose on change in SOFA score was 2·4 (4·1 to 0·7; p=0·005). The effect of high-dose nangibotide therapy on individual SOFA subscores was also evaluated with a consistent pattern of improvement shown across all subscores, albeit with a varying magnitude of effect (appendix pp 42–44). This magnitude of effect was similar at all evaluated cutoff values higher than the exemplar cutoff.

The high-dose group showed a similar pattern of activity on septic shock mortality compared with placebo (figure 3B, appendix p 40), all-cause mortality at day 28 (figure 3C, appendix p 40), and proportion of patients who were dead or on organ support at day 28 (figure 3D, appendix p 40)—although the study was not powered to show significance in these outcomes. At the exemplar cutoff of values greater than or equal to 532 pg/mL, in addition to the effect on SOFA score, high-dose nangibotide therapy was associated with a mean reduction in all-cause mortality of 4·8% (95% CI 21·2 to –11·7; p=0·57) and the proportion of patients with an outcome of death or ongoing organ support at day 28 of 7·4% (24·8 to –9·9; p=0·402). The effect of the high dose on the two outcomes following post-hoc adjustment for baseline characteristics was 7·3% (23·0 to –8·4; p=0·36) and 12·1% (28·5 to –4·3; p=0·15). When assessed as the change in IL-6 concentration between baseline and day 2, exploratory descriptive analysis displayed a pattern consistent with the observed clinical effect of a greater reduction at cutoff values higher than 500 pg/mL (figure 3E). Low-dose nangibotide therapy was associated with a similar pattern of effect on the change in SOFA score with a lower magnitude of effect size that did not achieve significance (appendix pp 45–47).

Discussion

To our knowledge, the ASTONISH trial is the first study to assess the effect of a TREM-1 modulation strategy on clinical outcomes in patients with septic shock, with the specific aim of identifying the degree of TREM-1 activation at which nangibotide exerts its greatest effect. Although well tolerated, nangibotide therapy did not achieve the primary efficacy endpoint of change in SOFA score at day 5 in the preliminary high sTREM-1 population (≥ 400 pg/mL) or in the overall population. For the high dose of nangibotide, the planned exploratory analyses revealed clinically relevant beneficial effects among septic shock patients with baseline sTREM-1 concentrations higher than 532 pg/mL.

The TREM-1 pathway is a synergistic regulator of toll-like and nod-like receptor signalling.[22] Nangibotide is a peptide that binds the TREM-1 ligand and thereby modulates the TREM-1 pathway in sepsis. In the normal inflammatory response, the endogenously produced soluble TLT-1 and sTREM-1 regulate this pathway. However, it was hypothesised that in a subgroup of patients with septic shock, this process is not sufficient to control the TREM-1-mediated inflammatory response. It is in this population that nangibotide might offer a relevant benefit.

This study builds on a preliminary cutoff value defined as a high sTREM-1 concentration of 400 pg/mL[17]. By necessity, the choice of threshold that defines a predictive biomarker must be based on the response to treatment in patients. In this case, the threshold was selected predominantly on the basis of the only randomised data available—a phase 2a study of 49 patients, which defined the enhanced population.[17] For this reason, the reassessment of the cutoff value that defines an optimal treatment responder subgroup was planned a priori.

This study was powered to detect efficacy in the form of decreasing SOFA score, but was not powered to detect mortality differences. Although a non-significant trend to improved SOFA score was delivered at the preliminary cutoff of ≥400 pg/mL, at higher thresholds the effect of nangibotide becomes clinically significant and is associated with an unadjusted p value of less than 0·05, in patients with sTREM-1 higher than 532 pg/mL (representing the 45th percentile of the overall study population), the difference in SOFA score between high-dose group and the placebo group at day 5 was 2·3 points. The treatment benefit associated with nangibotide at increasing sTREM-1 thresholds was seen across all six SOFA subscores with the largest effects observed in the cardiovascular, renal, and respiratory systems. This was consistent with preclinical observations of the activity of nangibotide[14] and was independent of the method used to control for missing data.

IL-6 is an important marker of the early inflammatory response and nangibotide is expected, via its mechanism of action, to regulate multiple inflammatory pathways downstream of toll-like receptor activation. The effect of nangibotide on IL-6 at the same sTREM-1 threshold as the observed clinical benefits, supports the hypothesis that the endogenous regulator sTLT-1 is overwhelmed at a certain degree of TREM-1 activation, contributing to septic shock syndrome in this subgroup of patients. It is at this point that modulation with a targeted anti-TREM-1 therapy might be protective.

Both doses of nangibotide showed a consistent pattern of effect on reducing SOFA score from baseline to day 5, but this change was not significant for either dose. This dose–response for nangibotide therapy provides some confidence in the biological activity of the drug. A numerically higher rate of mortality in the overall low-dose group was driven by baseline imbalances in the population, as shown by the effect of adjustment for baseline characteristics.

Nangibotide therapy was well tolerated without an increase in treatment-emergent adverse events or serious treatment-emergent adverse events compared with placebo. This ICU septic shock population suffers high rates of adverse events owing to underlying diseases. Nangibotide is catabolised by enzymatic processes in whole blood and clearance is independent of solid organ function. This study showed a consistent pattern of plasma drug concentrations and clearance to that observed in the previous phase 2a study.[17]

The lower proportion of patients with respiratory infection as the infectious source than that typically observed in septic shock trials is likely to be because of the low rates of non-COVID-19 respiratory infection observed in study countries during this period.

The limitations of our trial include the use of a composite SOFA morbidity score as the primary outcome. Although this approach is supported by the European and US regulatory agencies, it has in the past been associated with issues of missing data and mortality bias, which can make interpretation challenging.[23] The use of an endpoint like this in the setting of a phase 2 septic shock study is an essential part of the development journey, as it renders the assessment of drug efficacy feasible. In this study, we protocolised and shared the rules for the assessment of SOFA score[18,20] to minimise the degree of inter-rater reliability. We emphasised the importance of collection and assessment of SOFA data, which led to more than 99% of baseline SOFA scores being available for assessment of the primary outcome with a high degree of internal validity.

To address questions regarding the handling of missing data due to death and random missing data, multiple sensitivity analyses assessing different data handling methods showed that these had no relevant effect on the pattern of the results. In particular, the use of the last observation carried forward method for handling missing data could be a limitation. However, this approach had no effect on the evaluation of outcome compared with the other methods tested.

In addition, as this was an exploratory study, secondary endpoints and cutoff evaluation were not adjusted for multiple comparisons, however the highly consistent pattern of response to nangibotide therapy across multiple endpoints suggests that the observed effects are unlikely to be occurring by chance.

This study was not powered to detect a significant effect on mortality and point estimates of mortality benefit are confounded by wide CIs. The CIs reported do not rule out a clinically relevant effect, consistent with the observed improvement in morbidity. The imbalance in mortality in the low-dose treated population might be due to the higher baseline acuity of patients in the low-dose and high-dose groups compared with placebo. Adjustment for baseline covariates confirmed the effect of nangibotide on mortality and the proportion of patients free of organ support at day 28. This supports planned trials of nangibotide to answer the question of nangibotide efficacy in the subgroup of patients with septic shock and elevated sTREM-1 concentrations definitively.

In conclusion, the phase 2b ASTONISH study confirmed a favourable safety profile of nangibotide in patients with septic shock but could not confirm a significant effect on change in SOFA score in the study population. However, exploratory analyses suggest that high-dose nangibotide could provide a greater improvement in acute morbidity in patients with higher s-TREM-1 concentrations; further studies are needed to confirm this potential benefit.

Contributors

SL drafted the first version of the manuscript. BF, P-FL, ML, J-MG, and J-JG revised the first draft. SL, J-JG, MD, SG, VC, MS-M, J-MG, BF, P-FL, ML, MB, RF, and TH all contributed to the development and design of the protocol, data collection, and analysis. TF, VJ, NDS, JL, VP, J-PM, CB, EM, JV, VH, IV, and NA recruited ten or more patients to the trial and participated in data collection. SL and J-JG verified the data. SL, J-JG, J-MG, and BF had access to unblinded raw data only after database lock, and had the final responsibility to submit for publication. All authors reviewed the manuscript and approved the final version.

Declaration of interests

SL, MS-M, J-JG, MD, TH, and VC are employees of Inotrem. BF, ML, and P-FL are members of the steering committee. BF reports personal fees for consulting from Aridis, Enlivex, AM-Pharma, Combioxin, Nektar, GSK, and Inotrem outside of the submitted work. J-JG, MD, MS-M, SG, SL, and TH hold shares in Inotrem. J-PM reports payments for lectures and travel support from Fresenius and Roche and takes part in an advisory board with AM-Pharma, outside of the submitted work. JV reports sponsoring fees from Inotrem during the conduct of the study. MB reports grants from Novo Nordisk Foundation, Sygeforsikringen Danmark, and Svend Andersen Foundation, is the national coordinator for the REVIVAL study from AM-Pharma and is involved with the ESICM, outside of the submitted work. MD is designated as an inventor on a patent. P-FL reports consulting fees from Adrenomed and Inotrem outside of the submitted work. RF reports personal fees from Inotrem, during the conduct of the study and personal fees from Shionogi, Pfizer, MSD, Thermofisher, Menarini, Cytosorb, and Gilead, outside of the submitted work. SG reports consulting fees from Inotrem outside of the submitted work and is designated as an inventor on a patent related to Inotrem. SL reports personal fees from the UK COVID Therapeutics Advisory Panel and holds share in Critical Pressure outside of the submitted work. J-JG reports consulting fees from Inotrem during the conduct of the study and outside of the submitted work. CB, EM, IV, JL, J-MG, NA, NDS, TF, VH, VJ, and VP have no conflict of interests to report.

Data sharing

Data will be shared with investigators whose proposed approach is methodologically sound and is designed to achieve the aims of the proposed research. Proposals should be submitted to the corresponding author. To gain access, data requestors will need to sign a data access agreement.

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