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SCIENTIFIC LETTER

A mechanism-based prognostic enrichment strategy for the development of the TREM-1 inhibitor nangibotide in septic shock

Bruno François[1*], Mitchell Levy[2], Ricard Ferrer[3], Pierre-François Laterre[4] and Derek C. Angus[5]

Dear Editor,

Septic shock is a life-threatening organ dysfunction caused by a dysregulated host response to infection, and is a major health priority [1]. The lack of appropriate subpopulation biomarkers is cited as a key reason for the failure of several large trials evaluating novel interventions [2]. Biomarkers might perform best if they reflect the specific pathologic abnormalities that a therapeutic is targeting [2, 3]. Existing prognostic biomarkers may be less useful because they usually predict the risk of death, regardless of its mechanism. Recent subphenotyping efforts have been described as enrichment tools, though none have been validated for any specific therapy [4].

Here, through pre-planned analyses of the ASTONISH trial [5], we report the evaluation of an sTREM-1 biomarker-driven approach for a mechanism-based prognostic enrichment of the target population of the TREM-1 inhibitor nangibotide. Methods used are fully described in supplementary material. A baseline sTREM-1 optimal morbidity and mortality prognostic cut-off of 1050 pg/mL was derived from optimal cut-point of ROC curve analysis in placebo-treated patients (116/355 randomized), using the Elecsys® TREM-1 assay, aimed to become nangibotide's companion diagnostic. This cut-off identifies high-risk septic shock patients. The concordance between sTREM-1 Elecsys® and ELISA results reported in the previous ASTONISH publication has been demonstrated. The ROC-AUC was 69.0% [95% CI 58.9%; 79.1%] for the prediction of death or being on organ support at day 28 (AFOS28-NO) (electronic supplementary table S1). ROC-AUC robustness was also evaluated (electronic supplementary table S2). Fifty percent of ASTONISH patients had ≥ 1050 pg/mL sTREM-1 plasma levels. Interestingly, sTREM-1 was a better prognostic marker than baseline SOFA, APACHE-II or procalcitonin (based on ROC-AUC: 61.1% [95% CI 50.3%; 71.9%], 63.7% [95% CI 53.6%; 73.9%] and 48.8% [95% CI 37.1%; 60.4%] respectively; electronic supplementary table S1). High-risk patients displayed a hyperinflammatory profile, based on various inflammatory markers (electronic supplementary figure S1), and are distinct from subpopulations prognosticated for the same risks using baseline SOFA, APACHE-II or procalcitonin (Fig. 1A), suggesting that elevated sTREM-1 identifies patients at high-risk specifically associated with TREM-1 hyperactivation.

Efficacy results in ASTONISH primary and secondary endpoints favored nangibotide over placebo in this subpopulation (n = 62 1 mg/kg/h nangibotide- versus n = 56 placebo-treated patients). Briefly, the difference in change of SOFA score from baseline to day 5 (ΔSOFA5), the primary endpoint of ASTONISH, was -2.5 versus placebo (p = 0.007) (Fig. 1B). Results for other evaluated secondary endpoints also favored nangibotide over placebo in these high-risk patients with sTREM-1 ≥ 1050 pg/mL (electronic supplementary table S3). Patients with low-risk (< 1050 pg/mL sTREM-1) benefited similarly from standard of care whether treated with placebo or nangibotide (electronic supplementary figure S2).

The proportion of patients presenting treatment emergent adverse events (AE) was similar in the high-risk

*Correspondence: Bruno.Francois@chu-limoges.fr
[1] Intensive Care Unit & Inserm CIC 1435 & Inserm UMR 1092, Dupuytren University Hospital, Limoges, France
Full author information is available at the end of the article

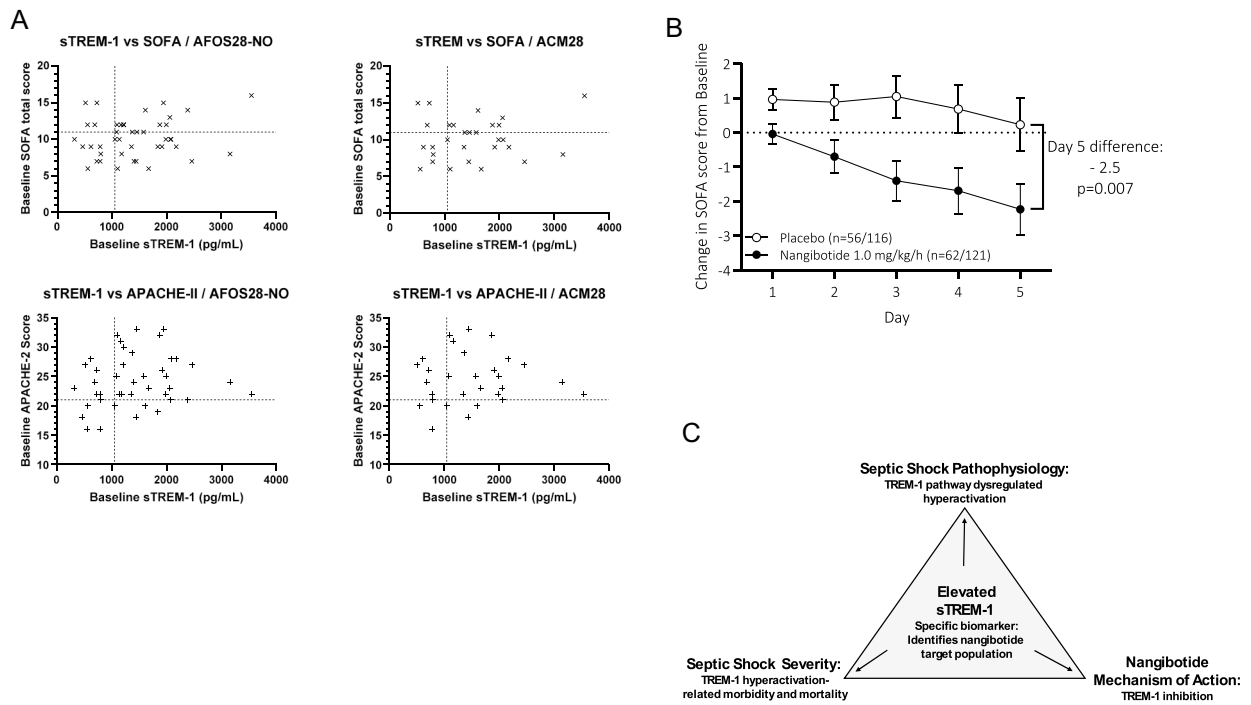

Fig. 1 Identification of nangibotide target population through prognostic enrichment for patients with high activation of the TREM-1 pathway. **A** Graph representation of baseline sTREM-1 level and baseline SOFA total score (top) or APACHE-II (bottom) for patients AFOS28-NO (left) or ACM28 (right). Each cross represents a patient who is dead or on organ support by day 28 (left graphs) or who has died by day 28 (right graphs). Dashed lines represent optimal prognostic cut-point determined by ROC-Curve analyses (Maximum Youden Index) for each prognostic marker. **B** Relative total SOFA score change from initial score at baseline through day 5 is shown for patients with sTREM-1 levels ≥ 1050 pg/mL, chosen as optimal prognostic cut-off based on ROC curve analysis (high risk sTREM-1 population or HRsTP). **C** Precision medicine approach model linking drug mechanism of action, targeted pathway dysregulation and pathway-dysregulation-related severity through the TREM-1 mechanism-based activation prognostic biomarker sTREM-1

and the low-risk patient groups (95% versus 95.4% respectively), while the proportion of patients presenting treatment emergent serious adverse events (SAE) was higher in the high-risk patients in comparison with low-risk patients (29.4% vs 18.3% respectively), which may relate to the higher severity of the septic shock in this population. Similar proportions of patients with AEs and SAEs were observed in the nangibotide- versus the placebo-treated group without any distinct pattern of AEs. This work confirms the value of sTREM-1 as the first biomarker linking drug mechanism of action, septic shock severity and targeted pathway dysregulation, thereby providing an attractive triad for a precision medicine approach (Fig. 1C). Another innovative drug, enibarcimab, an adrenomedullin stabilizing antibody, is developed using a drug-mechanism related biomarker: bio-active adrenomedullin (bio-ADM) [6]. However, post-hoc analyses suggested that the addition of a second biomarker (dipeptidyl-peptidase-3, DPP3) may be needed to identify the best enibarcimab target population [7].

High sTREM-1 levels identify patients at risk differently from traditional severity scores. Nangibotide treatment showed significant signs of efficacy in this subpopulation, while standard of care showed no benefit. We will therefore study prospectively the effect of nangibotide in a RCT enriched for TREM-1-associated high-risk patients aiming at concomitant registration of nangibotide and its sTREM-1 companion diagnostics. This approach constitutes a new paradigm for drug development in this field.

Supplementary Information

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Author details

[1] Intensive Care Unit & Inserm CIC 1435 & Inserm UMR 1092, Dupuytren University Hospital, Limoges, France. [2] Warren Alpert School of Medicine, Brown University, Providence, Rhode Island & Rhode Island Hospital, Providence, RI, USA. [3] Intensive Care Department, Vall d'Hebron University Hospital, Vall d'Hebron Institut de Recerca, Barcelona, Spain. [4] Department of Intensive Care, Centre Hospitalier Régional Mons-Hainaut, Mons, Belgium. [5] University of Pittsburgh Critical Care Medicine CRISMA Laboratory, Pittsburgh, PA, USA.

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Data availability statement
Data may 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.

Declarations

Conflicts of interest
BF, ML, PFL and RF were members of the ASTONISH study steering committee. BF reports personal fees for consulting from Inotrem, Eagle and Enlivex outside of the submitted work and support for attending a meeting from Inotrem. PFL 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, Cytosorbent, Gilead, Viatris, AOP and Grifols outside of the submitted work. DA reports consulting agreement with Inotrem outside of the submitted work. ML reports receiving a grant (1R01HL162954-01) outside of the submitted work. RF is Deputy Editor of the Intensive Care Medicine journal.

Ethical approval
The trial procedures and the informed consent form process were approved by the respective independent ethics committees following international standards, the national requirements of each participating country and the 1964 Declaration of Helsinki and its later amendments. The study was registered in the EU Clinical Trials Register (EudraCT number 2018-004827-36) and with Clinicaltrials.gov, NCT04055909.

Consent to participate
Informed consent was obtained from all individual participants included in the study.

Publisher's Note

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A mechanism-Based Prognostic Enrichment Strategy for the Development of the TREM-1 Inhibitor Nangibotide in Septic Shock

Supplementary material

M1

Study design and participants

ASTONISH study included 355 septic shock patients according to Sepsis-3 definition. One hundred and sixteen patients received placebo, 121 received 1 mg/kg/h nangibotide and 118 received 0.3 mg/kg/h nangibotide.

sTREM-1 plasma quantification

The baseline plasma sTREM-1 levels were measured with the to-be-marketed assay (Elecys® platform, Roche Diagnostics) intended to become nangibotide's companion diagnostics.

sTREM-1 method comparison between the initially used research-use-only ELISA method and the to-be-marketed Elecys®

Method comparison using weighted Deming regression is performed and parameter estimates (intercept and slope with jackknife confidence intervals) are reported. The comparison to ELISA is done, because ELISA was initially used to report biomarker related data from the ASTONISH trial.

Identification of patients at high morbidity and mortality risk by Receiver operating characteristic (ROC) curve analysis

The ROC curve analysis using sTREM-1 to prognosticate morbidity and mortality risk was determined using data from the 116 placebo patients, of whom 29 died and 14 were alive but required organ support on day 28.

ROC curves using sTREM-1 concentration (pg/mL), SOFA total Score, APACHE II Score and procalcitonin concentration (pg/mL) were plotted to evaluate their ability to prognosticate all-cause-mortality at day 28 (ACM28) and to prognosticate not being alive-and-free-of-organ-support (including cardiovascular, renal and mechanical ventilation support) at day 28 (AFOS28-NO). Youden indexes and minimal Euclidean distances were calculated to determine the optimal ROC curve prognostic cut-point.

Ninety-five % confidence intervals for the AUC estimates were primarily calculated using the asymptotic Wald method. As a sensitivity analysis, the intervals for sTREM-1 were also determined using stratified bootstrap with

2000 iterations. Both approaches yield similar results. In addition, the robustness of the ROC AUC estimates was investigated considering each subject's predicted probability from the logistic model fit excluding that subject, rather than using the fitted probabilities from the full data model. This yields an out-of-sample (cross-validated) predicted probability for every observation, which is then used to obtain a less biased estimate of ROC AUCs.

Efficacy analyses

Efficacy results shown correspond to the high-risk population above the optimal prognostic sTREM-1 cut-off of 1050 pg/mL treated with 1 mg/kg/h referred to as HRsTP. This population included 56 placebo-treated patients and 62 1.0 mg/kg/h nangibotide-treated patients. Efficacy results with 0.3 mg/kg/h dose are not shown since no superiority versus placebo was observed.

Analyses were performed on the safety set (as-treated patients) and were not adjusted for multiplicity. As the high sTREM-1 subgroup was not stratified for randomization, analyses were adjusted for the following baseline covariates (confounders): SOFA score, APACHE-II score, sTREM-1, IL6 (log10 transformed), Age, Gender, BMI and site of infection.

The ΔSOFA missing data not due to death were replaced using the Last Observation Carried Forward (LOCF) method and those due to death were replaced using the LOCF method plus a 4-point penalty (or 1-point for ΔSOFA subscores analysis) of 4 points. LSmeans and difference in LSmeans (95%CI) between nangibotide 1.0 mg/kg/h and placebo were estimated using an ANCOVA model.

For ACM28, ACM7, AFOS28 and shock reversal, the covariate adjusted estimate of the conditional treatment effect, expressed as the adjusted odds ratio, between nangibotide and placebo was obtained (with the 95% CI and p-value based on the Wald test) from a logistic regression model. A covariate adjusted estimate of the unconditional treatment effect, expressed as the adjusted difference in responder or risk rates between nangibotide and placebo was obtained (along with the 95% CI and p-value based on a z test) from a logistic regression model according to Ge et al (2011).

For Organ Support Free days and Length of stay at the ICU during study period (OSFD28 and ICU28) results were analyzed in an ANOVA model. LSmeans and difference in LSmeans were estimated. OSFD28 was calculated as the total number of calendar days from D0 to D28 without any organ support. In case of death, a value of 0 was imputed; if a patient withdrew alive and free of organ support prior to D28, the number of days between the withdrawal and D28 was imputed as OFFD. If a patient withdrew alive and on organ support prior to D28, the

number of days between the day of withdrawal and D28 was imputed as NOT OSFD. The number of ICU days until day 28 was capped at Day 28. Patients who died prior to or on Day 28 were assigned a penalty of 28-day stay. Number of ICU days until day 28 was analyzed in an ANOVA model. and LSmeans and difference in LSmeans (95% CI) were estimated.

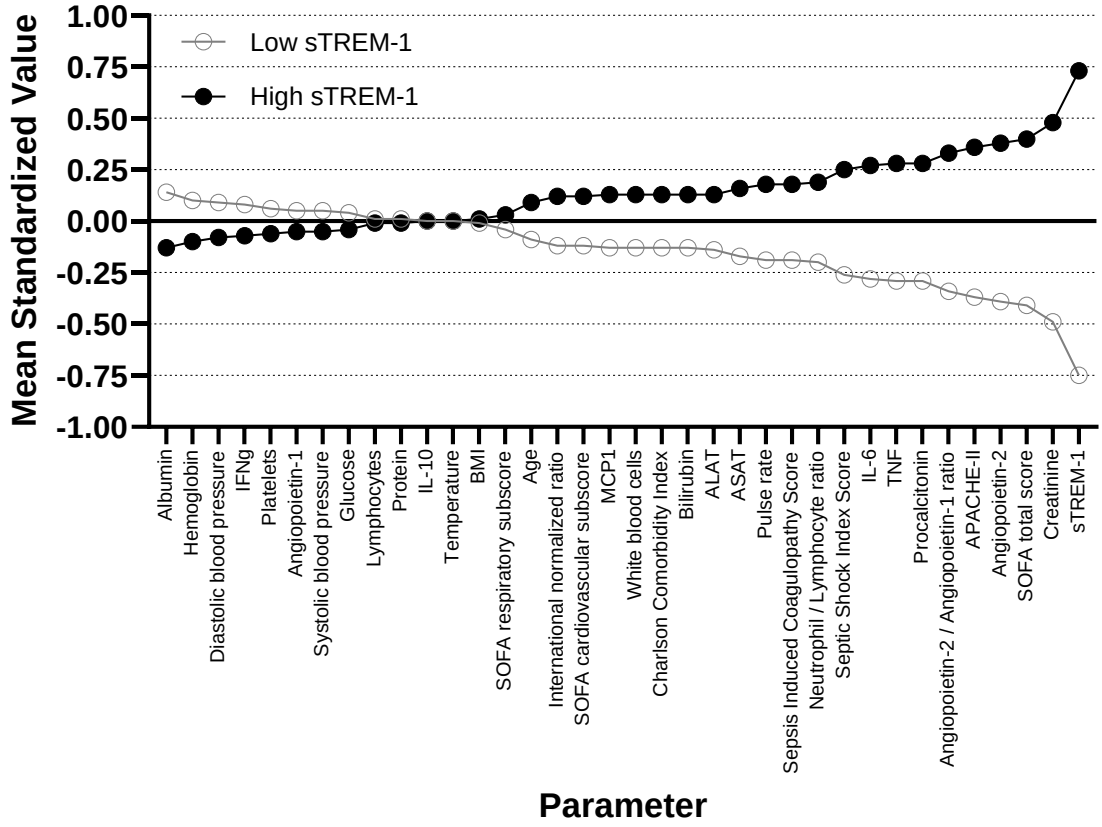

Figure S1. Mean standardized values per sTREM-1 subgroup ranked by increasing high sTREM-1 (≥1050 pg/mL) – low sTREM-1 (<1050 pg/mL) difference per parameter. The variables are standardized such that all means are scaled to 0 and SDs to 1. A value of 1 for the standardized variable value signifies that the mean value for the phenotype was 1 SD higher than the mean value for both phenotypes shown in the graph as a whole.

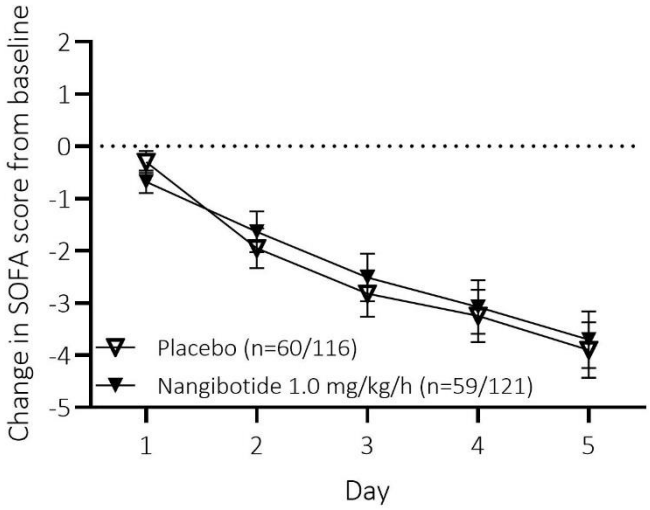

Figure S2. **Response to nangibotide in patients below the optimal prognostic sTREM-1 cut-off.** Relative total SOFA score change from initial score at baseline through day 5 is shown for patients with sTREM-1 levels below 1050 pg/mL which are patients with low risk of dying or needing organ support.

Table S1. **AUCs of Receiver operating characteristic curve (ROC) for prediction of death or being on organ support at day 28 (AFOS28-NO) or death at day 28 (ACM28)** based on plasma levels of sTREM-1 measured with the companion diagnostic-to-be Elecsys® assay, SOFA total baseline score, APACHE II baseline score and baseline procalcitonin plasma levels.

Biomarker		**AFOSD28-NO**	**ACMD28**
sTREM-1	ROC-AUC [95%CI]	69.0% [58.9%; 79.1%]	69.6% [58.6%; 80.6%]
	Logistic regression	Intercept=-1.78 slope=0.001 p=0.001	Intercept=-2.41 slope=0.001 p=0.002
SOFA Score	ROC-AUC [95%CI]	61.1% [50.3%; 71.9%]	57.7% [45.1%; 70.4%]
	Logistic regression	Intercept=-2.11 slope=0.16 p=0.04	Intercept=-2.25 slope=0.12 p=0.16
APACHE-II Score	ROC-AUC [95%CI]	63.7% [53.6%; 73.9%]	64.4% [53.5%; 75.2%]
	Logistic regression	Intercept=-2.82 slope=0.099 p=0.02	Intercept=-3.45 slope=0.10 p=0.03
Procalcitonin	ROC-AUC [95%CI]	48.8% [37.1%; 60.4%]	47.9% [33.9%; 61.9%]
	Logistic regression	Intercept=-0.66 slope=4E-6 p=0.42	Intercept=-1.29 slope=5.6E-6 p=0.29

Table S2. **ROC curve AUC robustness evaluation.** AUC and 95%CI of ROC curves plotted to prognosticate all-cause-mortality at day 28 (ACM28) and to prognosticate not being alive-and-free-of-organ-support (including cardiovascular, renal and mechanical ventilation support) at day 28 (AFOS28-NO) using primary and sensitivity/cross validation methods are tabulated**.**

	AFOSD28-NO	**ACMD28**
ROC-AUC [95%CI] Asymptotic (Wald method)	69.0% [58.9%; 79.1%]	69.6% [58.6%; 80.6%]
ROC-AUC [95%CI] Stratified bootstrap	68.9 % [58.4%; 78.8%]	69.5% [58.3%; 80.3%]
ROC-AUC [95%CI] Out-of-sample cross-validation	66.1% [55.5%; 76.8%]	65.9% [53.9%; 77.9%]

Table S3. **Summary of secondary endpoints results in for patients with sTREM-1 levels above 1050 pg/mL**, chosen as optimal prognostic cut-off based on ROC curve analysis (high-risk sTREM-1 population or HRsTP) expressed as difference between 1 mg/kg/h nangibotide or placebo treated arms.

	Placebo vs nangibotide 1.0 mg/kg/h (n=56 vs 62)	**Difference (nangibotide-placebo) [95% CI] p-value**
ΔSOFA5 total (Primary endpoint)	-0.1 vs -2.5	d=-2.5 [-4.2; -0.7] p=0.007
ΔSOFA5 respiratory	0.0 vs -0.4	d=-0.5 [-0.9; -0.1] p=0.02
ΔSOFA5 CNS	0.4 vs 0.3	d=-0.1 [-0.5; 0.3] p=0.61
ΔSOFA5 CV	-1.7 vs -2.4	d=-0.7 [-1.3; -0.1] p=0.02
ΔSOFA5 liver	0.0 vs -0.0	d=-0.1 [-0.4; 0.3] p=0.71
ΔSOFA5 renal	0.2 vs -0.5	d=-0.7 [-1.3; -0.2] p=0.006
ΔSOFA5 coagulation	0.9 vs 0.6	d=-0.3 [-0.7; 0.0] p=0.08
AFOS Day 28	42.0% vs 55.5%	d=13.5% [-3.4%; 30.4%] p=0.12
ACM Day 28	39.5% vs 30.7%	d=-8.8% [-25.2%; 7.6%] p=0.29
ACM Day 90	47.7% vs 35.7%	d=-12% [-28.0%; 4.0%] p=0.14
ICU Days through Day 28	20.9 vs 18.0	d=-2.9 [-6.3; 0.6] p=0.10
Organ Failure Free days until day 28	8.8 vs 12.4	d=3.6 [-0.5; 7.7] p=0.08
Shock Reversal	55.4% vs 72.6%	d=17.2% [0.1%; 34.3%] p=0.05

To account for between-group imbalance at baseline, analyses were adjusted for key co-variates: SOFA score, APACHE-II score, sTREM-1, IL-6, age, gender, BMI and site of infection.