Association between pretreatment lymphocyte count and efficacy of immune-enhancing therapy in acute necrotising pancreatitis: a post-hoc analysis of the multicentre, randomised, placebo-controlled TRACE trial

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Summary

Background Immune-enhancing thymosin alpha 1 (Tα1) therapy may reduce infected pancreatic necrosis (IPN) in acute necrotising pancreatitis (ANP). However, the efficacy might be impacted by lymphocyte count due to the pharmacological action of Tα1. In this *post-hoc* analysis, we tested the hypothesis that pre-treatment absolute lymphocyte count (ALC) determines whether patients with ANP benefit from Tα1 therapy.

Methods A *post-hoc* analysis of data from a multicentre, double-blind, randomised, placebo-controlled trial testing the efficacy of Tα1 therapy in patients with predicted severe ANP was performed. Patients from 16 hospitals of China were randomised to receive a subcutaneous injection of Tα1 1.6 mg every 12 h for the frst 7 days and 1.6 mg once a day for the following 7 days or a matching placebo during the same period. Patients who discontinued the Tα1 regimen prematurely were excluded. Three subgroup analyses were conducted using the baseline ALC (at randomisation), and the group allocation was maintained as intention-to-treat. The primary outcome was the incidence of IPN 90 days after randomisation. The fitted logistic regression model was applied to identify the range of baseline ALC where Tα1 therapy could exert a maximum effect. The original trial is registered with ClinicalTrials.gov, NCT02473406.

Findings Between March 18, 2017, and December 10, 2020, a total of 508 patients were randomised in the original trial, and 502 were involved in this analysis, with 248 in the Tα1 group and 254 in the placebo group. Across the three subgroups, there was a uniform trend toward more significant treatment effects in patients with higher baseline ALC. Within the subgroup of patients with baseline ALC≥0.8 × 10^9/L (n = 290), the Tα1 therapy significantly reduced the risk of IPN (covariate adjusted risk difference, −0.12; 95% CI, −0.21,-0.02; p = 0.015). Patients with baseline ALC between 0.79 and 2.00 × 10^9/L benefited most from the Tα1 therapy in reducing IPN (n = 263).

Interpretation This *post-hoc* analysis found that the efficacy of immune-enhancing Tα1 therapy on the incidence of IPN may be associated with pretreatment lymphocyte count in patients with acute necrotising pancreatitis.

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Translation For the Chinese translation of the Summary, see the Supplementary Materials section.

Keywords: Acute pancreatitis; Immunosuppression; Thymosin; Pancreatic necrosis; Infection

Research in context

Evidence before this study

We systematically searched PubMed, Embase, and the Cochrane Library, until December 10, 2022 by using keywords and medical subject heading (Mesh), including the following terms: ("acute pancreatitis" OR "acute necrotising pancreatitis") AND ("infected pancreatic necrosis" OR "infection") AND ("absolute lymphocyte count" OR "lymphopenia" OR "lymphocytopenia" OR "immunosuppression"). Full-text original research articles and reviews were included. Previous observational studies showed that early immunosuppression and lymphopenia might contribute to the development of infected pancreatic necrosis in patients with acute necrotising pancreatitis. However, studies investigating the clinical effect of immune-enhancing therapy on infection are scarce. The only large randomised trial is the TRACE trial showing that immune-enhancing thymosin alpha 1 (Tα1) treatment did not reduce the incidence of IPN.

Added value of this study

By performing a *post-hoc* analysis of the TRACE trial, we found that Tα1 therapy significantly reduced the incidence of infected pancreatic necrosis among patients with a pretreatment lymphocyte count greater than 0.8 × 10⁹/L. Further analysis revealed that those with a pretreatment lymphocyte count between 0.79 and 2.00 × 10⁹/L benefited most from Tα1 therapy.

Implications of all the available evidence

Our findings suggested that patients with predicted severe acute necrotising pancreatitis and no lymphopenia may be candidates for immune-enhancing Tα1 therapy to reduce the risk of infected pancreatic necrosis. The results show the potential of immune-enhancing therapy in acute pancreatitis and represent an important avenue for further research.

Introduction

Infected pancreatic necrosis (IPN) is a highly morbid and potentially lethal complication of acute necrotising pancreatitis (ANP).[1] Previous attempts to reduce the incidence of IPN using prophylactic antibiotics and enteral probiotics failed,[2,3] and therefore not widely used worldwide.[4] In recent years, it has been recognized that immunosuppression may develop early during the courses of severe acute pancreatitis and is associated with an increased risk of infection,[5–7] suggesting that immune-enhancing therapy might improve outcomes, including reducing the incidence of IPN.

The recently published multicentre randomised clinical (TRACE) trial investigated the efficacy of thymosin alpha 1 (Tα1) therapy on the incidence of IPN in patients with predicted severe ANP (APACHEII≥8).[8] Tα1 is a polypeptide hormone isolated from the thymus and has a wide range of immune-enhancing properties.[9] The primary analysis of the TRACE trial demonstrated that Tα1 treatment did not significantly reduce the incidence of IPN during the index admission or within 90 days of randomisation.[10,11]

The key pharmacological action of Tα1 is to promote antigen-presenting and stimulate the adaptive immunological responses, which are carried out by different classes of lymphocytes.[9] Previous studies also showed that absolute lymphocyte count (ALC) at admission and the trajectories of ALC during the early phase of acute pancreatitis are associated with the incidence of IPN.[12,13] On this basis, we hypothesized that the early immune-enhancing Tα1 therapy might be both effective and dependent on the ALC level before the initiation of treatment.

The aim of this *post-hoc* analysis of data from the TRACE trial was to (1) investigate the efficacy of Tα1 on the incidence of IPN in patients with or without lymphopenia at randomisation, and (2) determine the range of ALC where Tα1 exerts the maximum effect in reducing the incidence of IPN.

Methods

Study design

This *post-hoc* analysis was reported in light of the STROBE guidelines using data from the TRACE trial (ClinicalTrials.gov identifier, NCT02473406). This analysis was not pre-specified in the original trial protocol. The TRACE trial was a multicentre, double-blind, randomised, placebo-controlled, superiority trial, and the protocol[14] and results of this trial have been published.[8] The trial was approved by the local ethics committee at the 16 participating sites. Written informed consent was obtained from the patients or their next-of-kin before randomisation.

Study participants and data collection

The TRACE trial recruited patients with predicted severe ANP (APACHE II (acute physiology and chronic

health evaluation II) ≥ 8) admitted within seven days of the advent of abdominal pain. The complete eligibility criteria for the TRACE trial were published.[14] The intervention timing was chosen based on current evidence regarding the time course of immunosuppression in acute pancreatitis.[6,15,16] Patients were randomised to receive Tα1 (SciClone Pharmaceutical Co., Ltd, Hong Kong) treatment (1.6 mg every 12 h for the first week and 1.6 mg once a day for the following week) or matching placebo over a two-week period. The study treatment was initiated the day after randomisation. In this *post-hoc* analysis, all patients enrolled in the TRACE trial were considered for inclusion. Patients who discontinued the Tα1 regimen prematurely due to adverse events or withdrawal of consent, were excluded. The gender of the trial participants was determined according to the identity materials provided by the patients or their next-of-kin. Full details of data collection can be found in the published protocol of the original TRACE trial. All the data required in this analysis were extracted from the electronic database of the TRACE trial.

Outcomes

The primary outcome was the incidence of IPN within 90 days of randomisation. The diagnosis of IPN was made when one or more of the following criteria were present: gas bubbles within pancreatic/peripancreatic necrosis on CT; a positive culture from pancreatic and/or peripancreatic necrosis obtained by fine-needle aspiration, catheter drainage, or necrosectomy according to the latest guidelines.[17]

Secondary clinical outcomes included mortality at 90 days after randomisation, new requirement of invasive procedures, new-onset persistent organ failure as defined by the Revised Atlanta Classification,[17] and length of hospital and ICU stay during the index admission. Secondary laboratory and severity scoring endpoints consisted of C-reactive protein (CRP), lymphocyte count, monocyte human leukocyte antigen-DR (mHLA-DR), platelet count, APACHE II score, and SOFA (sequential organ failure assessment) score on day7 after randomisation.

Statistical analysis

Continuous data were reported as means and standard deviations when normally distributed or as medians and interquartile ranges when not normally distributed. The Shapiro–Wilk test was used to assess the normality. Categorical data are expressed as frequencies and percentages. Comparisons of categorical data between groups were performed using Pearson's Chi-square test. When one or more expected values were less than 5, Fisher's exact test was used. Student t-test (normal distribution) or Mann–Whitney's test (non-normal distribution) was adopted to analyse continuous variables. Statistical tests were two-sided, and p values < 0.05 were considered statistically significant. All data analyses were done in R 4.2.1 software.

The nonlinear relationship between the ALC at randomisation and IPN incidence was assessed by the locally weighted scatterplot smoothing (LOWESS). Three subgroup analyses were conducted based on three widely-used definitions of lymphopenia or severe lymphopenia at baseline (randomisation):

(1) ALC < 1.0×10^9/L[18];
(2) ALC < 0.8×10^9/L[19];
(3) ALC < 0.5×10^9/L.[20]

The subgroup × treatment interaction test was conducted by the Cox proportional hazards regression model that controlled for treatment and subgroups' main effects. Cox proportional hazards models were performed to calculate the hazard ratios and associated 95% confidence intervals, using R's "survival" package v3.4.0. We tested the assumptions of proportional hazard by checking the plots of Schoenfeld residuals over time.

For the primary and secondary outcomes, the generalised linear model (family = binomial (link = identity)) and quantile regression (R's "quantreg" package v5.94) were employed to compare group differences in the dichotomous and continuous outcomes, respectively, with potential risk factors (p < 0.2 for baseline characteristics) between two groups and site as covariates. The risk ratio (RR), together with its 95% confidence interval, was calculated. Kaplan–Meier curves were used to compare the cumulative incidence of IPN to 90 days after randomisation tested by log-rank test. Secondary laboratory and scoring endpoints at day7 after randomisation were analysed by ANCOVA (Analysis of Covariance) with the baseline value as the covariate. We performed data conversions (including log, reciprocal, and square root transformations) for the endpoints that did not meet the model assumptions of ANCOVA.

The fitted logistic regression model with broken-line relationships was additionally applied to identify the baseline ALC range where the Tα1 treatment could exert a maximum effect in reducing the risk of IPN, using R's "segmented" package v1.3.4. In the model, the 90-day IPN was the dependent variable and the independent variables included the use of Tα1, the baseline ALC, and their interaction.

Role of the funding source

This *post-hoc* study was funded by National Natural Science Foundation of China (NSFC). The NSFC had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

LK, WM, and WL have access to the dataset, and WL has final responsibility for the decision to submit it for publication.

Results

Baseline characteristic

Between March 18, 2017, and December 10, 2020, 508 patients were randomised at 16 participating sites across China to receive Tα1 treatment or placebo (254 patients in each group) in the original trial. Six patients in the intervention group were excluded due to premature discontinuation of the Tα1 therapy, leaving a study cohort of 502 patients. The baseline ALC data was available in all the study individuals, and there was no loss of patients over the 90-day follow-up period (Supplementary Fig. S1). The demographic and baseline characteristics of the study patients are shown in Table 1. The median age of the study individuals was 43, and 62.7% were male, with 248 in the Tα1 group and 254 in the placebo group.

Characteristics	Total (N = 502)	Tα1 group (N = 248)	Placebo group (N = 254)	*p*-value
Age, median (IQR), y	43.0 (35.0–53.0)	43.0 (34.3–52.8)	44.0 (35.0–54.0)	0.48
Gender				0.66
Women (%)	187 (37.3)	90 (36.3)	97 (38.2)	
Men (%)	315 (62.7)	158 (63.7)	157 (61.8)	
BMI, median (IQR), kg/m^2	26.3 (24.0–28.4)	26.2 (24.0–28.2)	26.5 (24.2–29.0)	0.45
Etiologies				0.99
Alcoholic	31 (6.2)	16 (6.5)	15 (5.9)	
Biliary	199 (39.6)	99 (39.9)	100 (39.4)	
Idiopathic	24 (4.8)	12 (4.8)	12 (4.7)	
Hypertriglyceridemia	248 (49.4)	121 (48.8)	127 (50.0)	
Charlson score, median (IQR)	0 (0–1)	0 (0–1)	0 (0–1)	0.59
Interval between onset and randomisation, median (IQR), d	4.0 (2.4–6.0)	4.0 (2.0–6.0)	4.0 (2.7–6.0)	0.92
The extent of pancreatic necrosis				0.28
<30%	312 (62.2)	161 (64.9)	151 (59.4)	
30–50%	127 (25.3)	55 (22.2)	72 (28.3)	
>50%	63 (12.5)	32 (12.9)	31 (12.2)	
Disease severity				
CTSI score, median (IQR)	6.0 (5.0–8.0)	6.0 (5.0–8.0)	6.0 (5.0–8.0)	0.15
APACHE II score, median (IQR)	10.0 (8.0–13.0)	10.0 (8.0–13.0)	10.0 (8.0–13.0)	0.98
SOFA score, median (IQR)	4.0 (2.0–6.0)	4.0 (2.0–6.0)	4.0 (2.0–6.0)	0.80
CRP, median (IQR), mg/L	164.7 (99.0–236.3)	168.9 (94.1–236.5)	160.6 (105.5–236.4)	0.93
Lymphocyte count, median (IQR), 10^9/L	0.9 (0.6–1.2)	0.9 (0.6–1.2)	0.9 (0.7–1.2)	0.31
Platelet count, median (IQR), 10^9/L	164.5 (123.8–212.0)	166.0 (130.0–209.5)	162.0 (119.8–216.3)	0.37

P > 0.05 for the comparison between the groups for all characteristics. Tα1 denotes thymosin alpha one. IQR denotes interquartile range. BMI denotes body mass index. CTSI denotes compute tomography severity index. APACHE II denotes acute physiology and chronic health evaluation II, which ranges from 0 to 71, with higher scores indicating more severe disease. SOFA denotes sequential organ failure assessment, which ranges from 0 to 24, with higher scores indicating more severe organ failure. CRP denotes C-reactive protein.

Table 1: **Baseline characteristics of the study individuals.**

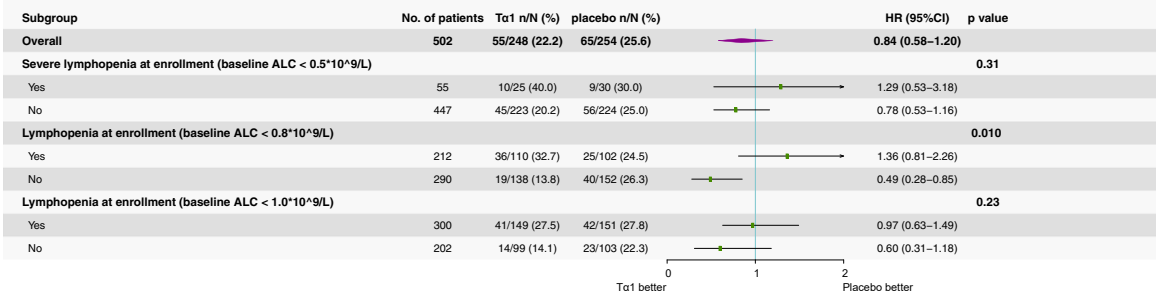

Fig. 1: **Subgroup analysis of the risk of infected pancreatic necrosis.** A risk difference of less than one indicates better results for the Tα1 group. Tα1 denotes thymosin alpha one.

Results of subgroup analysis

As shown in Supplementary Fig. S2, the LOWESS curve showed a nonlinear relationship between the baseline ALC and IPN incidence. To further investigate the efficacy of Tα1 on the incidence of IPN across different levels of baseline ALC, three subgroups were analysed, and the baseline characteristics of the subgroups are shown in Supplementary Tables S1–S3. In all the subgroup analyses, the proportional hazard assumptions were fulfilled. Across the three subgroups, patients with lymphopenia had an overall higher incidence of IPN, and there is a uniform trend toward more significant treatment effects in patients with higher baseline ALC. The impact of baseline ALC on the efficacy of Tα1 treatment is most significant when the study individuals are dichotomized at baseline ALC of $0.8 \times 10^{\wedge}9/L$ (p for interaction = 0.010, Fig. 1).

Primary and secondary clinical outcomes in different subgroups

Among patients with baseline ALC < $0.8 \times 10^{\wedge}9/L$, after adjusting for the computed tomography severity index (CTSI), CRP, platelet count at randomisation and site, both the primary outcome and secondary outcomes are comparable between groups (Table 2).

Among patients with baseline ALC≥$0.8 \times 10^{\wedge}9/L$, after adjusting for age and site, 19/138 (13.8%) developed IPN in the Tα1 group and 40/152 (26.3%) in the placebo group (difference, –0.12; 95% CI, –0.21,-0.02; p = 0.015). The cumulative incidence of IPN during 90 days after randomisation is shown in Fig. 2. The probability of developing IPN was significantly lower in the Tα1 group than in the placebo groups (Log-Rank p = 0.0093). Mortality occurred in 9/138 (6.5%) in the Tα1 group and 14/152 (9.2%) in the placebo group (difference, –0.02; 95% CI, –0.08, 0.04; p = 0.48) during the same period. Other secondary outcomes, including new requirement of invasive procedures, new-onset persistent organ failure, and length of hospital and ICU stay during the index admission, were not significantly different between groups (Table 2). Analysis results for the other two subgroups are shown in Supplementary Figs. S3 and S4 and Tables S4 and S5.

Laboratory and severity scoring outcomes in different subgroups

Among patients with baseline ALC < $0.8 \times 10^{\wedge}9/L$, the use of Tα1 did not result in differences in laboratory and severity scoring endpoints after adjusting for the baseline values. In contrast, among patients with baseline ALC≥$0.8 \times 10^{\wedge}9/L$, the ALC at day7 after randomisation was 1.62 (0.59)×$10^{\wedge}9/L$ in the Tα1 group and 1.45 (0.57) ×$10^{\wedge}9/L$ in the placebo group in the ANCOVA analysis after adjusting for the baseline value (F = 4.173, p = 0.042) (Fig. 3a). Other secondary laboratory and scoring endpoints, including CRP levels, mHLA-DR, platelet count, APACHE II score, and

Primary and secondary endpoints	Lymphopenia (N = 212)					Non- lymphopenia (N = 290)				
	Tα1 group (N = 110)	Placebo group (N = 102)	Risk difference (95% CI)	Unadjusted p value	Risk difference (95% CI)[a]	Adjusted p value	Tα1 group (N = 138)	Placebo group (N = 152)	Risk difference (95% CI)	Unadjusted p value	Risk difference (95% CI)[b]	Adjusted p value
90-day IPN, (n, %)	36 (32.7)	25 (24.5)	8.21 (–3.89, 20.32)	0.18	9.13 (–3.10, 21.35)	0.14	19 (13.8)	40 (26.3)	–12.55 (–21.61, –3.49)	0.0066	–11.51 (–20.78, –2.25)	0.015
90-day Mortality, (n, %)	13 (11.8)	9 (8.8)	2.99 (–5.17, 11.16)	0.47	5.22 (–3.75, 14.19)	0.25	9 (6.5)	14 (9.2)	–2.69 (–8.86, 3.48)	0.39	–2.17 (–8.25, 3.91)	0.48
New-onset persistent organ failure during the index admission, (n, %)	16 (14.5)	16 (15.7)	–1.14 (–10.80, 8.51)	0.82	0.75 (–9.23, 10.74)	0.88	23 (16.7)	31 (20.4)	–3.73 (–12.66, 5.20)	0.41	–3.68 (–12.71, 5.36)	0.42
New requirement of invasive procedures during the index admission, (n, %)	24 (21.8)	15 (14.7)	7.11 (–3.22, 17.45)	0.18	7.62 (–3.21, 18.45)	0.17	19 (13.8)	26 (17.1)	–3.34 (–11.64, 4.96)	0.43	–2.78 (–11.41, 5.86)	0.53
Length of hospital stay, median (IQR), days	15.5 (10.0–29.3)	16.0 (9.0–25.3)	0 (–10.00, 6.01)	1.0	–0.48 (–2.53, 3.71)	0.78	14.0 (8.0–21.3)	15.0 (8.0–24.0)	–1.00 (–7.02, 4.02)	0.51	–1.37 (–4.21, 0.75)	0.33
Length of ICU stay, median (IQR), days	11.0 (7.0–19.3)	9.0 (4.0–15.3)	2.00 (–6.50, 6.01)	0.18	2.24 (–0.55, 3.54)	0.090	7.0 (4.0–15.0)	9.0 (5.0–17.0)	–2.00 (–5.02, 2.02)	0.097	–1.55 (–3.35, 0.45)	0.17

ALC denotes absolute lymphocyte count. Tα1 denotes thymosin alpha one. CI denotes confidence interval. IPN denotes infected pancreatic necrosis. IQR denotes interquartile range. ICU denotes intensive care unit. [a]Adjusted for CTSI score, C-reactive protein, platelet count and site. [b]Adjusted for age and site.

Table 2: **Primary and secondary outcomes in patients with or without lymphopenia (baseline ALC < $0.8 * 10^{\wedge}9/L$).**

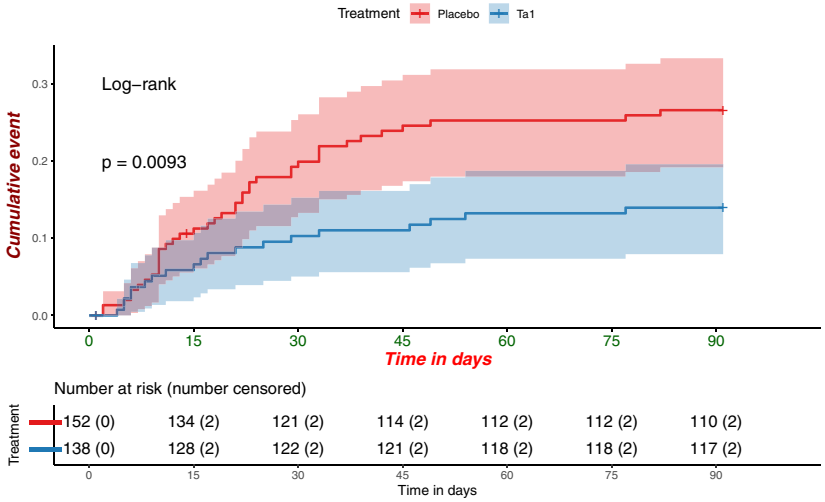

Fig. 2: **The Kaplan–Meier curves for the cumulative incidence of IPN from randomisation to day 90 in patients without lymphopenia (baseline ALC ≥ 0.8 *10^9/L).** IPN denotes infected pancreatic necrosis. Tα1 denotes thymosin alpha one. ALC denotes absolute lymphocyte count.

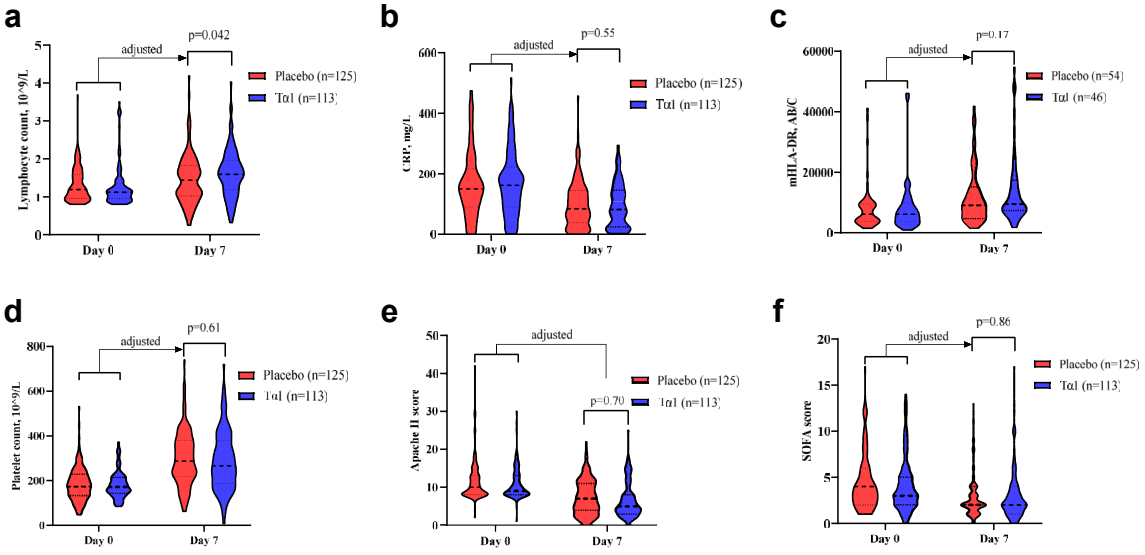

Fig. 3: **Secondary laboratory and scoring endpoints in patients without lymphopenia (baseline ALC ≥ 0.8 *10^9/L).** ALC denotes absolute lymphocyte count. Tα1 denotes thymosin alpha one. CRP denotes C-reactive protein. mHLA-DR denotes monocyte human leukocyte antigen-DR. APACHE II denotes acute physiology and chronic health evaluation II, which ranges from 0 to 71, with higher scores indicating more severe disease. SOFA denotes sequential organ failure assessment, which ranges from 0 to 24, with higher scores indicating more severe organ failure.

SOFA score, were comparable between groups (Fig. 3). Analysis results for the other two subgroups are shown in Supplementary Figs. S5 and S6.

Results of the fitted logistic regression model

The results of the fitted logistic regression model with segmented relations are shown in Fig. 4. The estimated optimal range of baseline ALC was between 0.79 and 2.00 × 10^9/L. Accordingly, 263 AP patients (127 in Tα1 and 136 in the placebo group) with baseline ALC within this range were enrolled in further analysis. The baseline characteristics of them are shown in Supplementary Table S6. During the 90 days after randomisation, 17/127 (13.4%) developed IPN in the Tα1 group and 38/136 (27.9%) in the placebo group (difference, –0.13; 95% CI, –0.23,-0.03; adjusted

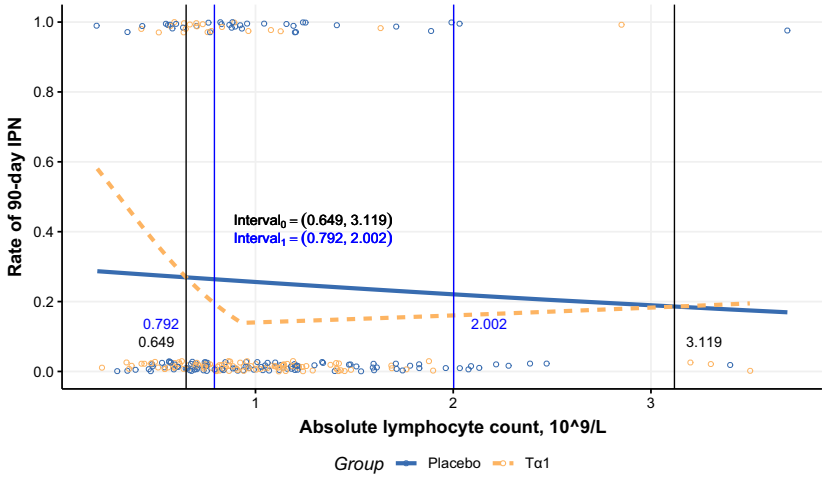

Fig. 4: **"Segmented" logistic regression estimation results**. Interval$_0$ is the estimated interval based on "segmented" results where the Tα1 group has lower rates of IPN than the placebo group. Interval$_1$, with the greatest difference between groups, is estimated by simultaneously shrinking the upper and lower limits of Interval$_0$ proportionally toward the estimated break-point. Tα1 denotes thymosin alpha one. IPN denotes infected pancreatic necrosis.

p = 0.010) (Table 3). Mortality and other clinical outcomes during the 90 days after randomisation was comparable between groups.

Discussion

In this *post-hoc* analysis of the TRACE trial, it was found that patients with predicted severe ANP and no lymphopenia (baseline ALC≥ 0.8×10^9/L) had a significant reduction in the risk of IPN (over 90 days) from early treatment with immune-enhancing Tα1 therapy. However, the reduction in IPN was not associated with other clinically relevant outcomes, which might be attributed to the insufficient sample size of the target subgroup.

The pharmacology of Tα1 may partly explain this phenomenon. Despite the pleiotropic nature of Tα1, it has a vital role as a toll-like receptor (TLR) agonist (TLR-9 and TLR-2) in myeloid and dendritic cells, which promotes antigen-presentation.[21] In this way, it stimulates the adaptive immune responses by increasing the production of multiple pro-inflammatory cytokines and enhancing the cytotoxic response, and the primary effector cells are T lymphocytes.[22,23] Moreover, it can stimulate antibody production, which is a B lymphocyte-dependent process.[24] Given these known pharmacological effects of Tα1, the lack of therapeutic efficacy in the subgroup of study patients with lymphopenia is likely due to an inadequate level of effector cells for effective adaptive immune responses. However, since the mHLA-DR expression was comparable between groups after the Tα1 therapy, the negative results may also be attributed to insufficient antigen-presenting stimulated by Tα1. A future prospective study is needed to clarify this.

The ALC is often used as a convenient marker of immunosuppression in many disease settings because of its wide availability.[25,26] In acute pancreatitis, the ALC during the early phase has been linked to the development of IPN, and prolonged ICU and hospital stay.[6,12,13]

Primary and secondary endpoints	Tα1 group (N = 127)	Placebo group (N = 136)	Risk difference (95% CI)	Unadjusted *p* value	Risk difference (95% CI)[a]	Adjusted *p* value
IPN within 90 days after randomization (n, %)	17 (13.4)	38 (27.9)	−14.56 (−24.14, −4.97)	0.0029	−13.20 (−23.21, −3.19)	0.010
Mortality within 90 days after randomization (n, %)	8 (6.3)	13 (9.6)	−3.26 (−9.76, 3.24)	0.33	−2.59 (−8.85, 3.67)	0.42
New-onset persistent organ failure during the index admission, (n, %)	19 (15.0)	29 (21.3)	−6.36 (−15.63, 2.90)	0.18	−5.90 (−15.21, 3.41)	0.21
New requirement of invasive procedures during the index admission, (n, %)	17 (13.4)	24 (17.6)	−4.26 (−12.99, 4.46)	0.34	−4.03 (−13.14, 5.08)	0.38
Length of hospital stay, median (IQR), days	13.0 (7.0–21.0)	15.0 (8.3–24.8)	−2.00 (−9.19, 3.69)	0.16	−2.59 (−4.45, 0.46)	0.094
Length of ICU stay, median (IQR), days	7.0 (4.0–14.0)	9.0 (5.0–17.0)	−2.00 (−5.38, 2.69)	0.13	−2.27 (−3.78, 0.23)	0.085

ALC denotes absolute lymphocyte count. Tα1 denotes thymosin alpha one. CI denotes confidenceinterval. IPN denotes infected pancreatic necrosis. IQR denotes interquartile range. ICU denotes intensive care unit. [a]Adjusted for age, C-reactive protein and site.

Table 3: **Primary and secondary outcomes in patients with baseline ALC between 0.792–2.002 * 10^9/L.**

This is consistent with the overall results of the TRACE trial in which patients with lymphopenia were more likely to develop IPN. Moreover, our results show that lymphopenia during the first week of disease onset was common in the TRACE participants with predicted severe acute pancreatitis. This finding is in line with a previous study suggesting that patients with more severe acute pancreatitis develop immunosuppression earlier.[6] Taken together, our findings provide a strong rationale for reassessing the effect of Tα1 therapy in patients at risk of IPN and no lymphopenia.

Based on the finding that patients with a normal or slightly decreased ALC may benefit from Tα1 treatment most, a fitted logistic regression model was used to determine the ALC range that was associated with maximum Tα1 efficacy. The results showed that in the control group, the incidence of IPN became progressively lower over increasing baseline ALC, which is in line with our previous study.[12] On the contrary, the Tα1 treatment resulted in a very different shape of the ALC-incidence relationship curve, and a sharp dive was detected in patients with modest baseline ALC (the yellow dotted line in Fig. 4). Of note, the optimal ALC range (0.79 to 2.00 × 10^9/L) for effective Tα1 therapy was defined by a *post-hoc* analysis. Thus the results were only explanatory, and the type I error rate may be inflated with multiple models. While this finding opens a new treatment approach, it will need to be confirmed by a prospective trial before any definitive clinical recommendations can be made.

According to the checklist provided by Sun et al.,[27] this subgroup analysis has several strengths: (1) the subgroup variable (lymphopenia as defined by reduced ALC) was measured at the baseline, and the definitions we used are from previously published studies[18–20]; (2) the comparison was undertaken within a single RCT; (3) tests of interaction were used; (4) adjusted analysis was applied within the subgroup to ensure the independent effect of treatment; (5) there is a strong pharmacologic rationale for the findings; (6) the size of the subgroup in which Tα1 therapy was found to be effective was relatively large (n = 290). Some limitations must be acknowledged: (1) the subgroup analysis and the "direction" of the treatment effect were not pre-defined; (2) the strength of interaction was inconsistent among multiple subgroups defined by using different definitions of 'lymphopenia'; (3) other measures of immune suppression such as IL-10, in addition to baseline ALC, were not used; (4) approximately half of the study patients were caused by hypertriglyceridemia, which may affect the generalisability of the results; and (5) Due to the difficulties in multisite lab standardisation, the mHLA-DR expression was only obtained in less than half of the study individuals, making the comparison likely underpowered.

In conclusion, this *post-hoc* analysis found that the efficacy of immune-enhancing Tα1 therapy on the incidence of IPN may be associated with pretreatment lymphocyte count in patients with predicted severe ANP. The results show the potential of immune-enhancing therapy in acute pancreatitis and represent an important avenue for further research.

Contributors

All co-authors have made substantial and intellectual contributions to the work and approved the submitted article. LK and WL conceived and designed the research, which was then revised and refined by ZT, JW and PM. Data analyses, interpretation and visualisation were performed by LK, WM, FS, JZ and MX. TC, WM and YL have verified the underlying data. The manuscript was written by LK, WM and revised by all co-authors. LK, WM and WL have accessed and verified the data, and WL was responsible for the decision to submit the manuscript.

Data sharing statement

Deidentified individual participant data are available indefinitely in the electronic database. Data can be accessed through capctg.medbit.cn with the approval of the authors. Request for data can be made to the corresponding author (ctgchina@medbit.cn) and will be discussed during a meeting of the Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG).

Declaration of interests

All authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.101915.

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