# Significance of the complete clearance of peripheral blasts after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia: the Tokyo Children's Cancer Study Group Study L99-15

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## ABSTRACT

### Background

Treatment response has become one of the most important prognostic factors in childhood acute lymphoblastic leukemia. We evaluated the significance of the complete clearance of peripheral leukemic blasts on survival in children with acute lymphoblastic leukemia.

### Design and Methods

Seven hundred and fifty-four children diagnosed with acute lymphoblastic leukemia, consecutively enrolled from 1999 to 2003 in the TCCSG L99-15 study, were eligible for analysis. Patients were stratified into three risk groups based on presenting features, such as age and the leukocyte count before starting the treatment, followed by reclassification into three categories 7 days after prednisolone monotherapy based on the peripheral blast count; 0/μL (*Day8NoBlasts*), 1-999/μL and ≥ 1,000/μL.

### Results

After 7 days of prednisolone monotherapy, 249 patients (33%) were classified as *Day8NoBlasts*, 392 patients (52%) had blast counts of 1-999/μL, and 113 patients (15%) had blast counts ≥ 1,000/μL. The event-free survival for all patients was 79.6±1.6 (SE)% at 4 years, whereas that for patients with *Day8NoBlasts* was 90.4±2.0% (n=249) and the event-free survival for the other patients was 74.2±2.2% (n=504) (log rank $p<0.001$). The event-free survival for *Day8NoBlasts* patients with B-lineage acute lymphoblastic leukemia and T-cell acute lymphoblastic leukemia was 89.8±2.1% (n=226) and 95.7±4.3% (n=23), respectively. In a multivariate analysis, age at diagnosis, the initial white blood cell count, immunophenotype, and gender did not remain as independent risk factors for treatment failure, whereas *Day8NoBlasts* and marked hyperdiploidy (more than 50 chromosomes) became statistically significant.

### Conclusions

Children with *Day8NoBlasts* constituted one third of all the cases with childhood acute lymphoblastic leukemia with an excellent outcome, and should be candidates for curative management with less intensive treatment.

Key words: lymphoblastic leukemia, children, clearance of blasts, steroid response.

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## Introduction

Early treatment response is one of the most useful prognostic indicators in childhood acute lymphoblastic leukemia (ALL). This response depends on numerous variables, including the clinicobiological features of the disease, chemotherapy dosages, and also the ability of individual patients to metabolize antileukemic drugs.[1,2] The level of circulating lymphoblasts after 1 week of chemotherapy is associated with the risk of relapse.[3-7] The Berlin-Franfurt-Münster (BFM) group has traditionally employed the response to prednisolone for 7 days and one dose of intrathecal methotrexate to stratify patients: a cut-off peripheral blood blast count of 1,000/μL is used to assign patients into two groups; that is, prednisolone good responders and prednisolone poor responders.[3,8] The utility of this method has been well appreciated and is now employed by other study groups.[9-11]

We analyzed the results of the L89-12 study of the Tokyo Children's Cancer Study Group (TCCSG), and found that a cut-off of 1,000 blasts/μL after a 7-day course of prednisolone monotherapy was useful for stratifying patients.[12] We also found that patients without detectable blasts in the peripheral blood had an even better prognosis (*unpublished data*). In the 99-15 study, we employed a cut-off of 0 blasts in addition to 1,000 blasts to stratify children with ALL. Here, we report the treatment outcome of the study, in which the utility of the above-mentioned stratification of the patients was examined.

## Design and Methods

### Patients

Seven hundred and seventy children (1 to 18 years of age) diagnosed with ALL were consecutively enrolled from February 1999 to July 2003 in the TCCSG L99-15 study. Children less than 1 year of age were excluded from this study and treated with an infant ALL protocol. Sixteen patients were not evaluable; therefore, 754 patients (male: female; 428 : 326) were eligible for analysis. Their median age was 5 years (range, 1-17). Written informed consent was obtained from parents or guardians and from the patients as appropriate for their age and conceptual ability.

The diagnosis of ALL was based on morphological, biochemical, and flow cytometric features of leukemic cells, including lymphoblast morphology on May- or Wright-Giemsa-stained bone marrow smears, negative staining for myeloperoxidase, and reactivity with monoclonal antibodies to B- or T-lineage-associated lymphoid differentiation antigens. Remission was defined as the presence of fewer than 5% blasts with the recovery of hematopoiesis.

### Day 8 risk classification

The patients were stratified into three risk groups based on presenting features (age and the leukocyte count before starting the treatment) and then reclassified into three categories 7 days later according to the sensitivity to oral prednisolone monotherapy, the dose of which was 30-60 mg/m$^2$/day (Table 1). A total dose of at least 210 mg/m$^2$ of prednisolone was to be administered in this 7-day prephase period. A diagnostic lumbar puncture was performed and initial intrathecal methotrexate was given on day 8.[12] The dividing counts of 0 blasts/μL and 1,000 blasts/μL were used to stratify patients. To count blasts in the peripheral blood, 200 cells were morphologically assessed under a microscope.

### Day 43 risk classification

The patients were finally stratified based on the bone marrow status examined between 43 and 50 days after the initiation of remission induction therapy and on cytogenetic findings. Patients who did not achieve remission and those with the Philadelphia chromosome or 11q23 rearrangements were allocated to the high risk group and underwent allogeneic stem cell transplantation, and those initially at standard risk who showed t(1;19) were switched into the intermediate risk group. The median follow-up period of patients was 3.8 years.

### Treatment protocol

The protocol was approved by the institutional review boards of the participating institutions or the equivalent organization. Treatment regimens are detailed in Table 2. A proportion of patients in the high risk group underwent stem cell transplantation in first



**Table 1.** Risk stratification.

| | | | |
|---|---|---|---|
| | *B-lineage ALL* | | |
| Initial risk WBC (×10$^9$/L) | 1-6 years | 7-9 years | >10 years |
| <20 | SR | IR | IR |
| 20-50 | IR | IR | IR |
| 50-100 | IR | IR | HR |
| ≥100×10$^9$/L | HR | HR | HR |
| | *Day 8 risk (final risk)* | | |
| Day 8 PB Blasts | 0 | 1-999 | ≥1,000/μL |
| Day 1 SR | SR | SR | IR |
| Day 1 IR | IR | IR | HR |
| Day 1 HR | IR | HR | Allo-SCT |
| | *T-ALL* | | |
| Day 8 PB blasts | 0 | 1-999 | ≥1,000/μL |
| All patients* | IR | HR | Allo-SCT |

*WBC: white blood cell count; PB: peripheral blood; SR: standard risk; IR: intermediate risk; HR: high risk; allo-SCT: allogeneic stem cell transplantation; *in T-ALL, patients were stratified only based on the day 8 PB blast count regardless of age and initial WBC. Patients with ALL, from 1 to 18 years of age at diagnosis. Philadelphia chromosome and MLL rearrangement: Allo-SCT, t(1;19): Shifted to IR if in SR. Cranial irradiation: patients with initial WBC > 100×10$^9$/L: 12 Gy for those aged 1-6 years, 18 Gy for the others.*

remission according to the protocol (n=58). Prophylactic cranial irradiation was given only to patients with an initial leukocyte count exceeding $100\times10^9$/L. The dose of irradiation was 12 Gy for patients aged between 1 and 6, and 18 Gy for the others. A maintenance phase, consisting of 6-mercaptopurine and methotrexate, was continued until week 146 in the standard risk group and until week 104 in the intermediate risk group, whereas no maintenance therapy was given to patients in the high risk group.

Two randomizations were performed. The first randomization concerned the schedule of L-asparaginase in the remission induction phase in the standard and intermediate risk groups: two doses a week *vs.* three doses a week for a total number of nine doses in both groups. The second randomization involved only intermediate risk patients: high-dose cytarabine at 2 g/m$^2$ 8 times *vs.* cytarabine at 75 mg/m$^2$ 15 times accompanied by cyclophosphamide at 1,000 mg/m$^2$ and 6-mercaptopurine at 60 mg/m$^2$ 21 times in the post-remission induction intensification phase. No differences in event-free survival had been documented previously;[13] we, therefore, analyzed the randomized patients as a single subset. The detailed results of these randomizations will be reported separately.

**Table 2.** Treatment regimens.

**Standard risk**
Induction: Pred 60 mg/m$^2$×5 weeks, VCR 1.5mg/m$^2$ x 5, Pirarubicin 20mg/m$^2$×2, L-asp 6,000U/m$^2$×9 (2 times/week *vs.* 3 times/week, randomized)
Intensification 1: CY 1,000 mg/m$^2$, Ara-C 75 mg/m$^2$×15, 6MP 60mg/m$^2$×21
Intensification 2: MTX 3 g/m$^2$×3
Interim maintenance: 6 MP 60 mg/m$^2$×14, MTX 25 mg/m$^2$×3
Reinduction: Pred 60 mg/m$^2$×14, VCR 1.5 mg/m$^2$×3, Pirarubicin 20 mg/m$^2$×3, L-asp 10,000U/m$^2$×4
Late intensification 1: CY 1,000 mg/m$^2$, Ara-C 75 mg/mm$^2$×10, 6MP 60 mg/m$^2$× 14
Late intensification 2 (3 cycles): MTX 500 mg/m$^2$, PSL/VCR/L-asp (2 wks)
Maintenance: 6 MP/MTX until week 146.
Total number of IT therapies: 11

**Intermediate risk**
Induction: Pred 60 mg/m$^2$×5 wks, VCR 1.5 mg/m$^2$×5, DNR 25 mg/m$^2$×4, CY 1,000 mg/m$^2$×2, L-asp 6000U/m$^2$×9 (2 times a week *vs.* 3 times a week)
Intensification 1 (Randomized): High-dose Ara-C (2 g/m$^2$×8), L-asp (10,000U/m$^2$) *vs.* CY 1,000 mg/m$^2$, Ara-C 75 mg/m$^2$×15, 6 MP 60 mg/m$^2$×21
Intensification 2: MTX 3 g/m$^2$×3
Interim maintenance: 6 MP 60 mg/m$^2$×14, MTX 25 mg/m$^2$×3
Reinduction 1: DEXA 6 mg/m$^2$×14, VCR 1.5 mg/m$^2$×4, DXR 25 mg/m$^2$×4, L-asp 10,000U/m$^2$×4
Late intensification 1: CY 1,000 mg/m$^2$, Ara-C 75mg/m$^2$×10, 6MP 60 mg/m$^2$× 14
Late intensification 2 (2 cycles): Ara-C 2 g/m$^2$×4 with L-asp 10,000U/m$^2$×1, MTX 500 mg/m$^2$
Reinduction 2: Pred 60 mg/m$^2$×14, VCR 1.5 mg/m$^2$×3, Pirarubicin 20 mg/m$^2$× 3
L-asp 10,000 U/m$^2$ × 4
Late intensification 3: CY 1,000 mg/m$^2$, Ara-C 75 mg/m$^2$ x 10, 6MP 60 mg/m$^2$ x 14
Maintenance: 6MP/MTX until week 104.
Total number of IT therapies: 10 or 11

**High risk**
Induction: Pred 60 mg/m$^2$×5 weeks, VCR 1.5mg/m$^2$×5, DNR 25 mg/m$^2$×4, CY 1,000 mg/m$^2$×2, L-asp 6,000U/m$^2$×9
Intensification 1: High-dose Ara-C (2 g/m$^2$×8) with L-asp (10,000 U/m$^2$) CY 1,000 mg/m$^2$, Ara-C 75mg/m$^2$×15, 6MP 60 mg/m$^2$×21
Intensification 2 (2 cycles): Ara-C 3 g/m$^2$×6, Etoposide 100mg/m$^2$×5, Mitoxantrone 10 mg/m$^2$
Allogeneic SCT if indicated. If not, proceed to the followings:
Intensification 3: high-dose MTX 3 g/m$^2$, CY 200 mg/m$^2$×5, VCR 1.5 mg/m$^2$
Repeat Intensification 2 twice, then repeat Intensification 3
Intensification 4 (2 cycles): Ara-C 3 g/m$^2$×4, L-asp 10,000 U/m$^2$
Cranial irradiation with 6MP 60 mg/m$^2$×14
No maintenance: Treatment stopped at week 48
Total number of IT therapies: 9-17

### Statistical analysis

The duration of event-free survival was defined as the time from the initiation of therapy to either treatment failure (relapse, death, or diagnosis of secondary cancer) or to the last day when the patient was confirmed to be in remission. In those patients who did not achieve complete remission after the first induction phase or who died before the confirmation of remission, treatment was considered to have failed at day 0. The probability of event-free survival was estimated by the Kaplan-Meier method, and was tested for significance using the log-rank test.

For multivariate analysis, the Cox proportional hazards model was employed to assess independent effects of risk factors on event-free survival. All calculations were performed by PC-SAS (SAS Institute Inc., PC-SAS, version 8, 2000, Cary, NC, USA).

## Results

### The numbers of peripheral leukemic blasts on day 8

The number of leukemic blasts was assessed in all patients (Table 3). Prephase prednisolone was administered to all the patients except six in whom vincristine and/or cyclophosphamide was used before day 8 because of insufficient cytoreduction. Overall, blasts were not detectable in 249 patients (33.0%) (*Day8NoBlasts*), 392 patients (52.0%) had a blast count of 1-999/μL, and 113 patients (15.0%) had a blast count ≥ 1,000/μL. In the subset of 90 patients with T-ALL, 23 (25.6%) fell in the *Day8NoBlasts* group, whereas 226 (34.0%) out of the 664 patients with B-lineage ALL belonged to the *Day8NoBlasts* group. Of note, 15

**Table 3.** The number of patients stratified by peripheral blood leukemic blast count on day 8.

| *Immunophenotype* | *The number of peripheral blood leukemic blasts per μL* | | |
|---|---|---|---|
| | *0* | *1-999* | *≥1,000* |
| B-lineage ALL | | | |
| Initial SR group | 132* | 148 | 22 |
| Initial IR group | 88 | 175 | 34 |
| Initial HR group | 6 | 34 | 25 |
| T-ALL | 23 | 35 | 32 |

*SR: standard risk; IR: intermediate risk; HR: high risk. *The numbers of patients in each category are shown.*

patients (9 with T-ALL and 6 with B-lineage ALL) in the initial high risk group achieved the status of *Day8NoBlasts*, despite high initial leukocyte counts exceeding 50,000/μL.

Subsets of patients with abnormal karyotypes were further analyzed: 25 (35.7%) of the 70 patients with *TEL/AML1* rearrangement achieved *Day8NoBlasts*, as did 69 (37.9%) of the 182 patients with high hyperdiploidy (more than 50 chromosomes), 3 (8.1%) of the 37 patients with *E2A/PBX1* rearrangement, 4 (36%) of the 11 patients with *MLL* rearrangement, and 2 (11.8%) of the 17 patients with the *BCR/ABL* fusion.

### Treatment outcome

Remission was achieved in 736 (97.6%) of the 754 patients: non-T ALL: 98.6%, T-ALL: 94.4%. The probability of event-free survival for all patients was 79.6±1.6% (SE) % at 4 years, whereas the event-free survival rates for patients with B-lineage ALL and T-ALL were 80.5±1.7% (n=664) and 66.0±5.1% (n=90), respectively (Figure 1). The event-free survival for patients stratified finally on day 43 (T-cell ALL included) were as follows: 92.4±1.8% in the standard risk group (n=262), 80.3±2.6% in the intermediate risk group (n=313), and 57.8±4.1% in the high risk group (n=179). The event-free survival for patients with *Day8NoBlasts* was 90.4±2.0% (n=249), which was significantly better than that for the other patients (74.2±2.2%; $p<0.01$) (Figure 2). The event-free survival for *Day8NoBlasts* patients with T-ALL (n=23) was 95.7±4.3%, which was comparable with that of patients with B-lineage ALL (n=226), 89.8±2.1% ($p=0.45$. Figure 3).Treatment failed in only one patient with T-ALL with *Day8NoBlasts* because of death due to pancreatitis during remission induction. The event-free survival for patients with *Day8NoBlasts* and blast counts of 1-999/μL in the initial standard risk group with B-lineage ALL did not differ (90.5±2.6% *vs*. 92.5±2.5%; $p=0.82$), whereas there was a significant difference in the initial intermediate risk group with B-lineage ALL (89.4±3.6% *vs.* 72.7±3.9%; $p=0.004$). Lastly, in the initial high risk group with B-lineage ALL, five out of six patients with *Day8NoBlasts* had a good outcome.

### Multivariate analysis

The significance of *Day8NoBlasts* was further analyzed using a Cox proportional hazards model. The results of the multivariate analysis are shown in Table 4. Age at diagnosis, the initial white blood cell count, immunophenotype (T-ALL *vs.* non T-ALL), and gender did not remain as statistically significant independent factors. Only *Day8NoBlasts* and high hyperdiploidy were statistically significant. We also assessed the prognostic value of *Day8NoBlasts* by dividing the counts into three categories; 0/μL, 1-999/μL, and ≥1000/μL. No difference was observed between the 1-999/μL and ≥1000/μL categories in the univariate analysis; the risk ratio for ≥1000/μL against 1-999/μL was 1.23 (95% CI: 0.98-1.56) whereas the risk ratio for ≥1000/μL against 0/μL was 0.46 (95% CI: 0.33-0.62). The results were similar when this prognostic factor was used as a three-category term in the multivariate analysis. These results led to the use of this prognostic factor as a two-category factor for simplicity and convenience for use in other studies.

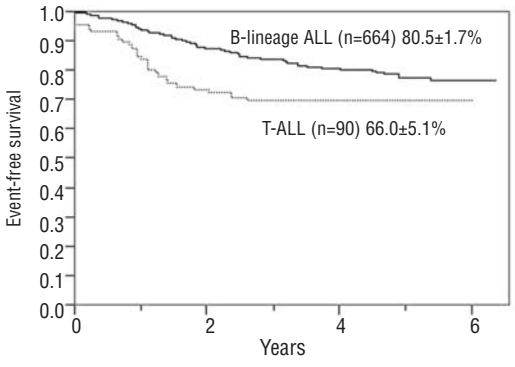


**Figure 1.** Kaplan-Meier plots of event-free survival according to immunophenotype (B-lineage ALL and T-ALL).

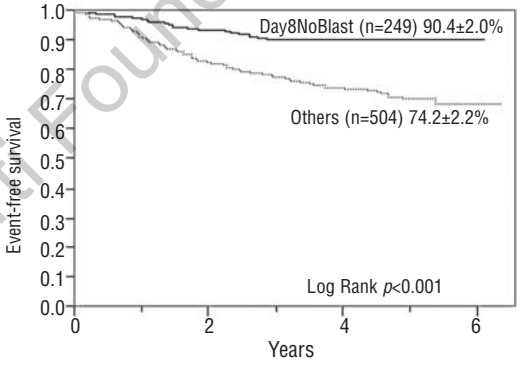


**Figure 2.** Kaplan-Meier plots of event-free survival of patients with no detectable blasts on day 8 and the other patients: the difference between the two curves at 4 years was highly significant ($p<0.001$ by the log-rank test).

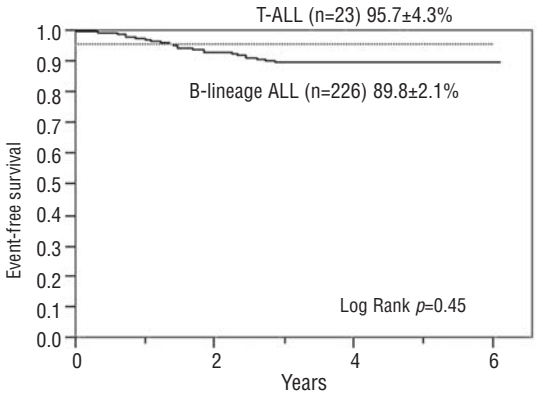


**Figure 3.** Kaplan-Meier plots of event-free survival of patients with no detectable blasts on day 8 divided according to the immunophenotype: the event-free survival at 4 years for *Day8NoBlasts* patients with T-ALL was comparable to that of patients with B-lineage ALL ($p=0.45$ by the log-rank test).

## Discussion

The concept that an early response to treatment is strongly predictive of relapse has been overwhelmingly emphasized. Here, we added novel information that patients whose peripheral blood blasts disappeared after 7 days of prednisolone monotherapy had an excellent prognosis, that is, a 4-year event-free survival of 90%. Of note, such patients constituted one third of all children with ALL, thus being quite a large group. *Day8NoBlasts* was also an independent prognostic factor when assessed by multivariate analysis. Of interest, a substantial proportion of patients with *TEL/AML1* rearrangement and high hyperdiploidy achieved *Day8NoBlasts* (36% and 38%, respectively), while a very small proportion (6%) of patients with *E2A/PBX1* did so, suggesting that the kinetics of reduction in leukemic blasts may be different in subsets of patients defined by genetic changes.

One of the limitations of our study was that the enumeration of blasts in the peripheral blood was done by microscopic evaluation, which is a subjective method. The results might, therefore, not be reproducible with confidence; however, the percentages of patients with blast counts of 0/μL, 1-999/μL and ≥ 1000/μL on day 8 in the present L99-15 study (33%, 52%, 15%, respectively) were almost identical to those of our most recent L99-1502 study (31%, 53%, 16%, respectively). On the other hand, there remains a possibility that patients having been staged down by this stratification system to less intensive treatment could even have fared better (i.e. over 90%) if treated according to the older stratification system. In the next study, we plan to employ more objective methods, such as flow cytometry.[14]

In this study, we confirmed the importance of the sensitivity of leukemic blasts to steroids. Since we did not administer intrathecal therapy until day 8, our assessment of the reduction of leukemic cells in peripheral blood on day 8 should exclusively reflect an early response of leukemic blasts to steroids.[12] Steroids function as antileukemic agents mostly by inducing ALL cells to undergo apoptosis, but little was known about critical molecules involving steroid-induced apoptosis of leukemic cells. Many investigators have addressed this issue by using gene expression profiling and several candidate genes, which might be able to predict steroid sensitivity, have been identified:[15-18] apoptotic pathway-associated genes (*MCL-1, DAPK1, CASP8A2, TXNIP, ZBTB16*), carbohydrate metabolism-associated genes, MAPK pathway-associated genes, and NF-κB-associated genes. Of these genes, *CASP8A2*, a caspase 8-related molecule, was identified as a crucial molecule differentially expressed by leukemic cells at diagnosis between patients who had high and low levels of minimal residual disease in bone marrow 18 and 45 days after the initiation of induction therapy, and it can predict both *in vitro* cell growth and prognosis.[16] One could identify the new molecule, which determines steroid sensitivity, using a similar methodology, for example, comparing gene expression patterns of leukemic cells from patients with *Day8NoBlasts* with those of patients with a classic poor response to steroid.

**Table 4.** The results of the multivariate analysis.

| *Factor* | *Risk ratio* | *95% CI* | *p value* |
|---|---|---|---|
| No blasts 0 at day 8 | 0.46 | 0.33-0.62 | <0.001 |
| High hyperdiploidy | 0.66 | 0.51-0.84 | <0.001 |
| *TEL-AML1* | 0.85 | 0.61-1.13 | 0.27 |
| T-ALL | 0.89 | 0.71-1.13 | 0.34 |
| Male | 0.97 | 0.82-1.16 | 0.75 |
| Age at presentation >10 years old | 1.00 | 0.82-1.25 | 0.96 |

The early response to steroids has been utilized to stratify children with ALL as a tradition by the BFM group since the early 1980s.[3,8] This group uses the cut-off of 1,000 blasts/μL, among other cut-offs, to identify the approximately 10% of patients with a very high risk of relapse;[19] however, the cut-off of 0 blasts has never been used.[20] We demonstrated that one-third of patients with a better prognosis could be identified by the use of this new cut-off of 0 blasts/μL. In this study, patients with no blasts on day 8 were identified not only in the initial low risk group but also in the initial higher risk groups: 94 of 362 patients with B-lineage ALL in the intermediate and high risk groups and 23 of 90 patients with T-ALL. Generally, children with T-ALL have a poorer outcome than those with B-lineage ALL and need more intensive treatment. We, however, showed that patients with T-ALL as well as those with B-lineage ALL had a favorable outcome if circulating leukemia cells could not be detected on day 8 of therapy. By using the cut-off of 0 blasts, we could select patients, including those with B-lineage ALL in the initial higher risk groups and those with T-ALL, who could be targeted for treatment reduction, as some previous studies showed that a subset of patients with ALL could be cured with less intensive regimens.[21,22]

## Authorship and Disclosures

AM and AO designed the research, analyzed data and wrote the paper; DH analyzed the data and reconstructed the text; KI, RH and MT designed the research; AO, KK, TS, NK, AK, HT and YH analyzed the data; MT is a chairman of the TCCSG.

The authors reported no potential conflicts of interest.

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