EMERGING INFECTIONS

Arginine deficiency is involved in thrombocytopenia and immunosuppression in severe fever with thrombocytopenia syndrome

Xiao-Kun Li[1]*, Qing-Bin Lu[2]*, Wei-Wei Chen[3]*, Wen Xu[3]*, Rong Liu[4], Shao-Fei Zhang[1], Juan Du[2], Hao Li[1], Ke Yao[5], Di Zhai[5], Pan-He Zhang[1], Bo Xing[1], Ning Cui[6], Zhen-Dong Yang[6], Chun Yuan[6], Xiao-Ai Zhang[1], Zhe Xu[3], Wu-Chun Cao[1,7]†, Zeping Hu[5]†, Wei Liu[1,7,8]†

Severe fever with thrombocytopenia syndrome (SFTS) caused by a recently identified bunyavirus, SFTSV, is an emerging infectious disease with extensive geographical distribution and high mortality. Progressive viral replication and severe thrombocytopenia are key features of SFTSV infection and fatal outcome, whereas the underlying mechanisms are unknown. We revealed arginine deficiency in SFTS cases by performing metabolomics analysis on two independent patient cohorts, suggesting that arginine metabolism by nitric oxide synthase and arginase is a key pathway in SFTSV infection and consequential death. Arginine deficiency was associated with decreased intraplatelet nitric oxide (Plt-NO) concentration, platelet activation, and thrombocytopenia. An expansion of arginase-expressing granulocytic myeloid-derived suppressor cells was observed, which was related to T cell CD3-ζ chain down-regulation and virus clearance disturbance, implicating a role of arginase activity and arginine depletion in the impaired anti-SFTSV T cell function. Moreover, a comprehensive measurement of arginine bioavailability, global arginine bioavailability ratio, was shown to be a good prognostic marker for fatal prediction in early infection. A randomized controlled trial demonstrated that arginine administration was correlated with enhanced Plt-NO concentration, suppressed platelet activation, and elevated CD3-ζ chain expression and eventually associated with an accelerated virus clearance and thrombocytopenia recovery. Together, our findings revealed the arginine catabolism pathway–associated regulation of platelet homeostasis and T cell dysregulation after SFTSV infection, which not only provided a functional mechanism underlying SFTS pathogenesis but also offered an alternative therapy choice for SFTS.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) caused by a novel bunyavirus, SFTSV (*1*), is an emerging infectious disease that was firstly identified in mainland China in 2009. The disease has also been reported in Korea, Japan, and similar cases in the United States, listed as one of the top 10 blueprint priority diseases by World Health Organization (*2*). SFTSV infection is associated with a wide clinical spectrum, with most of the patients having mild disease but more than 10% developing a fatal outcome (*3*). Currently, no specific antiviral therapy or vaccine is available for SFTS. Ribavirin, although has been recommended for clinical use in China, was found to be ineffective in altering clinical outcomes (*4*). On the other hand, a high oral dosage of ribavirin combined with plasma exchange successfully saved the lives of two patients in South Korea (*5*). Moreover, plasma exchange followed by convalescent therapy and intravenous immunoglobulin and corticosteroid therapy was also used for successful SFTS therapy, but limited to case report (*6*). The underlying pathogenesis of the disease remains understudied. Disordered host immunity that facilitated the progressive viral replication and severe thrombocytopenia that resulted in bleeding and disseminated intravascular coagulation are two of the known mechanisms that contribute to lethal outcome (*7*). Animal models demonstrate the integrity of the immune system to be critical in determining the outcome of SFTSV-infected mice (*8*). Only immune-deficient or immunosuppressive agent–treated mice were able to recapitulate the fatal illness after SFTSV infection (*9*), and the mortality and viral loads were higher in immunocompromised patients (*8*). The mechanism of SFTSV infection–induced thrombocytopenia has been ascribed to virus attachment and macrophage phagocytosis based on the findings from the mice infection model (*9*).

Up to now, it remained obscure how SFTSV infection perturbs host response to result in complications that were central to the disease. This has called for a comprehensive approach to globally capture the human responses to SFTSV. It has been well established that viral replication requires energy and macromolecular precursors derived from the metabolic network of the host cell (*10*). As the end products of cellular regulatory processes, host metabolism reflects the physiological state of a cell, tissue, or organism. Therefore, understanding the snapshot of the metabolic status or metabolomics is helpful to find metabolic pathways related to disease processes (*11*). Here, by performing metabolomics analysis on serum samples from prospectively observed SFTS cases, we determined arginine metabolism to be a key pathway that was involved in the interaction between SFTSV and host response. Arginine is a conditionally essential amino

[1]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, 20 Dongda Street, Fengtai District, Beijing 100071, P. R. China. [2]Department of Laboratorial Science and Technology, School of Public Health, Peking University, No. 38, Xueyuan Road, Haidian District, Beijing 100191, P. R. China. [3]The 302 Hospital, People's Liberation Army, No. 100, West 4th Ring Road, Fengtai District, Beijing 100039, P. R. China. [4]School of Basic Medical Sciences, Wuhan University School of Medicine, 185 Donghu Street, Wuhan 430071, P. R. China. [5]School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, P. R. China. [6]The 154 Hospital, People's Liberation Army, 104 Nan-Hu Road, Shihe District, Xinyang 464000, P. R. China. [7]School of Public Health, Shandong University, Jinan 250012, P.R. China. [8]Microbiology and Epidemiology, Beijing Key Laboratory of Vector Borne and Natural Focus Infectious Diseases, Beijing, P. R. China.
*These authors contributed equally to this work as co-first authors.
†Corresponding author. Email: liuwei@bmi.ac.cn (W.L.); zeping_hu@tsinghua.edu.cn (Z.H.); caowc@bmi.ac.cn (W.-C.C.)

acid for adult mammals. Its metabolism not only has important wound healing functions but also has been integrated with the host immune response, as it has been linked to myeloid-derived suppressor cell (MDSC) (*12*, *13*). The modulatory role of MDSC on arginine metabolism has been shown in a variety of chronic infection such as HIV and herpes simplex virus infection (*14*). However, MDSC-associated arginine deprivation or global metabolic changes have not been well described in acute viral infection. Here, after identifying the arginine pathway, we further determined its potential pathological effect from the perspective of platelet activation and cellular immune regulation. To determine whether arginine supplementation could reverse the virus-induced pathogenesis changes and offer clinical benefit, we performed a randomized controlled trial on SFTS patients and investigated the effectiveness of arginine administration in altering the clinical process.

RESULTS

Arginine metabolism alteration at acute SFTSV infection

To identity the metabolic perturbations associated with SFTSV infection, we analyzed metabolites relative abundance in serum from two independent groups of patients (see the "Study design" and "Study subjects" sections) using a liquid chromatography–tandem mass spectrometry (LC-MS/MS)–based targeted metabolomics method (*15*). We profiled 166 metabolites from 242 clinical samples (46 acute infection and paired convalescent samples versus 46 healthy controls and 46 non-SFTS, febrile cases in the first group; 10 fatal versus 10 surviving and 16 healthy controls in another group). This targeted metabolomics profiles were able to distinguish acute SFTSV infection from healthy controls and non-SFTS, febrile disease, and can also distinguish fatal from the surviving cases, by using orthogonal partial least squares–discriminant analysis (OPLS-DA) and principal components analysis (PCA) model (Fig. 1, A and B, and fig. S1, A and B). After filtering the metabolites [variable importance in projection (VIP) value of >1 or $P < 0.05$ in intergroup comparisons by one-way analysis of variance (ANOVA)], hierarchical clustering showed different metabolic signatures between groups (63 differential metabolites for the first group, 80 differential metabolites for the second group, with 54 differential metabolites that overlapped between two groups) (Fig. 1, C and D). A metabolic pathway analysis (MetPA) of altered metabolites was performed by using the MetaboAnalyst 3.0 online tool (*16*), revealing the change of the arginine metabolism pathway to be the most pronounced (Fig. 1E). By extracting the relative concentrations (RCs) of arginine-related

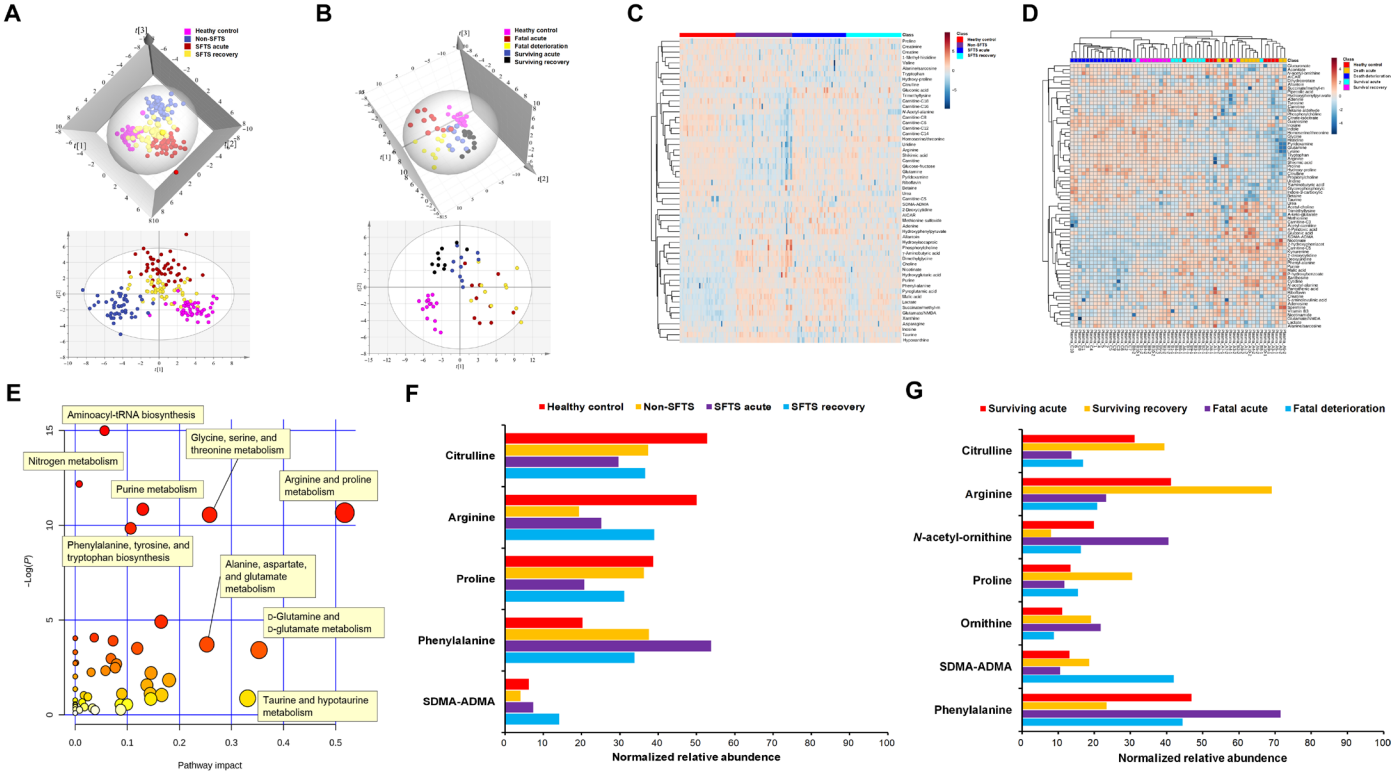

Fig. 1. Metabolomics profiling based on LC-MS/MS metabolomics data in two groups. The first group (group 1) comprised 46 SFTS patients at acute phase and recovery phase, 46 febrile patients without SFTSV infection, and 46 healthy controls. The second group (group 2) comprised 10 fatal SFTS cases at acute phase and deterioration, 10 surviving SFTS cases at acute phase and recovery phase, and 16 healthy controls. OPLS-DA score plots [two-dimensional (2D)/3D models] of LC-MS/MS metabolomics data on group 1 (**A**) and group 2 (**B**). Hierarchical clustering of identified differential metabolites (variables with VIP of >1 or $P < 0.05$ in one-way ANOVA) from group 1 (**C**) and group 2 (**D**) shown by heatmap. Each row shows relative ion intensity for a specific metabolite after mean centering and unit variance scaling of the data. Each column shows the serum metabolic profiles of the four groups of subjects. (**E**) MetPA. Pathway analysis was launched by MetaboAnalyst 3.0 using identified differential metabolites (variables with VIP of >1 or $P < 0.05$ in one-way ANOVA). The color and size of each circle are based on its *P* value and pathway impact value, respectively. (**F** and **G**) The variation of arginine-related metabolites based on group 1 (F) and group 2 (G). Non-SFTS, febrile patients without SFTSV infection. tRNA, transfer RNA; NMDA, *N*-methyl-D-aspartate; SDMA-ADMA, symmetric dimethylarginine-asymmetric dimethylarginine.

metabolites along the pathway, we found that arginine RC was significantly reduced in the acute phase of SFTS compared to healthy controls ($P < 0.001$) (Fig. 1F). Furthermore, the fatal cases had even lower arginine concentration than the surviving cases (Fig. 1G). An obvious recovery of arginine RC was seen during the convalescence of survived cases, but not in the deterioration stage of the fatal cases. In contrast, an opposite trend of phenylalanine was observed, suggesting that decreased arginine RC was due to altered downstream catabolism, instead of severely impaired protein degradation, because phenylalanine could act as a reflection of protein degradation (*17*). Arginine can be catalyzed to nitric oxide (NO) and citrulline by NO synthases (NOSs) (*18*), and the citrulline RC was also related to the clinical outcome ($P = 0.002$ for fatal deterioration versus surviving recovery) (Fig. 1G). Together, we hypothesized that arginine metabolism was altered in response to SFTSV infection, which could potentially promote the virus pathogenic process and worsen patient outcomes.

Arginine deficiency, decreased intraplatelet NO concentration, and thrombocytopenia

Because the blunted arginine/NO metabolism pathway might lead to platelet dysfunction (*19*–*22*), we examined whether NOS activity in platelets of patients influences disease progression. To do so, we further analyzed intraplatelet NO (Plt-NO) biosynthesis on single-cell level in platelets from SFTS patients. As expected, a significantly decreased Plt-NO was found ($P = 0.004$) (Fig. 2A), which was positively correlated with the platelet counts measured at the same time point (Fig. 2B). As the precursor of NO, the arginine concentration that was measured at 1 day early had a similar association with the platelet counts (Fig. 2C). It has been shown that arginine/NO is one of the most important pathways to inhibit platelet activation (*20*). We found a negative correlation between arginine concentration and platelet activation reflected by PAC-1 (activated integrin αIIb/β3) expression (Fig. 2D), which was also elevated in SFTS cases ($P = 0.009$) (Fig. 2E), suggesting that the platelet loss might be related to platelet activation in SFTSV infection. We further revealed a significant increase in the frequencies of platelet-monocyte complexes (PMC) and platelet apoptosis ($P = 0.007$ and $P < 0.001$) (Fig. 2, F and G), further suggesting that platelet hyperactivation might contribute to reduce platelet counts in circulation, especially considering the previous evidence in other diseases (*23*, *24*). Furthermore, arginine was shown to be able to moderately alleviate the SFTSV-induced platelet activation in vitro, whereas the NOS inhibitor, Nω-nitro-L-arginine methyl ester hydrochloride, could weaken the alleviation effect of arginine on platelet activation. (Fig. 2H). On the basis of these results, we propose that akin to chronic cardiovascular disease, the regulatory effect of arginine on platelet homeostasis might be attained through the arginine/NO

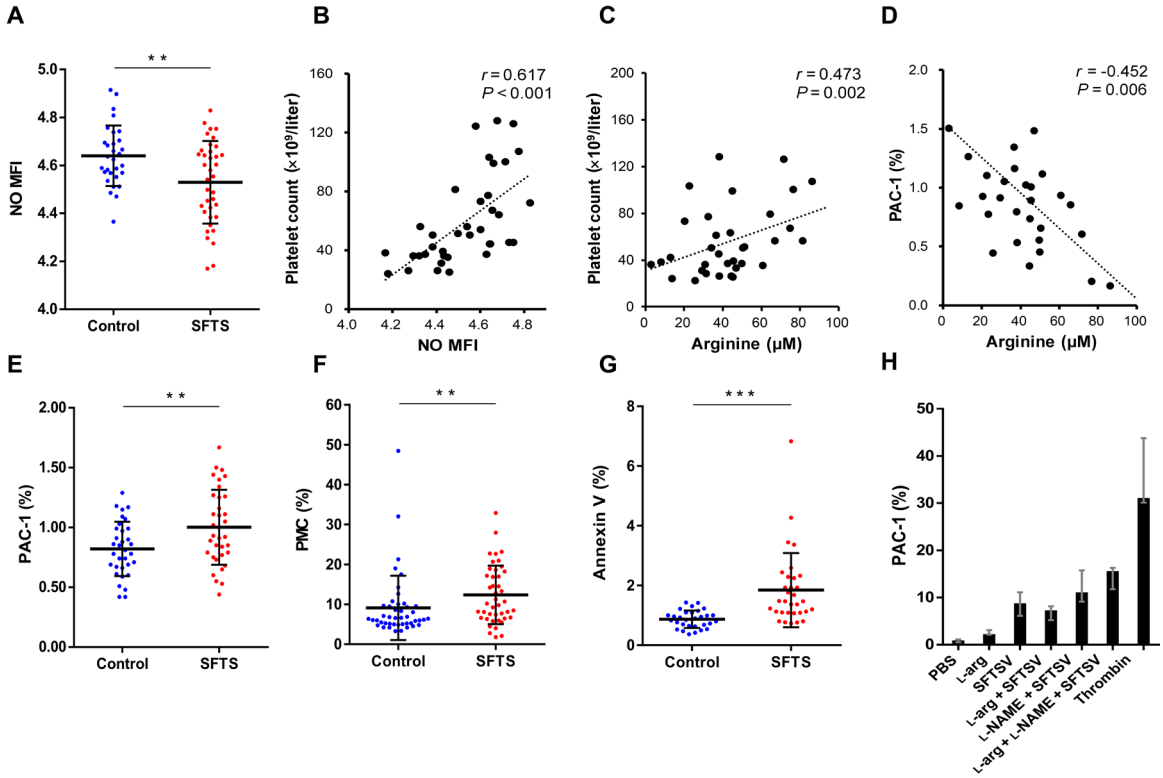

Fig. 2. The correlation of arginine/Plt-NO concentration and platelet count in SFTS. (**A**) Plt-NO expression in 30 controls and 38 SFTS cases and (**B**) correlation between Plt-NO expression and platelet counts in 38 SFTS cases. (**C**) Correlation between arginine concentrations and platelet counts in 36 SFTS cases. (**D**) Correlation between arginine concentration and platelet PAC-1 expression in 29 SFTS cases. (**E**) Platelet activation evaluated by the percentage of PAC-1 expression in 33 controls and 35 cases. (**F**) Cumulative dot plots showing PMC in 44 SFTS cases and 45 controls. PMC (%) was calculated as the percentage of $CD41^+$ cells in $CD14^+$ cells. (**G**) Platelet apoptosis presented as annexin V frequency in 30 controls and 32 cases. (**H**) The platelet activation stimulated by SFTSV in vitro was evaluated by the percentage of PAC-1 expression. The diluted platelet-rich plasma was pretreated with L-arg, L-NAME, both, or phosphate-buffered saline (PBS). The thrombin-induced platelet activation acted as positive control. L-arg, L-arginine; L-NAME, Nω-nitro-L-arginine methyl ester hydrochloride. The comparisons between controls and SFTS cases were tested by Student's *t* test (A, D, F, and G). Error bars, interquartile. Spearman correlation test (B, C, and E), $^{**}P < 0.01$, and $^{***}P < 0.001$. *r*, correlation coefficient.

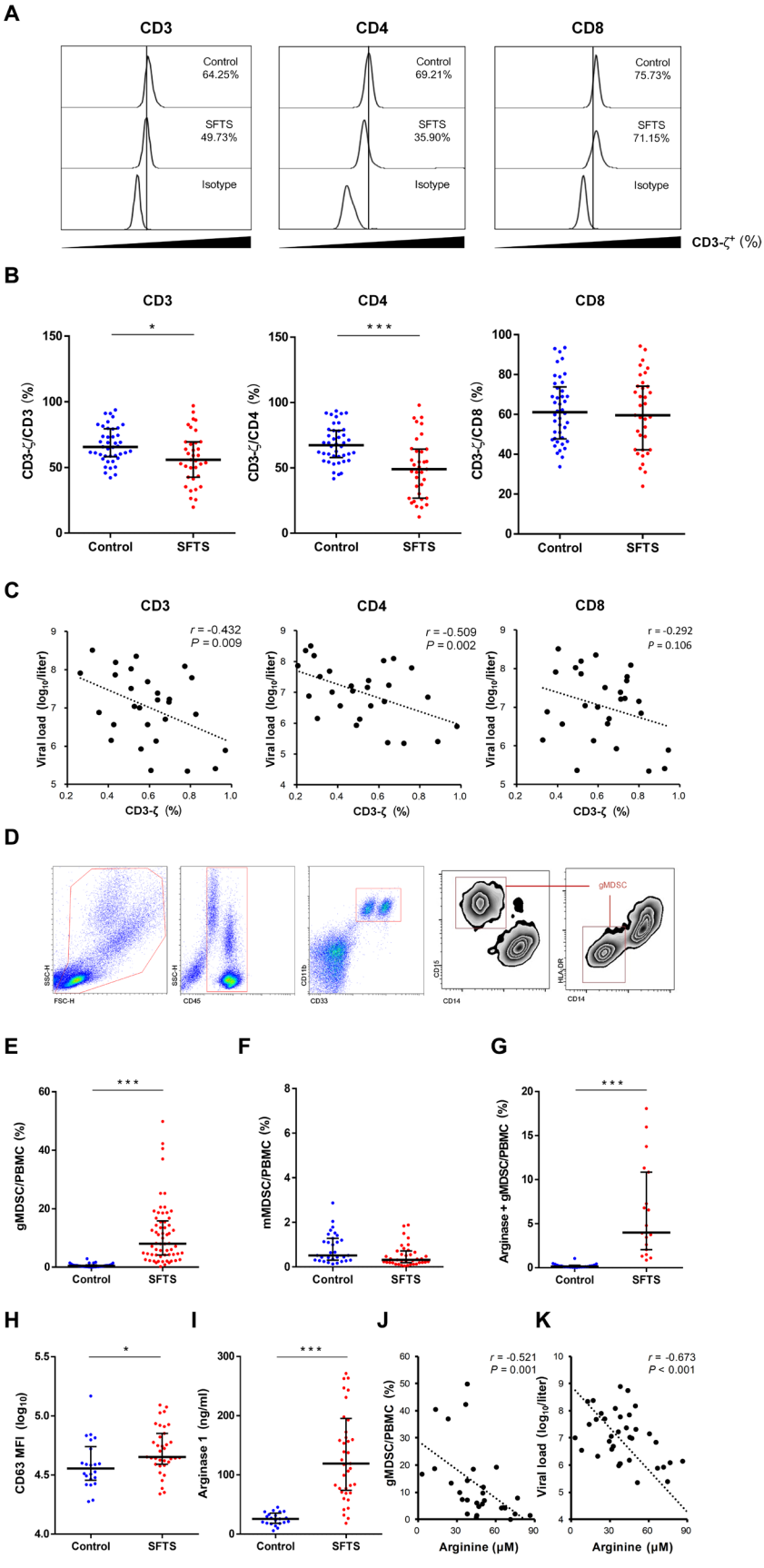

Fig. 3. CD3-ζ down-regulated in T cells and circulating arginine exhausted by an MDSC subset. Representative fluorescence-activated sorting plots (**A**) and cumulative dot plots (**B**) of CD3-ζ expression in T cell subsets of 42 controls and 35 SFTS cases. (**C**) Association between viral loads and CD3-ζ expression of different T cell subsets (CD3/CD4, $n = 28$; CD8, $n = 31$). (**D**) Sequential gating strategy for gMDSC identification (live, singlet, $CD45^+$, and $CD11b^{high}CD33^+HLA\text{-}DR^-CD14^-CD15^+$). (**E**) gMDSC and (**F**) mMDSC frequencies were calculated as a percentage of PBMCs ($CD45^+$) and compared between healthy controls and cases (35 controls and 27 cases). (**G**) The percentage of arginase I–expressing gMDSCs as a proportion of PBMC was compared between healthy controls and cases (22 controls and 19 cases). (**H**) Cumulative cell surface CD63 expression [mean fluorescence intensity (MFI)] on gMDSC (22 controls and 35 cases). (**I**) Serum arginase I concentration (nanogram per milliliter) of controls and cases ($n = 22$ controls and 41 cases). (**J**) Correlation of serum arginine concentration with circulating gMDSC frequencies. (**K**) Correlation of serum arginine concentration with viral loads. Error bars, median and interquartile. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. The comparisons between controls and SFTS cases were tested by unpaired *t* test. (C, J, and K) Spearman correlation test. FSC-H, forward scatter-height; SSC-H, side scatter-height.

pathway, and hypoargininemia might be associated with thrombocytopenia.

Involvement of MDSC expansion and arginine deprivation in compromised T cell immunity

Because arginine deprivation–associated T cell suppression is regulated by MDSC (*13*), we measured CD3-ζ chain expression in T cells, a hallmark of arginine deprivation that induces T cell receptor (TCR) disassembling (*25*), in parallel with MDSC frequencies. CD3-ζ chain was less expressed in T cells, especially in the CD4 subset of the patients (Fig. 3, A and B), which corresponded to the reversed ratio of CD4/CD8 in the same case series (fig. S2, A to D). The effect of CD3-ζ chain on the immune regulation was also observed, supported by the negative correlation between its expression in T cell subsets and viral loads (Fig. 3C).The frequencies of granulocytic MDSC (gMDSC) (gating strategy in Fig. 3D) and monocytic MDSC (mMDSC) (fig. S2, E) in fresh peripheral blood samples were evaluated in 39 patients with acute SFTSV infection and 35 healthy controls. An elevation of activated gMDSC (but not mMDSC) was found in patients, and to an even higher degree, with longer duration in fatal cases than in those survived (Fig. 3, E and F, and fig. S2, F and G). In contrast, the expansion of gMDSC returned

to normal at about 9 days after disease onset in the survived group (fig. S2F). This is indicative of short and transient effects of gMDSC, triggered only at acute SFTSV infection, a phenomenon that contrasted with the long-lasting effect in chronic infection (*26*, *27*). Congruent with previous reports on sepsis (*28*), among all the peripheral blood mononuclear cell (PBMC) subsets, expanded gMDSC expressed the highest intracellular arginase concentration, and an elevated proportion of arginase$^+$ gMDSC was observed in 18 SFTS patients (fig. S2H and Fig. 3G). A higher frequency of surface CD63 expression on gMDSC was also displayed in the cases (Fig. 3H), indicating the tendency of gMDSC to degranulate and release arginase I (*29*). Moreover, we noted a highly increased serum arginase I concentration (Fig. 3I), which occurred concurrently with the expansion of gMDSC. An inverse correlation between gMDSC frequency and circulating arginine concentration (Fig. 3J), as well as between circulating arginine concentration and viral loads (Fig. 3K), was simultaneously revealed in the same case group. Furthermore, in vitro study showed that SFTSV infection could directly induce gMDSC expansion in the whole-blood samples from healthy donors (fig. S2I). We propose that SFTSV-induced gMDSC deprives arginine through arginase activity, causing T cell CD3-ζ chain damage in SFTSV infection, which could subsequently lead to impairment of T cell response and prevent SFTSV clearance (*30*, *31*). These findings collectively implicated a role for arginase activity and arginine depletion in the impairment of anti-SFTSV T cell function.

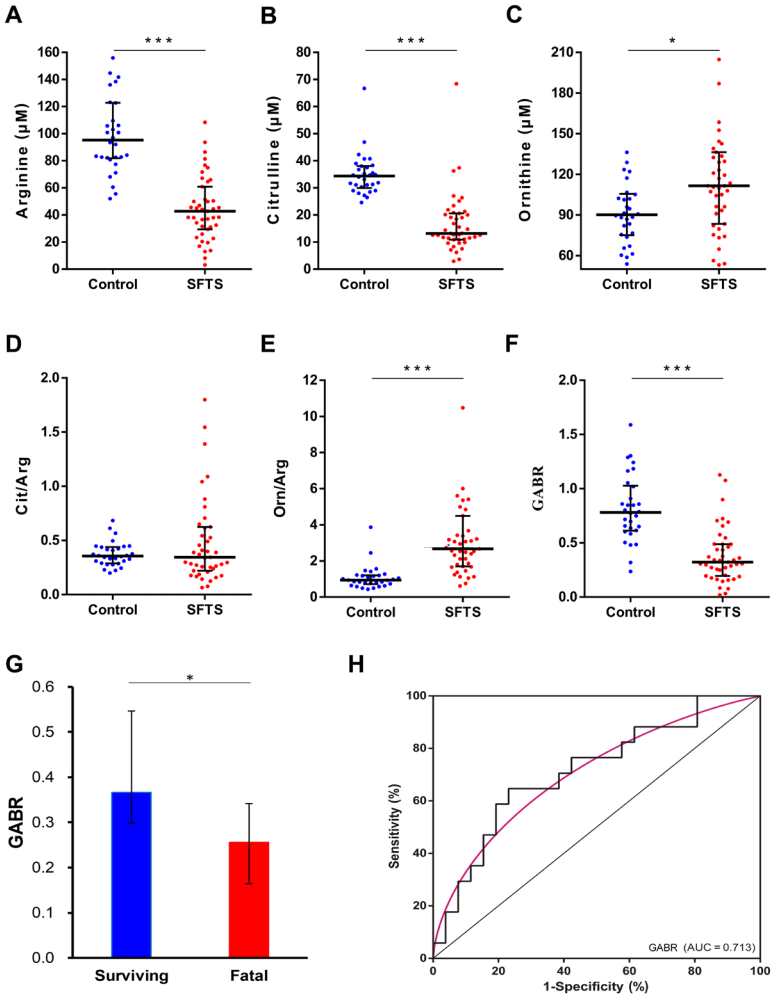

Fig. 4. Serum concentrations of arginine-related metabolites. The concentration of (**A**) arginine, (**B**) citrulline, (**C**) ornithine, (**D**) the ratio between serum concentration of citrulline (Cit) and arginine (Arg), (**E**) the ratio between ornithine (Orn) and arginine (Arg), and (**F**) the GABR defined as arginine/(ornithine + citrulline), compared between 30 controls and 43 SFTS cases. (**G**) The comparison of GABR between 17 fatal and 24 survived SFTS cases. (**H**) The ROC assessing GABR as a prognostic marker for mortality in SFTS. All comparisons were made by unpaired *t* test. Error bars, median and interquartile. *P < 0.05, **P < 0.01, and ***P < 0.001. AUC, area under the curve.

The potential of arginine metabolic parameter as a possible indicator of SFTS-related fatal outcome

We then investigated whether the arginine pathway could be useful in predicting clinical prognosis. To validate the systematic change of arginine and its related metabolites, we performed quantitative profiling of the serum amino acids in an independent group of 44 SFTS cases. Compared with 30 healthy controls that were tested in parallel, significantly decreased arginine and citrulline (both P < 0.001), while increased ornithine concentration (P = 0.043), were observed in SFTS patients at acute infection (Fig. 4, A to C). Consistently, the elevated ornithine/arginine ratio and slightly decreased citrulline/arginine ratio were revealed in SFTS patients (Fig. 4, D and E). These results corroborated the previous finding that arginine metabolism was skewed toward a preferential production of urea/ornithine instead of NO production. Global arginine bioavailability ratio (GABR), calculated as arginine/(ornithine + citrulline) (*32*) was found to be considerably reduced in SFTS patients (Fig. 4F) and to be even lower in the fatal SFTS patients (Fig. 4G). A multivariate analysis revealed that the decreased GABR remained associated with the fatal outcome after adjusting the effect from age, gender, and delay from disease onset to admission (P = 0.039). Further receiver operating characteristic (ROC) analysis disclosed that GABR value could be used as a prognostic marker for fatal prediction in early SFTSV infection with the area under the curve = 0.713 (Fig. 4H).

The benefit of arginine supplementation on the clinical and laboratory parameters

To corroborate the findings from these observational studies, we subsequently explored whether the supplementation of arginine could alter the disease progression or outcome of SFTSV infection by using randomized controlled trial. A total of 154 patients were assessed for randomized controlled trial eligibility, of whom 136 patients who met the inclusion criteria and were randomly assigned to

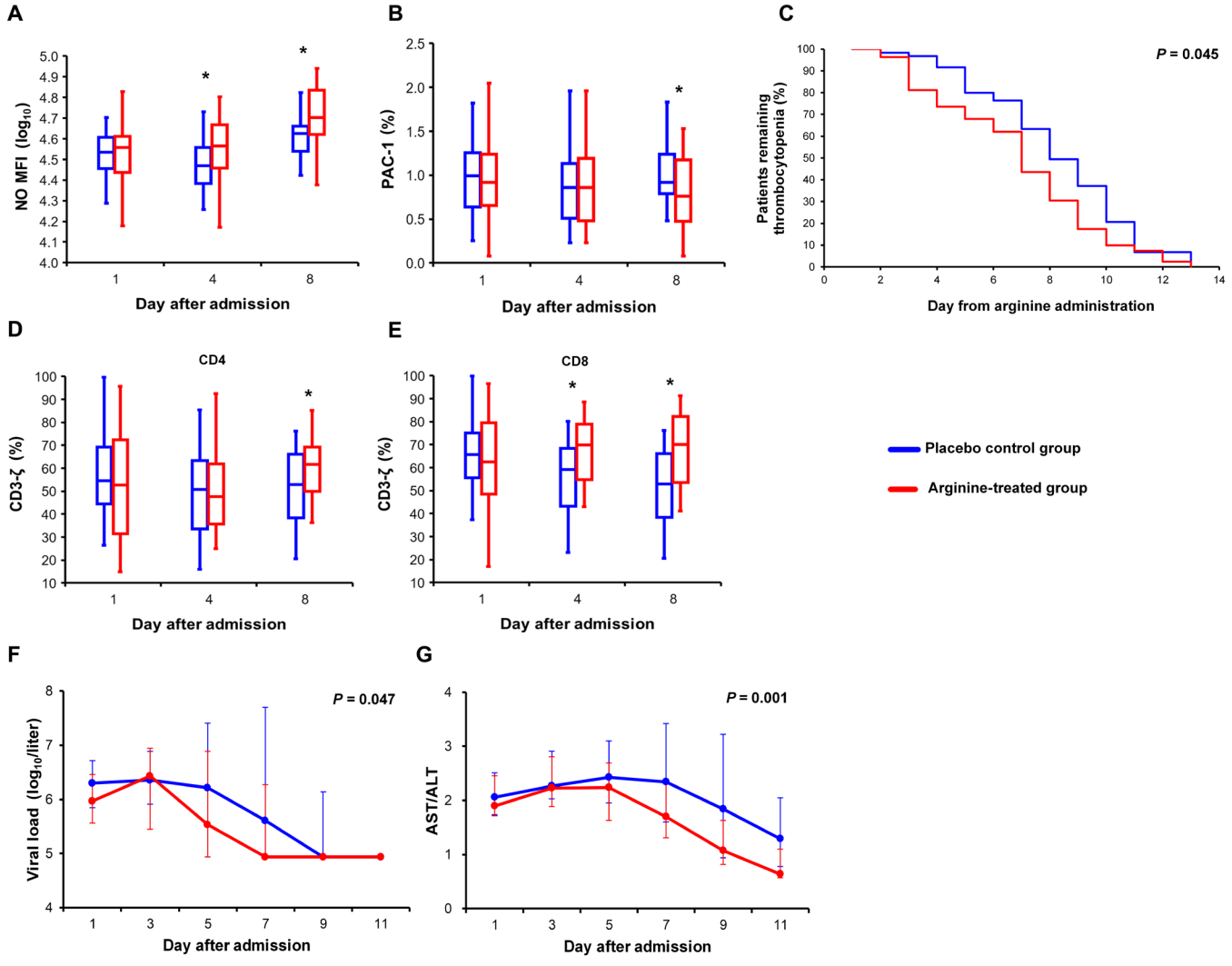

Fig. 5. The effect of arginine supplementation in a randomized controlled clinical trial. The serial evaluation of (**A**) Plt-NO, (**B**) PAC-1 expression, (**D**) CD3-ζ chain expression in CD4 cells, and (**E**) CD8 T cells from the 53 patients who received arginine supplementation and 60 patients who received supportive therapy only. (**C**) The proportion of patients with thrombocytopenia in arginine-treated and placebo control group shown by Kaplan-Meier curve. (**F**) The viral loads during the trial were determined every other day during treatment and compared by generalized estimating equations. (**G**) The AST/ALT concentrations during the trial were determined every other day during treatment. Boxplot shows the highest and lowest values as lines (whiskers) and the two interquartiles as a box and the median as a line in the box. Error bars, median and interquartile; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. The comparisons (A, B, D, and E) between placebo control group and arginine-treated group were tested by mixed-effect model.

receive arginine (20 g once a day, intravenous glucose guttae) plus supportive care ($n = 68$) or supportive care alone ($n = 68$). A total of 53 arginine-treated patients (15 were excluded: 5 with hospital duration less than 5 days, 10 with the use of L-arginine less than 3 days) and 60 placebo-controlled patients (8 were excluded with hospital duration less than 5 days) were included in the analysis (fig. S3). The two groups had comparable clinical and laboratory characteristics before intervention (table S1). The arginine concentration that was serially determined at four time points after exogenous supplementation showed only short-lived increased arginine concentrations (fig. S4A). This is an expected finding considering arginase was actively produced as a result of the SFTSV infection. The concentrations of arginine, citrulline, and ornithine were continuously monitored before, during, and after interventions (fig. S4, B to D). The Plt-NO biosynthesis was increased to a higher concentration in the arginine-treated group (Fig. 5A), whereas the platelet activation measured by PAC-1 expression was reduced at the same observation point (Fig. 5B). The survival analysis revealed an accelerated platelet recovery to normal value in the arginine-treated group compared with the placebo control group [adjusted hazard ratio (HR) = 1.508; 95% confidence interval (CI), 1.010 to 2.254; $P = 0.045$] (Fig. 5C). Moreover, a more pronounced elevation of CD3-ζ chain expression on T cells was found in arginine-treated patients (Fig. 5, D and E), indicating that restoration of the CD3-ζ chain expression of T cells might be associated with arginine supplementation. This could contribute to the accelerated SFTSV clearance in the arginine-treated group (Fig. 5F). The survival rate, however, displayed no intergroup difference, partially due to the low case fatality rate (CFR) of the recruited patients (5.7% versus 8.3%, $P = 0.058$). Another clinical benefit of arginine supplementation was achieved through a more rapid decrease of aspartate

Table 1. The basic characteristics and clinical manifestations of SFTS patients in the arginine-treated group and the placebo group during treatment. IQR, interquartile range.

Characteristics	Arginine-treated group (n = 53)	Placebo group (n = 60)	P value
Epidemiological characteristics			
Hospitalized day, days, median (IQR)*	8 (6–9)	8 (7–10)	0.241
Clinical manifestation	**No. (%)**		
Headache†	0 (0)	1 (1.7)	1.000
Dizziness†	1 (1.9)	3 (5.0)	0.621
Malaise‡	9 (17.0)	13 (21.7)	0.530
Myalgia‡	1 (1.9)	5 (8.3)	0.212
Chill‡	1 (1.9)	1 (1.7)	1.000
Cough‡	19 (35.9)	18 (30.0)	0.508
Sputum†	17 (32.1)	19 (31.7)	0.963
Gastrointestinal symptoms‡	18 (34.0)	20 (33.3)	0.944
Nausea‡	8 (15.1)	16 (26.7)	0.133
Vomiting‡	7 (13.2)	6 (10.0)	0.594
Diarrhea	10 (18.9)	10 (16.7)	0.760
Dyspnea†	2 (3.8)	6 (10.0)	0.279
Neurological symptoms†	10 (18.9)	17 (28.3)	0.239
Dysphoria‡	5 (9.4)	8 (13.3)	0.517
Convulsion‡	6 (11.3)	6 (10.0)	0.820
Somnolence†	2 (3.8)	9 (15.0)	0.058
Clouding of consciousness†	2 (3.8)	3 (5.0)	1.000
Lethargy†	2 (3.8)	0 (0)	0.218
Coma†	1 (1.9)	3 (5.0)	0.371
Hemorrhagic signs†	18 (34.0)	16 (26.7)	0.399
Melena†	3 (5.7)	4 (6.7)	1.000
Gingival bleeding†	12 (22.6)	8 (13.3)	0.196
Hemoptysis†	4 (7.6)	6 (10.0)	0.748
Hematemesis†	3 (5.7)	4 (6.7)	1.000
Hematuria†	1 (1.9)	0 (0)	0.469
Epistaxis†	0 (0)	0 (0)	1.000
Ophthalmorrhagia†	0 (0)	0 (0)	1.000
Plasma leakage†	6 (11.3)	4 (6.7)	0.511
Hydrothorax†	6 (11.3)	4 (6.7)	0.511
Hydropericardium†	0 (0)	0 (0)	1.000
Pelvisfluidify†	0 (0)	0 (0)	1.000
Ascites†	0 (0)	0 (0)	1.000
Hydropsia†	0 (0)	0 (0)	1.000
Outcome (fatal)	3 (5.7)	5 (8.3)	0.580

*Nonparameteric. †Fisher's exact test. ‡χ^2 test.

aminotransferase/alanine aminotransferase (AST/ALT) value to normal (Fig. 5G), but no significant effects on other laboratory tests (fig. S4, E to G). Some potential adverse effects were encountered, including elevated blood urea nitrogen concentration (fig. S4H), and vomiting was slightly overrepresented in the arginine treatment group (Table 1).

DISCUSSION

Here, we revealed arginine deficiency in SFTS cases, suggesting arginine catabolism by NOS and arginase to be an important pathway in SFTSV infection and related fatal outcome. Arginine deficiency was associated with decreased Plt-NO concentration, which might be involved in platelet activation and thrombocytopenia. The

expansion of arginase-expressing MDSC, which was related to T cell CD3-ζ chain down-regulation and virus clearance disturbance, was revealed after SFTSV infection, implicating a role of arginase activity and arginine depletion in the impairment of anti-SFTSV T cell function. The GABR measurement, which may be considered as a more comprehensive measurement of reduced NO synthetic capacity, was suggested to be a valid predictor of SFTS mortality than the arginine concentration. A randomized controlled trial on an independent group of SFTS patients demonstrated that arginine administration was associated with enhanced Plt-NO concentration, suppressed platelet activation, elevated CD3-ζ chain expression, and platelet counts, eventually attaining an accelerated virus clearance.

It is known that NO regulates platelets function (*33*) and stimulates soluble guanylyl cyclase in platelets, causing an increase in guanosine 3′, 5′-monophosphate (cGMP) and hence activation of cGMP-dependent protein kinase. This in turn causes inhibition of platelet activation through various pathways, for example, NO inhibits the activation of phosphatidylinositol 3-kinase, and causes activation of GPIIb-IIIa fibrinogen receptors (PAC-1 expression) (*22*). It has been reported that low plasma concentrations of arginine could directly impair Plt-NO synthesis and result in platelet hyperaggregability (*19*). However, the importance of this pathway has been mainly limited to cardiovascular disease (*34*), and there have been doubts concerning both expression and functionality of NOS in platelets (*35*). Our results provided important data showing that in SFTS patients, platelet arginine deficiency and subsequent NO down-regulation might contribute to the platelet activation, which was possibly related to platelet-monocyte aggregation and platelet apoptosis, thereby playing a role in platelet clearance and the development of thrombocytopenia.

L-arginine metabolic perturbation was also involved in MDSC expansion. It has been well established that MDSC expand in tumor infiltrates and down-regulate local and systemic immune responses by production of arginase I, which exhausts L-arginine (*36*). In recent years, researchers have also shown that MDSC effectively suppress antiviral host immunity by limiting the function of several immune cells, including T cells and natural killer cells (*37*). Here, we were able to determine the association between gMDSC and arginine consumption in SFTS and their suppressive effect on T cell subsets. Immunoregulation was accomplished by modulation of the CD3-ζ chain of the TCR, critical for signal transduction and correct assembly of the TCR complex (*25*, *38*). Our findings in the observational study were reinforced by the subsequent randomized controlled trial. Both platelet activation and suppressed T cell CD3-ζ chain expression were corrected after administration of L-arginine, revealing its potential beneficial effects on the recovery of laboratory abnormalities and alleviation of the clinical outcome. However, our randomized controlled trial design was restricted to treat mild cases for fear of affecting blood pressure; hence, the effect of arginine in reducing the CFR needs to be explored in the future.

On the basis of metabolic results, arginine metabolism has been focused on further analysis and verification; the effect of other metabolites on SFTS development had not been discussed. Most of the results were obtained only by a correlation analysis, but not based on experimental results, to prove the causal relationship between arginine deficiency and platelet deprivation or immunosuppression by SFTSV infection. Moreover, the diet intake of arginine was not measured in the patients, which might also interfere the arginine concentration, although with slight effect. Furthermore, considering the medication safety, the patients with severe complication (such as persistent bleeding) were not included in the clinical trial; therefore, the effect of arginine on these patients remained unclear.

Despite these limitations, we believe that the L-arginine–NO metabolic pathway perturbation is not unique to SFTSV but likely to be a key biochemical pathway that also plays part in other viral hemorrhagic fever (*39*–*41*). The potential of arginine in treating such infectious diseases as having similar clinical features as SFTS warrants exploration. Further metabolomics study holds the promise of digging new therapy targets for other emerging infectious disease as well.

MATERIALS AND METHODS

Study design

A case-control study was used to identify specific differential metabolites in SFTS infection. A total of 46 SFTS patients, 46 healthy controls, and 46 non-SFTS febrile controls were sampled for the metabolomics analysis. Similarly, a case-control study design was used to verify the perturbed arginine metabolism and its biological effect. Forty-four SFTS patients and 48 healthy controls were evaluated for the platelet and immunity phenotypes, as well as amino acid concentration. Finally, a randomized, single-blinded, placebo-controlled trial was performed to validate the effect of arginine administration, with 53 and 60 SFTS patients included into arginine-treated and placebo group, respectively. Primary data are in table S5.

Study subjects

The study was performed at the designated hospital, Chinese People's Liberation Army (PLA) 154 hospital for SFTS in Xinyang City, Henan Province, China. Xinyang is the most severely inflicted region with SFTS, where nearly one-third of the total nationwide SFTS patients have been reported (*42*). All the adult patients confirmed to be infected with SFTSV who were admitted to the hospital from April to August 2017 were screened by a trained research coordinator and recruited in the current study. Briefly, the patients were confirmed after SFTSV was detected in serum by real-time reverse transcript polymerase chain reaction (RT-PCR), according to China Ministry of Health guidelines (*43*). Considering that several infections, such as dengue virus infection, tuberculosis, and sepsis, can also perturb arginine metabolism, we have excluded the patients with such diseases before they were recruited. The recruited cases had their EDTA and sodium citrate anticoagulants and serum samples collected at the acute infection before any therapy was administered and serially throughout the hospitalization. Patients were discharged from hospital if they showed defervescence, stable vital signs, rising platelet trend, and good oral intake. The survived patients were followed up for over 1 month after disease to have their convalescent samples collected. All of the controls were asymptomatic age-matched healthy subjects who were recruited during the health examination in the same hospital. Before recruiting into the study, the controls had been determined to have normal clinical indicators and had been tested as SFTSV-negative by both RT-PCR and serological immunoglobulin M antibody test, as described below. The controls had their EDTA and sodium citrate anticoagulants and serum samples collected for later use. Two groups of subjects were sampled for the metabolomics analysis (group 1, 46 SFTS patients, 46 healthy controls, and 46 non-SFTS febrile controls; group 2, 10 surviving SFTS cases, 10 fatal SFTS cases, and 16 healthy controls). Together, 44 SFTS patients

and 48 healthy controls were used for the platelet activation and gMDSC T cell determination. Characteristics of all patients and controls in the observational studies are summarized in tables S2 to S4. The Committee for Ethical Review of PLA 154 hospital approved the protocol (20160305-SFTS-06).

Laboratory tests

The detailed protocols, methods, or references of all laboratory tests are summarized in the Supplementary Materials.

The randomized controlled trial

The effect of arginine supplementation was evaluated within the framework of a randomized, single-blind, parallel, placebo-controlled trial (ChiCTR-IPR-17012288, Chinese Clinical Trial Registry). The Chinese Committee for Ethical Review of clinical trials approved that the protocol meets the standards of the Declaration of Helsinki.

Patient enrollment and allocation

All details of subject inclusion/exclusion criteria could be tracked on the Chinese Clinical Trial Registry website. Briefly, confirmed SFTS patients that were recruited according to the criteria outlined above were enrolled in this clinical study after written informed consent was obtained. Cases were excluded for renal insufficiency or any other contraindication of arginine injection: If the interval from disease onset to the hospital administration was longer than 7 days; if the patients had already developed a severe complication, including persistent or recurrent bleeding; and if the patients had active peptic ulcer disease within 3 months and anticoagulant or antiallergic agent use within 4 weeks. The patients were allocated (1:1) into arginine-treated group or placebo group randomly using a random number list generated by Microsoft Excel program (version 2013). The researcher who allocated patients did not take charge of patients' enrollment.

Patient treatment

The supportive treatment of patients consisted of bed rest with light diet. The balance of fluid and electrolytes was monitored and managed by fluid therapy, especially for patients with hyponatremia. Parenteral nutrition therapy was provided to patients who could not eat by themselves. Fever and pain medications were allowed to be prescribed for patients who suffered high fever or pain. Antiemetic and antidiarrheal medications were given to patients with gastrointestinal symptoms. The arginine-treated group was transfused with arginine injection containing 20 g of arginine dissolved in 500 ml of 5% glucose solution daily. In addition, the placebo group received equal volume of 5% glucose injection with the same appearance. Patients and their family were blind to the intervention. The full course of treatment was 7 days continuously. The arginine treatment would be stopped if the patients developed severe adverse effects (such as vomited severely or renal insufficiency) during the course or, if the patients were discharged from the hospital when they showed defervescence, stable vital signs, rising platelet trend, and good oral intake. Patients were excluded from the final analysis if their intervention periods were less than 3 days, or if hospital durations were less than 5 days. Further supportive treatments were provided to patients who did not meet the discharge criteria at the end of interventions.

Clinical observation and laboratory tests

Demographic, clinical, and laboratory data were collected by the review of clinical notes, nursing charts, and laboratory records of patients. Clinical features, including symptoms related to SFTS and adverse effects related to the administration of arginine, were observed and recorded daily by doctors and nurses on duty. The adverse effects of arginine monitored in this study included renal insufficiency, hyperchloremic acidosis, severe vomiting, and other gastrointestinal problems. Routine blood tests and biochemical tests were collected at least every other day, and the results were reported by medical clinical laboratory of PLA 154 hospital. Fasting peripheral blood samples were collected at the morning of 1, 4, and 8 days after intervention. The samples were tested for arginine concentrations (*44*), gMDSC frequency and function, Plt-NO, platelet activation, and CD3-ζ chain expression on T cells by applying the same protocol as that of the observational study above.

Statistical analysis

Descriptive statistics were calculated for all variables; continuous variables were described as means and SDs accorded with normal distribution or as medians and interquartile ranges accorded with abnormal distribution, and categorical variables were described as frequencies and proportions. The metabolomics data were analyzed by OPLS-DA and PCA model. The data of the surface markers' MFI were $\log_{10}$-transformed. To determine the difference between SFTS cases and healthy controls or between arginine-treated group and placebo control group, an independent t test, a χ^2 test, a Fisher's exact test, or a nonparametric test (Mann-Whitney test) was used where appropriate. The differences of surface markers expressed by activated platelets, amino acid concentration, and T cell immune parameters for three time points were analyzed by the mixed-effect model, which was used to analyze the repeated measurements. The $\log_{10}$-transformed data on daily platelet counts, viral loads, and other laboratory indexes after treatment were analyzed over time with the generalized estimating equation models, which took into account the relationship between viral loads and platelet counts obtained at baseline and at follow-up points in the same patient. For the variables of CD3-ζ, platelet activation, and amino acids as the repeated measure data in the randomized controlled trial study, the mixed-effect model was performed to compare the differences between the arginine-treated group and the placebo control group. Considering that the variables of age, sex, and interval from disease onset to admission were related to the outcome of SFTS, these variables were adjusted in all multivariate analyses. Spearman correlation analyses were performed for the relationship between any two parameters of the platelet counts, viral loads, surface markers expressed by activated platelets, amino acids, and T cell immune parameters. We used ROC curve analyses for the fatal outcome using the surface markers expressed by activated platelets and amino acids. Kaplan-Meier curves were used to show the days that were needed for the platelet count to recover for the arginine-treated group and the placebo control group, respectively. Cox proportional hazard model was used to compare the recovery time of platelet counts in the two groups by estimating HR and 95% CI. A two-sided $P < 0.05$ was considered to be statistically significant. All analyses were performed using STATA 14.0 (Stata Corporation LP). The column scatter plots were made using GraphPad Prism 6.0, and the scatter plots of correlation and box plots were made using Microsoft Excel 2016.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/10/459/eaat4162/DC1
Materials and Methods
Fig. S1. PCA score plots (2D/3D models) of LC-MS/MS metabolomics data on two cohorts.
Fig. S2. The comparison of T cell subset and gMDSC between 44 SFTS cases at acute phase and 48 controls.
Fig. S3. Study profile of patients recruited in the randomized controlled trial.
Fig. S4. The evaluation of arginine-related metabolic parameters and laboratory indexes during treatment of the SFTS patients enrolled in the randomized controlled trial.
Table S1. The basic characteristics of patients in the arginine-treated group and the placebo group on admission.
Table S2. The basic characteristics and clinical manifestations of SFTS cases and healthy controls on admission.
Table S3. The basic characteristics and clinical manifestations of SFTS cases and healthy controls in the global metabolites study.
Table S4. The basic characteristics and clinical manifestations of SFTS fatal cases and surviving cases in the global metabolites study.
Table S5. Primary data.

REFERENCES AND NOTES

1. X.-J. Yu, M.-F. Liang, S.-Y. Zhang, Y. Liu, J.-D. Li, Y.-L. Sun, L. Zhang, Q.-F. Zhang, V. L. Popov, C. Li, J. Qu, Q. Li, Y.-P. Zhang, R. Hai, W. Wu, Q. Wang, F.-X. Zhan, X.-J. Wang, B. Kan, S.-W. Wang, K.-L. Wan, H.-Q. Jing, J.-X. Lu, W.-W. Yin, H. Zhou, X.-H. Guan, J.-F. Liu, Z.-Q. Bi, G.-H. Liu, J. Ren, H. Wang, Z. Zhao, J.-D. Song, J.-R. He, T. Wan, J.-S. Zhang, X.-P. Fu, L.-N. Sun, X.-P. Dong, Z.-J. Feng, W.-Z. Yang, T. Hong, Y. Zhang, D. H. Walker, Y. Wang, D.-X. Li, Fever with thrombocytopenia associated with a novel bunyavirus in China. *N. Engl. J. Med.* **364**, 1523–1532 (2011).
2. 2017 Annual review of diseases prioritized under the Research and Development Blueprint (WHO Meeting report, World Health Organization, 2017).
3. Q. Liu, B. He, S.-Y. Huang, F. Wei, X.-Q. Zhu, Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. *Lancet Infect. Dis.* **14**, 763–772 (2014).
4. W. Liu, Q.-B. Lu, N. Cui, H. Li, L.-Y. Wang, K. Liu, Z.-D. Yang, B.-J. Wang, H.-Y. Wang, Y.-Y. Zhang, L. Zhuang, C.-Y. Hu, C. Yuan, X.-J. Fan, Z. Wang, L. Zhang, X.-A. Zhang, D. H. Walker, W.-C. Cao, Case-fatality ratio and effectiveness of ribavirin therapy among hospitalized patients in China who had severe fever with thrombocytopenia syndrome. *Clin. Infect. Dis.* **57**, 1292–1299 (2013).
5. I. Park, H. I. Kim, K. T. Kwon, Two treatment cases of severe fever and thrombocytopenia syndrome with oral ribavirin and plasma exchange. *Infect. Chemother.* **49**, 72–77 (2017).
6. T. Takahashi, Severe fever with thrombocytopenia syndrome (SFTS) and SFTS virus. *Uirusu* **65**, 7–16 (2015).
7. Q.-B. Lu, N. Cui, J.-G. Hu, W.-W. Chen, W. Xu, H. Li, X.-A. Zhang, H. Ly, W. Liu, W.-C. Cao, Characterization of immunological responses in patients with severe fever with thrombocytopenia syndrome: A cohort study in China. *Vaccine* **33**, 1250–1255 (2015).
8. N. J. C. Robles, H. J. Han, S.-J. Park, Y. K. Choi, Epidemiology of severe fever and thrombocytopenia syndrome virus infection and the need for therapeutics for the prevention. *Clin. Exp. Vaccine Res.* **7**, 43–50 (2018).
9. C. Jin, M. Liang, J. Ning, W. Gu, H. Jiang, W. Wu, F. Zhang, C. Li, Q. Zhang, H. Zhu, T. Chen, Y. Han, W. Zhang, S. Zhang, Q. Wang, L. Sun, Q. Liu, J. Li, T. Wang, Q. Wei, S. Wang, Y. Deng, C. Qin, D. Li, Pathogenesis of emerging severe fever with thrombocytopenia syndrome virus in C57/BL6 mouse model. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 10053–10058 (2012).
10. J. Munger, S. U. Bajad, H. A. Coller, T. Shenk, J. D. Rabinowitz, Dynamics of the cellular metabolome during human cytomegalovirus infection. *PLOS Pathog.* **2**, e132 (2006).
11. J. K. Nicholson, J. C. Lindon, Systems biology: Metabonomics. *Nature* **455**, 1054–1056 (2008).
12. P. C. Rodriguez, A. C. Ochoa, A. A. Al-Khami, Arginine metabolism in myeloid cells shapes innate and adaptive immunity. *Front. Immunol.* **8**, 93 (2017).
13. V. Bronte, P. Zanovello, Regulation of immune responses by L-arginine metabolism. *Nat. Rev. Immunol.* **5**, 641–654 (2005).
14. K. S. Burrack, T. E. Morrison, The role of myeloid cell activation and arginine metabolism in the pathogenesis of virus-induced diseases. *Front. Immunol.* **5**, 428 (2014).
15. F. Huang, M. Ni, M. D. Chalishazar, K. E. Huffman, J. Kim, L. Cai, X. Shi, F. Cai, L. G. Zacharias, A. S. Ireland, K. Li, W. Gu, A. K. Kaushik, X. Liu, A. F. Gazdar, T. G. Oliver, J. D. Minna, Z. Hu, R. J. DeBerardinis, Inosine monophosphate dehydrogenase dependence in a subset of small cell lung cancers. *Cell Metab.* S1550-4131(18)30387-5 (2018).
16. J. Xia, D. S. Wishart, Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. *Curr. Protoc. Bioinformatics* **55**, 14.10.1–14.10.91 (2016).
17. M. S. Alkaitis, H. Wang, A. K. Ikeda, C. A. Rowley, I. J. C. MacCormick, J. H. Chertow, O. Billker, A. F. Suffredini, D. J. Roberts, T. E. Taylor, K. B. Seydel, H. C. Ackerman, Decreased rate of plasma arginine appearance in murine malaria may explain hypoargininemia in children with cerebral malaria. *J. Infect. Dis.* **214**, 1840–1849 (2016).
18. G. Wu, S. M. Morris Jr., Arginine metabolism: Nitric oxide and beyond. *Biochem. J.* **336** (Pt. 1), 1–17 (1998).
19. V. L. M. Pinto, P. F. C. de Souza, T. M. C. Brunini, M. B. Oliveira, M. B. Moss, M. A. de Sá Siqueira, M. R. Ferraz, A. C. Mendes-Ribeiro, Low plasma levels of L-arginine, impaired intraplatelet nitric oxide and platelet hyperaggregability: Implications for cardiovascular disease in depressive patients. *J. Affect. Disord.* **140**, 187–192 (2012).
20. G. Davì, C. Patrono, Platelet activation and atherothrombosis. *N. Engl. J. Med.* **357**, 2482–2494 (2007).
21. K. Ichiki, H. Ikeda, N. Haramaki, T. Ueno, T. Imaizumi, Long-term smoking impairs platelet-derived nitric oxide release. *Circulation* **94**, 3109–3114 (1996).
22. E. Gkaliagkousi, J. Ritter, A. Ferro, Platelet-derived nitric oxide signaling and regulation. *Circ. Res.* **101**, 654–662 (2007).
23. K. A. Metcalf Pate, C. E. Lyons, J. L. Dorsey, E. N. Shirk, S. E. Queen, R. J. Adams, L. Gama, C. N. Morrell, J. L. Mankowski, Platelet activation and platelet-monocyte aggregate formation contribute to decreased platelet count during acute simian immunodeficiency virus infection in pig-tailed macaques. *J. Infect. Dis.* **208**, 874–883 (2013).
24. A. Choudhury, I. Chung, A. D. Blann, G. Y. H. Lip, Platelet surface CD62P and CD63, mean platelet volume, and soluble/platelet P-selectin as indexes of platelet function in atrial fibrillation: A comparison of "healthy control subjects" and "disease control subjects" in sinus rhythm. *J. Am. Coll. Cardiol.* **49**, 1957–1964 (2007).
25. M. Baniyash, TCR ζ-chain downregulation: Curtailing an excessive inflammatory immune response. *Nat. Rev. Immunol.* **4**, 675–687 (2004).
26. R. S. Tacke, H.-C. Lee, C. Goh, J. Courtney, S. J. Polyak, H. R. Rosen, Y. S. Hahn, Myeloid suppressor cells induced by hepatitis C virus suppress T-cell responses through the production of reactive oxygen species. *Hepatology* **55**, 343–353 (2012).
27. B. A. Norris, L. S. Uebelhoer, H. I. Nakaya, A. A. Price, A. Grakoui, B. Pulendran, Chronic but not acute virus infection induces sustained expansion of myeloid suppressor cell numbers that inhibit viral-specific T cell immunity. *Immunity* **38**, 309–321 (2013).
28. F. Uhel, I. Azzaoui, M. Grégoire, C. Pangault, J. Dulong, J.-M. Tadié, A. Gacouin, C. Camus, L. Cynober, T. Fest, Y. Le Tulzo, M. Roussel, K. Tarte, Early expansion of circulating granulocytic myeloid-derived suppressor cells predicts development of nosocomial infections in patients with sepsis. *Am. J. Respir. Crit. Care Med.* **196**, 315–327 (2017).
29. L. J. Pallett, U. S. Gill, A. Quaglia, L. V. Sinclair, M. Jover-Cobos, A. Schurich, K. P. Singh, N. Thomas, A. Das, A. Chen, G. Fusai, A. Bertoletti, D. A. Cantrell, P. T. Kennedy, N. A. Davies, M. Haniffa, M. K. Maini, Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. *Nat. Med.* **21**, 591–600 (2015).
30. R. Geiger, J. C. Rieckmann, T. Wolf, C. Basso, Y. Feng, T. Fuhrer, M. Kogadeeva, P. Picotti, F. Meissner, M. Mann, N. Zamboni, F. Sallusto, A. Lanzavecchia, L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* **167**, 829–842.e13 (2016).
31. A. Das, M. Hoare, N. Davies, A. R. Lopes, C. Dunn, P. T. F. Kennedy, G. Alexander, H. Finney, A. Lawson, F. J. Plunkett, A. Bertoletti, A. N. Akbar, M. K. Maini, Functional skewing of the global CD8 T cell population in chronic hepatitis B virus infection. *J. Exp. Med.* **205**, 2111–2124 (2008).
32. W. H. Tang, Z. Wang, L. Cho, D. M. Brennan, S. L. Hazen, Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J. Am. Coll. Cardiol.* **53**, 2061–2067 (2009).
33. S. Moncada, A. Higgs, The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* **329**, 2002–2012 (1993).
34. J. E. Freedman, B. Ting, B. Hankin, J. Loscalzo, J. F. Keaney Jr., J. A. Vita, Impaired platelet production of nitric oxide predicts presence of acute coronary syndromes. *Circulation* **98**, 1481–1486 (1998).
35. S. Gambaryan, D. Tsikas, A review and discussion of platelet nitric oxide and nitric oxide synthase: Do blood platelets produce nitric oxide from L-arginine or nitrite? *Amino Acids* **47**, 1779–1793 (2015).
36. J. E. Talmadge, D. I. Gabrilovich, History of myeloid-derived suppressor cells. *Nat. Rev. Cancer* **13**, 739–752 (2013).
37. C. Zhang, S. Wang, C. Yang, R. Rong, The crosstalk between myeloid derived suppressor cells and immune cells: To establish immune tolerance in transplantation. *J. Immunol. Res.* **2016**, 4986797 (2016).
38. P. C. Rodriguez, A. H. Zea, J. DeSalvo, K. S. Culotta, J. Zabaleta, D. G. Quiceno, J. B. Ochoa, A. C. Ochoa, L-arginine consumption by macrophages modulates the expression of CD3ζ chain in T lymphocytes. *J. Immunol.* **171**, 1232–1239 (2003).
39. C. Galbán, J. C. Montejo, A. Mesejo, P. Marco, S. Celaya, J. M. Sanchez-Segura, M. Farré, D. J. Bryg, An immune-enhancing enteral diet reduces mortality rate and episodes of bacteremia in septic intensive care unit patients. *Crit. Care Med.* **28**, 643–648 (2000).
40. S. Yacoub, P. K. Lam, T. T. Huynh, H. H. Nguyen Ho, H. T. Dong Thi, N. T. Van, L. T. Lien, Q. N. T. Ha, D. H. T. Le, J. Mongkolspaya, A. Culshaw, T. W. Yeo, H. Wertheim, C. Simmons, G. Screaton, B. Wills, Endothelial nitric oxide pathways in the pathophysiology of dengue: A prospective observational study. *Clin. Infect. Dis.* **65**, 1453–1461 (2017).
41. B. E. Barber, T. William, M. J. Grigg, K. A. Piera, Y. Chen, H. Wang, J. B. Weinberg, T. W. Yeo, N. M. Anstey, Nitric oxide-dependent endothelial dysfunction and reduced arginine

bioavailability in *Plasmodium vivax* malaria but no greater increase in intravascular hemolysis in severe disease. *J. Infect. Dis.* **214**, 1557–1564 (2016).
42. K. Liu, H. Zhou, R.-X. Sun, H.-W. Yao, Y. Li, L.-P. Wang, D. Mu, X.-L. Li, Y. Yang, G. C. Gray, N. Cui, W.-W. Yin, L.-Q. Fang, H.-J. Yu, W.-C. Cao, A national assessment of the epidemiology of severe fever with thrombocytopenia syndrome, China. *Sci. Rep.* **5**, 9679 (2015).
43. Ministry of Health, Guideline for prevention and treatment of severe fever with thrombocytopenia syndrome (2010 vesion). *Chin. J. Clin. Infect. Dis.* **4**, 193–194 (2011).
44. A. L. Ksenofontov, A. I. Boyko, G. V. Mkrtchyan, V. N. Tashlitsky, A. V. Timofeeva, A. V. Graf, V. I. Bunik, L. A. Baratova, Analysis of free amino acids in mammalian brain extracts. *Biochemistry (Mosc.)* **82**, 1183–1192 (2017).

Acknowledgments: We thank all the tested individuals, their families, and collaborating clinicians for their participation. We also acknowledge M. K. Maini, F.-s. Wang, X. Tan, and M. Peng for their critical reading and feedback on our manuscript. **Funding:** This work was supported by the National Natural Science Foundation of China (81621005 to W.-C.C., 81473023 to W.L., 81703274 to Q.-B.L., and 81722041 to H.L.), the China Mega-Project on Infectious Disease Prevention (no. 2018ZX10713002 to W.L.), Tsinghua University grant (53332200517 to Z.H.), National Science and Technology Major Project for "Significant New Drug Development" (2017ZX09304015 to Z.H.), and Bayer Investigator Award (to Z.H.).
Author contributions: W.L., Z.H., W.-C.C., and X.-K.L. designed the study. Z.H. and W.L. conceived the project. Z.H. and K.Y. performed the metabolomic profiling and data analysis. X.-K.L., R.L., and Q.-B.L. performed the OPLS-DA, PCA, and clustering analysis. W.L., X.-K.L., W.-C.C., Z.H., and W.-W.C. designed the randomized controlled trial. N.C., C.Y., W.-W.C., Q.-B.L., P.-H.Z., R.L., X.-A.Z., and Z.X. supervised the clinical trial. X.-K.L., W.X., J.D., S.-F.Z., H.L., and B.X. collected and processed clinical specimens and performed clinical flow cytometry experiments. W.X., K.Y., X.-K.L., and D.Z. conducted the in vitro assays. X.-K.L., W.X., J.D., S.-F.Z., and Q.-B.L. sorted out clinical data. Q.-B.L., X.-K.L., and S.-F.Z. performed the statistical analysis. W.L, W.-C.C., X.-K.L, Q.-B.L., Z.H., and W.X. drafted the manuscript. W.L., X.-K.L., Q.-B.L., W.-C.C., Z.H., and W.-W.C. edited and revised the manuscript. All authors were involved in the discussion of results and critical reading of the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data associated with this study are present in the paper or the Supplementary Materials.

Submitted 24 February 2018
Resubmitted 1 May 2018
Accepted 14 August 2018
Published 19 September 2018
10.1126/scitranslmed.aat4162

Citation: X.-K. Li, Q.-B. Lu, W.-W. Chen, W. Xu, R. Liu, S.-F. Zhang, J. Du, H. Li, K. Yao, D. Zhai, P.-H. Zhang, B. Xing, N. Cui, Z.-D. Yang, C. Yuan, X.-A. Zhang, Z. Xu, W.-C. Cao, Z. Hu, W. Liu, Arginine deficiency is involved in thrombocytopenia and immunosuppression in severe fever with thrombocytopenia syndrome. *Sci. Transl. Med.* **10**, eaat4162 (2018).

Science Translational Medicine

Arginine deficiency is involved in thrombocytopenia and immunosuppression in severe fever with thrombocytopenia syndrome

Xiao-Kun LiQing-Bin LuWei-Wei ChenWen XuRong LiuShao-Fei ZhangJuan DuHao LiKe YaoDi ZhaiPan-He ZhangBo XingNing CuiZhen-Dong YangChun YuanXiao-Ai ZhangZhe XuWu-Chun CaoZeping HuWei Liu

Sci. Transl. Med., 10 (459), eaat4162. • DOI: 10.1126/scitranslmed.aat4162

Arginine arbitrates thrombocytopenia

SFTS virus is a bunyavirus named for the disease it causes, severe fever with thrombocytopenia syndrome. It has only recently been discovered, and little is known about its pathogenesis or how to intervene. Li *et al.* conducted an observational study in a hospital setting to identify differences between fatal cases and those that went on to recover and discovered that decreased arginine was associated with thrombocytopenia and death. They then performed a randomized controlled clinical trial to supplement patients with arginine. Patients receiving arginine had decreased platelet activation and faster viral clearance. This study may pave the way for a better understanding and treatment for SFTS virus.

Science Translational Medicine (ISSN 1946-6242) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science Translational Medicine* is a registered trademark of AAAS.