

Table 1. Post Hoc Analysis of Refractoriness to Treatment in the Safety Population of the TITAN Study.*

Definition of Refractoriness	Caplacizumab (N=35)	Placebo (N=37)
	number (percent)	
No platelet response after 7 days, despite daily plasma-exchange therapy†	2 (6)	8 (22)‡
Absence of platelet-count doubling after 4 days of standard treatment, with lactate dehydrogenase level >ULN§	0	4 (11)

* ULN denotes upper limit of the normal range.

† Definition is from Sayani and Abrams.²

‡ Two patients in the placebo group who discontinued the study prematurely (<7 days) without reaching the platelet-count criterion (i.e., platelet count, <150×10⁹ per liter) were counted as having disease that was refractory to treatment.

§ Definition is from Soucemarianadin et al.³

cizumab according to two definitions of refractoriness.^{2,3} Regardless of the definition, a lower percentage of patients in the caplacizumab group than in the placebo group had disease that was refractory to treatment, which suggests that caplacizumab might prevent refractory TTP and its associated worse outcomes.¹

To clarify, Table 3 of our article reports the cumulative number of patients with at least one adverse event that was considered to be related or possibly related to the investigational medicinal product, whereas the text separately reports

the number of patients with at least one adverse event that was considered to be either related or possibly related to treatment. We confirm that the data regarding patients with relapse during the 12-month follow-up include those with relapse during the 1-month follow-up: 11 patients in the caplacizumab group, as compared with 3 in the placebo group, had a relapse during the entire follow-up period. Overall, 13 patients in each treatment group had at least one recurrence of TTP (exacerbation or relapse).

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1. Benhamou Y, Boelle PY, Baudin B, et al. Cardiac troponin-I on diagnosis predicts early death and refractoriness in acquired thrombotic thrombocytopenic purpura: experience of the French Thrombotic Microangiopathies Reference Center. *J Thromb Haemost* 2015;13:293-302.

2. Sayani FA, Abrams CS. How I treat refractory thrombotic thrombocytopenic purpura. *Blood* 2015;125:3860-7.

3. Soucemarianadin M, Benhamou Y, Delmas Y, et al. Twice-daily therapeutical plasma exchange-based salvage therapy in severe autoimmune thrombotic thrombocytopenic purpura: the French TMA Reference Center experience. *Eur J Haematol* 2015 November 26 (Epub ahead of print).

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Convalescent Plasma for Ebola Virus Disease

TO THE EDITOR: In their study, van Griensven et al. (Jan. 7 issue)¹ found no significant survival benefit of using convalescent plasma with unknown levels of neutralizing antibodies in patients with Ebola virus disease (EVD). Survivors of EVD donated plasma anywhere from 2 months to 6 months or more after they had recovered. Substantial immune activation and robust B-cell and T-cell responses have been observed in patients with acute EVD and in some patients during convalescence,² although humoral response has not been thoroughly studied in EVD. We have found that in convalescent patients, specific neutralizing activity against Ebola virus glycoprotein (EBOV-GP) increases over time (≥9 months after infection), which suggests that affinity maturation of antibodies takes place long after clinical recovery.³

The time that has elapsed after recovery from EVD may be a proxy for the level of activity of EBOV-GP-specific neutralizing antibodies. Given these data, we would be interested in whether patients who received plasma that had been donated 6 months or more after recovery from EVD had a survival advantage over controls and over patients who had received plasma from survivors at earlier time points.

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1. van Griensven J, Edwards T, de Lamballerie X, et al. Evaluation of convalescent plasma for Ebola virus disease in Guinea. *N Engl J Med* 2016;374:33-42.
2. McElroy AK, Akondy RS, Davis CW, et al. Human Ebola virus infection results in substantial immune activation. *Proc Natl Acad Sci U S A* 2015;112:4719-24.
3. Luczkowiak J, Arribas JR, Gómez S, et al. Specific neutralizing response in plasma from convalescent patients of Ebola Virus Disease against the West Africa Makona variant of Ebola virus. *Virus Res* 2016;213:224-9.

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TO THE EDITOR: Although the study by van Griensven et al. suggests that the use of convalescent plasma in patients with EVD may have a small survival advantage only in particular populations, we identified several methodologic factors that could have increased the effectiveness of this treatment. First, the investigators did not include a characterization of neutralizing antibodies in the transfused products after viral inactivation and liquid storage for 40 days. Second, the storage of plasma at 2 to 8 C° for 40 days before freezing goes against international practices, which recommend freezing within 24 hours to preserve protein function from proteolysis or aggregation.¹ This increases the risk of transfusing plasma that does not contain enough neutralizing antibodies. Third, the investigators provided limited data on antibody cross-reactivity against Ebola virus strains during antibody-based therapies,² which further complicates interpretation of the data. Moreover, the decision not to screen Ebola survivors for transfusion-transmissible infections before blood donation contravenes the guidelines of the World Health Organization.³ We encourage the investigators to document lessons learned on safe plasma administration during epidemics in countries with poorly developed infrastructure, since the use of convalescent plasma may remain the first-line treatment during future Ebola outbreaks in developing countries.

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No potential conflict of interest relevant to this letter was reported.

1. World Health Organization. Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks: interim guidance for national health authorities and blood transfusion services. 2014 (<http://www.who.int/csr/resources/publications/ebola/convalescent-treatment/en/>).
2. WHO guidelines on good manufacturing practices for blood establishments. WHO Technical Report Series 2011;Annex 4:148-214.
3. Casadevall A, Pirofski LA. The Ebola epidemic crystallizes the potential of passive antibody therapy for infectious diseases. *PLoS Pathog* 2015;11(4):e1004717.

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TO THE EDITOR: We commend the effort of the Ebola-Tx trial team in investigating, under challenging circumstances, the use of convalescent plasma as a potential therapeutic option for EVD. However, in order to draw definitive conclusions, the investigators would have needed to evaluate the differences in clinical management in the two study groups. Although the efficacy of supportive care has not been evaluated in a clinical trial, multiple groups with clinical expertise during EVD outbreaks have agreed by consensus that such care is integral to decreasing EVD mortality.^{1,2} The authors acknowledge that patients in the convalescent plasma group may have received more intravenous fluids than patients in the control group owing to the previous placement of an intravenous catheter. However, the logistic-regression model for the study includes only the patient's age and threshold-cycle value and omits variables that might have accounted for key between-group differences in clinical management. Such differences include fluid resuscitation and point-of-care electrolyte testing. Although the conduct of studies such as the Ebola-Tx trial can be challenging, spurious interpretation of the incremental survival benefit of an adjunctive therapy such as convalescent plasma may occur when investigators do not account for the quality of clinical management across study groups.

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1. World Health Organization. Clinical management of patients with viral haemorrhagic fever. 2016 (<http://www.who.int/csr/resources/publications/clinical-management-patients/en/>).

2. Association of Medical Microbiology and Infectious Diseases Canada. Ebola clinical care guidelines: a guide for clinicians in Canada — updated report, 2015 (<https://www.ammi.ca/ebola-clinical-care-guidelines-now-available/>).

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THE AUTHORS REPLY: Arribas et al. suggest using the interval after recovery from EVD as a proxy for the level of neutralizing antibodies in the convalescent plasma. We had previously examined this variable and found no clear association with survival. In particular, patients who received plasma donated 6 or more months after cure did not have a survival advantage over other patients in the convalescent-plasma group (fatality rate, 45% and 27%, respectively). Further study is needed to determine whether there is an association between the level of neutralizing antibodies in the convalescent plasma and patient outcomes.

We agree with Burnouf et al. on the value of reporting on the feasibility of the collection and administration of convalescent plasma during an Ebola outbreak. We also agree that our finding that the use of convalescent plasma did not provide a significant benefit with respect to mortality demands further preclinical and clinical research. As to donor prescreening, we had planned to screen patients for transfusion-transmissible infections before proceeding to apheresis. However, the local association of Ebola survivors, which was very closely involved in our project, insisted that survivors should be given the option of undergoing testing for infections and apheresis in a single step as well as the option of not being told the test results. All six ethics committees that reviewed the protocol, including the WHO committee, thoroughly discussed this aspect of the study and agreed to the provi-

sion. Conditions for plasma storage were among those suggested in the relevant WHO guidance document.¹ Freezing within 24 hours has been associated with the most effective recovery of labile coagulation factors and much less so with the maintenance of stable plasma proteins such as antibodies. Antibodies against the Ebola virus make up the presumably active component of convalescent plasma.^{2,3} With respect to testing of donor plasma for the presence of neutralizing antibodies before administration, the WHO guidance document acknowledges that such testing may be impossible,¹ since it requires the use of biosafety level 4 laboratories. Samples of convalescent plasma have been shipped to France for analysis, including the measurement of antibody levels in Ebola patients before and after transfusion of convalescent plasma.

We concur with Fletcher et al. with respect to the challenges in obtaining detailed information on the level of supportive care from Ebola treatment centers that provide routine clinical care. This information was not reliably collected for the historical control group, which precluded adjustment in the analysis. We have acknowledged this lack of data as a study limitation.

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1. Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks: interim guidance for national health authorities and blood transfusion services. Geneva: World Health Organization, 2014.

2. Casadevall A, Pirofski LA. The Ebola epidemic crystallizes the potential of passive antibody therapy for infectious diseases. *PLoS Pathog* 2015;11(4):e1004717.

3. Position paper on collection and use of convalescent plasma or serum as an element in filovirus outbreak response. Geneva: WHO Blood Regulators Network, 2014.

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