

1. The International Adjuvant Lung Cancer Trial Collaborative Group. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004;350:351-60.

TO THE EDITOR: In the report by Olaussen et al., the data were not stratified according to age, sex, smoking status, or histologic type. Will a significant survival benefit persist after adjustment for all these factors?

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THE AUTHORS REPLY: Our analyses addressed, in three steps, the question of whether the characteristics of the tumors or of the patients influenced the observed differential effect of chemotherapy on survival among patients with ERCC1-negative tumors and those with ERCC1-positive tumors. First, we verified that there was no significant interaction between the treatment effect and any of the demographic and clinical characteristics of patients included in the IALT Biology (IALT Bio) study, a subgroup of the IALT study population.¹ This step was also taken in the analyses performed in the IALT. The absence of an interaction was verified in particular for such factors as histologic type, stage of disease, and the assigned treatment ($P=0.64$, $P=0.45$, and $P=0.26$, respectively). Second, when studying the interaction between ERCC1 status and treatment, we adjusted not only for stratification and prognostic factors but also for factors associated with ERCC1 status (histopathological type, the presence or absence of pleural invasion, and age). Third, we verified that the interaction with ERCC1 status would not disappear when any other interaction was added to the Cox model (P values for an interaction with ERCC1 status remained below 0.009 in the presence of an interaction with histologic type, stage of disease, or assigned treatment). Thus, the interaction be-

tween the ERCC1 status and treatment was robust enough to withstand the addition of any other interaction.

Wilcox points out the different histologic types of carcinoma among the patients with ERCC1-negative and ERCC1-positive tumors ($P<0.001$, in Table 1 of our article). As shown above, this difference does not explain the interaction between ERCC1 status and the treatment effect.

Cecere and colleagues request clarification of the role of the assigned drug and the stage of disease in the interaction between ERCC1 status and treatment. Our analysis shows that this interaction is the only one that remains significant when the model includes either of these two interactions (drug or stage of disease).

Grenader and Shavit are concerned that stratification according to age, sex, smoking status, and histologic type was not performed. Our analysis took all these factors into account, except smoking status. Data on smoking status were not collected in the trial. Smoking may affect the prognosis for patients with lung cancer and may be related to ERCC1 expression. However, since the proportion of smokers is quite high among patients with non-small-cell lung cancer (around 90%), only a very wide variation in the treatment effect between smokers and nonsmokers could explain the observed significant interaction between treatment and ERCC1 status. More important, adjustments for histologic type and sex (which are classically associated with smoking) and the corresponding interactions with treatment do not change the results.

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1. The International Adjuvant Lung Cancer Trial Collaborative Group. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004;350:351-60.

Cytokine Storm and an Anti-CD28 Monoclonal Antibody

TO THE EDITOR: The events of the phase 1 trial of TGN1412, as detailed by Suntharalingam et al. (Sept. 7 issue),¹ are a sobering reminder of the risks that research volunteers take when they participate in trials, but the article did not contain any com-

ments on the subjects' mental state as they emerged from this near-death experience. The volunteers did not just suffer insults to kidney and pulmonary function; they were afflicted emotionally and perhaps intellectually as well. When an experimental

drug has such serious adverse effects, it is worth reporting the psychological effects, which inform the ethical impact of testing experimental regimens and advance our understanding of the management of psychological harm. Without mention of the afflicted subjects' ability to return to their normal lives and their jobs or of psychological counseling offered to them, I view the article by Suntharalingam et al. as incomplete.

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1. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018-28.

TO THE EDITOR: We believe that the cytokine-release syndrome strongly resembles the toxic shock syndrome, which is induced by a staphylococcal superantigen that activates T cells, through their antigen receptor, to induce massive cytokine release.¹ However, mice with deletion of the CD28 gene are protected from superantigen-induced, toxic shock syndrome-like disease.² In addition, peptides that block binding to CD28 also block superantigen-dependent cytokine secretion by T cells.³ Together, these observations indicate that T cells activated by superantigen binding to both CD28 and the antigen receptor mediate the toxic shock syndrome.

CD28-deficient mice are protected from lethal superantigen challenge through the selective abrogation of the release of tumor necrosis factor (TNF).² Because TNF is also strongly induced by anti-CD28 antibodies and TNF infusion can induce toxic shock syndrome-like disease,⁴ short-term use of anti-TNF agents may benefit patients with the toxic shock syndrome and related syndromes involving "cytokine storm."

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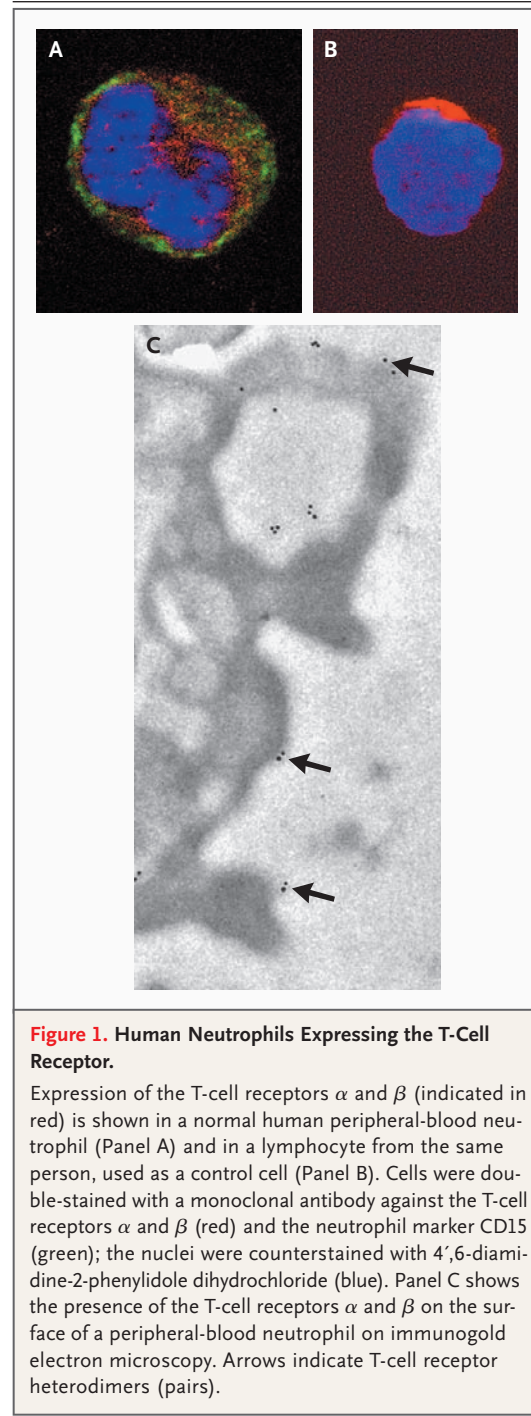
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TO THE EDITOR: CD28 expression by human neutrophils has been documented, and its activation alone results in the secretion of interferon- γ .¹ We now find that a subgroup of human neutrophils expresses the T-cell receptor (Fig. 1). We have also shown that costimulation of the T-cell receptor and CD28 triggers the release of interleukin-8 in



these cells.² Therefore, neutrophils may represent a quantitatively important target of TGN1412 and may link the pathogenesis of the cytokine-release syndrome to the innate immune system.

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TO THE EDITOR: The TGN1412-exposed patients described by Suntharalingam et al. had many of the clinical and laboratory features of the macrophage activation syndrome, also known as secondary hemophagocytic lymphohistiocytosis, a severe and sometimes fatal condition.^{1,2} Unfortunately, important diagnostic criteria for the macrophage activation syndrome,^{3,4} such as increased ferritin and soluble CD25 levels, enhanced activity of natural killer cells, and specific histologic characteristics of bone marrow, were not reported in these patients. Also, hepatosplenomegaly and lymphadenopathy, which are classic features of the macrophage activation syndrome, were absent in the volunteers exposed to TGN1412, perhaps because of the very acute nature of the reaction. If characterized further, TGN1412 may provide insight into the mechanisms that lead to the macrophage activation syndrome in other clinical settings, such as juvenile idiopathic arthritis or sepsis. This understanding may also help in the handling of future situations similar to the one described by Suntharalingam et al., in which evidence from clinical trials, including those involving patients with the macrophage activation syndrome, could help guide the care of the affected patients.³

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TO THE EDITOR: Suntharalingam et al. report severe adverse events in the phase 1 study of TGN1412. Monoclonal antibodies, which cannot be metabolized in the liver and cannot be eliminated from the kidney, usually have long half-lives.¹ Double filtration plasmapheresis, which selectively removes high-molecular-weight substances, including immunoglobulins and immune complexes, has been proven to be effective for the clearance of circulating monoclonal antibodies,² and some studies have shown the usefulness of continuous hemofiltration or continuous hemodiafiltration for ameliorating cytokine storm.^{3,4} We would like the authors to comment on whether these therapeutic measures might be beneficial for the treatment of severe adverse events associated with monoclonal antibodies.

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THE AUTHORS REPLY: The psychological effect of serious adverse events on healthy subjects involved in clinical trials is important; critical illness has its own known neuropsychological consequences.^{1,2} Our institution does provide follow-up after discharge of patients who stay in the intensive care unit for more than 3 days. However, our ar-

ticle was limited to the physiological and immunologic events that occurred within 30 days after exposure; the omission cited by Gardner does not make the report incomplete.

Corry and Lewis suggest that the cytokine storm induced by TGN1412 is similar to that resulting in the toxic shock syndrome, on the basis of the activation of CD28 on T cells. Although T cells were the intended targets of TGN1412, it is still unclear whether T-cell activation induced the cytokine storm. The trigger may have been on another type of cell expressing CD28, as suggested by Puellmann et al., or may have involved ligation of Fc receptors. Hence the macrophage activation syndrome was also considered. Since the neutrophil count was preserved and, as noted by Garcia-Bournissen et al., there was neither hepatosplenomegaly nor lymphadenopathy, we thought that the macrophage activation syndrome as a separate entity should be ruled out. Our article was a clinical report of the unintended consequences of a phase 1 trial and subsequent emergency care. Laboratory investigations were limited to those that were of direct clinical benefit.

Anti-TNF therapy was considered. When transfer to our care took place 12 to 16 hours after TGN1412 infusion, we decided that the TNF peak was likely to have passed and that downstream events had already been triggered. As cytokine data became available, showing persistently high TNF levels, this decision was reviewed. In our minds, in the setting of severe lymphopenia and monocytopenia of unknown duration, treatment with high-dose corticosteroids and anti-interleukin-2 receptor antagonist antibody, and the increased risk of secondary infections in the patients, the risk of anti-TNF therapy still outweighed its potential benefit. We would be yet more cau-

tious with a cause associated with infection, such as the toxic shock syndrome.

We agree with Takita et al. Plasmapheresis was considered, but the known pharmacokinetics of a low IgG4 antibody level suggested that the drug would be tissue-bound. We also agree that there may be a role for continuous venovenous hemodiafiltration in blunting the cytokine storm associated with a severe systemic inflammatory response. Furthermore, high dialysate rates³ and high-cutoff filters⁴ have previously been suggested for use in treating sepsis. Our patients received continuous venovenous hemodiafiltration at an initial dialysate rate of 1 liter per hour, which was subsequently raised to 4 liters per hour, using standard membranes. In our opinion, aggressive multiorgan support and early institution of high-volume diafiltration and treatment with high-dose corticosteroids, combined with the physiological resilience of these six young, healthy research volunteers, were key to their survival.

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T-Cell Costimulation

TO THE EDITOR: In their Perspective article, Sharpe and Abbas (Sept. 7 issue)¹ discuss possible explanations for the adverse events associated with a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412, as reported by Suntharalingam et al.² Sharpe and Abbas suggest that a difference in affinity for CD28 between species may be important.¹ However, an alternative explanation relates to differences in Fc-receptor binding between

species, which is dependent on the isotype of the monoclonal antibody.³ It is for this reason that anti-CD3 monoclonal antibodies (signal 1) have been engineered to reduce Fc-receptor binding. This process results in less cross-linking of CD3 on the T-cell surface (through Fc-receptor binding) and consequently in little release of cytokines.⁴ TGN1412 is a human IgG4 monoclonal antibody. Previous studies in nonhuman primates have dem-