mosaicism for Hsa21, confirming that phenotypically normal neonates with hematologic findings consistent with TMD should be screened for trisomy 21 because Gamis and colleagues’ study confirms others indicating they are similarly at risk of ML-DS.1,4,10 More than 75% of infants underwent bone marrow examination. Because it is unclear what additional information was obtained, it could be argued that marrow examination is unnecessary in infants with trisomy 21 where clinical and hematologic features are typical of TMD, particularly where GATA1 mutations confirm a (pre)leukemic clone.7,8,10

An important dilemma in TMD management is identifying which infants will benefit from treatment and what treatment is most effective in the short- and long-term. Gamis and colleagues approached the first question using prospectively defined criteria for the presence of one or more life-threatening symptoms (LTS), including hepatic dysfunction, hydrops fetalis, or blast count (> 100,000/μL), as sole criteria for instituting treatment at the physician’s discretion.1 Almost half of those with LTS treated according to the guidelines (13/29) succumbed to TMD or treatment complications. By contrast, TMD resolved completely without treatment in all patients without LTS, as found previously where similar treatment guidelines were used.3 These data support the conclusion that neonates without LTS (at diagnosis or while hematologic evidence of TMD persists) can safely be monitored without treatment because their outcome is favorable.

Previous reports confirmed here show that TMD patients with high-risk features defined as organ infiltration, especially hepatic, and/or high leukocyte count (> 100,000/μL) have a mortality of more than 30%.1,3,4 Klussmann et al’s study showed that treatment of patients with high-risk features (which also included ascites, prematurity, and failure of remission of TMD), improved survival in the first months of life. Gamis and colleagues did not find improved survival of TMD patients with treatment (cytarabine 3.33 mg/kg/24 hours by continuous infusion for 5 days), perhaps because their guidelines permitted treatment for less severe TMD (a single LTS) and/or because cytarabine-related toxicity was high (96% grade 3/4 toxicity). Although the main aim of treating high-risk TMD is improvement in short-term survival, eradicating the (pre)leukemic clone(s) and consequent reduction in risk of later ML-DS is a potential long-term benefit. Unfortunately, no studies, including that of Gamis and colleagues, have yet demonstrated a significant impact of treatment on the likelihood of developing ML-DS.1,4 Thus, further studies are needed to refine treatment intervention criteria for TMD and the most effective treatment regimen for short-term and long-term benefit.

The findings of Gamis et al are nevertheless helpful for clinicians caring for neonates and children with DS and go some way to answering important clinical questions. However, many other questions remain: What is the relationship between TMD, as clinically defined, and the presence of GATA1 mutation(s)? Does the presence of GATA1 mutation(s) in the absence of typical clinical and hematologic features constitute TMD and does this carry the same risk of transformation to ML-DS? Can patients without GATA1 mutations at birth develop ML-DS, and does this share the same characteristic time window of presentation (< 5 years of age) and immunophenotypic (typically megakaryoblastic) and genetic features, characteristically seen in patients with GATA1 mutations in the neonatal period? Answers to such questions continue to fascinate all interested in Hsa21 and its many enigmatic links to leukemia.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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These events were surprising given the benign preclinical safety testing. An extensive investigation concluded that the adverse events involved no contamination or errors in the manufacturing, formulation, dilution, or administration of TGN1412. Theories explaining the disparity between the initial preclinical studies and life-threatening outcomes observed in these 6 patients began to emerge. Findlay et al suggested that the initial in vitro studies missed the potential proinflammatory outcome because of functional differences between soluble and bound antibody. The initial in vitro studies of peripheral blood mononuclear cells (PBMCs) were done in a fashion similar to stimulation using soluble OKT3. Under these conditions, no inflammatory response was elicited; however, once artificially bound to a cell-culture surface, TGN1412 reproduced a similar inflammatory response in human PBMCs. In fact, the introduction of the soluble form actually inhibited the induction of CRS. TGN1412 showed promising results in the treatment of autoimmune disease, activating and expanding polyclonal Tregs in immunocompetent mice. These mouse studies most likely failed to re-produce the dramatic inflammatory response because of the differences of host immune system and cumulative antigen exposure history between young laboratory mice and humans. In mice, natural Tregs quickly act as IL-2 sinks and given a relative paucity of memory cells are able to quench the immune response induced by TGN1412. The much larger proportion of CD4+ effector memory cells present in an antigen experience human immune system most likely overwhelmed this same immunosuppressive safeguard.

Initial dose selection may explain the rapid onset of symptoms observed in all 6 patients. For the first-in-human trial the starting dose was calculated from the no observed adverse effect level (NOAEL), determined in preclinical animal models. Simply put, the maximum dose at which no statistically significant or biologically relevant adverse event was determined, and following various correction factors, a dose of 0.1 mg/kg/d, 16 times less than the maximum safe starting dose (MSD) and 500 times less than the dose administered to macaques, was chosen. However, these calculations depend on the presence of a similar biologic effects between test animal and humans. With no recorded adverse events described, even with doses up to 50 mg/kg, the macaque model poorly represented the activity of TGN1412 in humans. Later analysis would show that human subjects were actually given a near maximum dose with in silico calculations predicting 86% to 90% CD28 receptor occupancy. Because of these findings, most trials with biologics now select first-in-human dosing using the minimum anticipated biologic effect level (MABEL), which calculates initial administration based on the dose at the lowest end of the dose-response curve.

This failure of primates to respond to TGN1412 was later traced back to differences in immune regulation between humans and macaques. Eastwood et al demonstrated that unlike other antibodies bound to the surface of lymphocytes such as rituximab or alemtuzumab, which also induce a similar but significantly less dramatic cytokine release of TNF-α and IL-8, TGN1412 is unique in that it also causes robust secretion of IL-2 and IFN-γ. This difference was attributed to the unique effect of TGN1412 acting directly on CD4+CD45RO+ effector memory T cells, the predominant source of these inflammatory cytokines. Furthermore, they demonstrated that the macaque equivalent of human CD4+ effector memory T cells actually lacks CD28 expression, unlike other antibodies bound to the surface of lymphocytes such as rituximab or alemtuzumab, which also induce a similar but significantly less dramatic cytokine release of TNF-α and IL-8, TGN1412 is unique in that it also causes robust secretion of IL-2 and IFN-γ. This difference was attributed to the unique effect of TGN1412 acting directly on CD4+CD45RO+ effector memory T cells, the predominant source of these inflammatory cytokines. Furthermore, they demonstrated that the macaque equivalent of human CD4+ effector memory T cells actually lacks CD28 expression, thereby preventing CD28 superagonist effects. Other species specific effects of CD4+ T-cell differentiation have been uncovered in nonhuman primates, which explains in part the relative pathogenicity of HIV and SIV infections.

One last question remains as to how TGN1412 led to cytokine storm given that its effects on whole blood and PBMCs could not be reproduced in vitro unless antibody was artificially immobilized on a plastic surface. Romer et al may now have an explanation. They have shown that monocytes and T cells up-regulate functional activity during high-density culture mediated by adhesion-mediated up-regulation of TCR signal sensitivity as well as TCR priming by surface scanning for MHC class I and II molecules. Tissue-resident CD4+ effector memory T cells, which comprise upwards of 90% of the body’s lymphocytes, are therefore able to immediately respond to TGN1412 with cytokine release. This is supported by their work showing that lymph node cells, but not PBMCs, are able to mount a response to soluble TGN1412. This response was further
attributed to CD4⁺CD45RO⁺ cells and declined if cells were allowed to dissociate. Collectively, these data demonstrate the importance of appropriate preclinical modeling that adequately predicts lymphocyte biology. There are several other implications from this work by Römer and colleagues. First, most studies of lymphocyte signal transduction are conducted with cells at low density, and thus may not represent in vivo signaling, particularly in the case of cosignaling through CD28. One of the “dirty little secrets” of immunology is that lymphocyte cloning is often superior in round-bottom rather than flat-bottom vessels. Second, studies with large-scale lymphocyte cultures indicate improved growth at high density rather than low density. Finally, these studies imply that the peripheral blood T-cell compartments is likely a “safe haven” in that the T cells are hyporesponsive compared with their tissue-bound counterparts, and this may provide some protection to superantigen-induced CRS, which is dependent on CD28.

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Comment on Tacken et al, page 6836

Antibody co-targeting of DCs

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The 2011 Nobel Prize in Physiology or Medicine was awarded to Ralph M. Steinman, Jules Hoffman, and Bruce Beutler for their discovery of dendritic cells (DCs) and Toll-like receptors (TLRs). Their research paved the way for harnessing DCs to create vaccines.

An effective vaccine contains immunodominant antigens and adjuvants that are processed by in vivo DCs to elicit humoral and cellular (effector and memory) immunity. Uncoupling DCs and their adjuvants via route or scheduling of administration may reduce or eliminate the desired immune response. In this issue of Blood, Tacken et al show for the first time that in vivo DC vaccination benefits from co-targeting antigen with adjuvants via nanometer sized particulate carriers (see figure).

Steinman described DCs as the mandatory component linking antigen and immunogenicity. Early clinical trials demonstrated the safety and immunogenicity of autologous DCs loaded with exogenous antigens, yet revealed a defect in the migratory pathways of these cells and inconsistent clinical benefit against cancer. The recent FDA approval of Sipuleucel-T (Dendreon) admixing antigen presenting cells exposed to a fusion protein (composed of a prostate specific antigen and GM-CSF) to autologous lymphocytes in prostate cancer rekindled the interest of harnessing DCs for vaccine development. However, a more direct strategy using DCs residing in lymphoid organs has been investigated over the past 10 years.

The great potential for targeting antigen presenting cells (APCs) with antibody–opsonized antigens to induce humoral immune responses was brought up decades ago using anti-FcγR or MHC antibodies. Lately, the identification of DC subsets and their specific surface receptors as well as recent advances in understanding antigen capture, processing, and presentation have stimulated research on DC targeting in vivo. The quality of the immune response elicited by in vivo DC vaccination will depend on the expression pattern and biologic properties of the targeted receptor, the activation status of the DCs, and the formulation of the antigen. Numerous studies performed in
Predicting cytokine storms: it's about density
Matthew J. Frigault and Carl H. June