equivalent CR rates; however, no superiority in OS has been shown. Concerns about toxicity of high-dose (90 mg/m²) daunorubicin, the wide use of the 60-mg/m² dose as a newer “standard,” and similar remission rates of the 60-mg/m² dose led the UK NCRI AML Study Group to compare the 60- vs 90-mg/m² doses in induction in a prospective randomized trial.

At first glance, the conclusions from the study were that there is no benefit to the higher daunorubicin dosing, nor was there a subgroup where the higher dose benefited patients. However, one needs to look beyond the simple calculation of anthracycline dose in the first induction and consider the total dose of anthracycline given over several courses. Compared with the ECOG, the Dutch-Belgian Cooperative Trial Group for Hematology Oncology (HOVON), and Korean trials, where a total of 270 mg/m² of daunorubicin was given to a majority of patients, in this trial, the total dose of daunorubicin was 330 mg/m² for the 60-mg/m² group and 420 mg/m² for the 90-mg/m² group. Therefore, the total anthracycline in the lower-dose arm was slightly higher than the high-dose arms in the 3 randomized trials. There were also differences in the dose schedules of anthracycline, where pharmacokinetics and pharmacodynamics could have impacted leukemic cell exposure and outcomes.

The UK NCRI AML17 trial brings to light that the true benefit of anthracycline in AML may be in a defined therapeutic window. A threshold level is required for benefit in CR rates, but further dose escalation of the drug may not significantly improve, and may even worsen, outcomes. CR rates were similar in both arms, with no difference in mortality at 30 days. However, in addition to the lack of benefit of the higher dose, the trial was stopped prematurely because of the higher 60-day mortality noted in the 90-mg/m² arm. Perhaps this study defines the point of diminishing returns where the benefit of increasing anthracycline begins to erode.

The final factor in this trial is the short follow-up of the UK NCRI AML17 study group. The other published comparative trials had a much longer follow-up. Moreover, with time, certain interactions have become more evident. For example, with an 80-month follow-up in the ECOG trial, FLT3–ITD–positive AML patients did benefit from a higher dose of anthracycline. In the UK NCRI AML17 trial, there was a trend to a higher-dose daunorubicin benefit in the FLT3-positive patients. Longer follow-up is required to determine whether it becomes significant.

The appealing factor here for the UK NCRI AML17 study is that the lower dose of daunorubicin may allow for a third targeting drug to be more easily inserted into the regimen to treat FLT3-positive, core binding factor–positive, or other subgroups of AML patients. The foundation of therapy for this disease has been available for 40 years. Collectively, we are optimizing the appropriate dosing in induction. In this and future studies, these variations on the theme need to be perused and not just given a quick glance.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES
1. Burnett AK, Russell NH, Hills RK, et al, on behalf of the UK NCRI AML Study Group. A randomized comparison of daunorubicin 90 mg/m² vs 60 mg/m² in AML induction: results from the UK NCRI AML17 trial in 1206 patients. Blood. 2015;125(25):3878-3885.

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Comment on Goettel et al, page 3886

Daring to learn from humanized mice

Silke Paust and Matthew Bettini BAYLOR COLLEGE OF MEDICINE

In this issue of Blood, Goettel and colleagues introduce a novel humanized mouse model of immune dysregulation, polyclonallymphopenia, enteropathy, X-linked (IPEX) syndrome, whereby mice lacking murine major histocompatibility complex class II (MHC II) and expressing human HLA-DR1 (NOD. Prkdcr2Id2rry/– H2–Ab1–/–; NSGAb°DR1) are reconstituted with hematopoietic stem cells (HSCs) from a patient with IPEX syndrome to generate a humanized model for primary immune deficiency presenting as fatal autoimmunity.

“Humanized mice” allow the study of human immune cells in the context of human diseases for the evaluation of organ-specific pathologies for which human samples may not be available or accessible, and for the assessment of experimental approaches that may be harmful when performed in patients or human volunteers. Thus,
humanized mice have become a valuable translational research tool for the study of pathogen transmission, immunopathogenesis, viral latency, pathogen evolution, immune escape, prevention therapy assessments, and vaccine development. Humanization of tissues in mice is usually achieved by the transplant of human tissues and/or HSCs into lymphopenic hosts. The resulting cohort of humanized mice is genetically identical (individual members within a mouse strain are genetically identical due to a century of sibling inbreeding), and several dozen humanized mice can be generated from 1 human donor. Now, adoptive transfers of isolated human lymphocytes can be performed without rejection by the recipient immune system, and antibody-mediated in vivo lymphocyte depletion enables the assessment of the importance of individual immune cell types or pathways.

Several sophisticated humanized mouse models that differ in host genetics and/or their reconstitution methods have been established, including (1) mice that allow the evaluation of functional human innate and adaptive immune responses to infection and/or vaccination; (2) mice that enable the humanization of hepatocytes and the study of hepatotropic pathogens such as malaria parasites or hepatitis viruses; and (3) germfree mice, in which a human microbiome has been established by oral fecal transplant. However, the recapitulation of IPEX within mice using HSCs from IPEX patients has not been reported. In this issue, Goettel and colleagues successfully transplanted CD34+ cells into a NOD.Scid/Il2Rγ−/− (NSG) host that expresses the human MHC II HLA-DR1 (NSGAb°DR1), in the absence of murine MHC II (HLA-DR1-NSG; see figure). The lack of murine MHC II presumably allows for the full maturation of T and B cells and promotes T-cell help to B cells and full immune function. Indeed, Goettel and colleagues demonstrate that when CD34+ cells are engrafted into the HLA-DR1–expressing NSG mice, there is a significant improvement in both mature B- and T-lymphocyte development and function. Importantly, the HLA-DR1–expressing mice are able to develop delayed-type hypersensitivity, exhibiting increased IgG and IgE levels. To test whether this improved adaptive immune response could recapitulate a spontaneous and defined immunologic disorder, Goettel and colleagues transplanted HSCs from an IPEX patient.Remarkably, nearly all of the IPEX (DR1) (NSGAb°DR1) mice succumbed to multiorgan inflammation due to autoantibody production and the lack of a functional Treg compartment (see figure). It is important to note that IPEX(NSG) mice without the HLA-DR1–expressing allele displayed an increase in serum antibodies and an overall absolute increase in T-cell numbers.

Therefore, the immunopathology of patients with IPEX syndrome is swift, often presenting between 1 and 3 months of age, and includes autoimmune enteropathy; endocrinopathy; eczematous dermatitis, which manifests as excessive cytokine production; elevated immunoglobulin (Ig)E levels; and chronic inflammation, leading to death. The various mutations within FOXP3 can lead to multiple functional deficits, including a loss of transcriptional repression, messenger RNA stability, DNA binding, and FOXP3 dimerization. Goettel et al have significantly advanced the study of IPEX syndrome in vivo and, potentially, any hematopoesis-derived disease, in their newly generated NSGAb°DR1 mouse model.

Mice that carry the scurfy mutation have defective FOXP3 that closely resembles IPEX syndrome, including widespread autoimmunity and high quantities of lymphocyte infiltrates within the lung, pancreas, stomach, skin, liver, and gut. However, the recapitulation of IPEX within mice using HSCs from IPEX patients has not been reported. In this issue, Goettel and colleagues successfully transplanted CD34+ cells into an NOD.Scid/Il2Rγ−/− (NSG) host that expresses the human MHC II HLA-DR1 (NSGAb°DR1), in the absence of murine MHC II (HLA-DR1-NSG; see figure). The lack of murine MHC II presumably allows for the full maturation of T and B cells and promotes T-cell help to B cells and full immune function. Indeed, Goettel and colleagues demonstrate that when CD34+ cells are engrafted into the HLA-DR1–expressing NSG mice, there is a significant improvement in both mature B- and T-lymphocyte development and function. Importantly, the HLA-DR1–expressing mice are able to develop delayed-type hypersensitivity, exhibiting increased IgG and IgE levels. To test whether this improved adaptive immune response could recapitulate a spontaneous and defined immunologic disorder, Goettel and colleagues transplanted HSCs from an IPEX patient. Remarkably, nearly all of the IPEX (DR1) (NSGAb°DR1) mice succumbed to multiorgan inflammation due to autoantibody production and the lack of a functional Treg compartment (see figure). It is important to note that IPEX(NSG) mice without the HLA-DR1–expressing allele displayed an increase in serum antibodies and an overall absolute increase in T-cell numbers.

### Table: Generation of the HLA-DR1-NSG humanized IPEX syndrome mouse models

<table>
<thead>
<tr>
<th>Mouse genotype</th>
<th>Humanization protocol</th>
<th>Immunological and clinical phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NSGAb°DR1 (control)</td>
<td>None</td>
<td>No human immune cells present, No murine lymphocytes present, Clinical disease (autoimmunity): No</td>
</tr>
<tr>
<td>5. FOXP3+ (spontaneous mutation) IPEX mouse model</td>
<td>None</td>
<td>No human immune cells present, Murine lymphocytes are present, Functional murine Treg cells: No, Autoimmune murine T cells: Yes, Murine TCR diversity: High, Murine autoantibodies: Yes, Clinical disease (autoimmunity): Yes</td>
</tr>
</tbody>
</table>

Overview of the generation of the HLA-DR1-NSG humanized IPEX syndrome mouse models, appropriate controls, and disease phenotypes. TCR, T-cell receptor.
Mechanism of enhanced eosinophil survival in inflammation

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In this issue of Blood, Schwartz et al identify the nuclear factor (NF)-κB/Bcl-2 pathway as critical for regulation of eosinophil survival in inflammatory conditions.1

Cell death is essential for many physiologic processes, and its dysregulation characterizes numerous human diseases. This is especially true for eosinophils, because these cells do not undergo substantial extramedullary hematopoiesis, yet their tissue levels can be markedly and selectively increased by a combination of recruitment from the blood and regulation of their cell death within tissues. The field of eosinophil survival has come a long way since the original finding that human blood–derived eosinophils had prolonged survival (>14 days) when cocultured with endothelial cells and with the subsequent identification of interleukin (IL)-5, granulocyte macrophage–colony-stimulating factor (GM-CSF), and IL-3 as key eosinophil survival factors.2

It is now appreciated that these eosinophil-directed hematopoietins inhibit eosinophil apoptosis and that IL-5 has a special capacity to promote eosinophil development, which has led to several therapeutics that lower eosinophils by blocking IL-5, such as mepolizumab and reslizumab, which are at advanced stages of development.3

However, whether eosinophils are better “dead or alive” is still an unresolved question. Simplistically, both living and dead eosinophils can lead to positive and negative outcomes (see figure). For instance, eosinophils are homeostatically present in some tissues with no ill effect (figure panel A, upper left)4 and have been shown to contribute to a variety of homeostatic functions, including production of secretory immunoglobulin A in the intestine.5 Yet during many eosinophilic inflammatory diseases, eosinophils display an activated phenotype and likely induce tissue damage (figure panel A, upper right).6 Eosinophil cell death was previously viewed dichotomously, as either noninflammatory apoptotic (figure panel A, lower left) or proinflammatory necrotic (figure panel A, lower right) cell death, but recent studies have identified additional cell death pathways, including regulated necrosis.
Daring to learn from humanized mice

Silke Paust and Matthew Bettini