Finally, the authors performed several sensitivity analyses, restricting their main analyses to those patients aged 70 years or older (no change in effect) and to those with evidence of more advanced disease (the benefit of adding rituximab was lost).

As with all large retrospective database analyses, the study has important limitations. First, SEER-Medicare provides no data with respect to patient experiences, provider documentation, or test results, and the authors thus had to rely entirely on claims data, which can be incomplete. Moreover, treatment side effects are not measured in SEER-Medicare, but must be inferred from claims.[6] Second, SEER and SEER-Medicare are only able to capture CLL diagnoses that are reported to member registries, and diagnosis made in a private physician's office may be less likely to be reported. This issue has been a possible source of underascertainment for CLL in other large registries,[7] reflecting what might ironically represent these registries' own lack of real-world applicability.

On the other hand, one benefit of such large database analyses is that in addition to providing insight regarding the generalizability of clinical trial data, they can also help policymakers understand current patterns of care. For example, the authors found that the use of rituximab for CLL treatment increased from ~ 11% of patients in 2000 to 43% in 2005—a finding with obvious implications for health care utilization and funding. The authors also found that the likelihood of initial infused therapy for CLL was decreased in certain race-ethnic groups and in females. The first finding is of interest given a recent report of lower treatment rates for blacks versus whites for diffuse large B-cell lymphoma,[8] and suggests that race-ethnic disparities observed in the treatment of patients with solid tumors[9] may exist for hematologic malignancies as well. In addition, although there are gender differences in the incidence and outcomes of CLL,[10] it is unclear why, when matched for other covariates, women would be less likely to be treated with infused therapy. This finding is especially interesting given recent data demonstrating that women with CLL are likely to experience longer diagnostic delays.[11]

A final and significant feature of Danese and colleagues' work is the measured, careful way in which the authors report their results, even more important given that 2 of them are affiliated with the company that makes rituximab. Just as data from clinical trials imperfectly inform the best treatment strategy for an individual patient, data from heath services analyses must also be interpreted with caution. In this case, beyond the limitations of the SEER-Medicare dataset itself, the authors appropriately underscore the possibility of biases because of unobserved differences in patients who received different treatments, even after attempts to control for these differences. Despite these limitations, in a time when a drive toward truly understanding real-world comparative effectiveness has captured the nation and its policymakers, it is exciting to see such an analysis published in *Blood*. There will surely be more to come.

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● ● ● IMMUNOBIOLOGY

Comment on Grzywacz et al, page 3548

Plasticity of NK-cell differentiation

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In this issue of *Blood*, Grzywacz and colleagues provide in vitro experimental evidence that human natural killer (NK) lymphocytes may derive from myeloid precursors and that microenvironmental factors such as stromal cells and hydrocortisone (HDC) may play a key role in favoring such differentiation.[1]

In the past years the model of hematopoiesis has been revised. The statement that an early dicothomy between myeloid cell fate and lymphoid cell fate occurs during hematopoiesis, has been disclaimed both in mice and humans.[2,3] Current models of hematopoiesis suggest that hematopoietic stem cells (HSCs) may commit early to the erythroid/platelet lineages or to leukocyte lineages. However, once committed to the leukocyte lineage, hematopoietic precursors retain a high degree of plasticity. It has been conclusively shown that human multilymphoid progenitors (MLPs) retain myeloid differentiation potential.[3] The choice to terminally differentiate toward myeloid or lymphoid lineages would then depend on the role of lineage-specific transcription factors and on a permissive microenvironment.[2,3]

NK cells are lymphoid cells belonging to natural immunity. They play a dual role as effector cells and regulatory cells. NK cells are able to interact both with cells of the innate and of the acquired immune response.[4] They share common precursors with dendritic cells (DCs) and T lymphocytes,[5] and it has been proposed that they share precursors also with monocytes.[3,6]

Grzywacz et al confirm previous reports,[5-7] showing that stromal cells or HDCs, used alone or in combination, may greatly increase the in vitro NK-cell differentiation from

$CD34^+CD38^{-/+}$ cells in the presence of appropriate cytokines. More importantly, they demonstrate, at single-cell level, that common myeloid precursors (CMPs) and granulocyte-monocyte precursors (GMPs) retain the ability to differentiate into functional mature NK cells. They show that NK lineage maintenance is conserved until late steps of monocyte development, and that only the combined use of stromal cells and HDCs can efficiently recruit CMP and GMP cells to drive their differentiation toward NK cells.[1] Only in cases of bright expression of myeloid surface markers (CD13) or high concentrations of macrophage colony-stimulating factor (M-CSF) growth factor, myeloid precursors irreversibly differentiate toward monocytes.[1] These results imply that the microenvironment may play a key role. The presence of soluble factors (cytokines and/or hormones) and of peculiar stromal cells expressing NOTCH ligands[8] or membrane-bound interleukin-15[7] would make the difference in $CD34^+$ precursors differentiation. Notably, these factors/cells have been shown to play a crucial role also in NK-cell differentiation from NK-committed $CD34^+$ precursors present in human secondary lymphoid organs and human decidua.[5,9]

Grzywacz and colleagues suggest that the positive effect of the combined use of HDCs and stroma may induce up-regulation of the WNT pathway leading to the activation of the TCF/LEF transcription pathway, known to be involved in lymphocyte development.[1] However, previous reports suggested that corticosteroids could induce NK-cell differentiation from $CD33^+$ ($CD14^{+/-}$) myeloid cells even in the absence of stromal cells.[6,10] In particular, pharmacologic concentrations of Methylprednisolone induced differentiation of $CD33^+$ cell precursors toward NK cells.[10] These results suggest that corticosteroids may support NK-cell differentiation from myeloid precursors, possibly exerting a direct effect on these cells. In this context, it would be interesting to evaluate whether corticosteroids could be involved in the up-regulation of the expression of transcription factors such as E4BP4, shown to be involved NK-cell development.[11]

NK cells develop in a step-by-step process, characterized by phenotypically identified stages. They first acquire CD161, 2B4, CD56, and CD94/NKG2A surface molecules. The acquisition of LFA-1, NKp46, NKp30, NKG2D, and DNAM-1 activating receptors correlates with the gradual acquisition of cytotoxicity. The expression of CD16 and KIR occurs at later stages and is hardly observed in vitro.[5]

Grzywacz et al show that NK cells derived from $M\text{-}CSFR^+$ myeloid progenitors express higher percentages of $CD16^+KIR^+$ cells and display higher cytolytic activity against B-Epstein-Barr virus (B-EBV) cell lines than NK cells derived from more immature $M\text{-}CSFR^-$ progenitors. These results suggest that NK cells derived from $M\text{-}CSFR^+$ progenitors may differentiate more rapidly or follow an alternative differentiation pathway compared with NK cells derived from $M\text{-}CFR^-$ progenitors. In this context, it may be relevant to analyze the 2B4 receptor function. 2B4 acts as inhibitory rather activating receptor in immature NK-cell precursors, because of the lack of the cytoplasmic adaptor protein called signaling lymphocytic activation molecule–associated protein (SAP) that is not expressed at early stages of differentiation.[12] The inhibitory activity of 2B4 impairs the NK cell–mediated lysis of B-EBV cell lines. Thus, cytolytic NK cells derived from $M\text{-}CFR^+$ progenitors could display an activating 2B4 in agreement with their more advanced stage of differentiation. Conversely, $M\text{-}CSFR^-$ progenitor-derived NK cells could still express an inhibitory 2B4 receptor because of the lack of SAP.

The possibility that myeloid precursors, in the presence of stromal cells and HDCs, could generate more rapidly functional NK cells also offers important clues to several clinical observations. It is conceivable that NK cells, present in high percentages in peripheral blood of patients at the earliest time intervals after HSC transplantation may derive, at least in part, from myeloid precursors. NK cells have been shown to exert an important role in preventing leukemic relapses after transplantation,[13] and an increasing number of clinical trials are assessing the efficacy of NK cell–mediated immunotherapy. In this context, the study by Grzywacz and colleagues offers new insights in the physiology of the NK-cell development and underlines the need for further investigation on the effect exerted by conditioning regimens and corticosteroids on the myeloid/NK-cell differentiation pathways after HSC transplantation.

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