7. Hirahara N, Ito Y, Sasaki S, et al. Inoculation of NF1-related syndrome whose phenotype closely resembles that of NF-1. All of these conditions are secondary to germline mutations in genes encoding components of the Ras mitogen-activated protein kinase (MAPK) pathway. Somatic mutations in some of these genes play a fundamental role in the pathogenesis of juvenile myelomonocytic leukemia (JMML), as we and others have shown for PTPN11, KRAS, and NF1.
8. SPRED1 encodes a protein that is highly expressed in hematopoietic cells and negatively regulates Ras-MAPK signaling by suppressing Raf activation. The phenotypic similarity of the SPRED1 syndrome to NF-1, the fact that the NF1 gene product is a negative regulator of Ras-MAPK, the observation that children with NF-1 are at highly increased risk of JMML, and the recent letter by Pasman et al (exemplifying a link between SPRED1 disease and myeloid leukemia in children) led us to hypothesize that somatic mutations in SPRED1 might occur in cases of JMML that lack mutations in other JMML-associated Ras-related genes.
9. To test this possibility, we sequenced the SPRED1 gene in granulocyte DNA from 23 JMML patients without mutations in PTPN11, KRAS/NRAS, or CBL and without an NF-1 phenotype. All children were enrolled in the European Working Group of MDS in Childhood (EWOG-MDS) studies 98 or 2006. Approval was obtained from the Freiburg University institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki. The analyses comprised the entire coding sequence of SPRED1. No mutations were discovered in the 23 JMML samples, whereas 2 known exonic synonymous single nucleotide polymorphisms, c.291 G>A and c.1044 T>C, were identified in all 23 cases. At position c.291 the genotype was G/A in 4 cases and A/A in 19 cases, whereas the variation c.1044 T>C showed the genotype C/T in 5 cases and C/C in 18 cases. These findings are in accordance with documented allele frequencies in whites. We also reproduced the known intronic polymorphisms c.424-18 C>T and c.424-8 C>T (and the absence of splicing mutations c.50_684 delTTAA/insTTA GTAAA, c.50_684 delTTAA/insTTA GTAAA, and c.50_684 delTTAA/insTTA GTAAA, were found in 5 JMML specimens. In all 5 instances the variations were linked on one allele and the other allele retained wild-type sequence.

In summary, we found no evidence of leukemogenic SPRED1 involvement in JMML cases negative for mutations in PTPN11, KRAS/NRAS, or CBL and without NF-1 features. The assumption put forward by Dr Pasman and colleagues, that germline SPRED1 mutations predispose children to leukemia, is certainly plausible. However, the absence of SPRED1 mutations in an early childhood leukemia such as JMML indicates that the putative link between SPRED1 lesions and childhood myeloid malignancies requires further clarification.

To the editor:

**Does SPRED1 contribute to leukemogenesis in juvenile myelomonocytic leukemia (JMML)?**

Dr Pasman and colleagues recently reported a child with a neuro-cardio-facial-cutaneous (NCFC) syndrome caused by a germline SPRED1 mutation. The child developed acute myeloid leukemia. The relation between the molecular pathology of NCFC syndromes and that of myeloid malignancies in children is well-documented. NCFC syndromes include Costello, Noonan, LEOPARD, and cardiofaciocutaneous syndromes. The analyses comprised the entire coding sequence of SPRED1. Somatic mutations in some of these genes play a fundamental role in the pathogenesis of juvenile myelomonocytic leukemia (JMML), as we and others have shown for PTPN11, KRAS, and NF1.

SPRED1 encodes a protein that is highly expressed in hematopoietic cells and negatively regulates Ras-MAPK signaling by suppressing Raf activation. The phenotypic similarity of the SPRED1 syndrome to NF-1, the fact that the NF1 gene product is a negative regulator of Ras-MAPK, the observation that children with NF-1 are at highly increased risk of JMML, and the recent letter by Pasman et al (exemplifying a link between SPRED1 disease and myeloid leukemia in children) led us to hypothesize that somatic mutations in SPRED1 might occur in cases of JMML that lack mutations in other JMML-associated Ras-related genes.

To test this possibility, we sequenced the SPRED1 gene in granulocyte DNA from 23 JMML patients without mutations in PTPN11, KRAS/NRAS, or CBL and without an NF-1 phenotype. All children were enrolled in the European Working Group of MDS in Childhood (EWOG-MDS) studies 98 or 2006. Approval was obtained from the Freiburg University institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki. The analyses comprised the entire coding sequence of SPRED1. No mutations were discovered in the 23 JMML samples, whereas 2 known exonic synonymous single nucleotide polymorphisms, c.291 G>A and c.1044 T>C, were identified in all 23 cases. At position c.291 the genotype was G/A in 4 cases and A/A in 19 cases, whereas the variation c.1044 T>C showed the genotype C/T in 5 cases and C/C in 18 cases. These findings are in accordance with documented allele frequencies in whites. We also reproduced the known intronic polymorphisms c.424-18 G>A (G/G, 12 cases; A/A, 2 cases; A/G, 9 cases) and c.424-8 C>T (C/A, 6 cases; A/A, 17 cases). In addition, 3 known intronic polymorphisms, c.684 +49_684 +50 insTTAA/---, c.684 +50_684 +51 insT/-- and c.684 +53_684 +54 insT/TA, were found in 5 JMML specimens. In all 5 instances the variations were linked on one allele and the other allele retained wild-type sequence.

In summary, we found no evidence of leukemogenic SPRED1 involvement in JMML cases negative for mutations in PTPN11, KRAS/NRAS, or CBL and without NF-1 features. The assumption put forward by Dr Pasman and colleagues, that germline SPRED1 mutations predispose children to leukemia, is certainly plausible. However, the absence of SPRED1 mutations in an early childhood leukemia such as JMML indicates that the putative link between SPRED1 lesions and childhood myeloid malignancies requires further clarification.

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To the editor:

B cells in GVHD: friend or foe?

We were pleased to read the recent review by Shimabukuro-Vornhagen et al, “The role of B cells in the pathogenesis of graft-versus-host disease,” which highlights the importance of B cells after bone marrow transplantation, as B cells have tended to be overlooked as a contributor to transplantation immunology. This review comprehensively describes the use of the humanized chimeric CD20 monoclonal antibody, rituximab, for the prophylaxis and treatment of acute and steroid-refractory chronic graft-versus-host disease (GVHD). Three key observations have been made: (1) the use of rituximab as part of pretransplantation conditioning results in the in vivo depletion of donor B cells after transplantation; (2) pretransplantation rituximab is associated with reduced incidence and severity of acute GVHD in a cohort of patients; and (3) elevated B-cell numbers in donor grafts are associated with the development of both acute and chronic GVHD. Whether B-cell depletion per se is the mechanism underlying reduced GVHD rates, and whether this reflects a direct role of B cells in stimulating allogeneic T-cell expansion and effector function remains unknown.

It is important to also consider the potential non-specific effects of rituximab therapy on the activation of allogeneic T cells. Nonspecific IgG treatment, such as high-dose intravenous immune globulin, can inhibit interferon-γ (IFN-γ) responses in macrophages via a FcγRIII-dependent mechanism, and induce natural killer cell–mediated antibody-dependent cellular cytotoxicity of dendritic cells (DCs).2,3 Apoptotic lymphocytes also have a regulatory effect upon DCs, by down-regulating costimulatory molecules and inducing the production of the immunosuppressive cytokine interleukin-10 (IL-10).4,5 In GVHD, these events stimulate the generation and proliferation of regulatory T cells (Tregs), thus suppressing allogeneic T-cell activation.

Both T and B lymphocytes play a role in tolerance induction to autoantigens, whereby CD4+ T cells regulate early allogeneic T-cell activation and expansion, and B cells control their differentiation into effector T cells.6 Host B cells have also been shown to play a protective role in GVHD, via the secretion of IL-10 after total body irradiation, thus inhibiting allogeneic T-cell expansion and subsequent acute GVHD induction.7 Donor B cells can also inhibit acute GVHD in a major histocompatibility complex class II- and Treg-dependent manner. Mice receiving BM from B-cell-deficient mutant mice (B6.µ.MT) developed rapid-onset acute GVHD, contributed by faster donor CD8+ T-cell engraftment and production of IL-2 and IFN-γ (J.E.D., V. Watt, and D.R.S., manuscript in preparation), and indirect alloantigen presentation to CD4+ T cells.8 This is supported by recent in vitro human data, indicating that activated B cells directly suppress allogeneic CD4+ T-cell proliferation through the expansion of alloantigen-specific suppressor Tregs.9

While clinical observations indicate that rituximab has a beneficial effect in the prophylaxis of acute GVHD, it is worth considering that an alternate mechanism of its action may exist over and above simple B-cell depletion. Furthermore, the potential benefit of regulatory B cells may be lost if rituximab is adopted wholesale into pretransplantation conditioning regimens.

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