Neither of these malignancies has been clearly explained. Excesses of non-Hodgkin lymphoma, because they account for 3% of all cancer deaths, and, to a lesser extent, melanoma, because of the synergistic effect of procarbazine-containing chemotherapy and radiotherapy on stomach cancer, whereas breast cancer risks are reduced with cytotoxic chemotherapy due to its impact on ovarian function. Little is known about the spectrum of subsequent malignancy risks with current chemotherapy regimens such as ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine). Although the striking chemotherapy-related risks of acute myeloid leukemia are well established, these malignancies tend to occur relatively quickly following exposure, and thus they accounted for few second or third malignancies in the Dutch cohort, which was restricted to 5-year survivors.

Are some survivors particularly susceptible to developing subsequent malignancies? The observation of van Eggermond et al.1 that patients who develop a second malignancy have higher risk for developing third and additional malignancies could merely reflect differences in treatment exposures, which were not accounted for in the analysis. However, they did report higher subsequent malignancy risks for HL survivors treated at a younger age. One explanation may be increased radiosensitivity of developing tissues, particularly the breast. Alternatively, host susceptibility such as inherited genetic variation or HL-related immune dysfunction may contribute, independently or as a modifier of treatment-related risks. Indeed, a small genome-wide association study identified a susceptibility locus at chromosome 6q21 related to second cancers among HL survivors treated with radiotherapy. Those results support the importance of larger-scale studies of genetic susceptibility to subsequent malignancies, such as ongoing efforts in the Childhood Cancer Survivor Study. Additional investigation also is warranted to understand whether underlying immune dysfunction may predispose certain HL survivors to developing additional immune-related malignancies, which could explain excesses of non-Hodgkin lymphoma and, to a lesser extent, melanoma, because neither of these malignancies has been clearly related to radiation or chemotherapy exposures.

What is the impact of this research on long-term follow-up practices for HL survivors? Current screening protocols target surveillance based on survivors’ treatment exposures.

Breast cancer screening following chest radiotherapy is the most accepted. However, the risks and benefits of such screening for specific groups of survivors are not well understood, and screening for other malignancies such as lung or gastrointestinal tract cancers is even less established. The data from the Dutch cohort indicate that subsequent malignancy risks persist, and may even be higher, after the diagnosis of a second malignancy. Thus, survivors of second malignancies could realize even greater benefits from increased surveillance. These results are consistent with a previous report from the Childhood Cancer Survivor Study demonstrating particularly high subsequent malignancy risks among survivors who developed non-melanoma skin cancer.10

Unfortunately, cancer registry–based studies such as the current Dutch study do not systematically ascertain the occurrence of non-melanoma skin cancer and thus cannot confirm this finding. Plausibly, a non-neoplastic adverse outcome also could serve as a marker for identifying patients at high risk of developing subsequent malignancies if the outcomes share an underlying pathogenesis (eg, skin erythema or subcutaneous fibrosis, which could reflect underlying susceptibility to radiation-induced tissue damage). Future research should consider a range of adverse outcomes to further explore this possibility.

Because of their favorable prognosis, frequent diagnosis at a young age, and common receipt of cytotoxic chemotherapy and/or radiotherapy, HL survivors have long been harbingers of the late effects of treatments that also may affect other cancer survivors. The data from van Eggermond et al. are a valuable addition to the sparse literature on the occurrence of multiple subsequent neoplasms, and their findings remind us of the importance of continued efforts to reduce the late effects of treatment so that cancer survivors are not faced with double—or triple—jeopardy.

**REFERENCES**


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**Comment on Frankel et al., page 385**

**Targeted diphtheria toxin to treat BPDCN**

**David J. FitzGerald**  
**National Institutes of Health**

In this issue of *Blood*, Frankel et al describe a novel treatment of blastic plasmacytoid dendritic cell neoplasm (BPDCN) using an engineered version of diphtheria toxin that is targeted to malignant cells via a fusion with interleukin (IL)3 (see panel A). BPDCN is a rare hematological malignancy (known previously as blastic natural killer lymphoma or natural killer cell leukemia and other similar names) that commonly presents in the skin and progresses to a leukemic phase. Typically, malignant blasts are CD4+, CD56+,...
A

Diphtheria toxin domains

B

Diphtheria toxin-IL3 (SL-401)

(A) The fusion of IL3 at the C terminus of the truncated diphtheria toxin. The binding domain of diphtheria toxin, located at the C terminus, is deleted and replaced by IL3. Thus, the cytotoxicity of the fusion protein is targeted to cells expressing IL3 receptors (CD123). The domain names are as follows: A, active enzyme domain; T, translocation domain; B, binding domain. The binding domain is removed and replaced by IL3 to form DT-IL3, which is called SL-401 in Frankel et al. (B) The pathway of binding, entry, and killing by DT-IL3. DT-IL3 binds CD123 and is internalized, and the A chain is processed proteolytically to release the A chain. The A chain translocates from acidic endosomes to the cytosol. In the cytosol, the A chain ADP-ribosylates EF2 and inhibits protein synthesis. Cells die because they cannot make new protein.

and CD123. In a small phase 1-2 study, the authors report a high rate of complete remissions in patients diagnosed with BPDCN and receiving diphtheria toxin (DT)-IL3 as a single agent. This is a remarkable achievement. Apparently, malignant cells expressing IL3 receptors (CD123 is the α subunit of the IL3 receptor) bind and internalize the DT-IL3 fusion protein, leading to inhibition of protein synthesis and cell death (see panel B). In their paper, DT-IL3 is called SL-401.

In a landmark paper, >30 years ago, Thorpe et al suggested that DT could be engineered to kill leukemia cells. Frankel et al fulfill that promise and produced a functional example of a DT fusion protein that demonstrates clear clinical benefit for patients with a hematological malignancy. The results of more advanced trials in the BPDCN population and treated with DT-IL3 are now eagerly awaited. Further, because these results were achieved with a single agent, future studies will undoubtedly strive to identify suitable agents to combine with DT-IL3 and improve its efficacy.

The use of protein toxins such as diphtheria toxin, Pseudomonas exotoxin, and ricin to kill malignant cells is particularly attractive because of the potency associated with the enzyme domains of these toxins. The targeting of protein toxins (antibody-toxin chimeric proteins are frequently termed “immunotoxins”) was reviewed recently by Wayne et al, especially as it relates to the treatment of leukemia. In sum, protein toxins are not mutagenic, not subject to common pathways of drug resistance, and can be engineered easily into fusions or conjugates with cancer-binding antibodies or cytokines. The Achilles’ heel of toxin-based proteins is their immunogenicity. When given to patients with hematologic malignancies, several cycles of treatment can sometimes be administered if there is disease-induced immunosuppression or prior chemotherapies. However, in this study, Frankel et al remark on the problematic situation of prior DT vaccinations that apparently prime patient antibody memory responses and limit effective treatment to 1 cycle. In light of this, it should be noted that efforts to remove epitopes from protein toxins or quell antibody responses to toxins are being pursued in both preclinical and clinical settings and may ultimately allow multiple treatment cycles with toxin-based therapeutics. Thus, the prospects for repeated administrations of toxin-based therapeutics are apparently improving.

BPDCN qualifies for targeting by DT-IL3 by virtue of expressing the IL3 receptor (CD123) on the surface of malignant cells. Typically, the binding of IL-3 transmits growth and survival signals to the cell interior via phosphorylation of key effectors. DT-cytokine fusions may initially (minutes to hours) generate proliferation signals, but then as the toxin gains access to the cytosol, protein synthesis will be inhibited (hours) and cells will die (days) when they cannot make new proteins. Although BPDCN is relatively rare, expression of IL3 receptors is not. Specifically, CD123 is expressed on the surface of various B-cell and myeloid malignancies and as such could be targeted by agents such as DT-IL3 but also by such agents as chimeric antigen receptor T cells or immunotoxins directed to CD123—citations to these studies are found in Frankel et al. In addition, CD123 is expressed on various nonmalignant cells, and damage to these cells must also be addressed. Here the picture is not entirely clear. The preponderance of evidence suggests that CD123 is not expressed on precursor or stem cells but rather on more mature cells such as basophils, eosinophils, macrophages, and megakaryocytes. Whether or not targeting cytotoxic agents to CD123—expressing normal human cells will cause serious adverse events in the form of cell lineage depletion remains to be determined and will likely depend on careful evaluation of patients receiving treatments such as those described by Frankel et al. For now, however, the community should rejoice in the publication of a study reporting on major patient responses in a disease that is very difficult to treat with existing agents.

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REFERENCES


Comment on Gagne et al, page 437

Pearson syndrome in a Diamond-Blackfan anemia cohort

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In this issue of Blood, Gagne et al describe a cohort of 362 patients clinically classified as having Diamond-Blackfan anemia (DBA), in which 175 (48%) were found to have mutations and deletions in ribosomal protein genes or GATA1, and 8 of the remaining patients (2.2% overall) had mitochondrial gene deletions consistent with Pearson marrow-pancreas syndrome (PS). The authors propose that all patients with presumptive DBA should be tested for mitochondrial DNA (mtDNA) deletion during their initial genetic evaluation.1

Sometimes one syndrome turns out to be another: for example, newborn anemia that was first thought to be DBA (MIM #105650) is instead found to be PS (MIM#557000) on genetic evaluation. Should DBA and PS be distinguished clinically first? About 25% of patients with DBA may have characteristic physical features, such as short stature, low birth weight, abnormal thumbs, cleft lip or palate, and congenital heart disease among others.2 These features are not commonly seen in PS (except low birth weight), and the patients in the Gagne article do not show a specific pattern of physical features. Although both DBA and PS present in infancy with anemia and erythroid hypoplasia, the former do not usually have neutropenia and thrombocytopenia, which were present in all of the PS patients described by Gagne et al. There is a reasonably sensitive and specific blood test for untransfused patients with DBA—red cell adenosine deaminase (ADA)—which is elevated in about 85% of patients with DBA3; elevated ADA was reported once in PS but the type of specimen was not clear,4 and red cell ADA has not been examined systematically in PS.

Macrocytic red cells, low reticulocyte levels, and elevated hemoglobin F are common in any type of inherited bone marrow failure syndrome and do not distinguish DBA from PS; thus, bone marrow tests may be the most informative. In DBA, the marrow shows erythroid hypoplasia but is otherwise usually normal without significant dyspoiesis. In PS, however, the marrow is usually abnormal, with global hypocellularity and vacuoles in myeloid and erythroid precursors, as were found in the PS patients in the Gagne article (see figure). Vacuolated precursors are not a feature of DBA; they may be seen as an artifact or associated with infection, particularly infection due to parvovirus B19. Their absence in early marrows in the patients studied by Gagne et al is not clearly understood, because vacuoles were seen as early as 2 weeks of age in 1 of their patients. Another feature of marrow in mitochondrial disease is ringed sideroblasts resulting from iron deposition surrounding the nucleus of erythroblasts. The diagnosis of ringed sideroblasts requires at least 5 siderotic granules per cell surrounding at least one-third of the nucleus in at least 15% of erythroblasts (see figure). In general, patients who are heavily transfused and who have an overload of iron may have sideroblasts, but they are not ringed. The differential diagnosis of ringed sideroblasts includes genetic syndromes in addition to PS such as hereditary X-linked sideroblastic anemia, thiamine-responsive megaloblastic anemia, and autosomal recessive mitochondrial myopathy with lactic acidosis. Acquired causes are the myelodysplastic syndrome subtype, which is refractory anemia with ringed sideroblasts, alcoholism, chloramphenicol, and copper deficiency.

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<th>Vaquoes</th>
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<td>(Prussian blue)</td>
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Bone marrow from a patient with PS. Left: vacuoles in myeloid precursors; right: a ringed sideroblast. See Figure 1E in the article by Gagne et al that begins on page 437.
Targeted diphtheria toxin to treat BPDCN

David J. FitzGerald