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CLINICAL TRIALS

Comment on Schmiegelow et al, page 6077

Maintenance loses its innocence

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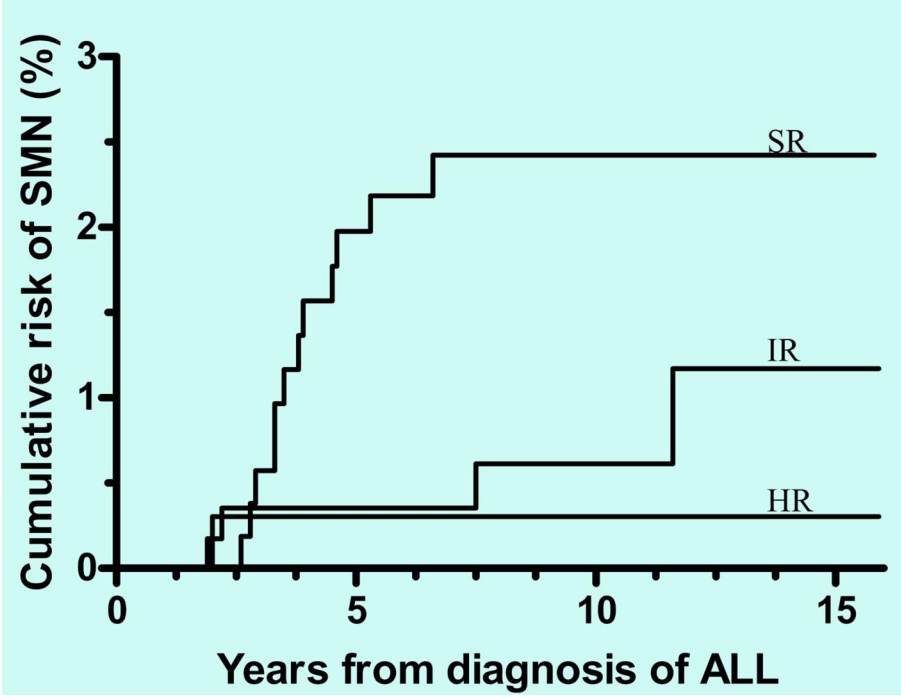
In this issue of *Blood*, Schmiegelow and colleagues on behalf of the Nordic Society for Paediatric Haematology and Oncology (NOPHO) present convincing evidence of a link between maintenance therapy with 6-mercaptopurine/methotrexate and the development of SMNs after treatment for pediatric ALL.

Given the toxicities associated with pediatric acute lymphoblastic leukemia (ALL) induction, consolidation, and delayed intensification courses, arrival at the maintenance phase was once thought to be a time to celebrate and relax. Unfortunately, results of comparative studies of 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP) in maintenance initiated a decade ago took the shine off maintenance "relaxation," as up to 11% of children treated with 6-TG developed veno-occlusive disease. A higher percentage of children developed a syndrome of periportal fibrosis and portal hypertension.[1,2] This left many groups with the seemingly more benign 6-MP at the core of their maintenance regimens. However, as Schmiegelow et al show, while use of 6-MP may avoid the early toxicities of its antimetabolite relative, because of the risk of late malignancies, its use in maintenance is not without consequences.[3]

Earlier reports of second malignant neoplasms (SMNs) after pediatric ALL therapy rounded up the usual suspects of alkylating agents, topoisomerase II inhibitors (anthracyclines, etoposide), and irradiation.[4,5] Many investigators subsequently modified their more intense, earlier phases of therapy to limit exposure to these higher-risk drugs and to radiation, with major progress occurring by eliminating cranial irradiation for a large percentage of children with ALL. Higher-risk disease regimens, however, still contain many of these agents, and have thus been associated with higher risks of second malignancies. With that in mind, this graph from the Schmiegelow paper jumps out at the reader (see figure). How is it that the cumulative risk of second cancers is 0.3% in high-risk patients and 8 times higher, or 2.4%, in standard-risk patients ($P = .007$)?

Using several lines of evidence, the article makes a case against 6-MP. The authors first describe a tight association with the duration of 6-MP/methotrexate (MTX) maintenance and the development of SMNs. The longer duration of 6-MP/MTX maintenance (and thus higher risk) was built into the NOPHO treatment plan for lower-risk disease, hence potentially explaining the results in the graph. They go on to show that exposure levels of 6-MP matter: patients geno-/phenotyped for polymorphisms with low thiopurine methyltransferase activity (TPMT, methylates 6-MP, low TPMT activity increases cytotoxic 6-thioguanine nucleotides [6-TGN]) had higher risk of SMNs, and patients with wild-type TPMT with SMNs received 10% to 15% higher doses of 6-MP compared with those patients with normal TPMT function who did not get SMNs. Two further suggestive, but not definitive, points are made: (1) the onset of SMNs trended toward occurring earlier in patients with low compared with normal TPMT activity ($P = .07$) and (2) higher 6-TGN levels (the result of low TPMT activity) were noted in patients with SMNs compared with those without



Cumulative risk of a second malignant neoplasms (SMN) by risk group for patients not receiving a transplant in first remission (SR: standard risk 2.4% ± 0.7%; IR: intermediate risk 1.2% ± 0.7%; HR: higher risk 0.3% ± 0.3%; $P = .007$). See the complete figure in the article beginning on page 6077.

($P = .11$). These observations add authoritative weight to a finding published a decade ago by the group at St Jude Children's Research Hospital. That finding stated that patients with low TPMT activity receiving 6-MP in combination with radiotherapy have increased risk of SMNs.[6]

The paper leaves many questions unanswered. Whether this association is caused by 6-MP therapy alone or whether high-dose MTX given along with 6-MP in maintenance contributes to the incidence of SMNs is unclear. Increased risk of SMNs in the NOPHO cohort is further associated with high hyperdiploidy, and the type of SMNs that occur differs from other reports, with a high incidence of tAML/MDS with aberrations of chromosomes 5 or 7. Although the authors speculate on reasons for these observations, the mechanisms behind these findings have yet to be elucidated. Finally, what other groups should do with the observations presented here is unclear. The NOPHO group initiated genotypic and phenotypic screening for low-activity TPMT polymorphisms in 2001, lowering 6-MP maintenance dosing from 75 to 50 mg/m^2 when low TPMT activity patients are detected by their screen. It is too soon to know whether this intervention has decreased SMNs. The approaches of other groups vary in dosing levels and length of administration of 6-MP, and an association of SMNs with different dosing schemes has yet to be described. These questions aside, this work provides convincing evidence that high dose levels or poorly methylated 6-MP in the context of extended maintenance with methotrexate is associated with SMNs. It poses a challenge to either identify patients at increased risk, or identify methods of administration of 6-MP that minimize or eliminate risk of SMNs without sacrificing ALL treatment efficacy.

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

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IMMUNOBIOLOGY

Comment on Lundqvist et al, page 6120

Reinforcing natural killers

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In this issue of *Blood*, Lundqvist and colleagues report enhanced antitumor effects of NK cells when adoptively infused into mice subsequent to treatment with bortezomib and depletion of regulatory T cells.

Natural killer (NK) cells were initially identified as lymphocytes capable of lysing tumor cells without prior sensitization. Meanwhile, numerous studies have established that NK cells play an important role in the immunosurveillance of tumors.[1,2] With regard to their antitumor activity, NK cells abide by the law of "missing-self," implying the preferential elimination of cells with a deficient expression of major histocompatibility complex (MHC) class I molecules. This recognition mode constitutes a fail-safe mechanism for MHC class I–restricted tumor recognition by cytotoxic T cells. In addition, triggering of NK-cell cytotoxicity requires stimulation via activating NK receptors that engage their cognate tumor-associated ligands. Apart from the intricate balance of activating and inhibitory signals provided by receptors recognizing ligands on tumor cells, additional factors, such as cytokines and interactions with other immune cells, determine NK-cell responsiveness. Ultimately, killing of tumor targets is achieved by secretion of granules containing both lytic perforin and granzymes, or by triggering death receptors (eg, by TNF-related apoptosis-inducing ligand [TRAIL]), respectively. Currently, the therapeutic potential of NK cells is exploited in the treatment of leukemia. Upon haploidentical stem cell transplantation, disparity of NK-relevant MHC class I molecules between donor and recipient is associated with an improved clinical outcome due to the failure of the recipient's leukemia cells to inhibit subsets of donor NK cells via their MHC class I–specific killer immunoglobulin-like receptors (KIRs).[1] However, available data indicate that solid tumors are largely resistant to therapeutic means employing NK cells.[1]

In this issue of *Blood*, Lundqvist et al provide experimental evidence (see figure) that antitumor efficacy of adoptively infused NK cells in mouse models of solid tumors can be enhanced by administration of bortezomib and depletion of regulatory T cells (Tregs).[3] The proteasome inhibitor bortezomib is well established in the treatment of multiple myeloma and other malignant lymphomas due to its ability to induce apoptosis in transformed cells. However, accumulating evidence indicates that bortezomib may also bolster NK-mediated antitumor immunity. In preceding studies, Lundqvist et al observed enhanced NK-cell cytotoxicity against bortezomib-treated human tumor cell lines.[4] This was attributed to increased susceptibility to TRAIL-mediated apoptosis, and sensitization to TRAIL was also reported to underlie the NK-cell stimulatory effect of bortezomib in another study employing murine NK cells.[4,5] Now, the authors report that augmented NK-cell killing of bortezomib-treated mouse tumor cells was not dependent on TRAIL but mediated through the perforin/granzyme pathway.[3] Could an increased susceptibility to perforin/granzyme-induced apoptosis be the central mechanism by which bortezomib augments NK-cell cytotoxicity against these mouse tumor cells, as the authors seem to suggest? Other studies indicate that bortezomib also promotes susceptibility of tumor cells toward NK-mediated cytolysis by a second, distinct mechanism: proteasome inhibition up-regulates the expression of ligands of NKG2D (NKG2DLs), thereby enhancing