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Secondary Myeloid Malignancy after Treatment of Acute Lymphoblastic Leukemia (ALL)

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Treatment of childhood acute lymphoblastic leukemia (ALL) has made tremendous strides over the past 20 years, but has been complicated by the induction of secondary tumors after some regimens. The cumulative incidence of therapy-related myeloid leukemia and myelodysplastic syndrome (referred to collectively as t-ML) varies widely among treatment protocols, from 1% to 12%.[1,2-7,8] Among children with ALL, t-ML secondary to topoisomerase II inhibitors (characterized by balanced translocations often involving the MLL gene on 11q23), has been the most common t-ML, but t-ML characteristic of that induced by alkylating agents (e.g. preceded by myelodysplasia, displaying monosomy 5 or 7)[2,5,9] and even secondary chronic myeloid leukemia (carrying a 9;22 translocation) have also been reported. It appears that the key event in leukemogenesis is formation of leukemogenic translocations, which for still unclear reasons, tends to occur most commonly in MLL (or at least to persist in MLL) following topoisomerase II agents. Because ALL is a disease for which excellent outcomes are achievable without the use of topoisomerase II agents, most ALL treatment regimens in the last 10 years have greatly reduced or eliminated the use of topoisomerase II inhibitors. However, the study of t-ML after ALL therapy has important implications for the field in that several important co-leukemogens and predisposing factors have been identified in studies of t-ML among patients with ALL, and that some of these factors appear to have relevance for secondary leukemia even in the absence of topoisomerase II agents. Multiple therapy-related and host-specific risk factors are likely to contribute to the development of t-ML,[2-5,7,8,10-18] in addition to the well-known contribution of topoisomerase II inhibitors.

At St. Jude, important work on factors that predispose to t-ML came from an analysis of the front line ALL trial, Total XI. Pui and colleagues 8,19 noted that the risk of t-ML was higher in patients who were assigned to a treatment arm consisting of 6 week blocks of exposure to topoisomerase II agents (Group III) than in those who received identical cumulative doses of all agents in the study, but whose schedule of administration was *rapidly rotating* topoisomerase II agents (Group II) (Figure 1). Although the mechanism for the difference in risk of t-ML remains unclear, multiple studies have shown that schedule and/or drugs given in addition to topoisomerase II agents substantially impact the risk of t-ML,[5,8,17,20-22] and the conclusion is that protocol-determined *cumulative dose* is not a helpful metric for assessing risk of t-ML. We *back-tracked* the molecular emergence of t-ML in one of our ALL patients and showed that t-ML emerged after only weeks of therapy, including only a total of 3 doses of topoisomerase II inhibitors (with G-CSF).[23] Subsequent studies have indicated that the combination topoisomerase II agents with cranial irradiation,[4,8,14,24] granulocyte colony stimulating factor (G-CSF),[24] asparaginase,[15,25] or thiopurines2,11 may increase the risk of t-ML (Table 1). Because many of these therapies may be specific to treatment of ALL, the risk factors for t-ML among patients with ALL may differ from those identified among other patient cohorts. In a front line study at St. Jude for ALL in which all patients received etoposide, we analyzed whether etoposide pharmacokinetics, formation of its CYP3A4-formed metabolites, protein binding, methotrexate exposure, or thiopurine methyltransferase (TPMT) activity differed in identically treated patients

	Total XI (1984-88)	
Week	Group II II	Group III III
1	VP + cyclo	VP + cyclo
2	MP + MTX	VP + cyclo
3	VM + AraC	VP + cyclo
4	Pred + VCR	VP + cyclo
5	VP + cyclo	VP + cyclo
6	MP + MTX	VP + cyclo
7	VM + AraC	MP + MTX
8	Pred + VCR	MP + MTX
9	VP + cyclo	MP + MTX
10	MP + MTX	MP + MTX
11	VM + AraC	MP + MTX
12	Pred + VCR	MP + MTX
13	VP + cyclo	VM + AraC
14	MP + MTX	VM + AraC
15	VM + AraC	VM + AraC
16	Pred + VCR	VM + AraC
to wk 120	etc.	
Cum.Dose	18 g	18 g
Risk t-ML	1.4%	8.2%

Figure 1. ALL continuation therapy for Group II vs Group III, showing higher risk of t-ML with Group III therapy.

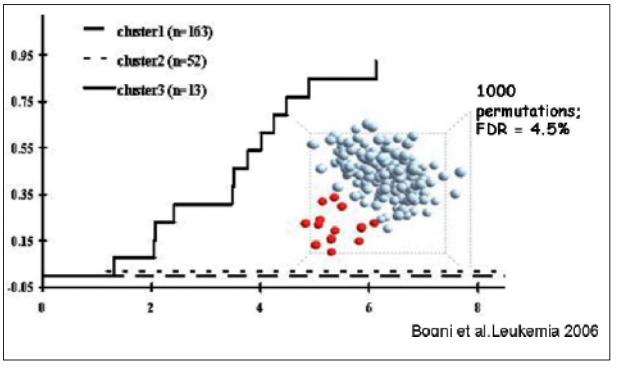

Figure 2. Cumulative incidence of t-ML in 3 clusters of patients, defined by expression of 83 distinguishing genes. Inset is a principal component plot, separating the patients with t-ML (red) from those who did not (blue).

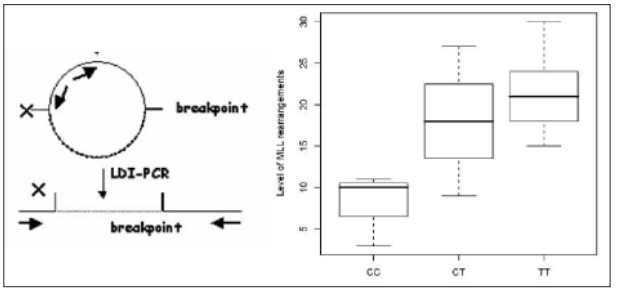

Figure 3. Experimental strategy to amplify MLL gene rearrangements using long-distance inverse PCR (left) that was used to assay etoposide-induced MLL rearrangements in 15 human lymphoid cell lines. Example of level of MLL fusions plotted vs germline genotype shown at right.

Table 1. Risk factors for t-ML.

Treatment-related	Host-related
asparaginase	thiopurine
thiopurines	methyltransferase
irradiation	glutathione
G-CSF	transferase
schedule	CYP3A4
potency of topo II inhibitor	Focal adhesion

who did and did not develop t-ML.[11] We found that TPMT activity was lower in those patients who developed t-ML, with onset of t-ML related to level of TPMT.[11] Shortly thereafter, similar findings were reported by the Nordic ALL treatment group (NOPHO),[26] even among patients whose only exposure to topoisomerase II agents was relatively low doses (120 to 250 mg/m^2) of anthracyclines. Taken together with other studies indicating a link between secondary malignancy and thiopurine exposure (Table 2), along with data indicating a mechanism whereby thiopurine incorporation into DNA could further stabilize topoisomerase II double-strand breaks in DNA,[27] it appears that thiopurines can act as co-leukemogens for t-ML, at least for some ALL regimens.

Despite associations between some candidate polymorphisms and t-ML (most of which are poorly penetrant and variably reproducible), it remains unclear which host genetic polymorphisms predispose to t-ML. To circumvent the limitations of a candidate gene approach, we have used genome-wide approaches in both clinical[30] and experimental systems to identify novel genes or pathways that may predispose to t-ML. We studied expression of over 10,000 genes in diagnostic ALL blasts to identify 83 genes whose expression differentiated patients who did develop t-ML from patients who did not (Figure 2).[30] We also interrogated over 100,000 single nucleotide polymorphisms (SNPs) to identify germline genotypes and acquired genetic abnormalities that differentiated patients with ALL who developed t-ML. In experimental cell lines, we modified our previously published technique to quantify *MLL* gene fusions[31] in human HapMap cell lines, lines that have been typed at over 1 million SNPs (Figure 3). The genes that were identified by multiple methods in clinical and experimental samples have been analyzed to reveal novel biological pathways that differ in patients with ALL who do vs do not develop t-ML, and these will serve as the basis to use a whole genome approach to identify novel genetic risk factors for the complication.

Table 2. Clinical evidence linking thiopurine use or defects in thiopurine methyltransferase to secondary leukemias.

Primary disease	*Other or primary leukemogens*	*Thiopurine challenge*	*Secondary Leukemia Findings*	*Reference*
ALL	etoposide, cyclophosphamide, asparaginase, ± irradiation, ± G-CSF	mercaptopurine	Higher frequency t-ML in pts with low TPMT	11
Renal transplant	none	azathioprine	Higher frequency of skin cancer in patients with high TGNs	28
organ transplant recipients	none	azathioprine	Higher risk of t-ML related to azathioprine dose	29
ALL	anthracyclines, asparaginase, ± cyclophosphamide	mercaptopurine ± thioguanine	Higher frequency of t-ML in patients with low TPMT	2

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