To the editor:

Outcome of children with hypodiploid ALL treated with risk-directed therapy based on MRD levels

Hypodiploid acute lymphoblastic leukemia (ALL) with <45 chromosomes has been associated with a dismal prognosis.[1-4] Near-haploid (24-31 chromosomes) and low-hypodiploid (32-39 chromosomes) ALL have particularly poor outcomes[5,6] and are distinct entities.[7] Near-haploid ALL is characterized by a younger age at diagnosis[5] and genetic alterations targeting receptor tyrosine kinase signaling, Ras signaling, and the lymphoid transcription factor gene *IKZF3*.[7] Low-hypodiploid patients are older[5] and have genetic alterations of *IKZF2*, *RB1*, and *TP53* that are often inherited.[7]

Despite overall improved treatment outcome of childhood ALL,[8] patients with hypodiploid ALL continued to fare poorly. In a Children's Oncology Group study of 41 hypodiploid cases treated between 2002 and 2006, the 4-year overall survival was 54% ± 8%, and the study could not evaluate the efficacy of allogeneic transplantation.[9] Because there are no known prognostic indicators in hypodiploid ALL, allogeneic transplantation continues to be recommended.[10]

We recently showed that minimal residual disease (MRD) levels during remission induction treatment have important prognostic and therapeutic implications in the context of MRD-guided therapy of ALL.[11] Notably, the adverse prognosis of pediatric Ph-like ALL can be significantly improved by this treatment approach.[12] We therefore sought to determine if the MRD-guided treatment strategy, as applied in 2 consecutive clinical trials, could also improve the outcome of hypodiploid ALL. In parallel, we comprehensively examined genetic features and their prognostic importance.

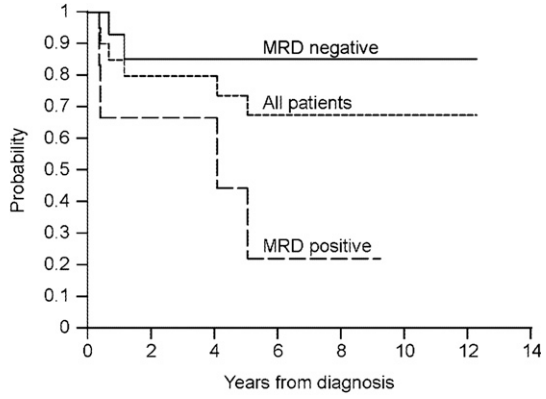
From June 2000 to October 2007, 498 patients (1 to 18 years of age) with newly diagnosed ALL were consecutively enrolled in the St. Jude Total Therapy Study 15,[13] and from October 2007 to June 2014, 410 patients (2 months to 18 years) in the Study 16.[14] The protocols were approved by the institutional review boards and registered at www.clinicaltrials.gov as #NCT00137111 and #NCT00549848, respectively. Written informed consent was obtained from the parents or guardians, and assent from the patients, as appropriate. All hypodiploid patients received intensive chemotherapy including high-dose methotrexate, dexamethasone, vincristine, and asparaginase. Patients with MRD ≥1%, as determined by flow cytometry and/or polymerase chain reaction analysis[15,16] after completion of induction therapy were offered the option of allogeneic transplantation.

Twenty (2.2%) patients had hypodiploid ALL. Eight patients had near-haploid ALL (median age 3.6 years, range 2.5-8.3), and 12 had low-hypodiploid ALL (median age 13.9 years, range 8.2-17.5 years, $P < .01$). There was no significant difference in presenting leukocyte count between near-haploid and low-hypodiploid ALL: median 8.1×10^9/L (range 3.0 to 52.0×10^9/L) vs median 5.6×10^9/L (range 1.5 to 36.9×10^9/L), $P = .21$.

All 20 patients achieved clinical remission. Negative MRD status (<0.01% leukemic cells among bone marrow mononucleated cells) was achieved in 6 of the 8 near-haploid and 8 of the 12 low-hypodiploid patients upon completion of remission induction ($P = 1.0$). Of the 6 patients with detectable MRD at the end of remission induction, 5 had MRD levels between 0.01% and 0.33%, and 1 had an MRD of 4.14%. Allogeneic transplantation was performed during initial remission in 1 MRD-negative patient with near haploidy because of the preference of the primary physician, and in the low-hypodiploid patient with MRD of 4.14% based on the protocol criterion.

The 5-year event-free survival was 73.6% (95% CI, 47.7-88.1) for all 20 patients (Figure 1) and was 68.2% (95% CI, 39.6-85.4) for the 16 treated in Study 15 as compared with 89.9% (86.5-92.5) for the other 409 B-cell ALL patients treated in Study 15 ($P < .001$). The result was not significantly different between the 8 patients with near-haploid and the 12 patients with low-hypodiploid ALL (72.9% [27.6-92.5] vs 75.0% [40.8-91.2], $P = .80$). However, it was significantly better for the 14 patients who achieved negative MRD status at the end of remission induction as compared with the 6 patients with detectable MRD: 85.1% (52.3-96.1) vs 44.4% (6.6-78.5), $P = .03$ (Figure 1). There were no other features associated with treatment outcome in this cohort. Twelve of the 13 hypodiploid patients with negative MRD status at the end of remission induction treated with chemotherapy only are alive in continuous complete remission for 0.7+ to 12.3+ years (median 7.0+ years); the 5-year event-free survival for this group was 91.7% (53.9-98.8). The near-haploid patient with negative MRD at the end of induction who was transplanted during initial remission died of transplant-related toxicity. Among the 6 patients with detectable MRD at the end of induction, the one with MRD of 4.14% remains in remission for 9.2+ years after transplantation, and 4 of the other 5 with lower levels of MRD (0.01% to 0.33%) treated with chemotherapy alone died of relapse with only 1 alive in remission for 1.4+ years.

Sixteen hypodiploid patients had suitable tumor samples, and all patients had remission samples for genomic analysis and sequencing, performed as previously described (Table 1).[17-21] The spectrum of genetic alterations was similar to those previously reported.[7] Somatic Ras pathway alterations were identified in 7 of 8 near-haploid patients, with a higher proportion of loss-of-function alterations of *NF1* than in our previous study (6 of 7 cases, with a *KRAS* mutation in 1 case). Four of 8 low-hypodiploid patients harbored *TP53*



Number at risk:

MRD negative	14	14	12	12	12	10	10	9	7	7	5	3	1
All patients	20	20	16	16	16	13	12	10	8	8	6	3	1
MRD positive	6	6	6	4	4	4	3	2	1	1	1	1	

Figure 1. Kaplan-Meier estimates of event-free survival for all 20 patients with hypodiploid ALL and according to the presence (n = 6) or absence (n = 14) of MRD.

Table 1. Cytogenetic and molecular genetics of the 20 patients with near-haploid and low-hypodiploid ALL

Patient	PCGP ID	Ploidy group	Next-generation sequencing	Karyotype	Genomic lesion
1	SJHYPO122	Near haploid	WES	*48(24,X,+21)x2[20]/46,XY[1]	NF1 Y2455*; RB1 L317fs; *CDKN2A/B* deletion
2	SJBALL156	Near haploid	WES	*50(25,X,+14,+21)x2,del(1)(q42),add(11)(p15)[5]/46,XY[4]	KRAS G12D and EP300 D1399N
3	SJBALL014876	Near haploid	WES	*53(26,X,+10,+14,+21)x2,del(9)(p22),i(9)(q10),+mar[4]/53, idem,+9,-mar[16]/46,XX[1]	CREBBP S1432fs, WHSC1 E1099K, USH2A I1326M, NF1 P678fs
4	SJHYPO143	Near haploid	WES	27,X,+X,+18,+21,+mar[6]/54,idemx2[3]/46,XX [15]	No mutations in key pathways
5	SJHYPO128	Near haploid	WES	27,X,+X,+14,+21,+22[13]/54,idemx2,-X,+10,-14,+17,+18,-21,-22,+mar[7]	NF1 T2600fs
6	SJHYPO123	Near haploid†,‡	WGS, RNA-seq	27,X,+Y,+13,+18,+21[8]/35,idem,+1,+2,+3,+5,+6,+7,+11,+15[6]/46,XY[14]	*NF1* deletion of exons 33-41; histone cluster (6p22) deletion
7	SJHYPO146	Near haploid†	WGS, WES	27,X,+Y,+17,+18,+21[5]/54,idemx2[6]/46,XY[8]	NF1 T2133fs and deletion; CREBBP E1240K and deletion; PAX5 G25E
8	SJHYPO124	Near haploid‡	WES	30,X,+Y,+4,+8,+10,+14,+18,+21[4]/60,idemx2[14]/46,XY[2]	NF1 E470*
9	SJHYPO141	Low hypodiploid§	WES (germ line)	33,X,+1,+2,+6,+10,+11,+12,+18,+19,+21,+22[6]/46,XX [14]	Not available for tumor
10	SJHYPO22048	Low hypodiploid§	WES (germ line)	34,X,+Y,+1,+5,+6,+7,+10,+11,+15,+17,+19,+21[13]/62, idemx2,-X,-Y,-1,+3,-5,-6,-7,-10,-11,+14,-15,-17,+18,-19,+20,+22[4]/46,XY[3]	Not available for tumor
11	SJBALL014875	Low hypodiploid	WES	35,X,+1,+5,+6,+9,+11,+12,+14,+18,+19,+20,+21,+22[11]/46,XY[9]	*MAPK* mutation
12	SJHYPO135	Low hypodiploid	WES	35,X,+X,+1,+2,+4,+5,+6,+8,+11,+12,+16,+19,+21[10]/67, idemx2,-2,-5,-6,+10,+10,-11,-12,-12,+14,-16,-16,+18,-19,+20,+22[5]/46,XX[2]	RB1 V654M; TP53 C283Y
13	SJHYPO125	Low hypodiploid‡	WES	35,X,+X,+1,der(1)t(1;7)(p36.1;q11.2)c,+5,+6,+8,+10,+11,+14,+18,+19,+21,+22[9]/67,idemx2,-5,-14,-19[9]	No mutations in key pathways
14	SJHYPO138	Low hypodiploid§	WES (germ line)	35,X,+Y,+1,+5,+6,+8,+9,+10,+11,+18,+19,+21,+22[20]	Not available for tumor
15	SJHYPO126	Low hypodiploid‡	WES	35,X,+1,+5,+6,+8,+10,+11,+14,+15,+18,+19,+21,+22[8]/64, idemx2,-5,-10,-11,-14,-15,-19[9]/46,XX[3]	*IKZF2* exon 5 deletion; TP53 I162fs mutation (germ line)
16	SJHYPO144	Low hypodiploid	WES	36,X,+Y,+1,+5,+6,+8,+10,+11,+14,+15,+18,+19,+21,+22 [16]/46,XY [4]	TP53 L32_E4splice
17	SJHYPO139	Low hypodiploid	WES	36,X,+Y,+1,+2,+5,+6,+8,+9,+10,+11,+17,+19,+21,+22[5]/72,idemx2[2]/46,XY[13]	TP53 R175H; IKZF2 deletion
18	SJHYPO140	Low hypodiploid†	WES	37,X,+Y,+1,+5,+6,+8,+9,+10,+11,+14,+18,+19,+20,+21,+22[11]/74,idemx2[10]/46,XY[3]	*RB1* mutation
19	SJBALL108	Low hypodiploid	WES	38,X,+1,+2,+5,+6,+8,+9,del(9)(p13),ins(10;11)(q26;q21q24),+11,+14,+15,+16,+18,+19,+20,+21,+22[7]/38,idem,del(2)(p13p23)[4]/46,XX[9]	*PAX5*, *CDKN2A*/B deletion
20	SJHYPER22044	Low hypodiploid*	NA	73(40,X,+Y,+1,+4,+5,+6,+8,+9,+10,+11,+13,+14,+15,+17,+18,+20,+21,+22)x2,-4,-5,-11,-13,-14,-15,-20[11]/46,XY[9]	No focal deletions; LOH of chromosomes 2,3,4,7,11,12,13,15,16,17

LOH, loss of heterozygosity; NA, not available; PCGP, Pediatric Cancer Genome Project; WES, whole exome sequencing; WGS, whole genome sequencing.
*Chromosome number reflected doubling of the hypodiploid clone.
†Also Ph-like.
‡Genome sequencing previously reported.
§No diagnostic material available for genomic analysis.

mutations, with 1 identified in the germ line. We also observed alterations of *IKZF2* in 2 low-hypodiploid patients and mutations of the histone acetyl-transferase genes *CREBBP* and *EP300* in near-haploid ALL.

Five patients had hematologic relapse, including 2 with near haploidy (nos. 2 and 7) and 3 with low hypodiploidy (nos. 15, 18, and 19). Both near-haploid patients harbored Ras-activating mutations, 1 *NF1* and 1 *KRAS*, and 1 also acquired *IKZF1* and *PAG1* deletions at relapse. Of the 3 low-hypodiploid patients that experienced relapse, 1 (no. 15) had alterations of *IKZF2* and *TP53*, the second (no. 18) had a sequence mutation of *RB1*, and the third (no. 19) had deletions of *PAX5* and *CDKN2A/B*. An additional 2 low-hypodiploid patients (nos. 9 and 16) had detectable MRD at the end of induction but did not experience relapse. Patient 16 had a somatic *TP53* mutation, and patient 9 lacked tumor material for analysis but did not have a germ-line *TP53* mutation. Three patients (nos. 6 and 7 with near haploidy and no. 18 with low hypodiploidy) also exhibited a Ph-like gene expression profile,[21] 2 of whom (nos. 7 and 18) relapsed. No associations between individual genetic alterations and outcome were observed.

In summary, our study demonstrates for the first time that MRD is the most important prognostic indicator for childhood hypodiploid ALL and that the outcome can be substantially improved by MRD-guided therapy. Importantly, hypodiploid patients with negative MRD status at the end of 6-week remission induction are highly curable with intensive chemotherapy alone. Studies are needed to determine if transplantation in first remission will improve the outcome of those with positive MRD at the end of remission induction. Genomic analysis confirmed the distinct genetic alterations characteristic of hypodiploid ALL[7] but, because of the small number of cases, did not identify any genomic features associated with treatment response or outcome. However, 1 near-haploid patient acquired a deletion of *PAG1* (also known as CBP, Csk-binding protein) at relapse. *PAG1* encodes a putative modulator of Ras signaling, and *PAG1* alterations were previously associated with treatment failure in hypodiploid ALL.[7] Additional studies are needed to develop effective targeted therapy for hypodiplod ALL. In this regard, inhibitors of phosphatidylinositol-3-kinase signaling were identified as potentially effective drugs in our previous study[7] and are being explored in preclinical studies as a new therapeutic strategy.

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To the editor:

Haploinsufficient loss of multiple 5q genes may fine-tune Wnt signaling in del(5q) therapy-related myeloid neoplasms

Wnt signaling in hematopoietic cells and the bone marrow microenvironment plays a critical role in maintaining the pool of hematopoietic stem cells (HSCs) and in regulating differentiation.[1,2] Wnt signaling is tightly regulated by the interplay of multiple cytoplasmic components, with Wnt activity being highest in HSCs and lower in more mature myeloid cells.[1] Moreover, graded Wnt signaling has differential effects, with high activation leading to bone marrow failure and less profound activation leading to HSC expansion.[1,3-5] Wnt activation has also been implicated in self-renewal of leukemia stem cells and is associated with a poorer outcome in acute myeloid leukemia (AML).[6]

However, our understanding of Wnt signaling in hematopoietic cells is incomplete; a holistic view is needed to understand how alterations to Wnt pathway components affect disease processes. This was most recently illustrated in a paper in *Blood* by Kühnl et al,[7] who examined the role of the putative tumor suppressor CXXC5, a CXXC-type Zn-finger protein that interacts with disheveled (DVL) and impairs Wnt signaling in leukemia cell lines. Contrary to expectations, in AMLs, downregulation of *CXXC5* expression via epigenetic silencing was associated with upregulation of cell cycling genes, coordinated with downregulation of genes implicated in leukemogenesis (eg, *WT1*, *GATA2*, *KMT2A/MLL*, *DNMT3B*, and *RUNX1*), and a better prognosis. The latter observation likely reflects the prevalence of Core Binding Factor AMLs in this series, which have low expression of *CXXC5*.

In previous studies, we and others have drawn attention to the role of loss of several genes on 5q, *APC* (5q22),[8] and *CSNK1A1* (5q32),[4] encoding negative regulators of the Wnt pathway, in the pathogenesis of therapy-related myeloid neoplasms (t-MN) or high-risk myelodysplastic syndromes (MDS)/AML with a del(5q), as well as MDS with an isolated del(5q).[9] The interstitial deletions of 5q are typically large, as virtually all patients have loss of 5q14-33, and confer haploinsufficient expression of many genes in the deleted interval.