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Toward Pediatric T Lymphoblastic Lymphoma Stratification Based on Minimal Disseminated Disease and NOTCH1/FBXW7 Status

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Abstract

While outcome for pediatric T lymphoblastic lymphoma (T-LL) has improved with acute leukemia-type therapy, survival after relapse remains rare. Few prognostic markers have been identified. NOTCH1 and/or FBXW7 (N/F) mutations identify good prognosis T-LL and high-level minimal disseminated disease (MDD) is reported to be of poor prognosis. We evaluated MDD and/or MRD status by 8-color flow cytometry and/or digital droplet PCR in 82 pediatric T-LL treated according to the EURO-LB02 prednisone reference arm. Both techniques gave identical results for values <0.1%, allowing compilation. Unlike historical studies, an MDD threshold of 1% had no prognostic significance. The 54% (42/78) of patients with MDD ≥0.1% had a relatively favorable outcome (5-y overall survival [OS] 97.6% versus 80.6%, P = 0.015, 5-y event-free-survival [EFS] 95.2% versus 80.6%, P = 0.049). MDD lower than 0.1% had no impact in N/F mutated T-LL, but identified the N/F germline patient with a high risk of relapse. Combining oncogenetic and MDD status identified 86% of patients (n = 49) with an excellent outcome and 14% of N/F germline/MDD <0.1% patients (n = 8) with poor prognosis (5-y-OS 95.5% versus 37.5%, P < 0.001; 5-y-EFS 93.5% versus 37.5%, P < 0.001). If confirmed by prospective studies, MDD and N/F mutational status would allow identification of a subset of patients who merit consideration for alternative front-line treatment.

Introduction

T-cell lymphoblastic lymphoma (T-LL) and T-cell acute lymphoblastic leukemias (T-ALL) are both characterized by the proliferation of malignant immature T-cell precursors but differ by the extent of bone marrow (BM) involvement, which is (arbitrarily) <25%, assessed by morphology, in T-LL. Despite the improvement of current therapy for children, achieving an event-free survival (EFS) at 5 years of 75%–85%,1–3 the survival rate of refractory or relapsed LL remains very poor, at 10%–30%.4,5 Relatively favorable results are obtained in the rare T-LL cases who achieve second remission and can undergo hematopoietic stem cell transplantation.6 The early identification of poor risk T-LL is thus mandatory.

Contrary to T-ALL,7,8 prognostic factors in T-LL are rare, partly due to its low incidence and difficulty in obtaining diagnostic material. Retrospective analyses identified the absence of NOTCH1 and/or FBXW7 (N/F) mutations and biallelic T-cell receptor-gamma (TRG) deletions to be associated to unfavorable outcome,9–11 and loss of heterozygosity at chromosome 6q (LOH6q) to an increased relapse risk.12,13

In T-ALL, one of the strongest prognostic factors is minimal residual disease (MRD).9 MRD analysis in peripheral blood (PB) and BM give comparable results.15 In T-LL, high-level minimal disseminated disease (MDD) at diagnosis and MRD positivity by multiparameter flow cytometry (MFC) and/or quantitative polymerase chain reaction (qPCR) for TR rearrangements identified high-risk patients,16–18 justifying intensification of chemotherapy and/or hematopoietic stem cell transplantation.6 The early identification of poor risk T-LL is thus mandatory.

Supplemental digital content is available for this article.

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dropout. An independent end-point PCR reaction allows absolute quantification, using Poisson statistics. Studies indicate that ASO-specific ddPCR is particularly adapted for lymphomas but ddPCR has not, to date, been evaluated in T-LL.

In France, pediatric T-LL was treated on the EURO-LB02 trial until its premature arrest due to excessive toxicity on January 1, 2008 and from then onward, according to the reference prednisone arm, with 24 months treatment. The present study compared T cell Receptor (TR) ddPCR and MFC to assess the clinical impact of MDD at diagnosis in patients on this reference arm. Since the recently opened European multicenter prospective pediatric LBL2018 protocol (NCT04043494) stratifies patients on their N/F mutation status, we also evaluated the correlation between these oncogenic markers and MDD on outcome. Unexpectedly, patients with MDD positivity above the correlation between these oncogenetic markers and MDD defines patients on their reference arm. Since the recently opened European multicenter prospective pediatric LBL2018 protocol (NCT04043494) stratifies patients on their N/F mutation status, we also evaluated the correlation between these oncogenic markers and MDD on outcome. Unexpectedly, patients with MDD positivity above the correlation between these oncogenetic markers and MDD defines patients on their reference arm. Since the recently opened European multicenter prospective pediatric LBL2018 protocol (NCT04043494) stratifies patients on their N/F mutation status, we also evaluated the correlation between these oncogenic markers and MDD on outcome.

Patients and samples

Between December 2004 and December 2007, pediatric T-LL patients were treated in France on the EURO-LB02 European trial, derived from the Berlin-Frankfurt-Münster BFM90 protocol, with CNS prophylaxis by high-dose systemic Methotrexate (HD-MTX) in the interim phase instead of prophylactic central nervous system irradiation (NCT00275106). The protocol included 2 randomizations (R) for T-LL: prednisone versus dexamethasone during induction (R1) and the total treatment duration of 18 versus 24 months (R2). Since January 1, 2008, pediatric T-LLs were treated according to the reference prednisone arm, with 24 months treatment according to EICNH group recommendations. Asparaginase treatment comprised E Coli native asparaginase (10,000 U/m2 per dose, 8 doses in induction, 4 doses during reinduction). All clinical data for French patients treated in Société Française des Cancers de l’Enfant (SFCE) centers were registered centrally (Lyon). As per the declaration of Helsinki, the Lyon ethics committee approved the study and the signed informed consent was obtained for all patients. Centralized prospective evaluation of oncogenetics on filtered diagnostic material in Paris-Necker and PB and BM MDD and MRD by 8-color multiparameter FC (MFC) in Lyon was set up in 2008. No qPCR MDD/MRD was planned initially, but leftover cells from FC were stored as pellets in Lyon, whenever possible, and later transferred to the Paris molecular platform.

MDD/MRD assessment by MFC

From 2008 to 2017, fresh PB and BM samples were collected at diagnosis and PB at day (d) 33 (end of induction). MFC analysis was performed with an 8-color panel on a FACS CANTOII with DIVA software (BD Bioscience), as described. Antibodies used are described in Supplemental Digital Material and Methods, http://links.lww.com/HS/A197. To exclude residual erythrocytes, dead cells, debris, platelet aggregates and doublets, a live gate was adjusted on forward scatter (FSC)/SSC and CD45/SSC. Positive MDD/MRD was defined as a cluster of >10 cells expressing at least 2 leukemia aberrant immunophenotype (LAIP) and SSC characteristics identified at diagnosis, amongst at least 500,000 viable cells (Supplemental Digital Figure S1B, http://links.lww.com/HS/A197). MFC sensitivity of 0.01% normal cells was possible in virtually all samples.

MDD/MRD assessment by ddPCR

To identify patient-specific clonotypic markers, genomic DNA was extracted from diagnostic tissue or effusions from 62 patients using a QIAampDNA mini kit (Qiagen Co., Hilden, Germany), TR clonality (TRD, TRB, and TRG) was assessed by one-step Next-Generation Sequencing using 100 ng DNA, EuroClonality-NGS amplicon primers and Vidjil software analysis. At least one allele-specific (ASO) CDR3 primer, selected on the basis of maximal clonotype amplification and minimal nonspecific positivity in normal PBL DNA, was designed for each patient. Thirty-eight patients with fresh or cryopreserved MDD samples (34 BM and 18 PB) and 18 patients with d33 MRD frozen samples (6 BM and 17 PB) were analyzed retrospectively (Paris-Necker). Quantification method is described in Supplemental Digital Material and Methods, http://links.lww.com/HS/A197.

NOTCH1/FBXW7 mutations

The mutational screening of 61 tumour samples (effusions, mediastinal mass, and lymph node) with at least 20% infiltration was performed by capture NGS, as described. The functionally silent SNVs of NOTCH1 and FBXW7 were not reported as mutations. Mutational status was not used to modify therapy.

Statistical analysis

Comparison of the 2 MDD/MRD quantification methods was performed by performing Spearman’s rank correlation coefficient. Group comparison for categorical and continuous variables was performed with Fisher’s exact and Mann–Whitney U tests, respectively. Overall survival (OS) was calculated from the date of prephase initiation to the last follow-up date by censoring alive patients. Events accounting for EFS were a nonresponse on day 33 (defined as >5% blasts in the bone marrow and/or blasts in the cerebrospinal fluid) and/or <35% tumor regression), relapse, secondary malignancy, or death from any cause. Survival analysis was performed using the Kaplan–Meier method, and the curves were compared using the log-rank test. Statistical analysis was performed with the R software, version 4.0.2. All P values were two-sided, with P < 0.05 denoting statistical significance.

Results

From January 2008 to December 2017, 205 pediatric T-LL were diagnosed in 30 SFCE centers (Flow Chart, Figure 1). Eighty-two T-LL patients, who did not differ from the overall group, had MDD and/or MRD analysis. MDD status was assessed for 78 patients, MRD status for 59, both for 53. Clinical and biological characteristics are detailed in Table 1 and Supplemental Digital Table S1, http://links.lww.com/HS/A195. As per the EURO-LB02 protocol, patients who received systemic corticosteroids for more than 8 days within 2 months before or at the time of MDD evaluation were excluded, although lower dose oral corticosterotherapy cannot be excluded for all patients. Two patients died in remission of toxic complications. Twelve patients diagnosed between 2008 and 2010 were previously published for N/F status.

MDD assessment by MFC

Sixty-two pediatric T-LL patients had MDD quantification by 8-color MFC from 98 samples (23 PB only, 3 BM only, 36 both) (flow chart, Figure 1). As described for MRD, optimal combinations were those containing TdT, CD99, CD1a, and CD34, in addition to a CD3/CD3/CD5 backbone. It was possible to compare pathological MDD phenotypes with those of the tumor in only 8 patients for whom a pleural or pericardiac effusion was
transmitted to Lyon for central MFC. Despite this, a pathological population of at least 0.01% was identified in 81/102 PB/BM samples (51/62 patients), with a median infiltration of 0.2% (range 0.01%–15%), when analyzing at least 500,000 viable cells (Figure 2A). Infiltration was similar in PB and BM MDD/MRD samples in the 36 patients with dual analysis (Supplemental Digital Figure S1A, http://links.lww.com/HS/A198).

MDD quantitation by ddPCR

To evaluate ddPCR MDD/MRD, TR immunogenetic status was evaluated in diagnostic DNA from 62 infiltrated tumor samples by genescan multiplex clonality analysis and/or EuroClonality amplicon NGS for TRD, TRG, and TRB (VDJ and DJ) rearrangements. Clonal rearrangement of at least one TR was identified in all but one case, for which MDD analysis was performed by MFC, with TRG clonality identified in 92% (57/62), TRB VDJ ± DJ in 84% (52/62), TRB DJ only in 8% (5/62), and TRD in 50% (30/60). At least one ASO CDR3 primer was developed for each of the 41 T-LL with MDD/MRD samples and first tested by ddPCR on diagnostic tissue samples. ddPCR quantified infiltration in the 22 pleural/pericardiac effusions transmitted to the Necker molecular platform varied from 8% to 100%, median 44% and from 14% to 80%, median 56%, in tissue samples. As such, diagnostic liquid and tissue T-LL samples cannot be presumed to be 100% infiltrated at the DNA level, even if histological interpretation suggests massive

Figure 1. Flow chart of MDD/MRD study in French pediatric T-LL from 2008 to 2017. MDD = minimal disseminated disease; MRD = minimal residual disease; T-LL = T lymphoblastic lymphoma.

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<th>Table 1. Clinical and Biological Characteristics of the MDD/MRD Cohort and the MDD Cohort According to MDD at 0.1% Threshold</th>
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Significant P values, <0.05, are indicated in bold. 
/ = not applicable; 5y-OS = 5-y overall survival; 5y-EFS = 5-y event-free survival; BM = bone marrow; BQL = below quantitative level by ddPCR; CNS = central nervous system; MDD = minimal disseminated disease; MRD = minimal residual disease at day 33.
involvement by tumor. This is relevant if tumor DNA is used to design qPCR calibration curves for ASO IG/TR MDD/MRD strategies.

ddPCR comparison with MFC

For MDD ddPCR quantification, we first evaluated cell pellets frozen after prospective MFC analysis. A total of 61 samples (18 PB MDD, 19 BM MDD, 5 effusions at diagnosis, 16 PB MRD and 3 BM MRD at d33) from 27 patients were evaluated by both methods (Figure 2B). MFC and ddPCR showed good correlation above the threshold of 0.1% (Spearman’s rank correlation coefficient, kappa = 0.93 [IC 95%: 0.81–1.00]). ddPCR gave quantifiable positivity in 10/61 samples (plus one BQL result) with no detectable infiltration by MFC, in keeping with reported sensitivity limits of 0.01% for MFC, compared to 0.001% by ddPCR, at least in MCL.22 For 7 samples (4 patients with 4 MRD and 3 MDD samples) with ddPCR quantification between 0.1% and 0.01%, MFC raw data review confirmed the absence of detectable T-lymphoblasts, albeit in patients whose tumour immunophenotype had not been assessed. In 5 samples (3 patients with 4 MDD and 1 MRD samples), ddPCR was positive/BQL below the sensitivity of MFC (0.01%). Taken together, these results demonstrate that ddPCR is an appropriate method for MDD/MRD assessment in T-LL, that MFC and ddPCR can be compiled for values above 0.1% and that ddPCR is more sensitive for low level positivity than MFC, as practiced here (0.5–1 million cells analyzed).

MDD infiltration at diagnosis

We extended our ddPCR study to all frozen PB/BM MDD/MRD samples for which a tumour sample with a clonal TR was also available (Figure 2C). In total, 41 patients were evaluated by ddPCR, including 38 with MDD quantification. ASO targets were TRB for 28 patients, TRD for 7 and TRG for 6. Only 2 patients had enough DNA to be quantified on 2 targets, when the most positive result was retained. All samples met the defined ddPCR criteria.22 The vast majority of MDD (34/38, 89%) were quantifiably positive, with 7 samples in the 0.001%–0.01% range and only 1 positive BQL (Figure 2C). Infiltration was similar in PB and BM in the 14 patients with dual analysis (Supplemental Digital Figure S1B, http://links.lww.com/HS/A198).
When combined with MFC results, 78 patients had MDD evaluation by MFC, ddPCR or both (Figure 2C). For each patient, the highest MDD value was retained, regardless of the sample type or assay used. We observed a continuum of infiltration in PB/BM, with 10% (8/78; 5 by MFC only, 3 by ddPCR ± MFC) of patients having undetectable disease, 36% (28/78) positivity <0.1%, 26% (20/78) positive ≥0.1%, and <1% and 28% (22/78) ≥1%. Overall, 54% of patients were MDD positive above 0.1%, at which level MFC and ddPCR were totally concordant.

**MDD ≥ 0.1% identifies patients with a good response to EURO-LB02 prednisone/24-month therapy**

To evaluate the prognostic relevance of MDD status, we first showed that the patients with MDD analysis were representative of the overall cohort treated with the prednisone/24-month treatment regimen (Supplemental Digital Table S1, http://links.lww.com/HS/A195). Applying the historically proposed 1% MDD threshold\(^{(16,17)}\) to the present series demonstrated no prognostic value (Supplemental Digital Figure S2A and B, http://links.lww.com/HS/A195). In contrast, when using a MDD cutoff of 0.1%, above which there was an absolute correlation between MFC and ddPCR, patients with MDD ≥0.1% had a favorable 5-year OS of 97.6% compared to 80.6% for patients with MDD <0.1% (HR 0.11, 95% confidence interval [CI]: 0.01–0.93, \(P = 0.015\) and a favorable 5-year EFS (95% versus 84%, HR 0.24, 95% confidence interval [CI]: 0.05–1.13, \(P = 0.049\)) (Figure 3A and B).

To investigate whether there were clinical differences in MDD (cutoff 0.1%) high and low/negative patients, we compared clinical and biological characteristics of the 2 groups (Table 1). Sex ratios were comparable. As expected, Stage IV and BM involvement were more common in patients with MDD >0.1% (Figure 3C). Patients with MDD >0.1% had significantly lower LDH values and tended to be younger and have more CNS involvement at diagnosis (Table 1).

**The prognostic impact of MDD status is restricted to NOTCH1/FBXW7 germline T-LL**

Since pediatric T-LL patients with NOTCH1/FBXW7 germline status (N/F\(^{GL}\)) are randomized for intensification in the LBL2018 protocol, we determined MDD impact as a function of N/F status. 21/82 (26%) patients were not evaluated due to absence of sufficiently infiltrated diagnostic material. 19/61 (31%) were N/F\(^{GL}\) and 42/61 N/F\(^{pos}\) (69%). The level of MDD was comparable in the 3 groups (Supplemental Digital Figure S3A, http://links.lww.com/HS/A195). As expected, N/F\(^{GL}\) patients had worse outcomes (Supplemental Digital Figure S3B and C, http://links.lww.com/HS/A195).

Using the MDD 0.1% threshold, MDD status had no prognostic impact in N/F\(^{pos}\) cases (5-y OS: 95% for MDD ≥0.1% versus 94.7% for MDD <0.1%; 5-y EFS: 90% versus 94.7%, respectively; Figure 4A and B). In contrast, among N/F\(^{GL}\) cases, 8/18 (44%) were MDD <0.1% and had inferior prognosis (5-y OS of 37.5% versus 100% for MDD ≥0.1%, \(P < 0.001\); a 5-y EFS of 37.5% versus 100%, \(P < 0.001\); Figure 4A and B). Details of the patients who progressed or relapsed are shown in Supplemental Digital Table S2, http://links.lww.com/HS/A196.

Overall, a molecular classifier combining MDD assessment and N/F genotype identified striking differences in outcome between the 8/57 (14%) high-risk patients (N/F\(^{GL}\) and MDD <0.1%) and low-risk patients (all others) with 5-year OS at 37.5% versus 95.9%, HR 20.70, 95% CI: 3.98–107.50, \(P < 0.001\); 5-year EFS of 37.5% versus 93.9% HR 14.27, 95% CI: 3.36–60.71, \(P < 0.001\), respectively (Figure 4C and D). Taken together, these results demonstrate that combined MDD and oncogenetic evaluation at diagnosis is likely to allow the identification of patients who should be considered for alternative treatment front line, given the very poor survival of relapsed pediatric T-LL.

**Prognostic impact of MRD**

MRD at the end of induction is a fundamentally important prognostic factor in T-ALL. We therefore evaluated MRD at d33 in 59 patients with available samples (41 by MFC only, 2 by ddPCR only, 16 by both; flow chart, Figure 1). One N/F\(^{mut}\) case (N° 6134, Supplemental Digital Table S2, http://links.lww.com/HS/A196) remained persistently MRD positive by ddPCR 4 months after diagnosis, so was switched to a high-risk T-ALL regimen with Nelarabine, and is in CR 3 years later. Samples from 9 patients (15%) were positive or BQL (2 were MFC+/ddPCR+, 4 ddPCR+/MFC− and 3 MFC+/ddPCR\(^{nd}\)), and 50 (85%) were undetectable, with a sensitivity of 0.01%. Details of the patients with pos/BQL MRD are shown in Supplemental Digital Table S2,
Prognostic evaluation showed an inferior outcome in MRD positive patients, with 4-year OS of 77.8% for MRD positive/BQL versus 92.0% for MRD undetectable, HR 0.31, 95% CI: 0.06–1.71, P = 0.157; a 4-year EFS of 66.7% versus 92.0%, respectively, HR 0.31, 95% CI: 0.06–1.71, P = 0.015, respectively (Figure 5A and B). MRD alone did not predict the majority of the 6 relapses/progression, whose clino-biological characteristics are detailed in Supplemental Digital Table S2, http://links.lww.com/HS/A196. The small sample size and the mixture of techniques used preclude reliable analysis of MRD significance within MDD or N/F defined subsets, but only 2/7 relapsing patients tested had MDD ≥0.1% and only 2/7 tested at diagnosis had N/Fmut T-LL, suggest that this should be addressed specifically and prospectively in a collaborative study.

Discussion

T-LL prognosis has greatly improved with ALL treatment strategies, achieving more than 85% OS. In contrast, refractory patients and those who relapse, predominantly within the first 2 years, have a dismal outcome, so their early identification is essential for timely therapeutic adaptation. We show that poor prognosis pediatric T-LL is virtually restricted to patients with MDD levels in PB/BM below 0.1% and absence of NOTCH1/ FBWX7 mutations at diagnosis. These results contradict prior data suggesting that MDD positivity is associated with poor prognosis.

We performed MDD and MRD quantification by MFC and ddPCR, following ddPCR approaches developed for Mantle Cell Lymphoma, which also frequently disseminates to PB and BM. Results were perfectly concordant for positivity levels above 0.1%, allowing compilation. ddPCR was, however, more likely to detect lower level positivity, since 10/32 samples were positive/BQL by ddPCR, but negative by MFC, compared to only 3 which were MFC positive (all at 0.01%) but ddPCR negative. In keeping with this, a disproportionate number of positive MRD samples were detected by ddPCR. As such, either technique is acceptable for detection of high level positivity, but evaluation of levels below 0.1% should either be performed by molecular clonotype quantification or by MFC of higher cell numbers than the 0.5–1 million events analyzed here. This is increasingly practiced in ALL MRD evaluation, but it is noteworthy that MFC is not recommended in isolation to determine MRD negativity in the European ALL-Together trial (EUDRACT 2018-001795-38). MDD evaluation by MFC may also be challenging without characterization of the tumour phenotype and should only be assessed by experienced reference platforms with sufficient T-LL recruitment.
Despite a comparable incidence of patients with high level MDD values, we did not confirm the inferior outcome of high level MDD of the COG-A5971 trial. On the contrary, when using a lower MDD cutoff at 0.1% (10⁻³), patients with circulating T lymphoblasts responded relatively well to ALL-type therapy. This is in keeping with the, at least, comparable outcome of Stage IV (5-y EFS 88%) compared to stage III (EFS 78%) patients in the EURO-LB02 trial. Reasons for this discrepancy are not evident, but may result from differences in treatment compared to A5971. This is in keeping with the loss of prognostic significance of MDD in the COG AALL0434 trial, interpreted to result from a modified interim phase with escalating intravenous methotrexate (Capizzi MTX)/pegasparaginase regimens. However, the EURO-LB02 used a HD-MTX regime similar to A5971, while achieving similar results to AALL0434. In light of our data, it would be interesting to compare MDD outcome in AALL0434 using a 0.1% cutoff in patients with known N/F status, although comparisons are hampered by use of different use of MFC and ddPCR for MDD assessment. MDD low/negative values here could be due to prior steroids, since only systemic corticotherapy was an exclusion criterion, but this seems unlikely to explain the poor prognosis, since corticosensitivity is usually associated with a relatively good outcome.

Given the relatively favorable outcome of N/Fmut in pediatric T-LL, this parameter is used to randomize patients for treatment intensification in the LBL2018 trial. The incidence of N/Fmut is slightly higher in the present cohort of patients compared to our initial assessment (66% versus 55%); at least partly due to the replacement of Sanger screening by next-generation-sequencing capture screening for N/F status.

The impact of MDD status was strikingly different in N/Fmut and N/FGL patients. Whereas there was no prognostic impact in N/Fmut patients, MDD below 0.1% clearly identified N/FGL patients at risk of relapse. These observations suggest that significant dissemination to PB/BM may correlate with sensitivity to ALL-type therapy. Conversely, purely tissue-based disease with low/undetectable MDD negativity may be less sensitive. One therapeutic possibility for the latter group might be proteasome inhibitors, given the demonstration in the COG AALL1231 trial that Bortezomb was of benefit for de novo pediatric T-LL but not T-ALL. Anti-CD38 immunotherapy could also be considered.

Our data encourage addition of MDD assessment for patients in the LBL2018 trial, preferably by central ddPCR or qPCR, given the superior sensitivity compared to flow cytometry, as practiced here, and since it is better adapted to retrospective analysis.

MRD evaluation in a limited number of patients, predominantly by MFC, confirmed previous reports for poor outcome. It failed, however, to detect the majority of relapses and will be difficult to undertake in purely nodal, MDD low/negative patients. Since a disproportionate number of positive results were from the minority of samples assessed by ddPCR, this technique would seem preferable to MFC for MRD assessment. Although it will be possible to perform MRD evaluation in the majority of patients, its value relative to combined oncogenetic/MDD stratification should be evaluated within N/F defined subgroups, as should its prognostic value compared to imaging evaluation. Whether patients with MDD low/negative status should be screened for MRD emergence (presuming that tumor immunogenotype/phenotype can be determined from diagnostic tissue) is unclear, but our data illustrate that this is unlikely to be as useful as in T-ALL, somewhat complicating inclusion of T-LL in MRD-driven T-ALL protocols.

In practical terms, pediatric T-LLs with N/Fmut respond very well to current standard protocols and MDD status adds little/nothing to their risk assessment. Most would be easily MRD accessible, since 82% demonstrated at least 0.01% PB/BM clonal dissemination at diagnosis, but their excellent outcome will make it difficult to demonstrate added value of MRD stratification, and MRD kinetics may differ in T-LL and T-ALL. Although the small number of relapsing cases precludes definitive assessment, of the 6 N/F evaluated relapses with MRD evaluation, only two were MRD positive (Supplemental Digital Table S2, http://links.lww.com/HS/A196). Among N/FGL patients, MDD <0.1% status represents a promising means of rapidly identifying patients with a very high relapse risk, if confirmed in other T-LL trials with larger patient numbers. Approximately 80% would be MRD accessible, and the impact of MRD in this small N/FGL, MDD low/neg. subgroup (8/57, 14%) should also be evaluated prospectively, although this will require large patient numbers and universal availability of diagnostic tissue for baseline molecular and immunophenotypic assessment. It will also be important to evaluate other oncogenetic markers in these patients, who merit consideration for alternative front line therapy.

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**Disclosures**

The authors have no conflicts of interest to disclose.

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