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Authors

Kirkey, Danielle

Robinson, Leila

Peplinski, Jack

et al.

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Prognostic impact of cooccurring mutations in *FLT3*-ITD pediatric acute myeloid leukemia

Katherine Tarlock,[1,2] Robert B. Gerbing,[3] Rhonda E. Ries,[2] Jenny L. Smith,[2] Amanda Leonti,[2] Benjamin J. Huang,[4,5] Danielle Kirkey,[1,2] Leila Robinson,[2] Jack H. Peplinksi,[2] Beverly Lange,[6] Todd M. Cooper,[1] Alan S. Gamis,[7] E. Anders Kolb,[8] Richard Aplenc,[6] Jessica A. Pollard,[9,10] Todd A. Alonzo,[3,11] and Soheil Meshinchi[2]

[1]Division of Hematology/Oncology, Seattle Children's Hospital, Seattle, WA; [2]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA; [3]Children's Oncology Group, Monrovia, CA; [4]Department of Pediatrics, University of California San Francisco, San Francisco, CA; [5]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA; [6]Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [7]Divisions of Hematology/Oncology, Children's Mercy Hospital and Clinics, Kansas City, MO; [8]Nemours Center for Cancer and Blood Disorders, Alfred I. DuPont Hospital for Children, Wilmington, DE; [9]Pediatric Oncology, Dana Farber Cancer Institute, Boston, MA; [10]Department of Pediatrics, Harvard Medical School, Boston, MA; and [11]University of Southern California Keck School of Medicine, Los Angeles, CA

Key Points

- Cooccurring mutational profile and not allelic ratio determines clinical outcomes for patients with *FLT3*-ITD.

- Therapy intensification improves survival for patients with *FLT3*-ITD; however, those with cooccurring poor-risk mutations still fare poorly.

We sought to define the cooccurring mutational profile of *FLT3*-ITD–positive (ITD^{pos}) acute myeloid leukemia (AML) in pediatric and young adult patients and to define the prognostic impact of cooperating mutations. We identified 464 patients with *FLT3*-ITD mutations treated on Children's Oncology Group trials with available sequencing and outcome data. Overall survival, event-free survival (EFS), and relapse risk were determined according to the presence of cooccurring risk stratifying mutations. Among the cohort, 79% of patients had cooccurring alterations across 239 different genes that were altered through mutations or fusions. Evaluation of the prognostic impact of the cooccurring mutations demonstrated that patients with ITD^{pos} AML experienced significantly different outcomes according to the cooccurring mutational profile. Patients with ITD^{pos} AML harboring a cooccurring favorable-risk mutation of *NPM1*, *CEBPA*, t(8;21), or inv(16) experienced a 5-year EFS of 64%, which was significantly superior to of 22.2% for patients with ITD^{pos} AML and poor-risk mutations of *WT1*, *UBTF*, or *NUP98::NSD1* as well to 40.9% for those who lacked either favorable-risk or poor-risk mutation (ITD^{pos} intermediate; *P* < .001 for both). Multivariable analysis demonstrated that cooccurring mutations had significant prognostic impact, whereas allelic ratio had no impact. Therapy intensification, specifically consolidation transplant in remission, resulted in significant improvements in survival for ITD^{pos} AML. However, patients with ITD^{pos}/*NUP98::NSD1* continued to have poor outcomes with intensified therapy, including sorafenib. Cooccurring mutational profile in ITD^{pos} AML has significant prognostic impacts and is critical to determining risk stratification and therapeutic allocation. These clinical trials were registered at www.clinicaltrials.gov as NCT00002798, NCT00070174, NCT00372593, and NCT01371981.

Introduction

Mutations in *FLT3*, specifically internal tandem duplications (*FLT3*-ITD), occur in 10% to 30% of pediatric and young adult acute myeloid leukemia (AML) cases.[1-6] *FLT3*-ITD mutations are associated with adverse prognosis, and allelic ratio (AR) is reportedly a mediating factor, with patients with high AR (HAR) *FLT3*-ITD having very poor survival when treated with chemotherapy alone.[2,7] Thus, AR has been used for risk stratification by many cooperative groups and in trials across age groups. Intensive consolidation with hematopoietic stem cell transplantation (HCT) improves survival for patients with HAR *FLT3*-ITD.[7-10] *FLT3* mutations have been effectively targeted with FLT3 inhibitors (FLT3i) for therapeutic intervention, with improved outcomes with the addition of FLT3i to chemotherapy and as maintenance after HCT.[11-16]

Despite intensive therapy with HCT and FLT3i therapy, many patients with *FLT3*-ITD still experience relapse.[8,17] Even among low AR (LAR) and HAR subgroups, the outcomes are heterogeneous, with many patients with LAR failing to achieve cure and many patients with HAR relapsing despite therapy intensification.[8,11,18] Thus, we hypothesized that factors beyond AR may be able to refine prognosis in pediatric and young adult patients with *FLT3*-ITD AML. In a large cohort of patients with *FLT3*-ITD, we sought to interrogate the mutational spectrum and to evaluate retrospectively the prognostic impact of additional mutations, specifically those that may otherwise be used for risk stratification, and in the context of AR. We also evaluated the outcomes of patients with *FLT3*-ITD across treatment trials and in the context of intensified and targeted therapy with the use of HCT in first complete remission (CR1) and FLT3i.

Materials and methods

Patients and treatments

Our cohort included 3033 pediatric and young adult patients (aged 1 month-29 years) with de novo AML enrolled on successive clinical trials from the Children's Cancer Group (CCG)/Children's Oncology Group (COG; CCG2961, [ClinicalTrials.gov identifier: NCT00002798; n = 610], COG AAML03P1 [ClinicalTrials.gov identifier: NCT00070174; n = 270], COG AAML0531 [ClinicalTrials.gov identifier: NCT00372593; n = 924], and COG AAML1031 [ClinicalTrials.gov identifier: NCT01371981; n = 1229]). Treatment protocol details have been described previously.[12,19-22] *FLT3*-ITD was used in the risk stratification of some patients on AAML0531 after an amendment, and for all patients on AAML1031 with an AR of >0.4 considered high risk, and who were allocated to HCT in CR1 if a donor was available. Additionally, on AAML1031 those same patients were also eligible to receive the FTL3i sorafenib in combination with chemotherapy and as post-HCT maintenance. Protocols were approved by the institutional review boards at each participating center. All studies were conducted in accordance with the Declaration of Helsinki.

Mutational analysis

Diagnostic bone marrow or peripheral blood from patients was tested for *FLT3*-ITD, *NPM1*, *CEBPA*, *WT1*, and *NUP98::NSD1* mutations and conventional karyotyping was performed on all patients with available specimen. Testing for the *NUP*98::NSD1

fusion, which can be cryptic, was performed on all FLT3-*ITD* samples from CCG2961, COG AAML03P1, and COG AAML0531 using reverse transcription polymerase chain reaction, as previously described, whereas all samples on COG AAML1031 had fusion detected by genomic sequencing.[23] Additionally, specimens underwent comprehensive sequencing with either targeted-capture sequencing using a panel of 338 genes (n = 788), whole-genome sequencing (n = 329), and/or transcriptome sequencing (n = 1782).[24] Among *FLT3*-ITD cases, samples underwent at least 1, and in some cases multiple, sequencing methodologies including targeted-capture (n = 125), whole-genome (n = 32), or transcriptome (n = 328) sequencing for identification of cooperating mutations and fusions (supplemental Figure 1). Determination of *FLT3*-ITD AR was performed after polymerase chain reaction amplification as previously described.[7]

Statistical methods

Patients were defined as being in CR if they had <5% blasts and absence of extramedullary disease after completion of first induction course. In cases for which measurable residual disease (MRD) data were available, remission without evidence of MRD was defined as <0.1% blasts in the bone marrow detected by flow cytometry. The Kaplan-Meier method was used to estimate survival outcomes.[25] Overall survival (OS) was defined as time from study entry to death; and event-free survival (EFS) was defined as time from study entry until death, induction failure, or relapse of any type. Disease-free survival (DFS) was defined as time from the end of induction 1 for patients in CR until relapse or death from any cause; and relapse rate (RR) was defined as time from end of induction 1 for patients in CR to relapse, for which deaths in the absence of relapse were considered competing events.[26] The significance of predictor variables was tested using log-rank statistic for OS, EFS, and DFS and Gray statistic for RR.[27,28] Outcome estimates at 5 years were summarized with their corresponding log-log 95% confidence intervals (CI). For analyses that violated the proportional hazards assumption, a direct comparison (landmark analysis) between the 5-year estimates was summarized instead of the log-rank statistic. Patients lost to follow-up were censored at the time of last contact. The significance of observed difference in proportions was analyzed by the χ^2 test between patient groups, and the Fisher exact test was used if the data were sparse. The Kruskal-Wallis test was used to determine the significance between differences in medians of groups. Cox proportional hazards models were used to estimate hazard ratios for multivariable analyses of OS and EFS.[29] Competing risk regression models were used to estimate the subgroup hazard ratios for multivariable analyses of RR.[30] Patients receiving HCT in CR were analyzed as a time-varying covariate.

Results

Patient characteristics

Of 3033 patients, *FLT3*-ITD mutations were identified in 464 (15.3%) patients treated on the following trials: CCG2961 (n = 74), AAML03P1 (n = 30), AAML0531 (n = 149), and AAML1031 (n = 211). Patients with a *FLT3*-ITD mutation (ITD^pos) were older than patients who did not have *FLT3*-ITD (non-ITD; median age 13.2 vs 9.1 years [$P < .001$]) and had higher diagnostic white blood cell counts and blast percentage (supplemental Table 1).

Mutational profile

Among 464 patients with ITD[pos] AML, cooccurring alterations were identified in 79% of the cohort in 239 distinct genes; 217 with single gene mutations and 22 altered by fusions; the median number of cooccurring mutations per patient was 3 (range, 0-25). A heterogeneous mutational profile was observed, with cooccurring missense and truncating mutations, copy number variants, as well as fusions detected (Figure 1). The most common cooccurring alterations were detected in *WT1* (n = 141, 30.4%), *NPM1* (n = 85, 18.3%; mutations, n = 81 and fusions, n = 4), and *NRAS* (n = 42, 9.1%). *WT1* and *NPM1* mutations were significantly more common in patients with ITD[pos] vs those with non-ITD, 30.7% vs 7.2% and 18.7% vs 6.3%; *P* < .001 for both. In addition, among patients with known results, we found *UBTF* alterations and *KMT2A*-partial tandem duplications significantly more common among patients with ITD[pos] vs those with non-ITD, 15.7% vs 10.1% and 3% vs 1.2%; *P* < .001 for both,. The most common fusions involved the nucleoporin *(NUP)* genes with *NUP98::NSD1* (n = 83, 17.9%) and *DEK::NUP214*/t(6;9) (n = 35, 7.5%), these were also significantly more common in patients with ITD[pos] vs those without (*P* ≤ .001 for both). Trisomy 8 was the most common recurring cytogenetic abnormality (n = 58, 12.5%) and significantly more common than in patients with non-ITD (*P* < .001, supplemental Table 1).

Outcomes for ITD[pos] vs non-ITD

Patients with ITD[pos] had significantly inferior end of induction I CR and higher MRD rates compared with patients with non-ITD (supplemental Table 1). Evaluations of outcomes across the entire cohort demonstrated that ITD[pos] status was associated with inferior outcomes compared with non-ITD status; 5-year EFS of 39.0% (95% CI, 34.4-43.5) vs 47.7% (95% CI, 45.7-49.6; *P* < .001) and OS of 53.8% (95% CI, 49.0-58.3) vs 63.3% (95% CI, 61.3-65.1; *P* < .001; supplemental Figure 2). Changes were made to therapy across the different treatment eras and studies; specifically, patients with HAR ITD[pos] were designated as being at high risk and were recommended for HCT in CR1 after an amendment to AAML0531, and those on AAML1031, in which they also were eligible to receive sorafenib. Evaluation according to treatment trial demonstrated that outcomes for patients with ITD[pos] improved significantly from CCG2961 to AAML1031, with a 5-year EFS of 26.9% (95% CI, 17.2-37.5) vs 46.5% (95% CI, 39.5-53.1; *P* = .007), and a corresponding drop in RR from 62.1% (95% CI, 46.4-74.4) to 32.7% (95% CI, 25.0-40.6); *P* = .002; supplemental Figure 3). In the 3 earlier studies, EFS and OS were significantly inferior for patients with ITD[pos], with a trend toward higher RR than for non-ITD; however, in AAML1031, outcomes were similar for patients with ITD[pos] and those with non-ITD (supplemental Figure 3).

Impact of cooccuring mutations on outcome

We stratified patients with ITD[pos] overall according to presence of cooccuring mutations. We initially evaluated the outcome of patients with ITD[pos] with mutations that have been previously recognized to be associated with either favorable-risk [*NPM1*, *CEBPA*, *RUNX1::RUNX1T1*/t(8;21), and *CBFB::MYH11*/ inv(16)/t(16;16)] or high-risk [*NUP98::NSD1*, *DEK::NUP214*/
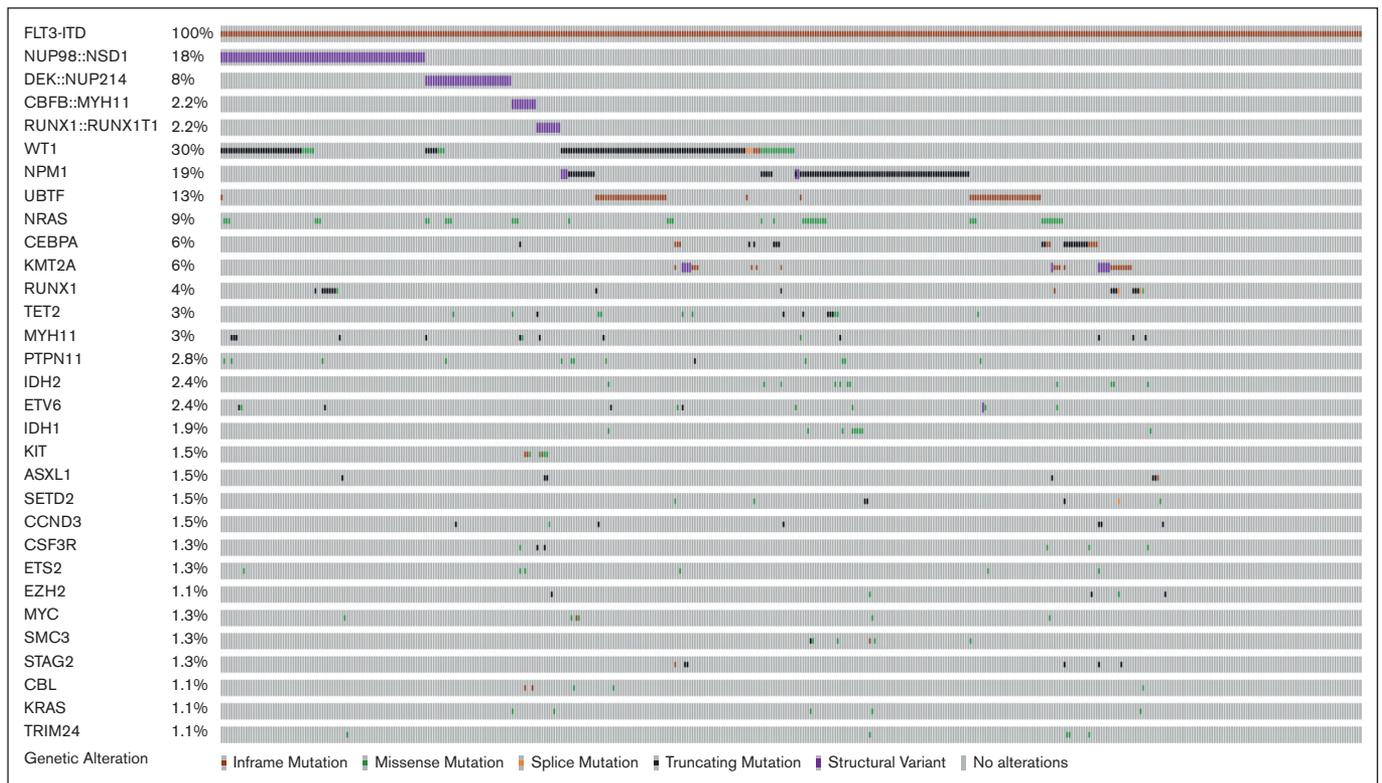

Figure 1. Cooccuring alterations in pediatric and young adult *FLT3*-ITD AML. Genes with alterations, including missense and truncating mutations and fusions with a frequency of >1%.
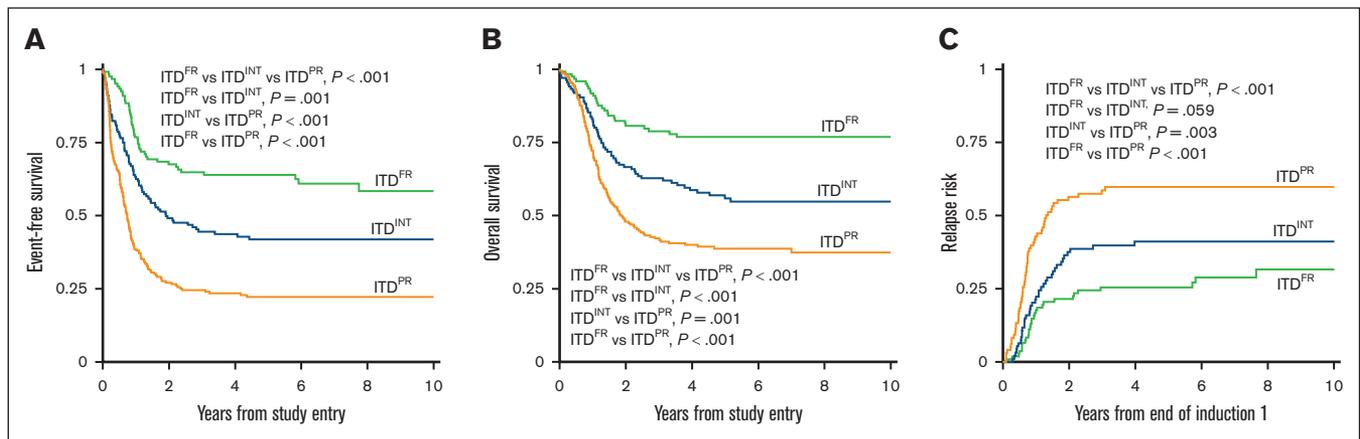
Figure 2. Outcomes for patients with ITD^pos according to cooccurring risk groups of FR mutation, INT, or PR mutations. (A) 5-year EFS, (B) 5-year OS, and (C) 5-year relapse risk.

t(6;9)] disease. We also evaluated the outcome of patients with a cooccurring *WT1* or *UBTF* because both of these are reportedly associated with inferior outcomes in *FLT3*-ITD AML.[31-33] Patients with ITD^pos with both *NPM1* and *WT1* mutations were included in the *WT1* cohort. There was also overlap of *WT1* and *UBTF* alterations, and those with both were included in the *WT1* cohort; thus, patients in the *UBTF* cohort lacked *WT1*. Outcomes (EFS, OS, and RR) varied significantly for patients with ITD^pos according to their cooccuring mutational profile and those lacking any of the above mutations (supplemental Figure 4). Based on outcomes according to these cooccurring mutations, we subsequently grouped patients with ITD^pos into 3 distinct groups for subsequent analyses. Patients with *NPM1, CEBPA, RUNX1::RUNX1T1,* or *CBFB::MYH11* and who lacked a cooccurring mutation that was considered to be unfavorable were grouped together for subsequent analyses and classified as favorable-risk ITD (ITD^FR; n = 122; 26.3%). In contrast, *WT1* and *UBTF* mutations and *NUP98::NSD1* fusions were found to associated with adverse outcomes, and we found that 44.3% of patients with ITD^pos (n = 206) had a cooccurring poor-risk (PR) mutation (ITD^PR). The remaining 29.3% (n = 136) of patients with ITD^pos lacked the above risk stratifying mutations and were defined as ITD^pos intermediate (ITD^INT). Our analyses found that patients with ITD^pos with cooccurring *DEK::NUP214* had significantly improved outcomes compared with those in the ITD^PR cohort. While Although this group overall, regardless of ITD status, has been associated with unfavorable outcomes in prior studies but improved with HCT in CR1,[34,35] nearly half of patients with *DEK::NUP214* in our analysis received HCT in CR. Thus, for our subsequent analyses, patients with *DEK::NUP214* were classified as ITD^INT.

Among the ITD^pos cohort, patients were stratified according to the cooccurring risk mutations of ITD^FR, ITD^INT, and ITD^PR (supplemental Table 2). Analysis by cooccurring mutational group demonstrated significantly different CR rates: ITD^FR, 91.6% vs ITD^INT, 70.1% vs ITD^PR, 49.7% (P < .001). CR1 rates were similar among the ITD^FR vs ITD^WT-FR cohorts (91.6% vs 87.9%, P = .238) and among ITD^INT vs ITD^WT-INT cohorts (70.1% vs 72.5%, P = .556). Analysis according to end of induction I MRD-negative status demonstrated similar findings among the risk-defined cohorts:

ITD^FR, 87.6% vs ITD^INT, 54.9% vs ITD^PR, 31.6% (P < .001). Again, no significant difference was observed between those in the ITD^FR vs ITD^WT-FR cohorts (87.6% vs 84.2%, P = .378).

Analysis of outcomes for patients with ITD^pos demonstrated striking differences when stratified according to the cooccurring risk-stratifying mutations. Patients with ITD^FR experienced superior outcomes compared with those with ITD^INT and ITD^PR (P < .001 for both OS and EFS; Figure 2). Notably, patients with ITD^PR experienced outcomes that were significantly inferior to both those with ITD^FR and those with ITD^INT. This inferior EFS was driven by relapse, with patients with ITD^PR experiencing significantly higher RR than those with ITD^INT (P = .003) and those with ITD^FR (P < .001; Figure 2). Outcomes of the ITD^FR cohort compared with patients without ITD with the same cooccurring FR features (non-ITD^FR) were nearly identical (EFS: 64.0% [95% CI, 54.6-71.9] vs 65.1% [95% CI, 61.9-68.1], P = .547), as were those for ITD^INT vs non-ITD without risk-stratifying lesions (non-ITD^INT; EFS: 41.9% [95% CI, 33-4-50.1] vs 38.4% [95% CI, 35.9-40.9], P = .230). There were also no significant outcome differences among those in the ITD^PR cohort compared with those in the non-ITD with cooccurring PR (non-ITD^PR) cohort, although there was a signal of inferior outcomes the patients with ITD^PR (EFS: 22.2% [95% CI, 16.7-28.2] vs 29.7% [95% CI, 22.1-37.6], P = .065; Table 1; supplemental Figure 5).

Impact of AR

We evaluated the impact of AR among the different cooccurring risk mutation groups with a cutoff of >0.4 and ≤0.4 considered HAR and LAR, respectively, to align with designated cutoffs of AAML0531 and AAML1031. The ITD^PR group had a higher prevalence of HAR (70.4%) vs LAR (29.6%) disease and had significantly higher prevalence of HAR disease than ITD^FR and ITD^INT subgroups (P < .001). In contrast, patients with ITD^FR had nearly equivalent prevalence of HAR vs LAR (49.2% vs 50.8%) and the prevalence of HAR disease was significantly less in patients with FR compared with those with non-FR disease (49.2% vs 67.8%, P < .001). Analysis in each of the ITD^pos subgroups (FR, INT, and PR) found no significant differences in EFS, OS, or RR in patients with LAR vs those with HAR (Figure 3). Multivariable regression analysis demonstrated that cooccurring mutational profile but not AR affected outcomes (Table 2).

Table 1. Outcomes for patients without *FLT3*-ITD and those with *FLT3*-ITD[pos] according to cooccurring mutation risk groups

	Non-*FLT3*-ITD		*FLT3*-ITD[pos]		
	N	**%, 95 CI**	**N**	**%, 95 CI**	**P value**
FR mutations					
5-year OS	931	81.5%, 78.9%-83.9%	122	76.9%, 68.1%-83.5%	.357
5-year EFS	931	65.1%, 61.9%-68.1%	122	64.0%, 54.6%-71.9%	.547
5-year relapse risk from EOI1	807	25.3%, 22.3%-28.4%	109	25.5%, 17.6%-34.1%	.506
INT risk mutations					
5-year OS	1502	53.2%, 50.6%-55.8%	136	55.9%, 46.8%-63.9%	.372
5-year EFS	1502	38.4%, 35.9%-40.9%	136	41.9%, 33.4%-50.1%	.230
5-year relapse risk from EOI1	1064	47.4%, 44.3%-50.4%	94	41.1%, 30.9%-51.0%	.104
PR mutations					
5-year OS	136	49.1%, 40.2%-57.4%	206	38.7%, 31.8%-45.5%	.093
5-year EFS	136	29.7%, 22.1%-37.6%	206	22.2%, 16.7%-28.2%	.065
5-year relapse risk from EOI1	90	53.5%, 42.4%-63.3%	98	59.8%, 49.2%-63.9%	.323

Cooccurring mutation risk groups stratified according to favorable (*NPM1, CEBPA, RUNX1-RUNX1T1, CBFB-MYH11*), poor (*WT1, UBTF, NUP98-NSD1*), and intermediate (all other) risk mutations.

EOI1, endo of induction 1.

Impact of treatment intensification with HCT and sorafenib

Analysis of outcomes according to treatment trial demonstrated overall improvements in survival in patients with ITD[pos]. Multivariable analysis with treatment analyzed according to the type of therapy received (eg, chemotherapy, gemtuzumab ozogamicin, sorafenib and HCT, and HCT alone) demonstrated the significant impact of specific interventions in patients with ITD[pos]. We found that patients treated on arm C of AAML1031 (sorafenib + HCT in CR1) had improved EFS and RR, and that HCT in CR on its own also resulted in significant improvements in OS, EFS, and RR (Table 2). Given our findings for patients with *DEK-NUP214* in the cohort overall, we analyzed outcomes specifically for patients with *DEK-NUP214* who received HCT in CR1, and they achieved a 5-year DFS of 84.6% (95% CI, 51.2-94.9).

Although outcomes improved overall for patients with ITD[pos] and were comparable with those for patients with ITD[WT] treated on AAML1031 and that we saw benefit of intensification approaches on arm C with sorafenib and HCT in CR1, we found significant outcome differences according to cooccurring mutations. Among patients with ITD[pos] treated on arm C, differences among cooccurring mutational risk groups persisted, with a 5-year EFS of 75.0% (95% CI, 50.0-88.7) for ITD[FR] vs 67.9% (95% CI, 44.1-83.2) for ITD[INT] vs 30.8% (95% CI, 19.0-33.5) for ITD[PR] (P < .001), with similar findings in OS and RR (supplemental Table 3). With continued inferior outcomes for patients with PR, we sought to determine whether any of the PR subgroups experienced differential benefit from therapy intensification. We found that patients with *NUP98::NSD1* continued to experience poor outcomes despite these intensifications in therapy, with a 5-year EFS of 7.9% (95% CI, 0.7-27.7) vs 46.2% (95% CI, 27.9-62.7; P = .021) in the group of patients with ITD[PR] who did not harbor a *NUP98::NSD1* (Figure 4); similar trends were seen for OS and RR (supplemental Figure 6). Analysis of the patients with ITD[FR] treated on AAML1031 found no differences according to treatment
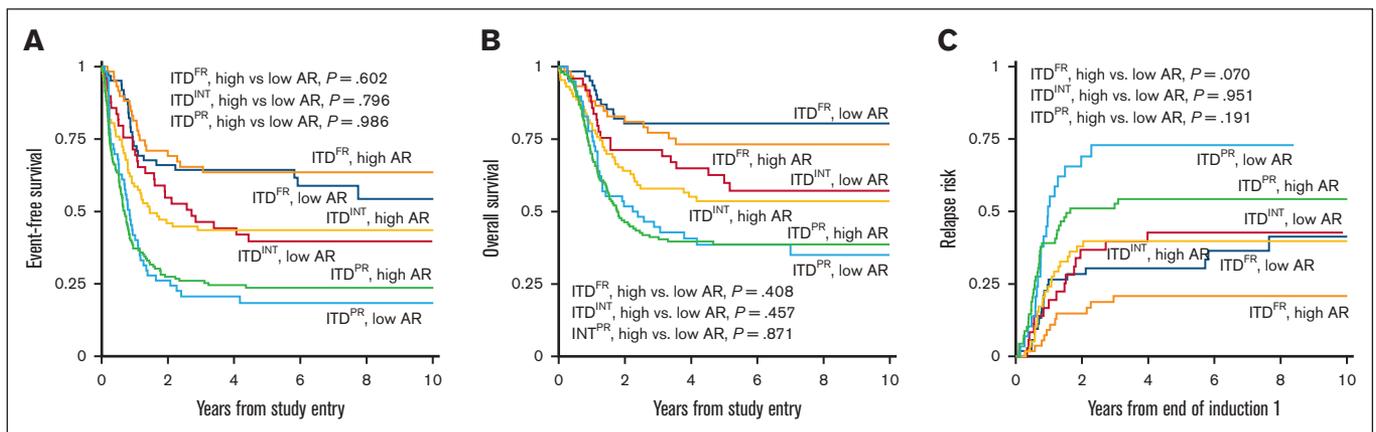

Figure 3. Outcomes for patients with LAR ITD[pos] (≤0.4) vs HAR ITD[pos] (>0.4) according to cooccurring risk group. (A) 5-year EFS, (B) 5-year OS, (C) 5-year relapse risk from end of induction 1.

Table 2. Multivariable regression analysis for EFS, OS, and RR

|		EFS			OS		Relapse risk end course 1		
	n	HR (95% CI)	*P* value	HR (95% CI)	*P* value	n	HR (95% CI)	*P* value
ITD^{FR}	118	1		1		105	1	
ITD^{INT}	134	1.91 (1.30-2.78)	**.001**	2.07 (1.3-3.31)	**.002**	92	1.74 (1.07-2.84)	**.027**
ITD^{PR}	202	3.70 (2.61-5.24)	**<.001**	3.48 (2.26-5.37)	**<.001**	95	3.87 (2.44-6.14)	**<.001**
LAR	166	1		1		112	1	
HAR	288	1.25 (0.83-1.45)	.097	1.29 (0.94-1.76)	.117	180	1.17 (0.79-1.73)	.431
Chemotherapy treatment	256	1		1		160	1	
Gemtuzumab ozogamicin treatment	110	1.10 (0.83-1.45)	.526	1.06 (0.77-1.47)	.723	70	0.69 (0.44-1.10)	.118
Sorafenib + HCT in CR1 (arm C AAML1031)	88	0.63 (0.43-0.93)	**.019**	0.81 (0.52-1.26)	.355	62	0.30 (0.15-0.61)	**.001**
HCT in CR not received	291	1		1		163	1	
HCT in CR received (TVC)	163	0.60 (0.43-0.83)	**.002**	0.62 (0.44-0.87)	**.006**	129	0.57 (0.37-0.90)	**.016**

Multivariable regression analysis for EFS, OS, and RR according to cooccurring risk mutation group (FR, INT, PR), LAR (≤0.4) vs HAR (>0.4), treatment received, and HCT in CR as TVC.
Patients in the chemotherapy treatment group included patients on CCG2961, AAML0531 arm A, and AAML1031 arm A/B, patients in the gemtuzumab ozogamicin treatment group included patients treated on AAML03P1 and AAML0531 arm B, and patients in the sorafenib + HCT in CR1 group were those on AAML1031 arm C.
HR, hazard ratio; TVC, time-varying covariate.

arm/intensity, with patients who received chemotherapy on arm A/B having similar outcomes to those treated with sorafenib and HCT in CR on arm C (supplemental Table 4). We subsequently compared outcomes for patients with ITD^{FR} HAR who were risk stratified to HCT in CR1 on AAML1031 or AAML0531 with those of patients treated on earlier studies (CCG2961, AAML03P1, and before amendment on AAML0531) in which AR was not used as a risk stratifying feature and found no differences in outcomes (supplemental Table 5).

Discussion

Our findings demonstrate that, in *FLT3*-ITD AML, cooccurring mutations significantly affect treatment responses and prognosis.

We demonstrate that the cooccurring mutational profile, not AR, is the most important prognostic feature in ITD^{pos} AML and that, in the setting of incorporation of mutational profile, AR loses its prognostic significance. We show that presence of a cooccurring FR mutation in patients with ITD^{pos} identifies a cohort with favorable outcomes that may not require HCT in CR1. Concurrent *NPM1* is generally considered a risk-modifying feature in *FLT3*-ITD AML in adults; however, findings regarding the impact on outcome are varied, especially when accounting for the impact of AR.[8,36-40] Favorable survival has been reported in a small cohort of pediatric patients with dual ITD/*NPM1* in the Japanese Pediatric Leukemia/Lymphoma Study Group AML-05 study.[41] Cooccurrence of ITD and *CEBPA* and core binding factor (*CBF*) fusions have rarely been reported in adults,[42,43] but in our pediatric cohort we

Figure 4. EFS for patients with ITD^{pos} treated on arm C of AAML1031 with sorafenib and HCT in CR1 according to cooccurring risk groups (FR, INT, and PR) and those with PR mutations further stratified according to presence of *NUP98-NSD1* fusion.
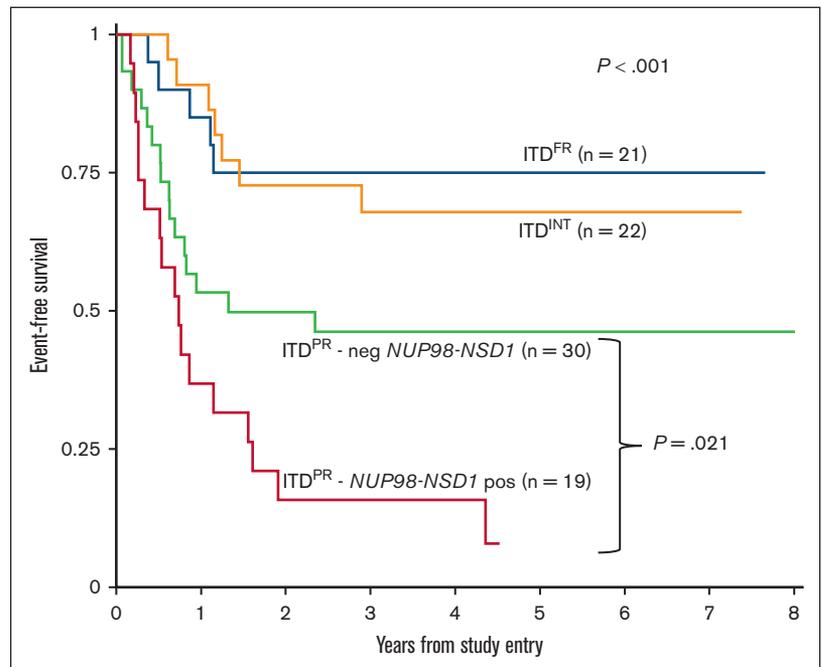
observed a nontrivial overlap with these lesions, highlighting the importance of recognizing favorable cooperating events outside of *NPM1*. Future studies that prospectively evaluate the outcomes of patients with ITD[FR] HAR with appropriate response to initial therapy treated with chemotherapy alone will help more definitively define the outcomes of these patients. Although *FLT3*-ITD may not act as the leukemia initiating event in patients with FR, biologically there is likely an effect that may derive benefit from FLT3i. For patients with dual *FLT3*-ITD/*NPM1*, a trend toward improved outcomes with midostuarin on the RATIFY trial has been shown, as well as improved outcomes with sorfenib when it was also used as post-HCT mainteance.[39,44]

Our findings demonstrate that cooperating mutational status and not AR affects outcomes for patients with ITD[pos]. The prognostic impact of diagnostic AR has been subject to inconsistency, with cooperative groups and clinical trials designating variable cutoffs of HAR vs LAR.[7,8,11] Determination of AR is affected by multiple factors including blast percentage and assay. Notably, FLT3i therapy, thus far, has resulted in therapeutic benefit across a wide range of ARs, including what has been considered lower ARs.[11,16,45] Further studies are important to determine whether AR may be important in predicting which patients derive the most benefit from FLT3i therapy. We found that HAR disease was more prevalent among patients with ITD[PR] and ITD[INT], thus AR may, in some situations, serve as a surrogate for other higher-risk disease features. Importantly, our findings show that that pediatric patients with ITD[pos] without a cooccurring FR lesion should be allocated to HCT in CR1 regardless of AR. This aligns with recent European Society for Blood and Marrow Transplation recommendations in adult AML.[46]

Treatment advances for patients with ITD[pos] including the incorporation of gemtuzumab ozogamicin, FLT3i therapy, and allogeneic HCT in CR1 have been shown to result in incremental improvements in survival.[8,10,11,16,47,48] Our findings support this; specifically, we show that HCT in CR1 and the combination of sorafenib and HCT in CR1 resulted in significantly improved outcomes in a multivariable analysis. However, our study also highlights that, among patients with ITD[pos], those in the ITD[PR] group have generally continued to experience significantly inferior outcomes compared with those with other cooccurring mutations; importantly, among this group, the gains have been uneven. Our findings regarding the prognostic impact of patients with ITD[pos]/*DEK::NUP214* being classified as having an INT and not a PR lesion, likely reflects the beneficial response to intensified therapy, specifically HCT in CR1 that this group experiences; thus, they should still receive intensified therapy and can achieve quite good outcomes with this therapy. Earlier studies have suggested that patients with *DEK::NUP214* experienced improved outcomes when *FLT3*-ITD HAR started being used as a risk-stratifying lesion and those patients were allocated to HCT in CR1.[34] Our findings align with a recent study in adults with ITD[pos]/*DEK::NUP214* AML that found HCT in CR1 significantly improved outcomes compared with chemotherapy.[49]

We demonstrate early dismal responses to therapy and poor survival in *NUP98::NSD1* AML. This supports recent findings that *FLT3*-ITD cooccurring with *WT1, UBTF*, or *NUP98-NSD1* is associated with significantly inferior prognosis.[23,31-33,50] Although there is significant overlap in *WT1* and *UBTF* among patients with

ITD[pos], we show that poor outcome was seen in patients with mutant *UBTF* independent of *WT1* status. Our findings highlight the particularly dismal responses to therapy and poor survival that has persisted despite therapy intensification among patients with *NUP98-NSD1* fusion. This, to our knowledge, is the first analysis of response of patients with ITD[pos]/*NUP98::NSD1* to FLT3i, and we show that sorafenib failed to have any benefit. Our findings suggest that, overall, FLT3 inhibition is not an effective target for therapeutic intervention in *NUP98::NSD1* AML. The unique biology of this group manifests clinically as poor responses to chemotherapy, including FLT3i. Our findings support previous studies demonstrating distinct gene expression profile for *NUP98::NSD1* AML.[50,51] Understanding the biology of this group may provide insights into potential targets for intervention.[52,53] Novel strategies are needed and should be prioritized early in therapy for these patients. The cohort of patients with ITD[PR] with *WT1* and *UBTF* alterations continued to have comparatively inferior outcomes to the ITD[FR] and ITD[INT] cohorts but were improved compared with those of patients with *NUP98::NSD1*. Further studies are needed to determine the relative degree of benefit of FLT3i in other PR subgroups.

The inclusion of patients across multiple studies receiving different treatments is a limitation of our study because there were significant evolutions in treatment for ITD[pos] AML over the study period. Some of the patients with cooccurring FR mutations and HAR treated on the later studies would have received HCT, which may have affected outcomes. However, inclusion of multiple studies allowed us to compare the impact of treatment changes, specifically intensification efforts with HCT consolidation and FLT3i. Our study did include post hoc analyses because outcome of *FLT3*-ITD AML was not a major aim of the studies except for patients with HAR ITD[pos] treated on AAML1031. However, given the frequency of the *FLT3*-ITD mutations in pediatric AML, a larger cohort than is generally included in a study was required to study the cooccurring mutational subgroups. Independent validation in additional cohorts is needed to validate our findings, and future studies that prospectively evaluated risk stratified treatments among patients with ITD[pos] will be important to confirm these findings.

We demonstrate that the incorporation of comprehensive cooccurring mutational profiling is the most critical factor in refining prognosis and appropriate risk and therapeutic stratification for patients with ITD[pos] and should be used instead of AR in determining risk allocation. We also show that therapy intensification, specifically the use of sorafenib and HCT in CR1 has resulted in significant improvements in outcome for patients with ITD[pos]. Although *FLT3*-ITD has generally been considered a high-risk feature for which HCT in CR1 is needed, we demonstrate that patients with cooccurring FR lesions may not require this degree of intensification. Additionally, although some patients with ITD[pos] AML greatly benefit from therapy intensification and can achieve very good outcomes, to date, patients with *NUP98::NSD1* fusions have not benefited from approaches, and further efforts to study the early intervention of novel and targeted therapies are urgently needed.

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Authorship

Contribution: K.T. and S.M. conceived of, and initiated, the study; R.B.G. and T.A.A. performed statistical analysis and helped generate figures; J.L.S., R.E.R., A.L., B.J.H., D.K., L.R., and J.H.P. performed genomic analyses; B.L., T.M.C., A.S.G., E.A.K., R.A., and J.A.P. oversaw the clinical trials and contributed data; K.T. wrote the initial manuscript draft; and K.T. and S.M. edited the manuscript with input from all authors.

ORCID profiles: K.T., 0000-0001-9717-2973; J.L.S., 0000-0003-0402-2779; B.J.H., 0000-0001-6996-0833; D.K., 0000-0001-5176-9416; J.H.P., 0000-0001-5097-0652; A.S.G., 0000-0003-1513-2893.

Correspondence: Katherine Tarlock, Division of Hematology/Oncology, Seattle Children's Hospital, M/S MB.8.501, PO Box 5371, Seattle, WA 98145-5005; email: katherine.tarlock@seattlechildrens.org.

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