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Association of leukemic molecular profile with efficacy of inotuzumab ozogamicin in adults with relapsed/refractory ALL

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Key Points

- Responses to inotuzumab were observed in all leukemic subtypes, genomic alterations, and risk groups.
- Inotuzumab demonstrated superior efficacy vs standard-of-care in patients with high-risk *BCR::ABL1*–like subtype.

The phase 3 INO-VATE trial demonstrated higher rates of remission, measurable residual disease negativity, and improved overall survival for patients with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL) who received inotuzumab ozogamicin (InO) vs standard-of-care chemotherapy (SC). Here, we examined associations between genomic alterations and the efficacy of InO. Of 326 randomized patients, 91 (InO, n = 43; SC, n = 48) had samples evaluable for genomic analysis. The spectrum of gene fusions and other genomic alterations observed was comparable with prior studies of adult ALL. Responses to InO were observed in all leukemic subtypes, genomic alterations, and risk groups. Significantly higher rates of complete remission (CR)/CR with incomplete count recovery were observed with InO vs SC in patients with *BCR::ABL1*–like ALL (85.7% [6/7] vs 0% [0/5]; $P = .0076$), with *TP53* alterations (100% [5/5] vs 12.5% [1/8]; $P = .0047$), and in the high-risk *BCR::ABL1*$^-$ (*BCR::ABL1*–like, low-hypodiploid, *KMT2A*-rearranged) group (83.3% [10/12] vs 10.5% [2/19]; $P < .0001$). This retrospective, exploratory analysis of the INO-VATE trial demonstrated potential for benefit with InO for patients with R/R ALL across leukemic subtypes, including *BCR::ABL1*–like ALL, and for those bearing diverse genomic alterations. Further confirmation of the efficacy of InO in patients with R/R ALL exhibiting the *BCR::ABL1*–like subtype or harboring *TP53* alterations is warranted. This trial was registered at www.ClinicalTrials.gov as #NCT01564784.

Introduction

The advent of genomics has transformed the landscape of acute lymphoblastic leukemia (ALL), with better-defined subtypes that are associated with prognosis and that drive risk stratification with conventional therapies.[1,2] The associations between ALL subtypes and outcomes with novel therapies are

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Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Additionally, subject to certain criteria, conditions, and exceptions, Pfizer may also provide access to the related individual deidentified participant data. More information is available at https://www.pfizer.com/science/clinical-trials/trial-data-and-results.

The full-text version of this article contains a data supplement.

poorly understood; however, greater understanding of these associations may lead to the identification of new therapeutic approaches and improvement of existing therapies.

Inotuzumab ozogamicin (InO) is a calicheamicin-conjugated antibody that targets cluster of differentiation 22 (CD22) on leukemic blast cells and has demonstrated clinical activity in patients with relapsed/refractory (R/R) ALL.[3-7] In the phase 3 INO-VATE trial, treatment with InO vs standard-of-care chemotherapy (SC) significantly improved rates of remission (80.7% vs 29.4%; $P < .001$) and measurable residual disease (MRD) negativity in responding patients with R/R ALL.[7] After ≥2 years of follow-up, the median overall survival (OS) with InO was 7.7 months vs 6.2 months with SC (hazard ratio [HR], 0.75; 97.5% confidence interval [CI], 0.57-0.99; 1-sided $P = .0105$), with more responding patients proceeding directly to hematopoietic stem cell transplantation (HSCT) with InO than with SC (39.6% vs 10.5%; $P < .0001$).[8] INO-VATE exploratory analyses have demonstrated InO clinical benefit across cytogenetic subgroups, including patients with *BCR::ABL1*$^+$ R/R ALL.[9,10] In the first of these analyses, rates of complete remission (CR) and CR with incomplete hematologic recovery (CRi) were higher in patients treated with InO than in those treated with SC, in all cytogenetic subgroups (*BCR::ABL1*$^+$ ALL, complex cytogenetics [≥5 abnormalities]; normal diploid karyotype; and other cytogenic abnormalities); however, analyses were based primarily on karyotype data, which limited the ALL subgroups that could be evaluated.[9] In the *BCR::ABL1*$^+$ leukemic subtype, CR/CRi and MRD negativity were higher with InO (CR/CRi, 73%; MRD, 81%) vs SC (CR/CRi, 56%; MRD, 33%); however, the study findings were limited by the small number of patients, limited availability of cytogenic and molecular data to fully classify leukemia subtypes, heterogeneity of prior therapy, and no stratification at randomization for *BCR::ABL1* status.[10]

Here, we further characterize the molecular profile of patients with R/R ALL in the INO-VATE trial and assess the impact of leukemic subtypes and alterations, and risk groups on the efficacy of InO vs SC.

Methods

Study design, patients, and treatment

INO-VATE (ClinicalTrials.gov identifier: NCT01564784) was an open-label, randomized, phase 3 trial; methods have been previously described.[7] Briefly, adult patients with R/R B-cell ALL (B-ALL; ≥5% bone marrow blasts based on local morphologic analysis), CD22$^+$, *BCR::ABL1*$^+$, or *BCR::ABL1*$^-$ ALL who were due to receive their first or second salvage-treatment were enrolled. Patients were randomized 1:1 to receive InO or an SC regimen of the investigator's choice, consisting of fludarabine/cytarabine (Ara-C)/granulocyte colony–stimulating factor, Ara-C/mitoxantrone, or high-dose Ara-C. Stratification at randomization was based on duration of first remission (<12 vs ≥12 months), salvage-treatment phase (1 vs 2), and age (<55 vs ≥55 years).

The study was conducted in accordance with the Declaration of Helsinki. Patients provided written informed consent, and the protocol was approved by the independent ethics committee and/or institutional review board at each study site. Differences between treatment groups were tested using χ^2 test or Fisher exact test. This post hoc analysis was based on the final data from 4 January 2017.

Laboratory investigations

Bone marrow samples were collected at screening from after protocol amendment 2 (24 June 2013). Total RNA was extracted from frozen bone marrow cell pellets using Direct-zol RNA Miniprep Kit (Zymo Research Corp, Irvine, CA) and checked for integrity (TapeStation, Agilent Technologies, Santa Clara, CA) and quantity (QUBIT RNA assay, Invitrogen, Waltham, MA). Total stranded transcriptome sequencing (RNA-sequencing) was performed using the TruSeq Stranded Total RNA library preparation kit and sequencing performed using NovaSeq 6000 platforms (Illumina Inc, San Diego, CA). Genetic subtypes were determined by integrating gene expression, rearrangement, and copy number, as previously described.[11,12] Single-nucleotide variants (SNVs) and short inserts and deletions (indels) were called from RNA-sequencing data by following the best practice workflow from the Genome Analysis Toolkit.[13] The following criteria were applied to filter mutations: (1) at least 3 reads supported the mutation, and the mutant allele frequency was >0.1; and (2) the mutation was not observed in the common single-nucleotide polymorphism database, dbSNP 150.

Outcomes

Outcomes were reported by leukemic subtype and genomic alterations at screening. Patients were assigned to risk groups based on a classification of leukemic subtypes previously described by Paietta et al[2]: high-risk *BCR::ABL1*$^+$ (*BCR::ABL1*$^+$); high-risk *BCR::ABL1*$^-$ (defined as *BCR::ABL1*–like, low-hypodiploid, and *KMT2A*-rearranged); standard to intermediate risk (defined as *DUX4*-rearranged, hyperdiploid, *MEF2D*-rearranged, PAX5alt, *TCF3::PBX1*, and *ZNF384*-rearranged); undetermined risk (defined as *CDX2/UBTF*, *ZEB2/CEBP*, and B-other [cases without an identified subtype-defining driver genetic alteration]); and tumors unable to be classified because of low-blast content (<30%) of samples.[2] Although recently published data suggest *CDX2/UBTF* to be a high-risk leukemic subtype,[12,14] the number of patients evaluated has been small, and in our analysis we used the classification scheme reported by Paietta et al that considered *CDX2/UBTF* to be in the undetermined risk group.[2]

Response and disease progression were assessed using modified Cheson criteria.[15] MRD negativity was assessed as <0.01% bone marrow blasts centrally analyzed using multicolor, multiparameter flow cytometry. Progression-free survival (PFS) was defined as the time from randomization to first event of disease progression, starting new induction therapy, or poststudy HSCT without achieving CR or CRi, or death from any cause. OS was defined as the time from randomization to death from any cause.

Statistical analysis

Analyses were carried out on the intent-to-treat (ITT) population. Median PFS and OS were estimated using the Kaplan-Meier method, with the HR and corresponding 97.5% CIs

calculated using a Cox proportional hazard regression model.[16] Unstratified HR and corresponding CI are displayed, except for "all patients (stratified)," which used the same stratification factors for randomization. Genes with an overall alteration prevalence cutoff of ≥8 patients were selected for correlative analysis. No additional candidate genomic alterations associated with efficacy differences emerged based on a further exploratory analysis based on alteration prevalence of 5 to 7 patients (not shown). *P* values were not corrected for multiple testing.

Table 1. Baseline characteristics of the molecular-profiling cohort

	ITT evaluable for molecular-profiling		ITT population	
	InO n = 43	SC n = 48	InO n = 164	SC n = 162
Age, median (range), y	49.0 (20.0-78.0)	54.5 (20.0-76.0)	46.5 (18.0-78.0)	47.5 (18.0-78.0)
Male, n (%)	26 (60.5)	28 (58.3)	91 (55.5)	102 (63.0)
Race, n (%)				
White	27 (62.8)	39 (81.3)	112 (68.3)	120 (74.1)
Asian	8 (18.6)	3 (6.3)	31 (18.9)	24 (14.8)
Black	1 (2.3)	1 (2.1)	4 (2.4)	3 (1.9)
Other	7 (16.3)	5 (10.4)	17 (10.4)	15 (9.3)
ECOG PS, n (%)				
0	14 (32.6)	20 (41.7)	62 (37.8)	61 (37.7)
1	25 (58.1)	21 (43.8)	81 (49.4)	80 (49.4)
2	4 (9.3)	6 (12.5)	21 (12.8)	20 (12.3)
Missing	0	1 (2.1)	0	1 (0.6)
Salvage status, n (%)				
1	30 (69.8)	37 (77.1)	111 (67.7)	102 (63.0)
2	13 (30.2)	11 (22.9)	51 (31.1)	59 (36.4)
Missing	0	0	2 (1.2)	1 (0.6)
Duration of first remission, n (%)				
<12 mo	24 (55.8)	28 (58.3)	96 (58.5)	106 (65.4)
≥12 mo	19 (44.2)	20 (41.7)	68 (41.5)	56 (34.6)
Response to prior induction regimen, n (%)				
Complete response	35 (81.4)	36 (75.0)	121 (73.8)	112 (69.1)
Partial response	1 (2.3)	1 (2.1)	11(6.7)	10 (6.2)
Stable disease	0	0	1 (0.6)	4 (2.5)
Resistant disease	7 (16.3)	11 (22.9)	28 (17.1)	30 (18.5)
Progressive disease	0	0	3 (1.8)	5 (3.1)
Unknown	0	0	0	1 (0.6)
Received prior HSCT, n (%)	10 (23.3)	6 (12.5)	29 (17.7)	32 (19.8)
WBC count, median (range), $\times 10^3$ cells/mm^3	3.6 (0.4-27.4)	4.4 (0.6-51.0)	4.1 (0.0-47.4)	4.0 (0.1-68.8)
Peripheral blood count, median (range), $\times 10^3/\mu L$	0 (0-7112.0)	18.4 (0-23 664.0)	0 (0-7112.0)	18.4 (0-23 664.0)
No circulating blasts, n (%)	23 (53.5)	23 (47.9)	71 (43.3)	74 (45.7)
Bone marrow blasts, n (%)				
<50%	17 (39.5)	12 (25.0)	53 (32.3)	48 (29.6)
≥50%	25 (58.1)	36 (75.0)	10.9 (66.5)	113 (69.8)
Missing	1 (2.3)	0	2 (1.2)	1 (0.6)
CD22 expression on ALL blasts, n (%)				
≥90	33 (76.7)	33 (68.8)	107 (65.2)	93 (57.4)
70% to <90%	9 (20.9)	8 (16.7)	30 (18.3)	18 (11.1)
<70%	0	6 (12.5)	5 (3.0)	18 (11.1)
Missing	1 (2.3)	1 (2.1)	22 (13.4)	33 (20.4)

ECOG PS, Eastern Cooperative Oncology Group performance status; WBC, white blood cell.

Results

Patients

Of 326 patients in the INO-VATE ITT population, 91 patients (InO, n = 43; SC, n = 48) had samples evaluable for molecular-profiling. Baseline characteristics were generally similar between the molecular-profiling cohort and the overall ITT population and were also comparable between study arms in the molecular-profiling cohort, although there were some variations because of the small sample size (Table 1).[8]

Molecular alterations

Thirteen different leukemic subtypes were identified (Table 2). The most common (≥5%) subtypes across treatment arms were *BCR::ABL1*$^+$ (14.3%), *BCR::ABL1*–like (13.2%), low hypodiploidy (12.1%), *KMT2A* rearranged (8.8%), and *DUX4* rearranged (5.5%); 25 of 91 (27.5%) patients could not be subtyped because of low-blast content of samples. The most common (≥5%) non–*BCR::ABL1* rearrangements were *IGH::CRLF2* (6.6%) and *KMT2A::AFF1* (6.6%) detected in the *BCR::ABL1*–like and *KTM2A*-rearranged subtypes, respectively (Figure 1). The majority of patients with *BCR::ABL1*–like subtype had CRLF2/JAK pathway fusions (9/12; Figure 1), rather than ABL-class fusions (1 patient had *TNIP1::PDGFRB*). Among nonrearrangement genomic alterations, sequence mutations of chromatin-modifying genes were common (*KMT2D*, 17.6%; *CREBBP*, 12.1%; *KMT2C*, 11.0%; and *ARID2*, 8.8%; Figure 2; supplemental Table 1). Other common gene alterations included *TP53* (14.3%), *NOTCH1* (11.0%), and *KRAS* (8.8%). Most of the *TP53* mutations (8/13 [62%]) were observed in low-hypodiploid B-ALL (Figure 2; supplemental Table 2).

Efficacy

In 91 patients evaluated for response by molecular genetic subset, the CR/CRi rate was 74.4% in the InO group vs 33.3% in the SC group ($P < .0001$; Figure 3A). CR/CRi rates were higher with InO than with SC across leukemic subtypes, with the difference reaching statistical significance in the *BCR::ABL1*–like subtype (6/7 patients [85.7%] vs 0/5 patients [0%], respectively; $P = .0076$; the patient who did not respond to InO had *IGH::CRLF2*$^+$ ALL). Among patients in the InO group who achieved CR/CRi, MRD negativity was typically achieved. MRD negativity was achieved by all 4 responding patients with the *BCR::ABL1*$^+$ subtype and all 6 responding patients with the *BCR::ABL1*–like subtype, compared with 1 of 4 and 0 of 0 patients, respectively, treated with SC. A trend toward a PFS benefit with InO was observed across leukemic subtypes and a trend toward OS benefit with most subtypes (Figure 3B-C).

It was evident that across genomic alterations, there was a potential for response to InO treatment (Figure 4A). Of note, CR/CRi rates were significantly higher in patients with *TP53* alterations who received InO (5/5 [100%]) than SC (1/8 [12.5%]; $P = .0047$). Although a trend toward a PFS benefit was observed across genomic alterations, no such trend was observed for OS (Figure 4B-C). Responses were observed in 1 of 3 (33.3%) patients with *BCR::ABL1*$^+$ ALL and the ABL1 T315I mutation treated with InO compared with 3 of 6 (50%) patients treated with SC (P = not significant).

In an evaluation of efficacy outcomes according to risk group based on a classification of leukemic subtypes (high-risk *BCR::ABL1*$^+$, high-risk *BCR::ABL1*$^-$, standard to intermediate risk, undetermined risk,

Table 2. ALL subtypes and risk groups by treatment arm and in the combined cohort

n (%)	InO n = 43	SC n = 48	Combined n = 91	Leukemic subtype–based classification of risk group*
BCR::ABL1$^+$	6 (14.0)	7 (14.6)	13 (14.3)†	High-risk *BCR::ABL1*$^+$
BCR::ABL1 like	7 (16.3)	5 (10.2)	12 (13.2)	High-risk *BCR::ABL1*$^-$
Low hypodiploidy	3 (7.0)	8 (16.7)	11 (12.1)	High-risk *BCR::ABL1*$^-$
KMT2A	2 (4.7)	6 (12.5)	8 (8.8)	High-risk *BCR::ABL1*$^-$
DUX4	3 (7.0)	2 (4.2)	5 (5.5)	Standard to intermediate risk
Hyperdiploid‡	3 (7.0)	0	3 (3.3)	Standard to intermediate risk
CDX2/UBTF	2 (4.7)	1 (2.1)	3 (3.3)	Undetermined risk
B-other	1 (2.3)	1 (2.1)	2 (2.2)	Undetermined risk
MEF2D	1 (2.3)	1 (2.1)	2 (2.2)	Standard to intermediate risk
PAX5alt	0	2 (4.2)	2 (2.2)	Standard to intermediate risk
TCF3::PBX1	1 (2.3)	1 (2.1)	2 (2.2)	Standard to intermediate risk
ZEB2/CEBP	0	2 (4.2)	2 (2.2)	Undetermined risk
ZNF384	1 (2.3)	0	1 (1.1)	Standard to intermediate risk
Low-blast§	13 (30.2)	12 (24.5)	25 (27.5)	Low blast

B-other, cases without an identified subtype-defining driver genetic alteration.
*Paietta et al[2].
†ABL1 T315I mutations were reported in 9 patients (InO, n = 3; SC, n = 6).
‡Confirmed by RNA-sequencing copy number variation (analysis) for absence of fusions and absence of CREBBP mutations.
§Low-blast: blasts of <30%; subtype undetermined.

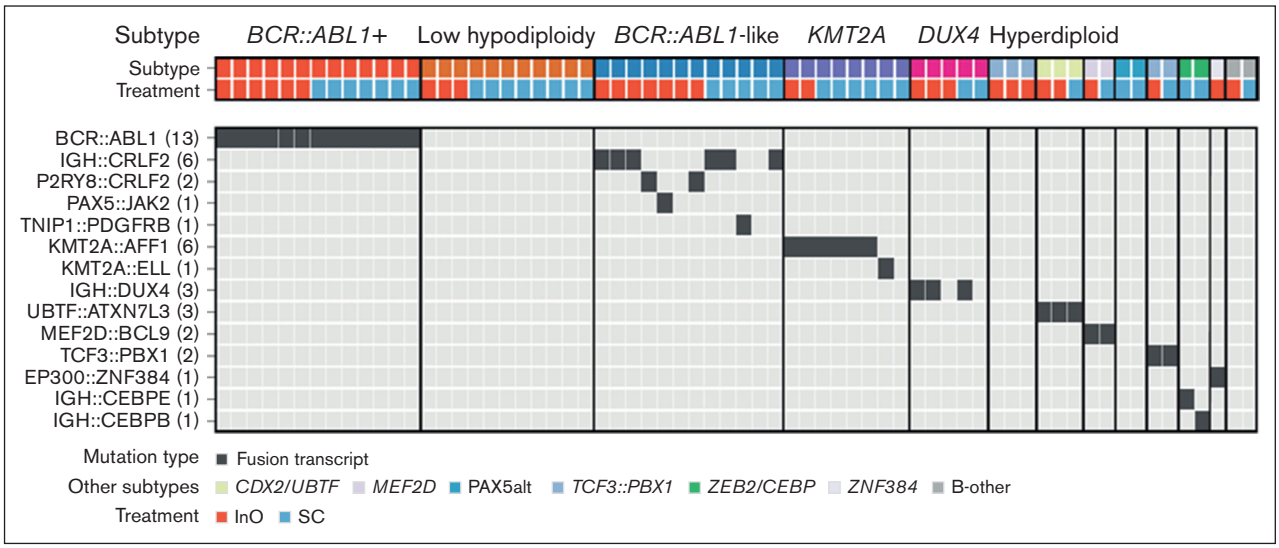


Figure 1. Gene fusions of interest by ALL subtype. The most common subtypes across treatment arms were *BCR::ABL1*$^{+}$, *BCR::ABL1*–like, low hypodiploidy, *KMT2A* rearranged, and *DUX4* rearranged. The most common non–*BCR::ABL1* rearrangements were *IGH::CRLF2* detected in the *BCR::ABL1*–like subtype, and *KMT2A::AFF1* detected in the *KMT2A* rearranged subtype. Oncoprint displays cases of both canonical and noncanonical fusion orientation. The numbers in parentheses denote the number of patients carrying that gene fusion.

and low-blast; Table 2 for full definitions of risk groups), response to InO was observed across the risk groups (Figure 5A). In the high-risk *BCR::ABL1*$^{-}$ group, CR/CRi rates were significantly higher with InO (10/12 [83.3%]) compared with SC (2/19 [10.5%]; $P < .0001$). A trend toward a PFS benefit was evident across most risk groups, which was significant in the high-risk *BCR::ABL1*$^{-}$ group (HR, 0.236; 97.5% CI, 0.081-0.686) and the low-blast content group (HR, 0.276; 97.5% CI, 0.086-0.884; Figure 5B). Similarly, a trend toward OS benefit was observed across most risk groups (Figure 5C). In general, trends for the low-blast content group appeared most similar to the overall "all patients" group that combined all risk groups (Figures 3 and 5), likely reflecting that the low-blast population had a similar fraction of subtypes as the overall cohort.

Discussion

This retrospective analysis of the INO-VATE trial demonstrated potential for benefit with InO for patients with R/R ALL across leukemic subtypes and for patients bearing different genomic alterations. Although the number of cases of ALL for several subtypes was small, the spectrum of gene fusions and other genomic alterations observed was generally consistent with that previously reported for adult ALL.[2,16]

Similar to previously reported findings of the INO-VATE trial, response rates were higher with InO than with SC across leukemic subtypes.[7-9] A notable finding was the low response rate observed in patients with *BCR::ABL1*–like ALL who were

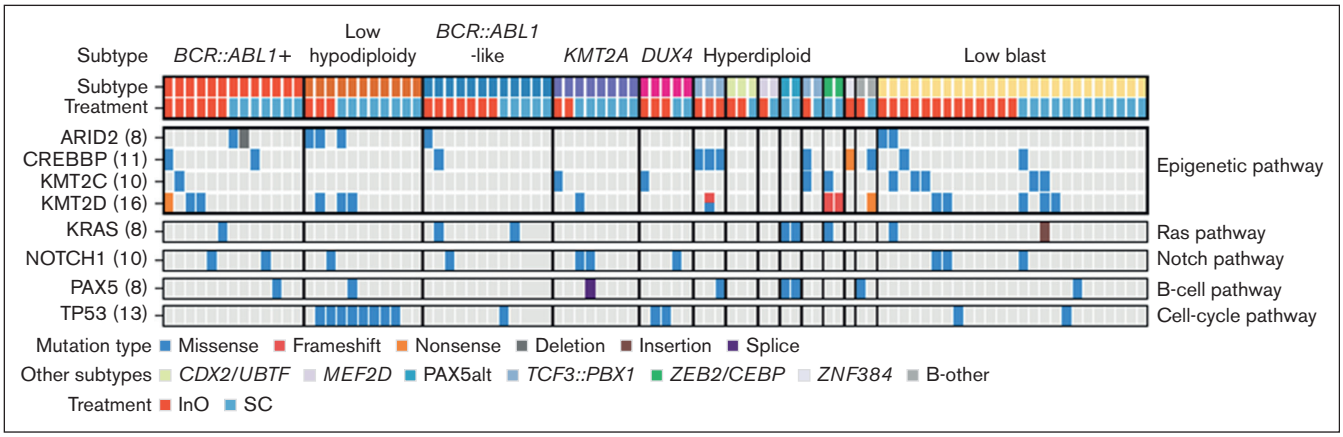


Figure 2. Common nonfusion genomic alterations by ALL subtype and molecular pathway. Among nonrearrangement genomic alterations, sequence mutations of chromatin-modifying genes were common. Other common gene alterations included *TP53*, *NOTCH1*, and *KRAS*. The numbers in brackets denote the number of patients carrying that genomic alteration.

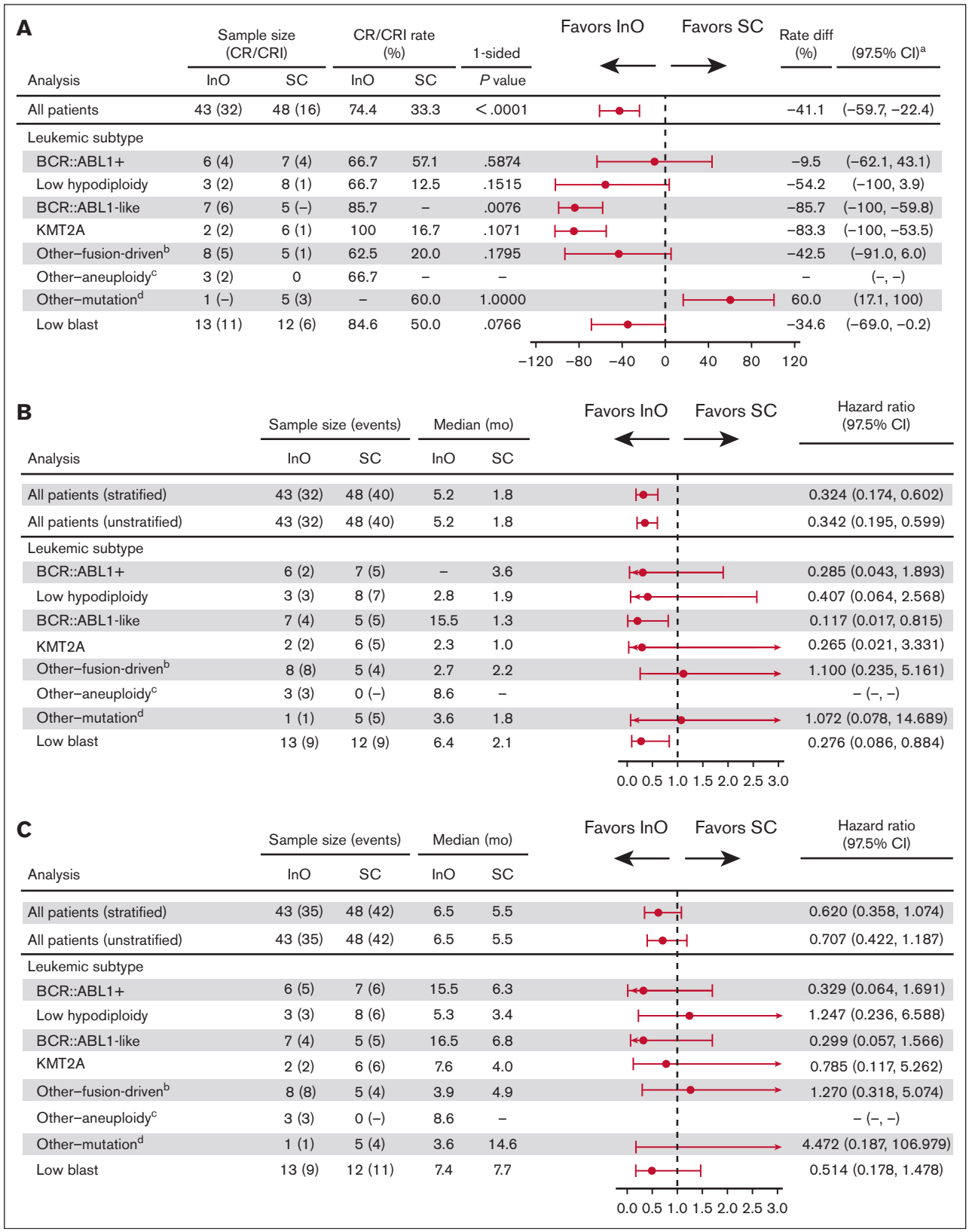
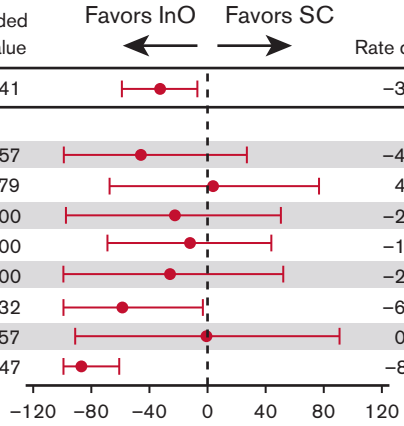


Figure 3. Efficacy outcomes with InO or SC by ALL subtype. (A) CR/CRi rates were higher with InO than with SC across leukemic subtypes, with the difference reaching statistical significance in the *BCR::ABL1*–like subtype. (B) A trend toward benefit with InO was observed across leukemic subtypes for PFS; and (C) across most subtypes for OS. [a]CIs were approximated using nominal distribution when ≥5 patients were in both the CR/CRi and non–CR/CRi subgroups. Where <5 patients were in either subgroup, exact method was used; [b]included *DUX4*, *CDX2/UBTF*, *MEF2D*, *TCF3::PBX1*, and *ZNF384*; [c]included hyperdiploid; [d]included B-other, *PAX5*alt, and *ZEB2/CEBP*.

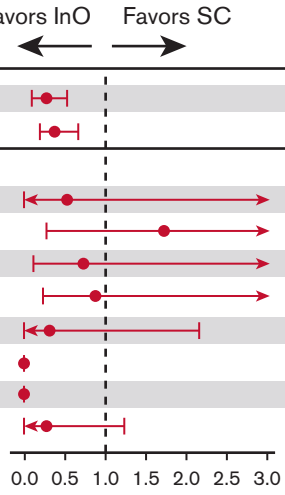
A

Analysis	Sample size (CR/CRI) InO	SC	CR/CRI rate (%) InO	SC	1-sided *P* value	Rate diff (%)	(97.5% CI)[b]
All patients[a]	31 (22)	34 (13)	71.0	38.2	.0041	−32.7	(−58.9, −6.6)
Genomic alteration							
ARID2	5 (4)	3 (1)	80.0	33.3	.2857	−46.7	(−100, 26.3)
CREBBP	8 (5)	3 (2)	82.5	66.7	.7879	4.2	(−67.9, 76.2)
KMT2C	7 (4)	3 (1)	57.1	33.3	.5000	−23.8	(−97.8, 50.2)
KMT2D	7 (4)	9 (4)	57.1	44.4	.5000	−12.7	(−68.7, 43.3)
KRAS	3 (2)	5 (2)	66.7	40.0	.5000	−26.7	(−100, 51.6)
NOTCH1	5 (4)	5 (1)	80.0	20.0	.1032	−60.0	(−100, −3.3)
PAX5	2 (1)	6 (3)	50.0	50.0	.7857	0.0	(−91.5, 91.5)
TP53	5 (5)	8 (1)	100	12.5	.0047	−87.5	(−100, −61.3)



B

Analysis	Sample size (events) InO	SC	Median (mo) InO	SC	Hazard ratio (97.5% CI)
All patients (stratified)[a]	31 (24)	34 (27)	3.6	1.8	0.422 (0.259, 0.689)
All patients (unstratified)[a]	31 (24)	34 (27)	3.6	1.8	0.545 (0.359, 0.828)
Genomic alteration					
ARID2	5 (5)	3 (1)	3.4	–	0.550 (0.034, 8.792)
CREBBP	8 (7)	3 (2)	4.5	8.4	1.708 (0.277, 10.518)
KMT2C	7 (6)	3 (2)	2.8	2.5	0.715 (0.107, 4.764)
KMT2D	7 (5)	9 (7)	3.4	4.1	0.879 (0.232, 3.330)
KRAS	3 (2)	5 (5)	3.6	1.0	0.310 (0.045, 2.134)
NOTCH1	5 (3)	5 (2)	7.4	–	0.000 (0.000,–)
PAX5	2 (2)	6 (4)	6.1	1.8	0.000 (0.000,–)
TP53	5 (5)	8 (7)	2.8	1.5	0.242 (0.048, 1.234)



C

Analysis	Sample size (events) InO	SC	Median (mo) InO	SC	Hazard ratio (97.5% CI)
All patients (stratified)[a]	31 (27)	34 (29)	5.3	5.5	0.781 (0.503, 1.210)
All patients (unstratified)[a]	31 (27)	34 (29)	5.3	5.5	1.108 (0.751, 1.634)
Genomic alteration					
ARID2	5 (5)	3 (1)	4.9	–	0.447 (0.019, 10.699)
CREBBP	8 (7)	3 (3)	7.6	15.6	1.873 (0.384, 9.133)
KMT2C	7 (6)	3 (2)	4.9	5.4	0.689 (0.098, 4.849)
KMT2D	7 (6)	9 (8)	6.1	9.5	1.067 (0.313, 3.629)
KRAS	3 (3)	5 (4)	4.9	6.2	1.261 (0.223, 7.133)
NOTCH1	5 (4)	5 (5)	7.4	5.5	0.543 (0.118, 2.490)
PAX5	2 (2)	6 (5)	6.1	11.2	1.874 (0.239, 14.685)
TP53	5 (5)	8 (7)	5.3	3.4	0.679 (0.169, 2.731)

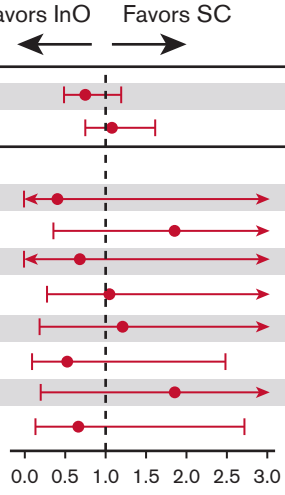


Figure 4. Efficacy outcomes with InO or SC by select genomic alterations. (A) Across genomic alterations, there was a potential for response to InO treatment, and CR/CRi rates were significantly higher in patients with *TP53* alterations who received InO. (B) A trend toward a PFS benefit was observed across genomic alterations; however, (C) no such trend was observed for OS. [a]All patients bearing an alteration in a gene with an overall alteration prevalence n of at least 8; [b]CIs were approximated using nominal distribution when ≥5 patients were in both the CR/CRi and non–CR/CRi subgroups. Where <5 patients were in either subgroup, exact method was used.

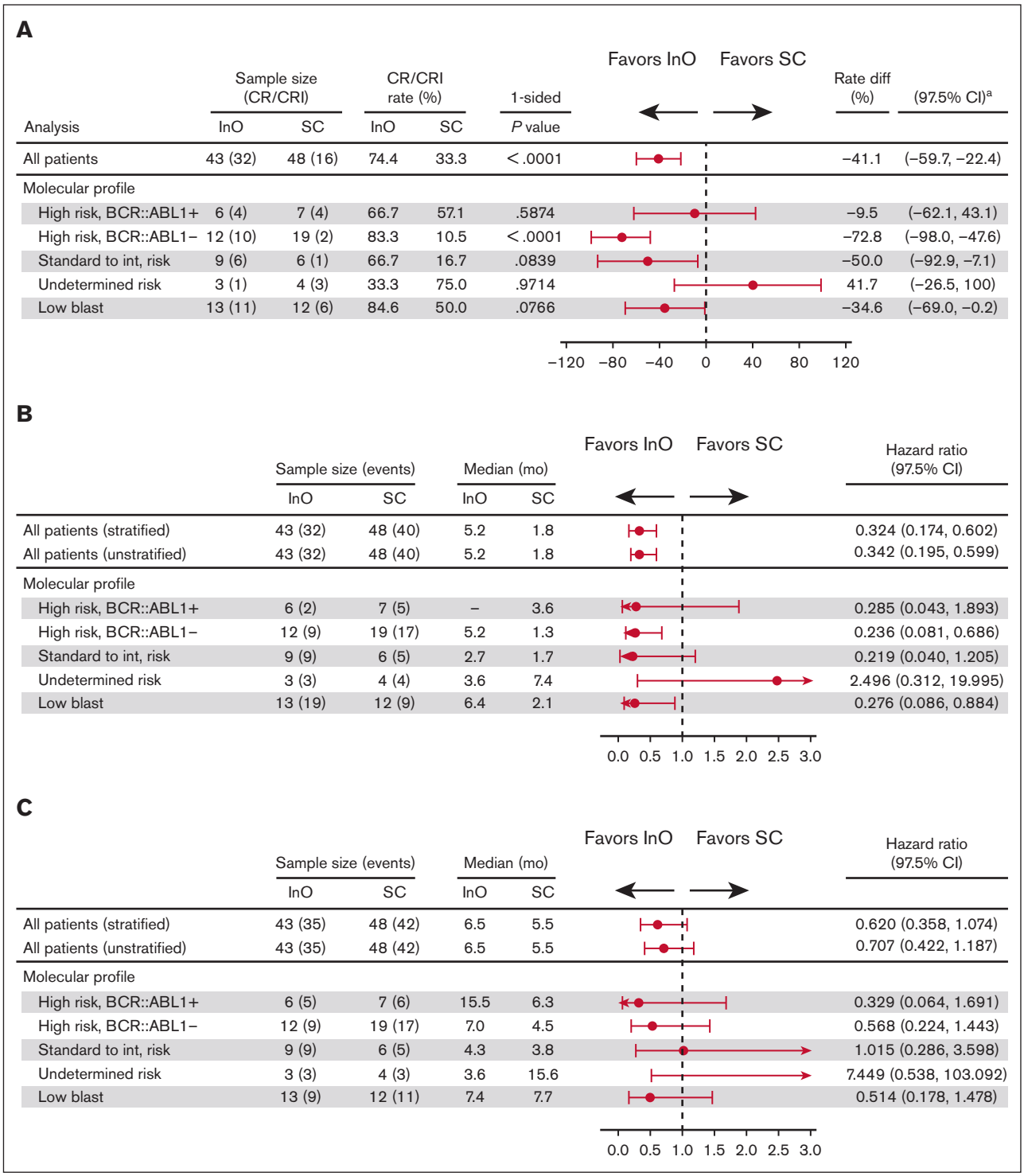


Figure 5. Efficacy outcomes with InO or SC by leukemic subtype–based risk group. (A) Response to InO was observed across risk groups group based on a classification of leukemic subtypes. In the high-risk *BCR::ABL1*$^{-}$ group, CR/CRi rates were significantly higher with InO compared with SC. (B) A trend toward a benefit with InO was evident across most risk groups for PFS; (C) OS. [a]CIs were approximated using nominal distribution when ≥5 patients were in both the CR/CRi and non–CR/CRi subgroups. Where <5 patients were in either subgroup, exact method was used. int, intermediate.

treated with SC (0/5 [0%]) vs InO (6/7 [85.7%]), with the difference reaching statistical significance. The lack of response in the SC group was not surprising given the small sample size and the poor prognosis of this patient group[2]; nevertheless, the results with InO for this high-risk subtype are encouraging.

The potential for response with InO was also evident across molecular alterations, including patients with ALL harboring *TP53* mutations, for whom the CR/CRi rate was significantly higher for patients receiving InO (5/5 [100%]) than those receiving SC (1/8 [12.5%]). Higher response rates with InO in patients with *TP53* alterations may reflect sensitization to calicheamicin-induced double-strand DNA breaks via abrogation of DNA repair and/or cell cycle checkpoints.[17] In contrast, in cellular models, *TP53* mutations have been reported to be associated with resistance to InO, which can be overcome via overexpression of wild-type *TP53*.[18] *TP53* alterations were significantly associated with low-hypodiploid ALL, with 8 of 13 (61.5%) *TP53* mutations occurring in this high-risk subtype, which is consistent with the literature.[2,19,20] *TP53* alterations have been identified as a hallmark of low hypodiploidy in both adults and children with ALL, with many of the alterations in children present in the germ line.[21] Our results warrant further evaluation of the interplay between *TP53* mutation, low-hypodiploid subtype, and InO efficacy in a larger cohort of patients.

Although all patients with *TP53* alterations treated with InO achieved a response, no significant PFS or OS benefit was observed, which may reflect the poor prognosis associated with *TP53* alterations,[19] especially in the R/R setting. In a study of patients with R/R disease with *TP53* mutations, treatment with mini-hyper-CVD (referring to low-intensity therapy administered on a hyperfractionated schedule) and InO was associated with high morphologic response rate (16/16 [100%]) but a similarly short OS (median OS, 5 months). The authors suggested that the timing of the mutation (baseline or R/R) was important, with inherently more aggressive *TP53* mutant clones expanding after chemotherapy.[22]

A previous analysis of patients with *BCR::ABL1*$^+$ R/R ALL in the INO-VATE trial demonstrated a substantial improvement in responses and HSCT rates with InO vs SC.[10] The authors discussed the potential ability of InO to target *BCR::ABL1*$^+$ ALL regardless of ABL-kinase mutational status, and highlighted the need to examine concurring mutations in patients with R/R *BCR::ABL1*$^+$ ALL (ABL- kinase mutation, particularly T315I). Our analysis identified the ABL1 T315I mutation in 9 of 13 (69.2%) patients with *BCR::ABL1*$^+$ ALL (data not shown); this is a "gatekeeper" mutation in the ABL-kinase domain and commonly seen in relapse after treatment with first- and second-generation tyrosine kinase inhibitors.[10,23,24]

The number of patients with the *KMT2A*-rearranged subtype treated with InO was small (n = 2), which limits the interpretability of these data. Interestingly, both patients had a relatively high CD22 positivity (90% and 84%, respectively), which is atypical because *KMT2A* rearrangements have been associated with lower CD22 positivity, and both patients achieved CR/CRi.[25] The percentage of CD22$^+$ blasts fell to <10% after only 1 cycle of InO treatment, which was a similar but more rapid decline compared with that typically seen for CD22 positivity in the INO-VATE study.[25] Regarding efficacy and progression in these patients, MRD in patient 1 was 90.8% at screening; 7.2% at cycle 1, day 20; and 1.4% at cycle 2, day 20, consistent with robust initial benefit. However, PFS was relatively short at 2.8 months. Likewise, for patient 2, MRD was 60.7% at screening; and 1.2% at cycle 1, day 21, with a PFS of 1.7 months.

The bispecific T-cell engager blinatumomab has also conferred activity in patients with high-risk *BCR::ABL1*$^+$ or *BCR::ABL1*–like R/R ALL. In the phase 2 ALCANTARA study, 16 of 45 (36%) patients with *BCR::ABL1*$^+$ R/R ALL achieved CR/CRi with blinatumomab, including 10 patients with the ABL1 T315I mutation, with a median OS of 7.1 months.[26] In a post hoc analysis from the phase 3 TOWER study, 3 of 9 (33%) patients with *BCR::ABL1*–like R/R ALL treated with blinatumomab achieved CR/CRi. A retrospective, single-center study has reported a higher response rate of 70% (16/23) in patients with *BCR::ABL1*–like R/R ALL after treatment with blinatumomab.[27,28] Acknowledging the small patient numbers, our results in *BCR::ABL1*$^+$ or *BCR::ABL1*–like subtypes compare favorably with these response rates.

The limitations of this analysis include its ad hoc, retrospective nature; the small sample size in subgroups; sample collection being initiated midway through the trial; and some suboptimal samples. In addition, we prioritized leukemic subtype classification, and RNA-sequencing was used to report gene fusions and SNV-indels but not gene deletions. Without DNA-based profiling and gene deletions, some alterations may be underestimated; for example, our study reported *IKZF1* SNVs/indels (4/91 [4.4%]), and a further investigation of *IKZF1* splicing isoforms identified IK6 isoform (which lacks exons 4-7)[29] at 13.2% (12/91) prevalence; because the most common *IKZF1* alterations are *IKZF1* deletions, this may explain the low rates of *IKZF1* alterations reported in our study relative to those reported in the UKALLXII/ECOG-ACRIN E2993 study (31% [41/131]).[2]

In summary, our results suggest a treatment benefit with InO to patients with R/R ALL across leukemic subtypes, molecular alterations, and risk groups, including poor prognosis, high-risk subtypes. Further confirmation of the efficacy of InO in patients with R/R ALL exhibiting the *BCR::ABL1*–like subtype or harboring *TP53* alterations is warranted.

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Authorship

Contribution: A.A., C.G.M., A.D.L., and E.V. conceived and designed the study; H.K., D.J.D., M.S., M.L., W.S., N.G., S.O., E.J., R.C.D., and A.A. provided study materials or recruited patients; Y.Z., K.G.R., R.Y., A.D.L., M.R.L., S.O., G.N., and C.G.M. performed experiments and/or assembled data; X.L. designed statistical analyses; and all authors analyzed and interpreted the data, wrote the manuscript, and approved the final manuscript.

Conflict-of-interest disclosure: H.K. received honoraria from AbbVie, Amgen, Daiichi-Sankyo, Pfizer, Bio Ascend, Novartis, Takeda, Adaptive Biotechnologies, Aptitude Health, Delta-Fly, Janssen Global, and Oxford Biomedical; research support from AbbVie, Amgen, Daiichi-Sankyo, and Pfizer; grants from Ascentage, Bristol Myers Squibb, Immunogen, Jazz Pharmaceuticals, and Sanofi; and serves on the advisory board of Actinium. D.J.D. received honoraria from Amgen, Autolus, Agios, Blueprint Pharmaceuticals, Forty Seven, Incyte, Jazz Pharmaceuticals, Kite Pharma, Novartis, Pfizer, Shire, and Takeda, and research support from AbbVie, GlycoMimetics, Novartis, and Blueprint Medicines. M.S. received research support and honoraria from Pfizer. M.L. received research support and honoraria from Pfizer and Amgen. W.S. received research support and/or honoraria from ADC Therapeutics, Agios, Amgen, Astellas, Gilead, Jazz, Kite, Pfizer, and Sigma-Tau. N.G. received research support and honoraria from Pfizer and Amgen. S.O. received research support and honoraria from Pfizer. E.J. received research support and consultancy fees from Amgen, Pfizer, Takeda, Adaptive Biotechnologies, AbbVie, Bristol Myers Squibb, Spectrum, and Genentech. R.D.C. reports consultancy for Amgen, Jazz, Kite/Gilead, and Pfizer; serves on boards and committees of PeproMene Bio and Autolus; received research support from Amgen, Kite/Gilead, Pfizer, Servier, and Vanda Pharmaceuticals; received honoraria from Kite/Gilead; and reports spouse employed by, and owns stocks in, Seagen. X.L. is a previous employee of Pfizer and is now an employee of Mirati Therapeutics Inc. C.G.M. reports research support from Pfizer and AbbVie; received honoraria from Amgen and Illumina; and reports equity in Amgen. A.A. reports consulting for Jazz Pharmaceuticals, Beam Theraputics, GlycoMimetics, Amgen, Pfizer, and Novartis, and received research support from Seattle Genetics, Immunogen, Glycomimetics, AbbVie, Pfizer, Amgen, Kite, and MacroGenics. A.D.L., R.Y., and E.V. are employees of, and own stock in, Pfizer. The remaining authors declare no competing financial interests.

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References

1. Arber DA, Orazi A, Hasserjian RP, et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200-1228.
2. Paietta E, Roberts KG, Wang V, et al. Molecular classification improves risk assessment in adult BCR–ABL1–negative B-ALL. *Blood*. 2021;138(11):948-958.
3. DiJoseph JF, Armellino DC, Boghaert ER, et al. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. *Blood*. 2004;103(5):1807-1814.
4. DeAngelo DJ, Stock W, Stein AS, et al. Inotuzumab ozogamicin in adults with relapsed or refractory CD22-positive acute lymphoblastic leukemia: a phase 1/2 study. *Blood Adv*. 2017;1(15):1167-1180.
5. Kantarjian H, Thomas D, Jorgensen J, et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol*. 2012;13(4):403-411.
6. Kantarjian H, Thomas D, Jorgensen J, et al. Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphocytic leukemia. *Cancer*. 2013;119(15):2728-2736.
7. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard care for acute lymphoblastic leukemia. *N Engl J Med*. 2016;375(8):740-753.
8. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: final report and long-term survival follow-up from the randomized, phase 3 INO-VATE study. *Cancer*. 2019;125(14):2474-2487.
9. Jabbour E, Advani AS, Stelljes M, et al. Prognostic implications of cytogenetics in adults with acute lymphoblastic leukemia treated with inotuzumab ozogamicin. *Am J Hematol*. 2019;94(4):408-416.
10. Stock W, Martinelli G, Stelljes M, et al. Efficacy of inotuzumab ozogamicin in patients with Philadelphia chromosome-positive relapsed/refractory acute lymphoblastic leukemia. *Cancer*. 2021;127(6):905-913.
11. Gu Z, Churchman ML, Roberts KG, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. *Nat Genet*. 2019;51(2):296-307.
12. Kimura S, Montefiori L, Iacobucci I, et al. Enhancer retargeting of CDX2 and UBTF::ATXN7L3 define a subtype of high-risk B-progenitor acute lymphoblastic leukemia. *Blood*. 2022;139(24):3519-3531.
13. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297-1303.
14. Passet M, Kim R, Gachet S, et al. Concurrent CDX2 cis-deregulation and UBTF::ATXN7L3 fusion define a novel high-risk subtype of B-cell ALL. *Blood*. 2022;139(24):3505-3518.
15. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol*. 2003;21(24):4642-4649.

16. Iacobucci I, Kimura S, Mullighan CG. Biologic and therapeutic implications of genomic alterations in acute lymphoblastic leukemia. *J Clin Med*. 2021;10(17):3792.
17. Salmoiraghi S, Rambaldi A, Spinelli O. TP53 in adult acute lymphoblastic leukemia. *Leuk Lymphoma*. 2018;59(4):778-789.
18. Tirrò E, Massimino M, Romano C, et al. Chk1 inhibition restores inotuzumab ozogamicin citotoxicity in CD22-positive cells expressing mutant p53. *Front Oncol*. 2019;9:57.
19. Stengel A, Schnittger S, Weissmann S, et al. TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis. *Blood*. 2014;124(2):251-258.
20. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. *Nat Genet*. 2013;45(3):242-252.
21. Comeaux EQ, Mullighan CG. *TP53* mutations in hypodiploid acute lymphoblastic leukemia. *Cold Spring Harb Perspect Med*. 2017;7(3):a026286.
22. Kanagal-Shamanna R, Kantarjian HM, Khoury JD, et al. Distinct prognostic effects of TP53 mutations in newly diagnosed versus relapsed/refractory (R-R) patients (pts) with B-acute lymphoblastic leukemia (ALL) treated with mini-HCVD-inotuzumab ozogamicin with or without blinatumomab regimens. *Blood*. 2020;136(Supplement 1):41-43.
23. Foà R, Vitale A, Vignetti M, et al. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome–positive acute lymphoblastic leukemia. *Blood*. 2011;118(25):6521-6528.
24. Saleh K, Fernandez A, Pasquier F. Treatment of Philadelphia chromosome–positive acute lymphoblastic leukemia in adults. *Cancers (Basel)*. 2022;14(7):1805.
25. Kantarjian HM, Stock W, Cassaday RD, et al. Inotuzumab ozogamicin for relapsed/refractory acute lymphoblastic leukemia in the INO-VATE trial: CD22 pharmacodynamics, efficacy, and safety by baseline CD22. *Clin Cancer Res*. 2021;27(10):2742-2754.
26. Martinelli G, Boissel N, Chevallier P, et al. Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome–positive B-precursor acute lymphoblastic leukemia following treatment with blinatumomab: results from a phase II, single-arm, multicenter study. *J Clin Oncol*. 2017;35(16):1795-1802.
27. Jabbour E, Patel K, Jain N, et al. Impact of Philadelphia chromosome-like alterations on efficacy and safety of blinatumomab in adults with relapsed/refractory acute lymphoblastic leukemia: a post hoc analysis from the phase 3 TOWER study. *Am J Hematol*. 2021;96(10):E379-E383.
28. Zhao Y, Aldoss I, Qu C, et al. Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL. *Blood*. 2021;137(4):471-484.
29. Marke R, van Leeuwen FN, Scheijen B. The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia. *Haematologica*. 2018;103(4):565-574.