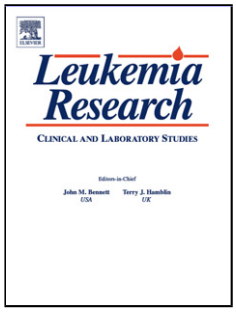

Impact of minimal residual disease status in patients with relapsed/refractory acute lymphoblastic leukemia treated with inotuzumab ozogamicin in the phase III INO-VATE trial

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Title: Impact of minimal residual disease status in patients with relapsed/refractory acute lymphoblastic leukemia treated with inotuzumab ozogamicin in the phase III INO-VATE trial

Running Title: Minimal residual disease with inotuzumab ozogamicin for ALL

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Highlights

- Subgroup data were analyzed in patients with ALL enrolled in INO-VATE (NCT01564784)
- The analysis was based on MRD status at end of treatment with inotuzumab ozogamicin
- MRD-negative patients with complete remission had improved survival vs MRD-positive
- MRD-negative patients treated in 1st salvage experienced the most survival benefit
- The best outcomes were seen in these patients who proceeded to stem cell transplant

Abstract

Minimal residual disease (MRD) negativity is a key prognostic indicator of outcome in acute lymphocytic leukemia. In the INO-VATE trial (clinicaltrials.gov identifier: NCT01564784), patients with relapsed/refractory acute lymphocytic leukemia who received inotuzumab *versus* standard chemotherapy achieved greater remission and MRD-negativity rates as well as improved overall survival: hazard ratio 0.75, one-sided *P*=0.0105. The current analysis assessed the prognostic value of MRD negativity at the end of inotuzumab treatment. All patients who received inotuzumab (*n*=164) were included. Among patients with complete remission/complete remission with incomplete hematologic response (CR/CRi; *n*=121), MRD-negative status (by multiparametric flow cytometry) was defined as $<1\times10^{-4}$ blasts/nucleated cells. MRD negativity was achieved in 76 patients at the end of treatment. Compared with MRD-positive, MRD-negative status with CR/CRi was associated with significantly improved overall survival and progression-free survival, respectively: hazard ratio (97.5% confidence interval; one-sided *P*-value) 0.512 (97.5% CI [0.313–0.835]; *P*=0.0009) and 0.423 (97.5% CI [0.256–0.699]; *P*<0.0001). Median overall survival was 14.1 *versus* 7.2 months, in the MRD-negative *versus* MRD-positive groups. Patients in first salvage who achieved MRD negativity at the end of treatment experienced significantly improved survival *versus* that seen in MRD-positive patients, particularly for those patients who proceeded to stem cell transplant. Among patients with relapsed/refractory acute lymphocytic leukemia who received inotuzumab, those with MRD-negative CR/CRi had the best survival outcomes.

Keywords: acute lymphoblastic leukemia, minimal residual disease, inotuzumab ozogamicin

1. Introduction

In patients with newly diagnosed acute lymphoblastic leukemia (ALL), minimal residual disease (MRD) assessment after induction chemotherapy can help determine prognosis and risk-stratify patients for appropriate post-remission therapies.[1-6] Patients with persistent MRD have a high risk of relapse and their prognosis is dismal.[7-9]

Modern innovative approaches, including new monoclonal antibody therapies, such as the anti-CD22 antibody-drug conjugate inotuzumab ozogamicin (InO),[10] the bispecific antibody construct blinatumomab[11] (either alone or in combination with cytotoxic chemotherapy[12]) and chimeric antigen receptor T-cell therapies,[13, 14] have recently shown promise in patients with relapsed or refractory (R/R) ALL. These agents improved outcomes compared with conventional chemotherapy and some patients experience long-term survival, particularly in those with deep response and no measurable disease burden. InO comprises a CD22 monoclonal antibody covalently linked to the potent cytotoxic agent calicheamicin.

InO has shown activity in adults with R/R ALL, including results in the global, open-label, phase III, randomized INO-VATE trial.[10, 15] In the final report of long-term follow-up, patients with R/R ALL who received InO *versus* standard chemotherapy (SC) maintained a greater rate of remission (74% *vs.* 31%).[15] As of the January 4, 2017 data cut-off date, overall survival (OS) was also improved for the InO arm *versus* the SC arm, with a stratified hazard ratio (HR) 0.75 (97.5% confidence interval [CI], 0.57–0.99) and one-sided *P*=0.0105, indicating a 25% reduction in risk of death.[15] The improvement in OS was most notable at later time points, wherein the 2-year survival was 23% among

patients in the InO arm *versus* 10% in the SC arm.[15] Greater rates of MRD negativity with InO *versus* SC (78% *vs.* 28%) were originally reported in the primary analysis from INO-VATE.[10] Herein, we report more detailed analyses. Analyses of hepatotoxicity and safety in this population have been previously reported.[10, 16, 17]

Prior studies have evaluated the prognostic role of MRD assessment in patients with newly diagnosed ALL; however, there are relatively few reports on the significance of MRD in patients with relapsed disease.[18-22] In these studies, lower levels of MRD in response to salvage treatment have been associated with improved outcomes. To assess the impact of MRD status on outcomes in adults with R/R ALL treated with InO in the INO-VATE trial, we conducted a *post hoc* analysis to assess the prognostic value of MRD negativity at the end of treatment (EOT) with InO.

2. Materials and Methods

INO-VATE (clinicaltrials.gov identifier: NCT01564784) trial details have been published.[10] The study was conducted in compliance with the ethical principles of the Declaration of Helsinki. The protocol was approved by the independent ethics committee or institutional review board at each study center. Written informed consent was provided by participants before any study procedures were conducted.

2.1 Trial Design, Patients and Treatments

Briefly, in this global (19 countries), open-label, randomized trial, patients aged ≥18 years with R/R (≥5% bone marrow blasts), $CD22^{+}$ and Philadelphia chromosome–positive or negative B-cell ALL who were due to receive first (S1) or second (S2)

salvage therapy were eligible.[10] Patients were randomized (1:1) to receive either InO or SC (investigator's choice); no crossover between groups was allowed.

The current analysis focused on patients in the InO arm who achieved complete remission/complete remission with incomplete hematologic response (CR/CRi). Among patients who achieved CR/CRi, MRD status was defined as negative (MRD^-) if $<1\times10^{-4}$ blasts/nucleated cells, or as MRD non-negative (MRD^+), based on the last assessment before or at EOT. Of note, six patients with no MRD assessment were included in the MRD^+ group. Additional analysis was conducted for OS by S1 *versus* S2 status at baseline when receiving InO as salvage therapy. Study design details are presented in Appendix A (Online Supplementary Materials S1).

2.2 Response Definitions

CR was defined as the presence of <5% blasts in the bone marrow (BM) aspirate, with $\geq1\times10^9$/L neutrophils and $\geq100\times10^9$/L platelets in the peripheral blood, and no evidence of extramedullary disease. Accordingly, CRi was defined as <5% blasts in the BM aspirate and no evidence of extramedullary disease but not meeting criteria for CR.

2.3 Minimal Residual Disease

MRD negativity achieved and maintained through EOT was determined to be most appropriate for this analysis because correlation with survival was desired. Best-response MRD (i.e. MRD negativity was achieved but not maintained through EOT) was not considered a sufficient parameter for MRD negativity because it would be less rigorous and outcomes analysis might be inaccurate if patients who were MRD^+ were

included. Therefore MRD negativity at EOT was a criterion for including patients in the MRD^- group. Additional details on MRD methods are described in Appendix A2 (Online Supplementary Materials S1).

2.4 Outcomes

Study outcomes have been previously described.[10, 15] Progression-free survival (PFS) was calculated from the time of randomization until an event, defined as treatment failure, relapse, or death from any cause. The OS was calculated from the time of randomization until death from any cause. Survival estimates were not censored at the time of allogeneic stem cell transplantation (ASCT).

2.5 Statistical Methods

The two primary endpoints were CR (including CRi) and OS. Secondary endpoints included safety measures, duration of remission, PFS, rate of subsequent ASCT and percentage of patients among those who achieved CR who had results below the threshold for MRD detection (MRD^-). Additional details are described in Appendix A4 (Online Supplementary Materials S1).

2.6 Data Sharing

Upon request, and subject to certain criteria, conditions and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines, and

medical devices (1) for indications that have been approved in the US and/or EU or (2) in programs that have been terminated (ie, development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

3. Results

3.1 Patient Characteristics

Between August 27, 2012, and January 4, 2015, 326 patients were randomized (intent-to-treat population). Baseline demographics and disease characteristics were generally similar between the InO (*n*=164) and SC (*n*=162) arms.[10]

Among the 164 patients who received InO, 121 (74%) achieved CR/CRi (92 achieved MRD negativity at any time during treatment). At EOT, 76 patients remained MRD^- and 45 were MRD^+, which constitutes the current analysis (Figure 1). Baseline characteristics are shown in Appendix B (Online Supplementary Table S2). Overall, the median age was 43 (range 20–78) years. Eighty-seven (72%) patients were treated as S1. Sixty-six (55%) patients had first CR duration <12 months; 20 (17%) had undergone prior stem cell transplantation. Thirty-five (29%) patients had a normal karyotype at the time of salvage treatment, 20 (17%) had complex karyotype, 16 (13%) had t(9;22) [i.e. Ph^+ or *BCR-ABL1*$^+$] and three (3%) had t(4;11).

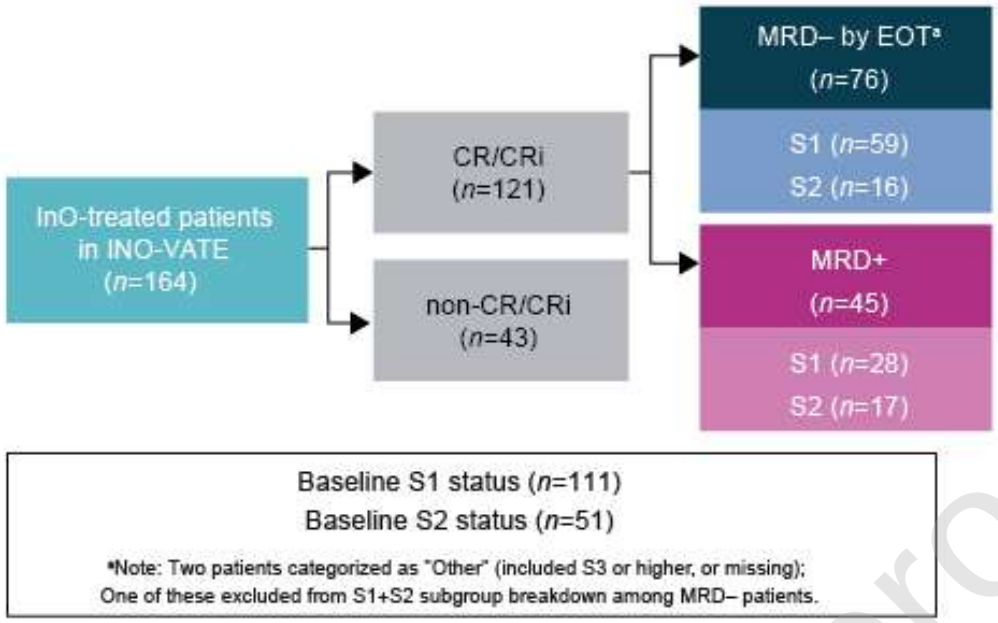

Figure 1 Subgroup breakdown for CR/CRi, MRD status and baseline salvage status (S1 vs. S2). *CR/CRi* complete remission/complete remission with incomplete hematologic response, *EOT* end of treatment, *InO* inotuzumab ozogamicin, *MRD* minimal residual disease.

3.2 MRD Response by Disease Status and Line of Therapy

Among patients treated with InO, MRD-negativity rates for patients with CR and CRi were 76% and 52%, respectively. In all, 76 (63%) patients achieving CR/CRi remained MRD$^-$ at EOT and 13 of 20 (65%) with prior ASCT achieved MRD negativity. Fifty-nine of 87 (68%) CR/CRi patients in S1 and 16 of 33 (48%) in S2 achieved MRD$^-$ status (one patient was listed as "Other," defined as S≥3 or missing). The majority of 121 patients with CR/CRi achieved best response (first MRD$^-$ status) after one or two cycles of InO (Appendix C: Online Supplementary Table S3). In all, 16 patients who had achieved MRD$^-$ best response were no longer MRD$^-$ at EOT.

In an exploratory univariate analysis of the pretreatment characteristics (Table 1), white race, S1 (*vs.* S2), baseline platelet count ≥100×10^9/L, baseline absolute circulating blast count <1×10^9/L, duration of first remission ≥12 months, normal cytogenetics and baseline lactate dehydrogenase <970 IU/L were associated with achievement of MRD-negativity (P<0.1). Exploratory multivariate analyses indicated that S2 compared with S1 status (odds ratio [OR] 0.499; 95% CI [0.243–1.024]; two-sided P=0.058) and platelets <100×10^9/L *versus* ≥100×10^9/L (OR 0.514; 95% CI [0.239–1.105]; two-sided P=0.088) were associated with lower likelihood of attaining MRD– status, whereas <1×10^9/L absolute circulating blast count at baseline (OR 3.231; 95% CI [1.546–6.750]; two-sided P=0.002) was significantly correlated with increased likelihood of attaining MRD$^-$ status.

Baseline Characteristic	Subsets, *n*	OR (95% CI)	*p* value (<0.1)*
Race, other / white	52 / 112	0.412 (0.208–0.815)	0.0108
Salvage status, 2 / 1	56 / 108	0.537 (0.279–1.036)	0.0638
Platelets, < / ≥100 $\times 10^9$/L	118 / 46	0.462 (0.229–0.930)	0.0306
Absolute circulating blasts, < / ≥1$\times 10^9$/L	108 / 55	3.251 (1.633–6.474)	0.0008
Duration of first remission, < / ≥12 months	109 / 55	0.468 (0.241–0.909)	0.0249
Cytogenetics, normal / other	35 / 98	2.257 (1.011–5.039)	0.0469
LDH (< / ≥970 IU/L)	128 / 31	2.534 (1.105–5.809)	0.0280

CI confidence interval, *LDH* lactate dehydrogenase, *OR* odds ratio.
Baseline variables included in logistic regression model are listed in Appendix D: Online Supplementary Table S4.
* Two-sided.

Table 1 Association of baseline characteristics and achievement of MRD negativity (by univariate analysis)

3.3 Survival Outcomes by MRD Response

Greater probability of PFS was seen in patients MRD^- *versus* MRD^+ over the study period (unstratified HR 0.423 [97.5% CI 0.256–0.699]; one-sided *P*<0.0001; Figure 2A). Median PFS (mPFS) was 8.6 months (95% CI 6.2–11.4) in patients MRD^- and 5.4 months (95% CI 3.9–6.2) in patients MRD^+; the corresponding 2-year PFS rates were 27% and 0%. Forty-seven (62%) MRD^- patients *versus* 40 (89%) of those MRD^+ experienced events: progressive disease/relapse from CR/CRi (25 [53%] *versus* 30 [75%]) and death (22 [47%] *versus* 10 [25%]). Of the 29 patients with MRD^- status who were censored (*versus* 5 MRD+ patients censored), the majority (21) had discontinued treatment with CR/CRi and without a PFS event; an additional 7 patients had an unacceptable gap (>28 weeks) between PFS event and most recent prior disease assessment. Five deaths from graft-versus-host disease occurred in the MRD^- group (*vs.* none with MRD^+); this higher incidence would be expected given that more MRD^- patients proceeded to ASCT.

Greater probability of OS was seen in patients with MRD^- *versus* MRD^+ status over the study period (unstratified HR 0.512 [97.5% CI 0.313–0.835]; one-sided *P*=0.0009; Figure 2B). Median OS (mOS) was 14.1 months (95% CI 8.6–23.0) in MRD^- and 7.2 months (95% CI 5.8–10.8) in MRD^+ patients; the respective 2-year mOS rates were 38% and 13%. In addition, patients who achieved MRD negativity after their first cycle of InO (*n*=42) had a similar OS probability as those who achieved MRD^- status after subsequent cycles (*n*=50): unstratified HR 0.889 (97.5% CI 0.507–1.558; *P*=0.3187).

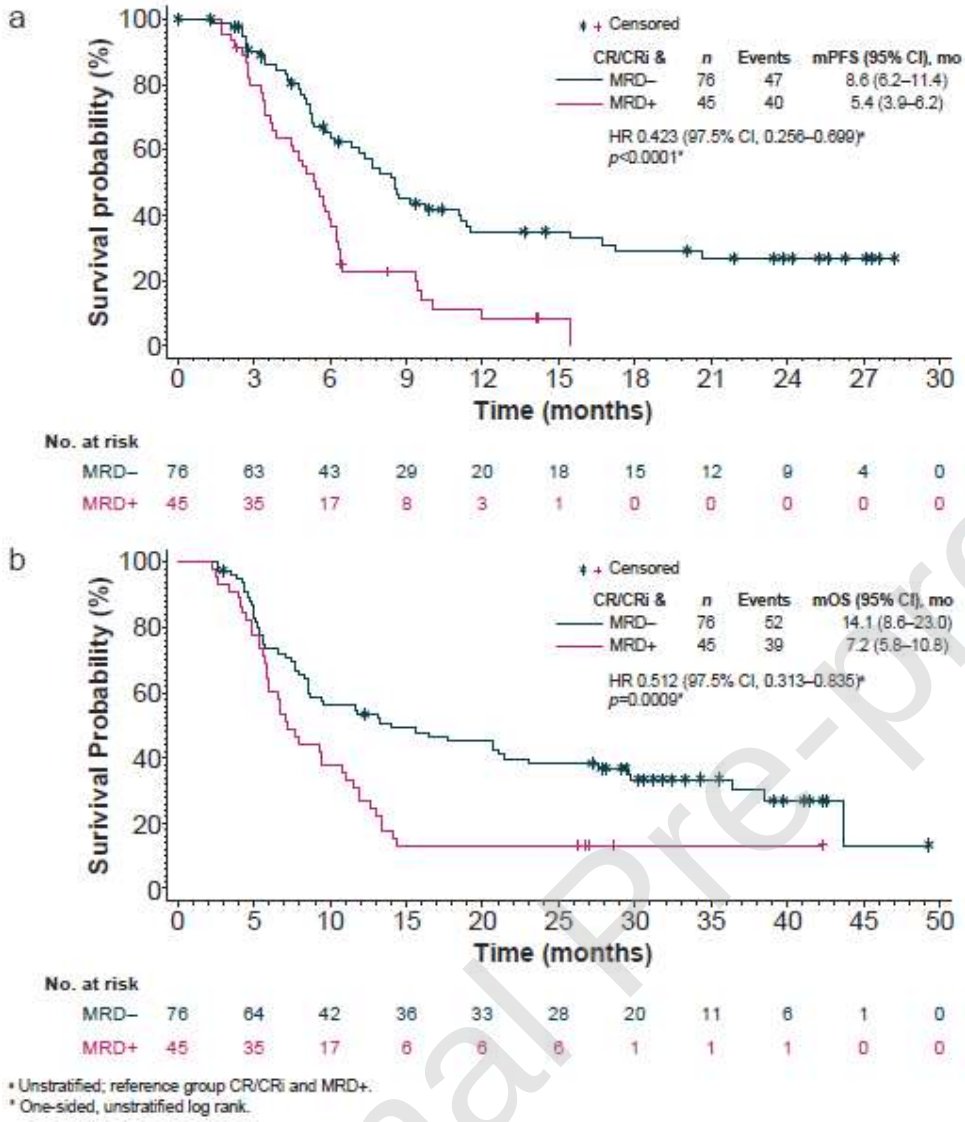

Figure 2 Outcomes by minimal residual disease (MRD) response among inotuzumab ozogamicin-treated patients. (**A**) Median progression-free survival (mPFS) and (**B**) median overall survival (mOS). *One-sided, unstratified log rank. [a]Unstratified; reference group. *CR/CRi* and MRD+. *CI* confidence interval, *CR/CRi* complete remission/complete remission with incomplete hematologic response, *HR* hazard ratio, *MRD* minimal residual disease.

3.4 Impact of Salvage Status on Outcomes

When patients were stratified according to salvage status (S1 *vs.* S2), a beneficial effect of attaining MRD negativity was observed in Kaplan–Meier analyses comparing MRD^- with MRD^+ in both subgroups (Figure 3A and 3B).

For PFS, the unstratified HR (97.5% CI) for patients with MRD^- *versus* MRD^+ status was 0.390 (0.210–0.723; one-sided *P*=0.0002) in S1 and 0.463 (0.183–1.173; one-sided *P*=0.0278) in S2. Among patients in S1 and S2, those with MRD^- had longer PFS than MRD^+ (Figure 3A). The 2-year PFS rates for MRD^- *versus* MRD^+ patients, respectively, were 29% *versus* 0% for S1 and 24% *versus* 0% for S2.

For OS, the unstratified HR (97.5% CI) for patients with MRD^- *versus* MRD^+ status, respectively, was 0.473 (0.259–0.863; one-sided *P*=0.0021) in S1 and 0.539 (0.213–1.366; one-sided *P*=0.0653) in S2. The mOS for patients who were MRD^- was longer than for those with MRD^+, for both S1 and S2 (Figure 3B). The respective 2-year OS rates were 40% *versus* 14% in S1 and 36% *versus* 12% in S2.

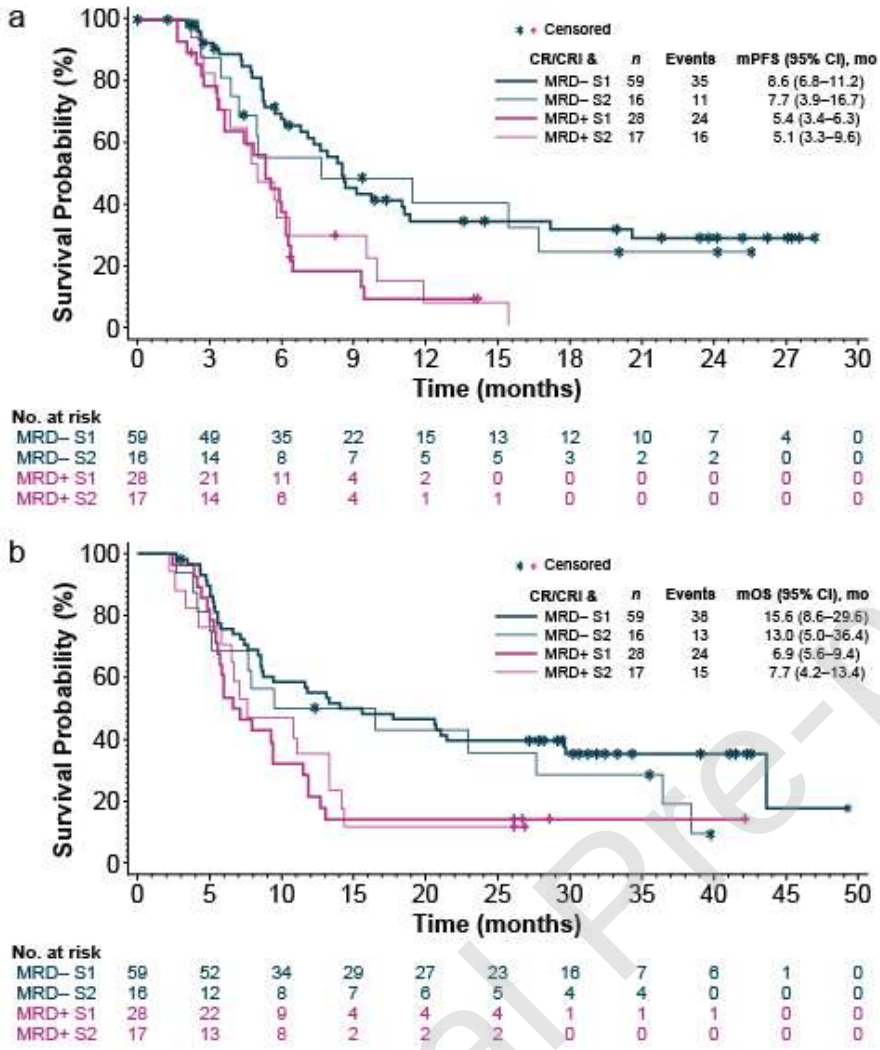

Figure 3 Outcomes with inotuzumab ozogamicin by minimal residual disease (MRD) response, stratified by salvage status. (**A**) Median progression-free survival (mPFS) and (**B**) median overall survival (mOS). *CI* confidence interval, *S1* salvage 1 status, *S2* salvage 2 status, *MRD* minimal residual disease.

3.5 Impact of follow-up ASCT on Outcomes

Of 121 patients who received InO and achieved CR/CRi, 65 (54%) underwent ASCT directly after achieving CR/CRi (48 of 76 patients with MRD^- and 17 of 45 MRD^+ status). The difference between MRD^- and MRD^+ groups for rates of direct ASCT was 25% (95% CI 7.5–43.2; *P*=0.0034). The median time from last MRD assessment to ASCT was the same for MRD– and MRD+ patients: 29 days in each group.

The overall ASCT rates for baseline S1 and S2 status were 61% and 52%, respectively. Overall follow-up ASCT rates according to both baseline salvage status and MRD at the EOT are shown in Table 2. The ASCT rate differences (95% CI) between MRD^- *versus* MRD^+ were 16% (–6.0, 38.2; *P*=0.0752) for S1 and 21% (–12.0, 54.6; *P*=0.1103) for S2. The greatest number of follow-up ASCT procedures occurred among patients in S1 who achieved MRD^- status at EOT (Table 2). Median survival follow-up time for patients who completed the study was 32.1 (range 27.2–49.3) months in the MRD^- (*n*=22) and 26.8 (range 26.2–42.3) months in the MRD^+ (*n*=6) groups.

	Salvage 1 (*n*=87)	Salvage 2 (*n*=33)
MRD– (*n*=75), *n* (%)	39/59 (66)	10/16 (63)
MRD+ (*n*=45), *n* (%)	14/28 (50)	7/17 (41)

ASCT allogeneic stem cell transplantation, *CR/CRi* complete remission/complete remission with incomplete hematologic response, *MRD* minimal residual disease.
*Defined as patients who achieved CR/CRi.
Note: First number is follow-up ASCT, second number is denominator for the breakdown of the respective MRD and salvage subgroup. One patient categorized as Salvage 3 and not included in this table.

Table 2 Rates of follow-up ASCT among responders* according to baseline salvage status and MRD status at end of treatment

Among patients who achieved MRD^{-} status, those who received follow-up ASCT had longer PFS than those who did not (Figure 4A); the unstratified HR was 0.495 [97.5% CI 0.255–0.960] with one-sided *P*=0.0075 and 2-year PFS rates were 38% and 9%. The OS was longer for those with follow-up ASCT *versus* those without (Figure 4B); the unstratified HR was 0.532 [97.5% CI 0.279–1.013] with one-sided *P*=0.0127 and the corresponding 2-year OS rates were 46% and 22%.

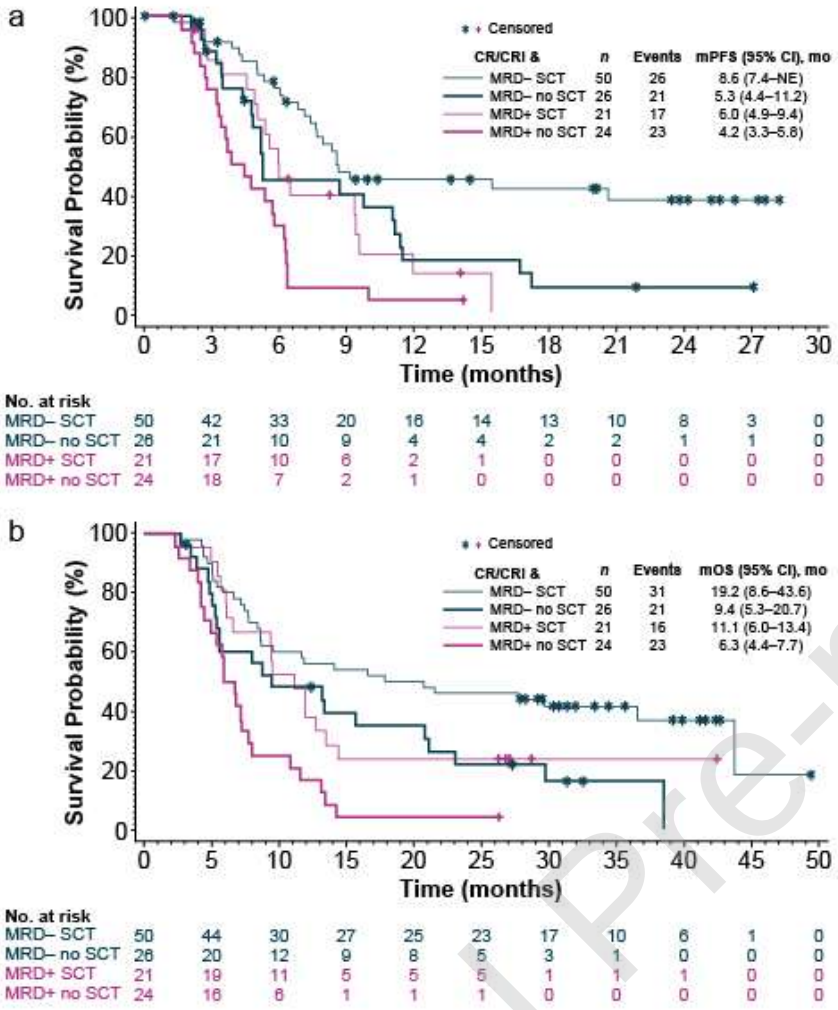

Figure 4 Outcomes with inotuzumab ozogamicin by minimal residual disease (MRD) response, stratified by follow-up allogeneic stem cell transplantation (SCT) status. (**A**) Median progression-free survival (mPFS) and (**B**) median overall survival (mOS). *CI* confidence interval, *MRD* minimal residual disease.

MRD$^+$ patients who received follow-up ASCT had longer mPFS than those who did not (Figure 4A); the unstratified HR was 0.506 [97.5% CI 0.241–1.064] with one-sided *P*=0.0183 and the 2-year PFS rates were 0%/not evaluable [NE] for each group, respectively. Similarly, patients MRD$^+$ with follow-up ASCT had longer OS than those without (Figure 4B); the unstratified HR was 0.446 [97.5% CI 0.211–0.938] with one-sided *P*=0.0062 and 2-year OS rates were 24% *versus* 4%. The OS outcomes in these patient subgroups according to MRD and ASCT status are shown in Table 3.

	ASCT		**No ASCT**		**ASCT vs. no ASCT**
	n	**mOS (95% CI), mo**	**n**	**mOS (95% CI), mo**	**HR (97.5% CI)**
MRD–	50	19.2 (8.6–43.6)	26	9.4 (5.3–20.7)	0.53 (0.28–1.01) *P*=0.0127*
MRD+	21	11.1 (6.0–13.4)	24	6.3 (4.4–7.7)	0.45 (0.21–0.94) *P*=0.0062*
HR (97.5% CI), MRD– vs. MRD+	0.63 (0.31–1.28) *P*=0.0704*		0.47 (0.23–0.98) *P*=0.0088*		–

ASCT allogeneic stem cell transplantation, *CI* confidence interval, *HR* hazard ratio, HSCT hematopoietic stem cell transplantation, *mOS* median overall survival, *MRD* minimal residual disease
*One-sided.

Table 3 Overall survival according to MRD and ASCT status

Patients in S1 who achieved MRD negativity after InO treatment and subsequently underwent ASCT appeared to have the best outcomes (Figure 5A). Among patients who became MRD^- in S1, those who underwent follow-up ASCT had longer PFS than those who did not (Figure 5A); the unstratified HR was 0.483 [97.5% CI 0.225–1.039] and the 2-year PFS rates were 40% *versus* 11%. Those in S1 who were MRD+ and underwent ASCT also had longer PFS than those who did not undergo ASCT (Figure 5A); the unstratified HR was 0.606 [97.5% CI 0.238–1.541]. Among patients who became MRD^- in S2, those who underwent follow-up ASCT (*n*=10) had longer PFS than those who did not (*n*=6). The mPFS (95% CI) was 11.6 months (3.9–NE) *versus* 7.5 months (2.6–16.7); the unstratified HR was 0.429 [97.5% CI 0.109–1.69] and the 2-year PFS rate was 38% *versus* 0%. Among patients MRD^+ in S2, those with ASCT (*n*=7) had an estimated mPFS (95% CI) more than double that for those without ASCT (*n*=10): 9.6 months (2.7–15.5) *versus* 4.3 months (2.3–5.8); the 2-year PFS rate was 0% with/without ASCT.

Likewise, OS in S1 for MRD^- patients with ASCT was longer than for those without (Figure 5B); the unstratified HR was 0.478 [97.5% CI 0.226–1.01] and the 2-year OS rate was 49% *versus* 21%. Patients in S1 who were MRD+ and underwent ASCT also had longer mOS than those who did not undergo ASCT (Figure 5B); the unstratified HR 0.488 [97.5% CI 0.193–1.237] and the 2-year OS rate was 21% *versus* 7%. Among MRD^- patients in S2 who underwent ASCT *versus* those who did not undergo ASCT, the mOS (95% CI) was 13.0 months (4.2–36.4) *versus* 15.5 months (2.7–38.4); the unstratified HR was 0.811 [97.5% CI 0.223–2.95] and the 2-year OS rate was 40% *versus* 25%. Among MRD^+ patients in S2 who underwent ASCT *versus* those

who did not undergo ASCT, the mOS (95% CI) was 13.4 months (2.7–NE) *versus* 6.9 months (2.3–10.8); the unstratified HR was 0.299 [97.5% CI 0.076–1.18] and the 2-year OS rate of 29% *versus* 0%.

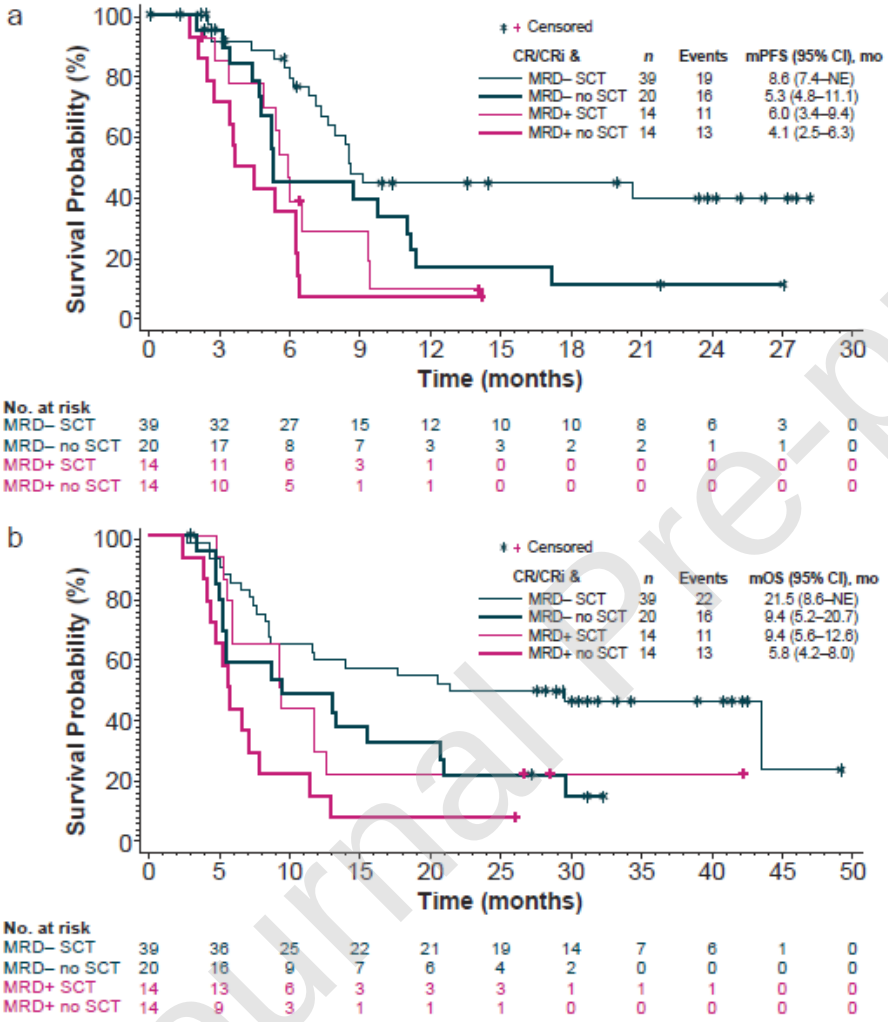

Figure 5 Outcomes of patients taking InO who achieved minimal residual disease negativity after first salvage treatment, stratified by allogeneic stem cell transplantation (SCT). (**A**) Median progression-free survival (mPFS) and (**B**) median overall survival (mOS). *CI* confidence interval, *MRD* minimal residual disease.

4. Discussion

The achievement of MRD negativity in response to frontline chemotherapy is predictive of improved survival among pediatric and adult patients with ALL.[1-6] In this study of adult patients with R/R ALL treated with InO, we found achievement of MRD negativity to be associated with improved outcomes in the salvage setting, resulting in an approximately two-fold increase *versus* patients who remained MRD^+ in both mPFS (9 *vs.* 5 months) and mOS (14 *vs.* 7 months). The rate of MRD negativity was higher among patients in early salvage (more than two-thirds of patients in S1 *vs*. almost half in S2). We identified that patients achieving MRD negativity and proceeding to ASCT had optimal outcomes (Figure 5), particularly in patients treated in S1 (Figure 3). However, when considering patients who did or did not have ASCT, patients with MRD^- status had improved OS compared to those with MRD^+ status.

The rate of MRD negativity among those with S1 *versus* S2 status who achieved CR/CRi after receiving InO was 68% *versus* 49%. Although the MRD negativity rate was lower among patients in S2, improved OS was still seen in S2 patients who were MRD^-. Patients in S1 or S2 who achieved MRD^- status had substantially improved survival outcomes compared with those who did not achieve MRD^-. Nonetheless, among patients in S1, achievement of MRD negativity was associated with the greatest increase *versus* MRD^+ in mOS (15.6 *vs.* 6.9 months) and a favorable 2-year OS rate (40% *vs.* 14%). Similar differences in outcomes were seen between MRD^- and MRD^+ in S2, although not as robust as observed for those in S1. Notably, patients who achieved MRD negativity in S1 had the highest rate of follow-up ASCT procedures (66%) and those who subsequently underwent ASCT had the best outcomes. Possibly because of

the smaller patient numbers in our subgroup analyses, a benefit for ASCT in S2 could not be clearly established. Nonetheless, as of the final data cut-off date of January 4, 2017, almost one-fourth (*n*=28 of 121) of InO-treated patients (MRD^- *n*=22; MRD^+ *n*=6) who achieved CR/CRi were still alive at a median follow-up of 2.6 years.

The survival time of patients who achieved MRD negativity in S1 (mOS: 15.6 months) and S2 (mOS: 13.0 months) compare favorably to the historical mOS of 4–5 months reported in adult patients with refractory or first relapsed ALL treated with SC-based salvage regimens.[7,23] Taken together, these findings suggest that patients with R/R ALL who achieve MRD negativity in S1 or S2 with InO treatment can have good long-term outcomes, especially if salvage ASCT is performed. Therefore, the use of InO in earlier lines of salvage (and, in particular, S1) is justified.[24, 25]

Our data also align well with observations from other studies in R/R ALL patients. For example, in an open-label, single-arm, phase II trial of blinatumomab (*n*=189, with 61% of patients in S1 or S2 and 39% in S≥3), median relapse-free survival was 6.9 months for MRD^- responders (*vs.* 2.3 months for MRD^+ nonresponders) and mOS was 11.5 months for MRD^- responders (*vs.* 6.7 months for MRD^+ nonresponders).[23] Our findings are also in agreement with a previous report by Jabbour *et al.* in patients with R/R ALL treated with one of three monoclonal antibody-containing regimens: InO, blinatumomab or low-dose chemotherapy plus InO.[21] In that study, better outcomes were obtained in patients treated in S1 who achieved MRD^- (though patients in later salvage fared more poorly when compared with S1 patients than we observed in the current study). InO and blinatumomab have both demonstrated superior efficacy compared with SC. The improved survival observed with these innovative strategies

may be mediated in part through the higher MRD-negativity rates achieved with these regimens as compared with standard cytotoxic chemotherapy.[10-12] Among patients who remained MRD^+ in first or subsequent remissions or who developed MRD relapse, those in their second CR or later who achieved complete MRD response with blinatumomab experienced a median relapse-free survival of 14 months and mOS of 19 months.[26] Furthermore, better outcomes were observed in patients with MRD^+ status treated in first remission as compared with patients treated in later remissions.

In general, MRD-negativity status has been shown to have a greater role in the outcomes of patients in S1 than in later salvage, i.e. outcomes were better when blinatumomab and InO were used earlier rather than later.[20,21] This was also observed when patients in S1 were treated with a combination of low-dose chemotherapy (mini-hyper-CVD) and InO.[12] Overall response and MRD^- rates were 91% and 93%, respectively, which translated into mOS of 25 months and a 1-year survival rate of 63% for patients in S1 who received ASCT.[12] These findings compare favorably with historical data of patients in S1 treated with SC who had a median survival of 6 months and a 5-year survival rate of 7%.[7, 13, 14, 27] In the current study, patients in S2 who achieved MRD negativity also had notable improvements in survival outcomes compared with MRD^+, although not as strong as those seen for patients in S1.

A lower disease burden was also associated with improved outcomes in patients treated with chimeric antigen receptor T-cell [CAR-T] therapies.[13,14] Adults with low disease burden had better event-free survival (median 10.6 *vs.* 5.3 months) and better OS (median 20.1 *vs.* 12.4 months) than that seen in patients with high-disease burden.[14] Our findings suggest that achieving a minimal measurable disease in the

salvage setting may translate into improved outcomes among patients treated with novel monoclonal antibody–based or immunotherapy-based regimens.

Our study has several limitations. Although we identified better outcomes in patients with MRD^- status (which in itself could be a marker of better disease), and particularly when ASCT was performed, we were not able to determine the optimal timing of MRD assessment in the salvage setting because treatment duration was variable and the number of MRD assessments depended on how long patients were on treatment; the potential for selection bias may have also contributed to difficulties in this assessment. Owing to the *post hoc* nature of this analysis of InO in patient subgroups from the primary study, smaller patient numbers also limit the interpretation of these results. In addition, this analysis could not control for the possibility that patients who received stem cell transplantation might have been younger or healthier (thereby more likely to meet eligibility criteria for undergoing transplantation) than those who did not proceed to ASCT. Lastly, this analysis was not adjusted for multiple testing.

In the frontline setting, the prognostic impact of MRD response varies based on the timing of MRD assessment, and therefore future studies to evaluate this and other issues in patients with R/R ALL are warranted. In addition, the finding that relapses were still frequent among patients who achieved MRD negativity highlights the importance of developing newer, more sensitive assays for MRD. Next-generation sequencing holds promise in identifying MRD with a higher level of sensitivity, although experience with this approach is relatively limited[28, 29] and potential consequences from detection of low-level MRD remain to be defined. Nevertheless, in the salvage setting, improved outcomes are often associated with ASCT, which generally has been shown

to be more successful among patients with MRD^- *versus* MRD^+ status. Even so, a recently published analysis from the TOWER study indicated that ASCT did not provide overall survival benefit among patients treated with blinatumomab who achieved complete remission; when patients were stratified by MRD response, no difference was detected in OS between those with ASCT *versus* no ASCT.[30] (As the authors of the paper noted, caution should be exercised when interpreting those data because of relatively small patient numbers and limited follow-up time.)

In conclusion, the achievement of MRD negativity after InO therapy in patients with R/R ALL is associated with improved survival outcomes. This was observed among patients in S1 or S2, though the data were more robust for patients with S1 status at baseline. Also, in this study, ASCT appeared to play a role in achieving optimal long-term survival. This was especially evident among MRD^- patients. In this analysis, achievement of MRD negativity appears to be an important therapeutic goal in the salvage setting.

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