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Running Head: Inotuzumab ozogamicin in pediatric ALL

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Study conception and design: CMZ and Pfizer Inc. through the development of the Pediatric Investigational Plan and its approval by the Paediatric Committee (PDCO) of the European Medicines Agency (EMA).

Data collection and patient management: CMZ, ACJA, EP, EB, BB, VHJVDV, SR, CDR, FJBS, ARSS, FR, CR, HBB, AP, BB, IO, AN, GE, CR, BB, FL

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ABSTRACT

Inotuzumab Ozogamicin (InO) is a CD22-directed antibody conjugated with calicheamicin. The Phase 1B of the ITCC-059 trial tested InO combined with chemotherapy in pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Relapsed /refractory CD22+ BCP-ALL pediatric patients were enrolled. The primary objective was to establish the Recommended Phase 2 Dose (RP2D). Secondary objectives included preliminary efficacy and tolerability. InO was combined with 1.5 mg/m^2 of vincristine (days 3, 10, 17, 24), 20 mg/m^2 of dexamethasone (two 5-day blocks, then amended), and intrathecal therapy. A rolling-6 design was used testing InO from 0.8 to 1.8 mg/m^2/cycle. Between May-2020 and Apr-2022, 30 patients were treated, and 29 were evaluable for dose limiting toxicities (DLTs). At 1.1 mg/m^2/cycle, two out of four patients had DLTs (liver toxicity). InO was de-escalated to 0.8 mg/m^2/cycle (n=6) without DLTs while awaiting a protocol amendment to reduce dexamethasone dose to 10 mg/m^2. Post amendment, InO was re-escalated to 1.1 mg/m^2/cycle (n=6, 1 DLT), then to 1.4 mg/m^2/cycle (n=3, no DLTs), and finally to 1.8 mg/m^2/cycle (n=7, 1 DLT). Three additional patients were treated in an expansion cohort. The pooled response rate was 80% (24/30; 95%CI: 61.4% to 92.3%) and, among responders, 66.7% achieved minimal residual disease negativity. The RP2D of InO combined with vincristine, dexamethasone and IT therapy was declared at 1.8 mg/m^2/cycle (1.5 mg/m^2/cycle after remission) in a fractionated schedule. This combination showed an response rate similar to the single agent cohorts of this trial, with liver toxicity issues at the initial higher dexamethasone dose. #NTR5736

1. INTRODUCTION

Approximately 10-15% of pediatric patients with acute lymphoblastic leukemia (ALL) experience disease relapse.[1] Following relapse, the estimated 10-year overall survival (OS) probability is around 50%, depending on the risk group.[2,3] The traditional treatment for relapsed patients is based on intensive chemotherapy.[4] A randomized trial in relapsed and refractory (R/R) pediatric patients comparing the two most used treatment strategies in Europe, the ALL-REZ BFM 2002 and the UKALL-R3, showed no significant differences in the 5-year probability of event-free survival (EFS) or OS.[5] Nevertheless, a subgroup analysis showed that patients with isolated bone marrow (BM) relapse had a significantly lower relapse rate (RR) if treated within the R3 arm (5-year cumulative incidence 6.5%, n=153) compared to the BFM arm (5-year cumulative incidence 12.5%, n=146); while the BFM approach resulted in superior outcome in patients experiencing isolated Central Nervous System (CNS) relapse (5-year EFS 81.6% , n=40 vs 43.3%, n=45).[5]

Increasing the intensity of chemotherapy to treat R/R patients is constrained by toxicity. For example, the UKALL-R3 reinduction block 1 (vincristine, mitoxantrone, dexamethasone, and asparaginase) results in non-negligible adverse events (AEs), especially in terms of severe infections (23.7%) and induction death (3%).[5] In B-cell precursors (BCP) ALL, toxicity can be reduced by using the CD19-directed T-cell engager blinatumomab, which proved efficacious in high-risk first relapse patients, while the reinduction remission rate in overt relapse ranged between 34% and 60%.[6–8] Moreover, CD19-specific chimeric antigen receptor (CAR) T cells therapies showed high complete remission rates in multiple relapsed BCP-ALL patients, and may be considered definitive therapy without allogeneic hematopoietic stem cell transplantation (HSCT) in some cases. Indeed, a 3-year EFS of 44% was reported for patients enrolled in the ELIANA trial.[9,10]

Despite improvements, new options for effective salvage of pediatric R/R ALL patients and for increasing the overall cure rates in this cancer are still needed. In the context of targeted chemotherapy, Inotuzumab Ozogamicin (InO) is a CD22-directed antibody-drug conjugate loaded with the cytotoxic agent calicheamicin which is already approved for adults with CD22-positive R/R BCP-ALL, based on the results from the INO-VATE ALL trial.[11,12] The safety and preliminary efficacy of InO as single agent in pediatric R/R BCP-ALL have been tested in phase I and phase II trials conducted by The Innovative Therapies for Children with Cancer (ITCC) consortium in Europe and by the Children's Oncology Group (COG) in the USA.[13–15] Namely, the estimated Overall Response Rate (ORR) in the phase II trials from COG and ITCC groups ranged from 58.3%

(90%CI: 46.5 - 69.3) to 81.5% (95%CI: 61.9%-93.7%), respectively, with approximately 70% minimal residual disease (MRD) negativity rate in responding patients.[14,16] Overall, InO appeared well-tolerated in children with R/R BCP-ALL and was associated with high response rates, potentially higher than with blinatumomab, despite no trial compared the two treatments in this population.

Studies in adults have investigated InO combined with chemotherapy, for examples with mini-hyper-CVD (cyclophosphamide, vincristine and dexamethasone in cycles 1, 3, 5, 7, and methotrexate plus cytarabine in cycles 2, 4, 6 and 8) or CVP (cyclophosphamide, vincristine and prednisone), and showed it is safe to combine these agents.[17,18] By contrast, in pediatrics, the safety of InO in combination with chemotherapy has not been assessed yet. Herein, we report the results from the phase IB of the trial ITCC-059 in the R/R setting, in which InO was combined with a modified UKALL-R3 regimen containing vincristine, dexamethasone and intrathecal (IT) therapy. This combination was developed with the aim to replace mitoxantrone with InO in the UKALL-R3 reinduction regimen, aiming at increasing efficacy while reducing toxicity.

Trial ITCC-059 is a phase I-II, multicenter, international, open-label clinical trial conducted in accordance with the International Council for Harmonization Guidelines for Good Clinical Practice, and the Declaration of Helsinki. Patients were treated under protocol version 3 and 4 following an amendment, after the single-agent recommended phase II dose (RP2D) was established in the single agent phase I part. Informed consent was obtained from all patients or their parents (as applicable) before enrolment. The study was sponsored by the Erasmus MC and funded by Pfizer inc. in the context of a Pediatric Investigational Plan. Netherlands Trial Registry nr NL5629 (EudraCT:2016-000227-71).

2. METHODS

2.1 PATIENTS AND TREATMENT

Patients aged ≥1 to <18 years, with CD22-positive BCP-ALL, M2/M3 bone marrow status, and either refractory disease, ≥2nd relapse, or any relapse post-HSCT were enrolled. Patients with isolated extramedullary disease were excluded (Table S1). The protocol received Institutional Review Board and/or Ethics Committee review and approval at all participating centers.

Four dose levels (DLs) of InO (fractionated on days 1, 8, and 15 of each cycle) with loading dose on day one (omitted once in remission) were tested (Table S2).[19] Namely, 0.8 mg/m^2/cycle (0.4 + 0.2 + 0.2 mg/m^2), 1.1 mg/m^2/cycle (0.5 + 0.3 + 0.3 mg/m^2), 1.4 mg/m^2/cycle (0.6 + 0.4 + 0.4 mg/m^2) and 1.8 mg/m^2/cycle (0.8 + 0.5 + 0.5 mg/m^2). InO was combined with vincristine 1.5 mg/m^2 (days 3, 10, 17 and 24), two 5-days blocks (days 1-5 and 15-20) of dexamethasone 20 mg/m^2 (later amended), and, depending on CNS status, IT therapy (days 1 and 8) with methotrexate alone or combined with cytarabine and steroids as per the UKALL-R3 regimen (Figure S1).[20] From cycle two, and per investigator's discretion, patients could receive either combination therapy or InO single agent at 1.8 mg/m^2/cycle, or 1.5 mg/m^2/cycle if already in remission.[13] A maximum of six cycles were allowed (of which maximum two combination cycles), except for those cases planned to proceed to HSCT, for which two cycles, or three in case the patient was not yet MRD negative after cycle two, were recommended. Criteria to proceed with the next cycle are reported in Methods S1.

2.2 STUDY DESIGN

The primary objective was to determine the RP2D. A rolling-6 design was used, assessing dose-limiting toxicities (DLTs) during cycle one (28 days) and capping the maximum escalation dose at 1.8 mg/m^2, based on the RP2D of InO single agent.[13,21] The primary end-point was the occurrence of DLTs, defined as any of the following toxicities related to InO: any grade 5 toxicity; absolute neutrophil count (ANC) <500/μL and/or a platelet count <50□000/μL lasting > 42 days in the absence of persisting leukemia; grade 3-4 non-hematologic toxicities persisting for > 48 hours (> 7 days for hepatic transaminases or bilirubin abnormalities). Secondary endpoints included frequency and severity of AEs based on the Common Terminology Criteria for Adverse Events version 4.03, and occurrence of toxic death. Preliminary efficacy end-points included ORR and MRD negativity

status (as best response after InO treatment and after cycle one), OS, EFS, duration of response (DOR).

All patients who received at least one dose of InO were considered for safety analysis. Those which also completed at least one baseline and one post-baseline disease assessment were considered for efficacy analysis (Methods S2). ORR was defined as the combined complete remission (CR; <5% BM blast, CNS1 without extramedullary leukemia), CR with insufficient platelet recovery (CRp; ANC > 500/μL but platelets ≤ 50,000/μL), and CR without recovery of counts (CRi; ANC ≤ 500/μL with or without platelets ≤50,000/μL). MRD negativity was centrally determined, and defined as either a PCR result below 10^{-4}, or a flow cytometry result below 0.01% when the QT-PCR was negative, but the quantitative range was > 10^{-4} (Methods S3).[22,23] EFS and OS probabilities (Methods S4) were estimated using the Kaplan–Meier method. SOS definition is provided in Methods S5.

3. RESULTS

Between 14-May-2020 and 11-Apr-2022, 37 patients were screened, 30 were treated, 29 were evaluable for the assessment of DLT, and 30 were evaluable for response (one patient received the wrong dose of InO on day 1 of cycle 1; the patient was excluded from the DLT assessment, but counted for response and overall safety as per protocol). Dataset cut-off date was 28-Feb-2023. Patient characteristics are reported in Table 1. Initially, four patients were enrolled at 1.1 mg/m^2/cycle of InO. Two DLTs occurred; namely grade 3 hepatic transaminases elevation lasting more than 7 days, and one case of grade 3 sinusoidal obstruction syndrome (SOS). InO was then de-escalated to 0.8 mg/m^2/cycle, and seven patients completed cycle one, of which one received in error 0.8 mg/m^2 on day 1, 0.5 mg/m^2 on day 8, and skipped the day 15 dose (instead of receiving 0.4 + 0.2 + 0.2 mg/m^2 on days 1, 8 and 15) and therefore was not evaluable for DLT (but included in the safety and efficacy dataset). No DLTs were recorded at 0.8 mg/m^2/cycle of InO. Nevertheless, the Steering Committee decided to amend the protocol to reduce the dexamethasone dose from 20 mg/m^2/day to 10 mg/m^2/day (divided in two daily administrations). The intent was twofold. First, mitigating liver toxicity which consisted of transient hepatic transaminases elevation probably caused by steroids and, secondly, allowing the use of higher doses of InO, closer to the RP2D already established for the single agent regimen (1.8 mg/m^2/cycle) also given the lower response rates observed at lower doses in phase 1A (DL1: ORR 75% and MRD negativity: 66%; DL2: ORR 85%, and MRD negativity 100%).[13,16]

Upon approval of the amendment the dose of InO was re-escalated. InO was first tested at 1.1 mg/m^2/cycle (n=6, one DLT: grade 3 hepatic transaminases elevation > 7 days); subsequently at 1.4 mg/m^2/cycle (n=3, no DLTs), and then at 1.8 mg/m^2/cycle (n=7 as two patients registered contemporary; one DLT occurred: ANC below 0.5 x 10^9/L > day 42). At the same dose level, three additional patients were enrolled in an expansion cohort (not assessed for DLT), increasing the total number of patients treated at 1.8 mg/m^2 of InO combined with chemotherapy to 10 (Table 2). The RP2D of InO in combination with 1.5 mg/m^2 of vincristine (days 3, 10, 17, 24) and 10 mg/m^2 of dexamethasone (two 5-day blocks) was declared at 1.8 mg/m^2/cycle (1.5 mg/m^2/cycle once in complete remission).

3.1 SAFETY

Sixteen patients received only one cycle of combination therapy, 10 patients one combination cycle plus one single agent cycle, three patients two combination cycles, and one patient received one combination cycle plus two single agent cycles.

All patients experienced at least one AE (Table S3). Alanine aminotransferase increase (ALT) occurred in 23 patients (76.%) of which 15 (50%) were ≥ grade 3. Aspartate aminotransferase (AST) increase occurred in 22 patients (73.3%) of which 10 (33.3%) were ≥ grade 3. Overall, 24 (80%) patients had either AST and/or ALT elevation. Seven patients (23.3%) had bilirubin increase; of which six (20%) at grade 1-2, and one (3%) at grade 3. None met Hy's law criteria.[24] Toxicities recorded before and after amending the dexamethasone dose are provided in Table 3.

Overall, 63% of patients reported infections. Four (13%) patients had sepsis, one (3%) had grade 3 skin infection, one (3%) grade 3 urinary infection, and two (7%) other grade 3 infections. Other eleven (36.7%) patients had grade 1-2 infections. Ten patients (33.3%) had grade 3 febrile neutropenia.

Platelet count decrease was experienced by 22 patients (73%) of which 20 (67%) at grade ≥ 3. Overall, ANC decrease was observed in 19 patients (63.3%) of which 18 (60%) at grade ≥ 3. Anemia was experienced by 24 patients (80%) of which 19 (63.3%) at grade ≥ 3. The full lists of AEs, treatment-relatedness, and laboratory abnormalities are provided in Tables S3-5. In total, five (17%) patients developed SOS. Four following HSCT (one grade 4 and three grade 3), after receiving a cumulative dose of 2.2, 2.9, 3.2 and 3.6 mg/m^2 of InO, and being transplanted 68, 38, 30 and 29 days since the last InO dose, respectively. The fifth case of SOS (grade 3) occurred on treatment after the administration of 0.8 mg/m^2 (0.5 + 0.3) of InO. Among those developing SOS post InO, one subject had a prior transplant. Four patients with SOS recovered completely, while in one case SOS was ongoing when the patient died due to sepsis after HSTC. Overall, SOS occurred in 21% (4/19) of the patients that received a HSCT any time after InO (including patients receiving additional treatment after InO and before HSCT). The median time to onset of SOS since the last InO dose was 47.5 days (range 36 - 119). Among the transplanted patients, six received prophylaxis with defibrotide per investigators' discretion, none of which developed SOS. A 11-year-old female subject who had received chemotherapy and two prior HSCT developed posterior reversable

encephalopathy syndrome while on treatment with InO at 1.8 mg/m^2/cycle and dexamethasone at 10 mg/m^2 at day 19 of the first cycle. The patient also received IT methotrexate on day 1 and 8 (15 mg) and vincristine on days 3, 10, 17. The subject recovered completely. The event was not considered related to InO but rather attributed to the background chemotherapy.[25] Four patients died while in CR after receiving HSCT. Two of them died due to infection (respiratory infection and post SOS septic shock), one had a multiorgan failure, and the fourth death was due to thrombotic microangiopathy (without prior SOS). The cumulative incidence of non-relapse death was 6.7% (95%CI: 1.1-19.5) at six months, and 10.2% (95%CI 2.5-24.3) at 12 months, including post HSCT follow-up.

3.2 EFFICACY

Combining all dose levels (n=30), 24 patients achieved complete response (ORR 80%; 95% CI: 61.4% to 92.3%) of which 22 (73%) after cycle 1; 20 were in CR, three in CRp and one in CRi. Response by dose level is provided in Table 2. Among responders, MRD negativity as best response was achieved by 16 (66.7%) subjects of which 13 after cycle one (Figure 1). Among those treated at 1.8 mg/m^2 in cycle 1 (n=10), 8 (80.0%) achieved response, and 6 (75.0%) also achieved MRD negativity after cycle one.

A total of 21 patients (70%) proceeded to consolidation therapy, 15 (50%) with HSCT (of which one after bridging with blinatumomab in presence of MRD positivity) and six (20%) with CAR T-cell therapy. Additionally, at the time of cut-off date, one responding patient received maintenance chemotherapy (then died due to relapse 10 months after last dose of InO) and other two responding patients did not receive consolidation treatment yet and relapsed a few months later (Figure 2). Other four patients received HSCT following additional therapy, of which three after relapse post InO, and one among the non-responders. Notably two of them received InO a second time and were able to proceed to HSCT (after relapse post CAR T).

The median follow-up was 15.9 months (Interquartile Range [IQR]: 12.4 – 18.4). At 6 months, the EFS probability was 66.5% (95%CI: 51.5-85.8) and the OS probability was 76.6% (95%CI: 62.9-93.4). At 12 months, the EFS probability was 41.7% (95%CI: 27.1-64.3) and the OS probability was 62.3% (95%CI: 46.9-82.8) (Figure 3). Median DOR was 8.38 months (IQR: 2.3-11.9). In a post-hoc analysis, we did not observe statistically significant differences in EFS and OS between responders

consolidated with HSCT or CAR-T cell therapy (Figures S2-S3). The cumulative incidence of relapse was 8.3% (95%CI: 1.0-27.0%) at 6 months and 13.6% (95%CI 2.9-34.0%) at 12 months. Overall, 10 patients relapsed of which five died (Figure 2), and three deaths occurred among the five non-responding subjects. Additionally, four patients died while in remission, for a total of 12 deaths.

4. DISCUSSION

This trial showed that in pediatric R/R CD22-positive BCP-ALL patients InO can be safely combined with 1.5 mg/m^2 of vincristine (days 3, 10, 17, 24), 10 mg/m^2 of dexamethasone (two 5-day blocks, BID) and IT therapy, at 1.8 mg/m^2/cycle, the same RP2D as per InO single agent.[13,19]

Despite this promising safety profile, our data suggest that the combination of InO with chemotherapy might increase the risk of transaminases elevation compared to the single agent treatment. Indeed, we observed 14.3% AST elevation ≥ grade 3 and 17.9% ALT elevation ≥ grade 3 in the single agent arm of this trial, compared to 33.3% ≥ grade 3 AST elevation and 50% ≥ grade 3 ALT elevation in the combination arm reported here. It is well known that transaminases are frequently increased by chemotherapy and by dexamethasone.[26] The clinical relevance of this data remains unclear as ALT/AST increase does not necessarily reflect or predict severe hepatotoxicity and, in our study, it was not associated with severe or long-lasting liver impairment, nor with clinically significant bilirubin increase, which only in one case was reported at grade 3 and none at grade 4.[26,27] By contrast, we confirm that one of the major risks associated with InO is SOS, and particularly in patients proceeding to HSCT as consolidation after InO treatment. Nevertheless, the addition of vincristine and dexamethasone to InO did not seem to further increase the incidence of SOS when compared to the single agent arms of the same trial (overall SOS incidence was 16.6% in phase IB vs 17.3% in phase IA and II combined; while among patients consolidating with HSCT after InO treatment it was 21% vs 26.1%, respectively), despite a rigorous comparison was not possible due to the non-randomized approach, the heterogeneity of the InO dose administered, SOS prophylaxis which was not uniformly performed, and the small sample size.[13,16] Beside, no significant differences in the incidence of AEs were observed before and after the amendment of the protocol (Table 3) in this limited sample size. Nevertheless, reducing dexamethasone dose prevented the occurrence of DLTs and allowed a higher escalation of InO under the rolling-6 rules.

The data reported above are in line with other trials in older R/R patients with CD22+ BCP-ALL. In trial SWOG 1312 (NSC-772518), InO at 1.8 mg/m^2 was safely combined with cyclophosphamide

750 mg/m^2, vincristine 1.4 mg/m^2 and max 2 mg, prednisone 100 mg orally days 1-5 for R/R CD22+ BCP-ALL, resulting in approximately 60% response.[18] Similarly, in the EWALL-INO study (NCT03249870), InO was safely combined at 1.8 mg/m^2/cycle with one triple IT injection, vincristine (1-2 mg, weekly) on day 1, 8, 15 and 22, and four 2-day blocks of dexamethasone (20 mg/day) and resulted in 87.7% response.[28]

In terms of efficacy, the ORR of InO combined with chemotherapy was comparable to the single agent arm of the trial (ORR 80% vs 81.5%).[16] In this phase 1B, though, it should be noticed that in cycle one we tested a much larger spectrum of dose levels, from 0.8 mg/m^2/cycle to 1.8 mg/m^2/cycle. In addition, the estimated ORR for the single agent cohort of this trial is already very high and it might be unnecessary to combine InO with toxic chemotherapy in heavily pre-treated patients to obtain a relatively small marginal improvement. Due to these considerations, it was decided not to proceed with the additional cohort 1B-ASP as originally planned, in which PEG-asparaginase on day 3 and 17 (1000 IU/m^2) would have been simultaneously added to the combination of InO and chemotherapy.

Furthermore, it is worth noting that recent data showed that low-intensity chemotherapy schemes without asparaginase when combined with multiagent immunotherapy can deliver very high ORR in both adults and children while sparing some of the toxicities related to chemotherapy.[29,30] For example, the MD Anderson Cancer Center is developing multiagent immune/target therapy regimens that combine low-intensity chemotherapy with blinatumomab, InO and rituximab in the so-called Pedi-cRIB regimen (NCT05645718). Early results have described that the combination of mini-hyper-CVD with cRIB (InO at 1.2 mg/m^2/cycle: 0.6 + 0.3 + 0.3 mg/m^2) is well-tolerated also in heavily pretreated pediatric patients.[31] In adults, mini-Hyper-CVD was administered with InO at a dose of 1.3 - 1.8 mg/m^2 in cycle 1, which was later amended to lower dosages to mitigate the risk of liver toxicities. rituximab was added in CD20+ patients only and patients subsequently received consolidation with blinatumomab. The combination yielded a remission rate of 89%, and the 5-year progression-free survival was 44.0% (95%CI: 31.2 - 54.3), in elderly newly diagnosed patients (n=80, median age 68, IQR: 63-72); while in younger subjects (n= 31, median age 25, range: 18-57) the remission rate and 1-year OS probability were both 100%, although 3 patients (10%) had isolated CNS relapse (NCT01371630).[32–34] Such regimens, developed due to the poor tolerance of high-intensity chemotherapy in elderly patients, are now being integrated into frontline setting followed by CAR T-cell consolidation. This represents a new paradigm for front-line ALL treatment which might

impact also future pediatric regimens currently still relying on conventional chemotherapy, particularly for the induction phase of the treatment.[35]

In the context of R/R pediatric patients, the trial NCT05748171 will randomize InO as single agent against the UKALL-R3 regimen in high-risk first relapse ALL patients. In newly diagnosed pediatric patients, a phase 3 randomized trial in high-risk CD22+ BCP-ALL (AALL1732) sponsored by the Children's Oncology Group, is evaluating two cycles of single agent InO at 1.2 mg/m^2 after standard induction and post-induction chemotherapy. Following consolidation, patients with MRD > 0.01% were randomized 1:1 (n=50) to chemotherapy (Arm A) or chemotherapy plus 2 cycles of InO (Arm B), one before the high-dose methotrexate interim maintenance and the other before proceeding to the delayed intensification blocks. From an interim analysis, no differences in grade ≥3 ALT or bilirubin elevations were recorded between arm A and B, but patients treated with InO showed a significant higher incidence of neutropenia (87.5% vs 50%) and sepsis during delayed intensification (10 cases in arm B, 1 case in arm A), as well as SOS (4 cases in arm B, 0 in arm A). The enrolment was halted and treatment was amended to mitigate toxicity during post InO chemotherapy blocks.[36] In Europe, the 'AllTogether1' group (NCT03911128) is testing InO given at 0.5 mg/m^2/week for six weeks as additional consolidation block in a randomized fashion within the intermediate-high risk patient group with high MRD levels. Patients randomized to receive InO, will be given two cycles of InO during consolidation.

In conclusion, preliminary efficacy and safety data underscore the possibility to combine InO up to 1.8 mg/m^2 with vincristine, dexamethasone and IT therapy in a safe manner. Nevertheless, a noticeable advantage of this combination regimen in terms of ORR when compared to the single agent arms of the same trial was not observed in these heavily pretreated patients. This study contributes to the knowledge on safety and efficacy of InO in pediatric patients, and opens the possibility to use less chemo-intensive treatments in pediatric ALL by either using InO as a single agent or in combination with immunotherapies such as blinatumumab and rituximab as already done in adults.

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Table 1. Patient Characteristics

Characteristic	N
Sex (%)	
Male	19 (63)
Female	11 (37)
Age at Enrollment (years)	
Median (range)	8.5 (1-17)
Status at Enrolment (%)	
First relapse post HSCT	2 (7)
≥ 2nd relapse	20 (67)
Refractory disease	8 (27)
Extramedullary Disease (%)	
CNS1	27 (90)
CNS2	2 (7)
CNS3	1 (3)
Testicular involvement	0 (0)
Lymph nodes enlarged	0 (0)
Other locations	1(3)
Other (range)	
Median WBC (10^9/L)	5.03 (1.27-63.60)
Median CD22 MFI†	1687 (359-7003)
Median CD22+ blast BM†	98% (66 – 100)
Selected Genetic Abnormalities (%)*	
High-hyperdiploid (51-67 chromosomes)	4 (13)
t(12;21)(p13.2;q22.1); ETV6::RUNX1	3 (10)
t(1;19)(q23;p13); TCF3::PBX1	3 (10)
t(4;11)(q21;q23); KMT2A::AFF1	1 (3)
TP53 mutation and/or deletion	1 (3)
TP53 mutation and/or deletion & t(12;21)(p13.2;q22.1); ETV6::RUNX1	2 (7)
IKZF1/7p12	1 (3)
t(9;22)(q34;q11.2); BCR::ABL1	2 (7)
Other	4 (13)
Normal	4 (13)
Not Available	5 (17)

* Known abnormalities detected either by karyotype and/or molecular methods (e.g. FISH, RT-PCR) at the local laboratory. † at screening as determined at the central laboratory on BM. WBC: White Blood Cells at screening; MFI: Mean Fluorescence Intensity; PB: Peripheral Blood; BM: Bone Marrow.

Table 2. Dose Escalation History

Dose of InO in cycle 1	Patients treated	DLTs	Notes	Achieved CR (%)
1.1 mg/m²	4	2 (SOS, AST ↑)	Both events resolved	3 (75)
0.8 mg/m²	7*	0		5 (71)‡
Amendment: Dexamethasone reduced to 10 mg/m² divided in 2 administrations per day (BID)				
1.1 mg/m²	6	1 (AST ↑)	AST normalized after 9 days	5 (83)
1.4 mg/m²	3	0		3 (100)
1.8 mg/m²	7**	1 (ANC ↓) > day 42)†	ANC recovered on day 45	6 (86)
1.8 mg/m²	3	NA	Expansion cohort	2 (67)

DLTs: Dose Limiting Toxicities; SOS: Sinusoidal Obstruction Syndrome of the liver; AST: Alanine Amino Transferase; ↑ Increase ≥ grade 3; ANC: Absolute Neutrophil Count; † < 500/μL. NA: Not Assessed. CR: Complete Remission. * One patient received a wrong dose of InO (1.3 mg/m²), therefore was excluded from the DLT calculation and replaced. ** Two patients were pre-registered contemporarily, therefore 7 instead of 6 were enrolled at this dose level. ‡ among the five responders one patient received 1.3 mg/m²/cycle (see results section).

Table 3. Most Frequent Treatment Emergent Adverse Events (>20%) Divided by Grade and Before and After Dexamethasone Amended Dose

AE Term	Full Dexamethasone dose (20 mg/m^2) n= 11		Reduced Dexamethasone dose (10 mg/m^2) n= 19		Total (n=30)
	Grade 1-2	Grade ≥ 3	Grade 1-2	Grade ≥ 3	
Anemia	2 (18%)	7 (63%)	3 (15%)	12 (63%)	24 (80%)
AST increased	2 (18%)	5 (45%)	6 (32%)	10 (53%)	23 (77%)
ALT increased	3 (27%)	3 (27%)	9 (47%)	7 (37%)	22 (73%)
Platelet count decreased	1 (9%)	8 (72%)	1 (5%)	12 (63%)	22 (73%)
ANC decreased	1 (9%)	6 (54%)	0	12 (63%)	19 (63%)
Constipation	3 (27%)	0	12 (63%)	0	15 (50%)
Fever	6 (54%)	0	7 (37%)	0	13 (43%)
Headache	4 (36%)	1 (9%)	8 (42%)	0	13 (43%)
Febrile neutropenia	0	3 (27%)	0	7 (37%)	10 (30%)
Hypokalemia	0	2 (18%)	4 (21%)	3 (15%)	9 (30%)
Abdominal pain	3 (27%)	0	5 (17%)	0	8 (27%)
Bilirubin increased	2 (18%)	0	4 (21%)	1 (5%)	7 (23%)
GGT increased	1 (9%)	1 (9%)	2 (11%)	3 (15%)	7 (23%)

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ANC: Absolute Neutrophil Count; GGT: Gamma Glutamyl Transferase

List of Figures' Legends

Figure 1. Proportions of Non-Responders, Responders and MRD negativity

Responders are defined as those with <5% of bone marrow blasts regardless the recovery of the neutrophil count and platelets. MRD negativity is defined as $<10^{-4}$. CR: Complete Remission; MRD: Minimal Residual Disease; SD/PD: Stable Disease/Progressive Disease

Figure 2. Swimmer Plot of Patients' Treatment and Response

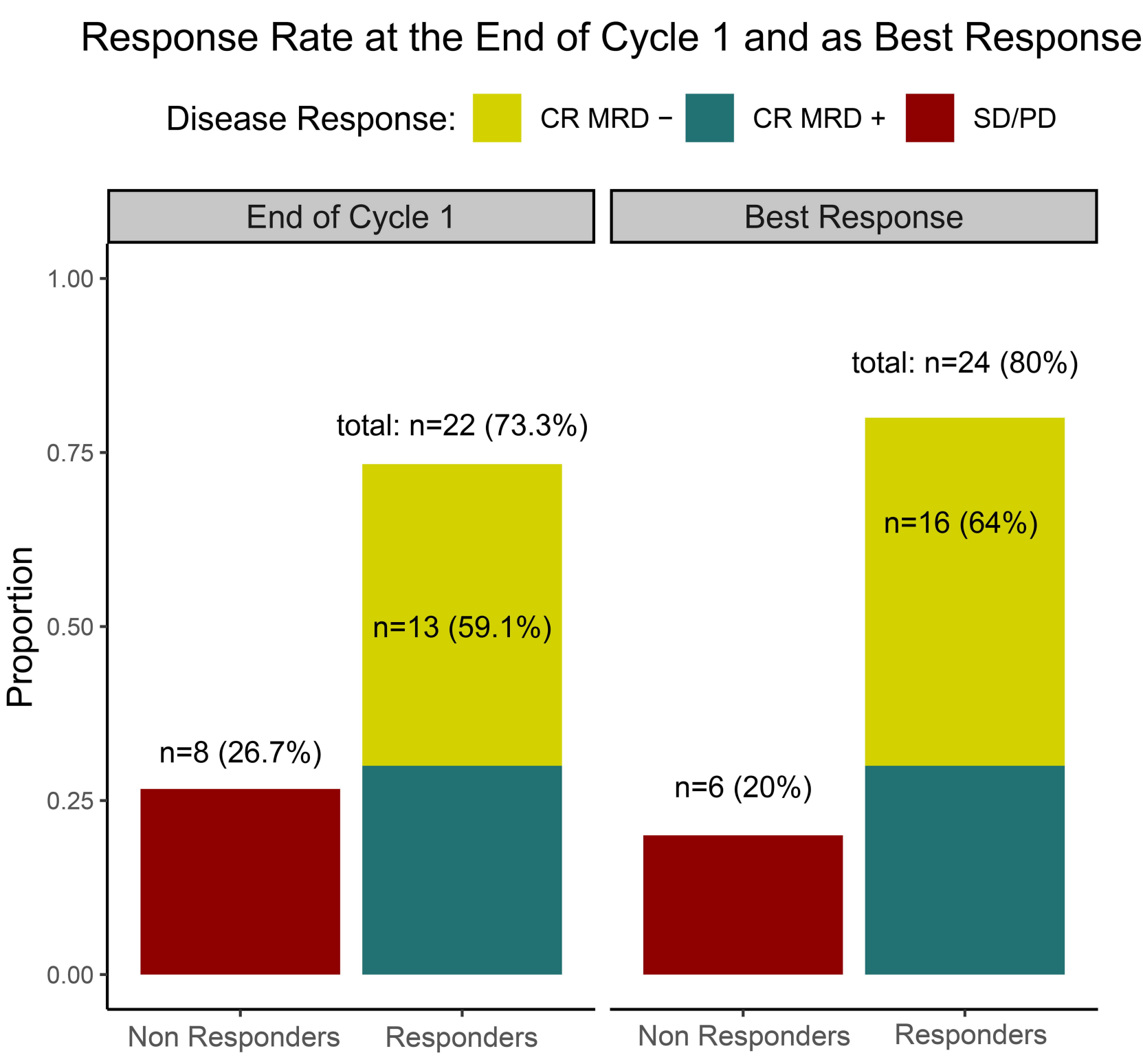
Each bar starts at day 1 of cycle 1. Yellow shaded areas represent the study treatment period. Green shaded areas represent the duration of response. InO Dose Levels: DL-1: 1.1 mg/m^2/cycle; DL-2: 0.8 mg/m^2/cycle; DL2: 1.8 mg/m^2/cycle; DL-1_amd: 1.1 mg/m^2/cycle (reduced dexamethasone); DL1_amd: 1.8 mg/m^2/cycle (reduced dexamethasone); DL2_amd: 1.8 mg/m^2/cycle (reduced dexamethasone). CR: Complete Remission. CCR: Continuous Complete Remission achieved on InO therapy. PD: Progressive Disease/Relapse. CAR-T: Chimeric Antigen Receptor T-Cells Therapy; HSCT: Hematopoietic Stem Cell Transplantation. SOS: Sinusoidal Obstruction Syndrome. NA: Not Applicable. FU: Follow up. 16 patients received only 1 cycle (combination), 10 patients 1 combination cycle + 1 single agent cycle, 3 patients 2 combination cycles, 1 patient received 3 cycles (1 combination cycle + 2 single agent cycles).

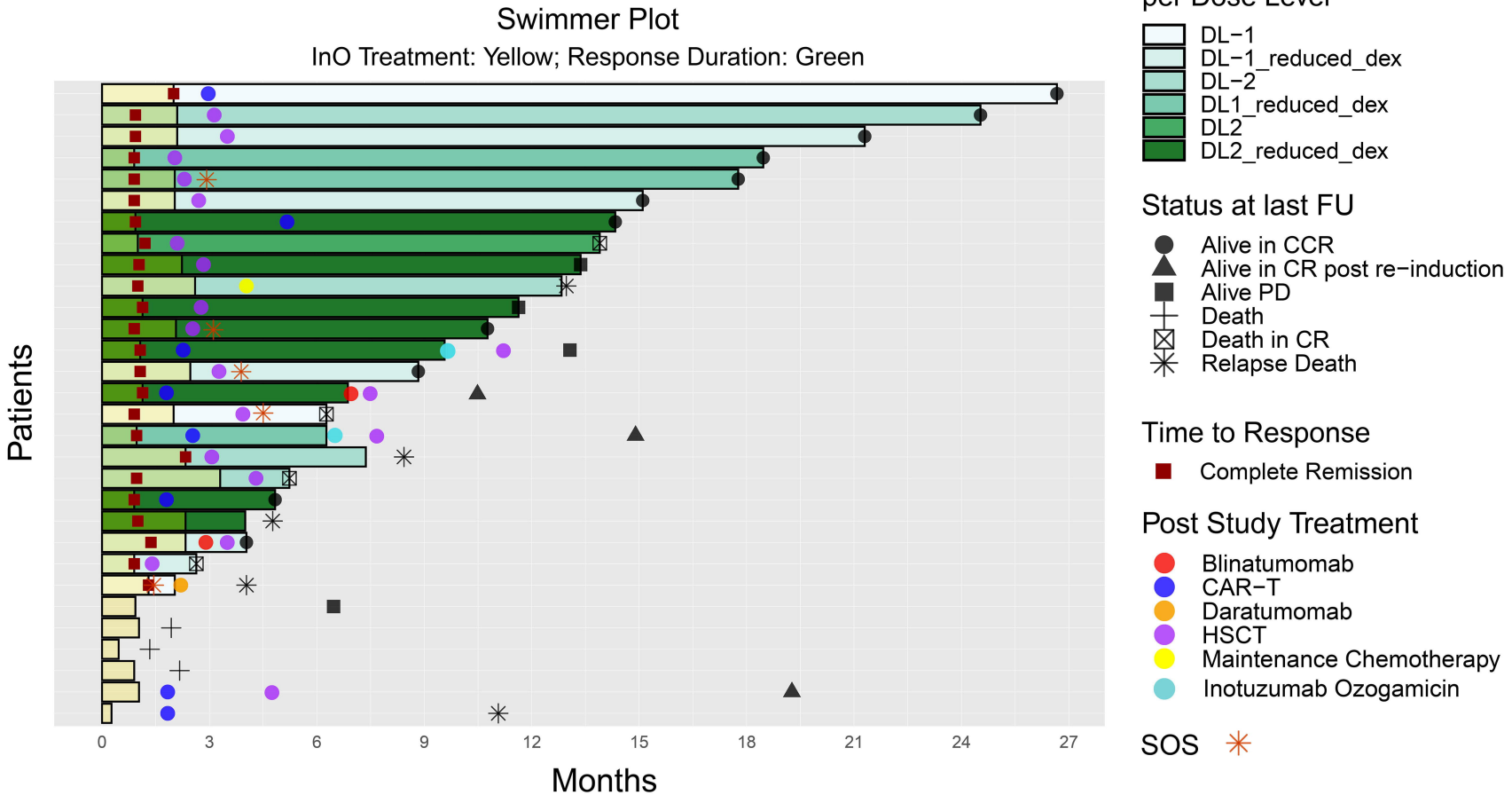
Figure 3. Overall Survival and Event-Free Survival

Probabilities were estimated using the Kaplan–Meier method. Events were defined as non-response (not achieving CR, CRi or CRp, considered as event at day 0), relapse after remission achieved as a result of InO treatment, death from any cause, or secondary malignancies. Crosses represent censored subjects. Shaded areas represent the 95% Confidence Interval.

Figure 4. Cumulative Incidence of Relapses and Non-Relapse Death

Probabilities were estimated using the Kaplan–Meier method. Patients not achieving remission were counted as event at time zero for the cumulative incidence of relapse (blue line). Patients dying while in remission achieved as a result of InO treatment were counted as event in the non-relapse death curve (red line).


Response Rate at the End of Cycle 1 and as Best Response
Disease Response:
CR MRD −
CR MRD +
SD/PD
End of Cycle 1
Best Response
Proportion
1.00
0.75
0.50
0.25
0.00
n=8 (26.7%)
total: n=22 (73.3%)
n=13 (59.1%)
n=6 (20%)
total: n=24 (80%)
n=16 (64%)
Non Responders
Responders
Non Responders
Responders


Swimmer Plot
InO Treatment: Yellow; Response Duration: Green
Patients
0
3
6
9
12
15
18
21
24
27
Months
Response Duration per Dose Level
DL−1
DL−1_reduced_dex
DL−2
DL1_reduced_dex
DL2
DL2_reduced_dex
Status at last FU
Alive in CCR
Alive in CR post re−induction
Alive PD
Death
Death in CR
Relapse Death
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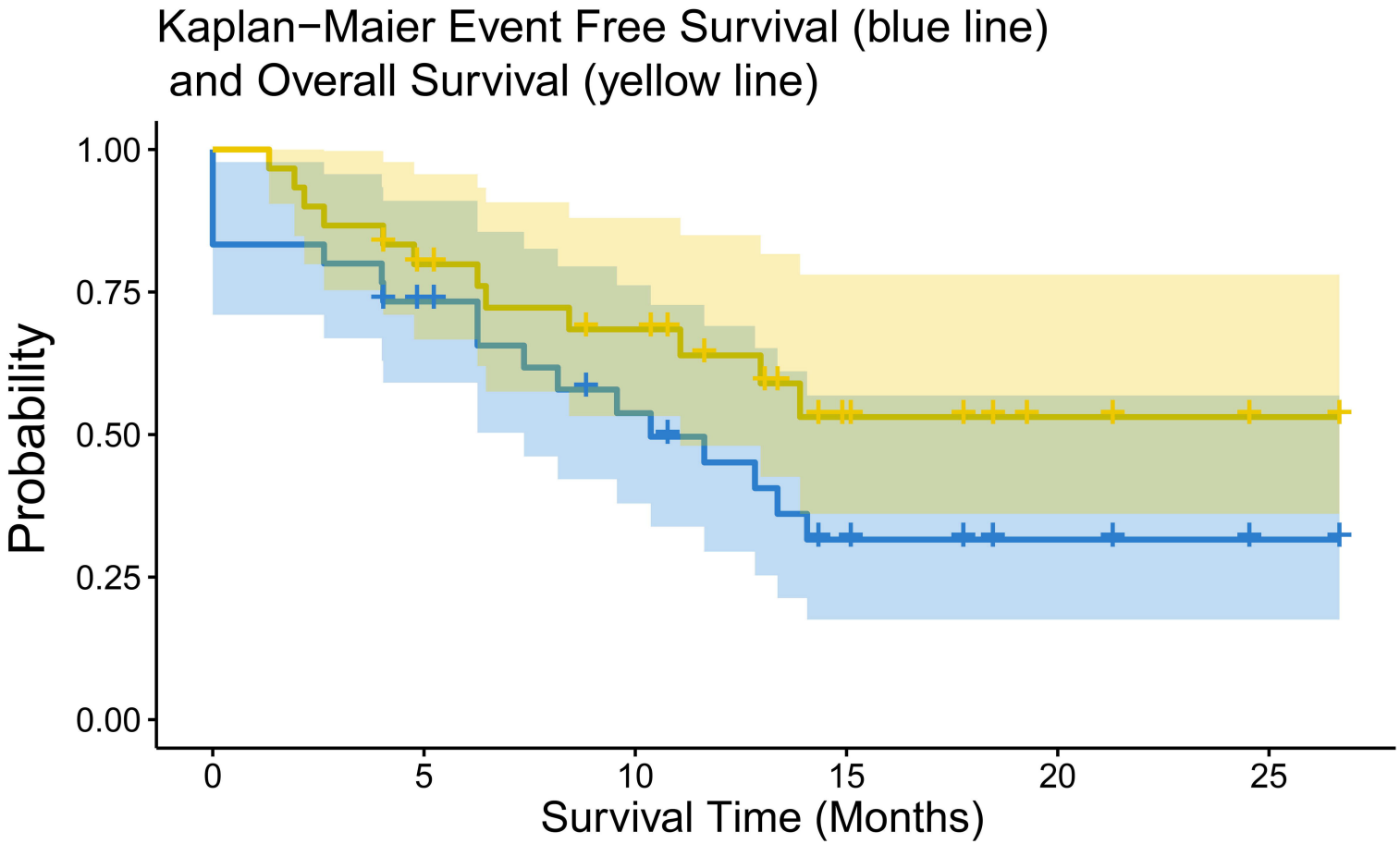
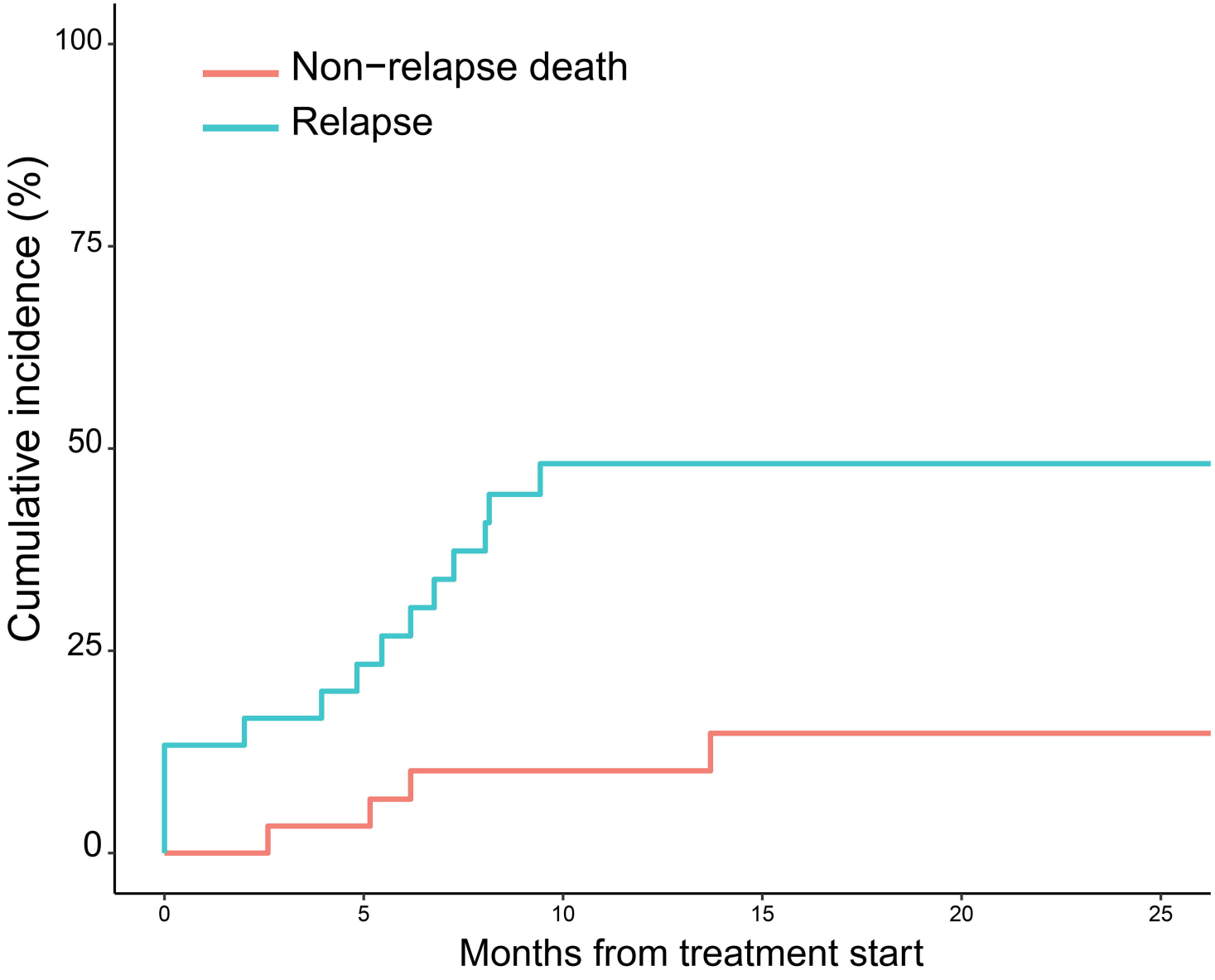
Complete Remission
Post Study Treatment
Blinatumomab
CAR−T
Daratumomab
HSCT
Maintenance Chemotherapy
Inotuzumab Ozogamicin
SOS



Number at risk

EFS::All	30	20	13	6	3	1
OS::All	30	22	17	7	3	1



Number at risk (number censored)

30	22	11	8	3	3

Supplementary Table 1: Inclusion and Exclusion Criteria.

Inclusion Criteria	
Age	• ≥1 and <18 years at time of enrollment • The first three patients on dose level 1 must be ≥6 and <18 years • Then ≥2 additional patients ≥1 year and <6 years at the same dose level
Diagnosis	• First relapse of BCP-ALL post allogeneic HSCT • Second or greater R/R BCP-ALL • Refractory disease (newly diagnosed patients who had induction failures after ≥2 previous regimens without attainment of remission, or patients with refractory first relapse after one previous reinduction regimen without attainment of remission) **AND**: • M2 or M3 marrow status (≥5% blasts by morphology) • Malignant clone CD22 surface antigen positive (in either bone marrow or peripheral blood) by institutional standards • The first six patients must have M3 marrow status (≥25% blasts by morphology)
Performance level and life expectancy	• Karnofsky >60% (>16 years) or Lansky >60% (≤16 years) • Life expectancy of ≥6 weeks
Prior therapy	Patients must have recovered from the acute toxic effects of all prior therapy, defined as resolution of non-hematologic toxicities to ≤Grade 2 per the CTCAE 4.03 prior to entering the study a. Chemotherapy ≥7 days since the completion of cytotoxic therapy (exceptions: hydroxyurea, 6-mercaptopurine and steroids which are permitted up until 48 hours prior to initiating protocol therapy) b. Radiotherapy ≥28 days since any prior radiation therapy c. Hematopoietic stem cell transplant ≥90 days since previous allo-HSCT No evidence of active graft vs host disease No GVHD prophylaxis or treatment d. Hematopoietic growth factors ≥7 days since the completion of therapy with GCSF or other growth factors, or ≥14 days since completion of therapy with pegfilgrastim (Neulasta®) e. Immunotherapy ≥42 days after the completion of any type of immunotherapy, e.g. CART therapy. Patients may not have received prior CD22-targeted therapy (immunotoxin or CART therapy) f. Monoclonal antibodies

	≥3 half-lives of the antibody must have elapsed after the last dose of a monoclonal antibody (rituximab = 66 days, epratuzumab = 69 days) Exclusion of blinatumomab: patients must have been off blinatumomab infusion for ≥14 days and all drug-related toxicity must have resolved to ≤Grade 2 g. Investigational drugs ≥7 days or five drug half-lives (whichever is longer) since prior treatment with any experimental drug (with the exception of monoclonal antibodies) under investigation. No residual toxicities should be observed following previous treatment h. Prior calicheamicin exposure Patient has not received prior treatment with a calicheamicin conjugated antibody (e.g. gemtuzumab ozogamicin)
Renal and hepatic function	• Serum creatinine ≤1.5 x institutional ULN according to age • AST and ALT ≤2.5 x institutional ULN • Total bilirubin ≤1.5 x institutional ULN unless the patient has documented Gilbert syndrome
Cardiac function	• Shortening fraction ≥30% by echocardiogram or an ejection fraction >50% by MUGA.
Reproductive function	• Female patients of childbearing potential: negative urine or serum pregnancy test confirmed prior to enrollment • Female patients with infants must agree not to breastfeed on study • Male and female patients of child-bearing potential must agree to use a *highly effective* method of contraception (≥8 months for females and for ≥5 months for males after the last dose of InO)

Exclusion Eligibility Criteria	
Isolated extramedullary relapse	• Patients with isolated extramedullary disease are excluded
VOD/SOS	• Any history of prior or ongoing VOD/SOS as per modified Seattle criteria, or prior liver-failure [defined as severe acute liver injury with encephalopathy and impaired synthetic function (international normalized ratio of ≥1.5)]
Infection	• Systemic fungal, bacterial, viral or other infection that is exhibiting ongoing signs/symptoms • The patient may not have: • A requirement for vasopressors • Positive blood culture within 48 hours of study enrollment • Fever above 38.2 degrees Celsius within 48 hours of study enrollment with clinical signs of infection. Fever that is determined to be due to tumor burden is allowed, with documented negative blood cultures for ≥48 hours prior to enrollment and no concurrent signs or symptoms of active infection or hemodynamic instability • A positive fungal culture within 30 days of study enrollment

	• Active fungal, viral, bacterial, or protozoal infection requiring intravenous or oral treatment. Chronic prophylaxis therapy to prevent infections is allowed
Other anti-cancer therapy	• Patients will be excluded if there is a plan to administer non-protocol anti-cancer therapy during the study period
Allergic reaction	• Patients with prior Grade 3/4 allergic reaction to a monoclonal antibody are excluded
Concurrent disease	• Significant concurrent disease, illness, psychiatric disorder or social issue that would compromise patient safety or compliance with protocol therapy, interfere with consent, study participation, followup, or interpretation of study results • Children with Down syndrome are excluded from participation in the dose finding parts of the study

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BCP-ALL: B-cell precursor acute lymphoblastic leukemia; CART: chimeric antigen receptor T cell; GCSF: granulocyte-colony stimulating factor; GVHD: graft versus host disease; HSCT: hematopoietic stem cell transplant; InO: Inotuzumab Ozogamicin; MUGA: multiple gated acquisition scan; R/R: relapsed/refractory; SOS: sinusoidal obstruction syndrome; ULN: upper limit of normal; VOD: veno-occlusive disease.

Supplementary Methods 1: Criteria to Proceed with the Next Treatment Cycle

M1 BM with absolute neutrophil count (ANC) ≥ 0.5 x 10^9/L and platelet count ≥ 30 x 10^9/L; or M3 BM at study entry attaining an M2 BM at the end of the cycle, irrespective of hematological parameters.

Supplementary Methods 2: Data Sets

The **full analysis dataset** and **safety analysis dataset** consisted of all enrolled patients who received at least one dose of study therapy. The **response analysis dataset** included all enrolled patients who received at least one dose of InO and completed at least one baseline and one post-baseline disease assessment.

A patient is considered evaluable for the dose escalation phase of the study if any of the following applies:

- The patient receives at least one dose of the planned dose of InO (together with the first dose of dexamethasone) and experiences a DLT at any time during the first cycle of combined study therapy.
- The patient does not experience DLT during the study therapy, and receives at least 2 out of 3 doses of the planned dose of InO during the first cycle and at least 3 days of dexamethasone, 1 dose of vincristine and 1 dose of intrathecal treatment.

A patient will be considered not evaluable for the dose escalation phase of the study if any of the following applies:

- The patient receives ≤1 dose of the prescribed dose of InO, < 3 days of dexamethasone, or no dose of vincristine or intrathecal treatment during the first cycle for reasons not related to toxicity or intolerability (e.g. early progressive disease/logistical reasons/non-compliance, etc), or for reasons possibly related to toxicity or intolerability not fulfilling the definition of a DLT as defined in section 4.6. (e.g. considered related to intrathecal therapy or specific ALL chemotherapy toxicities precluding ongoing treatment).
- Silent inactivation of asparaginase in a patient enrolled in Stratum 1B-ASP, in which case asparaginase is considered ineffective.

Note that patients who are not evaluable will be replaced.

Supplementary Methods 3: Minimal Residual Disease (MRD) Detection Methods

Molecular MRD levels were centrally determined by RQ-PCR of leukemia-specific rearranged immunoglobulin (IG) and T-cell receptor (TR) genes.[3] Quality control and standardized interpretation of RQ-PCR data were achieved following the guidelines of the European Study Group on MRD detection in ALL (EuroMRD).[4] For flowcytometric MRD analysis, also centrally performed, bone marrow samples were bulk-lysed and subsequently stained using 8 color stainings according to EuroFlow protocols.[5,6] Four million cells (if available) were acquired and MRD positivity was defined if at least 20 ALL cells could be detected. Flow MRD negativity was defined as MRD < 0,01% using an assay with a sensitivity of at least 0,01%. MRD negativity was defined as PCR below 10^{-4} or flow cytometry below 0.01% when PCR was negative but the Quantitative Range was above 10^{-4}.

Supplementary Methods 4: Definition of Event for EFS calculation and Duration of Response

Events were defined as: no response (not achieving CR, CRi or CRp, considered as event at day 0), relapse after remission achieved as a result of InO treatment, death from any cause, or occurrence of secondary malignancy. Duration of response was defined as the time between achieving response (CR, CRi or CRp) after starting study treatment and documented relapse or death.

Supplementary Methods 5: Diagnosis of Sinusoidal Obstruction Syndrome (SOS)

Two diagnostic systems are in common use, and are shown here: the modified Seattle criteria and the Baltimore criteria.[1,2] The Baltimore criteria are more stringent, with an absolute requirement for hyperbilirubinemia. In this protocol we used the Modified Seattle Criteria to define SOS. Formally these criteria describe SOS within 20 days post-HSCT, but since SOS may also occur post-InO and/or at a later time-point, for this study we considered all occurrences of SOS per the definition below:

Two of the following criteria must be present (Modified Seattle Criteria):

- Total bilirubin > 34.2 µmol/l (2mg/dL)
- Hepatomegaly or right upper quadrant pain
- Weight gain (> 2% from pre-transplant weight)

Other factors that may point at SOS include:

- ascites
- thrombocytopenia with refractoriness to platelet transfusion

- changes in the flow of vena portae

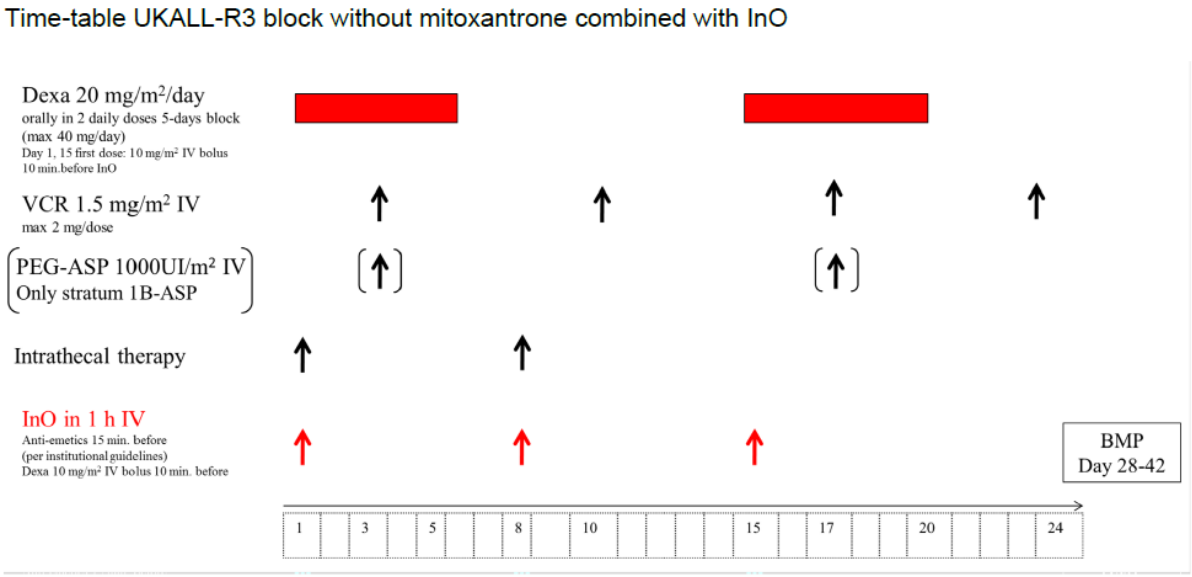
Therefore, when evaluating liver toxicity, the radiologist should be informed of the potential for hepatic vascular disease. When SOS is in the differential diagnosis, a right upper quadrant ultrasound with color flow doppler (including indices to hepatic artery flow and evaluation of hepatic venous outflow) should be performed. In addition, the radiology report should describe common bile duct, the degree of gall bladder wall thickening in millimeters, and the volume of ascites should be estimated as closely as possible (ie, small and localized, moderate and generalized, or large and generalized).

Supplementary Table 2. Dose Levels of InO for Patients Enrolled in Cohort 1B in Cycle 1 and Cycles 2 to 6

	Cycle 1*				Cycle 2-6#*			
Day	**1**	**8**	**15**	**Total Dose per Cycle**	**1**	**8**	**15**	**Total Dose per Cycle**
Level -2	0.4	0.2	0.2	**0.8 mg/m²**	0.2	0.2	0.2	**0.6 mg/m²**
Level -1	0.5	0.3	0.3	**1.1 mg/m²**	0.3	0.3	0.3	**0.9 mg/m²**
Level 1 (Start)*	0.6	0.4	0.4	**1.4 mg/m²**	0.4	0.4	0.4	**1.2 mg/m²**
Level 2	0.8	0.5	0.5	**1.8 mg/m²**	0.5	0.5	0.5	**1.5 mg/m²**

Dose de-escalation will not go below Level -2.
Following Cycle 1, in patients who have achieved a CR/CRi or CRp, the day 1 dose is decreased slightly due to no loading dose requirement. In patients who have not yet achieved a CR/CRi or CRp after cycle 1, a loading dose similar to cycle 1 will be given in cycle 2, but not in subsequent cycles.
***** Note that there will be no dose-capping for obese patients/patients with high BSA.

Supplementary Figure 1. Treatment Scheme



VCR: Vincristine; InO: Inotuzumab Ozogamicin; Dexa: Dexamethasone. Dexamethasone dose was then reduced to 10 mg/m²/day. IT methotrexate prophylaxis is recommended to be given intrathecally to patients with BCP-ALL who are CNS1 at study entry on day 1 and 8 of each cycle. Patients with BCP-ALL who are CNS 2 or 3 prior to enrollment may receive intensified IT therapy with triple IT agents (cytarabine plus either prednisolone or hydrocortisone) per local standard of care and based on which steroids are approved for IT use in a given country. PEG-ASP (Asparaginase) was not given in this cohort (1B) are reported in the main text.

Supplementary Table 3: List of Treatment Emergent Adverse Events (N=30).

AE term	Grade 1-2	Grade ≥ 3	Total
Anemia	5	19	24
Alanine aminotransferase increased	8	15	23
Aspartate aminotransferase increased	12	10	22
Platelet count decreased	2	20	22
White blood cell decreased	0	20	20
Neutrophil count decreased	1	18	19
Constipation	15	0	15
Fever	14	0	14
Headache	12	1	13
Febrile neutropenia	0	10	10
Hypokalemia	4	5	9
Abdominal pain	8	0	8
Blood bilirubin increased	6	1	7
GGT increased	3	4	7
Cough	5	0	5
Hypertension	5	0	5
Lymphocyte count decreased	0	5	5
Pain in extremity	5	0	5
Sinusoidal Obstruction Syndrome	0	5	5
Nausea	4	0	4
Sepsis	0	4	4
Bone pain	2	1	3
Creatinine increased	2	1	3
Diarrhea	3	0	3
Erythema multiforme	3	0	3
Generalized Edema	3	0	3
Hypertriglyceridemia	1	2	3

Hyperuricemia	3	0	3
Hypocalcemia	3	0	3
Rhinitis infective	3	0	3
Skin infection	2	1	3
Vitamin D deficiency	3	0	3
Vomiting	3	0	3
Allergic reaction	1	1	2
Anal fistula	2	0	2
Anxiety	2	0	2
Fatigue	1	1	2
Gastritis	2	0	2
Hyperglycemia	1	1	2
Hyperphosphatemia	2	0	2
Hypophosphatemia	2	0	2
Hypotension	2	0	2
Joint pain	2	0	2
Mucositis oral	2	0	2
Pain	2	0	2
Perianal Erythema	2	0	2
Pruritus	2	0	2
Sore throat	2	0	2
Upper respiratory infection	2	0	2
Acute kidney injury	0	1	1
Adenovirus infection	1	0	1
Allergic reaction to ambisome	1	0	1
Allergic rhinitis	1	0	1
Anal ulcer	1	0	1
Anaphylaxis	0	1	1
Arthralgia	0	1	1
Back pain	1	0	1

Bacteremia	1	0	1
Chest wall pain	1	0	1
Depressed level of consciousness	1	0	1
Disease progression	0	1	1
Dyspnea	1	0	1
E.coli infection	1	0	1
Facial pain	1	0	1
Flank pain	1	0	1
Folliculitis	1	0	1
Gastrointestinal pain	1	0	1
Herpes simplex reactivation	1	0	1
Herpes Zoster	0	1	1
Hypoalbuminemia	1	0	1
Hypomagnesemia	1	0	1
Hyponatremia	0	1	1
INR increased	1	0	1
Lactate dehydrogenase increased	1	0	1
Laryngeal inflammation	1	0	1
Lip infection	1	0	1
Lipase increased	0	1	1
Lung infection	1	0	1
Malaise	1	0	1
Mandible pain	1	0	1
Mandibular pain	1	0	1
Muscle weakness trunk	1	0	1
Neoplasms benign malignant*	1	0	1
Neuralgia	1	0	1
Non-cardiac chest pain	1	0	1
Omaya Catheter infection	0	1	1

Pain due to catheter removal surgery	1	0	1
Palmar erythema	1	0	1
Pancreatitis	1	0	1
Periorbital edema	1	0	1
Periorbital hyperemia	1	0	1
Peripheral motor neuropathy	1	0	1
Pharyngitis	1	0	1
Pneumonitis	1	0	1
Pyogenic granuloma	1	0	1
PRESS	0	1	1
Sars-Cov-2 Infection	1	0	1
Sinus bradycardia	1	0	1
Sinus tachycardia	1	0	1
Somnolence	1	0	1
Stomach pain	1	0	1
Toothache	1	0	1
Tumor lysis syndrome	0	1	1
Upper gastrointestinal hemorrhage	0	1	1
Urinary tract infection	0	1	1
Urinary tract pain	1	0	1

* inclusion cysts and polyps; PRESS: Reversible Posterior Leukoencephalopathy Syndrome

Supplementary Table 4: List of Adverse Events Considered Definitely, Probably or Possibly Related to Study Treatment (N=30).

AE term	Grade 1-2	Grade ≥ 3	Total	Percentage
Platelet count decreased	2	14	16	53%
ALT increased	5	11	16	53%
Anemia	4	12	16	53%
Neutrophil count decreased	0	12	12	40%
AST increased	5	6	11	37%
White blood cell decreased	0	7	7	23%
Febrile neutropenia	0	7	7	23%
Lymphocyte count decreased	0	4	4	13%
Sinusoidal Obstruction Syndrome	0	5	5	17%
Abdominal pain	2	0	2	7%
Blood bilirubin increased	3	0	3	10%
Constipation	3	0	3	10%
Gastritis	2	0	2	7%
Headache	2	0	2	7%
Sore throat	2	0	2	7%
Fever	2	0	2	7%
E.coli infection	1	0	1	3%
Facial pain	1	0	1	3%
Flank pain	1	0	1	3%
Hyperphosphatemia	1	0	1	3%
Hypertension	3	0	3	3%
Hyperuricemia	1	0	1	3%
Hypophosphatemia	1	0	1	3%
Malaise	1	0	1	3%
Mandible pain	1	0	1	3%
Tumor lysis syndrome	0	1	1	3%

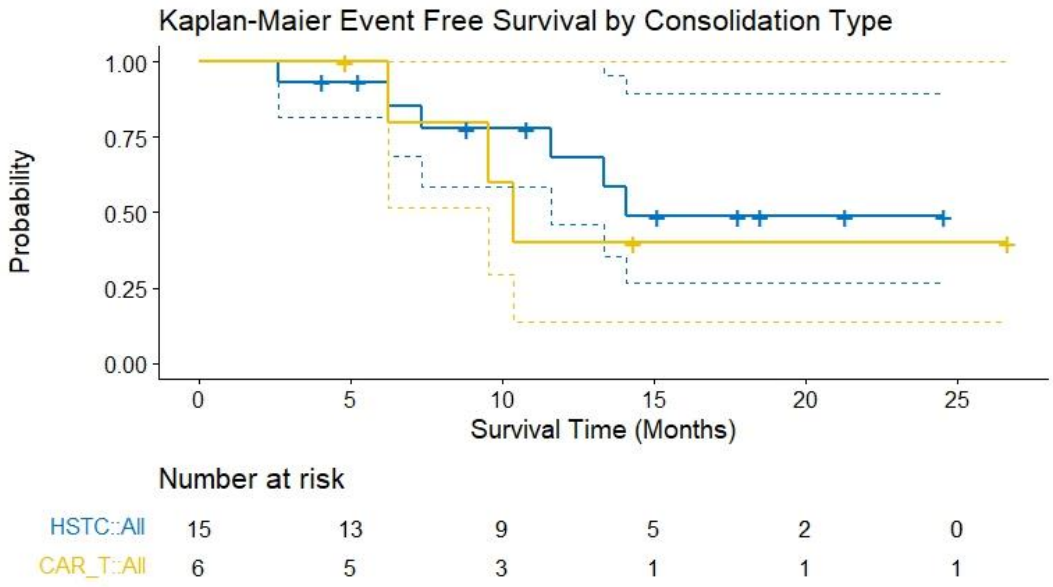
Urticaria	1	0	1	0%
Creatinine increased	1	0	1	3%
Herpes Zoster	0	1	1	3%
Hypertriglyceridemia	0	1	1	3%
Hyponatremia	0	1	1	3%
GGT Increased	1	0	1	3%
Lactate dehydrogenase increased	1	0	1	3%
Hypokalemia	1	0	1	3%
Lung Infection	1	0	1	3%
Muscle weakness trunk	1	0	1	3%
Nausea	1	0	1	3%
Neuralgia	1	0	1	3%
Pain in extremities	1	0	1	3%
Pancreatitis	1	0	1	3%
Pruritus	1	0	1	3%
Vomiting	1	0	1	3%

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ANC: Absolute Neutrophile Count; GGT: Gamma Glutamyl Transferase. The AE relatedness to study drug was based on the treating physician's judgment (definitely, probably, possibly, unlikely, not related or unknown).

Supplementary Table 5: List of Hematologic Laboratory Abnormalities (N=30) Based on the Local Upper Limit for Normality.

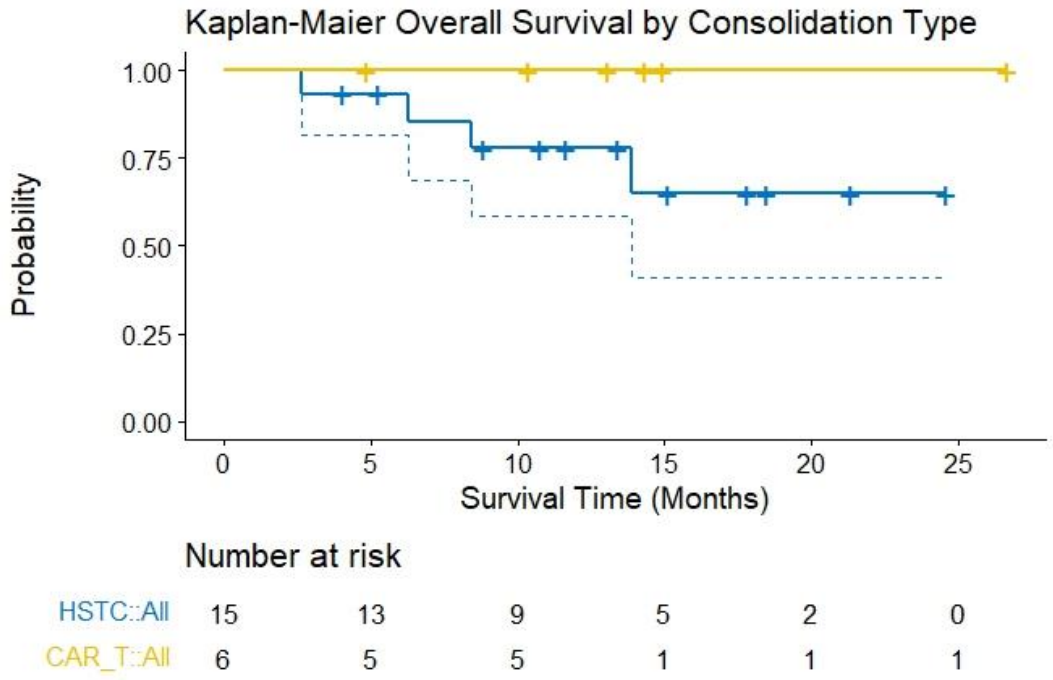
Grade	1	2	3	4	Total
Anemia	1	29	0	0	30
White blood cell count decrease	1	1	7	21	30
Absolute neutrophil count decrease	0	1	6	23	30
Platelet count decrease	1	1	6	22	30

Supplementary Figure 2. Event Free Survival among responders (n= 21) Consolidating Either by HSCT or by CAR-T Therapy (3 responders which did not consolidated after achieving remission are not reported)**.**



Event Free Survival among responders (n= 25) divided by consolidation treatment. HSTC: Hematopoietic Stem Cell Transplant; CAR-T: Chimeric Antigen Receptors T-Cell Therapy. Other three patients achieving remission with InO received either maintenance chemotherapy or no consolidation therapy at cut-off date (not shown in the figure). Dashed lines represent the 95% confidence interval.

Supplementary Figure 3. Overall Survival among responders (n= 21) consolidating Either by HSCT or by CAR-T Therapy (3 responders which did not consolidated after achieving remission are not reported)**.**



Overall Survival among responders (n= 25) divided by consolidation treatment. HSTC: Hematopoietic Stem Cell Transplant; CAR-T: Chimeric Antigen Receptors T-Cell Therapy. Other three patients achieving remission with InO received either maintenance chemotherapy or no consolidation therapy at cut-off date (not shown in the figure). Dashed lines represent the 95% confidence interval.

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