Combination low-intensity chemotherapy plus inotuzumab ozogamicin, blinatumomab and rituximab for pediatric patients with relapsed/refractory B-cell acute lymphoblastic leukemia

The 5-year overall survival (OS) for pediatric patients with relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (ALL) is <50%.[1] Standard of care re-induction therapy is a four-drug regimen derived from the mitoxantrone arm of UKALLR3 (R3), which achieves a 3-year overall survival (OS) of 69%.[2] The most recently completed Children's Oncology Group (COG) study for relapsed ALL (AALL1331) utilized this approach with a modified R3 backbone. Although active, this re-induction produced a significant amount of morbidity and treatment-related mortality (TRM), specifically a 39.8% rate of life-threatening infection and a 5% toxic death rate during block 1.[2-4] Promising immunotherapy agents may allow for less cytotoxic chemotherapy with maintained survival. This retrospective chart review identified ten patients <18 years old with R/R B-cell ALL treated at MD Anderson Cancer Center with an anthracycline-free reduced-intensity chemotherapy regimen that consisted of mini-hyper-fractionated cyclophosphamide, vincristine, and dexamethasone alternating with methotrexate and cytarabine cycles (mini-hCVD) plus condensed immunotherapy (inotuzumab-ozogamicin [INO], rituximab and /or blinatumomab), further termed Pedi-cRIB. We report the safety and efficacy in pediatric patients with overall response rate (ORR) of 75%.

Salvage therapy with immunotherapies INO, a CD22-directed humanized monoclonal antibody conjugated to calicheamicin, blinatumomab, a bispecific T-cell-engaging antibody with dual affinity for CD19 and CD3, and rituximab a CD20 directed monoclonal antibody, are associated with improved OS compared with conventional chemotherapy in R/R B-cell ALL; but these are often given as monotherapy or after intensive re-induction chemotherapy.[5-7] The combination of INO, +/- blinatumomab and rituximab, with mini-hCVD has been reported in adult patients and produced an ORR of 80% (complete remission [CR] in 57%) with 46% of patients proceeding to hematopoietic stem cell transplantation (HSCT).[8-10] Here, we report ten pediatric patients with R/R B-cell ALL who received mini-hCVD combined with condensed INO +/- blinatumomab and rituximab (further termed Pedi-cRIB). The chemotherapy schedule (*Online Supplementary Table S1*) was administered as described previously with dosing modified for pediatric patients.[11] Baseline characteristics are shown in Table 1. The median age was 8.5 years (range, 2-17 years). Six patients (60%) received Pedi-cRIB in first relapse, one (10%) had two prior lines of therapy, and three (30%) had ≥3 prior lines of therapy. Four patients had prior immunotherapy exposure (1 prior blinatumomab, 1 prior chimeric antigen receptor [CAR] T, 1 prior CAR T therapy and blinatumomab, and 1 prior CAR T and INO). No patients had a prior HSCT. Three patients demonstrated *CRLF2* rearrangement, three with *KRAS* mutation, two with PAX5 mutation, one with *KMT2A* rearrangement and one with *TCF3-PBX1* fusion. Patients received a median of two cycles (range, 1-4) of therapy. All patients received at least one cycle of INO with a median cumulative dose of 1.35 mg/m^2 (range, 0.9-4 mg/m^2). The median time from last INO dose to HSCT was 55 days. Blinatumomab and rituximab were given to 50% and 60% of patients, respectively, both for a median of one cycle (range, 1-2 cycles).

The most common hematological adverse events were thrombocytopenia, anemia, and neutropenia, as expected in a relapsed ALL population (*Online Supplementary Table S2*). One patient had prolonged thrombocytopenia ≥42 days; attributed to refractory disease rather than therapy. Grade 3 febrile neutropenia occurred in three patients (30%) and were effectively managed with antibiotics without complications. No cytokine release syndrome (CRS) or neurotoxicity were seen in patients receiving blinatumomab.

VOD/SOS developed in the first two pateints who received mini-hCVD + INO, without blinatumomab/rituximab. This led to revision of the regimen to include the additional immunotherapies with an aim to model what had been successful in adult patients and to extend the time from last INO to HSCT. Additionally, prophylactic defibrotide was given to high risk patients.[12] VOD/SOS was noted in the following two patients. Patient number 1 received an umbilical cord HSCT after a conditioning regimen of total body irradiation, fludarabine, and cyclophosphamide. Grade 3 VOD/SOS developed on day +14, with 1.12 months between HSCT and last INO dose. He received three cycles of mini- hCVD + INO with a cumulative INO dose of 2.1 mg/m^2. With defibrotide and aggressive diuresis, he recovered and remains alive and in remission with a follow-up of 2 years. Patient number 2 received a haploidentical HSCT after a conditioning regimen of busulfan, fludarabine, and thiotepa. On day +9 he developed grade 4 VOD/SOS. There were 1.81 months between HSCT and last INO dose. His therapy cumulative INO dose was 1.5 mg/m^2. He required defibrotide, peritoneal drain placement, continuous renal replacement therapy and BiPap. He recovered from this acute event and discharged from the hospital on day +41 from HSCT with only oral furosemide. Subsequently,

Table 1. Disease characteristics, treatment, and outcomes.

Patient #	Age in years/ sex Race	Cytogenetic alterations	Molecular alterations	Prior therapy N	Prior immunotherapy received	CD expression (%)/ immunotherapy received	Cycles N	Best response (cycle N achieved)	Life time INO mg/m²	HSCT type	Last INO to HSCT in days	Preparative Regimen	VOD / SOS	Vital status	Follow up time months
1	4/M White	Hyperdiploid, extra copy of *RUNX1*	*FLT3*	1	None	CD19 (99) CD20 (30) CD22 (97)/INO	3	CR, uMRD, NGS+ (2)	2.1	Cord	34	TBI/cyclo/flu	Yes, grade 3	Alive	31.2
2	16/M Hispanic	*CRLF2r* by FISH, del (7p)	*ASXL1, KRAS*	2	CD19 CAR T	CD19 (99) CD20 (6) CD22 (99)/INO	2	CR, uMRD (2)	1.5	Haplo	55	Bu/Flu/Thio	Yes, grade 4	Deceased (relapsed after 5 months)	13.9
3	17/M Hispanic	*CRLF2r* by FISH, +X, del (7p)	*KRAS, NRAS, PAX5*	1	None	CD19 (99) CD20 (97)/Ritux CD22 (99)/INO	4	CR, uMRD, NGS- (2)	4	MUD	59	TBI/etop/ rATG	No	Alive	39.4
4	17/F Hispanic	*BCOR/PAX5* fusion, +X, +9, 3 copies *CRLF2*	*CDKN2A*	1	None	CD19 (99) CD20 (95)/Ritux CD22 (99)/INO	1	NE	0.9	None	-	-	-	Deceased	11.4
5	5/M Asian	t(6;21), del(9P), Add (17p), *CRLF2r* by FISH	*PAX5, KRAS, TP53, SMC3*	4	CD19 CAR T	CD19 (99)/Blina CD20 (71)/Ritux CD22 (99)/INO	2	CR, uMRD (2)	1.2	MSD	36	TBI/cyclo	No	Alive	16.4
6	2/F Hispanic	t(11;17) (q23;p11.2) *KMT2Ar*	*CREBBP*	5	CD19 CAR T, Blina	CD19 (98)/Blina CD20 (0) CD22 (32)/INO	1	PD	1.2	None	-	-	-	Deceased	2.1
7	14/M Black	t(5;14) and t(11;19)	None	1	None	CD19 (93)/Blina CD20 (78)/Ritux CD22 (99)/INO	1	CR, uMRD, NGS- (2)	1.2	MSD	82	TBI/etop	No	Alive	22.9
8	8/F Black	None	None	1	None	CD19 (99)/Blina CD20 (10)/Ritux CD22 (99)/INO	2	CR, uMRD (2)	1.8	Cord	97	TBI/Flu/Cy	No	Alive	19.5
9	9/M White	*TCF3-PBX1*	None	3	Blina	CD19 (99) CD20 (14)/Ritux CD22 (73)/INO	1	PD	1.2	None	-	-	-	Deceased	5.1
10	7/M Hispanic	Hyperdiploid, trisomies 4, 17, 21	*PTPN11*	1	None	CD19 (98)/Blina CD20 (1) CD22 (98)/INO	2	CR, uMRD (1)	1.8	-	-	-	-	Alive	4.8

Patient baseline characteristics including cytogenetic and molecular alterations, number of prior therapies received, if patient was exposed to prior immunotherapy, which immunotherapy was received with hyper-fractionated cyclophosphamide, vincristine, dexamethasone, methotrexate, cytarabine (mini- hCVD), the total number of cycles given, best response achieved, lifetime inotuzumab ozogamicin mg/m² dose, type of hematopoietic stem cell transplant (HSCT) received, HSCT preparative regimen, veno-occlusive disease/sinusoidal obstructive syndrome (VOD/SOS) status and grade, vital status of patient at last follow-up and the total follow-up time since receiving mini-hCVD with immunotherapy. F: female; M: male; FLAG: fludarabine, cytarabine, granulocyte colony stimulating factor; INO: inotuzumab ozogamicin; Ritux: rituximab; Blina: blinatumomab; TBI: total body irradiation; rATG: rabbit anti-thymocyte globulin; cyclo: cyclophosphamide; flud: fludarabine; thio: thiotepa; etop: etoposide; cy: cytarabine; CRLF2r: cytokine receptor like factor 2 gene rearrangement; FISH: fluorescence *in situ* hybridization; CR: complete response; uMRD: undetectable minimal residual disease by multiparameter flow cytometry of bone marrow with less than 0.01% aberrant lymphoblasts; NGS: next generation sequencing by immunoglobin or T-cell receptor gene rearrangements; CAR T: chimeric antigen receptor T; PD: progressive disease; NE: not evaluable; HSCT: hematopoietic stem cell transplant; MUD: matched unrelated donor; MSD: matched sibling donor; cord: umbilical cord blood transplant; haplo: haploidentical transplant.

prophylactic defibrotide was administered during HSCT to all patients who received INO and were high risk for VOD/SOS risk. Of patients who received prophylactic defibrotide, none experienced VOD/SOS. Notably, there were no cases of transplant-related mortality or treatment discontinuations due to toxicity, and there were no new safety concerns observed.

There were nine evaluable patients for response (Figure 1), patient 4 was lost to follow-up after cycle 1 and was therefore not evaluable. The ORR after cycle 1 was 78% (CR=7). Minimal residual disease (MRD) negativity by flow cytometry was achieved in three of seven responders after cycle 1, and all seven responders after cycle 2. Three patients had next generation sequencing (NGS) MRD assessment of immunoglobin/T-cell rearrangements; two were found to be NGS negative. One patient remained NGS positive at 24 cells per million prior to HSCT but has not relapsed 28 months post HSCT. Two patients (patients 6 and 9) had no response and progressed during cycle 1 of therapy. These two patients demonstrated high-risk cytogenetics that are known to be resistant, one with *KMT2A* rearrangement and one with *TCF3-PBX1* fusion. At a median follow-up time of 17.1 months (range, 4.8-39.4 months), six (67%) patients remain alive and in remission. It is important to note that five of the six patients were in first relapse when they received this regimen. The median EFS and OS have not been reached (Figure 2A, B). Six of seven patients with CR proceeded to HSCT. One patient relapsed 5 months post HSCT and died of disease progression. This patient had *CRLF2* rearrangement and prior therapy with CAR T. One patient is still receiving therapy. After a median follow-up time of 17 months post-HSCT the median OS had not been reached (Figure 2C). Patients who responded to therapy during cycle 1 showed a better OS than patients who had no response during cycle 1 (Figure 2D).

This series demonstrates the safety and feasibility of giving an anthracycline-free reduced-intensity chemotherapy backbone with concurrent CD19, CD20 and CD22 targeted agents, and provides a novel therapy approach for patients who have failed other salvage attempts, have pre-existing cardiotoxicity, or serious infection history. Alternative regimens to the standard of care R3 backbone +/- blinatumomab (COG AALL1331) may produce high response rates with less acute toxicity.[7,13-15] Compared to AALL1331, Pedi-cRIB produced lower rates of infection and no toxic deaths, but there was an increase in manageable immunotherapy-related side effects.[2-4]

Despite the lower dosing and cumulative dose of INO administered here in comparison to the current COG trial standards, the most significant toxicity observed in this patient cohort remained VOD/SOS. This is consistent with toxicity data in adult patients that showed mini-hCVD + INO produced a 16% rate of VOD/SOS. In adult patients this was minimized to 3% by decreasing the dose of INO and incorporating blinatumomab to lengthen the time from INO dosing to HSCT.[10,11] Here, two patients developed VOD/SOS between days 9-15 post-HSCT. Interestingly, after the initial

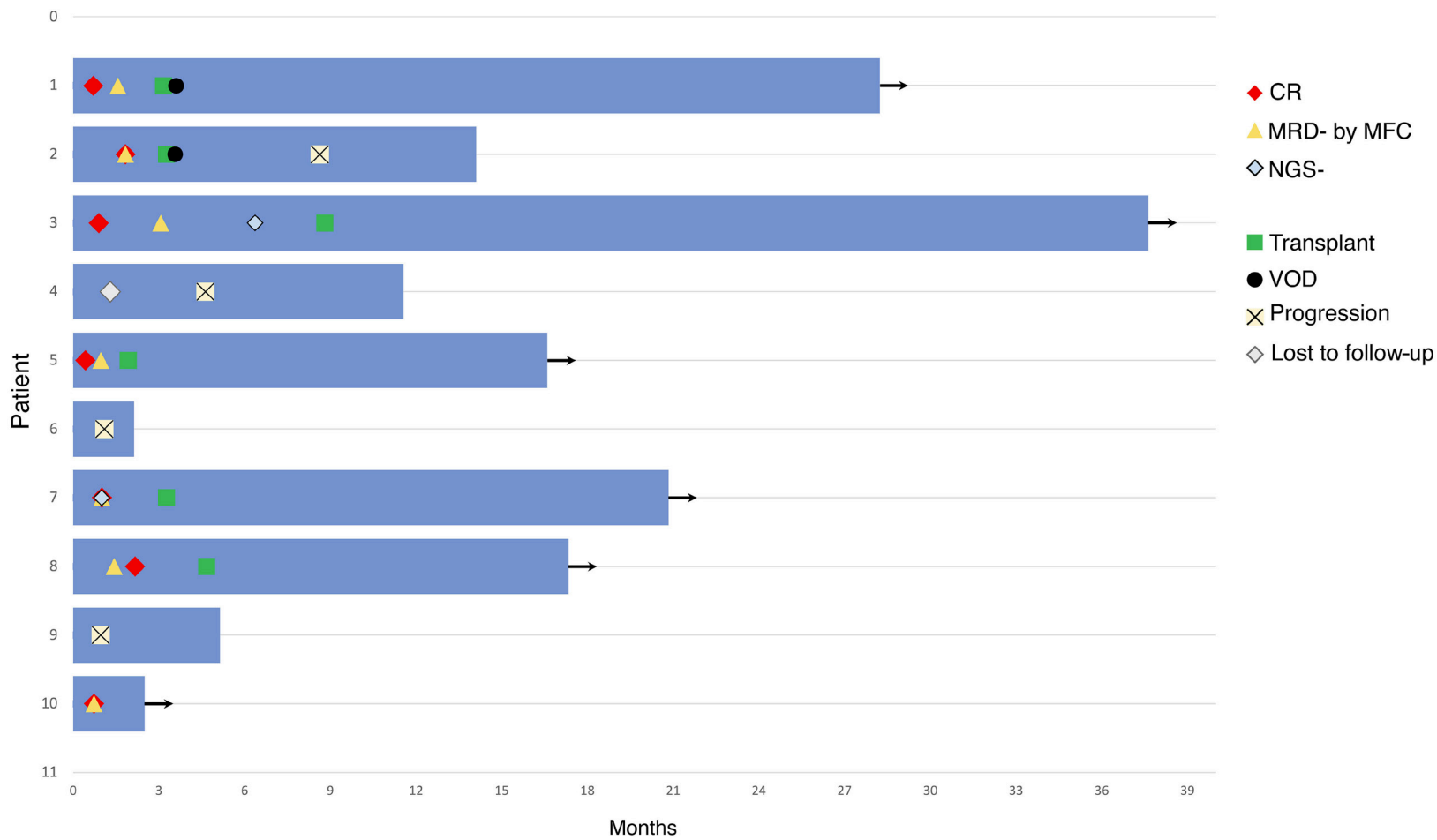

Figure 1. Patient outcome. Swimmers plot showing outcome of each patient and time points for complete response (CR), minimal residual disease negativity by multi-color flow cytometry (MRD$^-$ by MCF) next generation sequencing (NGS), transplant, veno-occlusive disease/sinusoidal obstructive syndrome (VOD/SOS), progression and lost to follow-up.

two cases, no additional occurrences of VOD/SOS were documented. Several factors may contribute to this phenomenon. First and foremost, the proactive use of defibrotide in VOD/SOS-susceptible patients could have prevented subsequent VOD/SOS. Secondly, taking a broader view, the incorporation of blinatumomab may have played a beneficial role. The incorporation of blinatumomab into the treatment regimen extended the interval between the last dose of INO and the subsequent preparative HSCT regimens and HSCT itself. This extended time frame could have facilitated recovery from hepatotoxicity and mitigated the risk of VOD/SOS, as previously postulated in prior studies.[16] This hypothesis requires validation through larger-scale studies to establish conclusive evidence. None of the patients experienced CRS as a result of blinatumomab therapy. Rituximab use in this series was variable, making conclusions difficult. There was no cut-off value of CD20 expression to give rituximab. Rituximab use was based on data from the adult population indicating that the addition of rituximab to HyperCVAD improved outcomes, and that chemotherapy can increase CD20 expression with rituximab giving a positive effect even among patients with low expression of CD20.[6,17] No patients had infusion reactions to rituximab here. A recent publication by Hoshitsuki *et al.* indicates higher infusion reactions, and no change in MRD at

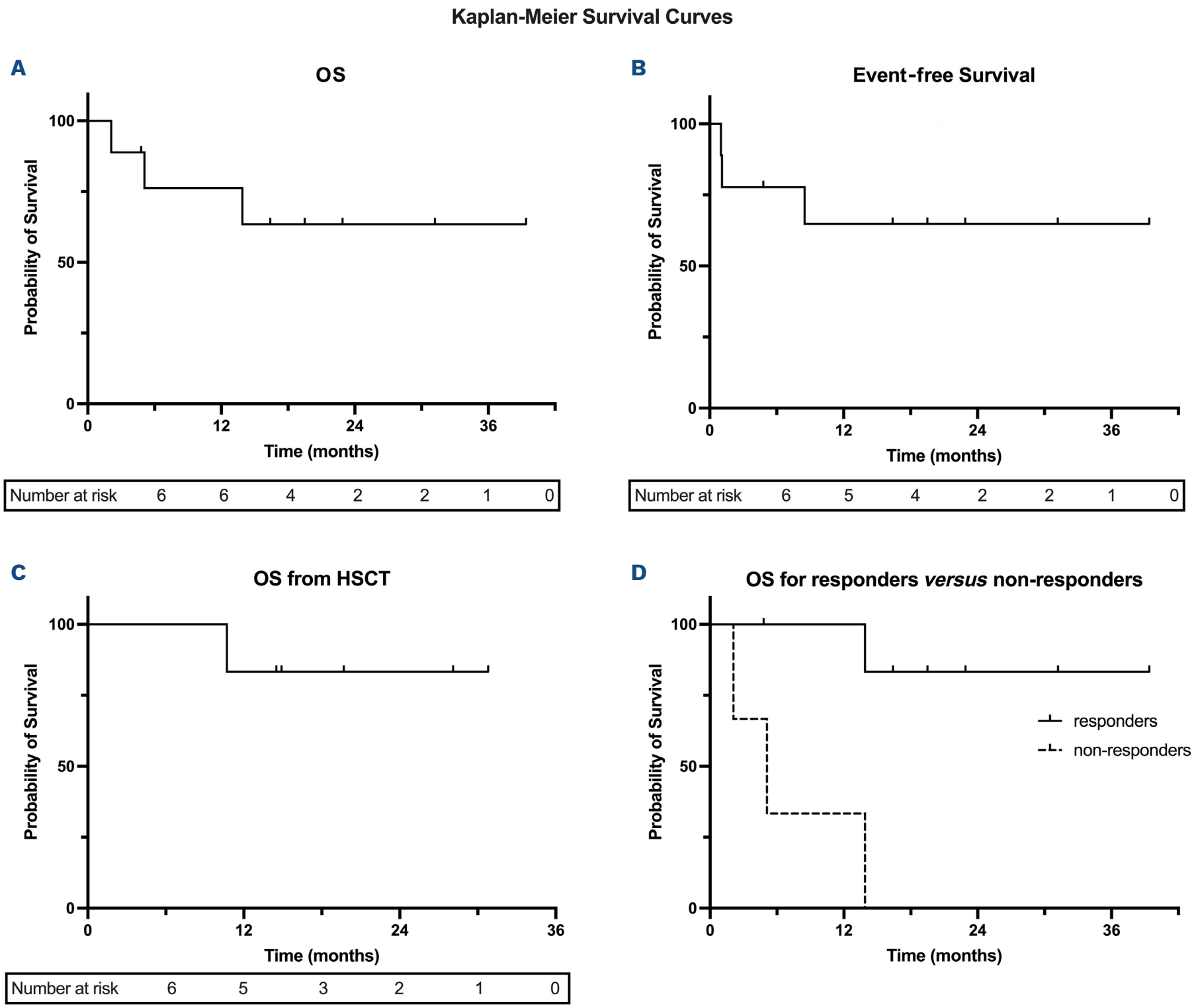

Figure 2. Survival curves for nine patients who were evaluable for response. Number at risk is listed below graphs(A-C). (A) Survival proportion graph from start of hyper-fractionated cyclophosphamide, vincristine, dexamethasone, methotrexate, cytarabine (mini-hCVD) + immunotherapy protocol until death. (B) Survival proportion graph from start of mini- hCVD + immunotherapy until major event, defined as progression, relapse or death. (C) Survival proportion graph from transplant until relapse or death for patients who proceeded to hematopoietic stem cell transplant (HSCT) (6 patients total). (D) Survival proportion for responders versus non-responders to mini-hCVD + immunotherapy treatment. OS: overall survival.

end of induction for patients who received rituximab, so this new data will need to be considered in future upfront trials.[18] This regimen showed notable success, achieving a 75% ORR including some heavily pretreated individuals who had undergone previous CD19 and CD22 directed therapies. Sustained MRD remissions were observed in the majority. While the specific contribution of each component is unclear, the synergistic effect likely reduces tumor burden and targets distinct phenotypes, potentially minimizing the emergence of resistance mechanisms.

Despite the limitations inherent in the study's size, heterogeneity in prior treatment, and retrospective nature, several pivotal insights can be gleaned. Considering relapsed patients frequently present with pre-existing comorbidities and may eventually undergo HSCT, the selection of a lower-intensity chemotherapy approach, when feasible, becomes imperative. This regimen emerges as a compelling candidate within this context, providing a rationale for forthcoming trials to explore the utilization of an anthracycline-free re-induction chemotherapy framework fortified with a combination of targeted immunotherapeutic agents. The use of this regimen must be discussed with each patient individually and the ethics of using a non-standard of care regimen in first relapse but be considered. An ongoing trial is currently underway to evaluate the Pedi-cRIB regimen (*clinicaltrials gov. Identifier: NCT05645718*).

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of MD Anderson Cancer Center.

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Contributions

Conceptualization by AG, CN, BC and DM. Data curation by LR and BK. Formal analysis by MR. Writing by AG, BC and DM. Review and editing by CN, LR, BK, MG, MR, RS, PT, AH, LT, RT, NJS, EJ, NJ, BC and DM.

Data-sharing statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to confidential patient information involvement.

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