Inotuzumab ozogamicin combined with chemotherapy in pediatric B-cell precursor CD22+ acute lymphoblastic leukemia: results of the phase IB ITCC-059 trial

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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

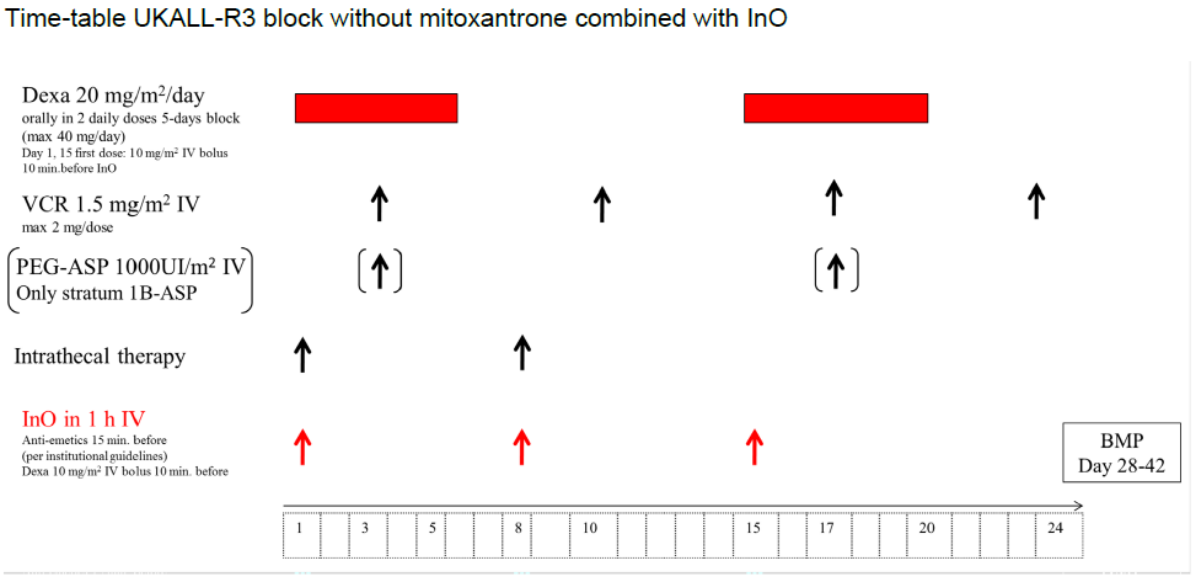
Therefore, when evaluating liver toxicity, the radiologist should be informed of the potential for hepatic vascular disease. When SOS is in the differential diagnosis, a right upper quadrant ultrasound with color flow doppler (including indices to hepatic artery flow and evaluation of hepatic venous 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%

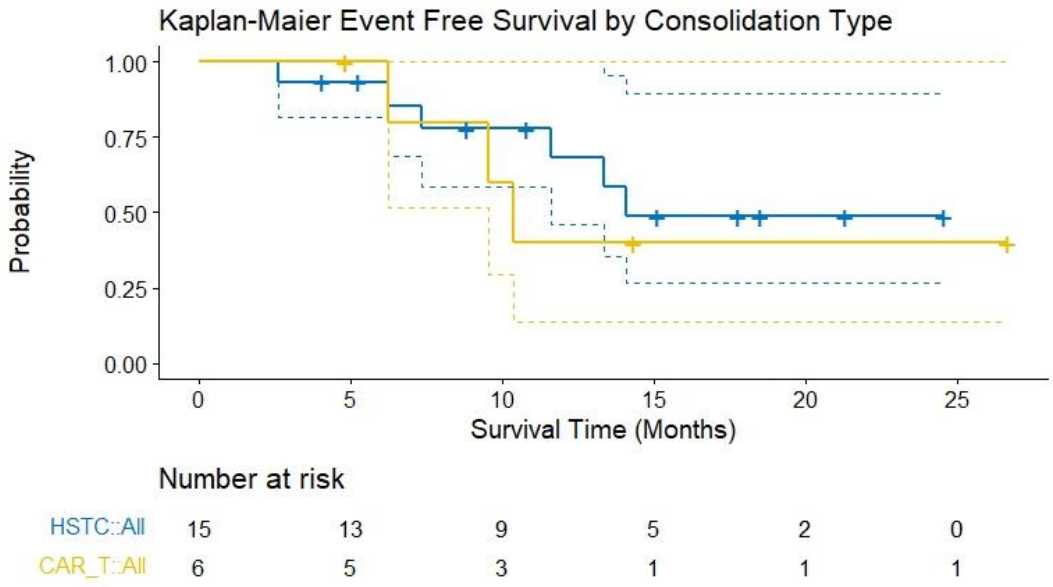
Urticaria	1	0	1	0%
Creatinine increased	1	0	1	3%
Herpes Zoster	0	1	1	3%
Hypertriglyceridemia	0	1	1	3%
Hyponatremia	0	1	1	3%
GGT Increased	1	0	1	3%
Lactate dehydrogenase increased	1	0	1	3%
Hypokalemia	1	0	1	3%
Lung Infection	1	0	1	3%
Muscle weakness trunk	1	0	1	3%
Nausea	1	0	1	3%
Neuralgia	1	0	1	3%
Pain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

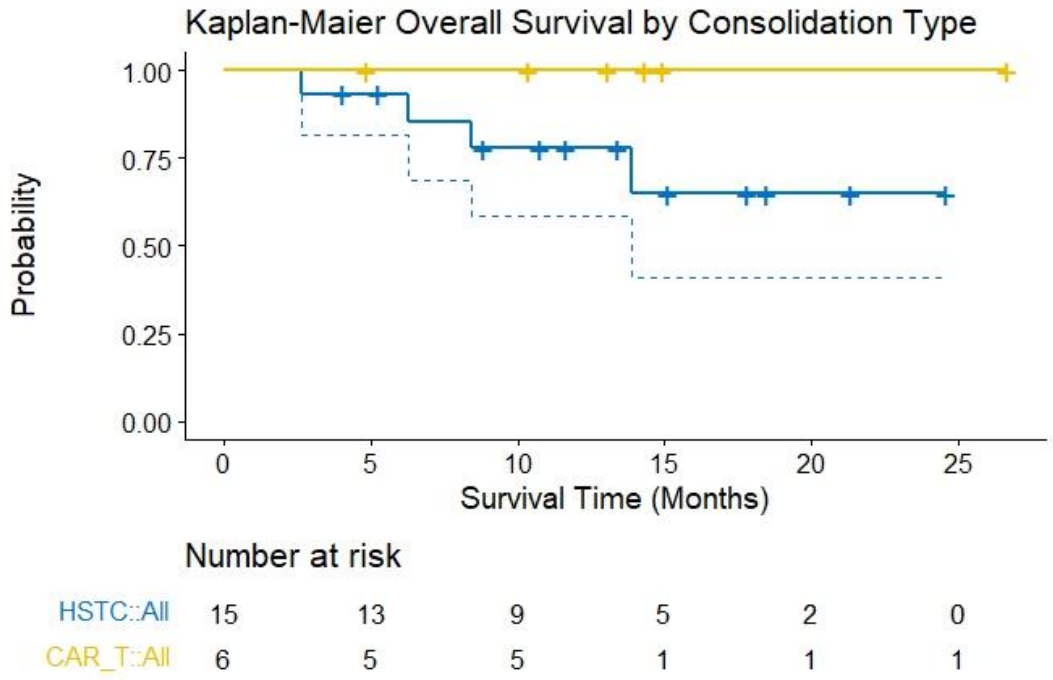
Grade	1	2	3	4	Total
Anemia	1	29	0	0	30
White blood cell count decrease	1	1	7	21	30
Absolute neutrophil count decrease	0	1	6	23	30
Platelet count decrease	1	1	6	22	30

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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