SUPPLEMENTARY APPENDIX

Pediatric-inspired chemotherapy incorporating pegaspargase is safe and results in high rates of minimal residual disease negativity in adults up to the age of 60 years with Philadelphia chromosome-negative acute lymphoblastic leukemia

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Supplementary methods:

Eligibility criteria:

Subject inclusion criteria:

1. Previously untreated Philadelphia chromosome (Ph) negative precursor B-cell or T-cell acute lymphoblastic leukemia (ALL) confirmed by conventional flow cytometry or immunohistochemical stain. Patients who have untreated B-cell or T-cell ALL confirmed by conventional flow cytometry or immunohistochemical stain, but Ph status is unknown, may also enroll.
2. Patients with T-cell or B cell lymphoblastic lymphoma (LBL) confirmed by conventional immature T–or pre-B cell markers even if the bone marrow is not involved are also eligible
3. Age 18 - 60 years
4. ECOG performance status of 0-2
5. Adequate renal function as demonstrated by a serum creatinine ≤ 2.0 mg/dl or a calculated creatinine clearance of > 60 ml/min.
6. Adequate hepatic function as demonstrated by a total bilirubin < 2.0 mg/dl (unless attributable to Gilbert‘s disease) and an alkaline phosphatase, AST, and ALT ≤ 4 times the upper limit of normal (unless clinically considered to be related to liver involvement with leukemia)
7. Normal cardiac function as demonstrated by a left ventricular ejection fraction ≥ 50% on echocardiogram or MUGA scan
8. Negative serum pregnancy test in women of childbearing potential
9. Men and women of childbearing potential must be willing to practice an effective method of birth control during treatment and at least 4 months after treatment is finished.

10. Patients with central nervous system involvement by ALL are eligible and may receive concomitant treatment with radiation therapy and/or intrathecal chemotherapy in accordance with standard medical practice. For patients with central nervous system (CNS) disease, dexamethasone may be temporarily administered instead of prednisone to reduce CNS pressure, at the discretion of the treating physician and after discussion with the MSK PI. Once dexamethasone is no longer needed, prednisone should be given as per protocol for 28 days.

Subject exclusion criteria:

1. Previous treatment for ALL, except for prior steroids and/or hydroxyurea
2. Patients known to have Ph+ ALL are not eligible. Leukemia cell samples will be obtained from all patients enrolled before starting protocol treatment and submitted for Philadelphia chromosome testing by either karyotyping, or for bcr/abl1 translocation by FISH or by PCR. Patients who are later found to have Ph+ ALL should have treatment on this trial discontinued and will not be considered in the evaluation.
3. Lymphoid blast crisis of chronic myelogenous leukemia
4. Mature B-cell (Burkitt-type) ALL
5. Active serious infections not controlled by antibiotics
6. Pregnant women or women who are breast-feeding
7. Concurrent active malignancy requiring immediate therapy
8. Clinically significant cardiac disease (NY Heart Association Class III or IV), including chronic arrhythmias, or pulmonary disease
9. Known HIV positive status

10. Other serious or life-threatening conditions deemed unacceptable by the principal investigator

Pegaspargase toxicity management, enzyme activity monitoring, and immunogenicity:

Liver biochemical tests, pancreatic enzymes, coagulation studies, and fibrinogen were followed at least twice weekly for two weeks following each dose of pegaspargase. Pegaspargase was permanently discontinued after clinical pancreatitis or anaphylaxis and in the event of the latter, substitution with 6 doses of Erwinia asparaginase 25,000 units/m^2 given every other day was permitted. Severe hyperglycemia was treated with insulin and serum hypertriglyceridemia (>1,000 mg/dL) was treated with gemfibrozil. Thrombosis was treated with low molecular weight heparin, with doses lowered or held during periods of severe thrombocytopenia; continuation of pegaspargase was permitted for non-CNS thrombosis. Cryoprecipitate was recommended for fibrinogen levels <50 mg/dL. For all other toxicities, including hepatotoxicity, subsequent doses were not reduced. Measurement of antithrombin III after pegaspargase was not required. See **Supplementary Table 1** for further details.

Asparaginase enzymatic activity was assessed in a subgroup of patients 7 days following the dose of pegaspargase (Next Molecular Analytics, Chester, VA). Anti-drug antibodies were assessed at similar timepoints using as multi-step process (BioAgilytix, Durham, NC).(1, 2) Samples were evaluated for the presence of antibodies specific to Oncaspar, including specific confirmation to 5 kDa polyethylene glycol (PEG). Each sample was evaluated at the minimum required dilution (25-fold) and run in duplicate. Following a screening assay, initial confirmatory assays were performed on samples and controls using 50 μg/mL Oncaspar® confirmatory buffer; samples that screened positive for Oncaspar® were then subject to pegaspargase specificity

confirmation. Samples and controls were tested in the presence and absence of 200 μg/mL 5 kDa pegaspargase confirmatory buffer. Samples that confirmed positive were subjected to up to 12 serial dilutions in negative control run on the same plate, until the signal fell below the normalized value plate specific dynamic titration cut point. The dilution factor above where the signal falls below the cut point for the first time (titer defining sample) was multiplied by the minimum required dilution to determine the titer.

Minimal (measurable) residual disease analysis (MRD):

Central MRD analysis was performed in one of two Children's Oncology Group (COG) accredited MRD reference laboratories (Johns Hopkins University [JHU] and Memorial Sloan Kettering Cancer Center [MSK]) as previously described for MSK evaluation,(3) for JHU methodology for B-ALL,(4) and for T-ALL.(5) Both laboratories used validated sensitivity of 0.01% abnormal cells among total white blood cells for T-cell and B-cell ALL and have established method concordance for B-cell ALL using 60 shared samples (23 positives, 37 negative) with perfect qualitative concordance (also see **Supplementary Figure 1A** for quantitative concordance). T-cell ALL methodology and approach were highly similar between institutions and concordance was confirmed using 10 split samples (8 positives, 2 negative) using either MSKCC or COG methodology with perfect qualitative concordance (see **Supplementary Figure 1B**). Furthermore, a subset of samples from the current trial (10 for B-ALL and 4 for T-ALL) was also analyzed in both laboratories with perfect qualitative concordance and high quantitative concordance for both B- and T-ALL. Specific panels used are listed in **Supplementary Table 7**. Both laboratories aimed to acquire at least 500,000 cells for analysis.

Of note, sensitivity of MRD analysis was reduced in selected samples due to paucicellular specimens (for example, day 15 of induction 1). In such instances, MRD analysis was performed using the same methods but reported with this limitation.

Early T-precursor ALL/LBL definition:

ETP-ALL/LBL immunophenotype was defined as follows: absent CD1a and CD8 expression; absent or dim CD5 expression; and expression of at least one myeloid (CD11b, CD13, CD33, CD117) or stem cell (CD34, HLA-DR) marker by flow cytometry.(6, 7)

Delays in treatment:

Delay in initiating a block of therapy was defined as initiation of protocol-specified therapy beyond the time frame advised by protocol (transition from induction I to induction II later than day 40 of induction I, or transition from intensification I/II to reinduction I/II later than day 35 of intensification I/II) or 4 weeks or more from the last date of protocol-specified chemotherapy in one cycle to the next (for transition from induction II to intensification I, or from reinduction I to intensification II). Delays were categorized as related to pegaspargase therapy if abnormal laboratory values or other sequelae attributed to pegaspargase precluded timely start of next protocol treatment based on protocol-specified parameters. Other delays were categorized as related to pegaspargase in part if the delay would not have been mandated by protocol, but the physician chose to delay treatment in part to allow further improvement in pegaspargase-related toxicity (for example, nausea). Delays related to myelosuppression or other factors independent of pegaspargase were categorized accordingly.

Molecular profiling:

Evaluation for Philadelphia chromosome-like ("Ph-like") ALL was performed when possible for patients with B-cell ALL/LBL using targeted RNA sequencing (FusionPlex, Archer, Boulder, CO) and fluorescence *in situ* hybridization. In selected patients, the FoundationOne Heme platform (Foundation Medicine, Cambridge, MA) was used for DNA and targeted RNA sequencing.

For selected patients treated at Memorial Sloan Kettering Cancer Center (MSK), MSK IMPACT-Heme was performed at diagnosis; the IMPACT-Heme (HemePACT) targeted deep sequencing assay has been described elsewhere(8-10). Specific mutations are detected by hybridization capture of DNA followed by massively parallel sequencing on an Illumina HiSeq2500 instrument. This assay is designed to detect single nucleotide variants and insertions and deletions (< 2,000bp) in protein-coding exons of the 400 gene panel. Mutations are called based on paired analysis using the submitted patient control sample and a pooled unmatched normal. This assay reports variants confirmed to be absent in the pooled unmatched normal and also confirmed to be somatic based on comparison of variant allele frequencies in the tumor sample and the matched blood or saliva or nail sample. This assay is at risk of false negatives when sequence coverage for an exon is below 100X.

Response definitions:

Complete response (CR) was defined as achievement of <5% blasts with approximately normal cellularity and trilineage hematopoiesis by BM aspirate and biopsy, resolution of CNS or other extramedullary disease, and recovery of peripheral blood counts (absolute neutrophil count [ANC] ≥1000/μL without growth factor support and platelets ≥100k/μL without transfusion); CR

with incomplete hematologic recovery (CRi) was defined as meeting criteria for CR, with the exception of achieving the above thresholds for ANC and/or platelet recovery. In patients with non-CNS extramedullary disease, CR for those sites was defined by Lugano criteria.(11)

MRD was assessed by multiparameter flow cytometry as outlined in the main **Methods** section of the manuscript and as noted previously in the **Supplementary Methods**. Key time points for MRD analysis included day 15 of Induction I and on recovery from Induction I and Induction II. Immunophenotyping was additionally performed on bone marrow aspirate specimens at hematopoietic recovery following reinduction I and reinduction II, every 3 months during years 1-2 of maintenance, and every 6 months during year 3 of maintenance

Study design:

We conducted a multicenter open-label phase II trial to study the efficacy of the novel pediatric-inspired regimen described herein in the treatment of adults up to age 60 years with newly diagnosed Ph-negative ALL/LBL. We utilized a Simon‘s Minimax two-stage design in which a 50% rate of molecular remission (minimal residual disease [MRD] negativity by local multiparameter flow cytometry [FACS] as described in **Methods**) was considered not promising, a 70% rate of molecular remission was considered promising, and the probabilities of a type I error and type II error were set at 0.10 and 0.10, respectively. The maximum trial size was set as 39 patients. The two-stage design was set such that in the first stage, 23 patients would be accrued. If at least 12 of these 23 patients with ALL or LBL achieved molecular remission (MRD negativity by local FACS) after Induction Phase I, then an additional 16 patients would be accrued to the second stage. At the end of the trial, if 24 or more molecular remissions were seen after Induction Phase I, the study would be considered promising for further investigation. The

study proceeded from the first to the second stage of enrollment after confirmation of molecular remission following Induction Phase I in 12 of the first 23 patients enrolled.

Consideration of allogeneic hematopoietic cell transplantation (alloHCT):

Patients achieving complete response (CR) post-induction were permitted to proceed to alloHCT at any point, if recommended by the treating physicians. There were not homogeneous criteria for alloHCT. Broadly, patients with t(4;11), Ph-like ALL, or persistent MRD beyond induction phase II were offered allogeneic transplant in CR1. Other patients underwent alloHCT in first CR based on donor availability and shared decision-making between the patient and involved physicians. Patients relapsing and therefore discontinuing study treatments were generally offered alloHCT in subsequent CR if attained.

Supplementary results:

Delays between courses of therapy:

In the absence of toxicity or complications, it was recommended patients proceed to Induction Phase II on day 32-40 of Induction Phase I, to Intensification I after hematopoietic recovery (ANC ≥ 1,000/mcL and platelets ≥75,000/mcL) following confirmation of CR post-Induction Phase II, to Reinduction I on day 28-35 of Intensification I, to Intensification II after hematopoietic recovery (ANC ≥ 1,000/mcL and platelets ≥75,000/mcL) following confirmation of CR post-Reinduction I, to Reinduction II on day 28-35 of Intensification II, and to Maintenance after hematopoietic recovery (ANC ≥ 1,000/mcL and platelets ≥75,000/mcL) following confirmation of CR post-Reinduction II.

Median time from initiation of one cycle to initiation of the next was 39 days from Induction Phase I to Induction Phase II, 71.5 days from Induction Phase II to Intensification I, 35 days from Intensification I to Reinduction I, 71 days from Reinduction I to Intensification II, 33 days from Intensification II to Reinduction II, and 82 days from Reinduction II to Maintenance.

Among the 24 patients who began Induction Phase II >40 days from initiation of Induction Phase I, the delay was directly attributed to pegaspargase in 7. Pegaspargase toxicity was cited by the treating physician as contributing in part to the delay in 3 patients, regardless of whether the patient otherwise met laboratory parameters to begin Induction Phase II chemotherapy. Time between Induction Phase I and Induction Phase II was not associated with inferior EFS or OS. Of the 13 patients who began Intensification I ≥71 days from start of Induction II, no delays were attributed to pegaspargase. Of the 11 patients who began Reinduction I >35 days from start of Intensification I, toxicities of pegaspargase were noted as a contributing factor in 2 patients. Of the 9 patients who began Intensification II ≥71 days from

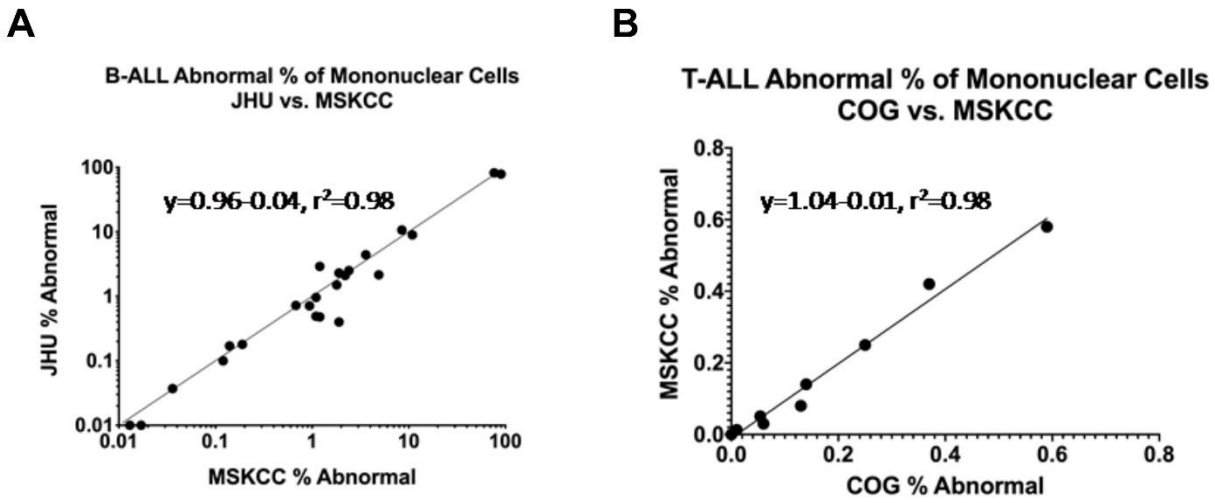
start of Reinduction I, no delays were attributed to pegaspargase. Of the 6 patients who began Reinduction II >35 days from start of Intensification II, toxicities of pegaspargase were considered a contributing factor in one case. Of the 7 patients who began Maintenance ≥71 days from start of Reinduction II, none had delays attributed to pegaspargase.

Local MRD assessment:

Local evaluation for MRD in BM was performed in 27 of 31 patients with ALL (i.e. not LBL) on day 15 of Induction I; at that time, 9 of 27 (33%) of patients had achieved MRD negativity. The proportion of patients with ALL exhibiting BM MRD negativity on local review increased following Induction I (13 of 30, 43%) and Induction II (21 of 25, 84%).

Supplementary figures:

Figure S1



Legend: Bone marrow aspirates from patient follow up samples for either B-cell ALL (A) or T-cell ALL (b) were collected at Memorial Sloan Kettering Cancer Center (MSK) in EDTA tubes and split equally between MSK and Johns Hopkins University (JHU), (a) or MSK and Children's Oncology Group reference laboratory (University of Washington, utilizing identical methodology to JHU), (B). Slope and intercept were calculated using Deming regression). 95% confidence intervals for slope and intercept included 1 and 0 respectively (not shown).

Supplementary tables:

Table S1: Pegaspargase toxicity monitoring, prevention, and management, based on reference (12)

Required labs at least twice weekly after each dose of pegaspargase for at least two weeks: amylase, lipase, fibrinogen, bilirubin, AST/ALT, alkaline phosphatase, glucose, triglycerides, PT/PTT, CBC				
Toxicity	**Prevention**	**Early detection**	**Treatment**	**Asparaginase resumption**
Hypersensitivity	-Steroids per protocol -Hydrocortisone premedication -Anaphylaxis kit at chairside/bedside during infusion		-Anti-histamine -Hydrocortisone or other corticosteroids -Epinephrine	-No E. coli asparaginase or pegaspargase after anaphylaxis -If Erwinia asparaginase available, replace each pegaspargase dose with 6 doses of Erwinia asparaginase 25,000 units/m^2 every other day including weekend days
Pancreatitis	-No EtOH -Avoid heavy meals	-Follow pancreatic enzymes at least twice weekly as above -Patient instructed to report abdominal pain/nausea/vomiting promptly -Serial labs for chemical-only pancreatitis	-Immediate admission for clinical pancreatitis -NPO -IV hydration -Antibiotics as indicated -CT abdomen	-No asparaginase products after clinical pancreatitis -Continue asparaginase for elevations of lipase/amylase without any symptoms
Thombosis	-Most patients have a decrease in ATIII. However, for history of recurrent past thrombosis, assess for thrombophilia.	-ATIII levels NOT followed routinely -Ultrasound if suspicion for VTE	<u>Central line-associated or DVT</u> -Change line -LMWH -Consider ATIII -Avoid plasma (repletes asparagine)	-Continue pegaspargase -LMWH (except when platelets <25-50k/mcL)
			<u>CNS or major vessel</u> -LMWH -ATIII -Avoid plasma (repletes asparagine)	-Anticoagulation as indicated -No E. coli asparaginase or pegaspargase
Bleeding	-No prophylactic cryoprecipitate unless fibrinogen <50 mg/dL	-Fibrinogen monitoring as above	-Cryoprecipitate -Platelet transfusions if indicated -Avoid plasma (repletes asparagine)	-Continue pegaspargase
Hyperglycemia		-Follow serum glucose at least twice weekly as above	-Insulin if needed	-Continue pegaspargase and corticosteroids

		-Educate patients to recognize signs and symptoms of hyperglycemia	-For DKA or HHNK, admit and treat immediately	
Hepatotoxicity	-Avoid EtOH	-Follow liver biochemical tests at least twice weekly as above		-Wait for bilirubin to drop before next cycle (treatment parameters per protocol)
Hypertriglyceridemia	-Avoid fatty meals	-Follow triglycerides at least twice weekly as above	-Start gemfibrozil 600 mg PO BID for triglycerides >1000 mg/dL	-Continue pegaspargase
Legend: EtOH=alcohol, NPO=nothing by mouth, ATIII=antithrombin III, VTE=venous thromboembolism, DVT=deep vein thrombus, CNS=central nervous system, LMWH=low molecular weight heparin, DKA=diabetic ketoacidosis, HHNK=hyperosmolar hyperglycemic non-ketotic state				

Table S2: Details of antibody panels used for flow cytometric assessment of minimal residual disease.

MSK B-cell ALL		
Antibody	**Flurochrome**	**Manufacturer**
CD20	FITC	BD
CD34	PE	BC
CD10	PC5.5	BC
CD33	PC7	BC
CD58	APC	BC
CD45	APC-H7	BD
CD19	BV421	BD Horizon
CD38	BV510	BD Horizon

MSK T-cell ALL		
Antibody	**Fluorochrome**	**Manufacturer**
Tube 1		
CD7	BB515	BD
CD16	PE	BC
CD56	PE	BC
CD34	PerCP-Cy5.5	BD
CD3	PC7	BC
CD5	APC	BD
CD4	APC A700	BC
CD8	APC-H7	BD
CD2	BV421	BD Pharm.
CD45	V500C	BD Horizon
Tube 2		
cyCD3	Alexa 488	BioLegend
CD7	PE	BD
CD56	PC5	BC
CD16	PC5	BC
CD5	PC7	BC
CD3	APC	BD
CD10	APC-R700	BD
CD38	APC-A750	BC
CD48	BV421	BD Horizon
CD45	V500c	BD

Legend: BD=Becton Dickinson; BC=Beckman Coulter; cy/s prior to marker=cytoplasmic/surface

JHU B-cell ALL		
Antibody	**Fluorochrome**	**Manufacturer**
Tube 1		
CD20	FITC	BD
CD10	PE	BD
CD38	PerCP-Cy5.5	BD Pharm.
CD19	PC7	BC
CD58	APC	BC
CD45	APC-H7	BD
Tube 2		
CD9	FITC	BD
CD13	PE	BD
CD33	PE	BD
CD34	PerCP-Cy5.5	BD
CD19	PC7	BC
CD10	APC	BD
CD45	APC-H7	BD
Tube 3		
Syto16	FITC	Molecular Probes
CD3	PerCP-Cy5.5	BD
CD19	PC7	BC
CD71	APC	BD
CD45	APC-H7	BD

JHU T-cell ALL		
Antibody	**Fluorochrome**	**Manufacturer**
Tube 1		
sCD3	BV421	BD Horizon
CD48	FITC	BD
CD7	PE	BD
CD16+56	PerCP-Cy5.5	BD
CD5	PE-Cy7	BD
cyCD3	APC	BD
CD45	APCH7	BD
Tube 2		
sCD3	BV421	BD Horizon
CD8	BV510	BD
CD10	BV605	BD
CD2	FITC	BD
CD7	PE	BD
CD16+56	PerCP-Cy5.5	BD
CD4	PE-Cy7	BD
CD34	APC	BD
CD5	APCR700	BD
CD45	APCH7	BD
Tube 3 (diagnosis only)		
CD99	FITC	BD
CD7	PE	BD
CD56	PerCPCy5.5	BD
CD1a	APC	BD
CD45	APCH7	BD
Tube for quantitation		
Syto16	FITC	Molecular Probes
CD7	PE	BD
CD71	APC	BD
CD45	APCH7	BD

Table S3: Cytogenetic classification system.

Classification(13)	Cytogenetic Findings
Unfavorable	t(4;11)(q21;q23) or any other balanced translocation involving band 11q23; Monosomy of chromosome 7 (−7); Trisomy of chromosome 8 (+8); Hypodiploid karyotype (≤43 with or without a near-triploid (i.e., with near 69 chromosomes) clone (or had a near triploid clone without a hypodiploid one)
Intermediate	Normal karyotype; structural abnormalities involving the short arm of chromosome 9 (9p); t(1;19)(q23;p13.3) or a derivative of chromosome 19 resulting from this reciprocal translocation [der(19)t(1;19)(q23;p13.3)]; deletions of the long arm of chromosome 6 [del(6q)] and the long arm of chromosome 13 [del(13q)]; trisomy of chromosome 21 (+21); high hyperdiploidy defined as chromosome number ≥50 (excluding near-triploidy and near-tetraploidy)
Favorable	Abnormalities involving bands 14q11, 7p14~15 or 7q34~36, and deletions and translocations involving the short arm of chromosome 12 (12p)
Not prognostically classified	Adequate karyotype but not one above
Not evaluable	Not adequate karyotype

Table S4: Reasons for pegaspargase discontinuation prior to completion of all 6 planned doses.

Rationale	**N**
AlloHCT in CR1	10
Changed to Erwinia asparaginase for hypersensitivity to pegaspargase	4
Death in CR1	2
Discretion of investigator	2
Early truncation of second reinduction course due to complications of pancytopenia	1
Hyperbilirubinemia attributed to pegaspargase	1
Morphologic relapse	1
Pancreatitis attributed to pegaspargase	1
Patient withdrawal of consent	1
Persistent MRD with change of therapy to blinatumomab	1
Removed from study for refractory disease	1
Transaminitis attributed to pegaspargase	1
Transaminitis attributed to HD-MTX leading to omission of planned dose of pegaspargase	1

Table S5: Serum asparaginase enzymatic activity post-PEG for patients age 40-60.

Time Point in Treatment	Samples (N)	Serum Asparaginase Enzymatic Activity 7 Days Post-PEG (IU/mL)	
		Mean	SD
Induction I	6	0.813	0.306
Induction Phase II	5	0.885	0.248
Intensification I	3	0.968	0.185
Reinduction I	4	1.118	0.286
Intensification II	3	0.688	0.289
Reinduction II	2	1.079	0.067
All Phases	23	0.909	0.274

Table S6: Summary of immunogenicity testing results in patients with positive confirmatory results at any point during treatment, as well as asparaginase activity at the corresponding time point.

Pt ID	Visit	Screening Result	Oncaspar® Confirmatory Result	5 kDa PEG Confirmatory Result	Titration Result	Asparaginase Activity Level (IU/mL)	Comments
12	Pre-treatment	Negative	N/A	N/A	N/A	<0.013	Pre-treatment; had not received any pegaspargase
	Induction I, 7 days post-pegaspargase	Negative	N/A	N/A	N/A	0.667	
	Induction II, immediate post-pegaspargase	Positive	Positive	Positive	100	0.106	Allergic reaction; pegaspargase stopped 5 minutes into infusion.
	Induction II, 7 days post-pegaspargase	Positive	Positive	Positive	200	<0.013	Had only received 5 minutes of pegaspargase infusion as above.
18	Pre-treatment	Positive	Positive	Positive	100	<0.013	Pre-treatment; had not received any pegaspargase
	Induction I, 7 days post-pegaspargase	Negative	N/A	N/A	N/A	0.904	
	Induction II, 7 days post-pegaspargase	Positive	Positive	Positive	50	0.807	
	Re-induction I, 7 days post-pegaspargase	Positive	Positive	Negative	25	0.942	
	Re-induction II, 7 days post-pegaspargase	Negative	N/A	N/A	N/A	1.131	
22	Pre-treatment	Positive	Positive	Negative	50	<0.013	Pre-treatment; had not received any pegaspargase
	Induction II, 7 days post-pegaspargase	Positive	Positive	Negative	25	0.340	
34	Induction I, 7 days post-pegaspargase	Positive	Negative	Negative	N/A	0.691	
	Induction II, 7 days post-pegaspargase	Positive	Positive	Positive	100	0.694	
	Intensification I, 7 days post-pegaspargase	Positive	Positive	Positive	50	1.139	
	Re-induction I, 7 days post-pegaspargase	Positive	Positive	Negative	200	0.954	
Legend: Pt ID=patient identification number; kDa=kilodaltons; PEG=polyethylene glycol							

Table S7: Numbers of patients with non-hematologic adverse events definitely, probably, or possibly related to protocol therapy as assessed by CTCAE v4.03. Maximal grade of each adverse effect is documented for each individual patient.

Toxicity	Maximal Grade									
	1		2		3		4		3 + 4	
	N	%	N	%	N	%	N	%	N	%
Fibrinogen decreased	1	3	6	15	21	54	2	5	23	59
Hypertriglyceridemia	3	8	6	15	11	28	12	31	23	59
ALT increased	3	8	10	26	15	38	1	3	16	41
Hyperglycemia	3	8	11	28	8	21	4	10	12	31
AST increased	10	26	6	15	10	26	0	0	10	26
Blood bilirubin increased	1	3	7	18	5	13	5	13	10	26
Febrile neutropenia	0	0	0	0	10	26	0	0	10	26
Alkaline phosphatase increased	4	10	2	5	4	10	0	0	4	10
Hypoalbuminemia	0	0	6	15	4	10	0	0	4	10
Fatigue	13	33	6	15	3	8	0	0	3	8
Peripheral sensory neuropathy	9	23	3	8	3	8	0	0	3	8
aPTT prolonged	14	36	4	10	2	5	0	0	2	5
Epistaxis	1	3	1	3	2	5	0	0	2	5
Hyponatremia	4	10	0	0	2	5	0	0	2	5
Nausea	13	33	12	31	2	5	0	0	2	5
Abdominal pain	3	8	3	8	1	3	0	0	1	3
Allergic reaction	0	0	2	5	1	3	0	0	1	3
Anaphylaxis	0	0	0	0	1	3	0	0	1	3
Anorectal infection	0	0	0	0	1	3	0	0	1	3
Anorexia	4	10	2	5	1	3	0	0	1	3
Arthralgia	0	0	0	0	1	3	0	0	1	3
Headache	4	10	3	8	1	3	0	0	1	3
Hepatic failure	0	0	0	0	1	3	0	0	1	3
Hepatic infection	0	0	0	0	1	3	0	0	1	3
Hyperkalemia	1	3	1	3	1	3	0	0	1	3
Hypokalemia	0	0	0	0	0	0	1	3	1	3
Hypophosphatemia	0	0	0	0	1	3	0	0	1	3
Hypotension	0	0	0	0	1	3	0	0	1	3
INR increased	15	38	2	5	1	3	0	0	1	3
Lipase increased	4	10	1	3	0	0	1	3	1	3
Muscle weakness lower limb	0	0	1	3	1	3	0	0	1	3
Pancreatitis	0	0	0	0	1	3	0	0	1	3
Pain in extremity	4	10	1	3	1	3	0	0	1	3
Sepsis	0	0	0	0	0	0	1	3	1	3
Thromboembolic event	0	0	0	0	1	3	0	0	1	3
Syncope	0	0	0	0	1	3	0	0	1	3
Vomiting	7	18	4	10	1	3	0	0	1	3
Alopecia	3	8	3	8	0	0	0	0	0	0

Anxiety	1	3	0	0	0	0	0	0	0	0
Avascular necrosis	0	0	1	3	0	0	0	0	0	0
Back Pain	1	3	0	0	0	0	0	0	0	0
Bloating	3	8	1	3	0	0	0	0	0	0
Blurred vision	1	3	1	3	0	0	0	0	0	0
Bone Pain	1	3	0	0	0	0	0	0	0	0
Breast pain	0	0	1	3	0	0	0	0	0	0
Chills	2	5	0	0	0	0	0	0	0	0
Constipation	11	28	4	10	0	0	0	0	0	0
Creatinine increased	2	5	1	3	0	0	0	0	0	0
Diarrhea	3	8	5	13	0	0	0	0	0	0
Dizziness	2	5	0	0	0	0	0	0	0	0
Dry mouth	1	3	0	0	0	0	0	0	0	0
Dry skin	3	8	0	0	0	0	0	0	0	0
Dysgeusia	4	10	3	8	0	0	0	0	0	0
Dyspepsia	2	5	1	3	0	0	0	0	0	0
Dyspnea	4	10	0	0	0	0	0	0	0	0
Depression	2	5	0	0	0	0	0	0	0	0
Edema limbs	3	8	2	5	0	0	0	0	0	0
Esophagitis	0	0	2	5	0	0	0	0	0	0
Fever	1	3	0	0	0	0	0	0	0	0
Flatulence	3	8	0	0	0	0	0	0	0	0
Gastroesophageal reflux disease	2	5	2	5	0	0	0	0	0	0
Gastrointestinal orders, other	2	5	0	0	0	0	0	0	0	0
Generalized muscle weakness	3	8	2	5	0	0	0	0	0	0
Gingival pain	2	5	0	0	0	0	0	0	0	0
Hearing impaired	2	5	0	0	0	0	0	0	0	0
Hemorrhoids	0	0	2	5	0	0	0	0	0	0
Hepatobiliary disorders, other	2	5	0	0	0	0	0	0	0	0
Hypercalcemia	1	3	0	0	0	0	0	0	0	0
Hypertension	1	3	0	0	0	0	0	0	0	0
Hypocalcemia	4	10	1	3	0	0	0	0	0	0
Hypomagnesmia	1	3	1	3	0	0	0	0	0	0
Hypoglycemia	2	5	0	0	0	0	0	0	0	0
Infusion related reaction	0	0	2	5	0	0	0	0	0	0
Insomnia	5	13	3	8	0	0	0	0	0	0
Malaise	3	8	0	0	0	0	0	0	0	0
Mucositis oral	3	8	1	3	0	0	0	0	0	0
Muscle weakness upper limb	1	3	0	0	0	0	0	0	0	0
Myalgia	1	3	1	3	0	0	0	0	0	0
Myositis	0	0	1	3	0	0	0	0	0	0
Nasal congestion	1	3	0	0	0	0	0	0	0	0
Neck pain	1	3	1	3	0	0	0	0	0	0
Neuralgia	1	3	0	0	0	0	0	0	0	0
Oral pain	1	3	1	3	0	0	0	0	0	0

Pain	1	3	1	3	0	0	0	0	0	0
Palpitations	1	3	0	0	0	0	0	0	0	0
Paresthesia	6	15	0	0	0	0	0	0	0	0
Photophobia	1	3	0	0	0	0	0	0	0	0
Peripheral motor neuropathy	2	5	3	8	0	0	0	0	0	0
Portal vein thrombosis	1	3	0	0	0	0	0	0	0	0
Postnasal drip	1	3	0	0	0	0	0	0	0	0
Pruritus	2	5	0	0	0	0	0	0	0	0
Rash acneiform	1	3	0	0	0	0	0	0	0	0
Rash maculo-papular	2	5	0	0	0	0	0	0	0	0
Renal and urinary disorders, other: Urinary discoloration	1	3	0	0	0	0	0	0	0	0
Renal and urinary disorders, other: Burning sensation on urinartion	1	3	0	0	0	0	0	0	0	0
Serum amylase increased	1	3	0	0	0	0	0	0	0	0
Sinus tachycardia	1	3	1	3	0	0	0	0	0	0
Skin & subcutaneous tissue disorders, other	0	0	1	3	0	0	0	0	0	0
Skin & subcutaneous tissue disorders, other: Irritation and tenderness near PICC line	1	3	0	0	0	0	0	0	0	0
Skin & subcutaneous tissue disorders, other: Rash not otherwise specified	1	3	0	0	0	0	0	0	0	0
Skin ulceration	1	3	0	0	0	0	0	0	0	0
Sore throat	1	3	0	0	0	0	0	0	0	0
Spasticity	1	3	0	0	0	0	0	0	0	0
Tinnitus	1	3	0	0	0	0	0	0	0	0
Upper respiratory infection	0	0	1	3	0	0	0	0	0	0
Urinary tract infection	0	0	1	3	0	0	0	0	0	0

Table S8: Management and clinical course of patients with persistent MRD following Induction Phase II

Pt ID	Age at Start of Treatment (years)	Disease Linage	Subsequent Therapies	Response to Next Therapies	AlloHCT	Current Status
1	44.9	B-cell	Continued on study protocol and received Intensification I	Reduction in MRD levels but persistent MRD positivity by FACS	Double umbilical cord blood + haploidentical HCT	Alive in ongoing MRD-negative CR
3	56.4	B-cell	Removed from study; received HDMTX + HiDAC	Achieved MRD negativity by FACS	Double umbilical cord blood + haploidentical HCT	Alive in ongoing MRD-negative CR
11	35.8	B-cell	Removed from study; received several lines of therapy. (1) Blinatumomab (2) IT cytarabine/MTX and HDMTX (3) HiDAC + mitoxantrone (4) Autologous CD19-targeted CAR T-cells	Responses to subsequent therapy as below: (1) Morphologic marrow and CNS relapse (2) Cleared CNS disease; persistent marrow-based disease (3) Transient cytoreduction to 7% blasts followed by further progression; no CNS involvement (4) CR2, MRD negative	Double umbilical cord blood HCT (following CAR T-cell therapy)	Died following relapse post-HCT
22	24.0	T-cell	Continued on study protocol and received Intensification I	Reduction in MRD levels but persistent MRD positivity by FACS	Double umbilical cord blood + haploidentical HCT	Alive in ongoing MRD-negative CR

Legend: Pt ID=patient identification number; BM=bone marrow; MRD=minimal residual disease; MNC=mononuclear cells; HDMTX=high-dose methotrexate; HiDAC=high-dose cytarabine; IT=intrathecal; CAR=chimeric antigen receptor-modified; FACS=fluorescence-activated cell sorting; alloHCT=allogeneic hematopoietic cell transplantation

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