Using stroma-anchoring cytokines to augment ADCC: a phase 1 trial of F16IL2 and BI 836858 for posttransplant AML relapse

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Supplemental Material

Supplemental Methods

Dose escalation and Bayesian logistic regression model (BLRM)

This study was an open-label, single-arm, prospective dose-escalation phase 1 trial conducted at two sites in Germany (Münster and Dresden) to find and investigate a safe dose of F16IL2 and BI 836858 in patients with AML relapse after allogeneic HSCT. Dose escalation was guided by a Bayesian logistic regression model (BLRM) that was fitted to binary toxicity outcomes, with provisional dose levels of 10 and 20 x 10^6 IU for F16IL2, and 10, 20, 40, 80, 160, and 320 mg for BI 836858. The estimate of parameters was updated as data accumulated using the BLRM and the toxicity probability at each dose combination level was calculated to determine the MTD. For any dose-escalation cohort, at least 3 patients were required. However, in the case that only 2 of 3 patients were evaluable and neither of the 2 patients had experienced a DLT within the first cycle (28 days), then the dose-escalation could procede based on these 2 patients as long as the escalation with overdose control (EWOC) criterion was still fulfilled.

During the dose escalation phase, enrollment into the first cohort was allowed after a minimum of 7 days from the first administration of the first patient, and subsequently between patient 2 and 3. If the cohort expanded to 6 patients, the 7-day observation period between the subsequent patients had to be followed. For subsequent cohorts, enrollment was allowed after a minimum of 3 days from the first administration of each patient. After all patients in a cohort have either experienced a DLT or have been observed for one cycle (28 days) without experiencing a DLT, the Bayesian model was updated with the newly accumulated data. The overdose risk was then calculated for each dose, and escalation was permitted to all doses, which fulfilled the EWOC criterion (escalation with overdose control) (probability for overdosing < 0.25). Based on the model and on additional information (e.g., pharmacodynamics, AE, patient profiles), the members of the DSMB defined the next dose level and cohort size. Dose escalation was restricted to a maximum of 100% from the previous dose for both compounds. If DLTs were observed in the first two consecutive patients of a previously untested dose level, subsequent enrollment to that cohort was stopped. The BLRM was re-run to confirm that the dose-level still fulfilled the EWOC criterion. Based on this information, the DSMB evaluated whether the next patients could be enrolled in the same dose level, or if they were enrolled to a lower dose level.

The DSMB made recommendations on stopping or continuing the dose escalation phase, after the criterion for MTD (section 6.2) was fulfilled. If no DLT was observed at a dose of which the efficacy was considered sufficient, the DSMB could recommend to include an additional number of patients at the same dose level and to declare this dose as the biological active dose. Dose escalation continued until the MTD was found or a biological active dose

was determined. Once the MTD or a biological active dose was defined, additional patients (up to 10) were to be treated with F16IL2 and BI 836858 dosed at the MTD in order to confirm the safety profile of the combination or to specify an RD.

Human anti-fusion protein antibodies (HAFA)

Diluted serum samples (1:500) were applied onto a microplate coated with F16IL2. Antibodies against F16IL2 were immobilized from the serum matrix onto the plate via the binding interaction with F16IL2. HRP-conjugated anti-human IgG (Sigma) was used for detection. Optical density (OD; 450-650 nm) was measured and analyzed considering a cut-off point determined during assay validation. The cut-off has been calculated as 3 times the average OD of negative samples. Samples with a mean OD value greater or equal than the cut-off value were considered positive. As positive control serum from monkeys dosed with F16IL2 in the preclinical study 0194-2007 (Nerviano Medical Sciences, Nerviano, Italy) and as negative control sera from healthy donors (SCIPAC, Sittingbourne, UK) were used.

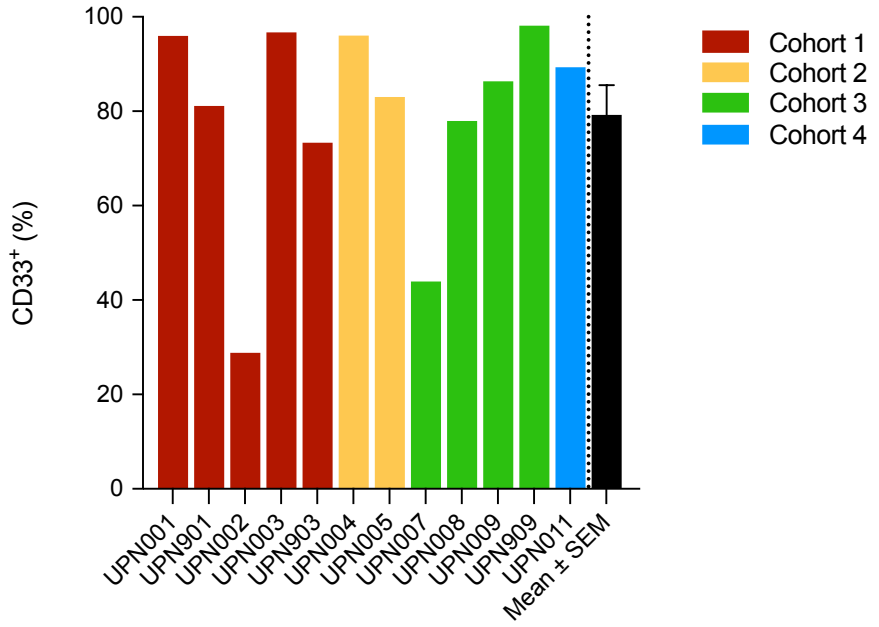
Immune effector cell analyses in bone marrow and peripheral blood

Lymphocyte phenotypes in BM and PB were investigated at screening, at day 15 (C1D15) and at the end of cycles 1 (C1) and 2 (C2) using fluorescence-conjugated mAbs as previously described (see ref. 21 in manuscript). Briefly, red blood cells (RBC) were lysed using 1x RBC lysis buffer (Biolegend 420301). Cell pellets were resuspended in phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA), stained using mAbs against CD3, CD4, CD8, CD16, CD25, CD45RO, CD45RA, CD56, CD127, CD197, CD279, CD335 (NKp46), CD337 (NKp30) and γδTCR (BD Pharmingen) and incubated for 15 minutes in the dark at room temperature. All antibodies were titrated and tested on known positive and negative cells prior to use. Following incubation, cells were washed and resuspended using PBS (0.5% BSA). Approximately 20,000 cells of each sample were analyzed with FACS Canto and FACS Diva software (BD Bioscience), subsequent analysis and figure creation was done with FlowJo software. For each experiment, established laboratory protocols for staining procedure, data acquisition, and gating strategy were used. PMT voltages and compensation were adjusted using unstained cells and stained BD CompBeads (552843). Analysis was done on single cells and gates were set as shown in supplemental Figure 5. A complete blood count on the same day as flow analysis provided percentage of lymphocytes and an absolute lymphocyte count (ALC) to calculate absolute cell numbers of NK and T cells.

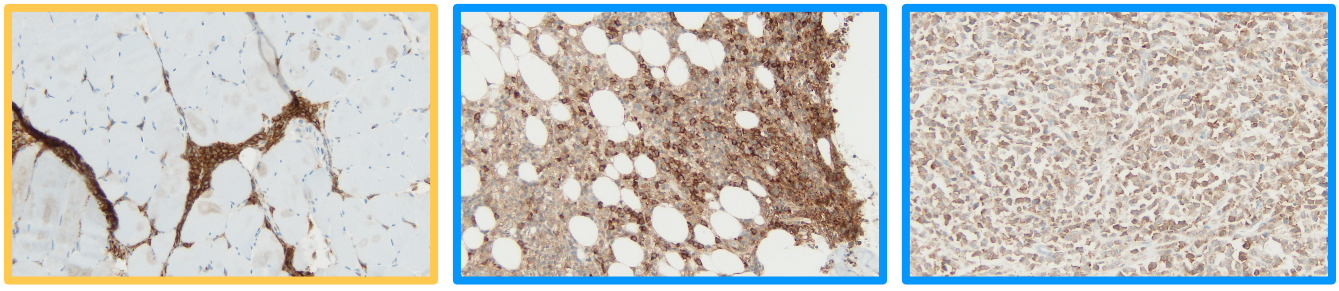
Supplemental Figures

Supplemental Figure 1. CD33 expression of leukemic blasts at study entry. All subjects, including those with extramedullary AML, had CD33$^+$ AML, as analyzed by flow cytometry (A) or immunohistochemistry (B).

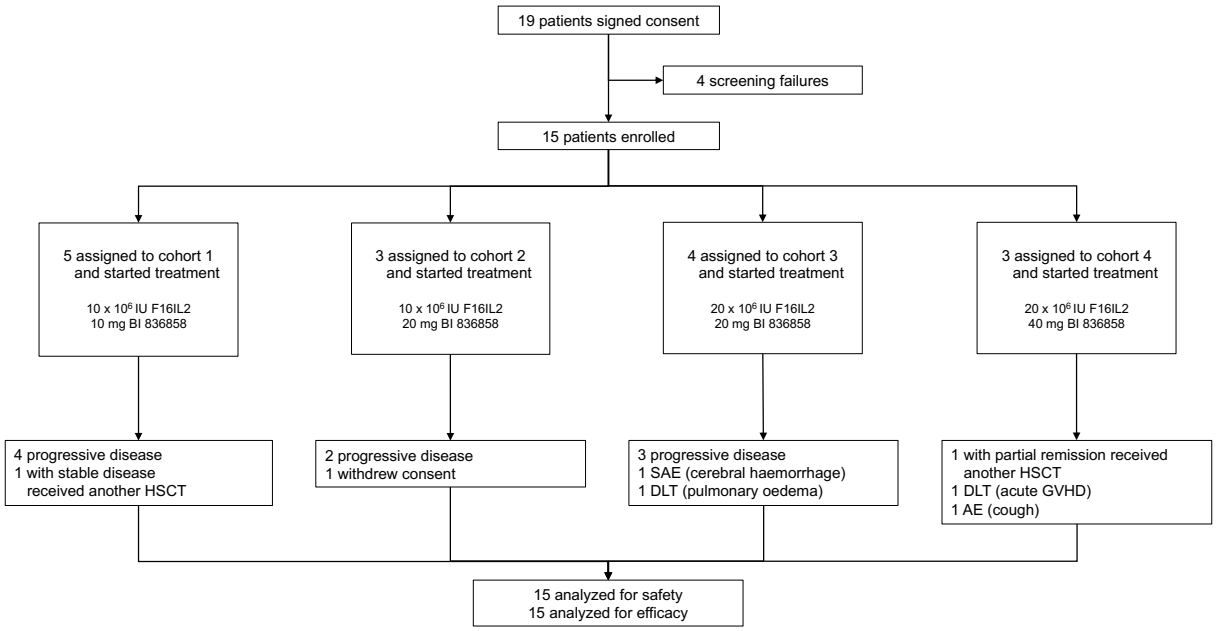
A



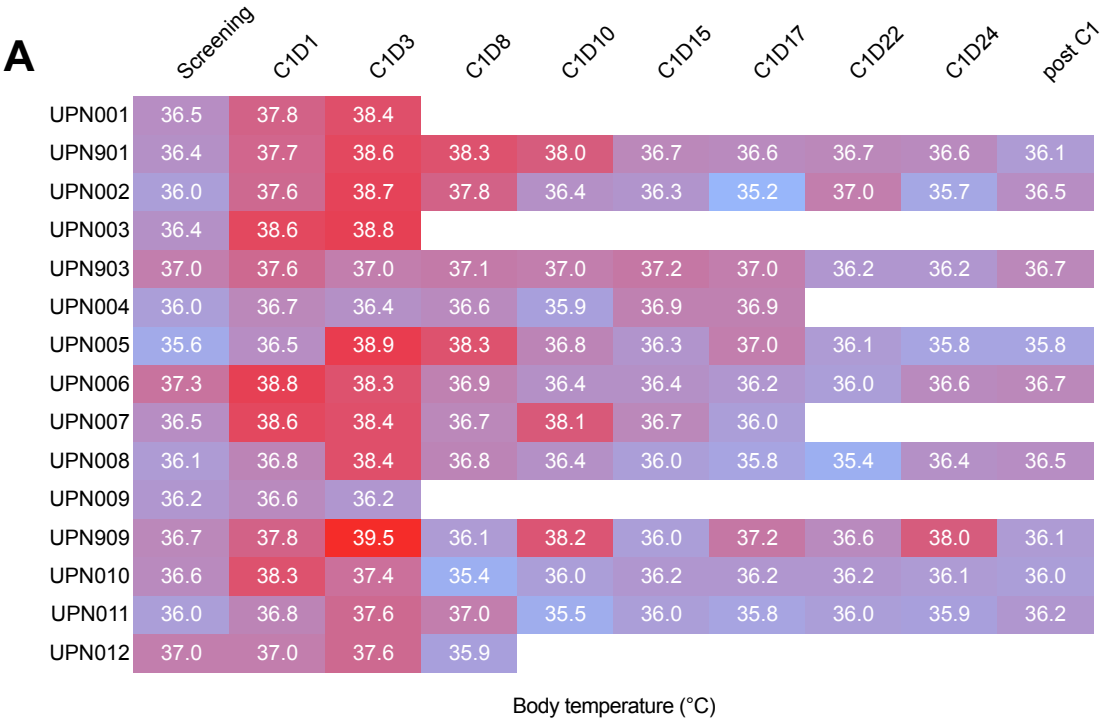
B

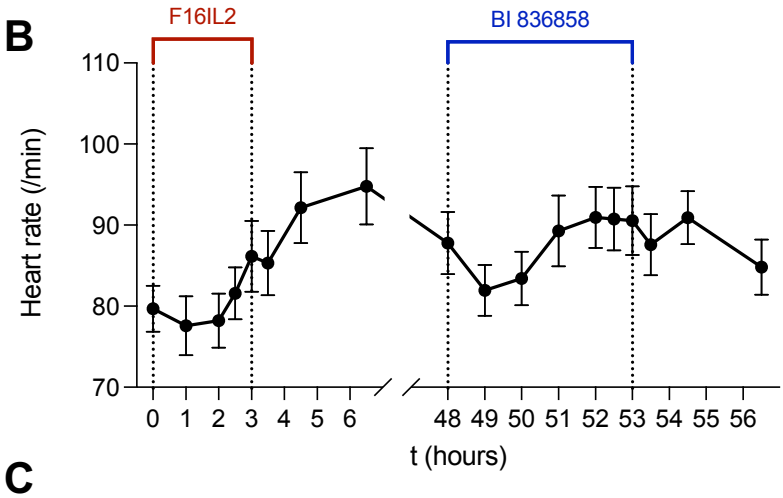


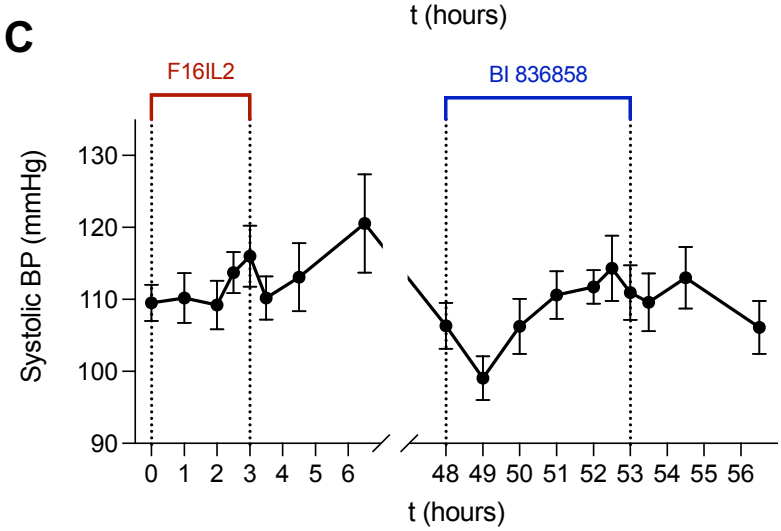
Supplemental Figure 2. Consort diagram. *SAE*, serious adverse event; *DLT*, dose limiting toxicity; *HSCT*, hematopoietic stem cell transplantation; *GVHD*, graft-versus-host disease.



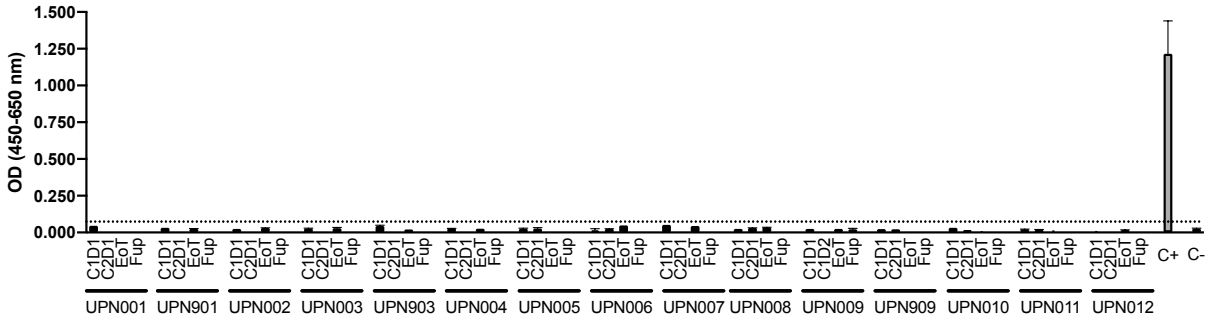
Supplemental Figure 3. Vital sign changes. (A) Heat map of maximum body temperatures (in °C) on indicated days of therapy during cycle 1. (B) Heart rate following the first administration of F16IL2 and BI 836858. (C) No relevant cytokine-induced hypotension was observed. *BP*, blood pressure. Mean +/- SEM.



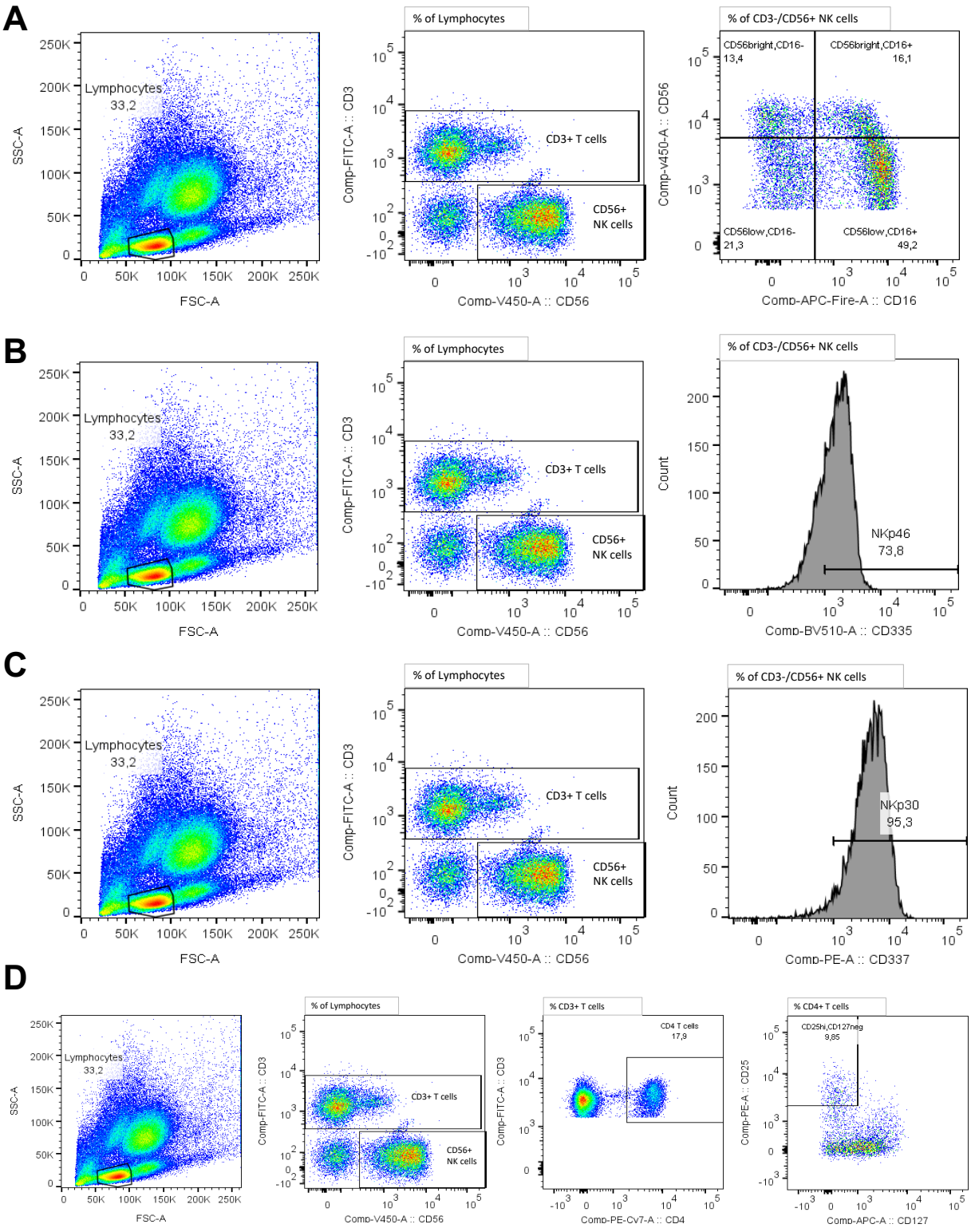




Supplemental Figure 4. Human anti-fusion protein antibodies (HAFA). No antibody formation against F16IL2 was observed throughout the study. *OD*, optical density; *EoT*, end of treatment; *Fup*, Follow-up; *C*+, positive control; *C*-, negative control; *C*, cycle; *D*, day.



Supplemental Figure 5. Gating strategies of flow cytometric analyses. Exemplary gating strategy of (A) CD16$^+$/CD56$^+$ NK cells, (B) NKp46$^+$ NK cells, (C) NKp30$^+$ NK cells, (D) Tregs, (E) effector phenotype of CD4$^+$ and CD8$^+$ T cells, (F) NK T cells, (G) γδ T cells, and (H) PD-1$^+$ expression on T cells.



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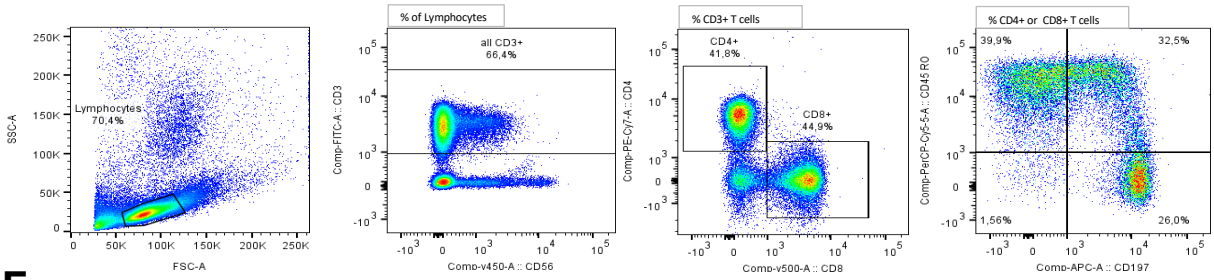
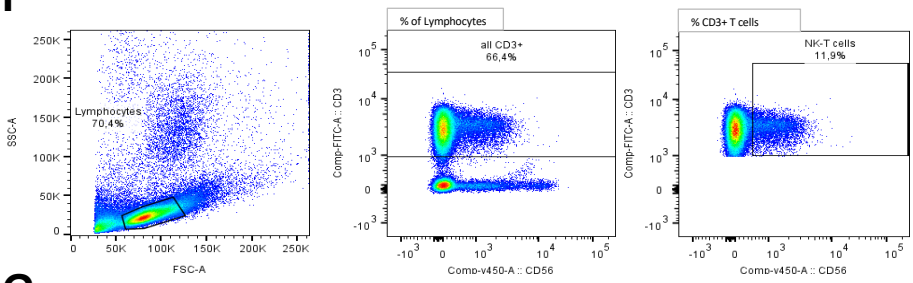
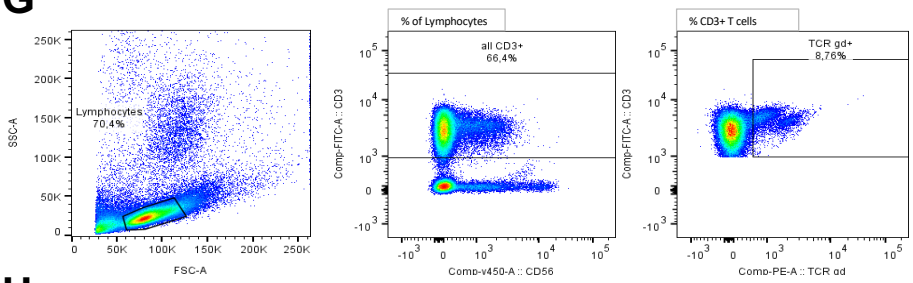

Lymphocytes
70,4%
SSC-A
FSC-A
% of Lymphocytes
all CD3+
66,4%
Comp-FITC-A :: CD3
Comp-v450-A :: CD56
% CD3+ T cells
CD4+
41,8%
CD8+
44,9%
Comp-PE-Cy7-A :: CD4
Comp-v500-A :: CD8
% CD4+ or CD8+ T cells
39,9%
32,5%
1,56%
26,0%
Comp-PerCP-Cy5-5-A :: CD45 RO
Comp-APC-A :: CD197

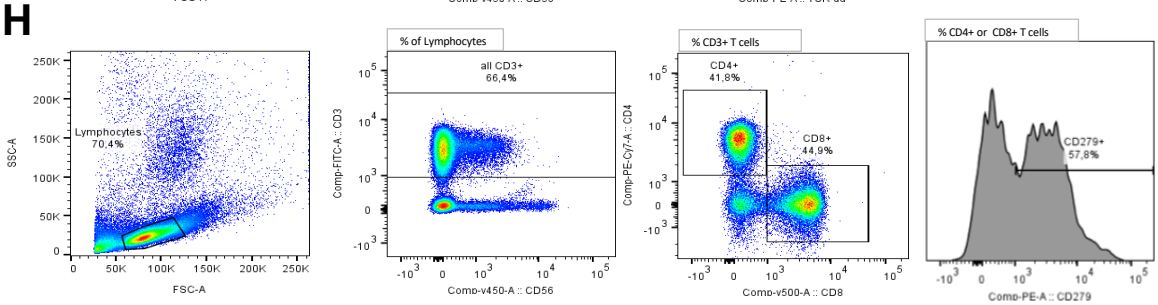
F


Lymphocytes
70,4%
SSC-A
FSC-A
% of Lymphocytes
all CD3+
66,4%
Comp-FITC-A :: CD3
Comp-v450-A :: CD56
% CD3+ T cells
NK-T cells
11,9%

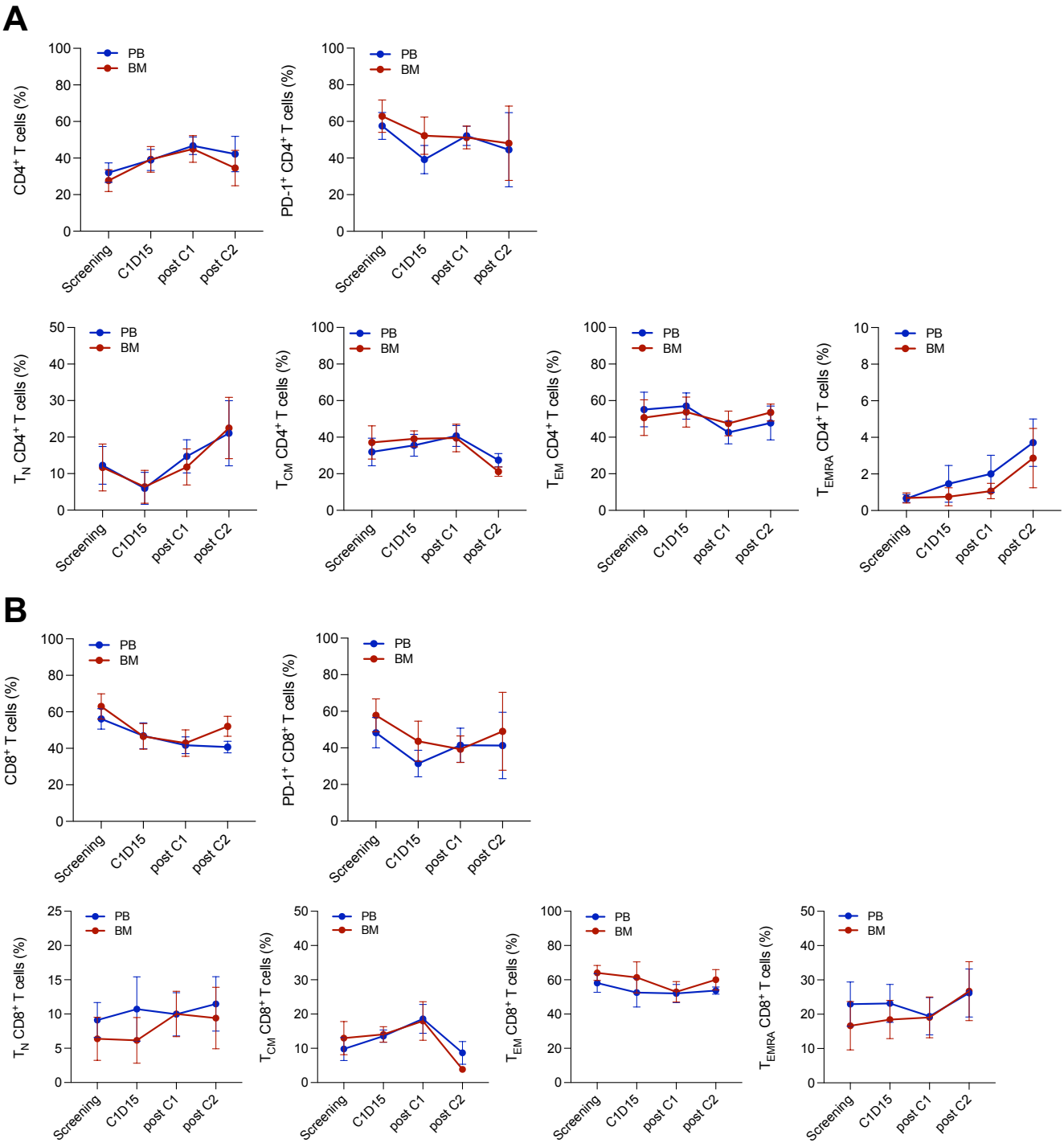
G


Lymphocytes
70,4%
SSC-A
FSC-A
% of Lymphocytes
all CD3+
66,4%
Comp-FITC-A :: CD3
Comp-v450-A :: CD56
% CD3+ T cells
TCR gd+
8,76%
Comp-PE-A :: TCR gd

H


Lymphocytes
70,4%
SSC-A
FSC-A
% of Lymphocytes
all CD3+
66,4%
Comp-FITC-A :: CD3
Comp-v450-A :: CD56
% CD3+ T cells
CD4+
41,8%
CD8+
44,9%
Comp-PE-Cy7-A :: CD4
Comp-v500-A :: CD8
% CD4+ or CD8+ T cells
CD279+
57,8%
Comp-PE-A :: CD279

Supplemental Figure 6. T cell subpopulations. No significant changes in $CD4^+$ (A) and $CD8^+$ (B) T cell frequencies, frequencies of respective $PD\text{-}1^+$ subpopulations or effector phenotypes were observed upon study treatment. Subsets were identified as naïve T cells (T_N) by $CD45RA^{high}/CD45RO^{low}/CD197^{high}$, as central memory T cells (T_{CM}) by $CD45RA^{low}/CD45RO^{high}/CD197^{high}$, as effector memory T cells (T_{EM}) $CD45RA^{low}/CD45RO^{high}/CD197^{low}$, and as terminally differentiated effector memory T cells (T_{EMRA}) by $CD45RA^{high}/CD45RO^{low}/CD197^{low}$ immune phenotypes. (C) Frequencies of NK T cells ($CD3^+/CD56^+$) and γδ T cells ($CD3^+/\gamma\delta TCR^+$) over time. Mean +/- SEM.



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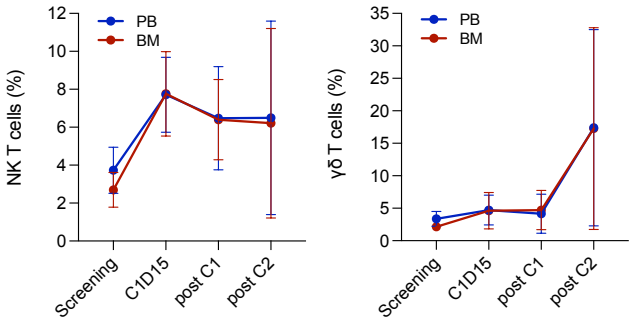
NK T cells (%)
PB
BM
0
2
4
6
8
10
12
Screening
C1D15
post C1
post C2
γδ T cells (%)
PB
BM
0
5
10
15
20
25
30
35
Screening
C1D15
post C1
post C2

Supplemental Tables

Supplemental Table 1. All adverse events and serious adverse events.

Adverse event	**All patients** (n = 15)			
	All		**Treatment-related**	
	Any grade	≥ Grade 3	Any grade	≥ Grade 3
Preferred term MedDRA v14.0	*Number of patients (percent)*			
All adverse events	15 (100)	11 (74)	15 (100)	5 (30.00)
Hematologic adverse events				
Platelet count decreased	7 (74)	5 (33)	4 (27)	2(13)
Anemia	3 (20)	2 (13)	1 (7)	1 (7)
Febrile neutropenia	3 (20)	3 (20)	1 (7)	1 (7)
Neutrophil count decreased	2 (13)	2 (13)	2 (13)	2 (13)
Acute myeloid leukemia	1 (7)	1 (7)	0	0
Eosinophilia	1 (7)	0	1 (7)	0
Leukocytosis	1 (7)	0	0	0
Thrombocytopenia	1 (7)	1 (7)	0	0
White blood cell count decreased	1 (7)	1 (7)	1 (7)	1 (7)
Nonhematologic adverse events				
Pyrexia	13 (87)	0	13 (87)	0
Chills	12 (80)	0	12 (80)	0
Infusion related reaction	9 (60)	0	9 (60)	0
Nausea	9 (60)	1 (7)	7 (47)	0
Vomiting	5 (33)	1 (7)	2 (13)	0
Back pain	4 (27)	0	0	0
Diarrhea	4 (27)	0	4 (27)	0
Dyspnea	4 (27)	1 (7)	3 (20)	1 (7)
Fatigue	4 (27)	0	3 (20)	0
Edema peripheral	4 (27)	0	2 (13)	0
Pain in extremity	4 (27)	1 (7)	1 (7)	0
Abdominal pain	3 (20)	1 (7)	1 (7)	0
Cough	3 (20)	1 (7)	1 (7)	1 (7)
Decreased appetite	3 (20)	1 (7)	1 (7)	0
Rash	3 (20)	1 (7)	2 (13)	0
Acute graft versus host disease in intestine	2 (13)	0	1 (7)	0
Alanine aminotransferase increased	2 (13)	0	2 (13)	0
Aspartate aminotransferase increased	2 (13)	0	2 (13)	0
Blood bilirubin increased	2 (13)	1 (7)	2 (13)	1 (7)
Chest discomfort	2 (13)	0	1 (7)	0
Epistaxis	2 (13)	0	0	0
Gamma-glutamyltransferase increased	2 (13)	1 (7)	2 (13)	1 (7)
Headache	2 (13)	0	2 (13)	0
Hypersensitivity	2 (13)	0	0	0
Hypoalbuminemia	2 (13)	0	2 (13)	0
Hypokalemia	2 (13)	2 (13)	1 (7)	1 (7)
Hypophosphatemia	2 (13)	1 (7)	2 (13)	1 (7)
Hypotension	2 (13)	1 (7)	1 (7)	0
Influenza like illness	2 (13)	0	2 (13)	0
International normalised ratio increased	2 (13)	0	2 (13)	0
Pain neck	2 (13)	0	1 (7)	0
Stomatitis	2 (13)	1 (7)	0	0
Syncope	2 (13)	2 (13)	0	0
Acute graft versus host disease	1 (7)	1 (7)	1 (7)	1 (7)
Acute graft versus host disease in liver	1 (7)	0	1 (7)	0

Acute kidney injury	1 (7)	0	1 (7)	0
Blood alkaline phosphatase increased	1 (7)	0	1 (7)	0
Bone pain	1 (7)	0	1 (7)	0
Cerebral hemorrhage	1 (7)	1 (7)	0	0
Chronic graft versus host disease in skin	1 (7)	0	0	0
Confusional state	1 (7)	0	0	0
Cystitis hemorrhagic	1 (7)	1 (7)	0	0
Cytokine release syndrome	1 (7)	0	1 (7)	0
Dizziness	1 (7)	0	1 (7)	0
Dry eye	1 (7)	0	1 (7)	0
Dry mouth	1 (7)	0	1 (7)	0
Dysgeusia	1 (7)	0	1 (7)	0
Folate deficiency	1 (7)	0	0	0
Gastrointestinal hemorrhage	1 (7)	1 (7)	0	0
Gastroesophageal reflux disease	1 (7)	0	0	0
General physical health deterioration	1 (7)	1 (7)	0	0
Groin pain	1 (7)	0	1 (7)	0
Herpes virus infection	1 (7)	0	0	0
Hot flush	1 (7)	0	1 (7)	0
Hyperphosphatemia	1 (7)	0	0	0
Hypomagnesemia	1 (7)	0	0	0
Hypoxia	1 (7)	1 (7)	1 (7)	1 (7)
Infection	1 (7)	1 (7)	0	0
Inflammatory marker increased	1 (7)	0	1 (7)	0
Infusion site extravasation	1 (7)	0	0	0
Mucosal inflammation	1 (7)	0	0	0
Muscle contractions involuntary	1 (7)	0	1 (7)	0
Muscle spasms	1 (7)	0	0	0
Nasopharyngitis	1 (7)	0	0	0
Orthostatic intolerance	1 (7)	0	0	0
Otitis media	1 (7)	0	1 (7)	0
Paraesthesia	1 (7)	0	1 (7)	0
Pericardial effusion	1 (7)	0	0	0
Petechiae	1 (7)	0	0	0
Pleural effusion	1 (7)	0	0	0
Pneumonia	1 (7)	0	0	0
Polydipsia	1 (7)	0	1 (7)	0
Presyncope	1 (7)	1 (7)	0	0
Proctalgia	1 (7)	0	0	0
Productive cough	1 (7)	0	0	0
Proteinuria	1 (7)	0	1 (7)	0
Pruritus	1 (7)	0	1 (7)	0
Pulmonary edema	1 (7)	1 (7)	1 (7)	1 (7)
Puncture site pain	1 (7)	0	0	0
Renal pain	1 (7)	0	1 (7)	0
Sinus tachycardia	1 (7)	0	0	0
Sinusitis	1 (7)	0	0	0
Skin infection	1 (7)	0	0	0
Thrombophlebitis	1 (7)	0	0	0
Toothache	1 (7)	0	0	0
Troponin increased	1 (7)	0	1 (7)	0
Troponin t increased	1 (7)	0	0	0
Vagus nerve disorder	1 (7)	0	1 (7)	0
Vertigo	1 (7)	0	0	0
Visual acuity reduced	1 (7)	0	0	0
Visual field defect	1 (7)	0	0	0
Vulvovaginal mycotic infection	1 (7)	0	0	0
Weight increased	1 (7)	0	1 (7)	0

Serious adverse event	All		Treatment-related	
	Any grade	≥ Grade 3	Any grade	≥ Grade 3
Preferred term MedDRA v14.0	*Number of patients (percent)*			
All serious adverse events	8 (53)	7 (47)	3 (20)	2 (13)
Abdominal pain	1 (7)	0	0	0
Acute graft versus host disease	1 (7)	1 (7)	1 (7)	1 (7)
Acute myeloid leukemia	1 (7)	1 (7)	0	0
Cerebral hemorrhage	1 (7)	1 (7)	0	0
Chest discomfort	1 (7)	0	0	0
Cytokine release syndrome	1 (7)	0	1 (7)	0
Febrile neutropenia	1 (7)	1 (7)	0	0
Gastrointestinal hemorrhage	1 (7)	1 (7)	0	0
Infection	1 (7)	1 (7)	0	0
Nausea	1 (7)	1 (7)	0	0
Presyncope	1 (7)	1 (7)	0	0
Pulmonary edema	1 (7)	1 (7)	1 (7)	1 (7)
Sinusitis	1 (7)	0	0	0
Syncope	1 (7)	1 (7)	0	0
Vomiting	1 (7)	1 (7)	0	0

Supplemental Table 2. Concomitant antimicrobial prophylaxis or therapy and steroids other than scheduled.

Patient ID	Medication	Application	Type	Start (day of treatment)	End (day of treatment)
UPN001					
	Sulfamethoxazol/Trimethoprim	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	3	10
	Linezolid	i.v.	Antimicrobial therapy	5	10
UPN002					
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Amphotericin B	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Ceftriaxone	p.o.	Prophylaxis	3	27
	Clarithromycin	i.v.	Antimicrobial therapy	20	27
UPN003					
	Sulfamethoxazol/Trimethoprim	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Amphotericin B	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Amoxicillin/Clavulanic acid	p.o.	Prophylaxis	Pre-treatment	Post-treatment
UPN004					
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Ciprofloxacin	p.o.	Prophylaxis	17	21
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	21	Post-treatment
UPN005					
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	44
	Levofloxacin	p.o.	Prophylaxis	3	44
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	9	13
UPN006					
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	34
	Metronidazole	p.o.	Antimicrobial therapy	Pre-treatment	0
	Meropenem	i.v.	Antimicrobial therapy	51	59
UPN007					
	Posaconazole	p.o.	Prophylaxis	Pre-treatment	Post-treatment

	Ciprofloxacin	p.o.	Prophylaxis	1	3
	Vancomycin	i.v.	Antimicrobial therapy	5	14
UPN008					
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Prevymis	p.o.	Prophylaxis	Pre-treatment	5
	Penicillin	p.o.	Antimicrobial therapy	12	Post-treatment
	Isavuconazol	p.o.	Antimicrobial therapy	14	Post-treatment
UPN009					
	Prednisolon	p.o.	Steroid	Pre-treatment	Post-treatment
	Amatadine	i.v.	Antimicrobial therapy	9	Post-treatment
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	16	Post-treatment
UPN010					
	Aciclovir	p.o.	Prophylaxis	Pre-treatment	Post-treatment
UPN011					
	Amphotericin B	p.o.	Prophylaxis	21	Post-treatment
	Aciclovir	p.o.	Prophylaxis	38	Post-treatment
	Amoxicillin/Clavulanic acid	p.o.	Prophylaxis	Pre-treatment	1
	Ciprofloxacin	p.o.	Prophylaxis	2	4
	Ciprofloxacin	p.o.	Prophylaxis	12	28
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	4	6
UPN012					
	Methylprednisolone	i.v./p.o.	Steroid	8	14
	Pentacarinate	inhaled	Prophylaxis	7	Post-treatment
	Amphotericin B	p.o.	Prophylaxis	10	Post-treatment
	Amoxicillin Clavulanic acid	p.o.	Prophylaxis	4	7
UPN901					
	Sulfamethoxazol/Trimethoprim	p.o.	Prophylaxis	Pre-treatment	16
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	8	15
UPN903					
	Amoxicillin Clavulanic acid	p.o.	Prophylaxis	15	24
	Amphotericin B	p.o.	Prophylaxis	Pre-treatment	Post-treatment

UPN909

	Isavuconazol	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Levofloxacin	p.o.	Prophylaxis	Pre-treatment	Post-treatment
	Piperacillin/Tazobactam	i.v.	Antimicrobial therapy	3	10

Supplemental Table 3. Disease characteristics and outcome of patients with an objective response.

	UPN009	**UPN909**	**UPN010**
Age, years	69	32	27
Sex	female	male	male
ECOG PS	0	1	0
ELN 2010 risk	adverse	intermediate	intermediate
WBC count, x 10^3/µL	1.77	0.7	1.6
PB blasts, %	0	10	0
BM blasts, %	10	90.5	0
Extramedullary AML	absent	absent	present*
Initial cytogenetics	39~44,XX,del(5)(q13q31), del(7)(q22), del(7)(q22), -17, del(18)(q21)	46,XY,del(9)(q13); FISH: partial Trisomy 11q23	46,XY,i(17)q10
Initial molecular genetics	No mutations detected	n.d.	Mutations in *NOTCH1*, *NRAS*, *PHF6*, *RUNX1*
Previous lines of therapy, no	2	4	5
Prior allogeneic HSCTs, no	1	1	2
Donor type in last HSCT	10/10 matched unrelated	9/10 matched unrelated	10/10 matched unrelated
Time since prior HSCT, days	130	427	860
Cohort	3	3	4
Time to response, days	60	27	24
Response duration, months	30.0 (ongoing)	0.5	7.4
Survival, months	32.0 (alive)	3.1	22.3

*see Figure 4