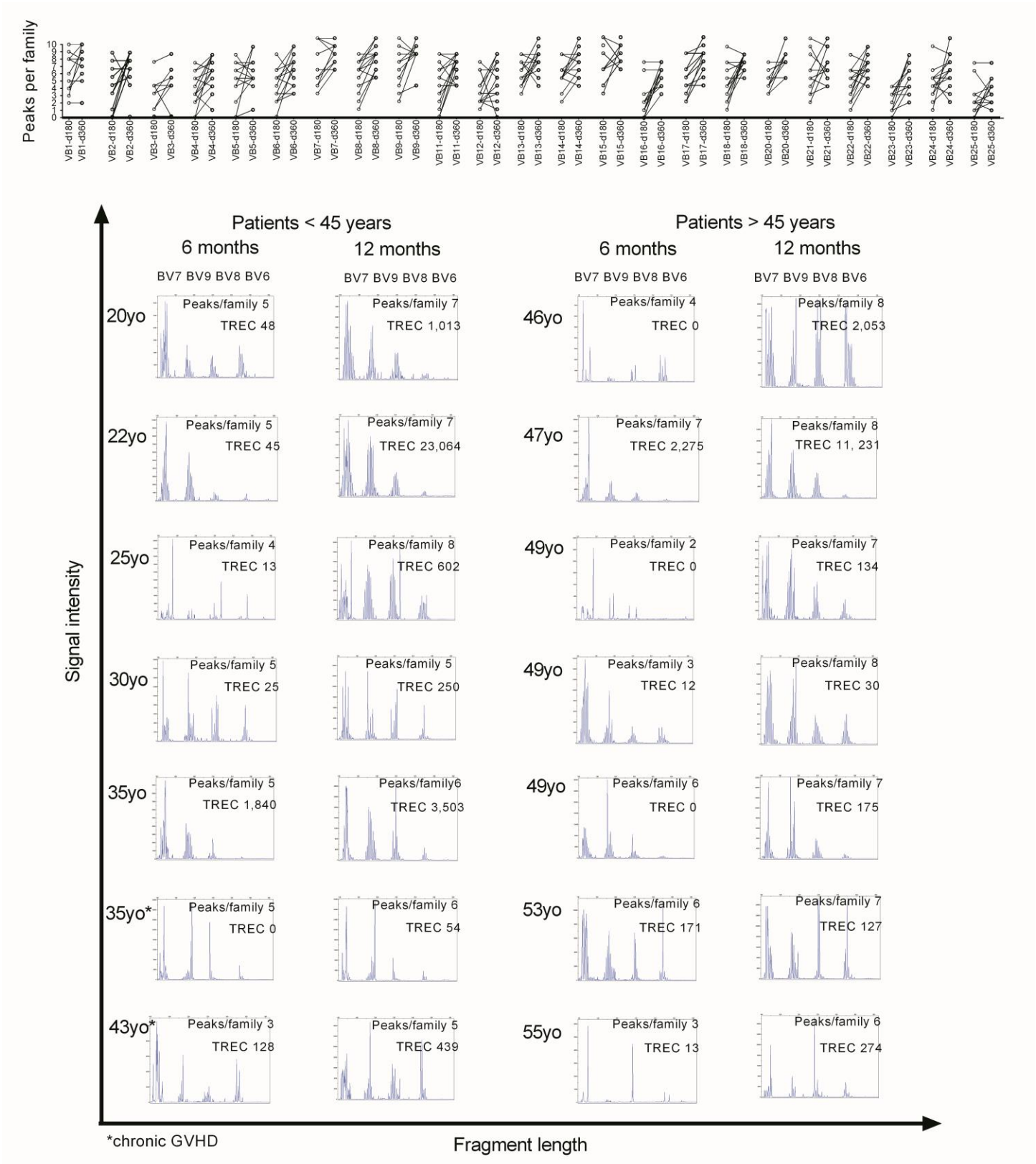
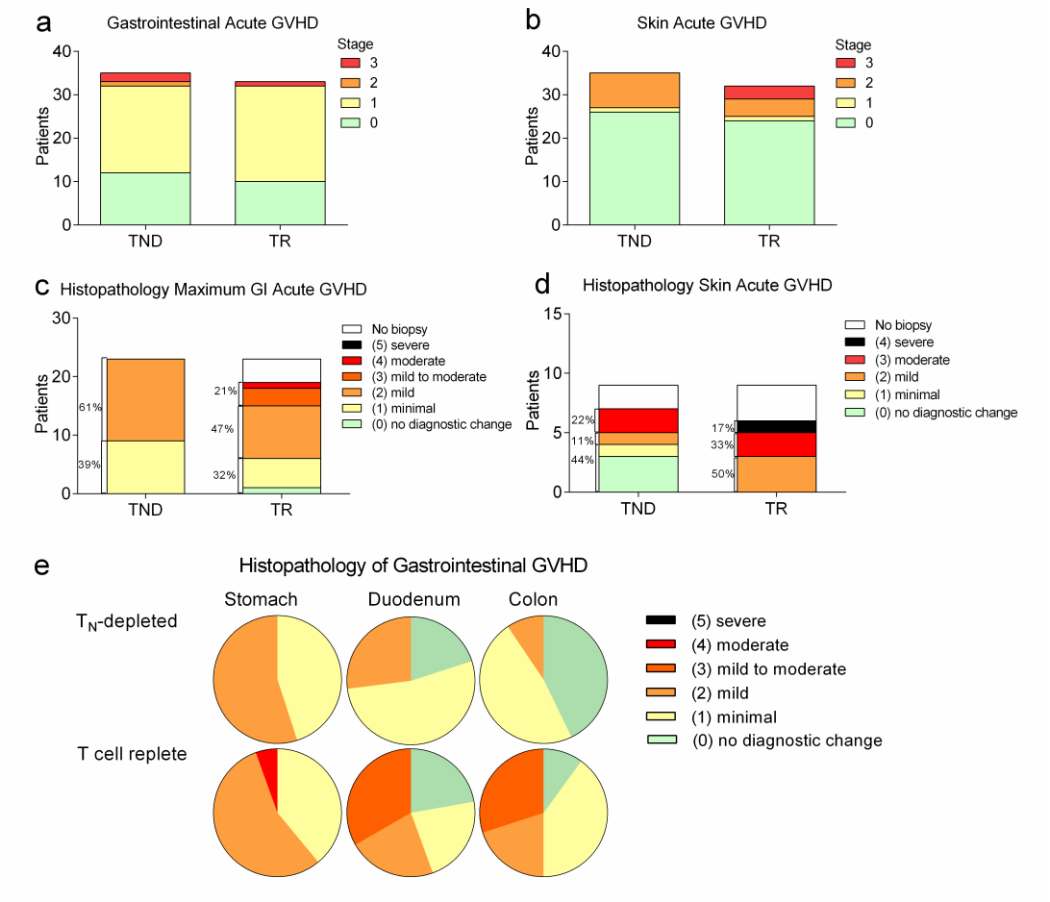
Supplementary Figure 1. V beta spectratyping



Supplementary Figure 1. V beta spectratyping. The top graph shows the number of peaks per Vβ family in the 23 Vβ families seen on spectratyping of cDNA derived from PBMC for 14 T_N-depleted HCT recipients at 6 and 12 months after HCT. The data presented in the lower section of the figure represents the spectratype in 4 representative Vβ families for 14 individuals at 6 and 12 months after T_N-depleted HCT. The information inset into each chart shows the median number of peaks/family across the Vβ families for each individual. TREC values at the same time point are presented immediately underneath and the patients age is listed along the left side.

Supplementary Figure 2. Acute GVHD in recipients of T_N-depleted PBSC and T cell-replete PBSC



Supplementary Figure 2. Acute GVHD in recipients of T_N-depleted PBSC and T cell-replete PBSC. The clinical severity of organ-specific aGVHD is compared between T_N-depleted (TND) and T cell-replete (TR) PBSC recipients: GI GVHD in panel (a) and skin GVHD in (b). The histological severity is compared in panels e-g: severity of GI GVHD at the site of maximal involvement (c); severity of skin GVHD (d)*; and histological severity of GVHD in the stomach, duodenum and colon (e). *Of note, the two T_N-depleted recipient classified as having moderate histological severity of skin GVHD had rashes and skin biospies performed early after HCT (days 19 and 20) and the histopathological features obsevered were also considered consistent with chemoradiotherapy conditioning.

Supplementary Table 1. Grade 3-5 non-hematologic adverse events up to day 100 in T_N-depleted HCT recipients. (National Cancer Institute Common Terminology Criteria for Adverse Events, V4.0)

Adverse event	Grade 3	Grade 4	Grade 5
Oral mucositis	31		
Anorexia	7		
Nausea	9		
Diarrhea	18		
C.Difficile infection	4		
CMV reactivation	18		
CMV GI tract	2		
Bacteremia	9	3	
Candidemia	1		
Catheter related infection	12		
Pulmonary infection (probably/possibly fungal)	4		
Sinusitis	1		
Bladder infection	2		
Acute kidney injury	2	1	
Liver transaminitis	1		
Elevated bilirubin (cholangitis lenta)	2		
Pulmonary (DAH/IPS)		1	1
Pulmonary -other respiratory failure		1	
Pulmonary-hypoxia from sedation/volume overload	2		
Laryngeal edema		1	
Pericarditis and pericardial effusion		1	
Prolonged QTc	1		
Hypertension	8	1	
Hypotension	4	1	
Hyperglycemia	12		
Hypophosphatemia	5		
Hyperphosphatemia	1		
Hyopokalemia	1		
Hypermagnesemia	2		
Hypocalcemia	1		
Hyponatremia	4		
Rash	5		
Pruritis	1		
Esophageal hemorrhage	1		
Menorrhagia	1		
Epistaxis	2		
Fatigue	2		
Weight loss	1		
Memory impairment	1		
Pain-abdominal	2		
Pain-headache	3		
Pain-musculoskeletal/extremity	3		
Pain-rectal	1		

Supplementary Table 2. Infectious complications up to day 100 in T_N-depleted HCT recipients.

Infection	Organism	N (%)
Bacteremia & CVL infections d0-d10 (pre-engraftment)	Streptococcus viridans[1]	6 (17)
	Vancomycin resistant enterococcus	2 (5.7)
	Gram negative (both Escherichia coli)	2 (5.7)
	Coagulase negative staphylococcus	7 (20)
	Other	2 (5.7)
Bacteremia & CVL infections d11-100 (post-engraftment)	Coagulase negative staphylococcus	5 (14.2)
	Rothia mucilaginosa (probable contaminant)	1 (2.9)
Urinary tract infection	Klebsiella oxytoca	1 (2.9)
Infectious diarrhea	Clostridium difficile	5 (14.2)
Respiratory virus	RSV, upper respiratory tract only	2 (5.7)
	Adenovirus, upper respiratory tract only	1 (2.9)
	Coronavirus, upper respiratory tract only	2 (5.7)
	Rhinovirus, upper respiratory tract only	2 (5.7)
	Parainfluenza, upper respiratory tract only	1 (2.9)
CMV reactivation	CMV >0 copies/ml	19 (54)
EBV reactivation[2]	EBV > 100 copies/ml	0 (0)
BK virus	BK viruria, mild & self-resolving	7 (20)
HSV	Herpes stomatitis	1 (2.9)
Fungal infections[3]	Probable pulmonary aspergillosis	1 (2.9)
	Possible pulmonary invasive fungal disease	3 (8.6)
	Candida glabrata in blood and lung	1 (2.9)

[1]Streptococcus viridans bacteremia was observed frequently among the first patients treated on the protocol, probably due to breaches in the oral mucosa due to conditioning-related mucositis[1,2] . However, the introduction of gram-positive organism antibiotic prophylaxis (penicillin and/or vancomycin, or ceftriaxone) effectively prevented streptococcus viridans bacteremia. Gram negative bacteremia occurred infrequently (5.7%) during the neutropenia pre-engraftment period and was not more frequent than previously reported after allogeneic HCT[3].

[2]One patient had one single test with a minimal EBV reactivation (41 copies/ml).

[3] Fungal infections: Four patients with a history of pulmonary fungal nodules pretransplant had manifestations of possible (3) or probable (1) recurrent or new fungal nodules in the first 100 days after HCT and changed anti-fungal therapy with no subsequent progression of nodules. One patient who required high-dose steroid for IPS/DAH developed candida glabrata in the lungs and blood stream.

Supplementary Table 3. Quantititave lymphocyte recovery. Comparison of observed values (median) for T_N-depleted recipients with previously reported values for T cell-replete and T cell depleted (TCD) HCT recipients

		TCD PBSCT*	T_N-depleted PBSCT	T cell-replete BMT**	T cell-replete PBSCT**
$CD8^+$ T cells normal range=250-1250	Day 28	12	177	46	84
	Day 80-100	97	129	59	134
	Day 180-200	113	278	258	605
	Day 360	258	485	233	440
$CD4^+$ T cells normal range=733-2250	Day 28	6	109	76	186
	Day 80-100	88	88	71	163
	Day 180-200	92	181	135	339
	Day 360	250	340	185	432
$CD19^+$ B cells normal range=160-690	Day 28	1	0	0.8	5.4
	Day 80-100	188	6	3.7	9.9
	Day 180-200	181	100	51	65
	Day 360	358	249	205	248
$CD56^+CD16^+CD3^-$ NK cells normal range=100-700	Day 28	384	257	131	250
	Day 80-100	269	182	115	163
	Day 180-200	181	225	147	160
	Day 360	157	161	200	184

* Devine. et all BBMT 2011 and **Storek et al. Blood 2001, and personal communication Drs. Jan Storek and Steven Devine and the BMT CTN 0303 study team.[4-6]

Supplementary Table 4. Clinical characteristics of T_N-depleted PBSCT recipients and concurrent T cell replete HCT comparison group.

Variable	T_N-depleted PBSCT (N=35)	T cell-replete PBSCT (N=33)
Recipient age		
Median (years)	37	38
Ranges (years)	19-55	17-55
Recipient sex, N (%)		
Male	14 (40)	23(70)
Female	21 (60)	10 (30)
Diagnosis, N (%)		
Acute lymphocytic leukemia	19 (54)	18 (55)
Mixed linage leukemia	2 (6)	0 (0)
Acute myeloid leukemia	10 (29)	9 (27)
Myelodysplastic syndrome	3 (9)	0 (0)
Chronic myeloid leukemia (lymphoid blast crisis)	1 (3)	6 (18)
Disease stage, N (%)		
Better risk		
CR1, MRD negative	16 (46)	13 (39)
Poor risk		
CR1, MRD positive	8 (22)	7 (21)
CR2/3, MRD negative	6 (17)	5 (15)
CR2/3, MRD positive	3 (9)	2 (6)
Relapse/refractory	2 (6)	6 (18)
Donor age		
Median (years)	37	39
Ranges (years)	17-57	17-57
Donor-recipient gender disparity, N (%)		
Female donor, male patient	5 (14)	10 (30)
Other combination	30 (85)	23 (70)
CMV status, N (%)		
Recipient or donor positive	26 (74)	23 (70)
Recipient and donor negative	9 (26)	10 (30)
Transplant date	12/2009-6/2014	04/2008-03/2014

Supplementary methods

Antimicrobial prophylaxis

Antimicrobial prophylaxis consisted of fluconazole or a mold-active azole from the start of conditioning until day 75 post-HCT, acyclovir from the start of conditioning to day 100 or 365 depending on patient varicella zoster virus (VZV) and herpes simplex virus (HSV) serostatus, levofloxacin from the onset of neutropenia (ANC <500/µl) until neutrophil engraftment, and in patients 14-35, penicillin and/or vancomycin or ceftriaxone for prophylaxis of gram-positive organisms (in particular streptococcus viridans) from day -2 to day 10 after HCT. Trimethoprim-sulfamethoxazole was given for pneumocystis jiroveci (PJP) prophylaxis preHCT until day -2 and re-starting around day 30 after HCT until at least 6 months after HCT.

Infection monitoring and preemptive management

Blood cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) surveillance was performed weekly with quantitative polymerase chain reaction (PCR) beginning pretransplant and through at least day 100. Patients with CMV PCR copy numbers of > 100 copies/ml were treated with ganciclovir or foscarnet according to standard institutional practice.

Definition of infections

Viral, Bacterial, and Fungal Infections through Day 100. CMV infection was defined as the presence of detectable viral DNA in plasma; CMV disease was defined as a dysfunction of an organ infected by CMV. Bacteremia was defined as one or more positive blood cultures for gram-positive or gram-negative organisms, unless the positive blood culture was drawn through central venous catheter and the organism typically colonizes catheters (such as coagulase negative staphylococcus), in which case the infection was classified as a central line infection. Invasive fungal infections were classified according to international consensus criteria.[7] Possible, probable or proven fungal infections were included in this analysis.

Chimerism evaluation

Hematopoietic chimerism was determined on peripheral blood samples obtained on days 28, 56, 80, 180 and at 1-year post-transplant and sorted for $CD3^+$ T cells and $CD33^+$ myeloid cells. The level of donor and recipient chimerism was determined by DNA genotyping for short tandem repeat (STR) polymorphisms using multiplex amplification of STR loci (PowerPlex 16; Promega, Madison, WI, USA) followed by capillary electrophoresis (ABI 3130 x 1; Applied Biosystems, Foster city, CA, USA).

Minimal residual disease evaluation

Multiparameter flow cytometry was performed on bone marrow samples (pretransplant, days 28, 56, 80 and 360) to determine the presence of residual leukemia as previously described.[8,9] Molecular testing by PCR was also performed to evaluate minimal residual disease in patients with established informative markers.

Supplementary methods continued

Acute GVHD Staging and Grading Assessment[10,11]

Severity of Individual Organ Involvement		
Skin	+1	a maculopapular eruption involving less than 25% of the body surface
	+2	a maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma involving >50% of the body surface
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	bilirubin (2.0-2.9 mg/100ml)
	+2	bilirubin (3-5.9mg/100ml)
	+3	bilirubin (6-14.9mg/100ml)
	+4	bilirubin > 15mg/100ml
Gut	Diarrhea is graded +1 to +4 in severity. Nausea and vomiting and/or anorexia caused by GVHD is assigned as +1 in severity. The severity of gut involvement is assigned to the most severe involvement noted. Patients with visible bloody diarrhea are at least stage +2 gut and grade +3 overall	
Diarrhea	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)†

**In the absence of infectious/medical cause*
†For pediatric patients

Severity of GVHD	
Grade I	+1 to +2 skin rash
	No gut or liver involvement
Grade II	+1 to +3 skin rash and/or
	+1 gastrointestinal involvement and/or +1 liver involvement
Grade III	+4 skin involvement and/or
	+2 to +4 gastrointestinal involvement and/or
	+2 to +4 liver involvement with or without a rash
Grade IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death

Supplementary methods continued.

Acute GVHD Histopathological Scale

GI tract

Numerical grading	Description	Features
0	No diagnostic alteration	
1	GVHD of minimal histologic activity, borderline GVHD	Rare apoptotic cells: 3-6 apoptotic epithelial cells in basal crypts per 10 sections.
2	GVHD of mild histologic activity	Occasional apoptotic epithelial cells: 1-3 per tissue section.
3	GVHD of mild to moderate histologic activity	Occasional to frequent apoptotic epithelial cells: 3 or more individual apoptotic cells per tissue section.
4	GVHD of moderate histologic activity	Occasional to frequent apoptotic epithelial cells cells with focal glandular destruction.
5	GVHD of severe histologic activity	Frequent apoptotic cells with diffuse glandular destruction, ulceration and exploding crypts.

Skin

Numerical grading	Description	Features
0	No diagnostic alteration or Non-specific inflammation	Perivascular inflammation and spongiosis without apoptotic keratinocytes
1	GVHD of minimal histologic activity, borderline GVHD	Rare individual apoptotic keratinocytes 1-2/section without significant spongiosis or basal vacuolization
2	GVHD of mild histologic activity	Spongiosis, basal vacuolization with rare individual apoptotic keratinocytes 1-3 per section
3	GVHD of moderate histologic activity	Spongiosis, basal vacuolization with occasional individual apoptotic keratinocytes 3-10 per section
4	GVHD of severe histologic activity	Spongiosis, lymphocytic exocytosis with frequent apoptotic keratinocytes, including vesiculation and desquamation in Steven Johnson/TEN pattern.

Supplementary methods continued.

Definition and Classification of Chronic GVHD.

Diagnosis of chronic GVHD requires the presence of at least 1 diagnostic clinical sign of chronic GVHD or the presence of at least 1 distinctive manifestation confirmed by pertinent biopsy or other relevant test in the same or other organ. Other possible diagnoses for clinical symptoms must be excluded. There is no time limit set for the diagnosis of chronic GVHD. Chronic GVHD includes classic chronic GVHD (without features or characteristics of acute GVHD) and an overlap syndrome in which diagnostic or distinctive features of chronic GVHD and acute GVHD appear together.

The criteria for the diagnosis of chronic GVHD thus requires:

i. Distinction from acute GVHD
ii. Presence of at least one diagnostic clinical manifestation OR at least one distinct manifestation confirmed by pertinent biopsy or other relevant tests (Table 1)
iii. Exclusion of other possible diagnosis for the clinical manifestations (e.g. drug effect, infection, other)

The severity of chronic GVHD is scored using a standard established scoring system (Table 2a & b).[12] Mild chronic GVHD involves only 1 or 2 organs or sites (except the lungs), with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). Moderate chronic GVHD involves (1) at least 1 organ or site with clinically significant involvement but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 is considered moderate chronic GVHD. Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater is considered severe chronic GVHD.

Supplementary methods continued.

Chronic GVHD Table 1. Classification of manifestations for the clinical diagnosis of chronic GVHD

ORGAN OR SITE	DIAGNOSTIC	DISTINCTIVE	COMMON
	(sufficient to establish a chronic GVHD diagnosis)	(insufficient alone to establish a chronic GVHD diagnosis)	(seen with both acute and chronic GVHD)
Skin	Poikiloderma Lichen planus-like Sclerotic Morphea-like Lichen sclerosis-like	Depigmentation	Erythema Maculopapular Pruritus
Nails		Dystrophy Longitudinal ridging, splitting or brittleness Onycholysis Pterygium ungus Nail loss (usually symmetric and affects most nails)	
Scalp and body hair		New alopecia not due to chemoradiotherapy Scaling, papulosquamous lesions	
Mouth	Lichenoid Hyperkeratotic plaques Diminished oral cavity opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseuodmembranes* Ulcers*	Gingivitis Mucositis Erythema Pain
Eyes		New onset of sicca (dryness, gritty or painful**) Cicatricial conjunctivitis Keratoconjunctivitis sicca** Confluent areas of punctate keratopathy	
Genitalia	Lichen planus-like Vaginal scarring / stenosis	Erosions* Fissures* Ulcers*	
GI tract	Esophageal web Esophageal strictures or stenosis in upper to mid third*		Anorexia, nausea, vomiting, diarrhea Failure to thrive / weight loss
Liver***			Bilirubin > 2x ULN* Alkaline phosphatase > 2x ULN* AST or ALT >2x ULN*
Lung	Bronchiolitis obliterans)## (biopsy confirmed)	Bronchiolitis obliterans)## (based on PFTs and CT scan imaging)	Cryptogenic organizing pneumonia (COP/BOOP)
Muscles, fascia, joints	Fasciitis Joint stiffness or sclerotic contractures	Myositis or polymyositis**	
MANIFESTATIONS ACKNOWLEDGED AS CHRONIC GVHD IF THE DIAGNOSIS IS ALREADY CONFIRMED			
Skin	Sweat impairment, ichthyosis, keratosis pilaris, hypopigmentation, hyperpigmentation		
Hair	Thinning scalp hair not otherwise explained (typically patchy, coarse or dull), premature graying		
Eyes	Photophobia, periorbital hyperpigmentation, blepharitis (eyelid erythema with edema)		
GI tract	Exocrine pancreatic insufficiency		
Muscles / Joints	Edema, muscle cramps, arthralgia or arthritis		
Hematology	Thrombocytopenia, eosinophilia, lymphopenia		
Immune	Hypo-or hyper- gammaglobulinemia, autoantibodies (AIHA, ITP)		
Other	Ascites, pericardial or pleural effusions, peripheral neuropathy, nephrotic syndrome, myasthenia gravis, cardiac conduction abnormality, cardiomyopathy		

AIHA, autoimmune hemolytic anemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BOOP, bronchiolitis obliterans with organizing pneumonia; COP, cryptogenic organizing pneumonia; CT, computerized tomography; ITP, immune thrombocytopenia; PFTs, pulmonary function tests; ULN, upper limit or normal

*In all cases, infection, drug effects, malignancy, and other cause must be excluded.

**Diagnosis of cGVHD requires biopsy or radiographic confirmation (or Schirmer test or slit lamp examination for eyes)

***Because liver histology in acute and chronic GVHD is not distinguishable the diagnosis of cGVHD cannot be made on the basis of biopsy alone and requires a distinctive manifestation in at least one other organ system.

Criteria for diagnosing bronchiolitis obliterans: Forced expiratory volume in 1 second/forced (or slow) vital capacity ratio <0.7 **and** forced expiratory volume in 1 second <75% of predicted **and** evidence of air trapping or small airway thickening or bronchiectasis on high-resolution chest computed tomography (with inspiratory and expiratory images) **or** residual volume >120%

Chronic GVHD Severity Table 2a. Global assessment of chronic GVHD severity

Global severity	No. organs/sites affected	Maximum score in all affected organ/site
Mild	One or two (except lungs *)	1*
Moderate	Three or more Or one or more	1* 2*
Severe	Any	3

*A lung score of 1 is considered moderate and a lung score of 2 is considered severe.

Chronic GVHD Severity Table 2b. Assessment of chronic GVHD severity

	Score 0	Score 1	Score 2	Score 3
Performance score	Asymptomatic and fully active (ECOG 0, KPS or LPS 100%)	Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	Symptomatic, limited self care, >50% waking hours in bed (ECOG 3-4, KPS or LPS <60%)
Skin Clinical features Maculopapular rash Lichen planus-like features Papulosquamous lesions or ichthyosis Hyperpigmentation Hypopigmentation Keratosis pilaris Erythema Erythroderma Poikiloderma Sclerotic features Pruritus Hair involvement Nail involvement	No symptoms	< 18% BSA with disease signs but NO sclerotic features	19-50% BSA OR involvement with superficial sclerotic features 'not hidebound' (able to pinch).	>50% BSA OR deep sclerotic features 'hidebound' (unable to pinch) OR impaired mobility, ulceration or severe pruiritis.
Mouth	No symptoms	Mild symptoms with disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs on examination **with** major limitation of oral intake
Eyes	No symptoms	Mild dry eye symptoms not affecting ADL (requiring eyedrops < 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctual plugs), W**ITHOUT** vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI tract	No symptoms	Symptoms such as nausea, vomiting,anorexia, dysphagia, abdominal pain or diarrhea without significant weight loss (<5%)	Symptoms associated with mild to moderate weight loss (5-15%)	Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
Liver	Normal LFT	Elevated Bilirubin, AP*, AST or ALT <2 x ULN	Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	Bilirubin or enzymes > 5 x ULN
Lungs	No symptoms	Mild symptoms (shortness of breath after climbing one flight of steps)	Moderate symptoms (shortness of breath after walking on flat ground)	Severe symptoms (shortness of breath at rest; requiring 02)
Joints and Fascia	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
Genital tract	No symptoms	Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); AST (aspartate aminotransferase); ULN (upper limit of normal); LFS (Lung Function Score); N/A (not applicable).

Supplementary Methods Reagents List (Antibodies and Immunomagnetic Beads)

Reagent type	Reagent	Manufacturer	Catalogue number/details
Immunomagnetic beads (Naïve T cell depletion trial)	CD45RA beads	Miltenyi Biotec	CliniMACs CD45RA microbeads (αCD45RA antibody (T6D11) conjugated to Miltenyi iron dextran beads). Now available (under IND/IDE) as catalogue number 701-46/130-020-003.
	CD34 beads	Miltenyi Biotec	CliniMACS CD34 microbeads. Catalogue 171-01/130-017-501.
Immunomagnetic beads ($CD34^+$ selection for T cell depletion in CTN 0303)	CD34 beads	Miltenyi Biotec	CliniMACS CD34 microbeads. Catalogue 171-01/130-017-501.
Antibodies for lymphocyte enumeration (Figure 3)	CD3 APC	BD Biosciences	Cat# 340661
	CD4 A594	BD Custom	Mat# 339403
	CD8a BV421	Biolegend	Cat# 301036
	CD3 PC5 CD4 ED1, CD8 ECD, CD45 FITC	Beckman coulter	Cyto-stat TetraChrome cocktail 1. Cat# 6607013
	CD3 PC5 CD56 RD1, CD19 ECD, CD45 FITC	Beckman coulter	Cyto-stat TetraChrome cocktail 2. Cat# 6607073
	CD4 FITC CD25 PC7 CD127 A647	BD Biosciences	Human Regulatory T cell Cocktails Cat# 560249
	CD45RA PE-Cy7	BD Biosciences	Cat# 337167
	CD45RO PE	Beckman Coulter	Cat# IM1307U
	CD62L FITC	Beckman Coulter	Cat# IM1231U
Antibodies for antigen-specific T cell evaluation (Figure 4)	iTag MHC pp65 NLVPMVATV tetramer PE	Beckman Coulter	Cat# T20100
	CD3 APC-Cy7	BD Biosciences	Cat# 557832
	CD4 AF700	BD Biosciences	Cat# 557922
	CD8 PERCP	BD Biosciences	Cat# 347314
	CD27 APC	BD Biosciences	Cat# 558664
	CD28 ECD	Beckman Coulter	Cat# 6607111
	Interferon γ FITC	BD Biosciences	Cat# 340449
	IL-2 APC	BD Biosciences	Cat# 554567
	CD28/CD49 co-stimulatory reagent	BD Biosciences	Cat# 347690

References Supplementary Material

1. Engelhard, D.*, et al.* Cytosine arabinoside as a major risk factor for Streptococcus viridans septicemia following bone marrow transplantation: a 5-year prospective study. *Bone Marrow Transplant* **16**, 565-570 (1995).
2. Blennow, O., Ljungman, P., Sparrelid, E., Mattsson, J. & Remberger, M. Incidence, risk factors, and outcome of bloodstream infections during the pre-engraftment phase in 521 allogeneic hematopoietic stem cell transplantations. *Transpl Infect Dis* **16**, 106-114 (2014).
3. Chien, J.W., Boeckh, M.J., Hansen, J.A. & Clark, J.G. Lipopolysaccharide binding protein promoter variants influence the risk for Gram-negative bacteremia and mortality after allogeneic hematopoietic cell transplantation. *Blood* **111**, 2462-2469 (2008).
4. Kanda, J.*, et al.* Immune recovery in adult patients after myeloablative dual umbilical cord blood, matched sibling, and matched unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant* **18**, 1664-1676 e1661 (2012).
5. Storek, J.*, et al.* Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. *Blood* **97**, 3380-3389 (2001).
6. Devine, S.M.*, et al.* Low risk of chronic graft-versus-host disease and relapse associated with T cell-depleted peripheral blood stem cell transplantation for acute myelogenous leukemia in first remission: results of the blood and marrow transplant clinical trials network protocol 0303. *Biol Blood Marrow Transplant* **17**, 1343-1351 (2011).
7. Ascioglu, S.*, et al.* Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Nichols, W Garrett Guthrie, Katherine A Corey, Lawrence Boeckh, Michael* **34**, 7-14 (2002).
8. Walter, R.B.*, et al.* Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. *J Clin Oncol* **29**, 1190-1197 (2011).
9. Wood, B.L. Flow cytometric monitoring of residual disease in acute leukemia. *Methods Mol Biol* **999**, 123-136 (2013).
10. Glucksberg, H.*, et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* **18**, 295-304 (1974).
11. Przepiorka, D.*, et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* **15**, 825-828 (1995).
12. Filipovich, A.H.*, et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* **11**, 945-956 (2005).