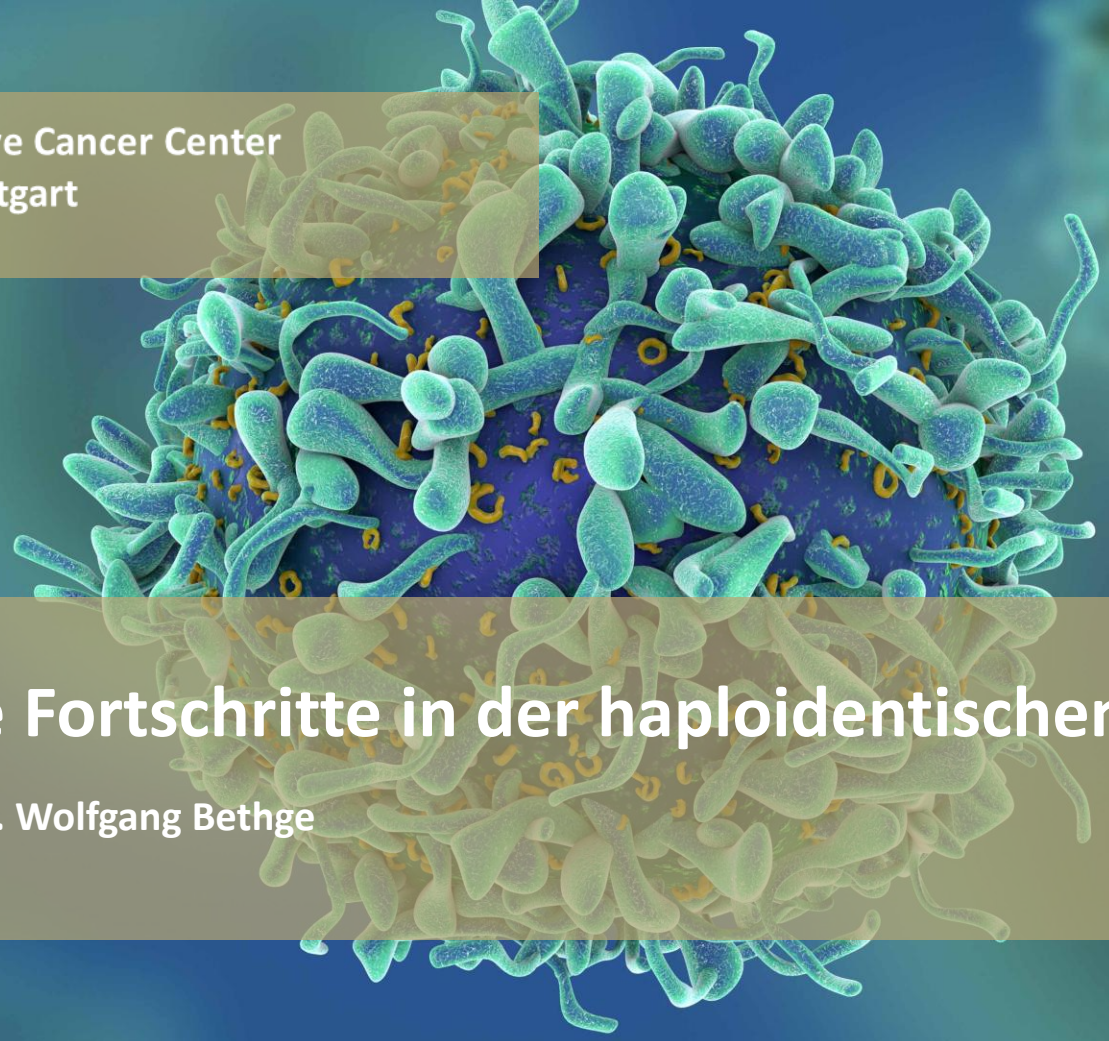
Comprehensive Cancer Center
Tübingen-Stuttgart



Aktuelle Fortschritte in der haploidentischen Stammzelltransplantation

Prof. Dr. med. Wolfgang Bethge

28. Juni 2025

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Cancer Center
Tübingen - Stuttgart

Disclosure of conflicts of interest

1. **Employment or Leadership Position**
   None

2. **Advisory Role or Expert Testimony**
   None

3. **Stock Ownership**
   None

4. **Patent, Copyright, Licensing**
   None

5. **Honoraria**
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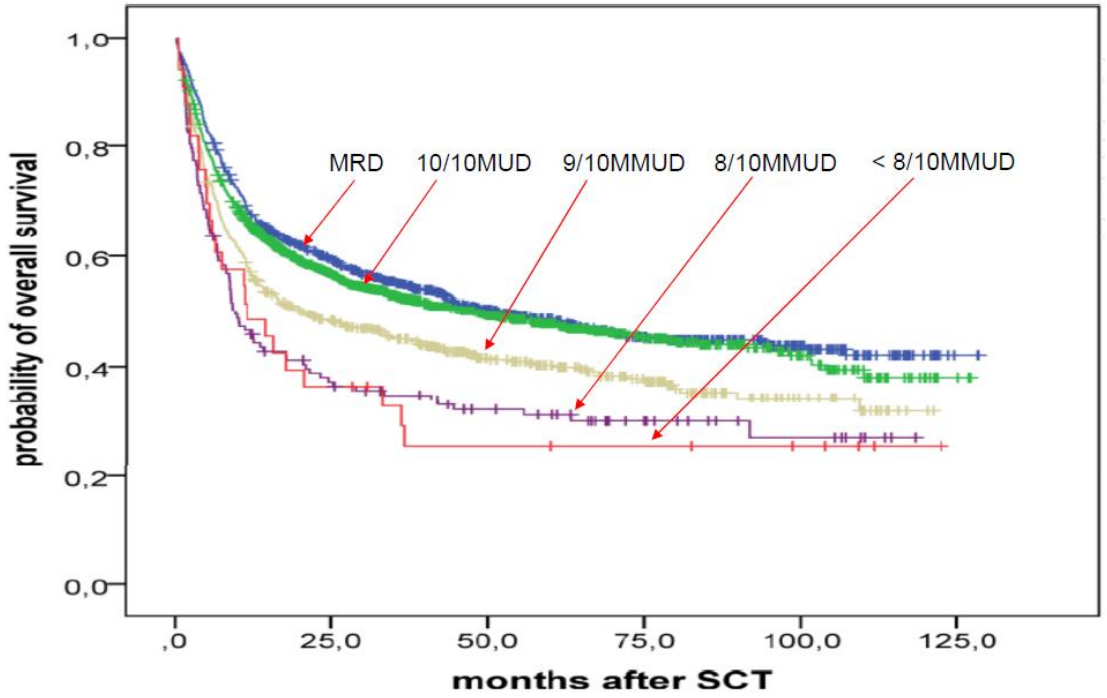
6. **Financing of Scientific Research**
   Miltenyi Biotec GmbH

7. **Other Financial Relationships**
   None

8. **Immaterial Conflicts of Interest**
   None

The fully matched donor

- Chances for 10/10 matched family donor ~25%
- Chances for 10/10 unrelated donor ~70-80%
- In ethnical minorities: ~10-25%
- Every mismatch increases complications:



Ayuk,…Bethge; BBMT 2018

Why Haploidentical Donor HCT ?

- Time matters: in case of relapse or refractory disease
- Only about 50% of HCT candidates in fact receive allogeneic HCT
- Increase graft-versus-tumor/leukemia effects
- Haploidentical donor readily accessible for post-HCT

  DLI, virus specific T-cells, donor CAR-T-cells etc.

Change for donor including alternative donors:

- Including HLA-mismatched 9/10 donor up to 90%
- Including haploidentical donor up to 95%
- Including cord blood virtually 100%

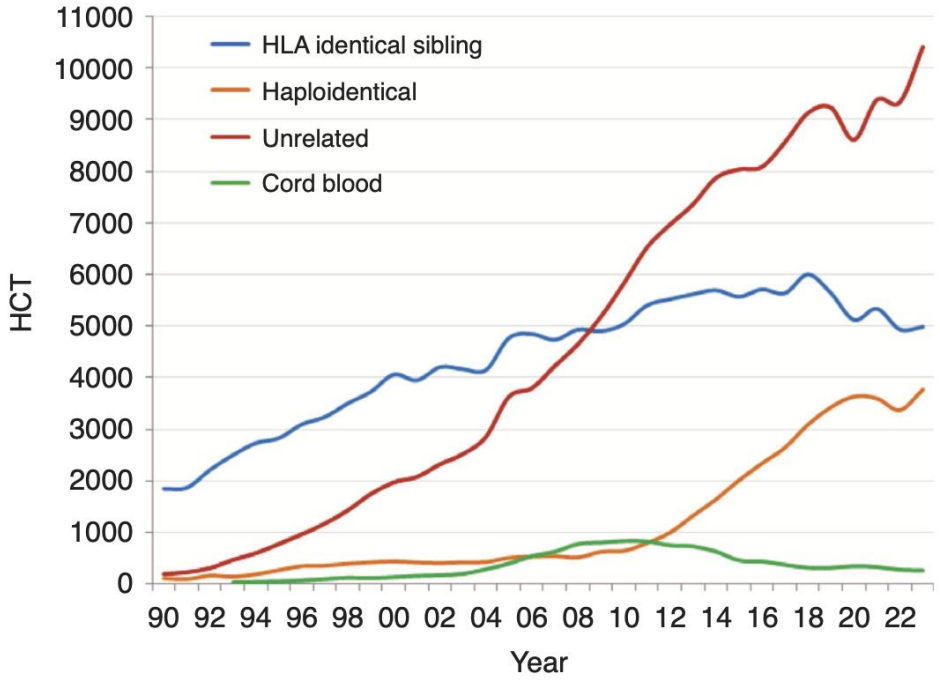
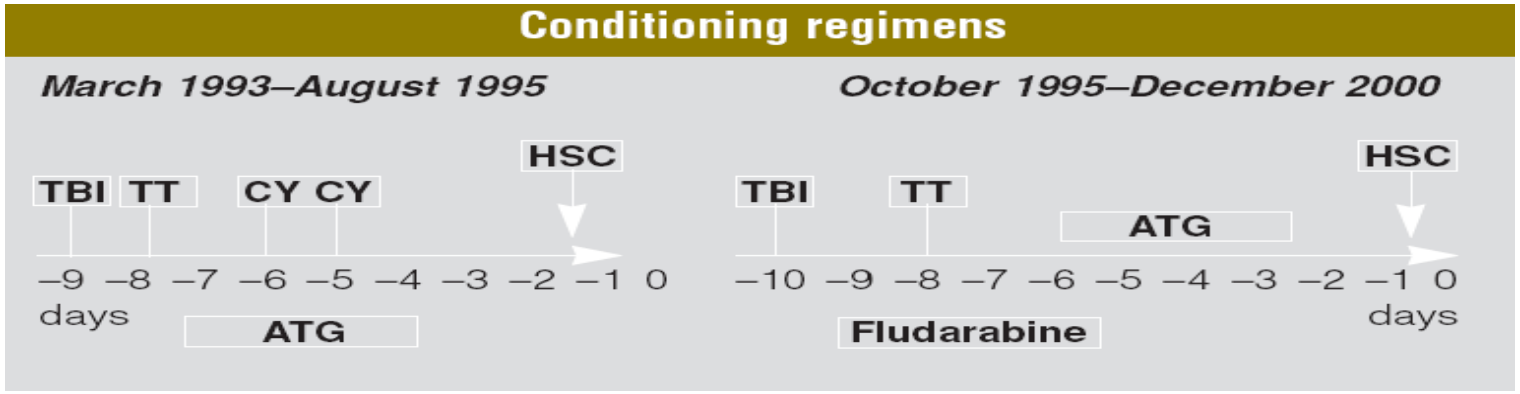


Fig. 4 Change in type of donor for first allogeneic HCT from 1990 to 2023.

Passweg et al. Bone Marrow Transplantation (2025) 60:519–528

Experiences Perugia

n=255 ALL=108 AML=147 Median Age=29 (range, 2-63)



TBI: 8 Gy Cy: 50 mg/kg/d Flu: 40 mg/m^2/d TT:10 mg/kg

Graft: Median CD34+/kg≥11.3x10^6

Median CD3+/kg=1x10^4

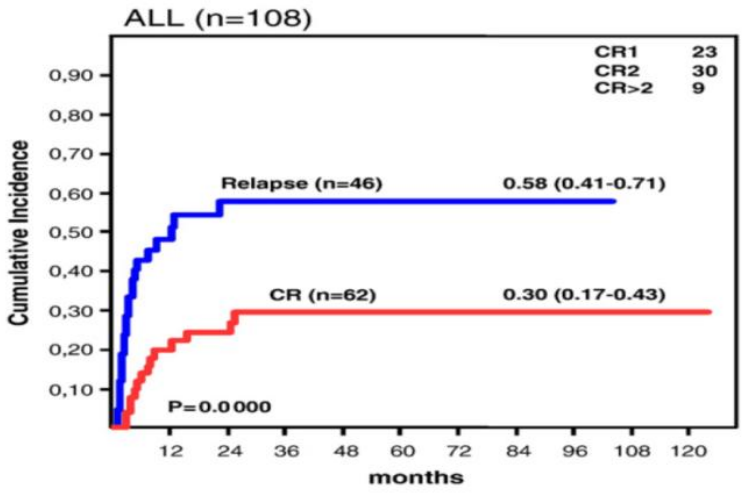
Primary engraftment: 95%

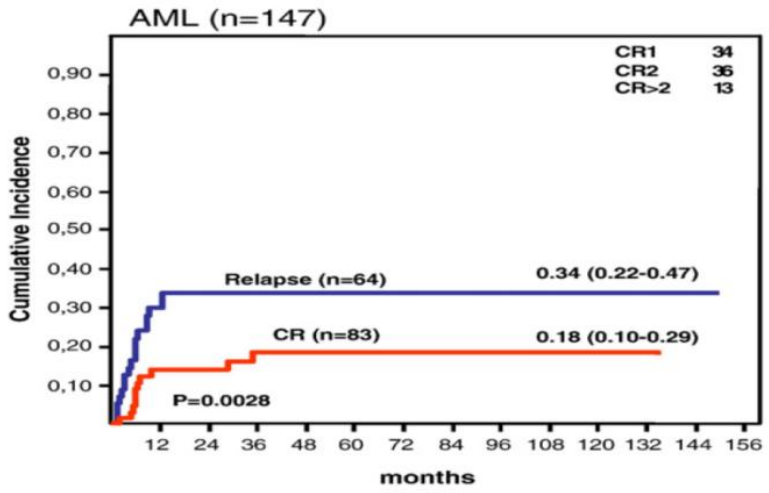
Aversa et al. Blood Cell Mol Dis, 40 (2008)

Outcome: aGVHD 8%
cGVHD 7%
TRM 41% (Infections)

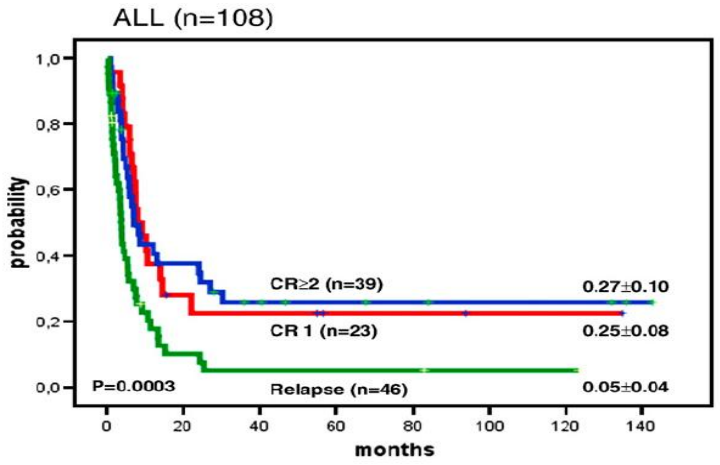
Experiences Perugia

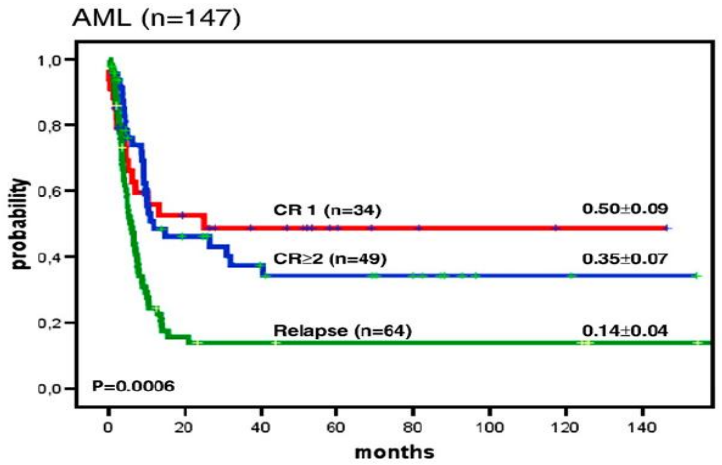
Relapse



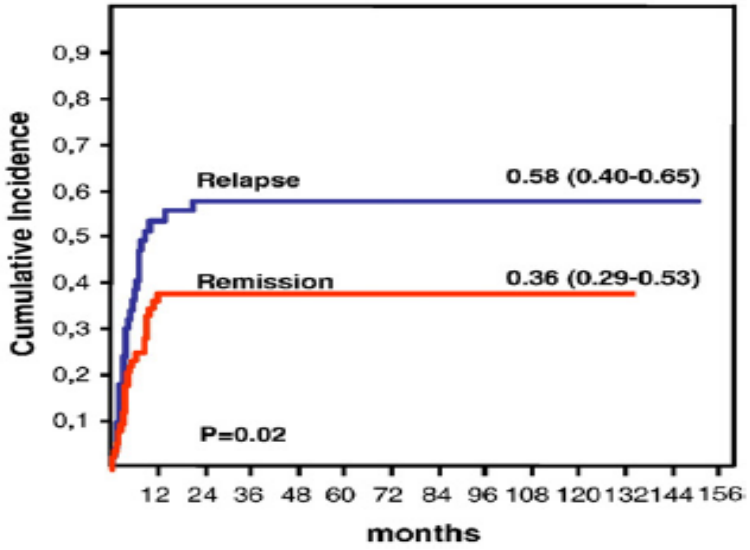


EFS





NRM



Aversa et al. Blood Cell Mol Dis, 40 (2008)

Problems of CD34 Selection

- Very intensive conditioning leading to significant toxicity especially in heavily pretreated and comorbid patients
- Megadose of CD34+ stem cells not easily achievable in adults
- Slow engraftment (PLTs) if graft contains $<8x10^6$ CD34+ cells/kg
- Slow and incomplete immune reconstitution
- High rate of infectious complications

Current Strategies for Haplo HCT

T-cell replete:

- Post-transplantation cyclophosphamide
- Intensive immunsuppression

  e.g. with CSA, MMF, mTOR inhibitors, ATG, Basiliximab, MTX, MabCampath…

With T-cell depletion:

- CD34 selection and myeloablative conditioning
- CD3/CD19 depletion and RIC
- αβTCR/CD19 depletion and RIC
  - No immunsuppression post HCT!
  - additional adoptive T-cell Tx post HCT if needed

T-replete Haplo HCT

1. Baltimore Approach:

NMA/MA

Post HCT Cyclophosphamide

BM

Post-HCT Immunosuppression:

TAC, MMF

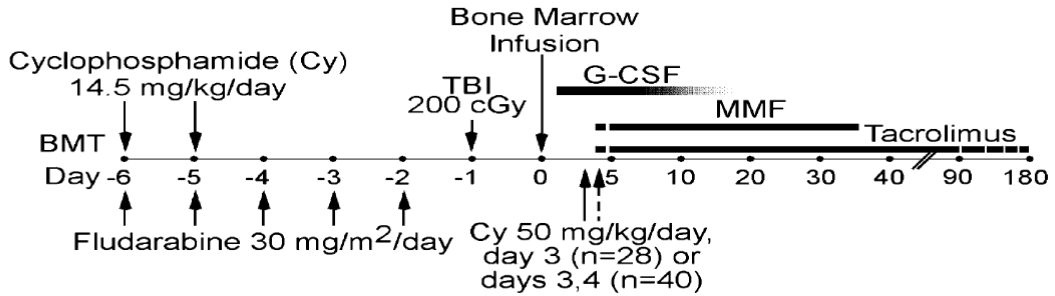


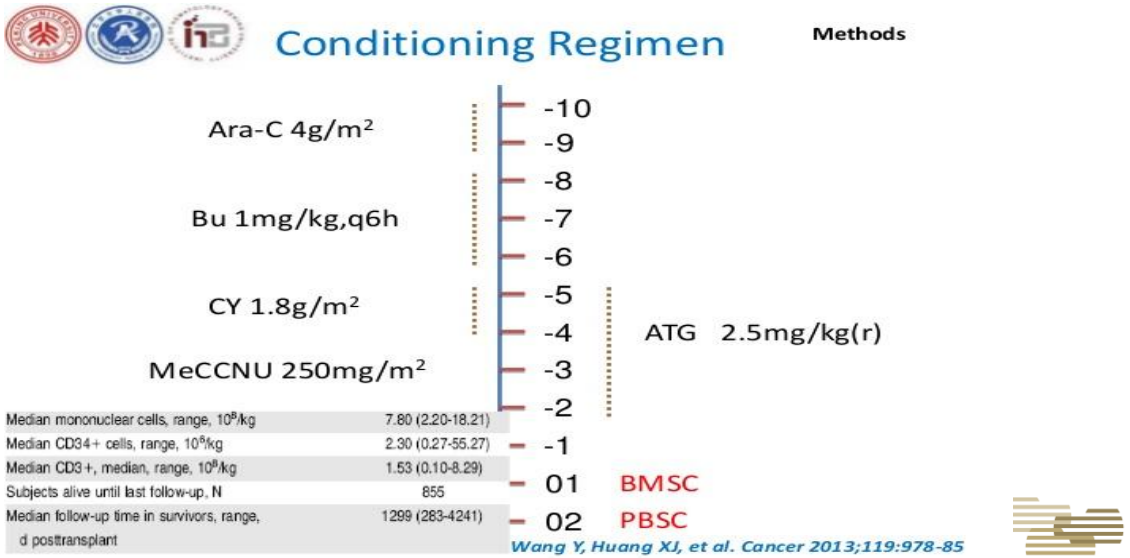
Figure 1. Nonmyeloablative haploidentical BMT conditioning and postgrafting immunosuppresive regimen.

Luznik et al., BBMT 2008

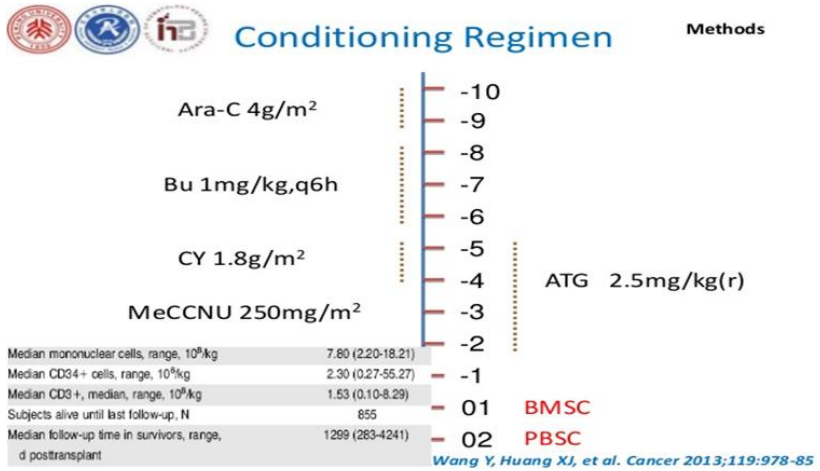
2. Peking Approach:

Myeloablative, G-CSF stimulated BM and PBSC

Post-HCT Immunosupression: ATG, CSA, MMF, MTX



T-replete Haplo HCT: Peking Approach



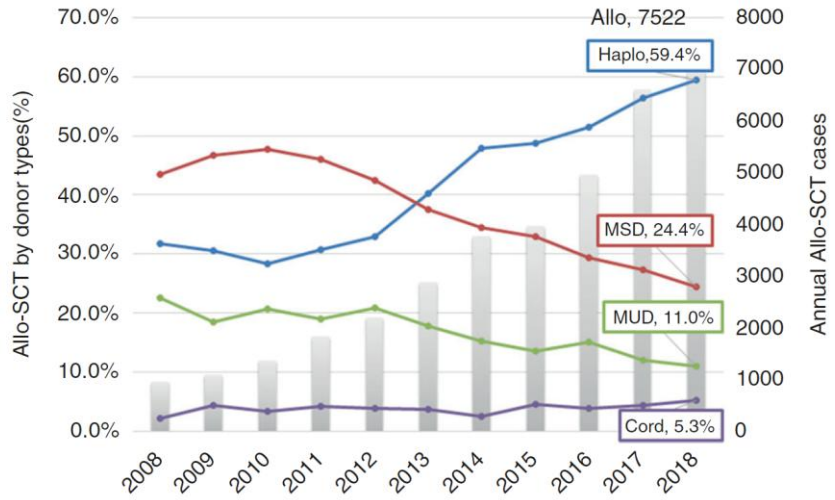
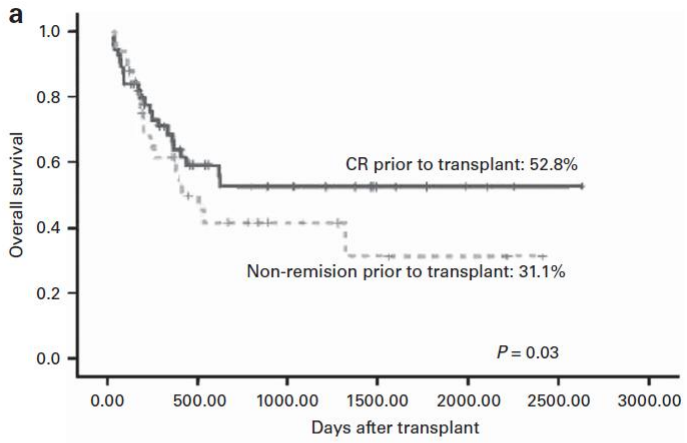
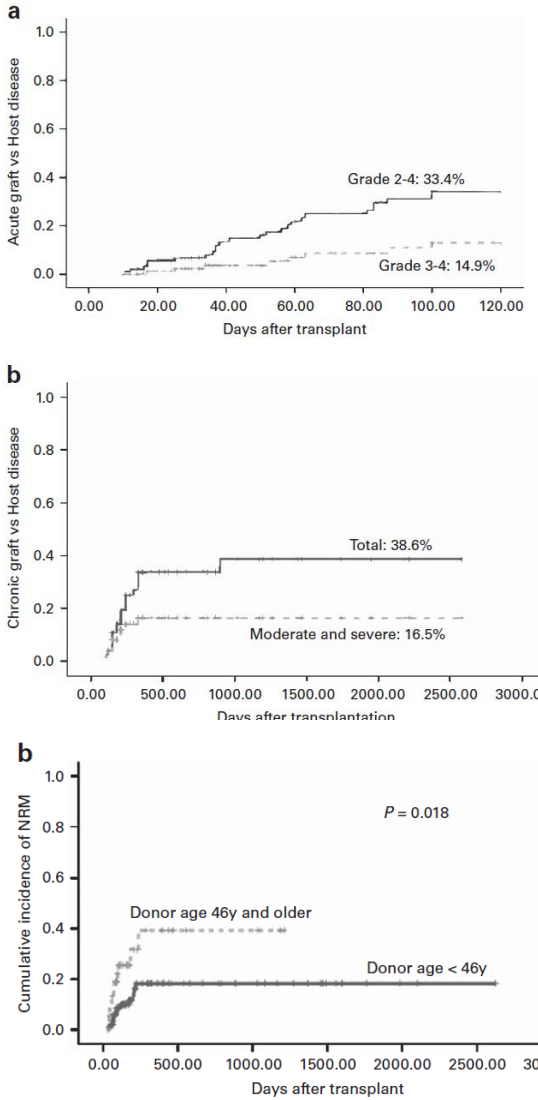


Fig. 1 Summary of Allo-SCT activities in Chinese registries (2008–2018)



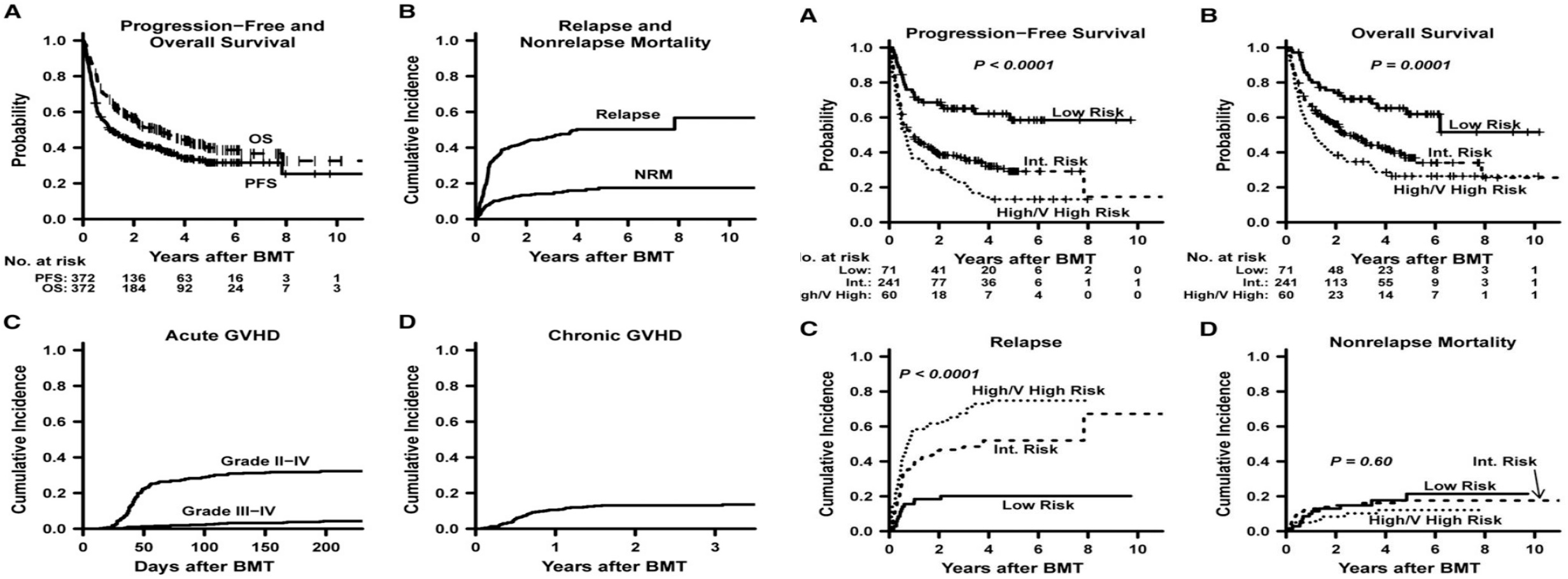


Lv et al. Bone Marrow Transplantation (2019) 54:703–707

Huang et al., Bone Marrow Transplantation (2016) 51

Results Post HCT-Cy Non Myeloablative

N=372, retrospective analysis



McCurdy et al., Blood 2015

Results Post HCT-Cy MAC

Table 1. Clinical characteristics of patients

n, Patients	148
Age; years (range)	47 (17–74)
Diagnosis	
AML	48
ALL	28
MDS	24
Myelofibrosis	16
Lymphoma	15
Other	18
Disease phase	
CR1	46
CR2	39
Active disease	63
Stem cell source	
BM	148
Conditioning regimens	
FLU-TBI	
9.9 Gy	*n*. 43
12 Gy	*n*. 13
TBF (BU3)	*n*. 53
TBF (BU2)	*n*. 39
Cell dose x 10^8/kg (range)	3.37 (1.4–7.8)
Follow up days (range)	301 (3–1332)

Abbreviations: BM = bone marrow; BU2 = 2 days of busulfan; BU3 = 3 days of busulfan; FLU = Fludarabine; MDS = myelòodisplastic syndrome; TBF = thiotepa, busulfan, fludarabine.

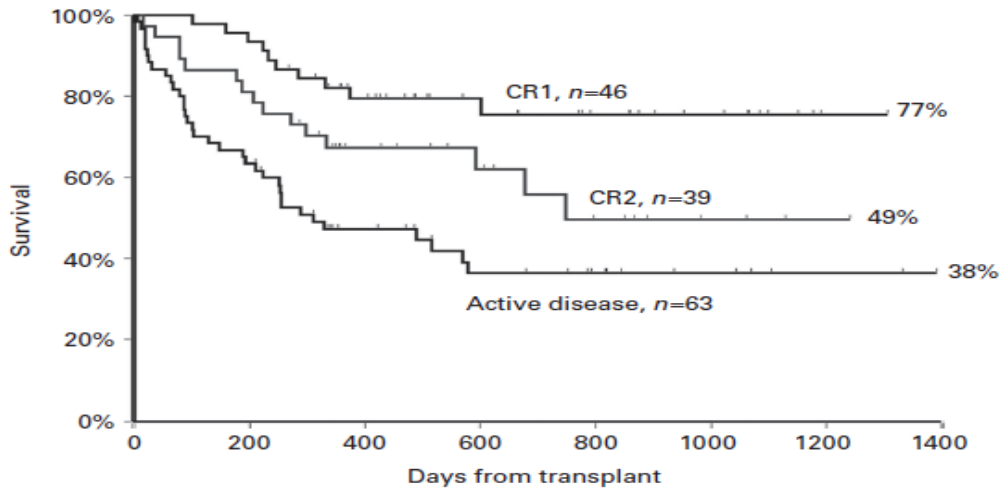
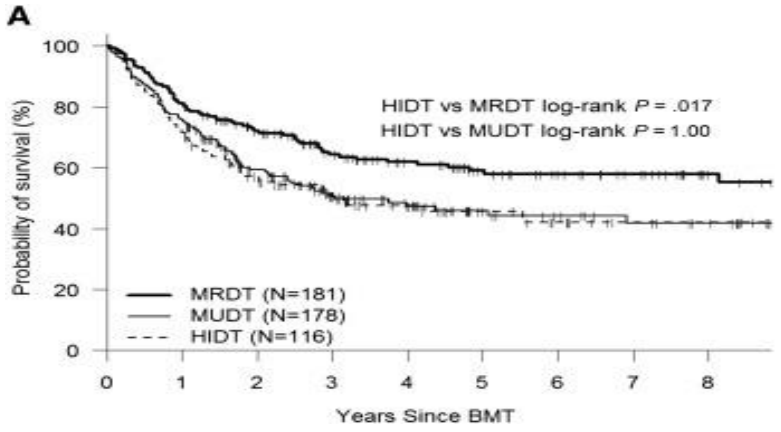


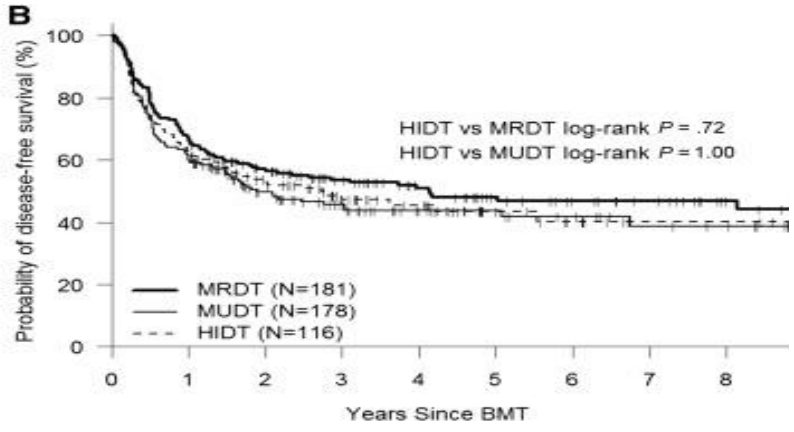
Figure 1. Actuarial survival of 148 patients receiving a myeloablative conditioning regimen, followed by haploidentical bone marrow transplantation and post-transplant cyclophosphamide, cyclosporine and mycophenolate for GVHD prophylaxis. Patients are stratified for disease phase.

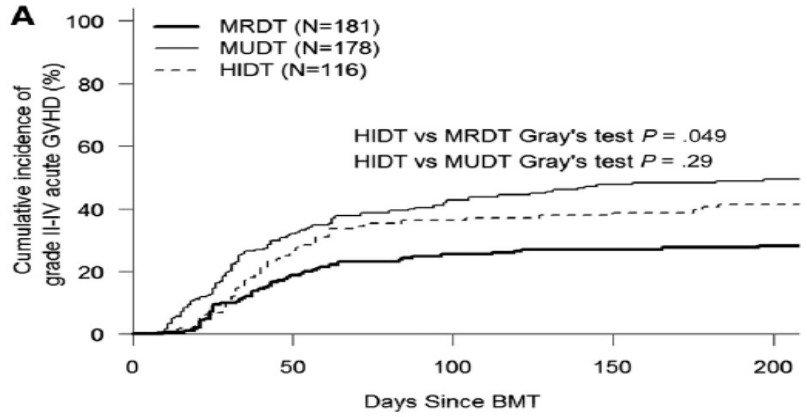
Bacigalupo et al., BMT 2015

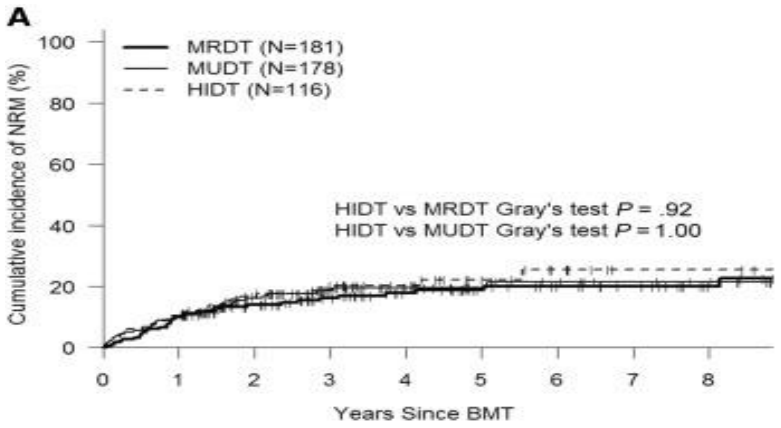
Post HCT-Cy vs MUD/MRD

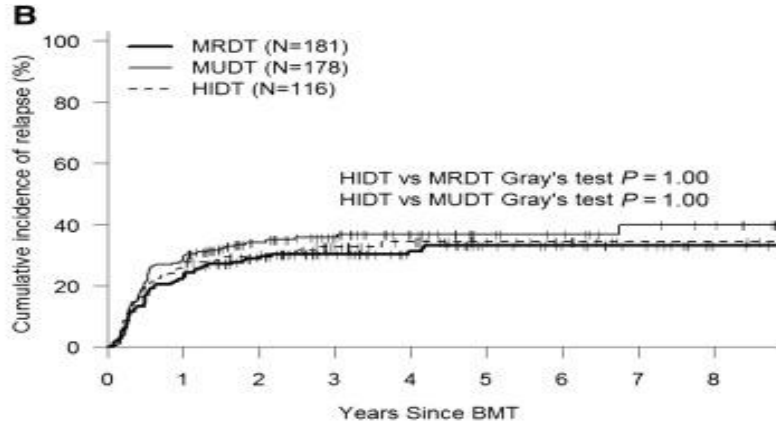
N=475, Haplo (n=116) vs MRD (n=181) vs MUD (n=178)
MAC, RIC and NMA Conditioning

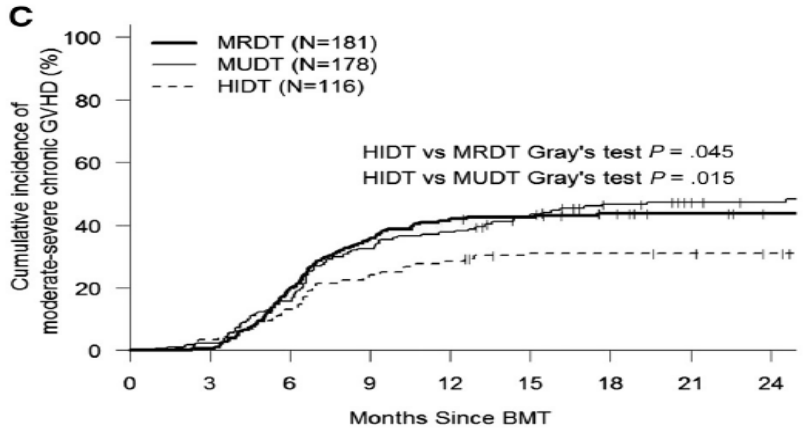












Bashey et al., BBMT 2015

Post HCT-Cy vs MUD ≥60 Jahre AML

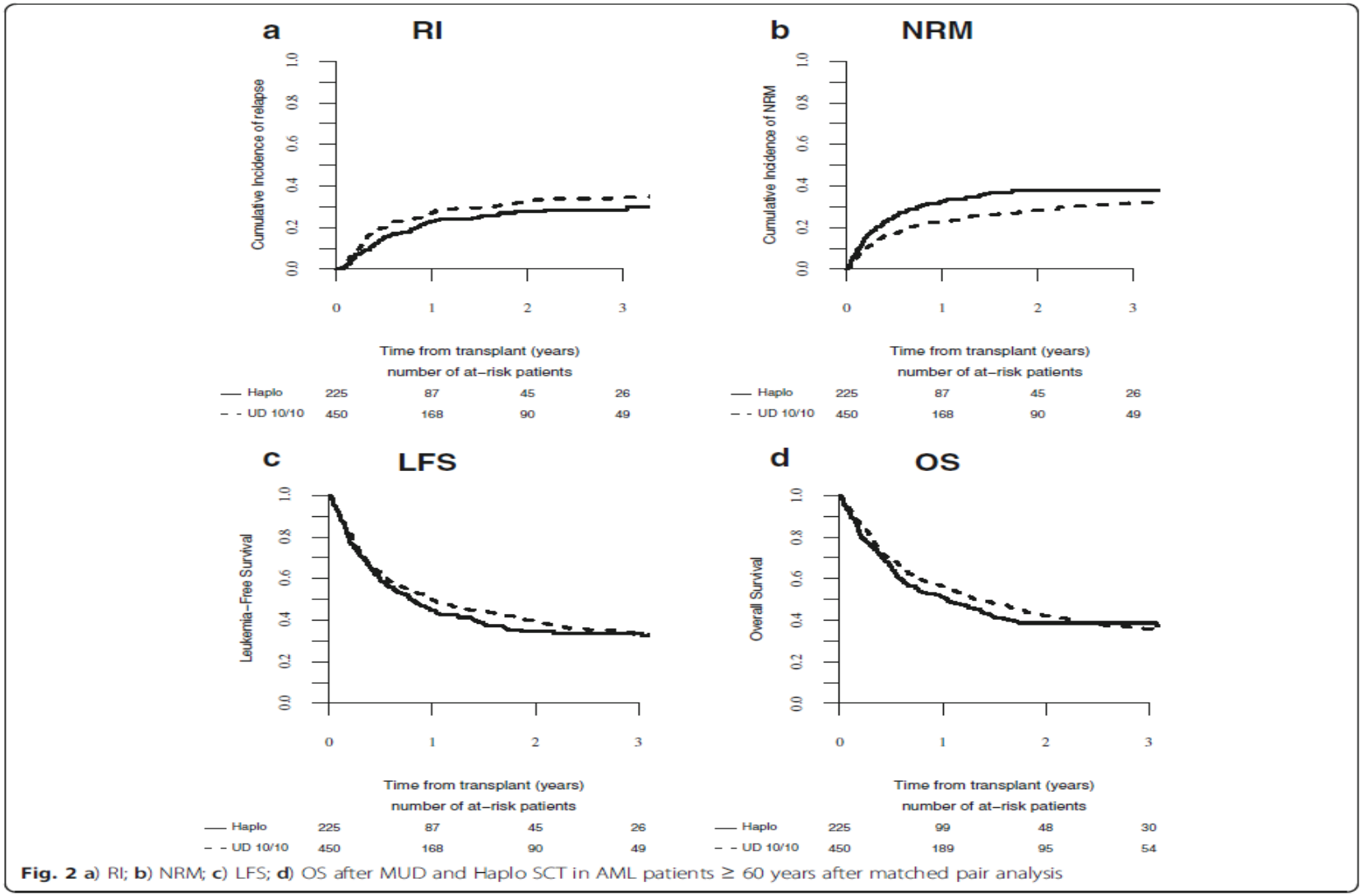
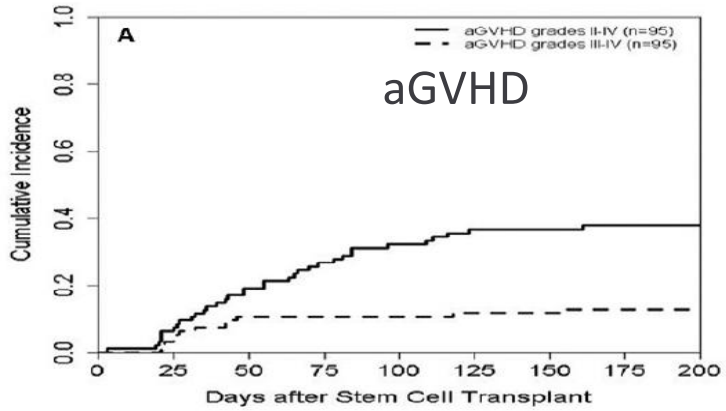
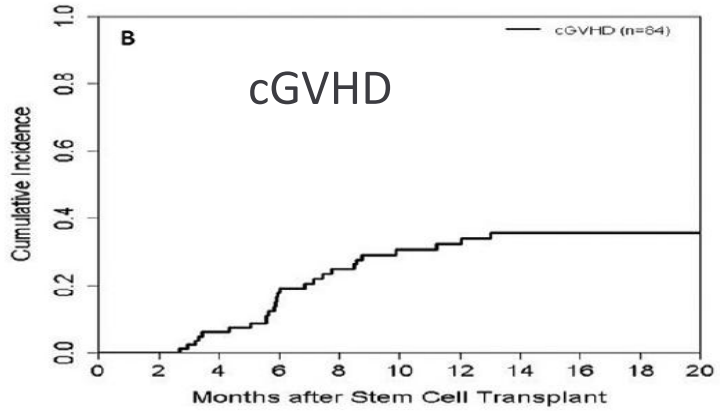


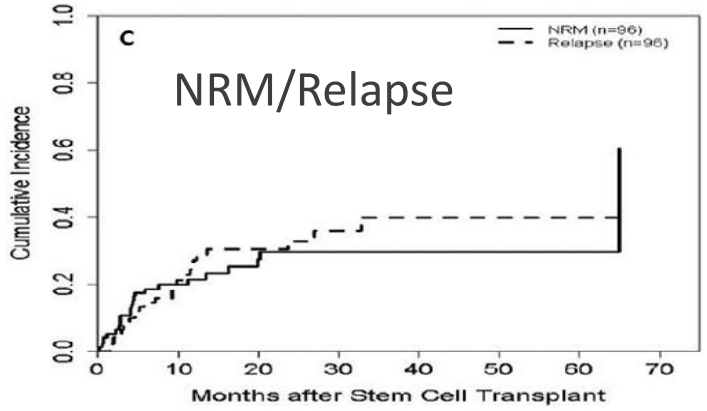
Fig. 2 a) RI; **b**) NRM; **c**) LFS; **d**) OS after MUD and Haplo SCT in AML patients ≥ 60 years after matched pair analysis

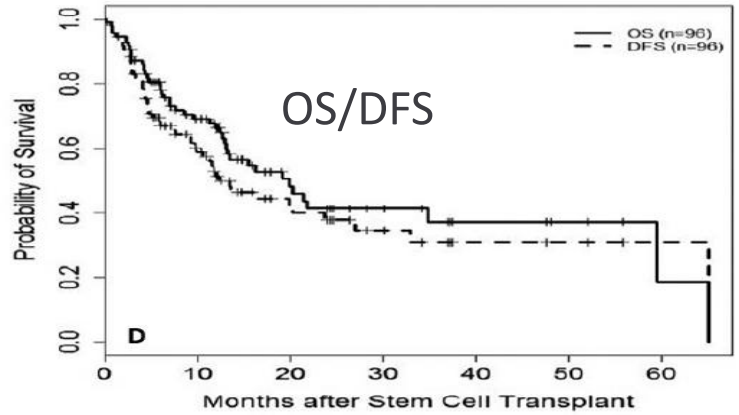
Santoro et al., Journal of Hematology&Oncology 2018

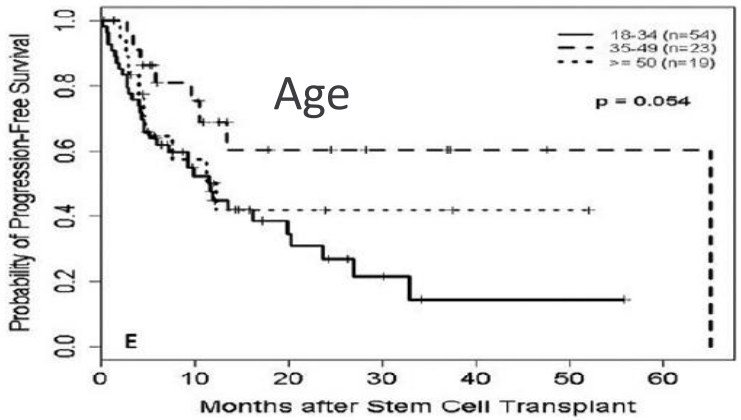
Post HCT-Cy Haplo ALL

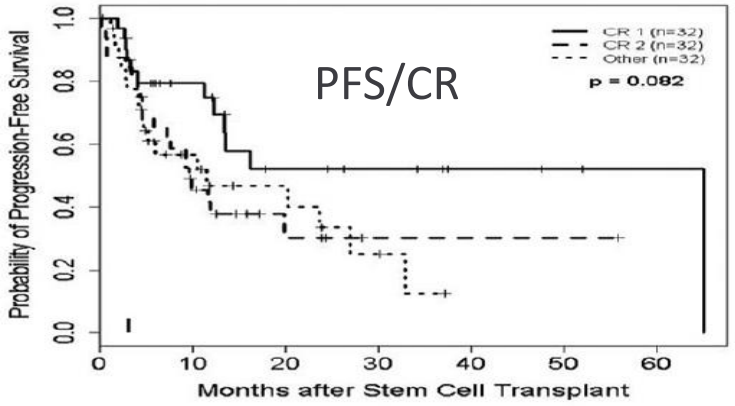












Srour et al., BBMT 2018

Sequential FLAMSA/Clo- Post HCT-Cy Haplo

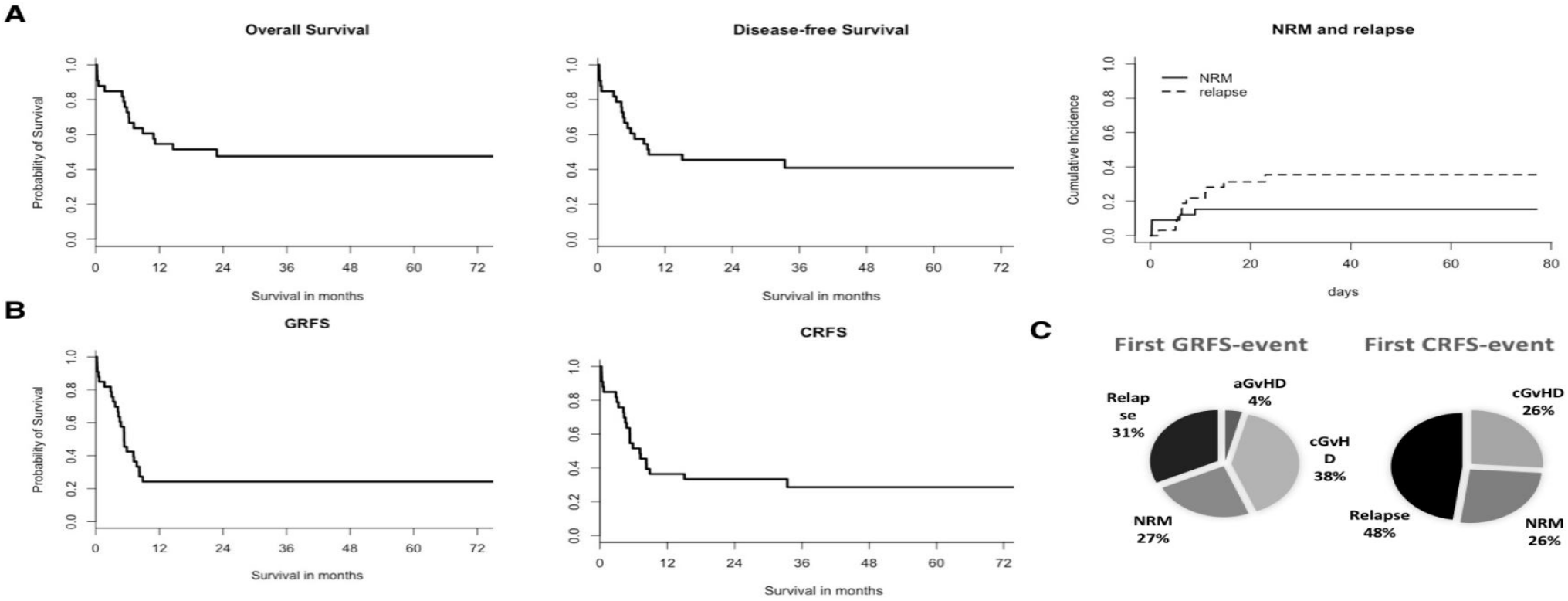


Figure 2: Kaplan-Meier curves of overall survival (OS), disease-free survival (DFS) and cumulative incidences (CI) of NRM and relapse (A), Kaplan-Meier curves of GvHD- and relapse-free survival (GRFS) and chronic GvHD- and relapse-free survival (CRFS) (B). Distribution of first event components of GRFS and CRFS (C).
Abbreviations: NRM, non-relapse mortality; aGvHD, acute Graft-versus-host disease; cGvHD, chronic Graft-versus-host disease

CY – MMF - Tac	33	100

Fraccaroli et al., Am. J. Hem 2018

Haplo vs MUD: Results Germany

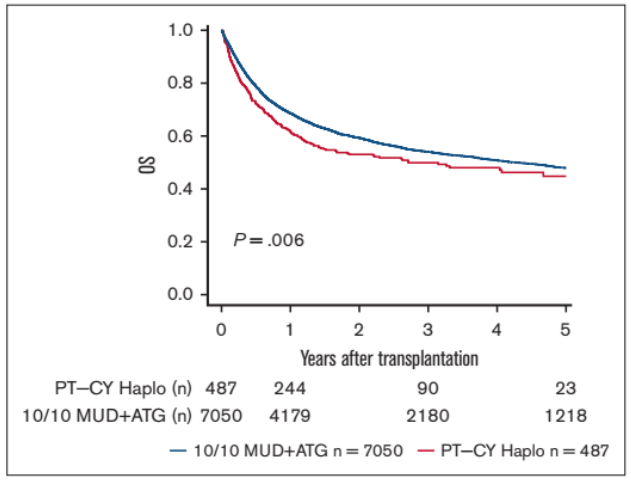


Figure 1. Overall Survival. Unadjusted 5-year OS according to transplant type (*P* = .006)

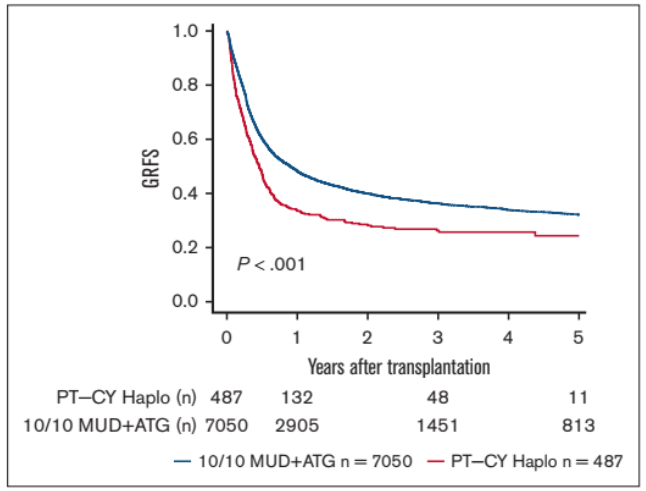


Figure 2. GvHD and Relapse Free Survival. Unadjusted 5-year GRFS according to transplant type (*P* < .001).

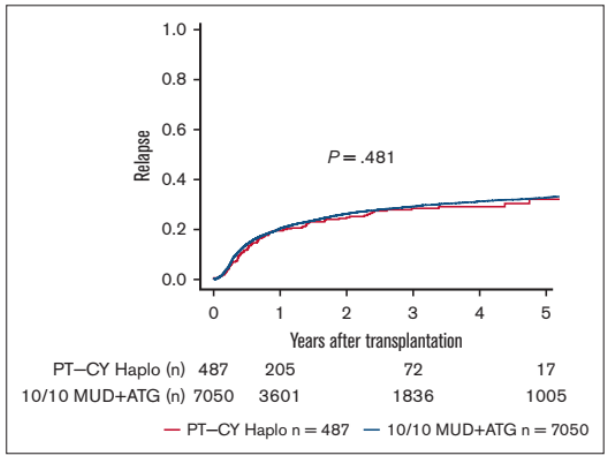


Figure 4. Non-relapse Mortality. Unadjusted 5-year NRM incidence according to transplant type (*P* = .001).

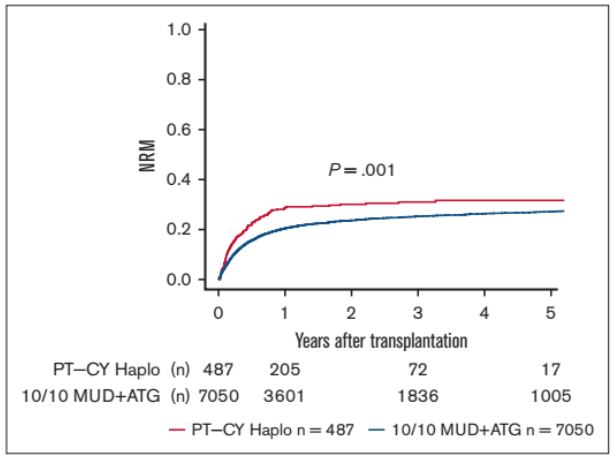
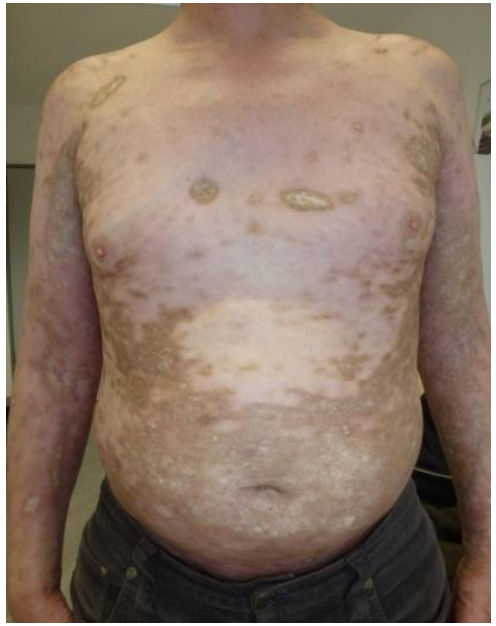


Figure 5. Relapse Incidence. Unadjusted 5-year relapse incidence according to transplant type (*P* = .481).

Arslan et al. Blood Advances; 2024, Vol. 8, 23

In vitro T-cell depletion: Haplo without GVHD?



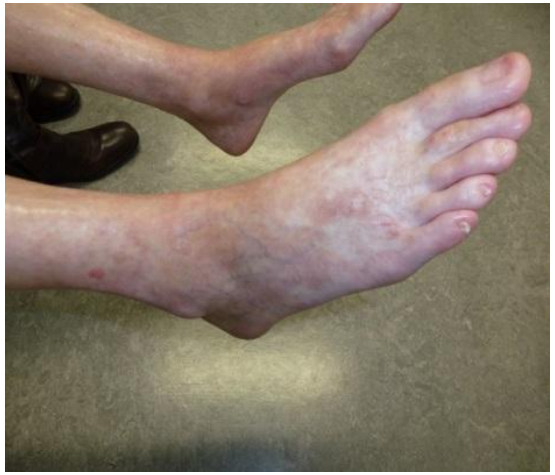
GVHD a major cause for morbidity and mortality

Incidence of GVHD with post-HCT Cy:

aGVHD II-IV = 30%, aGVHD III-IV = 11%

extensive cGVHD = 38%

Bashey et al., JCO 2013



Developments in graft-engineering for T-cell depletion

1995 CD34+ Selection of pure stem cells

2003 CD3/19 Depletion stem cells + effectors (NKs)

2012 TCRαβ/CD19 Depletion stem cells + effectors (NKs + γδT-cells)

- Better engraftment + immune reconstitution
- Retain low incidence of GVHD
- Lower CD34-dose in graft
- Improved GVT-effect (high NK-cell number)
- Reduced intensity conditioning

TCR-Alpha/Beta Haplo Study

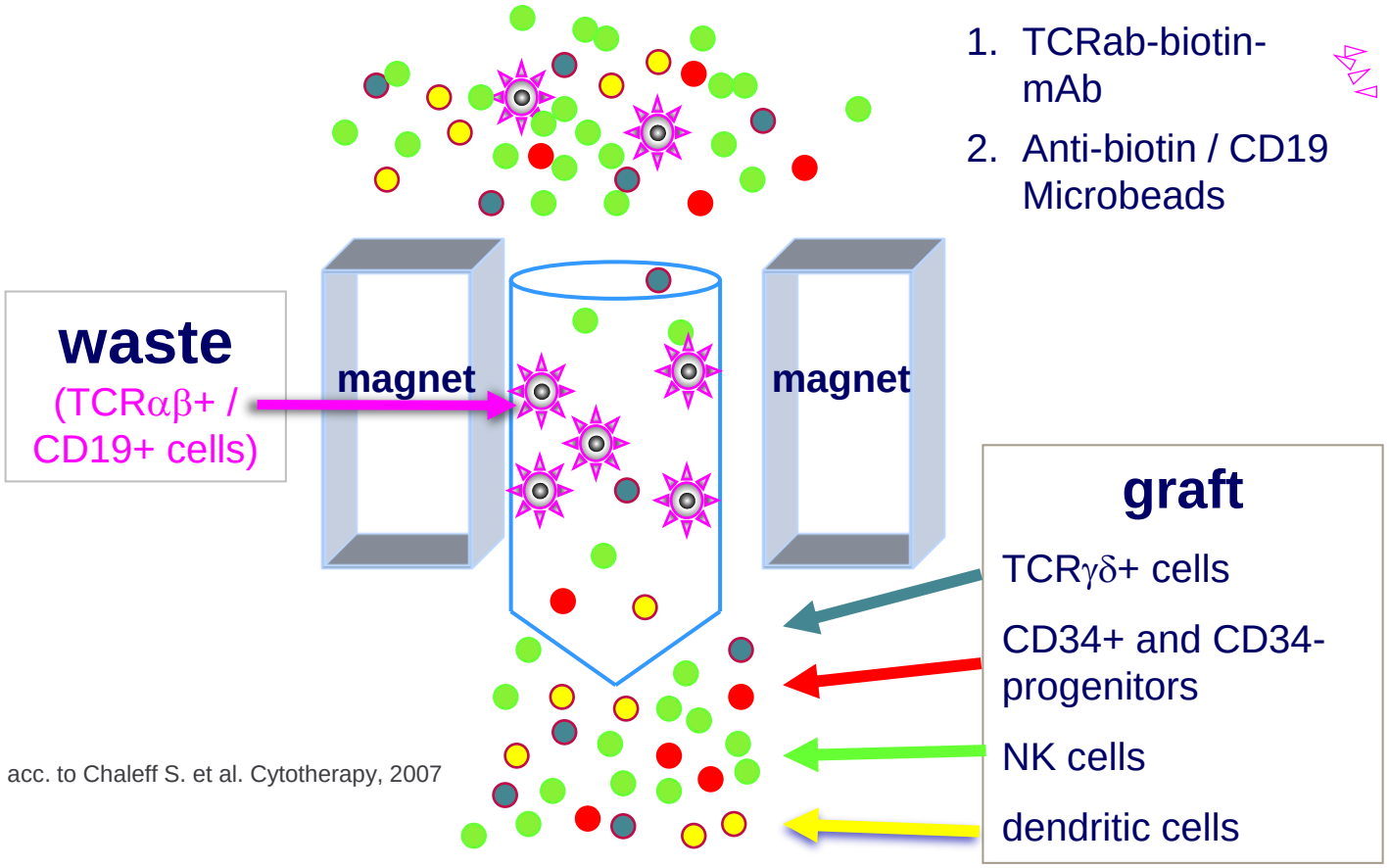
Results of a Prospective Multicenter Phase I/II Clinical Trial in Adult Patients Using TCR-Alpha/Beta and CD19 Depleted Haploidentical Stem Cell Transplantation Following Reduced Intensity Conditioning

Design	multi-center, open-label, single-arm, phase I/II
Patients	60 (30 pediatric, 30 adult)
IMP	manufactured in 6 GMP sites (decentralized), CliniMACS TCRαβ/CD19 depleted PBSC from haploidentical donors
Primary objective	Safety, tolerability and feasibility of TCRαβ/CD19 depleted PBSCs, defined as **Incidence of grade II-IV acute GVHD on day 100** post transplantation
Duration	2 year patient follow-up

Sponsor: Miltenyi Biotec

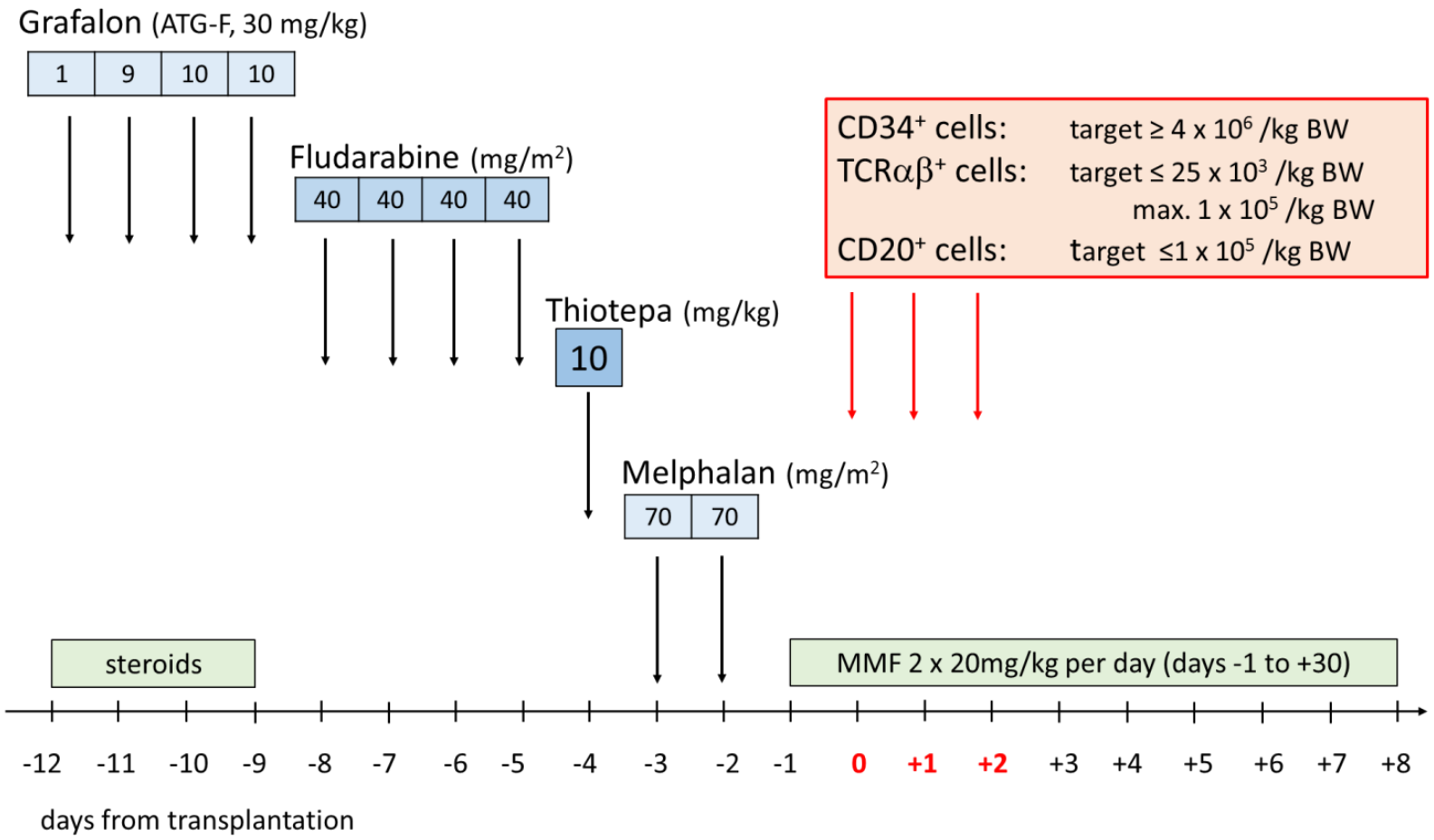
EudraCT No. : 2011-005562-38

Depletion of TCRab$^+$ and CD19$^+$ cells



acc. to Chaleff S. et al. Cytotherapy, 2007

Treatment protocol TCRαβ



Patients with therapy-refractory, hematological malignant disease:

➡ TNI (7Gy on day -1) instead of ATG possible

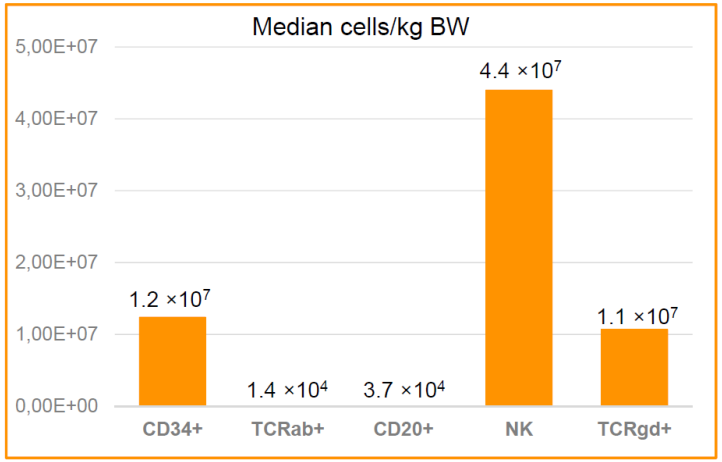
Patients and graft - TCRαβ

Patient demographics median 18.5 years (range 1 – 63)

May 2013-Nov 2016

Diagnosis	n = 60
AML	25
ALL	17
MDS/MPS	6
Solid tumors (rel. neuroblastoma, Ewings sarcoma, soft tissue sarcoma)	6
Non-malignant disorders (lysosomal storage disorder, SCID, WAS, sickle cell anemia)	4
Acute undifferentiated leukemia	1
Multiple myeloma	1
Disease status (malignant diseases)	**n = 56**
Complete remission	33
Partial remission (solid tumors)	2
Non-remission	21
2nd/3rd SCT (% patients with malignant diseases) (8 adults, 12 children)	20/56 (36%)

Graft composition



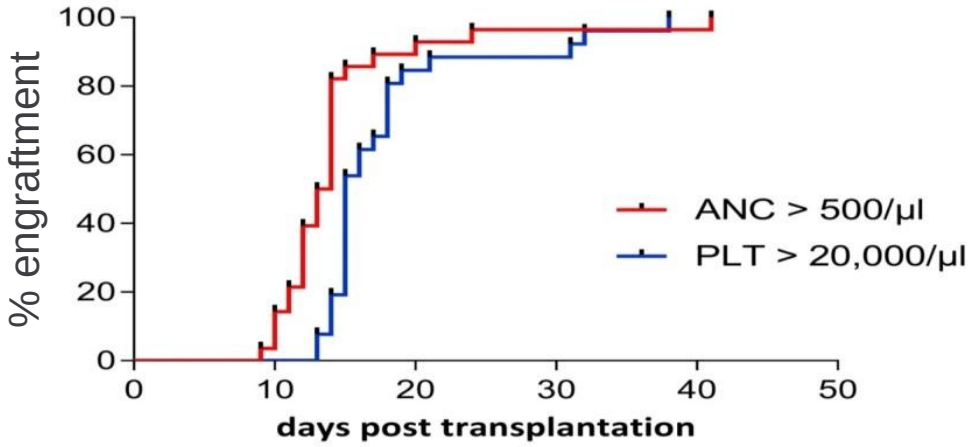
	CD34+	TCRab+	CD20+	NK	TCRgd+
min	0.4 ×10^7	0.06 ×10^4	0.2 ×10^4	0.68 ×10^7	0.11 ×10^7
max	5.5 ×10^7	6.43 ×10^4	72.7 ×10^4	18.2 ×10^7	4.5 ×10^7

TCRabHaplo-2010

Bethge et al., in submission

Engraftment

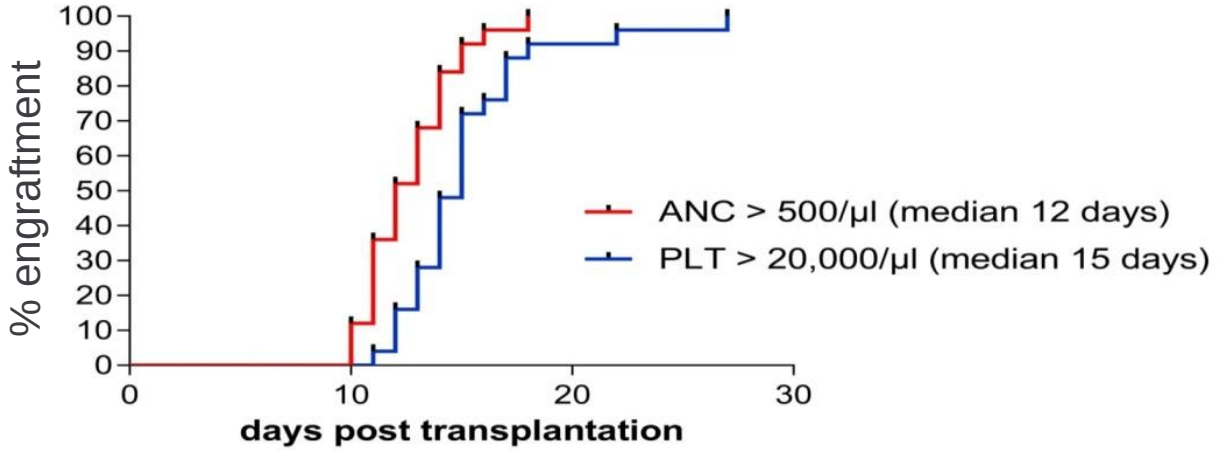
Adult patients



Median time to ANC: **13.5 days** (range 9 – 41)
Median time to PLT: **15 days** (range 13 - 38)

Final engraftment	30/30
Initial engraftment	28/30
- Engraftment (reconditioning, 2nd donation)	2/2

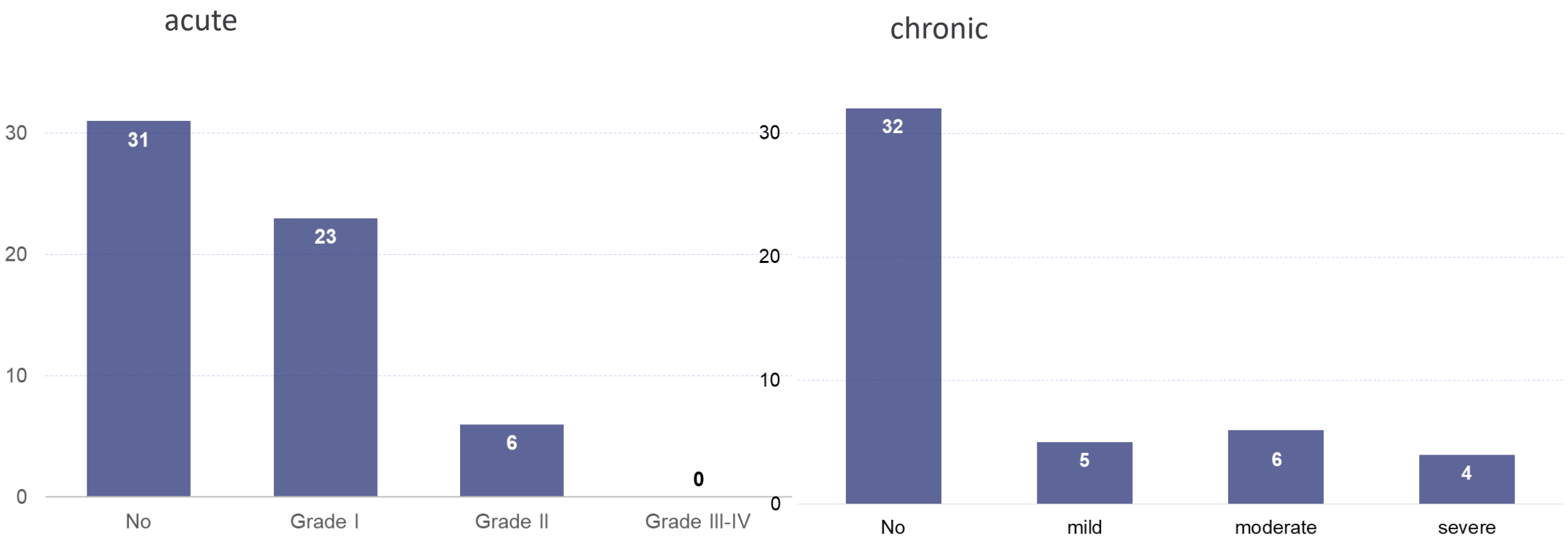
Children



Median time to ANC: **12 days** (range 10 – 18)
Median time to PLT: **15 days** (range 11 - 27)

Final engraftment	29/30
- TRM	1/30
Initial engraftment	23/30
- Engraftment (reconditioning, 2nd donation)	6/6

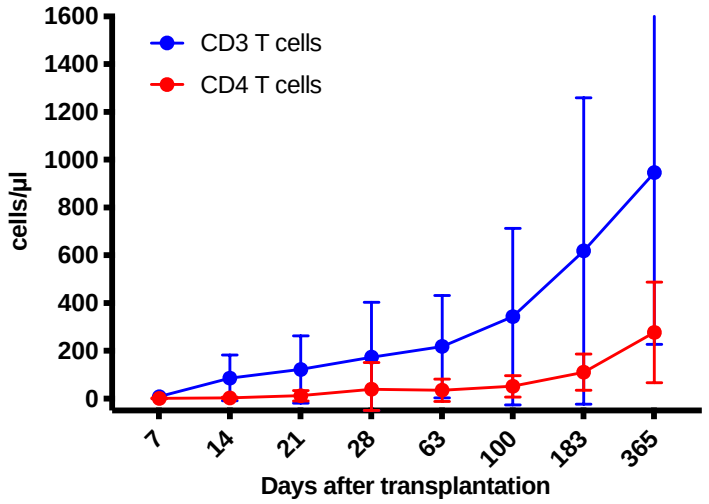
GVHD - TCRαβ



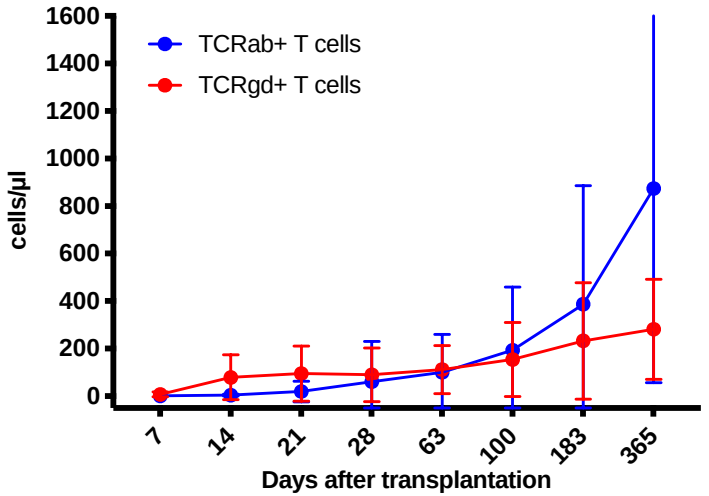
Bethge et al., Bone Marrow Transplant. 2022 Mar;57(3):423-430

Immune reconstitution

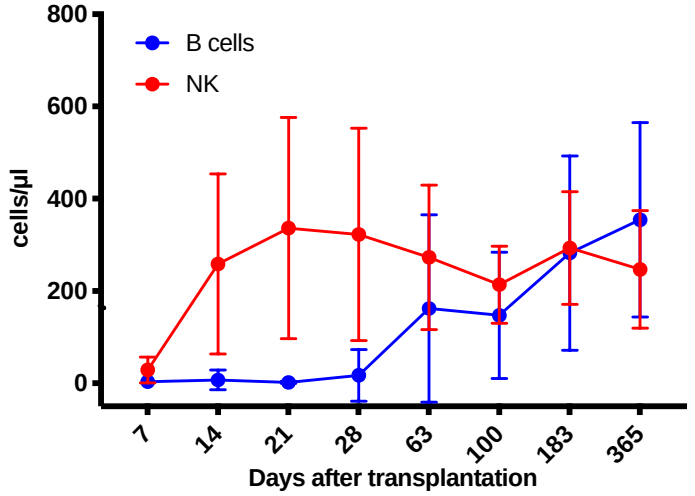
CD3/CD3CD4 T cells



αβ/γδ T cells



NK/B cells



Infections

		N (%)
CMV	Reactivation (PCR positive)	24 (40)
	Disease	2 (3)
Adenovirus	Reactivation (PCR positive, stool)	20 (33)
	Disease	11 (18)
HHV6	Reactivation (PCR positive)	18 (33)
	Disease	1 (2)
EBV	Reactivation (PCR positive)	1 (2)
	Disease	1 (2)
BK Virus	Reactivation (PCR positive)	6 (10)
	Disease	5 (8)
Infections (Other)	Sepsis	10 (17)

Outcome

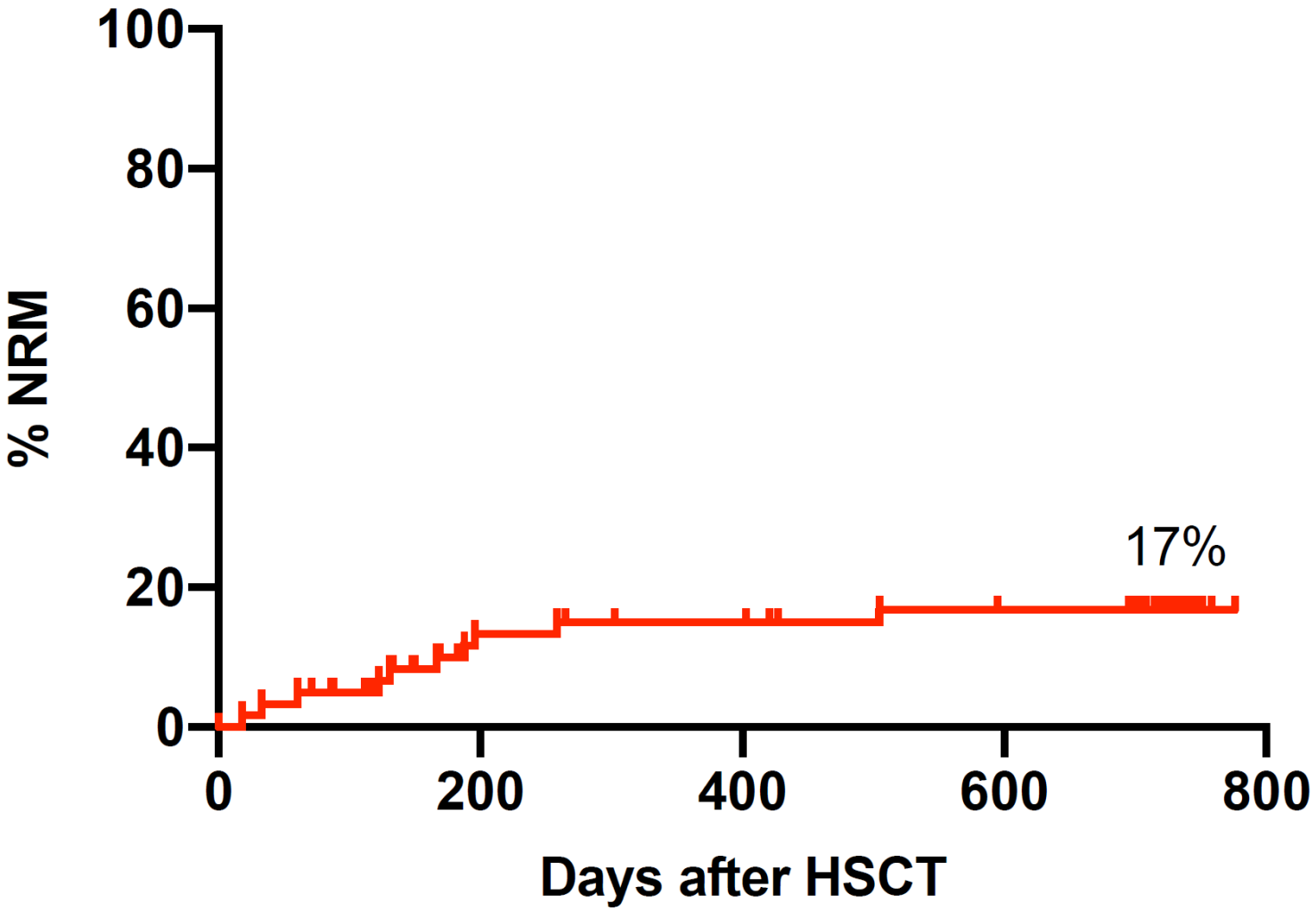
Outcome 60 patients	
Overall survival	**N (%)**
Patients alive	37 (62)
Causes of death*	
Relapse	12 (20)
Adenovirus infection	3 (5)
ARDS	3 (5)
Cardiac arrest	1 (2)
Multi organ failure	1 (2)
Sepsis due to graft failure	1 (2)
Demyelinating neuropathy	1 (2)

* For 1 patient cause of death was not reported

Median follow-up: 706 days (range: 18-776)

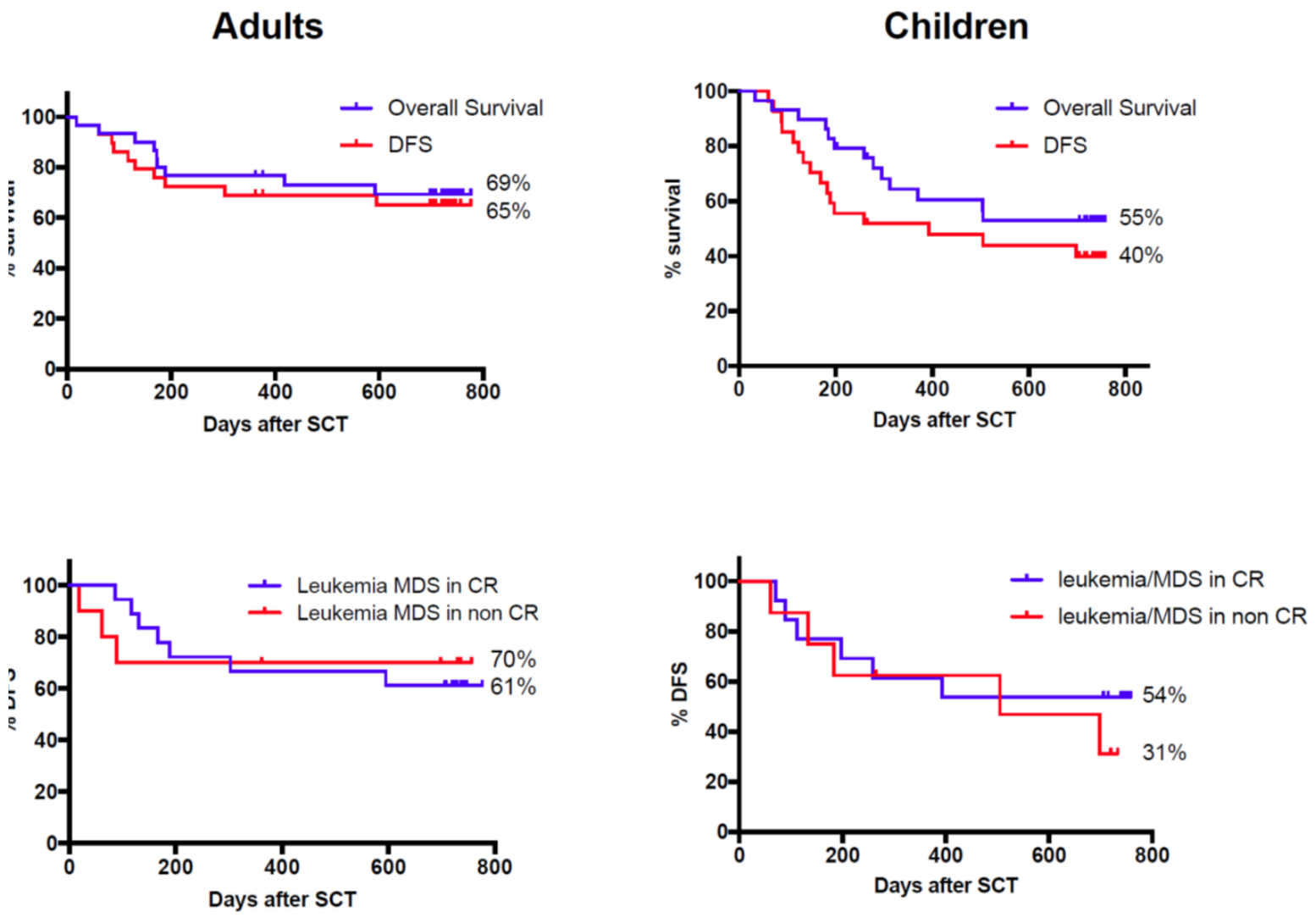
Bethge et al., Bone Marrow Transplant. 2022 Mar;57(3):423-430

Non Relapse Mortality



Bethge et al., Bone Marrow Transplant. 2022 Mar;57(3):423-430

Survival - TCRαβ



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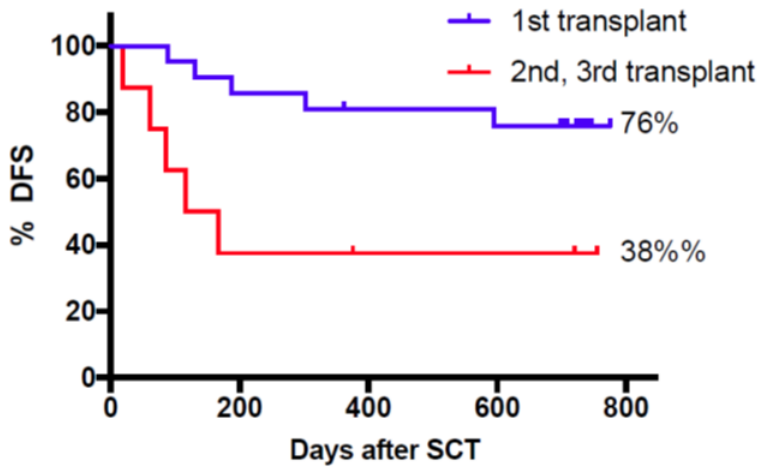
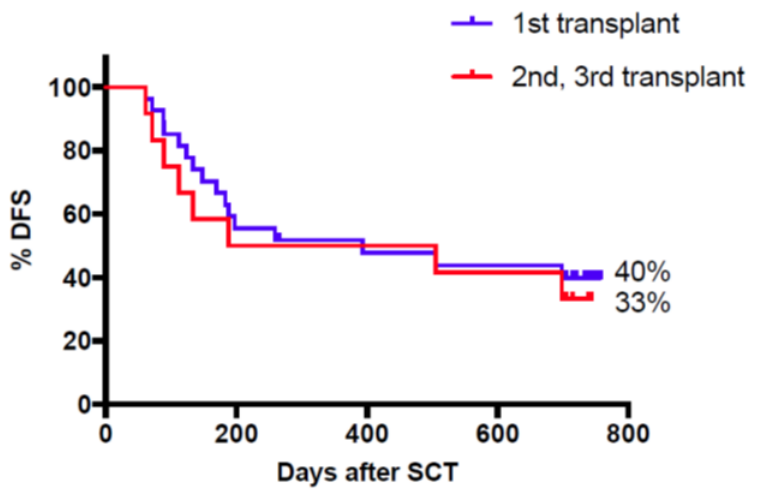
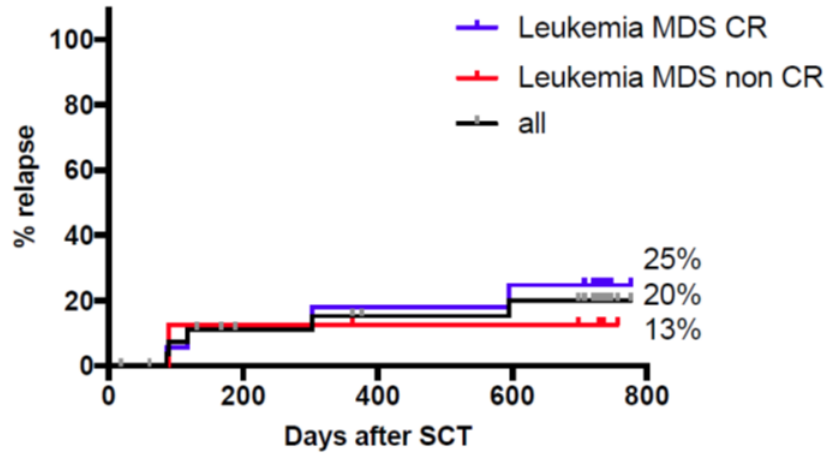
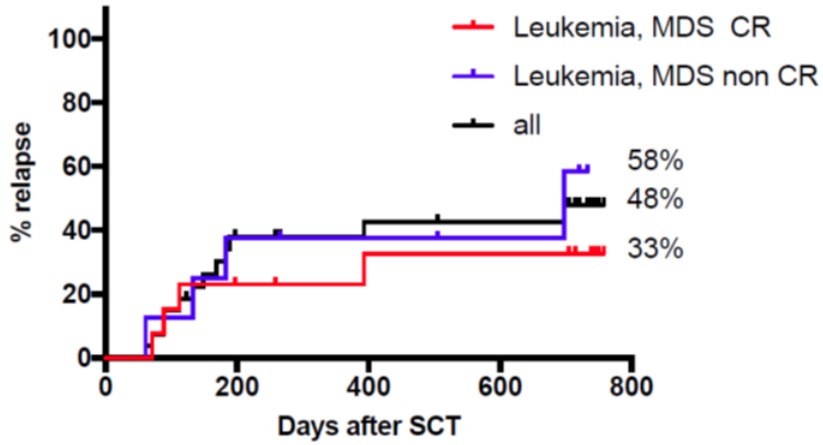
Survival - TCRαβ

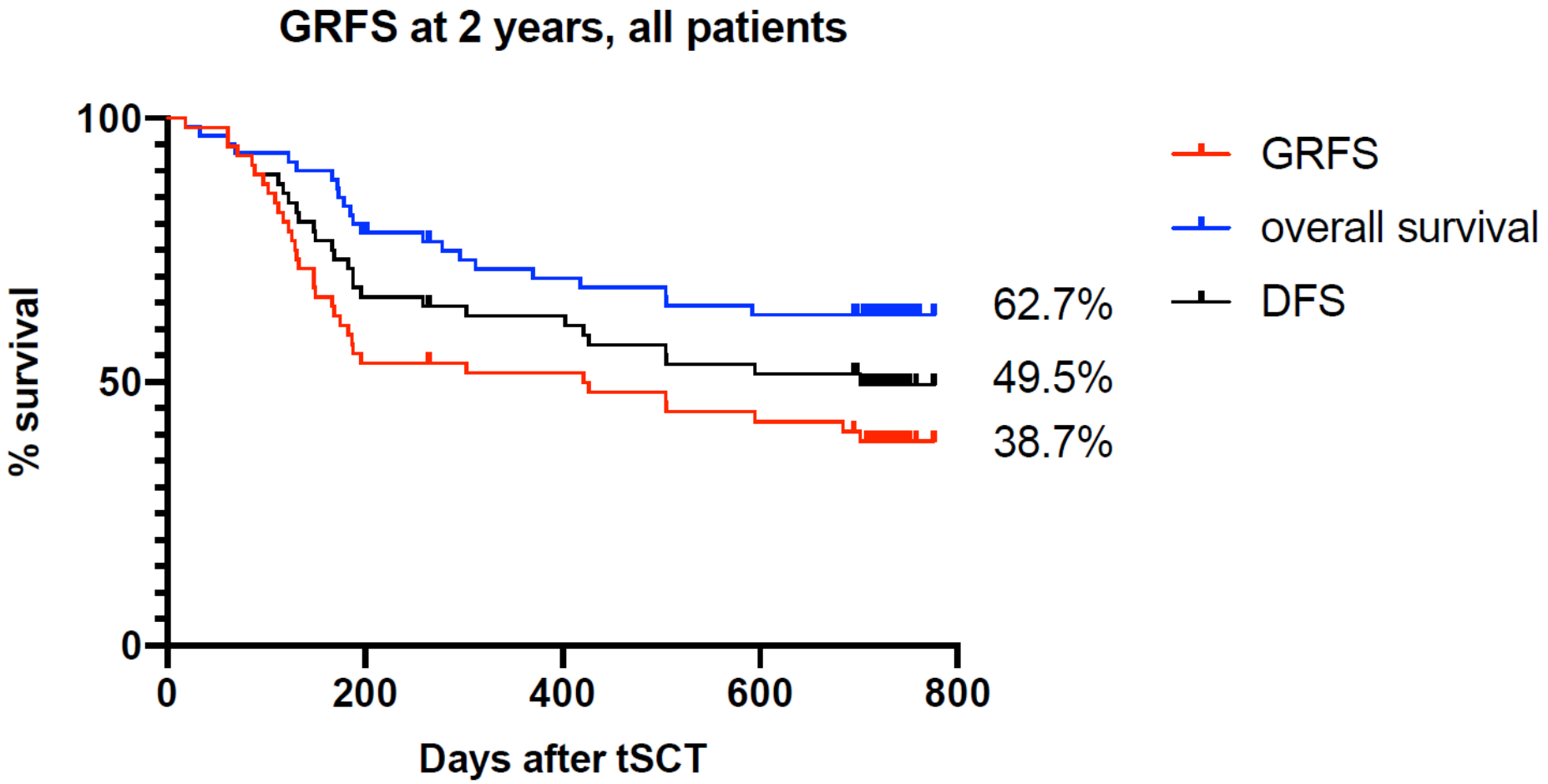






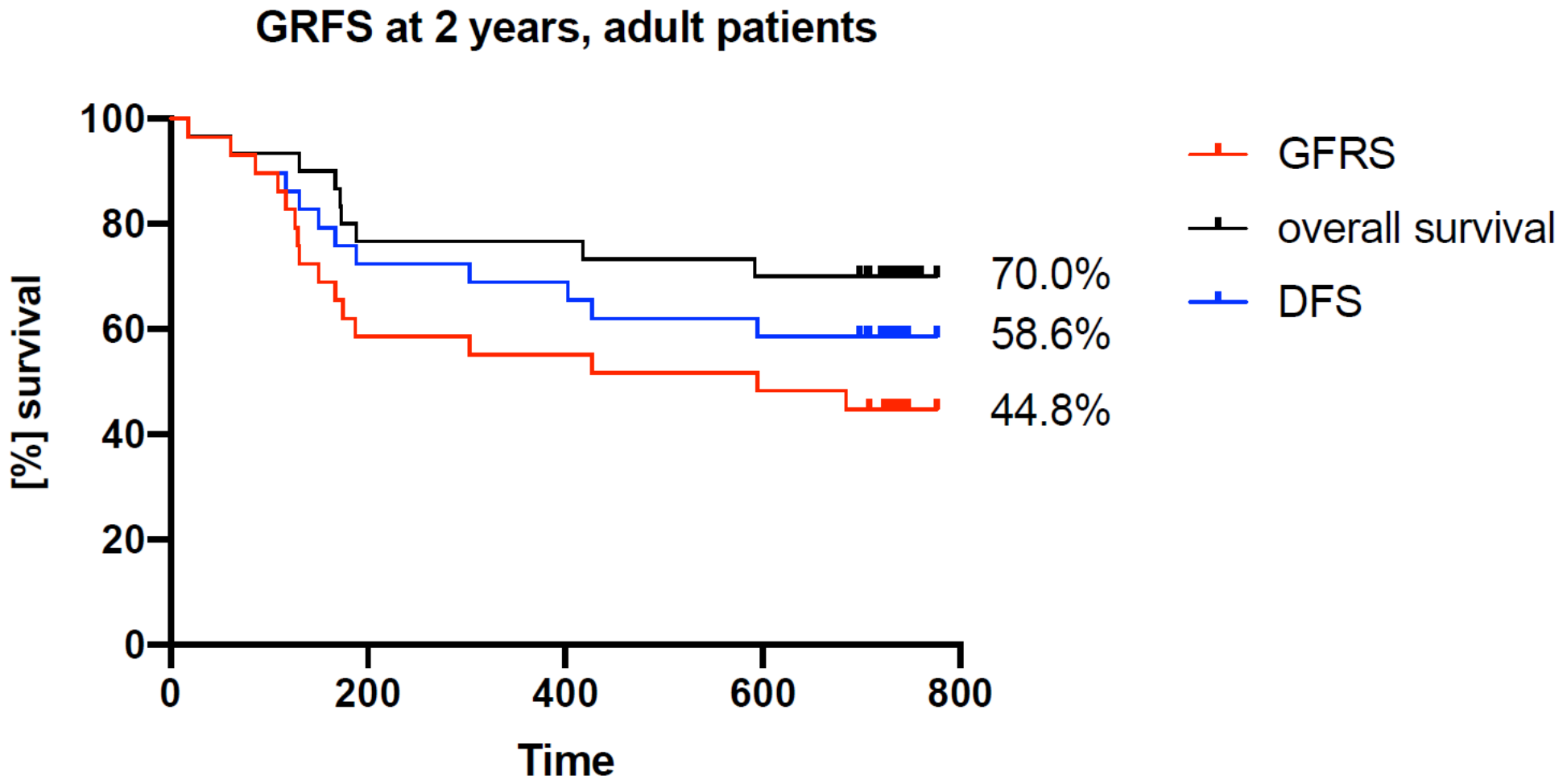


Survival - TCRαβ



Bethge et al., Bone Marrow Transplant. 2022 Mar;57(3):423-430

GVHD free, relapse free survival (GFRS)

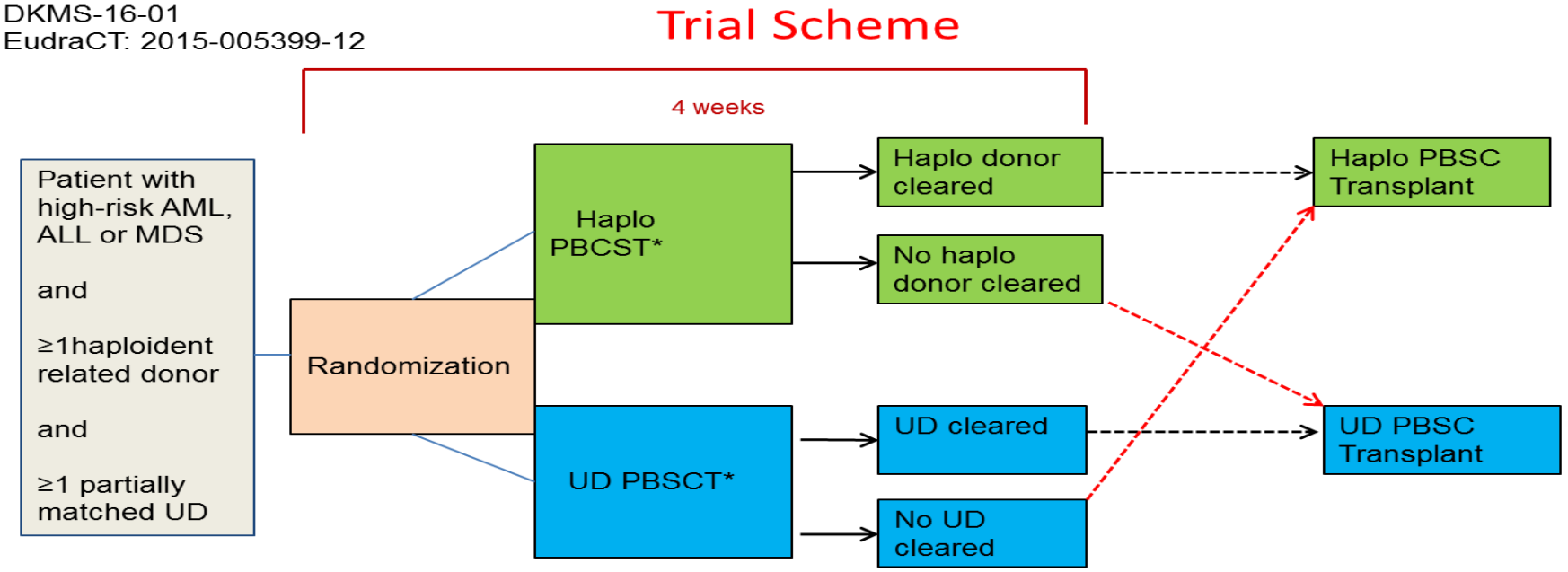


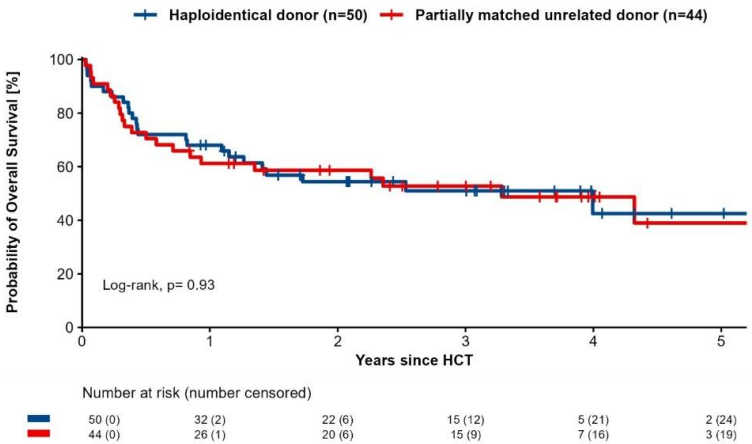
Bethge et al., Bone Marrow Transplant. 2022 Mar;57(3):423-430

HAMLET Study

HAploidentical versus **M**ismatched Unre**L**ate**E**d donor **T**ransplantation

HAMLET
DKMS-16-01
EudraCT: 2015-005399-12





First Results at EHA 2025:

Haplo-identical related donors and partially mismatched unrelated donors lead to comparable outcomes. No significant differences were found for any endpoint after transplantation with both donor types

CD45RADLIHaplo

A multicenter phase I/II trial of memory T cell donor lymphocyte infusions after transplantation of CliniMACS TCRα/β and CD19 depleted stem cell grafts from haploidentical donors for hematopoietic cell transplantation

Study design

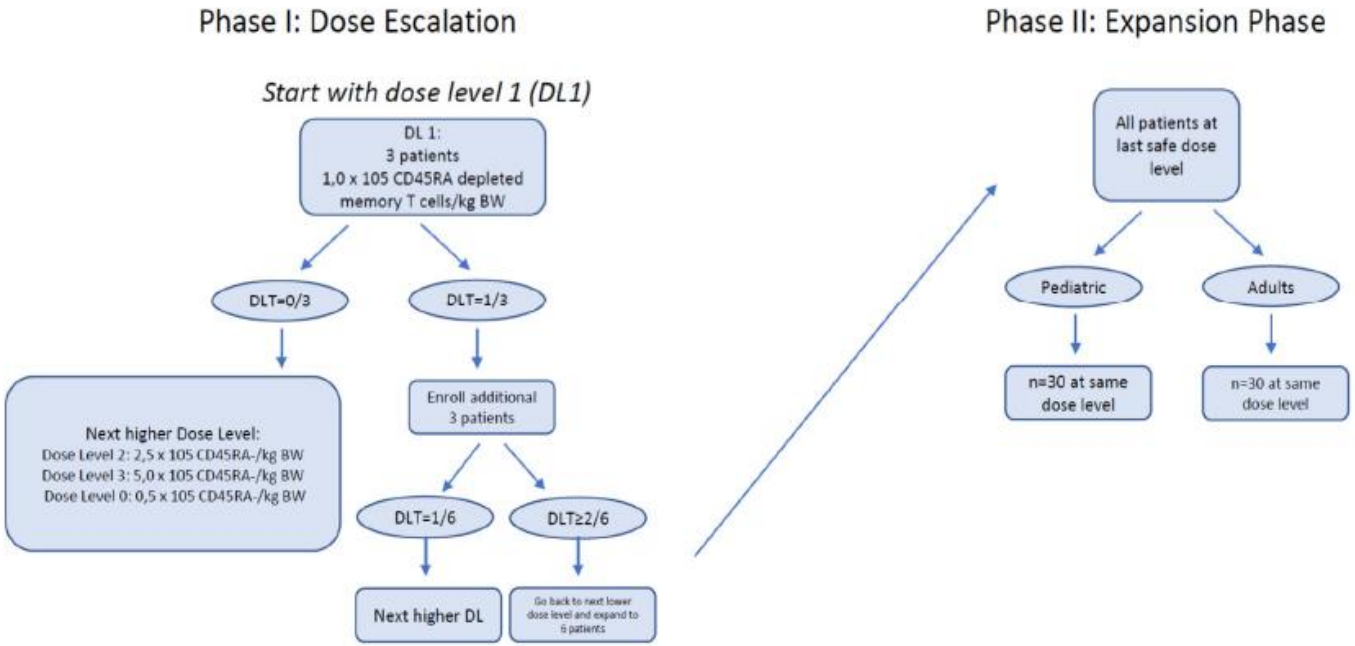
Phase I: " **3 + 3 design** " → Determination of the dose level for Phase II

Staged inclusion of three patients in each cohort:

- Follow-up of each first patient of each dose must be waited until day 100
- Release of additional patients by sponsor representative; simultaneous inclusion then possible
- Opening of new cohort only after day 100 of all patients
- A **DSMB** will be involved in each cohort as a decision-making body

Patients can be replaced,

- If you have withdrawn your consent
- In case of screening failures (patients only with visit 1)
- If their HHCT had less than 4x10



- Phase I: only adults and children >6 years
- Phase II: Study population is stratified into adults and children 30/30 (Phase I+II: total of 60 patients)
- Phase II: younger children ≥8 weeks can also be included

Inclusion criteria - indications

- Adult and pediatric patients with hematological malignancies in complete remission (CR), partial remission (PR), or with stable disease
  - Acute myeloid leukemia (AML) (patients with high-risk AML in CR1, patients with relapsed or primarily refractory AML)
  - Acute lymphoid leukemia (ALL) (patients with high-risk ALL in CR1, patients with relapsed or primary refractory ALL)
  - Hodgkin's disease (patients with relapsed or primary refractory Hodgkin's disease)
  - Non-Hodgkin lymphoma (patients with relapsed or primary refractory non-Hodgkin lymphoma)
  - Myelodysplastic syndrome (MDS) / myeloproliferative syndrome (MPS) (patients with refractory MDS/MPS)
  - Multiple myeloma (MM) (patients with relapsed or refractory MM)

Additional inclusion criteria:

- The decision for haploidentical HHCT according to hospital routine with TCR α/β and CD19-depleted stem cells was made before inclusion of the patient in this study.

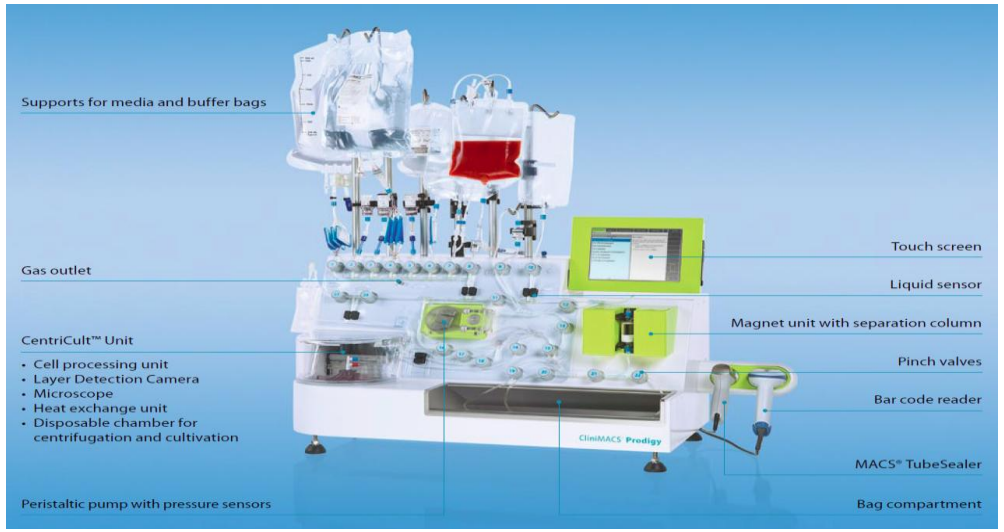
Conclusions

- ✓ Large body of retrospective evidence for T-cell replete haplo SCT using Post-Cy as almost equivalent to MUD
- ✓ Excellent equivalent results with TCRalpha/beta depleted Haplo SCT
- ✓ Haploidentical SCT is a feasible and safe source of stem cells with similar results as in 10/10 MUD SCT
- ✓ Pending results of prospective Study of Haplo vs 9/10 MM (HAMLET/Sponsor DKMS)
- ✓ Ongoing prospective Study of CD45 RA DLI after TCRalpha/beta depleted Haplo SCT
- ✓ Haplo excellent Platform for additional post SCT cellular therapies

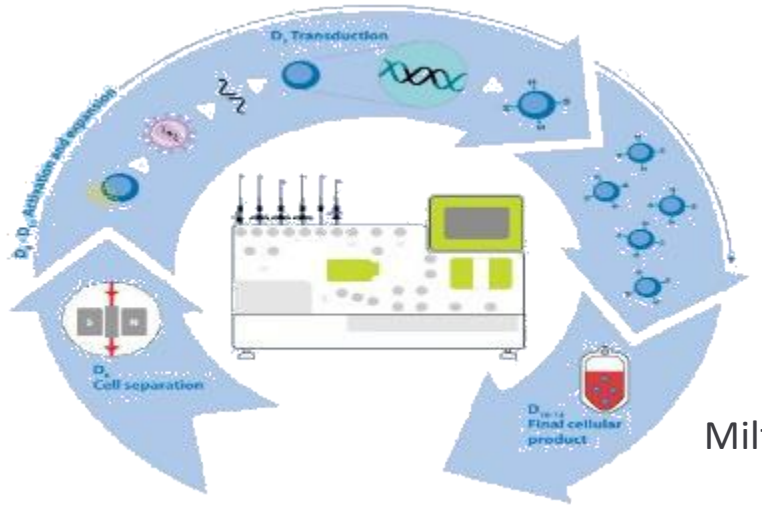
New concept for decentralized CAR-T production

Cell selection	Cell activation	Cell transduction	Cell expansion	Final formulation	IPC/QC
CliniMACS Buffer	MACS GMP TransAct	GMP Lentiviral Vector: FMC63 (murine anti-human CD19) scFv , 4-1BB /CD3 ζ)	MACS GMP TexMACS		
CliniMACS Reagents: CD4 / CD8	MACS GMP TexMACS		MACS GMP IL-7, IL-15		

CliniMACS Prodigy TS 520



12 days, fresh product



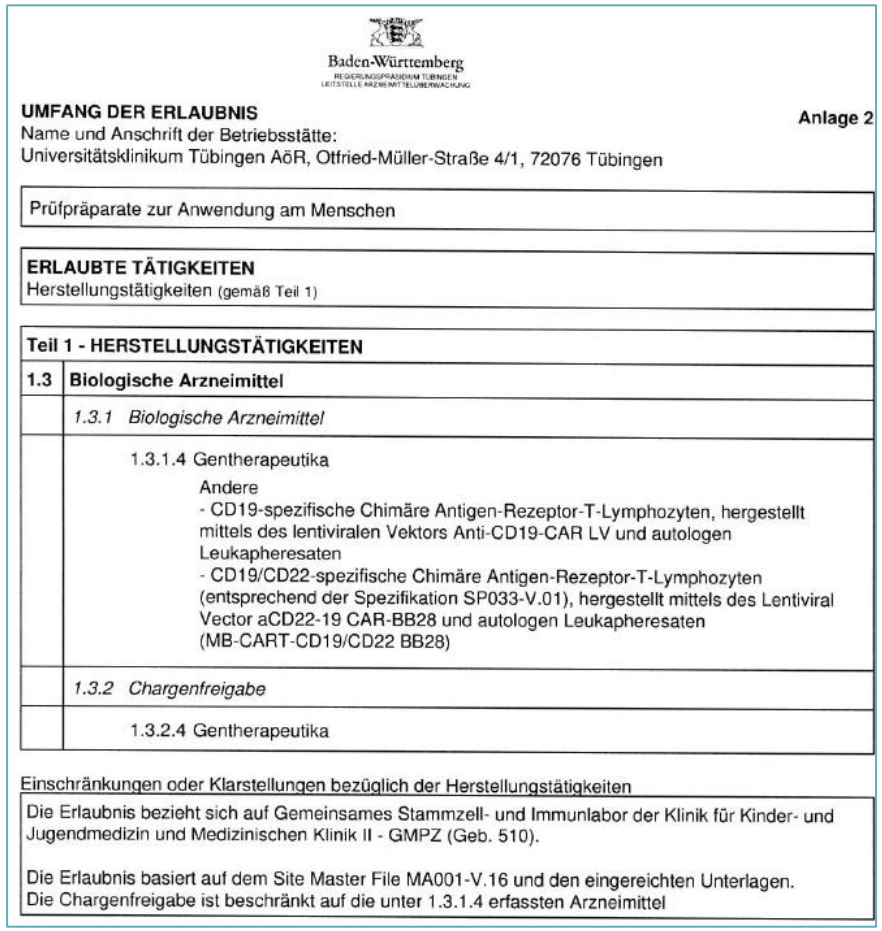
Miltenyi Biotech®

Joint GMP stem cell laboratory Children's Hospital/Medical Clinic

- 2 Class B clean rooms
- 4 CliniMACS columns
- 3 Miltenyi Prodigy
- at the GMP Center UKT: 1 Class B clean room + C Lab

- Manufacturing authorization for:
  - Stem cell preparation
  - CD34, CD3/CD19, TCAb /CD19 depletion
  - MSC
  - Virus-specific T cells
  - CAR-T CD19
  - CAR-T CD19/CD22

Physician-specific and study-specific manufacturing authorization for: CD 19 CAR-T - CD 19/22 CAR-T



Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN
LEITSTELLE ARZNEIMITTELÜBERWACHUNG

UMFANG DER ERLAUBNIS **Anlage 2**

Name und Anschrift der Betriebsstätte:
Universitätsklinikum Tübingen AöR, Otfried-Müller-Straße 4/1, 72076 Tübingen

Prüfpräparate zur Anwendung am Menschen

ERLAUBTE TÄTIGKEITEN
Herstellungstätigkeiten (gemäß Teil 1)

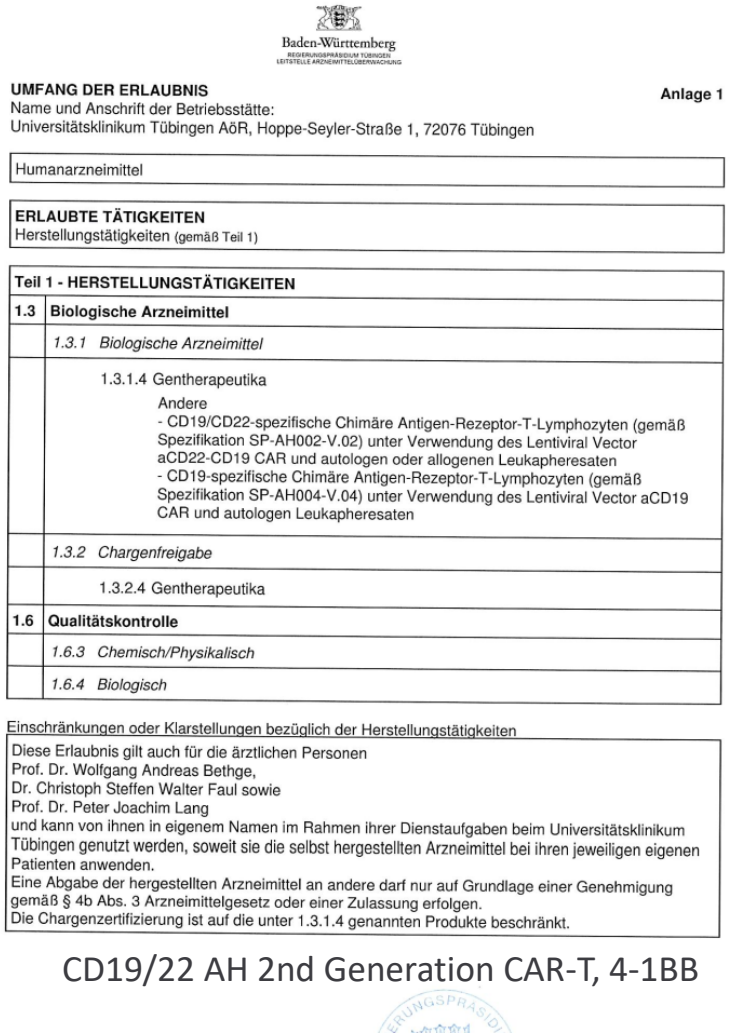
Teil 1 - HERSTELLUNGSTÄTIGKEITEN	
1.3	**Biologische Arzneimittel**
	1.3.1 Biologische Arzneimittel
	1.3.1.4 Gentherapeutika Andere - CD19-spezifische Chimäre Antigen-Rezeptor-T-Lymphozyten, hergestellt mittels des lentiviralen Vektors Anti-CD19-CAR LV und autologen Leukapheresaten - CD19/CD22-spezifische Chimäre Antigen-Rezeptor-T-Lymphozyten (entsprechend der Spezifikation SP033-V.01), hergestellt mittels des Lentiviral Vector aCD22-19 CAR-BB28 und autologen Leukapheresaten (MB-CART-CD19/CD22 BB28)
	1.3.2 Chargenfreigabe
	1.3.2.4 Gentherapeutika

Einschränkungen oder Klarstellungen bezüglich der Herstellungstätigkeiten

Die Erlaubnis bezieht sich auf Gemeinsames Stammzell- und Immunlabor der Klinik für Kinder- und Jugendmedizin und Medizinischen Klinik II - GMPZ (Geb. 510).

Die Erlaubnis basiert auf dem Site Master File MA001-V.16 und den eingereichten Unterlagen.
Die Chargenfreigabe ist beschränkt auf die unter 1.3.1.4 erfassten Arzneimittel

CD19/22 IMP 3rd Generation CAR-T
CD28 and 4-1BB (CD137) co-stimulatory domains



Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN
LEITSTELLE ARZNEIMITTELÜBERWACHUNG

UMFANG DER ERLAUBNIS **Anlage 1**

Name und Anschrift der Betriebsstätte:
Universitätsklinikum Tübingen AöR, Hoppe-Seyler-Straße 1, 72076 Tübingen

Humanarzneimittel

ERLAUBTE TÄTIGKEITEN
Herstellungstätigkeiten (gemäß Teil 1)

Teil 1 - HERSTELLUNGSTÄTIGKEITEN	
1.3	**Biologische Arzneimittel**
	1.3.1 Biologische Arzneimittel
	1.3.1.4 Gentherapeutika Andere - CD19/CD22-spezifische Chimäre Antigen-Rezeptor-T-Lymphozyten (gemäß Spezifikation SP-AH002-V.02) unter Verwendung des Lentiviral Vector aCD22-CD19 CAR und autologen oder allogenen Leukapheresaten - CD19-spezifische Chimäre Antigen-Rezeptor-T-Lymphozyten (gemäß Spezifikation SP-AH004-V.04) unter Verwendung des Lentiviral Vector aCD19 CAR und autologen Leukapheresaten
	1.3.2 Chargenfreigabe
	1.3.2.4 Gentherapeutika
1.6	**Qualitätskontrolle**
	1.6.3 Chemisch/Physikalisch
	1.6.4 Biologisch

Einschränkungen oder Klarstellungen bezüglich der Herstellungstätigkeiten

Diese Erlaubnis gilt auch für die ärztlichen Personen
Prof. Dr. Wolfgang Andreas Bethge,
Dr. Christoph Steffen Walter Faul sowie
Prof. Dr. Peter Joachim Lang
und kann von ihnen in eigenem Namen im Rahmen ihrer Dienstaufgaben beim Universitätsklinikum Tübingen genutzt werden, soweit sie die selbst hergestellten Arzneimittel bei ihren jeweiligen eigenen Patienten anwenden.
Eine Abgabe der hergestellten Arzneimittel an andere darf nur auf Grundlage einer Genehmigung gemäß § 4b Abs. 3 Arzneimittelgesetz oder einer Zulassung erfolgen.
Die Chargenzertifizierung ist auf die unter 1.3.1.4 genannten Produkte beschränkt.

CD19/22 AH 2nd Generation CAR-T, 4-1BB

NEW also for the following

Indications for physician-specific preparation:

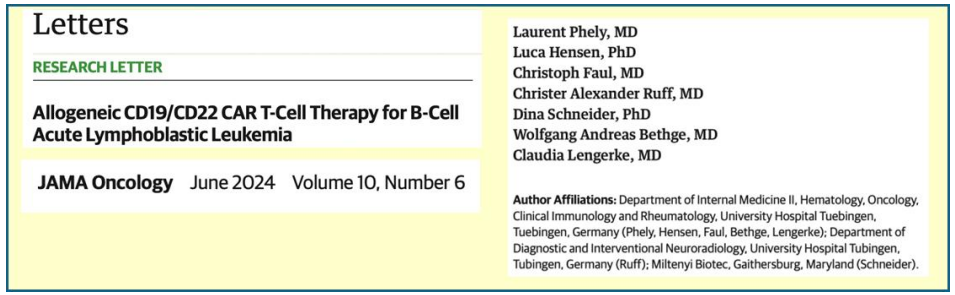
CD19/CD22 CAR-T:
- PTLD
- Burkitt lymphoma
- CLL
- CLL with Richter transformation in DLBCL
- Marginal zone lymphoma
- CD19 negative/CD22 positive
- R/R disease due to lymphoma/ALL after CAR-T or bispecific AK
- therapy-refractory ALL CD19 negative, CD22 positive
- CD19+/- CD22 positive disease (lymphoma/ALL) relapse after allo

CD19 CAR-T:
- Systemic lupus erythematosus
- Systemic scleroderma
- Multiple sclerosis
- Immune thrombocytopenia
- Myasthenia Gravis
- Autoimmune myositis
- Phemphigus Vulgaris
- ANCA-associated vasculitis
- Rheumatoid arthritis

Physician-specific CD 19 CAR-T - CD 19/22 CAR-T Applications and Individual Treatment Trials as Medical Emergency

Patient Cohort – Bispecific CD19/CD 22 CAR-T Cell Product 2nd Generation 4-1BB

- **First patient treated:** January 2021
- **Total patients treated:** 9
- **Patients with prior allogeneic transplant:** 7
- **Autologous (patient-derived) or allogeneic (donor-derived) lymphocytes were used**
- **Diagnoses:**
  - B-cell acute lymphoblastic leukemia (ALL) n=3
  - Mantle cell lymphoma (MCL) n=1
  - Richter transformation – DLBCL n=2
  - Primary mediastinal B-cell lymphoma (PMBCL) n=1
  - Post-transplant lymphoproliferative disorder (PTLD) n=1
  - Diffuse large B-cell lymphoma (DLBCL) n=1



Letters

RESEARCH LETTER

Allogeneic CD19/CD22 CAR T-Cell Therapy for B-Cell Acute Lymphoblastic Leukemia

JAMA Oncology June 2024 Volume 10, Number 6

Laurent Phely, MD
Luca Hensen, PhD
Christoph Faul, MD
Christer Alexander Ruff, MD
Dina Schneider, PhD
Wolfgang Andreas Bethge, MD
Claudia Lengerke, MD

Author Affiliations: Department of Internal Medicine II, Hematology, Oncology, Clinical Immunology and Rheumatology, University Hospital Tuebingen, Tuebingen, Germany (Phely, Hensen, Faul, Bethge, Lengerke); Department of Diagnostic and Interventional Neuroradiology, University Hospital Tubingen, Tubingen, Germany (Ruff); Miltenyi Biotec, Gaithersburg, Maryland (Schneider).

- **Repeated CAR-T cell infusions resulted in renewed responses (complete remissions) after relapse associated with CAR-T cell depletion.**

Outcome

	Diagnosis	CAR-T	Best Response	PFS (months)	OS (months)	Cause of death
#1 male 61y	MCL	1 x auto CAR	PD	0,9	0,9 †	Candida-Sepsis †
#2 female 58y	c-ALL	2 x auto CAR 1 x allo CAR	CR	4,1	58	
#3 male 61y	c-ALL	2 x auto CAR	CR	49	60 †	E.Coli Sepsis, COVID †
#4 female 25y	PMBCL	1 x allo CAR	PD	1,3	1,3 †	Progress, intestinal GvHD, fungal infection †
#5 male 43y	Richter-transformation (DLBCL)	1 x auto CAR	CR	13,6	26,3	-
#6 female 66y	PTLD	1 x autoCAR	CR	-	25,2	-
#7 male 66y	DLBCL	1 x auto CAR	PD	0,8	0,8 †	Progress †
#8 male 65y	Richter-t (DLBCL)	1 x auto CAR	CR	-	15,6	-
#9 female 61y	c-ALL	1 x auto CAR	CR	-	6,4	-

Dual CD19 and CD22-targeted in-house CAR-T cells in B-cell leukemias and lymphomas (Phase I study)

- Investigator -initiated trial (IIT)
- 36 patients (adults and children >12 years)
- Treatment period: 2025 to 2028
- Indications (Basket): B-cell leukemias (children) or lymphomas (adults) with CD19 and/or CD22 expression in specific clinical situations (indications outside of existing approved finished products)
- CAR-T cells: directed against two antigens (CD19 and CD22), third generation, CD28 and 4-1BB (CD137) co-stimulatory domains, manufactured in-house (GMP), manufacturing license for this study available
- Previous experience with CD19/CD22 second-generation CAR-T cells: Publication from the UKT: Phely et al, JAMA Oncology , 2024, a total of 21 patients treated in curative trials (adults and children)

Inclusion Criteria

On request PEI:
Separation of disease entities for children and adults
Children > 12 years
Start with adult cohort, then opening children

(1) Cohort 1: Adults ≥18 years: refractory/relapsed B-cell lymphoma, Richter's transformation/ Burkitt lymphoma /NHL/CLL

- After at least two of the more systemic therapies including therapy with approved bispecific antibodies or commercial CAR-T cells or with contraindication to such therapies
- Relapse after treatment with anti-CD19 or anti-CD22 targeted therapies (including CAR-T cells)
- Patients with CNS involvement if the disease is controlled at inclusion
- for auto /allo transplantation who cannot or do not want to receive this therapy (e.g. refractory diseases where CAR-T cells are used as a bridge to allo transplantation)

(2) Cohort 2 : Children 12-17 years: Acute Lymphatic Leukemia

- Disease progression with second **or** later relapse of ALL with >5% blasts **or** molecular recurrence without approved treatment option with CAR-T, bispecific or cytotoxic antibodies
- Relapse after allotransplantation or after treatment with anti-CD19 or anti-CD22 targeted therapies
- Patients recommended for allotransplantation who cannot or do not want to receive this therapy
- Patients in hematological remission after induction who cannot continue standard therapy (for medical or other reasons)
- Patients with extramedullary disease (CNS, testis , etc.), if the disease is controlled at inclusion
- Patients with Ph + ALL who are intolerant to tyrosine kinase inhibitors (TKIs), or with relapsed disease after at least 2 different TKIs

IIT Trial CD19/CD22 Phase I B-cell malignancies: study design

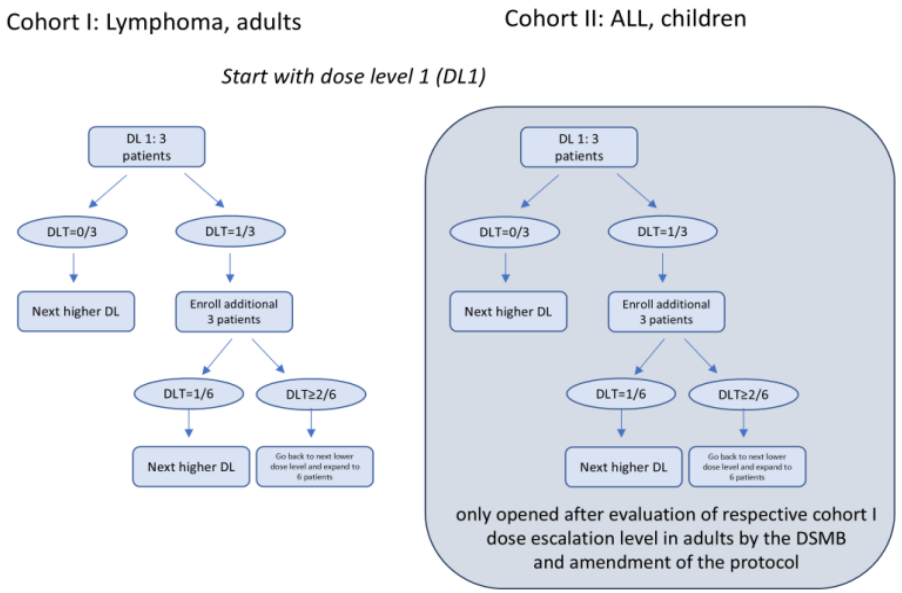


Figure 2: Dose escalation schema

Pre treatment phase			Treatment phase	*Follow-up*	
Days -45 to -15 Screening Outpatient	Day -14 Leukapheresis Outpatient	Days -5 to -3/ Days -6 to -4 Inpatient	Day 0 MB-CART19 CD19/22 Infusion Inpatient	Follow-up I Days 1 to 28 Inpatient at least until day 7	Follow-up II Months 2 to 6 Outpatient

Figure 3: Overall Study Design

Dose levels within the cohorts:

Dose level cohorts

1. $0.5x10^6$ CAR-transduced T cells/kg
2. $1x10^6$ CAR-transduced T cells/kg
3. $2x10^6$ CAR-transduced T cells/kg
0. $0.25x10^5$ CAR-transduced T cells/kg

Statistics, Safety Variables and Stopping Rules

Analysis populations

All patients who are treated with the IMP will be included in the analysis of safety/toxicity, clinical response and further secondary endpoints.

Statistical analyses

For each cohort and each dose level in each of the three dose level groups, results of dose-limiting toxicities and related safety/toxicities will be listed individually and if reasonable, also evaluated separately by using appropriate descriptive statistics. The safety and efficacy of MB-CART2219.1 in the will be further assessed in relation to prior published historical control group data, since the rarity of the patient population does not allow for fully powered randomized comparison. Safety and efficacy data of this trial will guide decisons about further development ofMB-CART2219.1 as an active agent in the respective indications.

CAR-T cell studies

Entity	Description	Framework conditions
Lymphomas/ Car-T cells	**Phase II study to evaluate the efficacy and safety of MB-CART2019.1 compared with standard therapy in participants with relapsed/refractory diffuse large B-cell lymphoma (RR DLBCL) |** DALY 2-EU	**Recruitment ended** Info: Prof. Dr. Bethge
Myeloma/ Car-T cells	**Line therapy for non-transplant eligible patients with VRD followed by ciltacabtagene Autoleucel vs VRD followed by lenalidomide maintenance |** Cartitude-5	**open** Info: Dr. Besemer
Lymphomas/ALL	**A phase I/II dose finding and efficacy study of MB-CART-CD19/CD22 in patients with relapsed/refractory B-cell malignancies**	**open** Infos: Prof. Dr. Bethge
Autoimmune Diseases	**An open-label phase I/IIa, multicentre, interventional single-arm trial of MBCART19.1 in patients with refractory SLE** ***Use of MB-CART19 also for autoimmune diseases (SLE, MS, myositis)***	**open** planned Info: Prof. Dr. Henes

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