The role of lymphocytes and IFD in the transplant setting

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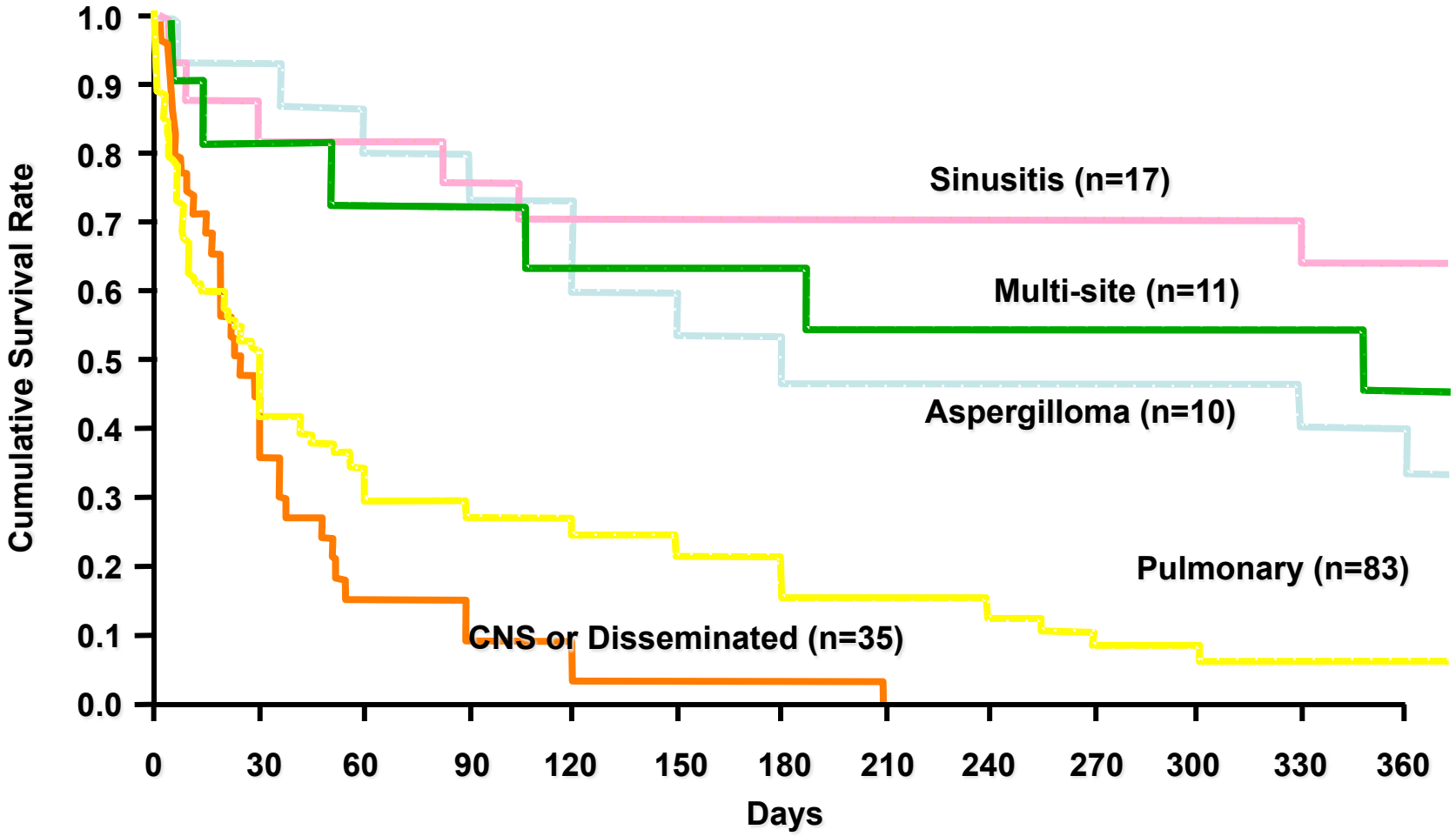
Focus on

ALLOGENEIC HSCT AND ASPERGILLUS DISEASE

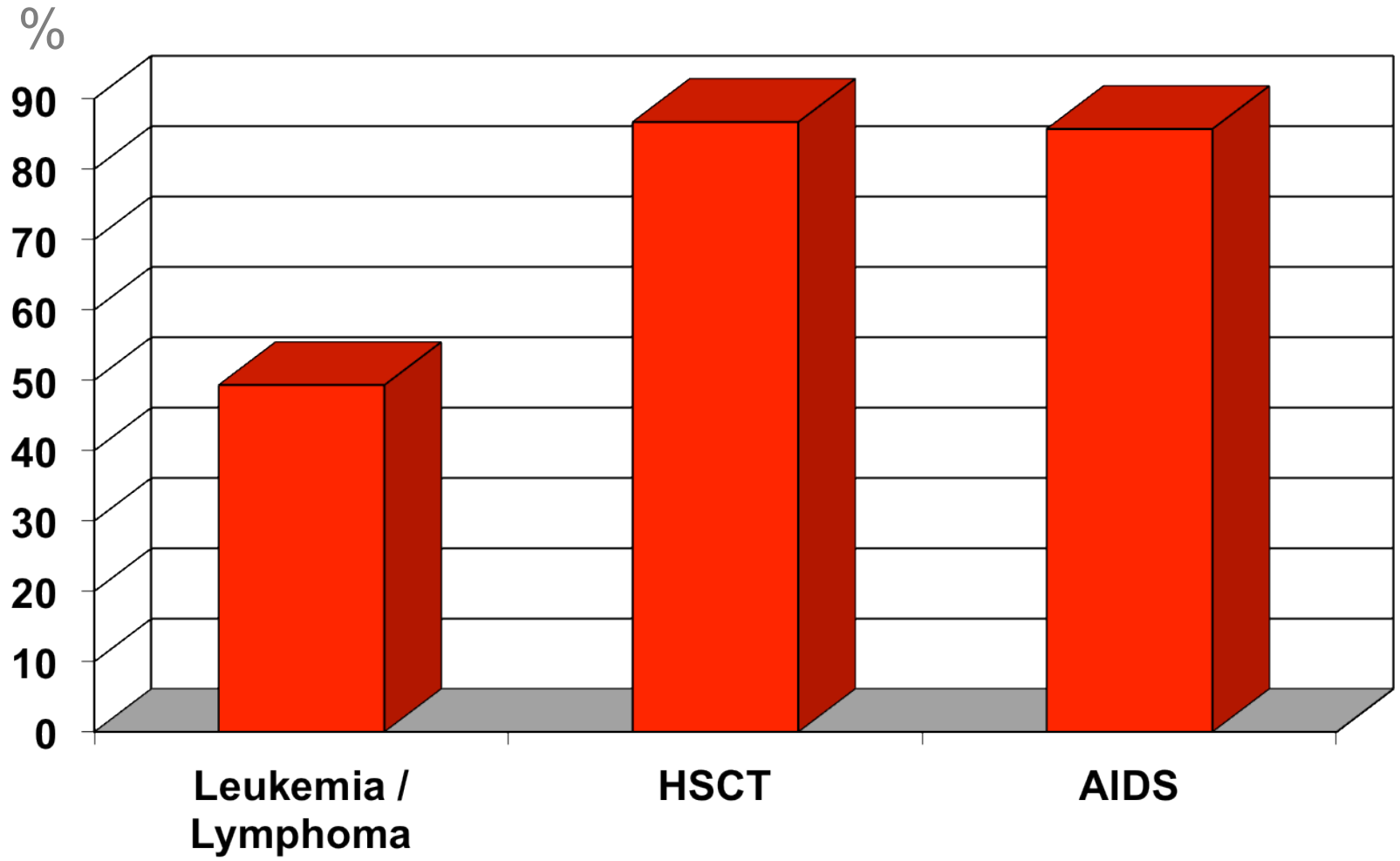
Survival: Aspergillosis with Amphotericin B

Site of Infection



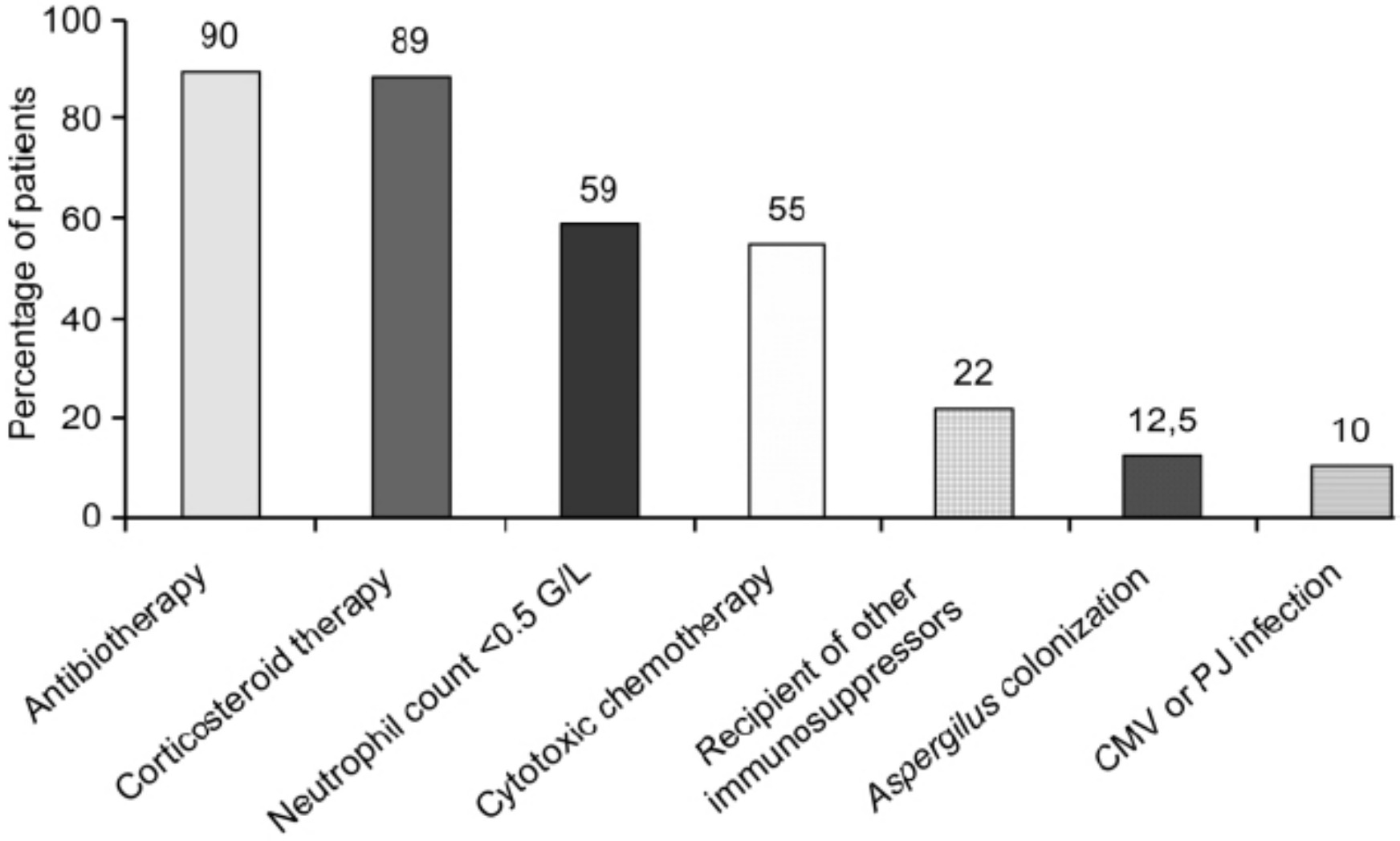
Lin et al, CID 2001; 32:358

Case fatality rate in invasive aspergillosis



Lin et al, CID 2001; 32:358

Risk factors for invasive aspergillosis



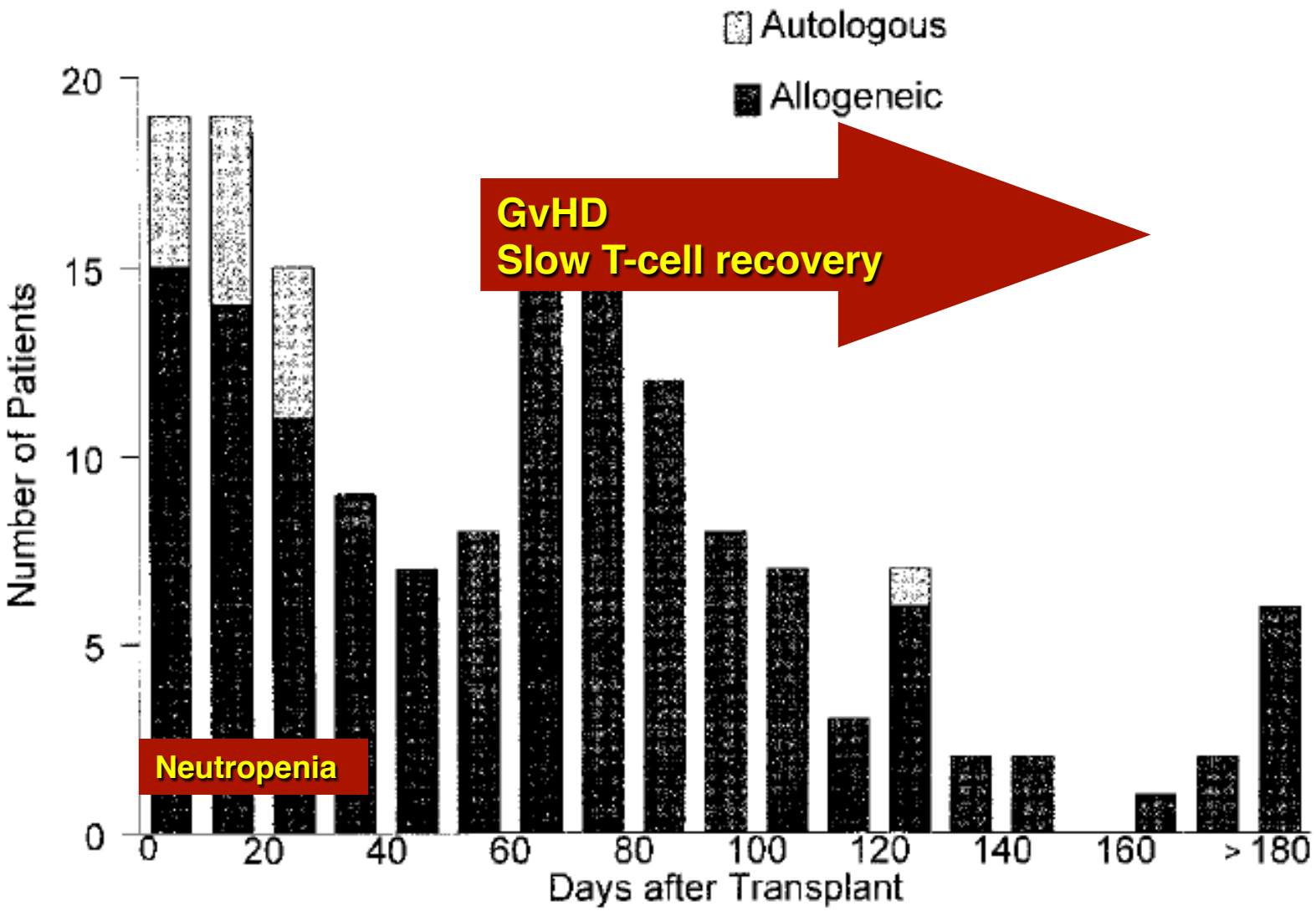
Cornillet et al. CID 2006

First Line Invasive Aspergillosis Trials

Allogeneic HSCT Patients

	Herbrecht et al, NEJM 2002	Herbrecht et al, NEJM 2002	Cornely et al. CID 2007	Herbrecht et al. BMT 2010
	Voriconazole N=37	cAmB N=30	L-AmB-3mg N=17	Caspo 70/50mg N=24
Disease Definition Proven/probable IA	**Modified** criteria Halo/air crescent sign only allowed	**Modified** criteria Halo/air crescent sign only allowed	**Modified** criteria Halo/air crescent sign only allowed	**NOT MODIFIED**
Favorable response	**32%**	**13%**	**47%**	**42%**
Complete response Defined as resolution:	> 90% all lesions and symptoms	> 90% all lesions and symptoms	All lesions and symptoms as Herbrecht	All lesions and symptoms as Herbrecht
Partial response	Clinical improvement and resolution 50% lesions	Clinical improvement and resolution 50% lesions	„Softer“ radiologic endpoints (Denning, CID 2007) halo sign disappear, lesions decrease size	Clinical improvement and resolution 50% lesions

Aspergillus: Time to Diagnosis of Aspergillosis after HSCT



Wald et al. JID 1997

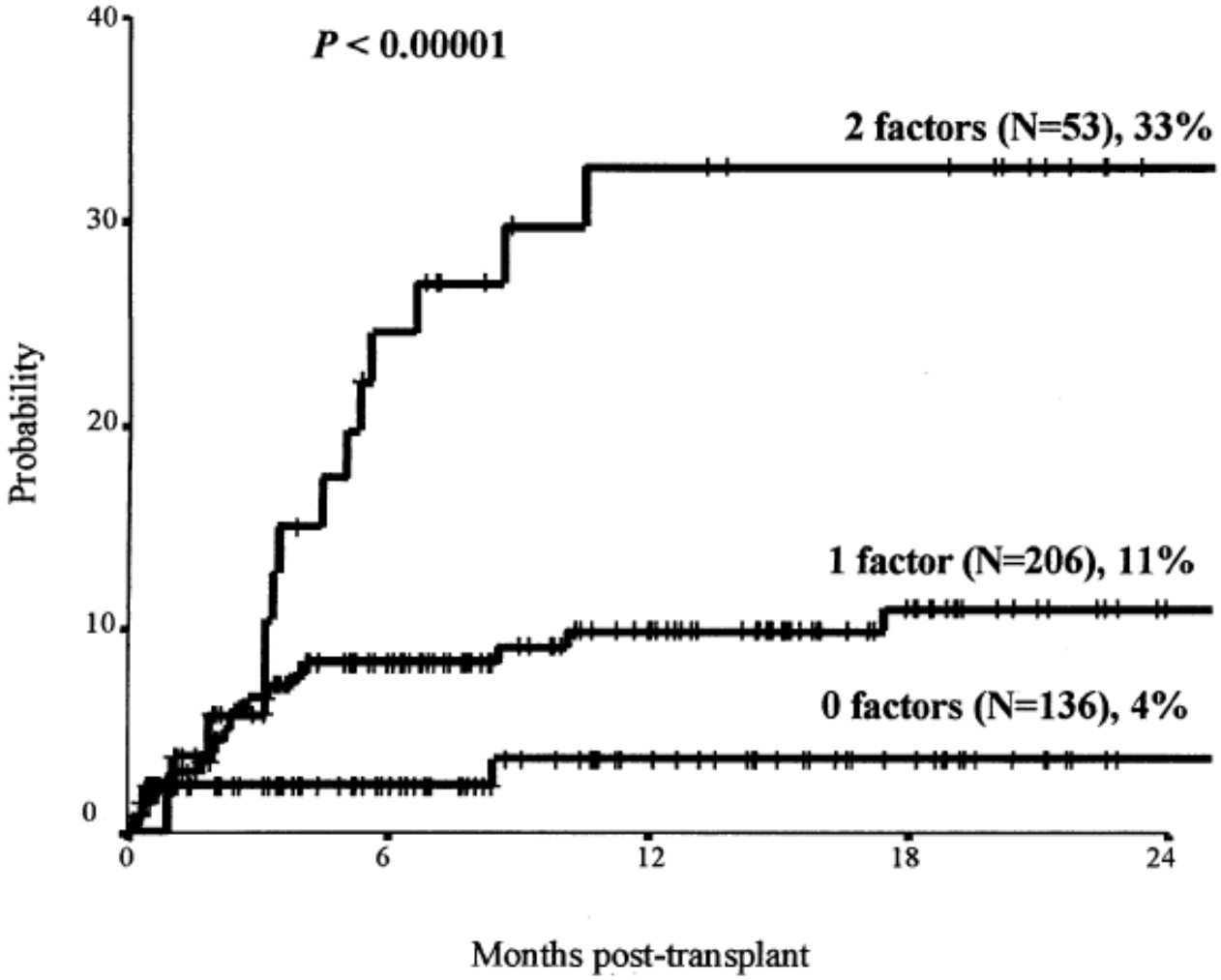
Invasive Fungal Infections in allogeneic HSCT

Risk Factors in Relation to Incidence

Overall incidence rate of IFI in 395 allogeneic HSCT: 14%

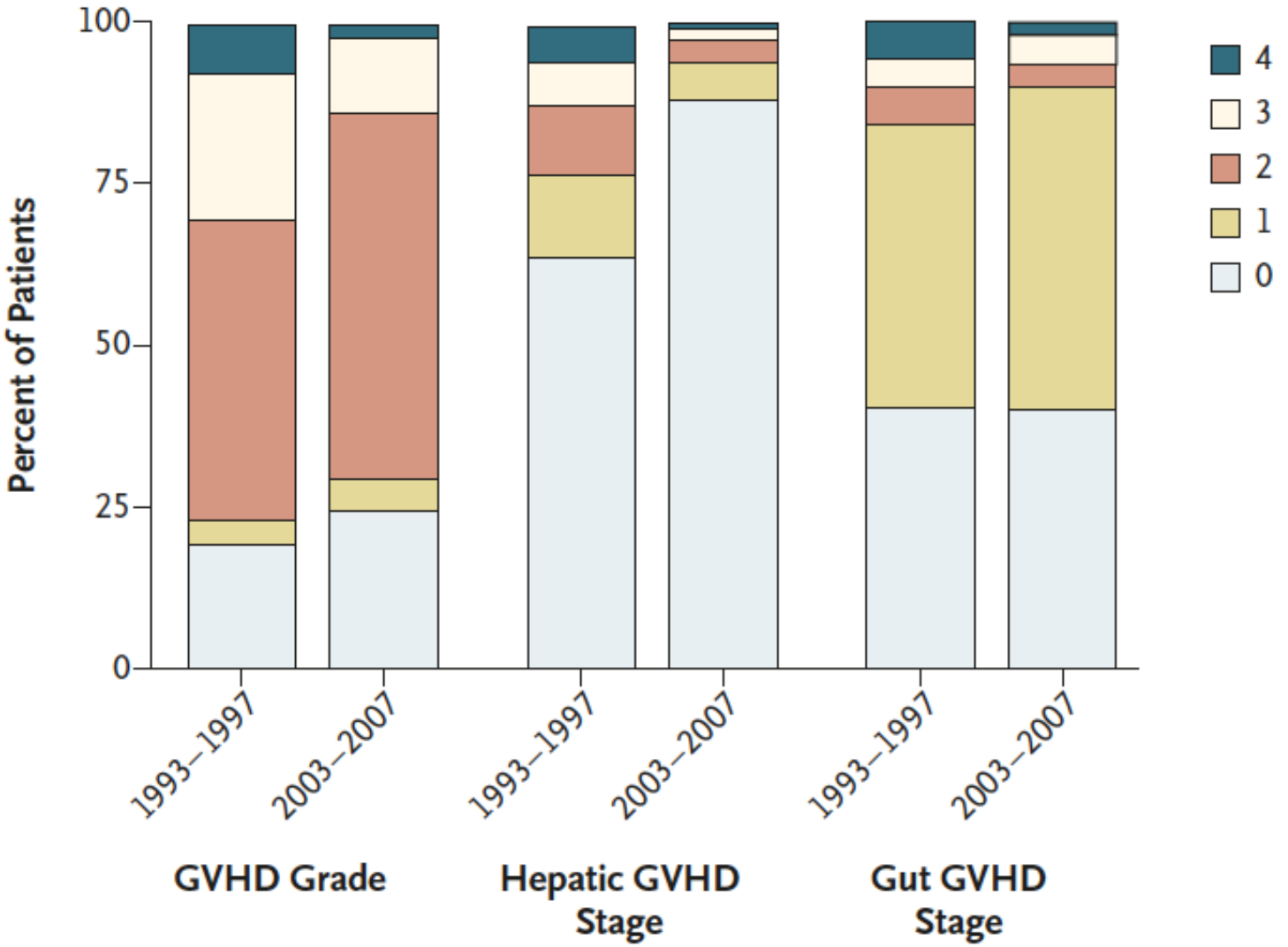
Risk Factors:

- **Steroid prophylaxis**
- **Moderate-to-severe GVHD**



Martino et al. Brit J Haema 2002

Changes of GVHD and IFI over time



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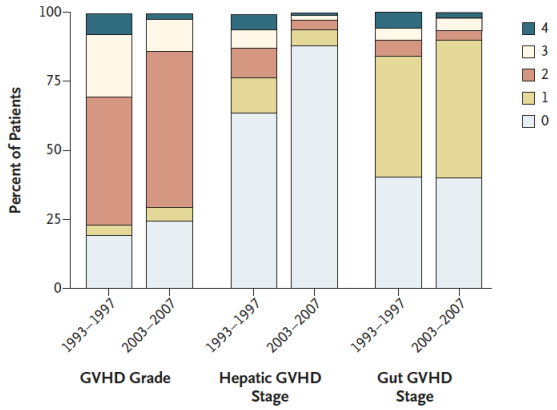
Gooley TA et al. NEJM 2010

Changes of GVHD and IFI over time



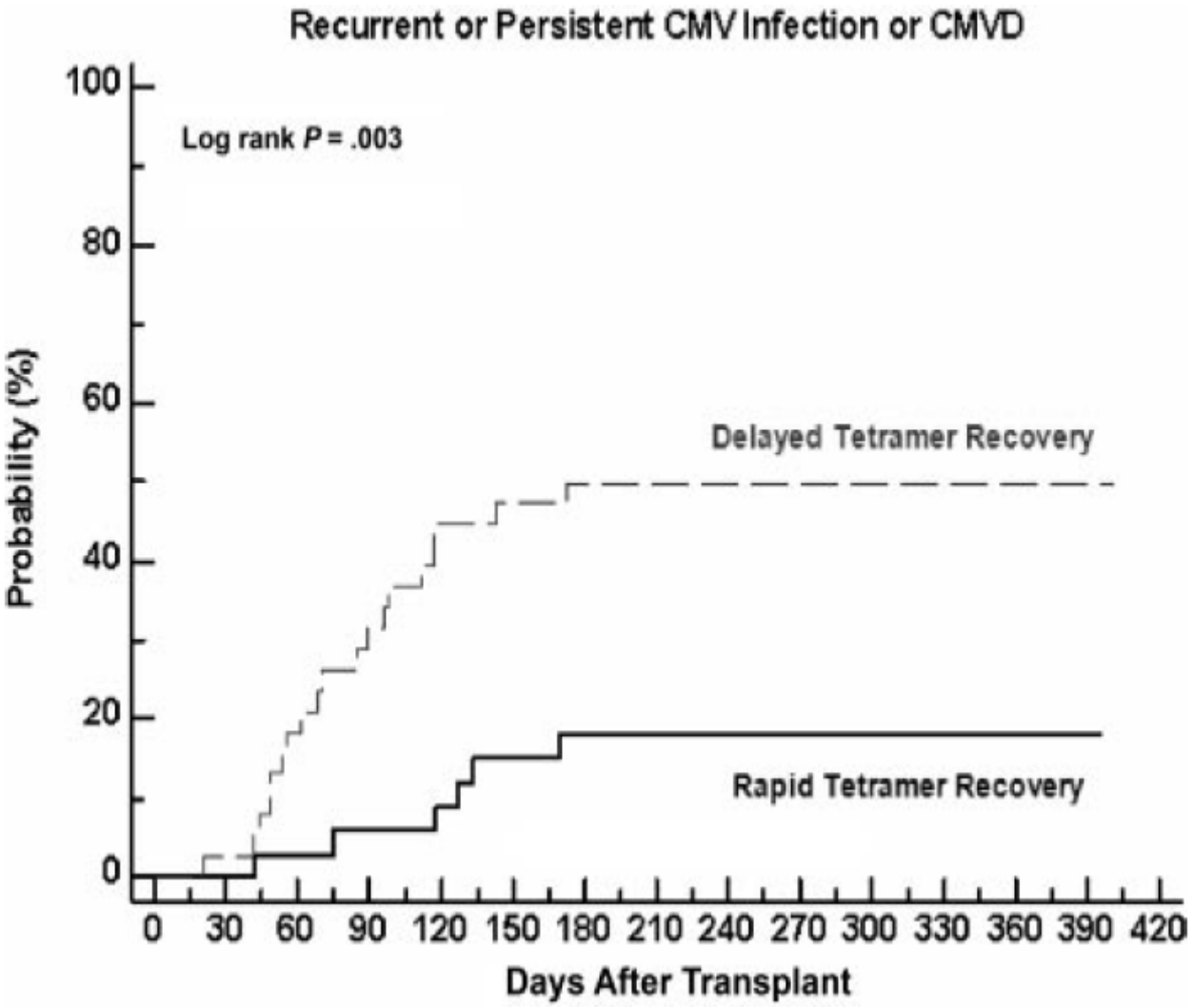
Variable	1993–1997 (N = 1418), n (%)	2003–2007 (N = 1148), n (%)	Adjusted Hazard or Odds Ratio (95% CI)	P-value	Patients Who Underwent Myeloablative Conditioning	P-value
Invasive mold infection	125 (9)	80 (7)	0.49 (0.35–0.71)	<0.001	0.55 (0.38–0.78)	<0.001
Invasive candida infection	99 (7)	10 (1)	0.12 (0.06–0.25)	<0.001	0.15 (0.08–0.29)	<0.001

Gooley TA et al. NEJM 2010

How can we learn from CMV?

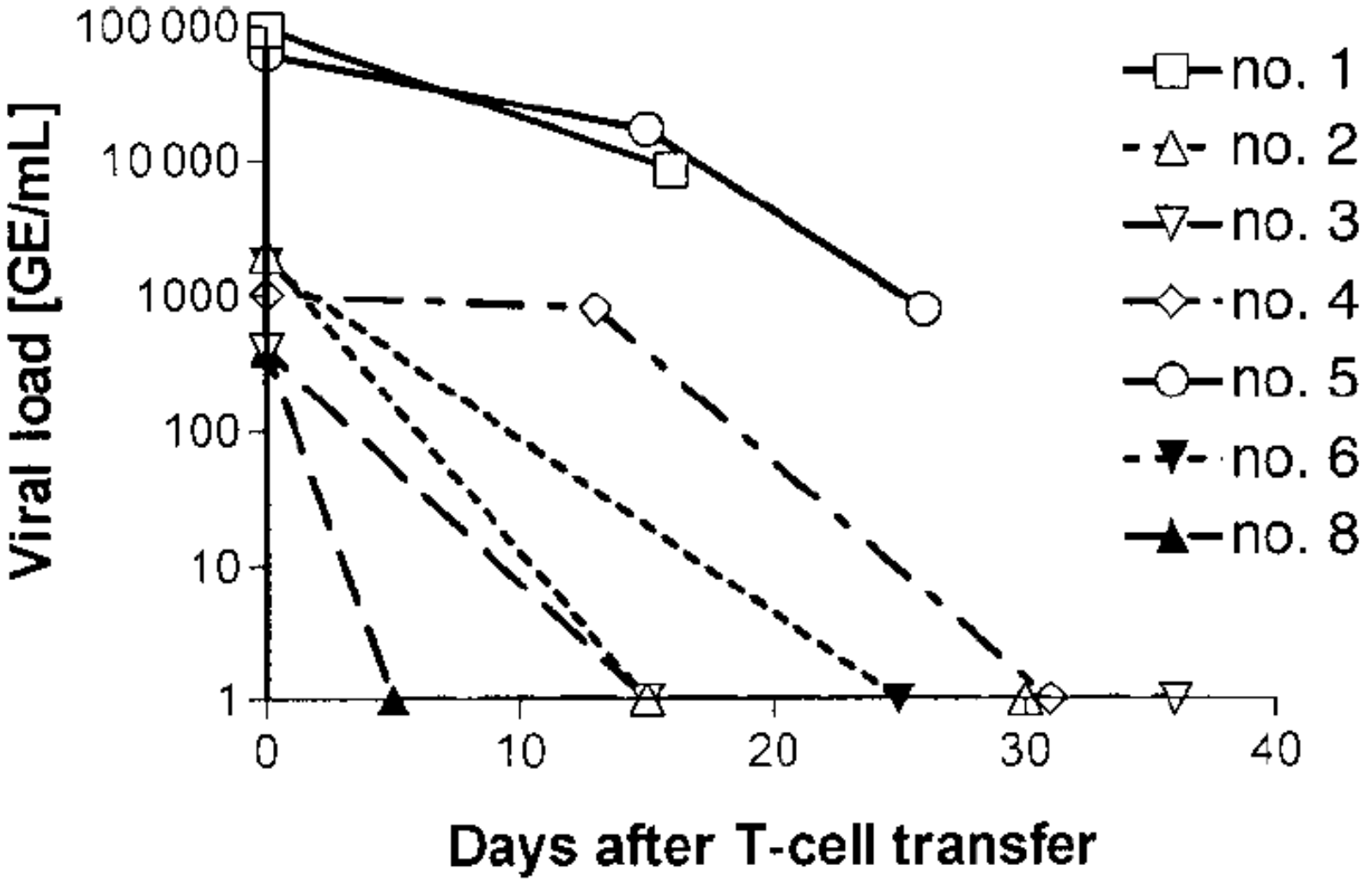
- Prophylactic / Preemptive Therapy
- Risk adapted Treatment
- Prevent and avoid Resistence
- Role of the Immune Response
- Immunotherapy

Role of the Immune Reconstitution



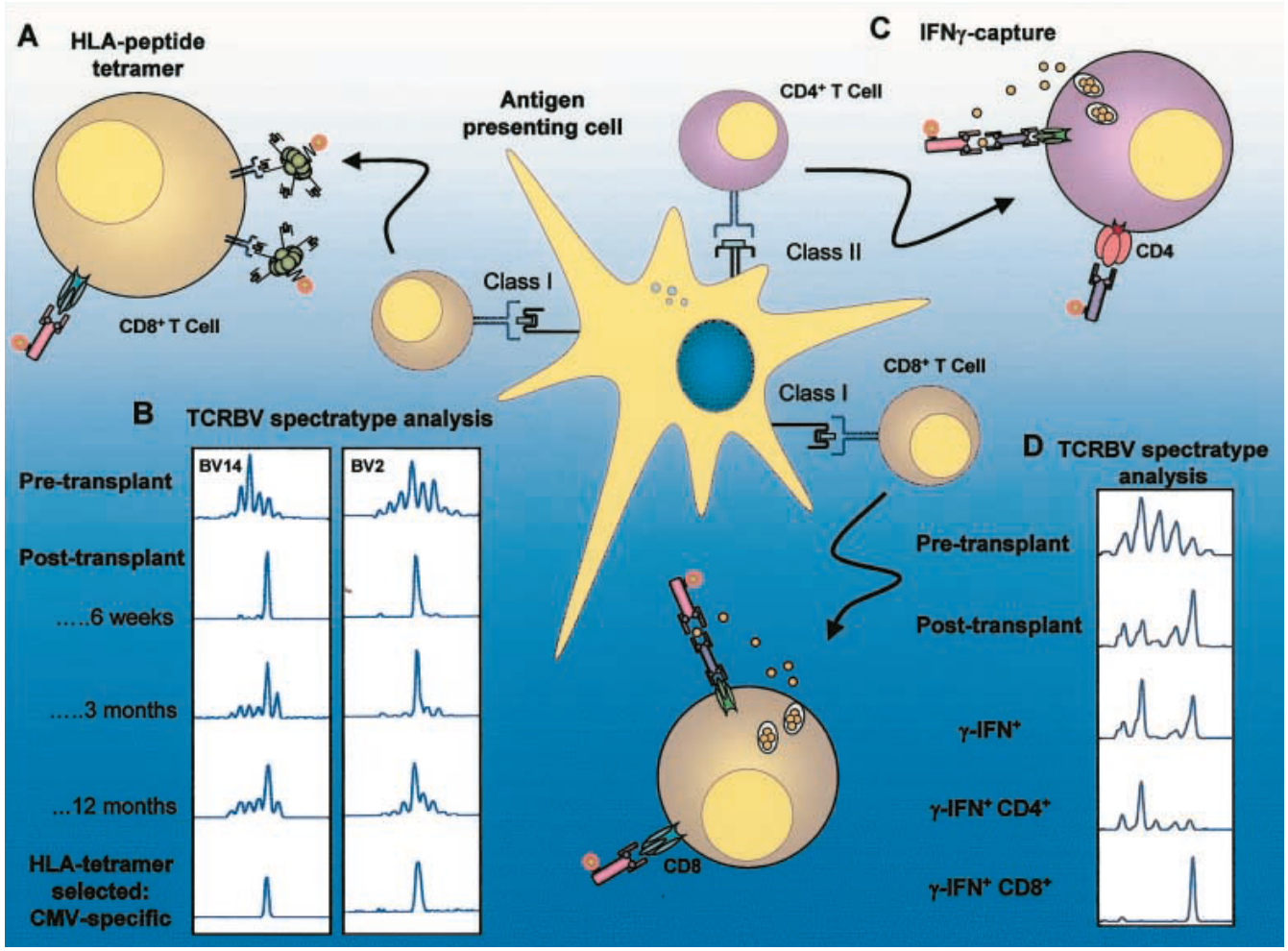
Gratama JW. et al *Blood* 2010

Decrease in CMV load following T-cell therapy



Einsele H et al. Blood 2002

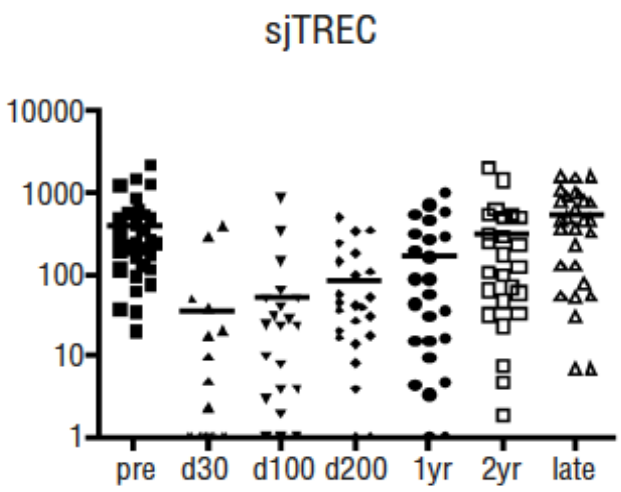
Reconstitution of antigen-specific responses following stem cell transplantation (Serial TCRBV spectratype analyses following transplantation show reduced spectratype diversity with gradual return to more diverse profiles over subsequent months/years)

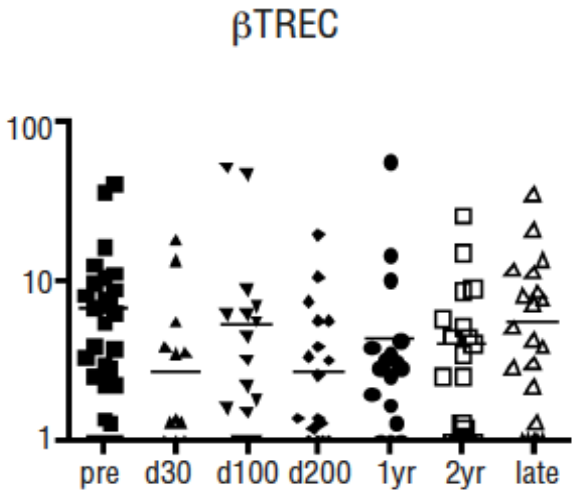


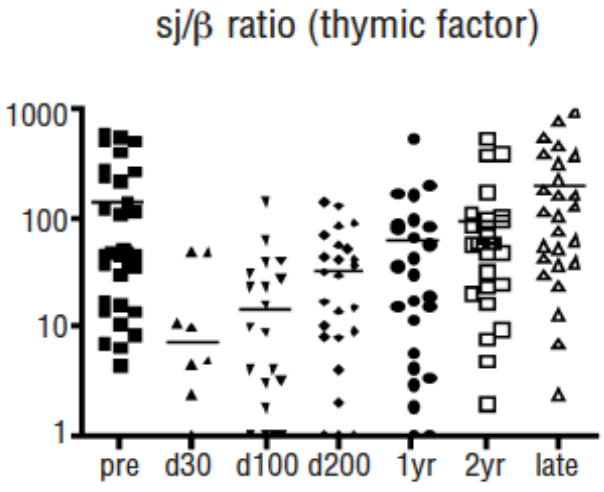
Peggs et al. 2004, British Journal of Haematology, 124, 407–420

T-cell neogenesis: Reconstitution kinetics of TREC and the thymic factor

Pooled data from the 66 patients in the cross-sectional study







Ringhoffer et al. Haematologica 2013

Major single nucleotide polymorphisms associated with susceptibility to fungal infections and diseases

Gene	SNPs or haplotypes	SNP effect	Disease	Outcome
CARD9	Q295X	Low numbers of T_H17 cells	Chronic mucocutaneous candidiasis	Susceptibility
CXCL10	+11101C/+1642G/ −1101A	Reduced chemokine production by DCs exposed to *Aspergillus fumigatus*	Invasive aspergillosis	Susceptibility
DECTIN1	Y223S	Reduced zymosan-binding capacity and IFNγ production	Oropharyngeal candidiasis	Resistance
	Y238X	Decreased cell surface expression, β-glucan-binding capacity and impaired cytokine production	Chronic mucocutaneous candidiasis, *Candida albicans* colonization and invasive aspergillosis	Susceptibility
DEFB1	−44G	Unknown	*C. albicans* carriage	Resistance
IFNG	+874TT	Increased levels of IFNγ	ABPA, CCPA	Susceptibility
IL1RN *IL1A* *IL1B*	VNTR2/−889C/−511T	Increased levels of C-reactive protein	Invasive aspergillosis	Susceptibility
IL4	−1098T/−589C/−33C	Unknown	Chronic disseminated candidiasis	Susceptibility
	−589T	Increased levels of vaginal IL-4 and reduced levels of nitric oxide and MBL	Recurrent VVC	Susceptibility
	−589T	Reduced levels of IL-4	Paracoccidioidomycosis	Susceptibility
IL4R	I75V	Upregulation of CD23 expression	ABPA	Susceptibility
IL10	−1082AA −1082A/−819C/−592C	Reduced levels of IL-10	Invasive aspergillosis	Resistance
	−1082A	Reduced levels of IL-10	CCPA	Susceptibility
	−1082GG	Increased levels of serum IL-10	ABPA, *A. fumigatus* colonization	Susceptibility

Luigina Romani Nat Rev Immun 2011

Major single nucleotide polymorphisms associated with susceptibility to fungal infections and diseases

IL15	+13689A	Increased levels of IL-15	ABPA, CCPA	Susceptibility
IL23R	R381Q	Impaired production of IL-17A	Invasive aspergillosis	Resistance
MASP2	D105G	Impaired MBL function	Invasive aspergillosis	Susceptibility
MBL2	O/O, A/O	Impaired MBL activity	Invasive aspergillosis, CCPA, VVC	Susceptibility
	LXA/O	Reduced levels of circulating MBL	Invasive aspergillosis	Susceptibility
	+1011A	Elevated plasma MBL levels and high peripheral blood eosinophilia	ABPA	Susceptibility
NLRP3	Length polymorphism (allele 7)	Impaired production of IL-1β	Recurrent VVC	Susceptibility
PLG	D472N	Predicted to enhance plasminogen binding to *A. fumigatus*	Invasive aspergillosis	Susceptibility
SFTPA2	A91P, R94R	Increased levels of total IgE and eosinophilia	ABPA, CCPA	Susceptibility
TGFB1	+869C	Decreased levels of TGFβ	CCPA	Susceptibility
TLR1	R80T, N248S	Unknown	Invasive aspergillosis	Susceptibility
TLR4	D299G/T399I	Predicted to impair the ligand–binding domain	Invasive aspergillosis, *A. fumigatus* colonization, CCPA, *C. albicans* systemic infections	Susceptibility
TLR6	S249P	Unknown	Invasive aspergillosis	Susceptibility
TLR9	T–1237C	Increased NF-κB binding affinity	ABPA	Susceptibility
TNF	–308G	Decreased levels of TNF	ABPA, CCPA	Susceptibility
TNFR1	+36G, –609T	Decreased levels of *TNFR1* mRNA	Invasive aspergillosis	Susceptibility
TNFR2	VNTR at –322	Unknown	Invasive aspergillosis	Susceptibility

Luigina Romani Nat Rev Immun 2011

CXCL10 Polymorphisms = Risk Factor for Invasive Aspergillosis

- CC chemokine which binds and activates CCR6
- **CXCL10 strongly chemotactic for lymphocytes and weakly attracts neutrophils**

-> 3 SNPs in *CXCL10 are significantly* associated with IA

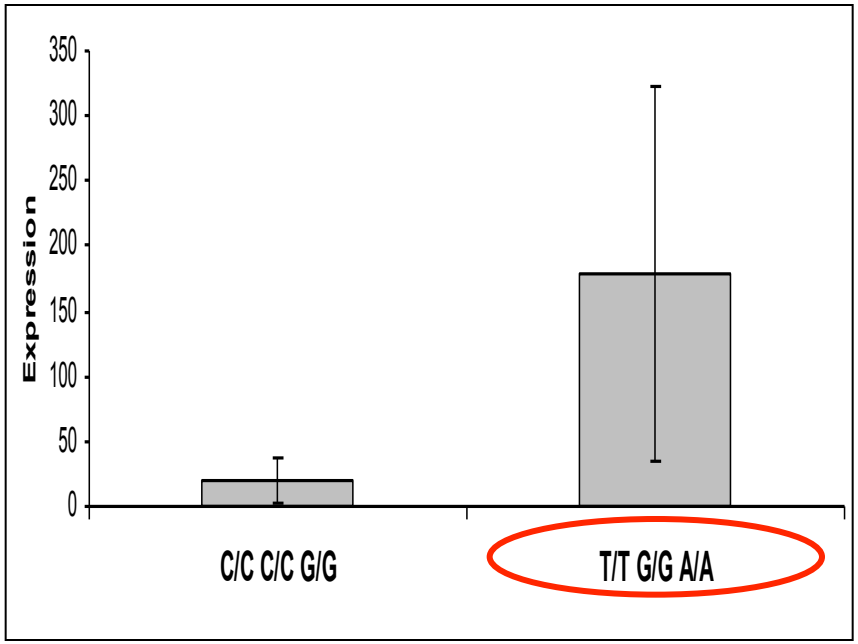
Gene	dbSNP number	Nucleotide position	Allele-frequency-difference-test (p-value)*
CXCL10	rs1554013	11101 C/T	**0.007**
	rs3921	1642 C/G	**0.003**
	rs4257674	-1101 A/G	**0.001**

*In cDNA library of patients after alloSCT:
58 without IA
81 with IA

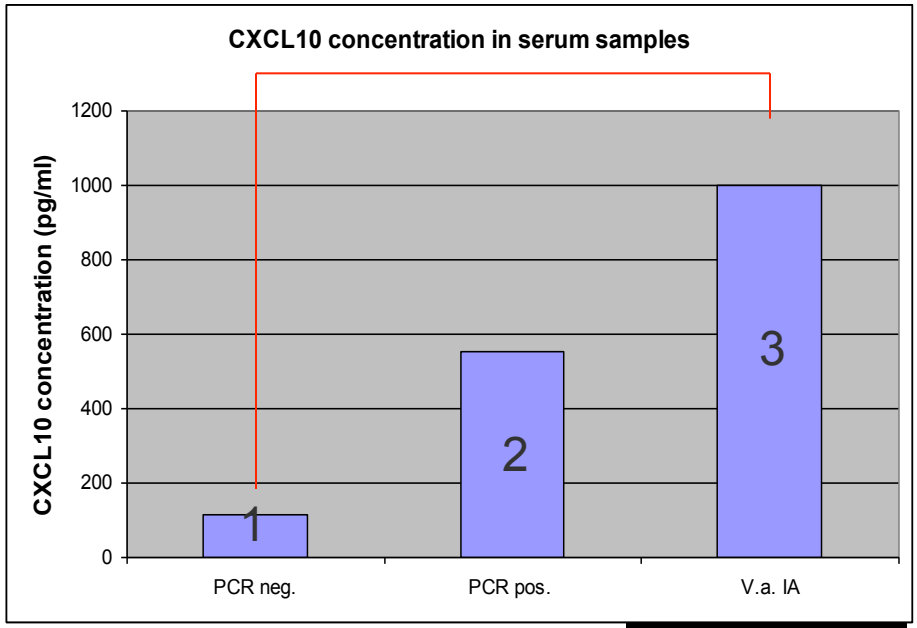
Mezger et al., *Blood* 2008

CXCL10 Polymorphisms = Risk Factor for Invasive Aspergillosis

CXCL10 gene expression after exposure to A.fumigatus



CXCL10 levels in serum



Aspergillus infection

Expression of *CXCL10* is significantly higher in DCs from persons carrying wildtype allels

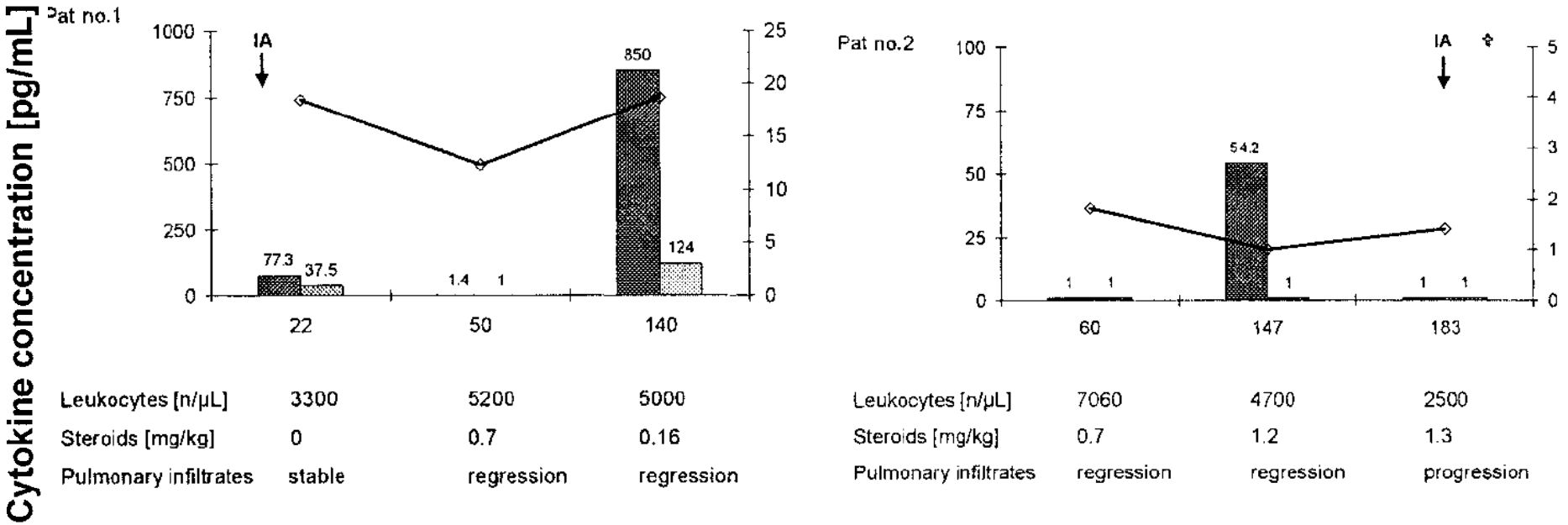
CXCL10 levels are significantly higher in patients exposed to A.fumigatus when compared to healthy controls

→ Patients with a CXCL10 wildtype show a higher production of CXCL10 upon exposure to A. fumigatus → better Protection against IA

New Options for Treatment of IFI

- Risk Stratification
- Treatment
  - Prophylaxis
  - early therapy
  - combination
- Challenge resistance
- **Immunotherapy for IFI?**

Longitudinal analysis of Aspergillus-specific T-cell responses in patients with invasive aspergillosis after allogeneic SCT



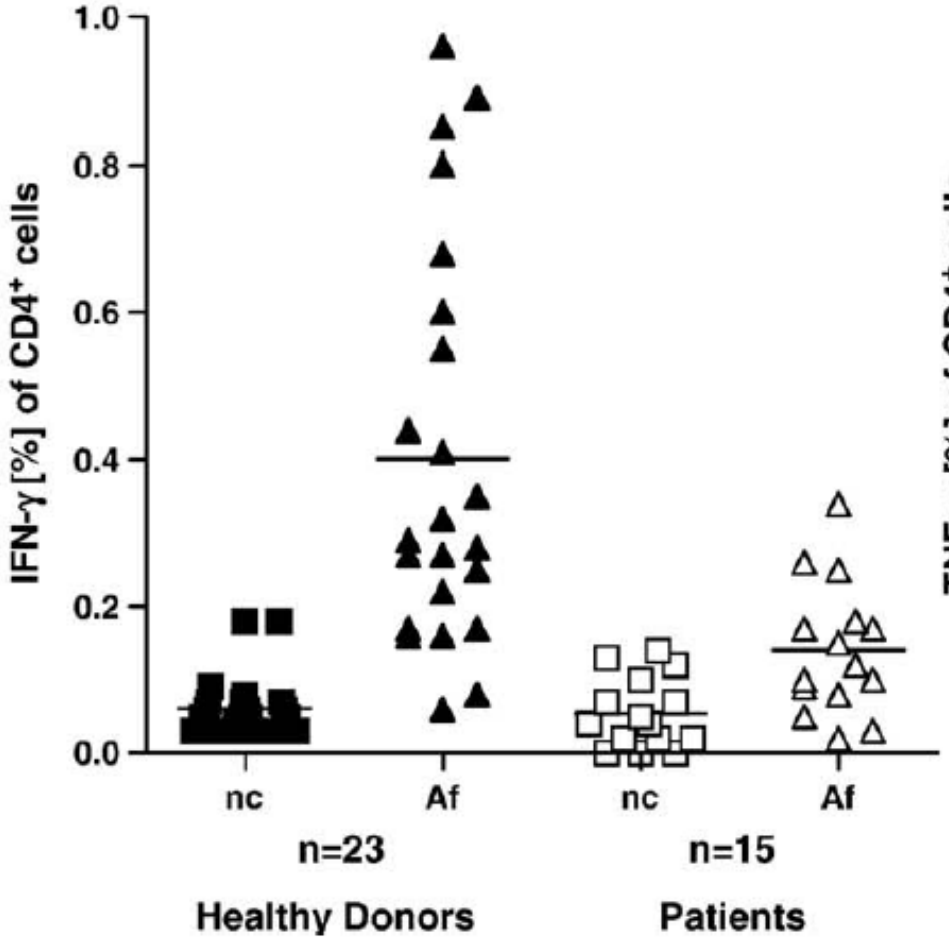
Long-term control of invasive aspergillosis was associated with a lymphoproliferative response to EC SAB and a dominant release of IFN-Y (patient no. 1), whereas a low SI (patient no. 2)

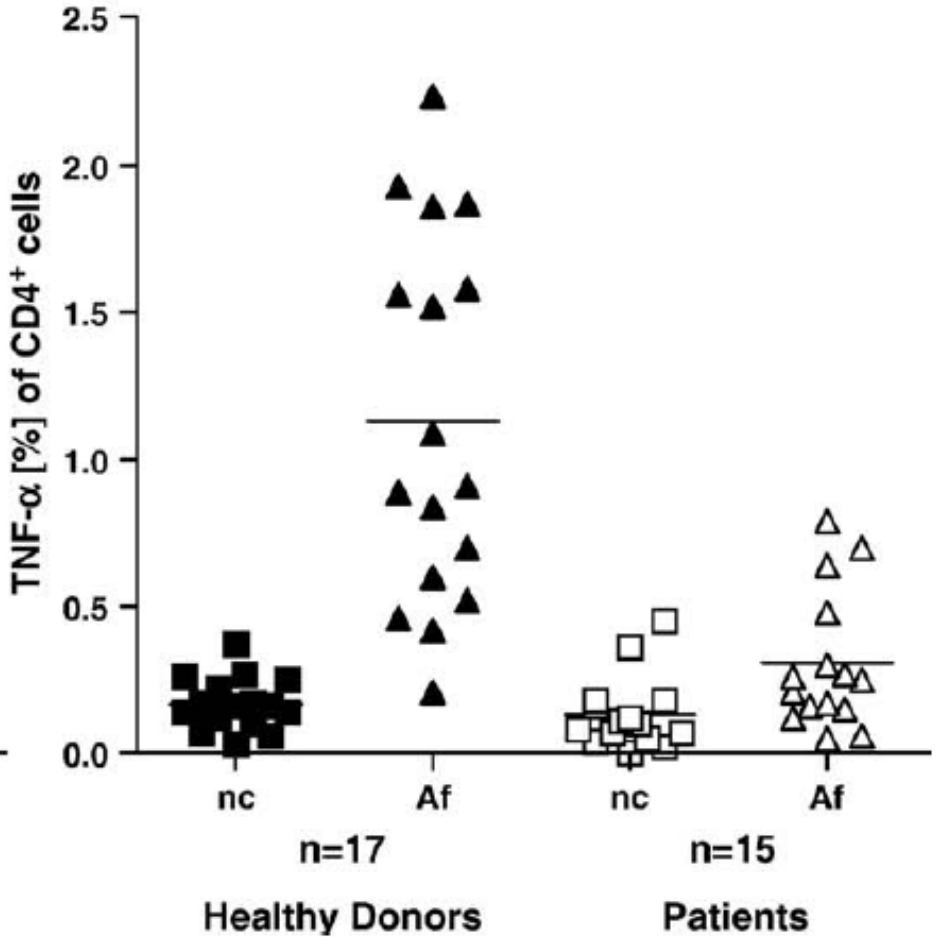
■ IFN-Y; and ■ IL-10

Hebert et al. Blood 2002

After one year: Frequency of CD4+ T-cells responding to *A. fumigatus* antigens with intracellular production of IFN-γ and TNF-α

CD3/CD4 cells





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Beck O et al, Journal of Immunological Methods 335 (2008) 41–45

T Cell Vaccination in Mice with Invasive Pulmonary Aspergillosis

Adoptive transfer of Aspergillus Ag-specific CD4+ T cells

CD4$^+$ T Cells from[a]	*A. fumigatus* infection[b] MST[c]	D/T[d]
Untreated	6.5	18/18
CCFA-treated	16*	14/16
Asp f 2-treated	5	16/16
—[e]	5	18/18

[a] Splenic CD4$^+$ T cells were from BALB/c mice either untreated or treated with *A. fumigatus* CCFA or recombinant 37-kDa protein (Asp f 2) before intranasal infection with *Aspergillus* conidia (see *Materials and Methods* for details). Two weeks later, cells were restimulated in vitro with the relevant Ag and purified spleen dendritic cells for 3 days before being transferred into naive recipients (5×10^5 cells i.v.).

[b] Mice were i.v. infected with 5×10^6 *A. fumigatus* conidia, 1 day after adoptive transfer of CD4$^+$ cells.

[c] MST, median survival time (days).

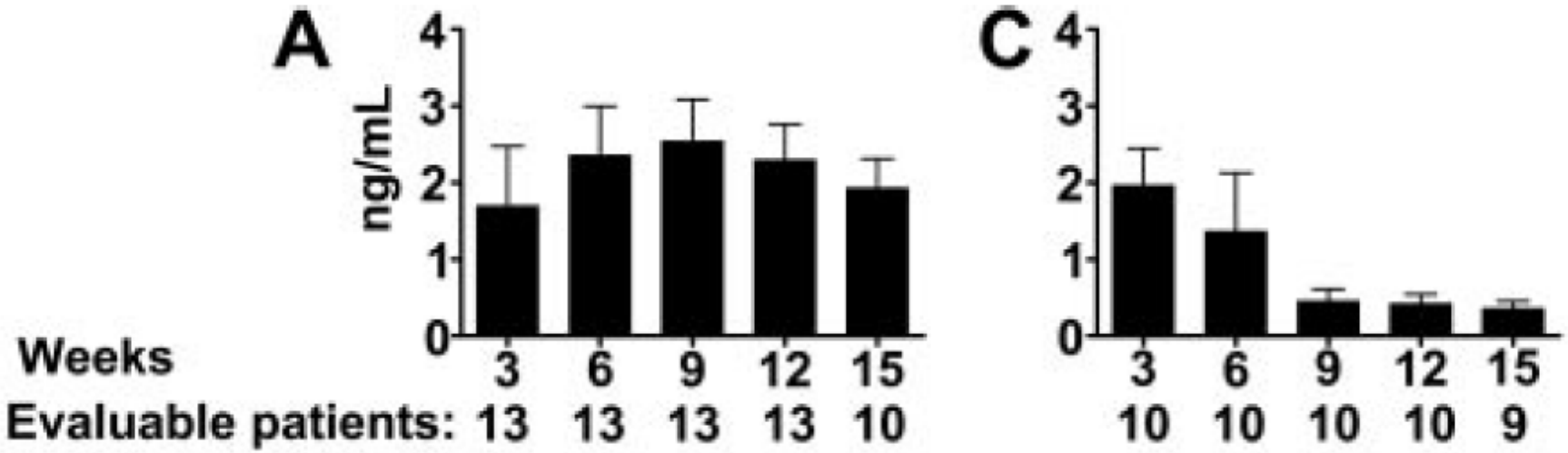
[d] D/T, number of dead animals over total animals infected.

[e] Mice that did not receive transfer of cells.

*, $p < 0.01$ (CCFA-treated vs untreated mice) according to Student's *t* test.

Cenci E. et al. J Immunol. 2000

Aspergillus antigenemias in 10 transplant patients who received T-cell therapy and in 13 control transplant recipients.



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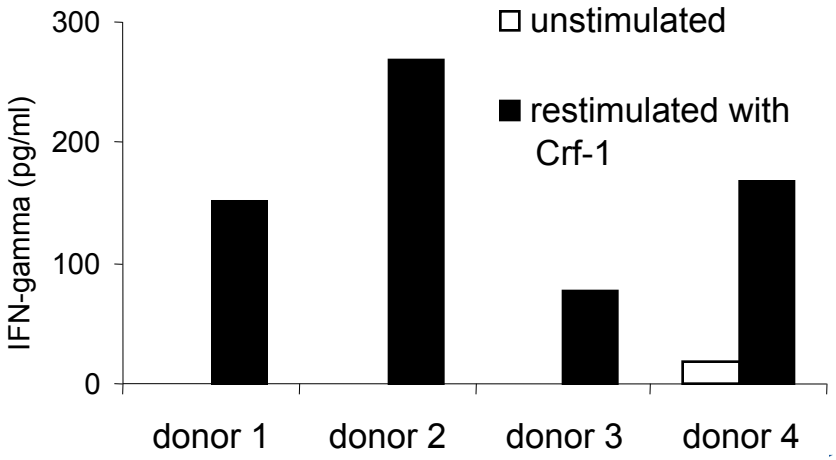
Perruccio K et al. Blood 2005

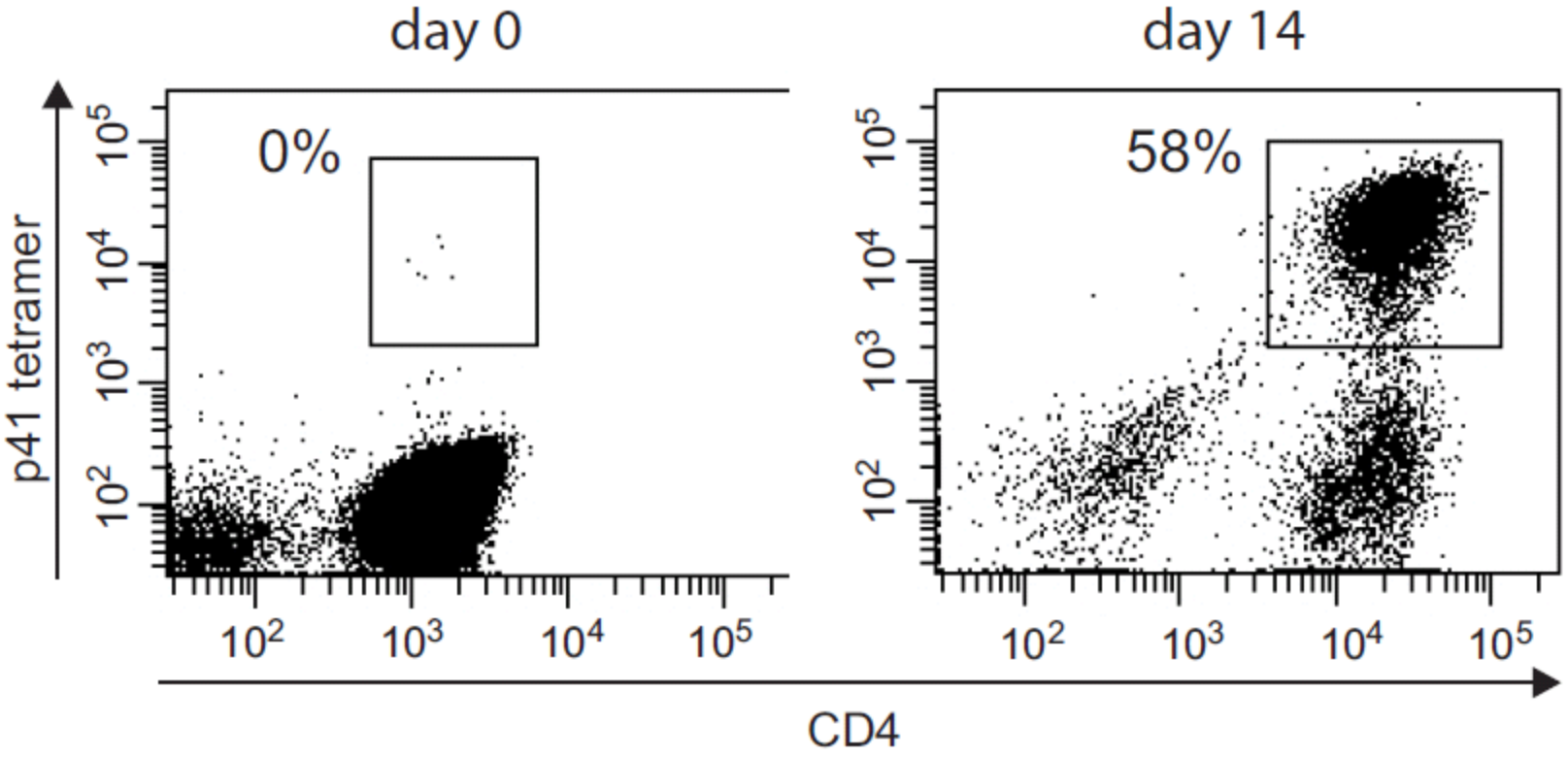
Crf-1 as candidate antigen

Antigen	IFN-γ response in healthy donors
catalase	1/8 donors weakly positive
Gel1	2/10 donors positive
superoxide dismutase	1/8 donors weakly positive
peptidase	5/10 donors positive
AspF1	1/9 donors positive
CipC	1/6 donors weakly positive
Crf1	**6/7 donors positive**



Stuehler et al., *Blood* 2011

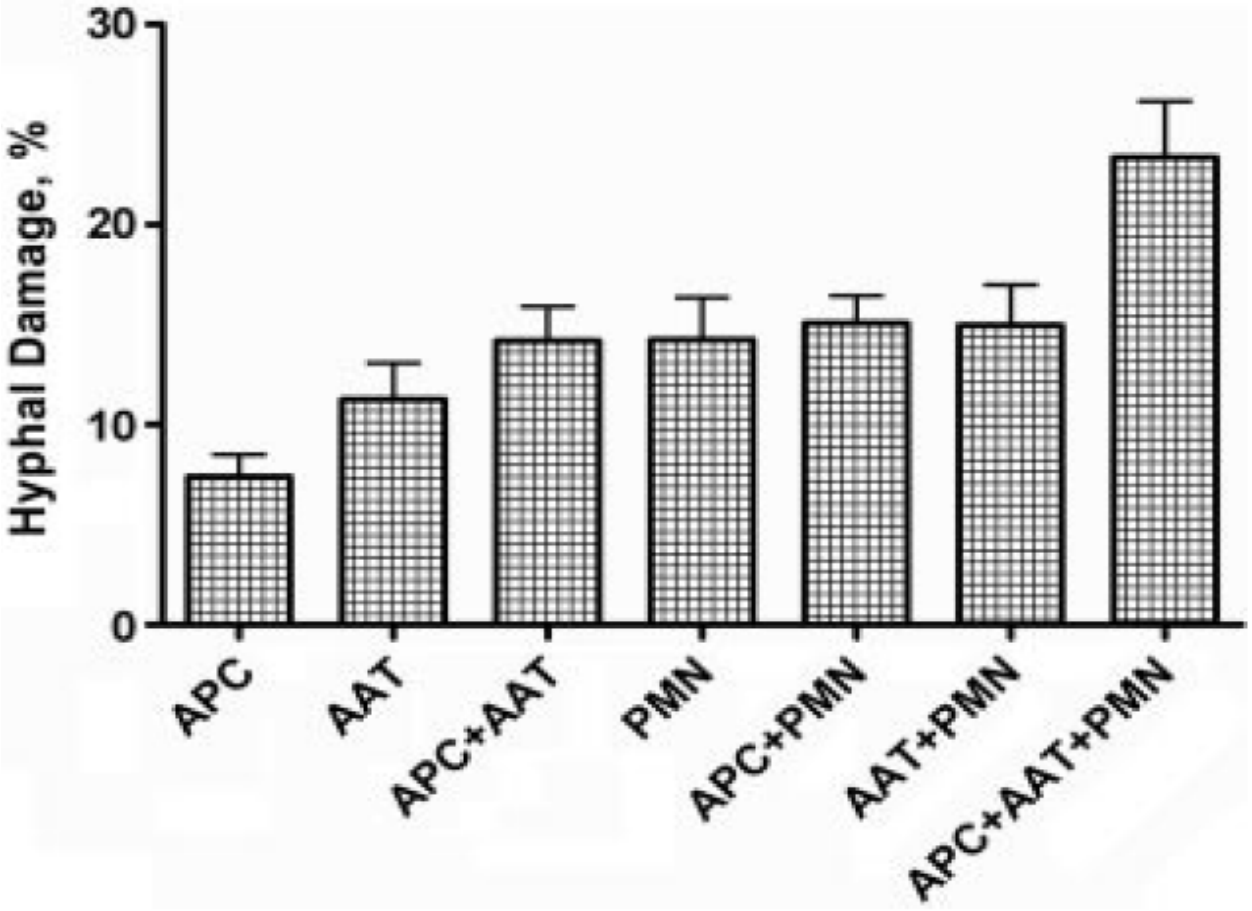
Protocol for generation of Aspergillus (Crf-1)-specific T-cell lines



Absolute cell count (x10^7), median (range)	**1**	**0.85 (0.53-1.18)**	**3.5 (3-49)**

Stuehler et al., *Blood* 2011

T cells and PMNs interact in inducing hyphal damage



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Beck O et al Blood 2006

Clinical-scale generation of multi-specific anti-fungal T cells targeting *Candida, Aspergillus* and mucormycetes

- Generation multi-specific human antifungal T cells after simultaneous stimulation with cellular extracts of *A. fumigatus*, *C. albicans*, and *Rhizopus oryzae*
- Activated memoryT_H1 cells and reproducibly responded with IFN-γ production to a broad-spectrum of medically important fungal pathogens
- Upon restimulation, the generated T cells proliferated and enhanced antifungal activity of phagocytes, and showed reduced alloreactivity as compared to the original cell fraction.

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Lars Tramsen et al. Cytotherapy 2012

Zusammenfassung

- Mortality remains high in targeted therapy
- Early treatment/prophylaxis is an alternative option
- Costs
- GvHD/lymphocytopenia is an additional risk factor for IA
- anti-Aspergillus T-cells are needed to fight of aspergillosis
- T_H1 appears to be protective
  - How to expand donor-derived anti-*Aspergillus* T-cells in invasive aspergillosis?
  - Which antigens?
  - Timing?
  - How many cells are needed?
  - How to prevent alloreactivity?
- Option as additional therapeutic option to the existing (on-going) antifungal therapy