Cultivated anti-*Aspergillus* T_H1 Cells

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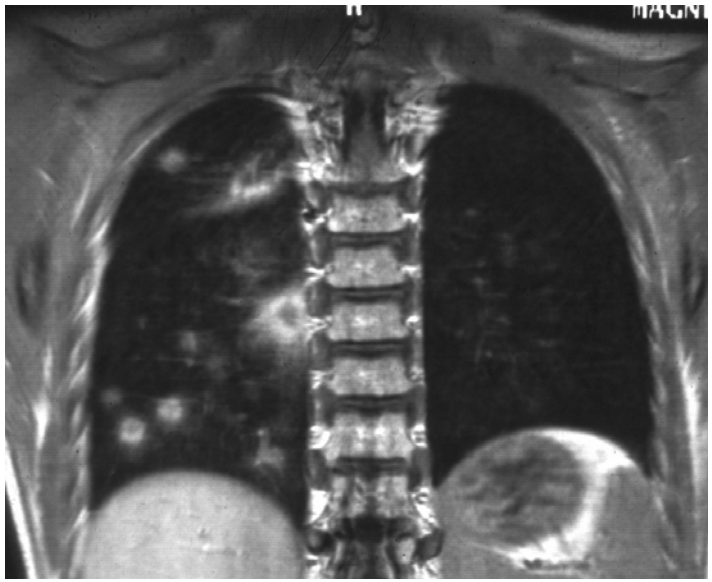
Invasive fungal infection after allogeneic SCT

Incidence of proven invasive fungal infections after allogeneic SCT ~15 %

Mortality 50% to 90 %

A.fumigatus, less frequently *A.flavus* or *A.terreus* seen as causing pathogen



Hebart et al. *Support Care Cancer* 2004
Lin et al. *Clin Infect Dis* 2001

Risk factors for invasive aspergillosis

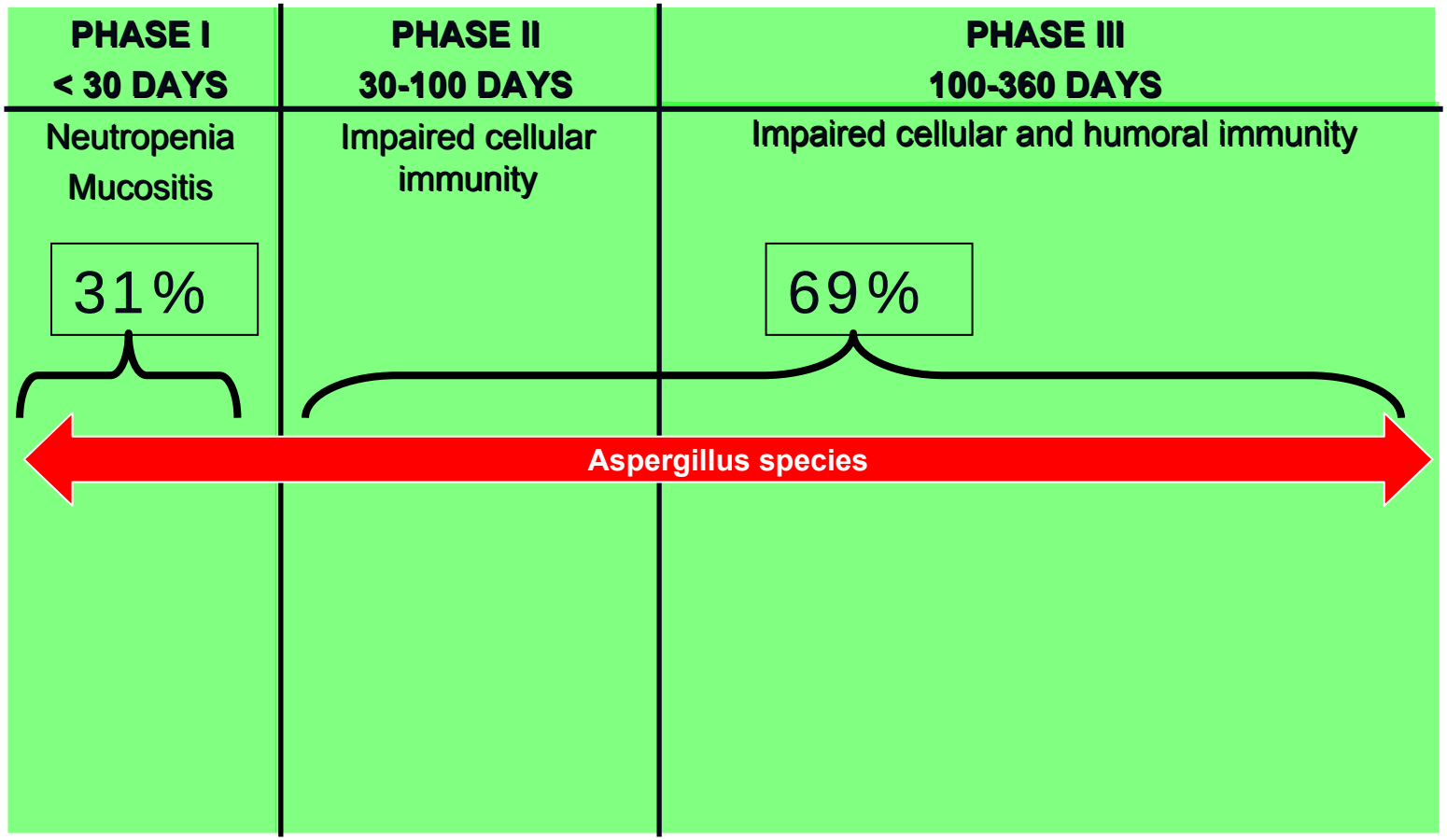
Exposure

Severe mucositis
Broad-spectrum antibiotics

Prolonged neutropenia
Defects of phagocyte function (e.g., steroids)

Defects of adaptive immunity (e.g., T-cell deficiency)

Invasive aspergillosis after SCT



American Society for Blood and Marrow Transplantation, 2000
Wald et al. J Infect Dis 1998

T-cells and invasive fungal infection

- *Aspergillus* fumigatus antigens are capable to induce T_H1 (IFN-γ, IL-2, TNF-α) or T_H2 response (IL-4, IL-5, IL-10)
- Patients with invasive aspergillosis and T_H1 response (increased IFN-γ, low IL-10) have a better outcome than patients with T_H2 response (low IFN-γ, increased IL-10)
- Adoptive transfer of dendritic cells pulsed with *Aspergillus* conidia increase resistance to invasive aspergillosis in mice

Kurup et al *Peptides* 1996
Hebart et al *Blood* 2002
Bozza et al *Blood* 2003

Principle of adoptive immunotherapy after SCT

Immunotherapy

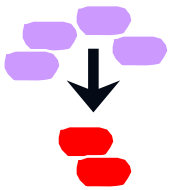
Collection of stem cells

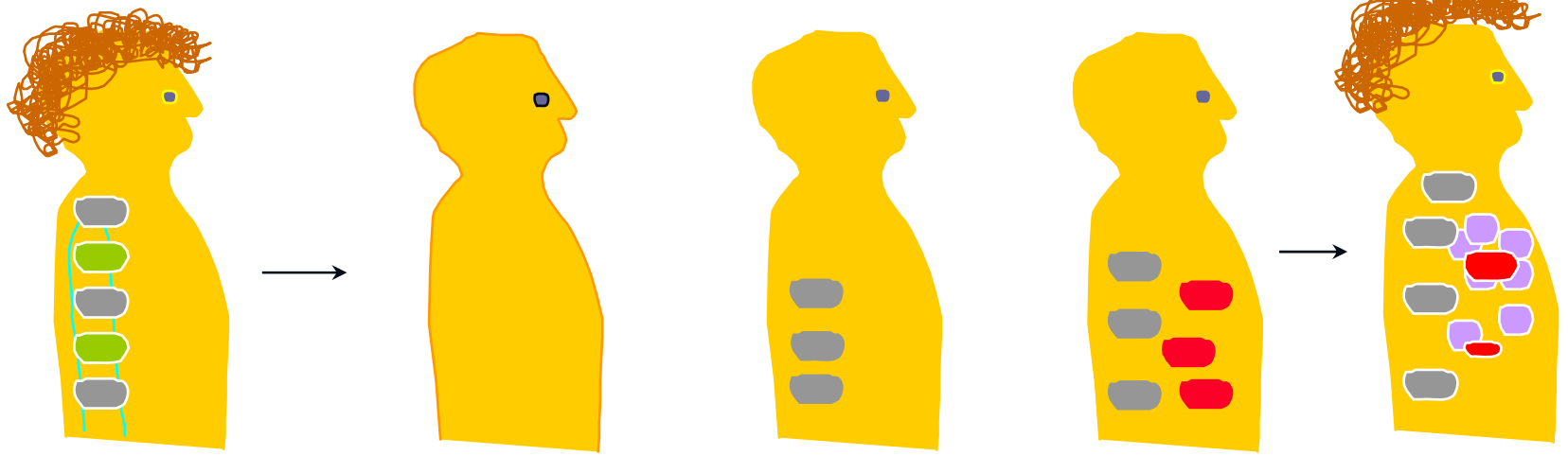
Transplantation

Infection

Effector cells

High dose chemotherapy





Normal cell

Leukemic cell

Aspergillus

Anti-*Aspergillus* T-cells in transplant patients

Transfusion of anti-*Aspergillus* T-cells in 10 patients after haploidentical SCT with evidence of invasive aspergillosis (e.g., pneumonia, positive galactomannan antigenemia)

Immunotherapy 17-37 days after transplantation

Galactomannan antigenemia resolved in all patients within 6 weeks of infusion ($P<.002$ versus controls)

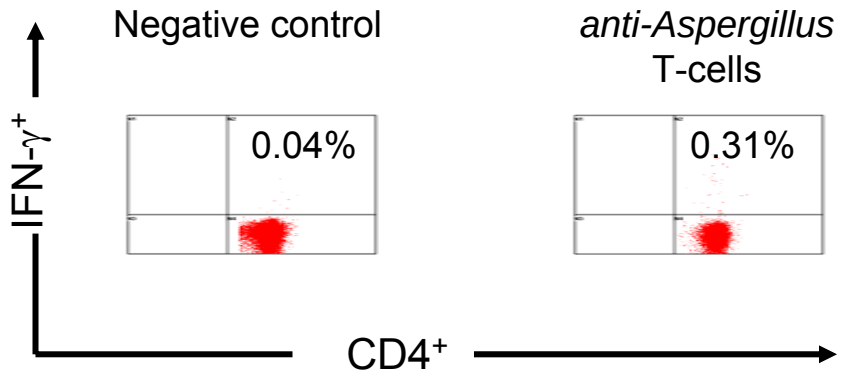
1/10 patients died vs 6/13 controls not receiving immunotherapy

Generation of anti-*Aspergillus* T_H1-cells by limiting dilution (minimum time required: 25 days)

Perruccio et al. *Blood* 2005

Objectives

- Rapid generation of T-cells against *Aspergillus* spp. possible?



Beck et al. submitted

- Specificity of generated T-cells?
- Alloreactivity (risk of GvHD) of selected T-cells?
- Antifungal activity of purified and expanded T-cells?
- Clincial-scale generation of anti-*Aspergillus* T-cells feasible?

Isolation and expansion of anti-*Aspergillus* T-cells

50-100 ml peripheral blood

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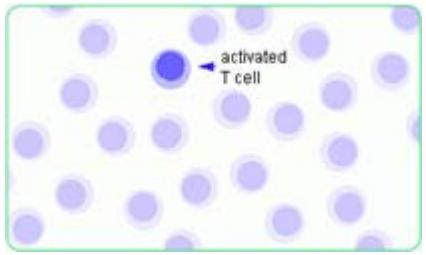
Stimulation with *Aspergillus*-antigen(s)



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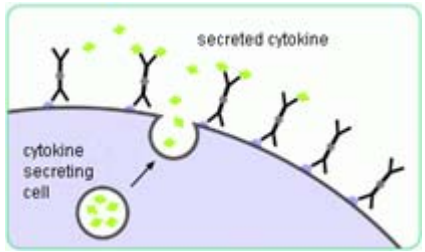
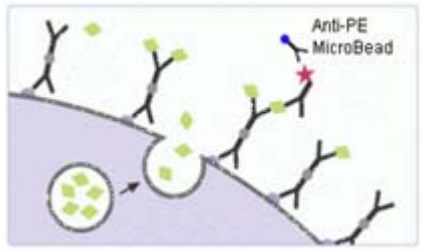
Only antigen-specific T-cells are activated to produce cytokines



Cytokine-secreting cells are magnetically labelled with MicroBeads



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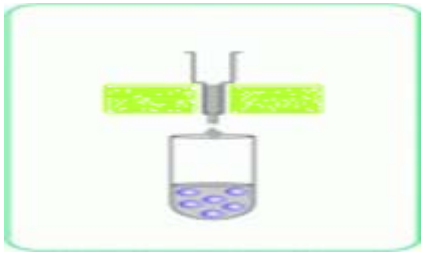
Selection over magnetic column



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anti-*Aspergillus* T-cells

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Culture and expansion

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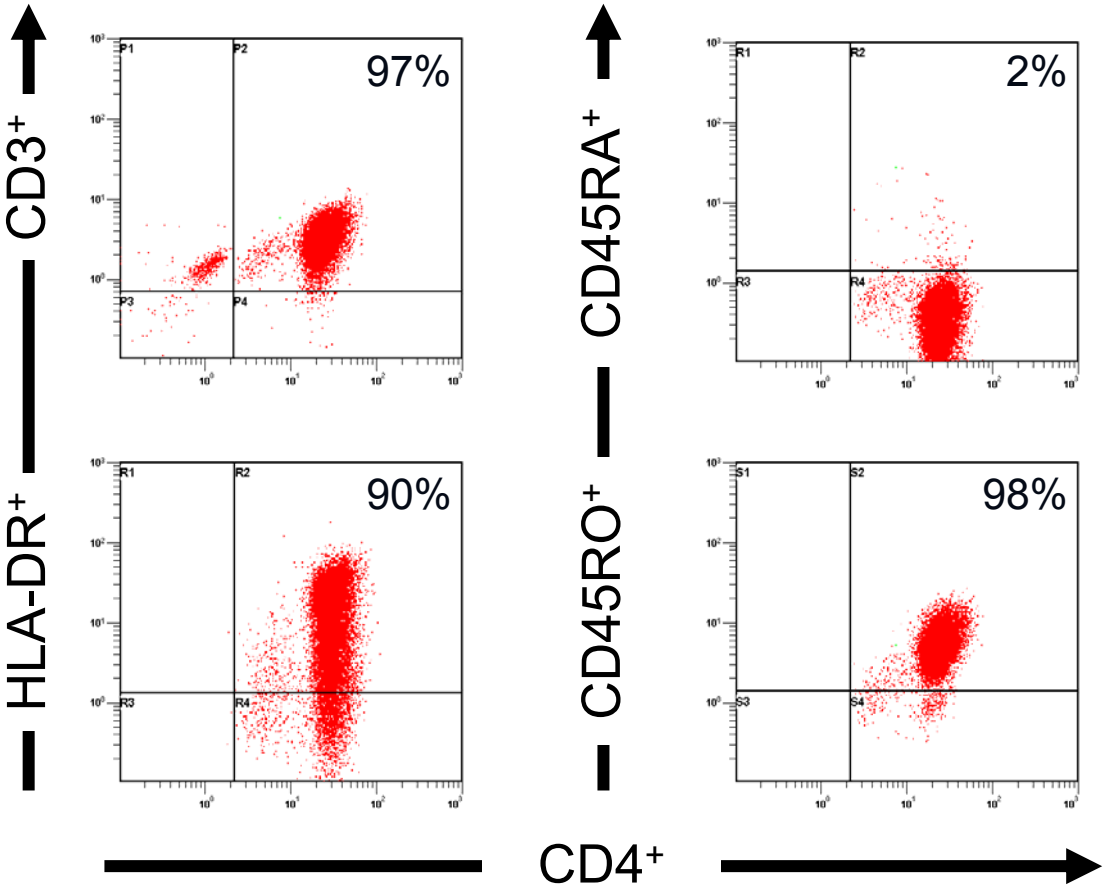
Characterization and functional tests

Immunophenotype of anti-*Aspergillus* T-cells



Number of generated cells after 10-14 days:

median 1.1 x10^7 (0.4 – 2.8 x 10^7; n=7)

Phenotype:

CD3: >97%

CD4: >97%

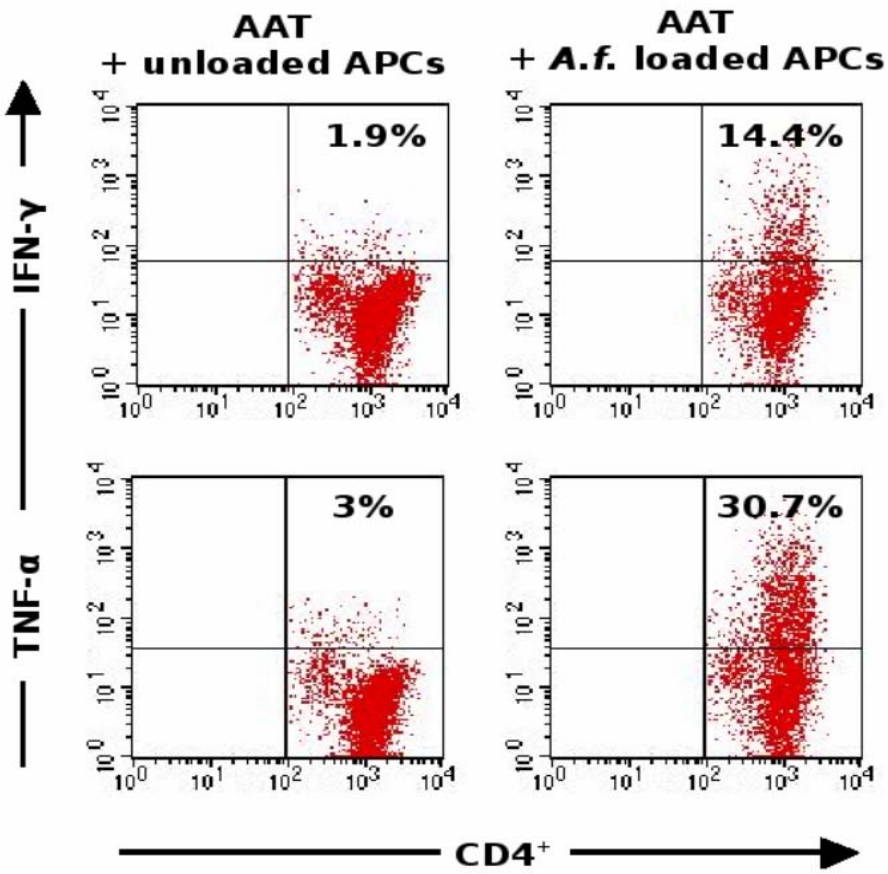
CD45RO: >97%

HLA-DR: >90%

→ activated memory T-cells

Beck et al *Blood* 2006

Cytokine secretion of anti-*Aspergillus* T-cells



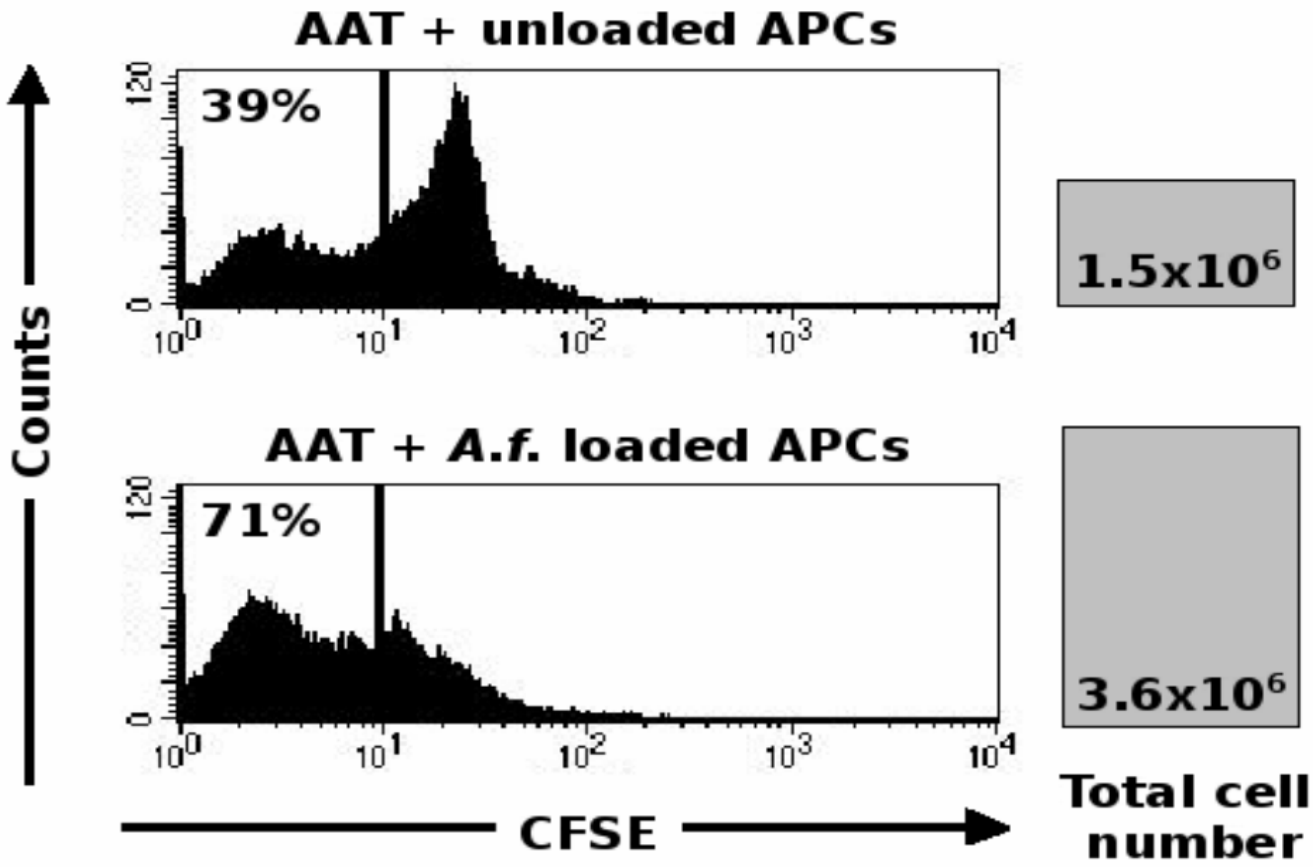
Cytokine secretion upon restimulation:

IFN-γ, TNF-α

No IL-4, IL-10

} T_H1 cells

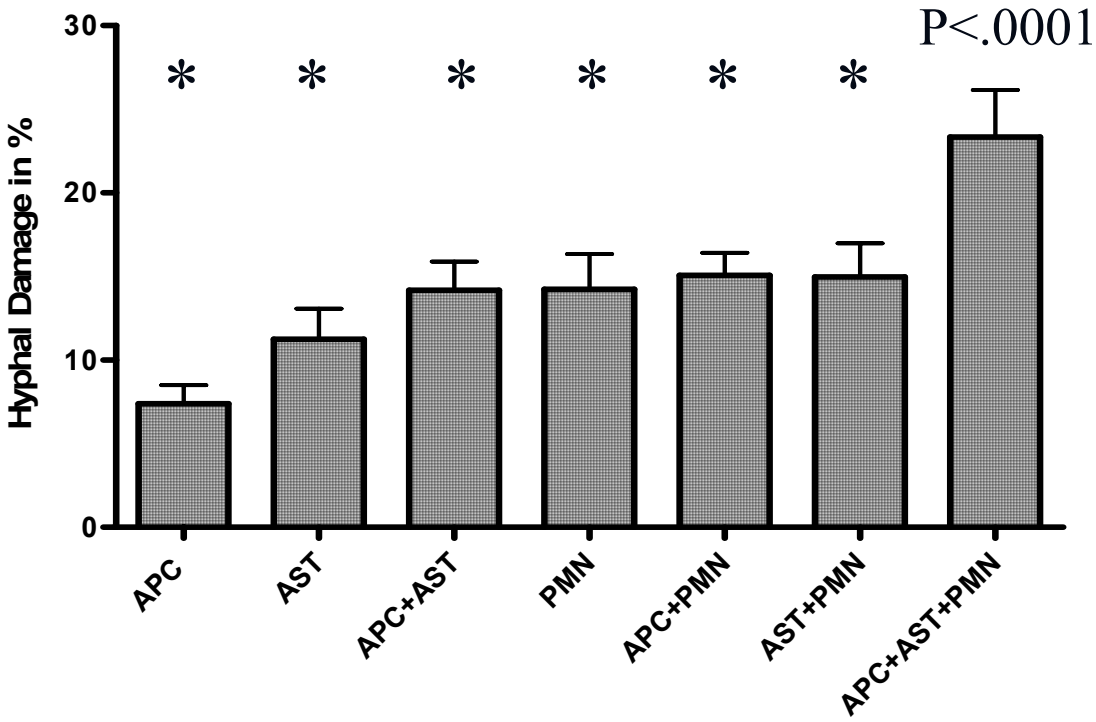
Proliferation upon restimulation



→ Generated T-cells not terminally differentiated

→ Further expansion of anti-*Aspergillus* T-cells *in vivo* to be expected if stimulated by *Aspergillus*-antigen presenting cells

Killing of *A.fumigatus* hyphae

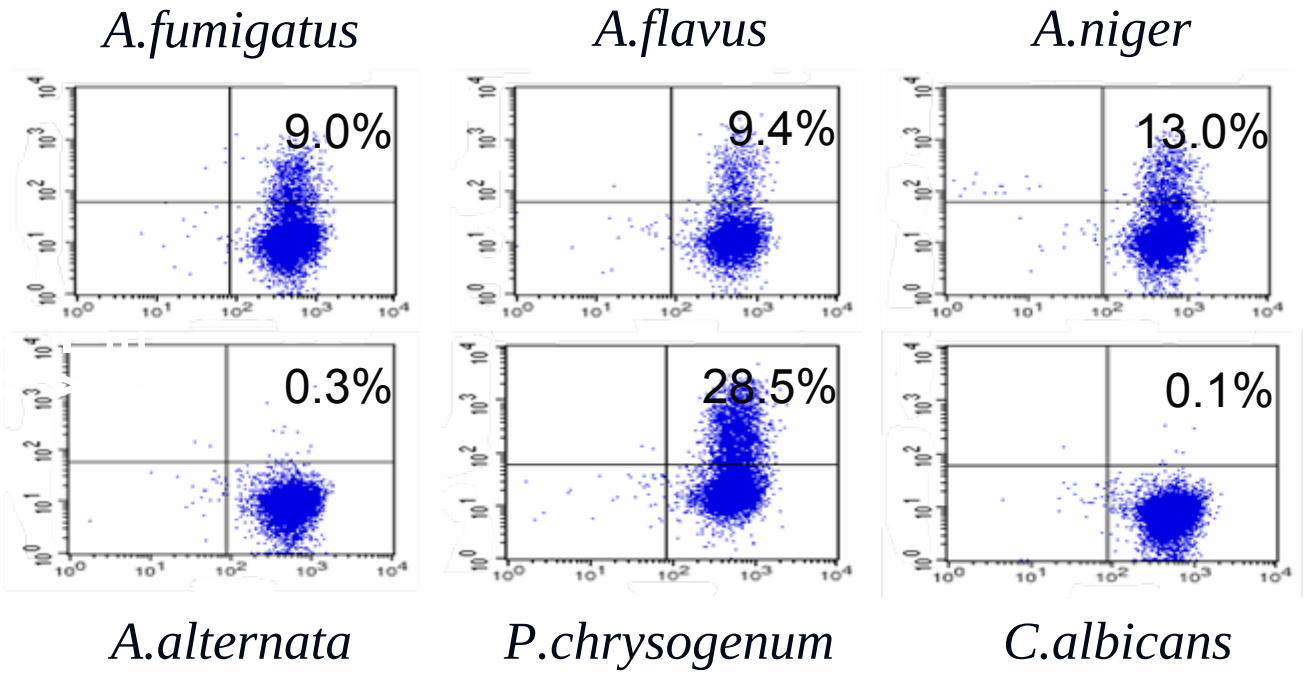


XTT assay; minimum of 12 tests (each in triplicate)

→ Combination of PMNs, T-cells and APCs exhibited highest hyphal damage

→ Hyphal damage also by T-cells alone (mechanism?)

Specificity of anti-*Aspergillus* T-cells



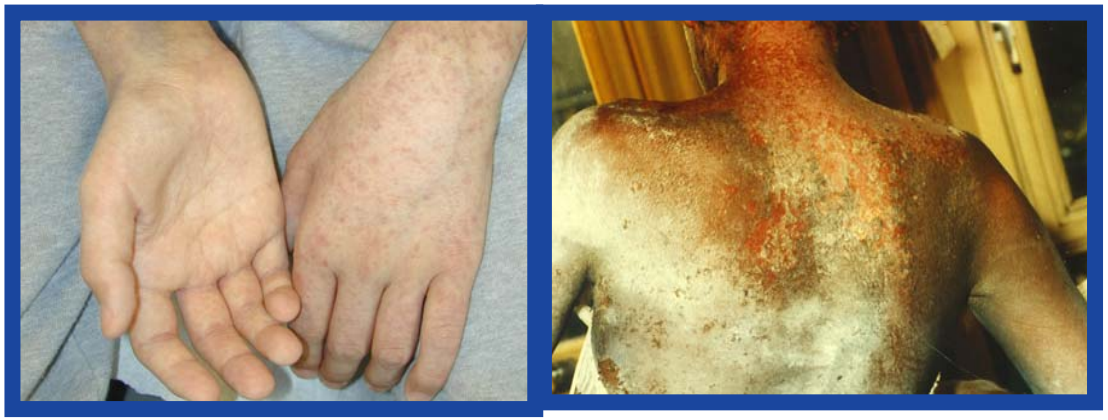
Cross-reactivity might be of clinical advantage, in particular since isolation of the pathogen not possible in most cases!

Donor T-cells and Graft-versus Host Disease

GvHD results from reactivity of donor T-cells against recipient (host) tissue

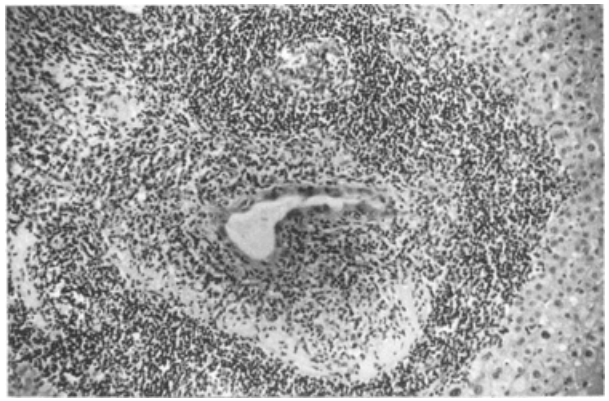
→ activation of alloreactive T-cells and production of inflammatory cytokines

Skin

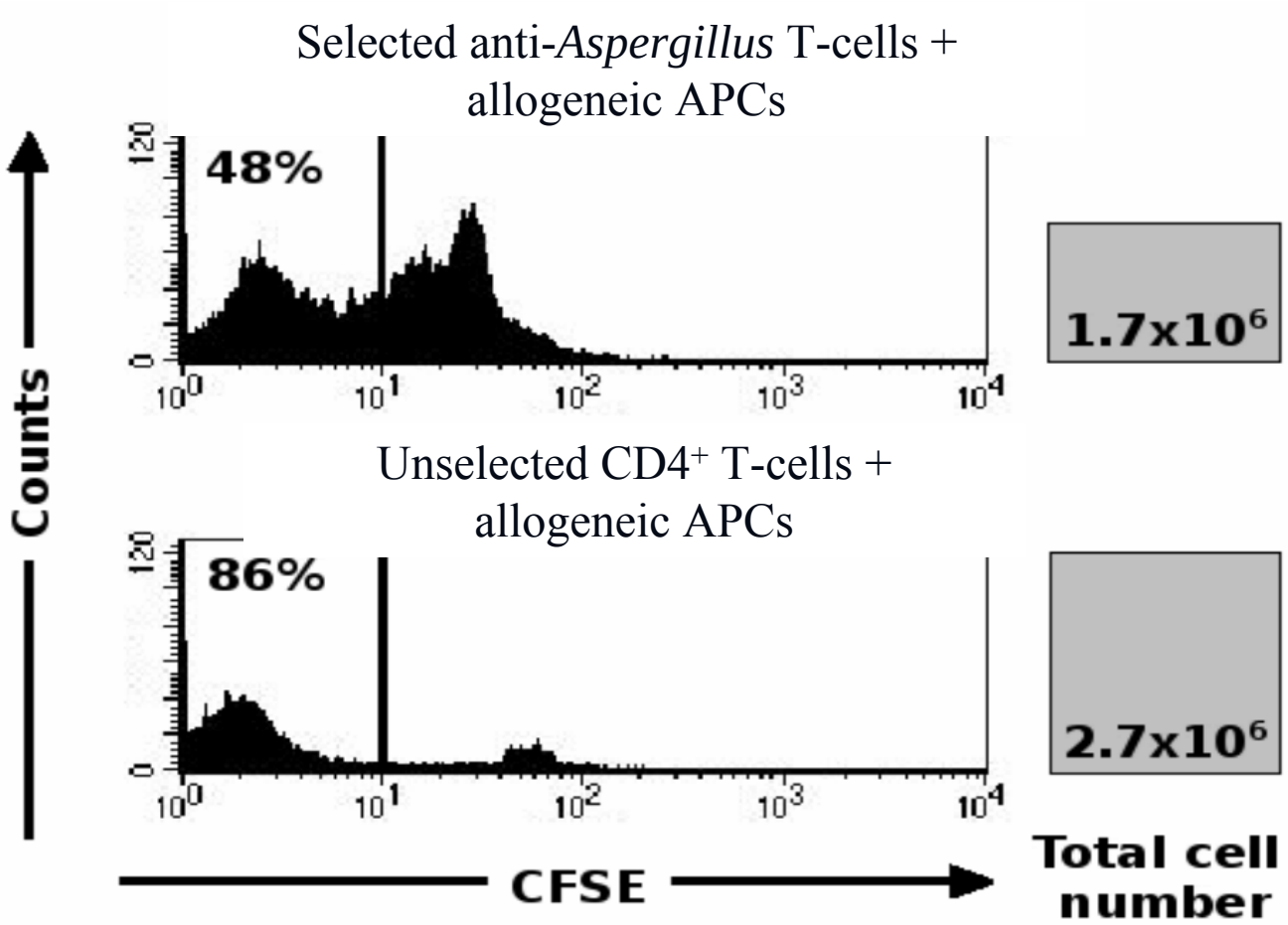


Liver (e.g., bilirubin↑)

Gut (e.g., diarrhea, pain)

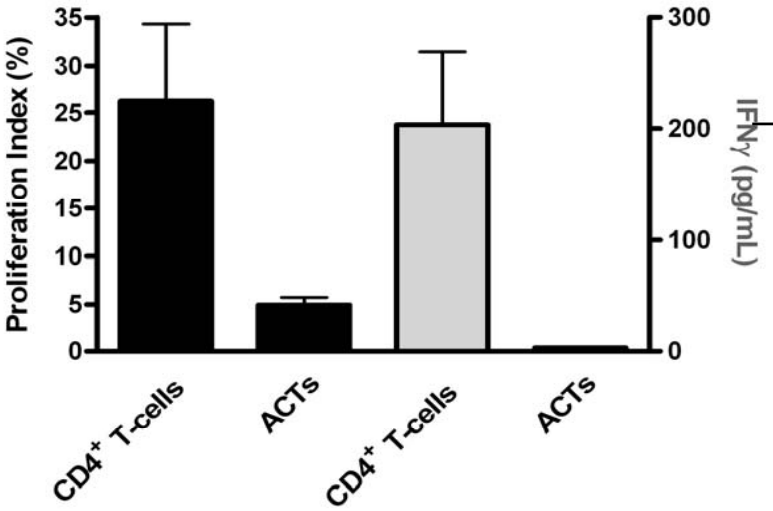


Alloreactivity of anti-*Aspergillus* T-cells



→ Purified anti-*Aspergillus* T-cells coincubated with allogeneic APC´s with lower proliferation response than unselected CD4+ cells

Reduced alloreactivity of anti-*Aspergillus* T-cells



→ Purified anti-*Aspergillus* T-cells coincubated with allogeneic APC´s with lower IFN-γ secretion than unselected CD4+ cells

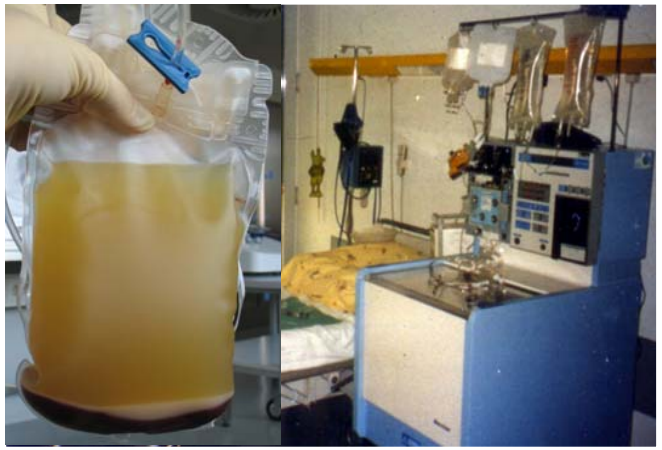
In vitro data indicate that purified anti-*Aspergillus* T-cells have a marked reduction of alloreactivity compared to unselected T-cells

Clinical-scale generation of anti-*Aspergillus* T-cells

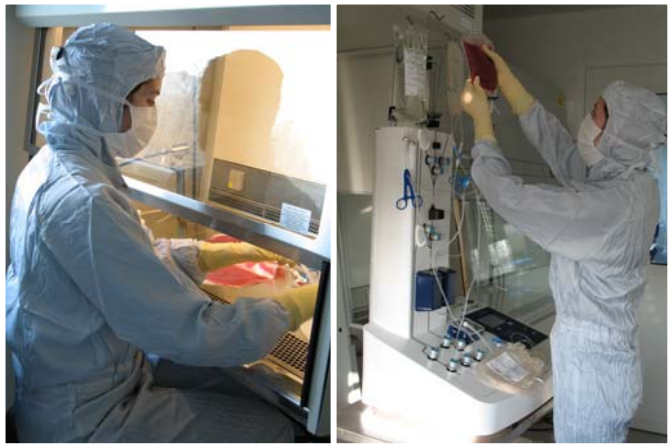
For testing adoptive immunotherapy with anti-*Aspergillus* T-cells („drug“) → generation of cells according to good manufacturing practice (GMP)

GMP-conditions include

- Special, approved facility (Institute of Transfusion Medicine, Frankfurt)
- Approved material (e.g., clincal-scale CliniMACS device, closed system, GMP-grade serum and cytokines)
- Extensive controls (e.g., endotoxin, contamination)

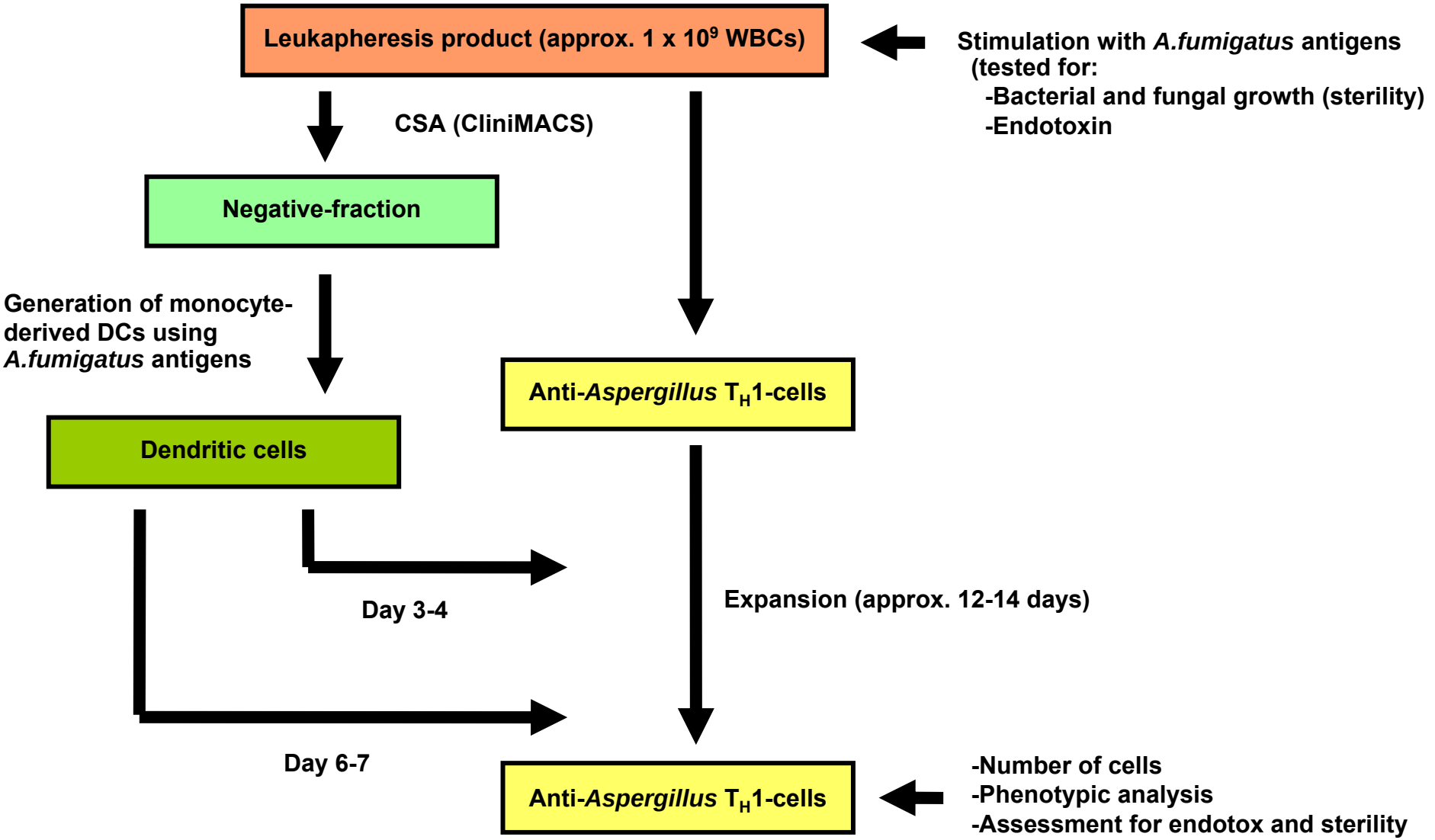


Leukapheresis



Isolation of anti-*Aspergillus* T-cells

Clinical-scale generation of anti-*Aspergillus* T-cells



Tramsen et al. submitted

Clinical-scale generation of anti-*Aspergillus* T-cells

Generated cells*	Total number of cells (WBCs-$CD45^+$) (median, range) [$x10^6$]	Viable** $CD3^+CD4^+$ T-cells (median, range) [$x10^6$]
After culture	22 (13-37)	19 (8-31)
After cryopreservation	8 (7-12)	6 (6-10)

* three independent experiments
** assessed by 7-AAD staining

Summary

- Generation of functionally active anti-*Aspergillus* T_H1-cells is feasible GMP conditions → clinical application in prophylaxis and therapy
- Anti-*Aspergillus* T-cells expand after restimulation with *Aspergillus* antigens
- Anti-*Aspergillus* T-cells can be stimulated by different *Aspergillus* species, but not by antigens of *Candida* spp or *Alternaria alternata*
- Anti-*Aspergillus* T-cells show reduced alloreactivity compared with that of the original cell population
- Anti-*Aspergillus* T-cells increase hyphal damage induced by human neutrophils

Open questions

- Which patient population will benefit from immunotherapy with anti-*Aspergillus* T-cells?
- When and how often to infuse anti-*Aspergillus* T-cells?
  - → (Secondary) prophylaxis for highest risk patients?
  - → Therapeutic strategy?
- Adequate number of anti-*Aspergillus* T-cells to be given?
  - → Efficacy
  - → Safety
- Interaction with/influence by antimycotic compounds?

Acknowlegment

University of Frankfurt

Lars Tramsen
Olaf Beck
Frauke Roeger
Mitra Hanisch
Ulrike Koehl
Thomas Klingebiel

Institute for Transfusion Medicine, Frankfurt

Torsten Tonn
Erhard Seifried

University of Thessaloniki

Emmanuel Roilides
Maria Simitsopoulou

Institut Pasteur, Paris

Jean-Paul Latgé
Jacqueline Sarfati

University of Würzburg,

Hermann Einsele
Max Topp

Deutsche Leukämie Forschungshilfe (DLFH)

Thank you for your attention!