T-Cells in Aspergillosis

- Clinical Aspects -

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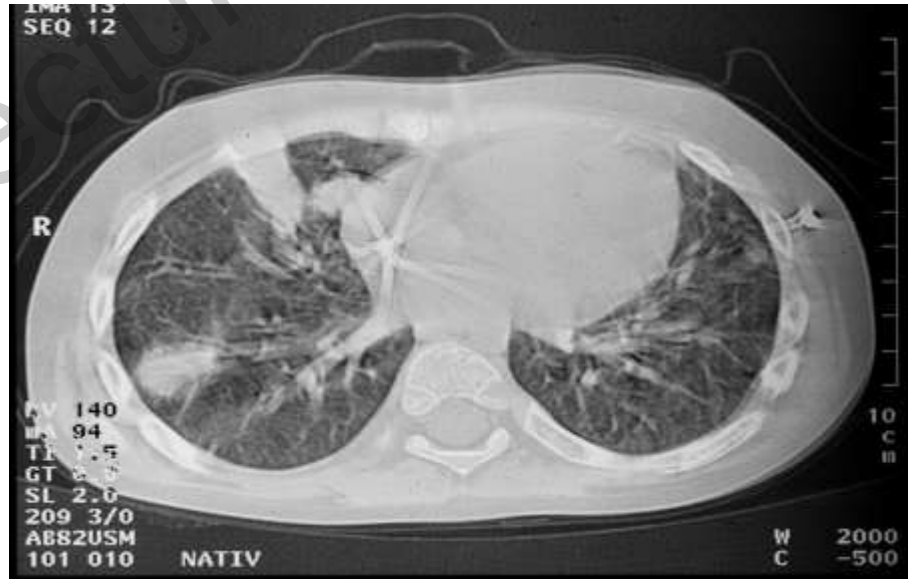
Case report

- 2-year-old girl, relapsed AML
- 3/2007 allogeneic HSCT (MUD)
- Post-Tx period uneventful, engraftment day +21, dismission day +32
- Antifungal prophylaxis with voriconazole (8 mg/kg BID)
- Day +56: dyspnea, coughing → admission to hospital
- GM serum ++, CT scan of the lung: infiltrates suggestive for IFD; no biopsy performed

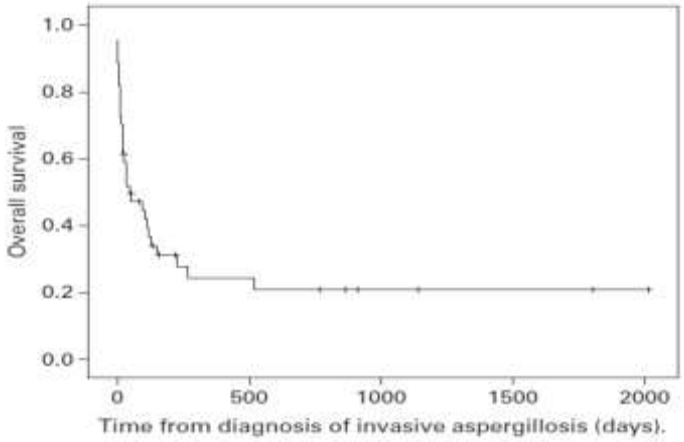


- Start of L-AmB (5 mg/kg/d)
- Clinical deterioration: caspofungin (50 mg/m^2 d1, then 70 mg/m^2) added after 10 d
- Exitus day +97; postmortem biopsy: *A. fumigatus*

Invasive fungal disease after allogeneic HSCT

Despite improvement of supportive care strategies (e.g., new antifungal compounds): mortality rates in allo transplant recipients unacceptably high

- Invasive aspergillosis up to 80%
- Candidiasis 55%
- Other molds (e.g. Fusarium) 80%
- Zygomycosis up to 90%

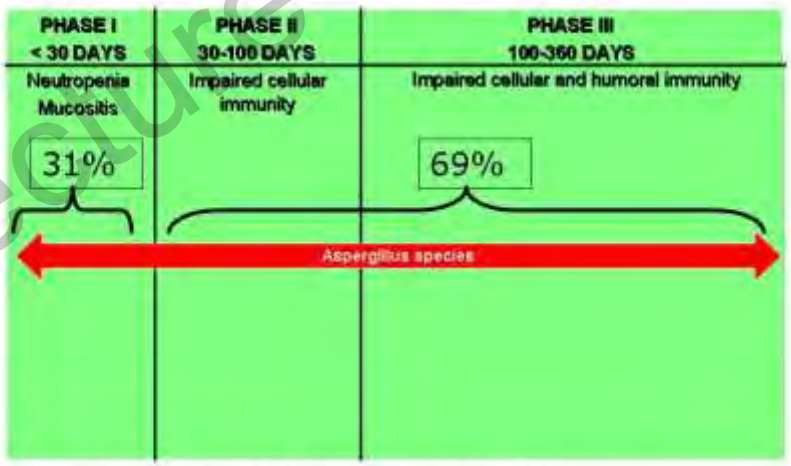


Pagano *CID* 2007; Neofytos *CID* 2009

Invasive fungal disease after allogeneic HSCT

Although neutropenia is the most important single risk factor for IFD, most IFDs in allo HSCT recipients occur after engraftment in non-neutropenic patients (as seen in our patient)

→ underlines importance of other arms of the immune system (e.g., cellular immunity)

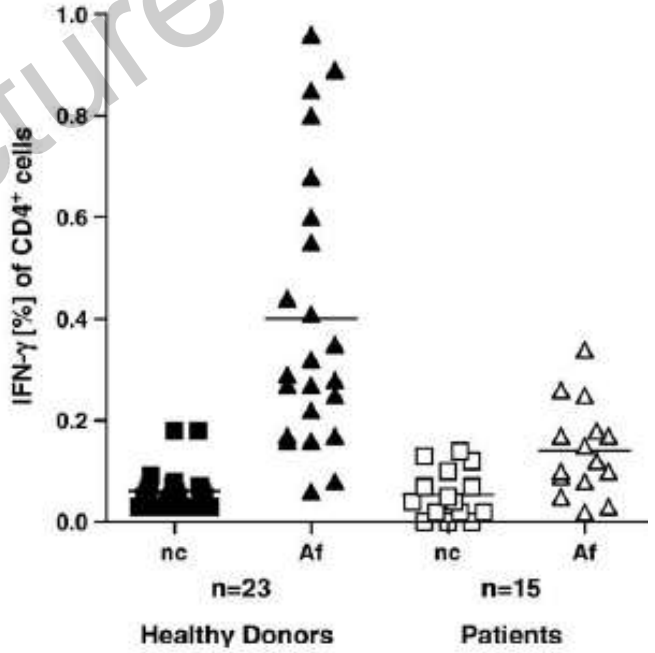


American Society for Blood and Marrow Transplantation, 2000
Wald et al. J Infect Dis 1998

T cells and IFD

- Low number of anti-*Aspergillus* T_H1 cells in HSCT recipients for months after SCT
- 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)

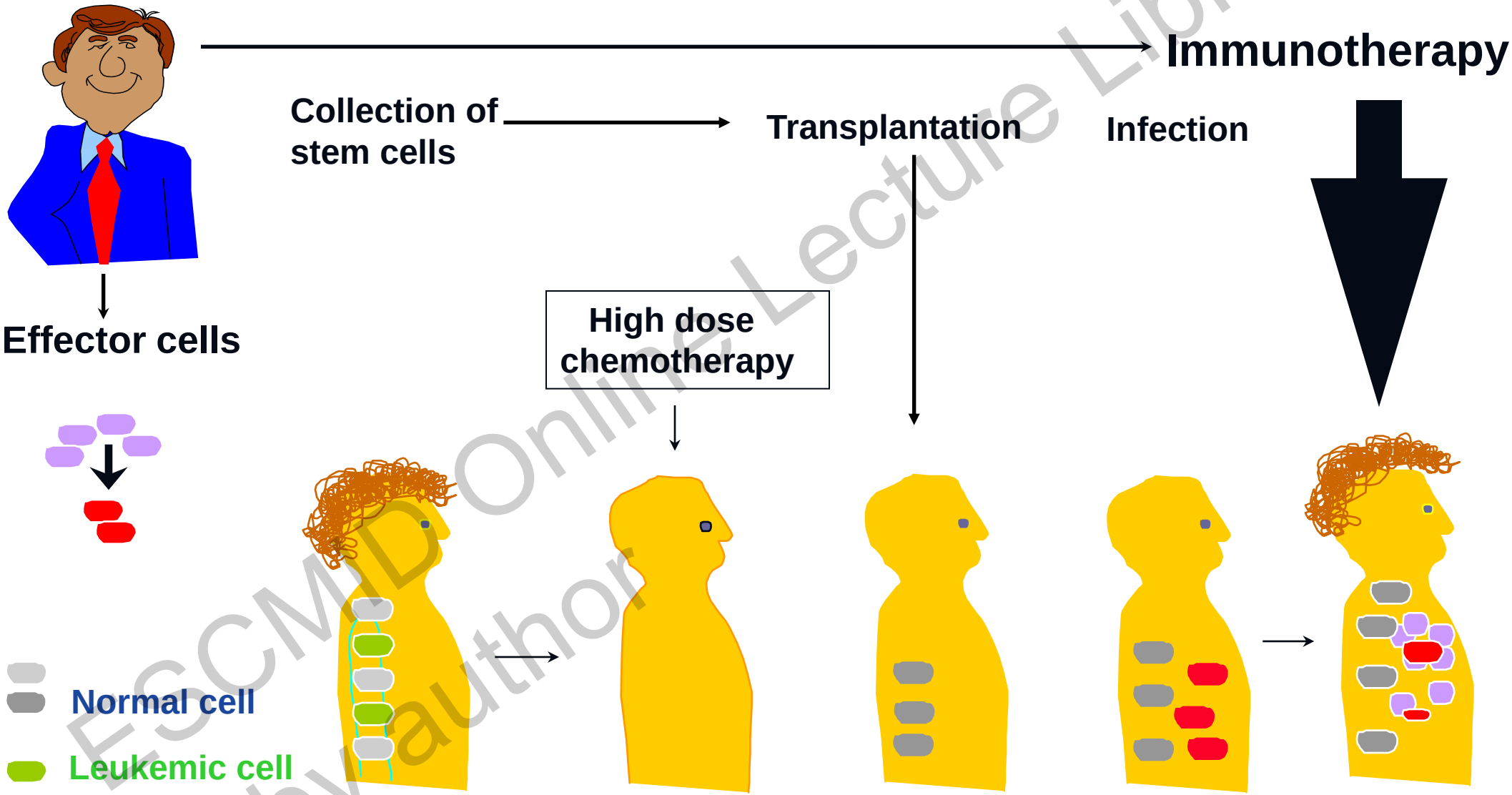
→ rationale of administration of antifungal T_H1 cells ("adoptive immunotherapy")



(median time after SCT: 212 days (92-1468))

Hebart et al, *Blood* 2002, Beck et al, *J Immunol Meth* 2008

Principle of adoptive immunotherapy after SCT



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)

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

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

Leukapheresis product (approx. 1 x 10^9 WBCs)

CliniMACS

24 hours

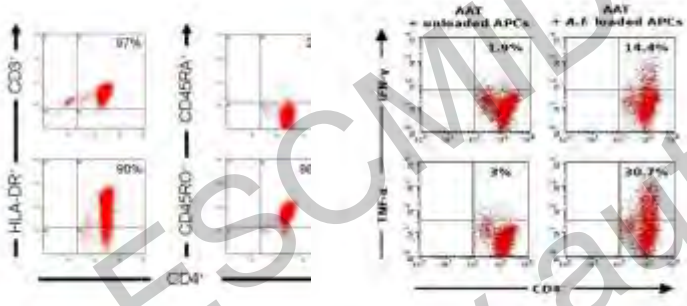
$2x10^6$ anti-*Aspergillus* T cells

Stimulation with *A.fumigatus* antigens
Tested for sterility (bacterial and fungal growth, endotoxin)

Tested for sterility (bacterial and fungal growth, endotoxin)

Leukapheresis

Isolation of anti-*Aspergillus* T cells



Cytokine-secretion upon restimulation:

IFN-γ, TNF-α

No IL-4, IL-10

} T_H1 cells

Tramsen *BMT* 2009

…..case report

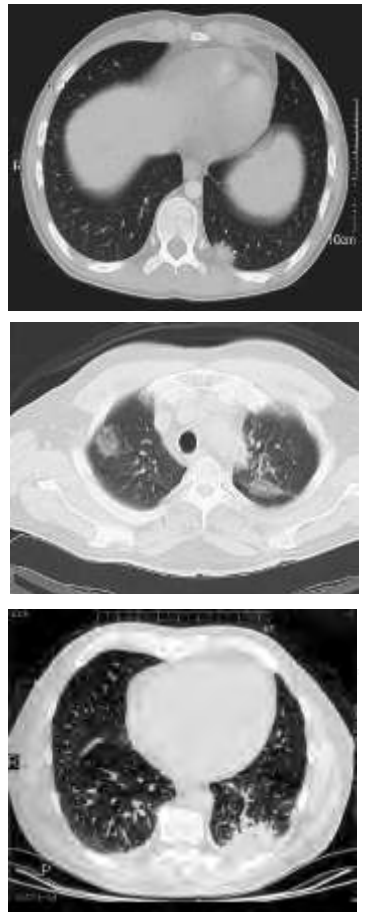
…2 weeks after post-mortem biopsy:

co-infection with *A. fumigatus* and *Rhizopus microsporus*

T cells against which pathogen(s) needed?

- IA most common IFD after allo HSCT, but in a considerable number of patients with IFD no isolation of a specific fungal pathogen is achieved
- No laboratory parameter or imaging study can determine which pathogen is causing a suspected infection
- Problem of co-infection with different pathogens

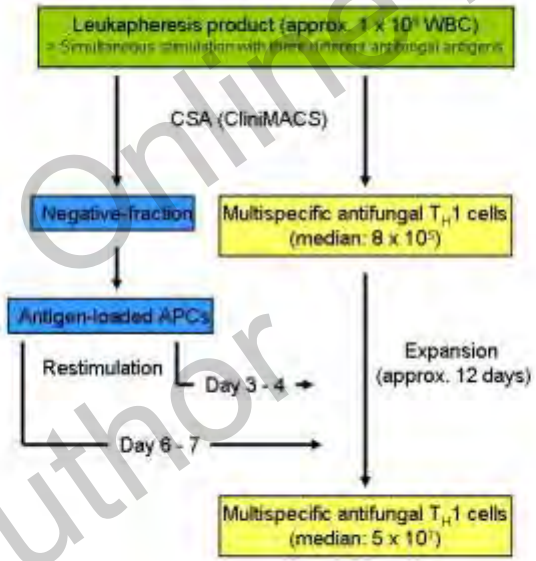
→ rationale for generating T cells that target a broad spectrum of fungal pathogens



Multipathogen-specific antifungal T cells

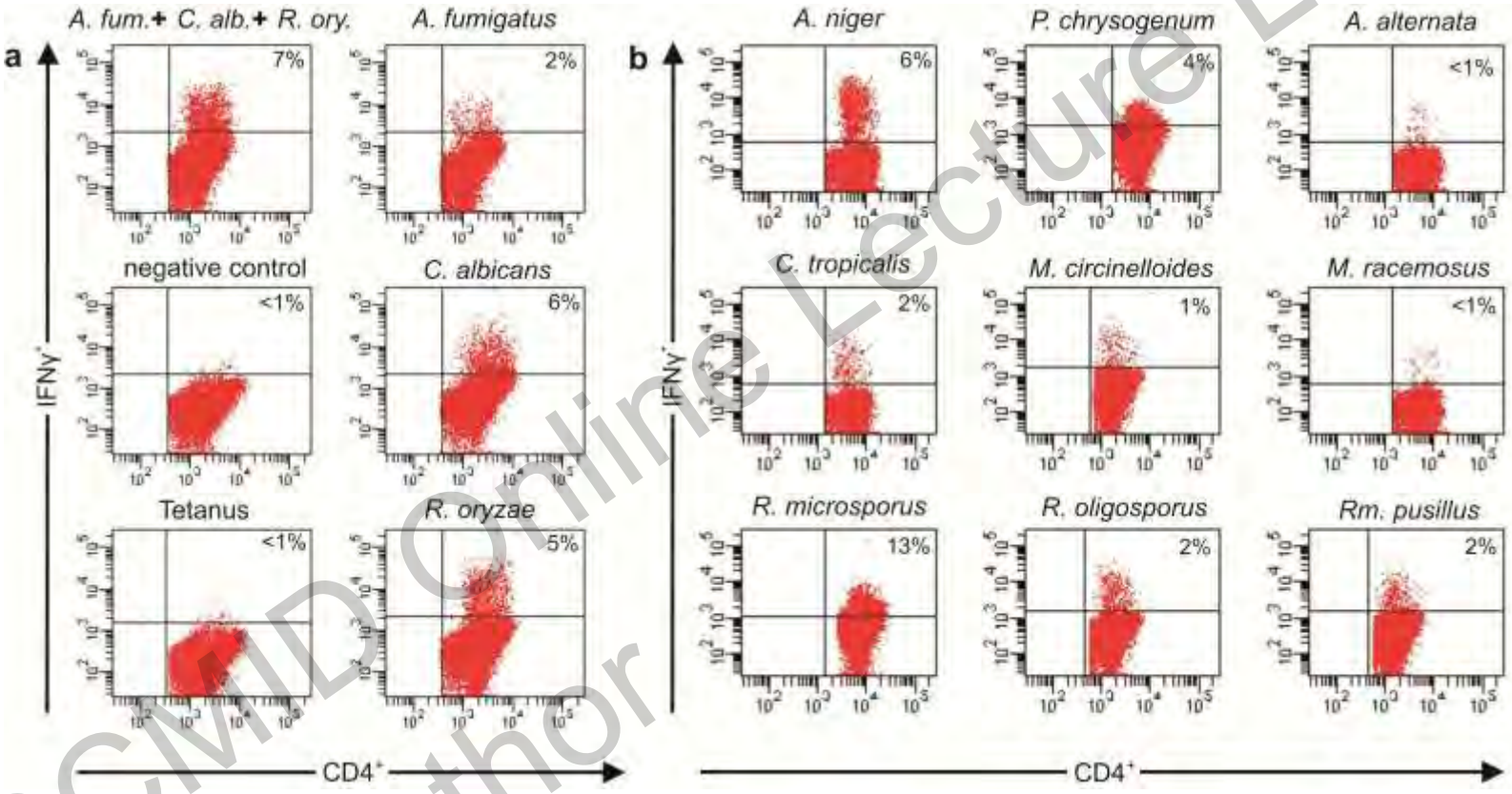
Simultaneous stimulation of WBC with antigen extracts of

- *A.fumigatus*
- *Rhizopus oryzae*
- *C.albicans*



Tramsen et al, *Cytotherapy* 2013

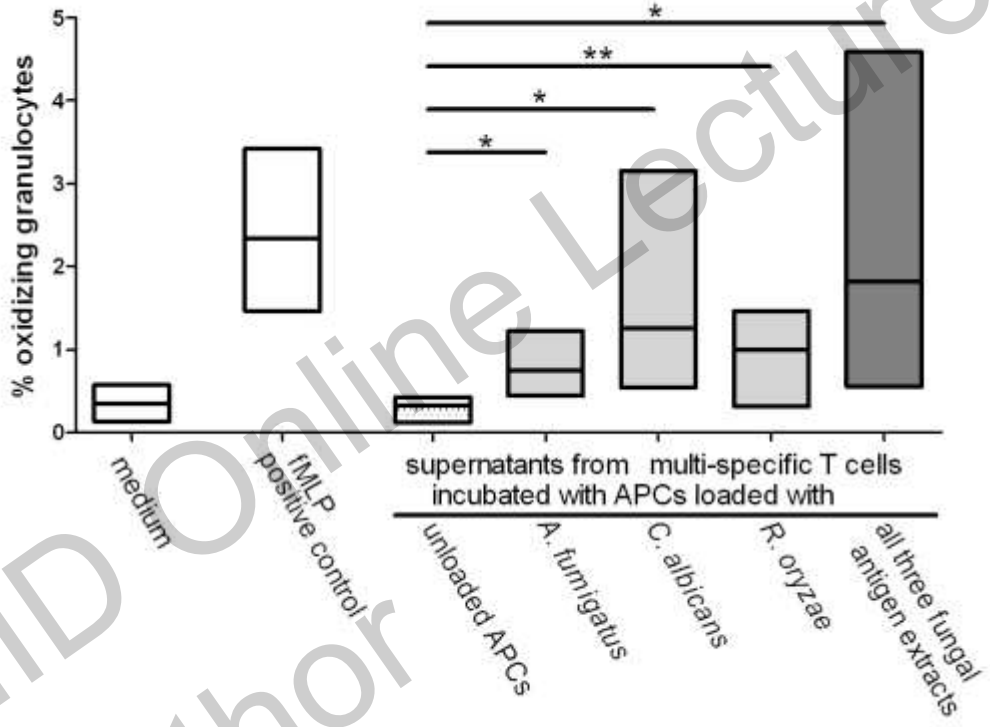
Response upon restimulation



→ broad activity of generated multipathogen-specific antifungal T cells

Tramsen et al, *Cytotherapy* 2013

Enhancement of antifungal activity of phagocytes



→ supernatant of restimulated multispecific T cells significantly enhances activity of granulocytes

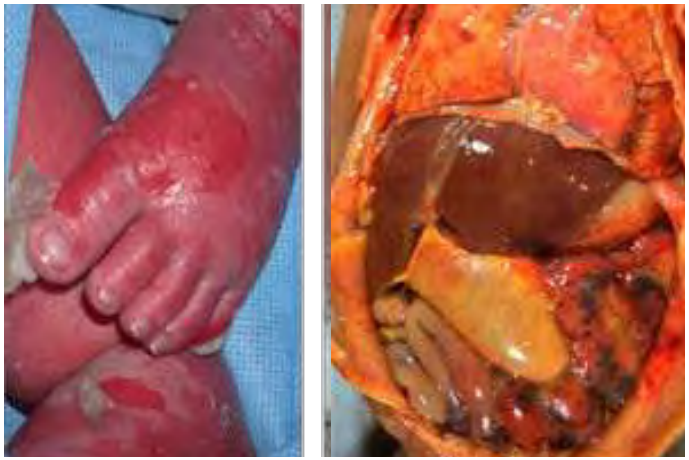
Tramsen et al, *Cytotherapy* 2013

Graft-versus Host Disease

Donor-derived T cells may recognize and attack normal tissues of the recipient as “foreign” (GVHD)

Pathophysiology of GVHD includes proliferation of T cells and production of inflammatory cytokines

GVHD can affect skin, liver, gut etc and is potentially lethal!

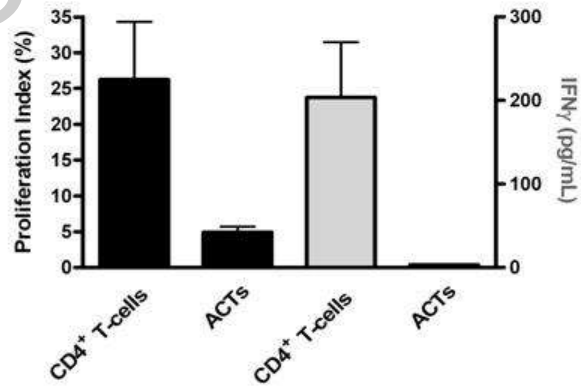


Risk of GVHD and antifungal T cells

Compared to unselected T cells

- generated T cells exhibit lower proliferation when co-incubated with third-party APCs
- generated T cells with lower IFN-γ production when co-incubated with third-party APCs

→ loss of alloreactive potential *in vitro*



In vito data corroborate clinical experience: no significant toxicity in 10 patients receiving up to $1x10^6$/kg anti-*Aspergillus* T cells

Perrucio et al, *Blood*, 2005; Tramsen et al, *Cytotherapy* 2013

Number of specific T cells needed

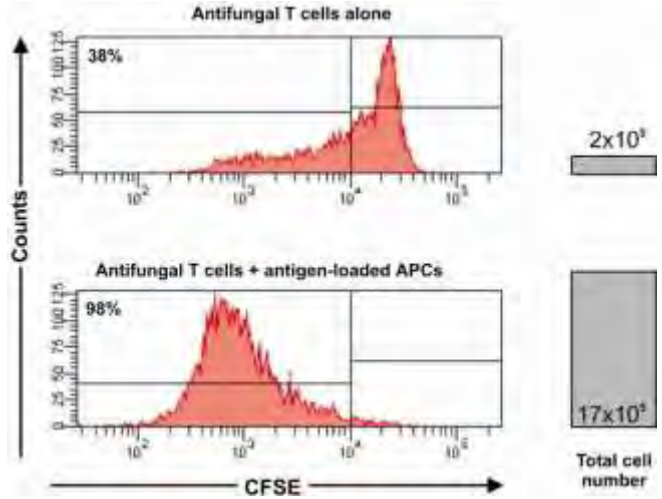
Successful treatment with virus-specific T cells:

CMV-specific T cells: 5 x 10^6 - 10^8 cells per m^2

Adenovirus-specific T cells: 1.2 - 5x10^3 cells per kg

Number of T cells needed for adoptive antifungal therapy?

- *In vitro* assays demonstrate that generated antifungal T cells are not terminally differentiated
- Expansion of multispecific antifungal T cells *in vivo* can be expected



Einsele et al, *Blood* 2002, Leen et al, *Nat Med* 2006, Feuchtinger et al, *BJH* 2006; Tramsen et al, *Cytotherapy* 2013

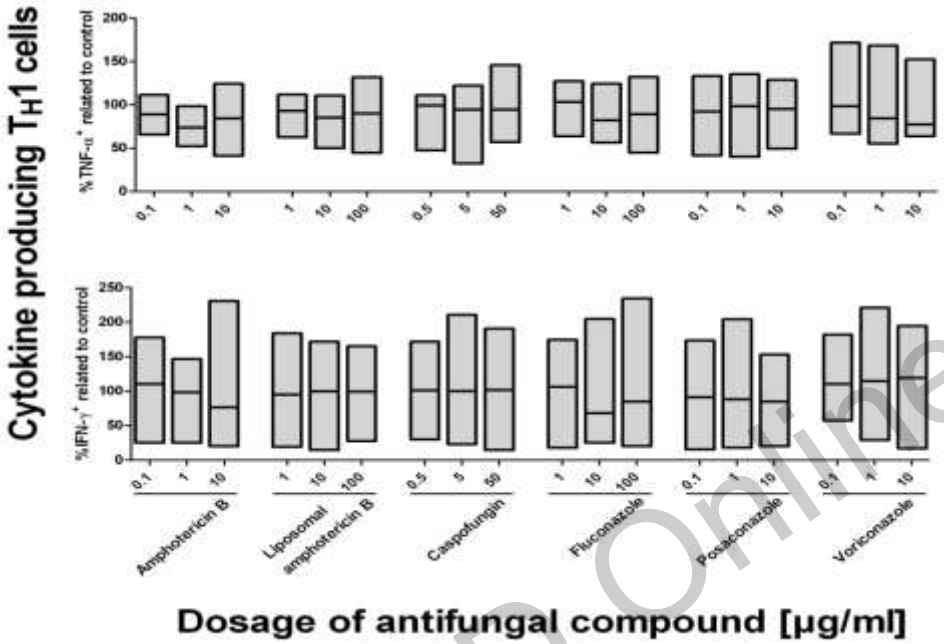
Antifungal T-cells and concomitant therapy

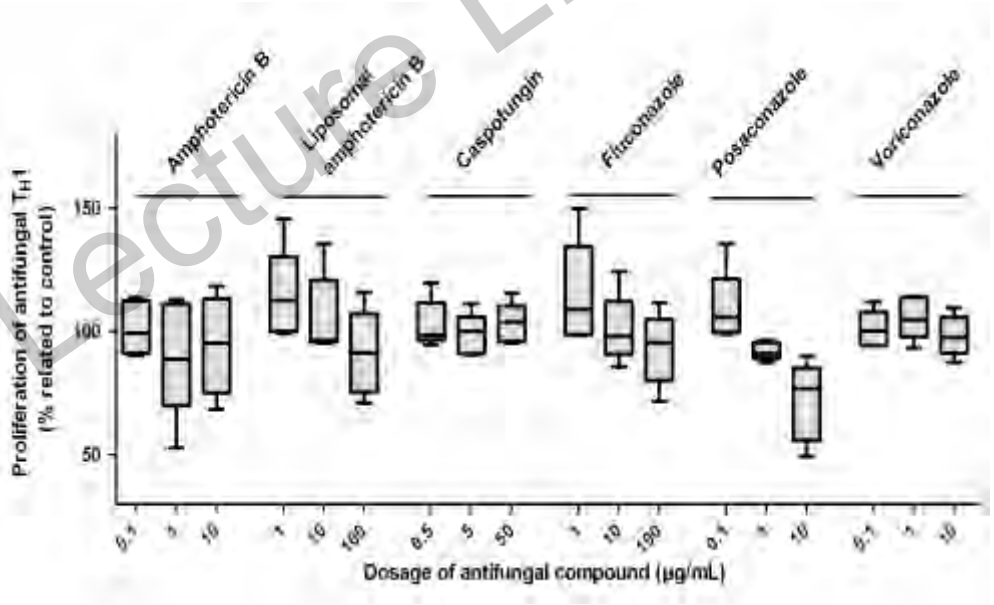
Most allo HSCT transplant recipients with IFD receive antifungal compounds and immunosuppressants

→ whether and to what extend do specific compounds influence important functional properties of anti-*Aspergillus* T cells such as

- IFN-γ production
- Proliferation

Influence of antifungal compounds on antifungal T cells



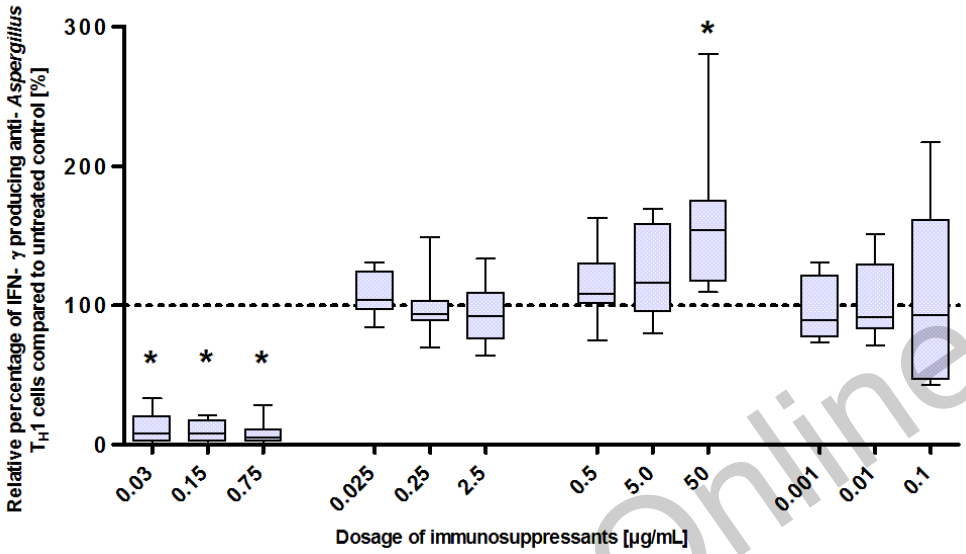


Concomitantly administered antifungal compounds

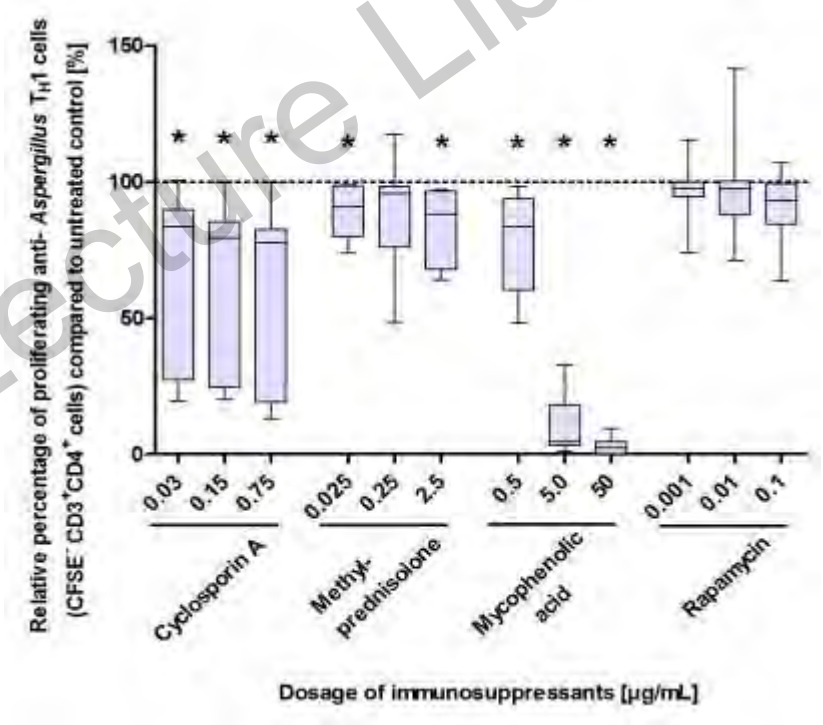
- do not influence cytokine production of antifungal T-cells
- only posaconazole at high dosages (>> recommended dosages) seems to negatively influence proliferation of antifungal T-cells

Tramsen et al, *Transpl Infect Dis* 2013

Influence of immunosuppressants on antifungal T cells



CsA Pred MPA Rapamycin



Antifungal immunotherapy in patients receiving immunosuppressants:

- CsA at smallest dosages inhibits cytokine production of antifungal T-cells
- CsA, methylprednisolone, and MMF at all dosages tested significantly decrease proliferation of antifungal T-cells

Tramsen et al, *Infect Immun* (in press)

Current Status and Perspectives

Anti-*Aspergillus* T cells given to a girl with uncontrolled invasive aspergillosis after allogeneic HSCT

- no immediate side effects
- death after 7 days due to fulminant relapse of AML → efficacy / long-term side effect not evaluable

Current Status and Perspectives

First multi-institutional clincial phase I/II trial in preparation

- PI: A.Ullmann, Würzburg (EudraCT # 2013-002914-11)
- Preparation of anti-*Aspergillus* T cells in Frankfurt
- GMP conform *Aspergillus* antigen extract
- ≥ 28 HSCT patients with probable/proven invasive aspergillosis
- Patients will receive a target dose of 0.5-3 x 10^3/kg BW of donor-derived anti-*Aspergillus* T-cells, but a maximum of 5 x 10^3/kg BW CD3 positive cells

Current Status and Perspectives

Primary end point:

- Incidence of occurrence of any GvHD

Secondary end points include:

- Response rate to antifungal therapy
- Mortality (overall and IFD attributable)

Other variables assessed:

- Surrogate markers of invasive aspergillosis (i.e., galactomannan)
- Assessing the general (e.g., CD3, CD4 and CD8 counts) and specific immune reconstitution (e.g., viral and fungal-specific T cells)
- Assessing the feasibility of the timely generation of donor-derived anti-*Aspergillus* T cells

Thank you for your attention!

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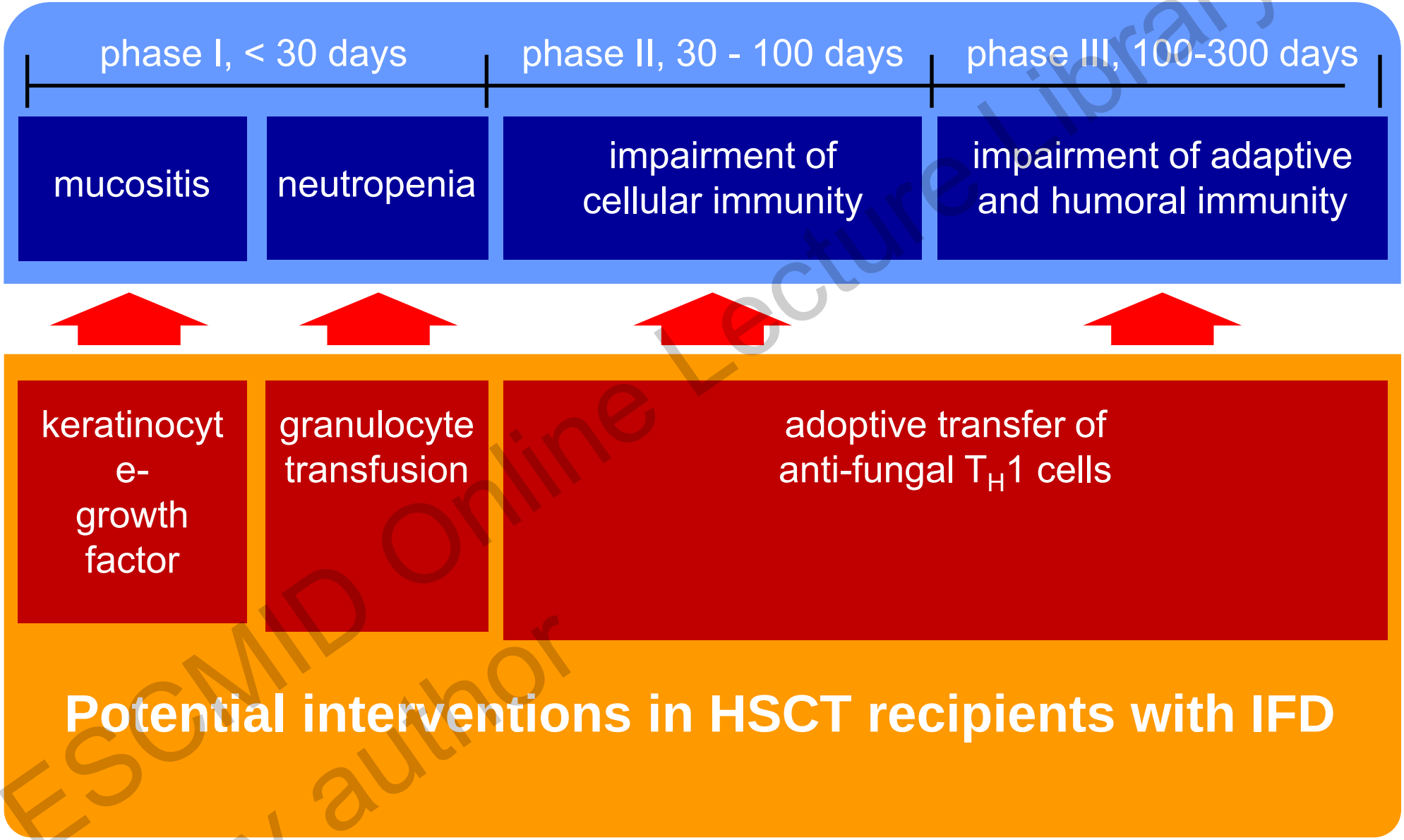

Summary

Clincial scale generation of multispecific antifungal T cells that target *Aspergillus*, *Candida*, and *Zygomycetes* is possible

- Out of $1x10^9$ WBCs, $5x10^7$ antifungal T cells can be generated within 12 days
- Generated T cells are activated memory T_H1 cells
- Generated T cells respond to a broad spectrum of fungal pathogens with IFN-γ secretion (not influenced by most antifungals)
- Generated T cells proliferate upon restimulation (not influenced by most antifungals)
- Generated T cells show loss of alloreactivity
- Cryopreservation does not impair function → prophylactic generation feasible in highest risk patients