Cultivated anti*-Aspergillus* $T_H^1$ 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

<table>
<thead>
<tr>
<th>PHASE I</th>
<th>PHASE II</th>
<th>PHASE III</th>
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<tbody>
<tr>
<td>&lt; 30 DAYS</td>
<td>30-100 DAYS</td>
<td>100-360 DAYS</td>
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<tr>
<td>Neutropenia</td>
<td>Impaired cellular immunity</td>
<td>Impaired cellular and humoral immunity</td>
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</table>

- Neutropenia: 31%
- Impaired cellular immunity: 69%

Aspergillus species

American Society for Blood and Marrow Transplantation, 2000
T-cells and invasive fungal infection

- *Aspergillus* fumigatus antigens are capable to induce T\textsubscript{H}1 (IFN-\textgreek{g}, IL-2, TNF-\textalpha) or T\textsubscript{H}2 response (IL-4, IL-5, IL-10)

- Patients with invasive aspergillosis and T\textsubscript{H}1 response (increased IFN-\textgreek{g}, low IL-10) have a better outcome than patients with T\textsubscript{H}2 response (low IFN-\textgreek{g}, 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

Collection of stem cells → Transplantation → Immunotherapy

- Effector cells
- Normal cell
- Leukemic cell
- Aspergillus

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

Beck et al. submitted
Isolation and expansion of anti-Aspergillus T-cells

50-100 ml peripheral blood

Stimulation with Aspergillus-antigen(s)

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

Cytokine-secreting cells are magnetically labelled with MicroBeads

Selection over magnetic column

anti-Aspergillus T-cells

Culture and expansion

Characterization and functional tests
Immunophenotype of anti-\textit{Aspergillus} T-cells

Number of generated cells after 10-14 days: median 1.1 \times 10^7 (0.4 – 2.8 \times 10^7; n=7)

Phenotype:
CD3: >97%
CD4: >97%
CD45RO: >97%
HLA-DR: >90%
→ activated memory T-cells

Beck et al \textit{Blood} 2006
Cytokine secretion of anti-Aspergillus T-cells

Cytokine secretion upon restimulation:
IFN-γ, TNF-α
No IL-4, IL-10

\( \text{T_H1 cells} \)
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

→ Combination of PMNs, T-cells and APCs exhibited highest hyphal damage
→ Hyphal damage also by T-cells alone (mechanism?)

**Graph:**
- XTT assay; minimum of 12 tests (each in triplicate)
- Hyphal Damage in %
- P<.0001

- APC
- AST
- APC+AST
- PMN
- APC+PMN
- AST+PMN
- APC+AST+PMN
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

Selected anti-*Aspergillus* T-cells +
allogeneic APCs

→ 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

*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., clinical-scale CliniMACS device, closed system, GMP-grade serum and cytokines)
- Extensive controls (e.g., endotoxin, contamination)
Clinical-scale generation of anti-\textit{Aspergillus} T-cells

Leukapheresis product (approx. $1 \times 10^9$ WBCs) → CSA (CliniMACS) → Negative-fraction → Generation of monocyte-derived DCs using \textit{A.fumigatus} antigens → Dendritic cells → Day 3-4 → Day 6-7 → Anti-\textit{Aspergillus} $T_H^1$-cells → Expansion (approx. 12-14 days) → Anti-\textit{Aspergillus} $T_H^1$-cells → Stimulation with \textit{A.fumigatus} antigens (tested for: - Bacterial and fungal growth (sterility) - Endotoxin)

- Number of cells
- Phenotypic analysis
- Assessment for endotox and sterility

Tramsen et al. submitted
**Clinical-scale generation of anti-Aspergillus T-cells**

<table>
<thead>
<tr>
<th>Generated cells*</th>
<th>Total number of cells (WBCs-CD45*) (median, range) [x10^6]</th>
<th>Viable** CD3^+CD4^+ T-cells (median, range) [x10^6]</th>
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</thead>
<tbody>
<tr>
<td>After culture</td>
<td>22 (13-37)</td>
<td>19 (8-31)</td>
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<tr>
<td>After cryopreservation</td>
<td>8 (7-12)</td>
<td>6 (6-10)</td>
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* three independent experiments
** assessed by 7-AAD staining
Summary

- Generation of functionally active anti-\textit{Aspergillus} T\textsubscript{H}1-cells is feasible under GMP conditions → clinical application in prophylaxis and therapy
- Anti-\textit{Aspergillus} T-cells expand after restimulation with \textit{Aspergillus} antigens
- Anti-\textit{Aspergillus} T-cells can be stimulated by different \textit{Aspergillus} species, but not by antigens of \textit{Candida} spp or \textit{Alternaria} alternata
- Anti-\textit{Aspergillus} T-cells show reduced alloreactivity compared with that of the original cell population
- Anti-\textit{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?
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Thank you for your attention!