

## Host response to fungal infections – how immunology and host genetics could help to identify and treat patients at risk

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### Summary

In spite of the ever-increasing incidence and poor outcome of invasive fungal infections in immune compromised patients, there is currently no reliable method to accurately predict the risk, to monitor the outcome and to treat these infections. Protective immunity against *Candida* and *Aspergillus* depends on a highly coordinated interaction between the innate and adaptive immune systems. Genetic and immunological defects in components of these networks result in increased risk of invasive fungal infections among patients undergoing chemotherapy or transplant recipients.

We review the most important genetic and immunological factors that influence human susceptibility to *Candida* and *Aspergillus* infections and discuss the potential role of basic research to promote precision medicine for infectious diseases. We discuss how immunogenetic studies can help to provide tools for improved identification of high-risk patients and the development of tailored antifungal therapies.

**Key words:** fungal infections; *Aspergillus*; *Candida*; genetic predisposition; host genetics; immunology; antifungal therapy; adoptive T cell therapy; immunotherapy

### Epidemiology of invasive fungal diseases in immune compromised patients

Invasive fungal infections caused by *Candida* species or *Aspergillus fumigatus* and other filamentous moulds are devastating in immune compromised patients. Patients at risk include transplant recipients, patients receiving chemotherapy, patients infected with human immunodeficiency virus-1 (HIV1) or patients with underlying autoimmune diseases. Allogeneic haematopoietic stem cell trans-

plant (HSCT) recipients and patients treated for acute leukaemia are predominately affected [1–5].

In these populations, *Candida* infections are associated with a mortality of around 20 to 40%, whereas invasive mould infections carry even higher mortality reaching up to 80% [6–11]. Prior to the routine use of antifungal prophylaxis, *Candida* species (spp.) accounted for the majority of fungal infections among these patients. However, over the last two decades, the incidence of *Aspergillus* infections has surpassed that of *Candida* infections. This mainly results from the use of effective antifungal prophylaxis targeting *Candida* spp. [12, 13].

In recent years, posaconazole and voriconazole prophylaxis have led to a great reduction of invasive mould and yeast infections in randomised controlled trials [14, 15] and are therefore recommended in high-risk populations (e.g., during neutropenia or graft-versus-host diseases [GVHD]). However, these prophylaxes are not uniformly adopted in Switzerland and other countries because of concerns about high costs, drug interactions, toxicity and, most importantly, limited clinical efficacy with the emergence of resistant fungal strains and breakthrough infections [14, 15]. Indeed, new and highly treatment-resistant fungal species, including yeasts resistant to azole therapies like *Candida krusei* and *Candida glabrata*, as well as highly resistant moulds such as *Aspergillus fumigatus* with mutations in the *cyp51A* gene, *Aspergillus terreus*, *Fusarium* spp., *Zygomycetes* spp. and *Scedosporium* spp. have emerged as serious pathogens in transplant recipients [13, 16–20]. Hence, reliable tools to identify patients at risk and tailored treatment strategies to improve patient outcome are urgently needed.

The introduction of precision medicine, which takes into account individual genetic and environmental factors in the choice of the most promising therapy for each patient, has led to impressive achievements, especially in the field of oncology [21]. Large-scale efforts, such as the United States Precision Medicine Initiative or the United Kingdom

100 000 Genomes Project, aim at enhancing the impact of this concept in oncology and extending its application to other clinical areas including infectious diseases [22, 23]. A comprehensive understanding of the genetic and functional basis of immune protection in fungal infection will promote precision medicine for infectious diseases through classification of patients according to a specific risk score and personalised therapy to restore the impaired host immunity in high-risk patients [24].

## Host immune response to fungal infection

The innate immune system plays a pivotal role in protection from acute fungal infections [25–27]. Innate immune cells including neutrophils, monocytes/macrophages and dendritic cells rapidly detect the presence of fungi and induce an antimicrobial response. Fungal recognition is mediated by a variety of surface-bound and soluble pattern recognition receptors (PRRs) recognizing fungal cell wall components and nucleic acids including Toll-like receptors (TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR9), C-type lectins (Dectin-1, Dectin-2, Mincle, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin [DC-SIGN], mannose binding lectin 2 [MBL2]), and long pentraxin 3 (PTX3) [28–33]. The importance of these PRRs for fungal control has been demonstrated in a variety of animal studies [34–39].

Neutropenia is a critical risk factor for the development of invasive fungal infections. Neutrophils possess a wide range of effector mechanisms that contribute to intra- and extracellular elimination of fungi, including production of reactive oxygen species, release of antimicrobial peptides, or the formation of neutrophil extracellular traps (NETs) to restrict fungal spread [27, 40–43]. However, recent findings show that neutrophil function is not only restricted to elimination of microorganisms. These cells can show extensive heterogeneity and plasticity, can greatly extend their life span and have important functions in immunoregulatory networks through contact-dependent mechanisms or by *de novo* production of cytokines [44, 45]. Neutrophils can further prevent morphotypic switching (e.g., transition from yeast to filamentous growth), a key virulence trait of *C. albicans* [46–48]. Taken together, although a large arsenal of different antifungal activities of neutrophils have been described, it is still not well understood which ones are most relevant *in vivo* in infected tissues.

Innate antifungal defence also relies on mononuclear phagocytes. Tissue-infiltrating monocytes have been described in the context of *Candida* and *Aspergillus* infections [49, 50] and tissue-resident macrophages, such as CX3CR1<sup>+</sup> macrophages in the kidney, were protective against systemic candidiasis [51]. Monocytes and macrophages display a remarkable ability to internalise fungi, to secrete several proinflammatory cytokines and chemokines and to exert significant fungicidal activity [27]. Their contribution to antifungal defence may be of particular relevance in neutropenic settings [52].

Natural killer (NK) cell recruitment was previously reported to be essential for antifungal defence in neutropenic mice [53, 54] and NK cell proliferation was associated

with inhibition of fungal growth [55]. Moreover, adoptive NK cell transfer led to enhanced fungal clearance in neutropenic animals [56]. NK cells might also be important in the context of invasive fungal infections in patients after HSCT, as we observed that patients with invasive aspergillosis had reduced NK cell counts, and lower NK cell counts were associated with a poor outcome [57]. NK cells may exert direct antifungal activity by secretion of interferon- $\gamma$  (IFN- $\gamma$ ) and perforin [58, 59], or contribute to fungal clearance by regulation of other innate and adaptive immune cells via cytokine production [60] such as granulocyte macrophage colony stimulating factor (GM-CSF), which promotes the mobilisation and antimicrobial activity of granulocytes and macrophages [54, 59, 61–63]. In humans, NK cells may directly respond to fungal stimuli via the NK receptor NKp30 and a still unknown fungal ligand [64] or become activated through accessory cells such as monocytes/macrophages or dendritic cells [65–67]. It will be interesting to determine whether particular NK cell subsets are important for fungal control, as we have previously shown for the control of viral infections [68].

Additionally to innate immunity, adaptive immune responses seem to play a crucial role in fungal control. The protective role of T cell responses in *Candida* and *Aspergillus* infections has been studied intensively in different experimental systems and some human studies [69–72]. Fungus-specific T helper (Th) 1 responses characterised by production of IFN- $\gamma$ , GM-CSF and tumour necrosis factor (TNF- $\alpha$ ) are protective, and impaired Th1 cell numbers and cytokine responses correlate with higher fungal burden [73–75]. A protective role of Th17 cells in fungal immunity has also been observed, in particular in mucocutaneous *Candida* infections [76]. Moreover, it has been recently demonstrated in a mouse model of oropharyngeal candidiasis that other sources of interleukin (IL)-17, including innate lymphoid cells, also contribute to fungal control [77]. Whereas the contribution of CD4<sup>+</sup> T cells in anti-fungal adaptive immunity is well characterised, recent studies have also unveiled protective CD8<sup>+</sup> T-cell immunity against *Aspergillus* and *Candida* infections [78]. We have recently shown that after HSCT patients have a defective *A. fumigatus*-specific T cell response for up to a year after transplantation, correlating with the period when patients are at highest risk for infection [57]. Moreover, *A. fumigatus*-specific T cell responses to different cell-wall and cytosolic antigens were higher in patients recovering from invasive aspergillosis than in patients with progressive disease [79]. Consistent with the observations in transplant patients, an inverse correlation between CD4<sup>+</sup> T cell numbers and the incidence of invasive fungal infections has been observed in HIV-infected patients [73, 80].

In summary, antifungal immunity relies on many different immune pathways and functional defects in many of those have been associated with the occurrence or severity of invasive fungal infections in animal as well as human studies.

## Association of genetic polymorphisms with increased risk for invasive candidiasis and aspergillosis

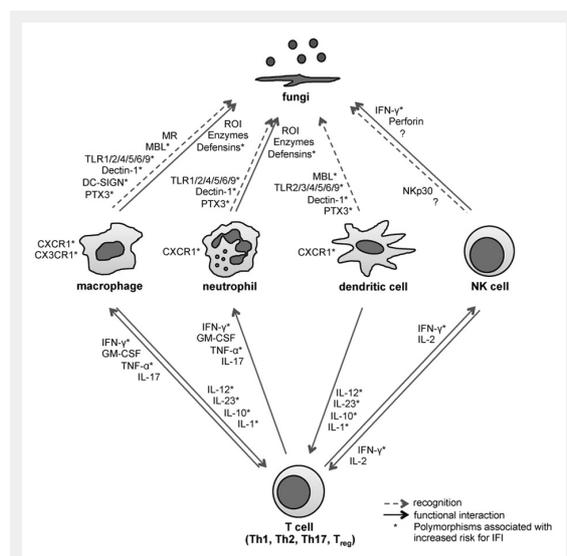
It is striking that despite similar clinical risk factors such as chemotherapy, graft source or GVHD and/or similar immunosuppressive conditions, some patients rapidly develop invasive fungal infections, while others seem to be protected and never do so. Such differences may result, at least in part, from the individual genetic makeup that would increase or decrease susceptibility to infection. Based on this hypothesis, many investigators analysed whether single nucleotide polymorphisms (SNPs) in genes involved in immune responses against fungal pathogens influenced susceptibility to infections (table 1 and fig. 1) [81].

Among the most studied candidate genes are those encoding PRRs, as well as those encoding cytokines, chemokines and their receptors and/or antagonists. So far, more than 35 association studies of such polymorphisms with invasive fungal infections have been published [81]. However, many studies were limited by several factors, including small sample size, lack of replication, and/or lack of functional evidence supporting the association. Many studies also had a problematic design, such as cases and controls not exposed to the same risk, inhomogeneous ethnic background, failure to account for relevant non-genetic risk factors or time at risk and/or lack of correction for multiple testing to obtain definite evidence. However, some studies reported relatively robust associations, which were either replicated by several investigators and/or are supported by strong functional evidence.

One of the first studies that included a replication group identified an association between two missense polymorphisms within TLR4 (D299G and T399I) and suscept-

ibility to invasive aspergillosis after HSCT [82]. The relevant polymorphisms were issued from the donor, i.e., affected the engrafted immune cells, but not the recipient, and could be combined with other risk factors such as cytomegalovirus serostatus for pretransplant risk stratification. Both polymorphisms were also associated with disseminated candidiasis in a small cohort of non-neutropenic patients [83]. However, the association of these TLR4 polymorphisms with invasive aspergillosis was not universally confirmed in other HSCT studies [84–86], possibly as a result of differences in the patients' characteristics, conditioning and/or antifungal prophylactic regimen across different studies or over time. The limited reproducibility could also be due to the very low frequencies of both TLR4 polymorphisms, thereby requiring very large patient numbers for replication.

A further study supported by strong functional evidence was the association of a stop-codon polymorphism in Dectin-1 (Y238X) in both recipient and donor with an increased risk for invasive aspergillosis after HSCT [87]. *In vitro* studies showed that Dectin-1 silencing in respiratory epithelial cells resulted in impaired *Aspergillus*-driven proinflammatory responses. The Dectin-1 polymorphism was associated with diminished IFN- $\gamma$  and IL-10 secretion in peripheral blood mononuclear cells (PBMCs) upon stimulation with this fungus *in vitro* [87]. *In vivo* mouse studies further revealed that both donor (haematopoietic cells) and recipient (nonhaematopoietic cells) Dectin-1 was needed to complement a protective role against invasive aspergillosis after HSCT [87]. In addition to its relevance for mould infections, the polymorphism in Dectin-1 was further associated with inherited forms of chronic mucocutaneous candidiasis [88] and oral and gastrointestinal *Candida* colonisation, but not with invasive candidiasis after HSCT [89].



**Figure 1**

Single nucleotide polymorphisms pathways of innate and adaptive antifungal immunity associated with increased risk for invasive fungal infections.

NK cell = natural killer cell; MR = mannose receptor; MBL = mannose-binding lectin; TLR = Toll-like receptor; PTX3 = pentraxin 3; ROI = reactive oxygen intermediates; IFN = interferon; GM-CSF = granulocyte macrophage colony stimulating factor; TNF = tumour necrosis factor; IL = interleukin; IFI = invasive fungal infection

Polymorphisms and/or haplotypic combinations of three IL-1 cluster genes including IL-1 $\beta$  (-31T/C and -511C/T), natural IL-1 receptor antagonist (IL1RN; 2018T/C, VNTR2) as well as IL-1 $\alpha$  (-889C/T) were associated with invasive aspergillosis in solid organ transplant recipients [90] and acute leukaemia patients [91]. IL-1 $\beta$  is a key proinflammatory cytokine, involved in promoting both innate and adaptive responses during infection with *A. fumigatus*, and its action can be controlled by its natural antagonist IL1RN. PBMCs from individuals carrying one or two copies of the -511C/T polymorphism in the promoter of IL-1 $\beta$  or the 2018T/C polymorphism in IL1RN showed decreased production of IL-1 $\beta$  and TNF- $\alpha$  upon stimulation with *Aspergillus* [90].

Moreover, polymorphisms in the chemokine receptors CXCR1 (CXCR1-T276) and CX3CR1 (CX3CR1-M280) were associated with an increased risk for disseminated candidiasis [92, 93]. These factors regulate neutrophil- and macrophage-mediated innate defence against fungal infections as discussed above.

The most promising results in the field of fungal immunogenetics have been provided by recent studies uncovering two frequent polymorphisms (281A/G and 734A/C) in the long PTX3 gene as susceptibility markers of invasive aspergillosis in two different populations: HSCT [94] and

solid organ transplant [95] recipients. The associations with invasive aspergillosis resulted from the presence of the risk allele in the donor for HSCT and in the recipient for solid organ transplants, which is consistent with the source

**Table 1:** Genetic risk factors for invasive fungal infections.

Gene	SNPs ID	Genetic association <sup>1</sup>		Replication	Functional evidence
		IA	IC		
<b>Pattern recognition receptors</b>					
TLR1	rs574361	↑	↑	Controversial	+/-
	rs4833095, rs5743618	↑	↓	Controversial	+/-
TLR6	rs5743810	↑	NA	Controversial	-
TLR4	rs4986790, rs4986791	↑	↑	Controversial	+/-
TLR2	rs5743708	NA	↑	Controversial	+
TLR3	rs3775296	↑	NS	No	+
TLR5	rs5744168	↑	NS	No	-
TLR9	rs5743836	NA	NA	No	-
CLEC7A	rs16910526	↑	NA	Controversial	+
	rs7309123, rs3901533	↑	NS	No	+
CD209	rs4804800, rs11465384, rs7248637, rs7252229	↑	NS	No	-
MBL	Low genotype	↑	↑	No	+/-
PTX3	rs2305619/rs3816527	↑	NS	Yes	+
<b>Cytokines and related genes</b>					
IL1A	rs1800587	↑	NS	No	+
IL1B	rs1143627, rs16944	↑	NA	Controversial	+
IL1RN	82bp VNTR	↑	NS	No	+
	rs419598	↑	NS	No	+
IL4	rs2243250, rs2070874, rs2243248	NS	↑	No	-
IL8	rs2227307	↑	NS	No	-
IL10	rs1800896, rs1800871, rs1800872	↑	↑	Controversial	+/-
IL12B	rs41292470	NA	↑	No	+
	rs3212227	↓	NS	No	-
IL23R	rs11209026	↓	NS	No	+/-
TNFA	rs1800629	NA	↑	No	-
TNFR1	rs4149570	↑	NS	No	+
TNFR2	-322 VNTR	↑	NS	No	-
IFNG	rs2069705	↓	NS	Yes	+
	rs2430561	NA	NS	No	-
CCL8	1-kg-17-29697448	NS	↑	No	+
CXCL10	rs3921, rs1554013, rs4257674	↑	NS	No	+
CXCR1	rs2234671	NS	↑	No	+
CX3CR1	rs3732378	NS	↑	Yes	+
<b>Other</b>					
VEGFA	rs3024994	↑	NS	No	-
	rs2146323, rs6900017	↑	NS	No	-
DEFB1	rs1800972	↑	↑	No	-
MASP2	rs72550870	↑	NS	No	-
RAGE	rs1800624	↑	NS	No	+
S100B	rs9722	↑	NS	No	+
PLG	rs4252125	↑	NS	No	+/-
STAT1	rs16833172	NS	↑	No	+
SP110	rs3769845	NS	↑	No	+
PSMB8	rs3198005	NS	↑	No	+
CD58	rs17035850, rs12025416	NS	↑	Controversial	+
LCE4A/C1orf68	rs4845320	NS	↑	Controversial	-
TAGAP	rs3127214	NS	↑	Controversial	+

C1orf68 = chromosome 1 open reading frame 68; CCL8 = chemokine C-C motif ligand 8; CD209 = cluster of differentiation 209 (known as DC-SIGN); CLEC7A = C-type lectin domain 7 (known as Dectin-1); CXCL10 = CXC-chemokine ligand-10; CXCR1 = Chemokine C-X-C Motif Receptor 1; CX3CR1 = Chemokine C-X3-C Motif Receptor 1; DEFB1 =  $\beta$ -defensin 1; IA = invasive aspergillosis; IC = invasive candidiasis; IL = interleukin; IL1RN = interleukin-1 receptor antagonist; IL23R = interleukin 23 receptor; IFNG = interferon gamma; LCE4A = late cornified envelope (LCE) protein 4 A; MASP2 = mannan-binding lectin serine peptidase 2; MBL = mannose binding lectin; PLG = plasminogen; PSMB8 = proteasome (prosome, macropain) subunit beta type 8; PTX3 = pentraxin 3; RAGE = advanced glycosylation end product-specific receptor; SNP = single nucleotide polymorphism; S100B = S100 calcium binding protein B; SP110 = speckled 110 kDa; STAT1 = signal transducer and activator of transcription 1; TAGAP = T cell activation RhoGTPase-activating protein; TLR = toll-like receptor; TNFA = tumor necrosis factor alpha; TNFR = tumour necrosis factor receptor; VEGFA = vascular endothelial growth factor A

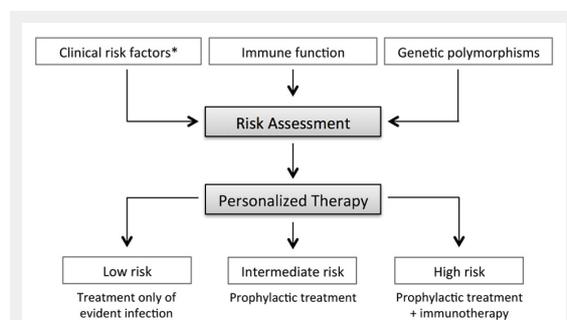
<sup>1</sup> Effect of minor allele on susceptibility to either invasive aspergillosis (IA) or invasive candidiasis (IC); the arrow symbol "↑" refers to variants that were associated with increased susceptibility; the arrow symbol "↓" refers to variants that showed protective effect; "NA" refers to variants that were studied but were not associated; "NS" refers to variants that were not studied.

of circulating immune cells in both situations. These data were strongly supported by functional work [94] and confirm the protective role of PTX3 observed in animal studies [34, 37, 38, 96]. The h2/h2 haplotype (composed of 281G and 734A alleles) was associated with lower expression of PTX3 by neutrophil precursors *in vitro* upon exposure to *Aspergillus*, most likely owing to changes in messenger RNA (mRNA) folding [94]. Lower PTX3 levels were consistently observed in bronchoalveolar lavage and lung biopsies from patients with invasive aspergillosis carrying the h2/h2 haplotype, compared with controls. PTX3 is released by circulating immune cells such as neutrophils upon infection with *Aspergillus* to promote fungal recognition and killing. Neutrophils from patients carrying the D48A PTX3 polymorphism had reduced ability to phagocytose and kill *Aspergillus* conidia as compared with other neutrophils [94].

In summary, diverse genetic studies linking the presence of polymorphisms with susceptibility to invasive fungal infections have considerably contributed to our understanding of antifungal immune mechanisms and might help to promote precision medicine for invasive fungal infections.

### How immunology and host genetics could help to identify and improve the treatment of patients at risk for invasive fungal infections

Basic research over recent years has provided important insights into the mechanisms of protection against invasive *Candida* and *Aspergillus* infections. These studies demonstrated the contribution of various immune cells including



**Figure 2**  
Possible risk assessment and personalised therapy scheme for patients at risk for invasive fungal infections.  
\*Clinical risk factors include graft-versus-host disease, steroid treatment, T cell-depleted graft and acute myeloid leukemia.

	advantages	disadvantages
 IFN-γ capture assay	<ul style="list-style-type: none"> <li>specific isolation of functional IFN-γ producing Th1 cells</li> <li>GMP compliant</li> <li>clinically applied for virus-specific T cells</li> </ul>	<ul style="list-style-type: none"> <li>loss of Th17 cells and T<sub>H2</sub></li> </ul>
 CD154 <sup>+</sup> isolation	<ul style="list-style-type: none"> <li>isolation of T-cells with different cytokine profiles</li> <li>high sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>loss of CD8<sup>+</sup> T cells</li> <li>potential isolation of non-protective Th2 cells</li> </ul>
 CD137 <sup>+</sup> isolation	<ul style="list-style-type: none"> <li>isolation of T cells with different cytokine profiles</li> <li>high sensitivity</li> <li>GMP compliant</li> </ul>	<ul style="list-style-type: none"> <li>isolation of "false positive" cells due to unspecific CD137 expression</li> <li>potential isolation of non-protective Th2 cells</li> </ul>

**Figure 3**  
Advantages and disadvantages of different enrichment strategies for antigen-specific T cells.  
GMP = Good Manufacturing Practice; IFN = interferon

neutrophils, macrophages, dendritic cells, NK cells and T cells to antifungal immunity, and partly revealed the molecular mechanisms of protection. Genetic studies demonstrated how genetic polymorphisms, especially in signalling pathways of innate immune cells, can predispose to the development of invasive fungal infections.

Techniques such as high throughput SNP genotyping (e.g., large arrays of selected SNPs) or genome-wide association studies (GWAS) have become more easily accessible in the clinical setting. Therefore, the chance to assign each patient an individual risk score based on the genetic background of the donor and/or the recipient is coming within reach. This knowledge might lead to personalised prophylaxis and treatment schemes comparable to the approach used in modern oncology and would counter overtreatment with antifungal therapy (fig. 2).

Early immunotherapeutic approaches such as granulocyte infusion [97] or administration of GM-CSF or IFN-γ tried to restore the deficient number and function of innate immune cells in patients with invasive fungal infections. Although these therapies were promising in patients with chronic granulomatous disease, they were used only reluctantly in transplant recipients because of their limited clinical efficacy and the potential to induce GVHD or graft loss [98, 99].

Another approach improving antifungal immunity might be administration of the soluble PRR PTX3 alone or in combination with antifungal drugs, especially in patients with the PTX3 h2/h2 haplotype. The protective effect of PTX3 alone or together with amphotericin B was shown in a murine model of invasive aspergillosis [34]. PTX3-mediated protection was associated with accelerated recovery of lung phagocytic cells and Th1 lymphocytes, and a concomitant decrease of inflammatory pathology. PTX3 administration also potentiated the therapeutic efficacy of suboptimal doses of amphotericin B. These encouraging results could be reproduced in rats treated with PTX3 alone or in combination with posaconazole or voriconazole [37, 38, 96]. Similar to the protective effect of PTX3, administration of MBL might be another therapeutic option. In mice with invasive aspergillosis, MBL administration significantly increased survival and production of the proinflammatory cytokines TNF-α and IL-1α [36], and human studies showed significantly lower serum MBL levels in patients with invasive aspergillosis than in control patients [100].

The identification of NK cells and T cells as important players in antifungal immunity also encourages cellular therapies such as adoptive transfer of NK cells or antigen-specific T cells. Many studies have assessed the potency of NK cell transfer in antitumour therapy [101, 102], but there is still limited knowledge of this approach in the context of infectious diseases. However, the observation that HSCT and solid organ transplant recipients with invasive fungal infections have lower NK cell counts compared with control patients, endorses adoptive NK cell transfer.

Adoptive transfer of donor-derived pathogen-specific T cells is to date the most promising and feasible immunotherapeutic strategy in transplant recipients restoring the lacking T cell function [103–105]. So far, only one study targeting fungal infections has been performed, in haploidentical HSCT recipients with invasive aspergillosis. In

this study, infusion of donor-derived *Aspergillus*-specific Th1 clones generated by stimulation with inactivated *A. fumigatus* conidia controlled *Aspergillus* galactomannan and helped to clear invasive aspergillosis in 9 of 10 patients [106]. However, reproducibility is difficult due to variation of stimuli and the elaborate production under good manufacturing practice (GMP). Recent research has therefore

focused on identification of suitable recombinant antigens for fungus-specific cells and on a simple, reproducible and reliable isolation or culture method to generate fungus-specific T cells.

The identification of fungal antigens is challenging and until now, only few immunogenic proteins and T cell epitopes specific for *A. fumigatus* have been characterised in

**Table 2:** Immunogenic *A. fumigatus* proteins in mice, healthy individuals and haematopoietic stem cell transplant recipients with invasive aspergillosis.

Antigen	Localisation	Study design	Responding T cells	Cytokine profile	Additional findings	Ref
Crf1 (Asp f9/16)	GPI-anchored cell wall protein	Rechallenge of vaccinated mice (CTX-treated or BMT) with <i>A. fumigatus</i>	CD4	IFN- $\gamma$ , IL-10, (IL-17)	Protective; cross-protective against <i>C. albicans</i>	[111, 120]
		Characterisation of PBMC, T cell lines or T cell clones from healthy donors	CD4, (CD8)	IFN- $\gamma$ , (TNF- $\alpha$ , GM-CSF, IL-10, IL-17, IL-4)	(Cross-reactive to <i>C. albicans</i> <i>in vitro</i> )	[79, 111, 115, 120–125]
		Characterisation of PBMC or T cell clones from HSCT recipients with IA	(CD4)	(IFN- $\gamma$ , IL-10, IL-17)	Patients with better IFN- $\gamma$ response show favourable outcome; specific T cells appear at regression of IA lesion	[79, 107, 123]
Gel1	GPI-anchored cell wall protein	Rechallenge of vaccinated mice (CTX-treated or BMT) with <i>A. fumigatus</i>	CD4	IFN- $\gamma$ , IL-10, (IL-17)	Protective; increased survival	[120]
		Characterisation of PBMC or T cell clones from healthy donors	CD4, (CD8)	(IFN- $\gamma$ , TNF- $\alpha$ , IL-10, IL-17)		[79, 120, 121, 123]
		Characterisation of PBMC from HSCT recipients with IA	n.d.	(IFN- $\gamma$ , IL-10)	Patients with better IFN- $\gamma$ response show favourable outcome	[79, 123]
Pmp20 (Asp f3)	Peroxisomal protein	Rechallenge of vaccinated WT, cortisone acetate-immunosuppressed or PMN-depleted mice with <i>A. fumigatus</i> or adoptive transfer of CD4 T cells from vaccinated mice to naive mice	CD4		Protective in WT mice, partly protective in immunosuppressed and PMN-depleted mice	[126, 127]
		Characterisation of PBMC from healthy donors	CD4, CD8	IFN- $\gamma$ , IL-4, IL-17, (TNF- $\alpha$ , IL-10)		[79, 121, 122]
		Characterisation of PBMC from HSCT recipients with IA	n.d.	(IFN- $\gamma$ )	Higher response in patients with favourable outcome	[79]
Pep1	Secreted protein	Rechallenge of vaccinated mice (CTX-treated or BMT) with <i>A. fumigatus</i>	CD4	IFN- $\gamma$ , IL-10, (IL-17)	Protective	[120]
		Characterisation of PBMC or T cell clones from healthy donors	CD4, (CD8)	IL-17 (IFN- $\gamma$ , IL-10)		[120, 123]
		Characterisation of PBMC from HSCT recipients with IA	n.d.	(IFN- $\gamma$ ), IL-10	Patients with IFN- $\gamma$ response show favourable outcome	[123]
Cat1	Secreted protein	Rechallenge of vaccinated mice with <i>A. fumigatus</i>	CD4, Th2	IL-4	Non-protective	[120]
		Characterisation of PBMC or T cell clones from healthy donors	CD4	IFN- $\gamma$ , (IL-4, IL-10, IL-17)		[115, 120, 128]
		Characterisation of T cell clones from HSCT recipients with IA	CD4	IFN- $\gamma$ (IL-17)	Specific T cells appear at regression of IA lesion	[107]
Sod	Secreted protein	Rechallenge of vaccinated mice with <i>A. fumigatus</i>	CD4, Th2	IL-4	Nonprotective	[120]
		Characterisation of PBMC or T cell clones from healthy donors	CD4 (CD8)	IL-17, IL-10, (IFN- $\gamma$ , TNF- $\alpha$ , IL-4)		[120, 121, 123]
		Characterisation of PBMC from HSCT recipients with IA	n.d.	(IFN- $\gamma$ ), IL-10	Patients with IFN- $\gamma$ response show favourable outcome	[123]

Crf1 = extracellular cell wall glucanase Crf1; Gel1 = 1 =3- $\beta$ -glucanosyl-transferase Gel1; Pmp20 = cytosolic peroxisomal peroxiredoxin Pmp20 (Asp f3); Pep1 = aspartic protease Pep1; Cat1 = mycelial Catalase 1; Sod = superoxide dismutase; GPI = glycosylphosphatidylinositol; CTX = cyclophosphamide; BMT = bone marrow transplanted; HSCT = hematopoietic stem cell transplantation; IA = invasive Aspergillosis; IFN = interferon; IL = interleukin; n.d. = not determined; PBMC = peripheral blood mononuclear cells; PMN = polymorphonuclear cells; TNF = tumor necrosis factor; WT = wild type  
Data in brackets apply only to a part of the studies.

healthy individuals and mice (table 2). Only recently, we and others have shown that T cell responses to some of these proteins correlate with a beneficial outcome in patients with invasive aspergillosis [107, 108]. Furthermore, it would be favourable if the transferred T cells would target various clinically relevant moulds [3, 109, 110]. We have previously shown that CD4<sup>+</sup> cells specific for the *A. fumigatus* Crf1/p41 epitope confer cross-reactivity to *C. albicans* in a mouse model, thereby targeting the two most important fungal pathogens in HSCT recipients [111]. In a further study, we showed that T cell lines specific for *A. fumigatus* Crf1, Gel1 and Pmp20 proteins not only efficiently recognised naturally processed *A. fumigatus*, but additionally cross-reacted with different clinically relevant *Aspergillus* and *Mucorales* species, suggesting that adoptively transferred T cells could very likely protect the recipients against a variety of fungal infections [79].

Various isolation and expansion methods for fungus-specific T cells have been assessed *in vitro* (fig. 3, reviewed in [112]). The cytokine capture assay is GMP-compliant and has been shown to be efficacious and safe for the isolation of virus-specific T cells [113]. This method has, however, limited sensitivity when fungus-specific peptide pools are used (unpublished data N.K.). Therefore other selection methods based on activation-dependent expression of CD154 or CD137 have been investigated [79, 107, 114, 115]. Although all isolation strategies were able to enrich antigen-specific T cells from PBMC, the relatively low specificity and cell number after isolation probably hinders direct infusion and additional *in vitro* expansion would be required [116–118].

A thorough understanding of antifungal immune pathways could further lead to novel treatment approaches such as the development of bioengineered T cells with antifungal activity. This was exemplified by Cooper and colleagues who showed that T cells expressing a chimeric antigen receptor based on the PRR Dectin-1 were able to inhibit hyphal growth of *Aspergillus* both *in vitro* and *in vivo* [119] and thereby provided an interesting alternative to conventional T cell therapy.

### Future challenges in invasive fungal infections

The identification of patients who are at increased risk for development of these infections, the lack of biomarkers to define the net state of immunosuppression and the problem of treating invasive fungal infections are remaining difficulties. It is debatable which patients would benefit the most from antifungal prophylaxis and who should be treated with combination therapies or even with immunotherapy. Ideally, these clinical decisions should be individualised based on clinical factors, genetic polymorphisms and immune function, which could be integrated into complex diagnostic algorithms or risk-stratification scores for personalised therapy. Well-designed intervention studies are needed to explore whether this concept can be translated into clinical practice, but previous experience in the field of oncology and the great progress of precision medicine raise confidence that the outcome of invasive fungal infections will be improved in the future.

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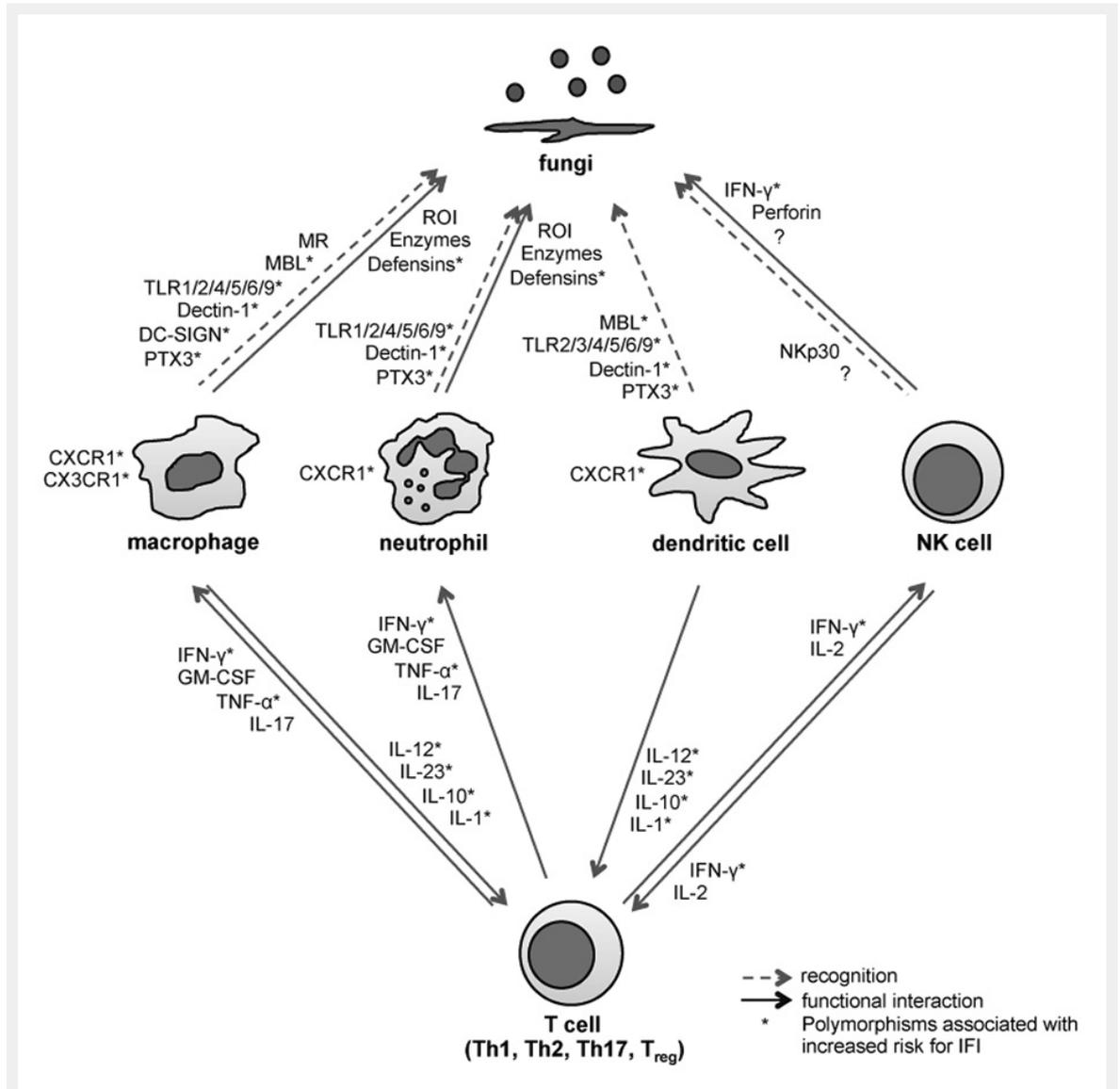
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Figures (large format)



**Figure 1**

Single nucleotide polymorphisms pathways of innate and adaptive antifungal immunity associated with increased risk for invasive fungal infections.

NK cell = natural killer cell; MR = mannose receptor; MBL = mannose-binding lectin; TLR = Toll-like receptor; PTX3 = pentraxin 3; ROI = reactive oxygen intermediates; IFN = interferon; GM-CSF = granulocyte macrophage colony stimulating factor; TNF = tumour necrosis factor; IL = interleukin ; IFI = invasive fungal infection

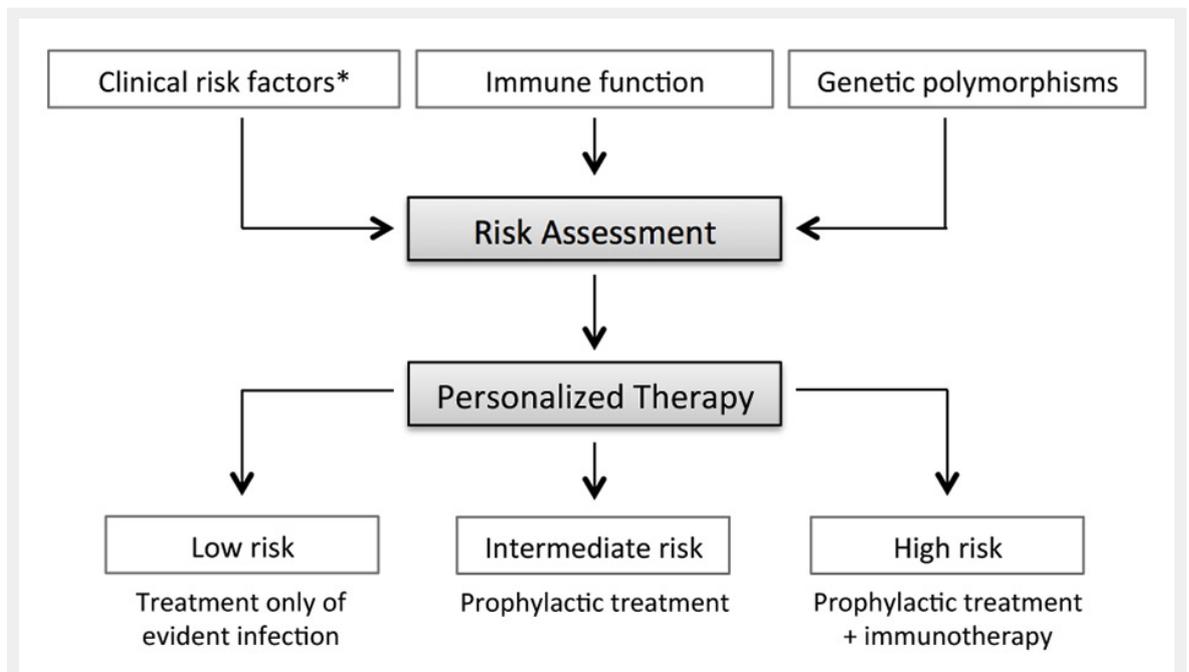


Figure 2

Possible risk assessment and personalised therapy scheme for patients at risk for invasive fungal infections.

\*Clinical risk factors include graft-versus-host disease, steroid treatment, T cell-depleted graft and acute myeloid leukemia.

	advantages	disadvantages
 <b>IFN-<math>\gamma</math> capture assay</b>	<ul style="list-style-type: none"> <li>specific isolation of functional IFN-<math>\gamma</math> producing Th1 cells</li> <li>GMP compliant</li> <li>clinically applied for virus-specific T cells</li> </ul>	<ul style="list-style-type: none"> <li>loss of Th17 cells and T<sub>reg</sub></li> </ul>
 <b>CD154<sup>+</sup> isolation</b>	<ul style="list-style-type: none"> <li>isolation of T-cells with different cytokine profiles</li> <li>high sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>loss of CD8<sup>+</sup> T cells</li> <li>potential isolation of non-protective Th2 cells</li> </ul>
 <b>CD137<sup>+</sup> isolation</b>	<ul style="list-style-type: none"> <li>isolation of T cells with different cytokine profiles</li> <li>high sensitivity</li> <li>GMP compliant</li> </ul>	<ul style="list-style-type: none"> <li>isolation of „false positive“ cells due to unspecific CD137 expression</li> <li>potential isolation of non-protective Th2 cells</li> </ul>

Figure 3

Advantages and disadvantages of different enrichment strategies for antigen-specific T cells.

GMP = Good Manufacturing Practice; IFN = interferon