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# Adoptive transfer of *Aspergillus*-specific T cells as a novel anti-fungal therapy for hematopoietic stem cell transplant recipients: Progress and challenges

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ABSTRACT

Although newer antifungal drugs have substantially altered the natural history of invasive aspergillosis, the disease still accounts for significant morbidity and mortality in hematopoietic stem cell transplant recipients. Both the evidence supporting a protective role of T cells against this fungal pathogen and the documented efficacy of adoptive transfer of antigen-specific T cells for prophylaxis and treatment of viral infections post-transplant have stimulated much interest towards development of *Aspergillus*-specific T cells (Asp-STs) for adoptive immunotherapy in the allogeneic transplant setting. In contrast to the remarkable progress with virus-specific T cells, clinical development of fungus-specific T cells is

*Abbreviations:* Ag, antigen; allo-HSCT, allogeneic hematopoietic stem cell transplantation; APCs, antigen presenting cells; Asp, *Aspergillus*; Asp-STs, *Aspergillus*-specific T cells; CAR, chimeric antigen receptor; cGMP, current good manufacturing practice; CMV, cytomegalovirus; CSFs, colony-stimulating factors; D-CAR, dectin-1 CAR; DCs, dendritic cells; DC SIGN, DC-specific ICAM3-grabbing non-integrin; DLIs, donor lymphocyte infusions; EBV, epstein-barr virus; GvHD, graft versus-host disease; HSCT, hematopoietic stem cell transplant; IA, invasive aspergillosis; ICS, intracellular cytokine staining; IFN-γ, interferon-gamma; IL, interleukin; NETs, neutrophil extracellular traps; PB, peripheral blood; PBMCs, peripheral blood mononuclear cells; PRR, pattern recognition receptor; SB, sleeping beauty; Tcon, conventional T cell; TCR, T cell receptor; TGF-β, transforming growth factor beta; Th, T helper; TLRs, toll-like receptors; TNF-α, tumor necrosis factor-alpha; Tregs, regulatory T cells; WBC, white blood cell.

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still in its infancy. Several groups have characterized Asp-STs in healthy individuals and patients with malignant hematological diseases, while others sought to develop GMP-compliant methods of expanding or bioengineering Asp-STs *ex vivo* as immunotherapy. This review highlights the recent advances in this field, and discusses critical issues involved in development and protocol design of Asp-ST immunotherapy.


## 1. Introduction

Infections by *Aspergillus* represent a leading cause of morbidity and mortality in patients with hematological malignancies; those who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) are particularly at risk (Kontoyiannis et al., 2010; Pagano et al., 2007; Dvorak et al., 2005). Immune competent individuals readily eliminate the ubiquitous conidial spores that are constantly inhaled, thus preventing their germination into disease-causing hyphae that feature prominently in invasive aspergillosis (IA). However, when host defenses are breached, as is the case in immunocompromised hosts, these conidia are free to precipitate devastating infection. Patients with prolonged and profound neutropenia after treatment with highly cytotoxic chemotherapy for hematological malignancies, those with primary or acquired immunodeficiencies, and hematopoietic stem cell or solid organ transplant recipients are considered to be at highest risk for IA (Vinh et al., 2010; Segal, 2009). In the current article, we provide an updated review of the literature on IA post allo-HSCT and discuss the recent advances and considerations for the use of adoptive T cell therapy using ex vivo expanded T cells in this special patient population.

### 1.1. Epidemiology and risk factors of IA in allo-HSCT recipients

*Aspergillus* is the most common fungal pathogen among adult allogeneic HCST recipients and accounts for 40–70% of all invasive fungal diseases. IA in allogeneic HSCT has a 12 month cumulative incidence of 1.6%, and a dismal overall 1 year survival of 25.4% among HSCT cohorts (Kontoyiannis et al., 2010). The high mortality rate (Kontoyiannis et al., 2010; Caira et al., 2008; Mikulska et al., 2009; Neofytos et al., 2009, 2013; Wiederhold et al., 2003) reflects contributions of both graft versus-host disease (GvHD) severity and intensity of immunosuppression to IA pathogenesis (Jantunen et al., 1997; Marr et al., 2002; Wald et al., 1997).

Over the last decades, the increased use of peripheral blood stem cells, growth factors and nonmyeloablative conditioning regimens in allo-HSCT decreased the frequency of IA early post HSCT (≤40 days) by shortening the duration of pre-engraftment neutropenia. However, the increase in the use of transplants from alternative (cord blood or haploidentical) donors (Passweg et al., 2015), older recipient age, increased chronic GvHD incidence (with concomitant therapy with corticosteroids) and cytomegalovirus (CMV) infection (resulting in prolonged lymphopenia), have dramatically shifted the incidence among HSCT recipients towards late (41–180 days) or very late (>6 months) onset post transplant (Marr et al., 2002).

### 1.2. The need for better treatment options in aspergillosis

Therapeutic interventions for IA are limited. The similarities between human and fungal cell organelles (such as nucleus, 80S ribosomes and Golgi apparatus) and mechanisms for DNA, RNA, and protein synthesis, greatly limit the number of potential antifungal drug targets, because the majority of compounds that inhibit fungi are also toxic to human cells (Shoham and Levitz, 2005). Among systemic antifungals available for the treatment of invasive fungal infections (Kauffman, 2006; Sucher et al., 2009; Moen et al., 2009), polyenes and azoles target components of the fungal cell membrane (ergosterol or ergosterol synthesis, respectively) (Haynes et al., 1996; Ghannoum and Rice, 1999) whereas echinocandins target the cell-wall, a structure not shared by mammalian cells, by inhibiting β-(1,3)-glucan synthesis (Denning, 2003).

Despite significant progress in developing antifungal drug therapies, not all allo-HSCT patients with IA are effectively treated (due to neutropenia, T cell suppression, or GVHD) and such therapies are associated with toxicity and drug–drug interactions (Marr, 2008). In addition, prolonged treatment can lead to uncommon or drug-resistant molds, whereas it significantly increases the financial burden of transplant care (Baddley et al., 2013; Kim et al., 2011; Dasbach et al., 2000).

There is thus an unmet need for the development of novel, alternative therapies for the management of IA after allo-HSCT. Given that only immunocompromised subjects succumb to IA, it is reasonably suggested that the enhancement of host defense by restoring or boosting *Aspergillus*-specific immunity could serve as a powerful treatment strategy.

### 1.3. The immune response to Aspergillus: a complex network of interactions

Inhaled conidia are initially repelled or damaged by the physical barrier of the respiratory tract and the produced reactive oxygen species and antimicrobial peptides of respiratory epithelial cells, respectively. Those escaping into the alveoli are mostly phagocytosed by cells of the innate immune system (Fig. 1). Alveolar macrophages constitute the first line of host defense against the spores whereas germinating conidia escaping macrophage surveillance and forming hyphae are cleared by monocytes and neutrophils recruited by the secretion of proinflammatory cytokines (Segal, 2009; Brakhage et al., 2010; Hasenberg et al., 2011). Because hyphae are too large to be engulfed, neutrophils exert a fungistatic effect and prevent further spreading through an array of extracellular killing mechanisms, including the formation of neutrophil extracellular traps (NETs) (Bruns et al., 2010). In addition, resting and germinating conidia, as well as hyphae, are potent activators of the complement cascade, which directs and modulates phagocytic functions (Brakhage et al., 2010). Natural killer cells are also recruited to the site of infection by secreted chemokines to support host defense (Morrison et al., 2003). Dendritic cells (DCs) bridge innate and adaptive immunity and shape T-cell responses to infection. Uptake and recognition of fungus by pattern recognition receptors (PRR) induces DCs maturation, presentation of fungal peptides to $CD4^+$ naïve T cells in draining lymph nodes and differentiation of naïve T-cells into T-helper (Th) cell subtypes (Bozza et al., 2002a). Polarization of the Th response by DCs is largely dependent on the type of PRR [soluble: pentraxin 3, complement; endocytic: dectin-1, DC-specific ICAM3-grabbing non-integrin (DC SIGN) or cell bound: toll-like receptors (TLR2, TLR4)] involved in recognition of *Aspergillus*; *Aspergillus* recognition via TLRs results in the induction of a Th1 response (Bozza et al., 2002a; Trinchieri, 2003), whereas recognition via dectin-1, promotes the secretion of interleukin (IL)-1β, IL-6 and IL-23 and induces Th17 differentia-

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