How I treat Adenovirus in haematopoietic stem cell transplantation recipients

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ABSTRACT

Adenovirus (Adv) infections are very common in the general pediatric population. The delayed clearance in young individuals imposes a threat to immunocompromised patients after haematopoietic stem cell transplant (HSCT), who can reactivate the virus, resulting in life-threatening disseminated disease. Although a definitive cure requires adequate immune-reconstitution, two approaches appear to be feasible and effective to improve the outcomes of AdV-infections. Strict monitoring with AdV quantitative-PCR followed by pre-emptive treatment with low dose (1 mg/kg) cidofovir 3-times a week, is effective in most cases to bridge the severely immuno-compromised period shortly post HSCT, with acceptable toxicity rates. For centers who have the access, Adv-specific cytotoxic T-cells (CTL) can be the other important corner stone of anti-adenovirus therapy with promising results so far. Methodologies to positively influence the reconstitution of the immune system post-HSCT and optimising new and currently available cellular immunotherapies will make HSCT safer against the threat of adenovirus infection/reactivation and associated disease.
INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is the last treatment option for a variety of diseases including certain haematological malignancies, inborn errors of metabolism, immune deficiencies and bone marrow failure syndromes. Although HSCT has become much safer over the last decade the major limitations remain transplantation related mortality (TRM) and relapse (in malignancies). In addition to the development of graft-versus-host disease (GvHD), infections are an important complication during HSCT procedures and contribute significantly to morbidity and TRM. They occur during the immunosuppressed period post-HSCT which is a consequence of both the preparative regimen (including serotherapy), a donor derived reconstituting immune system and the administration of immune-suppressive agents. Besides the risk of developing bacterial and fungal infections, there is a substantial risk of viral infection and reactivation. Of the latter, herpes viruses (VZV, HSV, Human Herpes Virus 6; HHV-6, Epstein Barr Virus; EBV, cytomegalovirus; CMV) and adenovirus (Adv) are the most important. In this review we will address the problem of adenovirus infections which can lead to lethal multi-organ involvement in the immunocompromised host and we will present a practical guideline for how we treat AdV-infections in HSCT recipients.

ADENOVIRAL INFECTIONS AND HOST DEFENSE

Adenovirus, belonging to the Adenoviridae family of DNA viruses, has 51 subtypes, of which (1, 2, 5, 7 and 14) are common causes of infections in the general population. In immunocompromised patients, mainly in post-HSCT patients, adenovirus disease may be life-threatening. For normal host defence against adenovirus both humoral and cellular responses seem to be important. In the first six months of life primary infections with adenovirus are uncommon, which is attributed to the presence of demonstrable levels of maternally derived serum IgG antibodies against several adenovirus subtypes in most infants at birth. Subtype-specific antibodies are formed against capsid as well as fiber proteins, which offer lifelong protection in the
immunocompetent host. Regarding cellular immunity directed against adenovirus, *in vitro* studies have demonstrated that adenovirus elicits both CD4+ and CD8+ T cell responses. However, *in vitro* removal of CD4+ T cells (but not CD8+ T cells) from a peripheral blood mononuclear cell (PBMC) population incubated with adenovirus, abrogates the lymphoproliferative response. In support of this finding, using MHC-class II-blocking antibodies, this lymphoproliferative response was demonstrated to be MHC class II restricted. Adenovirus activated CD4+ T cells have been demonstrated to produce IFNγ and can be cytotoxic, being able to lyse adenovirus infected cells *in vitro*. Approximately one third of the adenovirus genome is devoted to counteract innate and adaptive immune defences compromising the development of a protective immune response. The frequent re-infections and persistence of the virus in children on the one hand and the presence of adenovirus-specific CD4+ T cells in asymptomatic adults on the other hand, are a sign that the development of an adequate specific cross-reactive immune protection against adenovirus takes many years to develop. Adenovirus persists within lymphoreticular tissue: in macrophages of tonsils, adenoids, and intestines of infected hosts. Shedding can occur for months or years even in healthy children, making acquisition of the virus via horizontal transmission a major risk to immunocompromised patients. Although subtype-specific responses to various fiber epitopes have been detected, the majority of the most immunogenic epitopes are in the hexon protein of the capsid and appear to be conserved between several subtypes. Some of these have been shown to trigger both CD4+ and CD8+ T cell responses, which proved to be of great importance for the development of adenovirus targeting T-cell immunotherapy.

Permanent circulation of the virus among children explains why adenovirus infections are much more of a problem in pediatric patients (20-26%) undergoing HSCT, than in adults (9%). While adenovirus causes mild respiratory or gastrointestinal disease in healthy individuals, in the severely immunocompromised patient they are the cause of severe respiratory disease, hepatitis and colitis.
Other complications of the disease may involve hemorrhagic cystitis and adenoviral keratoconjunctivitis. Adenovirus primo-infected or reactivating patients can be divided in patients with subclinical viremia, viremia with disease symptoms, and disseminated disease. The incidence of disseminated disease is 1-7% with a reported mortality of 8-26% (Table 1). High mortality rates for disseminated disease were the reason for development of sensitive detection “monitoring” tools of subclinical adenovirus infections. Rapidly increasing of sustained viral load or sustained adenoviremia is associated with the occurrence of severe disease both in children and in adults\(^{13, 14, 15}\). Further complicating things the antiviral treatment options currently available have a considerable toxicity, which makes it particularly important to identify those patients most at risk of developing adenovirus disease. Monitoring of the adenoviral load by quantitative Adv PCR in the peripheral blood is far superior to other methods such as viral culture and the direct fluorescence assay, both in sensitivity and speed\(^ {16, 17}\). Performing weekly quantitative PCR (qPCRs) to monitor the adenovirus-, CMV-, EBV- and HHV6- DNA load, as well as immune-reconstitution monitoring (CD3 counts) post-HSCT are now widely used methods in many BMT-units\(^{14, 15}\). Weekly monitoring appears to be important because of the kinetics of AdV-replication, which can be rapid (Fig. 1)\(^{14, 15}\). Furthermore, Adv qPCR monitoring is used to evaluate therapeutic efficacy during treatment with antivirals. In the University Medical Center (UMC) Utrecht we use an in-house developed real-time PCR based adenovirus quantitative PCR test, but commercial tests are also available. The quality of these tests is similar and can be assured by joining the quality control for molecular diagnostics (QCMD) panel testing\(^{18, 19}\). Panels with three concentrations of DNA are sent out to different laboratories; results are submitted, analyzed, compared and the quality is reported back to the originating laboratories\(^ {18}\).

In some cases, adenovirus can be detected significantly earlier at local sites, such as in stool or nasopharyngeal aspirate (NPA). Lion et al. demonstrated that detection of adenovirus by PCR technology in stool is associated with disease and precedes Adv DNAemia by 11 days\(^ {20}\). In addition, we have recently shown
that adenovirus DNA positivity in NPA preceding HSCT, is a very strong predictor for adenovirus DNAemia in pediatric patients receiving an unrelated donor and we therefore perform screening as part of the standard pre-HSCT work-up. Presence of adenovirus in the NPA or stool might therefore be a decisive factor in postponement of an HSCT in pediatric patients with more elective non-malignant indications. 

**Risk factors and prevention of transmission**

Within the HSCT setting, there are several factors increasing the risk of adenovirus infection which are almost all related to a lack of cellular antiviral activity which is inherent to the first 100 days post-transplantation. Feuchtinger et al. demonstrated that the recurrence of adenovirus-specific T cells was crucial for clearance of infection, which was confirmed by others. In general, the number of CD3+ T cells, which is much more practical to determine, has been demonstrated to be valuable in the context of developing disease in case of viral reactivation and for the ultimate clearance of the virus. Absence of T cells (CD3+< 25/ul) or the failure of an adenovirus response shortly after adenovirus detection (CD3+ T cells < 300 / uL within 2 weeks of adenovirus detection) has been associated with a poor outcome and was therefore used in the presented guideline (Fig 2).

**Risk factors are:**

1) *Graft versus host disease* and the associated use of immunosuppressive agents (cyclosporine-A, methotrexate, steroids, MMF). However, we and others have shown that adenovirus DNAemia can also precede the occurrence of aGVHD. The hypothesis is that virus-induced tissue damage triggers allo-reactivity. Whether pre-emptive treatment of reactivating adenovirus will have an effect on the incidence or severity of aGVHD remains to be investigated.

2) *The use of serotherapy*, such as anti-thymocyte globulin (ATG) or Alemtuzumab (anti-CD52+) in conditioning regimens. Serotherapy is used to deplete the recipients T-cells *in vivo* preventing rejection of the graft as well as to
reduce the risk of aGvHD. However, thymoglobuline, ATG, has a depletory effect on the (antiviral) T-cells in the graft, due to the long half life of the polyclonal antibodies. Alemtuzumab (Campath-1H: anti-CD52), an alternative to ATG, holds an even a higher risk for adenovirus disease, probably due to deeper \textit{in vivo} T- and NK cell depletion \textsuperscript{29}.

3) \textit{Ex-vivo T cell depleted grafts} (e.g. CD34\textsuperscript{+} selection) \textit{and cord blood} are associated with delayed formation of memory T-cells and withhold a risk to all viral complications after HSCT \textsuperscript{30, 31}. In cord blood protocols, the use of similar doses of serotherapy as used in MUD setting, may result in even further \textit{in vivo} depletion of T-cells (from the graft) and thus an even more delayed T-cell immune reconstitution, which as a consequence then mainly relies on new-thymic output.

Furthermore all other donor sources are considered to be seropositive for adenovirus and are associated with a lower risk of adenovirus reactivation, because of protection by adoptive immunity from adenovirus specific T cells in the graft.

The presence of these risk factors determines individual susceptibility to develop adenovirus disease in case of reactivation. Poor results of therapeutic interventions, and severe side effects of the therapeutic options available in case of disease have motivated definition of specific risk groups each with a different stringency of monitoring and treatment (Fig. 1). Furthermore, in the prevention of \textit{de novo} infections from carrying contacts, protective isolation measures and building characteristics of the transplantation units may be crucial. It isn’t seldom that an HSCT unit faces nosocomial spread or even an adenovirus outbreak resulting in substantial morbidity and mortality \textsuperscript{2, 32}. For us (UMC Utrecht, The Netherlands) this was one of the main reasons for rebuilding the SCT unit (HEPA filtered rooms) with each having a separate front room. This, hygienic rules (parents, nurses and visitors), plus active surveillance (stool, nasopharyngeal aspirates) have prevented nosocomial spread or an outbreak so far (last 3 years) \textsuperscript{33, 34}. 
TREATMENT OPTIONS FOR ADENOVIRUS INFECTION AND DISEASE IN HSCT-PATIENTS

**Antiviral drugs**

Ribavirin and cidofovir are agents used in the treatment of Adv. Most evidence for efficacy against adenovirus however is present for cidofovir (Table 1). Cidofovir is a monophosphate nucleotide analogue of cytosine which is phosphorylated intracellularly to a diphosphate which can inhibit viral DNA polymerase and thus viral replication. It was first approved by the FDA in 1996 for the treatment of CMV retinitis. The antiviral selectivity of the acyclic nucleotide/nucleoside phosphonates is based on their higher affinity for the viral DNA polymerase compared to cellular DNA polymerases. Diphosphates of cidofovir (CDV-PP) compete with nucleoside triphosphates (dCTP) and are more efficiently incorporated in DNA strands, thereby inhibiting viral replication. Sustained effect of cidofovir in vitro has been shown to be present against all Adenovirus subtypes. Cidofovir resistant mutants have been described after serial passages in vitro and could be linked to mutations that affect nucleotide binding of viral DNA polymerase. A major disadvantage of cidofovir, is that bioavailability is low and that the antiviral effect is dependent on concentrations of the active phosphorylated metabolites present within infected cells. Pharmacological effects therefore do not correlate well with the prescribed dose as more than 90% of the drug is excreted unchanged in the urine.

The problem of most other antivirals that have been investigated, such as Acyclovir, is that they are nucleoside analogues which depend on viral kinases for their phosphorylation into their active form (nucleoside-diphosphate). They are much less efficiently phosphorylated by human intracellular kinases. Since adenoviruses, unlike herpesviruses such as CMV and HSV, do not encode a kinase themselves, they are relatively insensitive to classical acyclic nucleoside analogues. Of these nucleoside analogues Ribavirin is an agent for which in vitro anti-adenovirus activity differs widely against different subtypes (most active...
against group C, subtype 1,2,5) ⁴⁰. In contrast with what may be expected, there are several case reports suggesting therapeutic benefit for some patients with no response reported in others ⁴¹, ⁴², ⁴³. Due to availability of the more effective cidofovir, it is not included in our guideline. Though less efficient than nucleotide analogues, Ganciclovir can be tri-phosphorylated by cellular kinases which results in interference with the function of Adv DNA polymerase, thus inhibiting viral replication in vitro ⁴⁴. In retrospective studies lower incidences of adenovirus infections appeared to be reported in patients treated with ganciclovir as CMV prophylaxis ⁴⁵. However after adjusting for type of donor and age, ganciclovir administration did not convincingly lead to a reduction of adenoviral disease. Reports of positive outcome in patients with adenovirus infection treated with ganciclovir are fragmentary and do not justify incorporation in standard treatment protocols ⁴⁶.

So while cidofovir appears to be the most effective of the agents described, it is also the most toxic. Cidofovir is excreted in the urine by proximal tubule cells. The rate of drug uptake from the blood by organic anion transporters at the antiluminal (basolateral) membrane of renal tubular cells and the slower efflux into the tubule lumen is believed to be responsible for the intracellular accumulation of cidofovir to toxic levels, with the consequence of substantial tubular necrosis. Hyperhydration together with co-administration of the drug Probenecid has been shown to have a nephroprotective effect ⁴⁷, ⁴⁸. Probenecid, is an organic acid, which competes for the kidney’s organic anion transporter, and thereby protects tubule cells and increases cidofovir plasma level.

Prophylaxis, pre-emptive treatment, therapeutic treatment

Antiviral treatment can be used as prophylaxis, as pre-emptive treatment led by viral load cut off values, or as therapeutic treatment in case of Adv disease. Rationale for the first two strategies is to bridge the immuno-suppressed post-HSCT period until the antiviral defence is reconstituted from the graft. Prophylactic treatment has only been described by Greil et al. in an abstract
presented at the 2006 EBMT. Over a 2 year period all pediatric stem cell patients received ribavirin prophylaxis followed by pre-emptive cidofovir treatment when viral loads were detected. Comparing the outcome with historic controls in which no prophylaxis and cidofovir alone was given as a pre-emptive treatment, the combined strategy with ribavirin prophylaxis resulted in a significantly lower incidence of adenovirus infection (29% vs 66%) and adenovirus-associated mortality (0% vs 14%).

Although the documented studies with SCT-patients suffering from adenovirus disease vary in their detection methods and their definition of infection and disease, it appears that ribavirin, but even cidofovir, have only limited efficacy when started as therapeutic treatment for adenovirus disease. This underlines the importance of early detection of adenovirus viremia to be able to initiate therapy within a certain timeframe before the occurrence of disease symptoms associated with a significant increase in mortality. This window of opportunity may differ for individual patients depending on the presence of other risk factors. Our treatment guideline was designed from a pediatric perspective but may be as relevant to the less common adult patient with risk factors for adenovirus disease (Figure 1). The guideline takes these risk factors of patients in consideration dividing them in a low, intermediate and a high risk group each with a different treatment approach.

The observed dose-limiting nephrotoxicity of cidofovir, when given at the recommended dose of 5 mg/kg once weekly, has discouraged practitioners to use cidofovir as a pre-emptive treatment. Hyperhydration and co-administration of the drug Probenecid (2 grams, administered 3 hrs before cidofovir infusion) decreases nephrotoxicity. Of the very limited prospective studies on pre-emptive cidofovir available, it has been shown prospectively that cidofovir 1 mg/kg 3 times weekly in combination with probenecid is effective in the prevention of progression to end stage organ disease and in prevention of adenovirus disease when used as a pre-emptive treatment at the time the virus is detected by routine screening methods.
screening by plasma PCR in absence of disease symptoms to initiate pre-emptive treatment (Table 1)\textsuperscript{56}. However, regardless of their detection mode and thus timing of therapy, these studies demonstrated acceptable toxicity in a pediatric HSCT setting despite concomitant use of other nephrotoxic agents such as ciclosporin, which is in line with data from our pediatric transplant center in the Netherlands\textsuperscript{52,56,57}. In more than 25 patients treated (also for a duration > 3 weeks) over the last 6 years within our center, no nephrotoxicity, except some mild tubulopathy was observed (data not shown). Regarding the necessity of co-treatment with the nephro-protective probenecid during low dose cidofovir (1 mg/kg 3 times weekly), no data are currently available. Because of the limited side effects we recommend using it, although the risk of increasing plasma levels of other drugs (MTX, NSAIDs) should be taken into account.

In the UMC Utrecht, we do not limit cidofovir treatment to a three weeks period as reported by others\textsuperscript{55}, but prolong treatment until viral load has fallen below 400 cp/ml or until the CD3 >300/mL in high risk patients receiving pre-emptive treatment\textsuperscript{31,24}. However, in line with others, our experience is that clearance of the virus only occurs when T-cells reconstitute after HSCT\textsuperscript{21}. With the use of pre-emptive cidofovir the viral load usually stabilizes, buying time (weeks to months) for T-cells to reconstitute and form adenovirus-specific T-cells to clear the virus. It has become apparent that in case of adenovirus disease, the effect of cidofovir is limited resulting in a significant mortality (Table 1). Not all individuals die of adenovirus disease itself; the presence of aGVHD and a poor immune reconstitution which are often the case, contribute significantly to transplant related mortality in this group\textsuperscript{13,30}.

\textit{Immunotherapy}

As for many other virus infections in the post-transplant setting, an increased risk of adenovirus infection is clearly associated with the lack of recovery of endogenous virus-specific T cells. Firstly, one should try to taper
immunosuppressant therapy to help immunerecovery. However in a number of cases this is not possible due to aGVHD, or does not prevent disease symptoms from developing. Since the efficacy of antiviral drugs for the treatment of adenovirus disease is limited, cellular immunotherapeutic approaches to provide physiologic protection against adenovirus infection by adoptively transferring T cells with adenovirus specificity have been investigated.

Immunotherapies used and developed:

A) Donor lymphocyte infusions
The first adoptive T cell transfer protocols in the allogeneic HSCT setting were based on the premise that donor peripheral blood contained T cells able to mediate antitumor and/or antiviral activity in the HSCT recipient. Accordingly, donor lymphocyte infusions (DLI) have been used to provide antiviral immunity. As a proof of principle Hromas and colleagues reported a case of a 19-year-old man who underwent a T-cell-depleted allogeneic donor HSCT for T-cell lymphoblastic lymphoma. After presenting with hemorrhagic cystitis secondary to adenovirus infection and failing to respond to antiviral drugs he was given donor leukocytes (10^6 CD3 cells/kg) and subsequently cleared the virus. This initial success has been followed by a number of other case studies in which patients were infused with cell doses ranging from 1x10^5 – 3x10^7 CD3 cells/kg with similar positive outcomes. Despite this, DLI is often the last treatment of choice for clinicians since the efficacy of this approach is limited by the low frequency of T cells specific for many common “acute” viruses (such as adenovirus) and the relatively high frequency of alloreactive T cells which are associated with significant toxicity.

To enhance the safety of DLI, the infusion of T cell products from which the alloreactive cells have either been inactivated or selectively removed ex vivo, and/or which have been genetically modified with suicide genes to ensure that the high T cell doses required to provide protection against viruses
with a low frequency of reactive cells can be safely administered and eliminated in the event of adverse *in vivo* effects has also been investigated \(^{68,69}\).

**B) Adoptive Immunotherapy**

An alternative, safe and effective adoptive transfer approach to DLI involves the infusion of adenovirus-specific T cells that can be selected directly from peripheral blood or selectively expanded *in vitro*. To date this strategy has been investigated by two groups each with a different approach.

1) Feuchtinger and colleagues were able to directly identify and isolate donor peripheral blood T cells that secreted IFN-\(\gamma\) in response to stimulation with adenovirus antigen (Miltenyi Gamma catch\(^{\text{TM}}\)). The isolated T cells were transferred into nine pediatric recipients of allogeneic SCT with systemic adenovirus-infection, despite conventional therapy. Donor PBMCs were stimulated for 16 hours with adenovirus lysate and then labeled with an anti-IFN-\(\gamma\) monoclonal antibody conjugated to a CD45 antibody. Thereafter cells were magnetically labeled and selected with anti-IFN-\(\gamma\) microbeads (Miltenyi Gamma catch\(^{\text{TM}}\)), and infused without further in vitro expansion\(^{70}\). The frequency of adenovirus-specific T cells in donor peripheral blood increased from 1.1% (mean ± 1% SD) to 45.7% (mean ± 24% SD) following selection and the cells were polyclonal with a mixture of CD4+ and CD8+ T cells. None of the infusions (range 1,200 to 50,000 CD3+ cells/kg) were associated with toxicity *in vivo* and of six evaluable patients, five showed a significant decrease of adenoviral DNA in peripheral blood and stool with a corresponding increase in the frequency of adenovirus-specific T cells *in vivo*. In this small number of patients there is a suggestion that the T cell efficacy was independent of the infused cell dose, and that even low numbers of transferred adenovirus-specific T cells can expand sufficiently in vivo to reconstitute anti-viral immunity in the presence of antigen\(^{70}\).

2) Our group (BCM, Houston) has achieved similar success in recipients of HLA-matched related, matched unrelated and haploidentical HSCT, following the
adoptive transfer of \textit{in vitro} expanded adenovirus-specific T cells. To date we have performed two clinical studies using virus-specific T cells containing an adenovirus-specific T cell component. Both trials used CTL lines produced using antigen-presenting cells (APCs) transduced with adenovirus vectors. The adenovirus component was activated by virion proteins of the vector that were processed and presented to CD4+ and CD8+ T cells. Monocytes and EBV-transformed lymphoblastoid cell lines (EBV-LCL) were used as APCs so that EBV antigens were also presented. In one trial we infused bivirus CTL targeting adenovirus and EBV \textsuperscript{71} and in the other trial, the introduction of a CMV antigen into the adenovirus vector resulted in CTLs with specificity for all three viruses\textsuperscript{72}. Cells were infused from day 30 post-transplant in patients with ≤ grade II aGVHD. The infused doses ranged from $1.7 \times 10^5$ to $4.5 \times 10^6$ cells/kg, reconstituted immune responses to all three viruses and were able to control ongoing drug resistant virus infections. No toxicity or aGVHD was observed.

Of note, while we were routinely able to detect an increase in the frequency of T cells reactive against the latent viruses EBV and CMV (in patients treated with trivirus CTL) independent of detectable viral reactivation, adenovirus-specific T cells were detectable only in patients with recent or concurrent adenoviral infection. However, none of the treated patients developed a de novo adenovirus infection compared to an expected incidence of 68% in pediatric subjects receiving similar transplants in the absence of CTL \textsuperscript{23}. We demonstrated evidence of adenovirus-specific T cells for at least 8 weeks, even in CTL recipients without viral infection, implying that transferred cells were able to persist in sufficient numbers to mediate antiviral protection in the lymphopenic host\textsuperscript{71}. All patients with detectable adenovirus in blood, stool or tracheal aspirate (7/24) had a marked reduction in adenoviral load coincident with the rise in their adenovirus-specific T cells irrespective of infection serotype. This included one patient with progressive adenoviral pneumonia, requiring maximal ventilatory support, who after infusion had a progressive rise in adenovirus-specific T cells.
with a reduction in viral DNA-load and was completely weaned from ventilatory support within 10 days of receiving cells.

Though both systems described produce T cells that are safe in vivo and can effectively control active infections and provide broad spectrum antiviral protection in vivo, there are associated limitations. The Miltenyi Gamma catch™ system is expensive, requires large starting blood volumes, and access to clinical grade antigen as a T cell stimulus, while the in vitro expansion system requires the production of clinical grade viral vectors for antigen presentation, and a prolonged period of culture (10 to 12 week manufacturing process) with its attendant demands on technical skill and time. Thus, neither is ideal. Ultimately, the broader implementation of this therapeutic option requires the development of novel production processes which rapidly (< 2 weeks) and cost-effectively ensure the availability of T cell products which can be generated from small starting blood volumes, and this area is an area of intensive interest for many groups, particularly given the poor efficacy of conventional antiviral therapeutics for adenovirus. In the future, development of such as system will serve to move T-cell immunotherapy’s beyond highly specialized centers to a standard of care therapy available to all.

**Future perspectives**

1) **Boosting immune recovery**

In our setting (UMC Utrecht), rabbit polyclonal ATG (Thymoglobulin, Genzyme) is given prior to HSCT to prevent both graft-rejection and aGVHD. The long half-life and unpredictable pharmacokinetics of active-ATG make ATG an extremely important but uncertain variable influencing post–HSCT immune reconstitution. A future development might be tailor-made ATG-dosing prior to HSCT, which could be especially important for the cord blood setting, which already contains lower numbers of T cells in the graft. Therefore more insight in the pharmacokinetics-pharmacodynamics (PKPD) of (active)-ATG is warranted, and these studies
are currently being evaluated in our center. A more predictable immune-reconstitution may not only have a direct effect on the prevention of viral complications but is also of utmost importance for the efficacy of adjuvant immunotherapies.

Other experimental strategies that may boost immune-reconstitution are sex-hormone blocking to improve thymic function by increased expression of CCL25 (chemokine important for the immigration of thymocytes precursors from bone marrow into thymus) and thus enhancing the rate of T cell regeneration^73,74, or by the administration of IL-7 and keratinocyte growth Factor (KGF) which also enhance thymopoeisis and the output of recent thymic emigrants^74,75.

2) Antiviral Drug development

Novel drugs are being developed to circumvent the current problems associated with available drugs, such as nucleoside/nucleotide analogues which either do not need to be phosphorylated any further and are already in its active form or which can be adequately phosphorylated by intracellular kinases. Investigated candidates include ganciclovir triphosphate, which does not need further phosphorylation, and the S-2242 compound (N7-isomer of 6-deoxy-ganciclovir) which is adequately phosphorylated by cellular kinases 76. Furthermore, lipid esters of cidofovir are already available to some centers, for compassionate use only. They have been developed since they have a higher antiviral activity and particularly because they have a higher oral bioavailability and less nephrotoxicity at the EC50 of the drug 77, 78.

3) Dendritic Cell vaccination.

Besides ex-vivo generation of specific anti-viral cytotoxic T-cells, in vivo induction of specific CD4+ and CD8+ T cell responses to adenovirus using virus peptide/lysate loaded dendritic cell-vaccination has been proposed as an elegant and potentially more rapid strategy to be used early after
transplant. The method has already been performed in anti-tumor vaccination strategies. Development of an immuno-stimulatory adjuvant antiviral immunotherapy based on viral peptide/lysate pulsed dendritic cell (DC)-vaccination might be especially of interest in seronegative donors (such as cord blood). Dendritic cells generated from the graft can be pulsed with viral lysate, can be generated rapidly (<2 weeks), introduced into the patient by vaccination and may potentially induce a more polyclonal virus-specific T-cell response. This response is generated \textit{in vivo} and the treatment is not HLA-restricted. For CMV, a proof of principle study demonstrated that DC vaccination in allo-HSCT resulted in induction of hCMV specific CTL responses in about half of the evaluable patients (n=17), and control of CMV in almost all \textsuperscript{78}. In this study DC were generated from peripheral blood monocytes from CMV negative donors.

**THE TREATMENT GUIDELINE: HOW I TREAT ADENOVIRUS INFECTIONS IN PEDIATRIC HSCT RECIPIENTS**

The treatment guideline (Figure 1) is applicable to patients within 4 months post-HSCT and patients post HSCT on immune suppression. Although it was developed as a guideline in the pediatric HSCT setting, it can be as applicable to high risk adult patients as well, such as cord blood or haplo-graft-recipients and individuals with severe aGVHD\textsuperscript{13,30}.

The main pillars of this treatment protocol are:

1) No prophylaxis. However postponement of HSCT (e.g. in case of DNA positivity in NPA or stool) may be an option for elective HSCTs (e.g. non-malignant indications).

2) The definition of risk groups, and assignment of each patient to the low, intermediate or high risk group. The \textit{high risk group} has been defined as cord blood and T-depleted (<5\times10^4/kg) graft recipients within the first month; and patients on prednisone with a dose of more than 1mg/kg/day (for any indication) next to 1 or more lymphocyte proliferation inhibitor(s).
The intermediate group consists of recipients of CB and T-cell depleted grafts >1 month to <4 months post-HSCT, patients with immune suppression consisting of ≥2 lymphocyte proliferation inhibitors (e.g. cyclosporine-A and MMF) or 1 lymphocyte proliferation inhibitor + prednisone > 0.5 and < 1 mg/kg.

The low risk group consists of recipients of all other transplant sources within the first 4 months post HSCT, without GVHD and with maximum immune suppressive therapy of 1 proliferation inhibitor and/or prednisone ≤ 0.5 mg/kg/day.

3) Weekly monitoring of adenoviral load with qPCR to aim for a pre-emptive treatment. The frequency of monitoring is based on our experience with the quick rise in adenoviral load that can be seen early after detection (Fig. 1). A 1-3 log rise in load within the timeframe of a week is not uncommon. Weekly monitoring is also consistent with others. If adenovirus is detected (> 100 cp/mL) we monitor twice weekly to monitor response to antiviral medication. Unfortunately, there are no definite data available on how often and which groups of adults post HSCT should be monitored. We recommend that high risk adults are monitored weekly as well.

4) We start pre-emptive treatment with cidofovir 1 mg/kg 3 times a week (5 mg weekly as an alternative) when the adenoviral load exceeds a certain critical level depending on the risk group the patient is in. All high risk patients get treated when the load exceeds > 100 cp/mL (mainly based on the above mentioned fast kinetics in these highly vulnerable group of patients), all intermediate risk patients get treated when load exceeds > 1000 cp/mL (a cut off used in various guidelines for other viral reactivations: e.g. EBV, CMV). Patients in the low risk group are further divided in two groups on the basis of their immune status (number of CD3+ cells). Low risk patients with both detectable numbers of T cells (CD3+ > 25 /uL) present at the time of Adv detection and an adequate immune response (> 300/ul CD3+ T cells within 2 weeks of adenovirus
detection) only get treated if viral load exceeds > 10,000 cp/mL. A load > 10,000 cp/mL is regarded as failure of the wait and see policy and should result in initiation of treatment. In case of a suboptimal immune status, (absence of T cells, CD3+ < 25/uL or an inadequate increase of CD3+ cells within two weeks time) the low risk patient gets treated similar to the intermediate group, when the load exceeds 1000 cp/mL.

5) Probenecid (2 grams, administered 3hrs before cidofovir infusion) and hyperhydration are started as supportive care to limit cidofovir nephrotoxicity.

6) Treatment with cidofovir 1 mg/kg 3 times a week (or 5 mg weekly as an alternative) is started when there are signs of adenovirus disease irrespective of the load.

7) Evaluation after the first week and the second week of cidofovir treatment. When load increases by a log after 2 weeks of treatment, disease symptoms develop during pre-emptive treatment, or disease progresses during treatment for adenovirus disease, immunotherapy with adenovirus specific CD4+/CD8+ CTLs is indicated for patients in centers where this is an available therapeutic option.

8) Immunosuppressive therapy should be tapered as far as possible.

9) From detection adenoviral load detection by PCR is performed at least weekly for optimal timing of pre-emptive treatment and careful monitoring of the response to treatment.


11) Discontinuation of therapy only when adenoviral load has been < 400 cp/mL for two consecutive weeks and CD3+ T cells > 300/uL.

SUMMARY
Although HSCT has become much safer over the last decade, its major limitation remains “transplantation related mortality” (e.g. due to viral reactivations/disease) and relapse (in malignancies). The immune suppressed period during the first weeks to months after HSCT is the most important determining factor. The ideal treatment strategy would aim to enhance the desired (antiviral and antitumor) immunity during the first weeks while preventing the undesired allo-reactive immune response (aGVHD). HSCT is made safer by using uniform treatment guidelines, for which the validation has preferably been investigated in trials.

Adenovirus infection and disease pose an important problem with considerable mortality in immunocompromised patients. Reactivation within the recipient and horizontal transmission are most common routes of acquisition of the virus. Adenovirus infections are much more of a problem in pediatric HSCT than adult HSCT, possibly because of the reservoir of adenovirus in the general pediatric population who shed the virus for extended periods of time from lymphoreticular tissue before complete clearance can be secured. Antivirals are commercially available of which cidofovir is most effective as pre-emptive therapy. Due to toxicity and limited effectiveness cidofovir is still not ideal, but when used as a pre-emptive therapy is a reasonably safe option to buy time until immune-recovery. Besides drug therapy, CTL immunotherapy is promising and is probably more effective. Unfortunately this option is not available to all centers. However, we believe various issues currently preventing this treatment from introduction into general HSCT care will be addressed in the coming years. The treatment guideline presented in this paper is based on the evidence currently available and may be a practical guideline to treat and/or prevent adenovirus disease. In the guideline: a steering role for stringent monitoring of adenoviremia by qPCR, immune-reconstitution, pre-emptive treatment with cidofovir and administration of Adv specific CTLs for patients who do not adequately respond to cidofovir in centers for which CTL therapy is an option. Obvious limitations of current available therapies have been a motivation for development of new (immuno-)therapies which may make HSCT safer in the near future.
AUTHORSHIP

Contribution: CL, AL and JB equally contributed to the writing of the paper. Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


Table 1  Cidofovir for the treatment of adenovirus disease in HSCT patients

Table lists all clinical studies using cidofovir in HSCT patients either for pre-emptive treatment when symptoms are not yet present, or for therapeutic treatment when disease symptoms are present. Only studies with n > 5 have been presented in the table.
<table>
<thead>
<tr>
<th>Author (year of publication)</th>
<th>n</th>
<th>P/R</th>
<th>Indication</th>
<th>Drug therapy</th>
<th>Nephrotoxicity (%)</th>
<th>Success Adv clearance (%)</th>
<th>Mortality (%) Direct Adv-related mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-emptive Treatment</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Anderson (2008)</td>
<td>7</td>
<td>children</td>
<td>7 patients with Adv isolation from plasma, or from two other sites evaluated by PCR</td>
<td>CDV 1 mg/kg iv 3 times a week</td>
<td>0 (0%)</td>
<td>2 (28%)</td>
<td>2 (29%) 0 (0%)</td>
</tr>
<tr>
<td>Bhadri (2009)</td>
<td>20</td>
<td>children</td>
<td>Proven Adv in stool, urine, NPS, CSF. Routine stool screening rapid culture, antigen detection Screening for adv in other sites as clinically indicated</td>
<td>Induction with 5 mg/kg iv, followed by 3 mg/kg weekly. When prolonged treatment was needed 1 mg/kg 3 times a week</td>
<td>7 (35%)</td>
<td>17 (85%)</td>
<td>14 (70%) 2 (10%)</td>
</tr>
<tr>
<td>Greil et al. (2006)</td>
<td>10</td>
<td>children</td>
<td>Adv detection in 10 out of 34 pediatric pts treated with ribavirin prophylaxis</td>
<td>Ribavirin prophylaxis CDV pre-emptive</td>
<td>No data</td>
<td>No data</td>
<td>1/10 (0%) 0/0%</td>
</tr>
<tr>
<td>Ljungman (2003)</td>
<td>16</td>
<td>Adults and children</td>
<td>Asymptomatic patients (n=16) of a total of 45 patients with any detection of Adv from stool, upper airway specimens, blood, urine (detection by culture, antigen detection, PCR)</td>
<td>CDV 5mg/kg iv weekly (n=39) 1-4 mg/kg (n=6)</td>
<td>No data of this group. Overall in 45 pts: 17 (38%)</td>
<td>75%</td>
<td>6 (37%) 2 (13%)</td>
</tr>
<tr>
<td>Yusuf (2006)</td>
<td>14</td>
<td>children</td>
<td>14 asymptomatic pediatric patients(of a total number of 57 pts) Positive Adv detection by PCR</td>
<td>CDV 5 mg/kg iv weekly, for 2 weeks, followed by once every 2 weeks until viral clearance 3 negative samples. Median 5 doses (1-22)</td>
<td>0</td>
<td>100%</td>
<td>None of which developed reactivation No data for this group 29/57 (51%) for the whole group 1/57 (8%)</td>
</tr>
<tr>
<td><strong>Therapeutic treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robin (2007)</td>
<td>25</td>
<td>Adults and children</td>
<td>Disseminated Adv disease</td>
<td>CDV 5 mg/kg iv weekly</td>
<td>No data</td>
<td>6 (24%)</td>
<td>21 (84%)</td>
</tr>
<tr>
<td>Symeonidis (2006)</td>
<td>11</td>
<td>Adults and children</td>
<td>Positive Adv cultures and severe or persistent symptoms</td>
<td>CDV 5 mg/kg iv weekly OR CDV 1 mg/kg every other day</td>
<td>2 (18%)</td>
<td>No data</td>
<td>8 (54%) 6 (54%)</td>
</tr>
<tr>
<td>Ouachee-Chardin (2004)</td>
<td>16</td>
<td>children</td>
<td>Adv disease PCR for detection of Adv</td>
<td>CDV 3-5 mg/kg iv weekly</td>
<td>5 (31%) reversible tubulopathy</td>
<td>14 (87%)</td>
<td>3 (18%), 2 (13%)</td>
</tr>
<tr>
<td>Yusuf (2006)</td>
<td>43</td>
<td>children</td>
<td>43 patients with clinical symptoms attributable to Adv infection Adv detection by PCR (of a total number of 57 pts with positive Adv PCR)</td>
<td>CDV 5 mg/kg iv weekly, for 2 weeks, followed by once every 2 weeks until viral clearance 3 negative samples. Median 5 doses (1-22)</td>
<td>0</td>
<td>42 (97%)</td>
<td>No data for this group 29/57 (51%) for the whole group 1/57 (8%)</td>
</tr>
<tr>
<td>Hoffman (2001)</td>
<td>8</td>
<td>children</td>
<td>Of 17 pts with positive Adv cultures. 8 were enrolled in Phase II trial of CDV treatment. Detection with shell vial culture method</td>
<td>CDV 1 mg/kg iv 3 times a week</td>
<td>1 (13%) Not leading to discontinuation</td>
<td>8 (100%)</td>
<td>2 (25%) 0 (0%)</td>
</tr>
<tr>
<td>Ljungman (2003)</td>
<td>29</td>
<td>Adults and</td>
<td>Patients with probable and definite Adv disease (n=29) of a total of 45 patients with any detection</td>
<td>CDV 5mg/kg iv weekly (n=39)</td>
<td>No data of this group.</td>
<td>20 (69%)</td>
<td>10 (34%) 5 (17%)</td>
</tr>
<tr>
<td>Muller (2005)</td>
<td>10 children</td>
<td>R</td>
<td>Adenovirus isolation from more than 1 site and clinical symptoms, or isolation from 1 site with severe clinical symptoms</td>
<td>CDV 5 mg/kg iv weekly during 6 weeks, followed by once every 2 weeks. Total no: 9 doses</td>
<td>9 (90%), 1 required additional CDV therapy to clear the virus</td>
<td>0 (0%)</td>
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</tr>
<tr>
<td>Kampmann (2005)</td>
<td>11 children</td>
<td>R</td>
<td>Patients in which Adv was detected (n=26) were on ribavirin. In case of persistent adenoviremia and adv disease CDV was started (n=11).</td>
<td>CDV 5 mg/kg iv weekly for two weeks, followed by 5 mg/kg every two weeks</td>
<td>?</td>
<td>No data in this group. In the whole group 21 of 26 (81%) cleared the virus</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nagafuji (2004)</td>
<td>16 Adults</td>
<td>P</td>
<td>Post HSCT. pts with hemorrhagic cystitis and a urine sample positive for Viral culture, Adv PCR and immunochromatograph</td>
<td>CDV 1mg/kg iv 3 times a week, for 3 weeks</td>
<td>3 (19%)</td>
<td>12 (75%)</td>
<td>2 (13%)</td>
</tr>
</tbody>
</table>

Abbreviations: n: number of patients; P: prospective study; R: retrospective study; Adv: adenovirus; pts: patients; CDV: cidofovir; iv: intravenously
Figure Legends

Figure 1 Adenoviral load in relation to T cell numbers

A 2 year old girl was treated with unrelated CB HSCT for Hurler Syndrome (non-malignant, MPS1). She developed an adenovirus primo infection or reactivation, which was detectable by Adv qPCR (100 cp/mL) at day 19. Within a period of 4 days the viral load increases by 2 log and cidofovir is started. There are no signs of disease. Only as CD3 numbers (▬▬) are increasing, adenoviral load (▬▬) goes down. The downward pointing arrow indicates the timing of intensification of therapy with immunosuppressants when the post HSCT period is complicated by steroid refractory autoimmune–cytopenia: she receives Motefil Mycophenolate 45 mg/kg/day, prednisone 2 mg/kg/day, anti-CD20-therapy and Fludarabine infusions from day + 314. When symptoms stabilise, immunosuppressants are tapered over time and can be stopped at day + 455 post HSCT. Tapering of immunosuppressants is associated with CD3+ T cell recovery and clearance of Adenoviremia.

Figure 2 How I treat adenovirus in HSCT recipients: Treatment guideline

We refer to the text for a detailed explanation.

Lympho. Prolif. Inh.= Lymphocyte Proliferation Inhibitor (e.a. Cyclosporin A, CsA)

* alternative cidofovir 5 mg/kg i.v. weekly

** For centers that have the Adenovirus CTLs readily available, CTLs are immediately initiated for all high risk patients and for all patients with adenovirus symptoms before awaiting cidofovir effect.
Figure 1

Days Post HSCT

Adv load (cp/mL)

CD3+ T cells (x 10^9/L)
Figure 2

Critical load for pre-emptive treatment

Adenovirus disease

High Risk
- CD8 donor or T cell depleted graft recipient < 1 mo post SCT AND/OR
- Immune-suppression
  - Prednisolone 2 mg/kg/day and 
  - 1 Lympho Profil Inh

Intermediate Risk
- CB donor or T cell depleted graft recipient 1-4 mo post SCT AND/OR
- Immune-suppression
  - Prednisolone 0.5 mg/kg/day
  - >1 Lympho Profil Inh

Low Risk
- Donor source other than CB or T cell depleted graft; or > 4 mo post SCT for any donor source
- Immune-suppression: maximum
  - 1 Lympho Profil Inh and/or
  - Prednisolone ≤ 0.5 mg/kg/day

CD3 monitoring when Adv > 100 cp/mL

CD8 < 25/μL at detection, OR < 300/μL within 2 weeks
CD8 > 25/μL at detection AND > 300/μL within 2 weeks

Load
- > 100 cp/mL
- > 1,000 cp/mL
- > 1,000 cp/mL
- > 10,000 cp/mL

CIDOFOVIR (1 mg/kg/day* + hyperhydration + probenecid) 3 times a week*
And until viral load < 400 cp/mL (minimum 2x)
Tapering of the immune-suppressive drugs if possible

Immune recovery and viral clearance

Therapy failure
- Increase viral load > 1 log/week
- Adenovirus disease symptoms

Adenovirus CTLs Available**
- Immunotherapy

NO Adenovirus CTLs available
- Continue Ciclofovir treatment
How I treat adenovirus in haematopoietic stem cell transplantation recipients

Caroline A Lindemans, Ann M Leen and Jaap Jan Boelens