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REVIEW

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Management of adenovirus infection in patients after haematopoietic stem cell transplantation: State-of-the-art and real-life current approach

A position statement on behalf of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation

Prashant Hiwarkar[1] | Karin Kosulin[2] | Simone Cesaro[3] | Malgorzata Mikulska[4] | Jan Styczynski[5] | Robert Wynn[1] | Thomas Lion[2,6]

[1] Department of Haematology and Bone Marrow Transplantation, Royal Manchester Children's Hospital, Manchester, UK

[2] Division of Molecular Microbiology, Children's Cancer Research Institute, Vienna, Austria

[3] Paediatric Hematology-Oncology, Azienda Ospedaliera Universitaria Integrata Verona, Verona, Italy

[4] Division of Infectious Diseases, University of Genoa (DISSAL) and Ospedale Policlinico San Martino, Genoa, Italy

[5] Department of Paediatric Haematology and Oncology, Collegium Medicum, Nicolaus Copernicus University Torun, Bydgoszcz, Poland

[6] Department of Pediatrics, Medical University of Vienna, Vienna, Austria

Correspondence
Thomas Lion, Children's Cancer Research Institute, Zimmermannplatz 10, Vienna A-1090, Austria.
Email: thomas.lion@ccri.at

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Summary

The important insights gained over the past years in diagnosis and treatment of invasive adenoviral infections provide new paradigms for the monitoring and clinical management of these life-threatening complications. A meeting was held to discuss and subsequently disseminate the current advances in our understanding of the aetiology/pathogenesis and future treatment options facilitating effective control or prevention of adenovirus-related diseases in the allogeneic haematopoietic stem cell transplant setting. Invited experts in the field discussed recent progress with leading members of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation at the "State-of-the-art" Meeting in Poznan, Poland, in October 2017. In this review article, the panel of experts presents a concise summary of the current evidence based on published data from the last 15 years and on recent achievements resulting from real-life practice. The present position statement reflects an expert opinion on current approaches to clinical management of adenovirus infections in patients undergoing allogeneic haematopoietic stem cell transplant and provides graded recommendations of the panel for diagnostic approaches and preemptive therapy reflecting the present state of knowledge.

KEYWORDS

adenovirus infection, diagnostics, HSCT, preemptive therapy, stool monitoring

1 | INTRODUCTION

Human adenoviruses (HAdVs) are double-stranded DNA viruses with worldwide distribution. They represent a large family of genetically diverse pathogens displaying broad tissue tropism and causing a variety of clinical manifestations ranging from mild to severe diseases, even in immunocompetent individuals. The HAdV taxonomy divides the viruses into 7 species (A-G), currently comprising more than 80 different virus types, and the number has been steadily increasing over the past years as a result of common recombination events. Infections with HAdVs occur throughout the year, both in children and in adults. Transmission can occur through fomites, aerosolised droplets, faecal-oral spread, infected tissue, or blood. In immunocompetent individuals, the infections commonly cause conjunctival, respiratory, or

Abbreviations: ATG, anti-thymocyte globulin; CTL, cytotoxic T-lymphocytes; DLI, donor lymphocyte infusion; ECIL, European Conference on Infections in Leukemia; GI, gastrointestinal; GvHD, graft versus host disease; HAdV, human adenovirus; HSCT, haematopoietic stem cell transplantation.
Prashant Hiwarkar and Karin Kosulin are both first authors.
Robert Wynn and Thomas Lion are both senior authors.

gastrointestinal (GI) diseases. It is estimated that HAdVs cause 5% to 10% of all febrile illnesses and approximately 10% of pneumonias in infants and young children. By 5 years of age, 70% to 80% of individuals have serologic evidence of past exposure and by 10 years of age, most individuals have evidence of prior adenoviral infection.[1,2] The disease manifestations in the immunocompetent setting are mostly mild and self-limiting, although severe and even lethal courses of pneumonia or myocarditis have been reported.[3,4] Following primary infection, which most commonly affects young children, HAdVs can persist in different tissues from where active infection may recur in the presence of immunosuppression.[5-7] Well-documented sites of HAdV persistence include particularly tonsillar and adenoidal T-lymphocytes, which appear to represent a sanctuary for adenoviruses,[8] although other sites may also be involved.[9-11] Hence, in contrast to many other community-acquired respiratory viruses, adenoviral infections can occur both by exogenous acquisition and reappearance of persistent endogenous virus[12]; however, the latter appears to be far more prevalent in immunocompromised individuals.[13] Recipients of allogeneic haematopoietic stem cell transplantation (HSCT) represent one of the most vulnerable patient groups due to the severely impaired function of their immune system. This is mainly attributable to the lack of functional CD4 and CD8 T-lymphocytes in the early post-transplant period, particularly during the first 100 days after allografting. In this setting, exogenous exposure or viral recurrence can result in invasive infections potentially conferring high morbidity and mortality rates, particularly in children.[14] The reported frequency of invasive HAdV infections in the allogeneic HSCT setting is considerably higher in paediatric patients (6%-42%) than in adults (3%-15%),[13] but the clinical manifestations can be equally severe. The differences in the observed frequencies might be related to the permanent circulation of the virus among children[15] but could also be attributable to the more prevalent persistence of the virus in the childhood, providing a constantly available source of recurrence (unpublished observations). In immunocompromised patients, HAdV infection can cause a variety of clinical syndromes ranging from asymptomatic viraemia, through localised acute respiratory illness, gastroenteritis, conjunctivitis, or urinary tract infection, to disseminated disease. In this population of patients, HAdV infections can be very severe, and can cause respiratory failure, haemorrhagic cystitis, neurologic disease, and disseminated infection with lethal failure of individual or multiple organs.[3,13]

2 | SITES OF ADENOVIRUS PERSISTENCE AND RISK FACTORS FOR ADENOVIRUS RECURRENCE

The genetic heterogeneity of adenoviruses provides the basis for broad tissue tropism and the ability to infect several cell types. Current evidence indicates that HAdV can persist in a variety of susceptible cells following primary infection, such as tonsillar and intestinal T-lymphocytes, the central nervous system, or lung epithelial cells.[13] Recent findings revealed that the entire GI tract is a common location of HAdV persistence in children and the most important site for recurrence in the context of allogeneic HSCT in the paediatric setting.[16,17] The main risk factors for HAdV recurrence are well described and are generally related to immunosuppression, including allogeneic HSCT with unrelated donor or cord blood grafts, allogeneic HSCT with *in vivo* or *ex vivo* T-cell depletion, graft versus host disease (GvHD) grades III to IV, severe lymphopaenia (<300 CD3-cells/μl of peripheral blood), and treatment with the anti-CD52 antibody alemtuzumab or anti-thymocyte globulin.[13] Various other factors have also been reported, albeit with inconsistent statistical significance.[14,17,18] The absence of HAdV-specific T-cells plays an important role in the development of viral disease in allogeneic HSCT recipients.[13,19] Hence, screening for HAdV-specific T-cells in patients displaying any of the indicated risk factors or revealing a high load of the virus in stool exceeding the critical threshold level can provide information relevant for the potential benefit of early onset of preemptive treatment, including the option of immunotherapy.

3 | ROLE OF THE GI TRACT FOR INVASIVE ADENOVIRAL INFECTIONS POST-TRANSPLANT

Earlier findings in paediatric transplant recipients demonstrated that the onset of invasive HAdV infection is almost invariably preceded by the appearance and expansion of the virus in the GI-tract.[17] Rapidly rising HAdV copy numbers in serial stool specimens, exceeding the threshold of one million virus copies per gram, were shown to correlate with a high risk of invasive infection and disseminated disease.[14,17] This critical threshold was therefore integrated into an algorithm for diagnostic monitoring and treatment of impending HAdV-mediated complications.[13] The role of HAdV detection in stool above a certain threshold for the ensuing onset of viraemia has been confirmed by different centers.[20-22] It is important to bear in mind, however, that the threshold level of HAdV copy numbers in stool identified as critical for the risk of invasive infection is, to some extent, related to the method employed. Identification of the sources of invasive HAdV infections in the immunocompromised host may be regarded as a prerequisite for improved risk-assessment and the design of strategies aimed at the prevention of severe systemic manifestations. The occurrence of extremely high viral loads in serial stool specimens of paediatric patients with HAdV reactivation post-transplant, occasionally exceeding 10^{11} particles per gram, suggested the existence of a hitherto unknown compartment in the GI-tract harbouring persistent HAdV infection and providing a site of origin for invasive infections in the immunocompromised host. Despite this notion, the specific sites of HAdV persistence and proliferation were not well characterised. A recent study based on the investigation of biopsy materials obtained from generally immunocompetent paediatric patients undergoing elective upper and lower endoscopy of the GI-tract for a variety of indications, revealed persistence of HAdV in the GI-tract in >30% of cases, with the highest prevalence in the terminal ileum.[16] Lymphoid cells of the lamina propria were identified as the main site of HAdV persistence, whereas transplant recipients revealed high numbers of replicating virus in intestinal epithelial cells, as revealed by in-situ hybridisation and immunohistochemistry. Hence, it appears that intestinal lymphocytes represent a reservoir for HAdV persistence and recurrence, while the intestinal epithelium is the main site of viral replication preceding dissemination. HAdV persistence in the GI-tract was therefore identified as the most common origin of infectious complications in

immunocompromised children.[16] An earlier study in a large cohort of paediatric allogeneic HSCT-recipients revealed a 37% rate of intestinal HAdV recurrence.[17] The rates of HAdV persistence in the GI-tract of immunocompetent children and post-transplant recurrence of the virus in HSCT recipients were therefore similar. Moreover, the prevalence of HAdV species detected during intestinal persistence and recurrence was virtually identical.[16] This observation and the similarity between the observed frequencies of HAdV persistence in the GI-tract and virus recurrence in paediatric transplant recipients raised the possibility that individuals with persistent HAdV infection in the intestine are the main risk group for active viral infection post-transplant.

Moreover, recent data suggest that detection of intestinal HAdV shedding pre-transplant correlates with a high risk for invasive infection.[16] This finding was corroborated by a follow-up study based on HAdV monitoring of serial stool samples using RQ-PCR in more than 300 children undergoing allogeneic HSCT. Analysis of stool specimens was performed pre-transplant and at short intervals until day 100 post-HSCT. Peripheral blood screening was employed to determine the presence of systemic infection. The virus was detected already before HSCT in 14% of instances, and patients displaying HAdV shedding pre-transplant showed a markedly earlier and more rapid increase of intestinal HAdV titers above the critical threshold of 10E6 virus copies/g stool associated with high risk of invasive infection. In this subset of patients, critically high virus titers in stool mostly appeared within the first 3 weeks post-HSCT. Moreover, HSCT candidates with HAdV shedding before transplantation displayed a significantly higher occurrence of viraemia than patients without this finding ($P < .0001$). Multivariate data analysis considering other relevant risk factors for HAdV viraemia, such as GvHD, stem cell source, donor type, and lymphocyte reconstitution, confirmed that HAdV positivity in stool before HSCT confers a greatly increased risk for invasive infection and disseminated disease post-transplant.[23]

4 | IMPLICATIONS FOR DIAGNOSIS AND MONITORING

The indicated observations highlight the importance of early HAdV screening and timely preemptive therapeutic considerations in patients with intestinal shedding of the virus pre-transplant. The success of current treatment approaches with antiviral agents and HAdV-specific T-lymphocytes seems to correlate with early onset of therapy. The new insights may therefore have important implications for assessing the risk of life-threatening invasive HAdV infections and the clinical management of paediatric transplant recipients.

Current recommendations for adenovirus screening and monitoring as a basis for preemptive treatment in patients at high risk for HAdV disease are still relatively diverse, and further studies are needed to provide reliable data permitting the establishment of standardised approaches. Optimised diagnostics will greatly impact the rational and timely onset of antiviral treatment which was shown to be a prerequisite for successful therapy. Based on current data, mostly obtained in the paediatric allogeneic HSCT setting, HAdV screening should primarily include testing of serial stool specimens. The screening in children should be initiated prior to conditioning and continued at weekly intervals until adequate immune recovery (usually at least until day 100 post-transplant), in order to cover the period of greatest risk for recurrence and invasive infection. This general approach may be adapted to specific risk situations. Due to the current lack of adequate data from adult allogeneic HSCT recipients, the role of the GI-tract as an important site of HAdV reactivation and expansion remains unclear. Hence, in the adult transplantation setting, recommendations for HAdV screening in stool cannot be provided at the present time. Detection of HAdV in stool above the critical threshold level post-transplant was shown in paediatric patients to precede the onset of viraemia by a median of 11 days, and only exceptionally by less than 1 to 7 days.[17,23] Surveillance of HAdV in peripheral blood of patients undergoing screening of serial stool specimens at the indicated intervals may therefore be initiated when the critical threshold level in stool has been reached.[13] This approach can be pursued in the paediatric allogeneic HSCT setting where the temporal association between HAdV monitoring in stool and the risk of viraemia is well established. In the absence of serial stool screening data, HAdV testing in peripheral blood should be performed at least at weekly intervals starting immediately post-transplant until immune reconstitution, in line with the latest ECIL (European Conference on Infections in Leukemia) recommendations.[24] Quantitative molecular monitoring of virus levels in HAdV-positive patients should be used to assess the response to treatment.

Despite the recommendations for preemptive administration of antiviral drugs based on diagnostic HAdV screening provided by the ECIL several years ago,[24] unequivocal data indicating a beneficial effect on mortality have been missing, and the need for appropriate prospective studies was evident. The introduction of novel antiviral agents could be expected to further improve the efficacy of treatment and to reduce the toxicity of some commonly prescribed antiviral drugs. In addition to the favourable properties of new antiviral drugs, advances in antiviral immunotherapy with adenovirus-specific T-cells offer great potential for further improvement in the prevention or treatment of HAdV infections.

5 | PREEMPTIVE AND TARGETED TREATMENT STRATEGIES FOR HAdV: CURRENT EVIDENCE AND PRACTICE

Infectious complications due to reactivation of latent viruses in HSCT can be reduced by prophylactic, preemptive, and therapeutic antiviral therapies. Prophylactic and therapeutic pharmacological approaches for tackling adenoviral recurrence are not practised because of limited efficacy and toxicity of available anti-adenoviral drugs such as cidofovir and ribavirin.[25,26] Weekly viral monitoring for adenovirus using sensitive, quantitative PCR techniques permits rapid detection of viral recurrence and preemptive interventions such as reduction of immunosuppression and commencement of antiviral therapies which are currently the mainstay for prevention of morbidity and mortality associated with adenovirus infection.[27]

Adenoviraemia detected by sensitive PCR techniques might be asymptomatic and resolve spontaneously, especially when it is low level (<1000 HAdV copies per millilitre of blood) and where there is established or incipient donor T-cell reconstitution.[28] In contrast, lymphopenic hosts (lymphocyte count <300 per microlitre; and CD3+ T-cells <25 per microlitre) with viral copies above 1000 per millilitre are at risk of developing disseminated adenoviral disease and

disseminated adenoviraemia increases the risk of mortality to as high as 70%.[29-32] Therefore, reduction of immunosuppression and preemptive antiviral therapies for controlling the replication of adenovirus are widely practised in such immunocompromised patients. Cidofovir has been extensively used as a preemptive anti-adenoviral therapy and is the current standard of care treatment to control the replication of virus and prevent disseminated adenoviraemia.[29-32] Cidofovir is a monophosphonate nucleotide analogue of deoxycytidine. The cells phosphorylate cidofovir to its active diphosphate form and the active form competitively inhibits incorporation of deoxycytidine triphosphate into viral DNA by viral DNA polymerase, leading to viral DNA chain termination. However, poor cellular uptake of cidofovir leads to lower cellular concentration of the active drug and hence compromises its efficacy. Cidofovir is also a substrate for organic anion transporter 1, and therefore accumulates in the renal tubules leading to nephrotoxicity. These pharmacological properties of the drug underpin its clinical performance with relatively poor antiviral actions and significant associated toxicity. This dose-limiting nephrotoxicity of cidofovir had discouraged transplant physicians from using the recommended 5 mg/kg once weekly dose.[30] Hyperhydration and co-administration of probenecid (2 g administered before cidofovir infusion) reduce the incidence but do not abolish clinically significant nephrotoxicity. Three times weekly cidofovir at 1 mg/kg with probenecid and hydration has been used in some prospective studies with more acceptable toxicity,[29,33] but the 5 mg/kg weekly dose has remained the preferred treatment regimen at different centres, particularly in the adult setting. Cidofovir, however, has limited efficacy irrespective of dose. Its administration controls replication of virus and thus prevents progression to end-stage organ disease. However, cidofovir does not clear the virus in the absence of T-cell immune-reconstitution.[34,35] Nephrotoxicity and limited efficacy of cidofovir make it a less-than-ideal preemptive antiviral intervention.

A lipid conjugate technology platform of Chimerix Inc has resulted in the development of a lipid-linked derivative of cidofovir termed brincidofovir (CMX001). The lipid moiety not only improves oral bioavailability but also increases the intracellular concentration of the active drug. Brincidofovir is not a substrate for organic anion transporter 1 and therefore does not accumulate in the renal tubules, thereby reducing the risk of nephrotoxicity. In phase I and phase II trials as well as in retrospective studies, brincidofovir has been shown to be highly efficacious in controlling and clearing adenoviraemia. Two thirds of patients achieved a rapid decline in viraemia 2 weeks after initiation of brincidofovir, followed by complete resolution of viraemia at a median of 4 weeks.

Diarrhoea was the most frequently reported serious adverse event.[36] Grade 3 diarrhoea was reported in 20% of patients requiring interruption of drug in a phase II brincidofovir trial for adenoviraemia. However, rarely did diarrhoea or symptoms such as abdominal cramps require permanent discontinuation of brincidofovir.[36] In recipients of HSCT, diarrhoea is common and could also be a symptom of intestinal infection with HAdV or graft-versus-host disease. Hence, it is important to distinguish diarrhoea due to brincidofovir toxicity from gut graft-versus-host disease or adenovirus-associated enteritis. To meet this requirement, monitoring of sequential stool virus load, lymphocyte subsets, and histological examination of gastrointestinal biopsies needs to be routinely performed. It is noteworthy that nephrotoxicity was not observed in any of the studies performed to date.

The UK Paediatric BMT group reported contemporary experience from 7 paediatric HSCT centres indicating similar anti-adenoviral activity during the lymphopenic phase of HSCT and a good safety profile with brincidofovir.[37] In contrast, cidofovir did not lead to clearance of adenoviraemia in the absence of immune-reconstitution and was associated with nephrotoxicity.

Regarding the potential use of other antiviral agents, ribavirin was shown to display efficacy only against HAdV species C *in vitro*, and there is little evidence that inclusion of this drug in antiviral treatment, if HAdV species C is detected, may be beneficial.[38,39] Ganciclovir requires phosphorylation for conversion into an active compound, and the first phosphorylation step is not efficiently performed by cellular kinases. Since adenoviruses, unlike herpes viruses, lack the thymidine kinase gene, ganciclovir displays low efficacy against this virus family.[40,41] Finally, foscarnet was shown to have no effect against HAdV[41] thus underlining the limited antiviral treatment options against these viral pathogens.

6 | IMMUNOTHERAPY FOR INVASIVE HAdV INFECTION

The role of cellular immunity in preventing HAdV infection and controlling related disease is crucial, as demonstrated by studies reporting that a ≥ 2 log T-cell depletion of the graft, the use of mismatched or haploidentical donors requiring potent immunosuppression, and poor or delayed immune recovery represent risk factors for HAdV-mediated morbidity and mortality.[14,42,43] Indeed, the clearance of HAdV infection was associated with appearance of higher frequencies of HAdV-specific T-cells compared to patients who failed to achieve control of the HAdV infection.[13,44] Moreover, the survival of patients with HAdV viraemia is reportedly associated with recovery of the lymphocyte count and the presence of HAdV-specific T-cells.[24,44]

The adoptive transfer of HAdV immunity is possible by unselected donor lymphocyte infusion (DLI) or by using HAdV-specific T-cells. Although DLI are capable of clearing HAdV viraemia, their efficacy can be compromised by the low frequency of HAdV-specific T-cells contained in DLI and the risk of severe toxicity due to the high quantity of donor-derived alloreactive cells.[45] Several authors attempted to improve the safety of DLI by removing or inactivating alloreactive T-cells or by using genetically modified lymphocytes with suicide genes to control *in vivo* side effects such as GvHD.[46-48] More recent strategies to transfer HAdV-specific cellular immunity are based on the isolation of HAdV-specific T-cells from peripheral blood or the expansion of HAdV-specific T-cells *ex vivo*.[49,50] The former approach is based on the selection of T-cells secreting IFN gamma after stimulation with HAdV antigen. This method permits considerable concentration of HAdV-specific T-cells from 1% prior to selection to 45% in the final product containing polyclonal CD4 and CD8 cells, and the infusions were not associated with *in vivo* toxicity.[51] In another study based on IFN gamma capturing, 21 of 30 patients revealed an antiviral effect and complete clearance of viraemia was observed in 86% of patients with antigen-specific T-cell responses.[52] Moreover, the efficacy of the direct selection approach was recently demonstrated also by

third-party haploidentical donor usage in patients who had received an unrelated cord blood transplant.[53] The latter method is based on the production of cytolytic T-cell lines obtained by antigen-presenting cells transduced with HAdV vectors. With this approach, the inclusion of other viral antigens such as CMV or EBV facilitated the preparation of multi-specific cytotoxic T-lymphocytes (CTLs).[54] The main limitations of this method included the complexity of the manufacturing process, the costs, the long production time preventing the use of the CTL lines in cases of urgent medical need, and the availability of seropositive donors. More recently, the introduction of manufacturing processes for CTL-specific cell lines, generated by using seropositive third-party donors, provided the basis for the availability of banked ready-to-use antiviral T-cells, and the reported response rate of HAdV infections was greater than 70%.[55] General advantages and drawbacks of immunotherapy for viral infections after HSCT have been recently reviewed.[56] A phase II study in 38 patients using third-party-derived pentavalent CTLs specific for CMV, EBV, HAdV, HHV-6 and BKV showed an overall efficacy of 92%, with CTLs persisting in circulation for up to 12 weeks after infusion.[57] This recent study represents an important step to making adoptive immunotherapy broadly available which in turn can contribute to reducing virus-associated morbidity and mortality post HSCT as well as preventing organ toxicity associated with prolonged use of currently available antivirals. Although adoptive transfer of HAdV-specific T-cells from the original stem cell donor or third party donors is one of the most promising treatment approaches for invasive HAdV infections in high-risk patient populations, the clinical applicability still depends on timely access to virus-specific T-cells. Based on an established algorithm for the monitoring and treatment of invasive HAdV infections, the employment of immunotherapy was suggested in patients not responding to treatment with antivirals and lacking circulating HAdV-specific T-cells.[13] Broader availability of donor-derived or banked third-party virus-specific T-cells and/or T-cell lines represent a prerequisite for more general recommendations on antiviral immunotherapy in the transplant setting.

7 | RECOMMENDATIONS AND PERSPECTIVES

Based on the cumulative evidence considering very recent data and the clinical experience gained at leading centres in the field, the expert panel of authors provides recommendations for the management of HAdV infections in the HSCT setting. The recommendations are graded considering the strength and quality of evidence in line with the 4-level grading system for ranking recommendations in clinical guidelines currently used by the ECIL (Table 1).[58]

Recommendations for diagnostic screening and monitoring in the allo-HSCT setting are as follows:

1. Screening for HAdV shedding into the stool on at least two different days 1 to 2 weeks prior to conditioning by a sensitive quantitative PCR technique in paediatric patients (B II)/in adult patients, no recommendation is possible due to the current lack of pertinent data).
2. Weekly HAdV screening in stool specimens by a sensitive quantitative PCR technique until recovery of CD3+ T-cells above 300 per microlitre in paediatric patients (A II)/in adult patients, no recommendation is possible due to the current lack of pertinent data).
3. At least weekly adenoviral monitoring in peripheral blood using a sensitive quantitative PCR technique until recovery of CD3+ T-cells above 300 per microlitre and/or cessation of immunosuppression in HSCT recipients in the paediatric setting (A II)/in the adult setting (B III).
4. In patients testing positive for HAdV in peripheral blood and displaying <1000 virus copies per millilitre, in absence of systemic symptoms, twice weekly monitoring of HAdV levels using a sensitive quantitative PCR technique (A II).
5. Assessment of HAdV-specific T-cells particularly in patients displaying high-risk factors for HAdV-related disease or revealing high levels of the virus in stool above the critical threshold (A III).
6. In patients with HAdV viraemia undergoing antiviral treatment, monitoring of virus levels using a sensitive quantitative PCR technique once to twice weekly to permit surveillance of the response (A II).
7. In paediatric patients receiving preemptive anti-HAdV treatment in the presence of virus levels in stool above the critical threshold, in the absence of viraemia, monitoring of HAdV levels in stool using a sensitive quantitative PCR technique once to twice weekly to permit surveillance of the response (A III).
8. When treatment with brincidofovir is provided within clinical studies or for compassionate use, it is important to discriminate drug-mediated intestinal toxicity from gut graft-versus-host disease or HAdV-associated diarrhoea. Recommended diagnostics in this setting include daily recording of abdominal symptoms and diarrhoea grade, sequential measurement of stool virus load,

TABLE 1 ECIL-6 scoring system[a]

Strength of recommendation (SoR)	Definition
Grade A	ECIL strongly supports a recommendation for use
Grade B	ECIL moderately supports a recommendation for use
Grade C	ECIL marginally supports a recommendation for use
Grade D	ECIL supports a recommendation against use
Quality of evidence (QoE)	**Definition**
Level I	Evidence from at least 1 properly[b] designed randomized, controlled trial (orientated on the primary endpoint of the trial)
Level II	Evidence from at least 1 well-designed clinical trial (including secondary endpoints), without randomization; from cohort or case-controlled analytic studies (preferably from >1 center; from multiple time series; or from dramatic results of uncontrolled experiments
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

[a]Adapted from Cesaro et al.[58]

[b]Poor quality of design, inconsistency of results, indirectness of evidence, etc would lower the SoR.

quantitative analysis of lymphocyte subsets and histological examination of gastrointestinal biopsies, including viral immunohistochemistry (B II).

Recommendations for anti-adenoviral treatment are as follows:

1. Tapering of immunosuppression, whenever possible, (*a*) in the presence of viraemia >1000 HAdV copies per millilitre in a lymphopenic host with circulating CD3+ T-cells <25 per microlitre (A II) and (*b*) in the presence of HAdV positivity in stool with rapidly rising levels above the critical threshold (B II).
2. Cidofovir as preemptive antiviral therapy, preferably at the dose of 1 mg/kg three times weekly together with probenecid and hydration, (*a*) in the presence of viraemia >1000 HAdV copies per millilitre (A II) and (*b*) in the presence of HAdV positivity in stool with rapidly rising levels above the critical threshold (B II).
3. Use of ribavirin in addition to cidofovir in the presence of HAdV species C (C III). Treatment of HAdV infection with ganciclovir (D III) or foscarnet (D III).

Despite the demonstrated efficacy of brincidofovir in controlling adenoviraemia during the lymphopenic phase of HSCT and its favourable toxicity profile, approval of the drug for antiviral treatment by the Food and Drug Administration and/or European Medicines Agency is still pending. Currently, brincidofovir is only made available for clinical studies or for compassionate use. Based on present experience with brincidofovir as preemptive therapy for adenoviraemia in HSCT recipients, the recommended dose is 2 mg/kg twice weekly, with a maximum dose of 100 mg twice weekly (B II). In case of persisting or worsening diarrhoea, treatment should be interrupted until the GI symptoms improve or until the cause is identified (B II).

CONFLICT OF INTEREST

P. Hirwarkar, R. Wynn, and T. Lion: Honoraria from Chimerix; S. Cesaro: Honoraria from Chimerix and Gilead Sciences; the other authors declare no conflicts of interest in relation to the present study.

ORCID

Karin Kosulin http://orcid.org/0000-0001-7397-9692
Thomas Lion http://orcid.org/0000-0001-9346-0994

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