Original Article

Successful Treatment of Parainfluenza Virus Respiratory Tract Infection With DAS181 in 4 Immunocompromised Children

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Background. Parainfluenza virus (PIV), a common pediatric pathogen, is associated with significant morbidity in immunocompromised (IC) hosts. DAS181, a novel sialidase fusion protein inhibitor, seems to be effective against PIV in vitro and in vivo; its use in IC children has not been evaluated.

Methods. Patients were diagnosed with PIV infection using a quantitative reverse transcription-polymerase chain reaction. DAS181 was obtained under emergency investigational new drug applications and was administered via aerosol chamber or nebulizer. Patients were assessed daily for their clinical condition and adverse outcomes.

Results. Four pediatric hematopoietic cell transplantation (HCT) patients with PIV detected in respiratory specimens were identified and treated with DAS 181. Patients 1 and 2 were diagnosed with PIV lower respiratory tract infection (LRTI) by bronchoalveolar lavage at 9 months and 2 days after allogeneic transplantation, respectively. Patient 3 was on chemotherapy prior to planned autologous HCT at time of PIV diagnosis from a nasal swab. Patient 4 was diagnosed with PIV via nasal wash 2 days after HCT. Patients 1–3 had clinical symptoms and chest imaging consistent with LRTI. Inhaled DAS181 was administered for 5–10 days. All 4 patients tolerated therapy well. Clinical improvement in oxygen requirement and respiratory rate was observed in all patients who required oxygen at therapy initiation. Viral load decreased in all patients within 1 week of therapy and became undetectable by day 3 of therapy in patient 3.

Conclusion. DAS181 was used to treat 4 severely IC pediatric patients with PIV disease. The drug was well tolerated. Improvement in both viral loads and symptoms after initiation of therapy was observed in all cases. This report supports prospective, randomized studies in IC patients with PIV infection.

Key words. antiviral therapy; hematopoietic cell transplantation; parainfluenza virus

Parainfluenza virus (PIV) is a common respiratory pathogen typically associated with laryngotracheobronchitis (croup) in children, but it is also a frequent cause of upper respiratory tract infection (URTI), pneumonia, and bronchiolitis. In immunocompromised hosts, such as hematopoietic cell transplant (HCT) recipients and patients with hematologic malignancy, progression from URTI to lower respiratory tract infection (LRTI) is estimated at between 13% and 43%; mortality rates associated with LRTI are reported between 12% and 50% [1]. Currently, there are no known antiviral medications with proven efficacy against PIV nor are there preventive measures such as vaccines or prophylactic antibody therapies. Treatment remains primarily supportive in conjunction with reduction of immunosuppression [2].

DAS181, an investigational sialidase fusion protein, removes sialic acid-containing receptors from the surface of respiratory epithelial cells, thereby preventing PIV and influenza virus from binding to these cells [3]. DAS181 has been shown to be safe in phase I and phase II clinical trials evaluating treatment of influenza [4]. DAS181 also has documented in vitro and in vivo activity against PIV [5, 6]. Several case reports in adults have described the use of DAS181 in HCT and lung transplant recipients with PIV

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LRTI [7–9]. All adults had improvement in symptoms, oxygenation, and nasopharyngeal viral loads. In this report, we describe 4 severely immunocompromised pediatric patients with PIV infection treated with DAS181, with subsequent clinical and virologic improvement.

METHODS

Patients were diagnosed with PIV infection using a quantitative reverse transcription-polymerase chain reaction (RT-PCR) at the University of Washington Molecular Virology Laboratory [10]. We considered DAS181 (Ansun Biopharma, San Diego, CA) treatment based on the degree of immunosuppression and lung function. DAS181 was obtained under emergency investigational new drug applications that were individually approved by the US Food and Drug Administration and the Seattle Children's Hospital Institutional Review Board. The patients' parents provided informed consent. DAS181 was administered via dry powder inhaler (Cyclohaler; Teva Pharmaceuticals Ltd, North Wales, PA) or as a nebulized solution for either 5 or 10 days. The dry powder inhaler was administered once daily at a dose of 10 mg/day. For the nebulized solution, contents of 13 mg DAS181 capsules were mixed with 10 mL normal saline. The appropriate dose (see Table 1) was administered over 10 minutes. Dosing was based on manufacturer recommendations. Nasal wash (NW) samples were obtained in a standardized manner prior to starting therapy, daily while on therapy while hospitalized and following completion of therapy to monitor response. Samples were frozen and analyzed by quantitative RT-PCR at the University of Washington Molecular Virology Laboratory. Patients were assessed daily for their clinical condition and adverse outcomes. The following laboratory tests were conducted for patients while on therapy: complete blood count with differential, electrolytes, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, prothrombin time, activated partial thromboplastin time (PTT), and International normalized ratio. After completion of therapy, blood counts and liver function tests were monitored weekly for several months.

RESULTS

Four pediatric immunocompromised patients with PIV detected in respiratory specimens were identified. Underlying oncologic diagnoses for the 4 patients included the following: relapsed acute lymphoblastic leukemia (ALL), severe combined immunodeficiency (SCID), neuroblastoma, and acute myeloid leukemia (AML). Patients 1 and 2 underwent cord HCT; patient 3 underwent autologous HCT; and patient 4 underwent matched, unrelated donor peripheral blood HCT. Table 1 describes the timing of URTI and LRTI relative to transplant. Three patients (nos. 1, 2, and 3) were diagnosed as having lower respiratory tract PIV infection by detection of PIV following a bronchoalveolar lavage and by computerized tomography (CT) scan showing pulmonary involvement. Only 2 patients (patients 2 and 4) were severely neutropenic and lymphopenic at the time of PIV infection and treatment.

Patient 1 received 10 days of inhaled DAS181 via Cyclohaler at a dose of 10 mg/day. Patients 2 and 3 received nebulized drug at 0.14 mg/kg per day for 2 days followed by 0.2 mg/kg for 3 days; patient 3 received 0.14 mg/kg for 10 days. Patients 1 and 2 were weaned to their baseline oxygen requirement by days 2 and 5 after initiation of DAS181. All 4 patients had reduction of their clinical symptoms, including cough, tachypnea, and fever, after treatment. Patients 1, 2, and 4 had improvements in chest x-ray (CXR) abnormalities 10–30 days after DAS181 initiation (Supplemental Figure 1A). Patient 3 had improvements in CT abnormalities by day 9 after treatment (Supplemental Figure 1B). Parainfluenza virus quantitative viral loads from NW specimens were reduced 10-fold by day 10, day 20, day 1, and day 20 and were undetectable by day 44, day 44, day 3, and day 45 after initiation of DAS181 in patients 1, 2, 3, and 4, respectively (Figure 1).

All 4 patients tolerated treatment with DAS181 well. Patient 3 developed an elevated alkaline phosphatase during therapy (approximately twice the upper normal limit), with resolution to normal levels after discontinuation of drug. A transient prolonged PTT was observed in patients 2, 3, and 4. Patients 1, 3, and 4 also demonstrated a transient increase in alanine aminotransferase and aspartate aminotransferase. Descriptions of the individual patients and their clinical courses are summarized below.

Patient 1

A 12-year-old male developed upper respiratory symptoms 9 months after a double cord blood HCT for relapsed ALL. Parainfluenza virus 3 was detected in an outpatient NW (7.50 $\log_{10}$ copies/mL) with human metapneumovirus cycle time (CT) 35.6) and adenovirus (CT, 30.2). Human metapneumovirus was not detected in subsequent nasal wash samples. Twelve days later, the patient was admitted for increased work of breathing and daytime hypoxia, requiring 1.5–3 liters via nasal cannula (NC) to maintain oxygen saturations > 95%. Chest x-ray and chest CT results are shown in Supplemental Figure 1A. Bronchoalveolar lavage fluid sampling detected only PIV3 (7.93 $\log_{10}$ copies/mL). The patient developed worsening cough and rhinorrhea and increasing oxygen requirement (maximum 10 liters via face mask), and therefore treatment with DAS181 was considered.

Table 1. Summary of Clinical Parameters

	Patient 1	Patient 2	Patient 3	Patient 4
Age/sex	12-year-old male	7-month-old male	4-year-old male	3-year-old male
Underlying disease	Relapsed ALL	SCID	Stage IV neuroblastoma	AML
Transplant type	Double cord	Single cord	Autologous	MURD
PIV onset	URTI: TxD+260 LRTI: TxD+275	URTI: TxD-71 LRTI: TxD+2	URTI: TxD-26 LRTI: TxD-25	URTI: TxD+2
PIV type	PIV-3	PIV-2	PIV-3	PIV-3
Symptoms	New O_2 requirement, ↑respiratory distress	↑FiO_2, fever, wheezing	↑Cough, fever, crackles	Cough, rhinorrhea
Radiographic findings	Ground-glass opacities on CXR/CT	↑infiltrates on CXR/CT	New infiltrates on CXR/CT	
Co-pathogens	Adenovirus and hMPV (at time of URTI), HRV and adenovirus (at time of LRTI)	None	None	HRV
DAS181 treatment	10 mg/day × 10 days (dry powder)	0.14 mg/kg per day × 2 days, then 0.2 mg/kg per day × 3 days (nebulized)	0.14 mg/kg per day × 2 days, then 0.2 mg/kg per day × 3 days (nebulized)	0.14 mg/kg per day × 10 days (nebulized)
WBC (cells/μL)				
Start of therapy	2800	<200	9300	<200
Minimum while on therapy	2800	<200	3500	<200
End of therapy	4700	<200	3500	<200
Absolute neutrophil count (cells/μL)				
Start of therapy	2120	<200	6426	<200
Minimum while on therapy	1988	<200	1820	<200
End of therapy	3660	<200	1820	<200
Absolute lymphocyte count (cells/μL)				
Start of therapy	440	<200	1256	<200
Minimum while on therapy	372	<200	402	<200
End of therapy	752	<200	830	<200
Absolute monocyte count (cells/μL)				
Start of therapy	210	<200	1218	<200
Minimum while on therapy	140	<200	536	<200
End of therapy	282	<200	584	<200
Maximum alkaline phosphatase, IU/L (normal range)	477 (135–530)	298 (95–380)	598 (95–380)	247 (95–380)
Maximum alanine aminotransferase, IU/L (normal range)	126 (10–50)	35 (6–45)	73 (6–40)	152 (6–40)
Maximum aspartate aminotransferase, IU/L (normal range)	105 (5–41)	48 (3–74)	86 (5–41)	146 (5–41)
Maximum PTT, s (normal range)	30 (25–35)	51 (25–35)	90 (25–35)	47 (25–35)
Maximum INR	0.9	1.9	1.2	1.2

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CT, computerized tomography; CXR, chest x-ray; hMPV, human metapneumovirus; HRV, human rhinovirus; INR, International normalized ratio; LRTI, lower respiratory tract infection; MURD, matched unrelated donor; PIV, parainfluenza virus; PTT, partial thromboplastin time; SCID, severe combined immunodeficiency; TxD, transplant day; URTI, upper respiratory tract infection; WBC, white blood cells.

At the time of DAS181 initiation, the patient's oxygen requirement had decreased to 1–2 liters via NC. He tolerated the inhalation without difficultly and was weaned to his baseline oxygen requirement by day 2 (D+2) of therapy. Chest x-ray improved by D+10 and resolved completely by D+30 after therapy (Supplemental Figure 1A). His NW viral load decreased 10-fold by (D+10) of therapy and was undetectable by D+48 (Figure 1A). The patient continued to shed adenovirus from NW specimens after therapy (CT 36.2 on D+4 of therapy, CT 34.7 on D+48 after therapy). He was also found to be positive for human rhinovirus (HRV; CT 245.2 on D+9, CT 24.6 on D+28, CT 32.4 on D+48).

Patient 2

A 7-month-old male developed fever, tachypnea, increased work of breathing, and hypoxia 2 days after a cord blood HCT for SCID. The patient was originally diagnosed with PIV2 LRTI 70 days prior to transplant and was treated with 2 courses of inhaled ribavirin before transplant; however, PIV2 was persistently detected in NW specimens (9.25 $\log_{10}$ copies/mL 4 days prior to transplant). Two days after transplant, the patient developed fever and

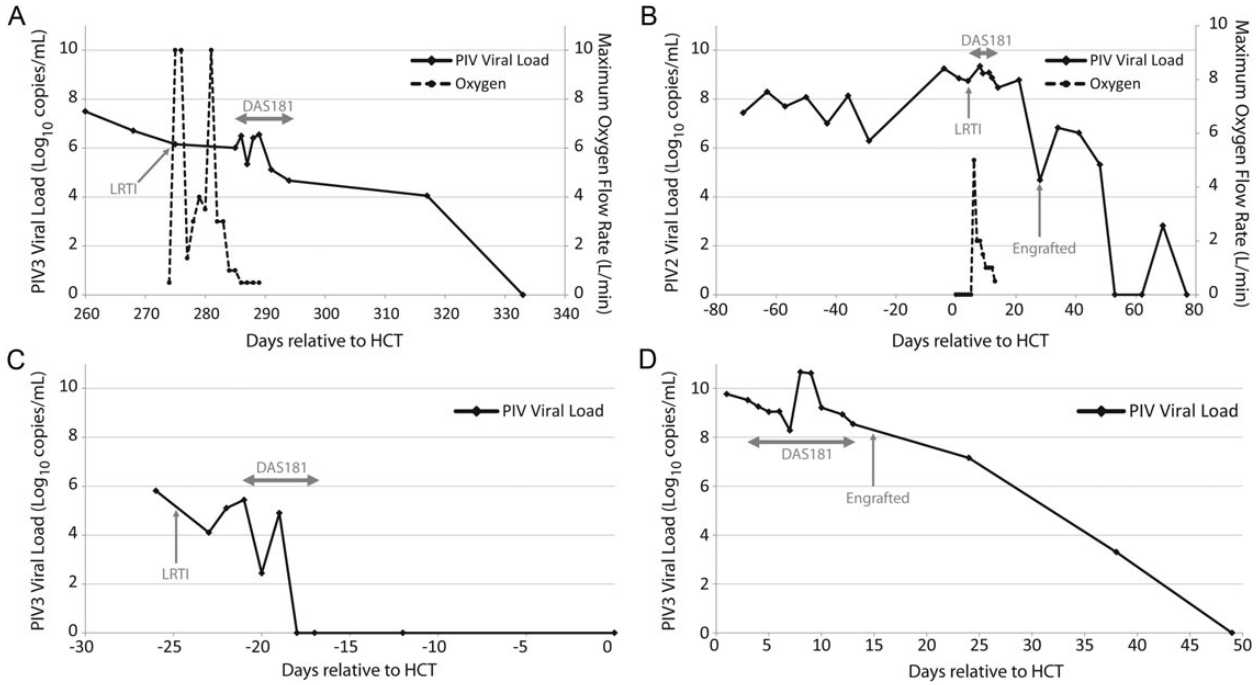

Figure 1. Quantitative nasal wash parainfluenza virus (PIV) viral loads and maximum oxygen flow rate for patient 1 (A), patient 2 (B), patient 3 (C), and patient 4 (D). HCT, hematopoietic cell transplantation; LRTI, lower respiratory tract infection.

increased respiratory symptoms. Treatment with inhaled ribavirin was again attempted, but it was poorly tolerated and discontinued after 3 days. The patient required up to 5 liters of supplemental oxygen via NC. Chest x-ray showed left lower lobe patchy opacities (Supplemental Figure 1A). Nasal wash samples continued to be positive for PIV2 with high viral load. DAS181 was administered via nebulized solution on 8 days post-HCT. He tolerated therapy without difficulty. He was weaned off of supplemental oxygen on D+5 after start of therapy and had marked improvement in tachypnea and work of breathing (Figure1B). He continued to have abnormal CXR findings until D+30 after DAS181 treatment, with PIV2 detected in NW samples until D+69, despite clinical improvement (Figure 1B).

Patient 3

A 4-year-old male developed a dry intermittent cough and clear rhinorrhea 1 week before a planned autologous HCT for stage IV neuroblastoma. He subsequently developed fever and a NW detected PIV3 (5.81 $\log_{10}$ copies/mL). Chest CT is shown in Supplemental Figure 1B. Physical exam revealed bilateral crackles and mild wheeze also suggestive of LRTI. He did not require oxygen. The first dose of DAS181 was administered while he was hospitalized, followed by 4 doses in the outpatient clinic. He received albuterol before each dose and tolerated therapy without difficulty. Chest CT on D+10 after initiation of DAS181 showed interval improvement (Supplemental Figure 1B). Parainfluenza virus 3 was undetectable by D+4 of DAS181 initiation. The patient went on to successful autologous HCT on D+21 without laboratory evidence of PIV3 infection.

Patient 4

A 3-year-old male with severe underlying lung disease developed mild cough and rhinorrhea 2 days following after a matched unrelated donor peripheral blood HCT for AML in second complete remission. Nasal wash was positive for PIV3 (9.76 $\log_{10}$ copies/mL) and HRV (CT, 27.6). The patient did not require oxygen nor did he have significant increased work of breathing; however, due to his pretransplant lung disease, the patient was considered for treatment with DAS181. The patient initially received 5 days of nebulized DAS181 but due to persistent leukopenia and high PIV viral load, another 5 days was administered (Figure 1D). Viral load began to fall on D+7 after initiation of treatment and was undetectable by D+45. Chest x-ray normalized by D+10. Nasal wash samples were positive for HRV from 33 days prior (D-33) through D-1 before DAS181 treatment (CT: 31.9, 21.4, 27.6, 30.4) but were then subsequently negative.

DISCUSSION

Parainfluenza viruses are responsible for up to 30%–40% of all acute respiratory tract infections in infants and children, and they are estimated to account for 7% of all hospitalizations for fever or acute respiratory illness in children

under 5 years in the United States [11]. The high incidence of morbidity and mortality associated with PIV infection in immunocompromised patients continues to be an important clinical problem. There are no known antiviral medications or vaccines with proven efficacy against PIV, and treatment is largely supportive including supplemental oxygen and reduction of immunosuppressive drugs. Neither inhaled ribavirin nor intravenous immunoglobulin has shown an appreciable effect on mortality due to PIV infection [2]. DAS181, a novel sialidase fusion protein, has shown in vivo and in vitro activity against PIV by effectively cleaving sialic acid from respiratory epithelial cells and preventing PIV infection. We report here 4 immunocompromised children treated for PIV infection with DAS181 using both powdered inhaler and liquid nebulized formulations.

Previous reports have described treatment of PIV LRTI with DAS181 in immunocompromised adults, including 2 HCT recipients and 2 lung transplant recipients [7–9]. Compared with these reports, patients described in this series presented with relatively moderate respiratory distress, with only 2 of 4 patients requiring oxygen at the time of treatment. Both patients were weaned to baseline oxygen requirement by completion of DAS181 therapy. As with previous reports, all patients described in this series demonstrated significant reduction in respiratory viral loads with improvement in radiography and clinical symptoms, although 2 patients in our series demonstrated clinical improvement prior to viral load reduction. All patients tolerated treatment with DAS181 well. All 4 patients successfully completed transplantation without recurrence of PIV infection.

The adverse effects of DAS181 treatment were mild and self-limited in our patients; duration of therapy was not impacted by any adverse event. Transient elevation in alkaline phosphatase described in previous reports [4, 9] is thought to be due to delayed clearance. Prolonged PTT has also been previously described [8] and has been documented to be related to interference of study drug with laboratory testing methods and not an actual abnormality in coagulation.

Although our study is limited to a description of only 4 patients who received DAS181 under emergent conditions, our patients were evaluated consistently using a standardized study protocol, and laboratory and clinical parameters were prospectively collected. Our data contribute to a growing body of evidence supporting the use of DAS181 for PIV infection in immunocompromised patients. The absolute effect of DAS181 versus other host, transplant, and viral factors (including coinfections) in reduction of symptoms and viral load cannot be determined in this study. Prospective, randomized clinical trials are currently underway in immunocompromised adults. Trials should be performed in immunocompromised patients of all ages to further evaluate clinical efficacy and safety in a population where PIV remains a significant and potentially deadly pathogen and no effective treatment options currently exist.

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Author contributions. A. W. performed the research, collected data, analyzed data, and wrote the manuscript; T. W., R. A., S. S., M. B., and R. M. critically reviewed the manuscript; J. K. provided resources and critically reviewed the manuscript; J. A. E. contributed to the analysis plan and wrote the manuscript.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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