Treatment of Parainfluenza 3 Infection With DAS181 in a Patient After Allogeneic Stem Cell Transplantation

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Parainfluenza virus (PIV) can cause significant morbidity after allogeneic stem cell transplantation (SCT). We report the first use of inhaled DAS181 for PIV in an allogeneic SCT recipient. Symptoms, oxygenation, and pulmonary function tests improved. Nasopharyngeal samples showed a reduction in viral load. DAS181 should be systematically evaluated for severe PIV infection.

Human parainfluenza virus (PIV) is a group of respiratory viruses that can cause a variety of infections in patients after allogeneic stem cell transplantation (SCT), ranging from self-limited upper respiratory tract infections to life-threatening lower respiratory tract infections [1, 2]. The severity of PIV manifestations usually depends on the type of transplant and the intensity of immunosuppression [3] and may be associated with long-term pulmonary dysfunction [4]. There is currently no effective vaccination or treatment against PIV, and standard therapy is supportive [5]. Although the majority of otherwise healthy patients who are infected with PIV will recover, an effective early treatment would be clinically beneficial for patients after allogeneic SCT. DAS181 is a novel inhaled sialidase fusion protein that has shown evidence of activity against multiple strains of influenza virus and PIV, including pandemic H1N1, multidrug-resistant influenza strains, and both clinical and laboratory isolates of PIV [6]. We report here the first case of DAS181 treatment of PIV in a patient after allogeneic SCT.

METHODS

An emergency investigational new drug application (EIND no. 109 449) was obtained from the US Food and Drug Administration to administer DAS181 to our patient with progressive PIV infection after allogeneic SCT. This single-patient protocol was also approved by our local institutional review board and the patient provided written informed consent. Treatment consisted of inhaled DAS181 provided in 10-mg dry powder capsules. DAS181 was administered by oral inhalation daily for 3 days using a designated dry powder inhaler (Cyclohaler; Teva). Three-day dosing was allowed based on the DAS181 human clinical safety data available at the time. The patient was evaluated daily. Pulmonary function tests, including standard spirometry and single-breath diffusion capacity of the lung for carbon monoxide (DLCO), were conducted before and after treatment. In addition, nasopharyngeal swab samples (Copan Diagnostics) were obtained daily, placed in 3-mL universal viral transport media vials (Becton Dickinson), and frozen at −80°C until analyzed by quantitative reverse-transcription polymerase chain reaction at the University of Washington Medical Reference Laboratory, Seattle, specific for PIV type 3 (PIV3) [7].

Specimens containing the patient’s PIV3 were inoculated into LLC-MK2 cells (American Type Culture Collection) and were passaged twice to obtain a large stock. LLC-MK2 cells were maintained in Minimum Essential Media (Invitrogen) containing Earle’s salts and L-glutamine supplemented with 10% fetal bovine serum, 1% biotic Antimycotic solution (Sigma) [8]. Cells were maintained at 37°C in a humidified atmosphere of 5% CO2. Viral titer was determined by plaque assay, and the presence of PIV3 after culture infection was confirmed using a PIV3-specific direct fluorescence antibody (DFA) reagent (Millipore; catalog no. 3120). In vitro characterization of the DAS181 sensitivity of this patient’s PIV3 strain was tested through inhibition of the median tissue culture infective dose (TCID50) and a modified plaque reduction assay. Briefly, LLC-MK2 cells were infected at the identified TCID50. Two hours after infection, LLC-MK2 cells were overlaid with agarose containing varying concentrations of
DAS181, ranging from 1000 to 0.1 nmol/L. Three to 5 days after infection, LLC-MK2 cells were fixed and then stained with the PIV3-specific DFA reagent and analyzed for viral spread by fluorescence analysis. A modified plaque reduction assay was conducted to determine the level of DAS181 sufficient to inhibit the infection by 50% (median effective concentration [EC₅₀]). LLC-MK2 cells were seeded the day before infection and then infected. Plates were overlaid with agarose in a 2X mixture of Eagles medium and Minimum Essential Media (1:1 ratio) containing appropriate concentrations of DAS181 (1000–0.1 nmol/L). Plaques were allowed to form for 2 days, at which point plates were fixed. After fixation, plates were stained with the DFA reagent.

RESULTS

A 63-year-old woman received a diagnosis of therapy-related acute myelogenous leukemia (AML) with monosomy 7 cytogenetics after being treated for many years with 6-mercaptopurine for Crohn’s disease. She underwent induction therapy with daunorubicin, cytarabine, and bortezomib as part of a national clinical trial (CALGB 10502) [9] and achieved complete remission. She then received 1 cycle of consolidation with intermediate dose cytarabine with bortezomib before proceeding to reduced-intensity allogeneic SCT from a matched unrelated donor (Figure 1). Conditioning was done with busulfan (6.4 mg/kg total dose), fludarabine, and anti-thymocyte globulin, and post-SCT graft-vs-host disease (GVHD) prophylaxis with tacrolimus and methotrexate. Forty days after SCT, the patient developed pancytopenia, and bone marrow biopsy showed findings of aplasia. She underwent a second SCT from the same donor. Conditioning was done with fludarabine and alemtuzumab, and post-SCT GVHD prophylaxis with tacrolimus. After this second SCT, engraftment was achieved. At day 24, stage III cutaneous GVHD developed, requiring treatment with corticosteroids and mycophenolate mofetil. Approximately 90 days after her second SCT, the patient developed nasal congestion and cough. A nasopharyngeal swab sample tested by DFA demonstrated PIV3 infection. She was treated supportively, but symptoms progressed over the next 35 days to worsening cough and wheezing, with a need for 3 L per minute of supplemental oxygen by nasal cannula. Chest computed tomography demonstrated scattered areas of tree-in-bud opacities and mild diffuse bronchial thickening consistent with viral bronchiolitis; there were no nodular or segmental consolidations, or ground-glass opacities, to suggest superimposed bacterial or fungal infections. Blood cultures were without growth. Normal oral flora grew in sputum samples; there was no fungal growth. Results of nasal and sputum DFA testing for influenza virus, adenovirus, and respiratory syncytial virus were negative, as were samples tested for herpes simplex virus culture and Mycoplasma pneumoniae polymerase chain reaction. Bronchoscopy with bronchoalveolar lavage was not performed. The patient was being treated for a previous episode of cytomegalovirus viremia with valganciclovir, was receiving prophylaxis with levofloxacin and posaconazole, and was receiving prednisone (10 mg daily) and mycophenolate mofetil (250 mg twice daily), both of which had been tapered for treatment of resolving GVHD. No additional empirical antimicrobial treatments were administered.

By day 2 of treatment by DAS181, the patient reported subjective improvement of her symptoms. This was noted at her physical examination with an improvement in breath sound auscultation and discontinuation of supplemental oxygen. While she was receiving treatment, serial nasopharyngeal swab samples were obtained for quantitative PIV3 viral load measurements, which showed a reduction over the 3 days of treatment from 1.72 × 10⁷ to 7.30 × 10⁵ copies/mL. At day 8, nasopharyngeal swab sample DFA was negative for PIV3. Pulmonary function tests were also performed before and after treatment. At initial testing, the forced expiratory volume in 1 second was 0.76 L, and the DLCO was 36% of predicted; after treatment, these values increased to 0.91 L and 56%, respectively. DAS181 was well tolerated without any appreciable toxic effects. The patient was able to go home without supplemental oxygen 6 days after the completion of DAS181 treatment (Figure 1).

DFA analysis of a pretreatment sample confirmed the strain to be PIV3 (data not shown), and the strain was confirmed again after minimal passaging in vitro. In vitro treatment of infected LLC-MK2 cells at the known TCID₅₀ with serially diluted concentrations of DAS181 showed that the concentration of DAS181 needed to inhibit viral infection was between 10 and 100 nmol/L (see Figure 2), whereas LLC-MK2 cells treated with 0.1–1 nmol/L DAS181 exhibited viral spread similar to that of the no-drug control. In concordance, a modified plaque reduction assay was also performed and showed the average EC₅₀ for DAS181 against this PIV3 strain to be 28 nmol/L (data not shown). We were unable to grow PIV3 from samples obtained subsequently. Therefore, we could not address changes in antiviral activity in vitro or potential emergence of DAS181 resistance.

Two weeks after completion of treatment, the patient’s symptoms recurred, and her nasopharyngeal swab sample DFA was again positive for PIV3. Additional findings at that time were consistent with relapsed AML and worsening skin GVHD, and a decision was made to not pursue further aggressive treatment. The patient ultimately died of relapsed AML.

DISCUSSION

There is no effective treatment for PIV infection after allogeneic hematopoietic SCT. Supportive care usually includes supplemental oxygen, bronchodilators, corticosteroids, antibiotics
to prevent superinfection, and intravenous immunoglobulin. Ribavirin had shown some activity against PIV in cell culture and thus has been used in some centers in some cases of PIV infection after SCT [3]. An early study described the use of both oral and intravenous ribavirin in combination with inhaled therapy for 13 patients with influenza, respiratory syncytial virus, or PIV, suggesting that ribavirin may be most effective when used before the development of hypoxia [10]. Another study described the outcomes in 24 patients who had PIV infection after SCT and suggested no benefit to early aerosolized ribavirin therapy [11]. In a larger study in 253 patients who contracted PIV pneumonia, 55 of these patients developed PIV pneumonia, and 31 were treated with a combination of aerosolized ribavirin, with or without intravenous immunoglobulin. Comparison between those treated and those not treated with ribavirin showed no difference in mortality or duration of viral shedding, leading the authors to conclude that ribavirin therapy had no clinically significant effect in the setting of PIV infection [5].

DAS181 is a novel inhaled recombinant sialidase protein containing the catalytic domain of Actinomyces viscosus sialidase and the heparin-binding domain from human amphiregulin, which prolongs DAS181 retention on epithelial surfaces [12]. The initial step in PIV infection is binding to the epithelium of the upper airway. This is achieved through interaction of the viral receptor-binding molecule (hemagglutinin-neuraminidase) with sialic acid–containing receptor molecules on the surface of the target cells. Inhibition of this initial interaction is thus an attractive target for therapy. In findings reported elsewhere, DAS181 has shown in vitro activity against human PIV and in vivo in a cotton rat model [8].

We report the first use of DAS181 to treat PIV infection after SCT. DAS181 was well tolerated without appreciable toxic effects. Treatment with DAS181 clearly brought about both subjective and objective improvement, allowing our patient to be discharged without any supplemental oxygen. Although only one patient sample obtained before dosing yielded successful viral amplification in culture to allow subsequent in vitro testing, resistance to DAS181 would not be expected during this course of treatment, given the limited duration of dosing and the drug’s host-targeted mechanism of action. It is possible that patients who are severely immunocompromised may require longer courses of DAS181 to derive maximal benefit. In addition, a nebulized version will need to be developed to treat

Figure 1. Patient timeline. Alemtuz, alemtuzumab; AML, acute myelogenous leukemia; ATG, anti-thymocyte globulin; BM, bone marrow; Bu/Flu, busulfan/fludarabine; CMV, cytomegalovirus; DLCO, diffusion capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 second; GVH, graft-vs-host disease; MMF, mycophenolate mofetil; Mtx, methotrexate; NC, nasal cannula; NP, nasopharyngeal; PIV3, parainfluenza virus type 3; SCT, stem cell transplantation; sx, symptoms; Tac, tacrolimus; URD, unrelated donor; URI, upper respiratory tract infection.
patients who require ventilatory support at the time of PIV diagnosis, but this is not currently available. Nevertheless, given the in vitro, animal, and now single-patient data, we believe that DAS181 warrants further testing to treat PIV-associated complications in patients after allogeneic SCT and in other immunocompromised populations.

Notes

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