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TNF-Receptor Inhibitor Therapy for the Treatment of Children with Idiopathic Pneumonia Syndrome. A Joint Pediatric Blood and Marrow Transplant Consortium and Children's Oncology Group Study (ASCT0521)

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A B S T R A C T

Idiopathic pneumonia syndrome (IPS) is an acute, noninfectious lung disorder associated with high morbidity and mortality after hematopoietic cell transplantation. Previous studies have suggested a role for TNFα in the pathogenesis of IPS. We report a multicenter phase II trial investigating a soluble TNF-binding protein, etanercept (Enbrel, Amgen, Thousand Oaks, CA), for the treatment of pediatric patients with IPS. Eligible patients were < 18 years old, within 120 days after transplantation, and with radiographic evidence of a diffuse pneumonitis. All patients underwent a pretherapy broncho-alveolor lavage (BAL) to establish the diagnosis of IPS. Systemic corticosteroids (2.0 mg/kg/day) plus etanercept (.4 mg/kg twice weekly × 8 doses) were administered. Response was defined as survival and discontinuation of supplemental oxygen support by day 28 of study. Thirty-nine patients (median age, 11 years; range, 1 to 17) were enrolled, with 11 of 39 patients nonevaluable because of identification of pathogens from their pretherapy BAL. In the remaining 28 patients, the median fraction of inspired oxygen at study entry was 45%, with 17 of 28 requiring mechanical ventilation. Complete responses were seen in 20 (71%) patients, with a median time to response of 10 days (range, 1 to 24). Response rates were higher for patients not requiring mechanical ventilation at study entry (100% versus 53%, $P = .01$). Overall survival at 28 days and 1 year after therapy were 89% (95% confidence interval [CI], 70% to 96%) and 63% (95% CI, 42% to 79%), respectively. Plasma levels of proinflammatory cytokines were significantly increased at onset of therapy, subsequently decreasing in responding patients. The addition of etanercept to high-dose corticosteroids was associated with high response rates and survival in children with IPS.

INTRODUCTION

Idiopathic pneumonia syndrome (IPS) describes an acute, noninfectious lung injury after hematopoietic cell transplantation (HCT). IPS responds poorly to conventional therapy, with mortality rates of 50% to 80% within 28 days of diagnosis

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[1-3]. Criteria for IPS include symptoms of respiratory distress plus radiographic evidence for diffuse alveolar injury in the absence of infection [4]. A recent update further categorized IPS by the primary anatomic site of cellular damage [5]. The incidence of IPS ranges from 2% to 12%, with a median onset 17 to 42 days after HCT and median time to death of 13 days from diagnosis [1,3,5-8]. Risk factors include acute graft-versus-host disease (GVHD) in both adult and pediatric HCT recipients, with a prior history of HCT or viral pneumonitis noted as additional risk factors in children [9-11].

Preclinical studies have revealed that inflammatory cytokines play a role in the development of IPS [5,12-15]. Specifically, TNFα contributes to endothelial cell injury and apoptosis and directs leukocyte recruitment by regulating pulmonary chemokine expression [12,13,15-18]. Increased levels of TNFα and its soluble receptors have also been noted in the bronchoalveolar lavage (BAL) fluid of humans with IPS [12-15,19].

The management of IPS traditionally involves supplemental oxygen, systemic corticosteroids, and advanced supportive care. Recent limited institution clinical trials using a soluble, dimeric, TNFα-binding protein (etanercept, Enbrel; Amgen, Thousand Oaks, CA), when given in combination with systemic corticosteroids, have noted significant improvements in response rate and early survival for patients with IPS [3,6,20]. In collaboration with the Pediatric Blood and Marrow Transplant Consortium and Children's Oncology Group, we conducted a multicenter phase II trial to determine whether the addition of etanercept to standard treatment would improve outcomes for children with IPS.

PATIENTS AND METHODS

Eligibility

Eligible patients were < 18 years old, received an allogeneic HCT within the previous 120 days, and met initial clinical and radiographic criteria for IPS. There were no exclusions to enrollment based on the underlying diagnosis, graft source, conditioning regimen, HLA match, or end-organ function. Patients with bacteremia within the prior 48 hours, cytomegalovirus (CMV) reactivation or CMV disease, mechanical ventilation >7 days, or a history of tuberculosis, prior tuberculosis exposure, or chronic active hepatitis B or C infections were ineligible. Patients receiving > 2.0 mg/kg/day methylprednisolone equivalent were ineligible. Written informed consent was required from all patients (or legal guardians). The trial was registered at ClinicalTrials.gov as NCT00309907.

Study Design

All patients underwent BAL at study entry to establish the diagnosis of IPS, including exclusion of infectious etiologies for the diffuse pneumonitis (Table 1). BAL samples were collected and subsequently subdivided for assays, outlined in Table 1. A clinical assessment of pulmonary dysfunction was obtained at study entry, recording the method of delivery and amount of supplemental oxygen. Other required observations at study entry included an echocardiogram (to exclude cardiogenic shock and pulmonary hypertension), chest x-ray (or computed tomography scan), CMV PCR assay (whole blood or plasma), and blood cultures. C-reactive protein (CRP), serology, and cytokine assays were performed at study enrollment and then weekly through day 28.

Study therapy (etanercept plus corticosteroids) was begun within 24 hours of the BAL, provided that required BAL fluid microbial stains (gram stain and fungal stain) were negative. The date therapy was initiated was defined as day 0 of study. Patients received etanercept (.4 mg/kg/dose, maximum 25 mg) twice weekly over 4 weeks (total of 8 doses). The day 0 etanercept dose was administered intravenously to expedite attainment of maximum plasma levels. Subsequent doses were administered subcutaneously 72 to 96 hours apart. If, at any point after initiation of therapy, pretherapy BAL fluid samples became positive for a pathogen, etanercept was discontinued and not reinstituted. The patient was considered nonevaluable for response and replaced on the study, though he or she was still followed for toxicity and survival.

Corticosteroids were begun at 2 mg/kg/day (methylprednisolone equivalent) on day 0. Intravenous corticosteroids were required the first 3 days, with subsequent change to oral dosing permitted thereafter. No dose reduction was allowed through day 7, with subsequent taper as clinically indicated. Patients already receiving corticosteroids before the study had dosing adjusted to 2 mg/kg/day on day 0. Other immunosuppressive agents were continued, without dosing adjustment, unless clinically indicated. Antimicrobial prophylaxis was given per local institutional practice.

Patients who developed sepsis syndrome, invasive fungal infections, disseminated viral infections, CMV reactivation (by PCR or antigenemia assay), or persistent bacteremia (>72 hours on appropriate antimicrobial therapy) while undergoing study therapy were removed from the study and not replaced. In each scenario, patients were followed for response, toxicity, and survival. Patients who had not met the response criteria before the time of study removal were deemed *nonresponders*.

Table 1
IPS Diagnostic Criteria

1. Presence of widespread alveolar injury:
   a. Diffuse radiographic infiltrates on CXR or CT
   b. SpO2 ≤ 93% on room air, or supplemental oxygen required to achieve SpO2 > 93%.
   c. Clinical signs and symptoms (cough, rales, dyspnea)
2. Absence of lower respiratory tract infection, based upon BAL studies:
   a. Gram stain, fungal stain, acid fast bacilli stain.
   b. Bacterial*, fungal, viral (RSV, parainfluenza, adenovirus, influenza A and B, CMV, rhinovirus) and mycobacterial cultures.
   c. *Pneumocystis jiroveci* assay (PCR, direct fluorescent antibody or cytology).
   d. Viral PCR assays for CMV, HSV, VZV, HHV-6 and community acquired respiratory viruses†.
   e. Galactomannan ELISA assay†.
3. Absence of cardiac dysfunction, acute renal failure, or iatrogenic fluid overload.

CXR indicates chest x-ray; CT, computed tomography; SpO2, peripheral capillary oxygen saturation; RSV, respiratory syncytial virus; HSV, herpes simplex virus; VZV, varicella zoster virus; HHV-6, human herpes virus−6.

* Quantitative bacterial culture ≥ 10^4 colony-forming units per milliliter considered positive.

† Per investigator discretion.

Plasma Biomarker Analysis

Whole blood samples for cytokine assays were collected in heparinized tubes on day 0 and then weekly through day 28. Frozen plasma samples were thawed and analyzed in batch using enzyme-linked immunosorbant (ELISA) assays for inflammatory cytokines, including TNFα, tumor necrosis factor receptor 1 (TNFR1), TNFR2, IL-6, IL-8, sCD14, IFNγ, angiopoietin-2 (Ang-2), and lipopolysaccharide-binding protein (LPB). Plasma samples were also obtained from healthy controls (n = 4) and allogeneic HCT recipients without complications (n = 5). For each assay, samples were diluted and analyzed in replicate per manufacturer's guidelines. Plasma samples from the transplantation controls were obtained from a separate institutional review board−approved study.

Statistical Analysis

The primary study endpoint was response to therapy, defined as survival to day 28 of study plus complete discontinuation of supplemental oxygen support for > 72 consecutive hours. The time to response was defined as the first of 3 consecutive days off all supplemental oxygen. Secondary endpoints included day-56 survival, overall survival (OS), and toxicity assessment using Common Terminology Criteria for Adverse Events version 3.0 (through June 30, 2011), then Common Terminology Criteria for Adverse Events version 4.0 thereafter. Patients were evaluable for response if they received at least 1 dose of etanercept and their pretherapy BAL studies remained negative for pathogen identification. OS was computed using the Kaplan-Meier method, with survival defined from the time of study entry to the date of death or last contact. Statistical comparisons of plasma protein levels were performed using the nonparametric Mann-Whitney test. The study was designed to have 90% power and type I error rate of 5% for detecting a 25% difference in response rates from 30% (historical controls) to 55%. The planned sample size was 40 patients evaluable for response. The protocol was approved by the Children's Oncology Group, Pediatric Blood and Marrow Transplant Consortium, and institutional review boards. A data safety monitoring board, appointed by the Children's Oncology Group, reviewed toxicity and response assessments.

RESULTS

Thirty-nine patients enrolled between 2006 and 2011 from 22 centers, with 28 patients evaluable for response assessment (Table 2). Eleven patients enrolled and began

Table 2
Demographics

Characteristic	No. of Patients (%)
Total enrolled	39
Total eligible	28
Age, yr	
Median (range)	14 (1-17)
Mean	11
Gender	
Female	14 (50)
Male	14 (50)
Primary disease	
ALL	10 (36)
AML/MDS	10 (36)
Lymphoma	1 (3)
Nonmalignant*	7 (25)
Stem cell source	
Peripheral stem cells	3 (11)
Bone marrow	14 (50)
Cord blood	11 (39)
Conditioning regimen	
TBI containing†	14 (50)
Non–TBI-containing	14 (50)
Myeloablative	27 (96)
Nonmyeloablative	1 (4)
Donor	
Unrelated	24 (86)
Related	4 (14)
GVHD prophylaxis	
Calcineurin inhibitor‡	22 (79)
Serotherapy§	15 (54)
No serotherapy	13 (46)
Oxygen support (% FiO2 at entry)	
Median FiO2 (range)	45 (25-100)
Mean FiO2	47
Oxygen support (method)‖	
Nasal cannula	6 (21)
Face mask/Bipap¶	5 (18)
Mechanical ventilation	17 (61)

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; TBI, total body irradiation.

* Nonmalignant diagnosis: aplastic anemia (1), hemoglobinopathy (2), immune-deficiency (4).

† TBI, total body irradiation (1200-1320 cGy).

‡ Tacrolimus or cyclosporine.

§ Serotherapy, includes anti-thymocyte globulin or anti-CD52 monoclonal antibody.

‖ Method of supplemental oxygen support at study entry.

¶ Bipap, biphasic positive airway pressure.

study therapy but were ultimately deemed nonevaluable because of identification of pathogens in their pretherapy BAL (n = 10) or because of corticosteroid dosing that exceeded study requirements (n = 1).

For the 28 evaluable patients, the median time to onset of study therapy was 20 days (range, 6 to 119) after HCT. The median duration of pretherapy supplemental oxygen support was 2 days (range, 1 to 20 days), with the median fraction of inspired oxygen (FiO2) of 45% (range, 25% to 100%). Seventeen of 28 patients required mechanical ventilator support at day 0. Twenty-three (82%) patients received all 8 etanercept doses. Five patients failed to complete etanercept dosing because of infectious (n = 2) or noninfectious (n = 1) complications or death (n = 2).

Response and Survival

Early study closure was recommended by the data safety monitoring board, as the primary endpoint was achieved in 20 of 28 (71%) patients. One additional complete response occurred after day 28, bringing the overall response rate by day 56 to 75%. The median time to complete response was 10 days (range, 1 to 24 days) requiring a median of 3 doses (range, 1 to 5) of etanercept. There were no differences in response by gender, recipient age, underlying diagnosis, graft source, HLA match, use of serotherapy for GVHD prophylaxis, or conditioning regimen (total body irradiation versus non–total body irradiation) (data not shown). Of note, 2 of the 8 nonresponders had a clear improvement in pulmonary status with study therapy, with both patients transitioned from mechanical ventilation to a nasal cannula during the course of study.

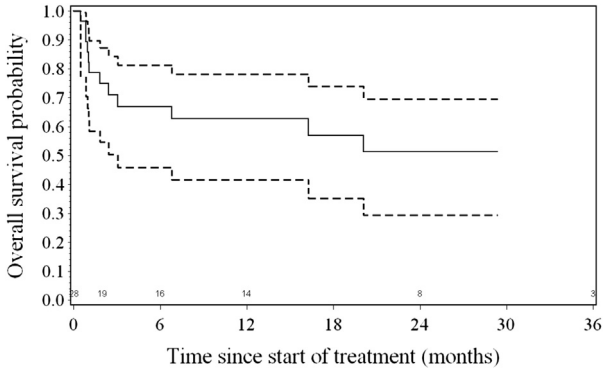

Figure 1. Overall survival (solid line) and 95% confidence intervals (dashed line) as a function of time since initiation of study therapy. Kaplan-Meier plot.

Patients requiring ≤ 45% FiO2 at study entry had higher response rates compared with patients requiring >45% FiO2 (87% versus 54%, $P = .05$). Complete responses were seen in all 11 (100%) patients not requiring mechanical ventilation at study entry, compared with responses in 9 of 17 (53%) patients on ventilatory support ($P = .01$). A trend toward improved response was seen in patients who initiated study therapy within 3 days of supplemental oxygen support (83% versus 50%, $P = .09$). Compared with expected rates, OS was high, 89% (95% confidence interval [CI], 70% to 96%) at day 28, 75% (95% CI, 55% to 87%) at day 56, and 63% (95% CI, 42% to 79%) at 1 year (Figure 1). Fourteen patients remain alive, 305 to 1761 days after initiation of study therapy. Eight of 17 patients (47%) requiring mechanical ventilation at study entry died within the initial 56 days of study. All 11 patients (100%) not requiring mechanical ventilation at study entry survived through day 56.

Toxicity

Grades 3 to 5 organ toxicities occurred in 8 of 28 patients between days 0 and 56. None of these toxicities (renal [n = 2], neurologic [n = 2], gastrointestinal [n = 2], cardiac [n = 1], and hepatic [n = 1]) were attributed to etanercept. Grades 3 to 5 infections occurred in 7 patients, including CMV (n = 2), herpes simplex virus (n = 2), virus-not specified (n = 1), *aspergillus* (n = 1), and bacterial enterocolitis (n = 1). CMV viremia developed in 2 patients on days 19 and 40 study, respectively, 1 associated with CMV pneumonitis. Five of the 7 grade 3 to 5 infections were pulmonary. No episodes of septicemia were observed during the study or the observation period (days 0 to 56). Eight deaths occurred by day 56 and were secondary to progression of IPS (n = 3), infectious pneumonitis (n = 3), multiorgan failure (n = 1), and cardiac arrhythmia (n = 1); all 8 events occurring in the cohort of patients requiring mechanical ventilation at study entry.

Four patients exhibited grade 2 to 4 acute GVHD at study entry; grade 2 (n = 2) and grade 3 (n = 2). Two of the 4

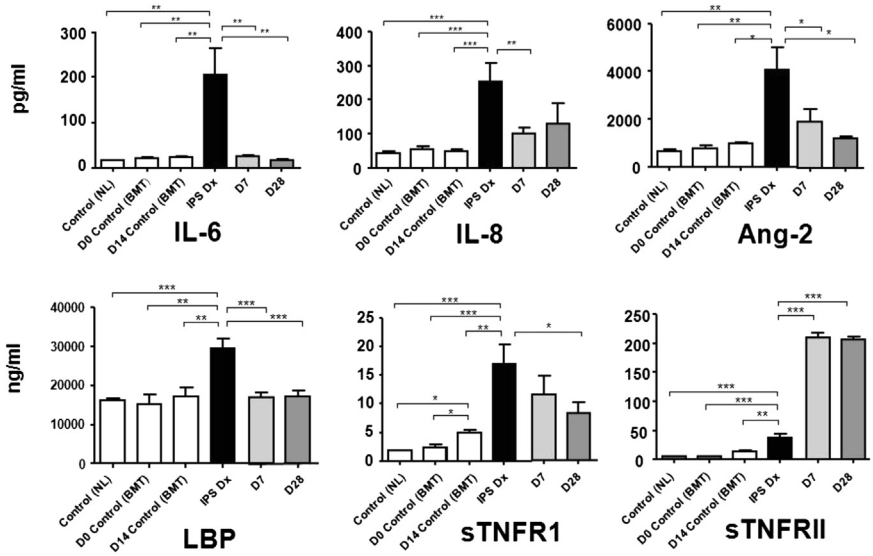

Figure 2. Plasma biomarkers in IPS. Plasma protein levels were analyzed from patient samples collected at the time of IPS diagnosis (IPS Dx), day 7 (D7), and day 28 (D28) of study. Two control groups were included, (1) healthy, nontransplantation patients, control (NL), and (2) allogeneic transplant recipients without complications, control (BMT). Plasma samples from control (BMT) were analyzed at day 0 and day 14 after transplantation, the day-14 time point chosen to approximate the time of onset of IPS. Data are expressed as mean ± SEM. Significant differences between groups are shown as $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

patients had complete resolution of GVHD during study therapy and 2 had no change in GVHD grading. One patient developed grade 2 GVHD while on study. The incidence and severity of chronic GVHD were not monitored.

Plasma Cytokine Analysis

Plasma samples were available for biomarker analysis in 26 of 28 patients. Mean levels of TNFR1, a surrogate marker for TNFα, were significantly higher in patients at study entry, when compared with non-HCT and HCT controls (Figure 2). The diagnosis of IPS was also associated with significantly higher plasma levels of IL-6, IL-8, Ang-2, LPB, and sTNFR2. Subsequent therapy for IPS led to reductions in day-7 and day-28 plasma levels of sTNFR1, IL-6, IL-8, Ang-2, and LPB. By contrast, plasma levels of sTNFR2 increased during study therapy, indicating uptake of etanercept, a TNFR2 analogue. Levels of sTNFR1, Ang-2, and IL-8 correlated well with response to therapy; mean plasma levels of all 3 cytokines declined by day 7 in responders but increased in nonresponders (Figure 3). The mean CRP level on day 0 was 28.1 ± 12.1, with levels decreased to 1.8 ± .6 in responders compared with 5.4 ± 3.3 in nonresponders by day 7. Though mean CRP levels were lower in responders than nonresponders at each time point (day 7, 14, 21, 28), none of the differences were significant.

Nonevaluable Patients

Eleven patients were deemed nonevaluable for response assessment. In 10 cases, pathogens were identified from the pretherapy BAL fluid cultures (or PCR assays) a median 2 days (range, 1 to 10 days) after initiation of study treatment. Viruses accounted for 9 of the 10 abnormal results, with CMV (n = 5) the most common virus identified. Two patients had already achieved a complete response to study therapy by

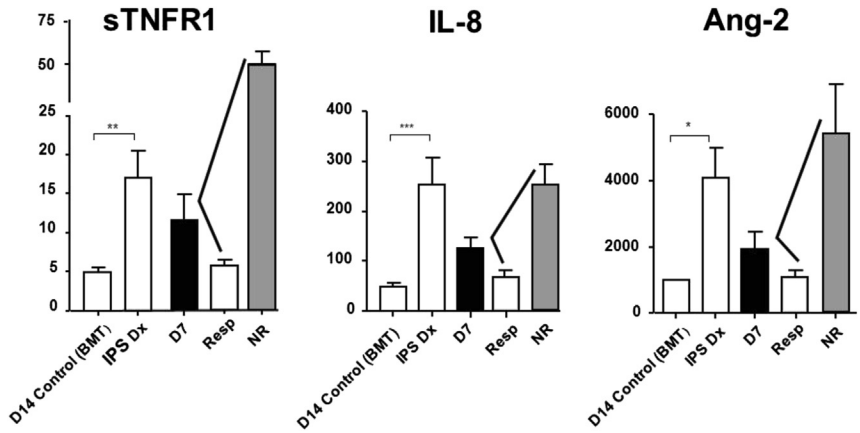

Figure 3. Plasma biomarker levels of IPS at the time of diagnosis and on day 7 of study therapy, in responders and nonresponders. Plasma protein levels were analyzed from patient samples collected at the time of IPS diagnosis (IPS Dx), and on day 7 (D7) of study. Day-7 levels are then subdivided by those patients who responded (Resp) or did not respond (NR) to study therapy. Control samples were obtained on day 14 after transplantation in allogeneic transplant recipients without complications, D14 control (BMT). Data are expressed as mean ± SEM. Significant differences between groups are shown as: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

the time CMV was detected in the prestudy BAL fluid, but given the abnormal BAL results, both patients were deemed nonevaluable for response assessment.

DISCUSSION

Improved therapy options for IPS are desperately needed, given the historically poor outcomes in both children and adults. Response rates of 18% to 30%, with day-28 survival <50%, have been reported when patients were treated with high-dose corticosteroids and supportive care measures [1-3,20]. In our current study, treatment with systemic corticosteroids and etanercept resulted in complete response rates of 71%, with day-28 survival of 89% and 1-year survival of 63%. Three other pediatric studies have confirmed that IPS remains an important complication in children, with cumulative incidence rates ranging from 6.7% to 11.8% after HCT [9,10,21]. In all 3 studies, transplantation-related mortality remained unacceptably high (>50%) and OS, poor [9,10,21]. Keates-Baleeiro examined 11 pediatric patients treated for IPS with systemic corticosteroids (n = 6), in which infliximab (n = 2) or etanercept (n = 3) were added for worsening pulmonary status. Though overall response rates were high in this report, the median survival was only 150 days [21]. Sano noted a median survival of only 9 days from IPS onset to death (range, 0 to 183 days) in children managed with corticosteroids and supportive care, with 100% mortality for those patients who required mechanical ventilation during therapy [10].

Early recognition of the disorder, before the development of severe pulmonary dysfunction, is key in the management of IPS in pediatric patients. Treatment responses were optimal when children were treated at lower baseline FiO2 values (≤45%) or before the requirement for mechanical ventilation. Day-56 survival was 100% in this subset of patients (n = 11), underscoring the importance of initiating therapy before the development of severe lung injury. Although outcomes were inferior in patients requiring mechanical ventilation at study entry, survival was still significantly higher than observed in other pediatric published reports [10].

Within this context, the current trial recommended that patients began treatment within 24 hours of performing the on-study BAL, provided that BAL fluid special stains were negative. We found no evidence that the early initiation of etanercept therapy increased therapy-related toxicity, even in patients later identified with pathogens on their pretherapy BAL. Interestingly, 2 patients with CMV pneumonitis had a complete response to study therapy before initiation of anti-CMV treatment. Although only 2 patients, such results suggest that TNF inhibition may play a potential role in CMV therapy. Elevated levels of TNFα have been reported in neonates with CMV pneumonitis but have not previously reported in HCT recipients [22].

A strength of our current study was the application of strict diagnostic criteria, with the requirement for BAL and echocardiogram in all subjects. In addition, a strict response definition was used, in which patient survival with “complete” discontinuation of all supplemental oxygen by day 28 were required. Clinical improvement rates in affected patients may have actually been higher, as significant reductions in supplemental oxygen support were noted in 2 additional study subjects by day 28, both subjects deemed nonresponders.

Comparison with Adult IPS Trial

The results of this trial warrant comparison with a parallel IPS study recently conducted in adults by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) [8]. Both trials had uniform eligibility (excluding age), dosing schedules for both etanercept and corticosteroids, and response assessments. The BMT CTN trial was a randomized, placebo-controlled trial of corticosteroids ± etanercept. There were no significant differences in response and survival based upon treatment assignment (etanercept or placebo). Response rates were 65% for the entire cohort, similar to the 71% response rate seen in our pediatric IPS study. However, in marked contrast to the dramatic survival rates noted in our pediatric IPS study, 1-year OS was extremely poor (<25%) for adults in both arms of the BMT CTN study.

Several differences between the 2 studies may account for these findings. Over 40% of patients in the BMT CTN trial received a reduced-intensity regimen, compared with only 4% in the pediatric IPS trial. Cytokine production, including TNFα, is diminished after reduced-intensity regimens [23]. Hence, it is possible that IPS developing in this context may initially be more responsive to corticosteroids alone. Cytokine analysis of BAL fluid and plasma from patients with IPS after reduced-intensity conditioning regimen are pending from the BMT CTN IPS trial and will help address this issue. Whereas adults in the BMT CTN trial received almost exclusively peripheral blood stem cells as the donor source, the vast majority of children in the pediatric IPS trial received marrow (50%) or cord blood (39%). Steroid responsive periengraftment syndrome has been reported after cord blood transplantation, with periengraftment respiratory distress syndrome recognized as a subset of IPS [24-26].

Interpretation of both studies is influenced by patient numbers. The pediatric IPS trial terminated early when it successfully met an efficacy stopping rule. In contrast, a major limitation of the BMT CTN IPS trial was its early termination because of poor accrual, with only 34 patients (out of a targeted 120) randomized [8]. Thus, the BMT CTN trial was drastically underpowered to draw any definitive conclusions. In addition, protocol adherence significantly affected the BMT CTN trial; 37% of patients on the etanercept arm received ≤ 2 etanercept doses, in several cases because of physician/patient discretion to discontinue “blinded” therapy. By comparison, over 80% of patients in the pediatric IPS trial received all 8 scheduled etanercept doses, regardless of therapy response. Hence, whereas compliance was high on the “open label,” phase II pediatric trial, compliance (and ultimately enrollment) on the phase III adult trial was significantly lower. Bronchoscopy was mandated (for diagnostic purposes) in both the pediatric and BMT CTN IPS trials. Critics have contended that the requirement for bronchoscopy may have also excluded the worst IPS cases from enrollment in the current trials, because of physician reluctance to perform a BAL in ventilated patients. The results from the pediatric IPS trial are thus even more impressive, as 17 of 28 (61%) patients required mechanical ventilation at study entry. The ability to ever perform the definitive phase III trial of etanercept in children with IPS is highly unlikely.

Biomarker Studies

A key finding in our pediatric study was the association of plasma biomarkers with response, providing further evidence that inflammatory cytokines contribute to the pathogenesis of IPS. Consistent with our previous work in animal models and humans [6], elevations in proinflammatory cytokines, including TNFRI, TNFRII, IL-6, IL-8, and LPB, a component of the LPS cascade, were present on day 0. Increased levels of Ang-2 have not been previously reported

in HCT patients with IPS. Angiopoietin (Ang)-1 and Ang-2 are peptide ligands for the receptor tyrosine kinase, Tie-2, that is expressed on the surface of endothelial cells (ECs) and are known to regulate vascular integrity [27]. Ang-2 sensitizes ECs to TNFα and regulates TNFα-induced adhesion molecule expression, findings that directly support pre-clinical data generated using murine IPS models [28]. Plasma levels of Ang-2 are increased in patients with acute respiratory distress syndrome and steroid refractory GVHD [29-32]. We found that Ang-2 levels in the plasma of patients with IPS were significantly elevated compared with controls. Importantly, levels of Ang-2 returned to baseline in IPS patients who responded to therapy, but they continued to rise in patients who progressed.

Although results of this pediatric phase II trial are encouraging, not all patients with IPS respond to anti-TNFα therapy. The reasons for this remain undetermined and limit the optimization of care. We recently analyzed the plasma proteome of patients with IPS and identified distinct similarities between IPS in humans and animal models, identifying a set of IPS-associated proteins that could predict at time of HCT which subjects would progress to IPS and respond to etanercept therapy [33]. Hence, patients who are "hot-wired" to respond to immunologic stress with high levels of TNFα secretion may be more likely to respond to medications like etanercept, whereas others may require alternative, novel strategies. For example, prior studies by our group suggest that other proinflammatory cytokines and chemokines, including IL-6 and MCP-1, are also markedly elevated at the onset of IPS [3,6]. Therapeutic agents targeting MCP-1 and the IL-6 receptor are now available for clinical use and could be considered as single agents or in combination with etanercept and corticosteroids.

In addition, an effort to categorize patients with IPS based upon the presumed anatomic site of primary injury in conjunction with mechanistic insights gained in the laboratory may lead to the use of other promising, noncross reactive therapeutic or preventive agents [5]. For example, it is conceivable that approaches to maintain EC integrity may be effective at preventing or treating IPS. The administration of molecules that function as survival factors for ECs has been successful in preventing endothelial damage and mortality from septic shock and radiation injury. Similarly, ongoing studies examining the role of surfactant replacement therapy might prove useful in overcoming the effects of epithelial injury and dysfunction. Finally, as IPS develops and progresses to respiratory failure despite conventional immune suppression, it is possible that novel strategies directed toward inhibiting pathways of leukocyte recruitment to the lung may serve as future adjuncts to standard therapy. Such strategies have been successful in early phase studies for GVHD prevention [34].

Etanercept Administration

Questions remain regarding the optimal route and administration schedule for etanercept in the management of IPS. The current trial used a 4-week course of therapy, with a median time to response of 10 days (3 doses of etanercept). Based upon results from the current trial, a shorter course of therapy, which could reduce the risks of opportunistic infections, may be warranted. Although "recurrent IPS" was not an issue in responders, whether this was because TNFα neutralization was continued for 4 weeks remains to be determined. Ideally, therapy would be guided by "real-time" monitoring of cytokine levels, with such technology currently under development at centers. In addition, the current study mandated that the first etanercept dose be given intravenously, with subsequent dosing via subcutaneous administration. Intravenous dosing was safe in our study, and not associated with infusion related events. Prior pharmaco-kinetic data noted a rapid attainment of maximal concentration (Cmax) after intravenous administration of etanercept, in comparison to subcutaneous dosing [35]. Given the critical nature of affected patients, the importance of rapid attainment of Cmax is evident, and intravenous etanercept dosing remains recommended at initiation of IPS therapy.

In conclusion, etanercept in combination with corticosteroids was associated with high response rates and OS in children with IPS. Therapy was well tolerated with an acceptable toxicity profile. Plasma biomarker studies further support the role for inflammatory cytokines in the pathogenesis of IPS, with reductions in biomarker levels coinciding with clinical response to therapy. Collectively, these results represent the culmination of a translational research endeavor that used established animal models, encouraging early phase clinical data and 2 collaborative pediatric consortium to bring novel insights into the pathophysiology of a lethal clinical disorder, from the laboratory to the clinic.

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