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Advances in the Clinical Management of GVHD

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Abstract

The principle cause of mortality and morbidity following hematopoietic cell transplantation (HCT) is graft-versus-host disease (GVHD). Studies in murine models have revealed that inflammatory mediators such as tumor necrosis factor- α (TNF- α) promote destruction of host tissue following HCT. Elevated plasma levels of soluble TNF receptor 1 have been associated with patients with GVHD and blocking TNF- α in experimental models has shown a reduced incidence of GVHD. Based on this finding, patients with new onset GVHD were treated with steroids plus the TNF- α inhibitor, etanercept, on a previously reported pilot trial (n = 20) and a phase 2 trial (n = 41) and their outcomes were compared with those of contemporaneous patients with GVHD (n = 99) whose initial therapy was steroids alone. Patients treated with etanercept were more likely to achieve CR than were patients treated with steroids alone. Plasma TNFR1 levels, a biomarker for GVHD activity, were elevated at GVHD onset and decreased significantly only in patients with CR. A four protein fingerprint of IL-2R α , TNFR1, IL-8, and HGF in the plasma has been identified to predict whether a patient will be at high-risk for GVHD based on biomarker analysis. In univariate and multivariate analysis, this four-protein fingerprint has shown a strong association with the grade of acute GVHD, and it can stratify patients into low- and high-risk groups and can be used as a laboratory-based screening method, to diagnose and perhaps treat patients preemptively.

Keywords

graft-versus-host-disease (GVHD); graft-versus-leukemia (GVL); tumor necrosis factor- α (TNF- α); TNF receptor I; etanercept; steroids; antigen-presenting cells; cytotoxic T cells; lipopolysaccharide; natural killer cells

Introduction

Graft-versus-host-disease (GVHD), a major toxic side effect associated with transplantation, reduces the incidence of response and survival in these patients. Older patients especially suffer increased mortality and morbidity from GVHD. Data generated on the pathophysiology of GVHD has come mostly from rodent models, but these findings have not yet translated to benefit clinical practice in humans. There are 3 steps to GVHD pathophysiology. In the first step, the host's antigen-presenting cells (APCs) are activated by the conditioning regimen. The host then experiences enhanced recognition of donor T cells due to the increased expression

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of major histocompatibility complex (MHC) antigens. In the second step, the donor T cells are activated after interaction with host APCs, resulting in the proliferation, differentiation, and secretion of cytokines. Cytokines such as IL-2 and IFN- γ amplify cytotoxic T cell (CTL) and natural killer (NK) cell responses which in turn prime mononuclear phagocytes to produce TNF- α and IL-1. These inflammatory cytokines help generate chemokines which mobilize effector cells to target organs. In the third step, mononuclear phagocytes are stimulated by lipopolysaccharide (LPS) that leaks through the intestinal mucosa damaged during step 1. Amplification of local tissue injury may occur via this inflammatory response, including the increased production of CTL and NK cells, and leads to target tissue destruction in the transplant host.^{1, 2}

GVHD is tightly associated with a major therapeutic benefit of allogeneic BMT, the graft versus leukemia (GVL) effect. GVL is mediated primarily by cytotoxic pathways of cytolytic T cells and natural killer cells. Cascades of inflammatory cytokines and the cellular response of CTLs and NK cells amplify GVHD and destroy target host tissue. Blockade of cascade checkpoints may be able to prevent GVHD but still protect the GVL effect. Furthermore, in well defined animal models we have shown that many of these anti-inflammatory activities can preserve cytolytic T lymphocyte function, curtailing only GVHD activity but not GVL effects. Studies in mouse models show both that TNF- α and IL-1 play prominent roles in these processes.³

TNF- α in Clinical GVHD

The role of TNF- α in augmenting GVHD was examined in a study where TNF Receptor 1 (TNFR1) levels were measured as a surrogate marker for TNF- α in 438 recipients of myeloablative HCT pre-transplant and day 7 post-transplant. This study also showed that the increase in TNFR1 levels from baseline correlated with eventual development of GVHD and with treatment-related mortality and levels at day 7 was a significant predictor for outcome.⁴

To determine the effect of specifically inhibiting TNF- α during the effector phase of GVHD, we added etanercept, a soluble, dimeric fusion protein of the TNF-receptor type II that inactivates TNF- α , to high-dose steroids (2 mg/kg) at the onset of clinical GVHD. We hypothesized that the addition of etanercept would increase the complete response (CR) rate.

In a pilot trial and a phase II trial at the University of Michigan, etanercept was given subcutaneously at 25 mg twice per week for 8 weeks.⁵ Methylprednisolone was given at 2 mg/kg for less than 72 hours, and the usual GVHD prophylaxis agents were continued. Steroids were continued for at least 7 days at the starting dose and then tapered as tolerated. The primary endpoint was complete response at 4 weeks. There were 61 patients who consented to the pilot (n = 20) and phase 2 (n = 41) etanercept and steroid trials, and 99 patients were not enrolled and received only steroids. These 99 patients were not enrolled for any of 4 reasons, including that a trial was not open, they had received treatment with steroids for over 72 hours, they refused, or they had a simultaneous uncontrolled infection. There were 191 control subjects who did not have GVHD. Of patients who received transplants from unrelated donors, significantly more patients received steroids alone than etanercept plus steroids ($P = 0.06$).

Complete Response Rates to Etanercept and Steroids

At 4 weeks after the initiation of therapy, there were significant differences in complete responses between the groups that received etanercept plus steroids vs. those who received steroids alone. In the etanercept plus steroids group, 81% of patients achieved a complete response in the skin, compared to 47% in the steroids-alone group ($P = 0.0008$). There were also significant differences in complete responses in the liver, but the numbers for liver complete responses are small. In animal models, GVHD in the gastrointestinal (GI) tract drives mortality. In the etanercept plus steroids group, 78% achieved CR, compared to 48% in the

steroids-alone group. Overall, 69% of the etanercept plus steroids group achieved a complete response, compared to 33% in the steroids-alone group.

In the subset of patients who received transplants from related donors, 40% of the steroid-alone group achieved a complete response 4 weeks, and the complete response rate continued to rise to around 70% at 8 weeks. Patients who are initially treated with steroids alone generally switched to another agent if a response was not observed after 2 or 3 weeks. However, the data showed that patients continued to achieve complete responses with steroids alone up to 2 months after transplant. Patients who received etanercept plus steroids did not receive additional agents if they achieved a complete response, and 79% of this group achieved a complete response at 4 weeks (Figure 1).

There was also a large and significant difference in response in recipients of transplants from unrelated donors. Patients who received steroids alone had only a 25% complete response rate by 4 weeks; but in an important difference to recipients of transplants from related donors, the complete response rate did not increase significantly after 4 weeks. In patients who received etanercept plus steroids, 53% achieved a complete response by 4 weeks (Figure 1). These data correlate with anecdotal observations and published literature that suggests that GVHD in patients who have had transplants from unrelated donors is difficult to treat.⁶

Infections

An important consideration in heavily immunosuppressed patients is the incidence of serious infections. In this regard, there were no significant differences in any of the classes of infections between the etanercept plus steroids and the steroids-alone groups (Figure 2). There was a non-significant increase in fungal infections in the etanercept plus steroids group, and all of these patients received voriconazole prophylaxis at the time of etanercept initiation, since it has been suggested that patients who are treated with TNF- α inhibitors are at increased risk for fungal infections⁷⁻⁹.

Flares, Relapses, and Survival

By 8 weeks after the initiation of primary treatment, there was no statistical difference between groups in GVHD flares (10% etanercept plus steroids vs. 12% steroids alone) or disease relapse (16% etanercept plus steroids vs. 21% steroids alone). However, because the groups were not balanced for disease-risk at the initiation of the trials, these data are inconclusive.

Many of the patients in the related donor group achieved a complete response, and there was no change in survival between etanercept and steroids only groups. By contrast, of patients with unrelated donors, those who received etanercept plus steroids had a significantly better 6-month and 1-year survival rate than those who received steroids alone. These results are consistent with observations in other studies that achieving a complete remission is a very important factor for survival in patients with GVHD^{10, 11}.

The Blood and Marrow Transplant Clinical Trials Network has recently completed a 4-arm, randomized phase II trial in multiple centers comparing the combination of corticosteroids with (1) etanercept, (2) mycophenolate mofetil, (3) denileukin diftitox, and (4) pentostatin. The primary endpoint for both trials is the cumulative incidence of complete responses at day 28. Secondary endpoints include GVHD flare, time to discontinuation of immunosuppression, and incidence of chronic GVHD. This completed accrual of 180 patients in March 2008 and results are expected to be available by late 2008.

TNFR1 Levels

In the University of Michigan study, we determined whether levels of TNFR1 in the plasma correlated with disease activity. In addition to the two treatment groups, TNFR1 was measured in 190 control patients who did not have GVHD. TNF- α is difficult to measure directly because it often circulates bound to its receptors, but TNFR1 is a good surrogate marker for TNF- α .¹² At the onset of GVHD, TNFR1 levels are approximately 3.5 times higher than levels in the control group. Patients who achieved a CR to either treatment option had a significant decrease in TNFR1 levels. For patients who did not achieve a CR, there was no change in TNFR1 level. The only difference between the etanercept plus steroids and the steroids-alone groups is that the CR group is twice as large in the etanercept plus steroids group. These results suggest a correlation between TNFR1 level in the plasma and disease activity, regardless of therapy.

Biomarker Panel to Diagnose GVHD

Other than the serum bilirubin serum level for liver involvement, there is no standard laboratory test that can confirm the diagnosis of GVHD. To determine what molecular markers might indicate a diagnosis with GVHD, a micro-array chip with 120 plasma proteins was initially evaluated with pooled serum from patients with and without GVHD. There were 20 proteins that were different between patients with severe GVHD and controls with no GVHD. In a training set of 282 patients and a validation set of 142 patients, sequential ELISA was performed to measure the levels of 8 proteins for which there were available antibodies and that showed the largest differences between groups. Plasma was taken at the onset of GVHD or weekly in patients without GVHD. In the training set, the most significant and informative proteins were IL-2R α and TNFR1. The differences in HGF and IL-8 were also significant, but to a lesser extent.¹³

We next determined whether the biomarker panel provided information in addition to standard GVHD risk factors. The training and validation sets were separated into high risk and low risk groups based on the results of the biomarker panel. High risk was defined as 95% specificity for GVHD or greater. Using this laboratory criterion to divide the patients, non-relapse mortality was significantly greater in the high risk group when compared to the low risk group. As a result, overall survival was also significantly higher in low risk patients.

We conducted both univariate and multivariate analyses using GVHD grade in a binomial comparison. In the univariate analysis, GVHD Grades I–II were given a risk of 1 and GVHD Grades III–IV were given a hazard ratio of 2.35. The multivariate analysis included donor relation, HLA match, age, and conditioning regimen. When comparing patients with biomarker levels 1.5 times the median value vs. patients who had median biomarker levels, the biomarker panel predicted risk in both the univariate and multivariate analyses.

Conclusions

Regarding the Phase II clinical trial at the University of Michigan, treatment with etanercept plus steroids resulted in a superior CR rate after 4 weeks, and higher complete response rates after both related and unrelated donor transplants. Survival was also superior at day 180 in the unrelated donor group treated with etanercept plus steroids. Furthermore, treatment with etanercept plus steroids did not result in an increase in infections or relapse.

Regarding the biomarker panel, which measures IL-2R α , TNFR1, IL-8, and HGF in the plasma, can accurately predict the presence of GVHD with good sensitivity and high specificity. This four-protein fingerprint has a strong association with initial and maximum grade of acute GVHD, and it stratified patients into low- and high-risk groups for GVHD, and predicted non-relapse mortality and long-term survival in these groups. In the future, this biomarker panel

may be used to help determine which patients will develop GVHD before a clinical diagnosis can be made.

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Figure 1. Incidence of complete responses from GVHD at 4 weeks after treatment

In all patients who had GVHD, those treated with etanercept + steroids (n=61) were more likely to achieve CR than were patients treated with steroids alone (n=99)(69% vs 33%; $P < .001$). This difference was observed in HCT recipients of both related donors (79% vs 39%; $P = .001$) and unrelated donors (53% vs 26%; $P < .001$).

Figure 2. Infection rates according to treatment groups

The infection rates in the first 100 days from initiation of GvHD treatment were similar between patients treated with steroids alone or with etanercept plus steroids for bacterial (gram positive shown), invasive fungal or viral infections. There were no mycobacterial infections observed in any patients.