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Graft-versus-host disease

Rapid response to alefacept given to patients with steroid resistant or steroid dependent acute graft-versus-host disease: a preliminary report

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Summary:

We evaluated the effect of alefacept (Amevive), a novel dimeric fusion protein, in steroid resistant/dependent acute graft-versus-host-disease (aGVHD). Seven patients were treated in eight aGVHD episodes. GVHD grade at treatment initiation and at peak ranged 2–4 (median 2.5) and 2–4 (median 4), respectively. System involvement at GVHD peak included skin ($n=7$), gastrointestinal tract ($n=5$) and liver ($n=3$). All patients responded. However, one patient with skin GVHD and two with gastrointestinal GVHD featuring an early initial response (IR) exacerbated and CR was not achieved. Skin GVHD responded rapidly with a median of 1 day to IR and 7 days to CR. Intestinal response was slower with median 7.5 days to IR. Of the four patients that achieved IR, CR was achieved in only one (40 days to CR). None of the patients had significant hepatic GVHD *before* treatment so no hepatic effect of alefacept could be determined. No immediate alefacept-related side effects were observed. Late side effects included infections (aspergillus sinusitis, pneumonia, bacteremia, pharyngeal thrush), pancytopenia and hemorrhagic cystitis. Three patients had CMV reactivation while on alefacept. We conclude that alefacept may have a beneficial effect in controlling aGVHD. Further investigations in larger cohorts of patients and controlled studies are warranted.
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Alefacept (Amevive) is a novel dimeric fusion protein that consists of the extracellular CD2-binding portion of the human leucocyte function antigen-3 (LFA-3) linked to the Fc portion of human IgG1 that selectively targets the memory T-cell population. It is successfully used in psoriasis, a T-cell-dependent autoimmune disease.[1,2] Onset of response to alefacept treatment was shown to begin 60 days after initiation of therapy while the median duration of response was 3.5 months.

Graft-versus-host disease (GVHD) is the most ominous side effect of allogenic stem cell transplantation (SCT). It causes a severe T-cell-dependent inflammatory process, which usually affects the skin, gut and liver. Despite the use of innovative immunosuppressive modalities, the prognosis of steroid resistant GVHD is usually poor.[3,4] Owing to its proven efficacy in another T-cell-dependent immune-mediated disease and considering the relatively low rate of side effects, we decided to investigate the role of alefacept in a cohort of patients with steroid-resistant or dependent acute GVHD.

Patients and methods

Patient's characteristics and GVHD policy

A total of seven patients were included in the preliminary study, five males and two females, with a median age of 41.5 years (range 7–53 years). Underlying diseases were MDS in transformation to leukemia ($n=3$), NHL ($n=2$), ALL ($n=1$) and myelofibrosis ($n=1$). Three patients were transplanted from HLA-A, B, C and high resolution DR fully matched siblings; two patients received stem cell grafts from fully matched unrelated donors (MUD) nonreactive in mixed lymphocyte culture and two patients were transplanted from partially mismatched donors. Patient characteristics are shown in Table 1. Treatment of each patient was individually authorized by the institutional review board as an off-label use of the drug.

Patients were eligible for inclusion if they developed grade 2–4 acute GVHD which was steroid-resistant (eg with progression after 3 days standard treatment, or unresponsive to at least 7 days standard treatment, or incomplete response to standard treatment after 14 days) or steroid dependent (eg responding to methylprednisolone 2 mg/kg/day but relapsing with an attempt to decrease the steroidal treatment). Standard treatment for acute GVHD included i.v. cyclosporin 3 mg/kg/day with doses adjusted

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Table 1 Patient characteristics

UPN	*Age (years)*	*Basic disease*	*Disease status at SCT*	*SCT conditioning*	*Pre-alefacept GVHD treatment*
1962	7	ALL	Relapse refractory	Flu x6, Bu x2, Eto x1, TBI 200 cGy x2	CSA, MP, FK, ATG, Cy, MMF
1924	51	MDS/AML	Relapse 1 untreated	Flu x6, Bu x2, TT x2, ATG x4	CSA, MP
1770	53	NHL	Relapse refractory	Flu x6, Bu x2, TBI 200 cGy x1, ATG x4	CSA, MP
1976	53	Myelofibrosis transformed to AML	Untreated	Flu x6, Bu x2, TT x2	CSA, MP
1972	32	MDS/AML	Untreated	Flu x 6, Bu x 2, TT x 2	CSA, MP
1980	14	MDS/ALL	Untreated	Flu x6, Bu x2, Eto x2, ATG x4	CSA, MP
1974	9	Burkitt's NHL	First resistant relapse	Flu x6, Cy x2, Bu x2, Eto x1	MP

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ATG = antithymocyte globulin; Bu = busulfan; CSA = cyclosporine; Cy = cyclophosphamide; Eto = Etoposide; Flu = fludarabine; FK = tacrolimus; MDS = myelodysplastic syndrome; MMF = mycophenolate mofetil; NHL = non-Hodgkin's lymphoma; TBI = total body irradiation; TT = thiotepa; UPN = unique patient number.

depending on serum levels and i.v. methylprednisolone (or equivalent steroid) $\geqslant 2$ mg/kg/day. No other immunesuppressive medications were used in these patients during the study. GVHD diagnosis was based upon clinical criteria supported by biopsy. An infectious cause for the gastrointestinal/liver symptoms was excluded in all patients. GVHD grading was according to the International Bone Marrow Transplant Registry (IBMTR) severity index.[5]

Alefacept treatment program

Alefacept (Amevive, Biogen inc. Cambridge, MA, USA) was a gift from Medison Pharma Ltd, Israel. The dose for children was 15 mg given intramuscularly (i.m.) once or twice weekly. The dose for adults was 30 mg i.m. twice weekly.

Response criteria

Therapeutic response was defined as the day of observed clinical improvement starting from the day of initiation of treatment with alefacept, for each of the involved systems, according to the following definitions:

Skin: amelioration of rush and pruritus (initial response); resolution of symptoms (complete response).

Liver: decrease of bilirubin level by 50% of basal levels (initial response); 51–75% (partial response 75); $<25\%$ (partial response 25) or achieving normal bilirubin levels (complete response).

Gastrointestinal tract: subjective amelioration of diarrhea volume and abdominal pain or disappearance of bleeding (initial response); resolution of diarrhea and all symptoms (complete response).

All possible adverse reactions that could be attributed to alefacept were monitored, including infectious complications (including routine weekly DNA-PCR test and/or pp65 antigenemia to detect cytomegalovirus (CMV) activation); basic disease status and chimerism, including weekly donor and host-specific DNA markers, using male and female amelogenine gene PCR bands,[6] and VNTR-PCR[7] and cytogenetic analysis when applicable.

Results

Seven patients were treated for eight acute GVHD episodes.

The first patient (UPN 1962) was a pediatric patient with grade 4 GVHD. He received the first dose of i.m. alefacept and was scheduled to receive i.m. alefacept once weekly as previously reported.[1] He responded quickly (see below) but exacerbation of the GVHD was seen after 5 days, the dose was repeated and he again responded (Figure 1). Following this patient, pediatric patients were treated with i.m. alefacept 15 mg/dose $\times$ 2/week and adult patients were treated with i.m. alefacept 30 mg/dose $\times$ 2/week.

The median time interval between SCT and GVHD appearance was 17 days (range 12–50 days) and median time interval from onset of GVHD to initiation of treatment was 20.5 days (median 5–62 days). GVHD grade at initiation of treatment and at peak ranged 2–4 (median 2.5) and 2–4 (median 4), respectively. System involvement at GVHD *peak* included skin ($n=7$), gastrointestinal tract ($n=5$) and liver ($n=3$). All patients responded to treatment with alefacept (Table 2). However, one patient with skin GVHD and two with gastrointestinal GVHD who featured an early partial response later developed exacerbation and CR was not achieved.

Skin GVHD responded rapidly with a median of 1 day to initial response (range 1–3, $n=8$) and 7 days to CR (range 1–30 days, $n=7$) (Figure 2). Median skin GVHD stage at completion of therapy was 0 (range 0–2). Intestinal response was slower with median 7.5 days to initial response (range 5–9, $n=4$). Of the four patients that achieved PR, CR was achieved in only one (40 days to CR). The other three patients continue with good PR (follow up 94, 68 and 36 days). The responding patient with gastrointestinal GVHD had a fluctuating course before achieving CR. Median gastrointestinal GVHD stage at completion of therapy was 2 (range 0–4). None of the patients had significant hepatic GVHD before treatment (but had such GVHD during alefacept treatment as mentioned above) so the role of alefacept on hepatic GVHD could not be clearly assessed.

No immediate alefacept related side effects were observed. None of the patients suffered from local complication at the site of injection (in spite of impaired immunity and low platelets count in some patients).

Later side effects included aspergillus sinusitis in one patient (UPN 1962), which was successfully treated with surgery and antifungal antibiotics. The same patient had another GVHD episode while off alefacept. He was

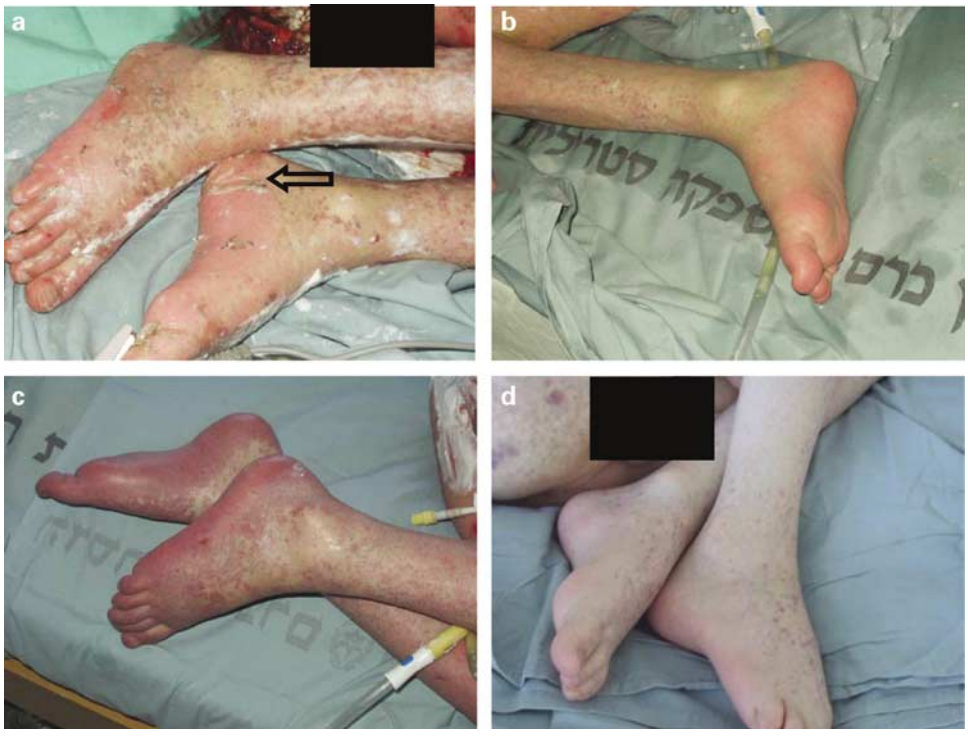

Figure 1 (**a**) Patient 1 (UPN 1962) at day +2 of alefacept treatment (7.12.04) with grade 4 GVHD of the skin including epidermolysis (arrow). (**b**) Same patient with almost complete epithelialization of the skin only mild GVHD activity at day +7 of alefacept treatment (12.12.04). (**c**) Exacerbation at day +9 of alefacept treatment (14.12.04) including markedly increased solar erythema. (**d**) Patient 1, day +19 of alefacept treatment in complete remission of grade 4 GVHD (26.12.04).

Table 2 GVHD response data

UPN	*GVHD involvement by system (grade) before alefacept treatment*	*Pre-alefacept steroid treatment*	*GVHD response to alefacept by system (days to CR)*	*Alefacept doses*	*Post-alefacept steroid treatment*
1962	First episode – skin (4) Second episode – skin (2)	I.v. 2 mg/kg MP	First episode – skin (13) Second episode – skin (2)	16	—
1924	Skin (2)	I.v. 2 mg/kg MP	No CR	4	I.v. 25 mg/kg MP
1770	Skin (3), GI (3)	I.v. 2 mg/kg MP	Skin (30), GI (40)	14	P.o. 2 mg/kg P
1976	Skin (1), GI (3)	I.v. 17 mg/kg MP	Skin (1), GI (no CR)	8	I.v. 2 mg/kg MP
1972	Skin (2)	P.o. 2 mg/kg P	Skin (7)	5	P.o. 0.3 mg/kg P
1980	Skin (2), GI (2)	I.v. 2 mg/kg MP	Skin (4), GI (no CR)	6	I.v. 2 mg/kg MP
1974	Skin (4), GI (3)	I.v. 0.17 mg/kg Dexa	Skin (12), GI (no CR)	5	I.v. 0.33 mg/kg Dexa

Dexa = dexamethasone; GI = gastrointestinal; MP = methylprednisolone; P = prednisone.

retreated with alefacept and responded but developed pancytopenia. Both complications may be attributed to other concurrent medications and the pancytopenia subsided while on alefacept. One patient (UPN 1972) had Gram-negative pneumonia, staphylococcus bacteremia, pharyngeal thrush and hemorrhagic cystitis all treated on an outpatient basis. Three patients had CMV reactivation while on alefacept and were treated with i.v. ganciclovir; two patients developed CMV reactivation before alefacept treatment and two had no reactivation at all. Full-donor chimerism (100% donor cells and no residual host-type DNA) was present in all patients and was stable throughout the treatment period in 6/7 patients. One patient developed mixed chimerism while receiving alefacept, which returned to full-donor chimerism with tapering of immunosuppression.

Two patients died, one from TTP and one with neurological complications (including progressive loss of sensation, paralysis and blindness, which were present before treatment with alefacept). Both deaths occurred while off alefacept. No relapse was observed in this group of patients until reporting (median follow-up 72 days, range 36–149).

Discussion

One of the first immunological events in GVHD, which is the major obstacle of SCT, is activation of donor T cells which encounter host-derived alloantigen-presenting cells.[8] It is therefore logical that prevention and management of GVHD should focus on T-cell elimination or suppression of T-cell function, or preferably induction of specific

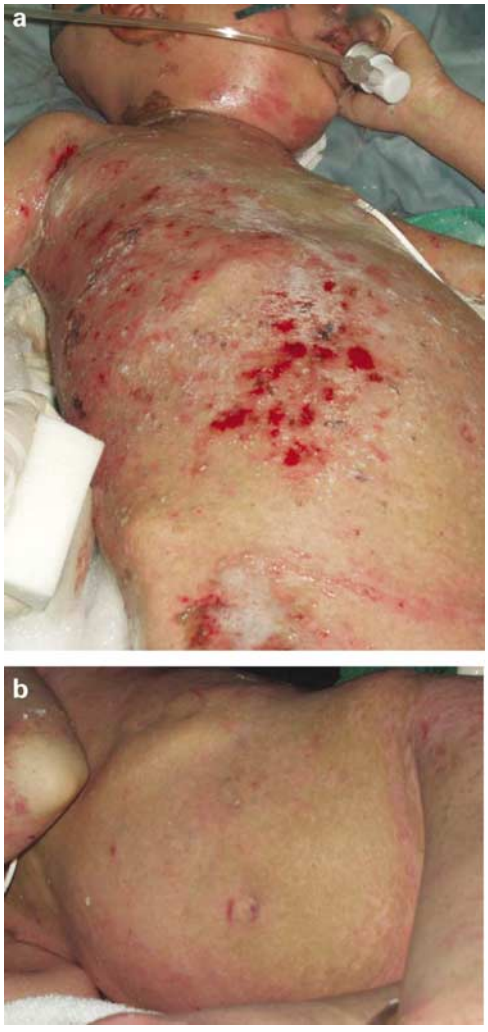

Figure 2 (**a**) Patient 1 (UPN 1962), day +2 of alefacept treatment (7.12.04) grade 4 GVHD of the skin. (**b**) Same patient, day +7 of alefacept treatment (12.12.04).

transplantation tolerance, provided that such treatment will not result in induction of unresponsiveness to putative tumor-specific or tumor-related antigens. The treatment of established GVHD consists of various immunosuppressive and immune-modulating drugs, including steroids, cyclosporine, methotrexate, antilymphocytic antibodies as well as an array of newer, potent immunosuppressive agents. In spite of all available modalities, not all patients achieve control of acute GVHD, which may rapidly lead to death.[3] Despite the use of innovative immunosuppressive modalities, the prognosis of steroid resistant GVHD is usually poor.[4] In view of failure of currently available anti-GVHD modalities, clinical application of monoclonal antibodies (MAbs) and chimeric proteins normally used for the treatment of T-cell-dependent autoimmunity is currently increasing.[9,10] Compounds used in the prophylaxis and treatment of GVHD include MAbs directed against CD3,[11] CD25,[12] CD52,[13] cytotoxic T-lymphocyte antigen (CTLA-4Ig), CD40 ligand or TNF-alpha.[14] Other agents such as IL-1 receptor antagonists did not prevent acute GVHD or improve survival.[15] Etanercept, a recombinant human soluble TNF receptor fusion protein, was anecdotally used in acute GVHD.[16]

Alefacept (Amevive) is an immunosuppressive dimeric fusion protein that consists of the extracellular CD2-binding portion of the human leucocyte function antigen-3 (LFA-3) linked to the Fc (hinge, C_H2 and C_H3 domains) portion of human IgG1. Alefacept is produced by recombinant DNA technology in a Chinese Hamster Ovary (CHO) mammalian cell expression system. It was shown to interfere with lymphocyte activation by specific binding to the lymphocyte antigen CD2, and inhibiting LFA-3/CD2 interaction. Alefacept was also shown to induce reduction in subsets of CD2+ T lymphocytes (primarily CD45RO+), presumably by bridging between CD2 on target lymphocytes and immunoglobulin Fc receptors on cytotoxic cells, such as natural killer cells.[17] Treatment of monkeys with cardiac allografts by alefacept was shown to prolong allograft survival.[18] It was also shown that alefacept treatment results in a reduction in circulating total CD4+ and CD8+ T-lymphocyte counts. In randomized, double-blind, once-weekly administration for 12 weeks (i.v. for study 1, i.m. for study 2) of placebo or alefacept in adults with chronic plaque psoriasis, the response to alefacept was significantly better.[19,20] In both studies, onset of response to alefacept treatment (defined as at least 50% reduction of baseline Psoriasis Area and Severity Index (PASI)) began 60 days after the initiation of therapy. I.m. administration of alefacept is well tolerated with similar adverse event rates in the placebo and active treatment groups. The most commonly reported adverse events in these studies included headache, pruritus, infection, rhinitis, injection site pain and injection site inflammation. Most of the headache and pruritus episodes were single events during the course of the study. Injection site reactions were typically classified as mild, were often restricted to single episodes per patient, and did not lead to discontinuation of therapy in any patient. The most significant laboratory abnormality with this medication is reduction of the CD4 count. However, no increases in infections have been seen in clinical trials of alefacept.

To our knowledge (by PubMed search), there is no previous report of the use of alefacept in GVHD. A phase II study of BTI-322, a rat monoclonal IgG2b directed against the CD2 antigen, in steroid-refractory acute GVHD that showed a total response rate of 55%,[21] supports of the rationale for our study.

We showed that a rapid response to alefacept could be seen within days even in grade 4 GVHD, especially in skin involvement. The outcome based on the first patient may suggest that dose-to-dose intervals should be shorter than the schedule used for the treatment of psoriasis. Our first patient, a 7-year-old boy had exfolliative grade 4 GVHD involving more than 75% of the body surface area (Figure 2a), failing all prior treatment prompted us to try the use of alefacept even at higher than the recommended dose for patients with psoriasis. He was therefore given 15 mg of alefacept i.m. (weighing 19.5 kg and body surface area of 0.85 m^2). The impressive and early effect (Figure 2b) justified doubling of the psoriasis recommended dose to the rest of the patients, which seemed rational due to the

severity and intensity of acute steroid-resistant GVHD as compared to psoriasis.

Comparing our initial results to the results of the randomized studies in the treatment of psoriasis,[1,2] the most striking difference is the short time to initial response and the CR observed. We speculate that this effect may be due in part to direct blockage of CD2 and LFA-3 on the one hand, and to the increased allogeneic NK cell activity[22] that in conjunction with the Fc end of the alefacept may induce cytotoxicity of alloreactive T cells, thus down-regulating GVHD activity.[17] Another possible theory to explain the rapid response against infiltrating T cells involved in GVHD may be related to neutralization of activated Fc positive dendritic cells and inflammatory genes as shown in psoriasis[23] especially combined with the other immunosuppressive agents given for GVHD. Although larger cohorts of patients with established GVHD require investigation for longer observation periods in well-controlled studies, we conclude based on our limited experience that alefacept may offer a significant nonharmful intervention in acute GVHD and further clinical studies are planned both in this setting and possibly using the agent for prevention of GVHD.

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