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Anti-LFA1 monoclonal antibody (25.3) for treatment of steroid-resistant grade III–IV acute graft-versus-host disease

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Abstract. The in vivo efficacy of 25.3 monoclonal antibody (mAb) directed against human LFA1 molecule was assessed in ten patients with steroid-resistant grade III–IV acute graft-versus-host disease (AGVHD). These patients received non-T-cell-depleted allogeneic bone marrow transplantation for aplastic anemia in two cases and hematologic malignancies in eight cases. Five grafts were fully matched, three were one antigen-mismatched, and two were two antigen-mismatched. Despite GVHD prophylaxis with cyclosporin A and short-term methotrexate, AGVHD occurred after a median of 24 days and clearly progressed under prednisone (median 2 mg/kg), given for a median of 12 days. 25.3 mAb was given at a dosage of 0.1 mg/kg in a 4-h perfusion for five daily doses without any clinical or biological side effects. Thirty percent of the patients experienced a reduction in the overall grading with two complete responses. Partial response in at least one involved organ (mostly skin) occurred in 80% of the patients. However, seven out of the eight responding patients experienced a new episode of AGVHD. This observation, which confirms that inhibiting a functional molecule is as efficient as a cytolytic therapy, offers an alternative strategy to antithymocyte globulin (ATG) and cytotoxic mAb in controlling steroid-resistant GVHD.

Key words: Anti-LFA1 monoclonal antibody, in GVHD – Monoclonal antibody, in GVHD – Steroid-resistant GVHD, monoclonal antibody – Graft-versus-host disease, steroid-resistant, monoclonal antibody

Allogeneic bone marrow transplantation (ABMT) is generally considered to be one of the most efficient treatments for severe hematologic disease. However, even in fully matched HLA donor-recipient pairs, acute graft-versus-host disease (AGVHD) remains a major cause of morbidity and mortality after BMT [3, 24, 28]. Postgraft immunosuppression with methotrexate (MTX) or cyclosporin (CyA) is effective in the prevention of AGVHD, the association leading to a reduction in the incidence from 50% to 30% [21, 23, 26, 27]. Despite this progress in AGVHD prevention, the treatment of established AGVHD remains problematic. When used singly, antithymocyte globulins (ATG) or corticosteroids can produce responses in 30%–50% of all patients receiving MTX prophylaxis, with moderate to severe AGVHD [4, 11–13, 25, 29]. The combination of both ATG and corticosteroids does not seem to be beneficial and has resulted in decreased survival, presumably because of excessive immunosuppression and associated complications. Nevertheless, 10%–30% of all patients still die from AGVHD [4]. Consequently, it has become crucial to develop and evaluate alternative approaches in the treatment of AGVHD. Murine monoclonal anti-T-cell antibodies have previously been reported showing varying efficiency and toxicity [2, 7–10, 14, 18, 19, 22].

We report here our clinical results in ten patients treated for steroid-resistant AGVHD with an antihuman leukocyte function antigen (HLFA1) monoclonal antibody (mAb) 25.3. Blocking the LFA1 adhesion molecule, this monoclonal antibody is highly efficient in inhibiting in vitro lymphocyte activation [5, 20] and has been shown to be active in vivo in preventing graft rejection, at least in pediatric patients, in the context of T-cell-depleted bone marrow transplantation [6, 16, 28, 29].

Patients and methods

The study group consisted of ten patients with a median age of 28 years (range 8–45 years); all of them were male. Two patients (nos. 1, 9) had aplastic anemia; three patients had acute leukemia (AL), two (nos. 5, 7) lymphoblastic leukemia (ALL) in relapse and one (no. 8) myeloblastic leukemia in relapse; two lymphoblastic lymphomas, one (no. 4) in first complete remission and one (no. 3) in second relapse; one refractory multiple myeloma (no. 10), and two chronic granulocytic leukemias, one (no. 6) in the chronic phase and one (no. 2) in the blastic phase.

The conditioning regimen consisted of cyclophosphamide (Cy) (60 mg/kg × 2 days), followed by thoracoabdominal irradiation

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Table 1. Clinical outcome after 25.3 therapy. AGVHD, Acute graft-versus-host disease; M, male; AA, aplastic anemia; CGL, chronic granulocytic leukemia; BP, blastic phase; CP, chronic phase; LL, lymphoplastic lymphoma; Rpse, relapse; CR, complete remission; ALL, acute lymphoblastic leukemia; MM, multiple myeloma; P, prednisone; IP, interstitial pneumonia; ATG, antithymocyte globulin

Patient no.	Age (years)	Sex	Diagnosis	HLA disparity	Onset of AGVHD (day)	Previous therapy (dose/duration)	Pre→post 25.3 AGVHD grading				Outcome
							Skin	Liver	Gut	Overall	
1	8	M	AA	none	10	P; 2 mg/kg × 5 days	1→0	3→0	1→0	III→0	Dead, day 220 from GVHD
2	45	M	CGL (BP)	B, Dr ≠	34	P; 2 mg/kg × 15 days	3→3	2→3	2→2	IV→IV	Dead, day 68 from GVHD + IP
3	30	M	LL 2nd Rpse	none	30	P; 2 mg/kg × 28 days	2→1	3→3	3→3	IV→IV	Dead, day 78 from GVHD + Aspergillosis
4	21	M	LL CR1	none	24	P; 2 mg/kg × 5 days	2→0	2→2	4→2	IV→II	Dead, day 65 from GVHD
5	25	M	ALL 1st Rpse	B, Dr ≠	15	P; 2 mg/kg × 11 days	4→0	2→3	2→4	IV→IV	Dead, day 40 from GVHD
6	37	M	CGL (CP)	Dr ≠	14	P; 2 mg/kg × 30 days; ATG × 5 days	4→0	0→0	1→1	III→0	Alive > 30 months with chronic GVHD
7	27	M	All 1st Rpse	none	40	P; 3 mg/kg × 30 days	2→0	3→4	0→0	III→III	Dead, day 150 from GVHD + IP
8	42	M	AML 1st Rpse	Dr ≠	20	P; 10 mg/kg × 2 days	3→3	3→3	4→4	IV→IV	Dead, day 30 from GVHD
9	19	M	AA	none	38	P; 3 mg/kg × 12 days	2→1	1→2	3→3	III→III	Dead, day 120 from GVHD + Aspergillosis
10	42	M	MM	Dr ≠	11	P; 2 mg/kg × 12 days	3→2	0→0	3→3	III→III	Dead, day 52 from GVHD + IP

(8 Gy single dose) for aplastic anemia (nos. 1, 9); in Cy (60 mg/kg × 2) and fractionated total body irradiation (TBI) for five patients (nos. 3, 4, 6, 7, 8); two patients (nos. 2, 5) received high-dose cytarabine associated with Cy-TBI. One patient (no. 10) received high-dose melphalan (140 mg/m^2) associated with CY-TBI. The total dose of TBI was 11 or 12 Gy, administered over 3–5 days with a low-dose rate of 3–4 cGy/min with pulmonary shielding after 7 Gy. All of the patients received non-T-cell-depleted bone marrow. Five grafts were fully matched, three (nos. 6, 8, 10) were one antigen-mismatched (DR locus), and two (nos. 2, 5) were two antigen-mismatched (loci B and DR).

All patients were managed in laminar air flow rooms with gastrointestinal decontamination and sterile alimentation. All blood products were irradiated at 15 Gy.

All patients received postgraft immunosuppression with short-term MTX and CyA, as previously described [6].

Monoclonal antibody (mAb) 25.3, a mouse IgG1 subclone, reacts with the high-molecular weight alpha chain of the HLFA1 molecule (anti-CD11a) [20]. This mAb is unable to fix complement but interferes in cell-cell adhesion, inhibits T-cell cytotoxicity, natural killer activity, and antibody-dependent cellular cytotoxicity in vitro [5, 20]. The IgG1 was purified from murine ascitis on protein A sepharose columns and diluted in sterile saline (Immunotech, Marseille, France). All of the preparations were checked for sterility and lack of pyrogenicity. The 25.3 mAb was given at a dosage of 0.1 mg/kg in a 4-h infusion for five daily doses. The sera of patient number 4 were collected after mAb infusion to dose mouse IgG by ELISA technique using a rat anti-mouse mAb. The minimal detection threshold was 10 ng/ml and correlated with the immunofluorescence cell binding assay on the HPB-ALL cell line. Patients were considered for 25.3 therapy for AGVHD grade III or IV as described by the Seattle criteria, namely:

Grade III: moderate to severe skin rash (score 2–3) with moderately severe gastrointestinal symptoms (score 2–3) (diarrhea > 1000 ml/day) or moderately severe liver function abnormalities (bilirubin > 50 μmol/l; score 2–3), moderate decrease in clinical performance.

Grade IV: severe skin rash (score 2–4) with occasional exfoliative skin lesions, severe gastrointestinal symptoms (diarrhea > 1500 ml/day; score 3–4) and severe liver function abnormalities (bilirubin > 100 μmol/l; score 3–4), and a pronounced decrease in clinical performance.

Protocols were approved by the ethics committees of the institutions, and informed consent was obtained from the patients.

Results

Criteria for 25.3 therapy

All patients received 25.3 therapy, while AGVHD was clearly progressing during steroid therapy; five patients had grade III and five grade IV GVHD. All of them had received previous therapy with prednisolone – median dosage 2 mg/kg (range 2–10 mg/kg) – for a median duration of 12 days (range 2–30 days). One patient (no. 6) had received five additional doses of ATG. CyA and prednisolone were continued through mAb therapy. The median onset of AGVHD was on day 24 (range day 10–day 40). AGVHD occurred earlier in mismatched patients (median day 13; range day 11–day 34) than in fully matched patients (median day 30; range day 11–day 40).

Pharmacokinetic data were available for one patient (no. 4) and showed that the peak level increased during the treatment (from 1200 ng/ml on day 1 to 2800 ng/ml on day 5) and that elimination was slowed after repeated infusions: trough levels 24 h after the infusions were 200 ng/ml on day 1 and 600 ng/ml on day 5.

Evaluation of AGVHD under 25.3 therapy (Table 1)

Three out of ten patients (nos. 1, 4, 6) showed improvement in the overall grading of AGVHD, with two complete responders (nos. 1, 6). Of these three patients, two (nos. 1, 4) experienced a new AGVHD progression within 9 and 11 days. On the other hand, partial responses were seen in eight out of ten patients. Skin involvement improved in eight patients; three patients (nos. 4, 5, 8) died rapidly without skin rash progression within 4, 8, and 10 days; four patients experienced secondary skin AGVHD progression within a median of 30 days (range 18–60 days). Liver involvement improved in one out of eight evaluable patients (no. 1) who experienced a new liver progression within 11 days. Gastrointestinal involvement improved in two out of nine evaluable patients but recurred within 9 days (no. 4) and 18 days (no. 1). Six patients (nos. 2, 3, 4, 5, 8, 10) died early after 25.3 therapy (median 6 days; range 1–30 days) with active AGVHD; three of them had clearly associated infections (two cytomegalovirus documented interstitial pneumonitis, one disseminated aspergillosis). Three patients (nos. 1, 7, 9) died from chronic GVHD and associated infections on days 120, 150, and 220. Only one patient (no. 6) is alive after 30 months with mild chronic GVHD.

Toxicity

Infusion of 25.3 mAb was perfectly well tolerated. No hematopoietic toxicity was observed. Leukocyte counts showed 2300 WBC/mm^3 (range 700–7800 WBC/mm^3) and 2350 WBC/mm^3 (range 1000–5000 WBC/mm^3) before and after treatment, respectively. Granulocyte/lymphocyte ratios were 1600/550 per mm^3 and 1700/350 per mm^3 before and after treatment, respectively. Seven out of the ten patients required platelet transfusions at the time of treatment. No significant decrease in platelet counts was observed in the other three patients who were not thrombocytopenic prior to 25.3 therapy.

Renal function was not significantly impaired with mean creatinine values of 100 Mmol/l (range 45–160) and 100 Mmol/l (range 50–300) before and after treatment, respectively. Liver function abnormalities were, in all cases, correlated with AGVHD.

Discussion

LFA1 is a protein present on the surface of T-lymphocytes, natural killer cells, polymorphonuclear cell leukocytes, and macrophages monocytes that helps the effector cell to bind closely to a target cell. 25.3 is a potent anti-LFA1 mAb that interferes with cell-cell interaction and inhibits in vitro T-lymphocyte activation, natural killer cell activity, antibody-dependent cell-mediated cytotoxicity, and mixed lymphocyte reactions [5, 20]. It has been reported that such a mAb has been used, with varying efficacy, to promote engraftment in T-cell-depleted BMT patients with inherited disorders or leukemia [6, 16, 17].

Results of this trial showed clear clinical reponses to anti-HLFA1 mAb 25.3 – without any hematologic or extra hematologic toxicity – of severe grade III–IV GVHD occurring under MTX and CyA prophylaxis, since we observed a diminution of overall grading (30%) in three out of ten patients, with two complete resolution of signs and symptoms. Improvement in at least one involved system occurred in eight out of ten patients. Responses were mostly observed in skin involvement (eight out of ten patients) without great benefit in liver or gastrointestinal involvement (one out of eight evaluable patients and two out of nine evaluable patients, respectively). Nevertheless, this efficiency was transient since seven out of the eight responding patients experienced a new GVHD progression and died.

The three clear responses seemed to be associated with a shorter time between AGVHD onset and mAb therapy (5 days; range 5–30 days) compared with the other patients (12 days, range 2–26 days). Two out of these three patients had received marrow from fully matched donors and one was mismatched on the DR locus. Only one out of these three patients is alive after 30 months; the other two died from AGVHD on day 65 and day 220, respectively.

We could not draw any definitive conclusions about the unique series of pharmacokinetic data, but the significant residual levels (range 200–600 ng/ml) did not suggest insufficient mAb doses.

No fully satisfying treatment has been reported to abrogate steroid-resistant established AGVHD. Overall, 30%–50% of all patients respond to treatment, mostly on skin involvement. Nevertheless, responses are transient and do not promote further tolerance in all cases. On the other hand, most of these reported therapies have been associated with significant, and sometimes unacceptable, toxicities [1, 7, 12–14, 18, 19, 22, 25, 29].

ATG raised from different animals has been reported to have a 50% response rate among patients receiving MTX prophylaxis when given as initial therapy, yet only 10%–20% of these patients have survived. Toxicity has mostly been hematologic with constant lymphocytopenia, frequent thrombocytopenia, and possible anaphylactic-like reactions and serum sickness manifestations [25, 29]. Randomized studies comparing ATG or methylprednisolone (MP) at conventional dosages for fully matched donor-recipient pairs with AGVHD under MTX prophylaxis have not shown any difference in response, new progression, or survival between the two therapies [13]. Subsequent attempts to treat AGVHD with higher doses of steroids (up to 10 mg/kg) have not increased the response rate but have been associated with a higher infection rate [1, 11, 12]. The combination of CyA and ATG has been reported to yield a 50% response rate when given initially among patients receiving MTX prophylaxis, with 67% survival beyond 6 months. The addition of steroids has not been beneficial and has resulted in decreased survival, probably due to excessive immunosuppression [4]. No published data are available at the present time about the efficiency of ATG and/or MP among patients receiving CyA and MTX, but fewer than 50% of the patients seem to respond to either therapy.

Thus, murine mAb treatment has been proposed for steroid-resistant AGVHD. Anti-CD3 mAb alone or associated with anti-CD2-CD5-CD6, given at escalating doses, has been reported to yield 45% improvement in at least one involved organ system (skin, liver, or gut) [15, 17]. The higher doses seem to correlate with the most complete responses. Infusions have been associated with anaphylactic-like reactions and transient disappearance in circulating T cells. However, responses have been transient since reactivation has often been observed. Although none of the patients has become immunized to murine immunoglobulin, this is probably a consequence of the severe immunodeficiency. The most striking complication has been the occurrence of fatal Epstein-Barr virus-related lymphomas in three patients [18]. Anti-CD5 mAb, coupled with ricin A chain – a specific cytotoxic agent – has been reported to yield a 30%–40% response rate in all of the involved organs without significant toxicities among patients with steroid-resistant AGVHD [2, 14]. Anti-CD8 mAb has recently been reported to have a 44% complete response rate among patients with AGVHD and to provide a significant number of long-term survivors (44% are alive after a median follow-up of 12 months; range 4–30 months) [8]. mAb BB 10 directed against the interleukin-2 receptor (anti-IL2R) has shown efficiency in 61% of all patients with severe AGVHD, steroid-resistant or not [9, 10].

The discrepancy between our results and these last two studies could be related to the differences in terms of AGVHD severity; no patient had grade II AGVHD in our series, as compared to about 40% grade II GVHD in the studies with anti-CD8 mAb or anti-IL2R mAb. Moreover, all of our patients were clearly progressing during steroid therapy.

Despite the fact that most of the responses to 25.3 were transient and incomplete, this therapy offers a response rate similar to ATG, high doses of steroids, or cytolytic monoclonal antibodies, but without the toxicity of these other strategies. This observation furthermore confirms that blocking a functional molecule (in this case, HLFA1) is as efficient as cytolysis in controlling severe immune disorders like AGVHD.

These preliminary results suggest that the use of higher dosages, earlier initiations, and longer durations of treatment should be examined in further clinical trials.

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