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Efficacy of anti-CD20 monoclonal antibodies (Mabthera) in patients with progressed hairy cell leukemia

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Background and Objectives. Recently, a chimeric monoclonal antibody (MoAb) directed against the CD20 antigen (rituximab) has been successfully introduced in the treatment of several CD20-positive B-cell neoplasias and particularly of follicular lymphomas. Based on these premises we evaluated the efficacy and the toxicity of chimeric anti-CD20 monoclonal antibody (MoAb) in relapsed/progressed hairy cell leukemia (HCL).

Design and Methods. Ten patients with relapsed/progressed HCL entered the study. Eight patients were males and two females with a median age of 55 years (range 41-78) and all of them had been previously treated with 2-chlorodeoxyadenosine and/or deoxycoformycin and α-interferon. Two out of 10 patients were anemic (Hb <10 g/dL), 4 thrombocytopenic (Plt $<100\times10^9$/L), 3 had fewer than 1.0×10^9/L neutrophils and 3 had circulating hairy cells (HC). All patients received 375 mg/m^2 i.v. of anti-CD20 MoAb once a week for 4 doses.

Results. All patients were evaluable for response, one patient showing a complete remission and 4 a partial response. Adverse reactions, such as fever, chills, bone pain, hypotension and thrombocytopenia, were transient and mild (grade 1-2) and occurred only during the first course of treatment. One month after the last infusion, patients who had had anemia, neutropenia or thrombocytopenia, recovered normal peripheral blood values. Circulating HC also disappeared within one month. Immunostained bone marrow biopsies were checked 1, 3 and 6 months after the end of therapy and in 5 out of 10 patients a >50% reduction of bone marrow HC infiltration was recorded.

Interpretation and Conclusions. On the basis of these preliminary results observed in 10 patients with progressed HCL, it appears that treatment with anti-CD20 MoAb is safe and effective in at least 50% of patients, particularly in those with a less evident bone marrow infiltration (≤ 50%) and in those previously splenectomized.

Key words: HCL, anti-CD20, rituximab, 2-CdA, DCF.

Hairy cell leukemia (HCL) is a chronic B-cell lymphoproliferative disorder characterized by splenomegaly and peripheral blood cytopenias. The diagnosis is based on distinctive hairy cell (HC) morphology, bone marrow (BM) histology and immunologic profile. In the last years the prognosis of patients with HCL has improved considerably following the use first of interferon-α (α-IFN)[1,2] and, more recently, of the purine analogs such as deoxycoformycin (DCF)[3-5] and 2-chlorodeoxyadenosine (2-CdA).[6,7] Although these molecules have been shown to be very effective in the treatment of patients with HCL, allowing higher complete remission (CR) rates, as well as a significant increase in the median survival, a considerable percentage of patients, ranging from 20 to 50%, tends to relapse and/or progress.

Recently, a chimeric monoclonal antibody (MoAb) directed against the CD20 antigen (rituximab) has been successfully introduced in the treatment of several CD20-positive B-cell neoplasias and particularly of follicular lymphomas.[8-10] On the basis of these encouraging results, coupled to the fact that HC express high levels of the CD20 receptor on their surface,[11] we have treated a series of progressed

HCL patients with chimeric anti-CD20 MoAb and here report the response rate and toxicity.

Design and Methods

Ten patients with progressed HCL who had been previously treated with 2-CdA or DCF and/or IFN and who showed a variable impairment of peripheral blood values were enrolled into this study after having given informed consent. Eight were males and 2 females with a median age of 55 years (range 41-78). Five had no palpable spleen and 2 had measurable splenomegaly, while the remaining 3 patients had been previously splenectomized. All patients were evaluable for clinical response. Table 1 illustrates the clinical characteristics of the patients.

The anti-CD20 MoAb (provided by Roche) was administered to each patient every week at a dose of 375 mg/m^2 iv for 4 consecutive doses. The first dose was infused at an initial rate of 50 mg/h and was then increased by 50 mg/h increments every 30 min, to a maximum of 400 mg/h. In the absence of important infusion-related toxicity, subsequent anti-CD20 MoAb infusions were administered at a rate of 100 mg/h and escalated by 100 mg/h increments at 30-min intervals. All patients were routinely premedicated with allopurinol for the prevention of tumor lysis syndrome and with oral acetaminophen and diprenydramine.

Complete blood counts with differentials and chemistry panels were performed before treatment and twice weekly during treatment. Thereafter, patients were monitored for the same parameters monthly for 6 months. Bone marrow biopsies were carried out before therapy, at 1, 3 and 6 months after therapy, and thereafter every 6 months. Samples were decalcified, embedded in paraffin and sections were prepared for routine histology (hematoxylin-eosin, Giemsa and Gomori staining) and for immunohistochemical studies with specific MoAb such as DBA44, CD45RA and CD20, as previously described.[7] Eight patients also had baseline abdominal ultrasonography.

Criteria for response were defined according to the consensus resolution, as previously described.[12] Briefly, CR was defined as the disappearance of HC from BM and peripheral blood, together with the regression of splenomegaly (when present) and a complete recovery of peripheral blood counts (Hb > 12 g/dL, PMN > 1.5×10^9/L, platelets > 100×10^9/L). In our analysis the absence of HC in the BM by immunostaining was an additional requirement for CR. Partial response (PR) was characterized by a decrease in BM infiltration of at least 50%, in addition to the normalization of peripheral blood values. Minor response (MR) was defined as restoration of at least one of the peripheral blood values, as indicated above.

Results

All 10 HCL patients who entered this study completed the planned treatment and were fully evaluable for clinical and hematologic response. As mentioned, all patients had been previously treated with at least 2 lines of therapy (Table 1). Responses were evaluated 1, 3 and 6 months after the

Table 1. Clinical and hematologic characteristics, response and outcome of 10 HCL patients treated with anti-CD20 MoAb.

Pts	*Age/Sex*	*Previous therapy*	*Hb (g/dL)*	*PMN (×10^9/L)*	*Plts (×10^9/L)*	*HC (×10^9/L)*	*BM HC %*	*Response§*	*Survival* (mos)*	*Current status*
#01	78/M	α-IFN, DCF	11.7 [12.6]°	0.9 [2.1]	90 [175]	0.06 [0]	30 [10]	PR	PR	Stable
#02	53/M	α-IFN, DCF, 2-CdA	11.1 [11.5]	0.8 [1.8]	101 [158]	0.05 [0]	75 [50]	MR	MR	Stable
#03	41/M	α-IFN, DCF	15.1 [14.3]	1.0 [1.0]	76 [66]	7.86 [1.4]	85 [80]	F	12	Progressed, treated with 2-CdA
#04	42/M	DCF, α-IFN	13.3 [13.2]	1.1 [1.2]	143 [165]	0	80 [80]	F	16	Progressed, treated with 2-CdA
#05	60/F	DCF, α-IFN	11.7 [11.1]	0.5 [1.3]	56 [70]	0	85 [70]	MR	17	Stable
#06	55/M	α-IFN, DCF, 2-CdA	9.4 [10.8]	2.4 [2.2]	184 [293]	0.07 [0]	80 [80]	MR	18	Stable
#07	51/M	α-IFN, DCF	11.2 [12.2]	1.0 [1.2]	157 [299]	0	80 [30]	PR	18	Stable
#08	60/M	2-CdA, DCF, α-IFN	10.9 [13.4]	1.5 [2.2]	190 [330]	0.18 [0]	30 [0]	CR	16	Stable
#09	73/M	α-IFN, 2CdA	16.6 [16.5]	0.9 [1.9]	84 [135]	0.06 [0]	50 [20]	PR	10	Stable
#10	42/M	α-IFN, DCF	12.4 [13.2]	0.8 [1.6]	98 [130]	0.13 [0]	70 [30]	PR	14	Stable

*§CR = complete remission; PR = partial remission; MR = minor response; F = failure. *From the end of therapy °In brackets hematologic results after therapy.*

completion of the anti-CD20 MoAb treatment and then every 6 months. Overall, 1 out of 10 treated patients achieved a CR and 4 a PR. Of the remaining 5 patients, 3 experienced a MR while the remaining 2 patients (#3 and #4) showed no improvement and both were retreated with a 5-day course of 2-CdA. All patients are alive and have been observed for 10 to 24 months (median 16 months) since the end of therapy; all patients who achieved a response, even minor, have so far maintained their response. One single patient, who had a marked leukemic picture (#3), failed to achieve any relevant or sustained response despite a significant but transient reduction of HC from 6.8 to 1.4×10^9/L at the end of the first infusion of anti-CD20 MoAb. Splenomegaly, when present (2 cases), as well as circulating HC, normalized within one month after the end of anti-CD20 MoAb administration. Clinical and hematologic responses occurred rapidly and, as reported in Table 1, 4 out of 6 patients with anemia (Hb ≤ 12 g/dL), 6 out of 7 with neutropenia (PMN ≤ 1.0×10^3/L) and 4 out of 6 with thrombocytopenia (Plt ≤ 100×10^9/L) had improved peripheral blood values within 3 months from the end of therapy, except for one case (#3) (Table 1). It is noteworthy that the best results were achieved in previously splenectomized patients and in those with less than 50% BM HC infiltration (Table 1), while the worse response was documented in the only patient who presented in leukemic phase and with a very high neoplastic mass (case #3).

Grade 1-2 fever, chills, hypotension and bone pain, together with a transient mono or pancytopenia, were observed in 6 out of 10 patients. Almost all the side-effects were infusion-related and of brief duration generally encountered during the first course of the anti-CD20 MoAb administration. No grade 3-4 toxicity was observed, and no infectious or hemorragic complications occurred.

Discussion

We report here that 5 out of 10 HCL patients who relapsed and/or progressed after 2 or more lines of therapy, and often with compromised hematologic conditions, experienced 1 CR and 4 PR after treatment with anti-CD20 MoAb without any major complication.

Despite the encouraging results achieved with the use of α-IFN and purine analogs, 20-30% of patients with HCL do not achieve CR with these agents and therefore tend to progress after a variable period of time.[13-16] New effective agents may thus prove very useful, mainly for neutropenic and immunocompromised HCL patients who have progressed after one or more courses of nucleoside derivatives.

The chimeric MoAb rituximab directed against the CD20 antigen which is expressed on the cell surface of most B-cell lymphoid neoplasias, has been recently employed in patients with follicular lymphomas and other chronic lymphoproliferative disorders.[8-10] The rationale for considering an immunotherapeutic approach with the anti-CD20 MoAb in the management of HCL patients, was based on the evidence that HC express high levels of CD20 antigen on their surface and also on the demonstration that a recombinant immunotoxin (LMB-2), composed of the Fv portion of the anti-Tac (anti-CD25) antibody, fused to a 38-kD truncated form of *Pseudomonas* exotoxin A, was particularly effective in 4 out of 4 HCL patients who progressed after conventional treatments with α-IFN and/or 2-CdA.[17] Therefore, the use of an effective and short-lasting treatment associated with a very low toxicity, as observed in patients with follicular non-Hodgkin's lymphomas,[8,9] appeared an attractive therapeutic option.

The preliminary results of our study on the role of anti-CD20 MoAb in the management of patients with progressed HCL appear promising since the majority of them had a sustained clinical and hematologic response with 50% of cases achieving a measurable response including one CR. In particular, it should be noted that the anti-CD20 administration produced an evident increase of the hemoglobin level in 4 out of 6 patients. An evident improvement in the neutrophil and platelet counts was observed also in almost 50% of cases and, more importantly, none of them experienced any infectious or hemorragic complications. The best results were achieved in previously splenectomized patients and in those with less (< 50%) HC BM infiltration. One single patient (#3) failed to achieve any relevant or sustained response following anti-CD20 MoAb therapy. In this typical HCL case, as witnessed also by the immunophenotypic profile ($CD103^+$, $CD25^+$, $FMC7^+$, $CD20^+$, CD5), despite a rapid and significant reduction in HC from 6.8 to 1.4×10^9/L, 72 hours after the end of the anti-CD20 MoAb infusion, HC rapidly rose to the pre-infusion values. This observation suggests that a large number of CD20 molecules on HC, together with a very high tumor burden, as in this case, may lead to rapid saturation and clearance of the antibody and to therapeutic failure.

Overall, anti-CD20 MoAb may represent an inter-

esting additional therapeutic option for HCL patients. In fact, the potential role of this immunotherapeutic approach has been further emphasized by the exciting results published by Kreitman *et al.*[18] during the editorial processing of our paper. These investigators reported 11 CR in 16 HCL patients resistant to purine analogs and treated with a recombinant immunotoxin (BL22) containing the variable portion of the anti-CD22 MoAb fused to a fragment of pseudomonas exotoxin. Therefore, on the basis of several studies, it is conceivable that in HCL, as in NHL,[19,20] immunotherapy with MoAb therapy may represent an additional therapeutic option both in relapse and after conventional therapy in order to reduce residual disease further.

In conclusion, confirming findings in a single recently reported case,[21] the anti-CD20 MoAb could be considered a safe and effective treatment option in a certain number of HCL patients. In view of its negligible toxicity, it may be considered particularly useful for the management of neutropenic and/or immunocompromised patients, as well as for patients with progressive disease who are very elderly and have a poor performance status.

Contributions and Acknowledgments

FL was the principal investigator, contributed to the conception of the study, its design and interpretation and wrote the paper: he should be considered as the principal author. ML, FF, SG, MLM, SM, and MT were involved in the recruitment of and day-to-day contact with patients and contributed to data handling. LA, DR, GM, MB, LB and PLZ contributed to the conception of the study, its design and were involved in the interpretation of results. RF contributed to the design of study, and was involved in the critical revision of the final version of the manuscript.

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Disclosures

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Potential implications for clinical practice

This therapeutic approach[22] may be considered in HCL patients with active disease in poor clinical conditions.

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