Importance of minimal residual disease in hairy cell leukemia: monoclonal antibodies as a therapeutic strategy

DEBORAH A. THOMAS, FARHAD RAVANDI, MICHAEL KEATING, & HAGOP M. KANTARJIAN

Department of Leukemia, University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Abstract

With the use of nucleoside analogs as frontline therapy, the prognosis of hairy cell leukemia (HCL) has improved dramatically. Unfortunately, disease recurrence remains problematic. Eradication of minimal residual disease (MRD) persisting after therapy may further improve outcome. The evolution of available techniques used to assess MRD, and the potential incorporation of novel agents such as monoclonal antibodies (MoAbs) into the treatment armamentarium for HCL mandate that MRD analyses be performed concurrently with routine assessments of disease status. Herein, the available data regarding the prevalence and clinical relevance of MRD after therapy for HCL is reviewed.

Keywords: *Minimal residual disease, rituximab, prognosis*

Introduction

Hairy cell leukemia (HCL) is a chronic B-lineage lymphoproliferative disorder which has a characteristic immunophenotype (expression of CD19, CD20, CD22, CD25, CD79a, CD11c, CD103, surface immunoglobulin [sIg]). The presence of these unique markers allows the use of flow cytometric (FC, sensitivity usually in range of 1 cell in 10 000) and immunohistochemical (IHC) techniques for detection of minimal residual disease (MRD) not readily discernable by routine microscopy. IHC staining of bone marrow biopsies (BMs) with anti-CD20 (lineage specific pan-B cell), DBA.44 (marks subsets of B-cells, hairy cells, and some B-cell lymphomas), PAX-5 [1] (B-cell follicles, B-lymphoid neoplasms) or CD79a antibodies facilitates identification of residual hairy cells in cases where complete remission (CR) as defined by conventional criteria has been achieved after treatment. The presence or absence of molecular disease after therapy can also be evaluated by assaying for immunoglobulin heavy chain (IgH) gene rearrangements via polymerase chain reaction (PCR) amplification, either using consensus V primers (usually for the framework

[FR] 1-3 regions of IgH, [cpPCR] with sensitivity ranging $1 \times 10^4 - 1 \times 10^5$) or more sensitive PCR techniques with clone specificity (sensitivity 1×10^6). Herein the prevalence and clinical relevance of detection of MRD after therapy for HCL is reviewed.

Comparison of techniques for the assessment of minimal residual disease in hairy cell leukemia

Ellison *et al.* [2] reported the use of IHC (L26 [CD20], DBA.44) on BMs to determine status of the disease at 3, 6, 12 and 24 months after therapy with cladribine (2-CdA) for HCL. A characteristic pattern of staining with L26 and/or DBA.44 was insufficient; presence of nuclear and cytoplasmic morphologic features of hairy cells was also required. A semi-quantitative assessment was used to classify BMs into categories: negative, indeterminate (IHC stains positive without morphological features), rare (>5 HCs meeting criteria), and positive (further categorized $<1\%$, $1-3\%$, $3-5\%$ or $>5\%$ of total cell population). Serial BMs were deemed positive for MRD by IHC an average of 50% of the cases assessed at the various time points, with the majority

in the <1% category. The MRD levels appeared stable over a 24-month observation period except in cases with higher proportion of MRD from the start.

Matutes *et al.* [3] studied the incidence of detectable MRD by FC in a cohort of patients with HCL ($n=23$) in CR after therapy with deoxycoformycin (DCF). Peripheral blood (PB) and BM samples collected at variable time points (median of 10 months) were assessed for expression of CD3, CD22, CD11c, CD25, HC2, and CD103. The incidence of detectable MRD either in the blood, marrow or both sites overall was 43%. Despite the long-term follow-up (median 72 months), no significant correlation was noted between the presence of MRD and subsequent relapse, owing to the usual limitations encountered when analyzing outcomes in a small number of patients.

Bengio *et al.* [4] compared the use of IHC (L26 [CD20], DBA.44) and FC (CD20, CD22, CD25, SIg, CD11c, CD103) to detect MRD after therapy with 2-CdA. The definition for positive MRD with IHC was 1–10% CD20/DBA44+ scattered or clustered cells with tricoleukocyte morphology, and with FC was any expression of CD11c/CD25/CD103 in the BM or >0.3% of the B-cells in PB. The MRD positivity rate for IHC was 46% compared with 64% for FC, suggesting that the latter was the more sensitive technique. MRD could not be correlated with relapse owing to the small cohort studied.

As discussed previously, PCR techniques offer another potential modality for the detection of MRD. Sausville *et al.* [5] performed a comparative analysis of PB multi-parameter FC (CD19, CD22, CD103, FMC7, CD23, CD19, CD20, CD11c, CD25, CD45, CD4, CD8, CD3, CD5, CD7, CD2) and cpPCR assays for the detection of MRD in previously treated HCL. FC was more sensitive for detection of disease because 31% of the cases positive by this method were negative by cpPCR. In contrast, only 1% of cases positive by cpPCR were negative by FC. Arons *et al.* [6] compared the incidence of MRD positivity by FC, cpPCR, and spPCR after therapy with the recombinant immunotoxin BL22 for previously treated HCL. The MRD positivity rates were 74%, 55%, and 98%, respectively. Quantitative levels of spPCR correlated with disease status. This report and the prior study of Sausville *et al.* [5] suggest that FC is clearly superior to cpPCR for the detection of MRD in HCL. Practically speaking, the use of spPCR may be most useful once negativity for MRD by FC has been established.

A surrogate marker of disease burden includes soluble serum interleukin-2 receptor (sIL-2R) levels [7,8]. Decreasing sIL-2R levels correlate with improvements in hematological parameters, reductions

in splenomegaly, and BM cytoreduction. The kinetics of sIL-2R levels vary with therapy; normalization occurs within 2–3 weeks of therapy with 2-CdA, within 4–6 months for DCF, and within 12 months for interferon- α (IFN- α). Increases in the levels of sIL-2R may precede manifestation of overt disease and could be assessed in conjunction with other MRD assessments in order to develop an algorithm for monitoring and intervention; no such prospective studies have been conducted to date.

Clinical relevance of minimal residual disease in hairy cell leukemia

The timing of marrow assessments may influence the interpretation of outcomes based on MRD status. Bastie *et al.* [9] assessed serial BMs by IHC staining with DBA.44 at 2–4, 6, 12–18, and 24 months after one course of standard 2-CdA therapy. The rate of MRD negativity (defined as <1% staining with DBA.44) improved from 57% at the 2-month assessment to 77% at the 6-month assessment, without significant changes thereafter, suggesting that it may take up to 6 months for the full effects of the nucleoside analog therapy to manifest. There was a correlation between DBA.44 immunostaining >5% on the 6-month marrow assessment and an increased incidence of relapse.

Wheaton *et al.* [10] assessed MRD via IHC staining (anti-CD20, DBA.44, anti-CD45-RO) of BMs after therapy with 2-CdA. Anti-CD45-RO was used to assay the T-cell population relative to cells that stained positive for CD20 or DBA.44, given prior observations that T-cells generally outnumber B-cells in nonneoplastic states. Hairy cell morphology had to be present in at least 50% of the cells that stained positive for CD20 or DBA.44. The incidence of detectable MRD by IHC at 3 months after therapy was lower than the Ellison *et al.* study [2], likely related to differences in the definition of MRD. Tallman *et al.* [11] then compared the outcomes by status of MRD as assessed by IHC in the cohort treated with 2-CdA ($n=39$) described in the Wheaton study [10] and a separate cohort treated with DCF ($n=27$). BM sampling varied by therapy owing to protocol stipulations: 3 months, then yearly after therapy with 2-CdA versus every 6 months after therapy with DCF. Relapse was defined as detection of HCL via H & E microscopy of BM. Findings are detailed in Table I. The prevalence of detectable MRD by IHC was similar between the two nucleoside analogs, although the BMs were collected at different time points. The 4-year relapse-free survival rate was significantly lower if MRD was detected (55% versus 88%, $p=0.0025$).

Summary of issues related to assessment of minimal residual disease in hairy cell leukemia

The prognostic implications of detectable MRD after therapy for HCL are unfortunately confounded by several factors: (1) variability in sensitivity of the various techniques used to assess MRD, with FC appearing to be the most informative and practical, (2) nonuniformity of the criteria used to define MRD, (3) variability in timing of MRD assessments after therapy, (4) limited numbers of patients in comparative groups, (5) limited duration of observation period in some series, and (6) variability in both detection of disease recurrence and time to retreatment owing to the relatively indolent nature of the disease. However, the preponderance of the data suggests that persistence of MRD after therapy with nucleoside analogs is predictive for eventual disease recurrence, further suggesting that interventions to eradicate MRD may improve outcome.

Monoclonal antibody therapy for eradication of minimal residual disease

Postremission consolidation therapy for MRD detected after achievement of CR by conventional criteria has been limited by the cumulative myelosuppressive and immunosuppressive effects of 2-CdA. The severe and prolonged CD4 lymphocytopenia can lead to opportunistic infections. Alternatively, persistence of MRD after treatment with DCF is unlikely to be eradicated by prolonged administration of this agent after achievement of CR. Alternative strategies to eradicate MRD include therapy with monoclonal antibodies (MoAbs) directed against antigens on the surface of HCs. The CD20 antigen is highly expressed on HCs (312×10^9 /L molecules per cell) compared with other leukemias (e.g. 65×10^9 /L molecules per cell

for chronic lymphocytic leukemia [CLL]) [12]. It is a human B lymphocyte-restricted differentiation phosphoprotein located on precursor and mature B lymphocytes in addition to indolent non-Hodgkin lymphomas (NHL) [13–15]. Upregulation of CD20 expression has been demonstrated with use of cytokines such as granulocyte-macrophage colony stimulating factor or IFN- α [16].

Rituximab is a high affinity chimeric IgG1 κ MoAb which contains murine light- and heavy-chain variable region sequences with human constant region sequences. It directly induces apoptosis in addition to complement- and antibody-mediated cellular cytotoxicity [14,17,18]. Initial clinical trials of standard dose rituximab in previously treated CLL were disappointing. The low response rates were attributed to the decreased expression of surface CD20 compared with low grade NHL. Clinical trials of dose escalated or dose intensive rituximab in relapsed or refractory CLL showed improvements in the response rates predominantly in the form of partial remissions [19,20]. However, when rituximab was administered in combination with chemotherapy such as fludarabine or fludarabine with cyclophosphamide, the outcome was significantly superior than with the chemotherapy alone [21,22].

Several clinical trials have been conducted using single agent rituximab as therapy for previously treated HCL with active disease (Table II) [23–27]. The possible reasons for the differential activity of rituximab observed in these clinical trials include differences in disease burden (more extensive marrow involvement was predictor for lower probability of response), dosing schema utilized (e.g. 4 *versus* 8 weeks of therapy), elevated soluble CD20 [28] levels resulting in antibody neutralization, and/or IgG FC- γ receptor polymorphisms [29]. Consideration should be given to include measurements of soluble CD20, rituximab, and CD20/rituximab complexes in the design of future clinical trials of this agent for HCL.

The use of rituximab to eradicate MRD after nucleoside analog therapy for HCL has been

Table I. MRD by IHC after 2-CdA or DCF [11].

	N (%)		p-value
	MRD	No MRD	
Incidence			
2-CdA (3 mos)	5/39 (13)	34/39 (87)	0.21
DCF (6 mos)	7/27 (26)	20/27 (74)	
Relapse rate [time of relapse, mos]			
2-CdA	2/5 (40) [24, 25]	3/24 (9) [NA]	NA
DCF	4/7 (57)	0/20 (0)	
	[18, 21, 44, 59]		
Overall	6/12 (50)	3/54 (6)	0.0025
% 4-year RFS	55 (± 15)	88 (± 6)	

MRD, minimal residual disease; IHC, immunohistochemistry; No, number; 2-CdA, cladribine; DCF, deoxycytosine; mos, months; NA, not available; RFS, relapse-free survival

Table II. Rituximab in relapsed/refractory hairy cell leukemia.

Study	N	No. of weeks*	CR (%)	PR (%)	OR (%)
Nieva <i>et al.</i> [23]	24	4	13	13	26
Hagberg and Lundholm [24]	11	4	55 [†]	10	64
Lauria <i>et al.</i> [25]	10	4	10	40	50
Angelopoulou <i>et al.</i> [26]	11	6	33	34	67
Thomas <i>et al.</i> [27]	15	8–12	66	13	80

No., number; CR, complete remission; PR, partial response; OR, overall response.

*Rituximab 375 mg/m² intravenously.

[†]CR rate 33% in 3 *de novo* HCL cases.

explored. Cervetti *et al.* [30] treated 10 HCL patients with standard dose rituximab for 4 weeks in an attempt to eradicate MRD detectable by cpPCR after one course of therapy with 2-CdA. Two patients were in CR, six had achieved PR, and two had failed to respond to therapy with 2-CdA. All patients with active disease achieved CR after rituximab, assessed within 2 months after therapy. Sequential assessments of cpPCR showed either a reduction in the levels or molecular negativity in several patients; in some cases this latter response was achieved as late as 12 months after completion of rituximab. A subsequent report included extended follow-up (median 36 months) of this cohort and inclusion of an additional 17 patients; durable complete molecular response rate was 70% after rituximab therapy (median of 4 months after 2-CdA, range 1.5–113 months) [31]. Two-year progression-free survival correlated with quality of response to rituximab therapy (94% for CR *versus* 50% for PR, $p < 0.001$) and MRD status (100% if negative *versus* 30% if positive, $p < 0.001$).

As discussed previously, several clinical trials conducted in CLL have exploited the *in vitro* synergy between rituximab and the nucleoside analogs with use of either sequential or concurrent regimens [21,22,32]. Ravandi *et al.* [33] reported the efficacy of 2-CdA 5.6 mg/m² by bolus infusion daily for 5 days followed by eight weekly doses of standard dose rituximab initiated on day 28 of the 2-CdA therapy. The CR rate was 100% in 13 patients with previously untreated or minimally treated (no more than one prior therapy) HCL. Eradication of MRD (assessed by four color multi-parameter FC and FR1-3 cpPCR) was achieved in all but two patients after completion of the sequential therapy. The study continues accrual. Clinical trials comparing the efficacy of concurrent *versus* sequential therapy with 2-CdA and rituximab have been designed.

Given the promising results observed with rituximab, alternative MoAbs (e.g. alemtuzumab, epratuzumab or ofatumumab) should be considered for incorporation into frontline therapy [34]. Alemtuzumab is a humanized rat IgG1 MoAb directed against the CD52 antigen, which is expressed in 90–100% of hairy cells [35]. Anecdotal activity has been reported [36]. Epratuzumab is a humanized murine IgG1 MoAb which targets the CD22 antigen; its efficacy and tolerance in NHL have been well established as a single agent and in combination with rituximab [37,38]. No clinical trials have been conducted in HCL to date. Ofatumumab is a fully humanized MoAb directed at a unique small loop epitope of CD20 with superior complement-dependent cytotoxicity compared with rituximab [39]. It has demonstrated promising activity in double refractory (fludarabine

and alemtuzumab) or bulky fludarabine refractory CLL and warrants further study in HCL [40].

Future directions

Therapeutic interventions toward a curative strategy will require a cooperative effort to develop prospective rationally designed clinical trials assessing MRD in a systematic fashion. The prognostic relevance of MRD in HCL can only be determined in the context of uniform definitions, methodology, and therapy. Clinical trials further exploring the potential for MoAbs to eradicate MRD and thereby improve disease outcomes in the setting of nucleoside analog therapy are underway.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Falini B, Mason DY. Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: clinical value of their detection by immunocytochemistry. *Blood* 2002;99:409–426.
2. Ellison DJ, Sharpe RW, Robbins BA, et al. Immunomorphologic analysis of bone marrow biopsies after treatment with 2-chlorodeoxyadenosine for hairy cell leukemia. *Blood* 1994;84:4310–4315.
3. Matutes E, Meeus P, McLennan K, et al. The significance of minimal residual disease in hairy cell leukaemia treated with deoxycoformycin: a long-term follow-up study. *Br J Haematol* 1997;98:375–383.
4. Bengio R, Narbaitz MI, Sarmiento MA, et al. Comparative analysis of immunophenotypic methods for the assessment of minimal residual disease in hairy cell leukemia. *Haematologica* 2000;85:1227–1229.
5. Sausville JE, Salloum RG, Sorbara L, et al. Minimal residual disease detection in hairy cell leukemia. Comparison of flow cytometric immunophenotyping with clonal analysis using consensus primer polymerase chain reaction for the heavy chain gene. *Am J Clin Pathol* 2003;119:213–217.
6. Arons E, Margulies I, Sorbara L, et al. Minimal residual disease in hairy cell leukemia patients assessed by clone-specific polymerase chain reaction. *Clin Cancer Res* 2006;12:2804–2811.
7. Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann Intern Med* 1990;113:619–627.
8. Arun B, Curti BD, Longo DL, et al. Elevations in serum soluble interleukin-2 receptor levels predict relapse in patients with hairy cell leukemia. *Cancer J Sci Am* 2000;6:21–24.
9. Bastie JN, Cazals-Hatem D, Daniel MT, et al. Five years follow-up after 2-chloro deoxyadenosine treatment in thirty patients with hairy cell leukemia: evaluation of minimal residual disease and CD4+ lymphocytopenia after treatment. *Leuk Lymphoma* 1999;35:555–565.
10. Wheaton S, Tallman MS, Hakimian D, et al. Minimal residual disease may predict bone marrow relapse in patients with hairy cell leukemia treated with 2-chlorodeoxyadenosine. *Blood* 1996;87:1556–1560.

11. Tallman MS, Hakimian D, Kopecky KJ, et al. Minimal residual disease in patients with hairy cell leukemia in complete remission treated with 2-chlorodeoxyadenosine or 2-deoxycoformycin and prediction of early relapse. *Clin Cancer Res* 1999;5:1665–1670.
12. Ginaldi L, De Martinis M, Matutes E, et al. Levels of expression of CD19 and CD20 in chronic B cell leukaemias. *J Clin Pathol* 1998;51:364–369.
13. Anderson KC, Bates MP, Slaughenhaupt BL, et al. Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell differentiation. *Blood* 1984;63:1424–1433.
14. Einfeld DA, Brown JP, Valentine MA, et al. Molecular cloning of the human B cell CD20 receptor predicts a hydrophobic protein with multiple transmembrane domains. *Embo J* 1988;7:711–717.
15. Valentine MA, Meier KE, Rossie S, et al. Phosphorylation of the CD20 phosphoprotein in resting B lymphocytes. Regulation by protein kinase C. *J Biol Chem* 1989;264:11282–11287.
16. Venugopal P, Sivaraman S, Huang XK, et al. Effects of cytokines on CD20 antigen expression on tumor cells from patients with chronic lymphocytic leukemia. *Leuk Res* 2000;24:411–415.
17. Tedder TF, Boyd AW, Freedman AS, et al. The B cell surface molecule B1 is functionally linked with B cell activation and differentiation. *J Immunol* 1985;135:973–979.
18. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells *in vivo* by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994;83:435–445.
19. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J Clin Oncol* 2001;19:2165–2170.
20. Byrd JC, Murphy T, Howard RS, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. *J Clin Oncol* 2001;19:2153–2164.
21. Byrd JC, Rai K, Peterson BL, et al. Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. *Blood* 2005;105:49–53.
22. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood* 2008;112:975–980.
23. Nieva J, Bethel K, Saven A. Phase 2 study of rituximab in the treatment of cladribine-failed patients with hairy cell leukemia. *Blood* 2003;102:810–813.
24. Hagberg H, Lundholm L. Rituximab, a chimaeric anti-CD20 monoclonal antibody, in the treatment of hairy cell leukaemia. *Br J Haematol* 2001;115:609–611.
25. Lauria F, Lenoci M, Annino L, et al. Efficacy of anti-CD20 monoclonal antibodies (Mabthera) in patients with progressed hairy cell leukemia. *Haematologica* 2001;86:1046–1050.
26. Angelopoulou MK, Pangalis GA, Sachanas S, et al. Outcome and toxicity in relapsed hairy cell leukemia patients treated with rituximab. *Leuk Lymphoma* 2008;49:1817–1820.
27. Thomas DA, O'Brien S, Bueso-Ramos C, et al. Rituximab in relapsed or refractory hairy cell leukemia. *Blood* 2003;102:3906–3911.
28. Giles FJ, Vose JM, Do KA, et al. Circulating CD20 and CD52 in patients with non-Hodgkin's lymphoma or Hodgkin's disease. *Br J Haematol* 2003;123:850–857.
29. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 2003;21:3940–3947.
30. Cervetti G, Galimberti S, Andreazzoli F, et al. Rituximab as treatment for minimal residual disease in hairy cell leukaemia. *Eur J Haematol* 2004;73:412–417.
31. Cervetti G, Galimberti S, Andreazzoli F, et al. Rituximab as treatment for minimal residual disease in hairy cell leukaemia: extended follow-up. *Br J Haematol* 2008;143:296–298.
32. Di Gaetano N, Xiao Y, Erba E, et al. Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line resistant to the cytotoxic activity of either drug alone. *Br J Haematol* 2001;114:800–809.
33. Ravandi F, Jorgensen JL, O'Brien SM, et al. Eradication of minimal residual disease in hairy cell leukemia. *Blood* 2006;107:4658–4662.
34. Thomas DA, Ravandi F, Kantarjian H. Monoclonal antibody therapy for hairy cell leukemia. *Hematol Oncol Clin North Am* 2006;20:1125–1136.
35. Quigley MM, Bethel KJ, Sharpe RW, et al. CD52 expression in hairy cell leukemia. *Am J Hematol* 2003;74:227–230.
36. Fietz T, Rieger K, Schmitt A, et al. Alemtuzumab (Campath 1H) in hairy cell leukaemia relapsing after rituximab treatment. *Hematol J* 2004;5:451–452.
37. Leonard JP, Coleman M, Ketas JC, et al. Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results. *Clin Cancer Res* 2004;10:5327–5334.
38. Leonard JP, Schuster SJ, Emmanouilides C, et al. Durable complete responses from therapy with combined epratuzumab and rituximab: final results from an international multicenter, phase 2 study in recurrent, indolent, non-Hodgkin lymphoma. *Cancer* 2008;113:2714–2723.
39. Castillo J, Milani C, Mendez-Allwood D. Ofatumumab, a second-generation anti-CD20 monoclonal antibody, for the treatment of lymphoproliferative and autoimmune disorders. *Expert Opin Investig Drugs* 2009;18:491–500.
40. Osterborg A, Kipps TJ, Mayer J, et al. Ofatumumab (HuMax-CD20), a novel CD20 monoclonal antibody, is an active treatment for patients with CLL refractory to both fludarabine and alemtuzumab or bulky fludarabine-refractory disease: results from the planned interim analysis of an international pivotal trial. *Blood (Suppl)* 2008;112(Abtract no. 328):126–127.