Multiple Myeloma

CD52 expression patterns in myeloma and the applicability of alemtuzumab therapy

Alemtuzumab has been proposed as a therapeutic agent in myeloma. CD52 was detected on plasma cells in 46/106 patients but levels were 30-fold lower than on alemtuzumab-responsive cells (n=138) and 8-fold lower than on alemtuzumab-resistant cells (n=57). The data suggest that myeloma plasma cells are unlikely to be depleted by alemtuzumab in most patients.

Haematologica 2006; 91:1577-1578

Alemtuzumab is a highly effective monoclonal antibody therapy for B-cell chronic lymphocytic leukemia (CLL).[1,2] There are several reports indicating that CD52 expression is detectable on myeloma plasma cells and that alemtuzumab therapy may be of value in myeloma.[3-5] However, the therapeutic efficacy of monoclonal antibodies is closely associated with the target expression level, as demonstrated by the modest efficacy of rituximab as a single agent in CLL.[6] The aim of this study was to determine the alemtuzumab-target antigen expression levels in patients with plasma cell disorders and in normal and neoplastic B-cells with known *in vivo* sensitivity to alemtuzumab therapy.

CD52 expression was assessed on plasma cells from patients with myeloma at presentation or relapse (n=106), monoclonal gammopathy of undetermined significance (MGUS, n=34) and from normal controls (n=19); on B cells from patients with CLL (n=91) and Waldenström's macroglobulinemia (WM, n=47) and control cases (lymphoma staging bone marrow samples with no evidence of involvement, n=27). In WM, CD52 was reported separately for plasma cells and B cells in which a discrete fraction of plasma cells was evident (20/47 cases).[7] CD52 expression was assessed as part of routine diagnostic procedures, using an in-house phycoerythrin conjugate of the murine anti-human CD52 antibody from which alemtuzumab was derived. Cells were prepared and analyzed as reported previously.[8,9] Populations were classified as being positive if over 20% of cells expressed CD52 above control (CD3) levels. Statistical analysis was performed using Stata v8.2 (Statacorp, Texas, USA). Differences in expression between groups were compared using the Mann-Whitney two-sample ranksum test. The proportion of normal and neoplastic B-lineage cells expressing CD52 above background and the relative CD52 expression levels are shown in Table 1. CLL and WM B cells are responsive *in vivo* to single-agent alemtuzumab therapy. These two disorders had the highest levels of CD52 expression of the neoplastic B-lineage cells tested, although the levels were slightly lower than those on normal B cells. Plasma cells in WM have a significantly lower level of CD52 expression, and these cells may persist throughout alemtuzumab therapy.[7] Similarly, B-progenitors show 3-fold lower levels of CD52 expression than do CLL/WM B cells, and are persistently present during alemtuzumab treatment in CLL patients even after complete depletion of neoplastic cells.[8]

All plasma cells showed much lower levels of CD52 expression than normal and neoplastic B cells. Plasma cell CD52 expression was detectable in 68% of normal controls (13/19), 50% of MGUS patients (17/34), and only 43% of myeloma patients (46/106). Expression was unimodal in all cases. There was lower expression of CD52 by myeloma plasma cells than by their normal counterparts (median 2.4-fold decrease) although this did not quite reach statistical significance (p=0.053). Myeloma plasma cell CD52 expression levels were approximately 8-fold lower than in cells that persisted during alemtuzumab monotherapy (WM plasma cells, n=20, 7.8-fold lower; normal B-progenitors, n=37, 7.5-fold lower, p<0.0001); and 30-fold lower than in cells that responded to alemtuzumab monotherapy (CLL, n=91, 28.1-fold lower; WM B-lymphocytes, n=47, 34.7-fold decrease, p<0.0001). There was a high degree of inter-patient variation of expression of CD52 on neoplastic plasma cells, but fewer than 10% of myeloma patients (7/106) had CD52 expression at a level similar to that on CLL cells. Approximately 70% of myeloma patients had plasma cells which expressed CD52 at a lower level than the lowest 5th percentile for B-progenitors and WM plasma cells (Figure 1).

These data support a broader application of alemtuzumab in Waldenström's macroglobulinemia, and promising activity has already been demonstrated in phase II trials for patients with relapsed/refractory disease.[7,10] However, as with rituximab therapy for Waldenström's macroglobulinemia, complete responses are rarely seen because the plasma cells and associated paraprotein secretion persist despite effective depletion of the B-cell component. Therefore, although generally applicable in Waldenström's macroglobulinemia, alemtuzumab therapy may not be appropriate for those patients whose primary symptoms relate to the parapro-

Table 1. Expression of CD52 by normal and neoplastic B-lineage cells.

Cell type	*Number of cases*	*Percentage CD52$^+$*	*Range*	*CD52 expression level*	*Range*
Normal plasma cells	19	38.8	3.1–86.5	86	21–240
MGUS plasma cells	34	21.7	3.1–96.0	70	20–1532
Myeloma plasma cells	106	12.1	0.5–98.3	36	11–1258
WM plasma cells	20	98	12.2–99.9	278	63–1557
Normal B-progenitors	27	98.7	27.1–100.0	256	54–864
B-CLL	91	99.9	99.2–100.0	1013	287–3178
WM B-lymphocytes	47	99.8	97.4–100.0	1243	312–3089
Normal B-lymphocytes	27	99.9	91.7–100.0	1773	589–4217

The percentage of cells expressing CD52 is defined as the percentage with higher CD52 fluorescence intensity than 99% of cells labeled with a phycoerythrin-conjugated anti-CD3 control antibody. The expression level equates to the CD52 geometric mean fluorescence intensity.

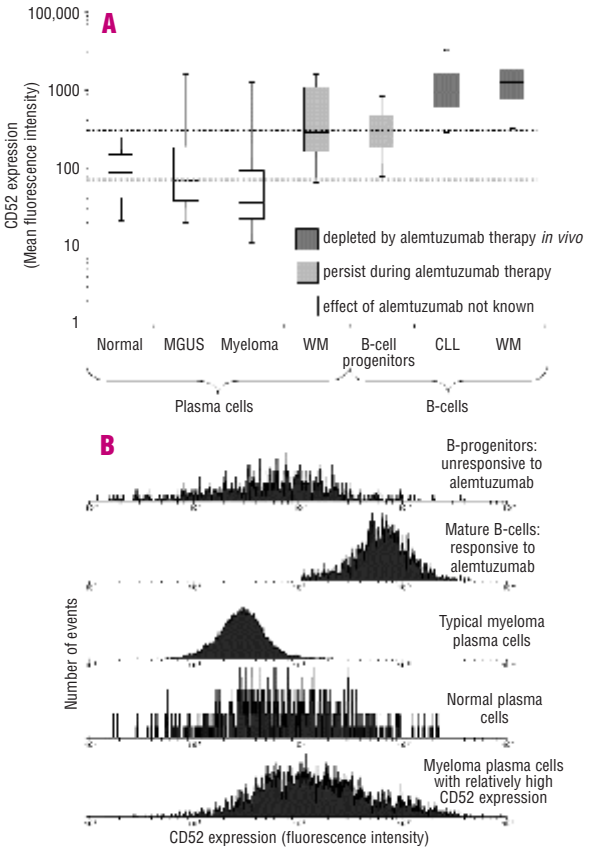

Figure 1. CD52 expression by normal and neoplastic plasma cells is significantly lower than that of cells known to be unresponsive to alemtuzumab therapy *in vivo*. Panel (A) shows the 5th, 25th, 50th, 75th and 95th percentiles for CD52 fluorescence intensity on different B-cell and plasma cell populations. The black dotted line indicates the lower limit of CD52 expression by cells that are depleted by alemtuzumab therapy *in vivo*, while the gray dotted line shows the lower limit of CD52 expression by cells that persist during alemtuzumab therapy. Panel (B) shows the CD52 expression pattern on individual B-cell populations. The typical myeloma plasma cell expression profile (middle histogram) is homogeneous with a weaker median than normal plasma cells and B-progenitors. Occasional atypical cases of myeloma (lowest histogram) show a more heterogeneous profile; although the median expression is higher, the majority of cells express CD52 at levels below those seen on cells that are unresponsive to alemtuzumab therapy.

tein, for example patients with hyperviscosity syndrome and autoimmune phenomena. Our data do not support the use of alemtuzumab therapy in myeloma because the level of CD52 expression of myeloma plasma cells is significantly lower than that of cells known to be resistant to alemtuzumab therapy. Alemtuzumab therapy may be of benefit in a small proportion of myeloma patients, but should only be considered in conjunction with comprehensive demonstration of strong CD52 expression.

Andy C. Rawstron, Giles Laycock-Brown,* Geoff Hale,° Faith E Davies,# Gareth J. Morgan,# J. Anthony Child,@ Peter Hillmen,* Roger G. Owen**

**HMDS, Algernon Firth Building, Leeds Teaching Hospitals NHS Trust, Leeds LS1 3EX, United Kingdom; °Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, United Kingdom; #The Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, United Kingdom; @The Academic Unit of Oncology and Haematology, The University of Leeds, Leeds LS2 9JT, United Kingdom.*

Funding: Research supported by the International Myeloma Foundation (UK).

Key words: CD52 expression, myeloma, Alemtuzumab.

Correspondence: Andy C. Rawstron, HMDS, Algernon Firth Building, Leeds General Infirmary, Leeds Teaching Hospitals, Leeds, LS1 3EX, United Kingdom.
E-mail: andy.rawstron@egu.york.ac.uk

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