Antibodies
AT10 bearing human IgG1 isotype and the N297Q mutation to reduce binding through the Fc region¹ were made in-house from published sequences and site-directed mutagenesis (Stratagene, UK). Mouse anti-human IgG, SB2H2² and rit m2a (Rituximab bearing mouse IgG2a)³ were produced and labelled in-house as previously described.

CLL prognostic markers
For CLL cells, mutation status of IgVH genes,⁴ CD38 expression,⁵ ZAP-70 expression⁶ and surface Ig expression⁷,⁸ were described previously.

FcγRIIa transfectants
FcγRII⁻ve Ramos cells were transfected using a recombinant expression plasmid (pcDNA3, Invitrogen) encoding human FcγRIIa and Amaxa Cell Line Nucleofector Kit (Lonza), solution T, programme 16. Transfected cells were cultured in supplemented RPMI media with selection in 1 μg/ml puromycin (Invitrogen).

RT-PCR
For RT-PCR, total RNA was isolated from purified cells and converted to cDNA (Invitrogen). PCR was performed using the cDNA and primers specific for human FcγRIIb1/2 or GAPDH as a control.

REFERENCES

Table S1. Demographic and clinical characteristics of MCL patients
The table describes the baseline characteristics and clinical responses of the MCL patients included in the study (Fig. 6).

<table>
<thead>
<tr>
<th></th>
<th>FcγRIIb− patients</th>
<th>FcγRIIb+ patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Median age, years (range)</strong></td>
<td>61 (51-84)</td>
<td>65.5 (52-82)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>2 females, 6 males</td>
<td>8 males</td>
</tr>
<tr>
<td><strong>Median ECOG performance status (range)</strong></td>
<td>0 (0-2)</td>
<td>1 (1-3)</td>
</tr>
<tr>
<td><strong>Median stage (range)</strong></td>
<td>4 (2-4)</td>
<td>4 (3-4)</td>
</tr>
<tr>
<td><strong>Median serum LDH, IU ml (range)</strong></td>
<td>465 (218-1889)</td>
<td>475 (114-1043)</td>
</tr>
<tr>
<td><strong>Median WBC, 10⁹/L (range)</strong></td>
<td>5.2 (1.6-9.5)</td>
<td>16.3 (1.4-209.8)</td>
</tr>
<tr>
<td><strong>Median MIPI score (range)</strong></td>
<td>4 (3-7)</td>
<td>5 (3-8)</td>
</tr>
<tr>
<td><strong>Total number of lines of therapy, range</strong></td>
<td>1-3</td>
<td>1-3</td>
</tr>
<tr>
<td><strong>Median line of therapy compared (range)</strong></td>
<td>1(1-3)</td>
<td>1(1-3)</td>
</tr>
<tr>
<td><strong>Quality of responses</strong></td>
<td>6 complete remission, 1 partial remission, 1 progressive disease</td>
<td>2 complete remission, 1 partial remission, 1 stable disease, 3 progressive disease, 1 died before formal assessment</td>
</tr>
<tr>
<td><strong>Median PFS, days (range)</strong></td>
<td>609 (36-2287)</td>
<td>144 (0-336)</td>
</tr>
<tr>
<td><strong>Median follow-up, days (range)</strong></td>
<td>1049.5 (234-2714)</td>
<td>904.5 (175-2546)</td>
</tr>
</tbody>
</table>
Figure S1. Correlation between phenotypic and prognostic markers and rituximab internalization in CLL

(A) The extent of internalization of rituximab was obtained by treating cells with Ritux-488 for 6 h followed by the quenching assay. Surface accessible anti-CD20 of the CLL samples was compared with IgVH mutational status, Zap-70, CD38 expression, slg isotype, the ability of cells to elicit calcium flux and viability (ns). (B) As in (A), internalization was compared with CD20 expression levels. A weak correlation was seen ($r$ value -.34, $P < .038$). Further multivariate regression analysis of CD20 and FcγRIIb expression against internalization showed that the weak correlation with CD20 was not significant. (C) slg expression of IgM$^-$ CLL cases showed no correlation with internalization of rituximab (ns). All data was analyzed by Mann-Whitney test or Spearman’s correlation analysis. (D) CD38-expressing CLL cases were cultured with Rit m2a-488 (mouse IgG2a) for 2 h and the quenching assay performed. The dot plots show variability in CD38 expression within the same CLL sample. Pre- (left) and post-fluorescence quenching (right) dot plots are shown. The corresponding histograms show CD20 expression in CD38$^+$ (solid peaks) and CD38$^-$ (hollow peaks) in the same pre- and post-quench samples. The experiment was repeated 3 times.

Figure S2. Characterization of FcγRIIb isoforms on human B cells

(A) Ramos FcγRIIib2 transfectants, purified normal B cells from healthy volunteers and CLL were examined by RT-PCR as described in “Supplemental methods”. mRNA levels of normal B cells and CLL cells from 3 different individuals are shown. The proportion of IIb1 and IIb2 expression appears dependent on the type of B cell. Normal B cells tended to express more IIb1 whereas CLL cells expressed more IIb2. (B) Surface FcγRII expression on Ramos vector control, FcγRIIa and FcγRIIb transfectants were examined by indirect staining. The cells were untreated or treated with 10 $\mu$g/ml AT10 hu IgG1 (N297Q) for 30 mins, then washed twice and treated with 10 $\mu$g/ml anti-human IgG-FITC (SB2H2). After a further wash, the samples were analyzed by flow cytometry. The lines represented are: gray filled (untreated Ramos cells), black line (Ramos vector control), red line (Ramos CD32b transfectants) and blue line (Ramos CD32a transfectants). No FcγRII was detected on the cell surface of Ramos vector control cells.

Figure S3. Activation of FcγRIIb by type I anti-CD20 mAb in CLL cells

CLL cells were stimulation with tositumomab, or rituximab ± AT10 (all 10 $\mu$g/ml) for 2 h and assessed for phosphorylated FcγRIIb by Western blotting.

Figure S4. Specificity of anti-human FcγRIIb mAb in IHC

FcγRIIa and FcγRIIb transfected Ramos cells were cytospun and paraffin-embedded and then stained for FcγRIIb expression by IHC as described in “methods”. IHC using mAb to human FcγRIIb demonstrated strong membrane staining in FcγRIIb but no membrane staining was seen in FcγRIIa-transfectants. Scale bar presents 50 $\mu$m.
Figure S1.

A

Surface accessible anti-CD20 (%)

IgVH\textsubscript{mutated} IgVH\textsubscript{unmutated}

ns

Surface accessible anti-CD20 (%)

% Zap70 positivity

% CD38 positivity

Surface accessible anti-CD20 (%)

% Viable cells

Surface accessible anti-CD20 (%)

% Calcium Flux

B

Surface accessible anti-CD20 (%)

CD20 expression

C

Surface accessible anti-CD20 (%)

slg expression

D

CD38 expression

CD20 expression

Pre-quench Quenched

Pre-quench Quenched
Figure S2.

A

200 bp →

Ilb1 (226 bp)

Ilb2 (169 bp)

500 bp →

GAPDH (500 bp)

MW marker

Ramos ForRIB2

Purified normal B cells

CLL cells

B

Counts

Anti-human IgG-FITC
Figure S3.
Figure S4.