Augmented Antitumor Activity against B-Cell Lymphoma by a Combination of Monoclonal Antibodies Targeting TRAIL-R1 and CD20

Sreeram Maddipatla, Francisco J. Hernandez-Illizaliturri, Joy Knight, and Myron S. Czuczman

Abstract

Purpose: Mapatumumab and lexatumumab are fully humanized, high-affinity immunoglobulin G1 monoclonal antibodies (mAb) that target/activate the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2), respectively, triggering the extrinsic apoptotic pathway. Theoretically, synergistic antitumor activity should be observed by combining TRAIL-R mAbs with agents (e.g., rituximab) that activate the intrinsic apoptotic pathway.

Experimental Design: To this end, targeted antigen expression in a NHL-cell panel was evaluated by flow cytometry. NHL cells were exposed to mapatumumab or lexatumumab followed by rituximab, isotype, or RPMI. DNA synthesis was quantified by [3H]-thymidine incorporation assays. Induction of apoptosis was detected by flow-cytometric analysis. For antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC) studies, standardized 51Cr-release assays were done. We inoculated severe combined immunodeficiency (SCID) mice with Raji cells i.v. The animals then were treated with various combinations of rituximab, mapatumumab, lexatumumab, and isotype alone or in combination.

Results: In vitro exposure to mapatumumab resulted in significant apoptosis (30–50%) and decreased DNA synthesis in sensitive lymphoma cells. Mapatumumab/rituximab combination resulted in a significant inhibition of cell proliferation (90% reduction) when compared with mapatumumab (60% reduction) or rituximab (5% reduction). In vivo, the median survival time of animals treated with mapatumumab and rituximab was longer (not reached) than those treated with rituximab monotherapy [33 days (95% confidence interval, 29–37), log-rank test, P = 0.05].

Conclusions: Mapatumumab induces apoptosis, cell growth arrest, ADCC, and CMC. The combination of mapatumumab plus rituximab is more effective in controlling lymphoma growth in vivo than either antibody. Rituximab and mapatumumab warrant further evaluation against B-cell lymphoma.

Background

Targeted therapy was largely developed as a consequence of advances in our understanding of the molecular biology of B-cell lymphomas, which identified tumor-associated antigens and/or key regulatory pathways that govern the development and maintenance of a given neoplastic process. Targeted therapies were also developed in response to the challenge of optimizing antitumor activity while decreasing nonspecific toxicity. Monoclonal antibodies against tumor-associated antigens are an attractive form of targeted biological therapy against cancer cells. In contrast to most traditional chemotherapies, conjugated or unconjugated monoclonal antibodies (mAb) can induce significant antitumor activity with improved therapeutic indices (1).

Rituximab, a chimeric mAb targeting the CD20 antigen present on B-cell lymphocytes and most of B-cell lymphomas, is already a universal biological agent used in the treatment of B-cell neoplasms. Although combinations of rituximab plus chemotherapeutic agents lead to augmented antitumor activity, a significant number of patients show limited responses, difficulty tolerating rituximab-chemotherapy combination regimens, and/or relapse post-therapy. There is a dire need to further develop innovative, less toxic combination therapies exploring the use of two or more biological agents against B-cell lymphomas.

More than a decade ago, it was shown that the deregulation of apoptosis is a key step necessary for the development, maintenance, and progression of various subtypes of B-cell lymphomas. In normal B cells, programmed cell death or apoptosis is a vital process that is tightly regulated in situ by complex intracellular and extracellular signals (2).

Cytokines such as the tumor necrosis factor (TNF) family members are an example of extracellular molecules capable of regulating apoptosis because they bind to specific cell surface
receptors on the target cells and regulate cellular proliferation and differentiation (3). TNF is the classic member of the family of cytokines that interact with the corresponding set of receptors from the TNF receptor (TNF-R) family and function as important mediators of immunoregulation and the inflammatory response by regulating programmed cell death via the extrinsic and intrinsic apoptotic pathways (4, 5). The activation of apoptosis via death receptors (DR) seems to be preserved in various subtypes of B-cell lymphomas, making the targeting of tumor necrosis-factor-related apoptosis-inducing ligand (TRAIL) receptors (TRAIL-R) an appealing strategy in cancer therapeutics. TRAIL-Rs are part of the TNF-R gene superfamily and have a broad range of biological functions like regulation of cell death and survival (6). TRAIL-DRs have a homologous cytoplasmic sequence termed as the death domain, in addition to a cysteine-rich extracellular domain, which regulates whether these receptors activate or prevent apoptosis (7). To date, six members of the TNF family receptors with death domains have been identified and include TNF-R1 (DR1), Fas (DR2), Apo-3 (DR3), TRAIL-1 (DR4), TRAIL-2 (DR5), and DR6.

In addition to TRAIL, other TNF-R family ligands have been identified such as Fas/Apo1L or CD95L (binds to DR CD95), and lymphotixin-α (binds to TNF-R1), or Apo3 ligand or Apo3L or TWEAK (binds to DR3), each capable of inducing apoptosis in transformed cells and activated lymphocytes. Their therapeutic use has been limited due to their toxic effects on normal tissues (i.e., systemic inflammatory response syndrome, or hepatotoxicity) in vivo (5, 7, 8). A novel member in the TNF family was recently identified and designated TRAIL or Apo-2L (for Apo-2 ligand; refs. 4, 9). TRAIL is a type II membrane protein resembling Fas/Apo1L in amino acid sequence expressed in a variety of human tissues that is capable of inducing apoptosis by binding to its appropriate DR (9).

TRAIL, like several other TNF family members, forms an oligomeric structure that helps to cross-link and trimerize its receptors and transduce a signal to the target cell, resulting in apoptosis (4, 10).

On the other hand, there are other TRAIL-Rs that lack functional death domains (TRAIL-R3/DcR1, TRAIL-R4/DcR2, osteoprotein/OPG); these are known as decoy receptors, and their function has not been clearly established. Triggering these decoy receptors may protect cells from signaling via the death-domain–containing TRAIL-Rs (5, 7, 11–13). TRAIL induces apoptosis in a wider range of cancer cells (1, 13). Upon binding of TRAIL, there is aggregation of TRAIL-Rs on the cell surface, triggering the proximal signaling of cell death (13). TRAIL-induced apoptosis in cancer cells is carried out by the activation of both extrinsic and intrinsic intracellular death signaling pathways (14). TRAIL promotes caspase-8 and caspase-10 activation after binding to appropriate cognate receptors (TRAIL-R1 and TRAIL-R2), which, in turn, results in the downstream activation of caspase-3, caspase-6, and caspase-7 (13). In some of the cancer cell lines, Apo-2L/TRAIL–induced activation of caspase-3 is further augmented through the activation of the intrinsic apoptotic pathway (1, 11). In vivo antitumor activity has been published using various xenograft cancer models with TRAIL ligands (12–18). Alternatively, TRAIL–mediated apoptosis can be triggered by mAbs against TRAIL-R1 or TRAIL-R2. Mapatumumab and lexatumumab are two fully human mAbs that target and activate TRAIL-R1 and TRAIL-R2, respectively; both of these antibodies are currently undergoing preclinical and clinical evaluation (13, 19). Agonist mAbs can induce apoptosis in human cancer cell lines in vitro and in vivo in murine xenograft cancer models (12, 20, 21). The theoretical advantages of agonist mAb against DR over TRAIL ligand are both (a) the specificity for only functional TRAIL-Rs (and not decoy receptors) and (b) their longer half-life as compared with soluble TRAIL.

Similarly to TRAIL, agonist mAbs mapatumumab and lexatumumab are capable of inducing effective antilymphoma effects in vitro and in vivo (20). Various groups of investigators had shown that the activation of the TRAIL-Rs by either ligands or mAbs sensitize cancer cells to the effects of various chemotherapeutic and/or biological agents. Combining two biologically active and well-tolerated agents with different mechanisms of action, such as rituximab and agonist mAbs against DR, is a potentially attractive treatment strategy for patients with B-cell lymphoma. In this report, we study the effects of targeting B-cell lymphomas in vitro and in vivo with agonist DR humanized antibodies (mapatumumab/lexatumumab) alone and in combination with rituximab.

Materials and Methods

Culture medium and reagents. Cells were maintained in RPMI 1640 (Sigma Chemical) supplemented with 10% heat-inactivated (60°C, 45 min) fetal bovine serum (FBS, Atlanta Biologicals), 5 mmol/L HEPES, 100 units/mL penicillin, and 100 μg/mL of streptomycin (Invitrogen Corp.). Ficoll-Hypaque used to isolate peripheral blood mononuclear cells (PBMC) was purchased from Sigma Chemical. Sodium chromate51 (51Cr; Perkin-Elmer Life Inc.) and [3H]-thymidine radioisotopes (Perkin-Elmer Life Inc.) were used in functional assays assessing antibody-associated cytotoxicity and cell proliferation, respectively. Triton X-100 was purchased from Sigma Chemical.

Cell lines. The Raji and Ramos cell lines are well-characterized B-cell lymphoblastic cell lines (phenotype: CD20+, CD19+, CD22+) (13) derived from a patient with Burkitt’s lymphoma (obtained from the American Type Culture Collection). The SU-DHL-4 and SU-DHL-10 cell lines were derived from patients with diffuse large-cell B-cell lymphomas and immortalized by concomitant EBV infection and were a kind gift from Dr. Steven Treon (Dana-Farber Cancer Institute, Boston, MA). Experiments were conducted in four rituximab-sensitive cell lines Raji, SU-DHL4, SU-DHL-10, and Ramos cells.

All cell lines were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS (Life Technologies BRL), 1% L-glutamine, and penicillin/streptomycin in a humid environment of 37°C and 5% CO2. Antibodies and reagents. Mapatumumab (TRAIL-R1 mAb) and lexatumumab (TRAIL-R2 mAb) are fully human immunoglobulin G1 (IgG1) mAbs and were provided by Human Genome Sciences. Rituximab (IDEC/Genentech Inc.) was obtained from the Roswell Park Cancer Institute (RPCI) Pharmacy Department at a stock concentration of 10 mg/mL. Unless otherwise specified, mAbs were used at a final concentration of 10 μg/mL for in vitro studies. Antibodies were dosed at 10 mg/kg and diluted in sterile PBS for tail vein injection into the severe combined immunodeficiency (SCID) mice for in vivo studies. Trastuzumab (Herceptin, Genentech Inc.) was used as an isotype control at the same dose.

51Chromium (51Cr) was used for antibody-dependent cellular cytotoxicity and complement-mediated cytotoxicity assays. [3H]-Thymidine was used for the assessment of DNA synthesis and cell proliferation. PBMCs were obtained from healthy donors on an Institutional Review Board–approved protocol and isolated by Phycoell centrifugation and used at an effector/target ratio of 40:1. Posiled human serum was used as a source of complement for complement-mediated cytotoxicity (CMC).
assays. Annexin V detection kit (Oncogene Inc.), FITC-conjugated mouse anti-human TRAIL-R1 and TRAIL-R2 mAb were obtained from BD Biosciences (clone numbers) and used for flow-cytometric studies.

Animals. For the in vivo experiments, 6- to 8-week-old SCID mice were bred and maintained at the Department of Laboratory Animal Resources facility at RPCI. The experimental design was approved by the Institutional Animal Care and Use Committee at RPCI under protocol P966M. All animals were housed and maintained in laminar flow cabinets or microisolator units and provided with sterilized food and water. Our laboratory facility has been certified by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulation and standards of the U.S. Department of Agriculture and the U.S. Department of Health and Human Services.

Evaluation of apoptosis following in vitro exposure of NHL cell lines to HGS ETR1 (TRAIL-R1 mAb) or lexatumumab (TRAIL-R2 mAb) with/ without rituximab. In vitro effects of either mapatumumab (TRAIL-R1 mAb) or lexatumumab (TRAIL-R2 mAb) at a final concentration of 10 µg/mL as single agent or in combination with rituximab were evaluated by flow-cytometric analysis. All samples were analyzed by multicolor flow-cytometric analysis using a fluorescence-activated cell sorter/FACStar Plus (Becton Dickinson) flow cytometer. Cells were scored as apoptotic if they were Annexin-V positive and propidium iodine negative/positive (early and late apoptosis, respectively). The experiments were done in triplicate, and the results were given as mean values ± SE.

The antiproliferative effects of mapatumumab or lexatumumab alone or in combination with rituximab on NHL cell lines. Lymphoma cell lines were placed in 96-well plates (1 × 10^5 cells per well) and exposed to 10 µg/mL concentrations of mapatumumab (TRAIL-R1 mAb) or lexatumumab (TRAIL-R2 mAb), alone or in the presence of rituximab (10 µg/mL) or isotype (10 µg/mL). The final concentration was adjusted to 200 µL with 10% RPMI 1640. Cell lines were then incubated at 37°C and 5% CO_2 for 24 and 48 h. Following 24 or 48 h, 1 µCi per well of [³H]-thymidine (New England Nuclear Products) was added, and cells were incubated for 16 h more. Cells were then harvested using the Harvest system (Tomac Cell Harvester, Tomtec) into the 96-well glass filters (Wallac Inc.), and [³H]-thymidine uptake was measured using an automated scintillation counter (Wallace). Each experiment was done in triplicate at three different times; results are presented as the mean ± SD of counts per minute (cpm) at 24 and 48 h.

³¹Cr release assays to study the effects of rituximab in combination with anti–TRAIL-R antibodies mapatumumab and lexatumumab to induce antibody-dependent cellular cytotoxicity or CMC in vitro. NHL cells (5 × 10^5 cells) were labeled with [³¹Cr] for up to 2 h as previously described (22). Subsequently, [³¹Cr]-labeled cell suspensions [2 × 10^6 cells/mL for CMC assays and 1 × 10^5 cells/mL for antibody-dependent cellular cytotoxicity (ADCC)] were plated in 96-well plates. Labeled cells were then exposed to mapatumumab or lexatumumab mAb at 10 µg/mL for 2 h and subsequently incubated in the presence of rituximab (10 µg/mL) or isotype control mAb (10 µg/mL) and human serum at a 1:4 dilution (CMC) or PBMCs at an effector/target ratio of 40:1 (ADCC). After a 6-h period of incubation at 37°C and 5% CO_2, the supernatant was harvested, and [³¹Cr] release was measured by standard gamma counter. The percentage of lysis was calculated as follows: % Lysis = [test cpm - background cpm]/[maximum cpm - background cpm].

Correlation between in vitro effects of mapatumumab and lexatumumab with surface expression of TRAIL-R1 and TRAIL-R2. To correlate

![Fig. 1. Changes in DNA synthesis and cell proliferation following 24 and 48 h exposure to anti–TRAIL-R1 and anti–TRAIL-R2 humanized mAbs as determined by [³H]-thymidine incorporation. In vitro exposure of Ramos cell lines to HGS-ETR1 (mapatumumab) resulted in a significant decrease in DNA synthesis at 24 and 48 h (P < 0.05) when compared with isotype or untreated controls (A). Similar finding were observed in Raji cell lines exposed to HGS-ETR1 (B). No antitumor activity was observed in HGS-ETR-2 (lexatumumab)–treated cells.](image-url)
the in vitro activity of mapatumumab or lexatumumab, with or without rituximab, we determined baseline expression of CD20, TRAIL-R1, and TRAIL-R2 in all cell lines tested. Surface CD20, TRAIL-R1, and TRAIL-R2 expression were determined by flow cytometry using FITC-labeling using antigen-specific mAbs in each cell line.

In vivo effects of mapatumumab or lexatumumab alone or in combination with rituximab against human B-cell lymphoma-bearing xenografts. These studies were carried out using a disseminated human lymphoma-bearing SCID mouse xenograft model. Raji cells were harvested from confluent cultures, and only suspensions with >90% viable cells were used for animal inoculation as previously described (22). On day 0, 6- to 8-week-old SCID mice were inoculated with 1 x 10^6 Raji cells via tail vein injection. After 72 h (to allow tumor engraftment), the animals were divided into seven cohorts. The first cohort (group A) served as control and received no treatment. Groups B and C consisted on animals treated with either HGS ETR1 (TRAIL-R1 mAb) at 10 mg/kg or HGS ETR2 (TRAIL-R2 mAb) at 10 mg/kg given via tail vein injection on days +3, +5, +7, +9, +11, +13, +15, and +17. Groups D and E were treated with rituximab and trastuzumab (isotype control) monotherapy given via tail vein injection at 10 mg/kg on days +3, +5, +7, +9, +11, +13, +15, and +17. Groups F and H were treated with either of mapatumumab (TRAIL-R1 mAb) at 10 mg/kg or lexatumumab (TRAIL-R2 mAb) at 10 mg/kg on days +5, +9, +13, and +17, alternating with rituximab at 10 mg/kg given via tail vein injection on days +3, +7, +11, and +15. After completion of therapy, animals were observed for up to a 90-day time period. The end point of the study was survival defined as the time to development of limb paralysis. Animals that reached the end point or survived after 3 months of observation were sacrificed by cervical dislocation. Pathologic examination of all organs (e.g., liver, lung and brain) was done to detect any residual disease. The experiments were repeated on three separate occasions.

Statistical analysis. In vitro experiments were repeated in triplicates, and the results are reported as the mean with standard error (ste). Statistical differences between treatment groups and controls were determined by χ² testing. In addition, differences in survival between treatment groups were calculated using the Kaplan-Meier curves (SPSS 11.0 for Windows 2000 software). P values were calculated by log-rank test. Differences were considered significant when the P value was <0.05.

Results

In vitro exposure of NHL cells to mapatumumab results in a significant decrease in DNA synthesis. Antiproliferation activity was observed in Raji and Ramos cells exposed to mapatumumab for 24 and 48 h. In standard proliferation assays, mapatumumab was able to inhibit the growth of Ramos cells by 81% and 73% at 24 and 48 h, respectively. When compared with untreated cells, in vitro exposure to mapatumumab resulted in a significant decrease in DNA synthesis. Mean cpm decreased from 124,085 cpm ± 6,733 ste and 315,800 cpm ± 4,458 standard error (ste) in untreated cells at 24 and 48 h, respectively, to 23,650 cpm ± 2,931 ste and 85,932 cpm ± 897 ste in mapatumumab-exposed Ramos cells (P = 0.007 and P < 0.001), respectively (Fig. 1A).

Similar antitumor activity was observed in Raji cells where in vitro exposure to mapatumumab resulted in growth inhibition by 44% and 56% at 24 and 48 h, respectively. The mean cpm of HGS-ETR1–exposed Raji cells were 2,008 cpm ± 147 ste (24 h) and 3,739 ± 384 ste (48 h) and were lower than control exposed cells, at 3,582 cpm ± 189 ste (24 h) and...
8,477 ± 739 ste (48 h), \( P = 0.014 \) and \( P = 0.025 \), respectively (Fig. 1B). No significant antiproliferative effects were observed with lextatumumab in the cell lines tested (Fig. 1).

\textit{In vitro} exposure of B-cell lymphoma cell lines to rituximab did not result in significant changes in DNA synthesis at the time intervals studied. The combination of rituximab and mapatumumab or lextatumumab did not result in additive or synergistic antiproliferative effects when compared with cells exposed to mapatumumab or lextatumumab alone in the majority of cell lines tested (Fig. 2A). Of interest, the \textit{in vitro} exposure of Ramos cells to rituximab plus mapatumumab for 48 h resulted in the near-complete inhibition of DNA synthesis as compared with Ramos cells exposed to mapatumumab (\( P = 0.001 \)) or rituximab (\( P < 0.001 \)) alone (Fig. 2B).

\textbf{Exposure of NHL cell lines to mapatumumab or lextatumumab induces a variable degree of apoptosis.} We found a variable degree of apoptosis following the \textit{in vitro} exposure of NHL cell lines to mapatumumab, but not HGS-ETR-2, which is in accordance to previous reports demonstrating that binding of DR receptors triggers apoptosis (6, 7, 9–11) in cancer cells. Direct apoptosis was detected only in Ramos (56.2\% ± 4.43 ste) after \textit{in vitro} exposure to mapatumumab for 48 h as compared with untreated cells. On the other hand, \textit{in vitro} exposure to rituximab did not result in the induction of apoptosis at the same time interval (4.13 ± 3.06\%). Although no significant improvement in apoptosis was noted by combining mapatumumab or lextatumumab with rituximab, the exposure of Ramos cells to both rituximab and lextatumumab resulted in improved activity as compared with Ramos exposed to rituximab or lextatumumab (15.7\% ± 3.67 ste versus 4.13 ± 3.06\% versus 0\%, \( P = 0.056 \); Fig. 3).

\textbf{Mapatumumab activates the complement cascade and is capable of inducing effective CMC in various lymphoma cell lines.} Depending on their Fc region, certain subclasses of mAbs can effectively activate the classic complement pathway and induce cell cytotoxicity. Mapatumumab and lextatumumab have an IgG1 backbone and can potentially activate CMC. In the cell lines tested by our group, we found that mapatumumab is capable of inducing a variable degree of cell death by complement activation as determined by \(^{51}\text{Cr} \) release assays. \textit{In vitro} exposure of NHL cells to mapatumumab alone in the presence of human serum resulted in significant cell lysis in Ramos (mean ± SE, 71.47 ± 1.21\%) and SU-DHL4 (27.65 ± 0.92\%) and cell lines as compared with untreated cells.

Additive effects were seen when mapatumumab was combined with rituximab in some cell lines. \textit{In vitro} exposure of Raji cells to rituximab resulted in 27\% cell lysis. The \textit{in vitro} addition of mapatumumab to rituximab increased the CMC lysis to 40.3 ± 1.48\% (\( P = 0.047 \); Fig. 4). Rituximab, as a single agent, was very effective in inducing CMC in Ramos (94 ± 2.1\%) and SU-DHL-4 (75 ± 2.2\%) and masked any potential augmented effect induced by combining it with mapatumumab mAb (Fig. 4).

\textbf{Mapatumumab can induce ADCC in NHL cells and results in additive effects when combined with rituximab in vitro.} As shown by other investigators, ADCC is believed to be one of the most important \textit{in vivo} mechanisms of action of rituximab and other mAbs (23–25). We further investigated the capacity of mapatumumab and lextatumumab to induce ADCC in \textit{in vitro}, with or without rituximab, in a panel of lymphoma cell lines. \textit{In vitro} exposure to single agent mapatumumab, but not lextatumumab, resulted in a variable degree of ADCC (primarily in Ramos and Raji cell lines). Following the exposure of Ramos cells to mapatumumab, ADCC was observed in 55 ± 9.1\% of the cells. Although the addition of rituximab to mapatumumab increased the ADCC to 69 ± 20.4\%, this was not statistically significant when compared with single agent mapatumumab (\( P = 0.68 \); Fig. 5).

\textit{In vitro} exposure of Raji cells to mapatumumab resulted in minimal ADCC (6 ± 3.1\%). However, when combined with rituximab, the combination resulted in higher cell lysis (20.7 ± 2.4\%) than either rituximab (6.1 ± 2\%) or mapatumumab monotherapy alone (\( P = 0.046 \); Fig. 5).

\textbf{The \textit{in vitro} antitumor activity of ETR-1 does not correlate with TRAIL-R1 antigen density expression on NHL cells.} It is a commonly accepted observation that there exists a direct correlation between the amount of antigen expression on a cancer cell and the degree of biological activity of its associated mAb. In the present work, we aimed to correlate the \textit{in vitro} responses to mapatumumab or lextatumumab (with or without rituximab) to the surface antigen expression of TRAIL-R1, TRAIL-R2, and CD20 antigen, respectively. The panel of cell lines studied expressed a similar degree of CD20, TRAIL-R1, and TRAIL-R2 antigens. No correlation was observed between sensitive cells and insensitive cells \textit{in vitro} to a given antibody given either alone or in combination versus antigen expression (data not shown). Our data suggest that the biological responsiveness of sensitive cells \textit{in vitro} to mapatumumab is independent to the TRAIL-R1 antigen density. Alternate mechanisms of action may explain differences in tumor response to therapy.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Induction of apoptosis following \textit{in vitro} exposure to HGS-ETR1 with or without rituximab or trastuzumab (isoype control). Ramos cells were exposed to HGS-ETR1 (mapatumumab) or HGS-ETR2 (lextatumumab) 10 \( \mu \)g/mL followed by either rituximab, isotype (10 \( \mu \)g/mL), or media. Induction of apoptosis was detected by multiparameter flow-cytometric analysis (FITC-Annexin V/P1 staining) after 48 h of incubation. HGS-ETR1 (mapatumumab) induced significant apoptosis in Ramos cells (56.2\% ± 4.43 ste) when compared with rituximab (4.13\% ± 3 ste) alone (\( P = 0.006 \)). The addition of rituximab to mapatumumab did not increase the percent of apoptotic cells (58.47\% ± 7.36 ste, \( P = 0.552 \)). The exposure of Ramos cells to both rituximab and HGS-ETR2 (15.7\% ± 3.67 ste, apoptosis) resulted in additive antitumor activity when compared with Ramos cells exposed to HGS-ETR2 alone (\( P = 0.056 \)).}
\end{figure}
responses between the cell lines tested; one possibility could be differences in heterodimerization with other TNF-R family members upon TRAIL-R1 binding by mapatumumab.

In vivo effects of anti–TRAIL-receptor antibodies in combination with rituximab: concurrent administration of mapatumumab with rituximab is more effective in controlling lymphoma growth and in prolonging survival than rituximab or mapatumumab monotherapy alone. Treatment of human lymphoma-bearing SCID mice with rituximab resulted in prolongation in survival as compared with placebo-treated controls. The median survival time for rituximab-treated animals was 33 days (95% CI, 29-37) as compared with a median survival of 21 days (95% CI, 20-22) for those animals receiving placebo (log-rank test, \( P = 0.002 \)). No significant antitumor activity was observed in animals treated with lexatumumab alone, and the median survival (95% CI, 20-22) was similar to control mice for lexatumumab treated (Fig. 6A and B). On the other hand, mapatumumab seems to be able to better control lymphoma growth as a single agent as compared with placebo controls. The median survival for mapatumumab treated animals was longer (26 days; 95% CI, 22-30) than placebo-treated animals, but did not reach statistical significance (\( P = 0.23 \)).

The administration of alternating doses of rituximab in combination with mapatumumab for a total of eight doses resulted in the most effective antitumor activity and prolongation of survival of human lymphoma-bearing SCID mice. Statistically, significant differences were observed between animals treated with rituximab versus mapatumumab plus rituximab (Fig. 6A and B). The median survival time of animals treated with mapatumumab and rituximab was longer (not reached after 90 days) than those treated with rituximab monotherapy alone [median survival of 33 days (95% CI, 29-37); log-rank test, \( P = 0.05 \) (Fig. 6A and B)]. Contrary to what was observed in animals treated with rituximab plus mapatumumab, lexatumumab when combined with rituximab did not improve the survival of human lymphoma-bearing SCID mice. Animals receiving rituximab plus lexatumumab had a median survival that was not different from those animals treated with rituximab monotherapy [49 days (95% CI, 17-81) versus 33 days (95% CI, 29-37)]. Despite a trend toward an improved survival, differences between rituximab-treated animals and those receiving the concurrent administration of rituximab plus lexatumumab did not reach statistical significance (log-rank \( P = 0.887 \); Fig. 6A and B).

After a follow-up period of 3 months, survival rates were the highest for animals treated with rituximab plus mapatumumab (60%) when compared with animals treated with rituximab alone (40%) or combination of rituximab plus lexatumumab (26.7%). Pathologic examination of surviving animals sacrificed at the end of the study failed to show any residual disease.

**Discussion**

Novel combination treatment strategies that attempt to maximize rituximab activity while minimizing nonspecific toxicity is a major focus of current preclinical and clinical lymphoma research (26–30). Integrating novel and active biological agents, such as mapatumumab, may potentially increase the treatment options for many lymphoma patients. In our current work, we evaluated the biological activity of two...
fully human mAbs targeting TRAIL-Rs alone or in combination with rituximab against a panel of various B-cell lymphoma cell lines.

As has been shown by other investigators, the DR pathway is intact and functional in various types of cancers, including B-cell lymphomas (19, 31). Moreover, binding and subsequent activation of the TNF death family receptors by either ligands or antibodies result in significant apoptosis and antitumor activity in a variety of solid tumor malignancies (5, 9, 12–15, 17–20). As shown by our data, mapatumumab, but not lexatumumab, is active and capable of inducing cell growth inhibition and apoptosis in NHL cell lines and xenografts. In addition, mapatumumab can activate the innate immune system and is capable of activating ADCC and CMC in sensitive B-cell lymphoma cells.

Of interest, the degree of antitumor activity of mapatumumab in vitro did not correlate with TRAIL-R1 antigen density. A plausible explanation for our results could be differences in the heterodimerization between various B-cell lymphoma cells upon TRAIL-R1 binding to mapatumumab. It has been published that following in vitro exposure to TRAIL ligand or mAbs targeting TRAIL-R1, there is recruitment by trimerization of other DRs, which results in signaling transduction and apoptosis (13). It is feasible that responses to mapatumumab are dependent on the type of DRs undergoing trimerization upon TRAIL-R1 binding rather than the degree of antigen density. The same principle could explain the absence of
antitumor activity of lexatumumab despite ample surface expression of TRAIL-R2 antigen in the cell lines tested. Additional studies are needed to further address such hypothesis.

A phase II clinical study with mapatumumab was recently reported in patients with relapsed/refractory B-cell lymphomas. Patients included on the study had relapsed/refractory NHL of any histologic subtype and were heavily pretreated. Mapatumumab was administered at two dose levels: 3 mg/kg (8 subjects) or 10 mg/kg (32 subjects) every 21 days in the absence of disease progression or prohibitive toxicity for up to six cycles. Preliminary reports on the efficacy and toxicity showed that mapatumumab was well tolerated with patients experiencing primarily grade 1 or 2 toxicities. In addition, objective antitumor responses were shown in a subset of patients, all with follicular histologies. Moreover, 30% of all patients had disease stabilization following mapatumumab therapy (32).

Because mapatumumab has activity in NHL and can elicit antitumor activity against B-cell lymphomas by different mechanisms of action than rituximab, we further studied its interactions with rituximab in the preclinical setting. Rituximab has been shown to be effective in activating the complement cascade via the classic pathway and in recruiting effector cells (e.g., neutrophils and natural killer cells) into the tumor bed, inducing ADCC (22–25). In addition, in vitro exposure of lymphoma cells to rituximab induces programmed cell death via the intrinsic apoptotic pathway. On the other hand, mapatumumab can trigger apoptosis by caspase-8 activation via the extrinsic apoptotic pathway. An earlier study (19) that showed resistance to mapatumumab and lexatumumab in lymphoma cell lines is due to lack of Bid expression, suggesting that the activation of intrinsic caspase pathway may be required for the amplification of the apoptotic effect. Based on these findings, we decided to study potential biological interactions between mapatumumab or lexatumumab and rituximab.

We believe that we are the first group to report on the augmented antilymphoma activity from the combination of rituximab plus mapatumumab in vivo and, to a lesser degree, in vitro. In our animal studies, the survival of lymphoma-bearing SCID mice was longest in animals receiving rituximab plus mapatumumab, as compared with animals receiving rituximab, mapatumumab, or isotype monotherapy alone. Because all the animals received a total of eight doses of antibody, either single agent or in combination, the prolongation in survival most likely is the result of biological interactions between two mAbs rather than excess activity from a single biologically active agent.

To a lesser degree, we found that mapatumumab, when combined with rituximab, results in additive effects with regard to cell growth inhibition and activation of effective ADCC and CMC in sensitive B-cell lymphoma cell lines. Because the mechanisms of action of each mAb tested (rituximab and mapatumumab) is diverse, in vitro testing using standardized immunologic (i.e., ADCC or CMC assays), apoptosis, and/or proliferation assays may largely underestimate the antitumor interactions between rituximab and mapatumumab. As a result of such limitations, in vivo animal studies may more accurately reflect and predict what may occur in clinical studies. Our findings support future evaluation of this unique combination (i.e., mapatumumab and rituximab) in clinical trials of patients with CD-20-positive, B-cell lymphomas.

References
7. Vaux DL, Cory S, Adams JM. Bcl-2 genepromotes


Augmented Antitumor Activity against B-Cell Lymphoma by a Combination of Monoclonal Antibodies Targeting TRAIL-R1 and CD20

Sreeram Maddipatla, Francisco J. Hernandez-Ilizaliturri, Joy Knight, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/13/15/4556

Cited articles
This article cites 32 articles, 11 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/13/15/4556.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/13/15/4556.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.