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CMC-544 (inotuzumab ozogamicin): A CD22-targeted immunoconjugate of calicheamicin



CMC-544 (Inotuzumab ozogamicin) is a CD22-targeted immunoconjugate of calicheamicin currently being evaluated in phase III clinical trials in patients with non-Hodgkin's B-cell lymphoma. CMC-544 is the product of a collaboration between Wyeth and UCB-Celltech. CMC-544 has demonstrated significant clinical activity in both follicular and diffuse large B-cell lymphoma patients who had failed multiple therapies.^{1,2} CMC-544 (Figure 1) is a humanized IgG4 anti-CD22 antibody (G5/44) covalently linked to N-acetyl gamma calicheamicin dimethyl hydrazide (CalichDMH) via an acid-labile 4-(4-acetylphenoxy)butanoic acid (AcBut) linker.^{3,4} CD22 is a B-lymphoid lineage-specific differentiation antigen expressed on the surface of both normal and malignant B cells. CD22 is not expressed on hematopoietic progenitors of the B-lymphoid lineage. CD22 efficiently internalizes upon binding to an antibody making it an ideal candidate for targeted drug delivery. Targeting malignant B-cells through CD22 by the CD22 specific antibody G5/44 could, therefore, be an efficient way to deliver calicheamicin to destroy malignant B-cells.

CMC-544 binds CD22 with subnanomolar affinity, and upon binding to CD22, is rapidly internalized delivering conjugated

calichDMH inside the cells. CalichDMH is a derivative of a potent cytotoxic agent gamma calicheamicin, a natural product synthesized by a strain of *Micromonospora echinospora*. Upon internalization, the conjugate is taken up by the lysosomes, where, under the acidic conditions in the lysosomes, the acid-labile hydrazone functional group within the AcBut linker is hydrolyzed allowing for the release of calichDMH. CalichDMH diffuses into the nucleus where it binds DNA in the minor groove and undergoes thiol-dependent structural changes in its enediyne moiety (war-head) to generate a diradical that abstracts hydrogens from the phosphodiester backbone of DNA producing double-strand DNA breaks leading to cell death.⁵⁻⁷

For calicheamicin conjugates, the linker stability was optimized with the selection of the spacer and the attached groups for plasma stability versus the ability to release calichDMH in the cellular lysosomal compartment.⁸ The AcBut linker with the hydrazone functional group produced the best balance between stability in pH 7.4 plasma and hydrolysis in the acidic (pH 4.5) lysosomal compartment. The stability of CMC-544 was confirmed in both human plasma and serum by monitoring the generation of

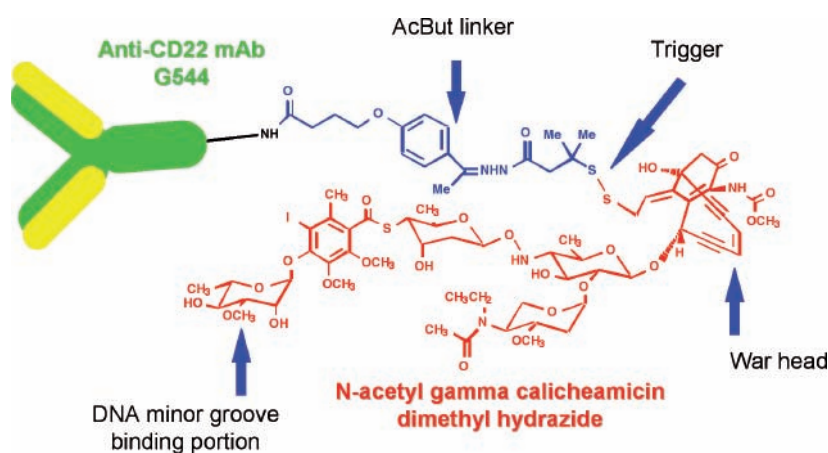


Figure 1. CMC-544: inotuzumab ozogamicin.

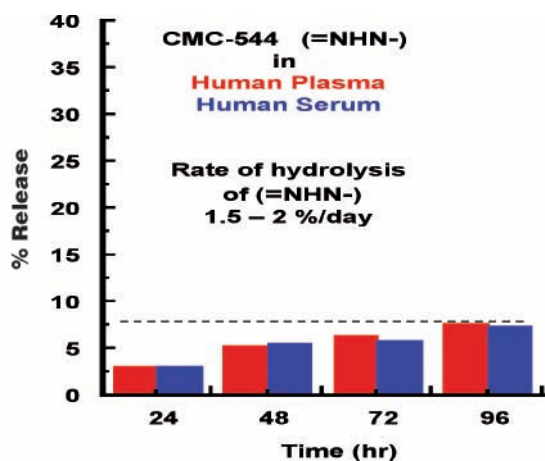


Figure 2. Stability of CMC-544 in human blood.

extractable unconjugated calichDMH from the antibody over a 4 day period using plasmon resonance analysis.⁹ In either human plasma or serum, CMC-544 remained relatively stable, hydrolyzing at a rate of 1.5-2% per day. (Figure 2).

CMC-544 has been studied extensively in preclinical models of human B-cell lymphoma. CMC-544 binds CD22 with a high affinity ($KD=235$ pM). It inhibited the *in vitro* growth of a number of CD22⁺ cell lines (IC_{50} s 6-300 pM) much more potently than unconjugated calichDMH, consistent with the active

CD22-mediated cellular internalization of the conjugate. In various *in vivo* models of human B-cell lymphomas, CMC-544 caused dose-dependent regression of both small and large established B-lymphoma xenografts.³ It was active over the dose range of 10 μ g of conjugated calichDMH/kg (minimum efficacious dose) to 160 μ g of conjugated calichDMH/kg (dose producing long term xenograft cures, for these studies CMC-544 was administered 3 times, Q4Dx3). CMC-544 caused long-term survival of SCID mice with systemically disseminated human B-lymphoma.¹⁰ In this disseminated human B-lymphoma model, when suboptimal doses of CMC-544 were combined with suboptimal doses of rituximab, superior anti-tumor activity was derived by the combination of these agents over either drug administered alone.¹¹ The targeting antibody G5/44 had no effect on the growth of B-cell lymphomas xenografts in nude mice over a wide range of dosages, consistent with its effector function-deficient IgG4 isotype. CMC-544 therefore, is regarded as an antibody-targeted chemotherapy agent rather than an immunotherapeutic agent such as rituximab. Largely due to its tumor-targeted drug-delivery capability, it is anticipated that CMC-544 will have a better therapeutic index than that of conven-

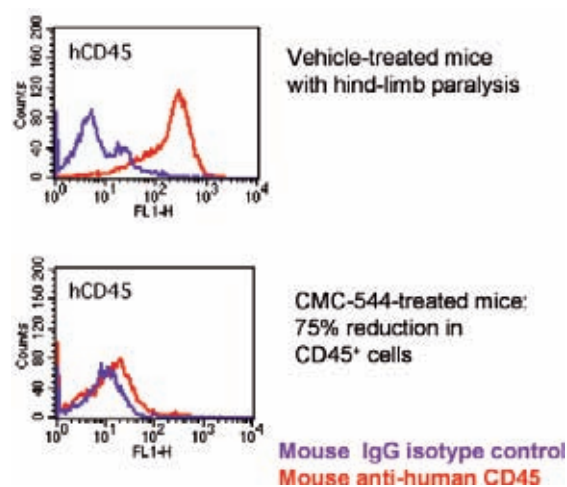


Figure 3. Effect of CMC-544 on human CD45⁺ cells engrafted in bone marrow of scid mice with disseminated ALL. Mice were injected intravenously with Reh ALL cells. At the time of the appearance of hind limb paralysis in the vehicle-treated mice, bone marrow cells were collected and analyzed by FACs for the presence of human CD45. Bone marrow from CMC-544 treated mice was also collected at the corresponding times for analysis.

tional chemotherapeutic agents.

To determine the serum levels of CMC-544 that were associated with the anti-tumor effects described above, tumor-bearing (RL B-lymphoma xenograft) or non-tumor-bearing nude mice received a single dose of CMC-544 ip (160 µg of conjugated CalichDMH/kg) and serum samples from these mice were assayed for the presence of CMC-544 using an enzyme-linked immunoassay.³ The immunoassay was specifically designed and validated to detect antibody conjugates of CalichDMH.¹² The mean (\pm SE) C_{max} values for non-tumor-bearing and RL BCL-bearing nude mice, respectively, were 2.6 ± 0.1 and 2.4 ± 0.2 µg/mL (CalichDMH equivalents of CMC-544), the $t_{1/2}$ were 34.2 and 35 h, and the corresponding mean $AUC_{0-\infty}$ values were 145 and 93 µg·h/mL in non-tumor-bearing and RL BCL-bearing mice, respectively. The difference between the $AUC_{0-\infty}$ values for non-tumor-bearing mice and RL tumor-bearing mice was statistically significant ($p < 0.05$). The lower systemic exposure

($AUC_{0-\infty}$) of CMC-544 in tumor-bearing mice than that in non-tumor-bearing mice is consistent with the tumor uptake of CMC-544.

Acute lymphocytic leukemia (ALL) is primarily a B-cell or pre-B-cell malignancy. ALL blasts differentially express a number of B-lymphoid specific antigens including CD19, CD20 and CD22. The CD22 expressed on these blasts may allow preferential targeting by CMC-544. In order to investigate the effect of CMC-544 in ALL, mice were injected with Reh cells (a CD22⁺ ALL derived cell line) in the lateral tail vein and monitored for disease symptoms.¹³ Mice treated with vehicle (PBS) developed hind-limb paralysis. All of the vehicle-treated mice succumbed to the disseminated disease by day 77. The average survival time for the group was 55 days. CMC-544, administered at a dose of 80 µg/kg of calichDMH (Q4Dx3) produced 100% survival of the treated mice over the 127 day observation period. At the dose of 4 µg/kg CalichDMH (Q4Dx3), 20 fold lower than the curative dose of 80 µg/kg, 90% of the mice still survived throughout the observation period.

In this disseminated disease model, four vehicle-treated mice were killed when they initially exhibited hind-limb paralysis (days 35, 41, 41, and 43). At equivalent time points (days 36, 41 and 43), 3 CMC-544-treated mice were randomly selected and killed. Flow cytometric analysis of bone marrow cells collected from the femur of these vehicle-treated mice demonstrated the presence of human CD45⁺ leukemic cells (Figure 3). CMC-544 (80 µg/kg, Q4Dx3) potently inhibited the engraftment of the human CD45⁺ cells.

In summary, CMC-544 is a potent antibody-targeted chemotherapeutic agent that has demonstrated potent anti-tumor activity in pre-clinical models of B-cell lymphoma and leukemia. CMC-544 should be considered as a targeted chemotherapeutic agent since the unconjugated targeting antibody G5/44 is inactive as a therapeutic agent. CMC-544 is

expected to have a better safety profile than non-targeted chemotherapeutics due to the preferential delivery of the potent cytotoxic agent calicheamicin to the tumor associated antigen via the targeting antibody G5/44. Phase I clinical data demonstrated that CMC-544 is active in non-Hodgkin's B-cell lymphoma with manageable toxicity. Expanded clinical trials (phase 2/3) are currently being conducted with CMC-544 in both follicular non-Hodgkin's lymphoma and diffuse large B-cell lymphoma.

At the time the studies were conducted, all authors were employees of Wyeth Research whose compound, inotuzumab ozogamicin, is discussed in this report.

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