

Answer 1:

### Bibliographic Information

**Rituximab blocks binding of radiolabeled anti-CD20 antibodies (Ab) but not radiolabeled anti-CD45 Ab.** Gopal, Ajay K.; Press, Oliver W.; Wilbur, Shani M.; Maloney, David G.; Pagel, John M. Department of Medicine, Division of Medical Oncology, University of Washington, Seattle, USA. *Blood* (2008), 112(3), 830-835. Publisher: American Society of Hematology, CODEN: BLOOAW ISSN: 0006-4971. Journal written in English. AN 2008:942115 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Rituximab therapy is assocd. with a long in vivo persistence, yet little is known about the effect of circulating rituximab on B-cell non-Hodgkin lymphoma (B-NHL) targeting by the other available anti-CD20 monoclonal antibodies (MoAbs) <sup>131</sup>Iodine-tositumomab and <sup>90</sup>Yttrium-ibritumomab tiuxetan. Therefore we assessed the impact of preexisting rituximab on the binding and efficacy of second anti-CD20 MoAbs to B-NHL and detd. whether targeting an alternative lymphoma-assocd. antigen, CD45, could circumvent this effect. We demonstrated that rituximab concns. as low as 5 µg/mL nearly completely blocked the binding of a second anti-CD20 MoAbs (P < .001), but had no impact on CD45 targeting (P = .89). Serum from patients with distant exposures to rituximab also blocked binding of anti-CD20 MoAbs to patient-derived rituximab-naive B-NHL at concns. as low as 7 µg/mL, but did not affect CD45 ligation. A mouse xenograft model (Granta, FL-18, Ramos cell lines) showed that rituximab pretreatment significantly reduced B-NHL targeting and tumor control by CD20-directed radioimmunotherapy (RIT), but had no impact on targeting CD45. These findings suggest that circulating rituximab impairs the clin. efficacy of CD20-directed RIT, imply that novel anti-CD20 MoAbs could also face this same limitation, and indicate that CD45 may represent an alternative target for RIT in B-NHL.

Answer 2:

### Bibliographic Information

**Acquirement of Rituximab Resistance in Lymphoma Cell Lines Is Associated with Both Global CD20 Gene and Protein Down-Regulation Regulated at the Pretranscriptional and Posttranscriptional Levels.** Czuczman, Myron S.; Olejniczak, Scott; Gowda, Aruna; Kotowski, Adam; Binder, Arvinder; Kaur, Harman; Knight, Joy; Starostik, Petr; Deans, Julie; Hernandez-Ilizaliturri, Francisco J. Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA. *Clinical Cancer Research* (2008), 14(5), 1561-1570. Publisher: American Association for Cancer Research, CODEN: CCRE4 ISSN: 1078-0432. Journal written in English. CAN 148:583652 AN 2008:270839 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Acquirement of resistance to rituximab has been obsd. in lymphoma patients. To define mechanisms assocd. with rituximab resistance, we developed various rituximab-resistant cell lines (RRCL) and studied changes in CD20 expression/structure, lipid raft domain (LRD) reorganization, calcium mobilization, antibody-dependent cellular cytotoxicity, and complement-mediated cytotoxicity (CMC) between parental and RRCL. Significant changes in surface CD20 antigen expression were shown in RRCL. Decreased calcium mobilization and redistribution of CD20 into LRD were found in RRCL. Western blotting identified a unique 35 kDa protein band in RRCL, which was not seen in parental cells and was secondary to an increase in surface and cytoplasmic expression of IgM light chains. CD20 gene expression was decreased in RRCL. In vitro exposure to PS341 increased CD20 expression in RRCL and minimally improved the sensitivity to rituximab-assocd. CMC. Our data strongly suggest that the acquisition of rituximab resistance is assocd. with global gene and protein down-regulation of the CD20 antigen affecting LRD organization and downstream signaling. CD20 expression seems to be regulated at the pretranscriptional and posttranscriptional levels. Proteasome inhibition partially reversed rituximab resistance, suggesting the existence of addnl. mediators of rituximab resistance. Future research is geared to identify drugs and/or biol. agents that are effective against RRCL.

Answer 3:

### Bibliographic Information

**A Novel Raji-Burkitt's Lymphoma Model for Preclinical and Mechanistic Evaluation of CD52-Targeted Immunotherapeutic Agents.** Lapalombella, Rosa; Zhao, Xiaobin; Triantafillou, Georgia; Yu, Bo; Jin, Yan; Lozanski, Gerard; Cheney, Carolyn; Heerema, Nyla; Jarjoura, David; Lehman, Amy; Lee, L. James; Marcucci, Guido; Lee, Robert J.; Caligiuri, Michael A.; Muthusamy, Natarajan; Byrd, John C. Division of Hematology-Oncology, The Ohio State University, Columbus, OH, USA. *Clinical Cancer Research* (2008), 14(2), 569-578. Publisher: American Association for Cancer Research, CODEN: CCREF4 ISSN: 1078-0432. Journal written in English. CAN 149:126265 AN 2008:106236 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

**PURPOSE:** To date, efforts to study CD52-targeted therapies, such as alemtuzumab, have been limited due to the lack of stable CD52 expressing transformed B-cell lines and animal models. We describe generation and utilization of cell lines that stably express CD52 both in vitro and in vivo. **Exptl. Design:** By limiting diln., we have established several clones of Raji-Burkitt's lymphoma cell line that express surface CD52. Immunophenotype and cytogenetic characterization of these clones was done. In vivo usefulness of the CD52high cell line to evaluate the therapeutic efficacy of CD52-directed antibody was investigated using a SCID mouse xenograft model. **RESULTS:** Stable expression of CD52 was confirmed in cells cultured in vitro up to 52 wk of continuous growth. The functional integrity of the expressed CD52 mol. was shown using alemtuzumab, which induced cytotoxic effects in vitro in the CD52high but not the CD52low clone. Compared with control antibody, alemtuzumab treatment in CD52high inoculated mice resulted in significantly increased median survival. Comparable levels of CD52-targeted direct cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cytotoxicity and anti-CD52 immunoliposome-mediated delivery of synthetic oligodeoxyribo nucleotides in CD52high clone and primary B-chronic lymphocytic leukemia cells implicated potential in vivo application of this model for evaluation of CD52-targeted antibody and immunoliposomes encapsulating therapeutic agents. **CONCLUSIONS:** These results show the in vitro utility of the cloned Raji cell lines that stably express high levels CD52. The disseminated leukemia-lymphoma mouse model described herein using these stable cell lines can serve as an excellent system for in vivo therapeutic and mechanistic evaluation of existing and novel antibodies directed against CD52 mol.

Answer 4:

#### Bibliographic Information

**Targeted cancer therapy with a novel low-dose rate  $\alpha$ -emitting radioimmunoconjugate.** Dahle, Jostein; Borrebaek, Joergen; Jonasdottir, Thora J.; Hjelmerud, Anne Kristine; Melhus, Katrine B.; Bruland, Oeyvind S.; Press, Oliver W.; Larsen, Roy H. Department of Radiation Biology, Norwegian Radium Hospital, Oslo, Norway. *Blood* (2007), 110(6), 2049-2056. Publisher: American Society of Hematology, CODEN: BLOOAW ISSN: 0006-4971. Journal written in English. CAN 148:489635 AN 2007:1050780 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

$\alpha$ -Emitting radionuclides are highly cytotoxic and are of considerable interest in the treatment of cancer. A particularly interesting approach is in radioimmunotherapy. However,  $\alpha$ -emitting antibody conjugates have been difficult to exploit clin. due to the short half-life of the radionuclides, low prodn. capability, or limited source materials. We have developed a novel technol. based on the low-dose rate  $\alpha$ -particle-emitting nuclide  $^{227}\text{Th}$ , exemplified here using the monoclonal antibody rituximab. In vitro, this radioimmunoconjugate killed lymphoma cells at Becquerel per mL (Bq/mL) levels. A single injection of  $^{227}\text{Th}$ -rituximab induced complete tumor regression in up to 60% of nude mice bearing macroscopic (32-256 mm<sup>3</sup>) human B-lymphoma xenografts at Becquerel per g (Bq/g) levels without apparent toxicity. Therapy with  $^{227}\text{Th}$ -rituximab was significantly more effective than the control radioimmunoconjugate  $^{227}\text{Th}$ -trastuzumab and the std.  $\beta$ -emitting radioimmunoconjugate for CD20+ lymphoma,  $^{90}\text{Y}$ -tiuxetan-ibrutinomab. Thorium-227 based constructs may provide a novel approach for targeted therapy against a wide variety of cancers.

Answer 5:

#### Bibliographic Information

**Tumor targeting with antibody-functionalized, radiolabeled carbon nanotubes.** McDevitt, Michael R.; Chattopadhyay, Debjit; Kappel, Barry J.; Jaggi, Jaspreet Singh; Schiffman, Scott R.; Antczak, Christophe; Njardarson, Jon T.; Brentjens, Renier; Scheinberg, David A. Molecular Pharmacology and Chemistry Department, Departments of Medicine and Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA. *Journal of Nuclear Medicine* (2007), 48(7), 1180-1189. Publisher: Society of Nuclear Medicine, CODEN: JNMEAQ ISSN: 0161-5505. Journal written in English. CAN 147:371466 AN 2007:1038611 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Single-walled carbon nanotubes (CNT) are mech. robust graphene cylinders with a high aspect ratio that are comprised of sp<sup>2</sup>-bonded carbon atoms and possessing highly regular structures with defined periodicity. CNT exhibit unique mechanochem. properties that can be exploited for the development of novel drug delivery platforms. We hypothesized that novel prototype nanostructures consisting of biologics, radionuclides, fluorochromes, and CNT could be synthesized and designed to target tumor cells. Methods: Tumor-targeting CNT constructs were synthesized from sidewall-functionalized, water-sol. CNT platforms by covalently attaching multiple copies of tumor-specific monoclonal antibodies, radiometal-ion chelates, and fluorescent probes. The constructs were characterized spectroscopically, chromatog., and electrophoretically. The specific reactivity of these constructs was evaluated in vitro by flow cytometry and cell-based immunoreactivity assays and in vivo using biodistribution in a murine xenograft model of lymphoma. Results: A sol., reactive CNT platform was used as the starting point to build multifunctional constructs with appended antibody, metal-ion chelate, and fluorescent chromophore moieties to effect specific targeting, to carry and deliver a radiometal-ion, and to report location, resp. These nanoconstructs were found to be specifically reactive with the human cancer cells they were designed to target in vivo in a model of disseminated human lymphoma and in vitro by flow cytometry and cell-based immunoreactivity assays vs. appropriate controls. Conclusion: The key achievement in these studies was the selective targeting of tumor in vitro and in vivo by the use of specific antibodies appended to a sol., nanoscale CNT construct. The ability to specifically target tumor with prototype-radiolabeled or fluorescent-labeled, antibody-appended CNT constructs was encouraging and suggested further investigation of CNT as a novel delivery platform.

Answer 6:

### Bibliographic Information

**Carbon nanotubes: potential benefits and risks of nanotechnology in nuclear medicine.** Reilly, Raymond M. University of Toronto, Toronto, ON, Can. *Journal of Nuclear Medicine* (2007), 48(7), 1039-1042. Publisher: Society of Nuclear Medicine, CODEN: JNMEAQ ISSN: 0161-5505. Journal; General Review written in English. CAN 147:433093 AN 2007:1038603 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

A review. The research of McDevitt et al. (2007) entitled "Tumor targeting with antibody-functionalized. radio- labeled carbon nanotubes" is reviewed with commentary and refs. McDevitt et al. explore the feasibility of targeted delivery of radionuclides to B-cell lymphomas using one new nanotechnol. platform: carbon nanotubes (CNTs). They show that CNTs can be functionalized on their surface with 1,4,7,10-tetraazacyclododecane- N,N',N'' ,N''' -tetraacetic acid chelators for complexing <sup>111</sup>In and also with the anti-CD20 antibody rituximab (Rituxan; Genentech and Biogen Idec) for targeting to malignant B-cells. The <sup>111</sup>In-labeled and rituximab-modified CNTs specifically localized in disseminated Daudi B-cell lymphoma xenografts in the bone marrow and spleen of severe combined immunodeficiency (acid) mice, after i.v. injection. These results suggest that CNTs may be useful vehicles for transporting radionuclides to malignancies; however, caution is advised because, among nanotechnol. platforms being investigated, least is known about the in vivo properties and potential risks of CNTs as delivery systems.

Answer 7:

### Bibliographic Information

**Imatinib mesylate reduces rituximab-induced tumor-growth inhibition in vivo on Epstein-Barr virus-associated human B-cell**

**lymphoma.** Nemati, Fariba; Mathiot, Claire; Grandjean, Isabelle; Lantz, Olivier; Bordier, Vincent; Dewulf, Sebastien; Ekue, Richard; Di Santo, James P.; Poupon, Marie-France; Decaudin, Didier. Laboratory of Pre-clinical Investigations, Institut National de la Sante et de la Recherche Medicale Unite, Paris, Fr. *Anti-Cancer Drugs* (2007), 18(9), 1029-1037. Publisher: Lippincott Williams & Wilkins, CODEN: ANTDEV ISSN: 0959-4973. Journal written in English. CAN 147:419446 AN 2007:914839 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

We have reported earlier an increase of tumor-growth inhibition following chemotherapy combined with concomitant administration of imatinib mesylate. Conversely, the combination of imatinib and rituximab has been reported in very few cases of patients and remains controversial. To explore this particular combination of targeted therapies, we therefore investigated the in-vivo impact of rituximab plus imatinib on B-cell lymphoproliferation. Combination of the tyrosine kinase inhibitor imatinib mesylate (STI571) and the anti-CD20 monoclonal antibody rituximab was evaluated on an Epstein-Barr virus-assocd. B-cell lymphoproliferative disorder xenografted into severe combined immunodeficient or Rag2 $\gamma$ c (B, T and NK) mice. Using severe combined immunodeficient mice, we found that STI571 diminished the efficacy of rituximab to inhibit tumor growth in vivo. Using alymphoid Rag2 $\gamma$ c mice, we showed that the effect of STI571 was not dependent on the presence of natural killer cells. In contrast, serum complement administered after STI571 treatment reversed this inhibitory effect. Finally, using nonimmunodeficient mice, we obsd. an in-vivo decrease of CD4-pos. T-cells and mature B-cell lymphocytes after imatinib administration. We found that STI571 decreased the in-vivo efficacy of rituximab via serum protein components that could influence complement-dependent cytotoxicity. In contrast, this effect was not dependent on the presence of natural killer cells.

Answer 8:

#### Bibliographic Information

**CD20-specific antibody-targeted chemotherapy of non-Hodgkin's B-cell lymphoma using calicheamicin-conjugated rituximab.** DiJoseph, John F.; Dougher, Maureen M.; Armellino, Douglas C.; Kalyandrug, Lyka; Kunz, Arthur; Boghaert, Erwin R.; Hamann, Philip R.; Damle, Nitin K. *Oncology Discovery*, Wyeth Research, Pearl River, NY, USA. *Cancer Immunology Immunotherapy* (2007), 56(7), 1107-1117. Publisher: Springer, CODEN: CIIMDN ISSN: 0340-7004. Journal written in English. CAN 147:203301 AN 2007:462343 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

Tumor-targeted delivery of a potent cytotoxic agent, calicheamicin, using its immunoconjugates is a clin. validated therapeutic strategy. Rituximab is a human CD20-specific chimeric antibody extensively used in B-NHL therapy. We investigated whether conjugation to calicheamicin can improve the anti-tumor activity of rituximab against human B-cell lymphoma (BCL) xenografts in preclin. models. BCL cells were cultured with rituximab or its calicheamicin conjugates and their in vitro growth was monitored. BCL cells were injected s.c. to establish localized xenografts in nude mice or i.v. to establish disseminated BCL in severe combined immunodeficient (scid) mice. I.p. treatment with rituximab or its calicheamicin conjugates was initiated and its effect on s.c. BCL growth or survival of mice with disseminated BCL was monitored. Conjugation of calicheamicin to rituximab vastly enhanced its growth inhibitory activity against BCL in vitro. Conjugation to calicheamicin had no deleterious effect on the effector functional activity of rituximab. Calicheamicin conjugated to rituximab with an acid-labile linker exhibited greater anti-tumor activity against s.c. BCL xenografts and improved survival of mice with disseminated BCL over that of unconjugated rituximab. Anti-tumor activities of rituximab conjugated to calicheamicin via an acid-stable linker were similar to that of unconjugated rituximab. Superior anti-tumor efficacy exhibited by a calicheamicin immunoconjugate of rituximab with an acid-labile linker over that of rituximab demonstrates the therapeutic potential of CD20-specific antibody-targeted chemotherapy strategy in the treatment of B-NHL.

Answer 9:

#### Bibliographic Information

**Recombinant Interleukin-2 Significantly Augments Activity of Rituximab in Human Tumor Xenograft Models of B-cell**

**Non-Hodgkin Lymphoma.** Lopes de Menezes, Daniel E.; Denis-Mize, Kimberly; Tang, Yan; Ye, Helen; Kunich, John C.; Garrett, Evelyn N.; Peng, Jing; Cousens, Lawrence S.; Gelb, Arnold B.; Heise, Carla; Wilson, Susan E.; Jallal, Bahija; Aukerman, Sharon L. Biopharma Research and Development, Chiron Corporation, Emeryville, CA, USA. *Journal of Immunotherapy* (2007), 30(1), 64-74. Publisher: Lippincott Williams & Wilkins, CODEN: JOIMF8 ISSN: 1524-9557. Journal written in English. CAN 146:266006 AN 2007:3045 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

Recombinant interleukin-2 (rIL-2) is a pleiotropic cytokine that activates select immune effector cell responses assocd. with antitumor activity, including antibody-dependent cellular cytotoxicity (ADCC). Rituximab is an anti-CD20 monoclonal antibody that activates ADCC in non-Hodgkin lymphoma (NHL). The ability of rIL-2 to augment rituximab-dependent tumor responses was investigated. The efficacy of rIL-2 in combination with rituximab was evaluated in 2 NHL tumor xenograft models: the CD20, rituximab-sensitive, low-grade Daudi model and the CD20, aggressive, rituximab-resistant Namalwa model. Combination of rIL-2 plus rituximab was synergistic in a rituximab-sensitive Daudi tumor model, as evidenced by significant tumor regressions and increased time to tumor progression, compared with rIL-2 and rituximab single agents. In contrast, rituximab-resistant Namalwa tumors were responsive to single-agent rIL-2 and showed an increased response when combined with rituximab. Using in vitro killing assays, rIL-2 was shown to enhance activity of rituximab by activating ADCC and lymphokine-activated killer activity. Addnl., the activity of rIL-2 plus rituximab F(ab')<sub>2</sub> was similar to that of rIL-2 alone, indicating a crit. role for IgG1 Fc-Fc $\gamma$ R-effector responses in mediating ADCC. Antiproliferative and apoptotic tumor responses, along with an influx of immune effector cells, were obsd. by immunohistochem. Collectively, the data suggest that rIL-2 mediates potent tumoricidal activity against NHL tumors, in part, through activation and trafficking of monocytes and natural killer cells to tumors. These data support the mechanistic and therapeutic rationale for combination of rIL-2 with rituximab in NHL clin. trials and for single-agent rIL-2 in rituximab-resistant NHL patients.

Answer 10:

#### Bibliographic Information

**High efficacy of combined rituximab and gemcitabine on Epstein-Barr virus-associated human B-cell lymphoma obtained after Hodgkin's xenograft in immunodeficient mice.** Decaudin, Didier; Marszak, Fanny Baran; Couturier, Jerome; Mathiot, Claire; Martin, Antoine; Nemati, Fariba; Lantz, Olivier; di Santo, James; Arnaud, Philippe; Bordier, Vincent; Vincent-Salomon, Anne; Poupon, Marie-France. Department of Clinical Hematology, Section de Recherche, Institut Curie, Paris, Fr. *Anti-Cancer Drugs* (2006), 17(6), 685-695. Publisher: Lippincott Williams & Wilkins, CODEN: ANTDEV ISSN: 0959-4973. Journal written in English. CAN 145:202256 AN 2006:798845 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

The objectives were to characterize an Epstein-Barr virus-assocd. human B-cell lymphoma obtained from Hodgkin's xenograft, and to evaluate the in-vivo combination of rituximab and/or gemcitabine. A lymph node biopsy sample from a patient with Hodgkin's disease was xenografted into Rag  $\gamma$ -/-c mice. Immunohistochem., cytogenetic and genetic analyses were performed on both the human biopsy and xenografted tumor from severe combined immunodeficient mice. Tumor-bearing mice were then treated with rituximab and/or gemcitabine. Histol. features of the patient's biopsy concluded on classical CD15/CD30-pos. Hodgkin's disease without expression of Epstein-Barr virus proteins. In contrast, morphol. and immunophenotypic examn. of the xenograft showed diffuse proliferation of large B cells with high Epstein-Barr virus protein expression. Comparative genomic hybridization showed a normal pattern in the first case and a gain of chromosomal 12 in the xenografted tumor. Finally, polymerase chain reaction detected an Ig heavy chain rearrangement in the xenografted tumor. Altogether, these results indicate that the xenograft grew from the patient's Epstein-Barr virus-infected B-lymphoid cells and could be assimilated to posttransplant lymphoproliferative disease. In-vivo treatments of xenografted tumors showed significant tumor growth inhibition induced either by rituximab or gemcitabine alone and an impressive efficacy of combined treatment. This result therefore indicates that combined rituximab and gemcitabine could be an alternative approach in patients with posttransplant lymphoproliferative disease.

Answer 11:

#### Bibliographic Information

**Antitumor efficacy of a combination of CMC-544 (inotuzumab ozogamicin), a CD22-targeted cytotoxic immunoconjugate of calicheamicin, and rituximab against non-Hodgkin's B-cell lymphoma.** DiJoseph, John F.; Dougher, Maureen M.; Kalyandrug, Lyka B.; Armellino, Douglas C.; Boghaert, Erwin R.; Hamann, Philip R.; Moran, Justin K.; Damle, Nitin K. *Oncology Discovery Research, Wyeth Research, Pearl River, NY, USA. Clinical Cancer Research* (2006), 12(1), 242-249. Publisher: American Association for Cancer Research, CODEN: CCREF4 ISSN: 1078-0432. Journal written in English. CAN 145:6153 AN 2006:12573 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

CMC-544 is a CD22-targeted cytotoxic immunoconjugate, currently being evaluated in B-cell non-Hodgkin's lymphoma (B-NHL) patients. Rituximab is a CD20-targeted antibody commonly used in B-NHL therapy. Here, we describe antitumor efficacy of a combination of CMC-544 and rituximab against B-cell lymphoma (BCL) in preclin. models. BCLs were cultured in vitro with CMC-544, rituximab, or their combination. BCLs were injected either s.c. or i.v. to establish localized s.c. BCL in nude mice or disseminated BCL in severe combined immunodeficient mice, resp. I.p. treatment with CMC-544 or rituximab was initiated at various times either alone or in combination and its effect on s.c. BCL growth or survival of mice with disseminated BCL was monitored. In vitro growth-inhibitory activity of CMC-544 combined with rituximab was additive. Rituximab but not CMC-544 exhibited effector functions, such as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Rituximab was less effective in inhibiting growth of established BCL xenografts than developing xenografts. In contrast, CMC-544 was equally effective against both developing and established BCL xenografts. Although CMC-544 and rituximab individually caused partial inhibition of the growth of BCL xenografts at suboptimal doses examd., their combination suppressed xenograft growth by >90%. In a disseminated BCL model, 60% of CMC-544-treated mice and 20% of rituximab-treated mice survived for 125 days. In contrast, 90% of mice treated with the combination of CMC-544 and rituximab survived for longer than 125 days. The demonstration of superior antitumor activity of a combination of CMC-544 and rituximab described here provides the preclin. basis for its clin. evaluation as a treatment option for B-NHL.

Answer 12:

#### Bibliographic Information

**Immunomodulatory Drug CC-5013 or CC-4047 and Rituximab Enhance Antitumor Activity in a Severe Combined Immunodeficient Mouse Lymphoma Model.** Hernandez-Ilizaliturri, Francisco J.; Reddy, Nishitha; Holkova, Beata; Ottman, Edris; Czuczman, Myron S. *Department of Medicine, State University of New York at Buffalo, Buffalo, NY, USA. Clinical Cancer Research* (2005), 11(16), 5984-5992. Publisher: American Association for Cancer Research, CODEN: CCREF4 ISSN: 1078-0432. Journal written in English. CAN 144:63981 AN 2005:864117 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

New thalidomide derivs. CC-5013 and CC-4047 (immunomodulatory drugs, IMiD) are up to 10,000 times more potent than Thalidomide. The biol. effects of IMiDs are presumed to be mediated by (a) activation of some components of the innate [natural killer (NK) cells] or adoptive immune system (T cells), (b) modification of cytokine microenvironment in the tumor bed, or by (c) inhibition of angiogenesis. In this article, we tested an innovative combination strategy involving rituximab and IMiDs in aggressive lymphoma cell lines and human lymphoma xenografts. Treatment of non-Hodgkin's lymphoma cells with CC-5013 resulted in a 40% to 70% growth inhibition when compared with controls ( $P < 0.05$ ). Exposure of lymphoma cells to CC-4047 resulted in a lesser degree of growth inhibition. Induction of apoptosis was shown in 10% to 26% of lymphoma cells 24 h following exposure to either IMiD. In vivo studies in severe combined immunodeficient mice showed synergistic activity between CC-4047 (and to a lesser degree, CC-5013) plus rituximab. Animals treated with the CC-4047/rituximab combination had a median survival of 74 days ( $P = 0.0012$ ) compared with 58 days ( $P = 0.167$ ) in CC-5013/rituximab-treated animals compared with 45 days in rituximab monotherapy-treated animals. The synergistic effect between IMiDs and rituximab in our mouse model was attributed to NK cell expansion. The enhancement of rituximab activity by IMiDs was abrogated by in vivo depletion of NK cells. Augmenting NK cell function by CC-4047 or CC-5013 exposure may increase the antitumor effects of rituximab against B-cell lymphomas and warrants further exploration in the context of a clin. trial.

Answer 13:

**Bibliographic Information**

**Efficient elimination of B-lineage lymphomas by anti-CD20-auristatin conjugates. [Erratum to document cited in CA142:273558].** Law, Che-Leung; Cervený, Charles G.; Gordon, Kristine A.; Klussman, Kerry; Mixan, Bruce J.; Chace, Dana F.; Meyer, Damon L.; Doronina, Svetlana O.; Siegall, Clay B.; Francisco, Joseph A.; Senter, Peter D.; Wahl, Alan F. Seattle Genetics, Inc., Bothell, WA, USA. *Clinical Cancer Research* (2005), 11(10), 3969. Publisher: American Association for Cancer Research, CODEN: CCREF4 ISSN: 1078-0432. Journal written in English. CAN 143:339085 AN 2005:604390 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

Figure 6 lacked symbol legends; the correct figure is given.

Answer 14:

**Bibliographic Information**

**Induction of Apoptosis Using Inhibitors of Lysophosphatidic Acid Acyltransferase- $\beta$  and Anti-CD20 Monoclonal Antibodies for Treatment of Human Non-Hodgkin's Lymphomas.** Pagel, John M.; Laugen, Christian; Bonham, Lynn; Hackman, Robert C.; Hockenbery, David M.; Bhatt, Rama; Hollenback, David; Carew, Heather; Singer, Jack W.; Press, Oliver W. Fred Hutchinson Cancer Research Center, Univ. Washington, Seattle, WA, USA. *Clinical Cancer Research* (2005), 11(13), 4857-4866. Publisher: American Association for Cancer Research, CODEN: CCREF4 ISSN: 1078-0432. Journal written in English. CAN 143:399001 AN 2005:585317 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

**PURPOSE:** Lysophosphatidic acid acyltransferase- $\beta$  (LPAAT- $\beta$ ) is a transmembrane enzyme crit. for the biosynthesis of phosphoglycerides whose product, phosphatidic acid, plays a key role in raf and AKT/mTor-mediated signal transduction. **Exptl. Design:** LPAAT- $\beta$  may be a novel target for anticancer therapy, and, thus, we examd. the effects of a series of inhibitors of LPAAT- $\beta$  on multiple human non-Hodgkin's lymphoma cell lines in vitro and in vivo. **RESULTS:** We showed that five LPAAT- $\beta$  inhibitors at doses of 500 nmol/L routinely inhibited growth in a panel of human lymphoma cell lines in vitro by >90%, as measured by [<sup>3</sup>H]thymidine incorporation. Apoptotic effects of the LPAAT- $\beta$  inhibitors were evaluated either alone or in combination with the anti-CD20 antibody, Rituximab. The LPAAT- $\beta$  inhibitors induced caspase-mediated apoptosis at 50 to 100 nmol/L in up to 90% of non-Hodgkin's lymphoma cells. The combination of Rituximab and an LPAAT- $\beta$  inhibitor resulted in a 2-fold increase in apoptosis compared with either agent alone. To assess the combination of Rituximab and a LPAAT- $\beta$  inhibitor in vivo, groups of athymic mice bearing s.c. human Ramos lymphoma xenografts were treated with the LPAAT- $\beta$  inhibitor CT-32228 i.p. (75 mg/kg) daily for 5 d/wk x 4 wk (total 20 doses), Rituximab i.p. (10 mg/kg) weekly x 4 wk (4 doses total), or CT-32228 plus Rituximab combined. Treatment with either CT-32228 or Rituximab alone showed an approx. 50% xenograft growth delay; however, complete responses were only obsd. when the two agents were delivered together. **CONCLUSIONS:** These data suggest that Rituximab, combined with a LPAAT- $\beta$  inhibitor, may provide enhanced therapeutic effects through apoptotic mechanisms.

Answer 15:

**Bibliographic Information**

**Rituximab therapy of lymphoma is enhanced by orally administered (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)-D- $\beta$ -glucan.** Modak, Shakeel; Koehne, Guenther; Vickers, Andrew; O'Reilly, Richard J.; Cheung, Nai-Kong V. Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA. *Leukemia Research* (2005), 29(6), 679-683. Publisher: Elsevier B.V., CODEN: LEREDD ISSN: 0145-2126. Journal written in English. CAN 143:131448 AN 2005:366655 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

By activating complement, antitumor monoclonal antibodies coat tumor cells with iC3b.  $\beta$ -glucans, naturally occurring glucose polymers, bind to the lectin domain of the leukocyte receptor CR3, prime it for binding to iC3b, and trigger cytotoxicity of iC3b-coated tumor cells. We studied the combination of the complement-activating antibody rituximab with barley-derived (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-glucan (BG) against CD-20 pos. lymphoma xenografts in SCID mice. Growth of established s.c. non-Hodgkin's lymphoma (NHL) (Daudi and EBV-derived B-NHL) or Hodgkin's disease (Hs445 and RPMI6666) was significantly suppressed in mice treated with a combination of i.v. rituximab and oral BG, when compared to mice treated with rituximab or BG alone. Survival of mice with disseminated lymphoma was significantly increased in the combination group as compared to other treatment groups. No clin. toxicity was obsd. The therapeutic efficacy and lack of toxicity of this combination supports further investigation into its clin. utility.

Answer 16:

### Bibliographic Information

**Enhanced efficacy of therapy with antisense BCL-2 oligonucleotides plus anti-CD20 monoclonal antibody in scid mouse/human lymphoma xenografts.** Smith, Mitchell R.; Jin, Fang; Joshi, Indira. Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA. *Molecular Cancer Therapeutics* (2004), 3(12), 1693-1699. Publisher: American Association for Cancer Research, CODEN: MCTOCF ISSN: 1535-7163. Journal written in English. CAN 142:211796 AN 2005:6117 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Monoclonal anti-CD20 antibody (rituximab) is active, but not curative, therapy for B-cell non-Hodgkin's lymphoma. BCL-2 is an antiapoptotic protein whose expression is dysregulated in most indolent B-cell malignancies. Antisense oligonucleotides (AS-ODNs) that down-regulate BCL-2 expression induce apoptosis and chemosensitize B-cell lymphoma cells. The authors hypothesized that BCL-2 down-regulation by AS-ODNs would sensitize cells to rituximab and improve therapeutic results. There is enhanced apoptosis and redn. in cell nos. when DoHH2 cells are treated in vitro with rituximab plus BCL-2 AS-ODNs, compared with either agent alone. There is little in vitro effect on WSU-FSCCL cells by rituximab, AS-ODNs that down-regulate BCL-2 by targeting the Ig portions of the BCL-2-Ig fusion mol., or a combination of the 2. The combination is more effective than either agent alone in clearing DoHH2 cells from ascites in scid mice. Combination therapy with AS-BCL-2-ODNs and rituximab significantly prolongs survival in both the DoHH2 and WSU-FSCCL models. With higher and repeated doses, this combination could be curative. The authors conclude that the combination of rituximab and antisense-mediated down-regulation of BCL-2 has enhanced activity against human lymphoma, prolongs survival, and could cure mice bearing human lymphoma. This merits investigation in clin. trials.

Answer 17:

### Bibliographic Information

**Potent and specific antitumor efficacy of CMC-544, a CD22-targeted immunoconjugate of calicheamicin, against systemically disseminated B-cell lymphoma.** DiJoseph, John F.; Goad, Mary E.; Dougher, Maureen M.; Boghaert, Erwin R.; Kunz, Arthur; Hamann, Philip R.; Damle, Nitin K. Oncology Discovery and Chemical and Screening Sciences, Wyeth Research, Pearl River, NY, USA. *Clinical Cancer Research* (2004), 10(24), 8620-8629. Publisher: American Association for Cancer Research, CODEN: CCRE4 ISSN: 1078-0432. Journal written in English. CAN 142:390643 AN 2004:1150196 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

CMC-544 is a CD22-targeted immunoconjugate of calicheamicin and exerts a potent cytotoxic effect against CD22+ B-cell lymphoma. This study evaluated antitumor efficacy of CMC-544 against systemically disseminated B-cell lymphoma. Scid mice received i.v. injections of CD22+ Ramos B-cell lymphoma cells for their systemic dissemination. CMC-544, G5/44, CD33-targeted CMA-676 (control conjugate) or rituximab were given i.p. 3, 9, 15, or 21 days after B-cell lymphoma dissemination. Diseased mice were monitored daily for hind-limb paralysis and death. Histopathol. examn. of CMC-544-treated and vehicle-treated diseased mice was also performed. Mice with disseminated B-cell lymphoma developed hind-limb paralysis within 35 days. When given up to 15 days

after B-cell lymphoma dissemination, CMC-544 extended survival of the diseased mice to >100 days, and these mice were considered cured. CMC-544 was efficacious when given during both the early initiation phase and the late established phase of the disease. A single dose of CMC-544 was effective in delaying the occurrence of hind-limb paralysis. In contrast, neither CMA-676 nor unconjugated G5/44 was effective. Rituximab was effective when given early in the disease process but not when the disease was established. Histopathol. anal. revealed B-cell lymphoma infiltration in brain, spinal cord, bone marrow, and kidney in vehicle-treated but not in CMC-544-treated diseased mice. Consistent with its efficacy against the disseminated B-cell lymphoma, CMC-544 also caused regression of established Ramos B-cell lymphoma xenografts in scid mice. CMC-544 confers strong therapeutic activity against systemic disseminated B-cell lymphoma and protects mice from hind-limb paralysis and death. These results support clin. evaluation of CMC-544 in the treatment of CD22+ lymphoid malignancies.

Answer 18:

#### Bibliographic Information

**Efficient elimination of B-lineage lymphomas by anti-CD20-auristatin conjugates.** Law, Che-Leung; Cervený, Charles G.; Gordon, Kristine A.; Klussman, Kerry; Mixan, Bruce J.; Chace, Dana F.; Meyer, Damon L.; Doronina, Svetlana O.; Siegall, Clay B.; Francisco, Joseph A.; Senter, Peter D.; Wahl, Alan F. Seattle Genetics, Inc., Bothell, WA, USA. *Clinical Cancer Research* (2004), 10(23), 7842-7851. Publisher: American Association for Cancer Research, CODEN: CCREFA ISSN: 1078-0432. Journal written in English. CAN 142:273558 AN 2004:1048126 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

The anti-CD20 antibody rituximab is useful in the treatment of certain B-cell malignancies, most notably non-Hodgkin's lymphoma. Its efficacy has been increased when used in combination with chemotherapy, yet anti-CD20 monoclonal antibodies (mAbs) directly conjugated with drugs such as doxorubicin (Dox) have failed to deliver drug or to demonstrate antitumor activity. We have produced anti-CD20 antibody-drug conjugates that possess potent antitumor activity by using the anti-mitotic agent, monomethyl auristatin E (MMAE), linked via the lysosomally cleavable dipeptide, valine-citrulline (vc). Two anti-CD20 conjugates, rituximab-vcMMAE and 1F5-vcMMAE, were selectively cytotoxic against CD20+ B-lymphoma cell lines, with IC50 values ranging from 50 ng/mL to 1 µg/mL. Unlike rituximab, which showed diffuse surface localization, rituximab-vcMMAE capped and was internalized within 4 h after binding to CD20+ B cells. Internalization of rituximab-vcMMAE was followed by rapid G2-M phase arrest and onset of apoptosis. Anti-CD20 antibody-drug conjugates prep'd. with Dox were internalized and localized as with rituximab-vcMMAE, yet these were not effective for drug delivery (IC50 > 50 µg/mL). Consistent with in vitro activity, rituximab-vcMMAE showed antitumor efficacy in xenograft models of CD20-pos. lymphoma at doses where rituximab or rituximab-Dox conjugates were ineffective. These data indicate that anti-CD20-based antibody-drug conjugates are effective antitumor agents when prep'd. with a stable, enzyme-cleavable peptide linkage to highly potent cytotoxic agents such as MMAE.

Answer 19:

#### Bibliographic Information

**Rituximab, cyclophosphamide, dexamethasone (RCD) regimen induces cure in a WSU-WM xenograft model and a partial remission in a previously treated Waldenstrom's macroglobulinemia patient.** Mohammad, Ramzi M.; Aboukameel, Amro; Nabha, Sanaa; Ibrahim, Dina; Al-Katib, Ayad. Division of Hematology and Oncology, Department of Internal Medicine, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA. *Journal of Drug Targeting* (2002), 10(5), 405-410. Publisher: Taylor & Francis Ltd., CODEN: JDTEAH ISSN: 1061-186X. Journal written in English. CAN 138:198246 AN 2002:629464 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

Waldenstrom's macroglobulinemia (WM) is an uncommon lymphoproliferative disease which remains incurable with current treatment protocols. A permanent WM cell line, WSU-WM, was previously established, which grows as a xenograft in severe combined immunodeficient (SCID) mice. This study investigated the antitumor effects of the rituximab (RTX), cyclophosphamide (CTX),

dexamethasone (DEX) [RCD] regimen in vivo in mice with a WSU-WM SCID xenograft and in a patient with WM. For the preclin. efficacy study, WSU-WM-bearing SCID mice received RTX (150 mg/kg/injection i.v.), CTX (90 mg/kg/injection, s.c.) as single agents, or diluent. The combination group received RTX at 150 mg/kg/injection, CTX at 150 mg/kg/injection, and DEX at 1.0 mg/kg/injection, i.v. Tumor growth inhibition, tumor growth delay, and log<sub>10</sub> kill (net) were 24.5%, 37 days, and 5.52 for RTX and 88%, 0.0 days, and 0.0 log<sub>10</sub> kill for CTX. No cures were obsd. with either agent; however, all the mice (6/6) with bilateral tumors were cured when treated with the RCD regimen. A 57-yr-old patient with relapsed WM was treated with the RCD regimen and showed an excellent partial remission for 7 mo. The patient tolerated the treatment very well, the Hb improved dramatically, platelets remained stable, the IgM level normalized and there was only minimal involvement of bone marrow. Based on these results, the RCD regimen is effective against WM and should be further evaluated in clin. trials.