

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) 131iodine-tositumomab and 90Yttrium-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. at 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

**Evaluation of CD20, CD22, and HLA-DR Targeting for Radioimmunotherapy of B-Cell Lymphomas.** Pagel, John M.; Pantelias, Anastasia; Hedin, Nathan; Wilbur, Shani; Saganic, Laura; Lin, Yukang; Axworthy, Donald; Hamlin, Donald K.; Wilbur, D. Scott; Gopal, Ajay K.; Press, Oliver W. Fred Hutchinson Cancer Research Center and the Department of Medicine, University of Washington, Seattle, WA, USA. *Cancer Research* (2007), 67(12), 5921-5928. Publisher: American Association for Cancer Research, CODEN: CNREA8 ISSN: 0008-5472. Journal written in English. CAN 147:26067 AN 2007:654953 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Despite the promise of radioimmunotherapy using anti-CD20 antibodies (Ab) for the treatment of relapsed patients with indolent non-Hodgkin lymphoma (NHL), most patients treated with conventional doses of 131I-tositumomab or 90Y-ibritumomab eventually relapse. We did comparative assessments using conventional radioimmunotherapy targeting CD20, CD22, and HLA-DR on human Ramos, Raji, and FL-18 lymphoma xenografts in athymic mice to assess the potential for improving the efficacy of radioimmunotherapy by targeting other NHL cell surface antigens. Results of biodistribution studies showed significant differences in tumor localization consistent with variable antigenic expression on the different lymphoma cell lines. Interestingly, the radioimmunoconjugate that yielded the best tumor-to-normal organ ratios differed in each tumor model. We also explored administering all three 111In-1,4,7,10-tetra-azacyclododecane N,N',N'',N'''-tetraacetic acid antibodies in combination, but discovered, surprisingly, that this approach did not augment the localization of radioactivity to tumors compared with the administration of the best single radiolabeled Ab alone. These data suggest that conventional radioimmunotherapy using anti-CD20, anti-HLA-DR, or anti-CD22 Abs is effective when used singly and provides targeted uptake of radiolabel into the tumor that is dependent on the levels of antigen expression. Improvements in tumor-to-normal organ ratios of radioactivity cannot be achieved using directly labeled Abs in combination but may be afforded by novel pretargeting methods.

Answer 3:

**Bibliographic Information**

**Conditionally Cleavable Radioimmunoconjugates: A Novel Approach for the Release of Radioisotopes from Radioimmunoconjugates.** Beeson, Craig; Butrynski, James E.; Hart, Michael J.; Nourigat, Cynthia; Matthews, Dana C.; Press, Oliver W.; Senter, Peter D.; Bernstein, Irwin D. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. *Bioconjugate Chemistry* (2003), 14(5), 927-933. Publisher: American Chemical Society, CODEN: BCCHES ISSN: 1043-1802. Journal written in English. CAN 139:303872 AN 2003:643330 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

One of the limitations of therapy with radiolabeled monoclonal antibodies (mAbs) is that significant toxicities can arise from circulating non-tumor-bound radiolabeled conjugate. Here, we describe a new method to reduce systemic radiation exposure from radiolabeled mAbs involving the attachment of the radioisotope through a linker that can be cleaved by an administered enzyme. To demonstrate the feasibility of this approach, we prepd. a conditionally cleavable radioimmunoconjugate (RIC) composed of <sup>131</sup>I-labeled cephalosporin conjugated to Tositumomab, a mAb against the CD20 antigen. The cleavable RIC bound antigen identically to directly iodinated antibody, and in the presence of  $\beta$ -lactamase, about 80-85% of the radioisotope was released. In vivo studies in mice revealed that the cleavable RIC and the directly iodinated anti-CD20 antibody had similar biodistribution patterns. Systemically administered  $\beta$ -lactamase induced a 2-3-fold decrease in the percent injected dose (ID) of the cleavable RIC/g of blood, marrow, spleen, lung, and liver 1 h after enzyme treatment, and a 4-6-fold decrease 20 h after enzyme treatment. This was accompanied by a 20-fold increase in % ID/g in urine 1 h after enzyme treatment, indicating that the released radiolabel was rapidly excreted through the kidneys. In mice with human tumor xenografts, there was no decrease in the %ID/g in tumor 1 h after enzyme treatment, but by 4 h after enzyme injection, decreases in tumor radioactive content began to diminish the targeting advantage. These studies demonstrate that the cleavable RIC substrate is able to bind to tumor antigens and localize within human tumor xenografts and that accelerated systemic clearance can be induced with  $\beta$ -lactamase.