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Perspectives

The Potential Effect of Statins on Rituximab Immunotherapy

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Background

CD20 is a cell surface marker expressed on mature B cells and most malignant B cells. It does not modulate rapidly, is not shed, and is highly expressed, leading to its use as a target for immunotherapy. In 1997, rituximab, a chimeric anti-CD20 monoclonal antibody (mAb), was approved for use in the treatment of cancer following its efficacy (46% response rate) in a phase II trial involving 37 patients with relapsed low-grade non-Hodgkin lymphoma [1]. Since then, rituximab has been approved for the treatment of numerous other B cell malignancies and is now being actively investigated for use in the treatment of autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus [2,3]. As a result, rituximab has been administered to more than a million patients worldwide, making it the most successful immunotherapeutic (commercially and clinically) used to date.

Importantly, much of the therapeutic efficacy of rituximab is seen when it is used in combination with other drugs. In the treatment of non-Hodgkin lymphoma, rituximab is routinely given in conjunction with chemotherapy, which greatly enhances the therapeutic outcome in terms of response rates, durations of remissions, and improvements in survival [1,4–6]. A similar synergy is observed between rituximab and cyclophosphamide or methotrexate [2,7,8] in the treatment of autoimmune conditions [3]. In part, these effective combinations are afforded by the distinct toxicity profiles of the different drugs and the relatively mild side effects observed with rituximab treatment. For these reasons rituximab is an ideal drug for investigating powerful combination therapies.

One group of drugs that could potentially be useful in combination with rituximab is the statins. Statins target 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR), which is the rate-limiting enzyme of the mevalonate pathway required for the synthesis of isoprenoids such as cholesterol [9]. As such, the main clinical use of statins to date has been in the treatment of hypercholesterolemia. However, in addition to inhibiting cholesterol synthesis, statins also have cytotoxic effects on tumor cells [10–13]. These cytotoxic effects are likely achieved through one of two downstream effects of HMG-CoAR inhibition. First, by reducing isoprenoid synthesis, statins impair protein prenylation, a critical process for the correct cellular localization and signaling activity of numerous proteins such as Ras that may be important for tumor cell survival. Second, reducing cholesterol synthesis can interfere with the formation of cholesterol-rich lipid microdomains, or "rafts," within the plasma membrane. These lipid rafts are thought to represent localized signaling platforms and may be particularly important in providing cell survival signals to tumor cells [14,15]. Moreover, as prenylated proteins commonly localize to lipid raft regions, statin treatment may therefore doubly compromise the biological activity of these proteins, providing potent cytostatic and cytotoxic effects. Clearly statins have an obvious anti-tumor potential, and it seems sensible to consider whether they provide a useful drug combination in conjunction with rituximab.

Linked Research Article

This Perspective discusses the following new study published in *PLoS Medicine:*

Winiarska M, Bil J, Wilczek E, Wilczynski GM, Lekka M, et al. (2008) Statins impair antitumor effects of rituximab by inducing conformational changes of CD20. PLoS Med 5(3): e64. doi:10.1371/journal.pmed.0050064

Jakub Golab and colleagues found that statins significantly decrease rituximab-mediated complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity against B cell lymphoma cells.

Statins Decrease the Efficacy of Anti-CD20 mAb by Preventing Their Binding

In a new study published in this issue of *PLoS Medicine* [16], Jakub Golab and colleagues address this very issue: namely, how do statins affect rituximab treatment? Perhaps surprisingly, they show that instead of enhancing the ability of rituximab to kill target cells, statins are inhibitory.

Rituximab has three main effector mechanisms: Complement-dependent cytotoxicity and antibody-dependent

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Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; HMG-CoAR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; mAb, monoclonal antibody

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cellular cytotoxicity (CDC and ADCC, respectively) and direct cytotoxic signaling [7,17–22] (reviewed in [23]). Using a number of well-established in vitro assays, Golab and colleagues clearly show that statins impair the ability of rituximab to lyse lymphoma cell lines with the help of either complement (CDC) or, to a lesser degree, effector cells (ADCC), and that this impairment occurs because surface binding of CD20 is greatly reduced in these cells [16]. The researchers did not examine the ability of statins to inhibit direct cytotoxic signaling, but presumably this was also reduced due to diminished mAb binding. Therefore, at least two (and likely all) of the main effector mechanisms of rituximab were reduced by statin treatment. After establishing that the loss of CD20 binding was due to a change in conformation of surface CD20 after statin treatment, the authors then performed experiments to examine the effect of statins on CD20 in vivo. Although the effects here were more marginal, again the evidence suggests that statins reduce the binding of rituximab to its target.

Statins as a Contraindication for Rituximab Treatment?

Given the potential implications of these findings, the next steps are to confirm them and then to establish the CD20 status of patients on long-term statin treatment. Subsequently, it will be important to address whether equivalent effects on CD20 binding are observed in patients suffering from malignant or autoimmune disease to determine whether B cells in these conditions are more or less susceptible to the effects of cholesterol depletion through statin treatment. Previous in vitro experiments with methyl-beta-cyclodextrin indicate that the effects of cholesterol depletion on CD20 mAb binding are highly dependent upon both the mAb and cell type [24,25]. Presumably, data on the effects of statins on rituximab use are already available through retrospective analysis, as it is likely that among the million patients treated with rituximab a proportion were also receiving statins. To date, very few cases of co-administration have been reported, but it is important to note that in at least one of these, long-term statin treatment did not appear to impair the normal therapeutic effect of rituximab [26]. These data, coupled with the limited effects seen in Figure 9 of Golab and colleagues' study [16], suggest that the pronounced effects of statin treatment on anti-CD20 binding observed on lymphoma cell lines in vitro may not translate to equivalent effects in vivo. However, note that the CD20 binding shown in [16] was assessed after only a three day treatment with atorvastatin; potentially greater effects would be observed after more protracted treatment. The definitive answer to the question of whether statins substantially affect CD20 binding and function in vivo awaits clinical investigation.

Assuming long-term statin treatment does indeed substantially reduce CD20 detection in vivo, two obvious changes to clinical management should be made. First, extensive use of statins for the treatment of hypercholesterolemia should be a contraindication for the use of CD20 as a diagnostic marker for mature B cells. Second, statins should be removed from the treatment of patients with either malignant or autoimmune disease who are required to undergo CD20-specific therapy.

Other Combinations?

Interestingly, Golab and colleagues reported (but did not show) that inhibitors of farnesyltransferase and geranylgeranyltransferase did not decrease CD20-mediated CDC [16], indicating that the effect of statins on anti-CD20 mAb binding is independent of their effects on impaired protein prenylation. As prenylation inhibition is a central component of the anti-tumor effect of statins [10,13,27,28], it is possible that farnesyltransferase and geranylgeranyltransferase inhibitors such as tipifarnib may work well in combination with rituximab. To discover if this is the case, a sensible way forward would be to examine the direct cell-killing activity of these reagents in combination with rituximab. Whether this combination will also fall foul of unexpected complications remains to be seen. ■

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