Correspondence

To the editor:

Treatment of viral hepatitis B infection in patients receiving intensive immunosuppressive therapies

We read with great interest the paper written by Raymond Liang,1 which represents an excellent and complete overview of the management of hepatitis B virus (HBV) infection during treatment of hematologic malignancies. Nevertheless, we would like to focus on the particular situation of HIV-HBV–coinfected patients. In our opinion, several points must be underlined because they lead to special attention when a hematologic malignancy, especially high-grade B lymphoma, is diagnosed in this population.

Prevalence of HBV infection is higher in HIV patients than in the general population. Moreover, in these cases, immunosuppression is generally more severe due to the coexistance of lymphoma and HIV. Addition of rituximab to the CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) regimen in HIV lymphoma patients has increased the response rate, but its immunosuppressive effects have, in some reports, led to an increase in infectious complications.2

Specific therapeutic considerations on the use of anti-HBV drugs must also be made. At first, it is important to remember that in HBV-HIV–coinfected patients, unlike HIV-negative patients, the use of 2 anti-HBV drugs is generally recommended.3 Tenofovir and lamuvidine are generally the recommended choices. Furthermore, several anti-HBV drugs (lamuvidine, tenofovir, emcitrabine, and entecavir) also have a potent activity against HIV. So these drugs should be used not alone, but as part of a highly active antiretroviral therapy against HIV. To omit this important aspect could lead to the appearance of mutations in HIV genome and, finally, to resistance to antiretroviral therapy.

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References


To the editor:

When is a predose a dose too much?

We read with great interest the review by Sharkey and colleagues1 that provides a fascinating and informative perspective on the practice of delivering a “cold” or predose of monoclonal antibody (mAb) before the delivery of the radioimmunconjugate in radioimmunotherapy (RIT) of B-cell lymphoma.

This review is important as it raises several clinically relevant questions to the application of RIT in the rituximab era. Predosing with unlabeled or “cold” anti-CD20 mAb has become standard practice in RIT targeting the CD20 antigen.2,3 The predose has been shown to increase tumor targeting of the labeled mAb by blocking “nonspecific” binding sites such as circulating and splenic B cells and is used in both licensed RIT approaches (90Y-ibritumomab tiuxetan [Zevalin] and 131I-tositumomab [Bexxar]). It is indeed timely to readdress the question of the optimal approach to predose, as in contrast to the pioneering studies, the majority of patients who are currently suitable for RIT have received rituximab.

The recent publication of the FIT study has provided compelling evidence for the efficacy of 90Y ibritumomab after induction chemotherapy with patients randomized to RIT enjoying more than a 2-year improvement in progression-free survival.4 However, the majority of patients in this study did not receive rituximab containing regimens. Therefore an important question in current clinical practice is whether predose is necessary as part of an RIT consolidation therapy after rituximab containing chemotherapy. This issue comes into sharper focus, if as suggested by Sharkey and colleagues, repeated doses of rituximab may prevent subsequent binding of radiolabeled anti-CD20 antibody to tumor and thus potentially compromise tumor targeting and clinical efficacy. Further uncertainty arises when examining the relative paucity of data on which the current licensed RIT approaches are given.2,3 The licensed predosing regimen for 90Y-ibritumomab was based on just 6 patients with differences observed in the biodistribution between 125 mg/m2 and 250 mg/m2 of rituximab and the higher dose was selected on the basis of the potential increased clinical activity of large doses of rituximab.3

Sharkey and colleagues cite recent preclinical evidence supporting the view that rituximab, if given in high enough doses, blocks the binding of the anti-CD20 radioimmunoconjugate in a Burkitt lymphoma xenograft model.5 In such xenograft models there is no cross-reactivity of the predose mAb targeting the normal host B-cell reservoir, leaving a finite antigen sink that is entirely limited to the small human tumors. In this context it is perhaps not surprising the tumors can be saturated with large enough doses of...
rituximab. Perhaps these important questions must ultimately be addressed in well designed clinical studies?

Currently there is a lack of evidence from clinical studies that prior rituximab compromises subsequent anti-CD20 based RIT. In stark contrast to the preclinical data, recent phase II clinical data using several doses of induction therapy with rituximab alone or as part of Rituximab containing chemotherapy have led to excellent clinical efficacy with high rates of conversion from partial to complete response after RIT.6 Our own recently published study attempts to address this predose question. We found that induction therapy with rituximab significantly increases the effective half-life of subsequent 131I-rituximab and correlated with increased effective half-life of the 131I-rituximab.7 Importantly, we demonstrated that multiple doses of rituximab did not appear to compromise the clinical efficacy or increase the myelotoxicity of subsequent anti-CD20 targeted RIT.

Targeting another antigen such as CD45, as suggested in the review,1 certainly bypasses the possible CD20 antigen competing effect from rituximab and is potentially an important approach to explore further. However, such an approach does not negate the predose issue, as the same dilemma remains as to how best to improve the targeting of radiolabeled anti-CD45 antibody targeting with a predose of anti-CD45.

The concern over excessive predosing adversely affecting tumor targeting in anti-CD20 based RIT remains an important theoretical concern. However decreased targeting leading to decreased efficacy of RIT has not thus far been observed in the clinic and if there is a deleterious effect with large amounts of mAb predosing, this does not appear to substantially affect the clinical efficacy.2 Perhaps of greater concern in improving outcomes for patients with follicular lymphoma is the gross under usage of RIT.

In an era where immunochemotherapy has substantially improved the outcome, it is perhaps easier to become complacent that using such a considered re-examination this will ensure that the future is not only more radiant for many patients with difficult to treat follicular lymphoma and potentially other NHL as well.

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References


To the editor:

APO866 activity in hematoologic malignancies: a preclinical in vitro study

Nahimana and coworkers have recently reported that the nicotinamide phosphoribosyltransferase (NAMPT) inhibitor APO866 elicited massive cell death in primary leukemia cells and in numerous leukemia/lymphoma cell lines.1 In particular, in 32 primary leukemias (including 12 B-cell chronic lymphocytic leukemias [B-CLLS]) these authors found that a 96 hour-exposure to 10 nM APO866 resulted in a median “fraction of dead cells” (Fdc, annexin-V [AV]+ cells) of 97%. Moreover, APO866 EC50, as measured by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-dipheny ltetrazolium bromide] colorimetric assay in 45 established hematologic cancer cell lines ranged between 0.09 and 27.2 nM. Similar experiments were performed by our group on 29 primary leukemia cell samples (23 B-CLLS, 1 T-cell chronic lymphocytic leukemia [T-CLL], and 5 acute myeloid leukemias [AMLs]). We determined cell viability by AV–propidium iodide (PI) staining and flow cytometry. Specific cell death (scd) was calculated with the formula: \( \text{scd} = \frac{x_n}{x_1/100 - x_0} \times 100 \), where \( x_n \) was the number of AV+ cells in response to a given APO866 concentration and \( x_0 \) were the AV+ elements among the untreated cells. In our hands, susceptibility to APO866 among primary leukemia cells was heterogeneous (Figure 1A,B). Most cases exhibited a minor decrease in cell viability after a 96-hour exposure to APO866, while only in 1 B-CLL sample the scd was 89%. In almost all of our titration experiments (1 nM-1 μM),
APO866 EC50 was not even reached. Finally, prolonging the exposure to APO866 (up to 10 days) did not substantially increase the efficacy of APO866 (data not shown). The observed failure to induce massive apoptosis by APO866 was not due to poor NAMPT inhibition by our compound batch as after a 48-hour treatment intracellular NAD^+ levels were typically reduced by more than 90% compared with the control cells in primary leukemias and in leukemia cell lines as detected by high performance liquid chromatography (HPLC).2 We also evaluated the response of various established cancer cell lines to APO866. Here, our results were similar to those of Nahimana et al except for the MOLT4 (T-acute lymphoblastic leukemia) cells that we found to be resistant to APO866 for doses up to 100 nM (Figure 1C). Importantly, in agreement with Hasmann and Schemainda,3 we observed that the MTT method tends to anticipate and to overestimate APO866 efficacy compared with the AV/PI flow cytometric assay. We ascribe this discrepancy to the NAD^+ dependent nature of tetrazolium-based assays, like MTT, XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] and WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2-tetrazolio)-1,3-Benzene Disulfonate).3,4 It is therefore conceivable that, by not considering this methodologic drawback, Nahimana et al underestimated, at least in some cell lines, APO866 EC50. In conclusion, we wonder if Nahimana et al reported their data on primary cell viability as an absolute value or as scd (ie, normalized to the untreated cells), because in our experience frozen hematologic cancer cells may exhibit high basal death rates. Finally, we suggest that for viability experiments the authors use a more reliable test, independent of intracellular NAD^+ levels such as sulforhodamine B.5 We believe APO866 is a promising agent in the treatment of hematologic malignancies. However, further studies are warranted to understand its mechanisms of action, as well as its potential in combination treatments.

Figure 1. Cytotoxic activity of APO866 in primary leukemia cells, MOLT4, and U266 cells. (A) Primary B-CLL (> 80% CD19^+ cells, n = 23), T-CLL (> 90% CD3, CD2, CD5, CD7^+, n = 1), and AML (> 70% blasts, n = 5) cells were isolated from peripheral blood (PB) samples by density gradient centrifugation. 10^6 cells/well were seeded in 24-well plates and cultured with or without 10 nM APO866 or 5 μg/mL fludarabine. Ninety-six hours later cells were harvested, washed, stained with fluorescein isothiocyanate (FITC)–conjugated annexin-V and PI, and analyzed by flow cytometry. (B) Quantification of early apoptotic (annexin-V^+/PI^-) and late apoptotic/necrotic (annexin-V^+/PI^+) cells on exposure to 10 nM APO866. The percentage of early apoptotic cells is shown as while late apoptotic cells are shown as (C) 3 × 10^4 MOLT4 and U266 cells/well were plated in 96-well plates and incubated with or without APO866 at the indicated concentrations for 96 hours. Thereafter, cell death was quantified by MTT colorimetric assay, and by AV/PI staining and flow cytometry. Results are presented as means of triplicate wells and SD.

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Response

NAD targeting efficiently kills hematologic cancer cells

We thank Cea and coauthors for sharing their results on APO866 activity in hematologic malignancies. We reported that primary cells and cell lines from patients with hematologic malignancies are highly sensitive toward APO866.1 Results were obtained from MTT (thiazolyl blue tetrazolium bromide), trypan blue dye exclusion, annexin V/7AAD stainings, clonogenic assays, and xenochimeric transplantation models. We are aware that NAD (nicotinamide adenine dinucleotide) synthesis inhibitors including APO866 potentially skew MTT assay results, the reason for which we confirmed results on cell lines by determining annexin V-7AAD expressions (Nahimana et al,1 Table 1) and in vivo cell viability assays (Nahimana et al,1 Figure 6). In addition, Cea and coworkers found similar results to ours on all cell lines except for Molt-4. Discrepancy concerning Molt-4 cell sensitivity to APO866 may be due to the time that cells are maintained in culture before testing. Indeed, we found that some cell lines (ML-2, Namalwa, and Jurkat) cultured for long period (ie, over 12 months [> 60 splittings]) developed resistance to APO866, possibly due to secondary genetic events (Figure 1A). We are currently dissecting the mechanisms of resistance of such cell lines.

Cea and colleagues found lower specific cell death on primary cells than what was published in our article (Nahimana et al,1 Table 2) and wondered whether the difference could be

Figure 1. Cytotoxic effect of APO866 in hematologic malignant cells. (A) ML-2, Namalwa, and Jurkat cells were cultured in RPMI+10% FCS+1% P/S after various splittings without or with 10 nM APO866. Cell death was assessed after 96 hours by flow cytometry using annexin V and 7AAD double staining. The percentage of early apoptosis cells (annexin V+ 7AAD-) are shown as [ ] and that of late apoptotic cells (annexin V+ 7AAD+) are shown as [ ]. Data are presented as means of triplicate ± SD. (B) Primary cells from patients with various hematologic malignancies (Nahimana et al,1 Table 2, patients 1-32): Acute myeloid leukemia (AML; n = 10); acute lymphoblastic leukemia (ALL; n = 3); chronic lymphocytic leukemia (CLL; n = 12); T-large granular lymphocyte leukemia (T-LGL; n = 1); T-lymphoma (TL; n = 1); marginal zone lymphoma (ML; n = 3); mantle cell lymphoma (MCL; n = 1); and follicular lymphoma (FL; n = 1) were cultured and cell death monitored as in panel A. Specific cell death (%) induced by drug was calculated according to the formula scd = [(S-C)/(100-C)] × 100; where S = treated sample cell death and C = untreated sample cell death. (C) 0.5–1 × 10⁶ Jurkat cells were cultured in various media as indicated on x-axis in the presence or absence of 10 nM APO866. Specific cell death calculated as mentioned above. #P indicates number of cell splittings.
To the editor:

Recipient plasmacytoid DCs are not required to prime allogeneic T-cell responses after BMT

We read with interest the elegant studies of Koyama and colleagues1 in which they demonstrate the ability of recipient conventional dendritic cells (cDCs) and plasmacytoid DC (pDCs) to prime allogeneic T-cell responses and initiate graft-versus-host disease (GVHD) when adoptively transferred into irradiated MHC class I/II recipients. Intriguingly, they report that the ability of recipient pDCs to prime allogeneic donor T-cell responses is dependent on their activation by the inflammatory environment generated by total body irradiation (TBI).

We recently investigated the role of pDCs in GVHD, and in the course of this study we were surprised to observe that recipient pDCs were exquisitely sensitive to myeloablative doses of TBI.2 As such, we were intrigued by the paradox presented by Koyama et al’s report, that is, that pDCs have the capacity to present host alloantigen only when activated by TBI; but in response to TBI, we have observed their complete elimination within 24 hours (Figure 1A).

To eliminate the possibility that the observed sensitivity of lymphoid organ pDCs to TBI simply reflects their trafficking into additional sites, we administered the pDC-depleting 120G8 mAb to irradiated B6D2F1 mice before the transplantation of allogeneic T cells. Equivalent donor T-cell responses were observed when mice treated with TBI were administered pDC-depleting or control antibody (Figure 1B,C). These data confirm that pDCs are systemically depleted by TBI (or alternatively, that residual pDCs remain in nonlymphoid tissue, but make no contribution to alloreactivity), and therefore strongly argue against the assertion that host pDCs play a significant role in the presentation of host alloantigen to donor T cells after TBI conditioning.

It is nonetheless possible that pDCs may play a role in priming allogeneic donor T-cell responses after non–TBI-based transplantation. We therefore also examined the effect of pDC depletion on the priming of allogeneic parental T cells when transferred into nonirradiated F1 recipients treated with NK1.1 to eliminate antidonor hybrid resistance. Consistent with the requirement for pDC activation by TBI demonstrated in the Koyama paper, no difference in donor T-cell responses were seen in the presence or absence of recipient pDCs (Figure 1B,C). As an intriguing aside, the only statistically significant finding was that donor cells injected into TBI-treated mice underwent significantly less proliferation than in the nonirradiated setting. This likely reflects the 10-fold greater numbers of host antigen-presenting cells (APCs) remaining at the time of bone marrow transplantation (BMT) in nonirradiated recipients.3,4 Because host pDCs fail to prime donor T cells in the absence of TBI conditioning1 and TBI itself rapidly depletes host pDCs, we suggest that this APC subset is unlikely to be an important population in the initiation of GVHD.

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Reference

Figure 1. Effects of recipient pDCs on donor T-cell priming. (A) B6D2F1 mice were irradiated with 1300 cGy in 2 split doses of 650 cGy, 3 hours apart. At the time of the second irradiation, mice were injected with 1 mg of the pDC-depleting mAb 120G8 or isotype control antibody MAC49. Twenty-four hours later (the usual time of transplant), bone marrow and spleen were examined for pDC and cDC content. Anti–PDCA-1 (Miltenyi Biotec, Bergisch Gladbach, Germany) and CD11c (BD Pharmingen, San Diego, CA) mAbs were used for flow cytometric analysis. DCs were enriched using density-gradient centrifugation before examination. Representative plots shown. (B) B6D2F1 mice received TBI or 1 mg anti-NK1.1 as pretransplant conditioning, followed by 120G8 or control mAb. Purified carboxyfluorescein succinimidyl ester (CFSE)-labeled B6 CD45.1+CD4+ T cells were injected and proliferation of splenic T cells analyzed by CFSE dilution 3 days later. Cells were stained with CD45.1, CD4, and the vital dye 7-AAD. Histograms shown are gated on live CD45.1+CD4+ cells. No difference in alloresponse was observed in the absence of pDC in either the irradiated or nonirradiated setting. (C) Modfit software (Verity Software House, Topsham, ME) was used to quantify the extent of donor T-cell proliferation. Calculated proliferation index = % all cells/computed number of parent cells. Data shown representative of 3 experiments, with n = 12 and n = 7 in respective TBI and NK1.1 groups. P = .31 nonirradiated control mAb versus nonirradiated 120G8. *P < .001 irradiated versus nonirradiated control and 120G8 mAb treated.

References

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