Response

We appreciate the opportunity to respond to Dr Masuda’s questions. His first line of questioning pertains to potential resistance mechanisms. We are interested in identifying and testing potential resistance mediators, including alternate angiogenic pathways, such as the FGF2–FGFR and EphB4–EphrinB2 pathways (1,2) and infiltrating vascular progenitor cells, such as CD11b-positive myelomonocytes (discussed in our article). Indeed, simultaneous blockade of EphB4–EphrinB2 and DLL4–Notch signaling has been shown to inhibit tumor growth more than blockade of either pathway alone (2). The discovery of resistance mediators is also important for the development of predictive biomarkers for DLL4–Notch inhibition therapy (3). We also agree that the potential contribution of cancer stem-like cell differentiation into tumor endothelial cells will be important to explore, especially given the demonstration by Ricci-Vitiani et al. (4) that Notch inhibition repressed the transition from CD133-positive CD144-negative cells to CD133-positive CD144-positive endothelial progenitor cells. Dr Masuda asks whether engraftment of tumors would be...
delayed or prevented if therapy was started immediately after tumor cell injection into mice. Although this experiment is potentially feasible, we do not believe that the information gained would be clinically relevant. Rather, we performed our experiments to mimic a common clinical situation in which therapies are initiated after patients have an established or advanced tumor, and the tumor has an established vasculature.

Dr Masuda’s concern that the gamma secretase inhibitor (GSI) dibenzazepine (DBZ) would be more effective on tumor growth delay if it was administered more frequently, or if the dosage was optimized, was addressed in the discussion: We were not able to use more frequent or prolonged dosing of DBZ due to gastrointestinal toxicity. It is important to highlight once again, however, that the DLL4 monoclonal antibody does not result in gastrointestinal toxicity and thus does not require dosing alterations to avoid this dose-limiting toxicity.

Lastly, Dr Masuda questions whether a longer exposure of cells to Notch inhibitors might be required to observe growth suppression in vitro. Previous studies investigating the effects of GSIs on in vitro proliferation of carcinoma or glioma cell lines used a 5-day treatment period, such as that used in our study. Concerning NOTCH1-activating mutations, to our knowledge, these have only been shown to be partly indicative of resistance to GSIs within the context of acute T-cell acute lymphoblastic leukemia and not for solid tumors. In addition, the complexity of resistance to Notch inhibitors has been demonstrated, and it is very likely that a “GSI-resistance signature” will be required to predict tumor response and resistance to GSIs (5). Dr Masuda also cites recent articles demonstrating that NOTCH1 is inactivated in 10%–15% of head and neck squamous cell carcinomas (6,7). However, once again, the NOTCH1 mutational status in FaDu cells is unlikely to be relevant to the response we saw because inactivation of NOTCH1 in these tumor cells would imply that the tumor radioreponse is due to DLL4–Notch inhibition in the stroma, which we have already posited.

References

Notes
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