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Letter to the Editor: Reply to E.L. Göttgens et al. regarding “Inhibiting BRAF Oncogene-Mediated Radioresistance Effectively Radiosensitizes BRAF^{V600E} Mutant Thyroid Cancer Cells by Constraining DNA Double-strand Break Repair”

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We thank E.L. Göttgens et al. on providing insightful comments on our publication. We agree that vemurafenib will commonly induce G1 arrest in BRAF mutant tumor cells, but that this typically happens over the course of 12–24 hrs or longer. We were observing higher levels of γ H2AX within just one hour after radiation with 3 hours of pre-treatment with vemurafenib (total= 4 hours of vemurafenib) in Figure 3A, arguing for a direct effect of vemurafenib on altering repair of double-stranded breaks. In addition, G1 arrested cells can be radioresistant in the earlier portions of G1, compared to late G1 when they are more radiosensitive (as they approach early S phase), so it's not clear that altered cell cycle state is playing a direct role in radiosensitization. However, we do agree that changes in cell cycle distribution towards a G1 arrested state could preferentially result in engagement of NHEJ DNA repair pathways which can account for our nuclear foci repair results. Despite this, we find that expression of mutant BRAF-V600E results in increased NHEJ repair activity in our GFP reporter system in the absence of vemurafenib, but not HR repair (Fig. 4C). In this experiment testing the effect of BRAF-V600E and vemurafenib on NHEJ or HR activity in our GFP reporter system, we are not inducing DNA damage by radiation, but rather by the endonuclease I-SceI, which induces double-strand breaks in our reporter DNA at the recognition sequence. The reason we treated with vehicle or vemurafenib at the same time as treatment with adenoviral I-SceI was to allow for time of infection and expression of I-SceI enzyme over the course of 24 hours. In effect, this served as a period of pre-treatment with vemurafenib prior to DNA damage induction by I-SceI. As shown in the right panel of Fig. 4C, we did not observe an increase in NHEJ repair activity of cells treated with 42 hrs (18 + 24 hrs) of vemurafenib in the empty vector (control) transfected cells, arguing against a role for an increase in G1 fraction accounting for the differences in DNA repair. However, in cells expressing BRAF-V600E, there was a higher level of baseline NHEJ repair activity in the absence of vemurafenib, which was significantly reduced when cells were treated for 42 hrs with vemurafenib.

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Our findings also suggest that a mechanism for heightened DNA repair in BRAF mutant cells may be due in alterations in XLF expression. The reason for selecting XLF as our focus for this manuscript was that out of the NHEJ repair pathway proteins identified to be upregulated in Table S2, we were able to verify that up-regulation of XLF RNA resulted in protein up-regulation by immunoblotting. We agree more in depth study is warranted to more comprehensively assess the effects of mutant BRAF on DNA repair, particularly NHEJ repair.

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