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Original Research Article

Dual blockade of PI3K and MEK in combination with radiation in head and neck cancer

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ABSTRACT

Background and purpose: In this study we have combined fractionated radiation treatment (RT) with two molecular targeted agents active against key deregulated signaling pathways in head and neck cancer.
Materials and methods: We used two molecularly characterized, low passage HNSCC cell lines of differing biological characteristics to study the effects of binimetinib and buparlisib in combination with radiation *in vitro* and *in vivo*.
Results: Buparlisib was active against both cell lines *in vitro* whereas binimetinib was more toxic to UT-SCC-14. Neither agent modified radiation sensitivity *in vitro*. Buparlisib significantly inhibited growth of UT-SSC-15 alone or in combination with RT but was ineffective in UT-SCC-14. Binimetinib did cause a significant delay with RT in UT-SCC-14 and it significantly reduced growth of the UT-SCC-15 tumors both alone and with RT. The tri-modality treatment was not as effective as RT with a single effective agent.
Conclusions: No significant benefit was gained by the combined use of the two agents with RT even though each was efficacious when used alone.

Background and significance

HPV-negative head and neck cancer (HNSCC) carries a poor prognosis despite optimal treatment with chemoradiation [1] which approaches the limit of tolerance. It is likely that improvements will be made by novel therapies based on the molecular mechanisms of HNSCC. Early success was achieved by targeting the EGFR pathway [2] but since then very few targeted agents have advanced to phase III trials with radiotherapy [3].

Genomic analysis of HNSCC from the TGCA proposed at least 15 significantly mutated genes including *CDKN2A, TP53, PIK3CA, FAT1, MLL2, TGFBR2, HLA-A, NOTCH1, HRAS, NFE2L2*, and *CASP8* [4]. Many of the genes converge on the EGFR/RAS/RAF/ERK/PI3K/AKT/mTOR cascade which has been reported to be one of the most frequently altered signaling pathways in HNSCC [5]. These pathways offer multiple targets for therapy. However, targeting these pathways with single agents has produced little success in translating this approach into clinical benefit [6].

This lack of success is likely due to the complexity of the signaling pathways in cancer where there are multiple nodes, feedback loops, crosstalk and redundancy. To overcome these issues it would seem rational that targeting these pathways at several points simultaneously might be more effective [7,8]. One of the mechanisms of cetuximab resistance is the presence of mutations, in particular *RAS*, that constitutively activate key downstream signaling mediators [9]. In addition, reports have also suggested that *BRAF* may also be frequently mutated in HNSCC [10]. This has led us and others [8] to target the EGFR signaling downstream at the level of MEK1 and 2. Another key pathway identified by the TCGA study was PI3K/AKT/mTOR signaling which has been shown to have an important role in the pathogenesis of HNSCC [11] and there is evidence PI3K antagonists are active HNSCC cells [12,13].

In this study, we investigated binimetinib, a potent oral inhibitor of MEK1/2 currently being studied in several clinical trials [14–16], and buparlisib, a specific oral inhibitor of the pan-class I PI3K family also under investigation in clinical trials [17–19] in two contrasting HNSCC models with different *EGFR, HRAS* and *PI3K* status and studied their combination with fractionated radiation.

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Materials and methods

Cell lines and drugs

The UT-SCC-14 and UT-SCC-15 cell lines were provided by Dr. Reidar Grénman (Turku University Hospital, Turku, Finland). Both are low passage HPV-negative cell lines and their culture and molecular characterization have been previously described [20]. Buparlisib and binimetinib were kindly provided by Novartis Pharma (Basel, Switzerland). A 10 mM solution of each was prepared in dimethyl sulfoxide and stored at −70 °C for *in vitro* experiments.

Irradiations

Cells were irradiated as previously described with an Xstrahl X-ray System, Model RS225 (Xstrahl, UK) [20].

3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Three-(4, 5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to assess the effects of the drugs on cell growth/viability. Cells were plated into 96 well plates and allowed to attach overnight. The next day the media was exchanged for media containing various concentrations of buparlisib and binimetinib and the plates returned to the incubator. After an additional 3 days, MTT (5 mg/mL in phosphate-buffered saline) was added to each well and the plate returned to the CO2 incubator for ~5 h. Media containing the MTT was then aspirated from the wells, and dimethyl sulfoxide was added to dissolve the purple formazan. After 5 min incubation at 37 °C, absorbance readings (at 560 nm and 670 nm) were taken on a Versamax multiplate reader (Molecular Devices, Sunnyvale, CA). To assess the effect of the combination of the drugs on cell growth, different single concentrations of buparlisib (0.1, 0.25 and 0.5 μM) were incubated with increasing concentrations of binimetinib. To study timing of drug exposure in combination with 4 Gy irradiation (RT), 0.5 μM buparlisib or 0.5 μM binimetinib were added to plated cells either 1 h pre-RT or 1, 4 or 24 h post-RT.

Clonogenic assay

Cells were irradiated and then plated into flasks containing 0.2 μM buparlisib, 0.1 μM binimetinib or their combination. Untreated cells and drug(s)-only treated cells were also assessed. Colonies were allowed to develop for 10–14 days, stained with crystal violet counted, and surviving fractions calculated. Data was normalized for plating efficiency to the unirradiated, non-drug treated controls and survival curves were fitted using the LQ model.

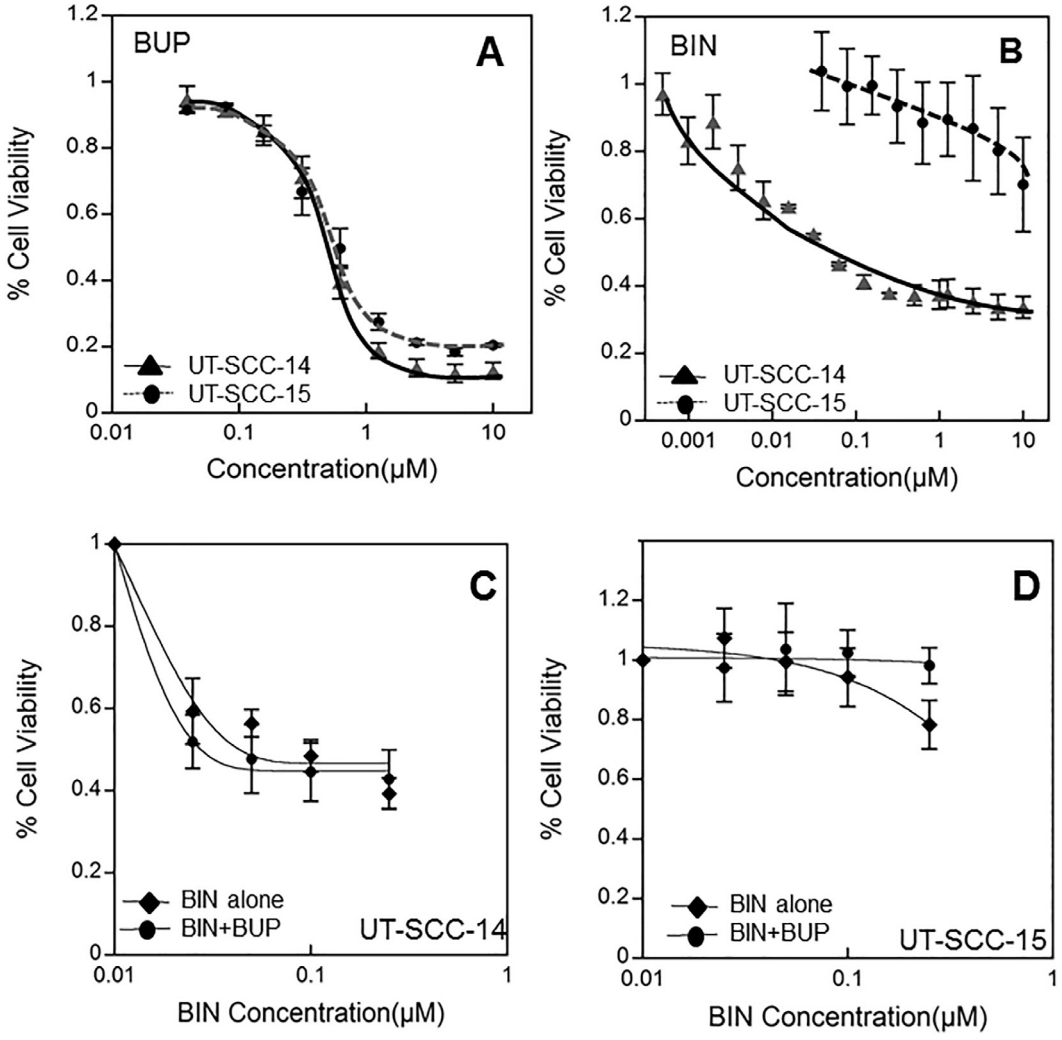

Fig. 1. The effect buparlisib (BUP) (A) and binimetinib (BIN) (B) on the growth of UT-SCC-14 and UT-SCC-15 cells *in vitro* as single agents or in combination (C and D). In C and D, the concentration of buparlisib was 0.25 μM.