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Assessing the role of Phosphoinositol-3 Kinase in Head and Neck cancer

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Background: Head and neck cancer is the sixth most common cancer worldwide. Though several frequent molecular pathological mechanisms have been identified, including high-risk HPV infections, inactivation of p16, p53-mutations or EGFR amplification, the tumorigenesis of head and neck cancers is still not enough understood. The phosphoinositide 3-kinase (PI3K) - AKT pathway is a key signal pathway for cell survival that is activated in many human cancers. As a major effector downstream of receptor tyrosine kinases, PI3K transduces signals by generating phospholipids that activate AKT, mTOR and other downstream effectors. Frequent genetic alterations of PI3K have been reported in head and neck cancers, indicating a pivotal role of PI3K-AKT in the tumorigenesis of HNSCC.
The purpose of this *in-vitro* study is to assess the role of PI3K-pathway by evaluating the efficacy of PI3K-pathway inhibition and determining possible biomarkers of response in head and neck cancer cell lines.

Methods: The status of MET, EGFR, PTEN and AKT were evaluated in 13 HNSCC cell lines by immunoblotting. In order to detect mutations, exons the p110-α catalytic subunit (PIK3CA) and the p-85-α regulatory subunit (PIK3R1) were sequenced in 11 cell lines and 20 tumor tissue samples. Effects of PI3K inhibition (LY294002), dual PI3K/mTOR inhibition (NVP-BEZ235), and mTOR inhibition (Rad001) on viability in 12 cell lines further were determined using resazurin viability assay. Moreover, apoptotic effect of NVP-BEZ235 was evaluated in 2 selected cell lines using Caspase 3/7 Assay. Apoptotic effects of the EGFR-inhibitor Erlotinib and the combination of NVP-BEZ235 and the MEK-inhibitor AZD6244 were compared to those of NVP-BEZ235 using Annexin V-Propidiumiodide Assay in 6 selected cell lines. Based on these results, effects of combination of NVP-BEZ235 and AZD6244 on EGFR expression in an EGFR-amplified cell line were studied using immunoblotting and Protein Phosphorylation Arrays. Last of all, synergistic effects of NVP-BEZ235 and the MET-inhibitor SU11274 / the EGFR-inhibitor Erlotinib were evaluated using resazurin viability assay.

Results: The PI3K-AKT pathway was activated in the majority of HNSCC cell lines, though AKT-phosphorylation is not directly associated with PIK3CA mutation status or sensibility to PI3K-inhibitors. Sequencing revealed two classical hotspot mutations in PI3KCA (E542K and H1047R), suggesting that PIK3CA mutations are rather uncommon in HNSCC. No non-synonymous mutations were detected in the iSH2-domain of PIK3R1. Resazurin viability assay showed that all 4 HPV16 positive cell lines were very sensitive to PI3K inhibition, which can be possibly explained by PIK3CA overexpression in HPV16+ HNSCC; 2 of 3 EGFR amplified cell lines showed poor response to PI3K inhibition; both cell lines with PIK3CA hotspot mutations were shown to be sensitive, as expected, to PI3K- but relatively resistant to mTORC1 inhibition. Increased sensitivity to PI3K inhibition is also observed in 3D soft agar culture. Caspase 3/7 Assays and Annexin V-Propidium Iodide Assay indicated that NVP-BEZ235 is a potent cytotoxic inhibitor in PIK3CA-mutated and HPV16 positive cell lines. Combinations of NVP-BEZ235 and AZD6244 showed additive or antagonistic effects on apoptosis in PIK3CA-mutated and HPV16+ cell lines, whereas the EGFR amplified cell line SQ20B showed clearly increased apoptosis when both inhibitors were combined. Treatment with NVP-BEZ235 and AZD6244 leads to inhibition of EGFR phosphorylation and expression and decreased phosphorylation of p53 in SQ20B. The combination of NVP-BEZ235 and the EGFR-inhibitor Erlotinib showed synergistic effects in all tested HNSCC cell lines and represents an effective therapeutic option. The combination of NVP-BEZ235 and the MET-inhibitor SU11274 is far less effective.

Conclusion and outlook: PI3K appears to be an attractive novel target for head and neck cancers with positive HPV16 status or PIK3CA mutation. Further in-vivo validation is indicated. In order to explain the strong sensitivity of HPV16+ cell lines to PI3K inhibitor, PIK3CA gain of copy number or amplification and PIK3CA gene expression should be evaluated. Combined inhibition of both PI3K- and MEK- pathway has shown to be an interesting therapeutic option, especially for cancer cells addicted to EGFR. This synergistic effect and the underlying mechanism need to be further studied in other EGFR-amplified or mutant HNSCC cell lines or tumors. The combination of NVP-BEZ235 and Erlotinib represents an effective therapeutic option in HNSCC.