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Tyrosine Kinase Inhibitors and Everolimus Reduce IGF1R Expression in HPV16-positive and -negative Squamous Cell Carcinoma

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Abstract. *Background/Aim: The effects of tyrosine kinase inhibitors (TKI) in head and neck squamous cell cancer (HNSCC) are not fully understood. We investigated the effects of selective TKIs erlotinib, gefitinib, nilotinib, and dasatinib and the mTOR-inhibitor everolimus on the expression of insulin-like growth factor 1 receptor (IGF1R) in HPV-positive and HPV-negative squamous cancer cell lines. Materials and Methods: HPV-negative UMSCC-11A and UMSCC-14C cells and HPV-positive CERV196 cells were treated with TKIs or everolimus. Protein concentration of IGF1R was measured using ELISA. Results: IGF1R expression was significantly reduced by all tested TKIs and everolimus in both HPV-negative cancer cell lines. In HPV-positive squamous cancer cells we observed significant protein inhibition. Conclusion: The crosstalk between epidermal growth factor receptors and IGF1R could be of central interest for the development of novel medical approaches for individualized therapy.*

Head and neck squamous cell cancer (HNSCC), most frequently occurring in the oral cavity and the oropharynx, comprises malignant tumors that can affect the entire upper aerodigestive tract. Main risk factors for HNSCC include tobacco and alcohol consumption (1). The prevalence of HNSCC is increasing worldwide (2, 3), due to an infection with high-risk subtypes of the human papilloma virus (HPV) (4, 5). The tumor-node-metastasis (TNM) staging for HNSCC published in 2017 has been updated due to related findings. Currently, $p16^{INK4A}$ immunostaining is included as a surrogate parameter for an HPV infection of HNSCC (6). The types of HPV are classified into low-risk and high-risk, according to the probability of inducing cancer. Approximately 20% of HNSCC cases present with HPV infection (7). The high-risk type of infections requires more time to overcome, and the risk of the integration of viral DNA into the host genome is higher. The integration results in an overexpression of the viral oncogenes E6 and E7 with subsequent stimulation of cell proliferation resulting in genomic instability (8). High-risk type (16 and 18) of infections are found in nearly 90% of HPV-associated oropharyngeal tumors (9). Patients with HPV-positive oropharyngeal cancer are younger (aged 30-50 years) and are often diagnosed with early occurrence of lymphogenic neck metastases (10-12). However, HPV-positive HNSCC carries a more favorable prognosis than HPV-negative cancers – although recent studies have not shown promising results regarding the de-escalation of treatment (13-15). Current treatment options for patients with HSNCC include a combination of chemotherapy, radiation, and surgery. Moreover, new target therapy approaches with the application of monoclonal antibodies (*e.g.* cetuximab), checkpoint-inhibitors, or tyrosine kinase inhibitors (TKIs, *e.g.* imatinib, nilotinib, dasatinib, erlotinib, and gefitinib) are intensively investigated and are already integrated in experimental therapy options.

The modulation of key signaling pathways for the development of HNSCC is intensively investigated (16). Deregulated tyrosine kinases play an important role in tumor progression by modifying cell signaling cascades. Selective TKIs inhibit key signaling tyrosine kinases by competing for the adenosine triphosphate binding site (17). Up to date, TKIs are established in the therapy of various tumors (18). Nilotinib and dasatinib inhibit BCR-ABL and are used for the treatment

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of chronic myelogenous leukemia (CML) (19, 20). Other TKIs, such as gefitinib and erlotinib, inhibit the epidermal growth factor receptor (EGFR) and are used in the treatment of non-small cell lung cancer (21-23). The mammalian target of rapamycin (mTOR) inhibitor everolimus (24) is also used in the treatment of malignant tumors (25). Recent studies conducted by our group revealed positive effects of everolimus on HPV-positive squamous carcinoma cells (26). However, tumor-specific reactions induced by the modulation of several TKIs and mTOR are not yet fully understood.

The insulin-like growth factor-1 receptor (IGF1R) is a receptor tyrosine kinase that is expressed in several human tissues. After IGF1R activation by IGF1, the tyrosine kinase domains of IGF1R become active *via* transphosphorylation, initiating downstream signaling through the phosphoinositide-3-kinase/Akt/mTOR and Ras/mitogen-activated protein kinase/ERK kinase/extracellular-signal-regulated kinase pathways. The activation of the IGF1R pathway can stimulate differentiation, migration, proliferation, survival, and angiogenesis in a context-specific way. IGF1R is overexpressed and investigated as a therapeutic target in several human cancers (27-31). Increased levels of circulating insulin-like growth factor-1 (IGF-1) are associated with an increased risk of colorectal, breast, prostate, and lung cancer (32-35). Increased level of circulating IGF1 is associated with increased risk of second primary malignancy in HNSCC (36). High levels of IGF1R are associated with high tumor (T)-stage, shorter disease-specific survival, and decreased overall survival (30). Inhibition of IGF1R can slow tumor growth in several human xenograft models (28, 37, 38). Furthermore, IGF1R signaling is associated with increased resistance to various antitumor therapies (39). This includes targeted EGFR inhibition, and it has been suggested that IGF1R contributes to EGFR-TKI (gefitinib) resistance *via* a pro-survival mechanism in HNSCC cells (40).

Up to date, eight HNSCC tumor sample studies are available, and all samples exhibited significant expression of IGF1R and IGF1R phosphorylation. The findings suggest that IGF1R may be relevant *in vivo*, and, thus, combined EGFR/IGF1R inhibition could be beneficial for some patients as a targeted molecular therapy (40).

However, there is lack of evidence regarding the influence of TKIs and everolimus on the expression of IGF1R. Therefore, we investigated the effect of the TKIs erlotinib, gefitinib, dasatinib, and nilotinib and the mTOR-inhibitor everolimus on IGF1R in an *in vitro* study with one HPV-positive and two HPV-negative squamous cancer cell lines.

Materials and Methods

Experiment design. Two human HPV16-negative squamous cancer cell lines [University of Michigan Squamous Cell Carcinoma (UMSCC)] provided by T.E. Carey, Ph.D. (University of Michigan, Ann Arbor, MI, USA) and one human HPV16-positive squamous cancer cell line (CERV196; Cell Lines Service GmbH, Eppelheim, Germany) were examined. The HPV16-negative cell lines were harvested from a skin metastasis of an oral cavity SCC of the floor of the mouth after surgery and radio-chemotherapy (UMSCC-14C) and from an untreated laryngeal SCC of the epiglottis (UMSCC-11A). The HPV16-positive cell line descended from a cervix SCC. The HPV-negative cells were cultured in Eagle's minimum essential medium (Gibco, Life Technologies, Carlsbad, CA, USA), containing 2 mM of L-glutamine and 10% fetal calf serum and antibiotics/antimycotics, according to the supplier's instructions. CERV196 cells were cultured in Eagle's minimum essential medium (Gibco, Life Technologies), supplemented with 2 mM of L-glutamine, 1.0 g/l sodium bicarbonate, 0.1 mM of non-essential amino acids, 1.0 g/l sodium pyruvate, and 10% fetal bovine serum (Gibco, Life Technologies). Incubation was performed under standardized conditions at 37˚C, 5% CO_2, and 95% humidity. Cells were subcultured by adding a PBS solution supplemented with 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA, Sigma Aldrich; Merck KGaA, Darmstadt, Germany) at 37˚C for 5 min.

Erlotinib, gefitinib, nilotinib, dasatinib, and everolimus were sponsored by Professor R.D. Hofheinz (Oncological Department, University Hospital Mannheim, Medical Faculty Mannheim, University of Heidelberg, Germany). All drugs were stored at room temperature and dissolved in dimethyl sulfoxide when needed. We added 20 μmol/l of each drug to the cultures, and the cells were incubated at 37˚C for 24, 48, 72, and 96 h. Untreated cells served as negative controls under identical conditions.

Enzyme-linked immunosorbent assay for IGF1R and proliferation assay. Experiments were repeated at least three times (n=3). The alamarBlue (AbD Serotec, Raleigh, NC, USA) cell proliferation assay was used to assess the proliferation of HNSCC cells, following the manufacturer's protocol. Protein concentrations were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions. DuoSet ELISA (DYC391, R&D Systems, Minneapolis, MN, USA) was used for IGF1R. MRX Microplate Reader (DYNEX Technologies, Chantilly, VA, USA) was used to measure optical density at a wavelength of 450 nm and wavelength correction of 540 nm. The detection range was 250-16000 pg/ml for IGF1R. The interassay coefficient of variation provided by the manufacturer was below 10%.

Statistical analysis. For statistical analysis, mean values were used. Mean values are presented±standard deviation. SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA) was used for the two coefficient variance test and Dunnett's test. A p-value of ≤0.05 was considered statistically significant. Professor C. Weiss (Head of the Department of Medical Statistics, Biomathematics and Information Processing, Medical Faculty Mannheim, University of Heidelberg, Germany) supported us in the statistical evaluation.

Results

IGF1R was detected in the three tested cell lines, with expression levels increasing over the culture time of untreated cells and after incubation with nilotinib. The

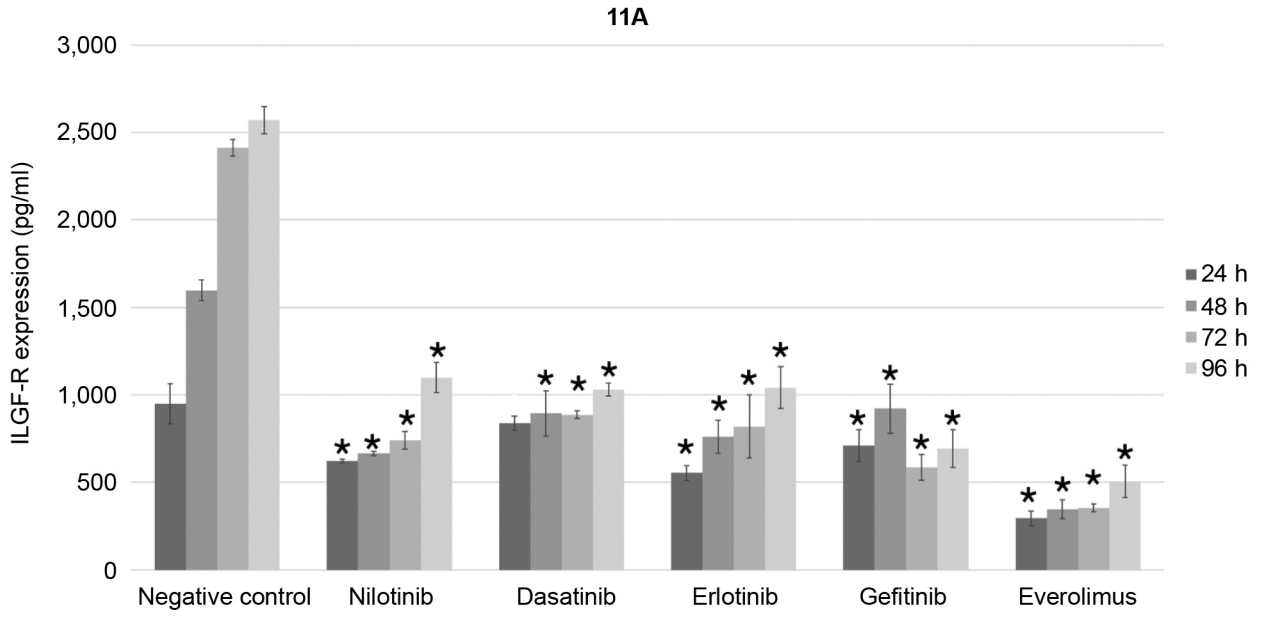

Figure 1. *IGF1R expression in UMSCC-11A cells after incubation with nilotinib, dasatinib, erlotinib, gefitinib, or everolimus relative to the negative control. Data are mean values with standard deviations. Significance (p-value of ≤0.001) is marked with *.*

highest IGF1R expression (3892 pg/ml) was measured in the untreated UMSCC-14C culture after 96 h of incubation. The addition of nilotinib, dasatinib, erlotinib, gefitinib, or everolimus led to a significant (all $p<0.001$) decrease in IGF1R levels in both HPV-negative cell lines, except for dasatinib treatment after 24 h ($p=0.2180$). The strongest effect was observed after incubation with everolimus in both HPV-negative cell lines. The expression patterns in the treated cells were similar in UMSCC-11A cells except for gefitinib treatment. The expression levels in treated cells were similar after incubation with any of the tested drugs in UMSCC-11A cells. The expression levels of IGF1R in the negative control were higher in UMSCC-14C cells relative to UMSCC-11A cells. The expression levels in treated UMSCC-14C cells were similar to those of treated UMSCC-11A cells. The strongest effect on IGF1R inhibition in UMSCC-14C was observed after incubation with everolimus. For the HPV-positive cell line, the data were not as uniform. Irrespective of drug treatment, expression levels of IGF1R were significantly lower in HPV-positive cancer cells compared with HPV-negative cancer cells. Interestingly, nilotinib-treated cells showed an almost identical expression compared with the negative control. Moreover, treatment effects could be seen only after 24 h. Nilotinib only led to a significant reduction ($p<0.001$) of IGF1R after 72 h, whereas no significant alteration was seen at the other measurement points. The expression patterns and the grade of expression of IGF1R were similar after incubation with dasatinib, erlotinib, gefitinib, or everolimus. The addition of dasatinib, erlotinib, gefitinib, or everolimus led to a significant reduction ($p<0.001$) of IGF1R expression after 48, 72, and 96 h, but no significant effect was seen after 24 h. Details are provided in Figures 1-3.

Discussion

Intensive research has been accomplished to improve therapy approaches that add to classic therapies (combinations of surgery, radiation, and chemotherapy). The monoclonal antibody cetuximab targeting EGFR has been established for HNSCC treatment (41), but other drugs also targeting EGFR such as the humanized antibody panitumumab and small molecule TKIs erlotinib and gefitinib have not yet reached significant effects compared with classic platinum-based therapy (42-52). Activation of EGFR, which is overexpressed in 90% of HNSCC, leads to receptor kinase activation followed by activation of signal transducer and activator of transcription 3 (STAT3) pathways, resulting in the promotion of cell proliferation, angiogenesis and invasion, and apoptosis inhibition (53, 54). EGFR-overexpression in HNSCC correlates with poor prognosis and radiation resistance (53, 55). Other TKIs inhibiting BCR-ABL such as nilotinib have not yet included in *in vivo* approaches but have been studied *in vitro* (56, 57). Further, mTOR inhibition with everolimus (mostly used in combination therapies) has not shown promising results in HNSCC treatment, and response rates remain moderate (58-60). Because numerous clinical studies could not

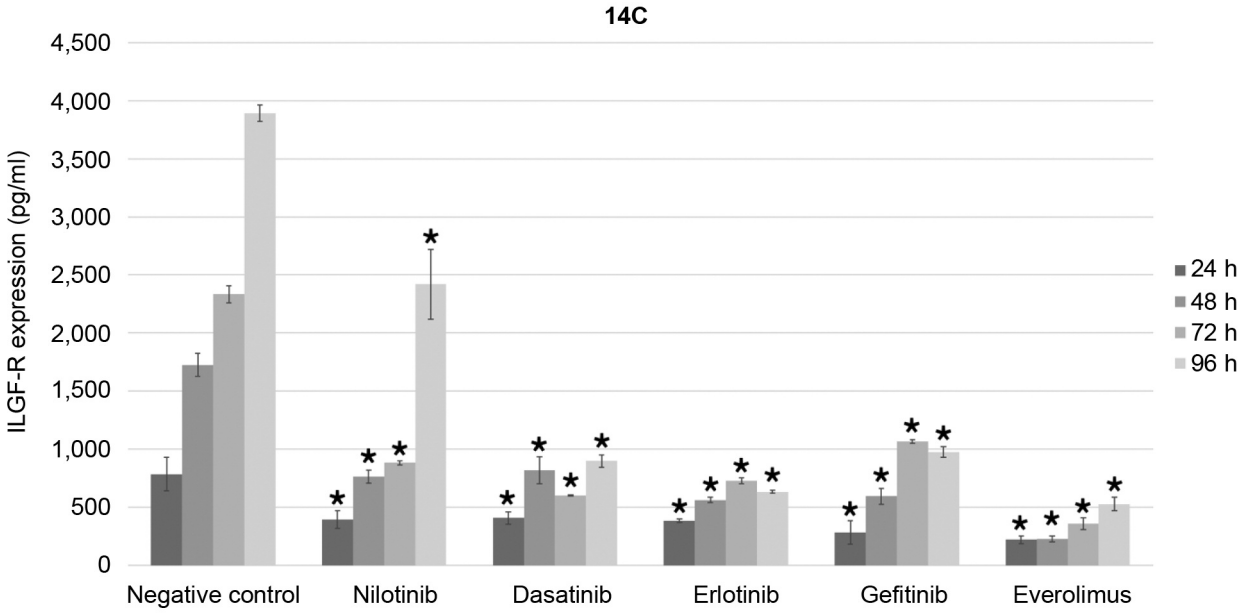

Figure 2. *IGF1R expression in UMSCC-14C cells after incubation with nilotinib, dasatinib, erlotinib, gefitinib, or everolimus compared with the negative control. Data are mean values with standard deviations. Significance (p≤0.001) is marked with *.*

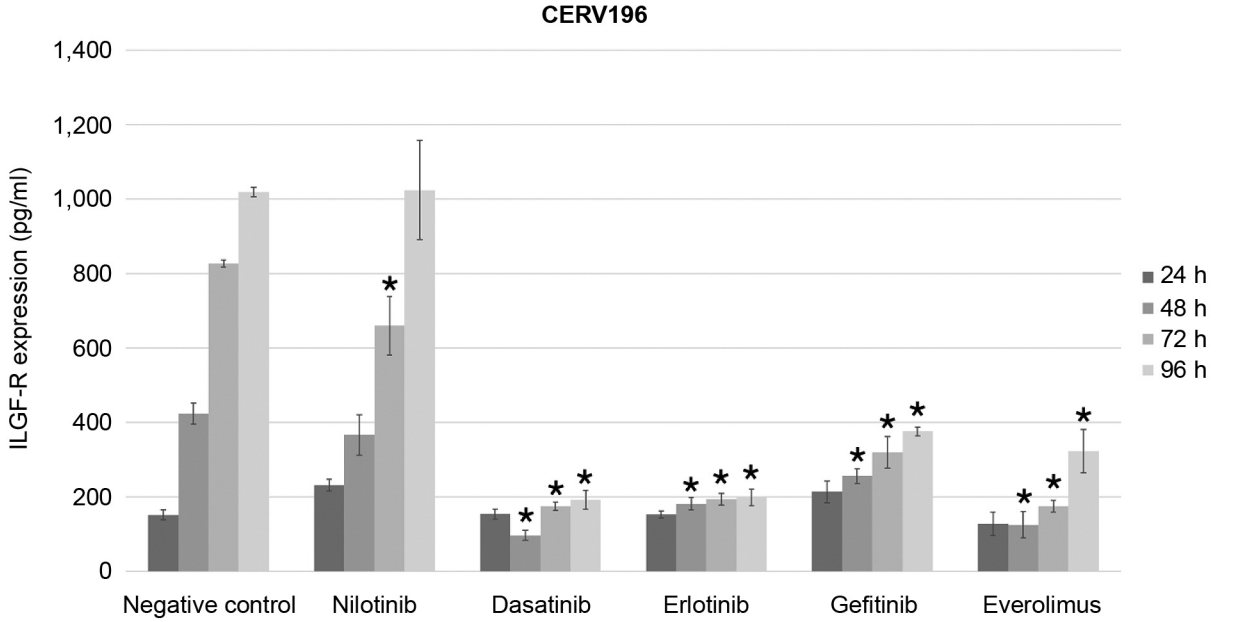

Figure 3. *IGF1R expression in CERV196 cells after incubation with nilotinib, dasatinib, erlotinib, gefitinib, or everolimus relative to the negative control. Data are mean values with standard deviations. Significance (p≤0.001) is marked with *.*

demonstrate beneficial effects in HNSCC treatment with TKI or mTOR inhibition, we designed this study to better understand the molecular mechanism of this failure because these drugs have been successfully established in the treatment of other tumor entities (*e.g.* CML and non-small cell lung cancer (20, 23). IGF1R is overexpressed in human cancers such as ovarian cancer, glioblastoma, and sarcoma and may therefore be suitable as a potential target protein

(27, 29, 31). In patients with HNSCC, it has been demonstrated that high expression levels of IGF1R are associated with high tumor (T)-stage, decreased overall survival, and shorter disease-specific survival (30). Because IGF1R can play a critical role in cancer development including proliferation and metastasis, TKIs directly targeting the IGF1R catalytic domain have been developed, and inhibitions of a broad range of human tumor types *in vitro* have been reported (61-68). Several current studies concentrate on the direct inhibition of IGF1R with TKIs and TKI combinations, but there is a lack of data regarding the direct effect of the tested TKIs on IGF1R in HNSCC. Therefore, this study was designed to close this gap by investigating effects of the small molecule TKIs erlotinib, gefitinib, dasatinib, and nilotinib and the mTOR-inhibitor everolimus on HPV-negative and HPV-positive SCC. Our results revealed a significant reduction of IGF1R expression in both TKI- and mTOR-inhibitor–treated HPV-negative and HPV-positive cultures relative to untreated cells. Gefitinib has been reported to induce nuclear accumulation of IGF1R in mucinous lung adenocarcinoma, and nuclear IGF1R induces G_1 arrest and promotes resistance (69). This mechanism might also play a role in HNSCC resistance to gefitinib. Yet, only few resistance mechanisms to EGFR-TKI have been identified. The ability to bypass EGFR-dependent signaling pathways (including the IGF1R pathway) as well as the presence of resistance mutations have been proven (70). In our results, IGF1R expression was significantly decreased by all the tested TKIs and by everolimus. The reduced expression may be due to survivin, which is a recently identified protein expressed in HNSCC and a possible molecular target correlating with clinical parameters and treatment outcome (71, 72). It has already been demonstrated that activation of IGF1R reduces sensitivity to EGFR-TKIs in HNSCC cell lines *via* a reduction of apoptosis, and it was demonstrated that resistance to lapatinib, an EGFR- and Her2/Neu-TKI (like gefitinib), correlates with enhanced survivin expression in HNSCC cells. This suggests that the regulation of survivin expression may be a key element in IGF1R-dependent therapeutic resistance (40, 73, 74). Additional experiments are necessary to investigate the expression of survivin in cell lines under the influence of TKIs and everolimus, as current data suggest a higher expression in cells resistant to the EGFR-TKI lapatinib (74). Nevertheless, the expression of IGF1R was suppressed by the tested small molecule TKIs in HPV-negative and HPV-positive SCC cells within 96 h. These results suggest a beneficial therapeutic effect in the tested cell lines, but a limitation of our study is that acquired resistance and IGF1R upregulation might appear later. Other groups have examined the effect of the exposure to gefitinib over several months and showed that acquired resistance to EGFR-TKIs in A431 SCC cells is mediated by a loss of IGF-binding proteins, and it was demonstrated that elimination of persistent IGF1R-induced Akt activity was required to reestablish gefitinib sensitivity (75). It has also been suggested that persistent IGF1R activity may predict resistance to anti-EGFR therapy in HNSCC (40). Also, cholangiocarcinoma cells escaped EGFR-TKI treatment with erlotinib by developing an adaptive mechanism undergoing an IGF1R-involving phenotypic switch (76). In our cultures, erlotinib reduced the levels of IGF1R within 96 h. The multi-TKI dasatinib (BCR-ABL, SRC, EGFR) has been demonstrated to suppress invasion and induce cell cycle arrest and apoptosis of HNSCC and non-small cell lung cancer cells *in vitro* and to increase radiation sensitivity by interfering with nuclear localization of EGFR and by blocking DNA repair pathways (77, 78). However, these effects could not be demonstrated in *in vivo* experiments (44). Promising results have been shown when combining the IGF1R inhibitor BMS754807 with either the human epidermal growth factor receptor family inhibitor BMS59962 or dasatinib, resulting in substantial synergy and growth inhibition *in vitro* (79). Everolimus treatment led to reduced IGF1R expression at all measurement points in the tested HPV-positive an HPV-negative cultures. The dual targeting of insulin and insulin-like growth factor 1 receptor enhances mTOR-inhibitor–mediated antitumor efficacy in hepatocellular carcinoma *in vitro*, but no comparable data are yet available in the literature regarding HNSCC (80). Up to date, everolimus has failed in HNSCC treatment (42). IGF1R could be influenced by everolimus treatment, as the IGF1 pathway might play a similar role in the EGFR bypass mechanism as mentioned above. For a subset of medullary thyroid carcinoma, it has been demonstrated that IGF1 influences the antiproliferative activity of everolimus (81).

In conclusion, these novel insights might contribute to a better understanding of the poor therapeutic effects of TKIs and everolimus in HNSCC. Future research investigating survivin in UMSCC-11A and -14C and in CERV196 and other TKIs would be of central interest. This is the first study analyzing the influence of TKIs and everolimus on IGF1R in HPV-negative UMSCC-11A and -14C and HPV-positive CERV196 cultures.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Benedikt Kramer: conception of the study, writing of the manuscript, generation of figures, data analysis; Angela Schell: data analysis, writing of the manuscript; Christoph Aderhold: performance of experiments, conception of the study, data analysis; Lena Huber: performance of the experiments, data analysis; Cornelia Emika Mueller: performance of the experiments, data

analysis; Nicole Rotter: providing conceptional design of the study, data analysis; Richard Birk: writing of the manuscript, conception of the study, generation of figures, data analysis. The manuscript was critically reviewed by all Authors.

References

1 Argiris A, Karamouzis MV, Raben D and Ferris RL: Head and neck cancer. Lancet *371(9625)*: 1695-1709, 2008. PMID: 18486742. DOI: 10.1016/S0140-6736(08)60728-X

2 Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M and Cozen W: Human papillomavirus and rising oropharyngeal cancer incidence in the united states. J Clin Oncol *29(32)*: 4294, 2011. PMID: 21969503. DOI: 10.1200/JCO.2011.36.4596

3 Gupta B, Johnson NW and Kumar N: Global epidemiology of head and neck cancers: A continuing challenge. Oncology *91(1)*: 13-23, 2016. PMID: 27245686. DOI: 10.1159/000446117

4 Castellsague X, Alemany L, Quer M, Halec G, Quiros B, Tous S, Clavero O, Alos L, Biegner T, Szafarowski T, Alejo M, Holzinger D, Cadena E, Claros E, Hall G, Laco J, Poljak M, Benevolo M, Kasamatsu E, Mehanna H, Ndiaye C, Guimera N, Lloveras B, Leon X, Ruiz-Cabezas JC, Alvarado-Cabrero I, Kang CS, Oh JK, Garcia-Rojo M, Iljazovic E, Ajayi OF, Duarte F, Nessa A, Tinoco L, Duran-Padilla MA, Pirog EC, Viarheichyk H, Morales H, Costes V, Felix A, Germar MJ, Mena M, Ruacan A, Jain A, Mehrotra R, Goodman MT, Lombardi LE, Ferrera A, Malami S, Albanesi EI, Dabed P, Molina C, Lopez-Revilla R, Mandys V, Gonzalez ME, Velasco J, Bravo IG, Quint W, Pawlita M, Munoz N, de Sanjose S, Xavier Bosch F, Head ICOIHi and Neck Cancer Study G: HPV involvement in head and neck cancers: Comprehensive assessment of biomarkers in 3680 patients. J Natl Cancer Inst *108(6)*: djv403, 2016. PMID: 26823521. DOI: 10.1093/jnci/djv403

5 Castellsague X, Mena M and Alemany L: Epidemiology of hpv-positive tumors in europe and in the world. Recent Results Cancer Res *206:* 27-35, 2017. PMID: 27699527. DOI: 10.1007/978-3-319-43580-0_2

6 Brierley JD, Gospodarowicz MK and Wittekind C: Tnm classification of malignant tumours. John Wiley & Sons, 2016.

7 Leemans CR, Snijders PJF and Brakenhoff RH: The molecular landscape of head and neck cancer. Nat Rev Cancer *18(5)*: 269-282, 2018. PMID: 29497144. DOI: 10.1038/nrc.2018.11

8 Doorbar J, Egawa N, Griffin H, Kranjec C and Murakami I: Human papillomavirus molecular biology and disease association. Rev Med Virol *25(Suppl 1):* 2-23, 2015. PMID: 25752814. DOI: 10.1002/rmv.1822

9 Kreimer AR, Clifford GM, Boyle P and Franceschi S: Human papillomavirus types in head and neck squamous cell carcinomas worldwide: A systematic review. Cancer Epidemiol Biomarkers Prev *14(2)*: 467-475, 2005. PMID: 15734974. DOI: 10.1158/1055-9965.EPI-04-0551

10 Deschler DG, Richmon JD, Khariwala SS, Ferris RL and Wang MB: The "new" head and neck cancer patient—young, nonsmoker, nondrinker, and hpv positive: Evaluation. Otolaryngol Head Neck Surg *151(3)*: 375-380, 2014. PMID: 24925311. DOI: 10.1177/0194599814538605

11 Young D, Xiao CC, Murphy B, Moore M, Fakhry C and Day TA: Increase in head and neck cancer in younger patients due to human papillomavirus (hpv). Oral Oncol *51(8)*: 727-730, 2015. PMID: 26066977. DOI: 10.1016/j.oraloncology.2015.03.015

12 Blitzer GC, Smith MA, Harris SL and Kimple RJ: Review of the clinical and biologic aspects of human papillomavirus-positive squamous cell carcinomas of the head and neck. Int J Radiat Oncol Biol Phys *88(4)*: 761-770, 2014. PMID: 24606845. DOI: 10.1016/j.ijrobp.2013.08.029

13 Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC and Lu C: Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med *363(1)*: 24-35, 2010. PMID: 20530316. DOI: 10.1056/NEJMoa0912217

14 Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A and Gillison ML: Improved survival of patients with human papillomavirus–positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst *100(4)*: 261-269, 2008. PMID: 18270337. DOI: 10.1093/jnci/djn011

15 Mehanna H, Robinson M, Hartley A, Kong A, Foran B, Fulton-Lieuw T, Dalby M, Mistry P, Sen M and O'Toole L: Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (de-escalate hpv): An open-label randomised controlled phase 3 trial. Lancet *393(10166)*: 51-60, 2019. PMID: 30449623. DOI: 10.1016/S0140-6736(18)32752-1

16 Moskovitz J, Moy J and Ferris RL: Immunotherapy for head and neck squamous cell carcinoma. Curr Oncol Rep *20(2)*: 22, 2018. PMID: 29502288. DOI: 10.1007/s11912-018-0654-5

17 Morin MJ: From oncogene to drug: Development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents. Oncogene *19(56)*: 6574-6583, 2000. PMID: 11426642. DOI: 10.1038/sj.onc.1204102

18 Wu P, Nielsen TE and Clausen MH: Fda-approved small-molecule kinase inhibitors. Trends Pharmacol Sci *36(7)*: 422-439, 2015. PMID: 25975227. DOI: 10.1016/j.tips.2015.04.005

19 le Coutre P, Schwarz M and Kim TD: New developments in tyrosine kinase inhibitor therapy for newly diagnosed chronic myeloid leukemia. Clin Cancer Res *16(6)*: 1771-1780, 2010. PMID: 20197479. DOI: 10.1158/1078-0432.CCR-09-2760

20 Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DW, Issaragrisil S, le Coutre PD, Etienne G, Dorlhiac-Llacer PE, Clark RE, Flinn IW, Nakamae H, Donohue B, Deng W, Dalal D, Menssen HD and Kantarjian HM: Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized enestnd trial. Leukemia *30(5)*: 1044-1054, 2016. PMID: 26837842. DOI: 10.1038/leu.2016.5

21 Bareschino M, Schettino C, Troiani T, Martinelli E, Morgillo F and Ciardiello F: Erlotinib in cancer treatment. Ann Oncol *18(suppl_6)*: vi35-vi41, 2007. PMID: 17591829. DOI: 10.1093/annonc/mdm222

22 Gridelli C, Bareschino MA, Schettino C, Rossi A, Maione P and Ciardiello F: Erlotinib in non-small cell lung cancer treatment: Current status and future development. Oncologist *12(7)*: 840-849, 2007. PMID: 17673615. DOI: 10.1634/theoncologist.12-7-840

23 Yang Z, Hackshaw A, Feng Q, Fu X, Zhang Y, Mao C and Tang J: Comparison of gefitinib, erlotinib and afatinib in non-small cell lung cancer: A meta-analysis. Int J Cancer *140(12)*: 2805-2819, 2017. PMID: 28295308. DOI: 10.1002/ijc.30691

24 Pópulo H, Lopes JM and Soares P: The mtor signalling pathway in human cancer. Int J Mol Sci *13(2)*: 1886-1918, 2012. PMID: 22408430. DOI: 10.3390/ijms13021886

25 Hasskarl J: Everolimus. *In*: Small molecules in oncology. Springer, pp. 101-123, 2018.

26 Aderhold C, Faber A, Umbreit C, Birk R, Weiss C, Sommer JU, Hoermann K and Schultz JD: Targeting mtor and areg with everolimus, sunitinib and sorafenib in hpv-positive and-negative scc. Anticancer Res *35(4)*: 1951-1959, 2015. PMID: 25862847.

27 King ER, Zu Z, Tsang YT, Deavers MT, Malpica A, Mok SC, Gershenson DM and Wong KK: The insulin-like growth factor 1 pathway is a potential therapeutic target for low-grade serous ovarian carcinoma. Gynecol Oncol *123(1)*: 13-18, 2011. PMID: 21726895. DOI: 10.1016/j.ygyno.2011.06.016

28 Ji Q-s, Mulvihill MJ, Cooke A, Feng L, Mak G, O'Connor M, Yao Y, Pirritt C, Buck E and Eyzaguirre A: A novel, potent, and selective insulin-like growth factor-i receptor kinase inhibitor blocks insulin-like growth factor-i receptor signaling *in vitro* and inhibits insulin-like growth factor-i receptor–dependent tumor growth *in vivo*. Mol Cancer Ther *6(8)*: 2158-2167, 2007. PMID: 17671083. DOI: 10.1158/1535-7163.MCT-07-0070

29 Bielen A, Perryman L, Box GM, Valenti M, de Haven Brandon A, Martins V, Jury A, Popov S, Gowan S and Jeay S: Enhanced efficacy of igf1r inhibition in pediatric glioblastoma by combinatorial targeting of pdgfrα/β. Mol Cancer Ther *10(8)*: 1407-1418, 2011. PMID: 21659463. DOI: 10.1158/1535-7163.MCT-11-0205

30 Dale OT, Aleksic T, Shah KA, Han C, Mehanna H, Rapozo DC, Sheard JD, Goodyear P, Upile NS and Robinson M: Igf-1r expression is associated with hpv-negative status and adverse survival in head and neck squamous cell cancer. Carcinogenesis *36(6)*: 648-655, 2015. PMID: 25896444. DOI: 10.1093/carcin/bgv053

31 Hughes DP: Novel agents in development for pediatric sarcomas. Curr Opin Oncol *21(4)*: 332-337, 2009. PMID: 19444103. DOI: 10.1097/CCO.0b013e32832c94e2

32 Li BD, Khosravi MJ, Berkel HJ, Diamandi A, Dayton MA, Smith M and Yu H: Free insulin-like growth factor-i and breast cancer risk. Int J Cancer *91(5)*: 736-739, 2001. PMID: 11267989. DOI: 10.1002/1097-0215(200002)9999:9999<::aid-ijc1111>3.0.co;2-#

33 Gao Y, Katki H, Graubard B, Pollak M, Martin M, Tao Y, Schoen RE, Church T, Hayes RB and Greene MH: Serum IGF1, IGF2 and IGFBP3 and risk of advanced colorectal adenoma. Int J Cancer *131(2)*: E105-E113, 2012. PMID: 21932422. DOI: 10.1002/ijc.26438

34 Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH and Pollak M: Plasma insulin-like growth factor-i and prostate cancer risk: A prospective study. Science *279(5350)*: 563-566, 1998. PMID: 9438850. DOI: 10.1126/science.279.5350.563

35 Yu H, Spitz MR, Mistry J, Gu J, Hong WK and Wu X: Plasma levels of insulin-like growth factor-i and lung cancer risk: A case-control analysis. J Natl Cancer Inst *91(2)*: 151-156, 1999. PMID: 9923856. DOI: 10.1093/jnci/91.2.151

36 Wu X, Zhao H, Do K-A, Johnson MM, Dong Q, Hong WK and Spitz MR: Serum levels of insulin growth factor (IGF-I) and igf-binding protein predict risk of second primary tumors in patients with head and neck cancer. Clin Cancer Res *10(12)*: 3988-3995, 2004. PMID: 15217929. DOI: 10.1158/1078-0432.CCR-03-0762

37 McKinley ET, Bugaj JE, Zhao P, Guleryuz S, Mantis C, Gokhale PC, Wild R and Manning HC: 18fdg-pet predicts pharmacodynamic response to osi-906, a dual IGF-1R/IR inhibitor, in preclinical mouse models of lung cancer. Clin Cancer Res *17(10)*: 3332-3340, 2011. PMID: 21257723. DOI: 10.1158/1078-0432.CCR-10-2274

38 Pitts TM, Tan AC, Kulikowski GN, Tentler JJ, Brown AM, Flanigan SA, Leong S, Coldren CD, Hirsch FR and Varella-Garcia M: Development of an integrated genomic classifier for a novel agent in colorectal cancer: Approach to individualized therapy in early development. Clin Cancer Res *16(12)*: 3193-3204, 2010. PMID: 20530704. DOI: 10.1158/1078-0432.CCR-09-3191

39 Riedemann J and Macaulay V: IGF1R signalling and its inhibition. Endocr-Relat Cancer *13(Suppl_1)*: S33-S43, 2006. PMID: 17259557. DOI: 10.1677/erc.1.01280

40 Jameson MJ, Beckler AD, Taniguchi LE, Allak A, VanWagner LB, Lee NG, Thomsen WC, Hubbard MA and Thomas CY: Activation of the insulin-like growth factor-1 receptor induces resistance to epidermal growth factor receptor antagonism in head and neck squamous carcinoma cells. Mol Cancer Ther *10(11)*: 2124-2134, 2011. PMID: 21878657. DOI: 10.1158/1535-7163.MCT-11-0294

41 Baselga J, Pfister D, Cooper M, Cohen R, Burtness B, Bos M, D'andrea G, Seidman A, Norton L and Gunnett K: Phase i studies of anti–epidermal growth factor receptor chimeric antibody c225 alone and in combination with cisplatin. J Clin Oncol *18(4)*: 904-904, 2000. PMID: 10673534. DOI: 10.1200/JCO.2000.18.4.904

42 Geiger JL, Bauman JE, Gibson MK, Gooding WE, Varadarajan P, Kotsakis A, Martin D, Gutkind JS, Hedberg ML, Grandis JR and Argiris A: Phase ii trial of everolimus in patients with previously treated recurrent or metastatic head and neck squamous cell carcinoma. Head Neck *38(12)*: 1759-1764, 2016. PMID: 27232378. DOI: 10.1002/hed.24501

43 Tang X, He J, Li B, Zheng Y, Li K, Zou S and Chen L: Efficacy and safety of gefitinib in patients with advanced head and neck squamous cell carcinoma: A meta-analysis of randomized controlled trials. J Oncol *2019:* 6273438, 2019. PMID: 31239839. DOI: 10.1155/2019/6273438

44 Brooks HD, Glisson BS, Bekele BN, Ginsberg LE, El-Naggar A, Culotta KS, Takebe N, Wright J, Tran HT and Papadimitrakopoulou VA: Phase 2 study of dasatinib in the treatment of head and neck squamous cell carcinoma. Cancer *117(10)*: 2112-2119, 2011. PMID: 21523723. DOI: 10.1002/cncr.25769

45 Cohen EE, Davis DW, Karrison TG, Seiwert TY, Wong SJ, Nattam S, Kozloff MF, Clark JI, Yan D-H and Liu W: Erlotinib and bevacizumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck: A phase i/ii study. Lancet Oncol *10(3)*: 247-257, 2009. PMID: 19201650. DOI: 10.1016/S1470-2045(09)70002-6

46 Elser C, Siu LL, Winquist E, Agulnik M, Pond GR, Chin SF, Francis P, Cheiken R, Elting J and McNabola A: Phase ii trial of sorafenib in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or nasopharyngeal carcinoma. J Clin Oncol *25(24)*: 3766-3773, 2007. PMID: 17704426. DOI: 10.1200/JCO.2006.10.2871

47 Gross ND, Bauman JE, Gooding WE, Denq W, Thomas SM, Wang L, Chiosea S, Hood BL, Flint MS and Sun M: Erlotinib, erlotinib–sulindac versus placebo: A randomized, double-blind, placebo-controlled window trial in operable head and neck cancer. Clin Cancer Res *20(12)*: 3289-3298, 2014. PMID: 24727329. DOI: 10.1158/1078-0432.CCR-13-3360

48 Stewart J, Cohen E, Licitra L, Van Herpen C, Khorprasert C, Soulieres D, Vodvarka P, Rischin D, Garin AM and Hirsch FR: Phase iii study of gefitinib compared with intravenous methotrexate for recurrent squamous cell carcinoma of the head and neck. J Clin Oncol *27(11)*: 1864-1871, 2009. PMID: 19289630. DOI: 10.1200/JCO.2008.17.0530

49 Argiris A, Ghebremichael M, Gilbert J, Lee J-W, Sachidanandam K, Kolesar JM, Burtness B and Forastiere AA: Phase iii randomized, placebo-controlled trial of docetaxel with or without gefitinib in recurrent or metastatic head and neck cancer: An eastern cooperative oncology group trial. J Clin Oncol *31(11)*: 1405, 2013. PMID: 23460714. DOI: 10.1200/JCO.2012.45.4272

50 Cohen EE, Haraf DJ, Kunnavakkam R, Stenson KM, Blair EA, Brockstein B, Lester EP, Salama JK, Dekker A and Williams R: Epidermal growth factor receptor inhibitor gefitinib added to chemoradiotherapy in locally advanced head and neck cancer. J Clin Oncol *28(20)*: 3336, 2010. PMID: 20498391. DOI: 10.1200/JCO.2009.27.0397

51 Ang KK, Zhang Q, Rosenthal DI, Nguyen-Tan PF, Sherman EJ, Weber RS, Galvin JM, Bonner JA, Harris J and El-Naggar AK: Randomized phase III trial of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III to IV head and neck carcinoma: Rtog 0522. J Clin Oncol *32(27)*: 2940, 2014. PMID: 25154822. DOI: 10.1200/JCO.2013.53.5633

52 Mesía R, Henke M, Fortin A, Minn H, Ancona ACY, Cmelak A, Markowitz AB, Hotte SJ, Singh S and Chan AT: Chemoradiotherapy with or without panitumumab in patients with unresected, locally advanced squamous-cell carcinoma of the head and neck (concert-1): A randomised, controlled, open-label phase 2 trial. Lancet Oncol *16(2)*: 208-220, 2015. PMID: 25596660. DOI: 10.1016/S1470-2045(14)71198-2

53 Burtness B: The role of cetuximab in the treatment of squamous cell cancer of the head and neck. Expert Opin Biol Th *5(8)*: 1085-1093, 2005. PMID: 16050785. DOI: 10.1517/14712598.5.8.1085

54 Ceresa BP and Peterson JL: Cell and molecular biology of epidermal growth factor receptor. Int Rev Cel Mol Biol *313*: 145-178, 2014. PMID: 25376492. DOI: 10.1016/B978-0-12-800177-6.00005-0

55 Rödel F and Balermpas P: Anti-epidermal growth factor receptor immunotherapy in combination with cisplatin chemoradiation for patients with advanced head and neck carcinoma—biological and clinical limitations of the triple treatment. Translat Cancer Res *5(2)*: 199-202, 2016. DOI: 10.21037/tcr.2016.03.03

56 Kramer B, Polit M, Birk R, Rotter N and Aderhold C: Hif-1alpha and mtor - possible novel strategies of targeted therapies in p16-positive and -negative hnscc. Cancer Genomics Proteomics *15(3)*: 175-184, 2018. PMID: 29695399. DOI: 10.21873/cgp.20075

57 Kramer B, Kneissle M, Birk R, Rotter N and Aderhold C: Tyrosine kinase inhibition in hpv-related squamous cell carcinoma reveals beneficial expression of ckit and src. Anticancer Res *38(5)*: 2723-2731, 2018. PMID: 29715092. DOI: 10.21873/anticanres.12514

58 Saba NF, Hurwitz SJ, Magliocca K, Kim S, Owonikoko TK, Harvey D, Ramalingam SS, Chen Z, Rogerio J and Mendel J: Phase 1 and pharmacokinetic study of everolimus in combination with cetuximab and carboplatin for recurrent/metastatic squamous cell carcinoma of the head and neck. Cancer *120(24)*: 3940-3951, 2014. PMID: 25103371. DOI: 10.1002/cncr.28965

59 Fury MG, Lee NY, Sherman E, Ho AL, Rao S, Heguy A, Shen R, Korte S, Lisa D and Ganly I: A phase 1 study of everolimus+ weekly cisplatin+ intensity modulated radiation therapy in head-and-neck cancer. Int J Rad Oncol Biol Phys *87(3)*: 479-486, 2013. PMID: 24074921. DOI: 10.1016/j.ijrobp.2013.06.2043

60 Day TA, Shirai K, O'Brien PE, Matheus MG, Godwin K, Sood AJ, Kompelli A, Vick JA, Martin D and Vitale-Cross L: Inhibition of mtor signaling and clinical activity of rapamycin in head and neck cancer in a window of opportunity trial. Clin Cancer Res *25(4)*: 1156-1164, 2019. PMID: 30420444. DOI: 10.1158/1078-0432.CCR-18-2024

61 Gao J, Chang YS, Jallal B and Viner J: Targeting the insulin-like growth factor axis for the development of novel therapeutics in oncology. Cancer Res *72(1)*: 3-12, 2012. PMID: 22215692. DOI: 10.1158/0008-5472.CAN-11-0550

62 Hewish M, Chau I and Cunningham D: Insulin-like growth factor 1 receptor targeted therapeutics: Novel compounds and novel treatment strategies for cancer medicine. Recent Pat Anti-Canc *4(1)*: 54-72, 2009. PMID: 19149688. DOI: 10.2174/157489209787002515

63 Carboni JM, Wittman M, Yang Z, Lee F, Greer A, Hurlburt W, Hillerman S, Cao C, Cantor GH and Dell-John J: Bms-754807, a small molecule inhibitor of insulin-like growth factor-1r/ir. Mol Cancer Ther *8(12)*: 3341-3349, 2009. PMID: 19996272. DOI: 10.1158/1535-7163.MCT-09-0499

64 Dinchuk JE, Cao C, Huang F, Reeves KA, Wang J, Myers F, Cantor GH, Zhou X, Attar RM and Gottardis M: Insulin receptor (IR) pathway hyperactivity in igf-ir null cells and suppression of downstream growth signaling using the dual igf-ir/ir inhibitor, bms-754807. Endocrinology *151(9)*: 4123-4132, 2010. PMID: 20610571. DOI: 10.1210/en.2010-0032

65 Huang F, Hurlburt W, Greer A, Reeves KA, Hillerman S, Chang H, Fargnoli J, Finckenstein FG, Gottardis MM and Carboni JM: Differential mechanisms of acquired resistance to insulin-like growth factor-i receptor antibody therapy or to a small-molecule inhibitor, bms-754807, in a human rhabdomyosarcoma model. Cancer Res *70(18)*: 7221-7231, 2010. PMID: 20807811. DOI: 10.1158/0008-5472.CAN-10-0391

66 Kolb EA, Gorlick R, Lock R, Carol H, Morton CL, Keir ST, Reynolds CP, Kang MH, Maris JM and Billups C: Initial testing (stage 1) of the IGF-1 receptor inhibitor bms-754807 by the pediatric preclinical testing program. Pediatr Blood Cancer *56(4)*: 595-603, 2011. PMID: 21298745. DOI: 10.1002/pbc.22741

67 Mulvihill MJ, Cooke A, Rosenfeld-Franklin M, Buck E, Foreman K, Landfair D, O'Connor M, Pirritt C, Sun Y and Yao Y: Discovery of osi-906: A selective and orally efficacious dual inhibitor of the igf-1 receptor and insulin receptor. Fut Med Chem *1(6)*: 1153-1171, 2009. PMID: 21425998. DOI: 10.4155/fmc.09.89

68 Wittman MD, Carboni JM, Yang Z, Lee FY, Antman M, Attar R, Balimane P, Chang C, Chen C and Discenza L: Discovery of a 2, 4-disubstituted pyrrolo [1, 2-f][1, 2, 4] triazine inhibitor (bms-754807) of insulin-like growth factor receptor (igf-1r) kinase in clinical development. J Med Chem *52(23)*: 7360-7363, 2009. PMID: 19778024. DOI: 10.1021/jm900786r

69 Guerard M, Robin T, Perron P, Hatat A-S, David-Boudet L, Vanwonterghem L, Busser B, Coll J-L, Lantuejoul S and Eymin B: Nuclear translocation of igf1r by intracellular amphiregulin contributes to the resistance of lung tumour cells to egfr-tki. Cancer Let *420:* 146-155, 2018. PMID: 29421153. DOI: 10.1016/j.canlet.2018.01.080

70 Chong CR and Jänne PA: The quest to overcome resistance to egfr-targeted therapies in cancer. Nature Medicine *19(11)*: 1389-1400, 2013. PMID: 24202392. DOI: 10.1038/nm.3388

71 Münscher A, Prochnow S, Gulati A, Sauter G, Lörincz B, Blessmann M, Hanken H, Böttcher A and Clauditz TS: Survivin expression in head and neck squamous cell carcinomas is frequent and correlates with clinical parameters and treatment outcomes. Clin Oral Inv *23(1)*: 361-367, 2019. PMID: 29671054. DOI: 10.1007/s00784-018-2444-8

72 Frassanito M, Saltarella I, Vinella A, Muzio LL, Pannone G, Fumarulo R, Vacca A and Mariggiò M: Survivin overexpression in head and neck squamous cell carcinomas as a new therapeutic target. Oncol Rep *41(5)*: 2615-2624, 2019. PMID: 30896830. DOI: 10.3892/or.2019.7082

73 Jameson MJ, Taniguchi LE, VanKoevering KK, Stuart MM, Francom CR, Mendez RE, Beckler AD, Carlson HT, Thomas CY and Khalil AA: Activation of the insulin-like growth factor-1 receptor alters p27 regulation by the epidermal growth factor receptor in oral squamous carcinoma cells. J Oral Path Med *42(4)*: 332-338, 2013. PMID: 23106397. DOI: 10.1111/jop.12014

74 Lehman CE, Mendez RE, Dougherty MI, Allak A, Adejumo OL, Taniguchi LE, Khalil A, Gioeli DG and Jameson MJ: Survivin in insulin-like growth factor-induced resistance to lapatinib in head and neck squamous carcinoma cells. Front Oncol *9:* 13, 2019. PMID: 30729097. DOI: 10.3389/fonc.2019.00013

75 Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, Rinehart C, Seidel B, Yee D and Arteaga CL: Acquired resistance to egfr tyrosine kinase inhibitors in cancer cells is mediated by loss of igf-binding proteins. J Clin Inv *118(7)*: 2609-2619, 2008. PMID: 18568074. DOI: 10.1172/JCI34588

76 Vaquero J, Lobe C, Tahraoui S, Clapéron A, Mergey M, Merabtene F, Wendum D, Coulouarn C, Housset C and Desbois-Mouthon C: The igf2/ir/igf1r pathway in tumor cells and myofibroblasts mediates resistance to egfr inhibition in cholangiocarcinoma. Clin Cancer Res *24(17)*: 4282-4296, 2018. PMID: 29716918. DOI: 10.1158/1078-0432.CCR-17-3725

77 Johnson FM, Saigal B, Talpaz M and Donato NJ: Dasatinib (bms-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non–small cell lung cancer cells. Clin Cancer Res *11(19)*: 6924-6932, 2005. PMID: 16203784. DOI: 10.1158/1078-0432.CCR-05-0757

78 Raju U, Riesterer O, Wang ZQ, Molkentine DP, Molkentine JM, Johnson FM, Glisson B, Milas L and Ang KK: Dasatinib, a multi-kinase inhibitor increased radiation sensitivity by interfering with nuclear localization of epidermal growth factor receptor and by blocking DNA repair pathways. Radiother Oncol *105(2)*: 241-249, 2012. PMID: 23010482. DOI: 10.1016/j.radonc.2012.08.010

79 Axelrod MJ, Mendez RE, Khalil A, Leimgruber SS, Sharlow ER, Capaldo B, Conaway M, Gioeli DG, Weber MJ and Jameson MJ: Synergistic apoptosis in head and neck squamous cell carcinoma cells by co-inhibition of insulin-like growth factor-1 receptor signaling and compensatory signaling pathways. Head Neck *37(12)*: 1722-1732, 2015. PMID: 24986420. DOI: 10.1002/hed.23822

80 Pivonello C, Negri M, De Martino MC, Napolitano M, de Angelis C, Provvisiero DP, Cuomo G, Auriemma RS, Simeoli C and Izzo F: The dual targeting of insulin and insulin-like growth factor 1 receptor enhances the mtor inhibitor-mediated antitumor efficacy in hepatocellular carcinoma. Oncotarget *7(9)*: 9718, 2016. PMID: 26756219. DOI: 10.18632/oncotarget.6836

81 Gentilin E, Di Pasquale C, Rossi M, Tagliati F, Gagliano T, Rossi R, Pelizzo M, Merante Boschin I, degli Uberti EC and Zatelli MC: Igf-i influences everolimus activity in medullary thyroid carcinoma. Front Endocrinol *6:* 63, 2015. PMID: 25999915. DOI: 10.3389/fendo.2015.00063

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