Sensitization of Head and Neck Cancer to Cisplatin Through the Use of a Novel Curcumin Analog

Waleed M. Abuzeid, MBBS; Samantha Davis, MD; Alice L. Tang, MD; Lindsay Saunders, BS; J. Chadwick Brenner, MSE; Jiayuh Lin, PhD; James R. Fuchs, PhD; Emily Light, MS; Carol R. Bradford, MD; Mark E. P. Prince, MD; Thomas E. Carey, PhD

**Objective:** To determine whether a novel small molecule inhibitor derived from curcumin (FLLL32) that targets signal transducer and activator of transcription (STAT) 3 would induce cytotoxic effects in STAT3-dependent head and neck squamous cell cancer (HNSCC) cells and would sensitize tumors to cisplatin.

**Design:** Basic science. Two HNSCC cell lines, UM-SCC-29 and UM-SCC-74B, were characterized for cisplatin [cis-diammineplatinum(II) dichloride] sensitivity. Baseline expression of STAT3 and other apoptosis proteins was determined. The FLLL32 50% inhibitory concentration (IC50) dose was determined for each cell line, and the effect of FLLL32 treatment on the expression of phosphorylated STAT3 and other key proteins was elucidated. The antitumor efficacy of cisplatin, FLLL32, and combination treatment was measured. The proportion of apoptotic cells after cisplatin, FLLL32, or combination therapy was determined.

**Results:** The UM-SCC-29 cell line is cisplatin resistant, and the UM-SCC-74B cell line is cisplatin sensitive. Both cell lines express STAT3, phosphorylated STAT3 (pSTAT3), and key apoptotic proteins. FLLL32 downregulates the active form of STAT3, pSTAT3, in HNSCC cells and induces a potent antitumor effect. FLLL32, alone or with cisplatin, increases the proportion of apoptotic cells. FLLL32 sensitized cisplatin-resistant cancer cells, achieving an equivalent tumor kill with a 4-fold lower dose of cisplatin.

**Conclusions:** FLLL32 monotherapy induces a potent antitumor effect and sensitizes cancer cells to cisplatin, permitting an equivalent or improved antitumor effect at lower doses of cisplatin. Our results suggest that FLLL32 acts by inhibiting STAT3 phosphorylation, reduced survival signaling, increased susceptibility to apoptosis, and sensitization to cisplatin.


ACCORDING TO THE AMERICAN Cancer Society, there were approximately 48,000 new cases of head and neck cancer resulting in 11,000 deaths in the United States in 2010. The overall 5-year survival for head and neck cancer has remained unchanged over the past 3 decades. This has driven the search for novel therapeutic agents that may obviate the need for, or, alternatively, enhance the effect of currently used treatment regimens.

Platinum-based agents, such as cisplatin, form the mainstay of currently used chemotherapeutic regimens for head and neck squamous cell carcinoma (HSNCC). However, head and neck cancers often demonstrate significant resistance to cisplatin, acquired through repeated treatment cycles or as an inherent characteristic of the cancer. Cisplatin resistance is a major factor in disease relapse. The resulting locoregional spread of disease and later recurrence are considered to be the main obstacles to improving outcome in head and neck cancer. Cisplatin resistance also has implications for ongoing treatment because relatively minor increases in resistance necessitate significant dose escalations, which result in increased toxic effects. The antitumor function of cisplatin is mediated by the development of DNA-platinum monoadducts and cross-links that can lead to DNA double-strand breaks during the process of replication. These, in turn, induce cell cycle arrest and apoptosis. Small molecule inhibitors of key pathways involved in apoptosis, differentiation, and cell growth may potentially improve the prognosis of head and neck cancer by sensitizing cancer cells, at a molecular level, to the antitumor effects of cisplatin.

Signal transducer and activator of transcription (STAT) proteins are key cytoplasmic transcription factors. STAT proteins contain multiple domains, including a DNA-binding site, Src homology-2 (SH2) domains, and a critical tyrosine residue (Y705) situated in the C-terminal domain. Cytokine and growth factor ligands bind to cell surface receptors, resulting in receptor dimerization and transphosphorylation. STAT proteins are recruited to activated cell...
surface receptors via their SH2 domain and become activated through phosphorylation of the critical Y705 residue by upstream kinases. In the case of cytokines, such as interleukin-6, whose receptors lack intrinsic tyrosine kinase activity, the Janus kinase (JAK) family of cytoplasmic tyrosine kinases perform the key STAT-activating phosphorylation step. Transmembrane growth factor receptors, such as the epidermal growth factor receptor (EGFR), harbor intrinsic tyrosine kinase activity and are able to phosphorylate STAT independently. Once activated, STAT monomers are able to dimerize through their SH2 domains in a process initiated and stabilized by the key Y705 residue. The activated STAT dimers translocate to the nucleus and bind to specific DNA-response elements in target genes to modulate gene expression.

The role of STAT proteins in critical cell fate decisions, such as cell growth, differentiation, and apoptosis, as well as metastasis and immune evasion, makes them attractive targets for anticancer therapy. STAT3 has been shown to be constitutively expressed in HNSCC both in vitro and in vivo. Approximately 82% of HNSCCs exhibit upregulation of STAT3 expression. These findings are likely secondary to the role of STAT3 in oncogenesis. Enhanced STAT3 expression has been correlated with increased antiapoptotic Bcl-XL protein levels and decreased levels of the proapoptotic BAX protein, enhancing HNSCC survival. STAT3 also induces vascular endothelial growth factor expression and, thus, contributes to tumor angiogenesis in HNSCC. Furthermore, overexpression of cell cycle regulators, such as cyclin D1, is induced by STAT3 in HNSCC. STAT3 is known to be constitutively activated in immortalized fibroblasts transformed by oncoproteins such as v-Src. In the absence of STAT3, v-Src is incapable of inducing neoplastic transformation. Overexpression of STAT3 induces tumors in nude mice.

 Interruption of STAT3 signaling has been shown to impede cancer cell growth and to enhance apoptosis in HNSCC. STAT pathway disruption can be achieved at an upstream level through inhibition of JAK2, targeting SH2-mediated dimerization or targeting downstream DNA-binding. Although significant literature exists regarding the use of STAT inhibitors as a monotherapy for HNSCC, relatively little has been published on the role of STAT inhibition in sensitizing HNSCC to standard treatment regimens. Recently, STAT3 expression has been correlated with cisplatin resistance in HNSCC. The aim of this study is to determine the role of STAT3 inhibition on sensitization of cancer cells to cisplatin, potentially paving the way for the use of lower, less toxic doses of the drug to achieve an equivalent or enhanced tumor kill.

**METHODS**

**CELL LINES**

The UM-SCC-29 and UM-SCC-74B cell lines were originally derived from human head and neck tumors explants, obtained from 2 patients with advanced HNSCC during surgical resection of the tumor. These patients gave informed written consent in studies reviewed and approved by the University of Michigan Medical School (Ann Arbor) institutional review board. These cell lines were originated and propagated in our laboratory. Cell line identity was confirmed through genotyping using a panel of 10 different short tandem repeat loci as previously described. UM-SCC-29 was derived from a primary lesion of the alveolus treated with 3 courses of neoadjuvant cisplatin and methylglyoxalbisguanylylhydrazone followed by surgical resection. UM-SCC-74B was derived from a patient with a persistent tumor of the tongue treated with 3 cycles of cisplatin and 5-fluorouracil in addition to external beam radiotherapy followed by surgical resection. Residual disease resulted in additional treatment with 5-fluorouracil and carboplatin followed by a second surgical extirpation. The UM-SCC-74B cell line is derived from samples obtained at the second surgery. These cell lines have previously been characterized for cisplatin sensitivity. UM-SCC-10B, UM-SCC-17B, and UM-SCC-33 are derived from local recurrence of laryngeal carcinoma, primary laryngeal carcinoma that extended through the cartilage into the soft tissues of the neck, and a paranasal sinus carcinoma, respectively. The ME-180-R cell line is derived from human cervical carcinoma and was made resistant to cisplatin by in vitro selection. The UM-SCC-29 and UM-SCC-74B cell lines were screened for STAT3, phosphorylated STAT3 (pSTAT3), EGFR, Bcl-XL, survivin, AKT, phosphorylated AKT (pAKT), SMAC, caspase-3, and p53 expression using Western blot. The cell lines were genotyped to confirm their unique identity. Cells were cultured in Dulbecco modified Eagle medium (dMEM; Sigma Chemical Co, St Louis, Missouri) containing 2mM L-glutamine, 1% nonessential amino acids, 1% penicillin-streptomycin (Invitrogen, Carlsbad, California), and 10% fetal bovine serum in a humidified atmosphere of 5% carbon dioxide at 37°C. Cells were tested for mycoplasma using the MycoAlert Detection Kit (Cambrex, Rockland, Maine) every 3 to 6 weeks to ensure that they were free of contamination.

**THERAPEUTIC REAGENTS**

Cisplatin [cis-diammineplatinum(II) dichloride] (CDDP) (Sigma-Aldrich, St Louis, Missouri) was dissolved in 0.9% sodium chloride to a stock concentration of 3.33mM. Curcumin, a plant-derived chemical, was used as the lead compound to develop a novel SH2 (Src Homology 2) (STAT3) inhibitor, termed FLLL32. Curcumin is known to inhibit multiple molecular pathways, including STAT3, but demonstrates poor bioavailability limiting its clinical potential. FLLL32 is a small molecule inhibitor that specifically targets the SH2 residue of STAT3 and concurrently incorporates improvements in bioavailability for use in biological systems. FLLL32 was reconstituted in dimethyl sulfoxide (DMSO) to a stock concentration of 1mM. FLLL32 was diluted in dMEM for all experiments such that the final DMSO concentration in all experiments was less than 1%. Both the FLLL32 and cisplatin were stored in dark conditions, and experiments were conducted in low light.

**FIFTY PERCENT INHIBITORY CONCENTRATION DETERMINATION**

Fifty percent inhibitory concentration (IC50) was derived for both cisplatin and FLLL32 in the UM-SCC-29 and UM-SCC-74B cell lines. For the cisplatin assays, cells were plated at a concentration of 5000 cells per well in 96 well plates. The cells were incubated overnight in humidified air with 5% carbon dioxide and 95% air. Plates were read using a spectrophotometer at a wavelength of 570 nm. Data were analyzed using SigmaPlot (version 10.0; SyStat Software, Chicago, Illinois) to determine the IC50.

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The IC₅₀ concentration was also determined for FLLL32. Cells were seeded in 96 well plates at a concentration of 2000 cells per well for UM-SCC-74B and 2500 cells per well for UM-SCC-29 and allowed to incubate overnight in 5% carbon dioxide at 37°C. The culture medium was then removed and replaced with cDMEM with 0.2% DMSO or medium containing FLLL32 (0.31µM-5.0µM). Cells were incubated for 96 hours, and MTT assays were performed as described herein to determine IC₅₀. Experiments were repeated in triplicate.

WESTERN BLOT

Cells were divided into 4 groups for each cell line: untreated, cisplatin (6.25µM) alone, FLLL32 at the IC₅₀ dose, and FLLL32 at the IC₅₀ dose with cisplatin (6.25µM). Protein was extracted 72 hours after treatment. Primary antibodies were rabbit anti-STAT3 monoclonal antibody (Cell Signaling, Boston, Massachusetts), rabbit anti-phospho-STAT3 (Tyr705) monoclonal antibody (Cell Signaling), mouse anti-JAK2 monoclonal antibody (Millipore, Billerica, Maine), rabbit anti-phosphorylated JAK2 monoclonal antibody (Millipore), rabbit anti-Bcl-xL monoclonal antibody (Cell Signaling), and rabbit anti-EGFR (Cell Signaling). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) detected by mouse monoclonal antibody was used as a loading control. The secondary antibody was either horseradish peroxidase–conjugated sheep anti-mouse antibody (Amersham Life Sciences, Arlington Heights, Illinois) or horseradish peroxidase–conjugated donkey anti-rabbit antibody (Amersham Life Sciences). Protein bands were observed using the ECL Plus Western Blotting Detection System (Amersham Life Sciences) and visualized on Hyperfilm (Eastman Kodak, Boston, Massachusetts). All experiments were repeated in triplicate. ImageJ, version 1.43, a widely used image analysis program publicly available through the National Institutes of Health (NIH), was used for densitometry analysis of protein bands as described in the software documentation.

IN VITRO FLLL32 EFFECT ON CELL SURVIVAL AND PROLIFERATION

Cells were divided into 12 groups for each cell line: untreated controls, cisplatin monotherapy (25.0µM, 12.50µM, 6.25µM, 3.13µM, and 1.56µM), FLLL32 monotherapy at the IC₅₀ dose, and FLLL32 at the IC₅₀ dose combined with cisplatin (25.0µM, 12.50µM, 6.25µM, 3.13µM, and 1.56µM). The UM-SCC-29 and UM-SCC-74B cell lines were first incubated overnight in 5% carbon dioxide at 37°C at seed concentrations of 2500 and 2000 cells per well, respectively, in 96 well plates. FLLL32-containing medium was then added into the appropriate treatment groups with all other groups receiving cDMEM with 0.2% DMSO. After a further 24 hours of incubation, protein was extracted 72 hours after treatment. Primary antibodies were rabbit anti-STAT3 monoclonal antibody (Cell Signaling, Boston, Massachusetts), rabbit anti-phospho-STAT3 (Tyr705), rabbit anti-Bcl-xL monoclonal antibody (Cell Signaling), and rabbit anti-EGFR (Cell Signaling). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) detected by mouse monoclonal antibody was used as a loading control. The secondary antibody was either horseradish peroxidase–conjugated sheep anti-mouse antibody (Amersham Life Sciences, Arlington Heights, Illinois) or horseradish peroxidase–conjugated donkey anti-rabbit antibody (Amersham Life Sciences). Protein bands were observed using the ECL Plus Western Blotting Detection System (Amersham Life Sciences) and visualized on Hyperfilm (Eastman Kodak, Boston, Massachusetts). All experiments were repeated in triplicate. ImageJ, version 1.43, a widely used image analysis program publicly available through the National Institutes of Health (NIH), was used for densitometry analysis of protein bands as described in the software documentation.

APOPTOSIS ASSAY

Cells were seeded at a density of 60,000 cells per well in 6 well plates for both cell lines and incubated overnight to humidified air with 5% carbon dioxide at 37°C. Each well represented 1 of the following groups: untreated controls, FLLL32 monotherapy (IC₅₀), cisplatin monotherapy (1.56µM and 3.13µM), or FLLL32 (IC₅₀) with cisplatin (1.56µM and 3.13µM). The FLLL32 was added after the first night of incubation, and the cisplatin was added the following day. After an additional 48 hours of incubation, cells were resuspended in Annexin V binding buffer (10mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] with 140.0mM sodium chloride and 2.50mM calcium chloride; pH 7.4) and stained with Annexin V-FITC (fluorescein isothiocyanate) (Invitrogen) and propidium iodide counterstain. The apoptotic and surviving fraction was then determined by flow cytometry.

STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS software (SPSS Inc, Chicago, Illinois). The 2-tailed t test for independent samples was used for the analysis of all data. The level of significance was set at P < .05.

RESULTS

FIFTY PERCENT INHIBITORY CONCENTRATION DETERMINATION

A number of HNSCC cell lines were treated with a range of cisplatin doses to determine the IC₅₀, including the UM-SCC-29 and UM-SCC-74B cell lines (Figure 1). The UM-SCC-29 cell line demonstrated marked resistance to cisplatin relative to UM-SCC-74B, as evidenced by an IC₅₀ of 12.5µM vs 4.8µM, respectively. These 2 cell lines were ultimately selected for further experimentation on the basis of their 2.6-fold difference in cisplatin sensitivity and comparable oral cavity squamous cell carcinoma histologic characteristics. The IC₅₀ was also determined for FLLL32 in the selected UM-SCC-74B and UM-SCC-29 cell lines (Figure 2). The values for the FLLL32 IC₅₀ for the UM-SCC-74B and UM-SCC-29 cell lines were 1.40µM and 0.85µM, respectively.

MOLECULAR CHARACTERIZATION OF CELL LINES

Both UM-SCC-29 and UM-SCC-74B were characterized for the expression of multiple proteins involved in apop-
tosis (Figure 3). Of importance is the presence of both the wild-type STAT3 protein and the active, phosphorylated variant, pSTAT3, in both cell lines. The phosphorylated variant is thought to serve as the target for FLLL32-mediated STAT3 inhibition. The UM-SCC-74B cell line demonstrates less EGFR expression than UM-SCC-29. Expression of p-AKT, the active form of the antiapoptotic protein AKT1, is also reduced in UM-SCC-74B relative to UM-SCC-29. UM-SCC-74B does not express the tumor suppressor protein p53, which is consistent with the wild-type p53 gene in this cell line. UM-SCC-29 does have mutant p53 and expresses p53 strongly as expected for the mutant protein. The antiapoptotic proteins Bcl-xL and survivin are expressed in both cell lines, as are the proapoptotic proteins SMAC and caspase 3. β-Actin control showed equal loading between cell lines.

**DOWNREGULATION OF pSTAT3 EXPRESSION AFTER FLLL32 EXPOSURE**

Phosphorylated STAT3 is the active form of the STAT3 protein. To determine whether FLLL32 is effective in downregulating pSTAT3 protein, a Western blot was performed incorporating cells subject to no treatment, cisplatin alone, cisplatin with FLLL32, and FLLL32 alone (Figure 4). Densitometry analysis demonstrated a significant downregulation of pSTAT3 protein following treatment with FLLL32 vs nontreated controls (P < .05). This downregulation was not observed in cells that were treated with cisplatin alone. FLLL32 had no effect on the expression of STAT3, JAK2, Bcl-xL, EGFR, or GAPDH protein, the latter of which was used as an internal positive control. Phosphorylated JAK2 protein could not be detected in either cell line.

**CYTOTOXIC EFFECTS OF FLLL32 ALONE AND ENHANCEMENT OF CISPLATIN CYTOTOXICITY ON HNSCC CELLS IN VITRO**

We performed MTT cell survival assays to assess the cytotoxic effect of FLLL32, alone and in combination with a range of cisplatin doses, on HNSCC cells from both cell lines (Figure 5). In the UM-SCC-74B cell line, the
percentages of viable cells relative to untreated control were 73%, 56%, 34%, 22%, and 16% for the cisplatin monotherapy groups at doses of 1.56µM to 12.50µM. Combining FLLL32 at the IC50 dose with cisplatin at doses of 1.56µM to 12.50µM resulted in relative cell viability rates compared with controls of 35%, 29%, 23%, 17%, and 15%, respectively. Monotherapy with FLLL32 resulted in 39% cell survival vs untreated controls. Comparison between treatment groups was statistically significant (P < .001 to P = .05) for all groups except FLLL32 with cisplatin (25.0µM) vs cisplatin (25.0µM), FLLL32 with cisplatin (6.25µM) vs cisplatin (12.50µM), and FLL with cisplatin (1.56µM) vs cisplatin (6.25µM). FLLL32 with cisplatin (1.56µM) induced a 38% reduction in cell viability vs cisplatin monotherapy (1.56µM; P < .001). In UM-SCC-74B cells, FLLL32 with cisplatin (1.56µM) induced tumor cytotoxic effects equivalent to those of cisplatin given alone at a 4-fold higher dose (6.25µM).

In the UM-SCC-29 cell line, cell viability rates relative to untreated controls was 84%, 61%, 41%, 26%, and 18% for the cisplatin monotherapy groups at doses of 1.56µM to 12.50µM. Combination therapy with FLLL32 at the IC50 dose and cisplatin at doses of 1.56µM to 12.50µM induced relative cell survival rates of 40%, 35%, 26%, 21%, and 16%, respectively, vs control. Monotherapy with FLLL32 alone resulted in a 48% cell survival rate vs control. Comparisons between treatment groups was statistically significant (P < .001 to P = .02) for all groups with the exception of FLLL32 with cisplatin (6.25µM) vs cisplatin (12.50µM), and FLLL32 with cisplatin (1.56µM) vs cisplatin (6.25µM). FLLL32 with cisplatin (1.56µM) induces a 44% reduction in cell viability vs cisplatin monotherapy (1.56µM) (P < .001). The key finding was that combination therapy with FLLL32 and cisplatin (1.56µM) induced a suppressive effect comparable with cisplatin monotherapy at 6.25µM and an enhanced cytotoxic effect vs cisplatin monotherapy at doses

![Western blots showing effects of FLLL32 with or without cisplatin (cis-diammineplatinum(II) dichloride) on the expression and phosphorylation state of key proteins. A. Downregulation of phosphorylated STAT3 (pSTAT3) in the UM-SCC-74B (74B) and UM-SCC-29 (29) cell lines following administration of curcumin analog FLLL32, with or without cisplatin. The expression level of nonphosphorylated, inactive STAT3 remains unchanged in both cell lines after the addition of FLLL32 or cisplatin, alone or in combination. JAK2 (Janus kinase) and nonphosphorylated STAT3 expression was stable across cell lines and treatment groups. Phosphorylated JAK2 protein (not shown) could not be isolated. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mouse monoclonal antibody was used as a positive loading control. B. FLLL32, with or without cisplatin, did not alter the expression of the proapoptotic proteins Bcl-xL or AKT or of the upstream signaling molecule epidermal growth factor receptor (EGFR). GAPDH mouse monoclonal antibody was used as a positive loading control.](image-url)
of 1.56µM to 3.25µM (P < .01) in cisplatin-resistant UM-SCC-29 cells. 

These findings suggest that FLLL32 sensitizes both cisplatin-sensitive cells and, critically, intrinsically cisplatin-resistant UM-SCC-29 HNSCC cells to treatment with low concentrations of cisplatin. FLLL32 effectively permits the use of up to a 4-fold lower dose of cisplatin than used in cisplatin monotherapy while achieving comparable or enhanced inhibition of tumor cell survival.

**FLLL32 POTENTIATES APOPTOSIS IN HNSCC CELLS**

To further investigate the mechanism of FLLL32 cytotoxic effects in HNSCC cells, as well as to elucidate potential underlying mechanisms for the enhancement of FLLL32 efficacy through combination therapy with cisplatin, apoptosis was evaluated using flow cytometry (Figure 6). As expected, untreated cells had low levels of apoptosis of 0.42% and 6.94% for the UM-SCC-74B and UM-SCC-29 cell lines, respectively. FLLL32 alone resulted in a modest increase in the proportion of apoptotic cells to 2.29% and 15.82% for the UM-SCC-74B and UM-SCC-29 cell lines, respectively. FLLL32 combination treatment with cisplatin (3.13µM) decreased the proportion of living cells to 45.34% and 39.06% and increased the percentage of apoptotic cells to 29.10% and 37.52% in UM-SCC-74B and UM-SCC-29, respectively. The effect was similar but reduced when FLLL32 was combined with a lower dose of cisplatin (1.56µM). This represents a significant improvement over the extent of apoptosis induced by cisplatin monotherapy alone at doses of either 1.56µM or 3.125µM. These data indicate that FLLL32 can potentiate apoptosis induced by cisplatin in both cisplatin-resistant and cisplatin-sensitive cell lines.

**COMMENT**

Platinum-based compounds are often used as first-line agents in the treatment of HNSCC. Cisplatin exerts a dose-dependent effect such that higher doses induce apoptosis in a larger fraction of tumor cells.29 The effectiveness of cisplatin is tempered by the development of enhanced DNA damage repair mechanisms in treated cancer cells such that they are able to escape apoptosis-inducing damage.3 Clinically, this necessitates the use of increasing doses of cisplatin and a significant increase in treatment-limiting toxic effects.30 Patients who survive treatment of their initial cancer often experience long-
term treatment-related morbidity and will often die of distant metastases. This is the underlying cause of the 5-year overall survival rate of approximately 50% and the lack of improvement in survival statistics over the past several decades.

Consequently, important research efforts have been directed toward understanding the molecular changes that underlie HNSCCs, targeting these mechanisms and either directly inducing cancer cell death or disrupting pathways that permit the survival of cancers subject to standard therapeutic approaches. One such molecular target is the JAK/STAT pathway, which is overexpressed in 82% of HNSCCs. The JAK/STAT3 pathway has been shown to drive HNSCCs independent of growth factors.
such as EGFR.\textsuperscript{17} Disruption of STAT3 signaling has been shown to be effective in a range of solid tumors, including HNSCC.\textsuperscript{7,10,11,21,26,30,31}

The current study investigates the role of FLLL32, a curcumin-based STAT3 inhibitor, on HNSCC cells in vitro in terms of efficacy as a monotherapy and as a means of sensitizing cancer cells to cisplatin. FLLL32 is specifically designed to inhibit STAT3 through blockade of the SH2 dimerization site.\textsuperscript{26} Consistent with a recently published report\textsuperscript{26} that used FLLL32 in breast and pancreatic cancer, we found that FLLL32 seems to be selective for the JAK/STAT pathway by downregulating activated STAT3 expression in HNSCC.

FLLL32 is able to induce a significant antitumor effect when used as a monotherapy in both cisplatin-sensitive and cisplatin-resistant cell lines. More important, FLLL32 induces a potent chemosensitization effect even in cisplatin-resistant HNSCC cells, permitting the use of cisplatin doses several-fold lower than those used in cisplatin monotherapy while achieving an equivalent tumor inhibition. Our data indicate that this is partially attributable to an increase in the proportion of apoptotic cells and a decrease in surviving fraction in HNSCC cells treated with FLLL32 and cisplatin in combination.

Translating these findings to the clinical realm will require further investigation. In particular, further studies will need to elucidate whether the observed in vitro chemosensitization effect is translatable to animal models of HNSCC. In addition, the absence of dose-limiting toxic effects seen with curcumin, the compound on which FLLL32 is based, suggests that STAT3 inhibitors may have a clinical role in the future.\textsuperscript{31,32} Continued investigation of the JAK/STAT pathway and the design of novel inhibitors, like FLLL32, that are capable of targeting this pathway may herald new therapeutic approaches that enhance or obviate the need for currently used chemo-therapeutic agents.

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Correspondence: Thomas E. Carey, PhD, Department of Otolaryngology–Head and Neck Surgery, University of Michigan, 5311 Medical Science I, West Medical Center Drive, Ann Arbor, MI 48109-5616 (careyte@umich.edu).

Author Contributions: Drs Abuzeid and Carey had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Abuzeid, Davis, Brenner, Lin, Fuchs, Prince, and Carey.

Acquisition of data: Abuzeid, Davis, Tang, Saunders, and Prince.

Analysis and interpretation of data: Abuzeid, Davis, Tang, Light, Bradford, Prince, and Carey.

Drafting of the manuscript: Abuzeid and Lin.

Critical revision of the manuscript for important intellectual content: Abuzeid, Davis, Tang, Saunders, Brenner, Fuchs, Light, Bradford, Prince, and Carey.

Statistical analysis: Abuzeid and Light.

Obtained funding: Bradford and Carey.

Administrative, technical, and material support: Abuzeid, Davis, and Tang.

Study supervision: Abuzeid, Davis, Brenner, Bradford, Prince, and Carey.

Chemical synthesis: Fuchs.

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