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IL6 is associated with response to dasatinib and cetuximab: Phase II clinical trial with mechanistic correlatives in cetuximab-resistant head and neck cancer

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ABSTRACT

Objective: Src family kinase (SFK) activation circumvents epidermal growth factor receptor (EGFR) targeting in head and neck squamous cell carcinoma (HNSCC); dual SFK-EGFR targeting could overcome cetuximab resistance.

Patients and methods: We conducted a Simon two-stage, phase II trial of the SFK inhibitor, dasatinib, and cetuximab in biomarker-unselected patients with cetuximab-resistant, recurrent/metastatic HNSCC. Pre- and post-treatment serum levels of interleukin-6 (IL6) were measured by ELISA. HNSCC cell lines were assessed for viability and effects of IL6 modulation following dasatinib-cetuximab treatment.

Results: In the first stage, 13 patients were evaluable for response: 7 had progressive and 6 had stable disease (SD). Enrollment was halted for futility, and biomarker analysis initiated. Low serum IL6 levels were associated with SD (raw $p = 0.028$, adjusted $p = 0.14$) and improved overall survival ($p = 0.010$). The IL6 classifier was validated in a separate trial of the same combination, but was unable to segregate survival risk in a clinical trial of cetuximab and bevacizumab suggesting serum IL6 may be specific for the dasatinib-cetuximab combination. Enhanced *in vitro* HNSCC cell death was observed with dasatinib-cetuximab versus single agent treatment; addition of IL6-containing media abrogated this effect.

Conclusion: Clinical benefit and overall survival from the dasatinib-cetuximab combination were improved among patients with low serum IL6. Preclinical studies support IL6 as a modifier of dasatinib-cetuximab response. In the setting of clinical cetuximab resistance, serum IL6 is a candidate predictive marker specific for combined dasatinib-cetuximab. The trial was modified and redesigned as a biomarker-enriched Phase II study enrolling patients with undetectable IL6.

Introduction

Despite over-expression of the epidermal growth factor receptor (EGFR) in the majority of head and neck squamous cell carcinomas (HNSCC), clinical responses to the EGFR-directed antibody, cetuximab, approximate 10% [1–3]. Unlike colorectal cancer, where activating *KRAS* and *BRAF* mutations predict cetuximab resistance [4], no selection biomarker exists in HNSCC [5,6]. In HNSCC preclinical models, activation of parallel growth factor receptors or downstream signaling nodes circumvents EGFR blockade [7]. Mechanistic identification of such a resistance node could establish

Abbreviations: EGFR, epidermal growth factor receptor; SFK, Src-family kinase; HNSCC, head and neck squamous cell carcinoma; IL6, interleukin-6; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; IRB, institutional review board; PD, progressive disease; ORR, overall response rate; AE, adverse events; HPV, human papilloma virus; SD, stabile disease; ISH, in situ hybridization; PFS, progression-free survival; OS, overall survival.

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a biomarker for clinical selection and/or a rational co-target, addressing an unmet clinical need.

Src family kinases (SFKs) play a key role in both EGFR-dependent and -independent signaling pathways, converging upon STAT3 [8,9]. As shown by our laboratory and others', activation of SFKs leads to EGFR inhibitor resistance [10–12]. Baseline tumoral phospho-Src expression was associated with resistance to the EGFR tyrosine kinase inhibitor erlotinib in patients with operable HNSCC [13]. Dasatinib is a potent multi-targeted inhibitor of at least five selected protein tyrosine kinases/kinase families including several members of the SFKs (SRC, LCK, YES, FYN), BCR-ABL, c-KIT, EphaA2 receptor and PDGFβ receptor [14]. Though the SFK spectrum inhibitor, dasatinib has negligible single agent activity in patients with recurrent/metastatic HNSCC [15], the potential for co-targeting EGFR and SFKs has not been exploited. Dual SFK-EGFR targeting could overcome cetuximab resistance by inhibiting EGFR-independent activation of STAT3 by Src. We conducted a Phase II trial evaluating the combination of dasatinib and cetuximab in patients with cetuximab-resistant, recurrent/metastatic HNSCC, after establishing its safety during a Phase I trial enrolling patients with refractory solid tumors [16].

HNSCC is molecularly heterogeneous [17], and responses to dual SFK-EGFR targeting are expected to vary depending upon genetic and biochemical profiles. Thus, we selected mechanistically relevant biomarkers to evaluate for associations with clinical benefit. Circulating cytokines and growth factors such as interleukin-6 (IL6) and vascular endothelial growth factor (VEGF) have been associated with response to cetuximab in HNSCC [16,18]. Activation of the IL6/JAK/STAT3 signaling axis is a known mechanism of acquired resistance to dasatinib [19]. Activation of MET, the receptor for hepatocyte growth factor (HGF), overcomes EGFR blockade in preclinical models of HNSCC and in HNSCC patients [12,20,21]. Moreover, serum HGF levels have been associated with resistance to EGFR inhibitors in colorectal and lung cancers [22–24]. Here, we report results for biomarker-unselected patients with cetuximab-resistant, recurrent/metastatic HNSCC treated with dasatinib-cetuximab, and identify serum IL6 as a biomarker of *de novo* resistance to this combination.

Patients and methods

Patients and biologic specimens

Primary inclusion criteria included: recurrent/metastatic HNSCC; progression after previous cetuximab; age ≥18; Eastern Cooperative Oncology Group performance status <2; adequate end organ function. Primary exclusion criteria included: prior exposure to SFK or EGFR inhibitor other than cetuximab. Blood was collected at baseline and following 6 weeks of treatment and archived tumor tissue was obtained. The protocol was approved by the University of Pittsburgh Institutional Review Board and registered with ClinicalTrials.gov (NCT01488318). Serum from two other protocols was analyzed retrospectively: a phase I trial of dasatinib-cetuximab in refractory solid tumors [16] (NCT00388427) and a phase II trial of cetuximab-bevacizumab, a VEGF-A targeting antibody, in recurrent/metastatic HNSCC (NCT004070810) [25].

Treatment

This single-arm, two-stage, phase II study evaluated the efficacy of dasatinib plus cetuximab in patients with cetuximab-resistant, recurrent/metastatic HNSCC (Supplemental Fig. 1). Cetuximab was dosed at 250 mg/m^2/week following a standard loading dose. Dasatinib 150 mg daily was initiated on day 3 [16]. Cycle length was 3 weeks; patients were treated continuously until progressive disease (PD) or intolerable toxicity.

Evaluations

The primary endpoint was objective response rate (ORR) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, performed every 2 cycles. Toxicity was graded using the National Cancer Institute CTCAE version 4.0 for toxicity and Adverse Event (AE) reporting [22].

Serum analyses

Blood samples were processed for isolation of sera and stored at −80 °C. Individual enzyme-linked immunosorbent assays (ELISAs) (Quantikine ELISA kits, R&D Systems) for IL6, VEGF, HGF and TGF-α were used to quantify each serum analyte in duplicate. Concentrations between the replicates varied by less than 10% and mean values were used for analysis. Analyte levels below the limit of detection (LOD) were set to one-half the LOD for statistical analyses.

Human papilloma virus (HPV) status and baseline tumor tissue expression of pSFK and pSTAT3

In situ hybridization (ISH) for HPV DNA was performed as previously described [18]. Tissues were evaluated by immunohistochemistry (IHC) for pSFK and pSTAT3 expression using anti-Phospho-SFK PY-416 (PK1109 1:50; Calbiochem) antibody and anti-Phospho-STAT3 PY-705 antibody (D3A7 1:500; Cell Signaling Technology). Tumor staining was semi-quantified using the Aperio imaging system. IHC scores were calculated as the product of average staining intensity (0–3) and the proportion of positive tumor cells (range 0–300). At least 8 areas all greater than 2 mm^2 were scored for each tumor. While the pSFK immunogen is a synthetic phosphopeptide corresponding to amino acids surrounding the tyrosine 416 phosphorylation site of Src, it is possible that this antibody may also recognize other SFKs phosphorylated at equivalent sites.

Cell line IL6 quantification

3×10^4 cells were plated in each well of 24-well plates and allowed to adhere overnight; media was refreshed the following day. After 24 h media was collected, centrifuged for 5 min at 5000*g*. IL6 levels in cell supernatants were determined using the Human IL6 Quantikine ELISA kit for PE/CA-PJ49, CAL27, CAL33, SCC9, BICR56 and FaDu HNSCC cell lines and cultured tumor-associated fibroblasts (TAFs). IL6 concentration is represented as the average of triplicate samples. TAFs were propagated from HNSCC patient tumors. Cell lines were authenticated by genotyping with a multiplex STR assay (Genetica) within 3 months of performed studies.

Viability assays

1.5×10^4 cells were plated in each well of 24-well plates and allowed to adhere overnight. Cells were treated with vehicle, dasatinib, cetuximab, or dasatinib plus cetuximab at indicated concentrations for 96 h in complete media. Cell viability was assessed by crystal violet assay; briefly, cells were washed once with PBS, fixed with 96% ethanol for 10 min and stained with 0.1% crystal violet solution for 30 min. Stain was solubilized with 1% SDS and absorbance at 570 nm quantified. To evaluate the effects of IL6 on cell viability, treatment conditions included supernatants from TAF