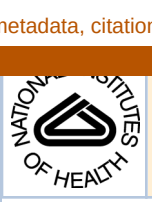

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INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR INHIBITOR, AMG-479, IN CETUXIMAB-REFRACTORY HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Abstract

Background—Recurrent head and neck squamous cell carcinoma (HNSCC) remains a difficult cancer to treat. Here, we describe a patient with HNSCC who had complete response to methotrexate (MTX) after progressing on multiple cytotoxic agents, cetuximab, and AMG-479 (monoclonal antibody against insulin-like growth factor-1 receptor [IGF-1R]).

Methods—The clinical information was collected by a retrospective medical record review under an Institutional Review Board–approved protocol. From 4 tumors and 2 normal mucosal epithelia, global gene expression, and IGF-1R and dihydrofolate reductase (DHFR) protein levels were determined.

Results—Effective target inhibition in the tumor was confirmed by the decreased protein levels of total and phospho-IGF-1R after treatment with AMG-479. Decreased level of DHFR and conversion of a gene expression profile associated with cetuximab-resistance to cetuximab-sensitivity were also observed.

Conclusion—This suggests that the combination of AMG- 479 and MTX or cetuximab may be a promising therapeutic approach in refractory HNSCC.

Keywords

IGF-1R inhibitor; cetuximab; head and neck squamous cell carcinoma; AMG-479; methotrexate

Recurrent and/or metastatic head and neck squamous cell carcinoma (HNSCC) remains 1 of the most difficult cancers to treat with limited chemotherapeutic options. Here, we describe

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a patient with HNSCC who had complete response to methotrexate (MTX) after progressing on multiple cytotoxic agents, cetuximab, and AMG-479 (monoclonal antibody against insulin-like growth factor-1 receptor [IGF-1R]). In a tumor tissue biopsy of this patient, we observed a decreased level of dihydrofolate reductase (DHFR) and conversion of gene expression profile from cetuximab resistance to cetuximab sensitivity after AMG-479 treatment, suggesting that AMG-479 may have sensitized the tumor to MTX and cetuximab. This represents a promising approach to the implementation of IGF-1R inhibitors in refractory HNSCC.

CASE REPORT

A 54-year-old woman was referred to our hospital for the management of recurrent HNSCC in 2004. She was a nonsmoker and was initially diagnosed with T1N1M0 SCC of the oral tongue in September 2001. She underwent a right partial glossectomy with right suprahyoid neck dissection without adjuvant chemotherapy or radiation therapy. She remained clinically disease free until May 2003, when she developed a new mass in the right lower alveolar ridge and was treated with radiation therapy. She developed local recurrences on 3 subsequent occasions. She underwent surgical resections for the first 2 recurrences in December 2003 and May 2004, but when the mass recurred for the third time in March 2005, it was deemed inoperable and she was referred for palliative systemic chemotherapy. She was treated with a combination of cisplatin and irinotecan on a clinical trial until her disease progressed after 10 weeks of therapy. She then received weekly docetaxel; however, the disease progressed after 3 weeks and treatment was discontinued. Due to poor performance status, in July 2005 she was not considered a candidate for further cytotoxic chemotherapy and was started on cetuximab monotherapy. After 11 weeks of cetuximab, she experienced a complete clinical response, but her disease progressed after 18 months (Figures 1A and 1D). Cetuximab was discontinued in November 2006. In January 2007, she was enrolled in a phase I clinical trial and received 3 doses of AMG-479 12 mg/kg every 2 weeks. AMG-479 was discontinued due to the rapid progression of disease (Figures 1B and 1E). As the last effort for palliation, she was started on a low dose of weekly MTX 40 mg/m^2 in April 2007. After 12 weeks of MTX, she achieved a near-complete response and remained without progression for 6 months (Figures 1C and 1F). In October 2007, she developed progressive disease while on MTX. At that point, the decision was made to re-challenge the tumor with cetuximab. The patient again experienced a significant clinical response as determined by consecutive measurements of the tumor size (decreasing from 10 × 6 cm to 3 × 3 cm within 3 weeks) and by palliation of her pain. However, this second response was brief. Unfortunately, her disease progressed in 8 weeks and she died in January 2008 under hospice care.

The clinical information was collected by a retrospective medical record review. Formalin-fixed paraffin-embedded tumor and adjacent normal mucosal epithelia from surgical resections in 2001 (original diagnosis) and 2004 (tumor recurrence after radiation therapy and before chemotherapies), and frozen tumors from biopsies post-cetuximab (pre-AMG-479) and post-AMG-479 treatments in 2007 were obtained under an Institutional Review Board–approved protocol. A signed consent form for publication of this case report was also obtained from the patient during her treatments. Biopsies from the tumor in 4 different time points and of normal mucosa from 2 different time points were available for translational studies.

Gene expression data were generated and analyzed in duplicate for each tumor, as previously described.1 The AMG-479 modulated genes were determined by comparing the pre-AMG-479 and post-AMG-479 treatment frozen tumor biopsies. The biological relatedness of the selected genes was examined using Ingenuity Pathways Analysis (IPA).

The protein levels of total and phosphorylated IGF-1R, protein kinase B (AKT), DHFR, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined by Western blots (IGF-1R, IGF-1R-Tyr1135/1136, AKT, and AKT-Ser473, Cell Signaling Technology, Danvers, MA; DHFR, BD Biosciences, San Jose, CA; and GAPDH, Santa Cruz Biotechnology, Santa Cruz, CA). Detection of human papillomavirus, and DNA mutation analyses of exons 18, 19, and 21 of epidermal growth factor receptor (*EGFR*), and codon 12 of *KRAS* in frozen tumors were determined, as previously described.[2–4]

RESULTS

Gene expression data were obtained from the tumor collected at 4 different time points: (1) at original diagnosis in 2001, (2) at recurrence after radiation therapy and before systemic chemotherapy or cetuximab treatment in 2004, (3) after cetuximab for 18 months and before AMG-479 treatment in 2007, and (4) after AMG-479 and before MTX treatment in 2007. Gene expression microarray data were also obtained from 2 areas of normal mucosal epithelium adjacent to the tumors: (1) at diagnosis in 2001 and (2) at recurrence after radiation therapy in 2004. Because the tumor rapidly grew during the AMG-479 treatment, effective target inhibition was confirmed by the decreased protein levels of total and phospho-IGF-1R and phospho-AKT after treatment with AMG-479 using Western blots (Figure 2). To examine the genes that were modulated by AMG-479 and the relatedness of the genes with various biological functions, differentially expressed genes with greater than 2-fold between pre-AMG-479 and post-AMG-479 treatment were determined by supervised analysis (Figure 3A and Supplemental Table, online only). Both the normal mucosa and tumor samples taken at the time of diagnosis before any treatment (samples obtained in 2001) differed in the expression signature when compared with samples at the time of recurrence (samples in 2004). This could be attributable to radiation effects because the recurrent tumor and normal mucosa samples were taken from the previously radiated field. Interestingly, after AMG-479 treatment, the expression signature reverted to the pattern observed in the pre-cetuximab-treated tumor which was sensitive to cetuximab.

The differentially expressed genes were further interrogated using IPA. The statistically significant networks of genes were those involved in DNA replication, recombination and repair, cell cycle, cellular assembly and organization, cell signaling, and immune response. One of the AMG-479-modulated genes with statistical significance was *DHFR* (Figure 3B) which was downregulated by AMG-479. DHFR is the binding target of MTX and its active metabolite, which results in S-phase cell cycle inhibition.[2] Eight additional genes in the folate biosynthesis pathway were not significantly altered by AMG-479. Decrease in the protein level of DHFR after AMG-479 treatment was confirmed by Western blot (Figure 2). The tumor was negative for human papillomavirus infection or mutations in *EGFR* tyrosine kinase (TK) domain or *KRAS*. Additionally, this tumor was 1 of the tumors analyzed for *EGFR* gene copy number by fluorescent in situ hybridization in our previous study, and shown to have normal *EGFR* gene copy number.[3]

DISCUSSION

Recently, the dearth of therapeutic options for HNSCC has motivated the search for molecularly targeted therapies. One of the most common approaches has been inhibition of receptor tyrosine kinases (RTK) using either small molecules that bind to the TK domain of the receptors, or antibodies against epitopes located at the extracellular domains or against their ligands. Although recent therapeutic successes of small molecule RTK inhibitors have been linked to oncogenic dependency, usually caused by mutations in the functional TK domains,[4,5] the mechanism(s) of response to RTK inhibition by antibody-based targeted agents such as cetuximab, bevacizumab, or trastuzumab is less clear. The mechanism of

action could be a direct inhibition of signal transduction through the inhibited receptors, but indirect mechanisms such as antibody-dependent cell-mediated cytotoxicity may play a role.6

The most studied molecular target in HNSCC has been EGFR due to frequent overexpression in tumors compared to normal mucosal epithelia.7 There is also increasing evidence for IGF-1R as a potential molecular target in HNSCC.8 The IGF-1R is highly expressed in HNSCC compared to normal mucosal epithelia, and IGF-1 stimulates S-phase transition in a phosphoinositide-3-kinase/AKT and mitogen-activated protein kinase-dependent manner in HNSCC cell lines.8,9 The findings in our patient were consistent with the current data indicating that AMG-479 downregulates IGF-1R and AKT.10 However, the tumor rapidly grew in the setting of effective target inhibition. It is unclear which signaling pathway is the dominant compensatory pathway for IGF-1R. When AMG-479-modulated genes were interrogated by IPA, multiple networks were identified suggesting the interconnected nature of the signaling pathways that are affected by inhibition of a single receptor. For example, the EGFR pathway may be involved considering the expression signature reverted to the pattern in the pre-cetuximab treated tumor after AMG-479. Although AKT activation through IGF-1R is independent of EGFR, activation of mitogen-activated protein kinase is thought to be mediated through crosstalk between EGFR and IGF-1R in oral carcinoma cells, probably through IGF-1 stimulation of matrix metalloproteinases (MMPs), release of EGFR ligands, and subsequent activation of EGFR.11,12 In addition, EGFR and IGF-1R are known to heterodimerize upon receptor activation, and a combination of EGFR and IGF-1R inhibitors is more effective in reducing cell proliferation and migration compared to each inhibitor alone.8 The clinical observation that the patient regained cetuximab sensitivity upon re-challenge after having progressive disease suggests that AMG-479 may modulate cetuximab sensitivity.

The dramatic response to MTX with an unusual degree of sensitivity has not been reported using MTX as the fifth-line treatment in a recurrent/metastatic setting. Possible explanations for this response are: (1) an MTX-specific response due to downregulation of DHFR after AMG-479, (2) cell cycle–specific effect due to increased proliferation as a compensatory response to IGF-1R inhibition, or (3) decreased AKT signaling by AMG-479 and subsequently increased proapoptotic response to a cytotoxic agent. One may speculate that the response was MTX-specific due to downregulation of *DHFR*, because increased expression or activating mutations in *DHFR* have been associated with MTX resistance.13 There is also evidence that a targeted agent such as gefitinib can affect the response to other cell cycle-specific cytotoxic agents such as paclitaxel. Solit et al14 reported that continuous inhibition of EGFR antagonized the effects of paclitaxel, a mitosis-specific cytotoxic agent, by causing cell cycle arrest.

In the development of novel targeted therapeutics, the antitumor effects of specific antibodies as a monotherapy in the treatment of solid tumors have been disappointing with minimal response rates.15,16 However, the therapeutic strategy to combine antibody-based agents with existing chemotherapies has been extremely successful in breast cancer, non-small cell lung cancer, colon cancer, and HNSCC.15,17–19 This report supports the rationale for combination therapies using antibody-based targeted agents as chemo-sensitizers for conventional cytotoxic agents. In contrast to the general perception of targeted agents being less toxic than cytotoxic agents, our findings suggest that the use of a targeted agent alone may even be harmful by activating compensatory pathways. This brings into question the validity of limiting the evaluation of targeted agents based on response rates in monotherapy phase II trials. This case report suggests that the clinical development of IGF-1R inhibitors in combination with cetuximab or MTX warrants further investigation in refractory HNSCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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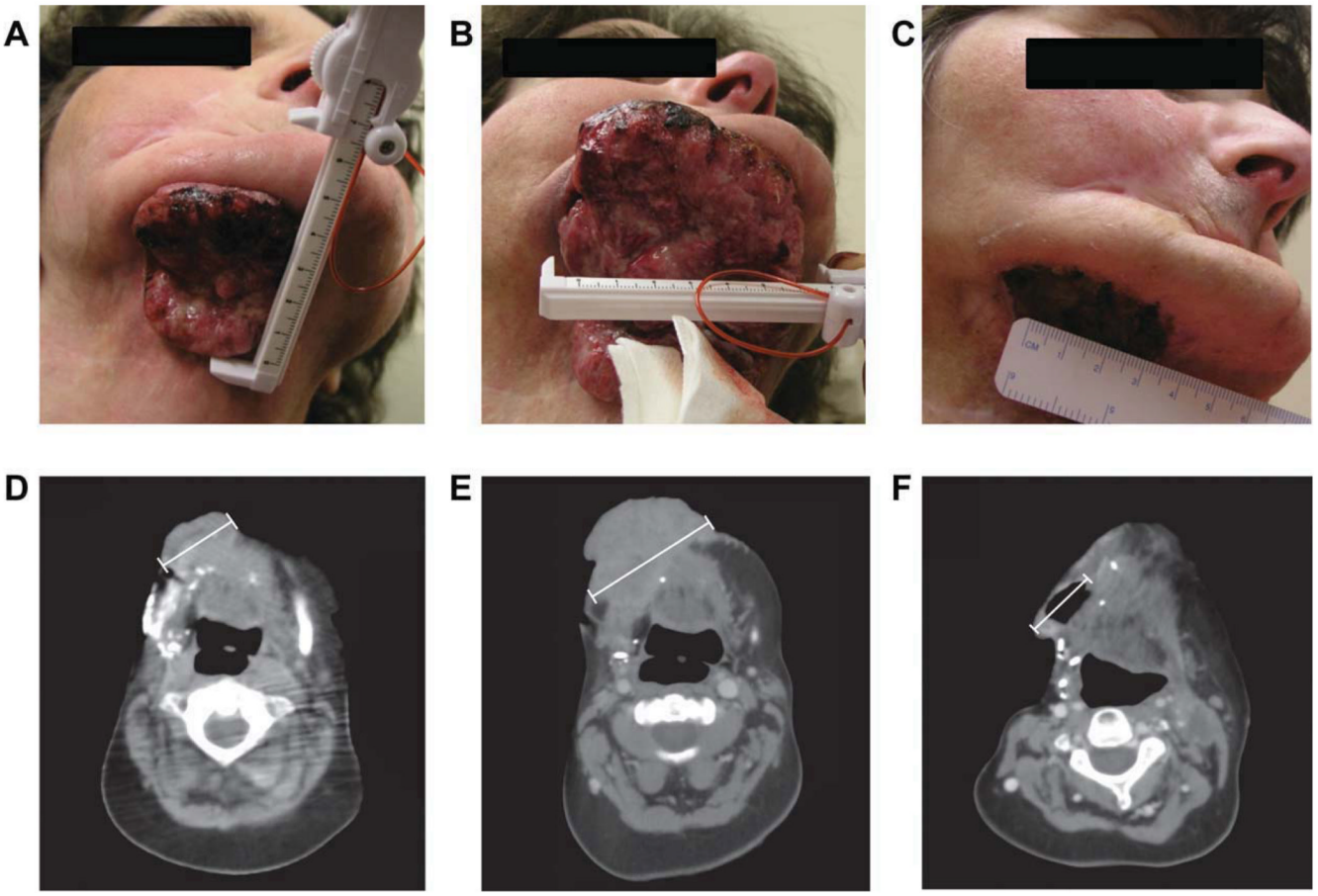

FIGURE 1.

Physical examination for tumor measurements: (**A**) after 18 months of cetuximab and on the day of first AMG-479 treatment, (**B**) after 4 weeks of AMG-479 treatment and determined to have progression of disease, and (**C**) after 12 weeks of methotrexate treatment. Radiographic examination for tumor measurements: (**D**) 4 days before AMG-479 treatment, (**E**) after 4 weeks of AMG-479 treatment, and (**F**) after 20 weeks of methotrexate treatment. The white line indicates the longest diameter of the measured tumor.

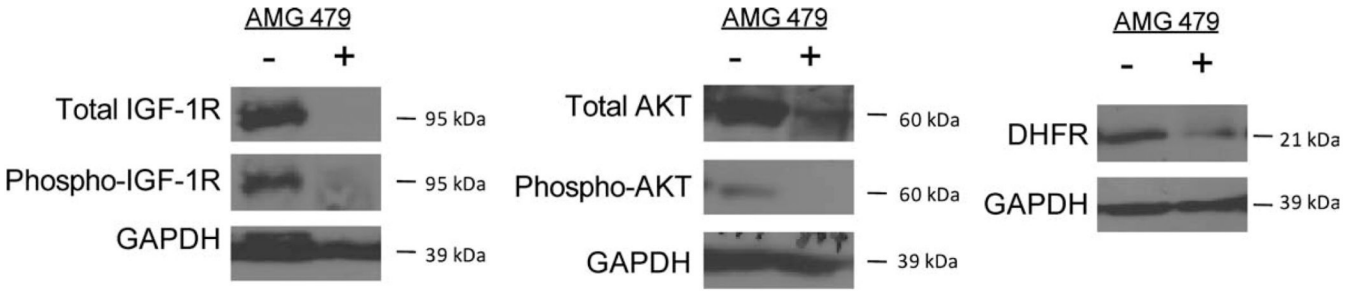

FIGURE 2.
Western blot comparison of protein levels in the tumors before and after AMG-479 treatment. IGF-1R, insulin-like growth factor-1 receptor; AKT, protein kinase B; DHFR, dihydrofolate reductase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

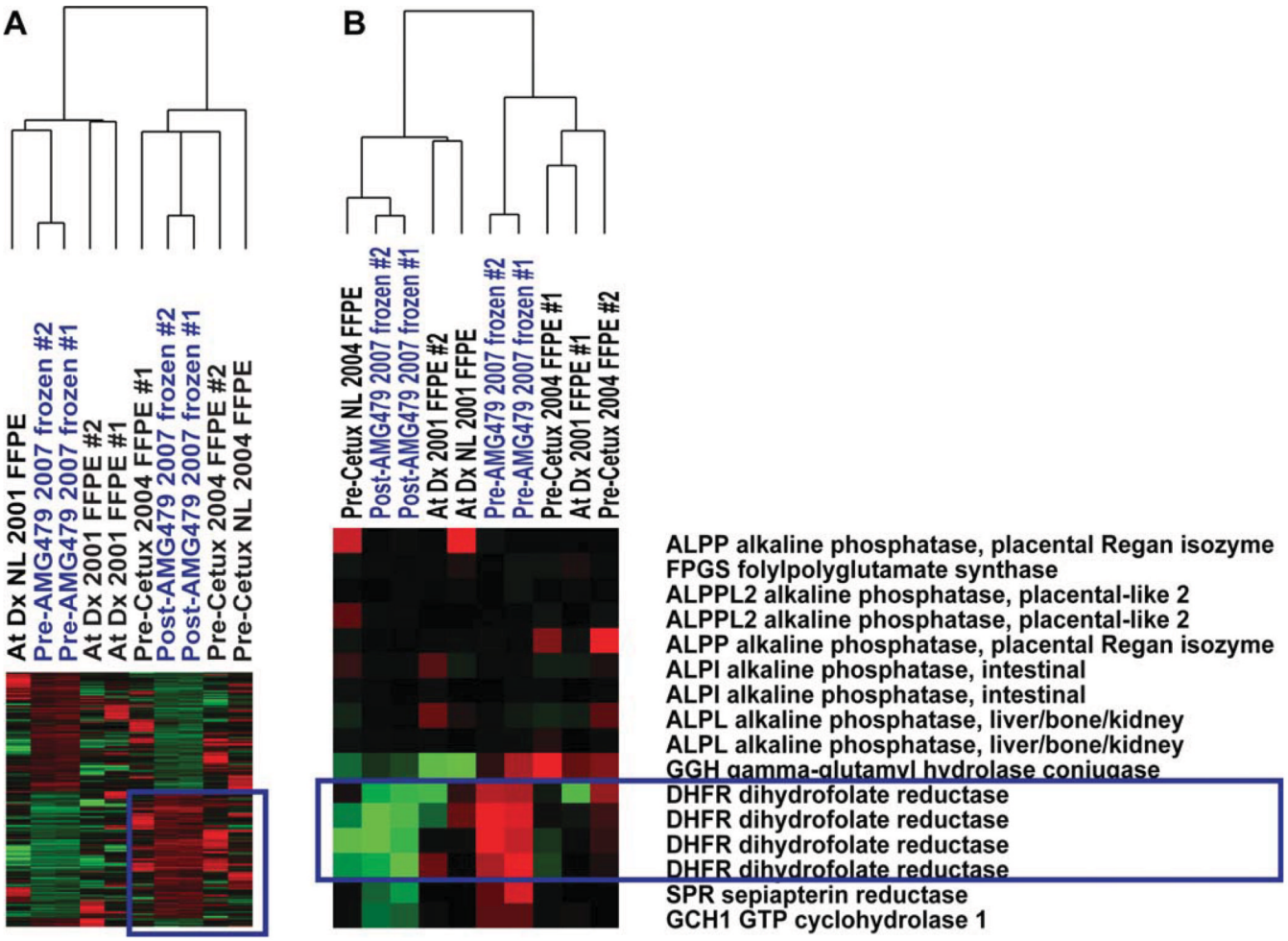

FIGURE 3.

Hierarchical clustering of tumors and normal mucosal epithelia taken at 4 different time points. The gene expression experiments were performed in duplicate; “At Dx NL 2001 FFPE” - normal mucosal epithelium taken from a formalin-fixed paraffin-embedded (FFPE) tissue at original diagnosis in 2001, “Pre-Cetux NL 2004 FFPE” - normal mucosal epithelium taken from a FFPE tissue at recurrence after radiation therapy and before systemic chemotherapy or cetuximab treatment in 2004, “Pre-Cetux 2004 FFPE” - tumor taken from a FFPE tissue at recurrence after radiation therapy and before systemic chemotherapy or cetuximab treatment in 2004, “Pre-AMG-479 2007 frozen” - tumor taken from a frozen tissue taken after cetuximab for 18 months and before AMG-479 treatment in 2007, and “Pre-AMG-479 2007 frozen” - tumor taken from a frozen tissue taken after AMG-479 and before methotrexate treatment in 2007. (**A**) Gene expression data were clustered using 2886 microarray probes that were differentially expressed between before and after the AMG-479 treatment. The genes indicated in a blue box strongly defined the expression pattern after the AMG-479 treatment. (**B**) Gene expression data were clustered using genes in folate biosynthesis obtained from Kyoto Encyclopedia of Genes and Genomes Pathway Database. Dihydrofolate reductase was down-regulated after the AMG-479 treatment. The intensity of the colors represents the range of gene expression levels: Red - higher expression, Green - lower expression, and Black - equal expression.