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Phase 1 Study of EGFR-Antisense DNA, Cetuximab, and Radiotherapy in Head and Neck Cancer With Preclinical Correlatives

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Abstract

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AUTHOR CONTRIBUTIONS

Julie E. Bauman: Data curation, investigation, methodology, writing–original draft, and writing–review and editing. **Umamaheswar Duvvuri:** Investigation and writing–review and editing. **Sufi Thomas:** Conceptualization, data curation, investigation, methodology, validation, visualization, and writing–review and editing. **William E. Gooding:** Conceptualization, data curation, formal analysis, methodology, software, and writing–review and editing. **David A. Clump:** Investigation and writing–review and editing. **Brian Karlovits:** Investigation and writing–review and editing. **Ahmad Wehbe:** Investigation and writing–review and editing. **Frank R. Miller:** Investigation and writing–review and editing. **Seungwon Kim:** Investigation and writing–review and editing. **Malabika Sen:** Conceptualization, data curation, investigation, methodology, validation, visualization, and writing–review and editing. **Dwight E. Heron:** Investigation and writing–review and editing. **Jennifer R. Grandis:** Conceptualization, funding acquisition, investigation, methodology, resources, supervision, validation, and writing–review and editing. **Athanassios Argiris:** Conceptualization, data curation, funding acquisition, investigation, project administration, resources, supervision, writing–original draft, and writing–review and editing.

CONFLICT OF INTEREST DISCLOSURES

Jennifer R. Grandis has a patent (Inhibition of Human Squamous Cell Carcinoma Growth in Vivo by Epidermal Growth Factor Receptor Antisense RNA Transcribed from a Pol III Promoter; US Patent No. 10/ 387,252) licensed by Benitec Biopharma. The remaining authors made no disclosures.

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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BACKGROUND: Cetuximab combined with radiation therapy (RT) is an evidence-based treatment for locally advanced head and neck squamous cell carcinoma (HNSCC); however, locoregional failure remains the primary cause of cancer-related death in this disease. Intratumoral injection of epidermal growth factor receptor (EGFR)-antisense plasmid DNA (EGFR-AS) is safe and has been associated with promising lesional responses in patients who have recurrent/metastatic HNSCC. For the current study, the authors investigated the antitumor effects of cetuximab and EGFR-AS in preclinical HNSCC models and reported their phase 1 experience adding intratumoral EGFR-AS to cetuximab RT.

METHODS: Antitumor mechanisms were investigated in cell line and xenograft models. Phase 1 trial eligibility required stage IVA through IVC HNSCC and a measurable lesion accessible for repeat injections. Patients received standard cetuximab was for 9 weeks. EGFR-AS was injected weekly until they achieved a lesional complete response. RT was delivered by conventional fractionation for 7 weeks, starting at week 3. Research biopsies were obtained at baseline and week 2.

RESULTS: When added to cetuximab, EGFR-AS decreased cell viability and xenograft growth compared with EGFR-sense control, partially mediated by reduced EGFR expression. Six patients were enrolled in the phase 1 cohort. No grade 2 or greater EGFR-AS–related adverse events occurred. The best lesional response was a complete response (4 patients), and 1 patient each had a partial response and disease progression. EGFR expression decreased in 4 patients who had available paired specimens.

CONCLUSIONS: In preclinical models, dual EGFR inhibition with cetuximab and EGFR-AS enhanced antitumor effects. In a phase 1 cohort, intratumoral EGFR-AS injections, cetuximab, and RT were well tolerated. A phase 2 trial is needed to conduct an extended evaluation of safety and to establish efficacy.

Keywords

antisense; cetuximab; epidermal growth factor receptor (EGFR); head and neck cancer; oligonucleotide; phase 2

INTRODUCTION

Epidermal growth factor receptor (EGFR) is a validated oncogene, prognostic biomarker, and therapeutic target in head and neck squamous cell carcinoma (HNSCC). Cetuximab, a monoclonal antibody that competitively inhibits EGFR at its extracellular binding domain, improves locoregional control (LRC), disease-free survival (DFS), and overall survival (OS) when combined with radiation therapy (RT) in patients with locally advanced HNSCC.[1,2] Combined cetuximab and RT (cetuximab-RT) is an established standard for locally advanced HNSCC[3] and is the predominant therapeutic strategy in the United States and Europe for patients who are elderly, frail, or unfit for cisplatin.[4,5] Because locoregional failure remains the major cause of death after cisplatin-based or cetuximab-based RT,[1,6] treatments to enhance LRC remain a major unmet clinical need.

Despite the near-universal expression of EGFR in HNSCC, cetuximab benefits only a minority of patients.[1,7,8] In the recurrent/metastatic setting, the response rate (RR) for

cetuximab is from 10% to 13%,[8] suggesting a rapid escape from extracellular inhibition. The EGFR-antisense plasmid DNA pNGVL1-U6-EG-FRAS (hereinafter referred to as EGFR-AS) is a 39-base-pair DNA plasmid that spans the translation start site for the *EGFR* gene and was designed to generate high expression of intracellular EGFR-antisense messenger RNA. We previously demonstrated that EGFR-AS decreased cellular proliferation compared with EGFR-sense control plasmid in well characterized HNSCC cell lines, and this decrease was mediated by decreased translation of *EGFR* and the sustained down-modulation of EGFR protein expression.[9,10] In vivo, intratumoral injection of EGFR-AS, but not EGFR-sense, inhibited tumor growth, coincident with increased apoptosis and suppressed EGFR protein expression in HNSCC xenografts.[11] We previously reported a phase 1 study evaluating intratumoral injection of EGFR-AS in patients with recurrent/metastatic HNSCC.[12] In that study, EGFR-AS caused no dose-limiting toxicities (DLTs) and yielded a promising lesional RR of 29%. This tolerability and efficacy profile raises the possibility that EGFR-AS injections could augment LRC if added to definitive cetuximab-RT.

We hypothesized that a dual anti-EGFR strategy of intratumoral EGFR-AS injections to reduce EGFR expression levels and systemic cetuximab to inhibit residual, extant EGFR may increase antitumor efficacy. We evaluated this combination in preclinical HNSCC models to characterize the mechanism and antitumor effects. We also evaluated the combination of EGFR-AS injections, cetuximab, and RT in a phase 1 cohort of patients with locally advanced HNSCC.

MATERIALS AND METHODS

Preclinical Methods

HNSCC cell lines and reagents, viability and immunoblotting assays, xenograft models, and statistical methods are described in Supporting Figure 1.

Clinical Trial Methods

Eligibility—The phase 1 trial was approved by the Institutional Review Boards of the University of Pittsburgh and the University of Texas San Antonio; both sites were nationally registered at clinicaltrials.gov (NCT00903461 and NCT01592721). All patients provided written, informed consent.

Key eligibility criteria included: stage IVA through IVC, histologically confirmed HNSCC of the oral cavity, oropharynx, hypopharynx, or larynx, as defined by the American Joint Committee on Cancer Staging Handbook, seventh edition; the presence of a primary tumor or lymph node that was measurable according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[13] and accessible for repeated injections and mandatory research biopsies; and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Radiation-naive patients who had stage IVC disease with asymptomatic distant metastases were eligible if local control was judged clinically necessary by the investigator; however, head and neck reirradiation was not permitted.

Treatment plan—The treatment schema is presented in Figure 1. Given the negligible toxicity of EGFR-AS injections at any dose during the phase 1 monotherapy trial,[12] the

highest dose of 1.92 mg/1.92 mL was selected for development in combination with cetuximab and RT. Treatment duration was 9 weeks. Cetuximab was administered as a loading dose of 400 mg/m^2 intravenously during week 1 followed by 250 mg/m^2 per week during weeks 2 through 9. Starting at week 1, EGFR-AS was injected weekly into the selected lesion for 7 weeks or until patients attained a complete response (CR). Patients underwent computed tomography-based treatment planning with intensity-modulated RT. The total radiation dose to gross disease was from 70 to 74 grays administered at 2 grays per fraction over 7 weeks starting at week 3. All locoregional disease was incorporated within the radiation field; distant metastases, if present, were not treated with radiation therapy.

Manufacture of investigational product—Clinical grade pNGVL1-U6-EGFRAS (EGFR-AS) was produced under good manufacturing practice conditions at the Center for Biomedicine and Genetics at the City of Hope (Duarte, Calif) to the City of Hope's Master File BB-MF-9778, as previously described.[12] Funding for drug manufacture was provided by the National Institute of Health's National Gene Vector Laboratory (NGVL) program.

Research biopsies—Pretreatment and posttreatment tumor specimens were obtained from the injected lesion at the time of diagnostic evaluation and after 2 doses of EGFR-AS and cetuximab, before the initiation of RT. A representative portion of each tumor was snap frozen. A reverse-phase protein array was performed on lysates from snap-frozen specimens, as previously described.[14]

Statistical Methods

This phase 1 trial was designed to enroll 11 patients to a fixed-dose combination of intratumoral EGFR-AS, cetuximab, and RT. Because no grade 2 or higher adverse event (AE) was observed during phase 1 development of intratumoral EGFR-AS, the highest previously tested dose of EGFR-AS was implemented without a plan for further escalation. The primary objective was to evaluate safety. The primary endpoint was DLT, defined as any grade 3 or 4 AE according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0 at least possibly related to EGFR-AS. The design sought to rule out an unacceptable DLT rate ≥20%. If no grade 3 or 4 AEs caused by EGFR-AS were observed, then the upper 90% confidence bound for the DLT rate would be <20%. Locoregional RECIST responses were categorized by computed tomography scans obtained 8 to 12 weeks after the completion of RT.[13] To qualify for a lesional CR, complete disappearance of the injected lesion was required. To qualify for a locoregional CR, complete disappearance of all locoregional disease within the radiated head and neck field was required. In patients with stage IVC disease, distant metastases were not included as target lesions when assessing lesional and locoregional response.

RESULTS

Dual EGFR Inhibition With Cetuximab and EGFR-AS Reduces Cell Viability

Both cetuximab and EGFR-AS reduce HNSCC proliferation and viability as monotherapy[15]; however, the combination has not been evaluated in preclinical models. We determined the proportion of HNSCC 15B cells that survived treatment with vehicle control, cetuximab,

EGFR-AS, or the combination. The combination significantly reduced viability compared with either agent alone ($P < .0001$), as indicated in Figure 2A.

The Combination of Cetuximab and EGFR-AS Enhances Antitumor Effects in Vivo

We previously demonstrated that EGFR-AS was as effective as cetuximab alone in reducing tumor growth in a 1483 xenograft model, whereas EGFR-sense treatment was not, indicating a sequence-specific treatment effect rather than a nonspecific effect of plasmid treatment.[16] After establishing that dual EGFR targeting with cetuximab and EGFR-AS reduced HNSCC cell line viability, we evaluated the combination in a 4-arm in vivo experiment. Two groups of 10 mice were inoculated in the flank bilaterally with 1483 cells, then randomized to intraperitoneal vehicle versus cetuximab. To control for animal heterogeneity in the comparison of EGFR-AS with EGFR-sense, each mouse served as its own control. EGFR-AS was injected into the left flank tumor, and EGFR-sense was injected into the right flank tumor. Thus, this was a 4-treatment experiment evaluating xenograft growth when treated with vehicle plus intratumoral EGFR-AS, vehicle plus EGFR-sense, cetuximab plus EGFR-AS, and cetuximab plus EGFR-sense. Cetuximab plus EGFR-sense decreased the rate of tumor growth relative to vehicle plus EGFR-sense ($P = .0009$), as illustrated in Figure 2B. However, the growth rate of tumors treated with the combination of cetuximab and intratumoral EGFR-AS was significantly lower than that of tumors treated with the combination of cetuximab and intratumoral EGFR-sense in the contralateral flank of the same mouse ($P = .0003$). The antitumor effects of cetuximab and EGFR-AS were additive ($P_{\text{interaction}} = .35$). Collectively, these findings suggest that the antitumor activity of the combination of cetuximab plus EGFR-AS depends on both the systemic effects of cetuximab and the antisense construct.

Total EGFR Expression Is Decreased in Tumors Treated With Cetuximab and EGFR-AS

The combination of cetuximab plus EGFR-sense plasmid DNA reduced tumor size relative to vehicle plus EGFR-sense in the 1483 HNSCC xenograft model, confirming that cetuximab has antitumor effects in this model. The combination of cetuximab plus EGFR-AS was significantly more effective than cetuximab plus EGFR-sense, indicating specificity of the antisense construct. To elucidate a possible mechanism, we evaluated paired, posttreatment tumor specimens from 4 mice treated with cetuximab plus intratumoral injection of both EGFR-AS (left flank) and EGFR-sense (right flank). Higher EGFR levels were observed in tumors treated with intratumoral EGFR-sense (Fig. 2C), suggesting that the combination of cetuximab plus EGFR-AS resulted in EGFR protein down-modulation.

Preliminary Safety of EGFR-AS, Cetuximab, and RT

Because of the safety profile of EGFR-AS during phase 1 development, the lesional RR, and preclinical evidence suggesting enhanced antitumor activity from combined cetuximab and EGFR-AS, we initiated a phase 1 trial evaluating the safety of intratumoral EGFR-AS, cetuximab, and intensity-modulated RT in patients with stage IVA through IVC HNSCC. Six patients were enrolled at the University of Pittsburgh and the University of Texas San Antonio from May 2013 to April 2014, when the trial closed prematurely because of exhaustion of the EGFR-AS supply. EGFR-AS had been produced under the NGVL program, which was subsequently terminated.[17] Patient characteristics are presented in Table

1. The Consolidated Standards for Reporting Trials (CONSORT) diagram for the 6 treated patients is presented in Figure 1B. The lesional RR for injected lesions was 83% (5 of 6 patients; 4 CRs and 1 partial response [PR]; 90% confidence interval, 56%−100%). The overall locoregional RR was 83% (5 of 6 patients; 3 CRs and 2 PRs).

Regimen-related toxicities are presented in Table 2. Acneiform rash and radiation dermatitis were consistent with toxicity patterns for cetuximab-RT.[1] The DLT rate was 0% (0 of 6 patients; 90% confidence interval, 0%−31%>). No grade 2 or higher AEs were attributed to EGFR-AS injections, in line with the phase 1 monotherapy experience. The protocol as designed required 11 accrued patients and a 0% DLT rate to conclude that the true DLT rate was <20%. Because we were unable to meet the accrual objective, we cannot make this claim. However, the absence of unacceptable AEs among 6 patients provides 90% confidence that the DLT rate is ≤31%.

Discordant CR of Large, Injected Neck Lesion

The locoregional RECIST response category for each patient was identical to the lesional response category, with 1 notable exception. Patient 5 initially presented in 2011 with stage IVC hypopharyngeal disease, including bilateral cervical adenopathy and pathologically confirmed pulmonary metastases. After an initial CR to frontline, systemic therapy (which included cisplatin, 5-fluorouracil, and cetuximab), he relapsed only within the hypopharynx and bilateral neck while remaining without radiologic evidence of pulmonary metastases. Because he had symptomatic locoregional disease, quiescent distant metastases, and no prior radiation therapy, he was deemed eligible and enrolled in the current trial. According to the protocol, all patients with locoregional disease received definitive cetuximab-RT. A hypermetabolic, 5-cm, left, level II/III lymph node was selected for injection of EGFR-AS, and 2 contralateral hypermetabolic lymph nodes were not injected (Fig. 3). After he completed protocol treatment, the patient demonstrated an anatomic and metabolic CR of the injected lesion, but only an anatomic and metabolic PR of the contralateral noninjected lymph nodes. A consolidative right neck dissection confirmed the presence of viable residual disease. Ultimately, the patient died from complications of right neck disease 3 years after completing protocol therapy, but he never relapsed within the left neck. The patient essentially served as his own control; the discordant and sustained CR in the larger injected lesion suggests that EGFR-AS contributed to immediate and long-term lesional control.

Total EGFR Expression in Paired Tumor Biopsies

Biopsies were obtained at baseline and after 2 weeks of protocol treatment in all 6 patients. Four of 6 biopsies (67%) provided sufficient tissue for paired proteomic analysis using a reverse-phase protein array. Because of the small sample size, we limited our descriptive analysis to total EGFR expression, for which the greatest body of mechanistic preclinical data exists, and no significance testing was performed. Total EGFR expression decreased numerically in these 4 patients, ranging from 1.2 to 2.3 at baseline and from 0.9 to 1.8 (17% −52% decrease) at week 2.

CONCLUSIONS

After attaining regulatory approval from the US Federal Drug Administration and the European Medicines Association's for cetuximab in 2006, the combination of cetuximab and definitive RT rapidly became an accepted standard for locally advanced HNSCC in both North America and Europe.[1,18,19] However, among patients who receive treatment with cetuximab-RT, the median LRC is only 24 months, and the major cause of cancer mortality remains locoregional failure.[1,18] We hypothesize that EGFR-AS injections may improve LRC without added toxicity. In the current study, we present preclinical evidence that combined extracellular EGFR blockade with cetuximab and transcriptional *EGFR* down-regulation with intratumoral EGFR-AS enhances antitumor activity and is associated with decreased tumor EGFR protein levels. Furthermore, we report the preliminary safety of intratumoral EGFR-AS, cetuximab, and RT in a phase 1 cohort.

This treatment strategy may be particularly relevant in elderly patients (defined as age ≥65 years), who represent >40% of incident HNSCCs, suffer more than one-half of HNSCC-related mortality, and predominantly receive treatment with cetuximab-RT.[5,20] In a US analysis for the period from 2001 to 2009, the use of combined-modality therapy in the elderly population doubled from 29% to 61%—a rise attributable to the adoption of cetuximab-RT.[5] This striking change in clinical practice likely reflects the favorable systemic toxicity profile. Although cetuximab increases in-field mucosal and dermatologic toxicity, systemic toxicities (including the hematologic, renal, and gastrointestinal AEs discordantly observed in elderly patients who receive cisplatin) are rare.[21,22]

Although EGFR is an established oncogene and prognostic biomarker in HNSCC, no molecular biomarkers have been identified that can predict clinical benefit from cetuximab, including *EGFR, RAS,* or *RAF* mutations; EGFR protein expression; or *EGFR* amplification.[2,23–25] Indeed, cetuximab and EGFR receptor tyrosine kinase inhibitors are near-universally active in well characterized HNSCC cell lines, representing an Achilles heel for clinical translation.[26,27] We previously demonstrated that EGFR-AS decreased *EGFR* messenger RNA transcripts and EGFR protein levels in HNSCC cells, a mechanism distinct from cetuximab or EGFR receptor tyrosine kinases.[9–11] Transcriptional deregulation of EGFR also may be mechanistically important in humans, because downregulation of EGFR expression was observed in pretreatment and posttreatment tumor biopsies during phase 1 development of intratumoral EGFR-AS.[12] Moreover, baseline overexpression of EGFR was associated with lesional response, suggesting that EGFR protein expression ultimately may be a relevant biomarker for EGFR-AS, also distinct from EGFR inhibitors currently in the clinic. The data from the current studies expand on these earlier findings. Specifically, intratumoral EGFR-AS reduced HNSCC xenograft size relative to control EGFR-sense in mice treated with systemic cetuximab, and this difference was associated with the down modulation of EGFR expression. In the current phase 1 cohort, EGFR expression was reduced by 17% to 52% during the 2-week window of dual EGFR-AS and cetuximab therapy, although mechanistic conclusions are inhibited because of the small sample size. Although the findings are anecdotal, 1 patient demonstrated a discordant, sustained, metabolic and anatomic CR in a 5-cm, injected lymph node compared with a 1.5-cm,

noninjected lymph node, suggesting that intratumoral EGFR-AS contributed to acute and long-term lesional control from cetuximab-RT.

The chief limitation of this phase 1 study was the inability to complete accrual to the 11-patient safety cohort because of exhaustion of the initial EGFR-AS drug supply and subsequent defunding of the NGVL.

The National Center for Research Resources inaugurated the NGVL in 2005 to encourage the manufacture of high-quality, clinical-grade vectors for gene-therapy trials. Major setbacks in the gene therapy treatment of ornithine transcarbamylase deficiency and X-linked severe combined immunodeficiency resulted in a severe contraction of gene therapy initiatives by the National Institutes of Health.[17] As systematic safety and monitoring measures are implemented for gene therapy clinical trials, and new clinical breakthroughs involving gene therapy are documented, there is resurgence in public and private investment in promising molecules. Rekindled development of EGFR-AS, recently licensed by Benetec Biopharma (Sydney, New South Wales, Australia), may be of clinical interest locally advanced HNSCC.

In summary, the morbidity and mortality associated with locoregional failure after cetuximab-RT in patients with locally advanced HNSCC should motivate therapeutic innovations to enhance LRC. EGFR-AS is a novel DNA plasmid that appears to transcriptionally deregulate *EGFR,* resulting in the down-modulation of EGFR protein expression. At least in part, this unique mechanism may underlie the enhanced antitumor activity of the combination of cetuximab and EGFR-AS observed in vivo and may contribute to the clinical activity of EGFR-AS. The combination of intratumoral EGFR-AS, cetuximab, and intensity-modulated RT was well tolerated and associated with promising local control in 6 patients. Therefore, this treatment strategy may be particularly relevant in elderly patients, for whom cetuximab-RT is the dominant therapeutic paradigm. To date, the safety of the combination of EGFR-AS, cetuximab, and RT and the identification of the patient population most likely to benefit remain unanswered questions. An expanded cohort or a de novo phase 2 trial will be required to fully evaluate safety and establish efficacy, with the primary endpoint of LRC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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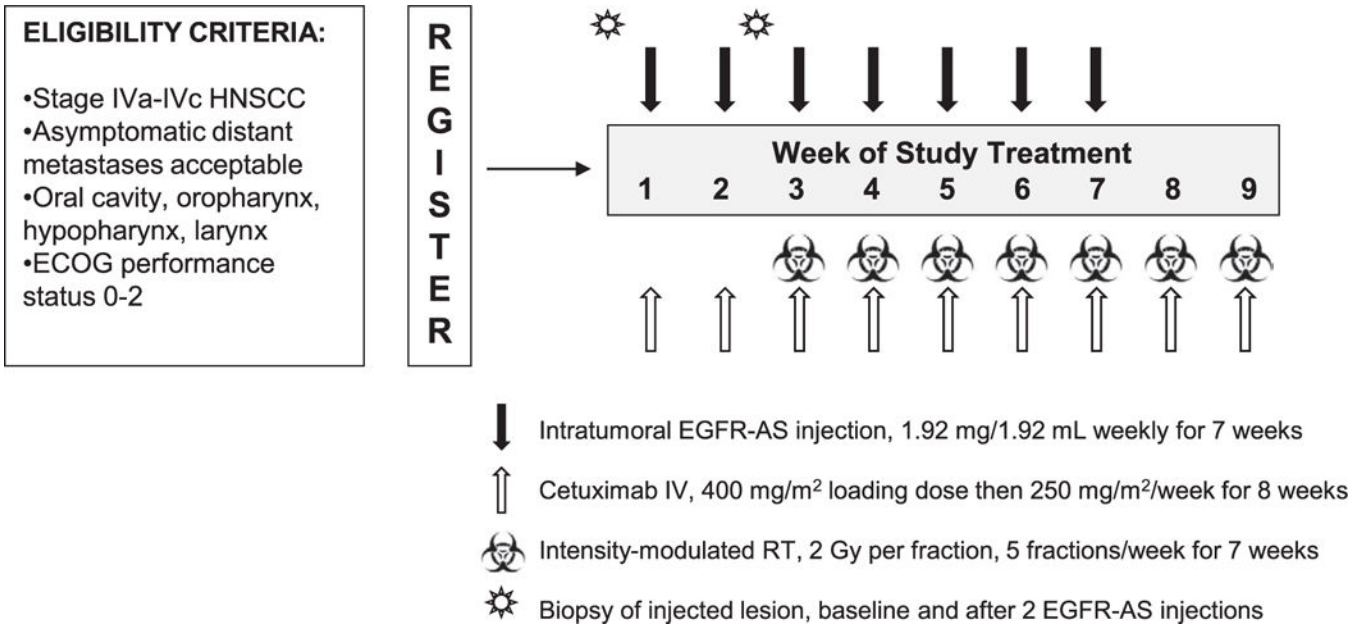

Figure 1.

The phase 1 schema is illustrated. ECOG indicates Eastern Cooperative Oncology Group; EGFR-AS, epidermal growth factor receptor-antisense plasmid DNA; RT, radiation therapy.

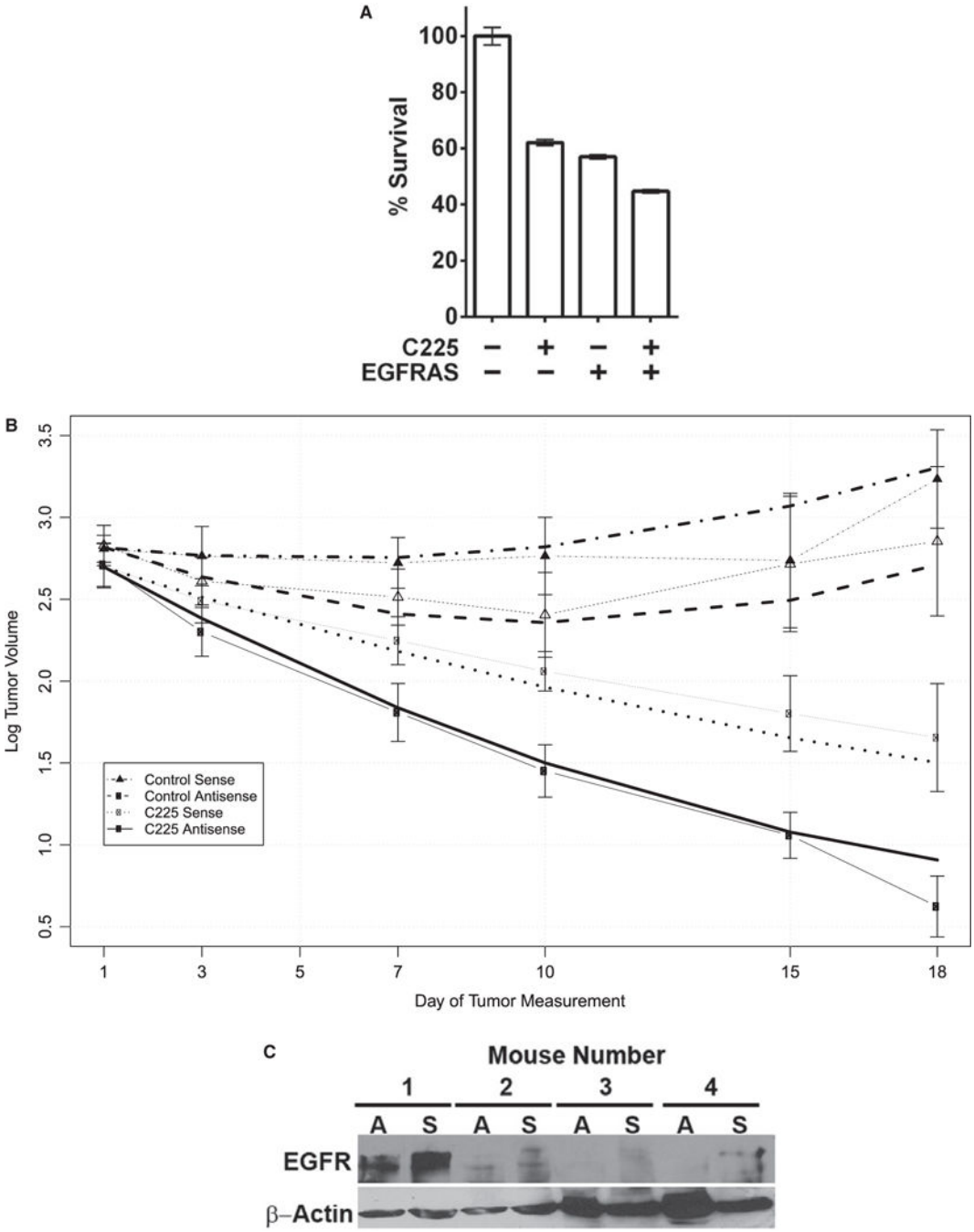

Figure 2.

Dual epidermal growth factor receptor (EGFR) Inhibition with EGFR-antlsense plasmid DNA (EGFR-AS) and cetuximab (C225) reduces viability and enhances antitumor effects in preclinical models of head and neck squamous cell carcinoma (HNSCC). (A) UPCI-15B cells (2 10^4cells per well) were plated in a 24-well plate in growth medium for 24 hours then transfected with EGFR-AS plasmid DNA. After 4 hours, cells were treated with RPMI containing either vehicle control (water) or cetuximab (60 μg/mL). After 72 hours, the percentage of surviving cells was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) assay. Viability differed significantly among the 4 conditions ($P < .0001$; exact 2-tailed Kruskal-Wallis test). Viability also was reduced significantly by the combination of cetuximab and EGFR-AS relative to EGFR-AS alone ($P = .05$; 1-tailed exact Wilcoxon test). (B) Female athymic nude mice were inoculated with 1 10^6 1483 cells on each flank. At day 8 postinoculation, 19 mice were randomly assigned to vehicle control (N = 9) versus cetuximab treatment (N = 10; 50 mg/kg twice weekly), stratified by tumor volume. Mice in both groups received intratumoral injections of EGFR-AS plasmid DNA in the left flank tumor and control EGFR-sense plasmid DNA in the right (25 μg per tumor, 5 days per week). Mice were killed after 18 days of treatment. A linear mixed-effects model was fit to the log-transformed daily tumor volume profiles in the 4 groups. On the basis of tests of interaction between systemic treatment arm and day, cetuximab decreased the rate of tumor growth ($P = .0009$). EGFR-AS decreased the rate of tumor growth relative to intratumoral plasmid ($P = .0003$) and independent of cetuximab (interaction test; $P = .35$). Thick lines indicate fitted model values, thin lines connect the mean values on each day, and vertical bars are standard errors. (C) Representative tumors from individual mice treated in the cetuximab arms illustrated in B were excised, snap frozen, and analyzed by immunoblotting at the conclusion of the experiment. Only 4 of 10 mice had sufficient residual tumor in the EGFR-AS-treated flank to conduct a paired analysis. In all 4 mice, tumors treated with EGFR-AS (A) versus EGFR-sense (S) plasmid DNA demonstrated lower expression of total EGFR protein.

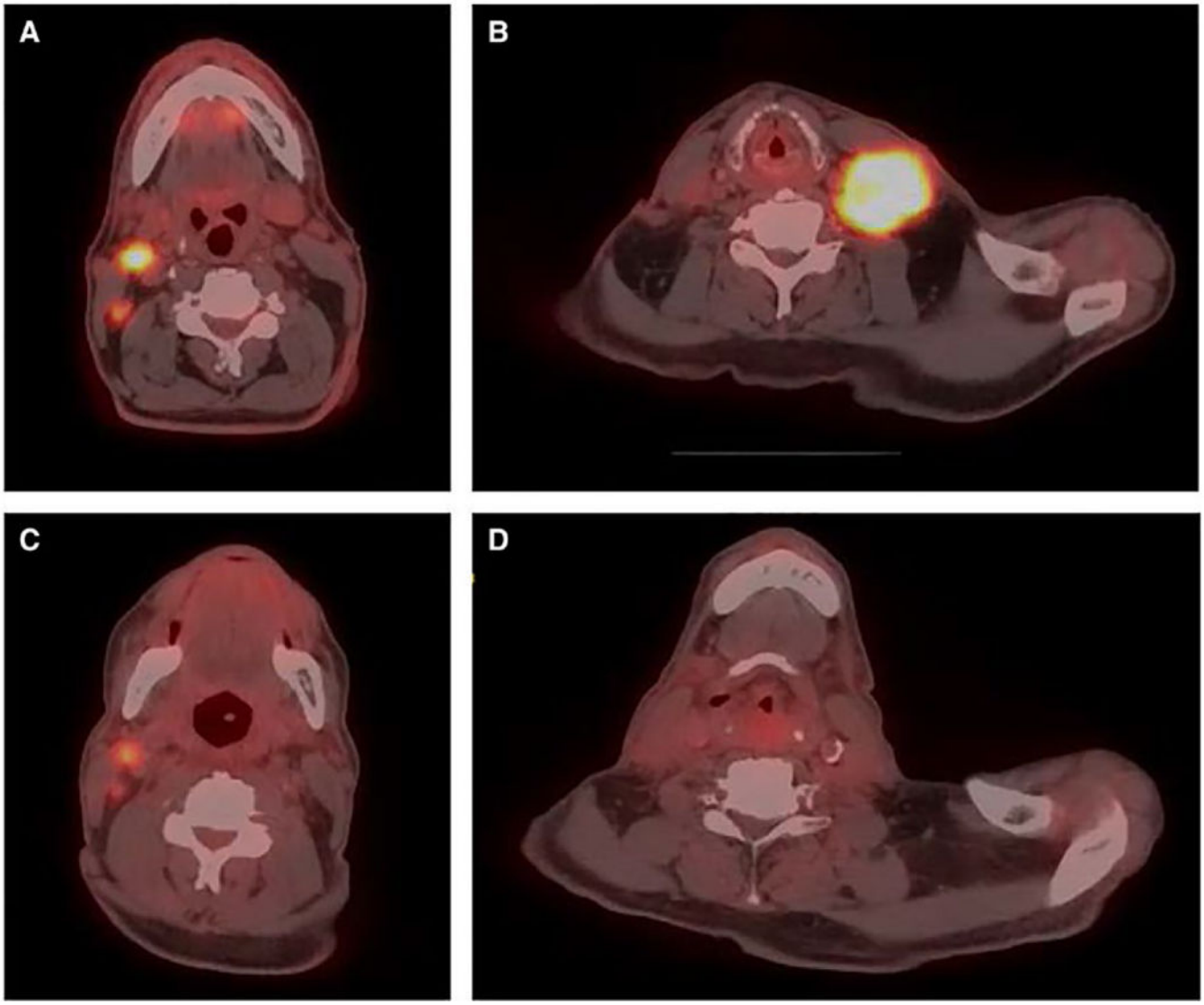

Figure 3.

(A–D) Patient 5 experienced a discordant complete response in a phase 1 trial, including (B,D) a complete anatomic and metabolic response in the large, injected left cervical lymph node; (A,C) however, only a partial anatomic and metabolic response was observed in 2 small, noninjected, contralateral cervical lymph nodes. Salvage right neck dissection confirmed the presence of residual viable tumor.

TABLE 1.

Individual Patient Data From the Phase 1 Cohort

	Patient No:					
Variable	**1**	**2**	**3**	**4**	**5**	**6**
Stage	IVA	IVA	IVA	IVA	IVC[a]	IVC[a]
Anatomic site	OP	OP	OC	OP	HP	OP
Injected lesion[b]	LN	OP	OC	OP	LN	OP
HPV status: p16 or ISH	+	Unk	–	Unk	–	–
Sex	Man	Man	Woman	Woman	Man	Man
Age, y	50	68	81	80	57	63
ECOG PS	0	1	0	2	1	1
Tobacco use	None	None	Prior	None	Current	Current
Best lesional response	CR	CR	CR	PD	CR	PR
Best locoregional response	CR	CR	CR	PD	PR	PR
PFS, mo	34[c]	12	16	4	12	9
Site of progression	NA	L,R	R	L	R,D	L,R,D
Lesional progression	No	Yes	No	Yes	No	Yes
OS, mo[d]	34	11	20	7	39	13

Abbreviations: –, negative; +, positive; CR, complete response; D, distant; ECOG PS, Eastern Cooperative Oncology Group performance status; HP, hypopharynx; HPV, human papillomavirus; ISH, in situ hybridization; L, local; LN, cervical lymph node; OC, oral cavity; OP, oropharynx; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; R, regional; Unk, unknown.

[a] These patients had distant metastatic sites in the lung (patient 5) and liver (patient 6).

[b] Injected primary tumors are indicated by anatomic site.

[c] Patient 1 had not progressed at the time of the last follow-up.

[d] Patient 1 was censored for OS at 34 months, and patient 4 was censored for OS at 7 months at the time she withdrew consent.

TABLE 2.

Regimen-Related Adverse Events

	AE Grade (CTCAE V.4): No. (%)		
Regimen Toxicity[a]	**1–2**	**3**	**4**
Acneiform rash	6 (100)	0(0)	0 (0)
Mucositis	3 (50)	3 (50)	0 (0)
RT dermatitis	4 (67)	2 (33)	0 (0)
Injection site discomfort	2 (33)	0 (0)	0 (0)

Abbreviations: AE, adverse event; CTCAE V.4, Common Toxicity Criteria for Adverse Events, version 4; RT, radiation therapy;

In models of head and neck squamous cell carcinoma, cetuximab with epidermal growth factor receptor-antisense plasmid DNA enhance antitumor effects. In a phase 1 cohort, the combination of intratumoral epidermal growth factor receptor-antisense DNA, cetuximab, and radiation therapy is well tolerated.

[a]No dose-limiting toxicities were attributed to epidermal growth factor receptor-antisense plasmid DNA injections, although epidermal growth factor receptor-antisense plasmid DNA was associated with grade 1 injection site discomfort in 2 patients. Observed rates of rash, mucositis, and RT dermatitis were consistent with the rates for cetuximab-RT.