Clinical Cancer Research

Phase Ib Trial of the Toll-like Receptor 8 Agonist, Motolimod (VTX-2337), Combined with Cetuximab in Patients with Recurrent or Metastatic SCCHN

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Abstract

Purpose: As Toll-like receptors (TLR) are key mediators of immune responses, TLR agonists may be important for augmenting the efficacy of therapies for squamous cell carcinoma of the head and neck (SCCHN). Motolimod (VTX-2337), a selective small-molecule agonist of TLR8, stimulates natural killer (NK) cells, dendritic cells, and monocytes. A phase Ib clinical trial assessed the safety and antitumor activity of motolimod in combination with cetuximab in patients with SCCHN. Correlative biomarkers of immune activity were explored.

Experimental Design: Thirteen patients with recurrent or metastatic SCCHN were enrolled in this open-label, dose–escalation study using a standard 3 + 3 design. Doses of motolimod (2.5, 3.0, or 3.5 mg/m^2) were given on days 1, 8, and 15, in combination with fixed weekly doses of cetuximab in 28-day cycles.

Results: There were no protocol-defined dose-limiting toxicities, drug-related deaths, or evidence of synergistic toxicities between motolimod and cetuximab. Clinical tolerability at the 3.5 mg/m^2 dose level was not optimal for repeated dosing and 3.0 mg/m^2 was identified as the MTD. Two patients achieved partial responses for an overall response rate of 15%. Five patients had disease stabilization equating to a disease control rate of 54%. Statistically significant increases in plasma cytokines and in the frequency and activation of circulating NK cells were observed.

Conclusions: Motolimod can be safely administered in combination with cetuximab with an acceptable toxicity profile. Encouraging antitumor activity and robust pharmacodynamic responses were observed. Motolimod is being further investigated in a phase II trial in patients with SCCHN (ClinicalTrials.gov ID: NCT01836029). *Clin Cancer Res; 1–9. ©2016 AACR.*

Introduction

Squamous cell carcinomas of the head and neck (SCCHN) broadly encompass malignancies of the oral cavity, nasopharynx, pharynx, and larynx and are the sixth most common cancer worldwide (1–3). An estimated 61,000 new cases of SCCHN will be diagnosed in 2016 in the United States alone, and approximately 13,000 patients will die of their disease (4).

Despite advances in the diagnosis and treatment of this disease, the prognosis for patients with SCCHN remains poor. Approximately two thirds of SCCHN cases are diagnosed in advanced stages, although metastatic disease at presentation is uncommon (2, 5, 6). Recurrent- and metastatic disease are often refractory or unable to be treated with further surgery and/or radiotherapy (7, 9). The 5-year survival rate for late-stage disease is estimated to be 50% or less (10, 11). Metastatic and recurrent SCCHN that is no longer amenable to local surgical/radiotherapy is associated with a high mortality rate and a median survival of 6 to 9 months (11, 12). For patients with disease progression after first-line therapy, or who are platinum intolerant or platinum refractory, conventional chemotherapy offers limited palliation (2).

The EGFR signaling pathway has been implicated in malignant transformation (13). More than 90% of SCCHNs express EGFR (3, 13, 14); therefore, targeting EGFR and its signaling pathway has significantly advanced the treatment of SCCHN. The EGFR-targeted mAb cetuximab was approved for use in recurrent or metastatic platinum-intolerant or platinum-refractory SCCHN patients by the FDA based on its demonstrated clinical benefit and tolerability in three phase II studies (15–17): Cetuximab was given in combination with platinum-based chemotherapy in two studies and alone in the third. Unfortunately, in this difficult-to-treat population, the response rates obtained with cetuximab therapy were low across these studies with objective responses in 10% to 13% of patients, disease control rates of 46% to 55%, and median overall survival (OS) of 5.2 to 6.1 months (15–17). Improved therapeutic approaches for metastatic and recurrent SCCHN are urgently needed.

Recently, immunotherapeutic approaches that enhance adaptive T-cell immunity have demonstrated encouraging antitumor

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Translational Relevance

Natural killer (NK) cell–mediated antibody-dependent cellular cytotoxicity (ADCC) is important for the antitumor activity of the EGFR-specific mAb, cetuximab. Motolimod, a small-molecule agonist of TLR8, stimulates NK cells, dendritic cells, and monocytes. Activation of these immune cell populations leads to increased ADCC activity of therapeutic mAbs, including cetuximab. The results of a clinical study in patients with SCCHN showed that rapid and statistically significant increases in G-CSF, IL6, MIP-1β, and MCP-1 were observed after combination treatment with motolimod plus cetuximab. There was no change in the magnitude of the pharmacodynamic response with repeated dosing, demonstrating that repeated treatments neither desensitized nor augmented the mediator response. Statistically significant treatment-related increases in the frequency and activation of NK cells were also observed. These data confirm that motolimod can induce robust immune responses in cancer patients who have been exposed to multiple prior chemotherapeutics that have myelo- and lymphotoxic side effects.

activity in several solid tumors, including head and neck cancer (18, 19). Activation of the innate immune response is another approach to inducing effective antitumor immunity.

Toll-like receptors comprise a family of 11 pattern recognition receptors that activate the innate immune system. The various TLRs have distinct patterns of cellular expression, subcellular distribution, and the range of natural ligand agonist. TLR8 is localized in endosomal compartments of monocytes and myeloid dendritic cells (mDC). TLR8 agonists stimulate the release of a distinct range of inflammatory mediators, including Th1-polarizing cytokines (20, 21). Activation of the TLR8 pathway also enhances natural killer (NK) cell function (22), augments antibody-dependent cellular cytotoxicity (ADCC; refs. 20, 23), and induces the production of IFNγ.

Motolimod (formerly known as VTX-2337) is a selective TLR8 agonist that induces a response comparable with that of the receptor's natural ligand of viral single-stranded ribonucleic acid (24). *In vitro* studies have demonstrated that motolimod effectively activates TLR8, leading to enhanced NK-cell function, and in combination with cetuximab, increases tumor-directed ADCC (23). In a phase I single-agent dose-finding study, motolimod was well tolerated and biologically active with a predictable pharmacokinetic profile and a characteristic immune-related adverse event profile. These features of motolimod make it an ideal immunotherapy to augment the activity of cetuximab in the treatment of SCCHN.

Here, we report the safety, tolerability, and antitumor activity of motolimod in combination with cetuximab in an open-label, phase Ib clinical trial in patients with SCCHN (ClinicalTrials.gov ID: NCT01334177). Effects on plasma cytokine levels and *ex vivo* NK-cell activation are also discussed.

Patients and Methods

This phase I, open-label, dose-finding study of motolimod in combination with cetuximab was conducted at a single study center in the United States from June 2011 to June 2014. The study was performed in accordance with Good Clinical Practice guidelines and the ethical principles outlined in the Declaration of Helsinki. Approval for the study was obtained from the Institutional Review Board, and all patients provided written informed consent before study enrolment.

Eligibility

Patients eligible for this study were adults with recurrent SCCHN not amenable to curative surgery or radiation, or patients with distant metastatic disease. Eligibility criteria also included patients previously treated with systemic therapy, but for whom platinum-based therapy was no longer appropriate due to the presence of platinum-intolerant/refractory disease, and/or patients who had completed definitive chemoradiation less than 3 months prior to study enrolment. Prior therapy with agents that target or block EGFR without a history of severe toxicity was allowed. Additional eligibility criteria included life expectancy of $\geq$12 weeks, Eastern Cooperative Oncology Group performance status (25) of 0 to 2, and adequate bone marrow, renal, hepatic, and cardiac function. Patients were ineligible if they had been treated with investigational therapy or had recent major surgery in the previous 4 weeks or had received chemotherapy, palliative radiotherapy, or oral or parenteral corticosteroids in the previous 2 weeks. Patients who required systemic immunosuppressive therapy and those with active central nervous system involvement, brain or meningeal metastases (unless stable for $\geq$28 days) were also ineligible. Additional exclusion criteria included active autoimmune disease or infection, clinically significant cardiac disease within 6 months, and current retinal vascular disorder and/or uveitis. Patients were also excluded if they had clinically detectable and actively progressing second primary malignancies.

Treatment and dose escalation

Cohorts of 3 to 6 patients were enrolled into each dose level and administered a fixed weekly intravenous dose of cetuximab (250 mg/m^2) in combination with escalating doses (2.5, 3.0, or 3.5 mg/m^2) of motolimod (VTX-2337) administered subcutaneously once weekly for 3 of 4 weeks of a 28-day cycle according to a standard 3 + 3 dose–escalation study schema (26). Motolimod doses were selected on the basis of data from the first-in-human study, which identified 2.5, 3.0, and 3.5 mg/m^2 as being in the range of biologically active, single-agent doses with acceptable tolerability (27).

For Cohort 1, a lead-in period of treatment with cetuximab alone for 4 weeks was implemented prior to the start of cycle 1 to establish a baseline for determining whether adding motolimod would increase the toxicities seen with single-agent cetuximab, particularly rash and diarrhea. The lead-in consisted of 1 intravenous cetuximab dose of 400 mg/m^2, followed by 3 doses of 250 mg/m^2 given weekly, per standard-of-care dosing in advanced and recurrent SCCHN (12). On the basis of the lack of toxicity observed with the combination of motolimod and cetuximab in Cohort 1, the protocol was amended to allow patients who had previously been treated with cetuximab to receive a limited lead-in treatment of only 1 dose of cetuximab (250 mg/m^2) at week -1 as long as cetuximab was given no more than 3 weeks prior to study initiation. If more than 3 weeks had elapsed since prior cetuximab therapy, the standard cetuximab loading dose of 400 mg/m^2 followed by 3 doses of 250 mg/m^2 was administered. Patients with toxicities $\geq$grade 3 [National Cancer Institute Common Terminology Criteria for Adverse Events (NCI

CTCAE) Version 4.0] attributable to cetuximab during the lead-in period were discontinued from study.

For each cohort, following the cetuximab lead-in period, 3 patients were administered the first cycle of fixed-dose cetuximab (250 mg/m^2) intravenously given on days 1, 8, 15, and 22, plus motolimod (at 1 of the 3 escalating prespecified dose levels of 2.5, 3.0, and 3.5 mg/m^2) given subcutaneously on days 1, 8, and 15 of a 28-day cycle. Subjects who did not complete a full 28-day cycle were not evaluable for dose-limiting toxicity (DLT). Upon review of safety data in each cohort, if none of the 3 patients in a cohort experienced a DLT during cycle 1 of the study, dose escalation proceeded to enroll the next cohort of 3 patients. If 1 of 3 patients experienced a DLT during cycle 1, the cohort was expanded to a total of 6 patients. The MTD was defined as the highest dose level at which $\leq$1 of 3 patients experienced a DLT. No intrapatient dose escalation was allowed. After successful completion of cycle 1, patients were eligible to receive subsequent treatment cycles until the criteria for study discontinuation or withdrawal were met, including disease progression, unacceptable toxicity, or death.

DLTs were defined as any study treatment-related adverse event (AE) occurring during cycle 1 that met one of the following criteria: hematologic toxicity of grade 4 severity, nonhematologic toxicity of grade 3 or higher severity (excluding grade 3 hypersensitivity reactions and localized injection site toxicities), grade 3 or higher diarrhea, nausea, or vomiting despite adequate antiemetics/diarrhea medications, any treatment delay due to toxicity that lasted more than 21 days since the last dose of cetuximab or motolimod, uveitis, or death.

Study assessments

Safety monitoring, including physical exams and assessment of AEs and DLTs, was conducted prior to cycle 1 and at each study visit during cycle 1 and each subsequent cycle. AEs were summarized using the Medical Dictionary for Regulatory Activities and graded for severity using NCI CTCAE Version 4.0. Hematology and chemistry laboratory tests were performed on all patients at screening, weekly prior to initiation of therapy in cycle 1, and on day 1 of all subsequent treatment cycles. Tumor imaging assessments were carried out at screening and at the end of every second cycle until disease progression. Patients were evaluated for response and progression per the RECIST) Version 1.1 (28). End of study and follow-up assessments included hematologic and chemistry laboratory evaluations, in addition to physical examinations and AE monitoring.

Correlative studies

Sample collection and measurement of immune mediators. Whole blood was collected predose and at 8 and 24 hours following motolimod administration on days 1 and 15. Plasma and peripheral blood mononuclear cells (PBMC) were processed from heparin tubes within 24 hours of blood draw. PBMCs were isolated using Ficoll–Paque density centrifugation and immediately used for the flow cytometric assays or cryopreserved for other assays. Plasma levels of immune mediators were analyzed using the HumanMAP v1.6 inflammation panel (Myriad-RBM).

Flow cytometric analyses. NK-cell activity was measured using a CD107a NK-cell degranulation assay. Freshly isolated PBMCs were cultured with or without stimulation using K562 cells that express transmembrane IL15 and 4-1-BB ligand (K562 15mb-41BBL; ref. 29) at a 10:1 ratio, or with plate-bound anti-CD16 mAb (BD Biosciences), all for 4 hours in RPMI (Life Technologies) with 10% human AB serum (Gemini). PBMCs were labeled with anti-CD107a-phycoerythrin (eBioscience) for 1 hour prior to the addition of Brefeldin A. Anti-CD3-AF488 and anti-CD56-APC (BioLegend) were also used to label NK cells after stimulation. Samples were fixed with 1% paraformaldehyde prior to analyses with a BD FACSCanto II and FlowJo software (Tree Star).

IFNγ ELISPOT assay. PBMCs from days 1 and 15, 0- and 24-hour time points for a single patient were thawed and tested at the same time to minimize variability between time points. ELISPOT assays were performed as described previously (30) with minor modifications. Details are outlined in Supplementary Methods.

Statistical analysis

Safety and efficacy analyses were conducted on all patients who received at least one dose of motolimod. Because of the exploratory nature of this phase I study, no confirmatory inferential analyses were conducted and missing data were not imputed. Data pertaining to patient characteristics, treatment compliance and administration, treatment efficacy and safety, in addition to pharmacodynamic and correlative study data, were analyzed and summarized using descriptive statistics. Categorical and continuous data were summarized using percentages and ranges, where appropriate. Predose plasma levels of select immune mediators (G-CSF, IL6, MIP-1β, and MCP-1) measured using the HumanMAP v1 were compared with levels at 8 and 24 hours using a two-sided *t* test, following the first (day 1) and third (day 15) doses of motolimod. The flow cytometry comparison of NK-cell frequencies and level of NK-cell degranulation (CD107a$^+$) in patient samples was performed using a log-linear mixed model.

Results

Demographics and baseline disease characteristics

Thirteen patients with recurrent or metastatic SCCHN were enrolled on this study (Table 1). One patient (8%) had locally recurrent disease only and 12 (92%) had distant metastases. Ten patients (77%) had received platinum-based systemic chemotherapy in the recurrent/metastatic setting prior to enrolling in the study, and 3 patients (23%) had received 2 or more lines of prior systemic chemotherapy. Ten patients (77%) had previously received cetuximab therapy, with 9 of these patients having receiving prior cetuximab for recurrent/metastatic disease. Twelve

Table 1. Baseline patient and disease characteristics

	(*N* = 13)
Age, years; median (range)	62 (39–78)
Gender, *n* (%)	
Male	10 (77)
Female	3 (23)
Recurrent/metastatic, *n* (%)	
Locally recurrent	1 (8)
Distant metastatic	6 (46)
Locally recurrent and distant metastatic	6 (46)
Prior interventions, *n* (%)	
Definitive chemoradiation	7 (54)
Adjuvant chemoradiation	4 (31)
1 prior chemotherapy	7 (54)
$\geq$2 prior chemotherapies	3 (23)
Cetuximab	10 (77)
Radiotherapy	12 (92)
Surgery	9 (70)

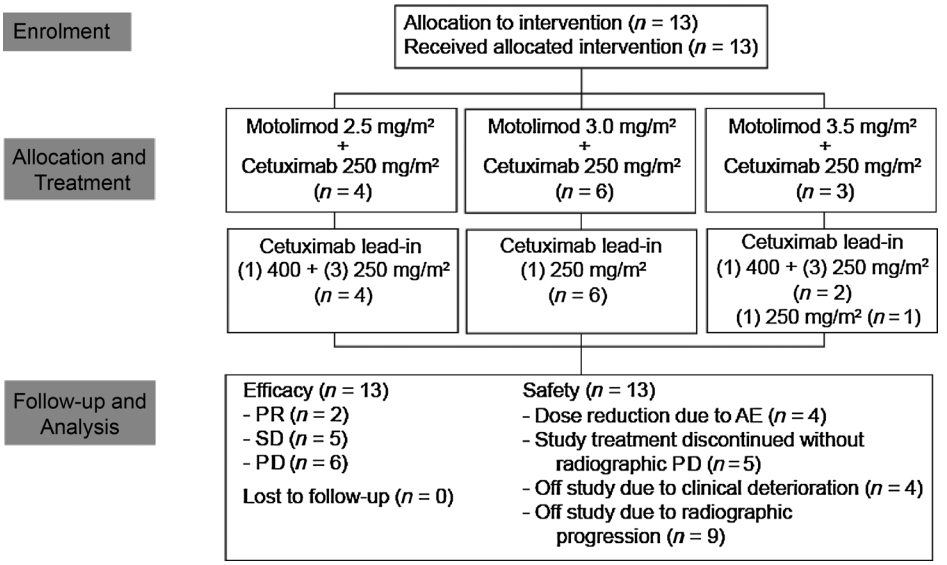

Figure 1.
Consort diagram. Phase Ib trial of the TLR8 agonist, motolimod (VTX-2337), combined with cetuximab in patients with recurrent or metastatic squamous cell carcinomas of the head and neck.

patients (92%) had been treated with radiotherapy and 9 patients (70%) had received surgery. Additional baseline characteristics are shown in Supplementary Table S1.

Safety

Cetuximab was administered in combination with three escalating dose levels of motolimod: 2.5 mg/m^2 ($n = 4$), 3.0 mg/m^2 ($n = 6$), and 3.5 mg/m^2 ($n = 3$). Across all dose cohorts, 6 of 13 patients (46%) received a lead-in treatment with cetuximab alone for 4 weeks prior to initiation of combination cetuximab and motolimod therapy; the remaining 7 patients (54%) had previously received cetuximab within 3 weeks of starting treatment and thus received a single lead-in dose. Eleven patients (85%) completed at least 2 cycles of combination therapy; the remaining 2 patients discontinued treatment during the first cycle because of rapid disease progression (PD). The majority of patients (10/13; 77%) remained on treatment until PD (Fig. 1).

As shown in Table 2, the most frequently reported AEs were flu-like symptoms and injection site reactions [each in 12 patients (92%)], fatigue [11 patients (85%)], anorexia [4 patients (31%)], and malaise [4 patients (31%)]. All AEs were grade 1 or 2 in severity with the exception of four grade 3 incidents considered to be nontreatment related (one each of fatigue, nausea, vomiting, and dyspnea). There were no SAEs or deaths due to study treatment. There were no serious, unexpected drug-related AEs or evidence of synergistic toxicities between cetuximab and motolimod at any dose level. However, 2 of the 3 patients treated at the 3.5 mg/m^2 dose level required dose reduction during the first cycle due to AEs (grade 2 flu-like symptoms and injection site reactions). Although these events did not meet the protocol-specified criteria for DLT, it was determined that the 3.5 mg/m^2 dose level was not tolerable and the 3.0 mg/m^2 cohort was expanded to obtain additional data from 6 total patients. No DLTs were observed and no dose reductions were required in this expanded cohort, making 3.0 mg/m^2 the recommended phase II dose.

Antitumor response

Two patients clinically progressed during the first cycle and were discontinued from study without radiographic evaluation (Fig. 2). Best response in the 11 radiographically evaluated patients using RECIST v1.1 was partial response (PR) in 2 patients, stable disease (SD) in 5 patients, and PD in 2 patients. The overall response rate (PR) was 15% (2/13 evaluable patients), and the disease control rate (PR + SD) was 54% (7/13 patients).

Table 2. Frequently reported AEs: AEs in ≥20% of patients[a]

Motolimod starting dose, *N* (% of all patients)	2.5 mg/m^2 ($n = 4$)	3.0 mg/m^2 ($n = 6$)	3.5 mg/m^2 ($n = 3$)	All ($n = 13$)
Flu-like symptoms	3 (23)	6 (46)	3 (23)	12 (92)
Injection site reaction	3 (23)	6 (46)	3 (23)	12 (92)
Fatigue	3 (23)[b]	6 (46)	2 (15)	11 (85)
Anorexia	1 (8)	3 (23)	0 (0)	4 (31)
Malaise	0 (0)	2 (15)	2 (15)	4 (31)
Arthralgia	1 (8)	1 (8)	1 (8)	3 (23)
Nausea	2 (15)[b]	2 (15)	1 (8)	5 (38)
Cough	1 (8)	3 (23)	1 (8)	5 (38)
Dehydration	1 (8)	2 (15)	1 (8)	4 (31)
Vomiting	1 (8)[b]	0 (0)	2 (15)	3 (23)
Oral pain	1 (8)	1 (8)	1 (8)	3 (23)
Rash	3 (23)	2 (15)	0 (0)	5 (38)
Dyspnea	1 (8)	1 (8)[b]	1 (8)	3 (23)
Dysphagia	0 (0)	3 (23)	0 (0)	3 (23)
Hypomagnesemia	2 (15)	1 (8)	0 (0)	3 (23)

[a]All AEs were grade 1 or 2 except where noted.
[b]Includes one incidence of grade 3 AE.

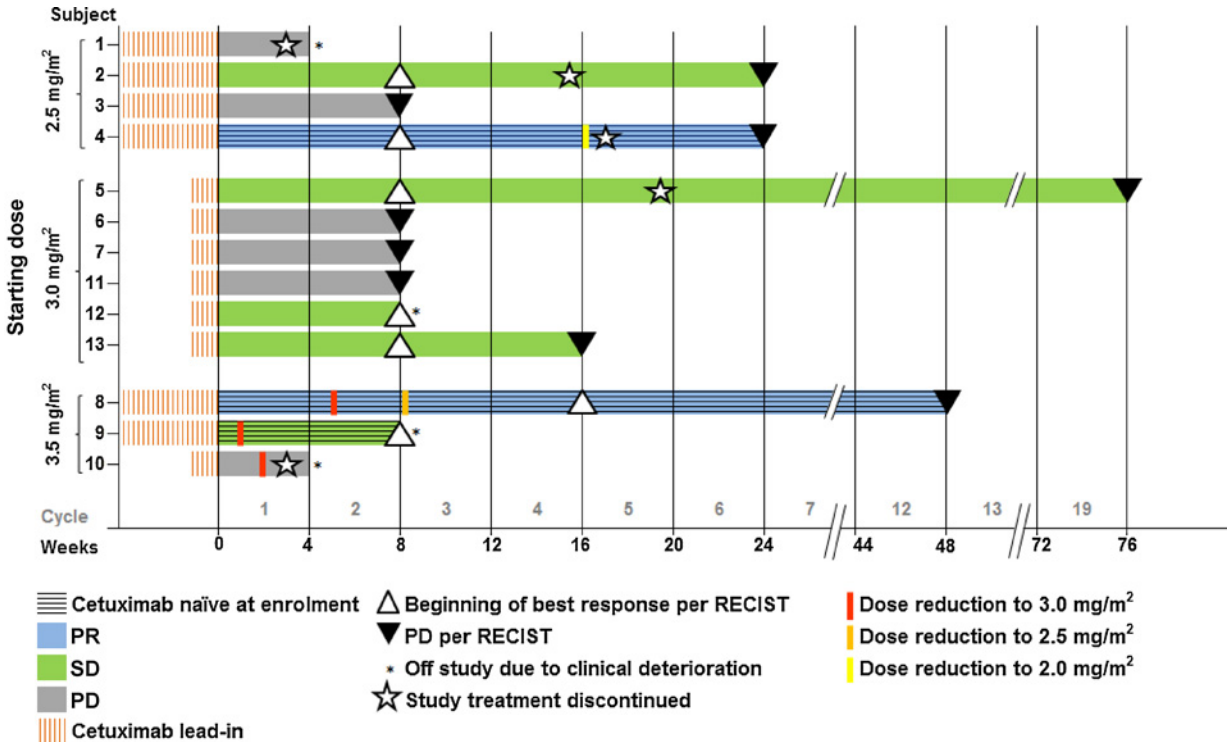

Figure 2.

Best response summary. Tumor imaging assessments, including CT and MRI scans of applicable sites of disease, were performed at screening and at the end of every second cycle until PD. Disease response was assessed using RECIST, Version 1.1. Two patients achieved PR for ≥6 cycles, 5 patients maintained SD for ≥2 cycles, 4 patients experienced PD after 2 cycles, and 2 patients were unevaluable, having failed to reach the first imaging time point. Patients are numbered in the order they were enrolled on study: 2.5 mg/m^2 cohort = patients 1–4; 3.0 mg/m^2 cohort = patients 5 to 7 and 11 to 13; 3.5 mg/m^2 cohort = patients 8 to 10.

The 2 patients who achieved PR were in the 2.5 mg/m^2 (patient 4) and 3.5 mg/m^2 (patient 8) dose cohorts (Fig. 2). Patient 4, who was cetuximab naïve prior to this study, achieved a PR after 2 cycles of combination study treatment, which was maintained until confirmed radiographic progression following cycle 6. Patient 8, who was also cetuximab naïve prior to this study, achieved a PR, which was maintained until cycle 12 (Supplementary Fig. S1).

Of the 5 patients who had disease stabilization, 3 patients were in the 3.0 mg/m^2 dose cohort and 1 patient each was in the 2.5 and 3.5 mg/m^2 cohorts. Four of these 5 patients had previously been treated with cetuximab before enrolment on this study. Notably, patient 5, with human papilloma virus–positive hypopharyngeal carcinoma with pulmonary metastases, had progressed after a prior chemotherapy and cetuximab-based regimen before enrolling in this study. He received 5 cycles of combination therapy on the 3.0 mg/m^2 cohort before discontinuing therapy due to tolerability but remained in SD until imaging showed progression after 76 weeks. He was subsequently treated for 7 months with anti-PD1 antibody on a clinical trial and maintained SD before discontinuing due to reversible transaminitis. The patient maintained SD without therapy for another year before passing away due to his disease 4 years after enrolling on this protocol.

Correlative studies

Levels of immune mediators. Administration of motolimod to study patients resulted in a dose-dependent increase in plasma levels of G-CSF, IL6, MCP-1, and MIP-1β (Fig. 3). Levels of these biomarkers of TLR8 activation peaked 8 hours following motolimod administration, returning to near baseline levels by 24 hours. The pharmacodynamic response on day 1 was comparable with the day 15 response. No obvious correlation was found between mediator response and clinical outcome.

Flow cytometric analysis of NK-cell frequency and activation. The mean ± 1 standard deviation (median) frequency of CD3$^-$CD56$^+$ NK cells increased to 17.1% ± 13.6 (12.2%) by 8 hours and 22.2% ± 19.6 (17.1%) by 24 hours after the day 1 dose from a pre-motolimod level of 7% ± 8.5% (2.6%). Mean NK-cell frequencies decreased to approximately baseline levels then increased again at both 8 and 24 hours after the day 15 dose (Fig. 4A). These treatment effects on NK-cell frequencies were statistically significant at both 8 ($P = 0.0023$) and 24 hours ($P < 0.0001$) compared with predosing but did not reach significance between the day 1 and 15 time points, or between motolimod dose levels. Relative to the day 1 predose NK-cell frequency, the peak fold increase occurred at 24 hours after the first dose (37.7-fold).

To determine the effect of motolimod and cetuximab on NK-cell activation, CD107a and IFNγ expression were measured on CD3$^-$CD56$^+$ NK cells at the same time points. After subtraction of background-unstimulated CD107a expression, NK-cell CD107a expression increased and peaked 24 hours after motolimod dosing

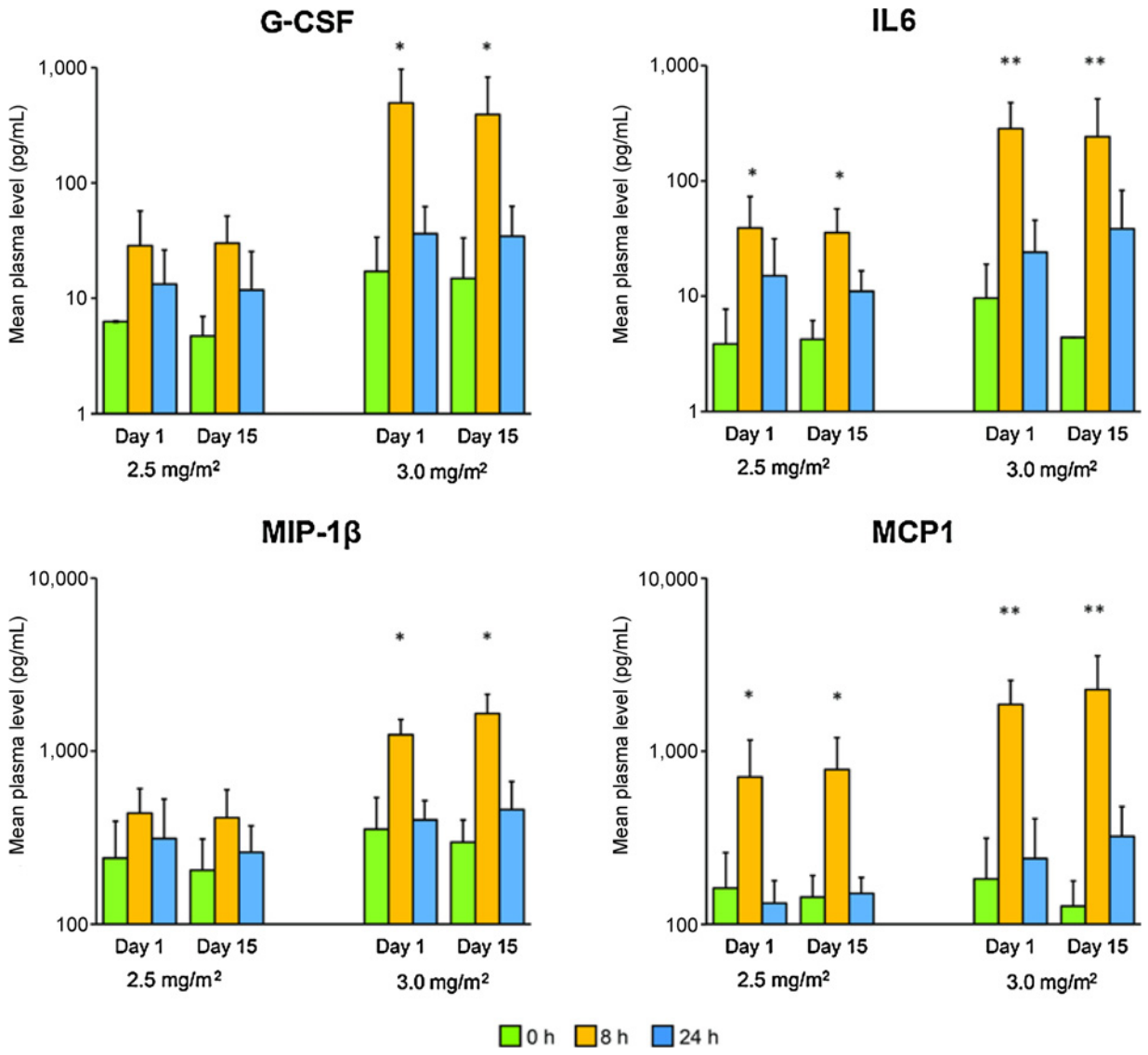

Figure 3.

Median levels of immune mediators after treatment with motolimod and cetuximab. Following the first (day 1) and third (day 15) motolimod doses given for cycle 1, G-CSF, IL6, MCP-1, and MIP-1β were measured at 0, 8, and 24 hours. Asterisks, statistical significance in change from the 0 time point (*, $P \leq 0.05$; **, $P \leq 0.01$); two-sided *t* test. The 3.5 mg/m^2 cohort is not shown due to incomplete sample data.

on both day 1 (mean 23% ± 13.7, median 17.1%) and 15 (mean 27% ± 12.3, median 24.1%; Fig. 4B). These increases were equivalent to mean fold increases of 4.6 compared with predosing 24 hours after day 1 (range, 0.9–21.6) and 4.9 after day 15 (range, 0.8–21.3) with K562 stimulation. The treatment effect at 24 hours was statistically significant ($P < 0.0001$), as was the increase at day 15 compared with day 1 ($P = 0.01$), but no motolimod dose effect was found. Similarly, mean fold increases of 13.4 (range, 0.3–120.2) and 5.5 (range, 0.8–34.4), respectively, 24 hours after day 1 and after day 15 were observed with anti-CD16 stimulation.

IFNγ ELISPOT analysis. To determine whether T-cell responses were stimulated with combination motolimod and cetuximab therapy, expanded IFNγ ELISPOT assays were conducted for the 7 patients with sufficient PBMCs available for testing (Supplementary Fig. S2A). Three of these 7 patients had qualitatively positive IFNγ responses to EGFR protein detected on day 15 (128, 313, and 62 spots per million cells input PBMC, respectively) that were not detected at day 0. PBMCs from 8 of 13 patients were also analyzed using less sensitive but quantitative unexpanded IFNγ ELISPOT assays (Supplementary Fig. S2B). Despite robust responses to control influenza A nucleoprotein and CEF peptide pools, no patients demonstrated IFNγ responses to EGFR or CD105 proteins. In addition, the frequency of IFNγ responses to influenza A or CEF peptides did not increase between days 1 and 15. This suggests that a low level EGFR-specific IFNγ response was present on day 15 after motolimod dosing that was not detectable with the standard ELISPOT assay.

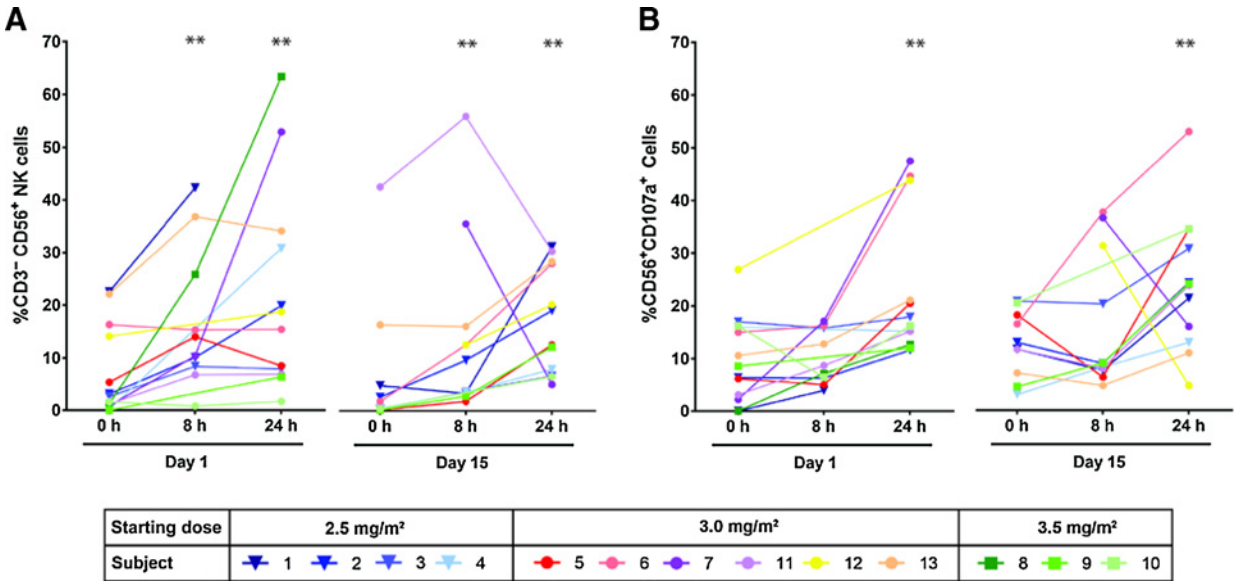

Figure 4.

Circulating $CD3^-CD56^+$ NK-cell frequencies and NK-cell degranulation after treatment with motolimod and cetuximab. $CD3^-CD56^+$ NK-cell frequencies and NK-cell degranulation ($CD107a^+$) were measured before and after dosing of motolimod on days 1 and 15 using flow cytometric analyses of freshly isolated PBMCs. The percentage of lymphocytes that were $CD3^-CD56^+$ (**A**) and $CD107a^+$ among $CD3^-CD56$ NK cells (**B**) are shown. **B,** Data from K562-stimulated NK cells (after subtraction of unstimulated control wells) are shown. Each colored shape indicates the results from a single patient. Bold black lines indicate the means with 1 standard deviation shown. Statistically significant treatment effects were seen on days 1 and 15 at 8 hours ($P = 0.0023$) and 24 hours ($P < 0.0001$) compared with baseline for NK-cell frequencies (**A**) and at 24 hours ($P < 0.0001$) compared with baseline for NK-cell degranulation (**B**). Asterisks, statistical significance in change from baseline (**, $P \leq 0.01$); log-linear mixed model.

Discussion

The objective response rate and median OS with single-agent cetuximab in patients with SCCHN is dismally low (15–17); therefore, there is a critical unmet need for combination regimens that enhance response and survival. The rationale for the current combination trial was based upon the knowledge that the therapeutic activity of cetuximab includes ADCC of EGFR-expressing tumor cells by NK cells (31). Moreover, *in vitro* studies have demonstrated that motolimod augments NK-cell function and enhances cetuximab-mediated ADCC (32, 33). In the phase I study of motolimod monotherapy in solid tumors, the best overall response was SD in 24% (8/33 subjects), indicating very modest antitumor activity as a single agent (27). However, the adverse event profile (injection site reaction, chills, pyrexia, fatigue, nausea, flu-like illness) had little overlap with the toxicity of cetuximab. Thus, the addition of motolimod to a cetuximab treatment regimen has the potential to substantially increase the clinical response rate in SCCHN with little synergistic toxicity.

This first-in-human study evaluating the combination of an immunotherapeutic TLR8 agonist with cetuximab in SCCHN shows that treatment was associated with a tolerable toxicity profile. Most patients treated at motolimod doses of 2.5 and 3.0 mg/m^2 were able to complete 2 or more cycles of combination therapy, with 1 patient completing 12 cycles. The majority of AEs were mild to moderate, and there were no treatment-related deaths or serious, unexpected, drug-related AEs. There was no evidence of synergistic toxicities between cetuximab and motolimod at any dose level, and the majority of AEs were those commonly associated with either agent alone, including flu-like symptoms, injection site reactions, and fatigue for motolimod (27), or infusion reactions and rash for cetuximab (17).

No DLTs were observed in any dose cohort; however, 2 of the 3 patients treated at the 3.5 mg/m^2 dose level experienced moderate AEs during the first or second cycle, including flu-like symptoms, injection site reactions, malaise, and fatigue, requiring dose delay and reductions. Although these events did not meet the protocol-defined criteria for DLT, it was determined that repeat dosing at 3.5 mg/m^2 was not tolerable, and 3.0 mg/m^2 was selected as the recommended phase II dose.

Preliminary antitumor activity was observed in patients treated with the combination of motolimod plus cetuximab. Two patients with radiographically evaluated disease achieved PR (15%) and an additional 5 patients had SD (38%). Including the 2 patients who clinically progressed before radiographic efficacy assessment, an overall disease control rate of 54% was observed. Furthermore, evidence of clinical benefit was seen across all dose levels evaluated in this study. Of note, all 3 of the cetuximab-naïve patients enrolled in this study achieved disease control, with 2 achieving a PR and 1 patient having SD. Four of the 8 previously cetuximab-exposed patients experienced SD, including 1 patient who had prolonged SD lasting approximately 19 months, despite receiving only 5 months of motolimod/cetuximab therapy, and having entered the study while progressing on cetuximab. Although this phase I study represents only a small number of patients, these efficacy results are encouraging and compare favorably with the antitumor activity of single-agent cetuximab.

Consistent with the phase I study of single-agent motolimod in which the biologically active dose levels used in the current study were identified (27), robust pharmacodynamic responses were seen. Rapid increases in G-CSF, IL6, MIP-1β, and MCP-1 were

observed after dosing, returning to near baseline levels within 24 hours. There was no apparent change in the magnitude of the pharmacodynamic response with repeat dosing, demonstrating that repeated, weekly treatments with motolimod do not result in either a desensitization or augmentation of the mediator response. Twenty-four hours after motolimod dosing, transient but statistically significant increases in the frequency and activation of NK cells were observed. Significant dose effects were not observed, however, which is likely related to the small number of patients studied in each dose cohort, combined with the inherent biological variation in cellular immune responses. Notably, 3 of 7 patients tested with the expanded assay had EGFR-specific IFNγ ELISPOT responses detected at day 15. This result is intriguing because the testing was conducted a relatively short time after initial motolimod dosing. Results from later time points after dosing would likely have been more informative regarding antigen-specific T-cell responses. Overall, no obvious correlation was found in this small exploratory analysis among plasma cytokine levels, NK-cell frequency or activation status, and clinical outcomes. However, these data confirm that motolimod can induce robust immune responses in cancer patients who have been exposed to multiple prior chemotherapeutics, which have numerous myelo- and lymphotoxic side effects. This finding is further supported by published data demonstrating that cancer patients treated with motolimod exhibit dynamic immune responses comparable with those seen in healthy volunteers (21).

Recently, the immune checkpoint inhibitors, such as anti-PD-1 antibodies, have shown antitumor activity, and produced durable responses, and improvements in survival in patients with SCCHN (18, 19), setting the premise and stage for the effectiveness of immunotherapy in this disease. As TLRs activate innate immune responses, which in turn are known to influence adaptive immunity, a TLR8 agonist, such as motolimod, could be exploited to potentiate antitumor T-cell responses. In fact, it was interesting that one patient survived an unprecedented 4 years after receiving motolimod plus cetuximab combination treatment and subsequent anti-PD-1 checkpoint blockade. The motolimod–cetuximab combination could potentially represent an intriguing sequenced or combinational approach with immune checkpoint inhibitors to enhance or improve responses and survival in SCCHN.

In summary, the current study shows that motolimod can be safely administered in combination with cetuximab with an acceptable toxicity profile. Robust pharmacodynamic responses were seen across all dose levels, and encouraging antitumor activity was observed in heavily pretreated patients with limited therapeutic options. Motolimod is being further investigated in a phase II randomized, double-blind, placebo-controlled trial of chemotherapy plus cetuximab in combination with motolimod in patients with recurrent or metastatic SCCHN (ClinicalTrials.gov ID: NCT01836029).

Disclosure of Potential Conflicts of Interest

L.Q.M. Chow is a consultant/advisory board member for Merck and Bristol Myers Squibb. R.M. Hershberg holds ownership interest (including patents) in VentiRx Pharmaceuticals. M.L. Disis reports receiving commercial research grants from Celgene, EMD Serono, Jansen, Seattle Genetics, and VentiRx Pharmaceuticals and holds ownership interest (including patents) in Epithany, University of Washington Patents, and VentiRx Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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