A Phase I Monotherapy Study of RG7212, a First-in-Class Monoclonal Antibody Targeting TWEAK Signaling in Patients with Advanced Cancers

Ulrik N. Lassen1, Didier Meulendijks2, Lilian L. Siu3, Vaios Karanikas4, Morten Mau-Sorensen1, Jan H.M. Schellens2, Derek J. Jonker5, Aaron R. Hansen3, Mary E. Simcox6, Kathleen J. Schostack6, Dean Bottino6, Hua Zhong6, Markus Roessler7, Suzana M. Vega-Harring7, Tiantom Jarutat7, David Geho6, Ka Wang6, Mark DeMario6, and Glenwood D. Goss5

Introduction

Tumor necrosis factor (TNF)–like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible molecule 14 (Fn14) are a ligand–receptor pair frequently overexpressed in solid tumors. TWEAK:Fn14 signaling regulates multiple oncogenic processes through MAPK, AKT, and NFκB pathway activation. A phase I study of RG7212, a humanized anti-TWEAK IgG1κ monoclonal antibody, was conducted in patients with advanced solid tumors expressing Fn14.

Experimental Design: Dose escalations, over a 200-7,200-mg range, were performed with patients enrolled in weekly (QW), bi-weekly (Q2W), or every-three-week (Q3W) schedules. Primary objectives included determination of dose and safety profile. Secondary endpoints included assessments related to inhibition of TWEAK:Fn14 signaling, tumor proliferation, tumor immune cell infiltration, and pharmacokinetics.

Results: In 192 treatment cycles administered to 54 patients, RG7212 was well-tolerated with no dose-limiting toxicities observed. More than 95% of related adverse events were limited to grade 1/2. Pharmacokinetics were dose proportional for all cohorts, with a t1/2 of 11 to 12 days. Pharmacodynamic changes included clearance of free and total TWEAK ligand and reductions in tumor Ki-67 and TRAF1. A patient with BRAF wild-type melanoma who received 36 weeks of RG7212 therapy had tumor regression and pharmacodynamic changes consistent with antitumor effects. Fifteen patients (28%) received 16 or more weeks of RG7212 treatment.

Conclusion: RG7212 demonstrated excellent tolerability and favorable pharmacokinetics. Pharmacodynamic endpoints were consistent with reduced TWEAK:Fn14 signaling. Tumor regression was observed and prolonged stable disease was demonstrated in multiple heavily pretreated patients with solid tumors. These encouraging results support further study of RG7212.

Abstract

Purpose: Tumor necrosis factor (TNF)–like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible molecule 14 (Fn14) are a ligand–receptor pair frequently overexpressed in solid tumors. TWEAK:Fn14 signaling regulates multiple oncogenic processes through MAPK, AKT, and NFκB pathway activation. A phase I study of RG7212, a humanized anti-TWEAK IgG1κ monoclonal antibody, was conducted in patients with advanced solid tumors expressing Fn14.

Experimental Design: Dose escalations, over a 200-7,200-mg range, were performed with patients enrolled in weekly (QW), bi-weekly (Q2W), or every-three-week (Q3W) schedules. Primary objectives included determination of dose and safety profile. Secondary endpoints included assessments related to inhibition of TWEAK:Fn14 signaling, tumor proliferation, tumor immune cell infiltration, and pharmacokinetics.

Results: In 192 treatment cycles administered to 54 patients, RG7212 was well-tolerated with no dose-limiting toxicities observed. More than 95% of related adverse events were limited to grade 1/2. Pharmacokinetics were dose proportional for all cohorts, with a t1/2 of 11 to 12 days. Pharmacodynamic changes included clearance of free and total TWEAK ligand and reductions in tumor Ki-67 and TRAF1. A patient with BRAF wild-type melanoma who received 36 weeks of RG7212 therapy had tumor regression and pharmacodynamic changes consistent with antitumor effects. Fifteen patients (28%) received 16 or more weeks of RG7212 treatment.

Conclusion: RG7212 demonstrated excellent tolerability and favorable pharmacokinetics. Pharmacodynamic endpoints were consistent with reduced TWEAK:Fn14 signaling. Tumor regression was observed and prolonged stable disease was demonstrated in multiple heavily pretreated patients with solid tumors. These encouraging results support further study of RG7212.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Ulrik Lassen, Department of Oncology 5072, The Finsen Centre, Rigshospitalet, Copenhagen, Denmark. Phone: 45-35458923; Fax: 45-35454391; E-mail: ulrik.lassen@rh.regionh.dk

doi: 10.1158/1078-0432.CCR-14-1334

©2014 American Association for Cancer Research.

Cancer Therapy: Clinical

A Phase I Monotherapy Study of RG7212, a First-in-Class Monoclonal Antibody Targeting TWEAK Signaling in Patients with Advanced Cancers

Ulrik N. Lassen1, Didier Meulendijks2, Lilian L. Siu3, Vaios Karanikas4, Morten Mau-Sorensen1, Jan H.M. Schellens2, Derek J. Jonker5, Aaron R. Hansen3, Mary E. Simcox6, Kathleen J. Schostack6, Dean Bottino6, Hua Zhong6, Markus Roessler7, Suzana M. Vega-Harring7, Tiantom Jarutat7, David Geho6, Ka Wang6, Mark DeMario6, and Glenwood D. Goss5

Abstract

Purpose: Tumor necrosis factor (TNF)–like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible molecule 14 (Fn14) are a ligand–receptor pair frequently overexpressed in solid tumors. TWEAK:Fn14 signaling regulates multiple oncogenic processes through MAPK, AKT, and NFκB pathway activation. A phase I study of RG7212, a humanized anti-TWEAK IgG1κ monoclonal antibody, was conducted in patients with advanced solid tumors expressing Fn14.

Experimental Design: Dose escalations, over a 200-7,200-mg range, were performed with patients enrolled in weekly (QW), bi-weekly (Q2W), or every-three-week (Q3W) schedules. Primary objectives included determination of dose and safety profile. Secondary endpoints included assessments related to inhibition of TWEAK:Fn14 signaling, tumor proliferation, tumor immune cell infiltration, and pharmacokinetics.

Results: In 192 treatment cycles administered to 54 patients, RG7212 was well-tolerated with no dose-limiting toxicities observed. More than 95% of related adverse events were limited to grade 1/2. Pharmacokinetics were dose proportional for all cohorts, with a t1/2 of 11 to 12 days. Pharmacodynamic changes included clearance of free and total TWEAK ligand and reductions in tumor Ki-67 and TRAF1. A patient with BRAF wild-type melanoma who received 36 weeks of RG7212 therapy had tumor regression and pharmacodynamic changes consistent with antitumor effects. Fifteen patients (28%) received 16 or more weeks of RG7212 treatment.

Conclusion: RG7212 demonstrated excellent tolerability and favorable pharmacokinetics. Pharmacodynamic endpoints were consistent with reduced TWEAK:Fn14 signaling. Tumor regression was observed and prolonged stable disease was demonstrated in multiple heavily pretreated patients with solid tumors. These encouraging results support further study of RG7212.
including colorectal cancer, pancreatic carcinoma, non–small cell lung cancer (NSCLC), and ovarian cancer (16–18). Moreover, Fn14 expression is considered a negative prognostic factor in glioblastoma multiforme (6), breast cancer (19), gastric cancer (20), and NSCLC (21). Therefore, targeting TWEAK:Fn14 signaling has led to the development of several anticancer therapeutic strategies, including the antibodies enavatuzumab, BIIB036, and RG7212 (22–24).

RG7212, a fully humanized IgG1κ monoclonal antibody, blocks TWEAK ligand binding to Fn14 (IC50 = 13 ng/mL; ref. 25). Single-agent tumor growth inhibition has been demonstrated for RG7212 in multiple in vivo models, including renal cell carcinoma (RCC), breast, and pancreatic cancer (25). This activity is accompanied by changes in TWEAK signaling pathways, including a reduction of AKT and ERK phosphorylation and a reduction of the transcriptional targets of NFκB. In addition, immunophenotypic changes associated with antitumor activity have been observed with anti-TWEAK antibody treatment in mice (25) and in TWEAK knockout mice (15). CD3+ T cells were significantly increased in blood and spleen and slightly, but not significantly increased in tumors, while monocytes/macrophages (CD11b+/F4/80+) were significantly decreased in blood and significantly increased in tumors from anti-TWEAK-treated mice (25). Increases in tumor and spleen T-cell numbers were reported for TWEAK knockout mouse studies (15). In vivo activity was greatest in models with high Fn14 receptor expression and absent in models lacking Fn14 expression (25). On the basis of these findings, a phase I multicenter trial of RG7212 monotherapy in patients with Fn14-expressing advanced solid tumors was initiated. Major objectives for this study were assessment of single-agent safety, recommended phase II dose, pharmacokinetics, pharmacodynamics, and preliminary efficacy.

Patients and Methods

Patients

This open-label phase Ia study of single-agent RG7212 was initiated in July 2011 at four centers. All patients were adults ages ≥18 years with Fn14-positive, advanced, refractory solid tumors for which no treatment options were available. Tumor Fn14 expression was determined by immunohistochemistry (IHC), as described below. Fn14 positivity was defined as ≥10% of tumor cells staining with at least weak intensity in cytoplasm and/or in membrane, hereafter referred to as "IHC ≥1+." Assessments were
permitted on either archival or fresh tumor biopsy samples. In addition, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate hepatic, renal, and bone marrow function at study baseline were required. Patients were ineligible if they had received a registered or investigational cancer therapy <21 days before the first study day. Other exclusion criteria included cardiovascular disease (congestive heart failure > New York Heart Association class II, uncontrolled arrhythmia, recent myocardial infarction, or cerebrovascular accident), other uncontrolled concurrent disease (e.g., diabetes, active infection), or immunosuppressive therapy. Patients with central nervous system or leptomeningeal metastases were ineligible, except those with clinically stable disease for >3 weeks before study treatment. The study was conducted in full conformance as outlined by institutional review boards of participant institutions. The trial followed the principles of the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonization. Accordingly, investigators obtained informed consent from each participant or each participant’s guardian. This study was registered as NCT01383733.

Study design

A 3+3 dose-escalation design was used. RG7212 was administered on weekly (QW), bi-weekly (Q2W), and every-three-week (Q3W) schedules (Supplementary Fig. S1). The individual doses examined are listed in Table 1. Patients were initially enrolled in the QW schedule at a dose of 200 mg, which provided a more than 15-fold safety margin from the NOAEL observed in preclinical 13-week GLP toxicology studies. The Q3W regimen was used subsequent to the QW schedule, beginning at an 800-mg dose based on the absence of dose-limiting toxicities (DLT) observed for QW schedule patients. Preliminary analysis of Q3W pharmacokinetic data revealed RG7212 half-life shorter than that anticipated by simulations of preclinical data. Therefore, an every 2-week schedule (Q2W) was later adopted to support an effective therapeutic steady state \( C_{\text{min}} \) at practical dose levels.

Treatment cycles were 21 days for schedules QW and Q3W, and 28 days for schedule Q2W. Patients received study treatments continuously in the absence of disease progression, DLTs, or withdrawal of study consent. Tumor response was assessed following every two cycles (6 or 8 weeks) of study treatment, according to RECIST 1.1. Maximum tolerated dose (MTD) was assessed according to DLTs observed during the first RG7212 treatment cycle (21 or 28 days).

Study objectives

The primary study objectives were to evaluate safety, determine MTD, and identify a recommended phase II dose (RP2D) for each of the three treatment schedules. Secondary objectives included evaluation of the pharmacokinetic and pharmacodynamic profile of RG7212 in peripheral blood (e.g., TWEAK and NF\( \kappa \)B transcription products), in tumor expression (including Fn14, Ki-67, and TRAF1), by \( ^{18} \text{F} \) fluoro-deoxyglucose (FDG)-PET imaging, and by evaluation of antitumor activity using anatomical imaging (RECIST 1.1).

Analytic and statistical methods

Descriptive statistical methods were used for efficacy and safety analyses. Paired \( t \) tests were performed to compare free and total TWEAK levels at baseline and posttreatment. RG7212 serum concentration was measured by a validated ELISA assay (ICON Development Solutions). Pharmacokinetic samples were collected at multiple time points in cycles 1 to 4 for all treatment schedules. Pharmacokinetic parameters were computed by noncompartmental methods (WinNonlin 5.2.1; Pharsight).

An Fn14 IHC assay was developed and validated at Roche Diagnostics GmbH (Penzberg, Germany). Archival or fresh, paraffin-embedded tumor samples received for eligibility screening were batched and assayed in a central Roche GCP laboratory at approximately 2-week intervals. Results were communicated in real time to the respective study center.

An electrochemiluminescence immunoassay (ECLIA) was developed at Roche Diagnostics GmbH, Penzberg Germany, for the detection of TWEAK in human plasma samples. The assay used biotinylated (capture) and ruthenylated (detection) anti-TWEAK monoclonal antibodies, which were added to plasma samples (35 \( \mu \)L) for immunocomplexing with TWEAK. Complexes were captured and quantified as chemiluminescent emission using a cobas e411 analyzer. Anti-idiotypic TWEAK mAb was incubated within the above samples for determination of total plasma TWEAK. The assay was calibrated over a 0 to 3.0 ng/mL range using recombinant TWEAK.

TRAF1 protein in tumor was determined by an IHC assay using a monoclonal rabbit antibody (clone 45D3 Cell signaling Technology). The assay was validated at Histogenex. Phosphorylated ERK expression was assessed by an anti-pERK rabbit monoclonal antibody (Cell Signaling Technology) IHC assay validated at Histogenex. Membrane, nuclear, and cytoplasmic staining were considered, using a 4 point intensity scoring range (0 to 3+). Ki67 (clone 30.9; Ventana) immunoreactivity was reported as

Table 1. Patient enrollment and drug administration

<table>
<thead>
<tr>
<th>Patients, n (%)</th>
<th>Overall</th>
<th>Schedule 1 (QW)</th>
<th>Schedule 2 (Q2W)</th>
<th>Schedule 3 (Q3W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n (%)</td>
<td>54</td>
<td>26 (48)</td>
<td>16 (28)</td>
<td>12 (22)</td>
</tr>
<tr>
<td>Median age, y</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (M:F), n (%)</td>
<td>22 (45:55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG baseline, n (%)</td>
<td>0</td>
<td>11 (20)</td>
<td>43 (80)</td>
<td></td>
</tr>
<tr>
<td>Dose range, mg</td>
<td>200–7200</td>
<td>200–3,600</td>
<td>3,600–7,200</td>
<td>800–3,200</td>
</tr>
<tr>
<td>Dose cohorts, n</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Doses administered, mg</td>
<td>192</td>
<td>105</td>
<td>39</td>
<td>48</td>
</tr>
<tr>
<td>Cycles, n</td>
<td>200; 400; 800; 1,600; 2,400; 3,600</td>
<td>3,600; 5,400; 7,200</td>
<td>800; 1,600; 3,200</td>
<td></td>
</tr>
<tr>
<td>Cycles per patient, n</td>
<td>2.0</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Median (range)</td>
<td>3.6 (1–16)</td>
<td>4.0 (1–12)</td>
<td>2.4 (1–4)</td>
<td>4.0 (1–16)</td>
</tr>
</tbody>
</table>

Clinical Cancer Research
percentage positive nuclear staining and mean densometric value of positively stained cells, with IHC assay validated at Histogenex. Tumor paired, paraffin-embedded biopsy specimens obtained from patients at study baseline and posttreatment (cycle 1) were batched for analysis at Histogenex and subsequent data reporting to Roche.

Pharmacodynamic parameters from tumor tissue and peripheral blood were analyzed as a function of post-dose change from study baseline for each patient. Changes to PD parameters relative to RG7212 exposure (AUCinf) were identified for individual study patients, for all patients in a given dose schedule, and for the entire study cohort. Because AUCinf and dose had a linear relationship over the dose ranges tested and interpatient variability within dose cohorts was low, AUCinf was used as the exposure variable. Changes to free and RG7212-bound (total) TWEAK ligand were analyzed for individual patients following each treatment cycle. In addition, changes to tumor Ki-67, TRAF1, phosphorylated ERK (pERK), and CD3 expression were analyzed as a function of RG7212 AUCinf for each of these groups following cycle 1 (biopsy day 17 for QW schedule; day 8 for Q2W and Q3W schedules).

Table 2. RG7212 treatment-related adverse events (≥5% incidence)

<table>
<thead>
<tr>
<th>AE</th>
<th>Overall incidence</th>
<th>NCI CTCAE grade 3*</th>
<th>Incidence by schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 54</td>
<td>N = 54</td>
<td>QW n = 26</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14 (26%)</td>
<td>3 (6%)</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>11 (20%)</td>
<td>1 (2%)</td>
<td>7 (27%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>10 (19%)</td>
<td>—</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>8 (15%)</td>
<td>—</td>
<td>7 (27%)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>7 (13%)</td>
<td>—</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6 (11%)</td>
<td>—</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>6 (11%)</td>
<td>—</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (9%)</td>
<td>—</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>5 (9%)</td>
<td>—</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>4 (7%)</td>
<td>—</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>4 (7%)</td>
<td>—</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>3 (6%)</td>
<td>—</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td>3 (6%)</td>
<td>—</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Flushing</td>
<td>3 (6%)</td>
<td>—</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Dysesthesia</td>
<td>3 (6%)</td>
<td>—</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Infusion-related reaction</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>


*No grade 4 or 5 treatment-related AEs were reported.
version 4. Neither patient discontinued study treatment due to these events. Subsequently, a prophylactic regimen of H1 antagonist (e.g., diphenhydramine 25 mg orally or intravenously) and antipyretic (acetaminophen 650–1,000 mg orally) was instituted for all patients before each of their first two drug infusions. Investigators had the option of continuing premedications subsequent to the second drug administration for any patient, but this was rarely done.

RG7212 pharmacokinetics

Pharmacokinetic parameters were examined for all 54 patients. The estimated average half-life of RG7212 based on Schedule Q3W data was 11.7 ± 4.5 days (CV% = 38, n = 12). Plasma exposures, assessed as Cmax and AUC0–inf, increased linearly over the 200–7,200 mg dose range for all three schedules. The relationship between Cmax and dose can be described by linear regression, with R² = 0.85, P < 0.0001 (Fig. 2A). Clearance values across the dose range of 200–7,200 mg also indicated linear pharmacokinetics for RG7212 (Fig. 2B). A slight reduction in clearance was observed at the 7,200-mg dose. This was likely due to data variation, but a trend of saturation of clearance at this dose level cannot be ruled out. The accumulation ratios for each schedule, calculated as AUCt following first infusion in cycle 2 versus AUC1 following cycle 1 first infusion were 2.17 ± 0.50 from schedule QW, 1.51 ± 0.42 from schedule Q2W, and 1.28 ± 0.35 from schedule Q3W.

An alternative administration schedule was also assessed. After day 22, with repeated dosing, drug exposures from 7,200-mg Q2W and 3,600-mg QW dosing cohorts reached a similar level (Fig. 2C), supporting the feasibility of the more convenient Q2W schedule.

Pharmacodynamic data

This study used multiple pharmacodynamic endpoints in peripheral blood and tumor and 18F FDG PET imaging to characterize RG7212 treatment–related changes.

TWEAK ligand

The modulation of circulating TWEAK levels was assessed following administration of RG7212. Following initiation of treatment, free TWEAK concentration dropped significantly compared with baseline for all cohorts and remained low at multiple postbaseline assessments in cycles (C) 1 through 4 [median = 0.13, 0, 0, and 0 ng/mL for baseline (n = 50), C1 (n = 39), C2 (n = 35), and C4 (n = 13), respectively; P < 0.001, P < 0.001, P < 0.001 from paired two-sample t tests comparing C1 with baseline, C2 with baseline, and C4 with baseline, respectively; Fig. 3A]. Similarly, total TWEAK dropped significantly from baseline after treatment for all cohorts and remained low at multiple postbaseline assessments in C1 through C4 [median = 0.14, 0.02, 0.02, and 0.02 ng/mL for baseline (n = 49), C1 (n = 44), C2 (n = 40), and C4 (n = 12) respectively; P < 0.001, P < 0.001, P < 0.001 from paired two-sample t tests as described above (Fig. 3A)].

TRAF1

TRAFs 1, 2, 3, and 5 are proteins known to complex with Fn14 at cytoplasmic domain binding sites (26, 27). TRAF binding may be important as a proximal event in Fn14-mediated signaling, promoting activation of signal transduction cascades involving the NFkB and ERK pathways (Fig. 1). A nonsignificant correlation toward decreased tumor TRAF1 protein expression relative to baseline with increased RG7212 exposure was observed among 31 patients with data from pre- and posttreatment tumor biopsy samples (R = −0.267; P = 0.147; Fig. 3B and C).

Ki-67

Ki-67 expression was assessed as a measure of tumor antiproliferative effects following RG7212 treatment. Three patients, two
1,600 mg QW and one 3,200 mg Q3W, had >40% posttreatment reduction in tumor Ki-67 expression (Fig. 3B). Among 33 patients with data from pre- and posttreatment tumor biopsy samples, a significant correlation of decreased Ki-67 expression with increasing RG7212 exposure was observed ($R = -0.381$, $P = 0.029$).
**18F FDG-PET imaging**

Preclinical studies have demonstrated that in vivo RG7212 activity is accompanied by reductions in tumor AKT phosphorylation (25). As previous clinical studies have suggested that 18F FDG-PET may be useful as a noninvasive pharmacodynamic marker for PI3K–AKT pathway inhibition, we examined metabolic responses (28). Partial metabolic responses in cycle 1 or cycle 2 were observed for 5 patients, as verified by independent, centralized review (ICON plc). Responses by FDG-PET were noted in patients from both the QW and Q3W schedules but were not well correlated with RECIST responses, duration of study treatments, or RG7212 exposure (data not shown).

**Immune infiltration**

CD3 and CD68 infiltration of tumors was assessed as a measure of tumor pharmacodynamic activity following RG7212 treatment. Patients in all schedules presented with increases or decreases in both CD3- and CD68-positive cell numbers after treatment. However, these changes were not statistically significant in relation to dose, schedule, type of disease, treatment duration, change in tumor volume or other pharmacodynamic measures (data not shown).

**Efficacy data**

Evaluation of tumor response to RG7212 treatment was a key secondary study endpoint. Forty-seven of 54 enrolled patients (87%) received the first scheduled (second cycle) RECIST tumor evaluation. A best tumor response of stable disease (SD) was noted for 23 of 54 patients (43%), of which 15 (28%) had prolonged SD (at least 16 weeks on study treatments), including patients with heavily pretreated NSCLC, squamous cell carcinoma of the head and neck, mesothelioma, breast cancer, melanoma, RCC, and cholangiocarcinoma.

A 72-year-old female with heavily pretreated BRAF wild-type, stage M1 melanoma had evidence of tumor regression during RG7212 treatment. Prestudy treatments included naptaxil, ipilimumab, and temozolomide completed 12, 6, and 2 months before RG7212 treatment, respectively. Tumor reduction was apparent in both examination of cutaneous-based disease and nodal metastases in the axilla and mediastinum. The patient's CT radiographs before study treatment and following the fourth treatment cycle are provided (Fig. 4A). A 14.5% overall reduction in assessable lesions (RECIST) was observed, whereas volumetric image analysis revealed a 50% reduction in a right axillary nodal lesion. Pharmacodynamic endpoints for this patient were also encouraging, including a partial metabolic response on cycle 2 18F FDG-PET imaging (Fig. 4A). A significant reduction in tumor Ki-67 expression (~70%) 1 week following first RG7212 dose, a 5-fold increase in T-cell infiltration (CD3) in this tumor biopsy and a 74% reduction in posttreatment pERK expression were also noted (Fig. 4B). This patient was treated in the 3,200-mg cohort of the Q3W schedule and received 12 treatment cycles (36 weeks) before terminating study treatments for disease progression.

**Discussion**

This phase Ia study demonstrated the feasibility of patient selection based on tumor Fn14 expression. Archival or fresh tissue samples for nearly 400 patients with advanced solid tumors were screened for this trial. Tumor Fn14 expression in at least 10% of cells (IHIC ≥ 1+) was noted for nearly 50% of screened patients. Tumors with the highest relative frequency of this Fn14 expression included hepatobiliary, gastric, gastroesophageal junction, and unknown primary adenocarcinomas.

The rationale for this selection strategy was based on preclinical data demonstrating that the most significant in vivo efficacy occurred in models with high Fn14 expression. Evidence of tumor regression was observed in a patient with melanoma whose archival tumor sample had 60% Fn14 expression. While the limited efficacy observed in this phase Ia study did not allow for a clear validation of the preclinical data, additional exploration of this selection strategy is warranted in future RG7212 studies for specific indications.

Monoclonal antibody drugs can be administered with either body-size–based dose or flat-fixed dose approaches depending on the interpatient variability in pharmacokinetic parameters (e.g., clearance) associated with the dosing strategy and therapeutic
window of the drug. Population pharmacokinetic model–based simulation results from the literature (29, 30) have demonstrated that neither dosing approach provided overall superiority in reducing the interpatient variability. Our in-house simulation analysis was consistent with these results. As it had a 30-fold safety margin, flat-fixed dosing of RG7212 was selected in this first-in-human study for its advantages in ease of dose preparation, reduced cost, and reduced chance of dosing errors.

RG7212 demonstrated favorable safety and pharmacokinetic properties. More than 190 treatment cycles were administered to 54 patients in three administration schedules. RG7212 was well tolerated. Fewer than 5% of related AEs were grade 2 or greater severity, and patients have received up to 16 treatment cycles without a significant increase in AE frequency or grade. Given the low toxicity observed, an MTD was not identified for these schedules based on safety findings. However, for each schedule, pharmacokinetic data demonstrated a linear dose-exposure relationship for all dose ranges tested. Concentrations at steady state suggested that preclinically identified efficacious exposures are attainable for both weekly and bi-weekly administration schedules. In terms of convenience, every 2-week administration is preferred for additional clinical study.

Pharmacodynamic data show that RG7212 treatment reduced free and total TWEAK ligand quickly and durably. TWEAK suppression has been demonstrated within the initial dose cohorts of all schedules. Additional data derived from biopsy samples indicated that RG7212 treatment resulted in exposure-dependent signaling effects, which may be indicative of higher antibody concentrations required to neutralize local TWEAK concentrations in tumors.

TRAF1 is a TWEAK-induced gene, and Fn14 contains a TRAF-binding domain, consistent with an important role for these proteins as proximal mediators of TWEAK:Fn14 signaling. Therefore, TRAF1 levels in tumor were examined. The observed trend of decreased tumor TRAF1 expression relative to baseline with increased drug exposures was consistent with results of preclinical studies (25), and supports RG7212 clinical mediation of this signaling pathway. This study examined treatment effects in tumor through biopsy following the first (Q2W and Q3W schedules) or third (QW schedule) drug administrations in cycle 1. Therefore, assessment of tumor TRAF expression following more prolonged RG7212 therapy would be an important objective for future trials. A significant correlation between reduced Ki-67 expression and increased RG7212 exposure supports antibody-mediated inhibition of tumor proliferation and is noteworthy, given the role of TWEAK:Fn14 signaling in MAPK pathway signaling.

In the absence of DLTs, pharmacodynamic data were taken into consideration with respect to defining RP2D. A trend toward a plateau in exposure-dependent reductions in Ki-67 and TRAF1 expression suggests that a 3,600-mg dose may provide maximal antitumor effect. Pharmacokinetic data demonstrate equivalent exposures overall for 3,600-mg QW and 7,200-mg Q2W dosing. Therefore, 7,200 mg was considered the RP2D for the Q2W schedule.

CT evidence of tumor regression was noted in a patient with treatment-refractory melanoma who received 36 weeks of therapy. In addition, evidence of prolonged SD (RG7212 treatment for 16 or more weeks) was achieved in nearly 30% of enrolled patients, including those with heavily pretreated NSCLC, squamous cell carcinoma of the head and neck, mesothelioma, breast cancer, melanoma, RCC, and cholangiocarcinoma. Eighty percent of patients receiving 16 or more weeks of therapy were treated within the 1,600 mg or greater dose cohorts.

Prolonged stable disease was not well correlated with the extent of tumor Fn14 expression at baseline. However, clear associations may not be evident from the small patient subset treated in this phase Ia study. Analyses of relative Fn14 expression and potential clinical benefit are therefore anticipated as key study endpoints for subsequent RG7212 trials. These studies will examine RG7212 efficacy in combination therapy regimens. Given the excellent safety profile of RG7212, combinations with cytotoxic chemotherapy should be feasible. Combination or sequenced therapies of RG7212 and EGFR-directed agents are also attractive, given an association between activating EGFR mutations and increased Fn14 expression in NSCLC (17).

Disclosure of Potential Conflicts of Interest

L.L. Siu reports receiving other commercial research support from Roche. D. Botino is an employee of Takeda Pharmaceuticals. T. Jarutat holds ownership interest (including patents) in Roche. K. Wang is an employee of Hoffmann-La Roche. M. DeMario is an employee of and holds ownership interest (including patents) in Hoffmann-La Roche. G.D. Goss reports receiving speakers bureau honoraria from Roche. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): U.N. Lassen, D. Meulendijks, L.L. Siu, V. Karanikas, M. Mau-Sorensen, J.H.M. Schellens, D.J. Jonker, A.R. Hansen, S.M. Vega-Harring, T. Jarutat, D. Geho, M. DeMario, G.D. Goss


Study supervision: U.N. Lassen, J.H.M. Schellens, K. Wang, M. DeMario

Other (e.g., study design): M. Mau-Sorensen

Other (e.g., discovery and development of pathway biomarker concepts implemented in the clinical trial): M. E. Simcox

Acknowledgments

The authors thank the patients who participated in this study and their families. The authors also thank Shirin Jadidi, Deborah Keller, Michael Leiss, Sabine Lohmann, Stacey Ukrainskyy, James Song, Jean Tессier, and Martin Weiser, as well as the nurses, investigators, and others on local study teams for their contributions to this trial.

Grant Support

This work was supported by Hoffmann-La Roche, Inc. Support for third-party writing assistance for this article was provided by Hoffmann-La Roche, Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 28, 2014; revised September 30, 2014; accepted October 16, 2014; published OnlineFirst November 11, 2014.
References
