Exposure and Tumor Fn14 Expression as Determinants of Pharmacodynamics of the Anti-TWEAK Monoclonal Antibody RG7212 in Patients with Fn14-Positive Solid Tumors

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Abstract

Purpose: The TWEAK–Fn14 pathway represents a novel anti-cancer target that is being actively investigated. Understanding the relationship between pharmacokinetics of anti-TWEAK therapeutics and tumor pharmacodynamics is critical. We investigated exposure-response relationships of RG7212, an anti-TWEAK mAb, in patients with Fn14-expressing tumors.

Experimental Design: Patients with Fn14-positive tumors (IHC+1/C21) treated in a phase I first-in-human study with ascending doses of RG7212 were the basis for this analysis. Pharmacokinetics of RG7212 and dynamics of TWEAK were determined, as were changes in tumor TWEAK–Fn14 signaling in paired pre- and posttreatment tumor biopsies. The objectives of the analysis were to define exposure-response relationships and the relationship between pretreatment tumor Fn14 expression and pharmacodynamic effect. Associations between changes in TWEAK–Fn14 signaling and clinical outcome were explored.

Results: Thirty-six patients were included in the analysis. RG7212 reduced plasma TWEAK to undetectable levels at all observed RG7212 exposures. In contrast, reductions in tumor Fn14 and TRAF1 protein expression were observed only at higher exposure (>300 mg/h/mL). Significant reductions in tumor Ki-67 expression and early changes in serum concentrations of CCL-2 and MMP-9 were observed exclusively in patients with higher drug exposure who had high pretreatment tumor Fn14 expression. Pretreatment tumor Fn14 expression was not associated with outcome, but a trend toward longer time on study was observed with high versus low RG7212 exposure.

Conclusions: RG7212 reduced tumor TWEAK–Fn14 signaling in a systemic exposure-dependent manner. In addition to higher exposure, relatively high Fn14 expression might be required for pharmacodynamic effect of anti-TWEAK monoclonal antibodies.

Introduction

TNF-like weak inducer of apoptosis (TWEAK) is a cytokine of the TNF superfamily which controls a range of cellular responses including cell proliferation, migration, and differentiation; apoptosis, angiogenesis, and inflammation. TWEAK plays an important role in tissue repair and remodeling (1), and deregulation of TWEAK signaling has been associated with autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis (2). In addition, over the past decade it has become clear that TWEAK is an important player in the progression of different types of cancer (2, 3).

TWEAK is expressed by hematopoietic cell types—mainly leukocytes—as a type II transmembrane protein which is cleaved to form a soluble cytokine of 156 amino acids (4). TWEAK forms a homotrimer, which binds with high affinity to its receptor, fibroblast growth factor inducible-14 (Fn14). Fn14, encoded by the gene TNFRSF12A, is the smallest type I transmembrane receptor of the TNF superfamily and is expressed by nearly all nonhematopoietic cell types. The cytoplasmic tail of Fn14 has no death domain, but contains a single binding site for TNF receptor-associated factor (TRAF), to which TRAFs 1, 2, 3, and 5 can bind.
Overexpressions of TWEAK and Fn14 are associated with poor patient outcome in many solid tumor types. Inhibition of the TWEAK–Fn14 pathway represents a novel anticancer strategy that is being actively investigated. Exposure-response relationships of monoclonal antibodies targeting soluble TWEAK in cancer patients are not well defined. Although preclinical studies suggest that tumor Fn14 expression is a prerequisite for antitumor effect, the implications of tumor Fn14 expression in cancer patients are not well understood. To address these issues, we investigated the pharmacokinetic-pharmacodynamic relationships of RG7212, an anti-TWEAK monoclonal antibody, in patients with Fn14-expressing cancers previously treated in a phase I study. Our analysis demonstrates that although plasma TWEAK was completely inhibited by RG7212 at all exposures tested, reductions of intratumoral TWEAK–Fn14 signaling were exposure dependent and occurred only at higher exposures. The presented data also suggest that higher tumor Fn14 expression might be required for pharmacodynamic effect of anti-TWEAK therapeutics.
RG7212 pharmacokinetics
RG7212 serum concentration was measured using a validated ELISA (ICON Development Solutions). Pharmacokinetic parameters were computed by noncompartmental methods using WinNonlin 5.2.1 (Pharsight; ref. 18). Area under the plasma concentration-time curves from time zero to infinity (AUC_{\text{inf}}) after the first infusion was used as the measure for exposure to determine exposure-response relationships.

Plasma TWEAK measurements
To measure plasma TWEAK levels, an electrochemiluminescence immunoassay was used, developed at Roche Diagnostics GmbH. The assay used biotinylated (capture) and ruthenylated (detection) anti-TWEAK monoclonal antibodies, which were added to 35 μl plasma samples 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 mentioned samples for determination of total plasma TWEAK. The assay was calibrated over a 0- to 3.0-ng/mL range using recombinant TWEAK.

Fn14 pharmacodynamic markers in tumor and serum
Paired pretreatment and on-treatment formalin-fixed paraffin-embedded tumor biopsies were analyzed for TNFRSF12A (the gene encoding Fn14) and TRAF1 gene expression using quantitative real-time PCR, and for Fn14, TRAF1, and Ki-67 protein expression using IHC. TNFRSF12A and TRAF1 gene expression was determined at Histogenex NV and data were reported as relative expression (on-treatment versus baseline). The IHC assay for Fn14 used a murine monoclonal antibody (clone 1.11.17, Roche Diagnostics GmbH) and was developed and validated at Roche Diagnostics GmbH. Samples were assayed in a central Roche GCLP certified laboratory. Fn14 immunoreactivity was determined semiquantitatively based on staining intensity of the cytoplasmatic compartment, using the following formula: (0 × "percentage cells 0") + (1 × "percentage 1 +") + (2 × "percentage 2") + (3 × "percentage 3 "), divided by 100, resulting in an immunoreactivity score between 0.00 and 3.00. TRAF1 protein expression in tumor was determined by IHC using a monoclonal rabbit antibody (clone 45D3 Cell signaling Technology), and the assay was validated at Histogenex. Ki-67 immunoreactivity was determined using clone 30.9 (Ventana Medical Systems) at Histogenex, and was reported as percentage positive nuclear staining and mean densimetric value of positively stained cells. Because of inherent limitations as to the amount of tumor tissue contained within each tumor biopsy, the number of evaluable samples differed slightly per pharmacodynamic assay (numbers given in the text or legends to the figures).

Serum concentrations of the TWEAK-inducible proteins MMP-9 and CCL-2 were determined using a validated ELISA at Histogenex. For MMP-9, serum concentrations at baseline were compared with concentrations 24 hours after the first infusion. For CCL-2, serum concentrations at baseline were compared with concentrations 7 hours after the first infusion.

Data analysis
All patients for whom paired tumor biopsies with sufficient tumor cell content available for laboratory analysis were included in the analysis (n = 36). To investigate exposure-response relationships of RG7212, patients were grouped as having low or high RG7212 exposure. As a cut-off, we chose the approximate mid-range of exposures observed in the patients who were enrolled (AUC_{\text{inf}} 300 mg·h/mL; ref. 6). Of the patients included in this analysis, 27 patients had low exposure (AUC_{\text{inf}} <300 mg·h/mL) and 9 patients had high exposure (AUC_{\text{inf}} ≥300 mg·h/mL). The median exposure was 119 mg·h/mL in the group of patients with low exposure (range 14-264 mg·h/mL) and 363 mg·h/mL in the group of patients with high exposure (range 306–616 mg·h/mL). To investigate the role of baseline tumor Fn14 expression in relation to changes in TWEAK-Fn14 signaling upon TWEAK inhibition, patients were grouped as having low or high baseline tumor Fn14 expression, as measured by IHC. Low expression was defined as having an immunoreactivity score lower than the population median, of 0.85, and high expression as immunoreactivity scores equal to or greater than the population median. The median score in patients with low expression was 0.50 (range 0.05–0.80, n = 18) and 1.50 in patients with high expression (range 0.90–2.50, n = 18).

Pharmacodynamic parameters from tumor tissue and peripheral blood were expressed as percentage change on-treatment versus baseline. To test whether pharmacodynamic parameters changed significantly from baseline to on-treatment, one-sample Wilcoxon signed rank tests were used (referred to in the text as "one-sample test"). For all group comparisons, for example, to test whether changes in pharmacodynamic parameters differed between patients with low versus high exposure, Mann–Whitney U tests were used. For all statistical tests, the significance level was set at P < 0.05. No adjustment for multiple comparisons was performed. All analyses were performed in R v3.1.1 (19).

Table 1. Patient and disease characteristics

<table>
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<tr>
<th>Patient characteristics</th>
<th>Patients, n</th>
<th>Median age, y (range)</th>
<th>Sex, n (%)</th>
<th>ECOG baseline, n (%)</th>
<th>Fn14 immunoreactivity score, median (range)</th>
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<tr>
<td>Patients, n</td>
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<td>61 (21–89)</td>
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<tr>
<td>Fn14 immunoreactivity score, median (range)</td>
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Tumor types

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<td>Melanoma</td>
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<tr>
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<tr>
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<td>Anal canal SCC</td>
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<td>Gall bladder (adenocarcinoma)</td>
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<td>Gastric cancer</td>
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<td>Pancreas</td>
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</table>

Abbreviations: NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma.
Results

Patients

Paired pretreatment and on-treatment tumor biopsies were available for 36 of 54 patients (67%) enrolled in NCT01383733. Insufficient tumor cell content to conduct pharmacodynamic analyses and no on-treatment biopsy due to early disease progression were the most common reasons for exclusion of subjects from the analysis. The patient and disease characteristics of the patients included in the analysis are summarized in Table 1.

Inhibition of soluble TWEAK reduces Fn14 expression in tumor

Pharmacokinetics of RG7212 and the effects of RG7212 on serum TWEAK have been described in detail previously (6). Briefly, free TWEAK concentrations dropped to below the lower limit of quantitation (LLQ) shortly after initiation of treatment and complete inhibition of TWEAK was maintained in cycles 1 through four across all dose levels. For the patients included in the current analysis, the median concentrations of free TWEAK at the time points closest to the biopsy were <LLQ, both for patients with low RG7212 exposure and for patients with high exposure (details not shown).

Given the available data to suggest that soluble TWEAK in tumor can induce expression of Fn14 (10), we examined the relationship between RG7212 exposure and change in TNFRSF12A mRNA expression in tumor tissue. Changes in TNFRSF12A expression ranged from −94% to +362%. In the overall population, there was a small, median reduction of 12% in TNFRSF12A mRNA levels on-treatment compared with baseline, which was nearly significant (P = 0.052, one-sample test). However, as shown in Fig. 1, when patients with low and high RG7212 exposure were considered separately, patients with high exposure had a significant reduction in tumor TNFRSF12A mRNA levels, of 25% (P = 0.010 compared with baseline, one-sample test), compared with an increase of 6% for patients with low RG7212 exposure (P = 0.885, one-sample test). High exposure led to significantly greater reductions in TNFRSF12A mRNA expression than low exposure (P = 0.022). The effect of RG7212 on change in TNFRSF12A mRNA expression appeared to be influenced by baseline Fn14 expression, as there was a trend toward greater reduction in tumors with higher baseline Fn14 expression (measured by IHC) than in tumors with low baseline Fn14 expression (−15% vs. +3%, P = 0.081). Consistent with this, a linear regression model of change in TNFRSF12A expression as a function of AUC was not significant (R² = 0.03, P = 0.359), but a model of change in TNFRSF12A expression as a function of both AUC and baseline Fn14 expression was significant (R² = 0.23, P = 0.016; P value for baseline Fn14 expression: 0.007).

The relationship between exposure to RG7212 and change in Fn14 immunoreactivity scores showed a pattern similar to that of changes in mRNA levels (Fig. 1). Changes in Fn14 immunoreactivity scores ranged from −100% to +400% for patients with low exposure, with a median increase of 40% (P = 0.074, one-sample test). High exposure led to significantly greater reductions in Fn14 immunoreactivity than low exposure (P = 0.033). Inconsistent with this, a linear regression model of change in Fn14 expression as a function of AUC was not significant (R² = 0.03, P = 0.359), but a model of change in Fn14 expression as a function of both AUC and baseline Fn14 expression was significant (R² = 0.23, P = 0.016; P value for baseline Fn14 expression: 0.007).

Figure 1.
Effect of RG7212 exposure on Fn14 expression, measured by gene expression and IHC. Change in TNFRSF12A (Fn14) gene expression (left, n = 34) and Fn14 protein expression as measured by IHC (right, n = 33) upon treatment with RG7212 according to exposure (low AUC: <300 mg·h/mL; high AUC: ≥300 mg·h/mL). Data are plotted as individual data points and as box-and-whisker plots. The upper and lower “hinges” of the box-and-whisker plot correspond to the first and third quartiles (the 25th and 75th percentiles). The upper and lower whiskers extend to the highest and lowest value, respectively, that is within 1.5 × the inter-quartile range. AUC, area under the plasma concentration-time curve.
RG7212 exposure, and from −50% to 0% for patients with high exposure. Higher exposure was associated with a median −17% decrease in Fn14 immunoreactivity scores ($P = 0.018$, one-sample test), compared with 0% for lower exposure ($P = 0.848$). Higher exposure led to a significantly greater decrease in Fn14 immunoreactivity scores than lower exposure ($P = 0.0497$).

TRAF1 expression is reduced at high RG7212 exposure

Fn14 binds TRAF proteins, including TRAF1, and TRAF1 therefore is a proximal component of the TWEAK-FN14 signaling pathway (20, 21). The TRAF1 gene was shown in gene array studies to be strongly induced in tumor cell lines treated with TWEAK, and levels of TRAF1 were reduced in tumor xenografts in RG7212-treated mice (15). Consistent with TWEAK inhibition and the observed changes in Fn14 expression, there was a significant decrease in TRAF1 immunoreactivity scores among patients with high RG7212 exposure ($−38\%, \ P = 0.047$, one-sample test, Fig. 2) but not with low exposure ($−2\%, \ P = 0.755$). Similarly, there was a trend toward a reduction in TRAF1 gene expression in tumor tissue for patients with high RG7212 exposure ($−51\%, \ P = 0.094$, one-sample test, Fig. 2), but not in patients with low exposure ($+4\%, \ P = 0.706$). Consistent with the role of TWEAK in the regulation of Fn14 and TRAF1 expression, reductions in TRAF1 gene expression correlated significantly with reductions in TNFRSF12A expression ($R^2 = 0.43$, $P = 0.003$).

Soluble TWEAK inhibition does not affect tumor TWEAK expression

There was no overall effect of RG7212 exposure on tumor TWEAK expression, with 11% increase at low exposure and 2% decrease at high exposure ($P = 0.364, n = 34$). In tumors expressing low levels of Fn14 at baseline, there was a 24% increase in TWEAK expression at low RG7212 exposure, versus 3% decrease at high exposure ($P = 0.736$). In tumors expressing high levels of Fn14 at baseline, there was 11% increase in TWEAK expression at low exposure, versus 59% increase at high exposure ($P = 0.138$).

RG7212 significantly reduces Ki-67 expression at higher exposure exclusively in tumors with high Fn14 expression

The effect of RG7212-induced TWEAK inhibition on tumor antiproliferative effects was investigated by measuring changes in tumor Ki-67 expression, as measured by IHC (6). In patients with low exposure to RG7212, tumor Ki-67 expression increased by 4%, whether there was 25% reduction in Ki-67 expression in patients that had high exposure ($P = 0.020$). When considering pretreatment tumor Fn14 expression, the effect of RG7212 on Ki-67 expression appeared to be restricted to tumors with high baseline Fn14 expression (Fig. 3). There was no significant effect of exposure on change in Ki-67 expression in tumors with low Fn14 expression ($−2\%$ and $−18\%$ for low and high exposure, respectively, $P = 0.371$). In
In inhibition of soluble TWEAK induces early changes in plasma levels of TWEAK-inducible proteins.

Because in vivo studies in mice have shown early changes in plasma concentrations of TWEAK-inducible proteins upon TWEAK inhibition, including MMP-9 and CCL-2, we investigated changes in serum concentrations of these proteins in patients treated with RG7212. There was no overall reduction in serum CCL-2 levels at higher compared with lower exposure (−4% vs. +9%, \(P = 0.643\)). However, in patients with high baseline tumor Fn14 expression, there was more reduction in CCL2 concentrations at higher RG7212 exposure compared with lower exposure (−26% vs. +6%, \(P = 0.035\), Fig. 4).

Overall, high exposure to RG7212 tended to induce a greater increase in MMP-9 concentrations than low exposure (±28% vs. −10%, \(P = 0.062\)). When baseline tumor Fn14 expression was taken into account, there was a significant increase in MMP-9 concentrations at high exposure but not at low exposure in patients with high baseline tumor Fn14 expression (±80% vs. −20%, \(P = 0.018\), Fig. 5).

Changes in TWEAK-Fn14 signaling in relation to disease characteristics and outcome

The four patients with high baseline tumor Fn14 expression who had high exposure to RG7212 (as shown in Figs. 3–5), showed pharmacodynamic response on all markers tested in tumor tissue, and showed early changes in serum concentrations of the TWEAK-inducible proteins CCL-2 and MMP-9. The tumor types of these four patients were: BRAF wild-type melanoma, adenocarcinoma of unknown primary, colon adenocarcinoma, and pancreatic adenocarcinoma. The first patient had been pretreated before enrollment with four lines of therapy that did not have a relevant treatment effect. During treatment with RG7212, however, this patient remained radiologically and clinically stable for 245 days, with a partial metabolic response on FDG-PET in cycle 2 (as reported previously; ref. 6) and substantial reductions in Ki-67 expression (−70%) in the on-treatment biopsy. The median time on study for the other three patients was 242 days, with a partial metabolic response on FDG-PET in cycle 2 (as reported previously; ref. 6) and substantial reductions in Ki-67 expression (−70%) in the on-treatment biopsy. The time on study for the overall population of patients analyzed was 53 days (0–245 days). Thus, there was no significant difference in time on study between the patients with changes in pharmacodynamic markers and the remaining population. There was also no difference in time on study between patients with high baseline tumor Fn14 expression compared with low expression (60 days vs. 53 days, \(P = 0.987\)). Because...
pharmacodynamic effects were seen at high RG7212 exposure but not at low exposure, we explored whether the duration on study was longer for patients with high exposure than for patients with low exposure. This analysis showed that patients with high exposure were on study for a median of 77 days (range 22–245 days), whereas patients with low exposure were on study for a median of 35 days (range 0–322 days), a difference that showed a trend toward significance (P = 0.086).

Discussion

Inhibition of TWEAK is being actively investigated as a novel anticancer strategy. We investigated exposure-response relationships of the anti-TWEAK monoclonal antibody RG7212 in patients with Fn14-positive cancers, hypothesizing that both exposure and relative Fn14 expression would be related to pharmacodynamic response. Our results indicate that the effects of RG7212 on tumor TWEAK–Fn14 signaling are exposure dependent, with higher exposure being required for more pronounced reductions in tumor TWEAK–Fn14 signaling. We observed a decrease in TNFRSF12A gene expression as well as Fn14 protein expression with high exposure. The fact that Fn14 expression decreased, in part, upon TWEAK inhibition, is in line with preclinical studies demonstrating that a positive feedback loop of TWEAK-stimulated Fn14 expression can occur in Fn14-expressing tumor cells. Thus far, this phenomenon had only been described by Tran and colleagues, who showed that addition of TWEAK to glioma cells increased Fn14 expression in vitro, likely through Fn14 promoter activation via Rac1/NF-kB activation by TWEAK (22). Our observations suggest that such a positive feedback loop might exist in tumors of patients, and in tumor types other than glioma. In in vitro studies of tumor cell lines responsive to RG7212 which were treated with TWEAK, however, no changes in Fn14 expression were observed, nor was TNFRSF12A mRNA expression reduced in xenograft tumors from mice treated with RG7212 (15). Another possible explanation is that the observed decreases in Fn14 expression are the result of tumor cells with high Fn14 expression being most RG7212-sensitive, and eliminated upon treatment with RG7212.

The fact that higher exposure was required for more prominent reductions in tumor TWEAK–Fn14 signaling could be explained by the fact that concentrations of antibody that reach the interstitial space of the tumor to capture TWEAK are much lower than circulating concentrations, as is typically seen with monoclonal antibody drugs (23, 24). It is also possible that high local (intratumoral) concentrations of TWEAK, or pools of TWEAK (such as membrane TWEAK which can be cleaved to release soluble TWEAK; ref. 25), contributed to some extent to the higher concentrations required for inhibition of TWEAK–Fn14 signaling.

Figure 4.
Changes in serum concentrations of CCL-2 in patients according to pretreatment tumor Fn14 expression. Data for CCL-2 on-treatment samples taken 7 hours after first infusion (n = 35). Data are plotted as individual data points and as box-and-whisker plots. The upper and lower “hinges” of the box-and-whisker plot correspond to the first and third quartiles (the 25th and 75th percentiles). The upper and lower whiskers extend to the highest and lowest value, respectively, that is within 1.5 * the inter-quartile range. Abbreviations: CCL-2, chemokine (C-C motif) ligand 2; AUC, area under the plasma concentration-time curve.
Although tumor TWEAK expression was measured, we did not measure tumor TWEAK protein concentrations to confirm this hypothesis. We are also not aware of any published data on tumor TWEAK concentrations.

We further investigated the effects of RG7212 on TWEAK–Fn14 signaling by measuring expression of TRAF1, which is transcriptionally induced by TWEAK. As with TNFRSF12A, we observed a trend toward reduced expression of TRAF1 at high exposure compared with low exposure, and a significant reduction in TRAF1 protein expression, in line with preclinical studies showing that levels of TRAF1 were reduced in tumor xenografts in RG7212-treated mice (15).

We determined the effect of RG7212 on tumor cell proliferation by measuring Ki-67 protein expression. Consistent with the role of TWEAK in inducing proliferation, and in line with the observed reductions in Fn14 expression at higher RG7212 exposure, we observed that Ki-67 decreased at high, but not at low exposure to RG7212. As in preclinical studies, where the level of antitumor activity was higher in models with higher Fn14 expression (15), there were indications that Ki-67 expression reduced significantly only in tumors with relatively high baseline Fn14 expression, although this concerned a very small number of patients. Although in vitro studies have shown that when cellular Fn14 levels are elevated to a certain threshold level, TWEAK-independent Fn14 signaling might also occur (2), our pharmacodynamic data show that blocking TWEAK leads to pathway inhibition, suggesting a lack of TWEAK-independent Fn14 signaling in the tumors of the patients studied. It remains to be explored whether highly Fn14-positive tumors are refractory to RG7212.

As binding of TWEAK to Fn14 activates the NF-κB pathway, it is not surprising that in vitro studies have shown that TWEAK affects the expression of NF-κB target genes, such as the secreted matrix metalloproteinase MMP-9 and the macrophage-recruiting chemokine CCL-2. Since in xenograft mouse models, early decreases in these markers were observed upon treatment with RG7212 (15), we investigated the circulating levels of these TWEAK-inducible proteins in our patients. Although no effect on CCL-2 plasma concentrations at 7 hours after first infusion was seen in patients with low baseline Fn14 expression, there was a small but significant decrease in patients with high baseline tumor Fn14 expression who had high RG7212 exposure.

These data suggest that high exposure and baseline tumor Fn14 expression are determinants of pharmacodynamic response to RG7212. The observed change in CCL-2 concentration was smaller in magnitude than in preclinical models in which tumor growth inhibition was observed, in which 3.5-fold to 18-fold decreases were observed (15).

In contrast with the observation of a decrease of CCL-2 levels, an increase in plasma MMP-9 concentrations was observed, and a significant change was observed only in patients with high...
baseline Fn14 expression. Although the observed decrease of CCL-2 is consistent with preclinical observations, the increase in MMP-9 levels is unexpected, as TWEAK significantly induces MMP-9 (3, 15, 26). Although it concerned a small number of patients, it is possible that this paradoxical increase is caused by cross-talk with other pathways that are linked to MMP-9 expression (e.g., other NF-κB-related pathways or the MAPK pathway), and might indicate that combination of anti-TWEAK with other treatment strategies may be required for more complete inhibition of Fn14’s downstream targets.

The observed pharmacodynamic effects with high RG7212 exposure and high pretreatment tumor Fn14 expression did not translate into a clear clinical benefit in terms of the time on treatment in this heterogeneous phase I population. It is possible that a clear relationship between Fn14 expression and treatment effect was not present due to heterogeneity among the tumor types treated and the fact that sample size was small. In addition, we cannot exclude that the fact that tumor biopsies were available for only part of the patients (67%) may have biased our results regarding associations with outcome. Studies in a larger and more homogeneous population are indicated to further address this issue. In the light of the observed dose dependency of RG7212’s pharmacodynamic effects, it is intriguing that we observed a trend toward longer time on treatment for patients who reached high RG7212 exposure compared with patients with low exposure. However, our analysis concerned a relatively small number of patients with different tumor types. In future studies with more homogeneous patient populations, it may be possible to establish associations between RG7212 pharmacokinetics and RG7212 pharmacodynamics and clinical outcome.

Overall, the observed pharmacodynamic effects were modest in magnitude. The fact that inhibition of tumor pharmacodynamic markers was less than complete could be the result of cross-talk from other pathways leading to activation of the investigated markers. This could mean that TWEAK inhibition alone does not result in sufficient target inhibition, and that combination treatment might be required in order to achieve meaningful inhibition of target pathways. Also, it will be an important objective of future studies to determine to which degree intratumoral TWEAK is inhibited, in order to confirm target engagement.

Because in the investigated population clinical benefit was observed in only several patients, it will be critical to determine which tumor (sub)types have TWEAK-Fn14 signaling as the predominant signal activation pathway that maintains tumor cell survival and proliferation, as these tumors might be most responsive to anti-TWEAK. Although overexpression of Fn14 has been demonstrated in many tumor types, and has been shown to be predictive for efficacy in animal studies to some degree, it is uncertain whether receptor overexpression per se, or expression alone, is predictive for treatment effect in patients, or that other factors, such as tumor TWEAK concentrations or a combination of tumor TWEAK concentrations and Fn14 expression, will be predictive of treatment effect in patients. Assessment of additional pharmacodynamic markers, such as baseline immune cell composition, could further contribute to a better understanding of the mechanism of action of RG7212 in humans and provide guidance for patient selection. Preclinical work to explore the efficacy of RG7212 in combination with standard-of-care agents, investigational targeted agents and immunomodulating drugs is also warranted.

Inhibition of TWEAK by RG7212 resulted in early reductions in tumor TWEAK-Fn14 signaling in an exposure-dependent manner, suggesting that higher exposure is required to achieve intratumoral pharmacodynamic effects. Higher pretreatment tumor Fn14 expression was associated with greater reductions in Fn14 expression in response to treatment, as well as with reductions of Ki-67 expression and early changes in serum concentrations of TWEAK-inducible proteins, indicating that higher tumor Fn14 expression is required for pharmacodynamic response. The observed systemic exposure dependency of RG7212’s pharmacodynamic effects, combined with the fact that RG7212 was safe at the higher dose levels tested and showed early signs of antitumor activity, support further clinical study with a dose of 7200 mg Q2W in patients with Fn14-expressing tumors.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Meulendijks, A.D.R. Huitema, S.M. Vega-Harring, G.D. Goss, J.H.M. Schellens


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