A Randomized, Double-Blind, Placebo-Controlled, Phase II Study of Oral Talactoferrin in Combination with Carboplatin and Paclitaxel in Previously Untreated Locally Advanced or Metastatic Non-small Cell Lung Cancer

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**Introduction:** The aim of the study is to investigate the activity and safety of oral talactoferrin (TLF) plus carboplatin and paclitaxel (C/P) in patients with previously untreated stage IIIIB/IV non-small cell lung cancer.

**Methods:** Patients \( n = 110 \) were randomly assigned to receive C/P plus either TLF (C/P/T) or placebo (C/P/P). The primary objective of this exploratory study was assessment of confirmed response rate (RR) in the prospectively defined evaluable population with a one-tailed \( p = 0.05 \). Secondary objectives included assessment of progression-free survival (PFS), duration of response, overall survival (OS), and safety.

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Disclosure: Y.W. and R.M. are employees of Agennix Inc. and A.V. designed the trial. Y.W. wrote the protocol and provided primary oversight of trial conduct. All the authors (except Y.W., R.M., and A.V.) recruited patients to the study. All data were collected by the participating centers, processed, and analyzed by Reliance Clinical Research Services (RCRS) and reviewed by Y.W., R.M., and A.V. The draft manuscript was written by Y.W., A.V., and R.M. with contributions, critical review, and approval from all authors.

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**Results:** The trial met the primary end point of improvement in confirmed RR in the prospectively defined evaluable population. Compared with the C/P/P group, RR increased in the C/P/T group by 18% (29–47%; \( p = 0.05 \)) and 15% (27–42%; \( p = 0.08 \)) in the evaluable and intent-to-treat populations, respectively. Compared with the C/P/P group, the C/P/T group had a longer median PFS (4.2 versus 7.0 months), OS (8.5 versus 10.4 months), and duration of response (5.5 versus 7.6 months), although the differences were not statistically significant. Adverse events (AEs) were consistent with C/P therapy. There were fewer total AEs (472 versus 569; two-tailed \( p = 0.003 \)) and grade 3/4 AEs (78 versus 105; \( p = 0.05 \)) in the C/P/T group compared with the C/P/P group.

**Conclusion:** TLF, in combination with C/P, demonstrated an apparent improvement in RR, PFS, and OS in patients with previously untreated stage IIIIB/IV non-small cell lung cancer and appears to enhance activity without significant additional toxicity. These results need to be confirmed in a phase III trial.

**Key Words:** Lactoferrin, Talactoferrin, Non-small cell lung cancer, Carboplatin, Paclitaxel.

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Lung cancer is the leading cause of cancer-related deaths worldwide and accounts for 28% of cancer-related deaths in the United States. Non-small cell lung cancer (NSCLC) is the most prevalent form of lung cancer constituting 85 to 90% of lung cancers. Despite aggressive treatment, improvement in long-term patient survival has been slow and an urgent need for new therapies remains.

Patients with stage IIIIB or IV disease have inoperable or unresectable lesions and are candidates for systemic anticancer therapy and, if appropriate, consideration for clinical trials. Platinum-based regimens have been associated with objective responses and improved survival and are a standard first-line therapy for these patients.

Talactoferrin alfa (TLF, also known as recombinant human lactoferrin) is a recombinant glycoprotein isolated from Aspergillus niger var. awamori. It is structurally similar...
to native human lactoferrin and is known only to differ in its glycosylation.3,4

TLF is an orally active, immunomodulatory protein with a novel mechanism of action. After oral administration, TLF interacts with gut-associated lymphoid tissue, recruiting circulating immature dendritic cells and inducing their maturation. In vitro studies demonstrate that dendritic cell maturation in the presence of tumor antigens and lymphoid effector cells induces strong innate and adaptive immune responses mediated by antitumor natural killer cells, CD8+ lymphocytes, and natural killer T cells. Such mechanism may result in the activation of tumor-draining lymph nodes, cellular infiltration of distant tumors, and tumor cell death.5–9 TLF is not systemically bioavailable.10,11 It is plausible to speculate that TLF’s initiation of the immune response in the gut-associated lymphoid tissue—using a physiologically important pathway that is anatomically distant from the primary tumor—may help to minimize the effect of the cancer’s local immunosuppressive defenses.

TLF has demonstrated antitumor activity in animal models as a single agent and in combination with chemotherapy.7–9 In in vivo studies, oral TLF inhibited tumor growth in squamous cell and adenocarcinoma tumor models in immunocompetent mice. In phase I trials in healthy volunteers10 and cancer patients,11 oral TLF was well tolerated without any drug-related serious adverse events (SAEs) or grade 3/4 adverse events (AEs). Doses of 1.5 to 9 g/d were well tolerated without any dose-limiting toxicities or definition of a maximum tolerated dose.11 TLF also showed apparent antitumor activity in a 36-patient phase IB cancer trial.12,13 This trial included 12 NSCLC patients whose disease had progressed after standard chemotherapy. The median progression-free survival (PFS) and median overall survival (OS) among these 12 NSCLC patients were 4.3 and 8.8 months, respectively.

Based on encouraging preclinical and clinical data, we conducted this randomized, double-blind, placebo-controlled, multicenter, phase II trial of TLF or placebo in combination with carboplatin/paclitaxel (C/P) in patients with locally advanced or metastatic NSCLC who had not received prior systemic anticancer therapy for NSCLC.

**METHODS**

**Study Objectives**

The objectives were to assess whether the addition of oral TLF to C/P in previously untreated patients with stage IIIB or IV NSCLC would result in enhanced antitumor activity and to evaluate the toxicity of this combination.

**Patient Population**

Eligibility criteria included histologically confirmed stage IIIB/IV NSCLC by tumor, node, metastasis staging, absence of previous systemic chemotherapy or biological therapy, the presence of measurable disease according to RECIST version 1.0, a performance status of Eastern Cooperative Oncology Group 0 or 1, and adequate organ function.

Exclusion criteria included brain metastases, active concurrent radiotherapy, prior radiotherapy to indicator sites, use of steroids or an investigational agent within 4 weeks of study. All patients provided written informed consent in accordance with institutional and governmental regulations.

**Materials and Methods**

The study was double-blind and placebo-controlled. Patients were randomly assigned (1:1) to one of two treatment arms without stratification:

- **Arm 1:** Carboplatin (area under the curve, 5.0 mg/ml/min) + paclitaxel (175 mg/m2); C/P (q 3 weeks × 6 cycles) + oral TLF (1.5 g in 15 ml twice daily for three 6-week cycles; C/P/T).
- **Arm 2:** C/P at the same doses as in arm 1 + oral placebo (15 ml twice daily for three 6-week cycles; C/P/P).

The rationale for choosing doses of C/P slightly lower than doses most commonly used in North America was the lack of prior experience with the combination of TLF and C/P and because these doses of C/P are commonly used in some parts of the world and community practices in the United States. Any C/P dose modifications were to be performed according to the manufacturer’s instructions for the appropriate agent. A TLF dose of 3 g/d (1.5 g twice daily) was chosen as the optimum study dose based on active doses in animal models; prior clinical studies showed no apparent increase in antitumor activity at higher doses.11 C/P was administered by intravenous infusion every 3 weeks for up to six cycles. TLF or placebo were administered orally for up to three 6-week cycles (35 consecutive days on the study drug followed by 1 week off the drug), starting from the day after C/P administration in the first, third, and fifth chemotherapy cycles. The C/P + TLF or placebo combination was administered until disease progression but not longer than 18 weeks (maximum of six 3-week chemotherapy cycles + three 6-week TLF or placebo cycles). Computed tomography (CT) scans were performed at 6, 12, and 18 weeks after completion of 2, 4, and 6 cycles of chemotherapy and at 7 and 10 months during the follow-up period if no disease progression had occurred.

**Study End Points and Statistical Analysis**

**Primary Analysis**

The primary efficacy parameter, upon which the sample size was based, was response rate (RR; partial response [PR] + complete response) in the evaluable population. RR was chosen as the primary end point for this study because TLF was administered in combination with chemotherapy, which produces responses in patients with advanced or metastatic NSCLC, and the study was designed to test whether adding TLF could increase the RR. In addition, this rationale was supported by preclinical data suggesting antitumor activity in combination with chemotherapy. All randomized patients were included in the intent-to-treat (ITT) population. The prospectively defined evaluable population included patients who received at least one dose of TLF/placebo in combination with at least one dose of C/P and had at least one CT scan after starting study drugs. Response was assessed by CT according to RECIST version 1.0.14 Responses required a confirmatory CT scan obtained at least 4 weeks after the first scan demonstrating a response. All CT readings, including those by the site radiologist(s), were blinded to treatment.
group. The investigators, who were also blinded, provided input into determining the response status.

**Secondary Efficacy Analyses**

PFS was calculated from the date of randomization until the date of radiological progression or death. OS was calculated from the date of randomization to the date of death. Duration of response (DOR) was calculated from the first date of a response until radiological progression or death.

**Safety Analysis**

The safety population consisted of all patients who received at least one dose of study drug. The safety end points included treatment-emergent and study agent-related AEs, SAEs, treatment discontinuations due to AEs, and grade 3/4 laboratory abnormalities using the National Cancer Institute Common Toxicity Criteria (version 2.0).

**Statistical Methods**

The sample size calculation was based on an assumption of a 40% target overall RR and a 20% null RR. A sample size of 50 evaluable patients per treatment group in this exploratory study provided a comparison between the test and the control group with 75% power and a one-tailed p value of 0.05. This was an exploratory phase II study, and a one-tailed test was thought to be appropriate in this setting to assess whether the addition of TLF to chemotherapy could increase the RR of the combination. Assuming a 10% rate of non-evaluable patients, the targeted enrollment was approximately 110 patients. For the RR analysis, where the patient's response status was not known, the patient was assumed to have progressed. The Fisher’s exact test was used for rate comparisons. The log-rank test was used for comparisons of PFS, DOR, and OS. As prospectively defined in the protocol, a one-tailed test was used for all efficacy analyses.

**RESULTS**

A total of 110 patients, 55 per arm, were enrolled and treated at 11 Indian sites between February 2004 and August 2005. The two arms appeared to be well balanced for known prognostic factors at baseline. Demographic characteristics at baseline are summarized in Table 1.

All 110 randomized patients were included in the ITT population. The prospectively defined evaluable population included all 100 patients who received at least one dose of study drug and had at least one CT scan after the start of treatment (scheduled at 6 weeks after the start of study drugs).

Seventy-three patients completed 18 weeks of study treatment, 40 (73%) and 34 (62%) in the C/P/T and C/P/P arms, respectively. Patient disposition is summarized in Table 2. The most common reason for discontinuation from the study before 18 weeks was disease progression, which was reported in 9% and 18% of patients in the TLF and placebo arms, respectively. Other reasons for discontinuation included an AE (2% TLF; 4% placebo) and consent withdrawal (4% TLF; 9% placebo). No patient withdrew because of TLF-related AEs.

Patients in the two arms received similar amounts of C/P, with 40 (73%) and 34 (62%) patients receiving six cycles of chemotherapy in the C/P/T and C/P/P arms, respectively. Compliance to TLF/placebo was very high at 97% in both arms. Compliance was assessed by counting vials returned by patients to the clinic during each visit and the patient recording vials taken in a diary.

**Confirmed RR**

The primary efficacy end point, confirmed RR, was analyzed in the Evaluable and ITT populations (Table 3). There were more responders in the C/P/T arm (23 PRs) compared with the C/P/P arm (15 PRs). One patient in the C/P/T arm with stage IV NSCLC and liver metastasis had a possible complete response. After treatment, there was a
small residual fibrotic lesion visible on a CT scan that was not biopsied. He remained disease-free for more than 2 years after the last dose of study drug. The RR in the 110-patient ITT population was 4.2 and 7 months in the placebo and TLF arms, respectively (HR = 0.85; p = 0.24). In the evaluable population, the median PFS in the placebo and TLF arms was 4.2 and 7 months, respectively (HR = 0.78; p = 0.14).

There was an improvement in the PFS rate at 18 weeks (end of treatment period), with an increase from 40% in the placebo to 53% in the TLF arm (p = 0.13) for the ITT population and from 43% in the placebo arm to 59% in the TLF arm (p = 0.08) for the evaluable population.

Overall Survival

In the ITT population, median OS increased from 8.5 months in the placebo arm to 10.4 months in the TLF arm (HR = 0.87; p = 0.26). The median OS in the evaluable patients increased from 8.5 months in the placebo arm to 11.3 months in the TLF arm (HR = 0.75; p = 0.11). The Kaplan-Meier curves for OS in the ITT and evaluable populations are shown in Figures 1A, B. Data on second-line and subsequent treatments are not available.

Safety Results

TLF appeared to be well tolerated. Patients who received C/P/T had fewer total AEs, grade 3/4 AEs, AEs related to chemotherapy, incidence of SAEs, and discontinuations due to AEs. The most frequent AEs were consistent with those typically observed in NSCLC patients undergoing chemotherapy. These included myelotoxicity, gastrointestinal (GI) disorders, respiratory disorders, and alopecia. The most frequently observed grade 3/4 AEs were myelotoxicity, GI disorders, and respiratory disorders (Table 4). Two patients in the placebo arm and one patient in the TLF arm discontinued due to an AE (Table 2).
The total number of AEs reported in the C/P/T arm was lower than in the C/P/P arm (472 and 569 AEs, respectively; Table 5). The difference was statistically significant using a two-tailed two-proportion binomial test (p = 0.003). The total number of grade 3/4 AEs was also lower in the C/P/T arm than in the C/P/P arm (78 and 105, respectively; p = 0.05).

A total of 605 AEs (297 in the TLF arm and 308 in the placebo arm) were considered to be related to C/P. There were fewer AEs considered related to study drug; 8 and 22 AEs in the TLF and placebo arm, respectively. None of the related AEs in the TLF arm were grade 3 or 4.

**DISCUSSION**

Oral TLF has previously demonstrated anticancer activity in animal models, both as a single agent and in combination with chemotherapy. More recently, single-agent activity of TLF was observed in a larger (100 patients) double-blind, placebo-controlled phase II trial in patients with refractory NSCLC. The addition of oral TLF to standard supportive care resulted in a 2.4 month, 65% improvement in median OS (3.7–6.1 months; one-tailed p = 0.04) relative to patients receiving placebo. Single-agent activity was also observed in a phase II trial in renal cell cancer patients who had failed previous chemotherapy.

Based on encouraging early data with oral TLF and its novel immunomodulatory mechanism of action, we initiated this randomized, double-blind, placebo-controlled phase II combination trial in NSCLC patients. The trial met its primary end point of a RR improvement in the prospectively defined evaluable population (one-tailed p = 0.05). Median PFS, OS, and DOR were also longer in the TLF arm although the differences were not statistically significant. The maximum duration of treatment was 18 weeks as TLF/placebo was discontinued with C/P discontinuation even in the absence of progression. This limited treatment duration was appropriate in phase II as the primary end point was RR and data on single-agent activity were limited at the time of study design. However, in a follow-on trial designed to detect improvements in PFS or OS, it would be desirable to continue TLF/placebo until disease progression, particularly in view of significant OS improvements reported recently with single-agent TLF.

The median age of patients enrolled in this trial (approximately 55 years of age) is consistent with that reported in the literature for Indian patients with NSCLC and is lower than the age of patients typically enrolled in clinical trials in Western populations.

An additional finding was the fewer number of total AEs and grade 3/4 AEs observed in the TLF arm. A similar reduction in AEs and grade 3/4 AEs was also observed in a second double-blind, placebo-controlled TLF trial—TLF monotherapy in patients with refractory NSCLC.

The majority of the AE reductions were in the four areas where reductions were expected based on lactoferrin’s

**TABLE 4. Grade 3 or 4 Adverse Events Occurring in >2% of the Population**

<table>
<thead>
<tr>
<th></th>
<th>Talactoferrin + C/P (N = 55), n (%)</th>
<th>Placebo + C/P (N = 55), n (%)</th>
<th>Total (N = 110), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>10 (18)</td>
<td>10 (18)</td>
<td>20 (18)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>5 (9)</td>
<td>6 (11)</td>
<td>11 (10)</td>
</tr>
<tr>
<td>Anemia</td>
<td>4 (7)</td>
<td>6 (11)</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5 (9)</td>
<td>1 (2)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Nonhematological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>5 (9)</td>
<td>9 (16)</td>
<td>14 (13)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0 (0)</td>
<td>6 (11)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0 (0)</td>
<td>4 (7)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>3 (5)</td>
<td>1 (2)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Hypoesthesia</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

C/P, carboplatin/paclitaxel.

**TABLE 5. Selected Body Systems Account for the Majority of the Adverse Events Reduction**

<table>
<thead>
<tr>
<th>All Adverse Events</th>
<th>Placebo + C/P</th>
<th>Talactoferrin + C/P</th>
<th>AE Decrease</th>
<th>Placebo + C/P</th>
<th>Talactoferrin + C/P</th>
<th>AE Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>All body systems*</td>
<td>569</td>
<td>472</td>
<td>97</td>
<td>105</td>
<td>78</td>
<td>27</td>
</tr>
<tr>
<td>Selected body systems**</td>
<td>Number of Patients with AEs</td>
<td>Number of Patients with AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoraco-respiratory system*</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Hematological**</td>
<td>44</td>
<td>25</td>
<td>19</td>
<td>25</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Diarrhea, loose stools, vomiting</td>
<td>41</td>
<td>29</td>
<td>12</td>
<td>8</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Musculoskeletal*</td>
<td>34</td>
<td>19</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nervous system disorders*</td>
<td>19</td>
<td>12</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

* Statistically significant decrease in total adverse events (AEs) (p = 0.003) and grade 3/4 AEs (p = 0.05) were observed by a two-tailed t-test.

** AE(s) including mainly dyspnea, pharyngolaryngeal pain, and tachypnea.

* AE(s) including mainly alopecia, leukopenia, lymphopenia, monocytopenia, neutropenia, and pancytopenia.

* AE(s) including mainly asthenia, back pain, and pain in extremity.

* AE(s) including mainly hypoaesthesia and paraesthesia.

C/P, carboplatin/paclitaxel.
biological activities and previously available data. One major group of AE reductions was in thoraco-respiratory signs and symptoms, which are largely attributable to the NSCLC disease process itself. These reductions are consistent with the apparent anticancer activity observed with TLF.

Other major groups of AE reductions included areas where lactoferrin has been shown to have protective effects: (i) hematological; (ii) GI, specifically relating to diarrhea, loose stools, and vomiting; and (iii) musculoskeletal/neurosensorial. As an immunomodulatory agent, lactoferrin has demonstrated acceleration in the reconstitution of the immune system after chemotherapy, with consistent reduction in hematological AEs in NSCLC patients receiving chemotherapy. The GI tract is an important target organ for cytotoxic chemotherapy. In animal and human studies, oral TLF protected the gut against irritant-induced enteropathy. This GI-protective effect of TLF is consistent with the reduction noted in some GI toxicities. Finally, the reductions in musculoskeletal and neurosensorial AEs are consistent with the known anti-inflammatory and antioxidant activities of TLF. As TLF is not systemically bioavailable, and the addition of TLF appeared to be associated with an increase in anticancer activity of the C/P/T combination, it is unlikely that the reduction in AEs is due to an impact on the pharmacokinetics of C/P.

The findings reported in this study on patients with previously untreated advanced or metastatic NSCLC suggest that addition of oral TLF to C/P chemotherapy results in enhanced activity without adding to chemotherapy toxicity. TLF is currently being evaluated in two phase III trials. The first study, conducted in patients whose disease has failed two or more previous treatments, has a primary end point of survival and compares TLF to placebo in patients who are also receiving best supportive care. The second study is a first-line NSCLC trial that compares the addition of TLF or placebo to C/P. Patients may receive up to six cycles of chemotherapy and if the disease has not progressed after completion of chemotherapy, TLF or placebo will be continued until disease progression. The study coprimary end points are PFS and OS.

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