NGR-hTNF and Doxorubicin as Second-Line Treatment of Patients with Small Cell Lung Cancer

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ABSTRACT

Background. Relapsed small cell lung cancer (SCLC) patients have limited treatment options and poor outcomes. NGR-hTNF is a vascular-targeting agent, which increases intratumoral chemotherapy penetration and T-lymphocyte infiltration.

Methods. Twenty-eight patients relapsing after at least one platinum-based regimen with a treatment-free interval shorter (n = 16; platinum-resistant) or longer (n = 12; platinum-sensitive) than 3 months received NGR-hTNF 0.8 μg/m² plus doxorubicin 75 mg/m² every 3 weeks. The primary endpoint of this single-arm phase II trial was progression-free survival (PFS), and safety, response rate, and survival were secondary endpoints.

Results. The most common grade 3–4 toxicities were neutropenia (53%) and anemia (21%). Median PFS was 3.2 months for all patients, 2.7 months for platinum-resistant patients, and 4.1 months for platinum-sensitive patients. Seven patients had partial responses (25%), including four (25%) with platinum-resistant and three (25%) with platinum-sensitive relapse. Mean changes from baseline in tumor burden (after two, four, and six cycles) did not differ between platinum-sensitive and platinum-resistant patients. Seven patients had partial responses (25%), including four (25%) with platinum-resistant and three (25%) with platinum-sensitive relapse. Mean changes from baseline in tumor burden (after two, four, and six cycles) did not differ between platinum-sensitive and platinum-resistant patients.

Discussion.

SCCL is characterized by high response rates to first-line platinum/etoposide-based chemotherapy. However, despite initial chemosensitivity, nearly all patients eventually experience relapse, which has historically been classified as platinum-resistant or platinum-sensitive according to a treatment-free interval shorter or longer than 3 months [1,2]. Salvage chemotherapy with topotecan yielded modest survival improvements in relapsed SCLC [3,4]. Since its discovery, tumor necrosis factor alpha (TNF) has shown powerful antitumor activity, but its early-stage development was hampered by severe toxicities, the maximum tolerated dose being 10-fold lower than the estimated effective dose. To increase the therapeutic index, NGR-hTNF was developed by conjugating TNF with the tumor-homing peptide NGR (asparagine-glycine-arginine-glycine), which selectively binds a CD13 isofom expressed by newly formed tumor blood vessels [5,6]. In preclinical models, NGR-TNF was 10-fold more active than untargeted TNF, with activity mostly noticed at low doses. Furthermore, a sequence- and time-dependent synergism between NGR-hTNF and chemotherapy was observed.

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when the former was administered 2 hours before the latter [7,8]. Phase I trials selected NGR-hTNF 0.8 µg/m² as the optimal dose in monotherapy [9] and in combination with doxorubicin [10], based on dynamic imaging changes, soluble TNF-receptors kinetics, and tolerability. In this single-arm phase II trial, the addition of NGR-hTNF 0.8 µg/m² to doxorubicin 75 mg/m² was associated with a manageable toxicity profile and similar antitumor activity in platinum-resistant and platinum-sensitive patients. Median progression-free survival was 3.2 months (95% confidence interval [CI] 1.8–4.7; 28 events) for all patients (n = 28), 2.7 months (1.8–3.6) for the platinum-resistant cohort (n = 16), and 4.1 months (2.4–5.8) for the platinum-sensitive cohort (n = 12). By radiologic tumor assessment, seven patients had partial response (PR; 25%; 95% CI 11–45) and eight stable disease (SD; 29%), for an overall disease control rate of 54% (95% CI 34–73). Among patients with PR and SD, median progression-free times were 6.3 months (range 2.7–7.9) and 4.6 months (range 3.2–7.0), respectively.

There were four PR (25%) and four SD (25%) in the platinum-resistant cohort and three PR (25%) and four SD (33%) in the platinum-sensitive cohort. Maximum percentage change in target lesion burden for individual patients are plotted in Figure 1A and on-treatment changes for all target lesions in Figure 1B. Reductions in tumor burden from baseline were noted in 14 (58%) of 24 patients who had at least one postbaseline assessment.

Consistently with NGR-hTNF and doxorubicin synergism shown in immunocompetent mice but not in nude mice [11], this study showed an association between overall survival and baseline lymphocyte count, with median survival of 13.1 months and 5.2 months in patients with counts above or below median (1.2/mL), respectively.

In this regard, the drug ability to increase the intratumoral T-cell infiltration [12], a prerequisite for response to immune checkpoint blockade [13], should facilitate the combination of NGR-hTNF with immune checkpoint inhibitors, to be evaluated in a randomized phase II setting.

**TRIAL INFORMATION**

| Disease | Lung cancer—SCLC |
|---------|------------------|
| Stage of Disease/Treatment | Metastatic/advanced |
| Prior Therapy | One prior regimen |
| Type of Study - 1 | Phase II |
| Type of Study - 2 | Single arm |
| Primary Endpoint | Progression-free survival |
| Secondary Endpoint | Overall response rate |
| Secondary Endpoint | Overall survival |
| Secondary Endpoint | Safety |

**Additional Details of Endpoints or Study Design**

According to a two-stage Simon’s optimal trial design (p0 = 35%, p1 = 60%, α = 10%, and β = 10%), the planned sample size was 27 patients, with 16 to be enrolled in the first stage. Study treatment was considered worthy of additional testing if 7 and 13 patients were progression free at 18 weeks after first and second stages, respectively.

**Investigator’s Analysis**

Active and should be pursued further

**DRUG INFORMATION**

| Drug 1 |
|--------|
| **Generic/Working Name** | NGR-hTNF |
| **Trade Name** | Zafiride |
| **Company Name** | MolMed |
| **Drug Type** | Biological |
| **Drug Class** | Angiogenesis—antivascular |
Dose: 0.8 mcg/m²
Route: IV
Schedule of Administration: Every 3 weeks until progressive disease

Drug 2
Generic/Working Name: Doxorubicin
Trade Name: Adriblastine
Company Name: Pfizer
Drug Type: Other
Drug Class: Anthracycline
Dose: 75 mg/m²
Route: IV
Schedule of Administration: Every 3 weeks up to 550 mg/m²

PATIENT CHARACTERISTICS
Number of Patients, Male: 19
Number of Patients, Female: 9
Stage: Metastatic or advanced
Age: Median (range): 63 (41–76)
Number of Prior Systemic Therapies: Median (range): 1 (1–3)
Performance Status: ECOG
0 — 14
1 — 12
2 — 2
3 —
Unknown —
Other
Best response to prior therapy, n (%)
Complete response (CR): 2 (7%)
Partial response (PR): 11 (39%) Stable disease (SD): 6 (21%) Progression disease (PD): 9 (32%)
Treatment-free interval, months
Median: 2.9
Range: 0.4–10.3

PRIMARY ASSESSMENT METHOD
Number of Patients Screened: 28
Number of Patients Enrolled: 28
Number of Patients Evaluable for Toxicity: 28
Number of Patients Evaluated for Efficacy: 28
Evaluation Method: RECIST 1.0
Response Assessment CR: n = 0 (0%)
Response Assessment PR: n = 7 (25%) Response Assessment SD: n = 8 (29%)
Response Assessment PD: n = 10 (36%)
Response Assessment OTHER: n = 3 (11%)
(Median) Duration Assessments PFS: 3.2 months, CI: 1.8–4.7
(Median) Duration Assessments OS: 5.6 months, CI: 5.3–5.9
(Median) Duration Assessments Response Duration: 6.3 months

Kaplan-Meier (Time Units: Months)
| Time of scheduled assessment and/or time of event | No. progressed (or deaths) | No. censored | Percent at start of evaluation period | Kaplan-Meier % | No. at next evaluation/No. at risk |
|-----------------------------------------------|---------------------------|-------------|--------------------------------------|----------------|----------------------------------|
| 1.2                                          | 1                         | 0           | 100.00                               | 96.43          | 27                               |
| 1.4                                          | 1                         | 0           | 96.43                                | 92.86          | 26                               |
| 1.7                                          | 3                         | 0           | 92.86                                | 82.14          | 23                               |
| 1.8                                          | 1                         | 0           | 82.14                                | 78.57          | 22                               |
| 2.0                                          | 1                         | 0           | 78.57                                | 75.00          | 21                               |
| 2.3                                          | 3                         | 0           | 75.00                                | 64.29          | 18                               |
| 2.5                                          | 1                         | 0           | 64.29                                | 60.71          | 17                               |
| 2.7                                          | 1                         | 0           | 60.71                                | 57.14          | 16                               |
| 3.2                                          | 2                         | 0           | 57.14                                | 50.00          | 14                               |
| 3.4                                          | 1                         | 0           | 50.00                                | 46.43          | 13                               |
| 4.1                                          | 2                         | 0           | 46.43                                | 39.29          | 11                               |
| 4.4                                          | 1                         | 0           | 39.29                                | 35.71          | 10                               |
| 4.6                                          | 1                         | 0           | 35.71                                | 32.14          | 9                                |
| 5.5                                          | 1                         | 0           | 32.14                                | 28.57          | 8                                |
| 6                                            | 2                         | 0           | 28.57                                | 21.43          | 6                                |
| 6.3                                          | 1                         | 0           | 21.43                                | 17.86          | 5                                |
| 6.4                                          | 1                         | 0           | 17.86                                | 14.29          | 4                                |
| 7                                            | 1                         | 0           | 14.29                                | 10.71          | 3                                |
| 7.1                                          | 1                         | 0           | 10.71                                | 7.14           | 2                                |
| 7.9                                          | 1                         | 0           | 7.14                                 | 3.57           | 1                                |
| 8.4                                          | 1                         | 0           | 3.57                                 | 0.00           | 0                                |
### ADVERSE EVENTS

| Adverse event          | Grade 1 n (%) | Grade 2 n (%) | Grade 3 n (%) | Grade 4 n (%) | All grades n (%) |
|------------------------|---------------|---------------|---------------|---------------|-----------------|
| Neutropenia            | —             | 2 (7)         | 2 (7)         | 13 (46)       | 17 (61)         |
| Anemia                 | 2 (7)         | 8 (29)        | 5 (18)        | 1 (3)         | 16 (57)         |
| Chills                 | 11 (39)       | 4 (14)        | —             | —             | 15 (54)         |
| Fatigue               | 1 (3)         | 7 (25)        | 5 (18)        | —             | 13 (46)         |
| Nausea                 | 7 (25)        | 5 (18)        | —             | —             | 12 (43)         |
| Lymphopenia            | 2 (7)         | 6 (21)        | 4 (14)        | 1 (3)         | 12 (43)         |
| Thrombocytopenia       | —             | 5 (18)        | —             | 1 (3)         | 10 (36)         |
| Appetite loss          | 5 (18)        | 2 (7)         | 3 (11)        | —             | 10 (36)         |
| Pyrexia                | 8 (29)        | —             | —             | —             | 8 (29)          |
| Mucositis              | 3 (11)        | 2 (7)         | 1 (3)         | —             | 6 (21)          |
| Dyspnea                | 2 (7)         | 2 (7)         | 1 (3)         | —             | 5 (18)          |
| Vomiting               | 3 (11)        | 2 (7)         | —             | —             | 5 (18)          |
| Constipation           | 3 (11)        | 2 (7)         | —             | —             | 5 (18)          |
| Cough                  | 3 (11)        | 1 (3)         | —             | —             | 4 (14)          |
| Diarrhea               | 4 (14)        | —             | —             | —             | 4 (14)          |
| Alopecia               | 1 (3)         | 2 (7)         | —             | —             | 3 (11)          |
| Conjunctivitis         | 2 (7)         | 1 (3)         | —             | —             | 3 (11)          |
| Sinus tachycardia      | 3 (11)        | —             | —             | —             | 3 (11)          |
| Dry skin               | 3 (11)        | —             | —             | —             | 3 (11)          |
| Feeling cold           | 3 (11)        | —             | —             | —             | 3 (11)          |
| Urinary infection      | 3 (11)        | —             | —             | —             | 3 (11)          |

Study-emergent adverse events in ≥10% of cases of safety population (n = 28), irrespective of treatment relationship, classified by preferred term and worst grade per patient.

Abbreviation: —, no data.

### ASSESSMENT, ANALYSIS, AND DISCUSSION

Completion: Study completed

Investigator’s Assessment: Active and should be pursued further

Patients eligible for this multicenter, single-arm, phase II trial were aged 18 years or older and had to have pathologically proven small cell lung cancer (SCLC), radiologically documented disease progression after at least one platinum/etoposide-based regimen, a performance status (PS) of 0–2, adequate bone marrow, hepatic and renal function, and measurable disease according to RECIST (version 1.0). A 4-week washout period for both radiotherapy and chemotherapy and 2 weeks for surgery were required before treatment initiation. Exclusion criteria included active brain metastases, significant cardiac dysfunction, including a left ventricular ejection fraction less than 55%, uncontrolled hypertension, and serious systemic disease or infection.

The primary study endpoint was progression-free survival, defined as the time from baseline to disease progression or death, whichever occurred first. Secondary endpoints included response rate, defined as the proportion of patients with complete response (CR) or partial response (PR), with radiologic assessments done at baseline and every other cycle (6 weeks); disease control rate, defined as the percentage of patients who had a best response of CR or PR or stable disease (SD); overall survival, defined as the time from baseline to death; and evaluation of toxicity using the Common Terminology Criteria for Adverse Events (version 3.0).

NGR-hTNF 0.8 μg/m² was given intravenously as a 1-hour infusion followed by doxorubicin 75 mg/m² as a 15-minute intravenous infusion 2 hours after NGR-hTNF dosing. Maximum cumulative doxorubicin dose was capped at 550 mg/m², whereas NGR-hTNF was continued until disease progression or intolerable toxicity occurred. For retreatment on next cycle, all the reported toxicities had to be recovered to grade 1 or resolved. For patients unable to meet retreatment criteria, a 1–3-week delay for both drugs was allowed. No formal dose reduction for NGR-hTNF was planned. If chills occurred during NGR-hTNF infusion, premedication with paracetamol was recommended for subsequent cycles. Doxorubicin dose modifications were applied according to the summary of product characteristics.

All analyses were based on the intent-to-treat principle. Time-to-event outcome variables were estimated by the Kaplan-Meier method. Exploratory Cox regression models assessed associations between overall survival and baseline characteristics, including age (> vs. ≤ median), sex, PS (0 vs. 1–
NGR-hTNF plus Doxorubicin in Pretreated SCLC

The 1-year overall survival rate was 30% (95% confidence interval 13–47; 27 events) for all patients, 27% (5–50) for platinum-resistant and 33% (7–60) for platinum-sensitive cohorts. By multivariate Cox regression analyses, the baseline lymphocyte count was the only factor significantly associated with overall survival. In patients with baseline lymphocyte counts higher or lower than the median value (1.2/mL), median overall survival times were 13.1 months versus 5.2 months among all patients, 15.7 months versus 5.2 months in platinum-resistant patients, and 5.6 months versus 4.6 months in platinum-sensitive patients, respectively.

In conclusion, this single-arm phase II trial showed a safe toxicity profile and promising activity of NGR-hTNF and doxorubicin combination in unselected patients with relapsed SCLC. Overall results seem promising, especially considering that more than half of cases presented with platinum-resistant relapse and one third with multiple prior treatment lines. The 1-year survival rate appears in line with that reported in relapsed SCLC with topotecan [3,4] or immune checkpoint inhibitors [14,15]. Indeed, a phase I/II trial testing immune checkpoint blockade with the programmed cell death protein 1 inhibitor nivolumab alone or combined with two doses of the cytotoxic T-lymphocyte-associated antigen 4 inhibitor ipilimumab showed response and 1-year survival rates of 10% and 33% in the monotherapy cohort and 19%–23% and 35%–43% in the combination cohorts, respectively [14]. Another early-stage trial with the programmed death-ligand 1 inhibitor pembrolizumab reported response and 1-year survival rates of 33% and 38%, respectively [15]. However, a phase III study of ipilimumab with or without chemotherapy among newly diagnosed SCLC patients was negative [16].

Finally, the mechanism of action of NGR-hTNF, which increases the intratumoral lymphocyte infiltration [12], might facilitate its combination with immune checkpoint inhibitors, which require high levels of tumor-infiltrating lymphocytes [13], with benefit to be assessed in a randomized phase II setting.

DISCLOSURES
Giulia Salini: MolMed (E); Antonio Lambiase: MolMed (E).
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(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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