Preliminary safety findings are presented from the open-label Phase I part of a combined Phase I/II study of BLP25 liposome vaccine (L-BLP25) in Japanese patients with unresectable Stage III non-small-cell lung cancer after primary chemoradiotherapy. Six patients received four or more once-weekly vaccinations with L-BLP25 1000 μg subcutaneously prior to a preliminary safety evaluation. Treatment continued with once-weekly vaccinations with L-BLP25 1000 μg subcutaneously until week 8, then maintenance vaccinations every 6 weeks until progressive disease. Cyclophosphamide (300 mg/m² i.v. single dose) was given 3 days before first vaccination. Median age was 63.5 years and performance status was 0–1. No serious adverse events occurred; none necessitated discontinuation. L-BLP25-related adverse events (Grade 1) were myalgia, arthralgia and nausea; cyclophosphamide-related adverse events comprised dysgeusia, anorexia and nausea. The first evaluation of L-BLP25 in Japanese patients shows that it is well tolerated, and the safety profile is consistent with that seen in previous studies of Caucasian patients.

Key words: immunotherapy – chemo-respiratory tract – lung medicine

INTRODUCTION

Lung cancer is the most common cause of cancer-related death worldwide (1) and at the national level in Japan (2). Non-small-cell lung cancer (NSCLC) accounts for the majority of lung cancers (~80%) (3,4), and survival rates for patients with Stage III/IV disease are poor (4). The standard of care for unresectable Stage III NSCLC is combined-modality therapy with chemotherapy and thoracic radiation therapy (TRT) (5). A Phase III study by the West Japan Lung Cancer Group showed that the combination of mitomycin, vindesine and cisplatin with concurrent TRT was associated with a median survival time of 16.6 months and a 5-year survival rate of 16% (6). There clearly remains an unmet need for novel treatment approaches to improve clinical outcomes in this patient population.

Therapeutic cancer vaccines contain tumor-associated antigens, which are supposed to stimulate the immune system to recognize the antigen expressed on cancer cells (7) and to respond with tumor cell destruction. One such vaccine in Phase III clinical development is the BLP25 liposome vaccine (L-BLP25, Merck KGaA), which targets mucin-1 (MUC1), a glycoprotein that is strongly expressed in many types of cancer (8,9). Cellular immune responses induced by L-BLP25 are characterized by the generation of cytotoxic T-lymphocytes capable of destroying MUC1-expressing tumor cells, the proliferation of CD4-positive T cells (10) and the production of pro-inflammatory cytokines (11).

A Phase IIb study in patients with Stage IIIB or IV NSCLC who had undergone primary chemo-and/or radiotherapy (n =
Stage IIIB locoregional disease (12). An updated analysis of L-BLP25 1000 mg (as measured by antigen mass) once every 6 weeks until disease progression [according to the study drug (12)]. These safety findings were supported by a subgroup analysis of 16 patients who received L-BLP25 for at least 2 years (14).

Previous studies of L-BLP25 recruited predominantly Caucasian patients; L-BLP25 has not been studied in Japanese populations. This open-label, non-randomized Phase I study combined with a double-blind, randomized, placebo-controlled Phase II study is being conducted in Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy. Preliminary Phase I safety data are reported.

**PATIENTS AND METHODS**

**STUDY OBJECTIVES**

The primary objective of the Phase I component of this combined Phase I/II study was to establish the safety of L-BLP25 1000 μg in Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy.

**STUDY DESIGN**

This was an open-label, non-randomized Phase I study (Study EMR: 63325–009) conducted at four centers in Japan, as part of a larger study that includes a Phase II placebo-controlled trial. This study was conducted in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice. The protocol was approved by institutional review boards and by the relevant authorities, in accordance with Japanese regulations.

**STUDY TREATMENTS**

L-BLP25 is a lyophilized preparation consisting of BLP25 lipopeptide, immunoadjuvant monophosphoryl lipid A and three lipids (cholesterol, dimyristoyl phosphatidylglycerol and dipalmitoyl phosphatidylcholine) forming a liposomal product. Patients received subcutaneous injections of L-BLP25 1000 μg (as measured by antigen mass) once weekly for 8 weeks, followed by a 1000 μg maintenance dose every 6 weeks until disease progression [according to Response Evaluation Criteria In Solid Tumors (RECIST)] or discontinuation. L-BLP25 was supplied as a sterile lyophilized preparation that was reconstituted with 0.9% sodium chloride. The 1000 μg dose consisted of four subcutaneous injections, each containing a quarter of the total dose, administered in the deltoid or triceps region of the upper arms, and the left and right anterolateral aspects of the abdomen.

Cyclophosphamide 300 mg/m² i.v. single dose (maximum 600 mg) was administered 3 days before the first vaccination in order to overcome the immune suppression seen in patients with cancer, thus enhancing the effect of immunotherapy (15,16).

**PATIENTS**

All patients provided written informed consent. Patients were aged ≥20 years with histologically or cytologically documented unresectable Stage III NSCLC. Inclusion criteria also required: documented stable disease or objective response (according to the RECIST criteria) after primary chemoradiotherapy (either concomitant or sequential) within 4 weeks before study entry (date of eligibility); primary thoracic chemoradiotherapy (two or more cycles of platinum-based chemotherapy, minimum radiation dose: ≥50 Gy) completed 4–12 weeks before study entry and Eastern Cooperative Oncology Group (ECOG) performance status 0–1.

Exclusion criteria included lung-cancer-specific therapy other than primary chemoradiotherapy; immunotherapy within 4 weeks prior to study entry; malignant pleural or pericardial effusion; any history of neoplasm other than lung carcinoma; autoimmune disease; immunodeficiency disease; splenectomy; and infectious conditions that could, in the investigator’s opinion, compromise the patient’s ability to mount an immune response.

**ASSESSMENTS**

Safety assessments included drug exposure; incidence and type of AEs and laboratory variables. Serum cytokines [interleukin 1β (IL-1β), IL-6, IL-8 and tumor necrosis factor alpha (TNFα)] and soluble IL-2 receptor alpha (sIL-2 Rα) were measured at a central laboratory. Cytokine levels were evaluated at the pretreatment evaluation visit (within 2 weeks of study entry) and at week 5.

**ANALYSIS**

An independent safety monitoring board reviewed safety data after six patients had received at least four doses of L-BLP25, which corresponded to the clinical data cut-off date 12 June 2009. All six patients were included in the safety analysis. Descriptive statistics on incidences of AE and serum cytokine monitoring are presented.
RESULTS

PATIENT CHARACTERISTICS AND DRUG EXPOSURE

Between 11 December 2008 and 10 May 2009, eight patients were screened at four study sites. Six received L-BLP25 and were included in the safety population. Median (range) age was 63.5 (59–69) years and five were male. ECOG performance status was 0 in five patients and 1 in one patient. At first diagnosis, five had Stage IIIA disease and one had Stage IIIB disease. Four patients were diagnosed with adenocarcinoma and two with squamous cell carcinoma. The median (range) duration of NSCLC (from diagnosis) was 5.7 (4.4–9.4) months. Primary chemoradiotherapy was concomitant in four patients and sequential in two, and resulted in stable disease in one patient and objective responses (partial or complete) in five.

As of 12 June 2009, median (range) duration of treatment (L-BLP25 including cyclophosphamide) was 7.7 (4.4–13.6) weeks, with a median (range) of 8 (5–9) L-BLP25 vaccinations. The median (range) total dose of cyclophosphamide was 300.0 (299.4–300.0) mg/m².

SAFETY

Of the six patients, five (83.3%) reported at least one AE (Table 1), all of which were Grade 1. No serious AEs were observed. No AEs led to discontinuation. One patient discontinued because of disease progression.

AEs related to L-BLP25 treatment were myalgia and arthralgia in one patient, and anorexia in another. AEs related to cyclophosphamide were dysgeusia in one patient, and anorexia and nausea in another.

No safety concerns were identified via serum cytokine monitoring (Fig. 1). Serum concentrations of IL-1β, sIL-2Rα, IL-6, IL-8 and TNFα all fell within the normal range at baseline and during the study, except for two patients: one whose IL-6 levels normalized during treatment, from 12.8 (pretreatment) to 11.3 pg/ml (normal range: 0.0–11.8 pg/ml), and another whose TNFα level increased from <2.2 (pretreatment) to 44.49 pg/ml during treatment (normal range: 0.00–7.46 pg/ml). There were no clinically significant changes in other laboratory variables.

DISCUSSION

Preliminary safety data reported here, in six Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy, suggest that L-BLP25 has an acceptable safety and tolerability profile in this patient population. These results were in accordance with previous findings in predominantly Caucasian populations (12,17). In a previous Phase Ib study of Caucasian patients with Stage IIIB or IV NSCLC, L-BLP25 was well tolerated with no unexpected safety issues. The most common side effects attributable to the vaccine were mild flu-like symptoms and mild injection site reactions (12). Follow-up of a subgroup of patients for ≥2 years showed that the good safety profile of L-BLP25 was maintained with prolonged treatment (14).

In March 2010 clinical trials of L-BLP25 were temporarily put on hold after a case of encephalitis occurred in a study of L-BLP25 for treatment of multiple myeloma. Subsequent work-up for the patient and overall safety analysis of L-BLP25 in NSCLC led to a lift of the clinical hold in June 2010. Trials of L-BLP25 in NSCLC restarted shortly afterwards. The data we present here were collected prior to, and so were not impacted by, the clinical hold.

Serum concentrations of pro-inflammatory cytokines were assessed in this study, in the expectation that according to its proposed mode of action, L-BLP25 induces an inflammatory and a T cell-driven immune response directed against the tumor. sIL-2 Rα is a cytokine receptor produced by activated T cells, while all other measured cytokines relate to inflammatory cells. All cytokines remained within the normal range for the majority of patients in this study, and these results did not indicate any safety concerns for L-BLP25.

Based on these Phase I findings, the Independent Safety Monitoring Board has recommended initiation of the Phase II Stage of this combined Phase I/II study without restrictions. In the Phase II component, 168 Japanese patients with unresectable Stage III NSCLC after primary

<table>
<thead>
<tr>
<th>(MedDRA preferred term)</th>
<th>Number of patientsa (n = 6)</th>
<th>Related to cyclophosphamide</th>
<th>Related to L-BLP25</th>
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</table>

L-BLP25, BLP25 liposome vaccine.

aSome patients experienced more than one adverse event.
bExperienced in the same patient on two separate occasions: the first event was considered to be related to cyclophosphamide and the second related to L-BLP25 treatment.
Figure 1. Serum concentrations of soluble interleukin (IL)-2 receptor alpha (sIL-2 Rα), IL-6 and IL-8 at the pretreatment evaluation visit and at week 5 of the open-label BLP25 liposome vaccine treatment period. Data not shown for serum concentrations of IL-1β (as all measurements were below the detection limit), or tumor necrosis factor alpha (as several measurements were below the detection limit). Dashed lines denote the corresponding normal ranges.
chemoradiotherapy will be randomized 2:1 to treatment with L-BLP25 plus BSC or placebo plus BSC, with once-weekly dosing for 8 weeks followed by maintenance doses every 6 weeks until disease progression or discontinuation (18). The primary objective of the Phase II stage is to compare overall survival time in the two treatment arms.

In conclusion, the first evaluation of L-BLP25 in Japanese patients with unresectable Stage III NSCLC after primary chemotherapy shows that it is well tolerated, and the safety profile is consistent with that seen in previous studies of Caucasian patients.

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Conflict of interest statement
S. Senger is employed by Merck KGaA and holds stock in Merck KGaA. N. Morsli is employed by Merck KGaA.

References