A Phase II Study of Tg4010 (Mva-Muc1-Il2) in Association with Chemotherapy in Patients with Stage III/IV Non-small Cell Lung Cancer

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Background: TG4010 is a recombinant viral vector expressing both the tumor-associated antigen MUC1 and Interleukine-2. This vector is based on the modified virus of Ankara, a significantly attenuated strain of vaccinia virus. TG4010 has been designed to induce or amplify a cellular immune response directed against tumor cells expressing MUC1.

Methods: A multicenter, randomized phase II study has explored two schedules of the combination of TG4010 with first line chemotherapy in patients with stage IIIB/IV non-small cell lung cancer. In Arm 1, TG4010 was combined upfront with cisplatin (100 mg/m^2 day 1) and vinorelbine (25 mg/m^2 day 1 and day 8). In Arm 2, patients were treated with TG4010 monotherapy until disease progression, followed by TG4010 plus the same chemotherapy as in Arm1. Response rate was evaluated according to RECIST. Median time to progression and median overall survival were calculated according to the Kaplan–Meier method.

Results: Sixty-five patients were enrolled, 44 in Arm 1 and 21 in Arm 2, in accordance with the two stage Simon design of the statistical plan. In Arm 1, partial response was observed in 13 patients out of 37 evaluable patients (29.5% of the intent to treat population, 35.1% of the evaluable patients). In Arm 2, two patients experienced stable disease for more than 6 months with TG4010 alone (up to 211 days), in the subsequent combination with chemotherapy, one complete and one partial response were observed out of 14 evaluable patients. Arm 2 did not meet the criteria for moving forward to second stage. The median time to progression was 4.8 months for Arm 1. The median overall survival was 12.7 months for Arm 1 and 14.9 for Arm 2. One year survival rate was 53% for Arm 1 and 60% for Arm 2. TG4010 was well tolerated, mild to moderate injection site reactions, flu-like symptoms, and fatigue being the most frequent adverse reactions. A MUC1-specific cellular immune response was observed in lymphocyte samples from all responding patients evaluable for immunology.

Conclusions: The combination of TG4010 with standard chemotherapy in advanced non-small cell lung cancer is feasible and shows encouraging results. A randomized study evaluating the addition of TG4010 to first line chemotherapy in this population is in progress.

Key Words: Phase II study, Recombinant viral vector, Chemotherapy, Non-small cell lung cancer, Vaccine, MUC1, MVA.

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Lung cancer is the leading cause of cancer related mortality in both men and women. The majority of new cases of lung cancer are non-small cell lung cancer (NSCLC). About 60% of cases are diagnosed as unresectable or advanced. In patients with unresectable localized NSCLC chemotherapy combined with radiation is a validated option. For patients with advanced or metastatic NSCLC, the usual treatment option is palliative chemotherapy which has demonstrated a positive impact on survival but, clearly, the treatment of these patients remains unsatisfactory, and more effective therapies needs to be developed.[1] Targeted therapies and immunotherapy are innovative approaches with the potential of improving the treatment of patients with advanced NSCLC. Most advanced targeted therapies based on either small inhibitory molecules or monoclonal antibodies interfere with tumor growth, angiogenesis, or both.[2] Immunotherapy is based mainly on therapeutic vaccinations designed to induce or amplify an immune response directed against the population of tumor cells.[3] A potential target for immunotherapy of lung cancer is the tumor-associated antigen, MUC1.[4] The MUC1 protein is a highly glycosylated mucin (MW >200 kD), normally found at the apical surface of mucin-secreting epithelial cells in many types of tissue, including the breast, prostate, lungs, pancreas, stomach, ovaries, fallopian tubes, intestine, and kidney[5,6] Cancer in secretory epithelial cells is

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often accompanied by excess expression of MUC1 by the tumor cells [7–9] and an aberrant glycosylation, revealing new peptide and carbohydrate epitopes.[10] TG4010 is a specific immunotherapy product targeting the tumor associated MUC1 antigen. It consists of a viral suspension of a modified virus of Ankara (MVA) containing the sequences coding for the human MUC1 protein and for the human interleukin-2 (IL2). The MVA is a vaccinia virus which belongs to the poxvirus family. This strain is significantly attenuated and was specifically developed to immunize patients at high risk for complication of vaccination against smallpox with classic strains.[11,12] TG4010 was developed for use in cancer patients whose tumors express the MUC1 antigen.[13] Two phase I dose escalation bridging studies have been completed with TG4010. The dose of 10^8 plaque forming units (pfu) per injection was considered a maximal feasible dose, regarding the volume and concentration, but not the maximal tolerated dose. Three patients had a metastatic NSCLC and in one of them multiple metastases shrank with a 2 months delay after the last vaccination.[14] The safety profile and the early signs of clinical and biologic activity served as a basis for a phase II program of TG4010 in several indications. The purpose of the present study was to assess whether the clinical activity of TG4010 in patients with NSCLC is sufficient to justify further clinical development, by evaluating the effects of vaccination (TG4010) in combination with chemotherapy.

PATIENTS AND METHODS

Study Population

Patients were enrolled according to the following criteria: histologically confirmed non-small cell carcinoma of the lung with documentation of MUC1 antigen expression on the primary tumor or on metastasis by a positive staining with the H23 monoclonal antibody[15] stage IIIB or IV NSCLC, with no prior treatment for advanced disease; at least one measurable lesion according to RECIST; ECOG Performance status 0 or 1; age more than 18; adequate hematological, hepatic, and renal function; written informed consent from the patient. Patients presenting one of the following criteria were excluded from the study: history of any form of systemic therapy for non-small cell carcinoma of the lung except for neoadjuvant treatment; uncontrolled brain metastases, prior history of other malignancy except for nonmelanoma skin cancer or other cured cancer for more than 5 years; any other serious concomitant systemic medical disorder incompatible with participation in the study; previous (within 4 weeks before baseline) or concomitant systemic steroids, immunosuppressive/immunomodulating drugs; positive serology for HIV, hepatitis B or C virus; pregnancy at the entry or breast-feeding women, patient without adequate protection against pregnancy during the conduct of the trial and for 3 months after the last injection; allergy to eggs; patients unable or unwilling to comply with protocol requirements.

Study Design

This was a randomized, open label, multicenter study testing 10^8 pfu (plaque-forming units) of TG4010 either in immediate combination with cytotoxic chemotherapy (vinorelbine-cisplatin) (Arm 1) or in monotherapy followed by combination with the same chemotherapy at progression (Arm 2).

Tumor response according to RECIST[16] was the primary end point of the study and was evaluated every 6 weeks. The tumor response taken into account was, for each patient, the best overall response obtained during the study and assessed by central reading. Stabilizations had to be maintained at least 12 weeks. Secondary endpoints were: safety of the product alone and in combination with chemotherapy, time to progression, survival, and cellular immune response against MUC1 ($CD4^+$ or $CD8^+$). Upon satisfaction of all of the inclusion and exclusion criteria, including obtaining written, informed consent, patients had baseline status completed, and were randomized into one of the two treatment Arms. The intent to treat (ITT) population was defined in the statistical analysis plan as the population of patients who were randomized and received at least one administration of the test product TG4010. The study was conducted in accordance with the ethical principles in the Declaration of Helsinki.

Treatment

In Arm 1, patients received up to 6 cycles, of 3 weeks each, of vinorelbine (25 mg/m^2 day 1–day 8)/cisplatin (100 mg/m^2 day 1) in addition to subcutaneous (SC) injections of TG4010 at the dose of 10^8 pfu, once every week for 6 weeks then once every 3 weeks, TG4010 was administered the same day as chemotherapy. Patients with complete response, partial response (PR), or stable disease after chemotherapy were treated until documentation of progressive disease (PD) with TG4010 as a maintenance therapy.

In Arm 2, patients received TG4010 alone, SC injections at the dose of 10^8 pfu, once every week for 6 weeks then once every 3 weeks, until documentation of PD. In case of progression, they were treated with chemotherapy in addition to TG4010, at the same regimen than in Arm 1, i.e., with up to 6 cycles, of 3 weeks each, of vinorelbine/cisplatin. After chemotherapy, if appropriate, they received TG4010 alone, once every 3 weeks, until disease progression. To avoid a possible interaction with the immune system, steroids, and other immunosuppressive drugs were not allowed during study. According to standard practice of each investigational center, chemotherapy was delayed by 1 week each time the investigator assessed that recovery of adverse events (AE) from previous cycle did not allow the administration of a new cycle. In this case, all assessments planned on that day and all following assessments were to be delayed by 1 week, including the TG4010 injections. TG4010 was administered by SC injection in a single injection, the volume of which depending on the specifications of each lot was usually 0.3 or 0.4 ml. Both before and after the study drug administration, the skin at the site of injection was to be disinfected with alcohol. Four injection sites were to be used: left and right arm, left and right thigh, according to a rotation schedule. Patients were to be monitored for one half hour after each study drug injection. Regarding the Genetic Modified Organism use (manipulation and patient administration) national regulatory

requirements of each country in which the study was conducted were to be applied.

Statistical Plan

To minimize the sample size, a "minimax 2 stage design" for phase II was used.[17] The study design required 36 evaluable patients for the first stage of the study (18 in each Arm), and 15 additional evaluable patients for each arm moving to the second stage. It was foreseen to replace patients not evaluable for the response. The purpose of the study was to reject the experimental treatment from further study if it was considered truly ineffective, and to accept it for further study if it was truly effective. The study design was based on the following assumptions: the ineffectiveness cutoff was chosen to be equal to 20% and the effectiveness cutoff equal to 40%. Hence the hypotheses of interest were H0: $r < 20\%$ against HA: $r > 40\%$, where r is the response rate (complete response plus PR according to RECIST), the type I error rate (α, probability of accepting an ineffective treatment, a false positive outcome) was set to 5%, the type II error rate (β, probability of rejecting an effective treatment, a false negative outcome) was set to 20%. Under these assumptions, an optimal design consisted of the following 2 stages: 18 evaluable patients per arm were to be obtained, if fewer than 5 responses were observed in a treatment arm it was stopped and the drug regimen of that arm declared ineffective, otherwise, patient enrollment continued to obtain 33 evaluable patients for this arm. If at least 11 responses were observed, the drug regimen of that arm was declared effective. Time to progression, duration of response and survival time were to be estimated by the Kaplan–Meier methods and plotted as curves.

Immune Response Analyses

Forty milliliters of blood were drawn into CPT tubes (Cell Preparation Tubes with Sodium Heparin, Beckton Dickinson) from patients at baseline, before the first injection of either TG4010 or chemotherapy; at day 43 (1 week after the sixth TG4010 injection and before the seventh injection) and on day 64 (3 weeks after the seventh injection). Blood was transported within 48 hours to a central laboratory (Claude Levy Laboratories, Paris) for Hypaque-ficol separation and peripheral blood mononuclear cells (PBMC) were frozen and stored in liquid nitrogen until used for immunology testing. Technical difficulties at the cell freezing laboratory resulted in loss of PBMC from half of the patients and therefore only 31 patients were evaluable for immune response.

MUC1-specific $CD4^+$ (T helper) cell responses were assessed by T cell proliferation as described previously.[18] Briefly, PBMC were seeded into 96 well, flat bottom plates in triplicate, at 10^5 cells per well, in AIM V culture medium (Life Technologies, Grand Island, New York) alone or in the presence of test peptide (5 μg/ml). Synthetic peptides used (NeoMPS, Strasbourg, France) corresponded to 23 amino acids each, from the human MUC1 sequence, overlapping by 5 amino acids. Peptides covering the sequence of the MUC1 variable number tandem repeat (VNTR) and the region from the VNTR to the extracellular N-terminus of the MUC1 sequence were used. Positive control antigens include Ultraviolet light inactivated MVA (10^5 or 10^6 pfu) viral particles (Transgene, Strasbourg, France), tetanus toxoid (Staten Serum Institute, Copenhagen) 20 μg/ml and IL-2 (1000 IU/ml). A 24 amino acid peptide corresponding to the murine MUC1 VNTR was used as a negative control. After 4 days of culture, ^{3}H-thymidine (1 μCi/well) was added and cells were harvested onto glass fiber filter paper and counted by liquid scintillation. Results are expressed as stimulation index, which is calculated as: Experimental counts per minute (cpm) (with peptide or antigen)/control cpm (medium only). A stimulation index of 2 or more is considered to be a positive response.

The MUC1-specific $CD8^+$ (Cytotoxic T Lymphocyte) cellular response was assessed by ELISpot assay as previously described.[19] Briefly, 5×10^5 PBMC were cultured for 6 days with 20 μg/ml short MUC1 peptides (below) or positive control viral peptides, in RPMI medium supplemented with 10% fetal bovine serum. IL-2 (50 IU/ml was added after 2 days of culture. After 6 days of culture, cells were collected and assessed for interferon gamma secreting cells by ELISpot using the kit from Diaclone (Besançon, France) and according to the manufacturer's instructions. Briefly, PBMC were harvested from the sensitization cultures and plated at 10^5 cells per well, in triplicate, in the ELISpot plates and cultured with MUC1 short peptides for 16 hours then assessed for ELISpots using an automatic ELISpot counter (Zeiss, Le Pecq, France). HLA binding MUC1 peptides were identified by a cell binding assay as described previously.[20,21] HLA-A2 represents over 40% of the Caucasian population and four 9 amino acid sequences from MUC1 which bind to HLA-A2 have been published. An HLA-binding analysis of 9 amino acid peptides was undertaken and peptides were found, which bind with moderate to high affinity to the most common HLA types (HLA-A1, A2, A3, A11, A24, B7, and B8). Fourteen such peptides, in addition to the 4 published sequences were used to test CTL activity, by ELISpot in PBMC from patients in this study (Table 1). Low stringency criteria were used to declare an ELISpot response positive: A response was said to be positive if there were at least 5 spots per 10^5 PBMC and if the value was at least 1.5 times the background ELISpot.

Some peptides have been described elsewhere (LLLLTVLTV and STAPPVHNV;[22] ALGSTAPPV, TLAPATEPA and FLSFHISNL;[20] NLTISDVSV.[23]

RESULTS

Study Population

Between May 2002 and February 2004 65 patients were recruited, randomized, and treated in 9 centers in Belgium, France, Poland, and Switzerland. In Arm 1, immediate combination of chemotherapy among the first 18 evaluable patients, 7 achieved an objective response, consequently the accrual was continued in the second stage of the Simon plan to obtain 33 evaluable patients. Forty-four patients entered this arm of the study and 37 patients were evaluable for response. Arm 2, TG4010 followed by combination therapy upon progression, did not meet the criteria for moving to the second stage of the Simon plan and therefore the recruitment was stopped after 21 inclusions.

TABLE 1. Sequences of the Peptides Used for Monitoring the Cellular Immune Response According to Patient HLA Haplotype

MUC1 peptide epitope sequences			
HLA Haplotype	**MUC1 Sequence**	**HLA Haplotype**	**MUC1 Sequence**
HLA-A1	SLEDPSTDYY DVETQFNQY ISEMFLQIY	HLA-A3	ALAVCQCRR
		HLA-A11	GVTSAPDTR GVTSAPDNR
HLA-A2	ALGSTAPPV SLSYTNPAV TLAPATEPA FLSFHISNL STAPPVHNV LLLLTVLTV NLTISDVSV	HLA-A24	YYQELQRDIS
		HLA-B7	APGSTAPPA
		HLA-B8	EAASRYNLT NIKFRPGSV QCRRKNYGQL

Control Viral CD8+ epitope sequences		
HLA Haplotype	**Virus**	**Peptide Sequence**
HLA A1	**CMV**	YSEHPTFTSQY
	Influenza	VSDGGPNLY
HLA A2	**CMV**	NLVPMVATVQ
	Influenza	GILGFVFTL
HLA A3	**CMV**	TTVYPPSSTAK
	EBV	RLRAEAQVK
HLA B7	**CMV**	TPRVTGGGAM
	EBV EBNA-3A	RPPIFIRRL
	EBV EBNA-2	VPDQSMHPL

Clinical Characteristics

Forty-five men (69.2%) and 20 women (30.8%) participated in the study. All patients were of Caucasian ethnicity, the mean age at study entry was 59. Forty-nine (75.4%) had a performance status classified ECOG-1 and 16 (24.6%) a performance status ECOG-0. At baseline the majority of patients, 49 patients (75.4%) were metastatic (TNM stage IV), the other 16 (24.6%) patients were TNM stage IIIB (locally advanced). Twenty-two (33.8%) patients underwent a surgical resection of their lung cancer before entering the trial, 8 (12.3%) had a radiotherapy and 2 had a neoadjuvant chemotherapy. The most frequent histologic subtype of NSCLC was adenocarcinoma found in 31 (47.7%) patients followed by squamous cell carcinoma in 14 (21.5%) and other subtypes in 20 (30.8%) patients. In the majority of the patients, 49 (75.4%), the immunohistochemistry for the MUC1 antigen has been carried out on a primary tumor sample, for the other patients this analysis has been done on a biopsy sample from a metastasis. The staining intensity was described as strong 3/3, in a majority of the patients 52 (80%), less intense 2/3 in 12 patients (18.5%), and faint 1/3 in one patient. A membrane staining was present in 42 patients (64.6%) and a cytoplasmic staining in 53 patients (81.5%)(Table 2).

Efficacy

In Arm 1, among the first 33 patients evaluable for response 13 achieved a PR, therefore the primary end point of 11 responding patients out of 33 defined in the statistical plan was achieved and the product considered worth of further development. The ITT response rate after central reading was 13/44 (29.5%) whereas the response rate of the evaluable patients was 13/37 (35.1%). Twelve other patients, 27.3% of

TABLE 2. Patients Characteristics

	Arm 1 TG4010 + Chemotherapy Immediately ***n* = 44**	**Arm 2 TG4010 in Monotherapy Before Combination with Chemo** ***n* = 21**	**Whole Study Population** ***n* = 65**
Age, yr			
Median	58.5	61	59
Range	33–76	37–77	33–77
Performance status (ECOG)			
0	10 (22.7)	6 (28.6)	16 (24.6)
1	34 (77.3)	15 (71.4)	49 (75.4)
Gender			
Male	31 (70.5)	14 (66.7)	45 (69.2)
Female	13 (29.5)	7 (33.3)	20 (30.8)
Stage			
IIIB	11 (25.0)	5 (23.8)	16 (24.6)
IV	33 (75.0)	16 (76.2)	49 (75.4)
Histological sub-type			
Adenocarcinoma	20 (45.0)	11 (52.4)	31 (47.7)
Squamous cell carcinoma	8 (18.2)	6 (28.6)	14 (21.5)
Other	16 (36.4)	4 (19.0)	20 (30.8)
MUC1 antigen staining			
Membrane staining	32 (72.7)	10 (47.6)	42 (64.6)
Cytoplasmic staining	33 (75%)	20 (95.2)	53 (81.5)
Staining intensity			
Faint (1/3)	0 (0)	1 (4.8)	1 (1.5)
Less intense (2/3)	9 (20.5)	3 (14.3)	12 (18.5)
Strong (3/3)	35 (79.5)	17 (81.0)	52 (80.0)

TABLE 3. Clinical and Immunological Responses by Patient

					ELISpot						
					Baseline		Day 43		Day 64		ELISpot Peptides
Patient	Stage at BL	HLA-A	HLA-B	Clin Resp	(+ve)	MUC1	(+ve)	MUC1	(+ve)	MUC1	Recognized
					A. Arm 1						
205/001	IIIB	01, 29	08	PR							
210/002	IV	02, 24	44, 62	PR							
210/009	IV	32, 66	07, 41	PR	−	−	+	+	−	−	B7
400/002	IV	01, 02	08, 39	PR	+	+	+	−			A2
400/005	IIIB	01, 02	08, 27	PR	+	−			−	−	
401/005	IIIB	03, 32	07, 18	PR	+	−				+	B7
401/006	IV	02, 03	44, 62	PR	+	+	+	+	+	−	A2
401/010	IV	01, 31	35, 58	PR	+	+	−	+	−	+	A1
401/011	IV	02	07, 44	PR							
402/004	IIIB	11, 25	07, 18	PR	+	+		−	+	−	A11
501/002	IV	02, 28	07, 35	PR							
501/003	IV	01	38, 51	PR							
205/003	IV	29	44, 61	SD							
210/007	IIIB	02, 03	27, 44	SD	−	−	+	−		−	
401/003	IV	01, 25	08, 18	SD	−	+	−	−			A1
401/004	IV	02, 30	44, 62	SD	+	+			−	−	A2
401/009	IV	11, 24	13, 61	SD	−	−			−	−	
402/002	IIIB	03, 33	38, 58	SD	−	−	+	−			
402/007	IIIB	02, 28	18, 44	SD	−	+	+	+		−	A2
402/009	IV	03, 33	07, 14	SD	+	−	−	−	−	−	
501/005	IV	02	17	SD					+	+	A2
501/006	IV	02, 24	18	SD	−	−			+	−	
602/002	IV	02, 03	35, 60	SD							
603/001	IV	24, 30	13, 57	SD		+		−			A24
205/006	IV	03, 29	07, 45	PD							
209/002	IV			PD							
209/009	IV	03, 28	40, 51	PD							
210/003	IV	02, 32	38, 63	PD							
210/008	IIIB	03	07, 14	PD	+	−	+	−			
400/003	IV	02	51, 57	PD	+	−					
400/004	IV	25, 26	18, 41	PD							
401/007	IV	02	44	PD							
402/008	IIIB	03, 11	07, 35	PD	−	−			−	−	
402/001	IIIB	02, 74	50, 58	PD	−	−	−	+	−	−	A2
402/005	IV	01, 03	51, 52	PD	−	−	−	−	−	−	
402/006	IV	03, 33	14, 44	PD							
402/004	IV	09, 24	37	PD							
401/001	IV	01, 25	18, 57	NE							
402/003	IIB	02	51, 62	NE							
402/008	IV	26, 30	18, 52	NE							
602/008	IV	03, 19	14, 51	NE							
602/005	IV	03	07, 18	NE							
603/002	IV	01, 28	08, 21	NE							
					B. Arm 2						
501/001	IV	01, 26	38, 51	CR							
602/001	IIIB	11, 26	38, 44	PR							
205/002	IIIb	01	08, 57	SD							
205/004	IIIb	01, 32	08, 49	SD	+	−		+	−	+	A1/B8
209/003	IV	02, 03	07, 14	SD							
400/001	IV	02, 24	07, 44	SD	+	−			−	−	

(*Continued*)

TABLE 3. (*Continued*)

Patient	Stage at BL	HLA-A	HLA-B	Clin Resp	ELISpot Baseline (+ve)	ELISpot Baseline MUC1	ELISpot Day 43 (+ve)	ELISpot Day 43 MUC1	ELISpot Day 64 (+ve)	ELISpot Day 64 MUC1	ELISpot Peptides Recognized
602/006	IV	19, 33	05, 51	SD	−	−	−	−	−	−	
205/005	IIIb	01, 31	08, 65	PD	−	+	−	+	+	−-	A1/B8
209/005	IV	10, 29	40, 44	PD							
209/007	IIIb	02	44, 37	PD	+		−	−	−	−	
209/008	IV	02, 24	07, 18	PD	−	−	−	−			
210/005	IV	01, 02	08, 18	PD							
602/003	IV	01, 01	08, 58	PD							
602/007	IV	02, 32	07, 18	PD	+	+	−	+			A2/B7
602/009	IV	01	17	PD	−	−	−	−	−	−	
209/001	IV	10, 29	18, 35	NE							
209/004	IV	01, 02	08, 16	NE							
209/006	IV	24, 31	27, 44	NE							
210/001	IV	02, 24	07, 35	NE							
210/004	IV	24	18, 57	NE							
401/002	IV	25, 30	18, 51	NE							

For each patient is given the stage of the NSCLC at baseline (BL), their HLA A and B groups.

The clinical response (CR indicates complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, non evaluable for response), the positivity (+) or negativity (−) against positive control and against MUC1 at 3 time-points (baseline, D43 and D85), in case of positivity the recognized ELISpot peptides are indicated.

the ITT population, had their disease stabilized for 12 weeks or more. Thus, 25 patients (56.8% of the ITT population and 67.6% of the evaluable patients) had either a response or a stabilization of the disease. Seven patients of this arm were not evaluable for response: 1 patient was in PR and 3 patients were stable at D43 (first evaluation) but had no confirmation at D85; 3 patients had an early progression before D43. In Arm 2 no objective response was observed with the vaccine alone but 2 patients remained stable more than 6 months. Fourteen patients in this arm received the combination therapy and were considered evaluable for response, 2 of them achieved a response, one complete and one partial (Table 3).

Mean duration of response was 4.3 months in Arm 1. The complete and PR observed in Arm 2 lasted respectively 4.2 and 3 months.

Nineteen patients, 17 in Arm 1 and 2 in Arm 2, stable or responding after 6 cycles of chemotherapy continued to be administered with TG4010 as a maintenance therapy for a mean duration of 3 months (range, 1–13.5).

Median time to progression (ITT) was 4.8 months in Arm1. In Arm 2, the determination of the time to progression was limited by the smaller number of patients but was estimated to be 1.4 months for the monotherapy phase with TG4010 and 7.2 months when taking into account the combination phase of TG4010 with chemotherapy (Figure 1). Median overall survival (ITT) was 12.7 months in Arm 1, 14.9 months in Arm 2, and 13.7 months for the whole study population (Figure 2). The 1 year survival rate was 53% in Arm 1, 60% in Arm 2, and 57% for the whole study population.

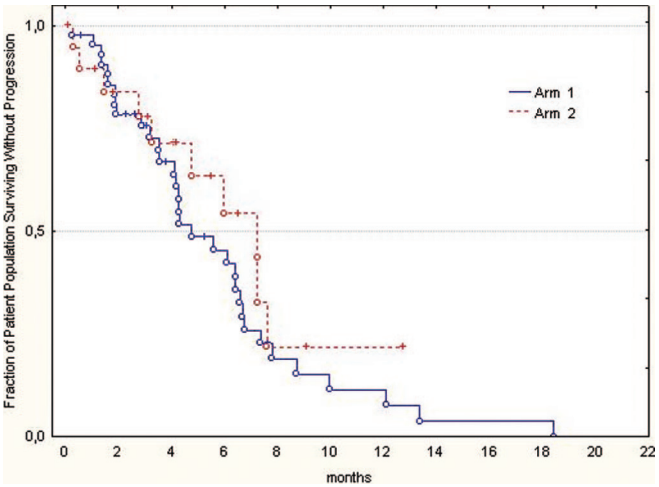

FIGURE 1. Time to Progression for the whole study period (ITT).

Safety

The safety population of the study consisted of 65 patients who received at least one injection of the study drug TG4010. The median number of injections was 8 in Arm 1 and 9 in Arm 2 corresponding respectively to a median duration of exposure to the study drug of 3 and 3.3 months. The median number of chemotherapy cycles was 4 in Arm 1 and 3 in Arm 2. Sixty-three (96.9%) out of the 65 patients of the safety population experienced at least one AE of which 48 (73.8%) had at least one severe adverse AE. Most frequent AEs in this study were anemia (34 patients, 52.3%), nausea (33 patients, 50.8%), vomiting (32 patients, 49.2%), fatigue (30 patients, 46.2%), and neutropenia (21 patients, 32.3%). Thirty 2 (49.2%) patients presented with a possibly or probably study drug related AE. One hundred and eight AE related to TG4010 were recorded in 32 patients, 106 of them

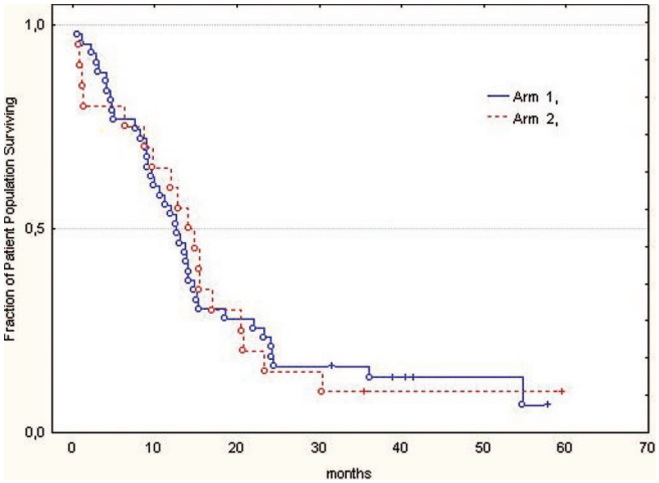

FIGURE 2. Overall Survival (ITT).

TABLE 4. Number of Patients with Adverse Events Related to TG4010 According to the Investigators and Reported in More Than 5% of the Patients

AEs	N	% (N/65)
Fatigue	9	13.8
Injection site reaction NOS	9	13.8
Injection site pain	6	9.2
Pyrexia	6	9.2
Anorexia	5	7.7
Erythema	4	6.2

were of grade 1 or 2. Most frequent related AEs were fatigue (in 9 patients, 13.8%), injection site reactions (in 9 patients, 13.8%), injection site pain (in 6 patients, 9.2%), and pyrexia (in 6 patients, 9.2%) (Table 4). Other flu-like symptoms such as arthralgia and myalgia are reported also but less frequently. Two AEs were classified severe (grade 3/4) and possibly/probably related, respectively one anemia and one papular rash. Fifty serious adverse event, none of them related to TG4010, were recorded in 33 (50.8%) patients and 23 (35.4%) patients were withdrawn from the study due to AEs. Fifteen patients died during their participation in the study, 11 from disease progression and 4 from other causes. No death, according to the investigators, was related to the study drug (Table 4).

Immunology

Blood samples from 31 patients were evaluable for immune response. Cellular immune responses in PBMC were measured by T cell proliferation (a measure of specific $CD4^+$ T cell response) and by ELISpot (a measure of specific $CD8^+$ T cell response) as described. The $CD8^+$ T cell population contains the MHC class I-restricted cytotoxic T cells which are considered to be necessary for the immune elimination of cancer cells. Positive T cell proliferation responses (Stimulation Indices $\geq$2.0) of PBMC cultured with MUC1 peptides ranged from 2 to 18.4 (median), with the magnitude of response increasing with treatment in 4 patients (all in Arm 1). There were 10 MUC1-specific T cell proliferative responses at baseline and 8 at either day 43 or day 64. MUC1-specific recognition by the $CD8^+$ T cells was more informative. Because an in vitro stimulation was required to reveal ELISpot responses to MUC1, the ELISpot data must be considered qualitative only (+ or −) indicating the presence or absence of MUC1-specific $CD8^+$ T cells in the PBMC samples. There were equal number of ELISpot positive tests in the prevaccination PBMC samples (10/31) as there were from day 43 or day 64 (10/31) (Table 3). This shows that some patients had an existing CTL response to MUC1 before immunotherapy with TG4010. ELISpot responses, like the T cell proliferative responses, were weak and transient in a majority of patients. PBMC from roughly half of the patients in each Arm showed evidence of MUC1-specific ELISpot reactivity (12/23 in Arm 1 and 3/8 in Arm 2). The magnitudes of the responses were not significantly different between study Arms (data not shown). Induced ELISpot responses (i.e., responses detected in the postbaseline samples but not in the baseline samples) were detected in 4 of 12 ELISpot responding patients in Arm1 and in 1 of 3 ELISpot responding patients in Arm 2. This suggests that concurrent chemotherapy had little if any impact on the generation of the cellular immune response. In the ELISpot evaluable group of patients, 12/21 patients with disease control (PR or stable disease) had MUC1-specific ELISpot responses at any time-point, whereas only 3/10 patients with PD had MUC1-specific ELISpot during the study. At least in Arm 1 there was an association between MUC1 ELISpot at baseline and disease control. Regarding patients who developed a MUC1 specific ELISpot response during the study 4 out of 5 enjoyed a disease control (Table 3).

In Figure 3, it can be seen that patients who had detectable MUC1-specific ELISpot response (at any time-point), had significantly longer time to progression (Figure 3*A*) and overall survival (Figure 3*B*).

These data suggest that a $CD8^+$ T cell response to MUC1 is associated with a better outcome in NSCLC patients in first line chemotherapy.

In previous clinical studies with either Vaccinia Virus-MUC1-IL2[2425] and with MVA-MUC1-IL2 (TG4010)[14] patient sera were tested at various timepoints for antibody responses to the vector and to MUC1. Although antibody responses to the vector were observed, no antibody responses to MUC1 was seen. For this reason, antibody responses to MUC1 were not assessed in this study.

DISCUSSION

Based on phase I observations with the product TG4010 and on the fact that 60 to 70% of NSCLC express the MUC1 antigen this phase II study was aimed to assess the efficacy of TG4010 in this indication. Advanced stages have been chosen because they represent the great majority of the NSCLC suffering patients and because the medical need in this setting is very high. Chemotherapy brings to these patients a clear but limited survival benefit and remains the standard of care.[1] The purpose of this study was to investigate whether specific immunotherapy against a tumor associated

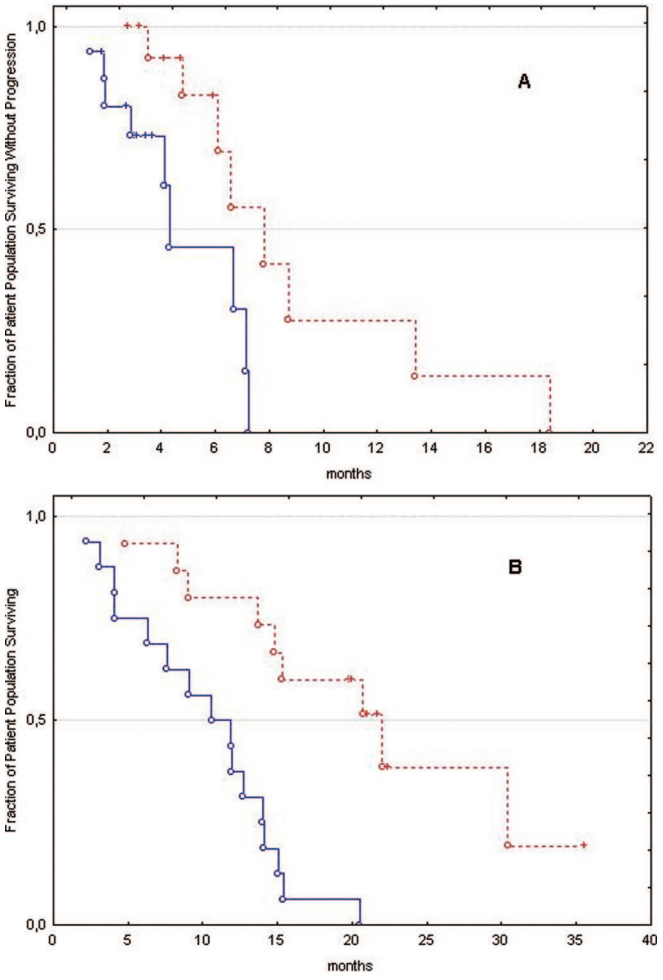

FIGURE 3. TTP and OS according to cellular immune response against MUC1 for the whole study population. Patients with (--------) or without (——) MUC1-specific ELISpot at any timepoint. *A*: Time to progression. *B*: Overall survival. o = complete data, + = censored. Differences between the 2 populations are statistically significant with A: $p = 0.025$ and B: $p = 0.001$.

antigen, in this case with TG4010 targeting MUC1, improves the results obtained with chemotherapy. The study was exploratory, with no intended comparison of the 2 Arms, but rather aimed to investigate 2 ways of combining TG4010 with chemotherapy, either immediately or after an initial monotherapy with TG4010. The statistical plan was optimized in that the number of patients to be included was limited in the case that a study arm would not achieve a sufficient level of efficacy.

The primary end point was chosen to be the response rate which is usual for the phase II evaluation of a new drug in oncology but it is clear that an immunotherapy product may induce biologic changes affecting the course of the disease in ways having an impact on other parameters than the objective response such as the disease control, the time to progression and survival. In this view it was also of importance to measure the immune response against MUC1 as a marker of biologic activity of TG4010. Although it is a serious limitation of the study that only half of the patients could be assessed for immune response, it is clear from the data in Figure 3, that patients who have a $CD8^+$ T cell response to MUC1 at some point during the study, were part of the group of patients with better time to progression and/or better survival. Interestingly also, MUC1 specific ELISpot in blood samples taken before therapy was associated with a better response rate (Table 3), as well as time to progression and survival (data not shown). However based on the available data it cannot be stated that TG4010 is the reason of this observation.

The fact that 49/65 (75.4%) patients were classified stage IV and PS1 rules out the possibility of an obvious positive selection bias. The distribution of the patients according to the histologic subgroups of NSCLC was also classic, therefore the study population is considered representative of the studied pathology and disease stage.

MUC1 expression on NSCLC is associated with a poorer prognosis due to several properties conferred by MUC1 to the tumor cells: increased capability to metastasize, reduced sensitivity to chemotherapy and specially to cisplatine.[26,27] The fact that the study population was selected for having MUC1 expressing tumors can therefore be interpreted as a possible negative selection bias. The safety profile of TG4010 with injection site reactions, flu-like symptoms and fatigue as main related adverse effects is in accordance with what is anticipated to be observed with a live attenuated viral vaccine and with the known side effects of vaccination with MVA.[17] The combination of AE from TG4010, the chemotherapy and the disease can make difficult the assessment of the relationship to the study drug for some symptoms such as fatigue. A more precise evaluation of the safety of TG4010 will be provided by a larger on-going randomized study allowing the comparison of 2 populations with the same disease and chemotherapy but differing for the administration or not of TG4010. The fact that the great majority of the AE related to TG4010 were of mild or moderate intensity allows the use of this product in patients with an advanced disease treated with chemotherapy. In no patient did the combination of TG4010 and chemotherapy need be stopped due to interactions between the 2 products, demonstrating the feasibility of this combination. The fact that the MVA is nonreplicative explains why even in the case of patients with chemotherapy induced neutropenia the injection of the vaccine did not induce exacerbated injection site manifestations.

The response rate observed in the immediate combination, 14 out of the first 33 evaluable patients, is higher than the 11/33 responding patients which was the statistical threshold to be achieved for considering TG4010 to be further developed in this indication. The response rate established after central reading was more stringent as the same evaluation at the investigator level. In Arm 2, monotherapy with TG4010 followed by combination with chemotherapy, the response rate in combination, 2 out of 14 evaluable, was not sufficient for moving to the second stage of the statistical plan.

In the case of targeted therapies and immunotherapy other endpoints than response rate may be of more impor-

tance as these new approaches are considered to induce stabilizations or reduction in the progression rate more frequently than tumor shrinkage.[28] Overall survival measured at, respectively, 12.7 and 14.9 months in Arms 1 and 2 compares favorably to those which have been reported in the literature with the same chemotherapy regimen used alone.[29,30–31] Even if observed in a smaller cohort of patients, the median TTP and OS in Arm 2 are consistent with the results obtained in Arm 1. These results, in addition, have been obtained in a population with MUC1 expressing tumors believed to be of worse prognosis as compared with unselected advanced NSCLC. Nineteen patients stable or responding after 6 cycles of chemotherapy continued to be administered with TG4010 as a maintenance therapy and up to 16 months of administration. These observations support an administration of TG4010 beyond chemotherapy as a maintenance therapy. Another MUC1 directed immunotherapy, Stimuvax, after encouraging phase IIb results is under clinical development as a maintenance therapy in NSCLC patients with a Stage IIIA disease stable or in response after chemotherapy-radiotherapy.[32]

The overall hypothesis raised by this study is that TG4010 may improve the outcome of advanced NSCLC patients with a MUC1 positive tumor treated with chemotherapy. Other products have been or are being developed with the aim of improving the results of first line chemotherapy in NSCLC. Small inhibitory molecules like gefitinib (Iressa) or erlotinib (Tarceva) have failed to demonstrate an improvement in this setting[33,34] while a monoclonal antibody, Bevacizumab (Avastin) has demonstrated a survival advantage despite some severe adverse events like bleeding or thrombo-embolism.[35] To demonstrate that TG4010 improves the outcome of patients with advanced MUC1 positive NSCLC receiving first line chemotherapy a further study has been initiated evaluating the outcome of this population of patients receiving a first line chemotherapy alone or in combination with TG4010. This study should provide information on the benefit and risks of combining TG4010 as a specific cancer directed immunotherapy with first line chemotherapy in this indication.

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