

III RESULTS

Tumor evolution and safety of vaccinated patients

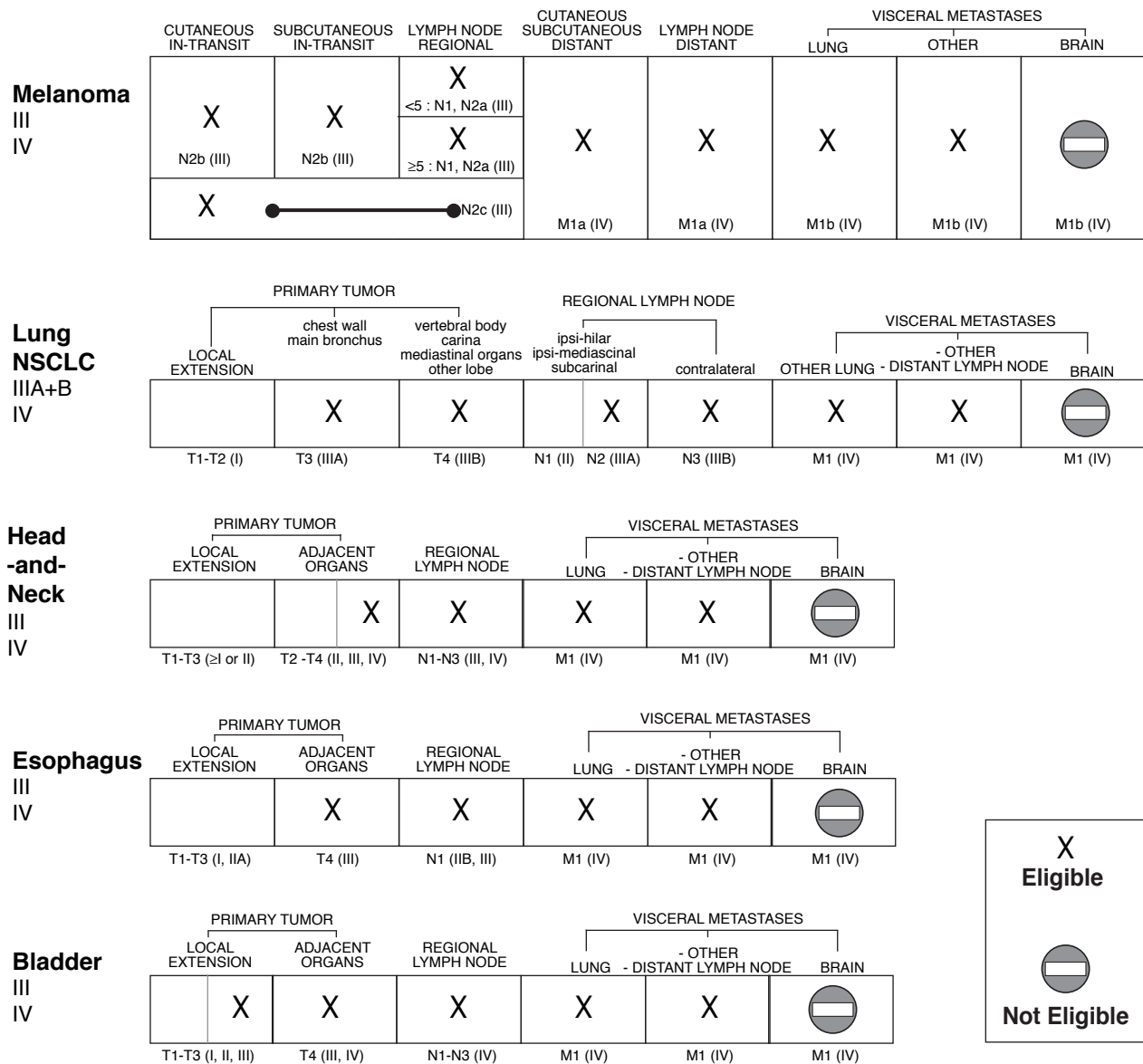
Our clinical research program focuses on therapeutic vaccinations with MAGE antigens. In October 1993, we started immunizing cancer patients with the first tumor antigen recognized by T lymphocytes that we had identified on a human tumor, i.e. MAGE-1.A1 (110). Our strategy was to immunize 6 patients with a given antigen and a given vaccination schedule. If no tumor response or CTL response was observed, another study was initiated, with another antigen or another vaccination schedule. In case we would observe either a tumor response or a CTL response or both among the 6 vaccinated patients, the number of patients in that study protocol would be increased, in order to confirm the clinical efficacy and/or the immunogenicity of the vaccine.

Since neither tumor response nor CTL response were observed in the 6 first patients immunized with the MAGE-1.A1 peptide, we started to immunize patients in March 1994 with another *MAGE*-encoded antigen that had just been identified, i.e. MAGE-3.A1 (*Section III. 1.1*) (132). Out of the first 6 melanoma patients that were immunized with this antigen, 2 displayed objective tumor responses and one additional patient had a MxR (220). The study protocol was then extended. Four out of the 25 melanoma patients who completed the study displayed objective tumor responses and 3 additional patients had MxR (*Section III. 1.1.1*) (197). Although no CTL response was detected with the methods available at that time, even in the blood of patients who displayed a complete tumor response to treatment, the MAGE-3.A1 peptide became our “reference antigen” for the design of new clinical trials, because some anti-tumor efficacy had been observed in a significant minority of patients. In an attempt to increase the anti-tumor efficacy of the MAGE-3.A1 peptide and to develop more sensitive methods to monitor the CTL response, we successively designed several small phase I/II trials, where the MAGE-3.A1 peptide was either mixed with an immunological adjuvant (*Section III. 1.1.2*), injected with another peptide (*Section III. 1.1.3*), injected according to a different vaccination schedule (*Section III. 1.1.4*), or integrated to a recombinant viral vector. Several other *MAGE*-encoded peptides, (*Section III. 1.2*) and a recombinant MAGE-3 protein (*Section III. 1.3*) have been tested as well and 3 of these trials are presently ongoing (*Annex 1*). Most of the patients that have been included in our 15 phase I/II immunization trials had advanced metastatic melanoma. A complete list of these trials is given in *Annex 1*.

We will focus here on the clinical results, i.e., tumor evolution and safety observed with *MAGE*-encoded peptides-based vaccines and with a recombinant MAGE-3 protein. These antigens were given with or without immunological adjuvants to melanoma patients, most of them having metastases detected at study entry. Because the MAGE-3.A1 peptide is our reference antigen, the results obtained with this peptide will be presented first (*Section III. 1.1*), followed by those obtained by other MAGE peptides (*Section III. 1.2*) and finally, those obtained with the recombinant MAGE-3 protein (*Section III. 1.3*) (198, 210). The recombinant viral approach will not be presented here (221).

Figure 9

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging



with or without evidence of disease

B. Accrual according to tumor type

Tumor type	Included			Treated					
				Completed protocol			Removed		
	Total	Tumor-bearing	NED	Total	Tumor-bearing	NED	Total	Tumor-bearing	NED
	53	43	10	37	28	9	16	15	1
Melanoma	45	39	6	30	25	5	15	14	1
NSCLC	2	1	1	2	1	1	0	0	0
Head & Neck	3	1	2	2	0	2	1	1	0
Esophagus	2	1	1	2	1	1	0	0	0
Bladder	1	1	0	1	1	0	0	0	0

Objectives

The objectives of this pilot study were to follow the tumor evolution of patients immunized with the MAGE-3.A1 peptide and to define the safety of this vaccine. In a small number of patients, we also examined whether they developed a specific CTL response (197, 220).

Treatment schedule

The treatment included 3 immunizations with the MAGE-3.A1 peptide at monthly intervals (*design summary*). The lyophilized synthetic MAGE-3.A1 peptide was diluted in 1 ml PBS at a concentration of 100 or 300 $\mu\text{g/ml}$. It was administered at 4 separate injection sites distant from the tumor, by s.c. (2 x 40%) and i.d. (2 x 10%) injections of 100 μg (8 patients) or 300 μg (45 patients) of peptide, on three occasions at monthly intervals.

Nine patients with a melanoma who did not require other systemic anti-cancer treatment received additional vaccinations at increasing intervals, for up to 5 years.

Eligibility criteria

Eligibility criteria relative to tumor type and stage are defined in *Fig. 9 A*. Patients with or without detected metastases were eligible (*for detailed eligibility criteria, see Annex 3*).

Patients' characteristics

Fifty-three HLA-A1 patients whose tumor expressed *MAGE-3* were included in the study from March 1994 to July 1997. Most of them had stage III or IV cutaneous melanoma. A few patients had other tumor types, i.e. NSCLC, head and neck carcinoma, esophageal carcinoma or bladder transitional cell carcinoma (*Fig. 9 B*). Forty-three patients had metastases and 10 had no evidence of disease at study entry. There were 28 males and 25 females. The median age was 54 years (range: 23 to 79 years).

Thirty-seven patients, including 28 patients with metastases, completed the cycle of 3 immunizations (*Fig. 9 B*). The latter were evaluable for tumor response.

Sixteen patients were withdrawn from the study before they received all 3 immunizations, because tumor progression or relapse necessitated other treatments.

Toxicity

No significant treatment-related toxicity was observed in the 53 patients who received at least 1 vaccination. Six patients had a mild inflammatory reaction at the site of superficial metastases (grade 1 toxicity) in the days following vaccinations. Six early deaths (≤ 6 weeks from the last vaccination) were reported. All six were due to tumor progression.

Table 11 Characteristics of melanoma patients with detectable metastasis at study entry

Patients		Previous		Study treatment							Time to	Further	Last Status ⁸		
Gender Code	Age	treatments ¹	AJCC 1992	Total	Dose	Toxicities ⁴			SAE	Tumor	Prog-	treat-	Tumor	Survival	
			Stage ² at	numb.	per	Maximum toxicity			(n) ⁵	response ⁶	ression	ments ⁷	Survival	Survival	
			study entry	injec-	injec-	severity or grade	Local	Flu	Other	(months)	(months)	(months)	(months)		
1) Patients having received complete protocol (≥ 3 vaccinations): n = 25															
7 patients with tumor regression															
LB-945	F	49	Sg	III N2b	24	300	0	0	0	0	CR	> 10 years	no	NED	> 10.5 years
LB-1448	F	67	Sg, ILP	III N2b	13	300	0	0	0	0	MxR	3	yes	PD	23
NAP-8	F	48	Sg, Ct, NSI	III N2b	18	300	0	0	0	0	PR	14	yes	PD	5 years
MI-28	M	59	Sg, Ct, NSI	III N2c	3	100	0	0	0	0	MxR	1	no	PD	3
NAP-10	F	65	Sg, Ct, NSI	III N2c	15	300	0	0	0	0	CR	41	yes	PD	7.5 years
KI-1	F	42	Sg	IV M1a	5	300	0	0	0	0	MxR	7	yes	PD	21
AVL-3	F	50	Sg	IV M1b	7	300	0	0	0	0	CR	6	yes	PD	33
18 patients with tumor progression															
LB-1348	F	73	Sg, ILP	III N2b	7	300	0	0	1	0	PD	2	yes	NED	> 9 years
NAP-11	F	41	Sg, Ct	III N2b	3	300	0	0	0	0	PD	2.5	yes	PD	14
NAP-9	F	75	Sg, NSI	III N2b	3	300	0	0	0	0	PD	2.5	yes	PD	4.5
LB-1045	F	68	Sg, ILP, NSI	III N2b	3	300	0	0	1	0	PD	< 1	yes	PD	6
NAP-13	F	61	Sg, NSI	III N2c	5	300	0	0	0	0	PD	< 1	yes	PD	36.5
NAP-1	M	43	Sg, NSI	III N2c	7	300	0	0	0	0	PD	1.5	yes	PD	40.5
HUG-2	F	70	Sg	III N2c	3	300	0	0	0	0	PD	3	yes	PD	19
VUB-27	F	62	Sg, ILP, H, Ct	IV M1a	3	300	0	0	0	0	PD	3	yes	PD	6
LB-1241	F	75	Sg, H, Ct, NSI	IV M1a	3	300	0	0	1	0	PD	1.5	yes	PD	15
HUG-1	M	56	Sg, Ct, NSI	IV M1a	3	300	0	0	0	0	PD	3	no	PD	4
LB-278	F	61	Sg, H, Ct	IV M1a	3	100	0	0	0	0	PD	2	no	PD	8.5
NAP-17	F	79	Sg, Ct	IV M1b	3	300	0	0	0	0	PD	< 1	yes	PD	5
AVL-5	F	54	Sg	IV M1b	3	300	0	0	0	0	PD	3	yes	PD	5.5
LB-1019	F	42	Sg, H, Ct	IV M1b	3	300	0	0	0	0	PD	1.5	yes	PD	12
LAU-177	M	42	Sg, NSI	IV M1b	3	300	0	0	1	0	PD	< 1	yes	PD	13
MI-275	M	46	Sg	IV M1b	3	300	1	0	0	0	PD	2	yes	PD	5.5
LB-1265	F	52	Sg, R, Ct	IV M1b	3	300	0	0	0	0	PD	1	yes	PD	5
MSR-3	M	47	Sg, R, H, Ct, NSI	IV M1b	6	300	0	0	0	0	PD	3	yes	PD	20
2) Patients who were withdrawn (1 to 2 vaccinations) : n = 14															
MZP-7	M	67	Sg, Ct, NSI, SI	III N2a	2	100	0	0	1	0	PD	1.5	yes	PD	9
NAP-12	M	50	Sg, NSI	III N2c	1	300	0	0	0	0	PD	< 1	yes	PD	6 *
UZG-2	M	49	Sg, H, Ct	III N2c	2	100	0	0	0	1	PD	< 1	no	PD	1
MI-47	M	34	Sg, NSI	IV M1a	2	100	0	0	1	0	PD	1	yes	PD	14.5
AVL-1	F	73	Sg, R	IV M1a	1	300	0	0	0	0	PD	< 1	yes	PD	4.5
MI-36	M	57	Sg, Ct, SI	IV M1a	2	100	0	0	0	0	PD	1	yes	PD	3.5 *
HA-8	M	33	Sg, Ct, NSI	IV M1b	2	300	0	0	0	0	PD	2.5	UK	PD	3
AVL-4	M	44	Sg	IV M1b	2	300	0	0	0	0	PD	1.5	yes	PD	6.5
EN-2	M	61	Sg, H, Ct	IV M1b	2	300	0	0	0	1	PD	1	UK	PD	1.5
AVL-9	M	23	Sg, R	IV M1b	2	300	0	0	0	0	PD	< 1	yes	PD	6.5
LAU-169	F	52	Sg, R, ILP, Ct, NSI	IV M1b	1	300	0	0	0	1	PD	1	yes	PD	1.5
CF-2	M	41	Sg, Ct	IV M1b	1	100	0	0	0	1	PD	< 1	yes	PD	1.5
LAU-167	M	69	Sg, Ct, NSI	IV M1b	1	300	0	0	0	1	PD	< 1	no	PD	0.5
MSR-2	M	36	Sg, Ct, NSI	IV M1b	1	300	0	0	0	0	PD	1	yes	PD	4

1) *Previous treatments:* Ct: Chemotherapy, H: Hormonal therapy, ILP: Isolated Limb chemotherapy Perfusion, NSI: Non-Specific Immunotherapy, R: Radiotherapy, Sg: Surgery, SI: Specific Immunotherapy.

2) *AJCC staging:* refers to the 4th edition of the AJCC staging manual, 1992 (see Table 5).

Melanoma staging: III = regional disease = N (N2b = in-transit metastasis; N2c = metastasis more than 3 cm in greater dimension in any regional lymph node(s) and in-transit metastasis); IV = distant metastasis = M (M1a = metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes; M1b = visceral metastasis). In-transit metastasis involves skin or subcutaneous tissue more than 2 cm from the primary tumor not beyond the regional lymph nodes.

3) *Total number of injections:* 9 patients who were thought by the investigator to have some benefit of the treatment received more than 3 vaccinations, on a compassionate use basis.

4) *Toxicities:* Maximum toxicity severity or grade reported according to the National Cancer Institute of Canada common toxicity criteria scale. Local: reaction at injection sites, Flu: Flu-like symptoms, Other: Any other toxicities.

5) *SAE:* number of Serious Adverse Events.

6) *Tumor response:* According to the WHO criteria: PR: Partial Response, SD: Stable Disease, PD: Progressive Disease. In addition, Mixed Responses were reported (MxR).

7) *Further treatments:* UK, unknown

8) *Last status:* Tumor: NED: No Evidence of Disease. Survival: *: lost to follow-up

Tumor response

Tumor response was assessed according to the WHO criteria, after the 3rd vaccination, as compared to baseline tumor measurements performed in the 2-week period preceding the first vaccination. “Mixed responses” (MxR), i.e. regression of some metastases while others either remain stable, progress or appear simultaneously, although formally classified as stable or progressive disease in the WHO classification, were documented as well.

Summary of tumor response

The 3 tumor-bearing patients with other tumor types than melanoma who received all 3 immunizations had progressive disease (PD) (Fig. 9 B). We will focus here on the observations made on the 39 melanoma patients with metastasis included in the study (*Table 11 and Fig. 10*). Out of these 39 melanoma patients, 14 were removed from the study before they received 3 immunizations because of tumor progression requiring other treatments and 25 completed the study treatment (*Table 11*).

Out of the 25 melanoma patients who received the complete treatment, 4 had an objective response (OR) including 3 CR and 1 PR, 3 had a MxR and 18 had PD (*Fig. 10*). Lesions that were sensitive to the vaccine were cutaneous or lymph node metastases, except in 1 patient who displayed complete disappearance of 5 lung metastases. Two CR led to a disease-free state that persisted for more than two years after beginning of treatment.

Our results provide no significant evidence that the higher dose of MAGE-3.A1 peptide (300 μ g) was more effective than the lower dose (100 μ g). The lower dose was administered to 1 out of 7 patients who showed OR or MxR, to 5/14 who were removed from the trial and to 1/18 who showed continuous tumor progression.

The main objective of our clinical trials is trying to understand the mechanisms involved in tumor regression. Therefore, we report below the detailed tumor evolution of the 7 patients who had OR or MxR.

Detailed narratives of responder patients

Patient n°1 (LB-945, female, 49 years) had about 100 small cutaneous in-transit metastases on the left leg at study entry (*Fig. 11*). The patient had received no treatment other than surgery. The MAGE-3.A1 peptide (300 μ g) was injected in the thighs and in the arms. By the second injection, the cutaneous nodules had become more numerous, but no further progression was observed at the time of the third injection. The stabilization was confirmed one month later. Four months after the third injection, all the nodules appeared flattened and dry (*Fig. 14 A*). One month later, 2 nodules were biopsied and found to contain a large number of macrophages containing melanin. A few remaining tumor cells were observed in one nodule and none in the other. Because of the favorable clinical

Outcome of the 25 melanoma patients with detectable metastases who received at least 3 vaccinations

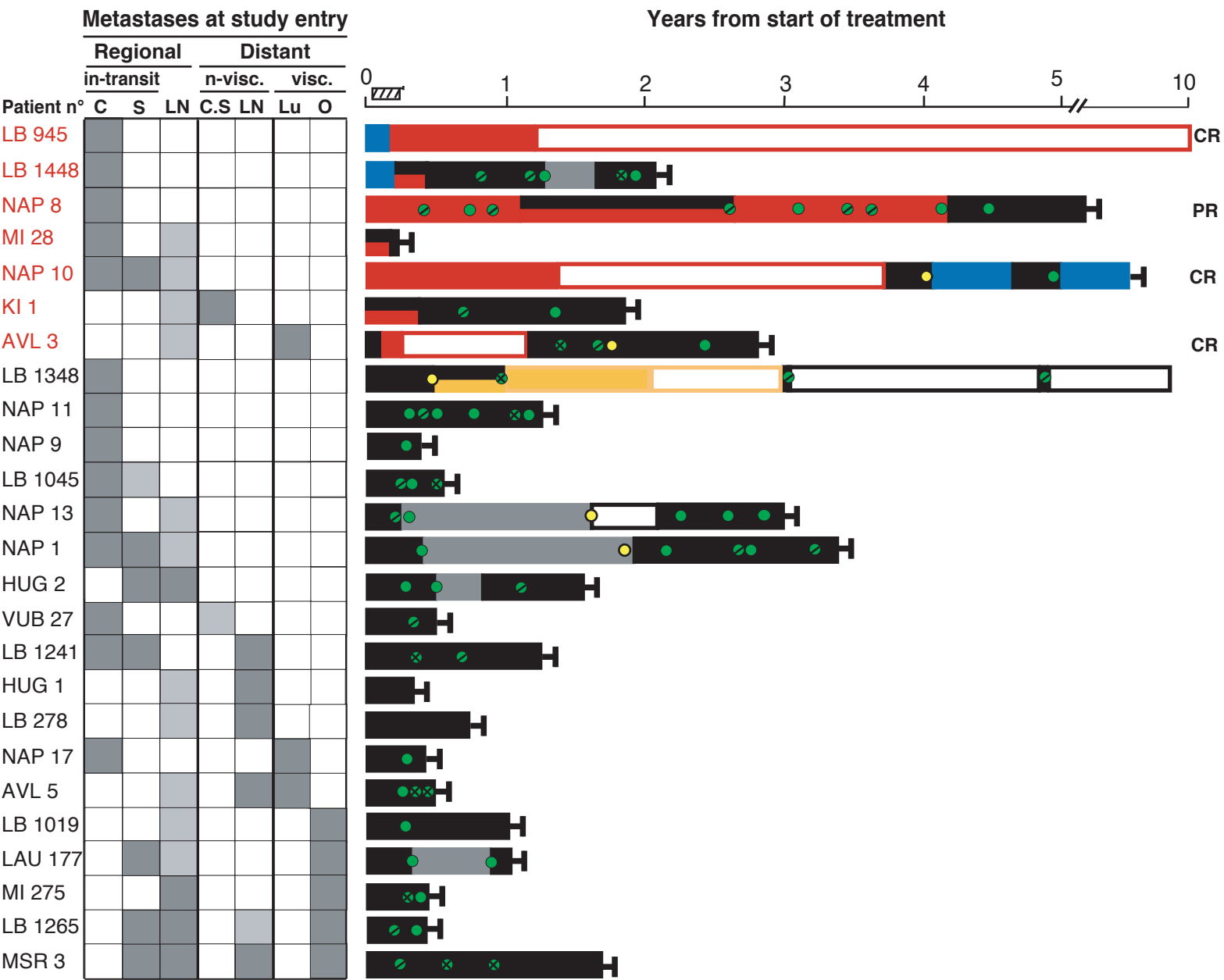
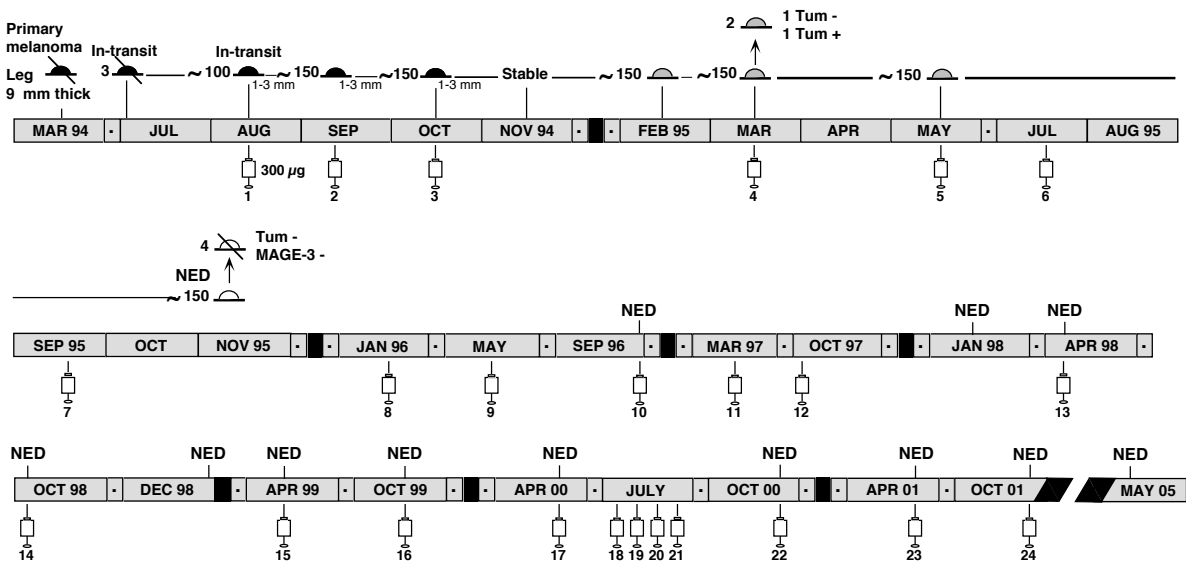
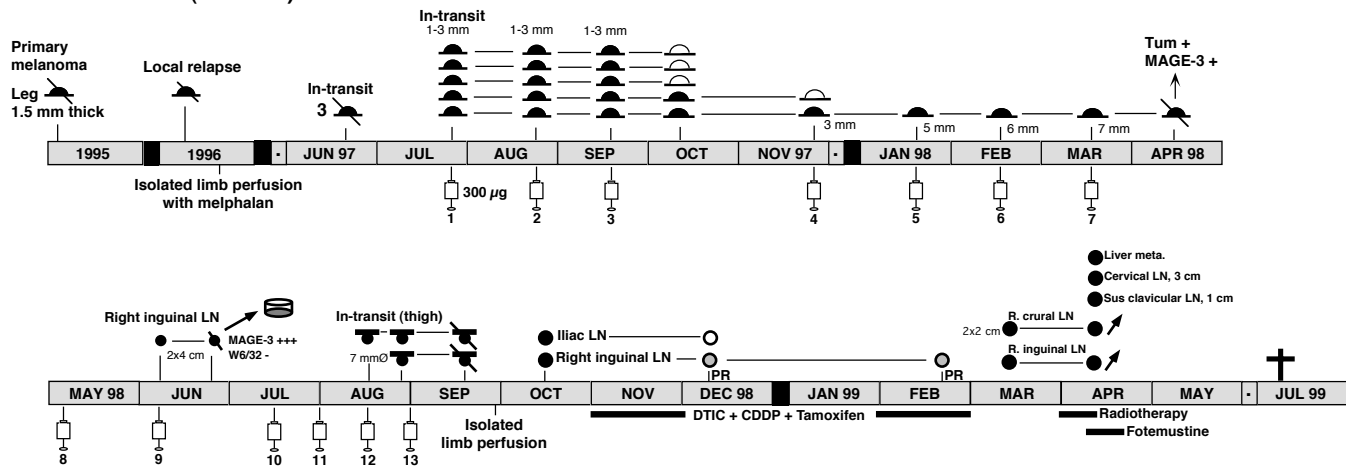


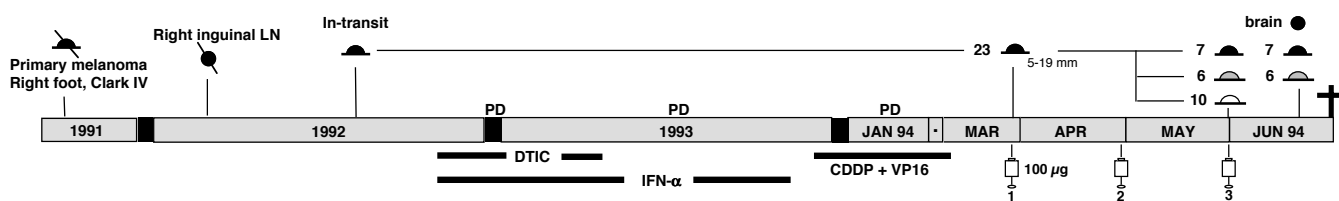
Figure 11
PATIENT n°1 (LB-945)



PATIENT n°2 (LB-1448)



PATIENT n°3 (MI-28)



LEGEND: Fig. 11 to 13

	cutaneous tumor	subcutaneous tumor	lymph node (LN) or visceral metastasis
new, stable or progressing			
regressing			
no longer detectable			
surgical removal			

Monthly injections of MAGE-3.A1 peptide (300 µg or 100 µg per vaccination day)

Frequent injections of MAGE-3.A1 peptide (300 µg)

Frequent injections of MAGE-1.A1 & MAGE-3.A1 peptides

NED: No evidence of disease

PD: Progressive disease

PR: Partial response

SD: Stable disease

NSCLC: Non small cell lung cancer

Tum +: tumor cells visible histologically; Tum -: not visible

MAGE-3 +: expression of gene MAGE-3 (RT-PCR); MAGE-3 -: no expression

Patient's death

CDDP: cisplatin

DTIC: dacarbazine

VP16: etoposide

IFN-α: interferon alpha

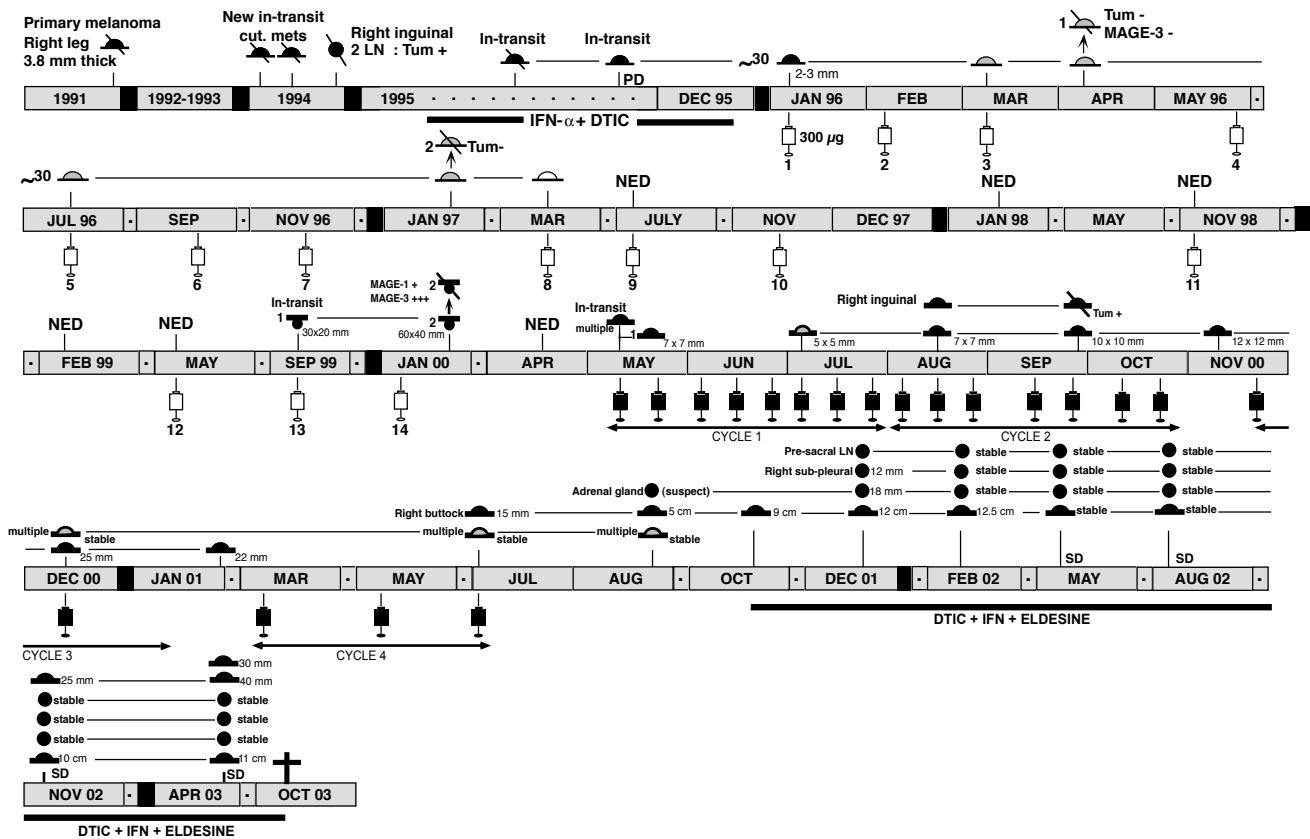
evolution, the patient received 4 additional injections of the same peptide at 2-month intervals. No other treatment was given. Fifteen months after the first injection, the patient appeared to be completely free of disease: all the skin lesions had become impalpable even though melanin tattooing was still visible at the sites of the former nodules. Biopsies of several sites revealed no tumor cells and no *MAGE-3* expression as assessed by RT-PCR. Physical examinations and CT-scans showed no evidence of disease. The patient received additional injections at increasingly large intervals. Forty-one months after the onset of treatment, the melanin tattooing had completely disappeared (Fig. 14 A). Vaccinations were stopped in October 2001 and no further treatment was given. In May 2005, she was still disease-free, more than 10 years after the onset of treatment.

Patient n°2 (LB-1448, female, 67 years) had undergone in 1996 surgical excision of a cutaneous local relapse on the right leg, followed by adjuvant isolated perfusion with melphalan (Fig. 11). In June 1997, 3 new in-transit cutaneous metastases appeared and were surgically removed. One nodule was tested for *MAGE-3* expression and found to be positive. One month later, the patient developed 5 new small cutaneous in-transit metastases on the right leg. In July 1997, vaccination was initiated with 300 µg of MAGE-3.A1 peptide. Two weeks after the third injection, 3 out of the 5 in-transit metastases had completely disappeared, while the 2 remaining ones appeared to be stable and no new metastases had appeared. Six weeks later, only one nodule of 3 mm diameter remained present. Two additional vaccinations were carried out at 2-month intervals. Nine months after study entry, the remaining nodule had progressed from 3 to 7 mm diameter. It was excised and found to express gene *MAGE-3*. In June 1998, a regional inguinal lymph node metastasis appeared and was resected. Despite multiple further treatments, the patient died with tumor progression in July 1999, almost 2 years after the start of MAGE-3.A1 peptide vaccinations.

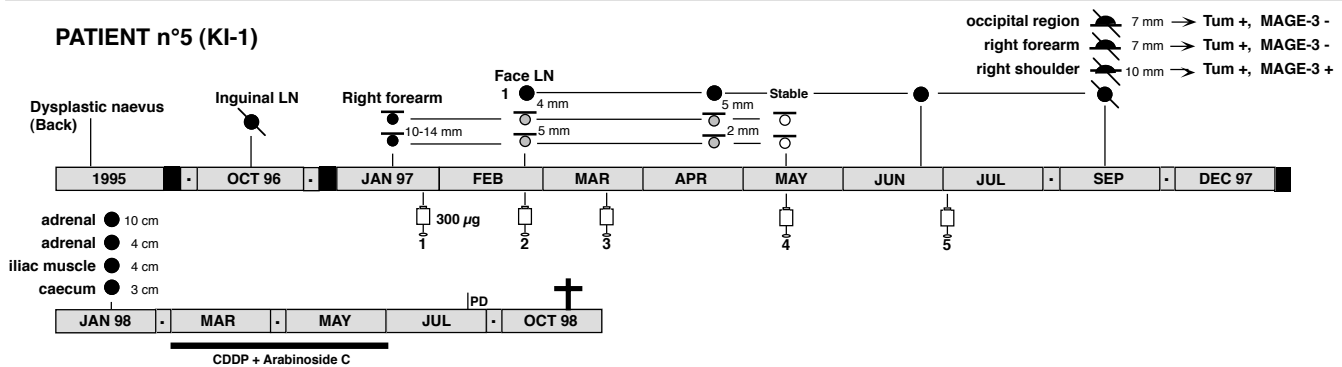
Patient n°3 (MI-28, male, 59 years) had melanoma localized to the right leg with 23 cutaneous or subcutaneous in-transit metastatic nodules ranging from 5 to 19 mm diameter at study entry (Fig. 11). These nodules had not responded to several cycles of chemotherapy involving dacarbazine (DTIC), interferon- α (IFN- α) and a combination of cisplatin and etoposide. The patient was vaccinated with 100 µg of peptide, starting four weeks after cessation of chemotherapy. By the third injection, 10 out of the 23 nodules, including the largest one, had regressed completely. Six other nodules had regressed partially. Six nodules were stable and one was progressing. Three weeks later, a brain metastasis was discovered and the patient died soon thereafter.

Patient n°4 (NAP-10, female, 65 years) had a primary melanoma of the right leg removed in 1991 (Fig. 12). In 1994, in-transit metastases and 2 invaded regional lymph nodes were removed. In 1995, despite adjuvant treatment associating DTIC and IFN- α , new in-transit metastases appeared and were removed and this treatment was interrupted by the end of the year. In January 1996, when vaccination

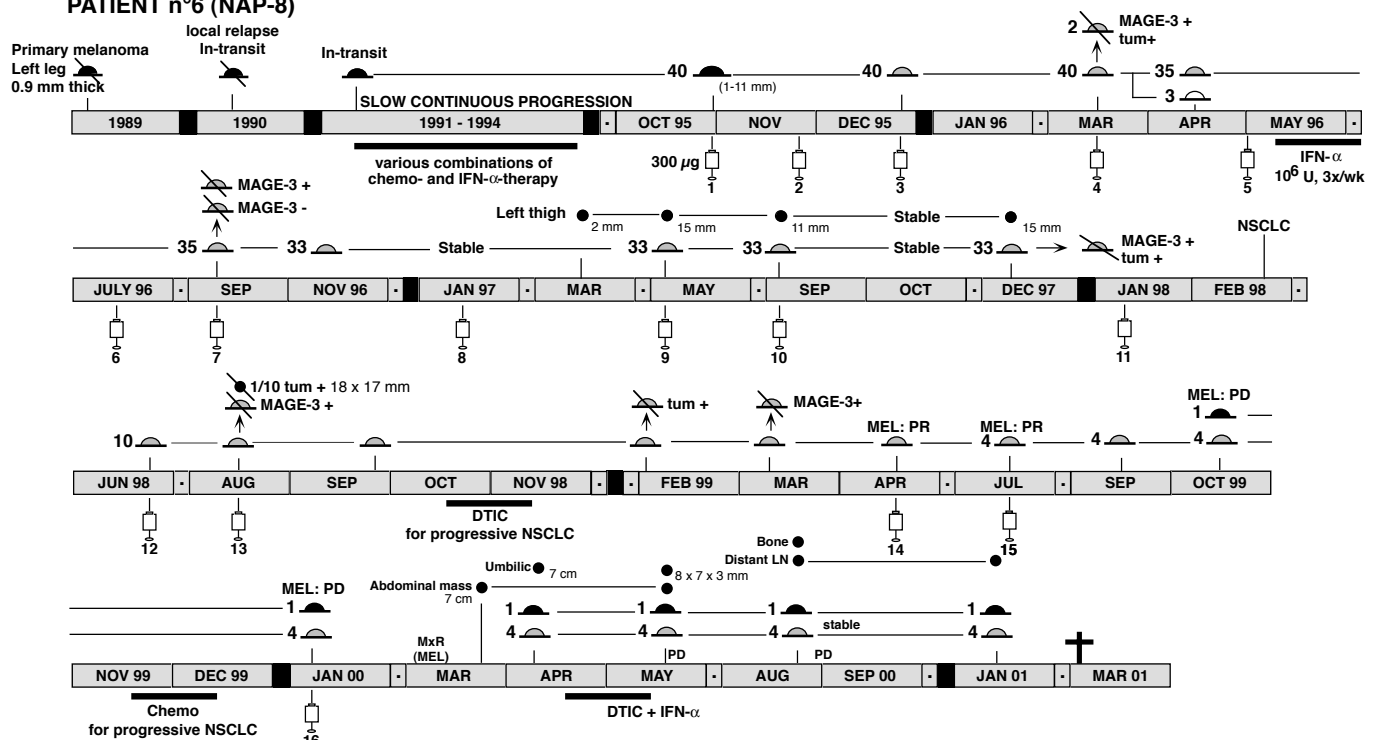
PATIENT n°4 (NAP-10)



PATIENT n°5 (KI-1)



PATIENT n°6 (NAP-8)



with 300 μ g of peptide was started, the patient displayed about 30 small cutaneous in-transit metastases disseminated on the right leg and thigh (Fig. 14 B). By the third vaccination, partial flattening of all the lesions was observed. One month later, one regressing lesion was biopsied and neither *MAGE-3* expression nor tumor cells were found. By September 1996, all the lesions were almost completely flat (Fig. 14 B). The patient received additional injections with the same amount of peptide, first at 2-month and then, at 4-month intervals. Fourteen months after the onset of treatment, all the lesions were impalpable. In September 1999, 41 months after the initiation of MAGE-3.A1 peptide vaccinations, in-transit relapse occurred and the patient was taken off study in January 2000. Because the in-transit metastases resected at that time still expressed *MAGE-3*, she participated in a protocol of vaccination where she received frequent injections of the MAGE-3.A1 peptide, from May 2000 to July 2001 (Study LUD 97-004). In August 2001, a visceral metastasis appeared and chemoimmunotherapy was initiated a few weeks later. In October 2003, the patient died of progressive disease, more than 7 years after the start of MAGE-3.A1 peptide vaccinations.

Patient n°5 (KI-1, female, 42 years) had a suspect lesion of the back removed in 1995 (Fig. 12). In October 1996, an inguinal lymph node was removed and was found to contain melanoma cells expressing *MAGE-3*. In January 1997, two new subcutaneous metastases measuring 10 and 14 mm diameter respectively appeared on the right upper arm. Vaccination with 300 μ g of peptide was initiated. By the second injection, the 2 subcutaneous metastases had reduced to 5 mm diameter, but a small metastatic maxillary lymph node had appeared. Two months after the third injection of peptide, the 2 subcutaneous lesions of the right arm had totally disappeared and the lymph node was stable. Two additional injections were given. Three months later, during which the lymph node had remained stable, three new cutaneous metastases had appeared. All the lesions were removed surgically and were found to contain tumor cells. Gene *MAGE-3* was expressed in only one of the three cutaneous lesions. Because of this tumor progression, the injections were stopped. The patient had no evidence of disease for a period of 4 months, after which she relapsed with several visceral metastases. Despite further chemotherapy, she died because of disease progression, 21 months after the onset of MAGE-3.A1 peptide vaccinations.

Patient n°6 (NAP-8, female, 48 years) had a primary melanoma of the left leg in 1989 (Fig. 12). Between 1990 and 1995, in-transit metastases appeared and displayed continuous slow progression, despite repeated treatments with DTIC, Vindesine and IFN- α . The patient was vaccinated with 300 μ g of peptide, starting in October 1995. At that time, she had about 40 small cutaneous in-transit metastases, the largest one measuring 11 mm diameter. At the time of the third injection, all the cutaneous nodules displayed some flattening. The patient received additional injections of peptide. In the following months, a few cutaneous nodules disappeared and the other lesions were either moderately regressing or stable. In May 1996, small doses of IFN- α were injected in an attempt to

favor the putative immune rejection of the tumor by increasing its expression of HLA class I molecules. The patient received 10^6 U IFN- α s.c. 3 times per week for 4 weeks. No acceleration of the regression process was observed. Vaccination with the MAGE-3 peptide was pursued. Between May 1996 and December 1997, the cutaneous metastases continued to regress slowly, but significantly. However, a small subcutaneous nodule appeared on the left thigh in March 1997.

In addition to the melanoma lesions of the leg, this patient displayed a suspect lung nodule of 15 mm diameter at the onset of treatment. This nodule remained stable throughout 1996, but increased gradually in size throughout 1997 to reach 25 mm diameter. In February 1998, it was excised and diagnosed as a peripheral lung adenocarcinoma. This second tumor was negative for the expression of the *MAGE-3* gene. Despite further chemoimmunotherapy, the patient died because of progression of both cancers in March 2001, more than 5 years after the onset of MAGE-3.A1 peptide vaccinations.

Patient n°7 (AVL-3, female, 50 years) had undergone removal of regional lymph node metastases in October 1994 (*Fig. 13*). She had not received any form of treatment other than surgery. In April 1995, she presented with 3 lung metastases and vaccination with 300 μ g of peptide was started. At the time of the third injection, a CT scan revealed an increase in the size of the known lung nodules as well as 2 new lung metastases. In addition, an enlarged mediastinal lymph node was visible. Two months later, 2 out of the 5 lung nodules had disappeared and the 3 remaining nodules had shrunk significantly. The mediastinal lymph node was stable. Because of this favorable evolution, the patient received an additional peptide injection and no other form of treatment was given. One month later, one of the 3 metastases had regressed further and the two others had disappeared. Two months later, in November 1995, all the metastatic nodules had disappeared and the mediastinal lymph node had returned to normal size (*Fig. 15*). The patient displayed no other evidence of disease. She received 3 additional injections with the same amount of peptide, at 2-month intervals and remained free of disease for 4 months more. In March 1996, a new metastatic mediastinal lymph node was observed. Injections were discontinued and local radiotherapy was given to this lesion, which partially regressed and then remained stable. Ten months later, a subcutaneous and an adrenal metastasis appeared. Because the subcutaneous metastasis expressed both the *MAGE-3* and *MAGE-1* genes, the patient was included in another study involving vaccination with both the MAGE-3.A1 peptide and a *MAGE-1*-encoded peptide that is also presented by HLA-A1 (110). However, the tumor continued to progress during vaccination. Subsequent chemotherapy with DTIC caused a transient partial response. The patient died from tumor progression, 33 months after entering the treatment with the MAGE-3.A1 peptide. It is noteworthy that during the 22 months following her first relapse the patient acquired progressive lesions in several sites but none in the five lung sites or in the mediastinal site that had regressed, suggesting that the regression process at these sites had removed all the tumoral cells, while smaller metastases had escaped that process.

Figure 13

PATIENT n°7 (AVL-3)

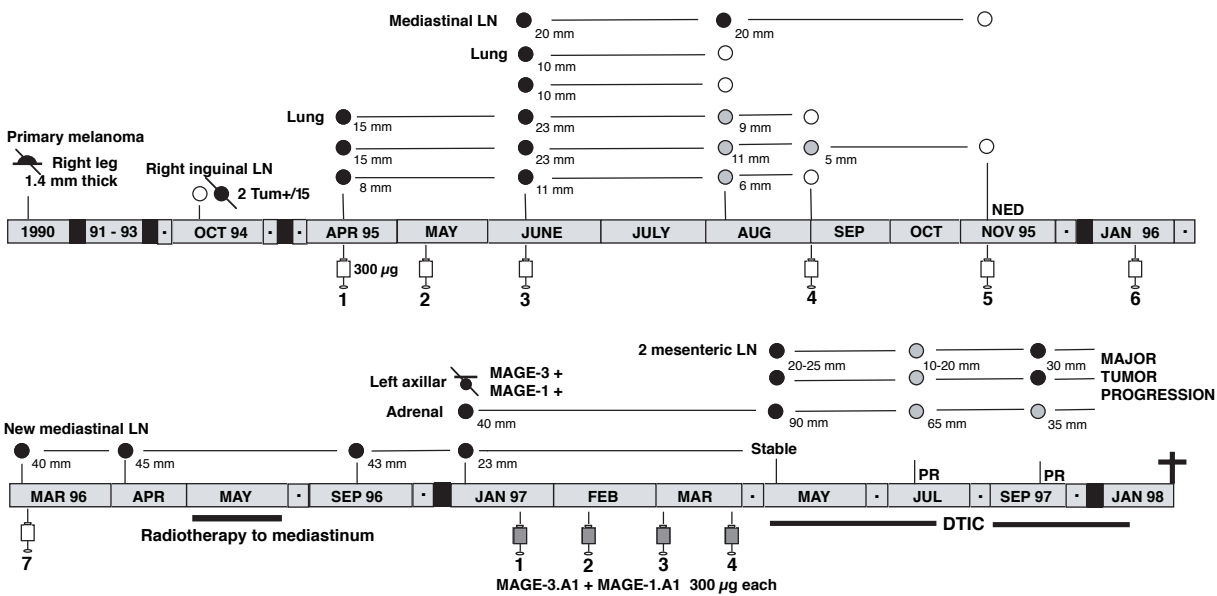


Figure 14 Evolution of cutaneous and subcutaneous in-transit melanoma metastases

A Patient n°1: Left leg, anterior aspect



B

Patient n°4:

Right leg



Right thigh

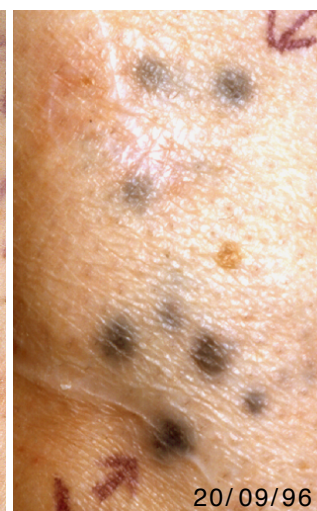
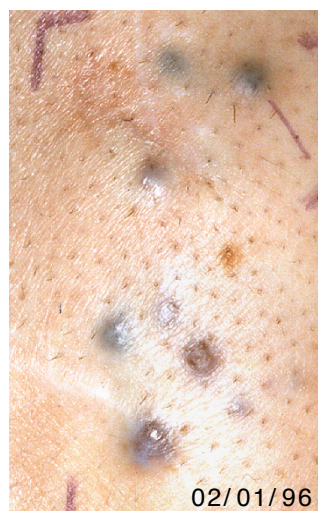
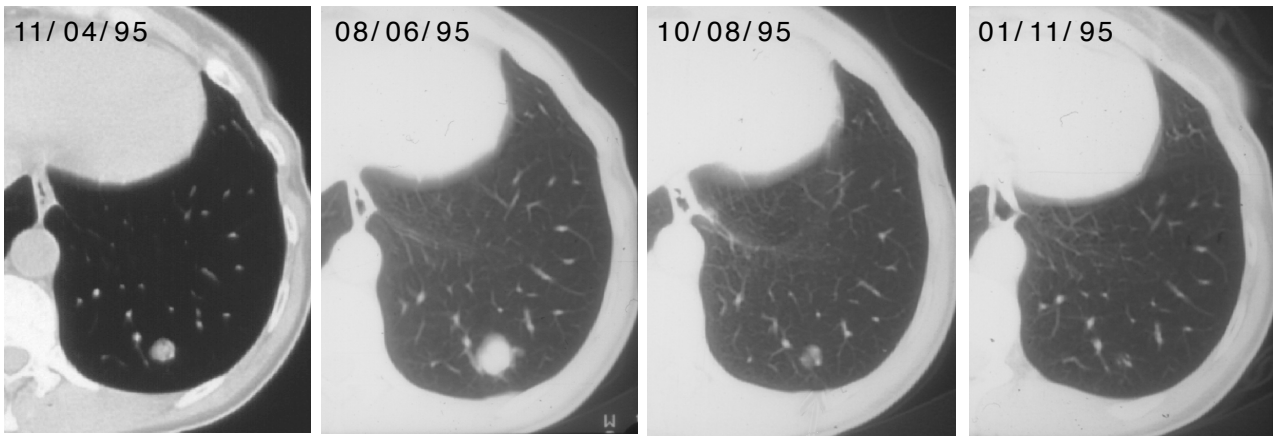


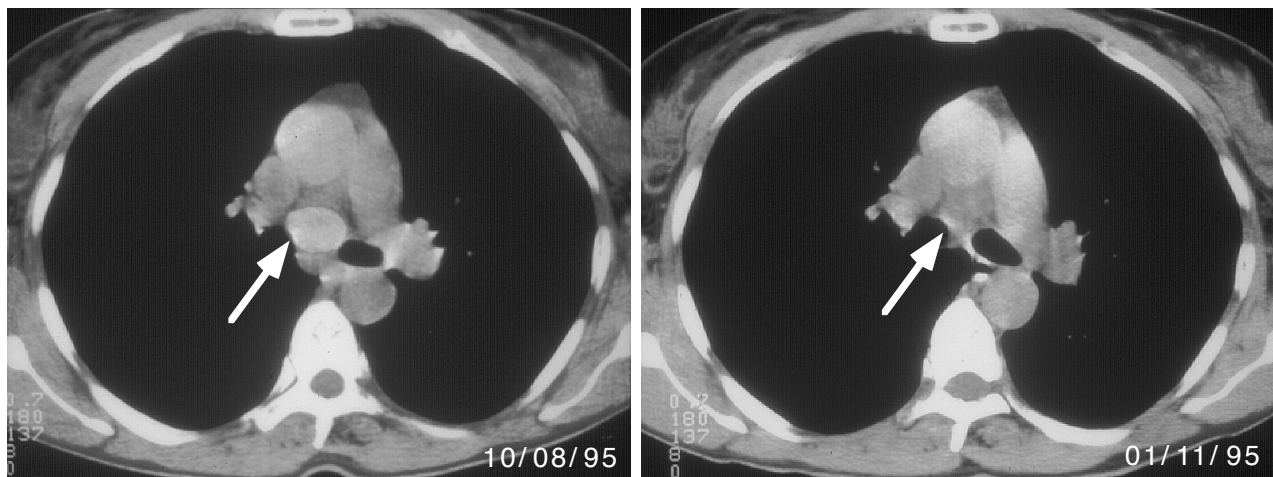
Figure 15

Evolution of distant melanoma metastases

Patient n°7: Left lung
Evolution of a lower lobe metastasis



Patient n°7: Mediastine
Evolution of a subcarinal adenopathy (indicated by an arrow)



Analysis of the anti-MAGE-3.A1 CTL response

No evidence for a cytolytic T lymphocyte response was found in the blood of the four patients who were analyzed, including two who displayed a complete tumor response. Thus, the complete tumor responses observed in patients n°1 and n°7 were not paralleled by a strong increase in circulating CTL precursors directed against the MAGE-3.A1 antigen.

We analyzed 5×10^5 CD8⁺ T lymphocytes in a blood sample collected from patient n°1 (LB-945) during the tumor regression (March 1995). These lymphocytes were stimulated three times *in vitro* with cells pulsed with the MAGE-3.A1 peptide (*Section II. 4.4.1, Method A*). No CTL activity was observed against targets expressing the MAGE-3.A1 antigen. A similar analysis was performed with 10^6 lymphocytes from patient n°7 (AVL-3) collected when the regression process was ongoing (August 1995) or complete (January 1996). The results were also negative. The lymphocytes of patients n°1 and n°7 produced significant CTL activity upon stimulation with an influenza peptide, indicating that the lymphocytes of these patients were still capable of responding to antigenic stimulation. The lymphocytes of patients LB-1045 and LB-278, who had progressive disease, were also restimulated with the MAGE-3.A1 peptide and no CTL activity specific for targets expressing the MAGE-3.A1 antigen was detected.

Stimulation of large numbers of CD8⁺ T lymphocytes (more than 10^7) by peptide-pulsed cells has made it possible to evaluate the frequency of anti-MAGE-3.A1 CTL precursors in cancer-free individuals (204). This frequency was estimated at $3 \cdot 10^{-7}$ in the CD8⁺ lymphocyte population. The presence of anti-MAGE-3.A1 CTL precursors was tested using this method in $2 \cdot 10^7$ blood CD8⁺ T lymphocytes of patient n°7 two months after documentation of a complete tumor response (*Section II 4.4.1, method B*). The observed frequency was 1.3×10^{-7} .

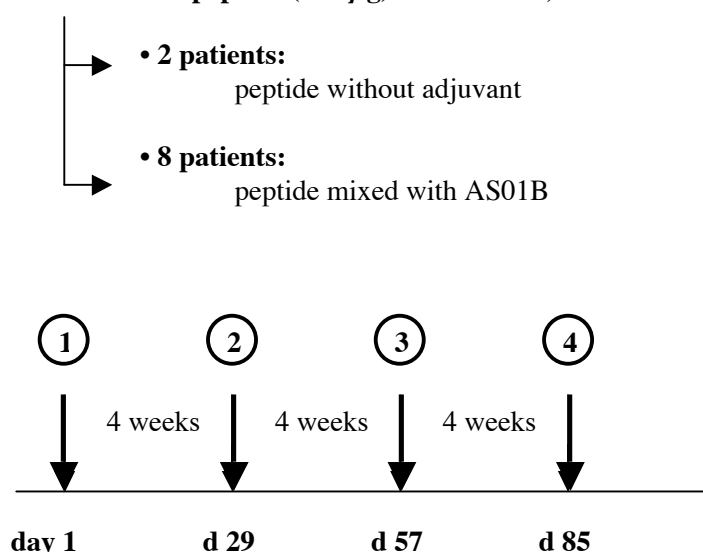
III. 1.1.2 Mixing the MAGE-3.A1 with the immunological adjuvant AS01B containing MPL and QS21

Study LUD 95-002

Pilot study of immunization with the MAGE-3.A1 peptide plus immunological adjuvants QS21 and MPL in patients with malignant melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma or bladder carcinoma.

DESIGN SUMMARY

Immunization: MAGE-3.A1 peptide (100 µg) at 1 i.m. site, distant from tumor



Coordinating investigator: Marie Marchand

Clinical sites:

- Cliniques universitaires Saint-Luc, Brussels
- LICR/CHUV Lausanne
- Rupprechts-Karls-Universität, Heidelberg

Principal investigators:

Y. Humblet
D. Liénard
U. Keilholz

Figure 16

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging

Melanoma
high-risk III
IV

CUTANEOUS IN-TRANSIT	SUBCUTANEOUS IN-TRANSIT	LYMPH NODE REGIONAL	CUTANEOUS SUBCUTANEOUS DISTANT	LYMPH NODE DISTANT	VISCERAL METASTASES		
		<5 : N1, N2a (III)			LUNG	OTHER	BRAIN
N2b (III)	N2b (III)	X	X	X	X	X	
		≥5 : N1, N2a (III)					
X	●————● N2c (III)		M1a (IV)	M1a (IV)	M1b (IV)	M1b (IV)	M1b (IV)

Lung NSCLC
IIIB
IV

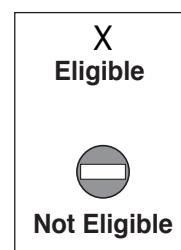
PRIMARY TUMOR			REGIONAL LYMPH NODE			VISCERAL METASTASES		
LOCAL EXTENSION	chest wall main bronchus	vertebral body carina mediastinal organs other lobe	ipsi-hilar ipsi-mediastinal subcarinal		contralateral	OTHER LUNG	- OTHER - DISTANT LYMPH NODE	BRAIN
T1-T2 (I)	T3 (IIIA)	T4 (IIIB)	N1 (II) N2 (IIIA)		N3 (IIIB)	M1 (IV)	M1 (IV)	M1 (IV)

**Head
-and-
Neck**
high-risk IV : M1
N3
T4 + N1, N2

PRIMARY TUMOR			VISCERAL METASTASES		
LOCAL EXTENSION	ADJACENT ORGANS	REGIONAL LYMPH NODE	LUNG	- OTHER - DISTANT LYMPH NODE	BRAIN
T1-T3 (≥I or II)	T2 -T4 (II, III, IV)	N1-N3 (III, IV)	M1 (IV)	M1 (IV)	M1 (IV)

Bladder
III
IV

PRIMARY TUMOR			VISCERAL METASTASES		
LOCAL EXTENSION	ADJACENT ORGANS	REGIONAL LYMPH NODE	LUNG	- OTHER - DISTANT LYMPH NODE	BRAIN
T1-T3 (I, II, III)	T4 (III, IV)	N1-N3 (IV)	M1 (IV)	M1 (IV)	M1 (IV)



with or without evidence of disease

B. Accrual according to tumor type

Tumor type	Included			Treated				
				Completed protocol			Removed	
	Total	Tumor- bearing	NED	Total	Tumor- bearing	NED	Total	Tumor- bearing
	10	9	1	9	8	1	1	0
Melanoma	8	7	1	7	6	1	1	1*
NSCLC	2	2	0	2	2*	0	0	0
Head & Neck	0							
Bladder	0							

* one of these patients received the peptide without adjuvant

Objectives

One method to improve the immunogenicity of the MAGE-3.A1 peptide is to include an appropriate combination of adjuvants in the vaccine. The MAGE-3.A1 peptide was mixed with the immunological adjuvant AS01B containing MPL and QS21, two molecules with demonstrated immunological adjuvant properties. QS21 is a carbohydrate extracted from the bark of the South American tree *Quillaja Saponaria Molina* (222, 223). Vaccines containing various protein antigens plus QS21 have been described to induce a MHC class I restricted CTL response against target cells expressing these antigens (224, 225). Monophosphoryl lipid A or MPL is a detoxified compound chemically derived from the lipopolysaccharide molecule, with preserved immunopotentiating effects (226). Experiments conducted in mice as well as primates, have shown that not only are MPL and QS21 potent immunostimulants on their own, but also that they can synergize when combined and induce even higher antibody and cellular mediated immune responses.

Accordingly, mixing MPL/QS21 adjuvants with the MAGE-3.A1 peptide would be expected to increase the immunogenicity of the latter by inducing a potent "second signal" which is required to effectively activate the T lymphocytes, increasing antigen processing and presentation by dedicated antigen presenting cells and switching the immune response towards CTL.

Treatment schedule

The treatment included 4 i.m. immunizations with the MAGE-3.A1 peptide (100 μ g) at 4-week intervals (*design summary*). Since the MAGE-3.A1 peptide had never been administered by i.m. route before, the 2 first patients received the peptide alone, without adjuvant, to test the safety of this administration route. Then, 8 patients received the same dose of peptide mixed with MPL and QS21 (50 μ g of each).

Eligibility criteria

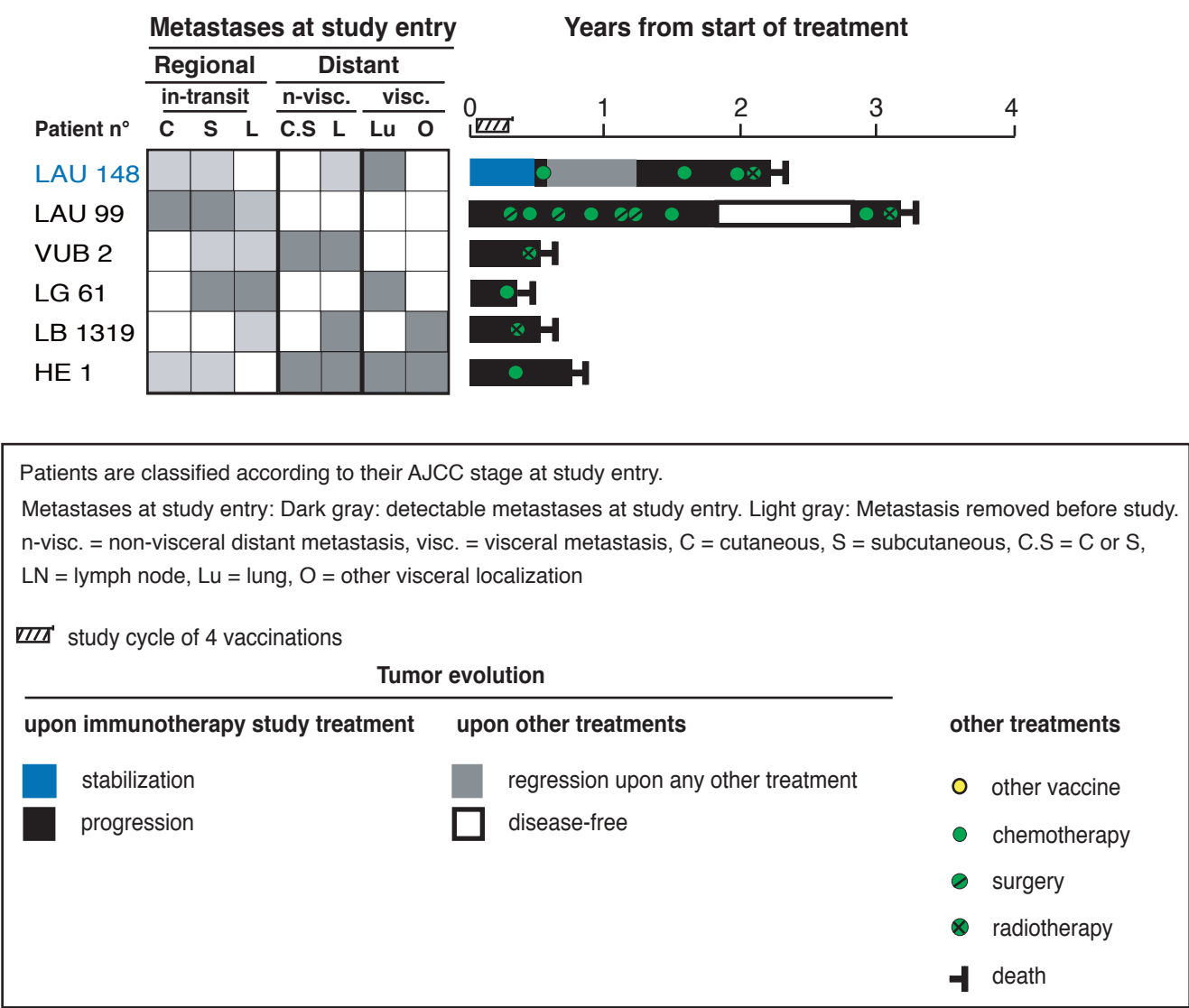
Eligibility criteria relative to tumor type and stage are defined in *Fig. 16 A*. Patients with or without detected metastases were eligible.

Patients' characteristics

Ten HLA-A1 patients whose tumor expressed *MAGE-3* were included in the study from November 1995 to September 1996. Eight patients had melanoma and 2 patients had NSCLC (*Fig. 16 B*). One melanoma patient had NED at study entry. There were 7 males and 3 females. The median age was 52 years (range: 31 to 72 years).

Nine patients, including 8 patients with detected metastases, completed the cycle of 4 immunizations (*Fig. 16 B*). The latter were evaluable for tumor response. Among the 9 patients who completed the study, 8 had received the peptide mixed with MPL/QS21 and 1 had received the peptide alone.

Outcome of the 6 melanoma patients with detectable metastases who received at least 4 vaccinations with the MAGE-3.A1 peptide mixed with MPL/QS21



One patient with a stage IV M1a melanoma, who received the peptide alone, was withdrawn from the study before he received all 4 immunizations, because of tumor progression necessitating other treatments.

Toxicity

No severe toxic reaction (grade 3 or 4) related to the treatment was observed in the 10 patients who received at least 1 vaccination. The melanoma patient who received the peptide alone had a mild inflammatory reaction at the site of a cervical lymph node metastasis (grade 1 toxicity) in the days following the first vaccination. This inflammatory reaction was not followed by any modification of tumor size at that site. There was no local reaction to the vaccine in the 2 patients who received the peptide alone. Out of the 8 patients who received the MAGE-3.A1 peptide mixed with MPL/QS21, 6 had grade 1 inflammatory reaction at injection sites and 2 had grade 1 or 2 flu-like symptoms. No early deaths (≤ 6 weeks from the last vaccination) were reported.

Tumor response

No objective response was observed. Out of the 2 patients who received the peptide alone (*Fig. 16 B*), one - with a stage IV NSCLC - completed the cycle of 4 immunizations. He had PD.

The 8 patients who received the peptide mixed with the adjuvant completed the study. Seven had metastases detected at study entry, including 6 patients with melanoma (*Fig. 17*) and one with a stage IV NSCLC. The 8th patient had high-risk stage III melanoma and had no evidence of disease at study entry. One patient with a stage IV melanoma displayed stabilization of 4 small lung metastases (maximum diameter, 10 mm). He received a total of 9 immunizations at 4-week intervals, until tumor progression occurred 7.5 months after the start of treatment. Seven patients had PD or tumor relapse after 4 immunizations.

Analysis of the anti-MAGE-3.A1 T cell responses

No evidence was obtained for the presence of anti-MAGE-3 CTL precursors in the blood of any patient included in this study, before or after immunization, as assessed by MLPC assay (*Section II. 4.4.1, method A*).

No delayed-type hypersensitivity (DTH) reaction was observed upon intradermal injection of 10 μ g of MAGE-3.A1 peptide without adjuvants in any of the patients, neither before, nor after immunization.

III. 1.1.3 Associating MAGE-3.A1 with another HLA class I-restricted MAGE peptide

Study LUD 96-007

Pilot study of immunization with the MAGE-1.A1 and MAGE-3.A1 peptides in patients with malignant melanoma, non-small cell lung carcinoma, head and neck squamous cell carcinoma, esophageal squamous cell carcinoma or bladder carcinoma.

DESIGN SUMMARY

Immunization:

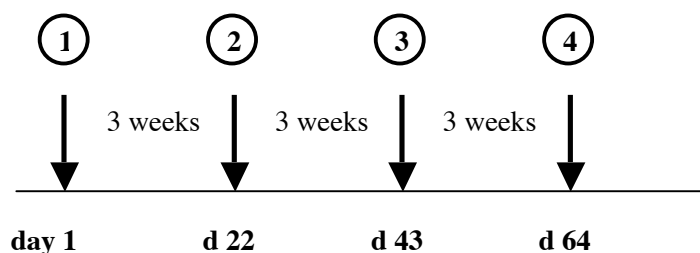
300 μ g MAGE 1.A1 peptide, 80% s.c. at 1 site, 20% i.d. at 1 site

300 μ g MAGE 3.A1 peptide, 80% s.c. at 1 site, 20% i.d. at 1 site

sites distant

from tumor

28 patients, including 17 melanoma with detected metastases



Coordinating investigator: Nicolas van Baren

Clinical sites:

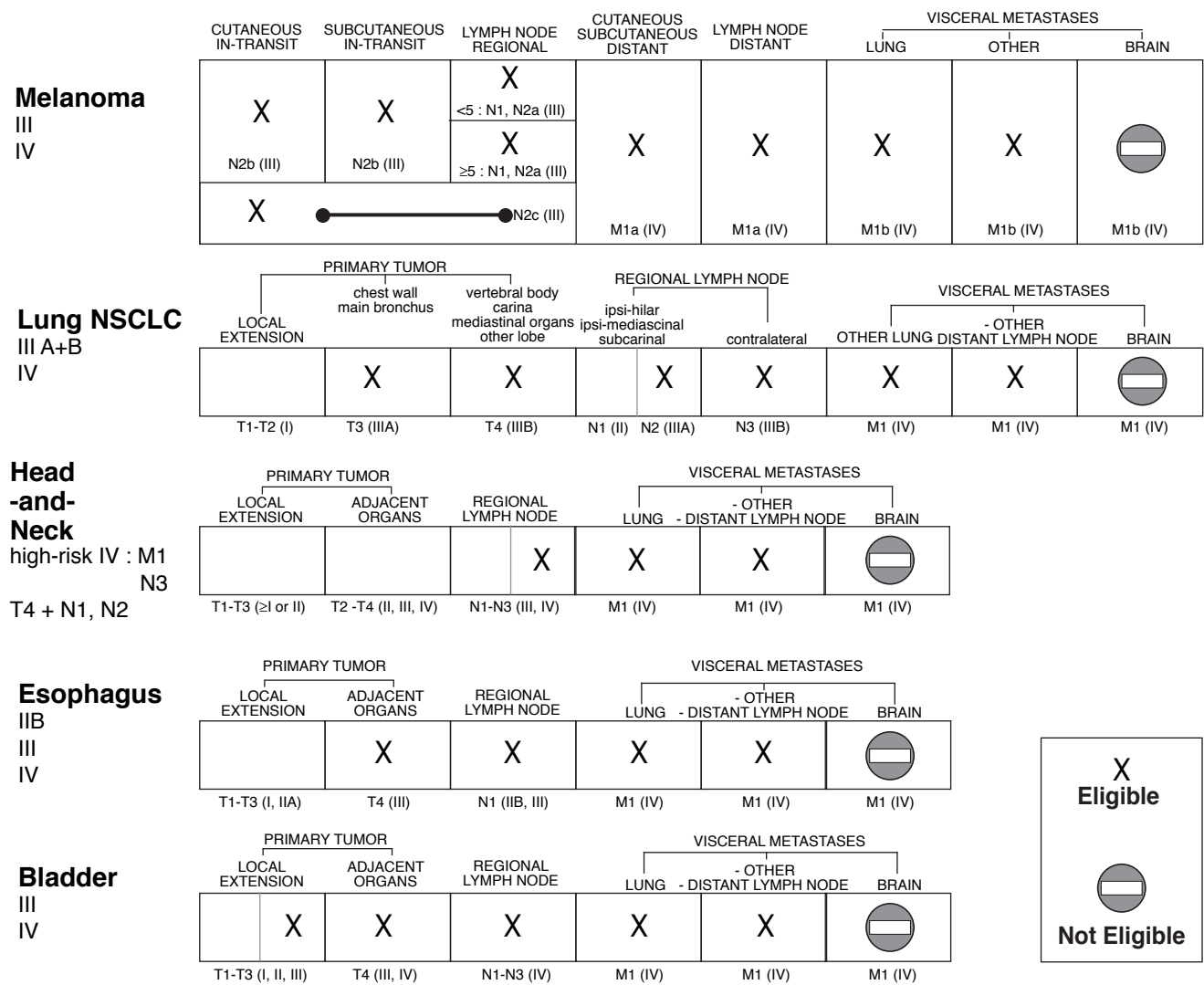
- Cliniques universitaires Saint-Luc, UCL, Brussels
- Hôtel Dieu, CHU, Nantes
- LICR/CHUV Lausanne
- Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano
- Institut Curie, Paris

Principal investigators:

Y. Humblet
B. Dréno
D. Liénard
G. Parmiani
V. Brichard

Figure 18

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging



with or without evidence of disease

B. Accrual according to tumor type

Tumor type	Included			Treated					
				Completed protocol			Removed		
	Total	Tumor-bearing	NED	Total	Tumor-bearing	NED	Total	Tumor-bearing	NED
	28	18	10	20	12	8	8	6	2
Melanoma	26	17	9	18	11	7	8	6	2
NSCLC	2	1	1	2	1	1	0		
Head & Neck	0								
Esophagus	0								
Bladder	0								

Objective

We wanted to see whether the addition of a second HLA class I-restricted peptide (MAGE-1.A1) would improve the tumor response rate and/or the CTL responses that has been reported with MAGE-3.A1 peptide alone (*Section III. 1.1.1*).

Treatment schedule

A combination of the MAGE-3.A1 and MAGE-1.A1 peptides (300 μ g of each) was administered on 4 occasions, at 3-week intervals, by s.c. and i.d. routes (*design summary*).

Nine patients who were thought by the investigator to have some benefit of the treatment received more than 4 vaccinations on a compassionate basis, for a period of up to 5 years.

Eligibility criteria

Eligibility criteria relative to tumor type and stage are defined in *Fig. 18 A*. Patients with or without detected metastases were eligible.

Patients' characteristics

Twenty-eight HLA-A1 patients whose tumor expressed *MAGE-1* and *MAGE-3* were included in the study from January 1997 to February 2000. Twenty-six patients had melanoma and 2 patients had NSCLC (*Fig. 18 B*). Ten patients had no evidence of disease at study entry. There were 14 males and 14 females. The median age was 56 years (range: 28 to 82 years).

Twenty patients, including 12 patients with detected metastases, completed the cycle of 4 immunizations (*Fig. 18 B*). The latter were evaluable for tumor response.

Eight melanoma patients were withdrawn from the study before they received all 4 immunizations, because of tumor progression or relapse necessitating other treatments.

Toxicity

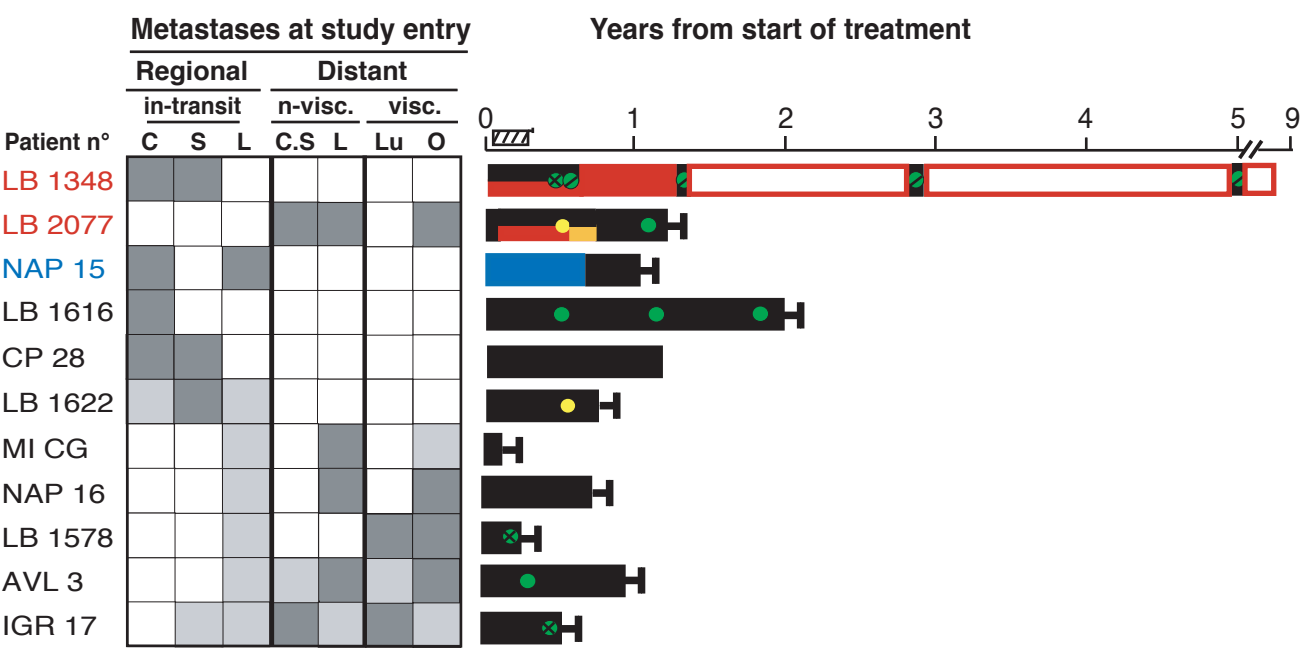
Treatment with id/sc injections of MAGE-3.A1 and MAGE-1.A1 peptides every 3 weeks was well tolerated, without severe toxicity.

Treatment-related adverse events were only reported in 5 out of the 28 patients who received at least 1 injection. One melanoma patient presented grade 3 headaches after each immunization. She was removed from the study after 3 immunizations but continued to suffer from headaches long time thereafter. Of note, this patient already reported headaches before study entry, during another experimental treatment with TIL and IL-2. Two patients experienced grade 2 inflammatory reaction at distant, superficial tumor sites after vaccinations. One patient had grade 2 flu-like symptoms and 2 had grade 1 local reactions at injection sites. Three early deaths related to cancer progression were reported.

Figure 19

MAGE-1.A1 + MAGE-3.A1 peptides (study LUD 96-007)

Outcome of the 11 melanoma patients with detectable metastases who received at least 4 vaccinations



Patients are classified according to their AJCC stage at study entry.
Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.
n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization

study cycle of 4 vaccinations

Tumor evolution

upon immunotherapy study treatment	upon other treatments	other treatments
regression	regression upon other immunotherapy	other vaccine
progression	regression upon any other treatment	chemotherapy
mixed response (prog./reg.)	mixed response (prog./reg.)	surgery
stabilization		radiotherapy
disease-free	disease-free	death

Tumor response

Summary of tumor response

No objective responses were observed.

Both patients with NSCLC completed the cycle of 4 immunizations (*Fig. 18 B*). One of them, with stage IIIB mediastinal adenopathies at study entry, had a SD period of 6 months until progression. The other one, with a resected stage IIIA tumor at study entry, remained free of relapse for more than 7 years.

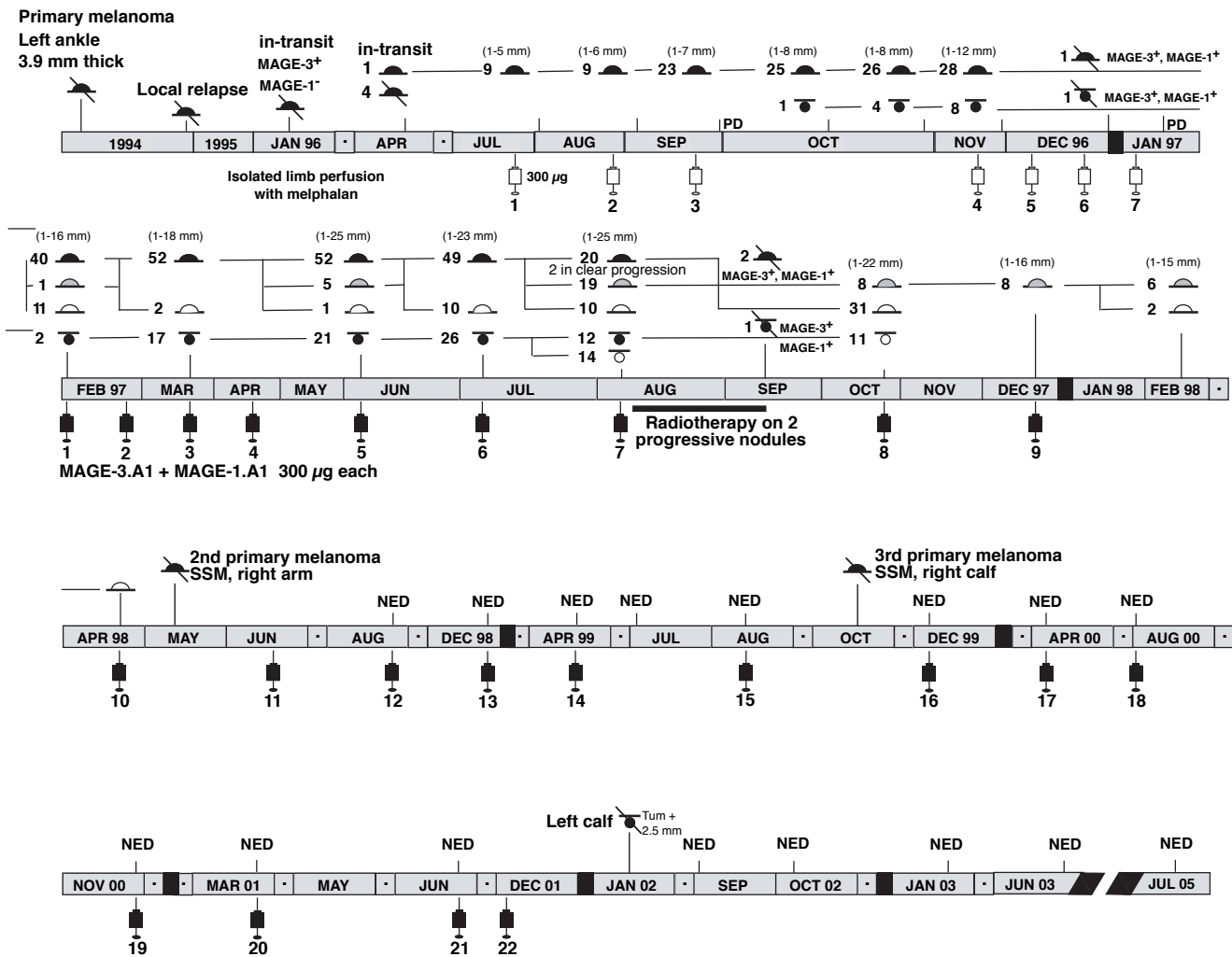
Eighteen melanoma patients received at least 4 immunizations (*Fig. 18 B*). Out of these 18 patients, 7 had no evidence of disease at study entry. Among the latter, 2 were still free of disease after 74 and 65 months, respectively. The 5 other patients presented a relapse of their disease during the study. Among the 11 patients with melanoma metastases who completed the study, 2 showed a MxR, 1 had SD for 6 months and 8 patients had PD (*Fig. 19*).

Detailed narratives of responder patients

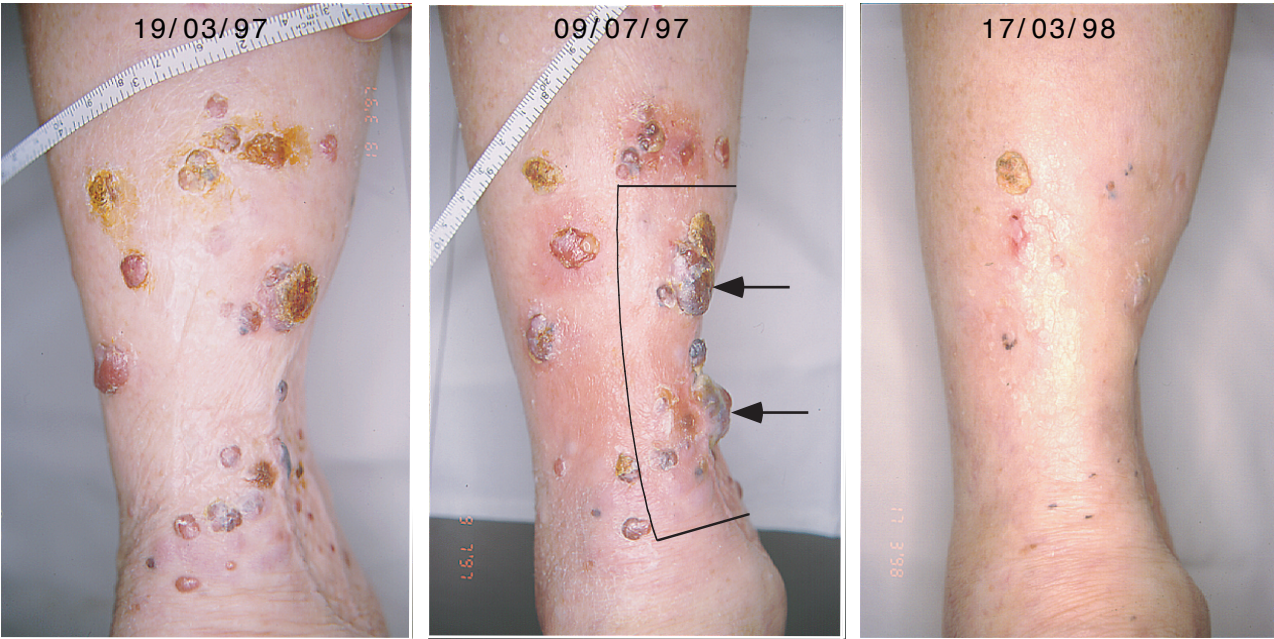
Interestingly, one of the 2 melanoma patients who displayed a MxR to treatment had a very favorable tumor evolution (*Fig. 20*). Patient LB-1348 had a primary melanoma of the left leg and a local relapsed lesion removed in 1994. A new in-transit metastasis was excised in January 1996 and found to express *MAGE-3* and not *MAGE-1*. Even though the patient was treated by isolated limb perfusion with melphalan, new cutaneous in-transit nodules appeared 3 months later. In July 1996, the patient displayed 9 small cutaneous nodules on the left leg and vaccination with 300 µg of the MAGE-3.A1 peptide was initiated (*Study LB 94-001, Section III. 1.1.1, Table 11*). By November, the number of the nodules had increased to 28, their size had increased and subcutaneous lesions had appeared. But a few nodules displayed a necrotic aspect and to favor this putative regressive process, four additional injections were given at 2-week intervals. By February 1997, forty cutaneous and twelve subcutaneous nodules were spread over the left leg. However, one small nodule had become completely flat, while an adjacent one showed clear signs of regression. Because a cutaneous nodule removed in December had been found to express not only *MAGE-3* but also *MAGE-1*, unlike the nodule tested in January 1996, the patient was included in this study involving immunization with both the MAGE-3.A1 and the MAGE-1.A1 peptides. Between February and April 1997, a few additional small cutaneous nodules became completely flat but new cutaneous and subcutaneous nodules appeared and others enlarged (*Fig. 20*). The treatment was pursued. By August, there was widespread regression: many cutaneous and subcutaneous nodules had completely disappeared, while many others had become flatter. However, two metastases necessitated local radiotherapy because of progression and ulceration. Following electron beam irradiation limited to an area surrounding the two lesions (*Fig. 20*), both of them flattened and became necrotic. During the following months, many cutaneous and subcutaneous nodules located far away from the irradiated region regressed. Additional

Figure 20

PATIENT LB-1348



Left calf, posterior aspect



Evolution of cutaneous and subcutaneous in-transit melanoma metastases.
The two nodules that were irradiated are indicated by arrows; the irradiated field is surrounded by the black line.

immunizations were performed. By March 1998, all the cutaneous nodules had completely regressed and the others were clearly regressing (*Fig. 20*). One month later, all the subcutaneous nodules had completely disappeared. Vaccinations with the MAGE-3.A1 and the MAGE-1.A1 peptides were pursued until December 2001. Two independent primary melanoma and 1 in-transit metastasis were resected in May 1998, October 1999 and January 2002, respectively. No other treatment was given and no evidence of disease has been detected since the last surgery. The patient was still free of disease in July 2005, 3.5 years after the last relapse. We cannot exclude that the combination of two peptides produced a regression that the MAGE-3.A1 peptide would not have produced alone. But it is also possible that a similar result could have been achieved by pursuing frequent injections with the single MAGE-3.A1 peptide from 1997 to 2001.

Patient LB 2077 experienced a partial regression of 1 out of 3 liver metastases measuring 20 mm diameter, while the 2 others measuring 9 and 10 mm diameter respectively, progressed under treatment (MxR).

Analysis of the anti-MAGE-3.A1 and anti-MAGE-1.A1 CTL responses

CTL responses against the MAGE-1.A1 and MAGE-3.A1 antigens were assessed by Tetramer assay in 6 patients, including patient LB-1348 who showed a MxR to treatment (*Section II. 4.4.1, method C*). No CTL response was detected.

Conclusion

Despite the lack of objective response, regression of some melanoma metastases in 2 patients suggests a possible effect of the vaccine, but does not support the hypothesis that the addition of a second peptide (MAGE-1.A1) improves the tumor response rate that has been reported with MAGE-3.A1 peptide alone.

The immunological analysis indicates that no strong CTL response was induced by the vaccine.

III. 1.1.4 Frequent and prolonged injections of MAGE-3.A1 peptide, alone or mixed with an HLA class II-restricted MAGE peptide

Study LUD 97-004

Phase I/II study of high-frequency and prolonged immunization with the MAGE-3.A1 and/or MAGE-3.DP4 peptide in stage III/IV patients with malignant melanoma.

DESIGN SUMMARY

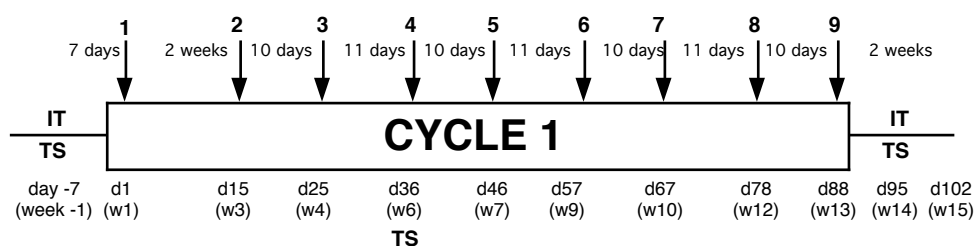
3 treatment arms:

arm A (n=25): HLA-A1 or B35 positive, HLA-DP4 negative patient: MAGE-3.A1 peptide alone

arm B (n=14): HLA-A1 and B35 negative, HLA-DP4 positive patient: MAGE-3.DP4 peptide alone

arm C (n=14) : HLA-A1 or B35 positive, HLA-DP4 positive patient: Both peptides, mixed

Immunization: 300 μ g MAGE-3.A1 and/or MAGE-3.DP4, 40% s.c. at 2 sites, 10% i.d. at 2 sites



IT: blood collection for immunological tests; **TS:** tumor staging

Coordinating investigator: Nicolas van Baren

Clinical sites:

- Krankenhaus Nordwest, Frankfurt am Main
- Cliniques universitaires Saint-Luc, UCL, Brussels
- Institut Gustave Roussy, Villejuif
- Ludwig Institute, Melbourne
- Hôtel Dieu, CHU, Nantes
- Hôpital Henri Mondor, Créteil
- Daniel den Hoed Cancer Center, Rotterdam
- Akademisch Ziekenhuis VUB, Brussels
- Institut Curie, Paris

Principal investigators:

A. Knuth, E. Jäger
 N. van Baren, V. Brichard
 M.-F. Avril, B. Escudier
 J. Cebon
 B. Dréno
 M. Bagot
 A. Eggermont, W. Kruit
 J. De Greve, B. Neyns
 T. Dorval, V. Brichard

Figure 21

A. Eligibility relative to maximum extent of disease according to AJCC 1997 staging

Melanoma advanced III IV	CUTANEOUS IN-TRANSIT	SUBCUTANEOUS IN-TRANSIT	LYMPH NODE REGIONAL	CUTANEOUS SUBCUTANEOUS DISTANT	LYMPH NODE DISTANT	VISCERAL METASTASES		
						LUNG	OTHER	BRAIN
	X	>5 X	>3 cm X	X	X	X	X	
	N2b (III)		N1, <u>N2a (III)</u>	M1a (IV)	M1a (IV)	M1b (IV)	M1b (IV)	M1b (IV)

with at least one metastasis detected

X

EligibleNot Eligible

B. Accrual per treatment arm

Treatment Arm	Included	Treated	
		Completed protocol	Removed
A : MAGE-3.A1	32	21	11
B : MAGE-3.DP4	2	0	2
C : Both peptides	11	7	4
TOTAL	45	28	17

Objectives

Two approaches were tested here to improve the antitumoral and immunological effectiveness of our vaccines (*design summary*).

- The MAGE-3.A1 peptide was injected every 10-11 days instead of every 3-4 weeks.
- The same peptide was associated with an HLA class II-restricted MAGE-3.DP4 peptide, in order to induce both CD8⁺ and CD4⁺ T cell responses.

Treatment schedule

The complete treatment (cycle 1) included 9 vaccinations at 10 or 11 days intervals. On each vaccination day, a total dose of 300 µg of MAGE-3.A1 peptide and/or MAGE-3.DP4 peptide (AA 243-258) was divided between 2 i.d. sites (2 x 30 µg) and 2 s.c. sites (2 x 120 µg).

According to their HLA type, patients received either the MAGE-3.A1/B35 peptide (treatment arm A), or the MAGE-3.DP4 peptide (Arm B), or both of them (Arm C) (*design summary*).

Eligibility criteria

Patients with stage III or IV melanoma were eligible provided they had at least one metastasis detected (*Fig. 21 A*).

Patients' characteristics

Forty-five patients whose tumor expressed *MAGE-3* were included in the study from December 1997 to August 2003 (*Fig. 21 B*). Thirty-two patients received either the MAGE-3.A1/B35 peptide alone, 2 the MAGE-3.DP4 peptide alone and 11 patients received both. There were 24 males and 21 females. The median age was 59 years (range: 20 to 86 years). Fifteen had stage III and 30 had stage IV melanoma at study entry.

Twenty-eight patients completed the cycle of 9 immunizations and were evaluable for tumor response (*Fig. 21 B*). Eighteen of them, whose tumor did not require another treatment, received additional maintenance vaccinations (10 to 24 in total).

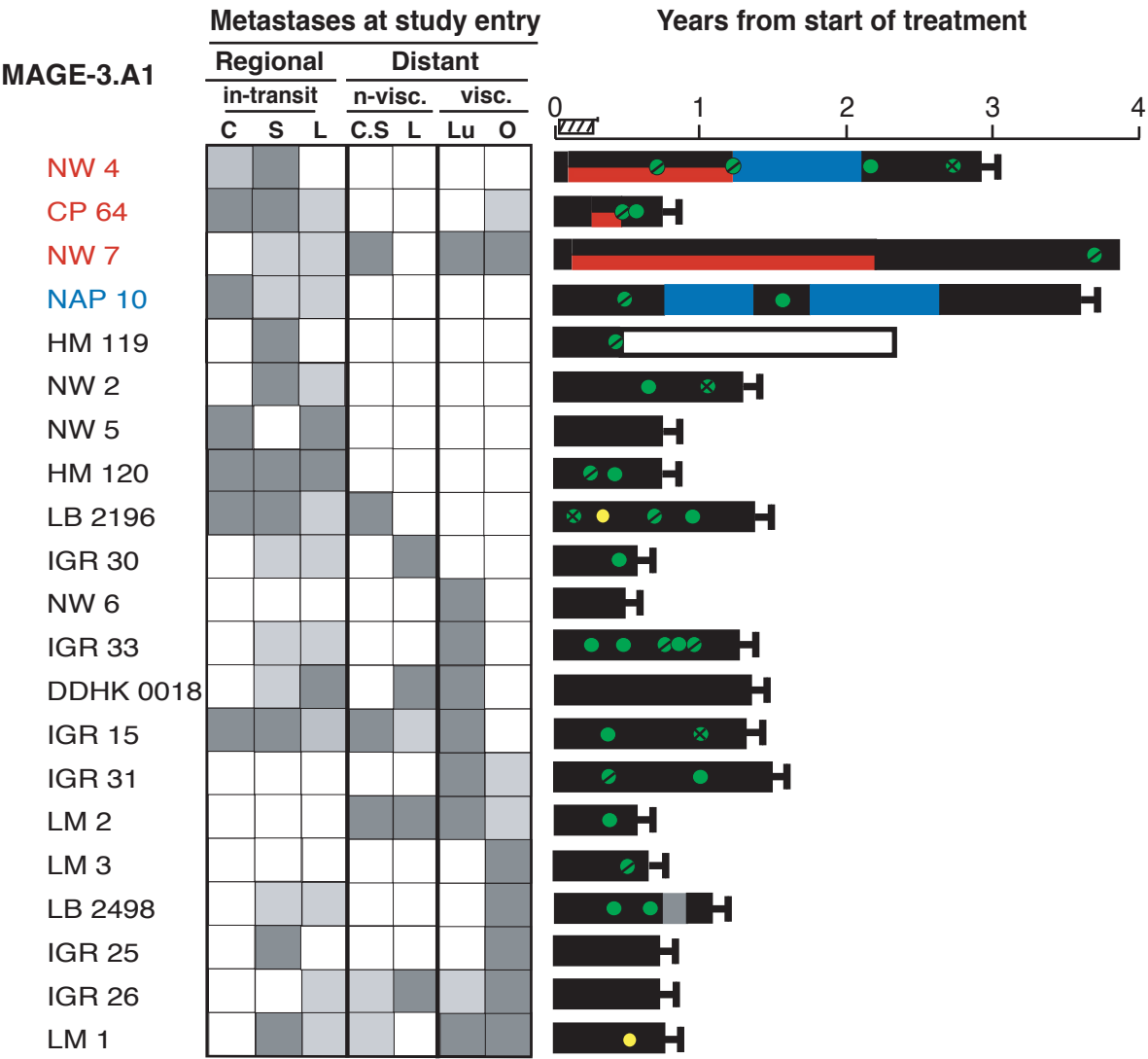
Seventeen patients, 6 with stage III and 11 with stage IV melanoma, were withdrawn from the study before they received all 9 immunizations, because tumor progression or relapse necessitated other treatments. Among them, were the 2 patients who received the MAGE-3.DP4 peptide alone.

Toxicity

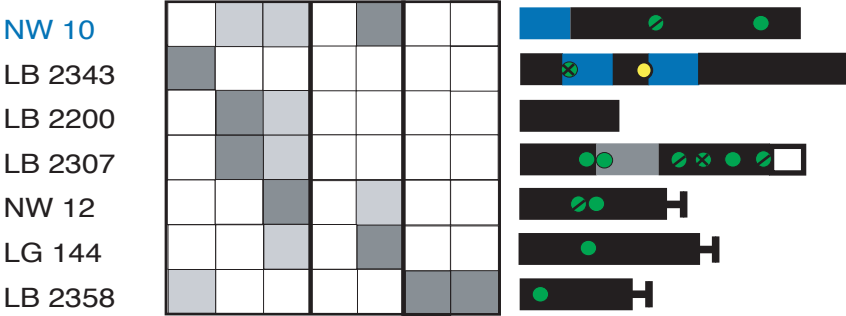
Treatment with frequent id/sc MAGE-3A1 and/or MAGE-3.DP4 peptide(s) injections were well tolerated, without severe toxicities (grade 3 or 4).

One patient who received the MAGE-3.A1 peptide had grade 2 pain at the site of cervical lymph node metastases in the days following vaccinations. Grade 1 or 2 local reaction at injection sites or flu-like symptoms were reported in 11 patients. There was no correlation between the intensity of

Outcome of the 28 melanoma patients who received at least 9 vaccinations



MAGE-3.A1 + MAGE-3.DP4



adverse reactions neither with the number of peptides received, nor with the number of vaccinations. Two early deaths were reported within the 6-week period following the last vaccination, that were both caused by cancer progression. No other serious adverse event was reported.

Tumor response

Summary of tumor response

No objective tumor response was observed. Mixed response was observed in a minority of patients, suggesting a limited effect of this vaccine.

Among the 21 patients who received the MAGE-3.A1 peptide alone, 3 had a MxR and 1 had SD for a 4-month period. Among the 7 patients who received both peptides, no OR or MxR was observed, but 1 patient had a SD for a 5-month period (*Fig. 22*).

Detailed narratives of responder patients

Patient NW 4 displayed complete regression of 4 subcutaneous in-transit metastases and partial regression of a regional metastatic lymph node. However, distant metastases appeared and progressed under treatment (MxR) (*Fig. 22*).

Patient CP 64 had several cutaneous metastases at study entry, one of them being a large tumor 10 cm in diameter in the cervical region. He received 15 injections of MAGE-3.A1 peptide, over a period of 5 months. The size of the cervical tumor first increased to 20 cm in diameter and then clearly decreased to reach a volume reduction of about 2-fold. At the same time, cutaneous and visceral metastases appeared and progressed steadily and the patient died from tumor progression 10 months after the first vaccination (MxR) (*Fig. 23*). The analysis of the anti-MAGE-3.A1 CTL response of this patient is presented in the next section (*Fig. 24*).

Patient NW 7 experienced complete regression of a single small lung metastasis of 12 mm diameter and complete or partial regression of several subcutaneous in-transit metastases, while an ovarian metastasis appeared and was resected during treatment (MxR) (*Fig. 22*).

Analysis of the anti-MAGE-3.A1 CTL response

A new approach with improved sensitivity was used to detect low-level specific CTL responses. We used the MAGE-3.A1 peptide to stimulate pre- and post-vaccination PBMC cultured in limiting dilution conditions, followed by HLA/peptide tetramer analysis, cloning of the tetramer-positive cells and TCR sequence analysis of the CTL clones that showed strict lytic specificity for the MAGE-3.A1 antigen (*Section II 4.4.1, method C*). This tetramer assay test was performed on PBMC collected from 10 patients, i.e. the 3 regressors and 7 non-regressors.

Figure 23

Patient CP64

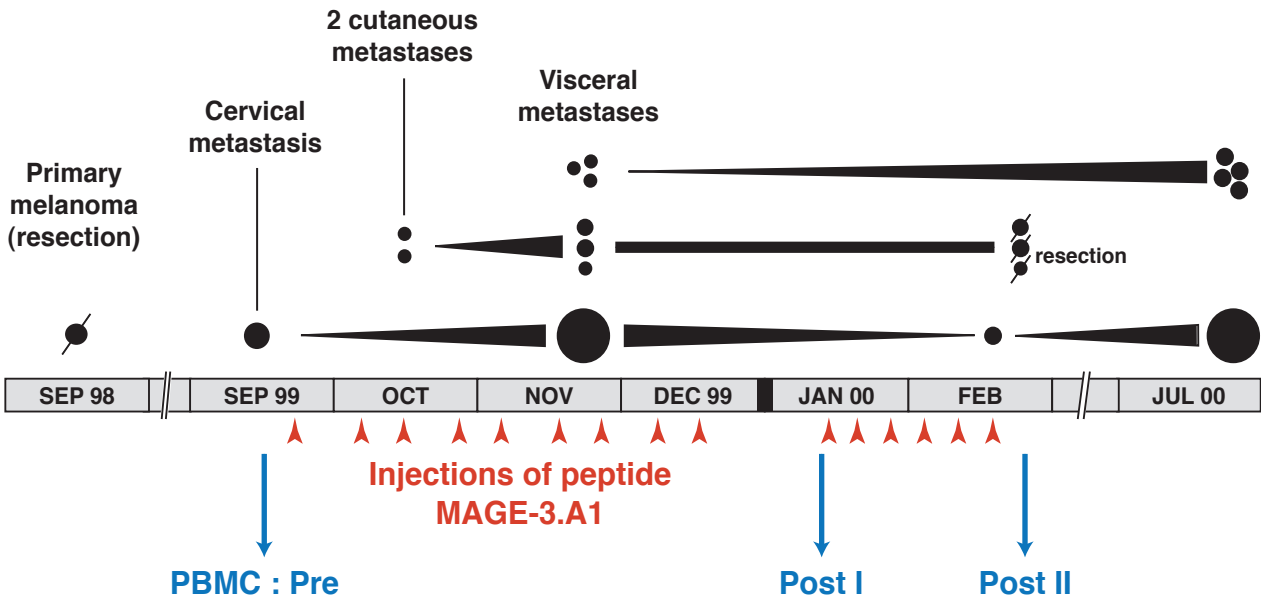
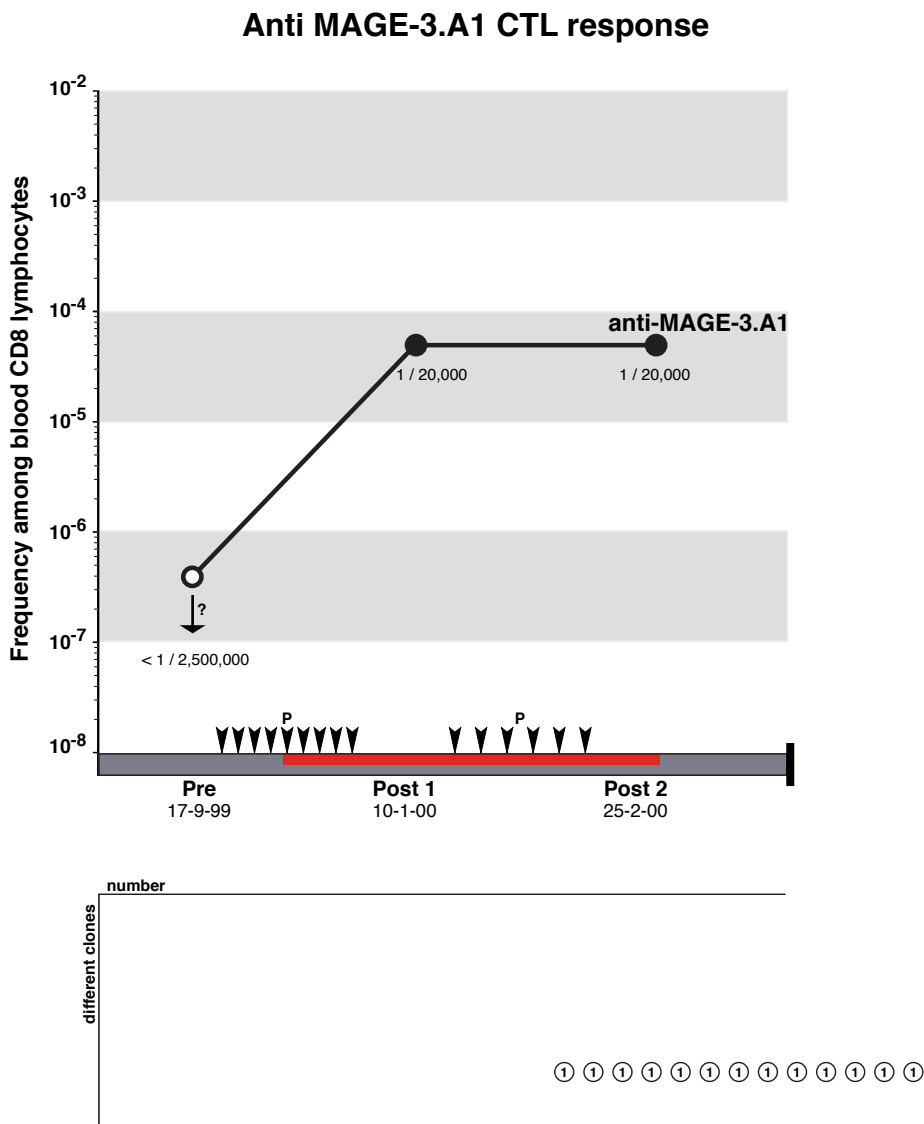


Figure 24

Patient CP64



A significant increase in CTL precursor (CTLp) frequency against the MAGE-3.A1 antigen was only observed in Patient CP 64 (*Fig. 23*). The frequency of precursors of tetramer-labeled cells (TETp) increased from $< 1.3 \times 10^{-6}$ of CD8⁺ cells before vaccination to about 2×10^{-5} after vaccination (*Fig. 24*). We analyzed the TCR V α and V β gene expression of 15 CTL clones derived from cells stained with the A1/MAGE-3 tetramer. All these anti-MAGE-3.A1 clones expressed identical V α and V β gene products, indicating that the anti-MAGE-3.A1 CTL response that we had detected was monoclonal (TCR 48). Direct evaluation of the frequency of TCR 48 among blood lymphocyte by PCR showed a frequency of T cells expressing TCR 48 of $< 4.10^{-7}$ and 5.10^{-5} among pre- and postimmune CD8⁺ cells, respectively (205). These results prove that vaccines containing only a tumor-specific antigenic peptide can elicit a specific CTL response in the presence of a large tumor mass. However, this CTL response was not followed by a significant clinical benefit. The possible causes of treatment failure are discussed in *Section IV. 2.2*.

The same approach was applied to the 2 other patients who had MxR after vaccination with the MAGE-3.A1 peptide and to 7 patients whose tumor progressed under vaccination. None of these 9 patients developed a specific anti-MAGE-3.A1 CTL response.

Conclusion

Increasing the frequency of vaccinations with the MAGE-3.A1 peptide, or adding the HLA class II-restricted peptide MAGE-3.DP4, is unlikely to improve clinical and immunological responses, as compared with monthly MAGE-3.A1 vaccinations. Therefore, enrolment of new patients was stopped.

III. 1.1.5 MAGE-3.A1 peptide in disease-free melanoma patients at high risk of relapse

Study LUD 01-006 a

Determination of MAGE-3.A1 peptide-induced immune response in patients with resected primary or regional melanoma at high risk of relapse.

DESIGN SUMMARY

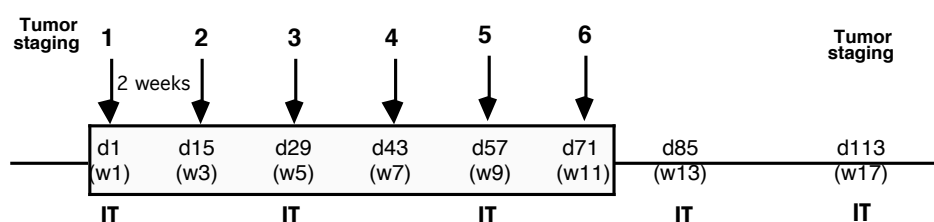
Immunization: 300 µg MAGE-3.A1 peptide **40% s.c. at 2 sites** **(distant from tumor)**
10% i.d. at 2 sites

Patients: 21 HLA-A1 or B35 melanoma patients without measurable disease

Group 1 : 7 non-metastatic patients (T2-4, N0), no RT-PCR analysis of the tumor

Group 2 : 7 patients with regional metastases (N1-3), tumor positive for MAGE-3

Group 3 : 7 patients with regional metastases (N1-3), tumor negative for MAGE-3



IT: blood collection for immunological tests

Coordinating investigator: Nicolas van Baren

Clinical sites:

- Cliniques Universitaires Saint-Luc, UCL, Brussels
- Krankenhaus Nordwest, Frankfurt am Main

Principal investigators:

N. van Baren, V. Brichard
E. Jäger

Figure 25

A. Eligibility relative to maximum extent of melanoma according to AJCC 2002 staging

PRIMARY TUMOR Breslow ≤1.0 mm	PRIMARY TUMOR Breslow 1.01-2.0 mm	PRIMARY TUMOR Breslow 2.01-4.0 mm	PRIMARY TUMOR Breslow >4.0 mm	1 REGIONAL LYMPH NODE METASTASIS a: micro b: macro	2 OR 3 REGIONAL LYMPH NODES METASTASES a: micro b: macro	REGIONAL SKIN METASTASES (IN-TRANSIT)	≥4 REGIONAL LYMPH NODES OR REGIONAL SKIN & LYMPH NODE METASTASES	DISTANT SKIN OR LYMPH NODE METASTASES	LUNG METASTASES	OTHER VISCERAL METASTASES OR ANY DISTANT METASTASIS AND ELEVATED LDH
T1	T2	T3	T4	N1	N2	N2c	N3	M1a	M1b	M1c
NO	YES	YES	YES	YES	YES	YES	YES	NO	NO	NO

without evidence of disease

B. Accrual

Tumor type	Included	Treated	
		Completed protocol	Removed
Melanoma	8	7	1

Objectives

The MAGE-3.A1 peptide was injected in the adjuvant setting to HLA-A1 or HLA-B35 patients with resected primary or regional melanoma at high risk of relapse, to see whether CTL responses can occur at high frequency in patients who have no evidence of disease after surgery. In these patients, potential immune tolerance induced by repetitive contacts between tumor cells and the immune system is less likely to happen than in patients with more advanced disease. The CTL responsiveness to the vaccine may be increased at earlier stages of the disease, either because a smaller tumor burden has caused less tolerization against tumor antigens, or because the tumor does not express the genes encoding these antigens at that stage. The CTL response could also be influenced by the expression status of the appropriate antigen by the tumor (*MAGE-3* positive versus *MAGE-3* negative).

Treatment schedule

The study treatment comprised 6 vaccinations at 2-week intervals. Upon each vaccination day, a total dose of 300 μg of MAGE-3.A1 peptide was divided between 2 i.d. sites (2 x 30 μg) and 2 s.c. sites (2 x 120 μg) (*design summary*).

Eligibility criteria

Eligibility criteria relative to tumor type and stage are defined in *Fig. 25 A*. Only patients with no evidence of disease were eligible.

Patients' characteristics

From December 2001 to September 2003, 8 patients enrolled in this study received the MAGE-3.A1 peptide (*Fig 25 B*). One patient had a stage II- and 7 had a stage III, surgically resected melanoma with no evidence of disease at study entry. There were 2 males and 6 females. The median age was 57 years (range: 40 to 72 years).

One patient was withdrawn before she completed the study treatment because of tumor relapse necessitating another treatment. Seven patients completed the cycle of 6 vaccinations and were evaluable for the CTL response. Their clinical outcome is given in *Fig. 26*.

Toxicity

The tolerance to the treatment was excellent. Four patients had mild (grade 1) inflammatory reaction at the injection sites. No serious adverse event was reported.

Analysis of the anti-MAGE-3.A1 CTL response

No CTL response was detected by our tetramer assay in these 7 patients, including 4 patients with a resected tumor that did not express *MAGE-3* and who are assumed to be immunologically naïve (*Section II. 4.4.1, method C*).

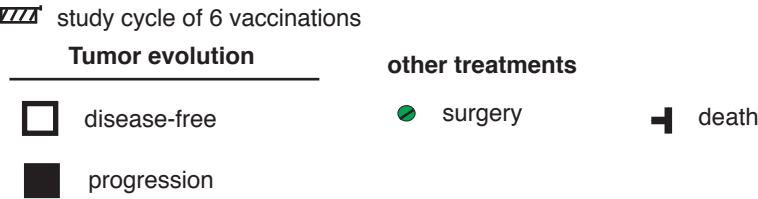
Outcome of the 7 disease-free melanoma patients
who received at least 6 vaccinations



Patients are classified according to their AJCC stage at study entry.

Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.

n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization



Conclusions

This clinical trial was designed to test the hypothesis that patients with primary or regional metastatic cutaneous melanoma, with no evidence of disease though a high risk of relapse, have a higher probability to mount an anti-MAGE CTL response of greater amplitude, as compared with the patients with detected metastases who had been previously investigated. The negative CTL results make this hypothesis very unlikely. Therefore, the trial was closed to accrual earlier than foreseen.

III. 1.2 Immunization with other MAGE-encoded single peptides

In addition to our clinical trials with the MAGE-3.A1 peptide, we also examined whether immunization with other class I restricted *MAGE*-encoded peptides could be of therapeutic benefit to cancer patients when they were administered alone, or combined with another MAGE peptide, or with an immunological adjuvant.

The following peptides were used:

<i>Name</i>	<i>AA sequence</i>	<i>AA position</i>	<i>Reference</i>
MAGE-1.A1	E A D P T G H S Y	161-169	(110)
MAGE-1.Cw*16	S A Y G E P R K L	230-238	(216)
MAGE-3.A2	F L W G P R A L V	271-279	(133)
MAGE-4.A2	G V Y D G R E H T V	230-239	(213)
MAGE-10.A2	G L Y D G M E H L	254-262	(214)

III. 1.2.1 The MAGE-1.A1 peptide

Study LB 93-001

Immunization with MAGE-1.A1 peptide of HLA-A1 positive cancer patients whose tumors express *MAGE-1* gene.

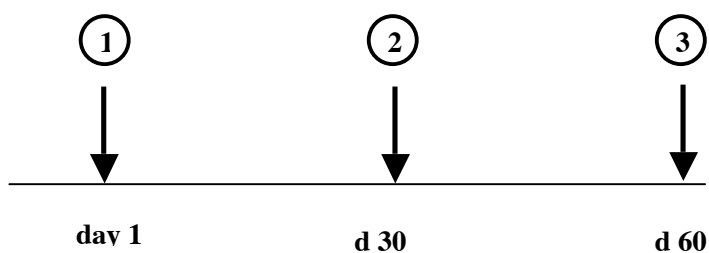
DESIGN SUMMARY

Immunization: MAGE-1.A1 peptide at 1 s.c. site, distant from tumor

2 patients: 30 μ g

If no toxicity: 2 patients: 100 μ g

If no toxicity: other patients: 300 μ g



Coordinating investigator: Marie Marchand

Clinical sites:

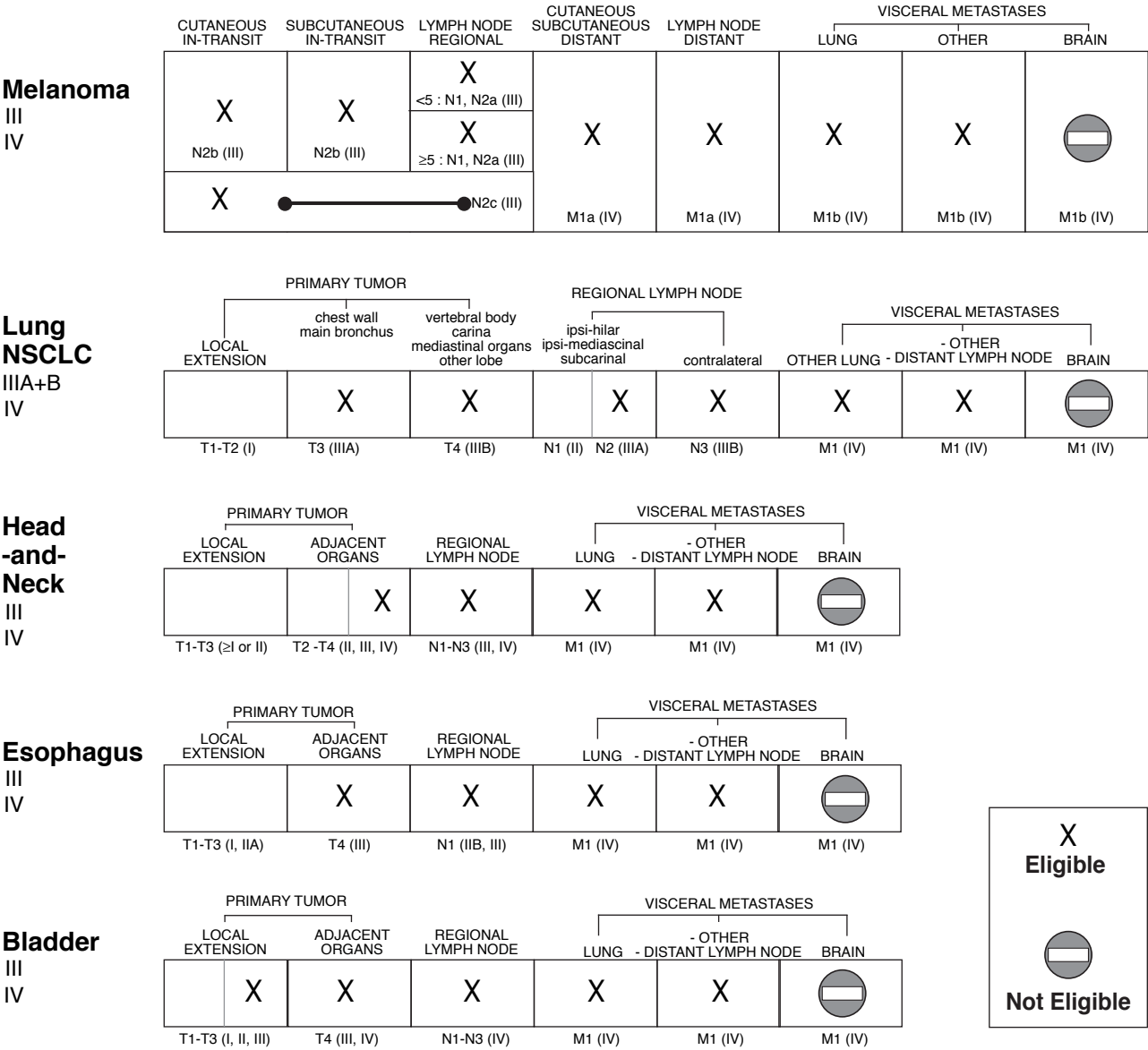
- Cliniques Universitaires Saint-Luc, UCL, Brussels
- Krankenhaus Nordwest, Frankfurt am Main
- Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano
- The Netherlands Cancer Institute, Amsterdam
- Gene Therapy Unit, H. San Raffaele, Milano

Principal investigators:

Y. Humblet
A. Knuth, E. Jäger
G. Parmiani
E. Rankin
V. Russo

Figure 27

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging



with or without evidence of disease

B. Accrual according to tumor type

Tumor type	Included			Treated				
				Completed protocol		Removed		
	Total	Tumor- bearing	NED	Total	Tumor- bearing	Total	Tumor- bearing	NED
	10	9	1	8	8	2	1	1
Melanoma	5	4	1	3	3	2	1	1
NSCLC	4	4	0	4	4	0		
Head & Neck	1	1	0	1	1	0		
Esophagus	0							
Bladder	0							

Objectives

The objectives of this pilot study were to follow the tumor evolution of patients immunized with the MAGE-1.A1 peptide and to define the safety of this vaccine. In a small number of patients, we also examined whether they develop a specific CTL response.

Treatment schedule

The treatment included 3 immunizations with the MAGE-1.A1 peptide at monthly intervals (*design summary*). The peptide was administered at 1 s.c. injection site, distant from the tumor. One patient received 30 μg of peptide per vaccination day, 1 received 100 μg , 6 received 300 μg and 1 patient successively received 3 immunizations with 30 μg and 3 immunizations with 100 μg of peptide.

Three patients, whose tumor evolution did not require another treatment immediately, received additional maintenance vaccinations (4 to 6 vaccinations in total).

Eligibility criteria

Eligibility criteria relative to tumor type and stage are defined in *Fig. 27 A*. Patients with or without detected metastases were eligible.

Patients' characteristics

From October 1993 to September 1994, 10 HLA-A1 patients with stage III or IV tumors that expressed *MAGE-1* were enrolled in the study (*Fig. 27 B*). There were 7 males and 3 females. The median age was 53 years (range: 24 to 69 years).

Out of the 10 patients included in the study, 2 were removed before they completed the cycle of 3 immunizations because of tumor progression or relapse necessitating other treatments.

Eight patients completed the study treatment and were evaluable for tumor response.

Toxicity

The vaccine was extremely well tolerated at all dose levels. In the days following vaccinations, three patients had mild (grade 1) inflammatory reaction at the site of superficial metastases distant from the injection sites. They had received 30, 100 and 300 μg peptide per vaccination, respectively. No other adverse reaction was reported.

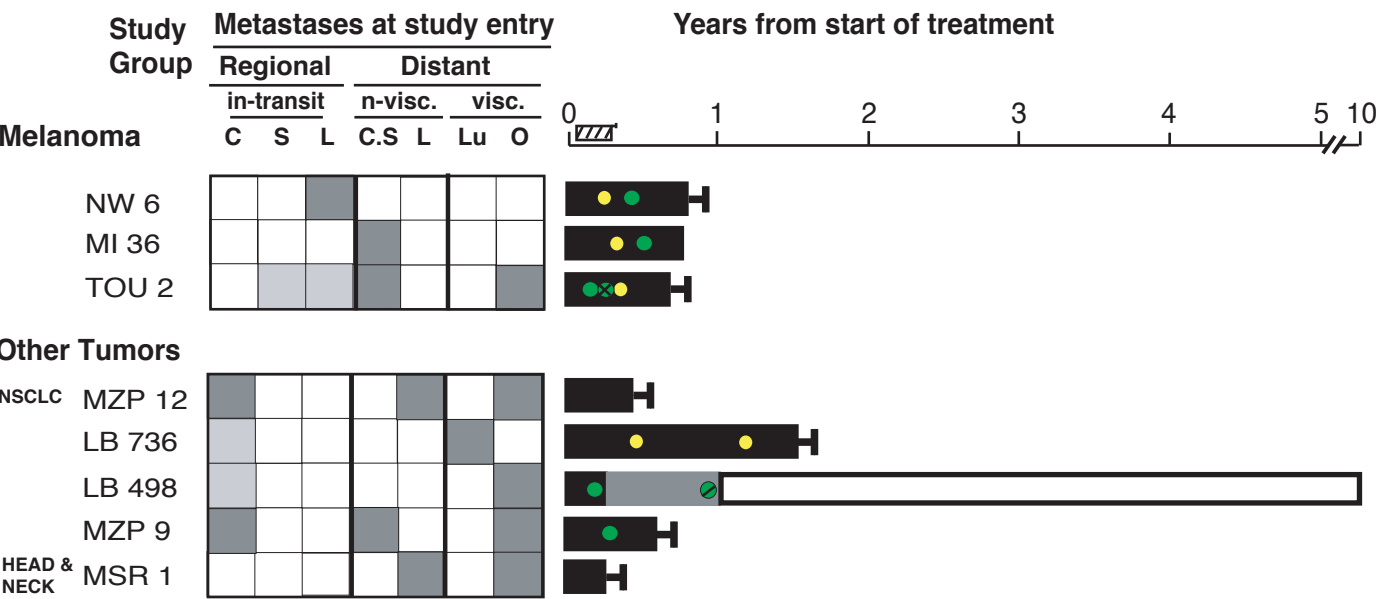
Tumor response

The 8 patients who completed the cycle of 3 immunizations had disease progression (*Fig. 28*).

Figure 28

MAGE-1.A1 peptide (study LUD 93-001)

Outcome of the 8 patients with detectable metastases who received at least 3 vaccinations



Patients are classified according to their AJCC stage at study entry.

Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.

n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization

study cycle of 3 vaccinations

Tumor evolution

upon immunotherapy study treatment

- regression
- progression
- mixed response (prog./reg.)
- stabilization
- disease-free

upon other treatments

- regression upon any other treatment
- disease-free

other treatments

- other vaccine
- chemotherapy
- surgery
- radiotherapy
- death

Analysis of the anti-MAGE-1.A1 CTL response

After 3 immunizations, no CTL response was detected in the PBMC of 3 patients by MLPC stimulation followed by a lysis assay (*Section II. 4.4.1, Method A*). One of them had a regional melanoma and received 100 μ g peptide per immunization. The 2 others had stage IV NSCLC and received 100 or 300 μ g peptide per immunization, respectively.

III. 1.2.2 The MAGE-1.Cw*16 peptide

Study LUD 94-002

Pilot study of immunization with MAGE-1.Cw*1601 peptide in patients with malignant melanoma, non small cell lung cancer, head and neck squamous cell carcinoma or bladder carcinoma.

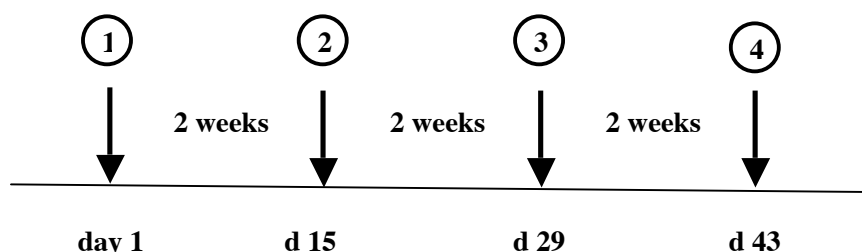
DESIGN SUMMARY

Immunization: MAGE-1.Cw*1601 peptide at 1 s.c. site, distant from tumor

2 patients: 10 μ g

If no toxicity: 2 patients: 30 μ g

If no toxicity: 2 patients: 100 μ g



Coordinating investigator: Marie Marchand

Clinical sites:

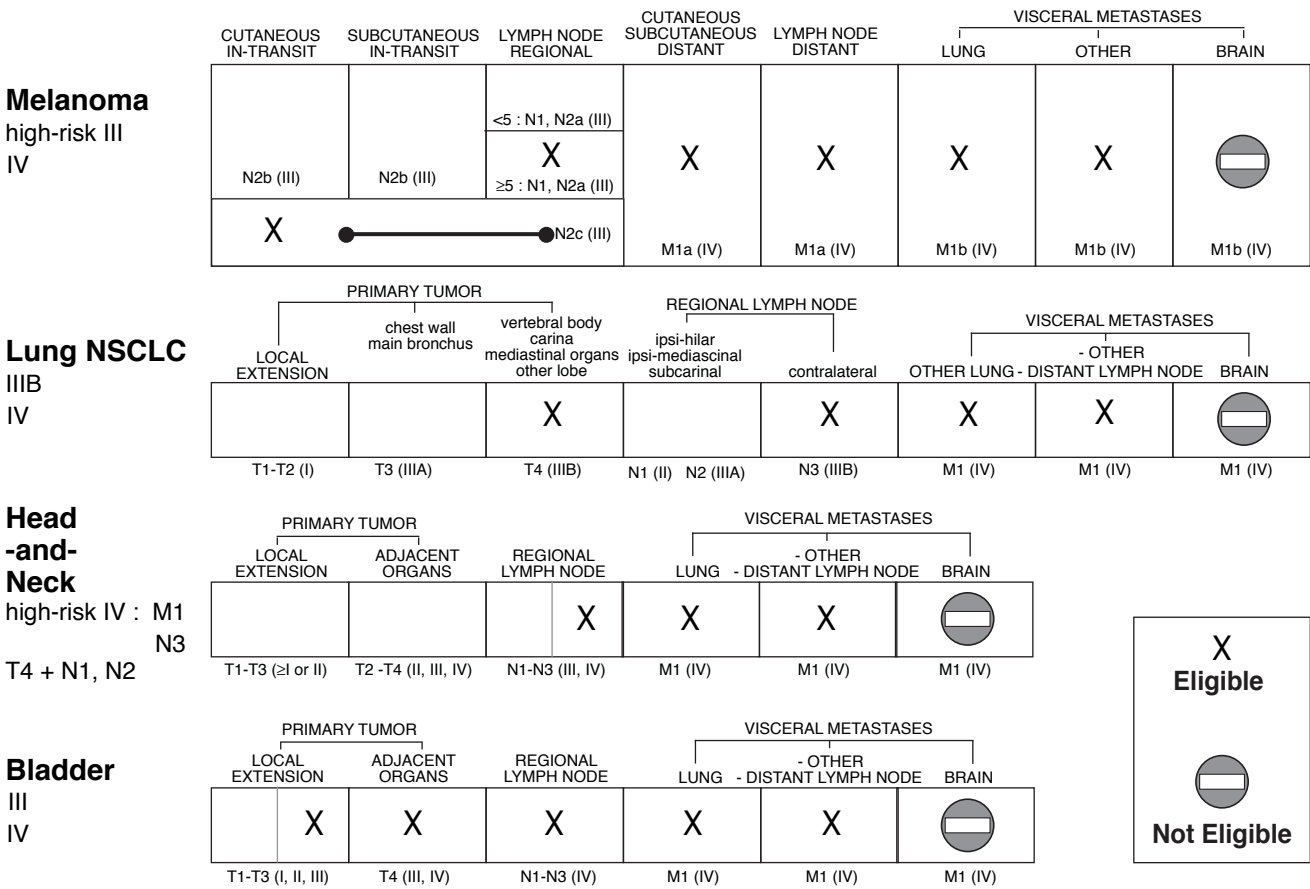
- Cliniques Universitaires Saint-Luc, UCL, Brussels
- Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano

Principal investigators:

Y. Humblet
G. Parmiani

Figure 29

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging



with or without evidence of disease

B. Accrual according to tumor type

Tumor type	Included			Treated	
	Total	Tumor-bearing	NED	Completed protocol	Removed
	3	3	0	2	1
Melanoma	2	2	0	2	0
NSCLC	1	1	0	0	1
Head & Neck	0				
Bladder	0				

Objectives

The objectives of this pilot study were to follow the tumor evolution of patients immunized with the MAGE-1.Cw*16 peptide and to define the safety of this vaccine.

Treatment schedule

The treatment included 4 immunizations with the MAGE-1.Cw*16 peptide at 2-week intervals (*design summary*). The peptide was administered at 1 s.c. injection site, distant from the tumor. Two patients received 10 μ g and 1 patient received 100 μ g peptide on each vaccination day.

One patient, whose tumor evolution did not require another treatment immediately, received 2 additional maintenance vaccinations.

Eligibility criteria

Eligibility criteria relative to tumor type and stage are defined in *Fig. 29 A*. Patients with or without detected metastases were eligible.

Patients' characteristics

From February 1996 to September 1999, 3 HLA-Cw*16 patients, with stage IV tumors that expressed *MAGE-1* were included in the study (*Fig. 29 B*). All three were males. One patient was 72 years old and 2 were 65 years old.

The patient with a NSCLC was removed from the study after 3 immunizations (100 μ g) for progressive disease necessitating another treatment. The 2 melanoma patients completed the study treatment and were evaluable for tumor response.

Toxicity

A mild inflammatory reaction of a cutaneous metastasis distant from the injection site was reported in one melanoma patient. No other adverse reaction was reported in any of the 3 patients.

Tumor response

No objective tumor response was observed.

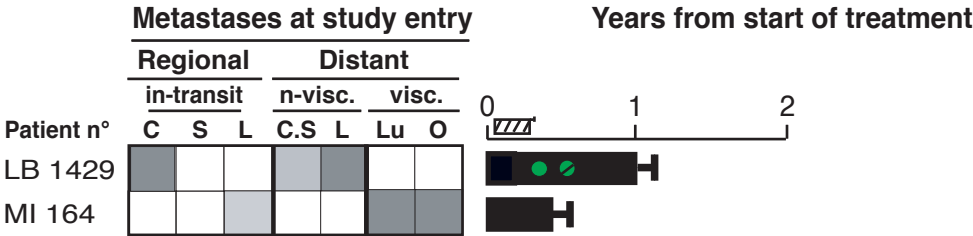
The first melanoma patient, with distant cutaneous and lymph node metastases at study entry, had a 3-month period stabilization of his tumor and the second one, with liver and kidney metastases, had progressive disease (*Fig. 30*).

Because of the infrequent expression of the MAGE-1.Cw*16 antigen in metastatic melanoma (3%) and in other tumors (1.5-2.5%), the accrual was so slow that the study was closed before the foreseen 6 patients had been enrolled.

Figure 30

MAGE-1.Cw16 peptide (study LUD 94-002)

Outcome of the 2 melanoma patients with detectable metastases who received at least 4 vaccinations



Patients are classified according to their AJCC stage at study entry.

Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.

n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization

 study cycle of 4 vaccinations

Tumor evolution

upon immunotherapy study treatment

 progression

other treatments

 chemotherapy

 surgery

 radiotherapy

 death

III. 1.2.3 The MAGE-3.A2 peptide

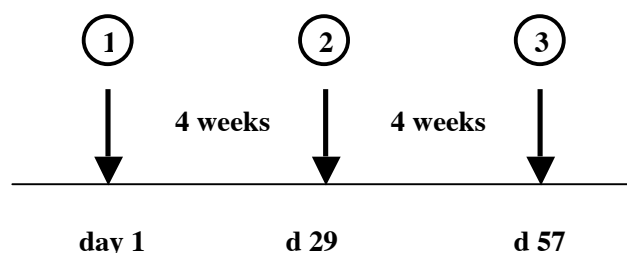
Study LUD 95-004

Pilot study of immunization with the MAGE-3.A2 peptide in patients with malignant melanoma, non small cell lung cancer, head and neck squamous cell carcinoma, esophageal squamous cell carcinoma or bladder carcinoma.

DESIGN SUMMARY

Immunization: 250 μg MAGE-3.A2 peptide	40% s.c. at 2 sites	(distant from tumor)
	10% i.d. at 2 sites	

Patients: 40 including at least 25 melanoma with detected metastasis



Coordinating investigators: Patrick Weynants and Marie Marchand

Clinical sites:

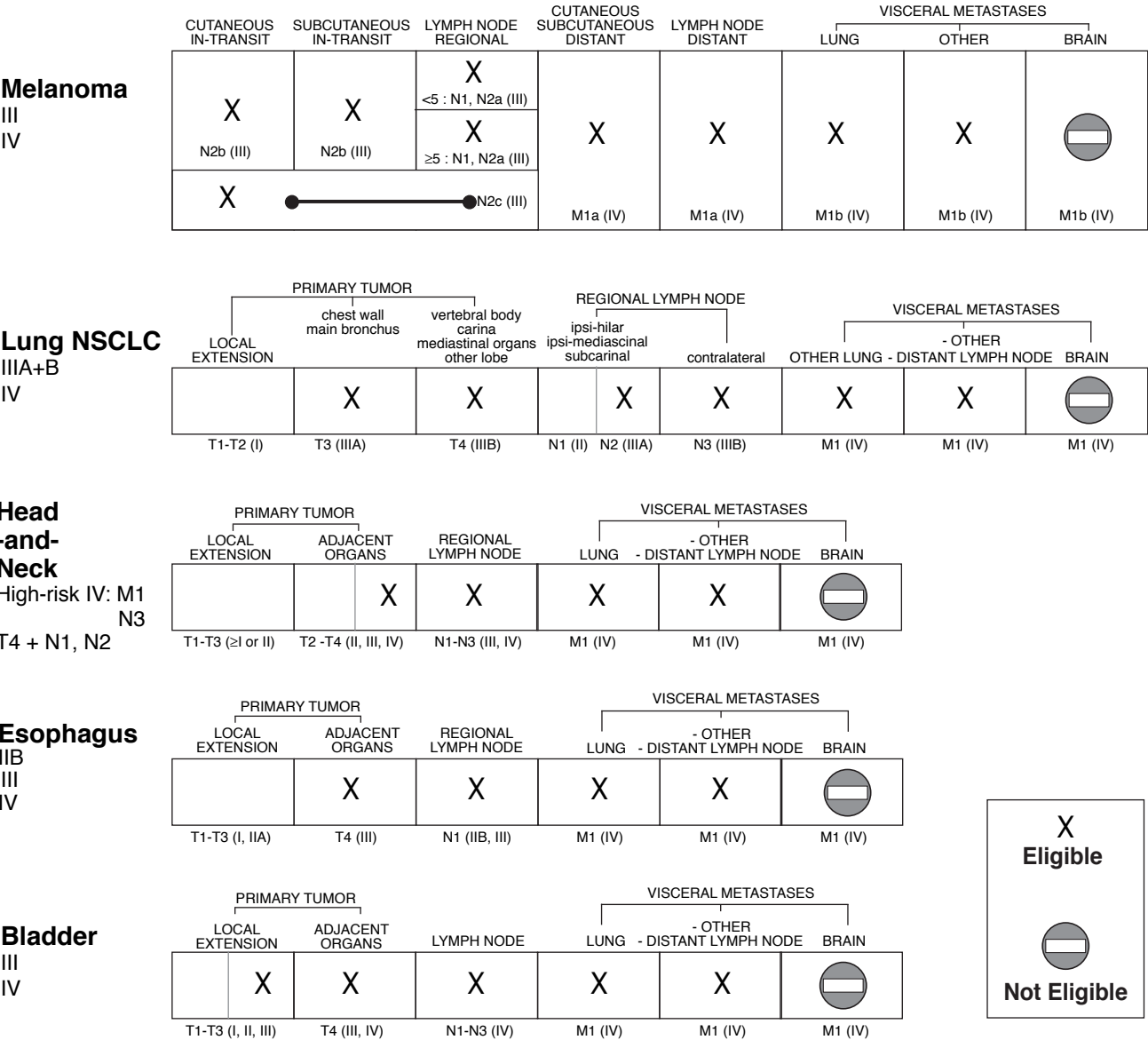
- Cliniques universitaires Saint-Luc, UCL, Brussels
- Institut Paoli-Calmettes & Hôpital Ste-Marguerite, Marseille
- Weston Park Hospital, Sheffield
- Hôpital cantonal universitaire de Genève, Genève
- Daniel den Hoed Cancer Center, Rotterdam
- Hospital Virgen de las Nieves, Granada

Principal investigators:

Y. Humblet
G. Gravis & J.-J. Grob
P. Lorigan, BW. Hancock
P.-Y. Dietrich
W. Kruit, A. Eggermont
S. Serrano Ortega,
M. Lopez Nevot

Figure 31

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging



with or without evidence of disease

B. Accrual according to tumor type

Tumor type	Included			Treated					
				Completed protocol			Removed		
	Total	Tumor-bearing	NED	Total	Tumor-bearing	NED	Total	Tumor-bearing	NED
	39	29	10	30	20	10	9	9	0
Melanoma	35	27	8	26	18	8	9	9	0
NSCLC	3	1	2	3	1	2	0		
Head & Neck	0								
Esophagus	1	1	0	1	1	0	0		
Bladder	0								

Objectives

The objectives of this pilot study were to follow the tumor evolution of patients immunized with the MAGE-3.A2₂₇₁₋₂₇₉ peptide (F L W G P R A L V) and to define the safety of this vaccine (133). In a small number of patients, we also examined whether they develop a specific CTL response.

Treatment schedule

The treatment included 3 immunizations with 250 μ g of the MAGE-3.A2 peptide at 4-week intervals (*design summary*). It was administered at 4 separate injection sites distant from the tumor, by s.c. (2 x 40%) and i.d. (2 x 10%) routes.

Twelve patients whose tumor evolution did not require another treatment, received additional maintenance vaccinations (up to 24 on a 6-year period).

Eligibility criteria

Eligibility criteria relative to tumor type and stage are defined in *Fig. 31 A*. Patients with or without detected metastases were eligible.

Patients' characteristics

Thirty-nine HLA-A2 patients whose tumor expressed *MAGE-3* were included in the study from June 1996 to November 1999 (*Fig. 31 B*). Most of them had stage III or IV cutaneous melanoma. A few patients had other tumor types, i.e. NSCLC or esophageal carcinoma. Twenty-nine patients had metastases and 10 had no evidence of disease at study entry. There were 19 males and 20 females. The median age was 49 years (range: 16 to 83 years).

Thirty patients, including 20 patients bearing metastases, completed the cycle of 3 immunizations (*Fig. 31 B*). The latter were evaluable for tumor response.

Nine patients were withdrawn from the study before they received all 3 immunizations, because tumor progression necessitated other treatments.

Toxicity

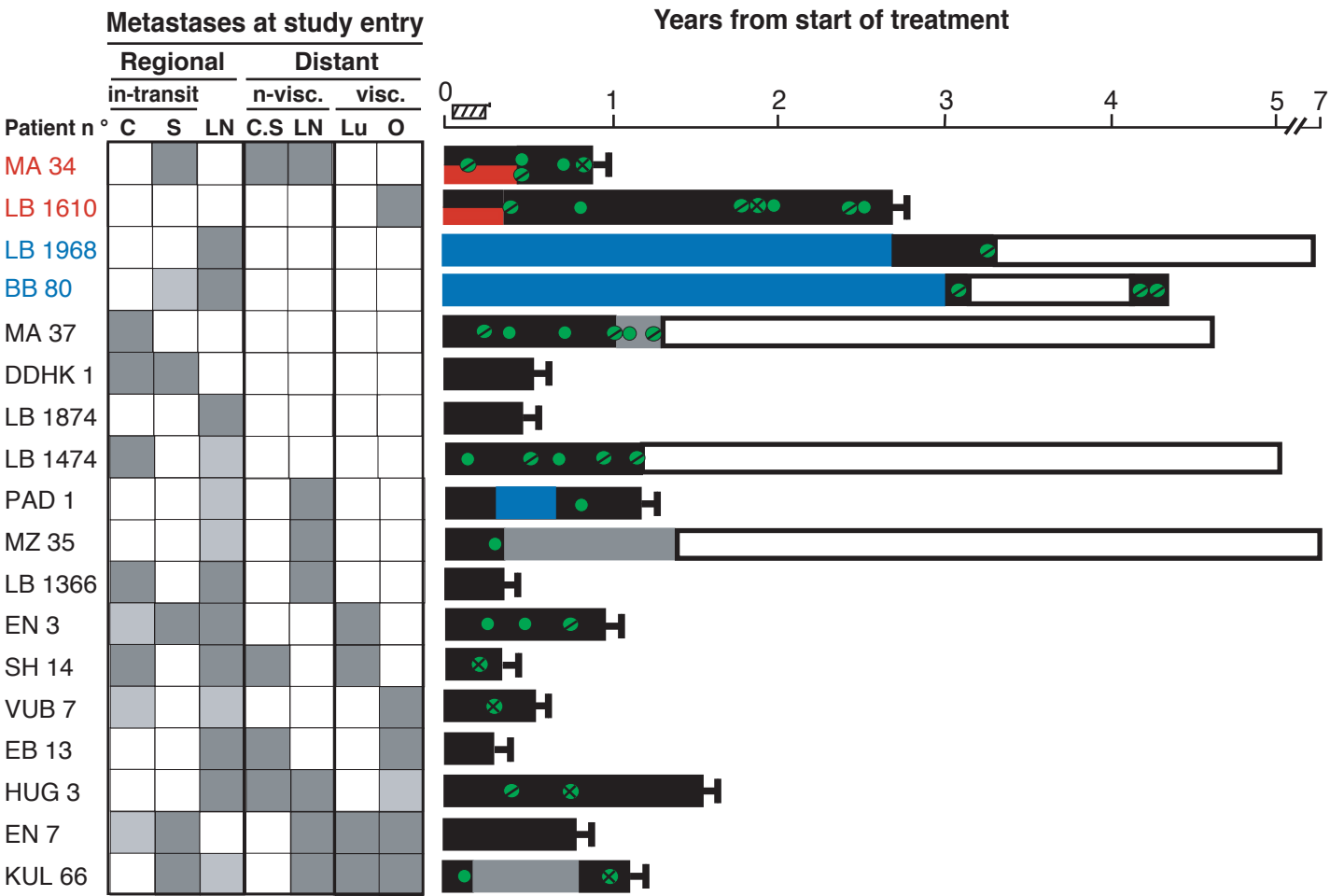
Treatment with id/sc injections of MAGE-3.A2 peptide at 4-week intervals was well tolerated, without treatment-related severe (grade III or IV) adverse events.

Grade 1 inflammatory reaction at injection sites and grade 1 flu-like symptoms were reported in 23 and 5 patients, respectively. Four early deaths were reported within the 6-week period following the last vaccination that were all caused by cancer progression. No serious adverse event related to the vaccine was reported.

Figure 32

MAGE-3.A2 peptide (study LB 95-004)

Outcome of the 18 melanoma patients with detectable metastases who received at least 3 vaccinations



Patients are classified according to their AJCC stage at study entry.
Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.
n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S,
LN = lymph node, Lu = lung, O = other visceral localization

study cycle of 3 vaccinations

Tumor evolution

upon immunotherapy study treatment	upon other treatments	other treatments
regression	regression upon other immunotherapy	other vaccine
progression	regression upon any other treatment	chemotherapy
mixed response (prog./reg.)	mixed response (prog./reg.)	surgery
stabilization	mixed response (prog./reg.)	radiotherapy
disease-free	disease-free	death

Tumor response

Summary of tumor response

No objective tumor response was observed. Six patients had a long-term (> 6 months) stable disease or had no evidence of relapse for more than 6 months after the start of the treatment.

Among the 18 melanoma patients bearing metastases who completed the cycle of 3 immunizations, 2 presented a disease stabilization for 33 and 36 months, respectively and 2 had a mixed response lasting for 5 and 3 months, respectively (*Fig. 32*).

Eight melanoma patients at high risk of relapse had no evidence of disease for a median period of 18 months (range: 1 month to 6.5 years+).

Four patients, 3 with a NSCLC and 1 with an esophageal tumor received at least 3 vaccinations. Two of them had PD after 3 vaccinations and the 2 others, who had no evidence of disease at study entry, relapsed after 10 and 24 months, respectively.

Detailed narratives of responder patients

Patient MA 34 had in-transit subcutaneous metastases at study entry, as well as distant subcutaneous and lymph node metastases. She experienced complete regression of a subcutaneous distant metastasis as well as complete regression of a distant cutaneous metastasis that had appeared, and then disappeared under treatment. However, after 6 vaccinations, a brain metastasis appeared and the patient was removed from the study (MxR).

Patient LB 1610 had a single breast biopsy-proven metastasis of a melanoma measuring 3 cm diameter at study entry. After 3 vaccinations, this lesion had completely disappeared. However, a new lung- and a new distant lymph node metastasis appeared in the mean time (MxR).

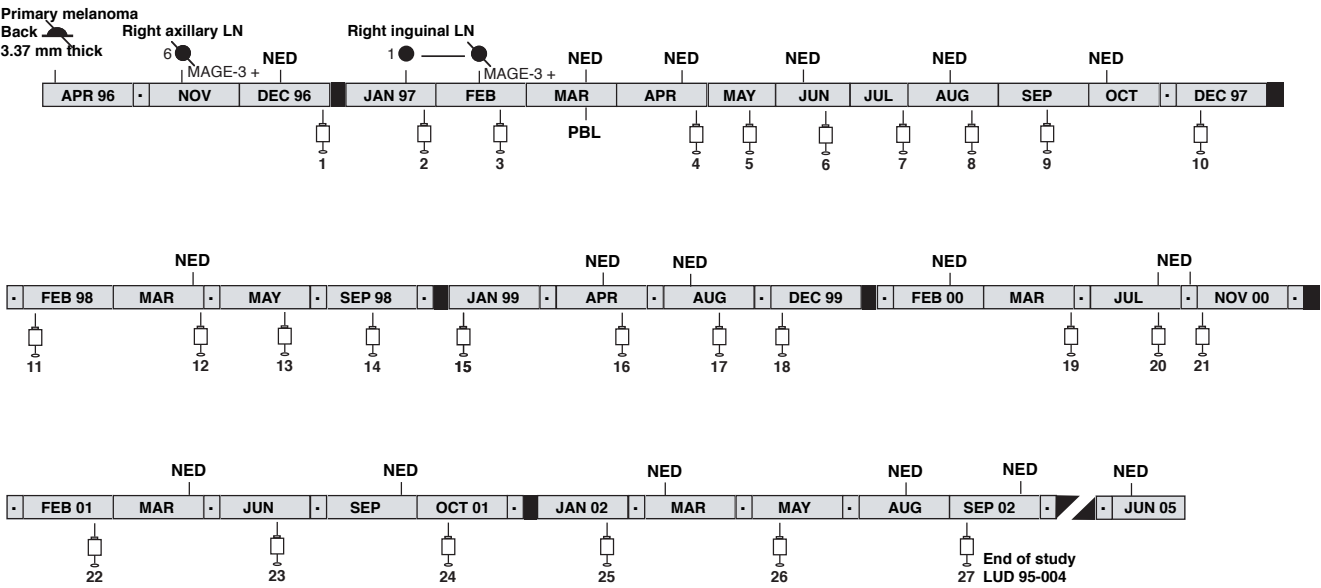
Patient LB 1580, one of the 8 patients with a melanoma at high risk of relapse, developed a regional lymph node metastasis shortly after inclusion. After excision of the adenopathy, vaccinations were pursued. She received the 27 vaccinations scheduled by the protocol on a 6-year period. More than 8 years after the start of treatment, she had not experienced tumor relapse (*Fig. 33*).

Detection of a CTL response against the MAGE-3.A2 antigen

Immunological tests, i.e. MLPC or enzyme-linked immunospot (ELISPOT) were performed in 10 patients: 6 had metastases at study entry and experienced tumor progression during treatment, whereas 4 had no evidence of disease at study entry and had no tumor relapse for several months. No specific cytolytic T-lymphocytes could be demonstrated in these 10 patients.

Figure 33

Patient LB 1580



LEGEND

cutaneous tumor

subcutaneous tumor

lymph node (LN) or visceral metastasis

new, stable or progressing

regressing

no longer detectable

surgical removal

Monthly injections of MAGE-3.A2 peptide (250 μg - subcutaneously and intradermally)

MAGE-3 +: expression of gene MAGE-3 (RT-PCR); MAGE-3 -: no expression

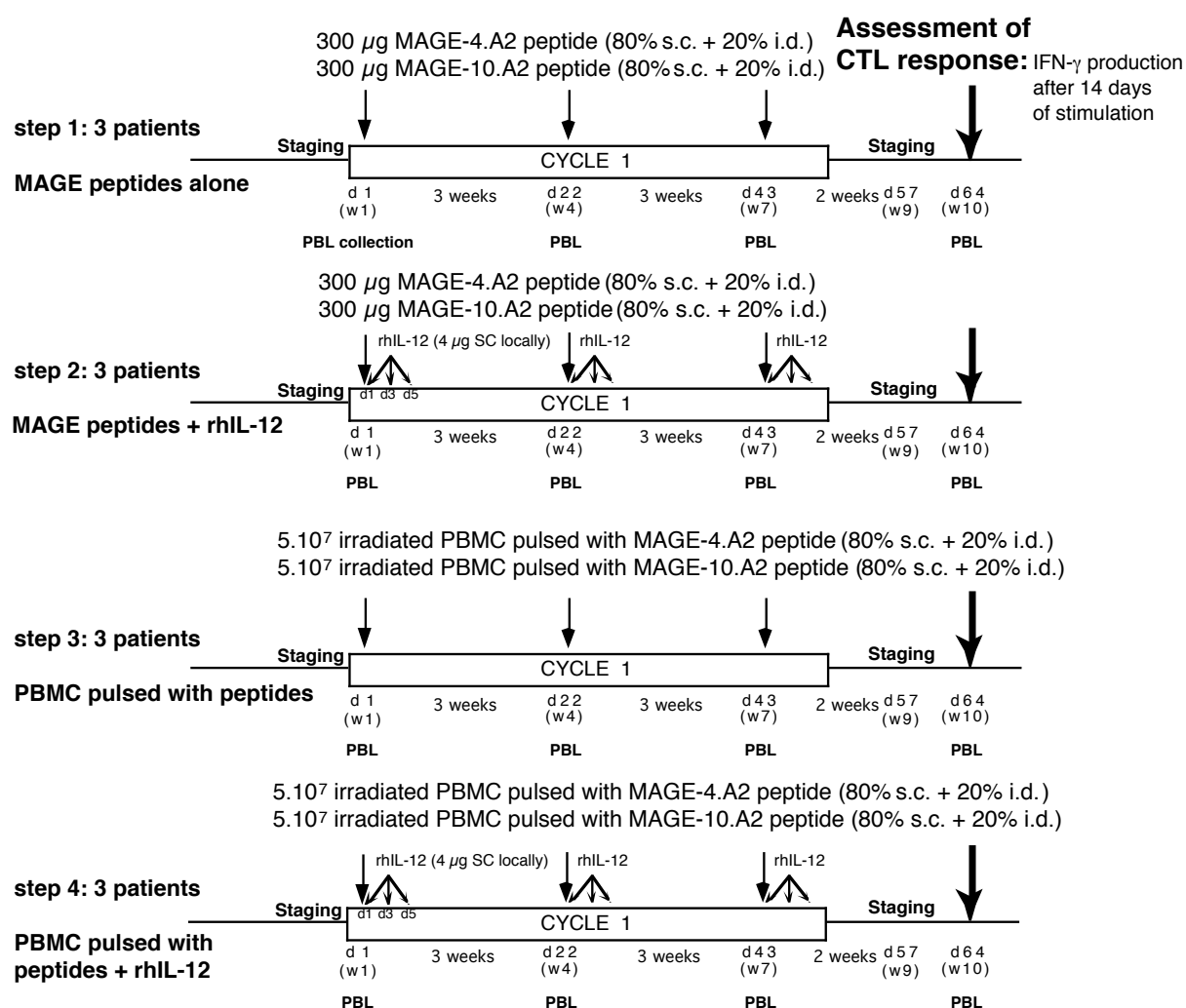
NED: No evidence of disease

III. 1.2.4 The MAGE-4.A2 and MAGE-10.A2 peptides

Study LUD 99-006

Phase I study of Immunization with autologous PBMC pulsed with the MAGE-4.A2 and MAGE-10.A2 peptides in combination with low-dose recombinant human Interleukin-12 in patients with advanced malignant disease

DESIGN SUMMARY



Coordinating investigator: Nicolas van Baren

Clinical site:

Cliniques universitaires Saint-Luc, UCL, Brussels

Principal investigators:

Y. Humblet, V. Brichard

Rationale and objectives

An efficient way to induce a CTL response against a tumor antigen in experimental mouse models is to inject autologous DCs loaded with the corresponding peptide. As compared with the free peptide, the use of DCs increases the immunogenicity by protecting the peptide from endogenous proteases, by providing costimulatory signals that may be crucial for the induction of a primary CTL response and by releasing IL-12. IL-12 is a heterodimeric glycoprotein (gp) that appears to play a critical role in both the initiation and maintenance of cellular immune responses. It is also secreted by monocytes, macrophages and B cells. Activities include induction of IFN- γ production by Natural Killer- and T cells, enhancement of their cytolytic activity, induction of the proliferation of CD4⁺ and CD8⁺ cells and differentiation of Th1 cells from uncommitted T cells. In murine models, IL-12 suppressed tumor growth *in vivo*, an effect mediated by CD8⁺ cells. Thus, rhIL-12 may have therapeutic benefit in diseases such as viral infections, diseases caused by other intracellular pathogens and cancer, either alone or as an adjuvant in combination with an immunogen (227). PBMC are much easier to obtain than dendritic cells and could have similar immunogenic potential provided exogenous IL-12 is added. In a murine model, we found that immunization with PBMC pulsed with the P1A peptide in the presence of IL-12 was a potent inducer of CTL responses against the P1A tumor antigen, thus bypassing the need to use DCs (228). To test this approach in human melanoma, Gajewski and colleagues designed a pilot clinical trial where 15 HLA-A2 patients with metastatic melanoma were immunized by s.c. injections of autologous irradiated PBMC pulsed with either the MAGE-3.A2₂₇₁₋₂₇₉ peptide or the Melan-A.A2₂₇₋₃₅ peptide (229). They received 3 immunizations at three-week intervals. Four patients received the PBMC without IL-12 and 3, 4 and 4 patients received the PBMC with respectively 30, 100 and 300 ng/kg of IL-12, injected s.c. on day 1 (together with the vaccine) and on days 3 and 5, close to the PBMC injection sites. No severe toxicity was observed in the patients receiving 0, 30 or 100 ng/kg of IL-12, whereas the 4 patients receiving the 300 ng/kg dose experienced significant fatigue, fever and/or neutropenia. One patient in the first group had a partial response after vaccination. One patient in the second group had a mixed response and one had a minor response. In the third group, two mixed responses and one complete response (lung metastasis) were observed. The 4 patients in the last group had progressive disease. Three of the responses occurred after Melan-A vaccination and the 3 others after MAGE vaccination. Among the 13 patients evaluable for immunological response, a significant CTL response was observed in 1 of the 4 patients without IL-12, in 3 of 3 patients with low dose IL-12, in 3 of 3 patients with intermediate dose IL-12 and in 1 of 3 patients with high dose IL-12. Five of the 6 patients with objective or mixed tumor response had a detectable CTL response. The 6th patient was not evaluable.

We wanted to see whether the encouraging results obtained by Gajewski and colleagues could be reproduced with 2 naturally well-processed tumor antigens recognized by T lymphocytes, i.e. MAGE-

Figure 34

A. Eligibility relative to maximum extent of disease according to AJCC 1997 staging

Melanoma
advanced stage III
stage IV

CUTANEOUS IN-TRANSIT	SUBCUTANEOUS IN-TRANSIT	LYMPH NODE REGIONAL	CUTANEOUS SUBCUTANEOUS DISTANT	LYMPH NODE DISTANT	VISCERAL METASTASES		
		<5 : N1, N2a (III)			LUNG	OTHER	BRAIN
N2b (III)	N2b (III)	X	X	X	X	X	
		≥5 : N1, N2a (III)					
X ●————●			M1a (IV)	M1a (IV)	M1b (IV)	M1b (IV)	M1b (IV)

with or without evidence of disease

X



Eligible

Not Eligible

B. Accrual per treatment step

Treatment step	Included			Treated	
	Total	Tumor- bearing	NED	Completed protocol	Removed
	5	5	0	5	0
1	3	3	0	3	
2	2	2	0	2	
3	0				
4	0				

4.A2 and MAGE-10.A2, that are expressed in approximately 13% and 21% of metastatic cutaneous melanoma, respectively (213, 214).

Treatment schedule

We designed a 4-step pilot study with 3 melanoma patients in each step, who were planned to be immunized with MAGE-4.A2 and MAGE-10.A2 peptides (step 1); the same peptides combined with low dose rhIL-12 (Genetics Institute, Munich, Germany) (step 2); autologous PBMC pulsed with both peptides (step 3); or PBMC pulsed with both peptides, combined with low dose rhIL-12 (step 4) (*design summary*).

The treatment included 3 immunizations at 3-week intervals. In step 1, 300 μ g of each peptide were administered at 2 injection sites (80% s.c. and 20% i.d.). In step 2, in addition to the peptides, patients received local s.c. injections of rhIL-12 (4 μ g), starting the day of each peptides vaccination and then twice at 48h intervals.

Eligibility criteria

Eligibility criteria relative to tumor stage are defined in *Fig. 34 A*. Patients with or without evidence of disease were eligible.

Patients characteristics

From March 2000 to August 2001, 5 HLA-A2 metastatic patients with stage III or IV melanoma that expressed *MAGE-4* and/or *MAGE-10* were included in the study. There were 2 males and 3 females. The median age was 62 years (range: 54 to 81 years).

Three patients were included in step 1 and 2 patients were included in step 2 (*Fig. 34 B*). All five received 3 immunizations and were evaluable for tumor response.

Because of the slow accrual rate, the pharmaceutical company decided to stop providing rhIL-12. The study was therefore closed to accrual before any patient was enrolled in steps 3 and 4.

Toxicity

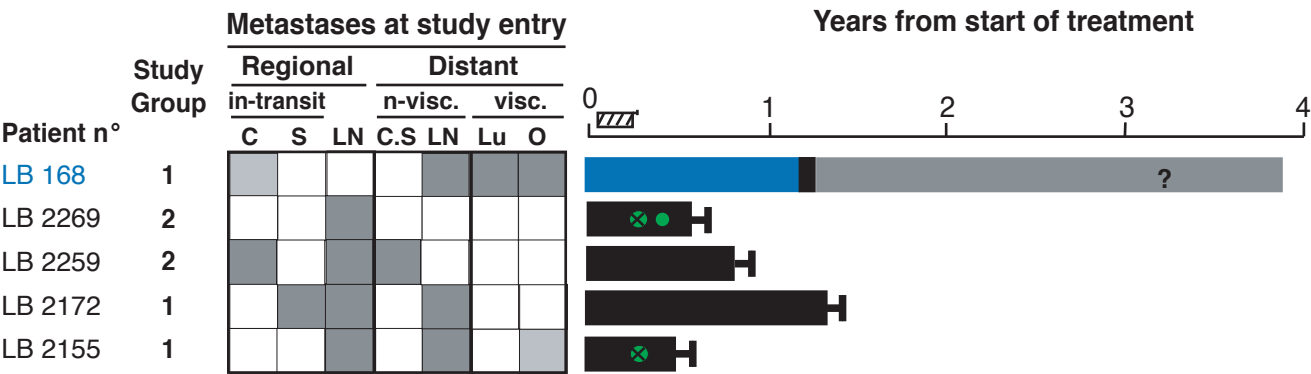
The tolerance to the vaccine was excellent. Two of the 3 patients who received the peptides alone had grade 2 flu-like symptoms after vaccinations (step 1). Of the two patients who received the peptides combined with low dose rhIL-12, one had grade 1 pain at the site of superficial distant metastases, occurring after each vaccination (step 2). No inflammatory reaction at injection sites was reported in any of the 5 patients. Neither treatment-related SAE nor early death were reported.

Tumor response

No objective response was reported (*Fig. 35*). One patient who was included in step 1 had a stabilization of multiple lung, liver and kidney metastases for a 16-month period. This patient

Figure 35 **MAGE-4.A2 + MAGE-10.A2 peptides (study LB 99-006)**

**Outcome of the 5 melanoma patients with detectable metastases
who received at least 3 vaccinations**



Patients are classified according to their AJCC stage at study entry.

Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.

n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization

study cycle of 3 vaccinations

Tumor evolution

upon immunotherapy study treatment

- stabilization
- progression

tumor status unknown

other treatments

- chemotherapy
- surgery
- radiotherapy
- death

received 7 additional vaccinations with both peptides at increasing intervals, until tumor progression occurred. Four patients had PD.

Detection of a CTL response against the MAGE-4.A2 and MAGE-10.A2 antigen

Four patients, including 1 patient who received the peptides combined with low dose rhIL-12, were evaluable for the analysis of the CTL response against the vaccine antigens by Tetramer assay. No CTL response was detected against MAGE-4.A2 or MAGE-10.A2 among the blood CD8⁺ T cells of these patients (*Section II. 4.4.1, Method C*).

Immunization with the MAGE-10.A2 peptide alone

In another study, the MAGE-10.A2 peptide (300 µg) was injected in the adjuvant setting to melanoma patients with resected primary or regional melanoma at high risk of relapse, to see whether CTL responses can occur at high frequency in patients who have no evidence of disease after surgery (*Section III. 1.1.5, study LUD 01-006 a*). The immunization schedule included 6 i.d. and s.c. vaccinations at 2-week intervals.

From December 2001 to September 2003, 11 patients enrolled in that study received the MAGE-10.A2 peptide. One patient had a stage I-, 1 had stage II- and 9 had a stage III, surgically resected melanoma with no evidence of disease at study entry. There were 6 males and 5 females. Their median age was 58 years (range: 39 to 82 years).

The tolerance to the treatment was excellent. One patient had moderate (grade 2) inflammatory reaction at the injection sites and 2 patients had grade 1 Flu-like symptoms.

Two patients were withdrawn before they completed the study treatment because of tumor relapse necessitating another treatment. Nine patients completed the first cycle of 6 vaccinations and were evaluable for the CTL response (*Fig. 36*).

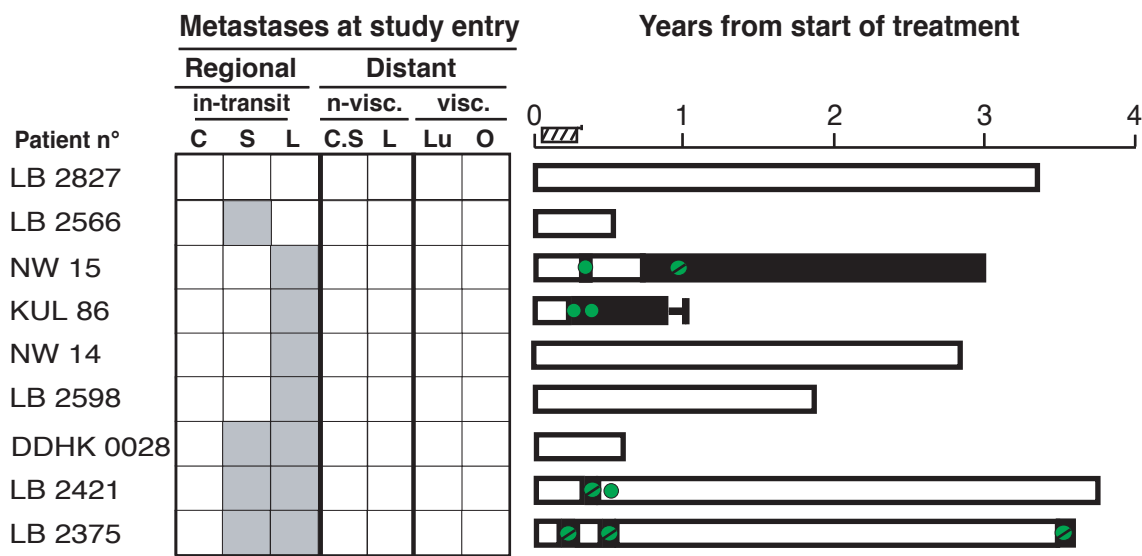
No CTL response was detected by our tetramer assay in these 9 patients, including 4 patients with a resected tumor that did not express *MAGE-10* and who are assumed to be immunologically naïve (*Section II. 4.4.1, Method C*).

This clinical trial was designed to test the hypothesis that patients with primary or regional metastatic cutaneous melanoma, in a disease-free status at high risk of relapse, have a higher probability to mount an anti-MAGE CTL response of greater amplitude, as compared with the patients with active metastatic disease. The negative CTL results make this hypothesis very unlikely. Therefore, the trial was closed to accrual earlier than foreseen.

Figure 36

MAGE-10.A2 peptide (study LUD 01-006 b)

Outcome of the 9 disease-free melanoma patients
who received at least 6 vaccinations



Patients are classified according to their AJCC stage at study entry.
Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.
n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization

study cycle of 6 vaccinations

Tumor evolution



disease-free



progression

other treatments

- chemotherapy
- surgery
- radiotherapy
- death

III. 2 IMMUNIZATION WITH A RECOMBINANT MAGE-3 PROTEIN

Because peptide-based vaccinations did not appear to be very effective, we wished to test potentially more efficient vaccines. Immunizing patients with a recombinant protein containing multiple class I and class II epitopes, which can be recognized by CD8⁺ and CD4⁺ T lymphocytes respectively, could induce simultaneous CD8⁺ CTL and CD4⁺ Th1 cell responses, thereby generating a more effective anti-tumor response.

The DNA recombinant MAGE-3 protein used in the 3 studies presented in this section is a fusion protein with a lipidated protein D derived from *Haemophilus influenzae* at its N-terminus and a sequence of several histidine residues at the C-terminus of the protein (ProtD-MAGE-3-His) (*Section II.8.2, Fig. 8*). The inclusion of the first 109 residues of the protein D as a fusion partner was expected to improve the immunogenicity and to provide the vaccine protein with additional bystander help properties, whereas the inclusion of a His tail facilitated the purification of the fusion protein. The protein was produced in *E. Coli* and extensively purified to eliminate bacterial contaminants.

We tested this protein either alone or in combination with immunological adjuvants, namely AS02B and Adjuvant System 15 (AS15) (*Section II. 8.3*).

III. 2.1 MAGE-3 protein by intramuscular route, mixed with the immunological adjuvant AS02B containing MPL/QS21 in an o/w emulsion

**Study LUD 97-002
EORTC 18972-13971**

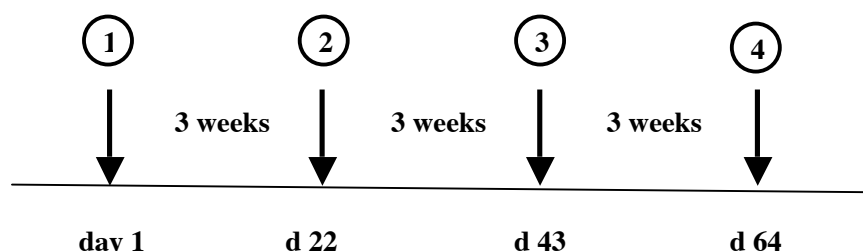
Phase I/Early II study of immunization with a MAGE-3 protein based vaccine plus immunological adjuvant AS02B in HLA class I selected patients with MAGE-3 positive tumors

DESIGN SUMMARY

Immunization: MAGE-3 protein plus adjuvant AS02B at 1 i.m. site, distant from tumor

Treatment groups:	n°1	3 patients:	300 µg protein	without adjuvant
If no toxicity:	n°2	12 patients:	30 µg protein	plus adjuvant
If no toxicity:	n°3	12 patients:	100 µg protein	plus adjuvant
If no toxicity:	n°4	12 patients:	300 µg protein	plus adjuvant

Patients: **39 tumor-bearing patients, including at least 24 melanoma patients**
At least 18 HLA-A1, the others non-HLA-A1 but either HLA-A2 or -B44



With the participation of the EORTC - Melanoma Cooperative Group (MCG)
 - Biological Treatment Development Group (BTDG)
 - New Drug Development Organization (NDDO)

Coordinating investigators: Marie Marchand and Gerit Stoter

Clinical sites:

- Cliniques universitaires Saint-Luc, UCL, Brussels
- University Medical Center St. Radboud, Nijmegen
- Norwegian Radium Hospital, Oslo,
- Institut Gustave Roussy, Villejuif
- Rotterdam Cancer Institute and University Hospital, Rotterdam,
- University Hospital Benjamin Franklin, Berlin
- University Hospital Linköping, Linköping
- Institut Jules Bordet, ULB, Brussels
- University Hospital "Vrije Universiteit", Amsterdam
- Cliniques universitaires UCL de Mont-Godinne, Yvoir
- The Netherlands Cancer Institute, Amsterdam
- LICR/CHUV Lausanne

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Figure 37

A. Eligibility relative to maximum extent of disease according to AJCC 1992 staging

	CUTANEOUS IN-TRANSIT	SUBCUTANEOUS IN-TRANSIT	LYMPH NODE REGIONAL	CUTANEOUS SUBCUTANEOUS DISTANT	LYMPH NODE DISTANT	VISCERAL METASTASES		
						LUNG	OTHER	BRAIN
Melanoma								
III	X	X	X	X	X	X	X	
IV	N2b (III)	N2b (III)	<5 : N1, N2a (III) ≥5 : N1, N2a (III)	M1a (IV)	M1a (IV)	M1b (IV)	M1b (IV)	M1b (IV)
	X							
			N2c (III)					
Lung NSCLC								
IIIA+B								
IV		X	X	X	X	X	X	
	T1-T2 (I)	T3 (IIIA)	T4 (IIIB)	N1 (II)	N2 (IIIA)	N3 (IIIB)	M1 (IV)	M1 (IV)
Head -and- Neck								
high-risk IV : M1 N3			X	X	X	X		
T4 + N1, N2	T1-T3 (≥I or II)	T2 -T4 (II, III, IV)	N1-N3 (III, IV)	M1 (IV)	M1 (IV)	M1 (IV)		
Esophagus								
III								
IV		X	X	X	X	X		
	T1-T3 (I, IIA)	T4 (III)	N1 (IIB, III)	M1 (IV)	M1 (IV)	M1 (IV)		
Bladder								
III								
IV	X	X	X	X	X	X		
	T1-T3 (I, II, III)	T4 (III, IV)	N1-N3 (IV)	M1 (IV)	M1 (IV)	M1 (IV)		

with at least one measurable metastasis

X
Eligible


Not Eligible

B. Accrual according to tumor type

Tumor type	Included	Not treated*	Treated	
			Completed protocol	Removed
	59	2	39	18
Melanoma	51	2	33	16
NSCLC	2	0	1	1
Head & Neck	1	0	1	0
Esophagus	2	0	1	1
Bladder	3	0	3	0

* Two patients were withdrawn before the first vaccination (see text).

Objectives

In order to further improve the immunogenicity of the MAGE-3 protein, AS02B was added to the vaccine. This adjuvant is a mix of the QS21 saponin and of monophosphoryl lipid A (MPL) in an oil/water emulsion (*Section II. 8.3.1*). QS21 is a natural saponine extracted from the bark of the South American tree *Quillaja Saponaria* Molina. It has been shown to activate MHC class I restricted CTLs to several subunit antigens, as well as to stimulate antigen specific lymphocytic proliferation (223). MPL is a compound chemically derived from lipopolysaccharide. Although less toxic, its ability to induce cell-mediated immune responses has been preserved (226). The capacity of AS02B to activate HLA class I restricted T lymphocytes as well as to stimulate antigen specific lymphocytic proliferation for a variety of antigens was demonstrated both in animal models and in clinical trials (230, 231).

In this phase I/II trial, 57 patients with *MAGE-3* positive metastatic cancer, most of them with melanoma, were vaccinated with escalating doses of a recombinant MAGE-3 protein combined with a fixed dose of the immunological adjuvant AS02B, or with the MAGE-3 protein alone (*Table 12*).

Treatment schedule

The vaccination schedule comprised 4 i.m. vaccinations at 3-week intervals (*design summary*) (198). Three escalating dose levels of ProtD-MAGE-3-His (30, 100 and 300 μ g) mixed with a fixed dose of AS02B were tested in 3 groups of patients (groups n° 2, 3 and 4, respectively). For safety reasons, the first 3 patients included in the study received the highest dose of MAGE-3 protein without AS02B (group n° 1). The vaccine was administered in the upper arms or thighs.

Patients whose tumor stabilized or regressed after 4 vaccinations received 2 additional vaccinations at 6-week intervals.

Eligibility criteria

Eligibility criteria relative to tumor stage are defined in *Fig. 37 A (for detailed eligibility criteria, see Annex 3)*. Only patients with detected metastases were eligible. HLA-A1, HLA-A2 or HLA-B44 positive patients with *MAGE-3* positive melanoma, NSCLC, head and neck squamous cell cancer, esophageal squamous cell cancer or bladder cancer were assigned to groups 1 to 4 by the NDDO data center, according to their HLA class I and tumor types. In each of groups 2-4, at least 6 patients with HLA-A1 type as well as 6 patients with melanoma were to be included. Patients removed from the study before having received 4 vaccinations for any other reason than treatment-related toxicity, were replaced.

Table 12 Patients' characteristics

Patients		Tumor type ¹		Class I HLA		Previous treatments	Study treatment					Further treatment	Last Status ⁸		
Gender				A	B		AJCC Stage ³	Study Group	Total numb.	Toxicities ⁵	SAE (n) ⁶	Tumor response ⁷		Tumor status	Survival (months)
Code	Age					Type ²	at entry		injections ⁴	Max. toxicity					
1) Patients having received complete protocol (≥ 4 vaccinations):												n = 39			
Group 1 (300 µg protein alone):												n = 3			
7002	F	30	MELA	A2,A68	B40,B53	Sg, NSI	IV M1a	1	14	1	0	PR	yes	PD	25.5
7023	F	76	MELA	A2, A19	B18	Sg	IV M1b	1	4	1	0	PD	yes	PD	6
7020	M	76	HD.NK	A2,A10	B7,B35	Sg, R, H	IV	1	4	1	2	PD	no	PD	5
Group 2 (30 µg plus AS02B):												n = 14			
7037	F	55	MELA	A1,A2	B8,B44	Sg, R, NSI	III N2c	2	6	2	0	MxR	yes	PD	26
7049	F	55	MELA	A2,A29	B44,B62	Sg, NSI	III N2c	2	4	2	0	MxR	yes	NED	> 7 years
7006	M	58	MELA	A1		Sg, ILP, Ct, NSI	III N2c	2	4	1	0	PD	no	PD	6
7040	F	38	MELA	A2,A3	B7,B62	Sg	IV M1a	2	4	2	1	PD	yes	PD	7.5
7051	M	41	MELA	A1		Sg, Ct, R	IV M1b	2	6	1	0	PD	yes	SD	> 7 years
7036	M	61	MELA	A1		Sg, R, Ct, NSI	IV M1b	2	4	2	0	PD	yes	PD	20.5
7046	M	52	MELA	A2,A24	B7,B62	Sg, H, Ct,NSI	IV M1b	2	12	1	1	PD	yes	PD	> 6 years
7007	M	73	MELA	A2		Sg, R, Ct, NSI	IV M1b	2	4	2	2	PD	no	PD	2.5
7004	F	53	MELA	A2	B44	Sg, Ct, H	IV M1b	2	4	3	0	PD	yes	PD	9
7029	F	47	MELA	A1	B8	Sg, R	IV M1b	2	4	2	0	PD	yes	NED	> 6.5 years
7013	M	47	MELA	A1	B7,B8	Sg, ILP, NSI	IV M1b	2	4	2	0	PD	yes	PD	5.5
7012	M	42	MELA	A2,A29	B7,B51	Sg, R, Ct, NSI	IV M1b	2	4	2	1	PD	no	PD	4
7009	F	49	MELA	A2	B44	Sg, Ct	IV M1b	2	4	3	0	PD	yes	PD	6.5
7028	F	49	NSCLC	A1,A30	B8,B13	Ct	IV	2	4	2	1	PD	yes	PD	5.5
Group 3 (100 µg plus AS02B):												n = 12			
7021	F	71	MELA	A1,A24	B62,	Sg	III N2b	3	4	2	0	PD	yes	PD	5 years
7025	F	62	MELA	A2,A30	B44,B7	Sg, ILP, NSI	IV M1a	3	10	2	0	SD	yes	NED	> 6.5 years
7050	M	59	MELA	A1,A29	B8,B15	Sg, ILP	IV M1a	3	4	3	2	PD	yes	PD	6
7024	M	68	MELA	A2,A24	B55,B61	Sg, R, Ct, NSI	IV M1a	3	4	2	1	PD	yes	PD	16
7019	M	53	MELA	A2,A10	B15,B40	Sg, R, Ct	IV M1a	3	4	3	0	PD	yes	PD	11
7018	M	75	MELA	A2	B44	Sg, Ct, NSI	IV M1b	3	4	2	1	PD	yes	PD	6
7039	F	53	MELA	A1	B44	Sg	IV M1b	3	5	2	0	PD	yes	PD	9.5
7047	F	30	MELA	A1		Sg, Ct, NSI	IV M1b	3	4	2	0	PD	yes	PR	58
7053	F	52	MELA	A1		Sg, Ct	IV M1b	3	4	2	1	PD	yes	PD	7
7015	M	61	BLAD	A2	B44	Sg, R, Ct	IV	3	7	3	0	PR	yes	PD	14.5
7022	M	54	BLAD	A2		Sg, Ct	IV	3	6	1	0	PD	yes	PD	17.5
7016	M	59	BLAD	A2	B44	Sg, Ct, NSI	IV	3	4	2	1	PD	no	PD	2.5
Group 4 (300 µg plus AS02B):												n = 10			
7057	F	55	MELA	A1, A2		Sg, R, Ct, NSI	III N2b	4	20	1	0	PR	no	PD	41.5
7048	F	69	MELA	A3,A23	B7,B44	Sg, ILP, NSI	III N2b	4	6	2	0	PD	yes	PD	6 years
7031	M	57	MELA	A2		Sg, Ct, NSI	IV M1b	4	4	1	0	PD	yes	PD	7.5
7038	M	38	MELA	A2,A3	B35,B51	Ct, NSI	IV M1b	4	5	3	1	PD	yes	PD	8
7055	F	27	MELA	A1,A11	B8, B39	Sg, Ct, NSI	IV M1b	4	4	2	0	PD	yes	PD	18
7054	F	84	MELA	A2	B44	Sg, H	IV M1b	4	4	2	0	PD	no	PD	6
7035	M	53	MELA	A2,A10	B7,B18	Sg, Ct	IV M1b	4	4	0	2	PD	no	PD	3
7042	F	47	MELA	A1,A2	B44	Sg, ILP	IV M1b	4	4	1	0	PD	yes	NED	> 6.5 years
7044	F	41	MELA	A2,A29	B44,B60	Sg, NSI, ILP	IV M1b	4	4	2	1	PD	yes	PD	7
7033	M	49	ESO	A1	B44	Sg, R, Ct	IV	4	9	2	0	PD	yes	PD	22.5
2) Patients removed (1 to 3 vaccinations):												n = 18			
7003	F	28	MELA	A2,A11	B35,B44	Sg, Ct, NSI	IV M1b	1	2	2	1	PD	yes	PD	4.5
7001	F	72	MELA	A2,A11	B35,B56	Sg	IV M1b	1	2	3	0	PD	yes	PD	10.5
7030	F	50	MELA	A2, A24	B55,B60	Sg, Ct	IV M1b	2	3	2	2	PD	no	PD	1.5
7032	M	56	MELA	A9	B35,B44	Sg, Ct, NSI	IV M1b	2	3	1	1	PD	yes	PD	8
7008	F	37	MELA	A1,A11	B7,B18	Sg	IV M1b	2	2	2	1	PD	yes	PD	8.5
7014	F	37	MELA	A1,A28	B14,B60	Sg, Ct	IV M1b	2	2	0	2	PD	no	PD	2.5
7010	F	42	MELA	A3,A24	B7,B44	Sg, R, Ct, NSI	IV M1b	2	3	2	0	PD	yes	PD	6.5
7041	M	59	MELA	A1,A2	B7,B57	Sg, Ct, NSI	IV M1b	2	3	1	1	PD	no	PD	2
7005	F	66	MELA	A2		Sg, R	IV M1b	2	3	2	0	PD	no	PD	3
7059	F	28	MELA	A1		Sg	IV M1b	3	2	0	1	PD	no	PD	4
7045	F	44	MELA	A1		Ct	IV M1b	4	2	3	1	PD	yes	PD	3
7043	F	52	MELA	A2		Ct	IV M1b	4	1	1	2	PD	no	PD	1
7026	F	34	MELA	A2,A3	B7,B27	R, Sg, Ct, NSI	IV M1b	4	3	2	3	PD	no	PD	1
7058	F	47	MELA	A1		Sg, Ct, NSI	IV M1b	4	2	2	0	PD	yes	PD	6
7056	M	43	MELA	A1		Sg	IV M1b	4	2	1	0	PD	yes	PD	22
7052	M	29	MELA	A1 A2	B44	Sg, Ct, NSI	IV M1b	4	2	3	0	PD	yes	PD	9
7027	M	54	NSCLC	A2	B44	Ct	IV	4	3	1	1	PD	no	PD	2
7034	F	55	ESO	A23,A24	B7,B44	Sg	IV	4	3	2	1	PD	yes	PD	2.5

Legend to Table 12

- 1) *Tumor type*: BLAD; bladder, ESO: esophagus, HD.NK: head and neck, MELA: melanoma, NSCLC: non-small cell lung cancer
- 2) *Previous treatments*: Ct: Chemotherapy, H: Hormonal therapy, ILP: Isolated Limb chemotherapy Perfusion, NSI: Non-Specific Immunotherapy, R: Radiotherapy, Sg: Surgery, SI: Specific Immunotherapy.
- 3) *AJCC staging*: refers to the 4th edition of the AJCC staging manual, 1992 (see Table 5).
Melanoma staging: III = regional disease = N (N2b = in-transit metastasis; N2c = metastasis more than 3 cm in greater dimension in any regional lymph node(s) and in-transit metastasis); IV = distant metastasis = M (M1a = metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes; M1b = visceral metastasis). In-transit metastasis involves skin or subcutaneous tissue more than 2 cm from the primary tumor not beyond the regional lymph nodes.
- 4) *Total number of injections*: 7 patients who were thought by the investigator to have some benefit of the treatment received more than 6 vaccinations, on a compassionate use basis.
- 5) *Toxicities*: Maximum toxicity severity or grade reported according to the National Cancer Institute of Canada common toxicity criteria scale.
Local: reaction at injection sites, Flu: Flu-like symptoms, Other: Any other toxicities.
- 6) *SAE*: number of Serious Adverse Events.
- 7) *Tumor response*: According to the WHO criteria: PR: Partial Response, SD: Stable Disease, PD: Progressive Disease.
In addition, Mixed Responses were reported (MxR).
- 8) *Last status*: Tumor: NED: No Evidence of Disease.

Patients' characteristics

From October 1997 to August 1999, 59 patients, 25 men and 34 women, were enrolled in the study in the 12 participating institutions (*Fig. 37 B*). Their mean age was 51 years (range: 27-76). Fifty-one patients had cutaneous melanoma AJCC stage III or IV. Among the other patients, 2 had NSCLC, 1 had head and neck carcinoma, 2 had esophageal cancer and 3 had bladder transitional cell carcinoma, all stage IV. Two melanoma patients were removed before the first vaccination due to rapidly progressive disease.

Fifty-seven patients received the vaccine (*Table 12*). Eighteen patients were removed from the study before they received 4 vaccinations due to early death for 3 of them and rapidly progressive disease for the others. All these 18 patients had stage IV disease with multiple visceral metastases at study entry.

Toxicity

The vaccine was generally well tolerated. The 57 patients who received at least 1 vaccination were evaluated for toxicity. A total of 222 vaccinations were performed as per protocol. In addition, a total of 35 vaccinations were given to 7 patients who were thought by the investigator to have some benefit of the treatment, on a compassionate use basis. No treatment-related adverse events, i.e. "toxicities", above grade 3 were reported. Toxicities occurred in all groups, after one or several vaccinations (*Table 12*).

The most frequently reported toxicities were mild to moderate (grade 1 or 2) swelling and redness at the injection site, flu-like symptoms and nausea. No major difference was observed between the 4 study groups (*Table 13*).

Thirteen vaccinations were followed by grade 3 toxicities, which were reported in 9 different patients (*Table 13*). The observed grade 3 toxicities were as follows: 9 vaccinations were followed by myalgia, nausea, (increase of) cancer-related pain and/or local reaction at the injection site in 6 patients; 2 vaccinations were followed by lymphopenia in a total of 2 patients and 2 vaccinations were followed by anemia in a single patient. Thirty-five serious adverse events (SAE) were reported in 26 patients (*Table 12*). However, only one SAE, a grade 3 anemia, was suspected to be related to the vaccinations (patient 7038, group 4). One event was of infectious origin and all others were related to tumor progression, including 3 deaths.

Tumor response and survival

Tumor response was assessed according to the WHO criteria, after the 4th and the 6th vaccinations, as compared to baseline tumor measurements performed in the 2-week period preceding the first vaccination. "Mixed responses", i.e. regression of some metastases while others either remain stable, progress or appear simultaneously, although formally classified as stable or progressive disease in the WHO classification, were documented as well.

Table 13 Incidence of most frequent toxicities

	Group 1 300 µg MAGE-3 prot. without adjuvant	Group 2 30 µg MAGE-3 prot. with adjuvant	Group 3 100 µg MAGE-3 prot. with adjuvant	Group 4 300 µg MAGE-3 prot. with adjuvant
Total number of vaccinations	18	81	57	66
<i>Local reaction at injection site</i>				
	9	59	41	44
<i>Flu-like symptoms</i>				
Fatigue	0	15	16	1
Myalgia	3	12	21	14
Headache	1	8	12	1
Fever	1	8	23	11
<i>Other general symptoms</i>				
Nausea	1	13	9	2
Total	15	115	122	73
Incidence	0.83	1.4	2.1	1.1

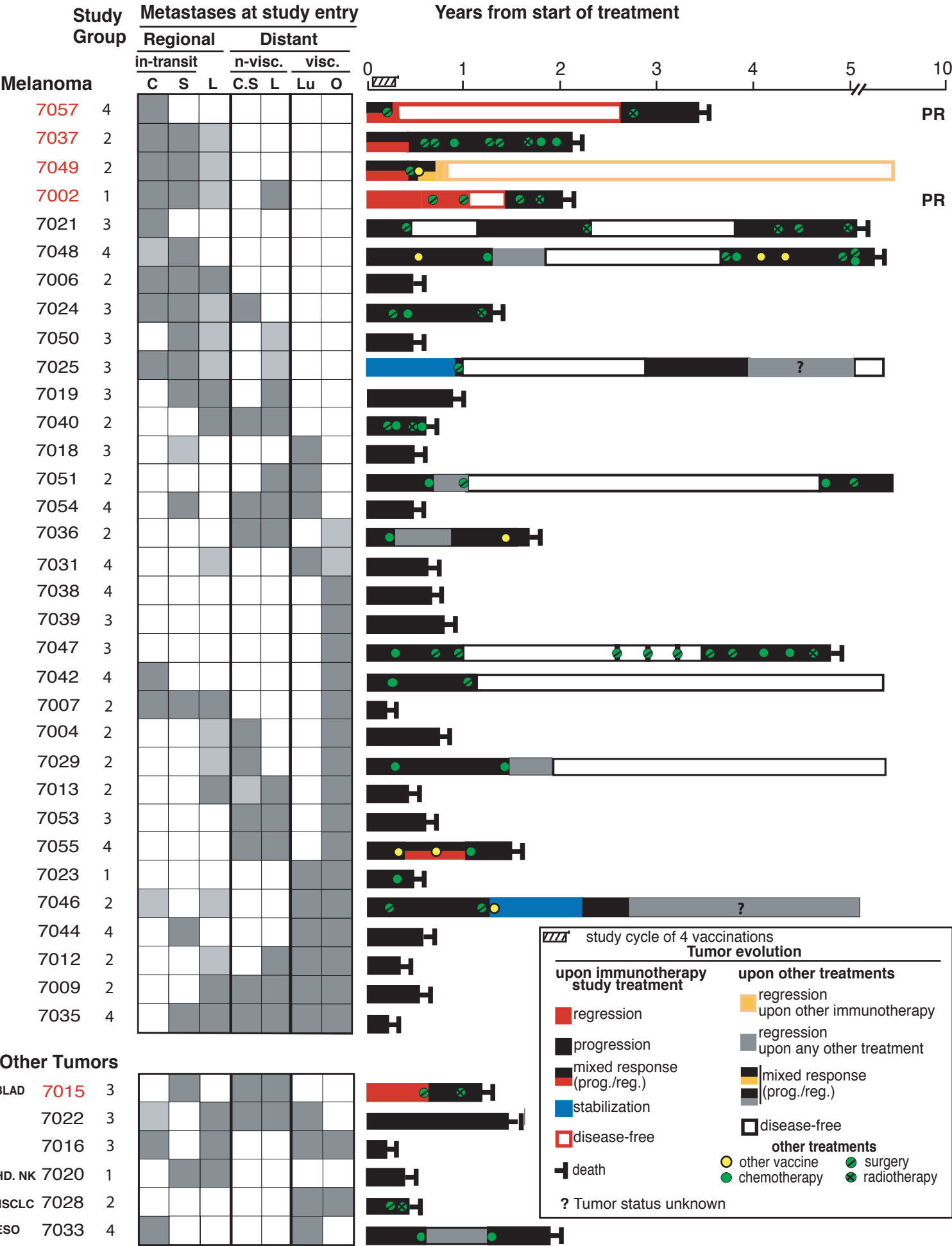
Only treatment-related toxicities reported with a frequency of at least 10% are shown here

Incidence of toxicities = Total number of toxicities reported/Total number of vaccinations

Figure 38

MAGE-3 protein ± AS02B (study LUD 97-002)

Outcome of the 39 patients who received at least 4 vaccinations



Summary of tumor response and survival

Because we did not expect full immunization of the patients before several injections, only the 39 patients who received at least 4 vaccinations were considered evaluable for tumor response. Their clinical evolution is summarized in *Fig. 38*. Among the 33 melanoma patients who were evaluable for tumor response, we observed 2 partial responses, 2 mixed responses and 1 stabilization, the latter lasting for 11 months. Six patients had other solid tumors types, namely, lung, head and neck, esophageal or bladder cancer. A partial response lasting 7 months was observed in 1 of the 3 metastatic bladder cancer patients included. None of the tumor responses described above involved visceral metastases.

For the 59 patients who entered the study, the median overall survival was 7 months (1 – 80.5+). For the 51 melanoma patients, it was 7.5 months (1 - 80.5+).

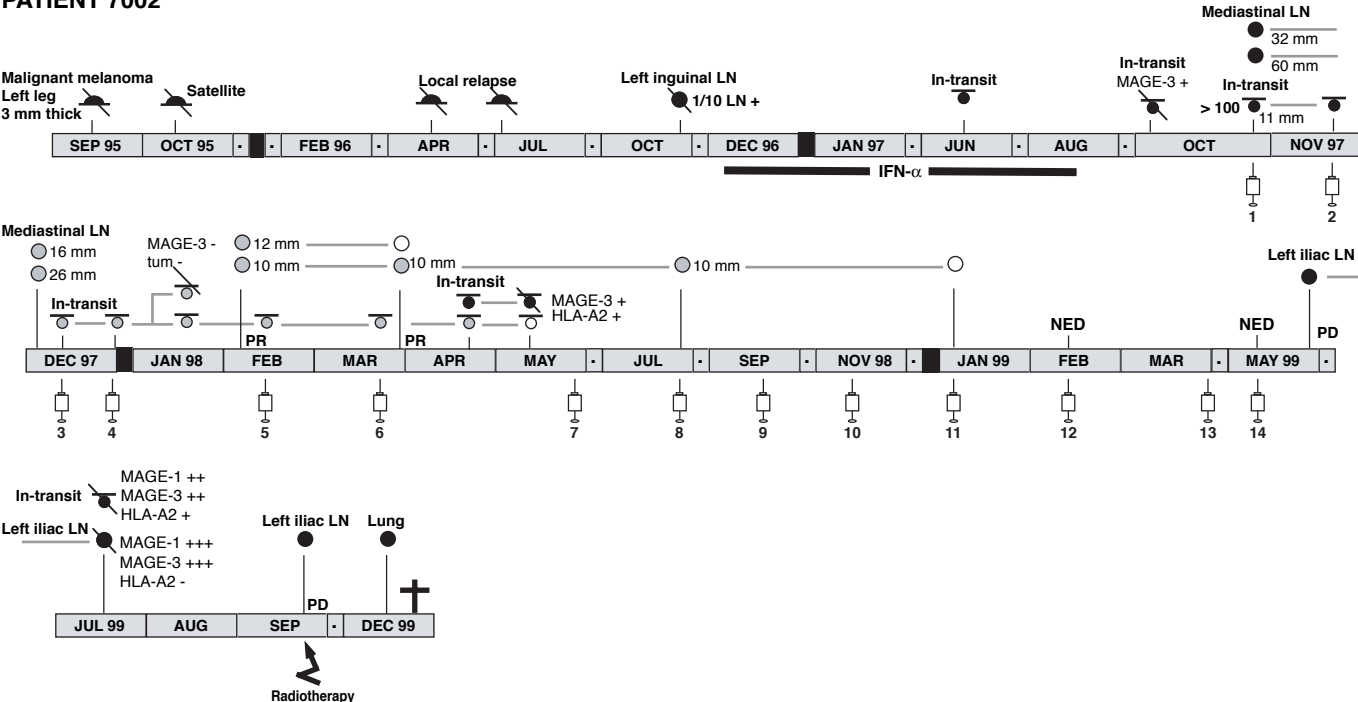
Detailed narratives of responder patients

Patient 7002, with a melanoma, received MAGE-3 protein without adjuvant (*Fig. 39*). She presented at study entry with more than 100 in-transit metastases as well as two mediastinal lymph node metastases. Two in-transit metastases were surgically removed during the treatment. In the first lesion, which was resected after the 4th vaccination because it showed clinical signs of regression, no tumor cells and no expression of *MAGE-3* were found. The other lesion, which appeared during treatment and was resected for curative purpose after the 6th vaccination, was found to express the *MAGE-3* gene. The presence of HLA class I molecules including HLA-A2 on the tumor cell membrane was confirmed by immunohistochemistry using the conformational antibodies W6/32 and AH86, respectively. Twelve months after the start of treatment, all the other lesions had completely disappeared, including the mediastinal nodes. However, 5 months later, new left iliac metastatic lymph nodes appeared, which could only be partially resected. The patient died 1 year later of disease progression.

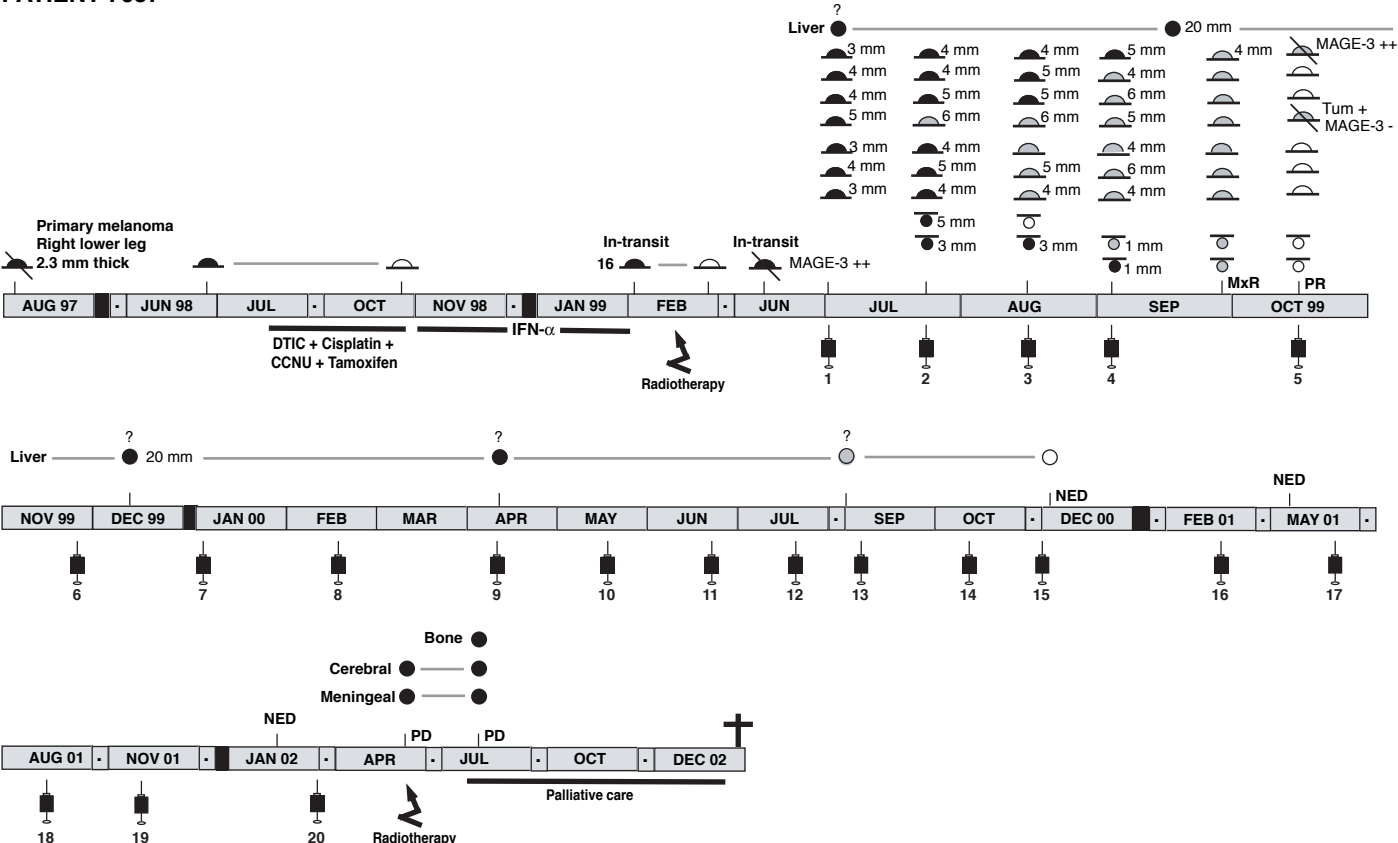
Patient 7057 showed 7 cutaneous in-transit melanoma metastases at study entry (*Fig. 39*). Regression of some lesions was already apparent 6 weeks after the 1st vaccination. Two regressing lesions were surgically removed during treatment, for scientific purpose. In both lesions, tumor cells were still present and expressed HLA class I molecules, including HLA-A2, on the cell surface. RT-PCR was positive for *MAGE-3* in one lesion and negative in the other one. However, the poor quality of the mRNA obtained from the latter makes this result difficult to interpret. After 4 months, all other in-transit lesions had completely disappeared. After the 4th and the 6th vaccinations, tumor response was first evaluated as mixed response, because a liver lesion was detected after 4 vaccinations. However, it was retrospectively found to have been present before treatment onset, it never grew and it became undetectable after 8 additional vaccinations. Our interpretation is that this lesion was not

Figure 39

PATIENT 7002



PATIENT 7057



LEGEND

new, stable or progressing

regressing

no longer detectable

surgical removal

Patient's death +



Injection of MAGE-3 protein (300 μ g) without adjuvant



Injection of MAGE-3 protein (300 μ g) + AS02E

CCNU: Lomustine

DTIC: Dacarbazine

IFN- α : Interferon alpha

MAGE-3 +: expression

NED: No evidence of disease

PR: Partial response

Tum +: tumor cells visible histologically; Tum -: not visible

? : suspect lesion, no biopsy was performed

cancerous. The patient had no detectable disease for a period of 29 months, then brain metastases occurred and despite radiotherapy on the brain metastases, the patient died due to tumor progression, almost 3.5 years after the start of MAGE-3 vaccinations.

Patient 7049, who had a mixed response of in-transit melanoma metastases followed after 4 months by progression of these lesions, was entered into another vaccination study involving the NA17.A2 peptide antigen (163). This resulted in a complete response, which has been lasting for more than 5 years (*Fig. 40*) (232).

Patient 7037 had a 4-month MxR of in-transit metastases followed by disease progression.

Patient 7015 presented at study entry with 2 distant metastatic lymph nodes of a bladder cancer progressing after two lines of chemotherapy (*Fig. 40*). The 2 lymph nodes regressed almost completely after 7 months. One of them was then surgically removed and was found to express *MAGE-3*, whereas the other became clinically undetectable. Tumor relapse occurred 2 months later.

Analysis of the anti-MAGE-3 and anti-ProtD antibody responses

To assess the antibody response, pre- and post-vaccination sera were available from 28 of the 39 patients who receive at least 4 vaccinations, including 3 patients who received the protein alone and 25 patients who received the MAGE-3 protein mixed with AS02B (*Table 14*) (208).

The specificity of the anti-MAGE-3 antibody responses was confirmed by Western blot on pre- and postvaccination sera of several patients (data not shown). Because the vaccine contained a recombinant MAGE-3 protein produced in *E. coli*, the Western blot was carried out with a MAGE-3 protein produced in a different expression system i.e. Chinese hamster ovary (CHO) cells, (MAGE-3^{CHO}). These antibodies were specific for native MAGE-3 protein because they recognized a MAGE-3^{CHO} antigen that had not undergone reduction/carboxyamidation and did not contain a His-tail.

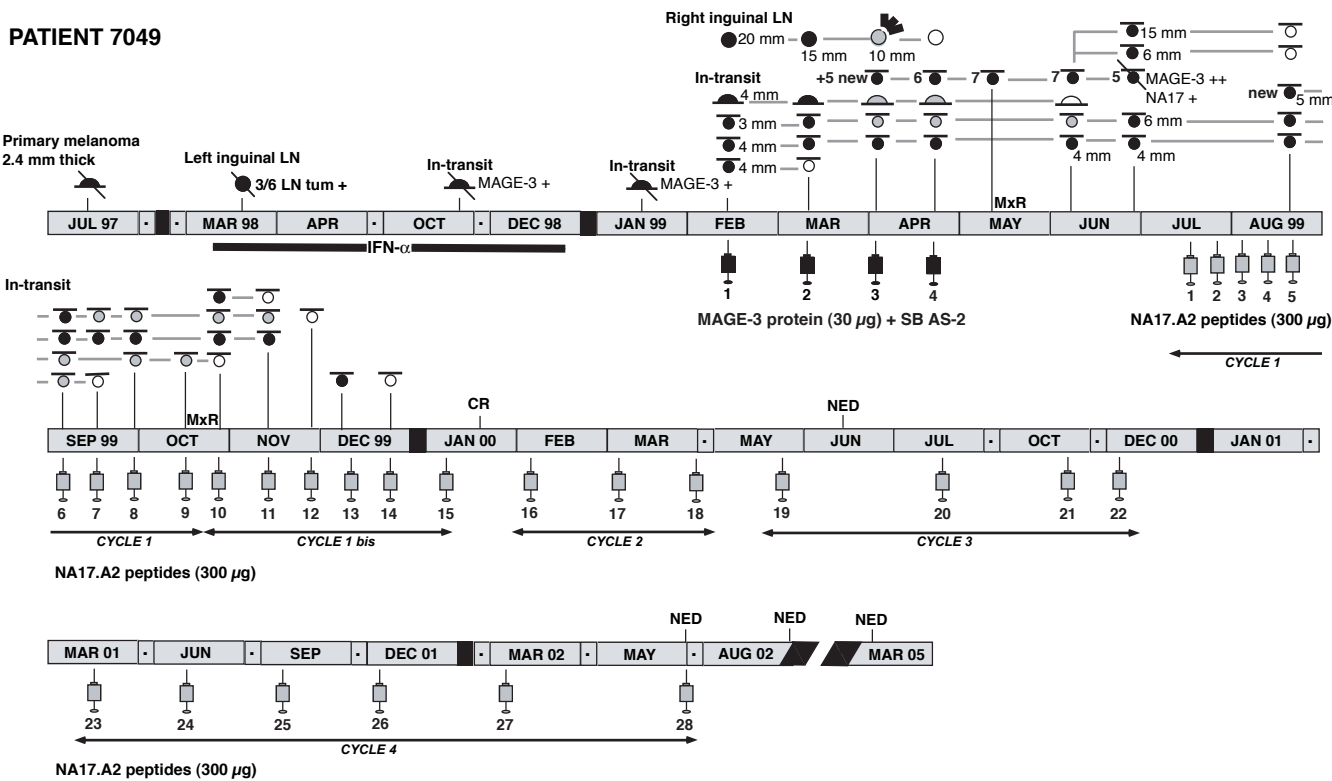
Summary of antibody response

Vaccination with ProtD-MAGE-3-His in AS02B adjuvant gave rise to statistically significant anti-MAGE-3 and anti-ProtD IgG responses. In the groups vaccinated with the ProtD-MAGE-3 protein and adjuvant, 23 out of 24 patients elicited a significant anti-MAGE-3 IgG antibody response after four vaccinations. The dose of MAGE-3 protein did not seem to affect the magnitude of the antibody response, whereas the presence of AS02B had a strong impact (208).

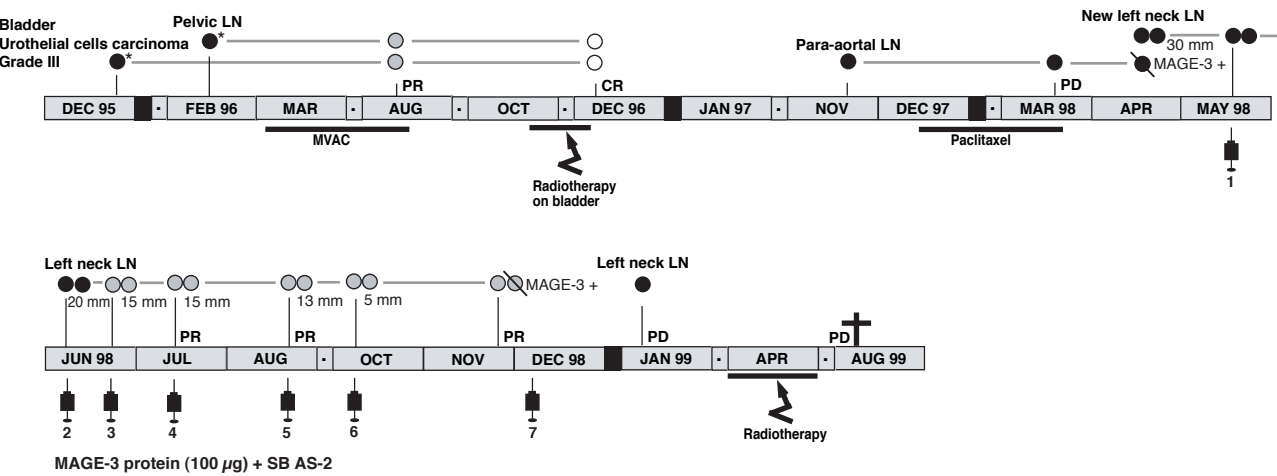
There was no apparent correlation either between the antibody responses and the tumor response or the AJCC stage in melanoma patients.

Figure 40

PATIENT 7049



PATIENT 7015



LEGEND

	cutaneous tumor	subcutaneous tumor	lymph node (LN) or visceral metastasis
new, stable or progressing			
regressing			
no longer detectable			
surgical removal			
incomplete resection			
Inflammatory reaction			
Patient's death			

Injection of MAGE-3 protein + AS02B i.m.

Injection of NA-17.A2 peptides - nonamer: VLPDVFIRC (300 µg) (i.d. / s.c.) - decamer: VLPDVFIRC (300 µg)

CR: Complete response
IFN-α: Interferon alpha
MAGE-3 +: expression of gene MAGE-3 (RT-PCR); MAGE-3 -: no expression
MVAC: Methotrexate, Vincristine, Doxorubicin (Adriamycin) and Cisplatin
MxR: Mixed response
NED: No evidence of disease
PD: Progressive disease
PR: Partial response
Tum +: tumor cells visible histologically; Tum -: not visible

Table 14 Antibody response against the MAGE-3 and the LipoD proteins

Vaccine	Patients	IgG Antibody Titers (EU/mL)				Tumor	
		Anti-MAGE-3 protein		Anti- ProtD		Response	Type
		Pre	Post-4	Pre	Post-4		
GROUP 1 300µg Prot D-MAGE-3 without AS02B	<u>7002</u>	10	10	178	100	PR	MELA
	7020	8	206	172	254	PD	HD.NK
	7023	8	16	100	100	PD	MELA
GEOMEAN		9	32	145	136		
GROUP 2 30µg Prot D-MAGE-3 with AS02B	7004	12	946	133	24532	PD	MELA
	7009	21	76	100	943	PD	MELA
	7013	8	517	100	2153	PD	MELA
	7036	10	1465	100	3498	PD	MELA
	<u>7037</u>	12	11742	244	320	MxR	MELA
	7040	11	285	100	376	PD	MELA
	7046	10	88	276	7487	PD	MELA
	<u>7049</u>	9	733	406	3421	MxR	MELA
	7051	10	23921	100	7481	PD	MELA
GEOMEAN		11	869	149	2514		
95% CI		9–14	192–3922	98–228	830–7614		
GROUP 3 100µg Prot D-MAGE-3 with AS02B	<u>7015</u>	9	973	100	1741	PR	BLAD
	7018	11	938	100	3922	PD	MELA
	7019	13	1467	100	7339	PD	MELA
	7022	<i>nt</i>	479	1849	18621	PD	BLAD
	7024	9	1033	100	782	PD	MELA
	<u>7025</u>	9	752	100	792	SD	MELA
	7050	9	1517	100	448	PD	MELA
GEOMEAN		10	961	152	2212		
95% CI		8–11	666–1385	55–521	631–7759		
GROUP 4 300µg Prot D-MAGE-3 with AS02B	7031	9	847	147	2743	PD	MELA
	7033	9	367	134	2595	PD	ESO
	7038	11	11	112	3472	PD	MELA
	7042	10	1524	100	1692	PD	MELA
	7044	10	3696	100	4358	PD	MELA
	7048	10	678	100	3624	PD	MELA
	7054	15	2104	135	926	PD	MELA
	7055	8	294	119	561	PD	MELA
	<u>7057</u>	8	157	100	365	MxR	MELA
GEOMEAN		10	477	115	1710		
95% CI		8–11	126–1802	102–130	863–3391		

The anti-MAGE-3 and anti-ProtD IgG antibody titers were quantified by an ELISA assay, using purified MAGE-3^{insect} protein or ProtD antigen and are expressed in Elisa Units (EU) / ml. All titers reported on the table are from pre-vaccination, post-4 vaccination (35 days post-4) or post-6 vaccination (8 days post-6, for patient 7015) samples.

A patient was considered a MAGE-3 or ProtD vaccine responder when the postvaccination titer was above the respective cutoff value and at least twice the patient's own baseline value.

Cutoff values: MAGE-3 : 27 EU / mL; Prot D : 100 EU/mL

The underlined patients' identification numbers indicate clinical responders.

Geomean = Geometric mean

95% CI = 95% Confidence Interval

nt : not tested.

Anti-MAGE-3 IgG antibody response

Twenty-three out of the 24 fully evaluated patients vaccinated with MAGE-3 protein in AS02B adjuvant elicited a significant anti-MAGE-3 IgG antibody response after 4 vaccinations (*Table 14*). Although spontaneous anti-MAGE-3 antibody production has been reported in about 10% of melanoma patients (209), there was no anti-MAGE-3 reactivity in the 27 pre-vaccination sera tested. Of the 3 patients who received the MAGE-3 protein alone, only one developed an anti-MAGE-3 antibody response.

Anti-ProtD antibody response

All the patients vaccinated with the adjuvanted ProtD-MAGE-3-His protein developed anti-ProtD IgG antibodies, whereas none of the 3 patients who were vaccinated with the protein alone did (*Table 14*). Anti-ProtD antibodies were present in 10 of the 28 pre-vaccination sera tested, indicating that these patients had previous immunizing contact with *H. Influenzae*, a bacteria that is frequently present in the environment.

Correlation between anti-MAGE-3- and anti-prot-D antibody responses

No correlation was found between the anti-MAGE-3 and the anti-ProtD antibody titers at individual levels, which could be ascribed to the heterogeneity of the preexisting antibody response to ProtD (*Table 14*). The mean antibody titers against the 2 moieties of the recombinant ProtD-MAGE-3-His protein were more than 10 times higher in patients receiving the adjuvanted protein than the mean titer of patients receiving the nonadjuvanted protein (group 1). No difference was seen between antibody responses after 4 or 6 vaccinations, suggesting that the immune response persisted at a plateau after the 4th vaccination (data not shown) (208).

III. 2.2 MAGE-3 protein by intradermal and subcutaneous routes, without immunological adjuvant

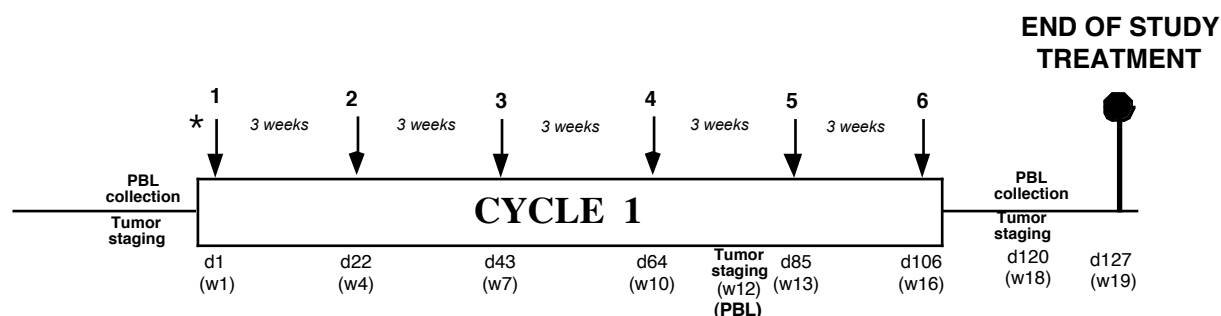
Study LUD 99-003

Phase I/II study of intradermal and subcutaneous immunization with the recombinant MAGE-3 protein in patients with *MAGE-3*-positive measurable non-visceral metastatic melanoma followed in selected patients by immunization with the MAGE-3 protein combined with antigenic peptides.

DESIGN SUMMARY

Immunization: 300 μ g MAGE-3 protein divided in one i.d. site (75 μ g), one s.c. site (75 μ g) and one i.d. + s.c. site (75 μ g + 75 μ g).

Patients: 28 melanoma patients whose tumor expresses gene *MAGE-3*



Coordinating investigators: Marie Marchand and Alexander M. M. Eggermont

Clinical sites:

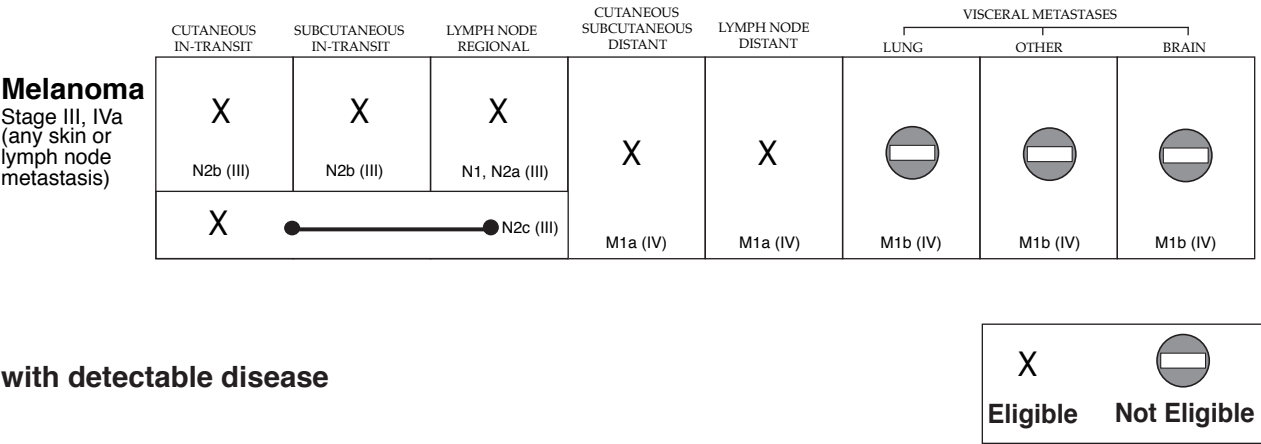
Erasmus Medical Center - Daniel den Hoed Cancer Center, Rotterdam
Cliniques universitaires St-Luc, UCL, Brussels
Institut Gustave Roussy, Villejuif
Institut Curie, Paris
Hôtel Dieu, CHU de Nantes, Nantes
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B. Dréno
P. Patel

Figure 41

A. Eligibility relative to maximum extent of disease according to AJCC 1997 staging



B. Accrual

Tumor type	Included	Not treated*	Treated	
			Completed protocol	Removed
Melanoma	32	2	26	4

* Two patients were withdrawn before the first vaccination (see text).

Objectives

In a previous study, where the recombinant MAGE-3 protein was mixed with AS02B (*Section III. 2.1*), all 5 clinical responses were observed in the group of 12 patients with non-visceral melanoma (*Fig. 37*). Interestingly, one of the two objective responses occurred in a patient vaccinated without the immunological adjuvant, indicating that the recombinant MAGE-3 protein alone is immunogenic, which might possibly be due to the help properties of its lipoprotein D portion.

This led us to set up the present study in which a fixed dose of ProtD-MAGE-3-His is injected without immunological adjuvant, i.d. and s.c. This injection route is believed to lead to more effective immunization than the i.m. route, possibly because of the presence of antigen presenting cells and of a rich lymphatic network in the skin. Because our previous observations suggested that non-visceral metastases are more sensitive to therapeutic vaccinations than visceral localizations, patients with visceral metastasis were excluded from the present study (197, 198).

Treatment schedule

The vaccination schedule comprised 6 vaccinations at 3-week intervals (*design summary*) (210). The day of each vaccination, a total dose of 300 µg of lyophilized ProtD-MAGE-3-His was reconstituted in 0.5 ml NaCl 0.9% and divided between three injection sites, one i.d. (75 µg), one s.c. (75 µg) and one i.d./s.c. (75 µg/75 µg).

After a first cycle of 6 vaccinations, patients whose tumor regressed, stabilized or progressed slowly, received additional vaccinations at increasing intervals for a maximum of five years, or until major tumor progression or relapse requiring other treatments. Six additional vaccinations were first given at 6-week intervals, followed by 3-month intervals between the following ones.

Eligibility criteria

Eligibility criteria relative to melanoma stage are defined in *Fig. 41 A* (*for detailed eligibility criteria, see Annex 3*). Only patients with metastases, but without visceral involvement by the tumor, were eligible.

Patients' characteristics

From December 1999 to March 2002, 32 patients, 13 men and 19 women, were enrolled in the study in the six participating institutions (*Fig. 41 B* and *Table 15*). Their median age was 62.5 years (range: 32-81 years). Two of them were removed from the study before the first vaccination because eligibility criteria were not fulfilled. For Patient 102, cytology did not confirm the neoplastic nature of a solitary cutaneous lesion and Patient 122 was found to have a second active tumor, i.e. a breast cancer. Twenty-nine patients had cutaneous and/or lymph node metastases (AJCC 1997 stage III or IVM1a). One patient (Patient 101) had former visceral metastases, i.e. lung and bone lesions that had

Table 15 Patients' characteristics

Patients		Previous treatments		Study treatment						Time to Further		Last Status ⁷		
Gender				AJCC 1997	Total	Toxicities ⁴			SAE	Tumor	prog-	treat-	Tumor	Survival
Code	Age	Type ¹		Stage ² at study entry	number	Maximum toxicity	(n) ⁵			response ⁶	ression	ments		(months)
					vaccina- tions ³	severity or grade	Local	Flu	Other		(months)			(months)
1) Patients having received complete protocol (≥ 4 vaccinations) :											n = 26			
101	M	56	Sg, Ct, NSI	IV pTX N2c M1b	9	1	1	3	0	MxR	3.5	yes	PD	20
103	F	61	Sg, ILP, SI	III pT3a N2b M0	6	3	2	2	0	PD	2	yes	NED	> 65.5
104	F	71	Sg, Ct, H	III pT3a N2b M0	29	3	3	2	0	MxR	> 58	yes	NED	> 63
106	F	71	Sg, NSI, H, Ct	III pT3a N2b M0	9	1	0	2	0	PD	< 1.5	yes	PD	29.5
107	F	56	Sg	IV pT0 N0 M1a	9	2	1	0	0	PD	2	yes	NED	> 34 *
108	F	81	Sg	IV pT3a N2c M1a	4	2	1	1	0	PD	< 1	yes	PD	4.5
109	M	65	Sg, Ct, NSI	IV pT4a N1 M1a	6	1	0	2	0	SD	4	yes	PD	13
110	F	74	Sg	IV pTX N2c M1a	7	3	1	1	0	PD	3	no	PD	10
111	M	79	Sg	IV pT3a N2b M1a	17	2	2	2	0	MxR	14	yes	PD	38
112	M	69	Sg	III pT4a N2b M0	28	2	1	0	1	PR	> 65	no	NED	> 65
113	F	72	Sg	IV pT3a N2c M1a	9	2	1	1	0	PD	3	yes	PD	29.5
114	M	67	Sg	III pT3a N2b M0	9	2	2	2	0	SD	3.5	yes	PD	43
115	M	32	Sg	III pT3b N2c M0	6	2	3	2	1	PD	< 1	yes	PD	28.5
116	F	50	Sg, NSI	III pT3a N2b M0	4	2	2	2	0	PD	< 1	yes	NED	> 63
117	F	41	Sg, Ct, NSI	III pT4a N2c M0	6	3	1	0	0	SD	2.5	yes	PD	15
118	M	56	Sg, NSI	III pT4a N2c M0	6	1	1	1	0	PD	1.5	yes	PD	34
120	F	62	Sg	III pT2 N2c M0	6	1	1	1	0	PD	< 1	yes	PD	29
121	F	55	Sg	III pT4a N2c M0	6	1	1	1	0	PD	< 1	yes	PD	28.5
123	F	39	Sg, NSI	IV pT3a N1 M1a	4	2	1	0	0	PD	< 1	yes	PD	18
124	F	68	Sg, Ct, NSI	III pT4a N2c M0	9	3	0	2	0	PD	1.5	yes	NED	> 45.5
125	F	63	Sg, Ct	III pT3a N2c M0	6	2	0	1	0	PD	1.5	yes	PD	14
126	M	74	Sg, Ct, R	IV pT4b N2c M1a	4	3	0	0	0	PD	2.5	yes	PD	26
127	F	68	Sg	III pT4a N2b M0	6	2	0	0	0	PD	1.5	yes	PD	17
128	M	45	Sg, NSI	IV pT3a N2c M1a	12	2	0	1	0	MxR	8	yes	PD	29
130	M	42	Sg	III pT3a N2b M0	19	2	1	0	0	PD	< 1	yes	NED	> 49
131	M	78	Sg	III pT4a N2c M0	4	0	1	0	0	PD	< 1	yes	PD	9.5
2) Patients who were withdrawn (1 to 3 vaccinations) :											n = 4			
105	M	53	Sg, NSI, SI	IV pT3b N2b M1a	2	3	3	2	1	PD	< 1	yes	PD	4.5
119	M	40	Sg	IV pT0 N0 M1a	3	1	0	0	1	PD	2	yes	PD	4
129	F	51	Sg	IV pT4a N2c M1a	3	1	1	3	3	PD	2	yes	PD	8
132	F	44	Sg, NSI	IV pT2 N2c M1a	3	2	0	0	0	PD	< 1	yes	PD	4
3) Patients who were enrolled but not vaccinated :											n = 2			
102	F	79	Sg	III pTX N2c M0	0	NA	NA	NA	NA	NA	NA	NA	NED	> 57
122	F	77	Sg	III pT3b N2c M0	0	NA	NA	NA	NA	NA	NA	NA	PD	27

1) *Previous treatments*: Ct: Chemotherapy, H: Hormonal therapy, ILP: Isolated Limb chemotherapy Perfusion, NSI: Non-Specific Immunotherapy, R: Radiotherapy, Sg: Surgery, SI: Specific Immunotherapy.

2) *AJCC staging*: refers to the 5th edition of the AJCC staging manual, 1997 (see Table 5).

Melanoma staging: III = regional disease = N (N2b = in-transit metastasis; N2c = metastasis more than 3 cm in greater dimension in any regional lymph node(s) and in-transit metastasis); IV = distant metastasis = M (M1a = metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes; M1b = visceral metastasis). In-transit metastasis involves skin or subcutaneous tissue more than 2 cm from the primary tumor not beyond the regional lymph nodes.

3) *Total number of injections*: 12 patients who were thought by the investigator to have some benefit of the treatment received more than 6 vaccinations, on a compassionate use basis.

4) *Toxicities*: Maximum toxicity severity or grade reported according to the National Cancer Institute of Canada common toxicity criteria scale. Local: reaction at injection sites, Flu: Flu-like symptoms, Other: Any other toxicities.

5) *SAE*: number of Serious Adverse Events.

6) *Tumor response*: According to the WHO criteria: PR: Partial Response, SD: Stable Disease, PD: Progressive Disease. In addition, Mixed Responses were reported (MxR).

7) *Last status*: Tumor: NED: No Evidence of Disease. Survival: *: lost to follow-up

completely responded to a biochemotherapy treatment including IL-2 and IFN- α (AJCC 1997 stage IV M1b). However, because no visceral involvement was detected at study entry, the decision was taken by the study chairmen to include this patient in the study.

Thirty patients received the vaccine. Four patients were withdrawn from the study before the 4th vaccination, due to rapidly progressive disease. These four patients had stage IV M1a disease at study entry. Twenty-six patients received at least 4 vaccinations and were considered evaluable for tumor response.

Toxicity

The 30 patients who received at least one vaccination were evaluated for safety and toxicity. The vaccine was generally well tolerated.

A total of 251 vaccinations were administered. No treatment-related adverse events, i.e. “toxicities”, above grade 3 were reported (*Table 15*). Toxicities occurred after one or several vaccinations. The most frequently reported ones were mild to moderate redness and swelling at the injection sites and flu-like symptoms (*Table 16*).

The observed grade 3 toxicities were as follows (*Table 15*). Inflammatory reactions at the injection sites, i.e. erythema, local heat, pain and/or swelling were observed following 15 vaccinations in a total of seven patients, without significant difference between the i.d. and the s.c. injection sites. Flu-like myalgia, sweating, arthralgia and/or fatigue/lethargy were observed following four vaccinations in a total of three patients. For Patient 101, a vaccination was followed by a moderate hypertension that was treated by metoprolol. Patient 129 had severe constipation after one vaccination, which was most likely related to tumor progression.

Seven SAE were reported in five patients (*Table 15*). Only one was definitely related to the study treatment. After the first vaccination, Patient 105 had a grade 2 allergic reaction with erythema at injection sites as well as some urticaria-like patches on the chest accompanied by itching of the whole body. These symptoms resolved spontaneously within two weeks without anti-allergic medication. Because of this moderate allergic reaction, a preventive hospitalization, which is the justification to report this event as a SAE, was required by the clinical investigator for the administration of the second vaccine, in order to monitor possible anaphylactic reactions after the second vaccine. Given the absence of general symptoms after a 1-day hospitalization, the patient was allowed to leave the hospital. Two SAE were of infectious origin, one was related to previous cardiac ischemia and the three others to tumor progression.

MAGE-3 protein i.d. / s.c.

study LUD 99-003

Table 16 **Incidence of most frequent toxicities**

	Number of vaccinations after which symptoms were reported	Incidence of toxicities
Local reactions at injection site		
Redness	199	0.79
Induration/swelling	120	0.48
Heat	90	0.36
Pain	84	0.33
Flu-like symptoms		
Headache	40	0.16
Myalgia	34	0.14
Fatigue/lethargy	33	0.13
Fever	29	0.12
Total number of vaccinations		
	251	

Only treatment-related toxicities reported with a frequency of at least 10% are shown here.

Incidence of toxicities = Total number of toxicities reported/Total number of vaccinations.

Tumor response, time to progression and survival

Tumor response was assessed according to the WHO criteria, after the 4th and the 6th vaccinations, as compared to baseline tumor measurements performed in the 2-week period preceding the first vaccination. “Mixed responses”, i.e. regression of some metastases while others either remain stable, progress or appear simultaneously, although formally classified as stable or progressive disease in the WHO classification, were documented as well.

Summary of tumor response, time to progression and survival

Because we did not expect effective immunization of the patients before several injections, only the 26 patients who received at least 4 vaccinations were evaluated for tumor response (*Fig. 42*). Among these 26 patients, we observed 1 partial response and 4 mixed responses. In addition, short-lasting tumor stabilization was observed in three patients, followed by tumor progression after 2.5, 3 and 4 months, respectively. Eighteen patients had tumor progression.

Time to progression in the 30 patients who received at least one vaccination varied from 3 weeks to 58+ months (median = 7 weeks, *Table 15*). Three patients had a time to progression above one year. These three patients had experienced a partial (1) or a mixed (2) tumor response. For the five patients who showed complete (1) or mixed tumor response (4), the median time to progression was 14 months (range: 3.5 to 58+ months).

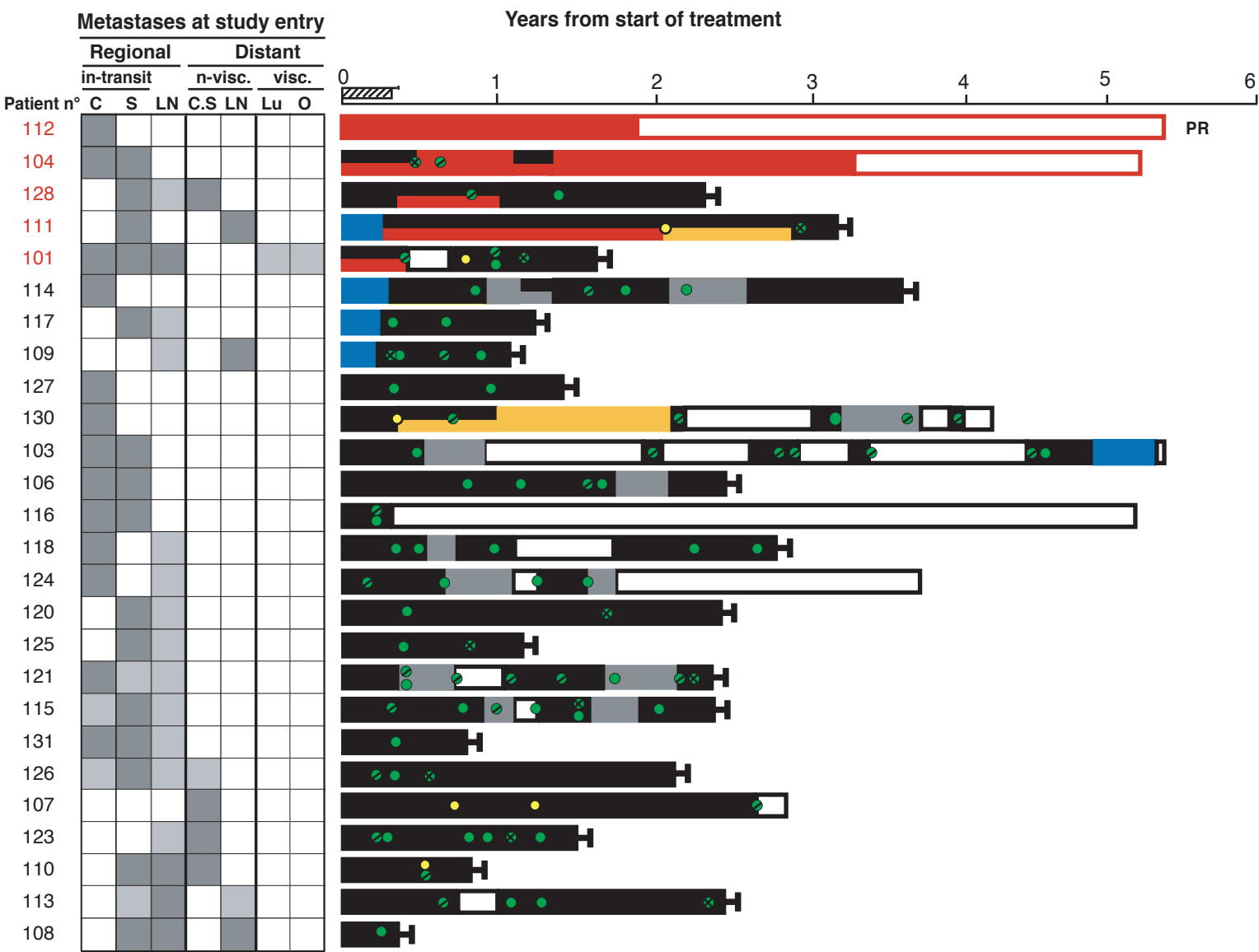
For the 32 patients who entered the study, the median overall survival was 28.5 months (range: 4 to 65.5+ months). For the 26 patients who received at least four vaccinations, it was 29 months (range: 4.5 to 65.5+ months).

Detailed narratives of responder patients

Patient 112 experienced a partial response to treatment (*Fig. 43*). He had a 4.1 mm thick primary melanoma of the left auricle resected in February 2000. In August 2000, in-transit metastases appeared. Although some of them were resected on two occasions, new lesions developed. At study entry, in November 2000, the patient presented with four small in-transit metastases of the left auricle. Their largest diameter was 2 to 8 mm. Tumor regression started in two lesions after the first vaccination, but the process was very slow to complete. After six vaccinations, the tumor response was evaluated as partial and the patient received additional vaccinations according to the protocol. Twenty-two months after the start of treatment, the response had become complete: all four tumor lesions and residual pigmented deposits in the skin had completely disappeared. In December 2005, maintenance vaccinations were stopped at the end of the 5-year period foreseen by the protocol and follow-up was pursued without any other treatment. In April 2006, 65 months after the start of vaccinations, he was still in complete remission and no severe long-term toxicity was reported (*Table 15*).

Figure 42

Outcome of the 26 melanoma patients who received at least 4 vaccinations



Patients are classified according to their AJCC stage at study entry.

Metastases at study entry: Dark gray: detectable metastases at study entry. Light gray: Metastasis removed before study.

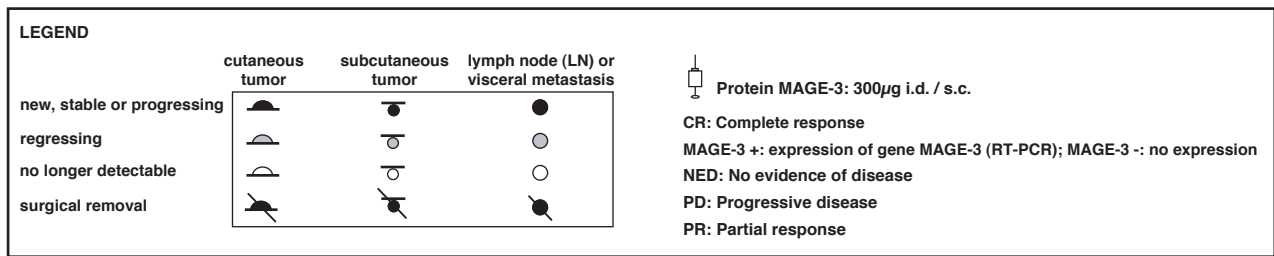
n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, LN = lymph node, Lu = lung, O = other visceral localization

study cycle of 6 vaccinations

Tumor evolution

upon immunotherapy study treatment	upon other treatments	other treatments
regression	regression upon other immunotherapy	other vaccine
progression	regression upon any other treatment	chemotherapy
mixed response (prog./reg.)	mixed response (prog./reg.)	surgery
stabilization	disease-free	radiotherapy
disease-free		death

PATIENT 112



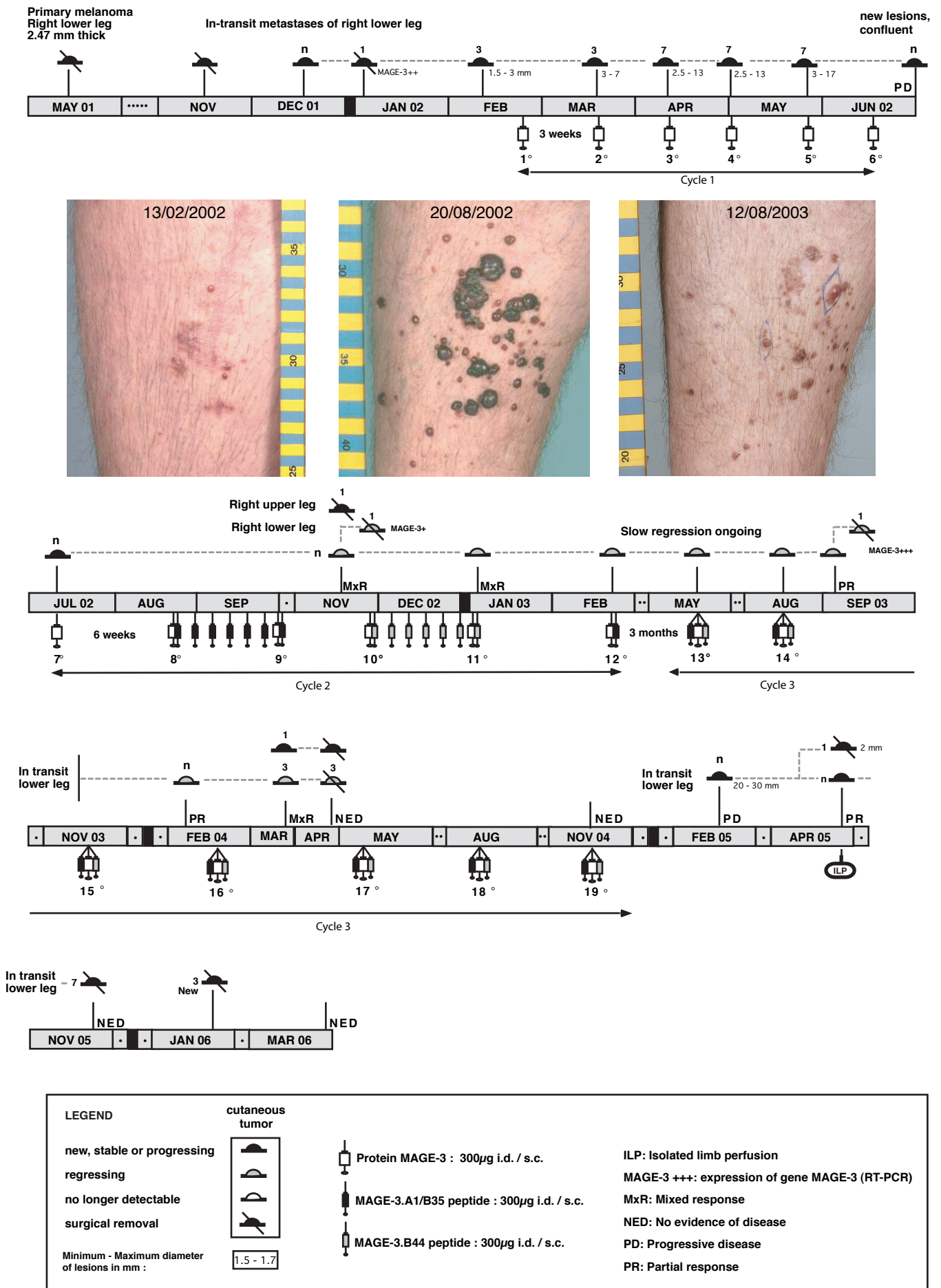
Four patients experienced a mixed response. Patient 104 had regression of in-transit metastases, patient 128 had regression of distant cutaneous metastases, patient 111 had regression of both cutaneous and distant lymph node targets, and patient 101 had regression of in-transit metastases and regional lymph nodes.

Late tumor regression in a patient who received the MAGE-3 protein and peptides during the maintenance phase of the treatment

In an attempt to increase the efficacy of ProtD-MAGE-3-His vaccine, we amended the maintenance phase of this study. After the first cycle of 6 vaccinations, patients with halted incomplete regression or slow progression were to receive not only MAGE-3 protein vaccinations at 6-week and then at 3-month intervals, but also weekly injections of a MAGE peptide selected according to their HLA type and to the *MAGE* genes expressed by their tumor. Because of administrative delays, only one patient received this modified maintenance treatment.

Patient 130, who expressed HLA-A1, B-35 and -B44, showed progression of cutaneous in-transit metastases after the first cycle of ProtD-MAGE-3-His vaccinations (*Fig. 42*). He then received a second cycle of six maintenance vaccinations with ProtD-MAGE-3-His at 6-week intervals (*Fig. 44*). The total dose and the injection route were the same as those described for the first cycle of vaccinations. Between the 8th and the 9th MAGE-3 protein vaccinations, he received 7 weekly vaccinations with the MAGE-3.A1₁₆₈₋₁₇₆ peptide at a dose of 300 µg per vaccination, divided between 1 i.d. (60 µg) and 1 s.c. site (240 µg). Peptide vaccinations 1 and 7 were given the same day as MAGE-3 protein vaccinations 8 and 9, respectively, but at distant injection sites. Interestingly, three months after the onset of this combination regimen, signs of tumor regression appeared in several in-transit lesions, but proceeded very slowly (mixed response). Between the 10th and the 11th MAGE-3 protein vaccinations, the MAGE-3.B44₁₆₇₋₁₇₆ peptide replaced the MAGE-3.A1 peptide, with the same vaccination schedule. During the third cycle of vaccinations, the patient received ProtD-MAGE-3-His with both peptides, at 3-month intervals. One year after the start of this combined vaccinations regimen, a partial response was achieved that lasted for 8 months. Neither specific anti-MAGE-3.A1 nor anti-MAGE-B35 CTLp could be detected by tetramer assay among the PBMC collected after the 6th and after the 9th vaccinations with the MAGE-3 protein (P. Coulie, personal communication). Five regressing nodules were resected at different time points during the combined vaccination regimen. Two of them, resected after the 9th and the 14th protein vaccinations, respectively, were analyzed for the expression of *MAGE-3*, as well as HLA class I and β 2-microglobulin molecules and were found to be positive. Nineteen months after the start of the combined vaccinations regimen, several new in-transit metastases appeared and vaccinations were discontinued. In April 2005, the patient was treated

Figure 44 Clinical evolution of Patient 130



by isolated limb perfusion with melphalan and TNF. He achieved a PR to ILP treatment and a few residual in-transit metastases were resected. In March 2006, the patient had no evidence of disease, more than 4 years after the start of the in-transit dissemination.

Analysis of the CD4⁺ T-cell response

van der Bruggen and colleagues recently developed an approach which involves overnight stimulation of blood T cells with autologous dendritic cells loaded with MAGE-3 protein, sorting by flow cytometry of the CD4⁺ T cells that produce IFN- γ , cloning of these cells and evaluation of the number of T cell clones that secrete IFN- γ upon stimulation with the antigen (*Section II. 4.4.2, Method B*) (207). We summarize here the results obtained in the patients who displayed an objective (1) or a mixed tumor response (4) and in one patient who had PD after the first cycle of MAGE-3 protein vaccinations (*Table 17, Fig. 42 and 44*) (207). Before vaccination, no anti-MAGE-3 CD4⁺ T cells were found among the PBMC of the four patients tested. After vaccination, anti-MAGE-3 CD4⁺ T cells were detected in only one out of the six patients tested (Patient 101 who experienced a MxR). The frequency of the anti-MAGE-3 CD4⁺ T cells in the post vaccination blood samples of this patient peaked at $4.7 \cdot 10^{-6}$ of the CD4⁺ T cells after the 6th vaccination, representing at least an 18-fold increase of the frequency found before immunization. All 13 anti-MAGE-3 T cell clones which were derived and which corresponded to five different TCR clonotypes, were found to recognize the same MAGE-3.DR1₂₆₇₋₂₈₂ peptide (145).

Analysis of the antibody responses against the ProtD-MAGE-3-His recombinant protein

Summary of antibody responses

The majority of patients had no anti-MAGE-3 antibody response.

Anti-MAGE-3 IgG antibody response

Twenty-six patients who received at least 4 vaccinations were evaluable for the anti-MAGE-3 IgG antibody response (209). ELISA assays were performed on the pre-vaccination and post-vaccination sera that were available for 21 and 24 patients, respectively.

Only one out of 24 patients (Patient 114) developed a weak antibody response against the MAGE-3 protein (AA 57-219) after 6 vaccinations (serum titer 1: 400). Although the reactivity was weak, we consider it as significant, because of the low background against non-relevant antigens and the conservative cutoff point determined for MAGE-3 (data not shown). No other post-vaccination sera were available for this patient who was removed from the study after the 9th vaccination because progression of in-transit metastases necessitated another treatment. No MAGE-3 reactivity was found in any of the 21 pre-vaccination sera tested, including that of patient 114 (210).

Table 17

Anti-MAGE-3 CD4⁺ frequency among blood CD4⁺ lymphocytes

Patients code	Before vaccinations	After 6	Tumor response
112	ND	$< 7.10^{-7}$	PR
104	$< 9.10^{-7} *$	$< 10^{-6} **$	MxR
128	ND	$< 5.10^{-7} **$	MxR
111	$< 10^{-6}$	$< 2.10^{-6}$	MxR
101	$< 3.10^{-7}$	5.10^{-6}	MxR
130	$< 5.10^{-7}$	$< 7.10^{-7}$	PD

ND = not done

* Lower limit of detection for each experiment

** After 9 vaccinations.

Anti-ProtD antibody response

The anti-ProtD antibody response was not assessed in this study, because in the previous study using the same ProtD/MAGE-3 recombinant protein, the anti-ProtD antibody response was negative in the three patients who received the recombinant protein without immunological adjuvant (*Section III. 2.1, Table 12*) (208).

Analysis of the antigen presentation capability by the tumor

In order to test a possible explanation for the treatment failures observed in most of the patients included in the study, we examined the tumors for possible causes of lack of presentation of the MAGE antigens, such as loss of expression of *MAGE-3* or loss of surface expression of either β 2-microglobulin or HLA class I molecules.

Because *MAGE-3* expression by the tumor was an inclusion criterion to this study, the 32 enrolled patients had a *MAGE-3* positive tumor biopsy at study entry. Eleven out of the 30 patients vaccinated underwent the resection of a superficial metastasis during or after treatment. Eight out of these 11 lesions were progressive or new metastases that appeared during study treatment. All eight expressed *MAGE-3*. Out of the three regressing metastases, only one contained analysable tumor tissue. It was also positive (data not shown).

Frozen tumor biopsy slides were examined by immunohistochemistry for the binding by the conformational anti-HLA class I antibody W6/32 and by an anti- β 2-microglobulin antibody. Sixteen pre-vaccination tumor biopsies sampled from 13 patients and 12 post-vaccination tumor biopsies from 11 patients were made available for this analysis. For 7 patients, samples of both types were obtained. No complete loss of expression of HLA class I molecules or β 2-microglobulin was observed in any of the samples analyzed. Some heterogeneity in HLA class I molecules and/or β 2-microglobulin expression was noted in the post-vaccination tumor sample of patient 124 and in the pre- and post-vaccination tumor samples of patient 126. Both patients experienced tumor progression after vaccination (*data not shown*).

Conclusion

The clinical and immunological responses generated by the vaccine are rather limited. Nevertheless, given the potential anti-tumor efficacy and the very mild toxicity of vaccinations, further studies combining MAGE proteins and/or peptides with potent immunological adjuvants are warranted, not only in metastatic melanoma, but also in the adjuvant setting.

III. 2.3 MAGE-3 protein by intramuscular route, mixed with AS15 - a liposome-based adjuvant containing CpG/QS21/MPL - and combined with peptides

Study LUD 02-002

Phase I/II study of immunization with recombinant MAGE-3 protein combined to AS15 adjuvant containing CpG/QS21/MPL and with peptides, in patients with MAGE-3 positive metastatic cutaneous melanoma.

DESIGN SUMMARY

Immunization:

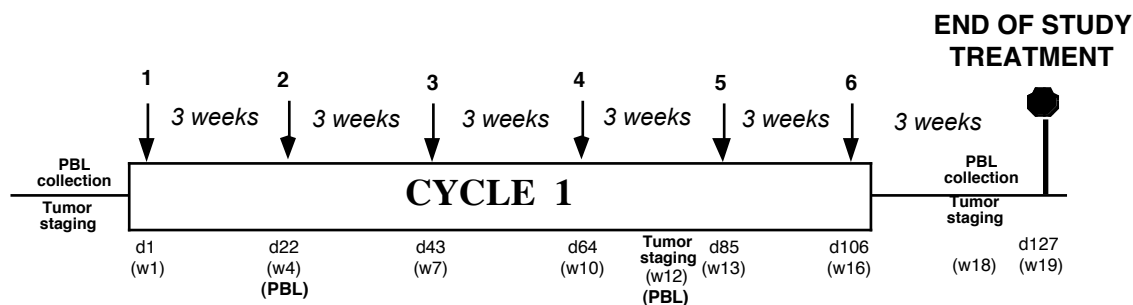
- 300 μ g MAGE-3 protein mixed with AS15 and injected at a single i.m. site, combined with:
- 300 μ g of each selected peptides* injected i.d. (60 μ g) and s.c. (240 μ g) at sites close to the protein/AS15 injection site

*Peptides will be selected according to the patient's HLA type and tumor antigen expression, in the following order: MAGE-3.A1/B35, NA17.A2, MAGE-10.A2, MAGE-3.A24, MAGE-3.B44/B18, MAGE-12.Cw7, MAGE-3.DP4 and MAGE-3.DR13.

Patients will receive a minimum of 1 and a maximum of 3 peptides

Study size:

28 patients should receive at least four vaccinations



Coordinating investigator: Marie Marchand

Clinical sites:

Cliniques universitaires St-Luc, UCL, Brussels
Hôtel Dieu, CHU de Nantes, Nantes
Cancer Research UK Clinical Centre, St James' University Hospital, Leeds.

Principal investigators:

J.-F. Baurain
B. Dréno
P. Patel

Rationale

Injection of bacterial DNA into mice activates innate and acquired immune mechanisms. Particularly, it induces endogenous release of IL-12 and IFN- γ , by activating macrophages and NK cells. This Th1-type response can also be obtained with synthetic oligonucleotides, provided they contain unmethylated CpG dinucleotides, which are abundant in bacterial but not vertebrate DNA. CpG-containing oligonucleotides activate antigen-presenting cells after binding to Toll-Like Receptor 9 (TLR9). Thus, they can be used as immunological adjuvants to enhance Th1-type immune responses. Added to a peptide or protein vaccine, they might favor the induction of a CTL response to that antigen (233). In order to evaluate the antigenicity and immunogenicity of the human MAGE-3 protein, the vaccine candidate was injected in C57BL/6 mice, for which potential MHC class-I and class-II binding peptide motives was theoretically predicted. Four intramuscular vaccines made of 10 μ g of ProtD-MAGE-3-His protein either mixed in saline or in the AS15 adjuvant, a combination of CpG 7909, a synthetic, single stranded phosphorothioate oligodeoxyribonucleotide optimized for *in vivo* use in humans, with QS21 and MPL, were given 14 days apart. Both the specific humoral and cellular immune responses induced by the vaccines were analyzed 14 days after the second and the fourth injection. The preliminary results obtained by Catherine Gérard, GlaxoSmithKline, Rixensart, Belgium, suggest that the ProtD-MAGE-3-His is weakly immunogenic by itself in mice. However, the immunogenicity of this antigen was greatly improved by the use of AS15 as shown by the induction of both MAGE-3 specific humoral and cellular responses.

The use of a protein is based on the diversity of class I and class II epitopes provided by a single agent. However, immunization with the MAGE-3 protein alone might generate a specific CD4⁺ T helper response, but no CD8⁺ T-cell response. In our previous studies with the MAGE-3 protein, we have been unable to identify specific CD8⁺ CTL clones against MAGE-3 epitopes, whereas specific CD4⁺ clones were only detected in Patient 101 who was immunized with the MAGE-3 protein id/sc and who showed a mixed response to that treatment (145, 207). The addition of class I peptides might generate an additional CD8⁺ response, improve the immune response and, hopefully, the clinical response. Accordingly, the first choice of peptides among those listed in the design summary were peptides presented by HLA class-I molecules. Priority was given to peptides MAGE-3.A1, NA17.A2, MAGE-10.A2 and MAGE-3.A24 because HLA class I-peptide tetrameric complexes were available for these four peptides, which should greatly facilitate the detection of an immune response to vaccination (205). If no class I epitope could be added, a MAGE-3 class II epitope was added to the protein. This might improve the recognition of this class II epitope by CD4⁺ T helper cells in case the presentation of the whole protein to the CD4⁺ T cells would result in the dominant recognition of other epitopes. The combination of several epitopes should not only increase the likelihood of occurrence of an immune response. It should also increase the diversity of the response, thereby reducing the emergence of antigen-loss variants.

Figure 45

A. Eligibility relative to maximum extent of disease according to AJCC 2002 staging for Melanoma

I and II		III			IV		
PRIMARY TUMOR ONLY	1 REGIONAL LYMPH NODE MET. ONLY	2 OR 3 REGIONAL LYMPH NODES METS ONLY	REGIONAL SKIN METS ONLY	≥ 4 REGIONAL LYMPH NODES METS <i>or</i> REGIONAL SKIN AND LYMPH NODE METS	DISTANT SKIN <i>or</i> LYMPH NODE MET	LUNG MET	OTHER VISCERAL MET, EXCEPT BRAIN OR ELEVATED LDH
<i>T1-4</i>	<i>N1</i>	<i>N2 a, b</i>	<i>N2c</i>	<i>N3</i>	<i>M1a</i>	<i>M1b</i>	<i>M1c</i>
NO		YES					

with at least one detectable metastasis

B. Accrual

Tumor type	Included	Treated	
		Completed protocol	Removed
Melanoma	11	10	1

Objectives

In the present trial, the MAGE-3 protein mixed with AS15 was administered in combination with selected class I or class II peptides, in order to see whether this vaccination procedure could result in the simultaneous activation of both CD8⁺ and CD4⁺ specific T lymphocytes. Tumor evolution and safety of the vaccine were examined as well.

Treatment schedule

The vaccination schedule of this phase I/early II study comprised 6 vaccinations at 3-week intervals (*design summary*). The day of each vaccination, a fixed dose of ProtD-MAGE-3-His was mixed with AS15 - a liposome-based adjuvant containing CpG/QS21/MPL - and administered by i.m. route. In addition, patients received 300 µg of each selected peptide solubilized in 0.5 ml NaCl 0.9%, without adjuvant. For the peptides, the i.d. and s.c. routes were privileged because of the presence of Langerhans cells in the skin and the main role played by dendritic cells in antigen presentation. Peptides were selected according to the patient's HLA type and tumor antigen expression, in the following order: MAGE-3.A1/B35, NA17.A2, MAGE-10.A2, MAGE-3.A24, MAGE-3.B44/B18, MAGE-12.Cw7, MAGE-3.DP4 and MAGE-3.DR13. Patients received a minimum of 1 and a maximum of 3 peptides injected i.d. (60 µg) and s.c. (240 µg) at a distance of approximately 1 cm from the MAGE-3/AS15 injection site. These close injection sites should presumably be drained by the same lymph node, in which the immune response was expected to occur. All injections were given in the arms or the anterior aspect of the thighs.

After a first cycle of 6 vaccinations, patients whose tumor regressed, stabilized or slowly progressed, received additional vaccinations at increasing intervals until major tumor progression or relapse requiring other treatments. Six additional vaccinations were first given at 6-week intervals, followed by 3-month intervals between the following ones.

The study was initially designed to include 28 patients. However, because a 1-year delay in the accrual related to various constraints, GSK took the decision of an early closure of the study.

Eligibility criteria

Eligibility criteria relative to melanoma stage are defined in *Fig. 45 A (for detailed eligibility criteria see Annex 3)*. Only patients with detected metastases were eligible.

Patients' characteristics

From December 2003 to May 2004, 11 patients whose tumor expressed *MAGE-3* were enrolled in the study in the 3 participating institutions (*Fig. 45 B* and *Table 18*). There were 5 males and 6 females. Their median age was 53 years (range : 36 - 77 years).

Table 18 Patients' Characteristics

Patients			Previous treatments ¹	AJCC 2002 Stage ² at study entry	Total number injec- tions ³	Study treatment			SAE (n) ⁵	
Gender Code	Age	Toxicities ⁴								
		Maximum toxicity severity or grade								
						Local	Flu	Other		
1) Patients having received complete protocol (≥ 4 vaccinations) :										n = 10
1	M	39	Sg, SI	IV pTX N0 M1c	6	2	3	2	0	
3	F	74	Sg, ILP, NSI, SI, Ct	IV pT4b N3 M1c	4	2	3	1	0	
4	F	56	Sg, NSI	IIIC pTX N3 M0	8	2	2	2	0	
5	F	36	Sg, R, Ct	IV pT4 N2c M1c	4	2	3	1	0	
6	M	77	Sg	IV pT4b N1b M1c	4	2	3	1	1	
7	F	66	Sg	IV pT2a N1b M1c	4	1	3	2	0	
8	M	64	Sg, NSI	IV pT0 N2b M1c	4	2	3	1	0	
9	F	48	Sg	IV pT3a N3 M1a	6	2	2	1	0	
10	M	48	Sg, Ct	IV pT2a N0 M1c	11	2	2	0	5	
11	F	53	Sg	IIIB pT2a N2c M0	17	2	2	2	0	
2) Patients who were withdrawn (< 4 vaccinations) :										n = 1
2	M	47	Sg	IV pT1 N3 M1c	1	0	0	0	1	

- Previous treatments:* Ct: Chemotherapy, H: Hormonal therapy, ILP: Isolated Limb chemotherapy Perfusion, NSI: Non-Specific Immunotherapy, R: Radiotherapy, Sg: Surgery, SI: Specific Immunotherapy,
- AJCC staging:* refers to the 6th edition of the AJCC staging manual, 2002 (see Table 6).
Melanoma staging: III = regional disease = N (N1b = 1 palpable node; N2b = 2–3 palpable nodes; N2c = in-transit met(s) / satellites(s) without metastatic nodes; N3 = ≥ 4 nodes or in-transit met(s) / satellites(s) with metastatic node(s)); IV = distant metastasis = M (M1a = metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes; M1b = lung metastasis; M1c = any other visceral metastasis and/or elevated LDH). In-transit/satellite metastases involve skin or subcutaneous tissue between the primary tumor and the regional lymph node.
- Total number of injections:* 3 patients who were thought by the investigator to have some benefit of the treatment received more than 6 vaccinations, on a compassionate use basis.
- Toxicities:* Maximum toxicity severity or grade reported according to the National Cancer Institute common toxicity criteria scale, version 2.0 April 30, 1999. Local: reaction at injection sites, Flu: Flu-like symptoms, Other: Any other toxicities.
- SAE:* number of Serious Adverse Events.

They all received the vaccine. One patient was withdrawn from the study before the 4th vaccination, due to rapidly progressive disease. Ten patients received at least 4 vaccinations and were considered evaluable for tumor response.

The selected peptides received by each patient in combination with the MAGE-3 protein/AS15 vaccine are reported in *Table 19*. MAGE-3.DP4, the most frequently expressed MAGE antigen in metastatic melanoma (57%), was given to 9/11 patients (*Table 10*).

Toxicity

All patients were evaluated for toxicity. The vaccine was generally well tolerated.

A total of 69 vaccinations were administered. No treatment-related adverse events, i.e. “toxicities”, above grade 3 were reported (*Table 18*). Toxicities occurred after one or several vaccinations. The most frequently reported ones were mild to moderate local reactions at the injection sites and flu-like symptoms (*Table 20*).

Grade 3 fatigue was reported in 6 patients after 15 vaccinations (*Tables 18* and *20*). Seven SAE were reported in 3 patients (*Table 18*). These events were unrelated to the study treatment. The 3 patients were hospitalized for the following reasons: uncontrolled pain in the sacral region (Patient 2); hematuria (Patient 6); surgical excision of intestinal metastases; detection of a brain metastasis requiring γ -knife treatment, epileptic convulsions and degradation of performance status (Patient 10). In all cases, the symptoms were related to tumor progression. Hematuria was reported as grade 3 by the investigator, whereas the other SAE were of grade 4.

Tumor response, time to progression and survival

Upon GSK request, clinical responses will not be disclosed here.

Analysis of the CTL responses

Immunological readouts are ongoing.

Table 19 Peptides received by each patient

Patients code *	Peptides n°		
	1	2	3
1	MAGE-3.A1	MAGE-12.Cw7	MAGE-3.DP4
2	NA17.A2	MAGE-3.DP4	MAGE-3.DR13
3	MAGE-3.B44	MAGE-12.Cw7	MAGE-3.DP4
<u>4</u>	MAGE-10.A2	MAGE-3.A24	MAGE-3.DP4
5	MAGE-3.A1	MAGE-10.A2	MAGE-12.Cw7
6	NA17.A2	MAGE-10.A2	MAGE-3.B44
7	MAGE-10.A2	MAGE-12.Cw7	MAGE-3.DP4
8	MAGE-3.DP4	NA	NA
9	MAGE-3.A1	MAGE-12.Cw7	MAGE-3.DP4
<u>10</u>	MAGE-3.A24	MAGE-3.DP4	NA
<u>11</u>	MAGE-3.B44	MAGE-3.DP4	NA

* The underlined identification numbers indicate patients who displayed tumor regression.

NA: not applicable

Table 20 Incidence of most frequent toxicities

	Number of vaccinations after which symptoms were reported	Incidence of symptoms
Local reactions at injection site		
Pain	40	0.77
Redness	37	0.71
Heat	22	0.42
Induration/swelling	19	0.37
Flu-like symptoms		
Chills	42	0.81
Fever	30	0.58
Myalgia	28	0.54
Headache	26	0.50
Fatigue/lethargy	21	0.40
Nausea	19	0.37
Arthralgia	14	0.27
Malaise	12	0.23
Total number of vaccinations		
	52	

III. 3 CONCLUSIONS

The clinical observations made in cutaneous melanoma patients with detected metastases immunized either with MAGE peptides or with a recombinant MAGE-3 protein can be summarized as follows:

Immunization with MAGE peptides or the MAGE-3 recombinant protein is devoid of significant toxicity.

Among the 157 patients who completed the first cycle of vaccinations according to the relevant protocol, 4% displayed objective tumor responses (OR = CR and/or PR), and 10% had MxR (*Table 21*). Long lasting, i.e. more than 3-year complete responses remain the exception. The intent-to-treat analysis on 225 patients generates a little lower response rates, i.e. 3% for OR and 7% for MxR. Although the rate of OR observed is low, it is about ten times higher than the reported incidence of spontaneous regression of melanoma metastases, that is lower than 0.4%, suggesting that the regression of metastatic lesions observed here are linked to the vaccinations (102).

Except for 3 patients who had complete regression of some visceral metastases – which does not necessarily mean that they had a complete tumor response to treatment - the lesions that regressed were regional or distant cutaneous or lymph node metastases (*Table 22*). Cutaneous and lymph node melanoma metastases are also those who better respond to chemotherapy, radiotherapy and cytokine treatment (19) (pp. 499, 516, 524).

The regression process is often slow to occur and slow to proceed, which suggests that prolonged immunization of patients with a slowly progressing disease might be helpful. Regression of skin metastases is usually not accompanied by local inflammatory reactions. It can be restricted to a subset of the lesions, whereas the others increase in size or remain stable (MxR). Sometimes, new skin nodules can appear during the regression process and then disappear rapidly.

Complete tumor responses can be followed by the appearance of multiple new metastases at new sites in the absence of relapses at the original site. Analysis of some relapsing lesions, or lesions that progressed during mixed responses, has shown persistence of the expression of the MAGE gene that encodes the antigen used in the vaccine. Loss of expression of the MAGE gene is therefore not the main cause of relapse.

The observation made with Patient LB-1348 (*Section III 1.1.3*, study LUD 96-007) suggests that therapeutic efficacy might be improved by numerous and frequent vaccinations (197). Likewise, those patients who display a complete tumor response may have to be immunized regularly for several years to maintain a sufficient level of immune protection against emerging metastases.

Approximately 30% of melanoma patients who were vaccinated in the above-mentioned studies were withdrawn before the first cycle of vaccinations was completed because other treatments were deemed necessary to cope with the progression of the disease. Most of these patients had distant metastases and had failed to respond to multiple previous treatments. Since the clinical course of

Table 21

Tumor response in melanoma patients with measurable disease at study entry

Study	Immunogen	Patients (n)			Tumor responses (n)			
		Received at least 1 vaccine	Withdrawn	Completed study treatment	Objective responses (CR + PR)*	Stable disease (SD)*	Mixed responses (MxR)*	Objective Responses + SD + MxR
1) Peptide-based vaccines								
LB 94-001	MAGE-3.A1	39	14	25	4	0	3	7
LUD 95-002	MAGE-3.A1 + AS01B	7	1	6	0	1	0	1
LUD 96-007	MAGE-1.A1 + MAGE-3.A1	17	6	11	0	1	2	3
LUD 97-004	MAGE-3.A1, frequent ± MAGE-3.DP4	45	17	28	0	0	3	3
LB 93-001	MAGE-1.A1	4	1	3	0	0	0	0
LUD 94-002	MAGE-1.Cw*16	2	0	2	0	0	0	0
LUD 95-004	MAGE-3.A2	27	9	18	0	2	2	4
LUD 99-006	MAGE-4.A2 + MAGE-10.A2	5	0	5	0	1	0	1
TOTAL (n)		146	48	98	4	5	10	19
Patients who received at least 1 vaccine (%) :								
		100%	33%	67%	3%	3%	7%	13%
Patients who completed study treatment (%) :								
				100%	4%	5%	10%	19%
2) Protein-based vaccines								
LUD 99-003	MAGE-3	30	4	26	1	0	4	5
LUD 97-002	MAGE-3 + AS02B	49	16	33	2	1	2	5
TOTAL (n)		79	20	59	3	1	6	10
Patients who received at least 1 vaccine (%) :								
		100%	25%	75%	4%	1%	8%	13%
Patients who completed study treatment (%) :								
				100%	5%	2%	10%	17%
1 + 2) Peptide- and Protein-based vaccines								
TOTAL (n)		225	68	157	7	6	16	29
Patients who received at least 1 vaccine (%) :								
		100%	30%	70%	3%	3%	7%	13%
Patients who completed study treatment (%) :								
				100%	4%	4%	10%	18%

* CR = Complete response ; PR = Partial response ; SD = Stable disease ; MxR = Mixed response.

Table 22 Regression of metastases in melanoma patients with measurable disease at study entry

Study	Immunogen	Number of patients who received ≥ 1 vaccine	Localization of evaluable metastases at study entry ¹		Number of patients who completed study treatment	Localization of evaluable metastases at study entry ¹		Number of patients who had regression of metastases	
			non-visceral	visceral		non-visceral	visceral	non-visceral	visceral
Peptide-based vaccines									
LB 94–001	MAGE-3.A1 peptide	39	36	16	25	23	8	7	1 (lung, complete regression of 5/5 mets.)
LUD 95–002	MAGE-3.A1 + AS01B	7	5	4	6	4	4	0	0
LUD 96–007	MAGE-1.A1 + MAGE-3.A1	17	13	10	11	10	5	2	1 (liver, partial regression of 1/3 mets.)
LUD 97–004	MAGE-3.A1, frequent ± MAGE-3.DP4	45	30	30	28	16	12	3	1 (lung, complete regression of 1/1 meta.)
LB 93–001	MAGE-1.A1 peptide	4	4	1	3	3	1	0	0
LUD 94–002	MAGE-1.Cw*16 peptide	2	1	1	2	1	1	0	0
LUD 95–004	MAGE-3.A2 peptide	27	26	13	18	16	7	1	1 (breast, complete regression of 1/1 meta.)
LUD 99–006	MAGE-4.A2 + MAGE-10.A2	5	5	1	5	5	1	0	0
Protein-based vaccines									
LUD 99–003	MAGE-3 protein	30	30	NA*	26	26	NA	5	NA
LUD 97–002	MAGE-3 protein + AS02B	49	38	36	33	26	20	4	0
TOTAL (n)		225	188	112	157	130	59	22	4
Regression of metastases in patients who received at least 1 vaccine (%)								12%	4%
Regression of metastases in patients who completed study treatment (%)								17%	7%

1) The majority of patients with visceral metastases also had non-visceral localizations. NA = not appropriate.

melanoma is rather unpredictable, it is impossible to exclude that our treatment accelerated the course of the disease in some patients, but we feel that there is no significant evidence to support this.

Regression of metastases are not limited to melanoma. A patient with metastatic bladder cancer experienced a PR on a distant lymph node metastasis after immunization with the MAGE-3 protein (Study LUD 97-002, Patient 7015, *Section III. 2.1*) (198).