PERGAMON

European Journal of Cancer 39 (2003) 70–77

European Journal of Cancer

www.ejconline.com

Immunisation of metastatic cancer patients with MAGE-3 protein combined with adjuvant SBAS-2: a clinical report☆

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Received 1 July 2002; accepted 4 July 2002

Abstract

Fifty-seven patients with MAGE-3-positive measurable 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 SBAS-2, which contained MPL and QS21. The immunisation schedule included 4 intramuscular (i.m.) injections at 3-week intervals. Patients whose tumour stabilised or regressed after 4 vaccinations received 2 additional vaccinations at 6-week intervals. The vaccine was generally well tolerated. Among the 33 melanoma patients who were evaluable for tumour response, we observed 2 partial responses, 2 mixed responses and 1 stabilisation. Time to progression in these 5 patients varied from 4 to 29 months. In addition, a partial response lasting 10 months was observed in 1 of the 3 metastatic bladder cancer patients included. None of the tumour responses described above involved visceral metastases. Immunological responses to the vaccine will be reported separately.

Keywords: Antibodies; Cancer; Immunological adjuvant SBAS-2; Immunotherapy; MAGE-3 protein; Melanoma; T lymphocyte

1. Introduction

Gene *MAGE-3* codes for several antigenic peptides capable of binding to human leucocyte antigen (HLA) class I or class II molecules [1]. These complexes can be recognised by T lymphocytes on the tumour cell surface [2]. Gene *MAGE-3* is expressed in 76% of metastatic melanomas and in many other tumours of various histological types. Since the *MAGE-3* encoded antigens are not expressed in normal tissues, they represent potentially safe targets for cancer immunotherapy. In a previous study, a MAGE-3 peptide presented by HLA-A1 (MAGE-3.A1) was administered to 25 patients with metastatic melanoma, on 3 occasions at monthly intervals, by intradermal and subcutaneous routes [3,4]. No significant toxicity was observed. Seven patients showed significant tumour regressions, including 3 complete responses to treatment. However, single peptides may

☆ Preliminary results of this study were presented at the Annual Meeting of the American Society for Clinical Oncology (ASCO), New Orleans, LA, USA, May 2000. For the EORTC Melanoma Group and Biological Treatment Development Group (EORTC study 18972-13971).

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PII: S0959-8049(02)00479-3

not be the best immunogens and various other approaches are currently investigated to develop safe and more effective therapeutic vaccines [5]. Immunising patients with a recombinant protein containing multiple class I and class II epitopes, which are recognised by cytolytic T lymphocytes (CD8+ CTL) and T helper lymphocytes (CD4+ Th-1 cells), respectively, could induce simultaneous CD8+ and CD4+ T cell responses against tumour-specific antigens, thereby generating a more effective anti-tumour response [2,6].

The DNA recombinant MAGE-3 protein used in the present study is a fusion protein with a lipidated protein D derived from *H. influenzae* at its N-terminus, and a sequence of several histidine residues at the C-terminus of the protein (Prot.D MAGE-3/His). 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 affinity tail facilitated the purification of the fusion protein. The protein was produced in *E. coli* and extensively purified to eliminate bacterial contaminants. In order to further improve the immunogenicity of the MAGE-3 protein, adjuvant SBAS-2 (SmithKline Beecham Biologicals Adjuvant System 2) 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. SBAS-2 has potent inflammatory properties and its capacity 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 [7,8].

We report here, the clinical observations made in 57 patients who received escalating doses of the MAGE-3 protein combined with a fixed dose of SBAS-2. Because the evaluation of the immunological responses is not yet completed, it will be reported separately.

2. Patients and methods

2.1. Eligibility criteria

Patients were required to have stage III or IV malignancies with measurable lesions, of one of the following histological types: cutaneous melanoma, non-small cell lung cancer (NSCLC), carcinoma of the head and neck region, oesophageal or bladder cancer [9]. They were also required to have at least 1 of the 3 HLA class I types known at that time to present MAGE-3 peptides, i.e. HLA-A1, HLA-A2 and/or HLA-B44, with tumour expression of the gene*MAGE-3* as assessed by reverse transcriptase-polymerase chain reaction (RT-PCR) on mRNA extracted from a frozen tumour biopsy [1]. Patients had to be over 18 years old, with a World Health Organization (WHO) performance status $\leqslant 1$, normal organ functions, and without brain metastasis, second neoplasm or other serious disease. No chemotherapy, radiotherapy or immunotherapy was allowed in the 4-week period before the first vaccination. All patients provided written informed consent prior to inclusion. The study was approved by the regulatory boards of the participating institutions.

2.2. Vaccine production

The MAGE-3 recombinant protein was provided by GlaxoSmithKline Biologicals, Rixensart, Belgium. The DNA recombinant MAGE-3 protein was expressed in *E. coli* as a fusion protein with a lipidated protein D (Prot.D) derived from *H. influenzae* at the N-terminus, and a sequence of several histidine residues at the C-terminus of the protein (Prot.D MAGE-3/His). The encoding plasmid was designed in such a way to express a precursor protein consisting of the 18 amino acids signal sequence and the first 109 residues of the processed protein D, 2 unrelated amino acids (Met and Asp), the amino acid residues 2–314 of the MAGE-3 protein, and 2 Gly residues functioning as a hinge region to expose the subsequent 7 His residues. Thus, the Prot.D MAGE-3/His fusion protein produced comprises 433 amino acid residues.

SBAS-2 was also provided by GlaxoSmithKline Biologicals. QS21 was provided to GlaxoSmithKline Biologicals by Cambridge Biotech Corporation, Worcester, USA, and Monophosphoryl lipid A (MPL), by Ribi ImmunoChem, Hamilton, Montana, USA. QS21 and MPL were mixed together in the oil/water emulsion SB62 to obtain SBAS-2.

2.3. Study design

The vaccination schedule of this phase I/early II study comprised 4 vaccinations at 3-week intervals. Patients whose tumour stabilised or regressed after 4 vaccinations received 2 additional vaccinations at 6-week intervals. Three escalating dose levels of Prot.D MAGE-3/His (30, 100 and 300 μg) mixed with a fixed dose of SBAS-2 containing 100 μg MPL, 100 μg QS21 in 250 μl oil/water emulsion were tested in 3 groups of 12 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 SBAS-2 (group n° 1). Due to the potent inflammatory properties of SBAS-2, the vaccine was administered by the intramuscular (i.m.) route in the upper arms or thighs. 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. From October 1997 to August 1999, 59 patients were enrolled in the

study in the 12 participating institutions. They were assigned to groups 1–4 by the NDDO Oncology project manager, according to their HLA class I and tumour types.

2.4. Evaluation of patients

Adverse events were graded according to the National Cancer Institute of Canada Clinical Trial Group Common Toxicity Criteria scale (NCIC CTG CTC) [10]. Relationship to treatment was evaluated by the clinical investigators as probable, suspected, unlikely or not related. Adverse events were considered as being related to the treatment if the relationship was reported as probable or suspected. Treatment-related adverse events are reported here with the term "toxicities".

Tumour response was assessed after the 4th and the 6th vaccinations, compared with baseline tumour measurements performed in the 2-week period preceding the 1st vaccination. Tumour response was defined according to the WHO criteria [11,12]. For cutaneous melanoma, "mixed responses", i.e. regression of some target lesions while others progress or appear simultaneously, although formally classified as progressive disease in the WHO classification, were documented as well. A long-term follow-up of all patients included was realised every 6 months until death. This trial was performed according to the Good Clinical Practice guidelines.

3. Results

3.1. Patients' characteristics

Fifty-nine patients, 25 men and 34 women, entered the study. Their mean age was 51 years (range: 27–76 years). Fifty-one patients had cutaneous melanoma American Joint Committee on Cancer (AJCC) stage III or IV. Among the other patients, 3 had bladder transitional cell carcinoma, 2 had NSCLC, 2 had oesophageal cancer and 1 had head and neck 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 1). 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.

3.2. Adverse events

The 57 patients who received at least 1 vaccination were evaluated for safety and toxicity. A total of 222 vaccinations were performed, which were generally well tolerated. No treatment-related adverse events, i.e. "toxicities", above grade 3 were reported (Table 1). Toxicities occurred in all groups, after one or several vaccinations.

The most frequently reported toxicities were mild to moderate (grades 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 2).

Thirteen vaccinations were followed by grade 3 toxicities, which were reported in 9 different patients. 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 anaemia in a single patient. Thirty-five serious adverse events (SAE) were reported in 26 patients (Table 1). However, only one SAE, a grade 3 anaemia, was suspected to be related to the vaccinations (patient 7038, group 4). One event was of infectious origin and all others were related to tumour progression, including 3 deaths.

3.3. Tumour response to treatment and survival

For the 59 patients who entered the study, the median overall survival was 7 months (1–44+ months). For the 51 melanoma patients, it was 7.5 months (1–44+ months). Because we did not expect full immunisation of the patients before several injections, only the 39 patients who received at least 4 vaccinations were considered evaluable for tumour response. Their clinical evolution is summarised in Fig. 1.

Thirty-three patients had melanoma. The median overall survival in this subgroup was 9.5 months (2.5–44+ months). Tumour regression was observed in 4 patients. Two of these patients experienced objective clinical responses. Patient 7002, who received MAGE-3 protein in the absence of adjuvant, 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 tumour 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 tumour 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 par-

Table 1
Patients' characteristics

Patients			Tumour type	Class I HLA		Previous treatments (type)	Study treatment						Further treatment	Last status	
Code	Gender	Age (years)		A	B		AJCC stage at entry	Study group	Total number injections	Toxicities maximum toxicity	SAE (n)	Tumour response		Tumour status	Survival (months)
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 SBAS-2) (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	>39
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	NED	>37
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	>33
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	>42
7013	M	47	MELA	**A1**	B7,B8	Sg, ILP, NS	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 SBAS-2) (n = 12)															
7021	F	71	MELA	**A1**,A24	B62	Sg	III N2b	3	4	2	0	PD	yes	NED	>38
7025	F	62	MELA	**A2**,A30	**B44**,B7	Sg, ILP, NSI	IV M1a	3	10	2	0	**SD**	yes	PD	>44
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	NED	>33
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	18
7016	M	59	BLAD	**A2**	**B44**	Sg, Ct, NSI	IV	3	4	2	1	PD	no	PD	2.5
Group 4 (300 μg plus SB AS-2) (n = 10)															
7057	F	55	MELA	**A1, A2**		Sg, R, Ct, NSI	III N2b	4	20	1	0	**PR**	no	PD	>34
7048	F	69	MELA	A3,A23	B7,**B44**	Sg, ILP, NSI	III N2b	4	6	2	0	PD	yes	NED	>38
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	>39
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 (1–3 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

(*continued on next page*)

Table 1 (*continued*)

Patients			Tumour type	Class I HLA		Previous treatments (type)	Study treatment						Further treatment	Last status	
Code	Gender	Age (years)		A	B		AJCC stage at entry	Study group	Total number injections	Toxicities maximum toxicity	SAE (n)	Tumour response		Tumour status	Survival (months)
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	23
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

Tumour type: BLAD, bladder; ESO: oesophagus, HD.NK, head and neck; MELA, melanoma; NSCLC, non-small cell lung cancer. *Previous treatments*: Ct, chemotherapy; ILP, isolated limb chemotherapy perfusion; H, hormonotherapy; NSI, non-specific immunotherapy; R, radiotherapy; Sg, surgery. *AJCC staging*: refers to the 4th edition of the American Joint Committee on Cancer (AJCC) staging manual, 1992 [9]. 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 tumour not beyond the regional lymph nodes. *Total number of injections*: six patients who were thought by the investigator to have some benefit of the treatment received more than six vaccinations, on a compassionate basis. *Toxicities*: Maximum toxicity grade reported according to the National Cancer Institute of Canada Common Toxicity Criteria scale. *SAE*: number of serious adverse events. *Tumor response*: according to the WHO criteria: PR, partial response; SD, stable disease; PD, progressive disease. In addition, mixed responses were reported (M×R). *Last status*: NED, no evidence of disease. M, male; F, female; HLA, human leuocyte antigen.

tially resected. The patient died 1 year later of disease progression. Patient 7057 showed 7 cutaneous and 3 subcutaneous in-transit lesions at study entry. Regression of some lesions was already apparent 1 month after the 1st vaccination. Two regressing lesions were surgically removed during treatment, for scientific purposes. In both lesions, tumour 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, tumour 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 cancerous. The patient had no detectable disease for a period of 29 months, then brain metastases occurred. Two other patients with regional disease experienced a mixed response. Patient 7049, who had a mixed response of in-transit metastases followed after 4 months by progression of these lesions, was entered into another vaccination study involving the NA17.A2 peptide antigen [13]. This resulted in a complete response, which has been lasting for more than 2 years [14]. Patient 7037 had a 4-month mixed response of in-transit metastases followed by disease progression. In addition to the 4 patients who showed tumour regression, patient 7025, with cutaneous and subcutaneous in-transit metastases, experienced tumour stabilisation for a period of 11 months.

Six patients had other solid tumours types, namely, bladder, head and neck, lung or oesophageal cancer. Tumour regression was observed in 1 patient. This patient (7015) presented at study entry with 2 distant metastatic lymph nodes of bladder cancer progressing after two lines of chemotherapy. 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. Tumour relapse occurred 2 months later.

Time to progression in the 5 patients who had some clinical benefit from the treatment varied from 4 to 29 months.

4. Discussion

In this first study involving immunisation of metastatic cancer patients with a MAGE protein, we found that vaccinations with or without immunological adjuvant SBAS-2 were generally well tolerated. This is inline with the observations that were made in melanoma patients in a vaccination trial involving MAGE-3.A1 peptide alone and in another study involving a combination of MAGE-3.A1 peptide in incomplete Freund's adjuvant [3,4,15]. A similar lack of toxicity has also

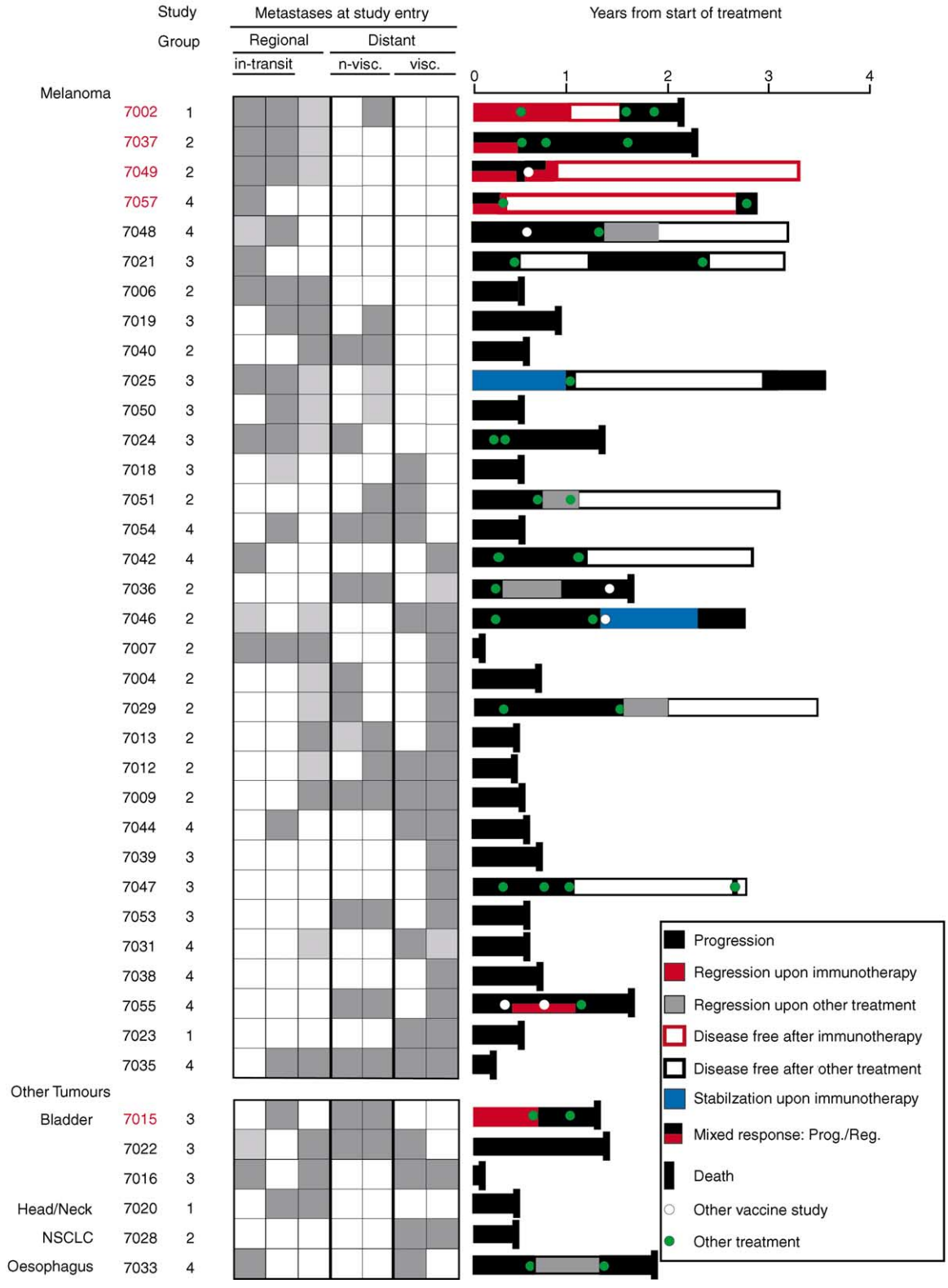

Fig. 1. Patients are classified according to their tumour type and their AJCC stage at study entry. *Metastasis at study entry*: dark grey: measurable metastases at study entry; light grey: metastasis removed before study. n-visc. = non-visceral distant metastasis, visc. = visceral metastasis, C = cutaneous, S = subcutaneous, C.S = C or S, L = lymph node, Lu = lung, O = other visceral localisation. Patient 7046 (group 2) entered after this study into another vaccination protocol involving the MAGE-4.A2 and the MAGE-10.A2 peptides. This treatment resulted in a 10 months stabilisation period.

Table 2
Incidence of most frequent toxicities

	Group 1 300 μg MAGE-3 protein without adjuvant	Group 2 30 μg MAGE-3 protein with adjuvant	Group 3 100 μg MAGE-3 protein with adjuvant	Group 4 300 μg MAGE-3 protein with adjuvant
Total number of vaccinations	18	81	57	66
Local reaction at injection site	9	59	41	44
Flu-like symptoms				
Fatigue	0	15	16	1
Myalgia	3	12	21	14
Headache	1	8	12	1
Fever	1	8	23	11
Other general symptoms				
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.

been observed by other groups who immunised melanoma patients with dendritic cells pulsed with MAGE epitopes [16–19]. Vaccinations with NY-ESO-1 derived peptides, another tumour-specific antigen, combined with granulocyte macrophage-colony stimulating factor (GM-CSF), did not generate any severe toxicity either [20].

For patients with melanoma, the intent-to-treat objective tumour response rate was 4% (2/51). However, we analysed separately the group of patients who received at least 4 vaccinations, because we did not expect full immunisation of the patients before several injections. Among the 33 melanoma patients who received at least 4 vaccinations, we observed 2 partial responses and 2 mixed responses. Thus the fraction of patients with evidence of clinical effect of the vaccine after 4 vaccinations is 4/33. The 4 patients who showed tumour regression belonged to the subgroup of 12 patients who had no history of visceral metastases at study entry. In contrast, none of the 21 patients who had visceral metastases showed benefit from the treatment, including 3 patients who had lung as the unique visceral organ involved by the tumour. In a previous study, we observed tumour regressions in 7/25 melanoma patients who were immunised with the MAGE-3.A1 peptide [3,4]. The lower proportion of tumour regressions observed in the present trial (4/33) is likely to be due to the higher proportion of patients with visceral metastases, compared with the MAGE-3.A1 peptide study (21/33 versus 8/25 patients, respectively). Among the 7 patients who showed tumour regression in the MAGE-3.A1 peptide study, only 1 had visceral (lung) metastases. These observations suggest that non-visceral metastases are more sensitive to MAGE-3 therapeutic vaccination than visceral localisations.

In this trial, we observed only one tumour stabilisation lasting for 11 months, in a patient who had a stage IV M1a melanoma at study entry, and in other studies involving MAGE epitopes, we rarely observed significant, i.e. more than 6-month disease stabilisations. This is in contrast with the results obtained by Jäger and colleagues, who immunised 9 melanoma patients with NY-ESO-1.A2 peptides combined with GM-CSF [20]. Although no objective tumour responses were observed in that study, the treatment resulted in tumour stabilisation in 5/9 patients, including 3 patients whose melanoma stabilised for 8, 9 and 10 months, respectively. One of these 3 patients had a lung metastasis at study entry ([20] and E. Jäger, personal communication). A difficult problem in the comparison of the various trials in this respect is the low number of patients included in these studies.

Interestingly, of the 6 patients with other tumour types than melanoma who received at least 4 vaccinations in the present study, 1 partial response was observed in a patient with cervical metastatic lymph nodes of bladder cancer. Recently, Nishiyama and colleagues treated 4 patients with metastatic bladder cancer using autologous dendritic cells pulsed with the MAGE-3.A24 peptide (amino acid position in MAGE-3, 195–203). Following this treatment, 3/4 patients showed tumour regression involving metastatic lymph nodes and a liver metastasis [21]. These observations suggest that MAGE-3 is an interesting target for vaccination studies in bladder cancer patients.

Of the 39 patients who were evaluable for tumour response in this study, 17 underwent resection of a metastatic lesion which progressed or appeared during vaccination. *MAGE-3* expression was found in all samples, indicating that tumour progression or relapse was

not due to a loss of expression of gene *MAGE-3* by the tumour.

In the 5 patients who experienced tumour regression, no macroscopic inflammatory reaction was observed at regressing cutaneous or superficial lymph node metastatic sites. In the 3 patients who had partial response to treatment, there was no simultaneity in the regression of the different metastases.

Tumour responses were observed at all doses of MAGE-3 protein tested, both with and without the adjuvant SBAS-2. The fact that 1 of the partial responses occurred upon vaccination without adjuvant indicates that the recombinant MAGE-3 protein given alone could have some capacity to immunise, which might be due to the properties of its Lipoprotein D portion. This is presently being investigated in a trial where Prot.D MAGE-3/His is given intradermally and subcutaneously without adjuvant to melanoma patients with cutaneous or lymph node metastases.

In conclusion, vaccination with the MAGE-3 protein with or without adjuvant resulted in tumour responses in a minority of patients. At least in melanoma, the extent of disease, i.e. non-visceral versus visceral, appears to be a prognostic factor for clinical response. The objective response in another tumour type, bladder cancer, is encouraging. Of note, this patient was also without visceral metastases. Given the very mild toxicity of vaccinations with shared tumour-specific antigens and the potential antitumour efficacy in melanoma and bladder cancer patients, further studies are warranted, not only in metastatic disease, but also in the adjuvant setting, to develop a more effective vaccine therapy.

Acknowledgements

We gratefully acknowledge the following Institutions and clinical investigators for their participation in this study: Amsterdam Cancer Institute (G.C. de Gast); Amsterdam Free University (W. Gerritsen); Institut Jules Bordet, Brussels (F. Lehmann); Centre pluridisciplinaire d'Oncologie and Ludwig Institute for Cancer Research, Lausanne Branch, Lausanne (D. Liénard); and Cliniques Universitaires de Mont Godinne, University of Louvain, Yvoir (J. Kerger). This work was supported by the Fortis Bank-Assurances and VIVA (Belgium).

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