

# Immunotherapy of distant metastatic disease

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Immunotherapy of metastatic melanoma consists of various approaches leading to specific or non-specific immunomodulation. The use of FDA-approved interleukin (IL)-2 alone, in combination with interferon  $\alpha$ , and/or with various chemotherapeutic agents (biochemotherapy) is associated with significant toxicity and poor efficacy that does not improve overall survival of 96% of patients. Many studies with allogeneic and autologous vaccines have demonstrated no clinical benefit, and some randomised trials even showed a detrimental effect in the vaccine arm. The ongoing effort to develop melanoma vaccines based on dendritic cells and peptides is driven by advances in understanding antigen presentation and processing, and by new techniques of vaccine preparation, stabilisation and delivery. Several agents that have shown promising activity in metastatic melanoma including IL-21 and monoclonal antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (anti-CTLA-4) or CD137 are discussed. Recent advances of intratumour gene transfer technologies and adoptive immunotherapy, which represents a promising although technically challenging direction, are also discussed.

**Key words:** adoptive immunotherapy, anti-CTLA-4, interferon, interleukin, metastatic melanoma, vaccine

## Introduction

Depending on tumour thickness, mitotic index, presence of ulceration, lymphocyte infiltration, age, gender and anatomical site, 20–25% of all primary melanoma will spread. Dissemination to distant visceral organs is, with the exception of rare cases of surgery for oligometastatic disease, almost invariably a sign of incurable, stage IV disease that has a median survival time of ~6–12 months and a 3-year survival rate of only 10–15% [1]. This is mainly because no treatments of distant metastatic melanoma have demonstrated over the last three decades any survival benefit. Innovative systemic treatment approaches including immunotherapy have focused on metastatic melanoma and are being tested with an increasing intensity. The term ‘immunotherapy’ is used for non-specific as well as specific immunomodulation that encompasses a number of different approaches summarised below.

## Cytokines

### Interleukin-2 and interferon $\alpha$

Interleukin (IL)-2 and interferon (IFN)  $\alpha$  are the most widely used immunomodulating drugs in metastatic melanoma.

Analysis of eight clinical trials of high-dose IL-2 in 270 patients conducted between 1985 and 1993 [2] reported an overall objective response rate of 16% and a complete response in 6% of patients; importantly, ~4% of patients remained progression-free, which indicates that, in some patients, IL-2 therapy can achieve durable complete remission of the disease. IL-2 (Proleukin) was approved for distant metastatic disease based on a series of phase II studies by the FDA in 1998 in the USA, but not by the EMEA in Europe. Treatment with IL-2 is associated with significant toxicity that includes severe hypotension and vascular leak syndrome, resulting in interstitial and pulmonary oedema, renal and hepatic dysfunction, cardiovascular failure, neurological disturbances, nausea, vomiting and thrombocytopenia [3]. This has limited its use to selected patients with good organ function who are treated by experienced clinicians at selected specialised centres.

Several studies have identified pre-treatment factors predicting response to IL-2 therapy [4, 5]. Univariate analyses revealed that the ECOG performance status, number of involved organs, site of metastases and serum lactate dehydrogenase (LDH) were all predictive of survival in stage IV melanoma. The pre-treatment peripheral neutrophil count was identified as an independent prognostic factor [6]; the data were validated on blood samples from patients in the EORTC 18951 biochemotherapy trial [7]. These results indicated that selection of patients in stage IV melanoma studies is highly

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critical; selection of patients for high-dose IL-2 therapy based on performance status, serum LDH and peripheral neutrophil counts seems to be of importance. However, prospective randomised clinical trials are needed to demonstrate whether the patient selection itself or its combination with high-dose IL-2 leads to the observed significant clinical benefit in a subset of patients.

The most effective IL-2 therapy appears to be the original high-dose bolus regimen used by the National Cancer Institute (NCI) [2]. Both long-term, low-dose administration and different subcutaneous schedules have all failed to produce acceptable new regimens with reduced toxicity and higher efficacy compared with that of high-dose IL-2 therapy alone. The continuous infusion schedule has been used in most of the randomised biochemotherapy trials published. A recent report showed a response rate of 19.2% in patients receiving high-dose bolus IL-2 who progressed on biochemotherapy [8]. Overall, despite the limited number of patients who benefit from IL-2-based immunotherapy, its curative potential makes it an essential component of immunological strategies in the treatment of metastatic malignant melanoma [9].

IFN $\alpha$  is only rarely used as a single agent in stage IV melanoma, but has achieved objective response rates of ~10–15% independent of the dose and schedule [10]. Pegylated (PEG) IFN $\alpha$ , an improved IFN $\alpha$  formulation with increased bioavailability [11], was recently used in a trial of stage IV metastatic melanoma patients as a single agent and showed dose-dependent response rates in the range 6–12% [12]. In

recent phase II trials, a combination of PEG-IFN $\alpha$ 2a with dacarbazine (DTIC) [13] and PEG-IFN $\alpha$ 2b with temozolomide [14] increased response rates to 24% and 18%, respectively. Combining IFN $\alpha$  and IL-2 did not demonstrate significantly better response rates and survival than those with either agent alone [15, 16].

Biochemotherapy, a combination of IL-2 and/or IFN $\alpha$  with chemotherapeutic agents such as DTIC, temozolomide, fotemustine, cisplatin, carboplatin, vinblastine, paclitaxel or docetaxel, has also not demonstrated a better survival than that with the agents alone and has been associated with increased toxicity (Table 1) [17, 18]. In summary, biochemotherapy improves response rates but not OS and cannot be recommended for treatment of metastatic malignant melanoma.

### interleukins 15 and 21

The common cytokine receptor  $\gamma$ -chain is a critical component of the receptors for IL-2, 4, 7, 9, 15 and 21. IL-21 and IL-15 have sequence homology with IL-2.

IL-21 is produced by activated CD4+ T cells and NK-cells. IL-21 has pronounced effects on B cell differentiation and antibody production, mostly via CD40. Furthermore, activation of the IL-21 receptor leads to multiple effects on T cells, including proliferation, differentiation and activation of cytokine and chemokine production. IL-21 has effects on both CD8+ T cells and CD4+ T cells, and synergises with IL-15 in inducing an optimal and sustained antigen-specific CD8+ T cell

**Table 1.** Randomised trials with IFN- and/or IL-2-containing regimens

Year (author)	Regimen	No. patients	Median survival (months)	Signif.	Ref.
1991 (Falkson)	D $\pm$ IFN	64	9.6 versus 17.6	$P < 0.01$	[19]
1993 (Thomson)	D $\pm$ IFN	170	7.6 versus 8.8	NS	[20]
1994 (Bajetta)	D $\pm$ IFN <sup>a</sup>	242	11 versus 11 versus 13	NS	[21]
1998 (Falkson)	D versus D/IFN versus D/T versus D/IFN/T	258	10 versus 9 versus 8 versus 9.5	NS	[22]
2000 (Middleton)	D/IFN versus DCBT	105	6.5 versus 6.5	NS	[23]
2001 (Young)	D $\pm$ IFN	61	7.2 versus 4.8	NS	[24]
2005 (Kaufmann)	TMZ $\pm$ IFN	282	8.4 versus 9.7	NS	[25]
2005 (Vuoristo)	D/nIFN versus DCBT/rIFN versus D/rIFN versus DCBT/rIFN	108	11 versus 10 versus 9 versus 7.5	NS	[26]
1993 (Sparano)	IL-2 $\pm$ IFN	85	10.2 versus 9.7	NS	[15]
2002 (Agarwala)	IL-2 $\pm$ histamine	305	9.1 versus 8.2	NS	[27]
1997 (Keilholz)	IL-2/IFN $\pm$ C	133	9 versus 9	NS	[28]
1998 (Johnston)	CDBT $\pm$ IFN/IL-2	65	5.5 versus 5.0	NS	[29]
1999 (Dorval)	C/IL-2 $\pm$ IFN	117	10.4 versus 10.9	NS	[30]
1999 (Rosenberg)	CDT $\pm$ IFN/IL-2	102	15.8 versus 10.7	$P < 0.06$	[31]
2001 (Hauschild)	D/IFN $\pm$ IL-2	290	11 versus 11	NS	[32]
2002 (Eton)	CVD $\pm$ IFN/IL-2	183	9.2 versus 11.9	$P < 0.06$	[33]
2002 (Atzpodien)	D/B/C/T $\pm$ IFN/IL-2	124	13 versus 12	NS	[34]
2002 (Ridolfi)	CVD $\pm$ IFN/IL-2	176	9.5 versus 11.0	NS	[35]
2005 (Keilholz)	CD/IFN $\pm$ IL-2	363	9 versus 9	NS	[36]
2006 (Bajetta)	CVD $\pm$ IFN/IL-2	139	12 versus 11	NS	[37]
2008 (Atkins)	CVD $\pm$ IFN/IL-2	416	8.7 versus 8.4	NS	[38]

<sup>a</sup>Dose 3 or 9 MIU.

D, dacarbazine; C, cisplatin; V, vinblastine; B, BCNU (carmustine); T, tamoxifen; IFN, interferon  $\alpha$ ; IL-2, interleukin-2; TMZ, temozolomide; NS, not significant; n, natural; r, recombinant.

response [39]. In contrast to IL-2, IL-21 does not enhance the proliferation of T regulatory cells. IL-21 may therefore promote autoimmunity and consequently also antitumour immunity in cancer patients.

IL-15 was initially identified based on its ability to stimulate proliferation of IL-2-dependent T cell lines in the presence of neutralising anti-IL-2 antibodies. IL-15 mediates functions very similarly to IL-2, as these two cytokines share receptor  $\beta$ -subunits. However, distinctly different  $\alpha$ -subunits lead to differences in *in vivo* immune function [40].

IL-21 is being investigated in clinical phase I/II studies as a single drug in patients with metastatic melanoma, and recent reports indicate that the treatment is biologically active and well tolerated [41, 42].

## monoclonal antibodies

### anti-cytotoxic T lymphocyte-associated antigen 4

Activation or 'priming' of naïve T cells requires recognition of the antigen by the T cell receptor (TCR) and provision of co-stimulatory signals. The engagement of the molecule B7 on the antigen-presenting cell with its ligand CD28 on the T cell launches a signalling cascade that is required for full T cell activation [43]. Following antigen stimulation of the T cell, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) receptors are up-regulated and move to the cell surface. These receptors have greater affinity for B7 than CD28, and their binding induces an inhibitory signal that down-regulates T cell activation in response to a stimulus. CTLA-4 serves as a natural breaking mechanism that returns T cells to homeostasis following an immune response; it controls the duration and intensity of the immune response.

Monoclonal antibodies that bind to CTLA-4 can block the interaction between B7 and CTLA-4. Inhibition of this negative switch may break peripheral tolerance to self-tissues and induce antitumour responses [44]. Two fully human IgG monoclonal antibodies recognising CTLA-4, ipilimumab (MDX-010) and tremelimumab (CP-675,206), have been tested, alone or in combination, in numerous phase II trials and in four phase III trials. Comprehensive reviews were recently published on targeting the CTLA-4 receptor as a strategy for melanoma treatment [45, 46], and on clinical development of ipilimumab [47] and tremelimumab [48].

### ipilimumab

Activity of ipilimumab in patients with metastatic melanoma was examined alone, in combination with chemotherapy or vaccines and in various dose regimens.

In a randomised phase II trial, 72 chemotherapy-naïve patients were treated with ipilimumab (3 mg/kg every 4 weeks for 4 months) alone or in combination with DTIC. The median OS for the monotherapy and combination groups was 351 [95% CI, 208–596] and 386 [95% CI, 253–571] days, respectively, and ~10% of patients were alive after 2 to >4 years of follow-up in both therapy arms, indicating that the treatment can achieve long-term control of the disease [49].

To assess the activity of ipilimumab in combination with a vaccine, 56 patients were randomised in a phase II study of 3

mg/kg every 3 weeks or a 3 mg/kg initial dose of ipilimumab followed by 1 mg/kg every 3 weeks, with both cohorts receiving concomitant vaccination with two modified HLA-A\*0201-restricted peptides [50]. Overall response rate was 13%; better clinical responses were observed in patients with grade 3/4 autoimmune toxicity. This result was later confirmed by a subsequent analysis of additional data, which reported that some of the immune-related adverse events were observed in 62% of 139 overall treated patients and were associated with a greater probability of objective antitumour response ( $P = 0.0004$ ) [51]. The most common grade 3/4 immune-related adverse events were colitis/diarrhoea and dermatitis, which responded to systemic steroids without significantly affecting the efficacy of ipilimumab therapy [52]. These studies indicate that induction of manageable autoimmunity in patients with metastatic melanoma treated with ipilimumab could be a surrogate marker of objective and durable clinical response.

Although earlier studies used ipilimumab at doses of 3 mg/kg every 3–4 weeks, results from more recent dose-escalation trials showed an increase in overall response and long-term survival benefits with increasing dose, and suggested 10 mg/kg every 3 weeks for 4 months (Q3Wx4) as an optimal treatment [53]. This induction regimen along with a maintenance treatment of 10 mg/kg ipilimumab every 12 weeks starting at week 24 (Q12W) has been used in most ongoing phase II and phase III clinical trials.

The Q3Wx4 regimen of ipilimumab with Q12W maintenance showed clinical activity in the form of either objective response or stable disease in 27% of 155 patients with metastatic melanoma who developed progressive disease on a median number of two prior therapies [54]. Immune-related grade 3/4 adverse events occurred in 21.9% of patients. The study reported median OS of 10.2 months and 1-year survival of 46.7% (95% CI, 38.6–55.6). Four patterns of response were observed: (a) response in baseline lesions; (b) stable disease with slow, steady decline in total tumour burden; (c) response after initial increase in total tumour burden; (d) response in index and new lesions after the appearance of new lesions [54]. It is of note that 7 of 26 patients who developed new lesions after 12 weeks of treatment experienced shrinking and stabilisation of the new lesions during additional follow-up. This result demonstrated that development of new lesions in patients receiving ipilimumab might not always indicate progressive disease and treatment failure as defined by modified World Health Organization (mWHO) criteria [55]. Novel, immune-related response criteria (irRC) that may more accurately describe response to immunotherapy and avoid premature treatment cessation in patients with disease progression before response were presented at ASCO 2008. Contrary to mWHO criteria, irRC (a) only consider measurable lesions (>1 cm), (b) define total tumour burden as the sum of index lesions identified at baseline and new lesions detected after baseline and (c) aim for follow-up after progressive disease to detect late activity [56].

Overall positive results of ipilimumab in patients with metastatic melanoma led to the initiation of a pivotal trial in first-line treatment comparing DTIC with or without ipilimumab. Another phase III trial of ipilimumab alone or in

combination with a peptide vaccine as second-line therapy is also ongoing.

### tremelimumab

Early-phase clinical studies of tremelimumab demonstrated acceptable toxicity (diarrhoea/colitis, dermatitis, fatigue, pancreatitis, Grave's disease) and similar efficacy of 10 mg/kg monthly and 15 mg/kg quarterly doses of the antibody [57] with median survival of 10.3 and 11 months, respectively [58]. Survival outcomes were better than the historical median survival of 7 months and independent of the patients' objective response [58].

Activity of tremelimumab as a single agent (15 mg/kg quarterly,  $\geq 1$  dose, 44% of patients  $\geq 2$  doses) in 246 previously treated patients with metastatic melanoma was assessed in a single-arm phase II study. Although the objective response rate was only 8.3%, the duration of the response (183+ to 540+ days) and the median OS of 10.1 months indicate a role for tremelimumab in this patient population [59].

The quarterly dose regimen (15 mg/kg) was chosen for a phase III clinical trial since it was associated with a lower incidence of serious adverse events and comparable efficacy [57]. That trial, a randomised study investigating tremelimumab as a single agent in comparison with a standard chemotherapy (DTIC or temozolomide) in previously untreated 324 and 319 patients, respectively, was stopped based on a second interim analysis for futility in March 2008. Median OS by intent-to-treat was 11.8 months in the tremelimumab arm, and 10.7 months in the chemotherapy arm, with a hazard ratio (HR) (chemotherapy over tremelimumab) of 1.04 (95% CI, 0.84, 1.28). The study concluded that the antibody failed to demonstrate an improvement in OS as a first-line treatment in patients with metastatic melanoma when compared with standard chemotherapy [60]. Data from subsequent phase II and phase III studies will be useful to further evaluate the dosing regimen and the relationship between tumour response and survival.

A combination of tremelimumab (15 mg/kg quarterly) administered concurrently with high-dose IFN $\alpha$ 2b was assessed in a phase II trial of 16 previously treated patients with metastatic melanoma and showed acceptable toxicity and an overall response rate of 19% [61].

Collectively, development of therapies based on selective inhibition of the CTLA-4 receptor with monoclonal antibodies is a promising direction in treatment of stage IV melanoma, but the recently reported lack of efficacy of tremelimumab as a single agent in first-line treatment indicates that future investigative efforts of anti-CTLA-4 agents may focus on combination studies and patients with refractory tumours. Also, the unique kinetics of response to ipilimumab therapy will likely lead to revision of the criteria that describe response to CTLA-4 antibodies.

### anti-CD137 (4-1BB)

Inducible receptor-like protein 4-1BB is expressed by both CD4+ and CD8+ T cells after activation. Cross-linking of 4-1BB, either by 4-1BB ligand binding or antibody ligation, delivers a co-stimulatory signal to enhance T cell activation and

proliferation. Pre-clinical studies demonstrated that the administration of 4-1BB (CD137) monoclonal antibodies can induce antitumour immune responses. In a pre-clinical B-16 mouse melanoma model, a combination of granulocyte monocyte colony-stimulating factor (GM-CSF)-secreting tumour cell immunotherapy and anti-4-1BB monoclonal antibody treatment resulted in rejection of established tumours [62]. In a pre-clinical B16F10 mouse melanoma model, a combination treatment with anti-CD4+ and anti 4-1BB monoclonal antibodies potentiated the observed anti-cancer effects [63]. A phase I dose-escalation study of BMS-663513, an agonist anti-CD137 human monoclonal antibody, in 54 metastatic melanoma patients reported manageable toxicity (up to 15 mg/kg), with fatigue, transaminitis and neutropenia being the most common adverse events, and clinical activity that justifies its further development both as a single agent and in combination [64]. A large, randomised phase II clinical study with BMS-663513 in previously treated melanoma patients with stage IV disease is currently ongoing.

### anti-integrin

Human monoclonal antibody targeting  $\alpha_v$  integrin, CNTO 95, was found to inhibit growth of human melanoma tumours in nude mice (10 mg/kg, 3 times a week) by  $\sim 80\%$  and in nude rats by  $>99\%$  [65]. Based on these preclinical data, a dose-escalating phase I clinical trial assessed the safety and pharmacokinetics of CNTO 95 in 24 patients with advanced refractory solid tumours. The antibody was well tolerated up to weekly doses of 10 mg/kg, and had a dose-dependent half-life ranging from 0.19 to 11.81 days over the doses 0.1–10 mg/kg, indicating tissue binding at low doses [66]. An ongoing phase I/II trial is investigating the safety and efficacy of CNTO 95 alone or in combination with DTIC in 138 patients with metastatic melanoma.

Volociximab (M200) is a chimeric monoclonal antibody that specifically recognises  $\alpha 5\beta 1$  integrin and inhibits tumour angiogenesis by interrupting the binding of endothelial cells to fibronectin in extracellular matrix. A pilot, single-arm phase II study of 40 patients with metastatic melanoma tested a combination of volociximab (10 mg/kg i.v. bi-weekly) with DTIC (1000 mg/m<sup>2</sup> monthly) and reported stable disease in 53% of patients at 8 weeks with median time to progression 72 days. The combination therapy was relatively well tolerated, with reported adverse events including nausea, constipation, vomiting, hypertension and thrombosis [67]. A recent phase II study of volociximab (15 mg/kg weekly for 8 weeks) as a single agent in 19 patients who failed at least one prior therapy reported poor clinical activity at 8 weeks (overall response rate of 5%) but indicated a potential correlation between the expression of  $\alpha 5\beta 1$  integrin in tumours or stroma and clinical response [68].

Etaracizumab (Abegrin), formerly known as Vitaxin or MEDI-522, is a humanised monoclonal antibody that specifically targets the integrin  $\alpha v\beta 3$  overexpressed on various tumour cells, angiogenic blood vessels and osteoclasts, and results in antitumour, antiangiogenic and antiosteolytic activities [69]. Results from a phase II study in 112 patients with distant metastatic melanoma examined antitumour activity and safety of etaracizumab (8 mg/kg/week) with or

without DTIC (1000 mg/m<sup>2</sup> once every 3 weeks). The therapy with or without DTIC reported median progression-free survival of 2.6 or 1.4 months, respectively, and was generally well tolerated with adverse events including neutropenia, thrombocytopenia, anaemia and leukopenia. Approximately 70% of patients were alive at 6 months in each arm [70]. Later evaluation of the trial data showed a 12.7-month median survival for patients treated with etaracizumab alone and a 9.4-month median survival for patients treated with etaracizumab plus DTIC, indicating that the antibody may prolong survival in melanoma patients. However, a 24-month follow-up was less positive and resulted in withdrawal of the agent from further development in metastatic melanoma at the end of 2006.

## vaccines

The rationale for developing a melanoma therapeutic vaccine is based on several observations: (a) the ability of melanoma to initiate an immune response that can induce spontaneous regression; (b) the effectiveness of vaccines for treating melanoma in animal models; (c) the association of the presence of lymphocytic tumour infiltration with prognosis in melanoma patients; (d) the identification of numerous human melanoma-associated antigens; (e) relatively low toxicity and few side-effects of vaccines in humans with melanoma.

Rosenberg and co-workers [71] stated that despite great progress in the field of tumour immunology over the past decade, optimism regarding the clinical application of currently available cancer vaccine approaches is based more on surrogate end points than on clinical tumour regression. In the NCI surgery branch cancer vaccine trials involving 440 metastatic cancer patients (96% melanoma), the objective response rate was very low (2.6%) and was comparable to the response rate of 4.0% obtained by others in 40 studies including 756 patients [71]. These disappointing results in patients with distant metastatic melanoma are often softened by arguments that such immunosuppressed patients are unsuitable for vaccine development studies and that vaccines will likely be successful only in immune-competent patients after full resection of their tumour(s) (adjuvant setting). However, it is precisely in this setting (large adjuvant trials in resected stage II–IV melanoma) where results with vaccines have not been positive or even indicated that the vaccines are potentially detrimental.

### allogeneic and autologous vaccines

The vaccination of tumour patients with autologous and allogeneic cell-based vaccines has a long tradition [72]. The largest two studies to date, which assessed the efficacy of an allogeneic cancer vaccine from three cell lines (Canvaxin) in patients with stage III or IV melanoma, were closed prematurely based on the advice of the Independent Data Monitoring Committee (IDMC) [73]. In these trials, >1400 patients were randomised to Canvaxin + bacillus Calmette–Guerin (BCG) or placebo + BCG after resectional surgery. There was a survival disadvantage with Canvaxin treatment in both studies. The median survival in the stage III study had not been reached, but the 5-year survival rate was 59% for the Canvaxin patients and 68% for the placebo patients. In the

stage IV study, the median survival was 32 months for the Canvaxin patients and 39 months for the placebo patients, with a 5-year survival of 40% and 45%, respectively.

In contrast to allogeneic vaccines, autologous vaccines are prepared from tumours of individual patients. Vitespen (Oncophage, formerly HSPCC-96) is a tumour-derived, HSP-peptide complex vaccine based on the pioneering work of Pramod K. Srivastava [74]. It was earlier examined in a trial with 64 stage IV melanoma patients [75] and showed no toxicity of the treatment and increased T cell activity associated with the vaccine application.

The most recent, phase III trial comparing Vitespen with a physician choice therapy (2:1 randomisation) in 322 stage IV cutaneous melanoma patients not previously treated for metastatic disease found similar overall survival (OS) in both arms ( $P = 0.32$ ; HR = 1.16; 95% CI, 0.69–1.71) based on intention-to-treat analysis of survival for all patients [76]. Non-significant difference was also found for OS of patients stratified by substage (M1a, M1b, M1c). However, exploratory landmark analysis of survival of patients who received >10 vaccines showed, after adjustment for survival bias, a separation of both arms in Kaplan–Meier plots in favour of the Vitespen-treated patients for all patients and statistical significance for combined data from M1a + M1b patients ( $P = 0.03$ ; HR = 0.45; 95% CI, 0.21–0.96). The study also reported a correlation between the number of immunisations and improved survival in favour of the vaccine in all patients, as well as in M1a and M1b, but not M1c, substages [76]. Whether these results will be convincing enough for further clinical development is still open.

### MAGE-3

A number of clinical trials involving antigens encoded by genes of the MAGE family, particularly MAGE-3, have been documented and demonstrated tumour regression in melanoma patients [77]. These tumour regressions appeared to be mediated by low-level cytolytic T lymphocyte (CTL) responses. A recombinant MAGE-A3 fusion protein—Prot.D MAGE-A3/His—was engineered as a fusion protein with a lipidated protein D derived from *Haemophilus influenzae*. A recent, proof-of-concept, randomised, open study with the MAGE-A3 protein combined with different immunological adjuvants—AS02B or AS15—assessed the adjuvants for toxicity and clinical and immunological responses. The MAGE-A3 protein was administered as first-line treatment to 68 patients with unresectable stage III or stage IV M1a melanoma; in combination with AS15 it yielded higher anti-MAGE-3 antibody titre, stronger T cell induction and long-lasting clinical response [78]. A randomised trial in patients with resected stage IIIB and IIIC melanoma is planned to commence in late 2008.

### peptide-based vaccines

The identification of tumour-specific antigens recognised by autologous cytolytic CD8+ T cells has led to the use of defined antigens in therapeutic vaccination of cancer patients. Although overall response rates to all peptide-based vaccines have been disappointing in distant metastatic melanoma

patients [71], a new interest is stimulated by insights into tissue-specific processing of the immunogenic epitopes of proteins and by the discovery of unusually long cytotoxic T lymphocyte epitopes that may lead to identification of new targets and an improvement in peptide immunogenicity [79]. Longer peptides harbouring particular peptide epitopes allow adequate epitope processing and consequently better peptide vaccine efficacy [80]; also, they can be synthesised with known post-translational modifications and/or protease-resistant peptide bonds to regulate their processing independently of tissue-specific proteolysis, and to stabilise them *in vivo* [79].

### dendritic-cell-based vaccines

Dendritic cells (DCs) play a crucial role in the induction of antigen-specific T cell responses and are considered to be promising adjuvants for use in active immunotherapy of metastatic malignancies. The generation of immune responses against tumour antigens following DC immunisation has been demonstrated, and favourable clinical responses have been reported in some patients [81]. However, the data on the usefulness of DCs for melanoma immunotherapy remain inconclusive because of varying DC preparation and vaccination protocols, the use of different antigens and a lack of rigorous criteria for defining clinical responses. Issues regarding the optimal dose and clinical setting for the application of DC vaccines remain to be resolved.

In one of the earliest studies [82], DCs pulsed with MAGE-3A1 tumour peptide and a recall antigen (tetanus toxoid or tuberculin) were injected into 11 advanced stage IV melanoma patients. A significant expansion of MAGE-3A1-specific CD8+ cytotoxic T lymphocyte precursors were induced in eight of those patients, and regressions of individual skin metastases were observed in six patients. Regressing skin metastases in two of the patients demonstrated erythema and CD8+ T cell infiltration, whereas non-regressing lesions lacked CD8+ T cells as well as MAGE-3 mRNA expression.

In a randomised phase III trial conducted by Schadendorf et al. [83], patients received autologous peptide-loaded DC vaccination or DTIC (850 mg/m<sup>2</sup>) at 4-week intervals. DC vaccines loaded with MHC class I and II-restricted peptides were applied subcutaneously at 2-week intervals for the first five vaccinations and every 4 weeks thereafter. At the time of the first interim analysis, 55 and 53 patients had been enrolled into the DTIC- and DC-arm, respectively. Since the overall response was low (DTIC: 5.5%, DC: 3.8%) and not significantly different in the two arms, the study was closed. Many factors could have affected the efficacy of the vaccine. These include the low and variable maturation status of DCs, administration of lower than the expected number of DCs per class I peptide, subcutaneous instead of intradermal route of administration and a lack of non-specific helper proteins like KLH or tetanus toxoid. Although various small clinical phase I/II studies were conducted using DCs, no results of other randomised trials in melanoma have been published [84]. Current technologies try to benefit from improved understanding of DC biology and use mostly transfection techniques of RNA from the whole tumour or RNA of specific tumour antigens.

In summary, vaccine development in melanoma has gone through a difficult phase and, to date, no vaccination procedure has shown statistically significant efficacy in the adjuvant or metastatic setting [85]. Currently available data do not justify the use of vaccination for the treatment of patients with cutaneous melanoma outside of a clinical trial.

### intratumoural gene transfer therapy

The first clinical trial of gene therapy for cancer was performed in the early 1990s in patients with melanoma, and several intratumoural gene-therapy-based protocols have been attempted since then. Conclusive evidence demonstrating the clinical efficacy and therapeutic response using this approach for melanoma is yet to be established.

A phase I/II study [86] was recently conducted to evaluate the safety, efficacy and biological effects of intratumoural injections of adenovirus-IL-2 (TG1024) in patients with advanced solid tumours including melanoma. Twenty-five patients with metastatic melanoma were treated with intratumoural TG1024 injections in combination with DTIC. Objective responses were observed in five metastatic melanoma patients, of whom two demonstrated complete responses [86].

Intratumoural injections of a plasmid harbouring the IL-12 gene in nine stage IV melanoma patients [87] resulted in clinical benefit in three patients, and eight patients exhibited transient responses. Successful results were also observed when intratumoural injections of a similar plasmid were used to treat lesions in 12 metastatic melanoma patients in a phase I/IB trial [88]. The size of the treated lesions decreased by >30% in 5 of the 12 patients. No responses were seen in untreated lesions.

Results from a recent phase II study with intratumoural injections (up to 18 cycles) of OncoVEX<sup>GM-CSF</sup>—an oncolytic herpes simplex virus vector encoding GM-CSF—into 43 stage IIIc and IV patients who failed previous therapies reported an encouraging 28% objective response and very minor side-effects [89]. Injected tumours routinely responded, often with local complete response, within 2 months of therapy. More importantly, systemic long-term responses were observed. The responses were observed late and were independent of the disease stage: six complete responses, six partial responses and seven stable diseases were reported. A phase III trial in 360 previously treated, unresectable melanoma patients is planned and will be initiated at the beginning of 2009.

These results show that intratumoural injections of a cytokine gene can produce beneficial clinical effects and that intratumoural gene therapy may be a promising therapy for patients with metastatic melanoma.

### adoptive immunotherapy

Adoptive cell transfer (ACT) immunotherapy is based on *ex vivo* activation and expansion of tumour reactive lymphocytes taken from the tumour-bearing host and their re-infusion back into the patient [90].

The availability of the T cell growth factor in the form of recombinant IL-2 in the 1980s promoted the investigation and use of cell therapy in human cancer with lymphokine activated killer (LAK) cells and IL-2, demonstrating non-specific

antitumour activity [91]. On the other hand, tumour-infiltrating lymphocytes (TILs) are T cells that have specific antitumour activity, and in melanoma these cells can be reliably generated [92].

Eighty-six patients with metastatic melanoma were treated with TILs and high-dose (HD) IL-2 in the early 1990s. The overall response rate was 34%, and responses were also documented in patients failing prior HD IL-2 therapy [93]. In 2002 Dudley et al. [94] reported that ACT of highly selected tumour-reactive T cells into 13 HLA-A2<sup>+</sup> refractory metastatic melanoma patients after a non-myeloablative conditioning regimen approach resulted in the persistent clonal repopulation of T cells in these cancer patients, proliferation of transferred cells *in vivo*, functional activity of T cells and trafficking to tumour sites. Six of the 13 patients had objective clinical responses to treatment, and four patients demonstrated mixed responses. This study established the proof of principle that normally expressed 'self-antigens' can be useful targets for human tumour immunotherapy if the autoimmune consequences of such treatment are not of major concern.

Mackensen et al. [95] generated Melan-A-specific CTLs by *ex vivo* stimulation of purified CD8<sup>+</sup> peripheral blood lymphocytes with mature Melan-A pulsed DCs and reported that 3 of 11 patients experienced objective clinical responses after infusion of the T cells with a 6-day course of low-dose IL-2. Regression of melanoma lesions was observed in subcutaneous and lymphatic, but not in visceral metastases. Powell et al. [96] treated nine patients with distant metastatic melanoma who were vaccinated with GP-100:209–217 peptide. The patients received autologous peripheral blood mononuclear cells stimulated *ex vivo* with the peptide and were pretreated with non-myeloablative chemotherapy; the cells were given concomitantly with high-dose IL-2. Two patients experienced some evidence of melanocyte-directed autoimmunity [96].

A recent report [97] demonstrated a durable clinical remission of a patient with refractory metastatic melanoma who was treated with autologous CD4 T cell clones specific for melanoma-associated antigen NY-ESO-1, isolated and expanded *ex vivo*; the treatment also led to endogenous responses against melanoma antigens other than NY-ESO-1 [97].

Studies carried out at the surgery branch of NCI demonstrated that, in addition to IL-2, lymphodepletion with chemotherapy was crucial to support the transferred cells. Lymphodepletion was believed to eliminate T regulatory (suppressor) cells and to reduce competition for homeostatic cytokines (IL-7, IL-15) vital for T cell survival. Bulk TIL populations that were rapidly expanded *in vitro* were preferred over cloned TIL populations [98]. The protocol used a non-myeloablative but lymphodepleting regimen consisting of i.v. administration of cyclophosphamide (60 mg/kg/day for 2 days) followed by fludarabine (25 mg/m<sup>2</sup>/day for 5 days). This was followed by the administration of bulk TILs and HD IL-2. A response rate of 51% was achieved in the first 35 metastatic melanoma patients, with three patients achieving a durable complete response ongoing for >3 years after therapy. Further improvement in the clinical efficacy of the TIL programme was achieved by adding total body irradiation with up to 1200 cGy

in three divided doses. The patients treated with this programme received peripheral stem cell support, and the response rate reached 70% [99].

Conversion of normal peripheral blood lymphocytes (PBLs) into antitumour cells by transduction with genes encoding one of the TCRs relevant to tumour cells is under study [100]. This approach is being explored for the immunotherapy of patients with melanoma and common epithelial cancers. Thirty-one patients with metastatic melanoma were treated by autologous PBLs transduced by MART-1-specific TCRs following non-myeloablative conditioning. Four patients had objective tumour regression [99].

Studies to optimise ACT include examination of the role of cytokines such as IL-7, IL-15 and others, and the addition of other immunomodulatory agents such as anti-CTLA-4. One of the most important steps toward developing a technically more simple way of producing effective TILs is by using 'young', unselected TILs. This technology uses the entire tumour to rapidly expand TILs for administration without testing for antitumour reactivity. This simple method may facilitate the adoption of ACT by more cancer centres and provide this effective therapy to more patients [101].

## conclusion

The poor efficacy and high toxicity of current immunotherapeutic approaches used to treat distant metastatic melanoma underscore an urgent need for novel therapies that take advantage of recent advances in understanding the biology of the disease. In parallel, introducing novel criteria for the selection of potential therapy responders would result in a more targeted use of current therapies and spare future non-responders from unnecessary toxicity.

Early clinical trials with monoclonal antibodies targeting CTLA-4, CD137 or integrins show generally manageable toxicity, and results from large randomised studies are needed to assess the future role of these agents in metastatic melanoma. Combinations with cytotoxic agents appears to be the likely direction, mainly in patients with refractory tumours. Development of a vaccine that would show significant clinical benefit in melanoma has not been successful, but the extent of research activity in the field and a number of novel approaches indicate that such an approach remains attractive. Intratumoural gene transfer represents a promising direction of yet uncertain clinical benefit. Adoptive therapy has demonstrated a remarkable response rate and efficacy in a number of small studies, but it is a complex and labour-intensive technology that would require extensive optimisation before its introduction into mainstream clinical practice.

## conflict of interest disclosures

S. M. Algarra has had an advisory role with Pfizer and has received honoraria from Schering-Plough; L. Bastholt has had an advisory role with Schering Plough, Celgene, Pfizer, Genmab and AstraZeneca, and has received honoraria for educational lectures from Schering-Plough and AstraZeneca; G. Cinat has served as advisor for Pfizer and received lecture honoraria from Pfizer; B. Dreno has been a member of a French national board

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