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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 α, 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 α

Interleukin (IL)-2 and interferon (IFN) α 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].

IFNα 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) IFNα, an improved IFNα 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-IFNα2a with dacarbazine (DTIC) [13] and PEG-IFNα2b with temozolomide [14] increased response rates to 24% and 18%, respectively. Combining IFNα 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 IFNα 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 γ-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 ± IFN	64	9.6 versus 17.6	$P < 0.01$	[19]
1993 (Thomson)	D ± IFN	170	7.6 versus 8.8	NS	[20]
1994 (Bajetta)	D ± IFN[a]	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 ± IFN	61	7.2 versus 4.8	NS	[24]
2005 (Kaufmann)	TMZ ± 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 ± IFN	85	10.2 versus 9.7	NS	[15]
2002 (Agarwala)	IL-2 ± histamine	305	9.1 versus 8.2	NS	[27]
1997 (Keilholz)	IL-2/IFN ± C	133	9 versus 9	NS	[28]
1998 (Johnston)	CDBT ± IFN/IL-2	65	5.5 versus 5.0	NS	[29]
1999 (Dorval)	C/IL-2 ± IFN	117	10.4 versus 10.9	NS	[30]
1999 (Rosenberg)	CDT ± IFN/IL-2	102	15.8 versus 10.7	$P < 0.06$	[31]
2001 (Hauschild)	D/IFN ± IL-2	290	11 versus 11	NS	[32]
2002 (Eton)	CVD ± IFN/IL-2	183	9.2 versus 11.9	$P < 0.06$	[33]
2002 (Atzpodien)	D/B/C/T ± IFN/IL-2	124	13 versus 12	NS	[34]
2002 (Ridolfi)	CVD ± IFN/IL-2	176	9.5 versus 11.0	NS	[35]
2005 (Keilholz)	CD/IFN ± IL-2	363	9 versus 9	NS	[36]
2006 (Bajetta)	CVD ± IFN/IL-2	139	12 versus 11	NS	[37]
2008 (Atkins)	CVD ± IFN/IL-2	416	8.7 versus 8.4	NS	[38]

[a]Dose 3 or 9 MIU.

D, dacarbazine; C, cisplatin; V, vinblastine; B, BCNU (carmustine); T, tamoxifen; IFN, interferon α; 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 β-subunits. However, distinctly different α-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, ≥1 dose, 44% of patients ≥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 IFNα2b 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 α_v integrin, CNTO 95, was found to inhibit growth of human melanoma tumours in nude mice (10 mg/kg, 3 times a week) by ~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 $\alpha5\beta1$ 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^2 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 $\alpha5\beta1$ 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 $\alpha v\beta3$ 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^2 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 HSPPC-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^2) 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 intratumour injections (up to 18 cycles) of OncoVEX$^{GM\text{-}CSF}$—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-$A2^+$ 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+ 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^2/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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references

1. Balch CM, Buzaid AC, Soong SJ et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001; 19: 3635–3648.
2. Atkins MB, Lotze MT, Dutcher JP et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999; 17: 2105–2116.
3. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004; 351: 998–1012.
4. Keilholz U, Conradt C, Legha SS et al. Results of interleukin-2-based treatment in advanced melanoma: a case record-based analysis of 631 patients. J Clin Oncol 1998; 16: 2921–2929.
5. Phan GQ, Attia P, Steinberg SM et al. Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma. J Clin Oncol 2001; 19: 3477–3482.
6. Schmidt H, Bastholt L, Geertsen P et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer 2005; 93: 273–278.
7. Schmidt H, Suciu S, Punt CJ et al. Pretreatment levels of peripheral neutrophils and leukocytes as independent predictors of overall survival in patients with American Joint Committee on Cancer Stage IV Melanoma: results of the EORTC 18951 biochemotherapy trial. J Clin Oncol 2007; 25: 1562–1569.
8. Tarhini AA, Kirkwood JM, Gooding WE et al. Durable complete responses with high-dose bolus interleukin-2 in patients with metastatic melanoma who have experienced progression after biochemotherapy. J Clin Oncol 2007; 25: 3802–3807.
9. Petrella T, Quirt I, Verma S et al. Single-agent interleukin-2 in the treatment of metastatic melanoma: a systematic review. Cancer Treat Rev 2007; 33: 484–496.
10. Legha SS. The role of interferon alfa in the treatment of metastatic melanoma. Semin Oncol 1997; 24: S24–S31.
11. Bukowski RM, Tendler C, Cutler D et al. Treating cancer with PEG Intron: pharmacokinetic profile and dosing guidelines for an improved interferon-alpha-2b formulation. Cancer 2002; 95: 389–396.
12. Dummer R, Garbe C, Thompson JA et al. Randomized dose-escalation study evaluating peginterferon alfa-2a in patients with metastatic malignant melanoma. J Clin Oncol 2006; 24: 1188–1194.
13. Hauschild A, Dummer R, Ugurel S et al. Combined treatment with pegylated interferon-alpha-2a and dacarbazine in patients with advanced metastatic melanoma: a phase 2 study. Cancer 2008; 113: 1404–1411.
14. Spieth K, Kaufmann R, Dummer R et al. Temozolomide plus pegylated interferon alfa-2b as first-line treatment for stage IV melanoma: a multicenter phase II trial of the Dermatologic Cooperative Oncology Group (DeCOG). Ann Oncol 2008; 19: 801–806.
15. Sparano JA, Fisher RI, Sunderland M et al. Randomized phase III trial of treatment with high-dose interleukin-2 either alone or in combination with interferon alfa-2a in patients with advanced melanoma. J Clin Oncol 1993; 11: 1969–1977.
16. Marincola FM, White DE, Wise AP, Rosenberg SA. Combination therapy with interferon alfa-2a and interleukin-2 for the treatment of metastatic cancer. J Clin Oncol 1995; 13: 1110–1122.
17. Hamm C, Verma S, Petrella T et al. Biochemotherapy for the treatment of metastatic malignant melanoma: a systematic review. Cancer Treat Rev 2008; 34: 145–156.
18. Ives NJ, Stowe RL, Lorigan P, Wheatley K. Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients. J Clin Oncol 2007; 25: 5426–5434.
19. Falkson CI, Falkson G, Falkson HC. Improved results with the addition of interferon alfa-2b to dacarbazine in the treatment of patients with metastatic malignant melanoma. J Clin Oncol 1991; 9: 1403–1408.
20. Thomson DB, Adena M, McLeod GR et al. Interferon-alpha 2a does not improve response or survival when combined with dacarbazine in metastatic malignant melanoma: results of a multi-institutional Australian randomized trial. Melanoma Res 1993; 3: 133–138.
21. Bajetta E, Di Leo A, Zampino MG et al. Multicenter randomized trial of dacarbazine alone or in combination with two different doses and schedules of interferon alfa-2a in the treatment of advanced melanoma. J Clin Oncol 1994; 12: 806–811.
22. Falkson CI, Ibrahim J, Kirkwood JM et al. Phase III trial of dacarbazine versus dacarbazine with interferon alpha-2b versus dacarbazine with tamoxifen versus dacarbazine with interferon alpha-2b and tamoxifen in patients with metastatic malignant melanoma: an Eastern Cooperative Oncology Group study. J Clin Oncol 1998; 16: 1743–1751.
23. Middleton MR, Lorigan P, Owen J et al. A randomized phase III study comparing dacarbazine, BCNU, cisplatin and tamoxifen with dacarbazine and interferon in advanced melanoma. Br J Cancer 2000; 82: 1158–1162.
24. Young AM, Marsden J, Goodman A et al. Prospective randomized comparison of dacarbazine (DTIC) versus DTIC plus interferon-alpha (IFN-alpha) in metastatic melanoma. Clin Oncol (R Coll Radiol) 2001; 13: 458–465.
25. Kaufmann R, Spieth K, Leiter U et al. Temozolomide in combination with interferon-alfa versus temozolomide alone in patients with advanced metastatic melanoma: a randomized, phase III, multicenter study from the Dermatologic Cooperative Oncology Group. J Clin Oncol 2005; 23: 9001–9007.
26. Vuoristo MS, Hahka-Kemppinen M, Parvinen LM et al. Randomized trial of dacarbazine versus bleomycin, vincristine, lomustine and dacarbazine (BOLD) chemotherapy combined with natural or recombinant interferon-alpha in patients with advanced melanoma. Melanoma Res 2005; 15: 291–296.
27. Agarwala SS, Glaspy J, O'Day SJ et al. Results from a randomized phase III study comparing combined treatment with histamine dihydrochloride plus interleukin-2 versus interleukin-2 alone in patients with metastatic melanoma. J Clin Oncol 2002; 20: 125–133.
28. Keilholz U, Goey SH, Punt CJ et al. Interferon alfa-2a and interleukin-2 with or without cisplatin in metastatic melanoma: a randomized trial of the European Organization for Research and Treatment of Cancer Melanoma Cooperative Group. J Clin Oncol 1997; 15: 2579–2588.
29. Johnston SR, Constenla DO, Moore J et al. Randomized phase II trial of BCDT [carmustine (BCNU), cisplatin, dacarbazine (DTIC) and tamoxifen] with or without interferon alpha (IFN-alpha) and interleukin (IL-2) in patients with metastatic melanoma. Br J Cancer 1998; 77: 1280–1286.
30. Dorval T, Negrier S, Chevreau C et al. Randomized trial of treatment with cisplatin and interleukin-2 either alone or in combination with interferon-alpha-2a in patients with metastatic melanoma: a Federation Nationale des Centres de Lutte Contre le Cancer Multicenter, parallel study. Cancer 1999; 85: 1060–1066.
31. Rosenberg SA, Yang JC, Schwartzentruber DJ et al. Prospective randomized trial of the treatment of patients with metastatic melanoma using chemotherapy

with cisplatin, dacarbazine, and tamoxifen alone or in combination with interleukin-2 and interferon alfa-2b. J Clin Oncol 1999; 17: 968–975.
32. Hauschild A, Garbe C, Stolz W et al. Dacarbazine and interferon alpha with or without interleukin 2 in metastatic melanoma: a randomized phase III multicentre trial of the Dermatologic Cooperative Oncology Group (DeCOG). Br J Cancer 2001; 84: 1036–1042.
33. Eton O, Legha SS, Bedikian AY et al. Sequential biochemotherapy versus chemotherapy for metastatic melanoma: results from a phase III randomized trial. J Clin Oncol 2002; 20: 2045–2052.
34. Atzpodien J, Neuber K, Kamanabrou D et al. Combination chemotherapy with or without s.c. IL-2 and IFN-alpha: results of a prospectively randomized trial of the Cooperative Advanced Malignant Melanoma Chemoimmunotherapy Group (ACIMM). Br J Cancer 2002; 86: 179–184.
35. Ridolfi R, Chiarion-Sileni V, Guida M et al. Cisplatin, dacarbazine with or without subcutaneous interleukin-2, and interferon alpha-2b in advanced melanoma outpatients: results from an Italian multicenter phase III randomized clinical trial. J Clin Oncol 2002; 20: 1600–1607.
36. Keilholz U, Punt CJ, Gore M et al. Dacarbazine, cisplatin, and interferon-alfa-2b with or without interleukin-2 in metastatic melanoma: a randomized phase III trial (18951) of the European Organisation for Research and Treatment of Cancer Melanoma Group. J Clin Oncol 2005; 23: 6747–6755.
37. Bajetta E, Del Vecchio M, Nova P et al. Multicenter phase III randomized trial of polychemotherapy (CVD regimen) versus the same chemotherapy (CT) plus subcutaneous interleukin-2 and interferon-alpha2b in metastatic melanoma. Ann Oncol 2006; 17: 571–577.
38. Atkins MB, Hsu J, Lee S et al. Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol 2008; 26: 5748–5754.
39. Davis ID, Skak K, Smyth MJ et al. Interleukin-21 signaling: functions in cancer and autoimmunity. Clin Cancer Res 2007; 13: 6926–6932.
40. Fehniger TA, Cooper MA, Caligiuri MA. Interleukin-2 and interleukin-15: immunotherapy for cancer. Cytokine Growth Factor Rev 2002; 13: 169–183.
41. Thompson JA, Curti BD, Redman BG et al. Phase I study of recombinant interleukin-21 in patients with metastatic melanoma and renal cell carcinoma. J Clin Oncol 2008; 26: 2034–2039.
42. Frederiksen KS, Lundsgaard D, Freeman JA et al. IL-21 induces in vivo immune activation of NK cells and CD8(+) T cells in patients with metastatic melanoma and renal cell carcinoma. Cancer Immunol Immunother 2008; 57: 1439–1449.
43. Inman BA, Frigola X, Dong H, Kwon ED. Costimulation, coinhibition and cancer. Curr Cancer Drug Targets 2007; 7: 15–30.
44. Melero I, Hervas-Stubbs S, Glennie M et al. Immunostimulatory monoclonal antibodies for cancer therapy. Nat Rev Cancer 2007; 7: 95–106.
45. O'Day SJ, Hamid O, Urba WJ. Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): a novel strategy for the treatment of melanoma and other malignancies. Cancer 2007; 110: 2614–2627.
46. Cranmer LD, Hersh E. The role of the CTLA4 blockade in the treatment of malignant melanoma. Cancer Invest 2007; 25: 613–631.
47. Weber J. Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. Oncologist 2007; 12: 864–872.
48. Ribas A, Hanson DC, Noe DA et al. Tremelimumab (CP-675,206), a cytotoxic T lymphocyte associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. Oncologist 2007; 12: 873–883.
49. Hersh EM, Weber JS, Powderly JD et al. Disease control and long-term survival in chemotherapy-naive patients with advanced melanoma treated with ipilimumab (MDX- 010) with or without dacarbazine. 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 9022.
50. Attia P, Phan GQ, Maker AV et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005; 23: 6043–6053.
51. Downey SG, Klapper JA, Smith FO et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. Clin Cancer Res 2007; 13: 6681–6688.
52. Beck KE, Blansfield JA, Tran KQ et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 2006; 24: 2283–2289.
53. Hamid O, Chin K, Li J et al. Dose effect of ipilimumab in patients with advanced melanoma: Results from a phase II, randomized, dose-ranging study. 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 9025.
54. O'Day SJ, Ibrahim R, DePril V et al. Efficacy and safety of ipilimumab induction and maintenance dosing in patients with advanced melanoma who progressed on one or more prior therapies. ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 9021.
55. Wolchok JD, Ibrahim R, DePril V et al. Antitumor response and new lesions in advanced melanoma patients on ipilimumab treatment. ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 3020.
56. Hodi FS, Hoos A, Ibrahim R et al. Novel efficacy criteria for antitumor activity to immunotherapy using the example of ipilimumab, an anti-CTLA-4 monoclonal antibody. ASCO Annual Proceedings Part I. J Clin Oncol 2008; 26: Abstr 3008.
57. Ribas A, Antonia S, Sosman J et al. Results of a phase II clinical trial of 2 doses and schedules of CP-675,206, an anti-CTLA4 monoclonal antibody, in patients (pts) with advanced melanoma. ASCO Annual Meeting Proceedings Part I. J Clin Oncol 2007; 25: Abstr 3000.
58. Gomez-Navarro J, Antonia S, Sosman J et al. Survival of patients (pts) with metastatic melanoma treated with the anti-CTLA4 monoclonal antibody (mAb) CP-675,206 in a phase I/II study. ASCO Annual Meeting Proceedings Part I. J Clin Oncol 2007; 26: Abstr 8524.
59. Kirkwood JM, Lorigan P, Hersey P et al. A phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melanoma. ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 9023.
60. Ribas A, Hauschild A, Kefford R et al. Phase III, open-label, randomized, comparative study of tremelimumab (CP-675,206) and chemotherapy (temozolomide [TMZ] or dacarbazine [DTIC]) in patients with advanced melanoma. 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr LBA9011.
61. Tarhini AA, Moschos SS, Schlesselman JJ et al. Phase II trial of combination biotherapy of high-dose interferon alfa-2b and tremelimumab for recurrent inoperable stage III or stage IV melanoma. 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 9009.
62. Li B, Lin J, Vanroey M et al. Established B16 tumors are rejected following treatment with GM-CSF-secreting tumor cell immunotherapy in combination with anti-4-1BB mAb. Clin Immunol 2007; 125: 76–87.
63. Choi BK, Kim YH, Kang WJ et al. Mechanisms involved in synergistic anticancer immunity of anti-4-1BB and anti-CD4 therapy. Cancer Res 2007; 67: 8891–8899.
64. Sznol M, Hodi FS, Margolin K et al. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA). 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 3007.
65. Trikha M, Zhou Z, Nemeth JA et al. CNTO 95, a fully human monoclonal antibody that inhibits alphav integrins, has antitumor and antiangiogenic activity in vivo. Int J Cancer 2004; 110: 326–335.
66. Jayson GC, Mullamitha S, Ton C et al. Phase I study of CNTO 95, a fully human monoclonal antibody (mAb) to av integrins, in patients with solid tumors. 2005 ASCO Annual Meeting Proceedings Part I. J Clin Oncol 2005; 23: Abstr 3113.
67. Cranmer LD, Bedikian AY, Ribas A et al. Phase II study of volociximab (M200), an $\alpha 5\beta 1$ anti-integrin antibody in metastatic melanoma. 2005 ASCO Annual Meeting Proceedings Part I. J Clin Oncol 2005; 24: Abstr 8011.
68. Linette G, Cranmer L, Hodi S et al. A multicenter phase II study of volociximab in patients with relapsed metastatic melanoma. 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008: 26: Abstr 3505.
69. Mulgrew K, Kinneer K, Yao XT et al. Direct targeting of alphavbeta3 integrin on tumor cells with a monoclonal antibody, Abegrin. Mol Cancer Ther 2006; 5: 3122–3129.
70. Hersey P, Sosman J, O'Day S et al. A phase II, randomized, open-label study evaluating the antitumor activity of MEDI-522, a humanized monoclonal antibody directed against the human alpha v beta 3 (avb3) integrin, ± dacarbazine (DTIC) in patients with metastatic melanoma (MM). 2005 ASCO Annual Meeting Proceedings Part I. J Clin Oncol 2005; 23: Abstr 7507.

71. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004; 10: 909–915.
72. Sondak VK, Sabel MS, Mule JJ. Allogeneic and autologous melanoma vaccines: where have we been and where are we going? Clin Cancer Res 2006; 12: 2337s–2341s.
73. Morton DL, Mozzillo N, Thompson JF et al. An international, randomized, phase III trial of bacillus Calmette-Guerin (BCG) plus allogeneic melanoma vaccine (MCV) or placebo after complete resection of melanoma metastatic to regional or distant sites. 2007 ASCO Annual Meeting Proceedings Part I. J Clin Oncol 2007; 25: Abstr 8508.
74. Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 2002; 20: 395–425.
75. Belli F, Testori A, Rivoltini L et al. Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: clinical and immunologic findings. J Clin Oncol 2002; 20: 4169–4180.
76. Testori A, Richards J, Whitman E et al. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. J Clin Oncol 2008; 26: 955–962.
77. Coulie PG, Karanikas V, Colau D et al. A monoclonal cytolytic T-lymphocyte response observed in a melanoma patient vaccinated with a tumor-specific antigenic peptide encoded by gene MAGE-3. Proc Natl Acad Sci USA 2001; 98: 10290–10295.
78. Kruit WH, Suciu S, Dreno B et al. Immunization with recombinant MAGE-A3 protein combined with adjuvant systems AS15 or AS02B in patients with unresectable and progressive metastatic cutaneous melanoma: a randomized open-label phase II study of the EORTC Melanoma Group (16032-18031). 2008 ASCO Annual Proceedings Part I. J Clin Oncol 2008; 26: Abstr 9065.
79. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov 2007; 6: 404–414.
80. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer 2008; 8: 351–360.
81. Osada T, Clay TM, Woo CY et al. Dendritic cell-based immunotherapy. Int Rev Immunol 2006; 25: 377–413.
82. Thurner B, Haendle I, Roder C et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med 1999; 190: 1669–1678.
83. Schadendorf D, Ugurel S, Schuler-Thurner B et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. Ann Oncol 2006; 17: 563–570.
84. Lesterhuis WJ, Aarntzen EH, De Vries IJ et al. Dendritic cell vaccines in melanoma: from promise to proof? Crit Rev Oncol Hematol 2008; 66: 118–134.
85. Lens M. The role of vaccine therapy in the treatment of melanoma. Expert Opin Biol Ther 2008; 8: 315–323.
86. Dummer R, Rochlitz C, Velu T et al. Intralesional adenovirus-mediated interleukin-2 gene transfer for advanced solid cancers and melanoma. Mol Ther 2008; 16: 985–994.
87. Heinzerling L, Burg G, Dummer R et al. Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: clinical efficacy. Hum Gene Ther 2005; 16: 35–48.
88. Mahvi DM, Henry MB, Albertini MR et al. Intratumoral injection of IL-12 plasmid DNA—results of a phase I/IB clinical trial. Cancer Gene Ther 2007; 14: 717–723.
89. Senzer NN, Kaufman HL, Amatruda T et al. Phase II clinical trial with a second generation, GM-CSF encoding, oncolytic herpesvirus in unresectable metastatic melanoma. 2008 ASCO Annual proceedings Part I. J Clin Oncol 2008; 26: Abstr 9008.
90. Gattinoni L, Powell DJ, Jr, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol 2006; 6: 383–393.
91. Mule JJ, Shu S, Schwarz SL, Rosenberg SA. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science 1984; 225: 1487–1489.
92. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 1986; 233: 1318–1321.
93. Rosenberg SA, Yannelli JR, Yang JC et al. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst 1994; 86: 1159–1166.
94. Dudley ME, Wunderlich JR, Robbins PF et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002; 298: 850–854.
95. Mackensen A, Meidenbauer N, Vogl S et al. Phase I study of adoptive T-cell therapy using antigen-specific CD8+ T cells for the treatment of patients with metastatic melanoma. J Clin Oncol 2006; 24: 5060–5069.
96. Powell DJ, Jr, Dudley ME, Hogan KA et al. Adoptive transfer of vaccine-induced peripheral blood mononuclear cells to patients with metastatic melanoma following lymphodepletion. J Immunol 2006; 177: 6527–6539.
97. Hunder NN, Wallen H, Cao J et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med 2008; 358: 2698–2703.
98. Dudley ME, Wunderlich JR, Yang JC et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 2005; 23: 2346–2357.
99. Rosenberg SA, Restifo NP, Yang JC et al. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer 2008; 8: 299–308.
100. Morgan RA, Dudley ME, Wunderlich JR et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006; 314: 126–129.
101. Tran KQ, Zhou J, Durflinger KH et al. Minimally cultured tumor-infiltrating lymphocytes display optimal characteristics for adoptive cell therapy. J Immunother 2008; 31: 742–751.