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REVIEW

Review of clinical studies on dendritic cell-based vaccination of patients with malignant melanoma: assessment of correlation between clinical response and vaccine parameters

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Abstract During the past years numerous clinical trials have been carried out to assess the ability of dendritic cell (DC) based immunotherapy to induce clinically relevant immune responses in patients with malignant diseases. A broad range of cancer types have been targeted including malignant melanoma which in the disseminated stage have a very poor prognosis and only limited treatment options with moderate effectiveness. Herein we describe the results of a focused search of recently published clinical studies on dendritic cell vaccination in melanoma and review different vaccine parameters which are frequently claimed to have a possible influence on clinical response. These parameters include performance status, type of antigen, DC maturation status, route of vaccine administration, use of adjuvant, and vaccine induced immune response. In total, 38 articles found through Medline search, have been included for analysis covering a total of 626 patients with malignant melanoma treated with DC based therapy. Clinical response (CR, PR and SD) were found to be significantly correlated with the use of peptide antigens ($p = 0.03$), the use of any helper antigen/adjuvant ($p = 0.002$), and induction of antigen specific T cells ($p = 0.0004$). No significant correlations between objective response (CR and PR) and the tested parameters were found. However, a few non-significant trends were demonstrated; these included an association between objective response and use of immature DCs ($p = 0.08$), use of adjuvant ($p = 0.09$), and use of autologous antigen preparation ($p = 0.12$). The categorisation of SD in the response group is debatable. Nevertheless, when the SD group were analysed separately we found that SD was significantly associated with use of peptide antigens ($p = 0.0004$), use of adjuvant ($p = 0.01$), and induction of antigen specific T cells ($p = 0.0003$). No specific route of vaccine administration showed superiority. Important lessons can be learned from previous studies, interpretation of these findings should, however, be done with reservation for the many minor deviations in the different treatment schedules among the published studies, which were not considered in order to be able to process and group the data.

Keywords Cancer · Dendritic cells · Malignant melanoma · Immunotherapy · Tumour vaccines

Abbreviations

CR	Complete response
PR	Partial response
SD	Stable disease
PD	Progressive disease
MR	Mixed response
NED	No evidence of disease
NEV	Not evaluable
AG	Antigen
Inj	Injection
NP	Number of patients
Ref	Reference number
ATL	Autologous tumour lysate
ATH	Autologous tumour homogenate
ALTL	Allogeneic tumour lysate
ATC	Autologous tumour cells, NA17-A and Colo 829 are tumour cell lines
KLH	Keyhole limpet haemocyanin

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Flu-MP	Flu-matrix protein
HBs	Hepatitis B surface protein
HBsAg	Hepatitis B antigen
PPD	Tuberculin
TT	Tetanus toxoid
MCM	Monocyte-derived conditioned medium
MM	Malignant melanoma
PBMC	Peripheral blood mononuclear cells
GM-CSF	Granulocyte/macrophage-colony stimulating factor
IL	Interleukin
TNF	Tumour necrosis factor
INF	Interferon
PGE2	Prostaglandin E2
CM	Complete medium
CD40-L	CD40-ligand
iDC	Immature dendritic cells
mDC	Mature dendritic cells
i.n.	Intranodal
i.d.	Intradermal
s.c.	Subcutaneous
i.v.	Intravenous
i.l.	Intralymphatic
NNL	No new lesions
ND	Not defined
Rand	Randomised
a	GM-CSF + IL-4
b	GM-CSF + TNF
c	GM-CSF + TNFα
d	GM-CSF + IL-13

Introduction

Cancer remains a frequent cause of death even though several standard and experimental treatments have been developed. These include surgery, chemotherapy, radiotherapy, biotherapy and immunotherapy. Especially melanoma has incidence rates that rise faster than for any other malignancy in Caucasian populations over the past 30 years [25]. According to a World Health Organization estimate there are 132,000 new cases of melanoma per year worldwide. Survival has been found to be strongly correlated with thickness and ulceration of primary tumour at the time of diagnosis with an approximate 5-year survival rate of 30–50% for patients with melanoma thicker than 4 mm.

Metastasising melanoma might occur in spite of primary surgery and the mortality in this group of patients is very high. As indicated conventional treatments are generally insufficient since metastases are often inoperable and resistant to chemo- and radiotherapy. Immunotherapy with interleukin-2 (IL-2) and interferon-alpha (IFN-α) has a response rate of 10–20% and long time remission is seen in only 3–5% of the cases [4, 20, 69]. In addition this treatment frequently has quite severe side effects.

These circumstances call for new treatment modalities for melanoma patients; one potential treatment strategy under evaluation is dendritic cell based vaccination. The rational of this treatment is to stimulate the patient's immune system to recognise the cancer cells and eliminate them. The aim of this paper is to review data from published clinical studies on dendritic cell vaccination against melanoma and to perform an assessment of influence of different vaccine parameters on clinical response, in order to optimise the future work on DC vaccines in patients with malignant melanoma.

Background

Dendritic cells

Dendritic cells (DC) are sentinels of the human immune system. They are considered the most potent stimulators of T cell responses, superior to other antigen presenting cells (APC) such as B cells and macrophages. However, the DC are not just stimulators, but more correctly regulators of the immune response [7]. Consequently, DC can actually contribute to diminishing an immune response against an antigen if inappropriate circumstances are present. In peripheral tissue the immature DC take up antigens, process them and go through a maturation process. Thereafter the DC migrates to the draining lymph node where they meet and activate cognate T cells [60]. DC present the antigen on the surface through their major histocompatibility complex (MHC); MHC class I interacts with $CD8^+$ T cells whereas MHC class II interacts with $CD4^+$ T cells. DC goes through licensing after interaction with the CD40L on $CD4^+$ (Th1) cells [26]. Licensing allows the DC to communicate with $CD8^+$ T cells through 4-1BBL (DC)–4-1BB (T cell) interaction. Also, licensed DC upregulate OX40L which interacts with OX40 on $CD4^+$ T cells and enhances their survival and proliferation.

Activation of T cells through the T cell receptor is antigen specific but requires co-stimulation; one important co-stimulatory pathway involves CD28 engagement by CD80 or CD86. However, other ligand–receptor complexes can also provide co-stimulation and may have important functions in modulating the T cell response. Activated $CD8^+$ T cells differentiate into cytotoxic T lymphocytes (CTL) while $CD4^+$ T cells differentiate into T helper 1 (Th1) and T helper 2 (Th2) cells, which interact with macrophages and B cells, respectively. The goal of utilising DC to deliver a cancer vaccine is to induce a Th1 immune response and also activate CTLs in order to eliminate the tumour.

Preparation of dendritic cells

DC are present in peripheral blood but comprise less than 1% of mononuclear cells [22], therefore, in order to prepare a DC based vaccine isolation and in vitro culturing of DC is required. DC are cultivated from $CD34^+$ progenitor cells or, more commonly, peripheral blood mononuclear cells (PBMC) (for review see [5]). This is typically done by incubating the precursor cells (monocytes) with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) for approximately 7 days which induce differentiation of the monocytes into immature DC (iDC) [66]. Recently, rapid 2-day protocols have been developed which seems to be as effective as the 7-day method [15]. Differences in maturity status of the DCs may have a significant impact on the induced response [4, 22] and an important question in DC immunotherapy is whether to use immature or mature DCs. It has been proposed, that immature DCs are inferior to mature DCs, at least with respect to their T cell stimulatory capacity [16] and might even have a direct role in establishing tumour tolerance [48]. Mature DC (mDC) can be characterised both phenotypically and functionally. Phenotypically mDC is described using maturation markers, such as C83, CD1a, CD40, CD36, CD11c, CD80 (B7.2), CD86 (B7.1), CCR-7 and MHC expression while the functional criteria for a matured DC is defined by IL-12 production, migration capacity, and the ability to induce antigen-specific T cell responses [48, 49, 73]. IL-12 is thought to be important in the licensing process; it polarises towards a Th1 response and consequently induces a $CD8^+$ CTL response.

The optimal method for maturation of DCs is to date a subject of debate. The most commonly used factors for DC maturation are IL-6, prostaglandin E_2 (PGE_2), IL-1β and TNF-α [35]. Used in combination these are referred to as the standard maturation cocktail (sDC). PGE_2 is believed to enhance the migratory/homing capacity of the DC [67] by upregulating the expression of chemokine receptor CCR7. However, PGE_2 possibly also mediates Th2 polarisation and secretion of the immunosuppressive cytokine IL-10 [46]. Furthermore, Banerjee et al. [9] showed that cytokine cocktail-matured DC were more prone to expand immunosuppressive T regulatory cells than immature DC.

Improved protocols for maturation of DC, e.g. by the "multi-cytokine cocktail" protocol consisting of TNF, IL-1β, PolyI:C, IFN-α and IFN-γ (αDC) has been proposed [45]. αDC cocktail-matured DC display superior immunogenic abilities compared to standard cocktail-matured DC and have retained lymph node migratory capacities in vitro even though PGE_2 was not added. Nevertheless, we have demonstrated that the published advantages of αDC1 maturation are not reproducible when DCs are prepared in plasma containing medium [79]. These observations underline that αDC1 maturation can not be directly adapted to alternative protocols for DC generation. Also, clinical trials are needed to determine whether αDC1 matured DC are more effective in vivo.

As an alternative to the well-defined but expensive cytokine cocktails monocyte-derived conditioned medium (MCM) has been used for DC maturation in some studies [53]. Presumably, this supernatant from activated monocyte cultures contains critical maturation factors [63].

Antigen pulsing

Antigens can be added to DC in several ways, e.g. exogenously as peptides, whole protein, tumour lysate, apoptotic tumour cells, endogenously by transfection with mRNA or cDNA encoding the antigen, or by DC–tumour cell fusion. Each method and type of antigen comprise of advantages and limitations (for review see [26]).

Tumour specific peptide antigens are precisely defined by the amino acid sequence representing immunogenic parts of a defined tumour protein. They can easily be produced in large scale, and in addition it is a possibility to modify the peptides by exchange of single amino acids in order to improve potency. The peptides are human leucocyte antigen (HLA) type specific and consequently restricted to a subgroup of patients [65], and normally only a limited number of peptides are included in a vaccine.

Tumour lysate/apoptotic tumour cells have the advantage over tumour specific antigen peptides that they consist of a broad panel of proteins making this method feasible regardless of HLA type. Multiple epitopes are potentially presented by the DC and a broader spectrum of T cells might, therefore, respond to the variety of epitopes including T cells recognising epitopes not yet identified. Either autologous tumour or allogeneic tumour cells are used.

Another strategy that circumvents the problems of HLA mismatch and introduces a broad spectrum of epitopes for polyvalent stimulation of T cell responses is transfection of DC with full length mRNA encoding either specific antigens [74] or whole tumour cell mRNA [37]. The use of electroporation to transfect the DC is an attractive approach for delivery of mRNA into the DC, and development of effective electroporation protocols has made the technique clinically suitable with a broad applicability. This method provides a supply of antigen that lasts for a longer time than peptide or protein loading which is important to bear in mind since there seems to be a correlation between antigen persistence in the DC and magnitude of the immune response [29, 42].

Route of injection

DC can be administered in several different ways, including intravenously (i.v.), intradermally (i.d.), subcutaneously

(s.c.), intralymphatically (i.l.), and intranodally (i.n.). DC tracing studies have been carried out showing that DC injected i.v. primary accumulated in the lungs and subsequently redistributed to the liver, spleen and bone marrow while DC migrated to the regional lymph nodes after i.d. or s.c. injection [47]. In another study, it was demonstrated that i.d. and i.l. administration induced a T cell IFN-γ response, whereas i.v. injection resulted in a humoral response [23], suggesting that the nature of the immune response varies with the route of injection. Intranodal injections have previous been claimed superior to peripheral injection [10], however, recently Kyte et al. [36] found i.d. injection to be significantly better at inducing an immune response compared to i.n. Thus the most favourable combination of DC maturation status and route of injection still need to be clarified.

Adjuvant and helper antigen

Interleukin-2 (IL-2) is a frequently used DC vaccination adjuvant. It is a potent stimulator of T cell growth and in some clinical trials administered in combination with DC vaccination for enhancement of T cell proliferation and differentiation into effector cells in order to improve vaccine efficacy [41]. On the other hand, high dose IL-2 therapy has been found to promote expansion of regulatory T cells and could thereby potentially limit the antigen specific immune response [1].This effect could be associated with the intensive regime; however, we have recently found that even low doses of IL-2 for a period of two weeks are able to expand $CD4^+CD25^+$ Treg cells in vivo in cancer patients [75].

Helper antigens in the form of microbial components such as keyhole limpet haemocyanin (KLH), tetanus toxoid (TT) and hepatitis B virus antigen (HBsAg) can also be added to the vaccine. These compounds interact with the Toll like receptors and promote cytokine secretion and inflammation. In addition, KLH amplifies a Th1-type cellular tumour-specific response when added to the lysate [69], which suggests that helper T cell epitopes contained within KLH may enhance the ability of DC to induce CTL responses.

Material and methods

Recent literature (1996–2007) was reviewed for clinical DC vaccination trials in patients with disseminated malignant melanoma. Medline database was searched for "melanoma dendritic cell immunotherapy" with limitations full text, humans, English, cancer, clinical trial. A total of 38 articles have been included for analysis (see Table 1). The analysis focus on the assessment of correlation between clinical response and study parameters such as patient performance status, antigen, DCs, adjuvant, and immune response. Due to pronounced inter-trial variation in parameters such as type of antigen and adjuvant as well as DC preparation it was necessary to perform an overall grouping of these data for statistical analysis. Objective response was defined as CR and PR, while clinical response included SD in addition to objective response. SD as defined by the author of the individual trials is described in Table 2. The definition of SD is very heterogeneous and in 10 out of 38 articles the criteria for this response category was not further specified.

In tests for overall association between clinical response and the independent variables response was treated as being on an ordinal scale. Mann–Whitney tests/Kruskal–Wallis tests were used when the independent variable consisted of two factors/more than two factors. A Spearman correlation test was used when they consisted of more than two ordered factors.

In analysis where the response where dichotomised (e.g. PR or better versus SD or worse) a Fisher's exact test was used. All tests were two-sided and a p value below 0.05 was considered significant.

Results

A total of 38 articles [2, 6, 8, 10, 11, 14, 16, 18, 20, 21, 27, 28, 30, 31, 34, 37–40, 44, 50–57, 61, 62, 64, 68, 70–72, 77, 78, 80] have been included for analysis in this review; including 626 MM patients treated with DC based vaccination therapy. The objective response rate (CR and PR) was 9% with 20 (3%) complete responses (CR) and 37 (6%) partial responses (PR). The clinical response rate (CR, PR, and SD) was 30% with 133 patients (21%) having stable disease (SD). Data from the clinical studies are compiled in Table 1.

Performance status

Only a limited number of the reports from clinical trials (8) describe Eastern Cooperative Oncology Group (ECOG) performance status (PS) of the patients in details in particular PS > 1 was not indicated. Data on 123 patients from 8 trials are summarised in Table 3. We found no correlation between PS and objective response. Regarding clinical response 24 out of 91 (26%) patients with PS = 0 had CR, PR or SD, while 4 out of 25 (16%) patients with PS = 1 had CR, PR or SD. This is a non-significant difference.

These data indicates that patients with PS 0 are not more prone to obtain objective or clinical response than patients with PS 1 having minor symptoms of disease. Interestingly one patient with PS 3 had partial response [80].

Table 1 Published clinical studies on dendritic cell vaccination in melanoma patients

Author	Year	NP	CR	PR	SD	PD	MR	NED	NEV	Type	Maturation	Maturation medium	Antigen	Helper AG	Inj. route	Stage
Akiyama [2]	2005	6	1	1	1	3	0	0		PBMC	iDC	a	Peptide	KLH	SC	IV
Banchereau [6]	2001	17	3	4	3	7	0	0		CD34	iDC	b	Peptide	KLH, Flu-MP	SC	IV
Banchereau [8]	2005	19	0	0	11	8	0	0		CD34	mDC	c, Flt3-L	Peptide	KLH, Flu-MP, INFα2b	SC	IV
Bedrosian [10]	2003	26	0	0	9	14	3	0		PBMC	mDC	GM-CSF, IL-2, IL-12	Peptide		IV/IN/ID	IV
Bercovici [11]	2008	40	0	0	13	27	0	0		PBMC	iDC/mDC	d, FMKp, INFγ	ALTL		SC + ID	IV
Chang [14]	2002	11	0	1	3	7	0	0		PBMC	iDC	a	ATL	KLH	ID	IV
de Vries [16]	2003	16	0	1	3	11	1	0		PBMC	7 iDC/ 9 mDC	a, MCM, TNFα, PGE2	Peptide	KLH	IV + SC/ IV + ID	IV
Dillman [18]	2004	20	0	0	0	5	0		15	PBMC	iDC	a	ATL	GM-CSF	SC	IV
Escobar [20]	2005	18	0	0	11	7	0	0		PBMC	mDC	a, TNFα	ALTL	KLH +/− IL-2	ID	III–IV
Escudier [21]	2005	15	0	1	3	10	1	0		PBMC	iDC/exosome	a	Peptide		ID + SC	III–IV
Gilliet [27]	2003	5	1	1	1	2	0	0		PBMC	Semi-mature	a, CM	Peptide	KLH	IN	IV
Grover [28]	2006	6	0	1	0	5	0	0		PBMC	Intermed-mDC	a, CD40-L	Peptide		IL	IV
Hersey [31]	2004	26	0	3	9	13	1	0		PBMC	iDC	a	Peptide/ATL	KLH	IN	IV
Hersey [30]	2007	22	0	3	6	13	0	0		PBMC	mDC	a, IL-1β, IL-6, PGE-2, TNFα, KLH	ATL/peptide	IL-2 +/−	IN	IV
Jonuleit [34]	2001	8	0	0	5	3	0	0		PBMC	iDC + mDC	a	Peptide	TT, PPD	IN	IV
Kyte [37]	2006	22	0	0	2	18	0	0	2	PBMC	mDC	a, IL-1β, IL-6, PGE-2, TNFα	AT-mRNA		ID/IN	III–IV
Lau [38]	2001	16	1	0	2	11	2	0		PBMC	iDC	a	Peptide		IV	IV
Lesimple [39]	2006	12	0	0	2	10	0	0		PBMC	mDC	d, INFγRibomonyl, KLH	NA17-A/peptide		IL + IN	IV
Linette [40]	2005	12	0	2	3	7	0	0		PBMC	iDC, irrad.	a	Peptide	TT	IV	III–IV
Mackensen [44]	2000	14	0	1	6	6	0	1		CD34	mDC	a, IL-3, IL-6, TNFα	Peptide		IV	IV
Nagayama [50]	2003	10	0	0	1	7	2	0		PBMC	mDC	c	ATL	IL-2	ID	IV
Nakai [51]	2006	11	0	0	3	6	0	2		PBMC	mDC	a, TNFα, polyI:C	Peptide/ATL		ID	III-IV
Nestle [52]	1998	16	2	3	0	10	1	0		PBMC	iDC	a	Peptide/ATL	KLH	IN	IV
O'Rourke [53]	2003	17	3	3	0	11	0	0		PBMC	mDC	a, MCM	Irrad. ATC		ID	IV
O'Rourke [54]	2007	34	3	3	0	28	0	0		PBMC	mDC	a, MCM	Irrad. ATC		ID	IV
Palucka [55]	2003	18	0	0	7	6	1	3	1	CD34		b, Flt3-L	Peptide	KLH, Flu-MP	SC	IV
Palucka [56]	2006	20	1	1	2	15	0	0	1	PBMC	mDC	a, TNFα, CD40L	Irrad. allog. Colo	KLH	SC	IV
Panelli [57]	2000	7	0	1	0	6	0	0		PBMC	iDC	a	Peptide	IL-2	IV	IV
Ribas [61]	2004	9	1	0	1	1	1	0	5	PBMC	iDC	a	Peptide		ID	II-IV
Ridolfi [62]	2006	21	1	1	6	11	2	0		PBMC	8 iDC/ 13 mDC	a, PGE-2, TNFα, IL-1β, IL-6	ATL/ATH	KLH/IL-2	ID/SC	IV
Salcedo [64]	2006	9	1	0	1	6	0	0	1	PBMC	iDC	d	ALTL	TT, HBs	SC + ID + IN	III–IV
Schadendorf [68]	2006	45	0	2	8	35	0	0		PBMC	mDC	a, IL-1β, IL-6, PGE-2, TNFα	Peptide		SC	IV
Schuler-Thurner [70]	2002	16	1	0	8	7	0	0		PBMC	mDC	a, TNFα, IL1β, IL-6, PGE-2	Peptide	KLH	SC	IV
Slingluff [71]	2003	13	0	1	1	11	0	0		PBMC	iDC	a	Peptide	TT, IL-2	IV + SC	IV
Smithers [72]	2003	19	1	2	1	15	0	0		PBMC	iDC	a	AT peptides	HBsAg	ID	IV
Thurner [77]	1999	11	0	0	0	11	0	0		PBMC	mDC	a, MCM	Peptide	TT, tuberculin	SC + ID + IV	IV
Trakatelli [78]	2006	8	0	0	1	4	0	0	3	PBMC	mDC	INFβ, IL-13, polyI:C	Peptide		SC + ID	III–IV
Vilella [80]	2004	11	0	1	0	8	2	0		PBMC	mDC	a, IL-1, IL-6, TNFα, PGE-2	ALTL		IN	IV
Total no.		626	20	37	133	385	17	6	28							
Percentage of total (%)			3	6	21	62	3	1	4							

Table 2 Stable disease definition

Author	Year	NP	CR	PR	SD	PD	MR	NED	NEV	SD definition	Phase	By incl.
Akiyama [2]	2005	6	1	1	1	3	0	0		<25%, NNL, 4 weeks	I	
Banchereau [6]	2001	17	3	4	3	7	0	0		<25%, NNL, 10 weeks		
Banchereau [8]	2005	19	0	0	11	8	0	0		<20%, NNL, 10 weeks	I	
Bedrosian [10]	2003	26	0	0	9	14	3	0		"RECIST"	I	
Bercovici [11]	2008	40	0	0	13	27	0	0		"RECIST"	I/II	
Chang [14]	2002	11	0	1	3	7	0	0		<25%, NNL, 8 weeks	I	
de Vries [16]	2003	16	0	1	3	11	1	0		"WHO criteria"		PD
Dillman [18]	2004	20	0	0	0	5	0		15	"RECIST"		
Escobar [20]	2005	18	0	0	11	7	0	0		<25%, NNL, 6 weeks	I	
Escudier [21]	2005	15	0	1	3	10	1	0		<20%, NNL, 6 weeks	I	
Gilliet [27]	2003	5	1	1	1	2	0	0		ND		
Grover [28]	2006	6	0	1	0	5	0	0		ND		
Hersey [31]	2004	26	0	3	9	13	1	0		"RECIST", no PD 3 months	I/II	
Hersey [30]	2007	22	0	3	6	13	0	0		"RECIST", no PD 3 months	I/II	
Jonuleit [34]	2001	8	0	0	5	3	0	0		ND		PD
Kyte [37]	2006	22	0	0	2	18	0	0	2	RECIST, 3 months	I/II	PD
Lau [38]	2001	16	1	0	2	11	2	0		ND	I	
Lesimple [39]	2006	12	0	0	2	10	0	0		<25%, 3 months	I/II	
Linette [40]	2005	12	0	2	3	7	0	0		"RECIST"	I	
Mackensen [44]	2000	14	0	1	6	6	0	1		ND	I	PD
Nagayama [50]	2003	10	0	0	1	7	2	0		"WHO criteria", 10 weeks	I	
Nakai [51]	2006	11	0	0	3	6	0	2		<20%, NNL		
Nestle [52]	1998	16	2	3	0	10	1	0		<25%, NNL, 6 weeks	I	
O'Rourke [53]	2003	17	3	3	0	11	0	0		Included as PD	I	
O'Rourke [54]	2007	34	3	3	0	28	0	0		Included as PD		
Palucka [55]	2003	18	0	0	7	6	1	3	1	ND	I	
Palucka [56]	2006	20	1	1	2	15	0	0	1	"RECIST", 5 months	I	
Panelli [57]	2000	7	0	1	0	6	0	0		ND	I	
Ribas [61]	2004	9	1	0	1	1	1	0	5	ND	II	
Ridolfi [62]	2006	21	1	1	6	11	2	0		"In partial accordance with RECIST"		
Salcedo [64]	2006	9	1	0	1	6	0	0	1	<25%, NNL, 4 weeks	I/II	
Schadendorf [68]	2006	45	0	2	8	35	0	0		"RECIST" 12 weeks	III, rand.	
Schuler-Thurner [70]	2002	16	1	0	8	7	0	0		ND	I	PD
Slingluff [71]	2003	13	0	1	1	11	0	0		"RECIST"	II, rand.	
Smithers [72]	2003	19	1	2	1	15	0	0		"RECIST"	I/II	
Thurner [77]	1999	11	0	0	0	11	0	0		ND	I	PD
Trakatelli [78]	2006	8	0	0	1	4	0	0	3	"RECIST"	I	
Vilella [80]	2004	11	0	1	0	8	2	0		<25%, 4 months	I	PD
Total no.		626	20	37	133	385	17	6	28			
Percentage of total (%)			3	6	21	62	3	1	4			

Type of antigen

Antigens grouped as either synthetic tumour specific peptides (MART-1, MAGE-1, 2 or 3, gp100, tyrosinase) or autologous tumour preparations were included in the analysis. Data on 517 patients from 32 trials are compiled in Table 4. A slightly higher fraction of patients attained objective response when autologous tumour antigens were included in the vaccine; 13% compared to 9% with synthetic tumour specific peptides. This tendency was, however, not significant ($p = 0.12$). When clinical response rates were compared synthetic peptides resulted in a significantly higher RR (34%) than autologous tumour antigens (23%) ($p = 0.03$). This difference between objective

Table 3 Performance status, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
0	3 (3%)	6 (7%)	15 (16%)	62 (68%)	3 (3%)		2 (2%)	91
1	1 (4%)		3 (12%)	18 (72%)	3 (12%)			25
2				6				6
3		1						1
All	4 (3%)	7 (6%)	18 (15%)	86 (70%)	6 (5%)		2 (2%)	123

Included: [16, 21, 39, 56, 62, 64, 72, 80]

Table 4 Type of antigen, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
Peptide	9 (3%)	21 (6%)	84 (25%)	199 (59%)	10 (3%)	4 (1%)	9 (3%)	336
Autol. tumour	9 (5%)	14 (8%)	19 (10%)	117 (65%)	5 (3%)		17 (9%)	181
All	18 (3%)	35 (7%)	103 (20%)	316 (61%)	15 (3%)	4 (1%)	26 (5%)	517

Not included: [11, 20, 56, 64, 80] because allogeneic tumour lysate was used and [51] because of combined ATL and peptides

Table 5 Maturation stage, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
iDC	7 (4%)	15 (8%)	28 (14%)	117 (60%)	6 (3%)		21 (11%)	194
mDC	9 (3%)	16 (5%)	76 (22%)	218 (64%)	10 (3%)	3 (1%)	6 (2%)	338
All	16 (3%)	31 (6%)	104 (20%)	335 (63%)	16 (3%)	3 (1%)	27 (5%)	532

Not included: [6, 11, 27, 28, 35, 55]

response and clinical response is due to a high rate of SD in patients who received peptide pulsed DC's.

Thus, autologous tumour antigens might be better than synthetic peptide antigens at inducing objective clinical responses, whereas the use of synthetic peptides is associated with a high incidence of SD. On the other hand, definite conclusions regarding clinical efficacy are hampered by the diversity of the individual antigen preparations.

Maturation stage (phenotype) of dendritic cells

The maturation status of DCs and with that, their ability to induce immune responses has been investigated extensively and is still discussed. The general dogma is that mDCs are superior at inducing an immune response. Whether the DCs need to be matured in vitro in order to induce a response in vivo is a different question though. For analysis, data on DC preparations from 32 trials, including 532 patients, were grouped as either immature or mature (including any kind of maturation) as presented in Table 5. Overall comparison of data from the trials included in this review, offered no clear answer as the response rates were not significantly different in patients treated with mature DC compared to immature. Grouping the responses into objective responses as compared to SD, PD and MR indicated a trend ($p = 0.08$), that iDC are actually superior to mDC at inducing an objective response. The difference in clinical response rates (26% iDC vs. 30% mDC) is on the other hand not significant when compared to PD ($p = 0.76$). For these calculations the trials were solely grouped according to the employment or omission of any kind of DC maturation in vitro regardless of differences in maturation factors employed. In addition, further analyses were performed comparing the use of more uniformly prepared DCs. Thus, response rates in patients vaccinated with iDCs differentiated with medium "a" (GM-CSF + IL-4) were compared with patients vaccinated with mDCs differentiated with medium "a" and matured with IL-1, IL-6, TNFα and PGE2; including 307 patients in 19 different trials. Dose and the type of antigen are summarised in Tables 6 and 7. This subgroup analysis did not reveal any significant differences in the response (clinical or objective) between patients vaccinated with mDC and iDC.

Route of vaccine injection

DC injection i.d. and i.n. is usually considered superior to other routes of vaccine injection regarding immune as well as clinical response [10, 36], furthermore, i.v. injection is regarded as inferior. The analysis included 494 patients from 30 studies (Table 8) and an overall comparison of data did not show that a specific injection route were superior. We also found that i.d., s.c. and i.n. injection did not induce more objective or clinical responses than the i.v. route ($p = 0.4$, $p = 0.9$). Furthermore, i.n. injection did not turn out to be superior as compared to i.d. and s.c. ($p = 1.0$, $p = 0.7$).

Table 6 Studies on immature dendritic cells (subpopulation maturation medium "a")

Author	Year	NP	CR	PR	SD	PD	MR	NED	NEV	Mat. Med.	Antigen
Akiyama [2]	2005	6	1	1	1	3	0	0		a	Peptide
Chang [14]	2002	11	0	1	3	7	0	0		a	ATL
Dillman [18]	2004	20	0	0	0	5	0		15	a	ATL
Escudier [21]	2005	15	0	1	3	10	1	0		a	Peptide
Hersey [31]	2004	26	0	3	9	13	1	0		a	Peptide/ATL
Jonuleit [34]	2001	8	0	0	5	3	0	0		a	Peptide
Lau [38]	2001	16	1	0	2	11	2	0		a	Peptide
Linette [40]	2005	12	0	2	3	7	0	0		a	Peptide
Nestle [52]	1998	16	2	3	0	10	1	0		a	Peptide/ATL
Panelli [57]	2000	7	0	1	0	6	0	0		a	Peptide
Ribas [61]	2004	9	1	0	1	1	1	0	5	a	Peptide
Slingluff [71]	2003	13	0	1	1	11	0	0		a	Peptide
Smithers [72]	2003	19	1	2	1	15	0	0		a	AT peptides
		178	6	15	29	102	6	0	20		
(%)			3	8	16	57	3	0	11		

Table 7 Studies on mature dendritic cells (subpopulation maturation medium "a")

Author	Year	NP	CR	PR	SD	PD	MR	NED	NEV	Mat. Med.	Antigen	IL-1 (ng/ml)	IL-6 (ng/ml)	TNFα (ng/ml)	PGE2 (μg/ml)
Hersey [30]	2007	10	0	0	2	8	0	0		a	ATL	10	800	10	1
Hersey [30]	2007	12	0	3	4	5	0	0		a	Peptide	10	800	10	1
Kyte [37]	2006	22	0	0	2	18	0	0	2	a	AT-mRNA	10	1,000	10	1
Ridolfi [62]	2006	13	1	1	3	6	2	0		a	ATL/ATH				
Schadendorf [68]	2006	45	0	2	8	35	0	0		a	Peptide	2	1,000	10	1
Schuler-Thurner [70]	2002	16	1	0	8	7	0	0		a	Peptide	10	100	10	1
Vilella [80]	2004	11	0	1	0	8	2	0		a	ALTL	10[a]	1,000	10	1
Total no.		129	2	7	27	87	4	0	2						
Percentage of total (%)			2	5	21	67	3	0	2						

Table 8 Route of injection, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
ID	9 (6%)	10 (6%)	27 (17%)	101 (62%)	6 (4%)	2 (1%)	7 (4%)	162
SC	6 (4%)	16 (10%)	32 (20%)	86 (53%)	1 (0.6%)	3 (2%)	17 (11%)	161
IV	1 (2%)	4 (7%)	15 (26%)	34 (60%)	2 (4%)	1 (2%)		57
IN	3 (3%)	12 (11%)	23 (21%)	64 (59%)	6 (6%)			108
IL		1 (17%)		5 (83%)				6
All	19 (4%)	43 (9%)	97 (20%)	290 (59%)	15 (3%)	6 (1%)	24 (5%)	494

Ref. [11, 16, 21, 39, 62, 64, 71, 77, 78] (Ridolfi iDC) are not included because of combination of route of injection or not identified patients

Helper antigen/adjuvant

In many of the trials, helper antigens and adjuvant are added to induce inflammation, which is thought to promote a more potent immune response. The data were grouped as "any use of helper antigen or adjuvant" or "no use of helper antigen or adjuvant" and included 626 patients in 38 trials (Table 9). A trend towards a higher objective response rate ($p = 0.09$) when using helper antigen/adjuvant was found while the correlation with clinical response was highly significant ($p = 0.002$); patients developing either CR, PR or SD in trials using helper antigen/adjuvant constituted 35% compared to 24% in trials not using helper antigen/adjuvants. However, as was the case for choice of antigen there are large variations in the helper antigen and adjuvant used in the different studies.

Table 9 Helper antigen/adjuvants, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
Any	12 (4%)	25 (7%)	83 (24%)	191 (56%)	8 (2%)	3 (1%)	18 (5%)	340
None	8 (3%)	12 (4%)	50 (17%)	194 (68%)	9 (3%)	3 (1%)	10 (3%)	286
All	20 (3%)	37 (6%)	133 (21%)	385 (62%)	17 (3%)	6 (1%)	28 (4%)	626

Included: [2, 6, 8, 10, 11, 14, 16, 18, 20, 21, 27, 28, 30, 31, 34, 37–40, 44, 50–56, 61, 62, 64, 68, 70, 72, 77, 78, 80]

Table 10 DTH against ATL, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
DTH+	2 (4%)	3 (6%)	8 (17%)	22 (47%)	2 (4%)	1 (2%)	9 (19%)	47
DTH−	2 (2%)	5 (5%)	12 (13%)	61 (67%)	3 (3%)		8 (9%)	91
All	4 (3%)	8 (6%)	20 (14%)	83 (60%)	5 (4%)	1 (1%)	17 (12%)	138

Included: [14, 18, 30, 31, 37, 50–52, 54, 62, 72]

Table 11 INFγ secreting cells, *n* (%)

	CR	PR	SD	PD	MR	NED	Not eval	Total
Increased	4 (5%)	4 (5%)	38 (43%)	32 (36%)	4 (5%)	3 (3%)	3 (3%)	88
No change	1 (1%)	5 (6%)	17 (19%)	55 (63%)	4 (5%)	2 (2%)	4 (5%)	88
All	5 (3%)	9 (5%)	55 (31%)	87 (49%)	8 (5%)	5 (3%)	7 (4%)	176

Included: [2, 6, 10, 20, 31, 40, 51, 55, 61, 62, 64, 72, 77]

Delayed-type hypersensitivity responses against autologous tumour lysate

Delayed-type hypersensibility (DTH) is a cell-mediated immunity elicited in the skin and mediated by $CD4^+$ Th1 cells. The general consensus is that induration $\geq$5 mm and erythema 48 h after intradermal injection is regarded a positive response.

The reports on DTH are highly inconsistent; some authors have reported correlation and others lack of correlation between DTH and clinical outcome. DTH technique differs among the studies as some use peptides or lysate in solvents, where as others use peptide or lysate pulsed DC's.

In this review, only DTH responses against autologous tumour lysate (ATL) has been gathered from 138 patients in 11 studies for analysis, since it is not appropiate to compare DTH responses against ATL, tumour specific peptides, keyhole limpet haemocyanin (KLH), hepatitis B virus and tetanus toxoid or other substances used for inducing a DTH response.

DTH outcome did not correlate to objective response ($p = 0.51$). As can be seen from Table 10, 13 out of 47 (27%) patients with a positive DTH response had CR, PR or SD compared to 19 out of 91 (20%) patients in the DTH negative group. Thus, only a weak trend of correlation between positive DTH and clinical response could be demonstrated ($p = 0.18$). The feasibility of DTH response in the clinic as a tool for determining DC vaccine efficacy is also questionable since almost half of the DTH positive patients had PD.

Induction of antigen specific T cells

IFN-γ ELISpot is an in vitro test monitoring IFN-γ secretion from T cells as an indicator of antigen specific activation. This test is proposed as a standard in vitro test to determine the in vivo immunogenic effectiveness of cancer vaccines [32, 33]. As for the DTH response, data on the correlation between ELISpot and clinical outcome is highly variable in the individual trials.

ELISpot data were defined as positive if an increase post-vaccination compared to pre-vaccination were described otherwise they were defined as negative. Data from 176 patients in 13 trials were available (Table 11). Regarding correlation with objective response no difference was shown ($p = 0.8$). Increased ELISpot reactivity can, therefore, not be considered a reliable indicator of objective response to DC vaccination in melanoma. In contrast, 53% of the patients in the group with increased ELISpot reactivity had clinical response in opposition to 26% in the group with negative ELISpot ($p = 0.0004$).

Stable disease

When we analysed the SD group separately we found that the use of synthetic peptides were associated with a significantly higher response rate ($p = 0.0004$). The use of helper antigen or adjuvant ($p = 0.01$) and induction of antigen specific T cells ($p = 0.0003$) were also found to be associated with a higher frequency of SD.

Table 12 Studies with progressive disease as inclusion criteria

Author	Year	NP	CR	PR	SD	PD	MR	NED	NEV
de Vries [16]	2003	16	0	1	3	11	1	0	
Jonuleit [34]	2001	8	0	0	5	3	0	0	
Kyte [37]	2006	22	0	0	2	18	0	0	2
Mackensen [44]	2000	14	0	1	6	6	0	1	
Schuler-Thurner [70]	2002	16	1	0	8	7	0	0	
Thurner [77]	1999	11	0	0	0	11	0	0	
Vilella [80]	2004	11	0	1	0	8	2	0	
Total		98	1	3	24	64	3	1	2
Percentage of total (%)			1	3	24	65	3	1	2

The SD patient category, however, constitutes a rather heterogeneous group; in some trials not only SD induced during treatment but also sustained SD is accepted in this response group. In addition, the criteria for SD are frequently poorly defined and essential information is lacking in some publications. In order to analyse the potential influence of these differences in response definition we identified all trials where only patients with verified PD at time of inclusion were included and the described SD, therefore, attained during treatment (Table 12). When analysing this patient group separately, we still found a significant correlation to the use of synthetic peptides ($p = 0.03$) and a weak trend that the use of adjuvant/helper antigen were correlated to SD ($p = 0.2$). Thus, these findings are consistent with the findings of the total SD group, with the reservation of a smaller number of patients in the latter group.

Discussion

During the past decade numerous types of DC vaccines have been tested in more than 600 melanoma patients. Establishment and conduction of clinical DC trials are highly complex and frequently important study details are determined based on traditions and assumptions and not necessarily on well documented data. The aim of this review was to gather experiences from all clinical DC trials in melanoma and assess the significance of specific vaccine parameters which are frequently claimed to have a possible influence on clinical response, i.e. patient performance status, antigen, DCs, adjuvant, route of injection and immune response. Statistical analyses were performed in order to clarify whether it was possible based on these results to draw any conclusions which could point the direction for the next generation of DC-based melanoma vaccines. Our analyses showed that even though there are trends that specific vaccine procedures are superior, the clinical evidence is not always that clear. This is important to bear in mind in the planning of future trials to avoid that some methods are abandoned and others appointed the gold standard before solid scientific data exists.

Due to pronounced inter-trial variation in parameters such as type of antigen and adjuvant as well as DC preparation methods it was necessary to perform an overall grouping of the data for statistical analysis. The results should, therefore, be interpreted with the reservation that they do not take into account all trial-specific details. It is also important to consider that the parameters described have an influence on each other. Nevertheless, even with these reservations in mind we believe that several important lessons can be learned from the available studies.

Our statistical analyses showed significant correlation between clinical responses defined as CR, PR or SD and the use of peptide antigens, the addition of helper antigen or adjuvant, and treatment induced antigen specific T cells response. Overall, only 9% of the patients had objective response (CR and PR) to the treatment, and maybe therefore, we were not able to detect any significant correlations between objective response and the tested parameters. However, a few non-significant trends were demonstrated including an association between objective response and the use of immature DCs, the addition of adjuvant ($p = 0.09$), and use of autologous antigen preparation.

The choice of antigen is of course very essential and might influence several other vaccine parameters such as choice of DC preparation method and adjuvant. In the analysis, antigens were grouped as either synthetic tumour specific mainly HLA class I restricted peptides (MART-1, MAGE-1, 2 or 3, gp100, tyrosinase) or autologous tumour preparations. Interestingly, we found a significant correlation between the use of peptide antigens and clinical response while objective response more tended to be associated with the use of autologous tumour antigens. These findings indicate that autologous tumour antigens might be better than synthetic peptide antigens at inducing objective responses, whereas synthetic peptides more frequently are associated with SD. On the other hand, the results might be biased if disease status of the patient groups were not comparable, i.e. if patients treated with autologous tumour antigens predominantly had subcutaneous or nodal metastases whereas patients treated with peptide antigens had more severe systemic disease. In addition, general conclusions

require that the differences in antigen preparation are further scrutinised.

Surprisingly, a trend towards iDC being superior to mDC in producing objective responses was found. This finding raises the important question how to define a mature DC. As mentioned previously DC can be described both phenotypically and functionally. Most of the studies in this review have only classified DC from a phenotypic perspective. Because phenotyping is performed prior to injection the use of helper antigens perhaps skew the picture as they might induce further maturation of the DC in vivo. Interestingly, a remarkable high response rate is found in a study by O'Rourke et al. [53, 54] where they used monocyte conditioned medium (MCM) to mature the DC. MCM is a supernatant derived from cultures of autologous monocytes plated onto petri dishes pre-coated with human immunoglobulin and possess a variable ability to induce maturation of DC. However, due to the considerable clinical effectiveness, it would be interesting to look further into the cytokine profile of MCM and also further characterise the MCM prepared DC.

In many of the trials, helper antigens such as KLH or viral proteins were added. In addition, the cytokine IL-2 and GM-CSF (in one trial) were used as vaccine adjuvant. Data were grouped as "any use of helper antigen or adjuvant" or "no use of helper antigen or adjuvant" independently of the specificity of the helper antigen or adjuvant. The use of helper antigen or adjuvant was non-significantly associated with objective response and significantly correlated to clinical response which could indicate that helper antigens and adjuvant in general increase vaccine potency. However, due to the large variations in helper antigen and adjuvant used in the different studies it is not possible to make firm conclusions on the effectiveness of the individual compounds. Remarkably in one study achieving one of the highest objective RR no helper antigen or adjuvant were used [53].

The best route for vaccine administration in melanoma patients could not be established from the available data. We found no significant differences among the different injection sites; especially i.n. administration did not prove to be superior. This finding is in contrast to results from Bedrosian et al. [10] who compared i.n., i.d. and i.v. administration and found that i.n. administration resulted in superior T cell response. Conversely, results from another DC vaccination study by Kyte et al. [37] showed a better immune response in malignant melanoma patients vaccinated with DCs transfected with tumour mRNA when the vaccine was administered i.d. compared to i.n. Recently, we found that renal cell carcinoma patients treated with a DC-based vaccine were more likely to attain SD when the vaccine was administered i.d. compared to i.n. [12].

Induction of immunity against tumour antigen after DC vaccination has been discussed as a potential surrogate endpoint in clinical trials. All though no significant correlation with objective response was found, clinical responses (CR, PR, and SD) correlated highly significant with an induction of vaccine specific T cells as measured by ELISpot. These findings are very encouraging for the ongoing work to optimise immune monitoring in clinical vaccination trials including defined standards for monitoring CD8+ T cell response against the used tumour antigen during vaccination [13, 33]. One problematic aspect of this work is underlined by the in vitro demonstration of vaccine specific memory $CD8^+$ T cells able to secrete IFN-γ and proliferate in patients without objective clinical responses [8]; raising the question if these T cells are in fact able to kill their tumour target in vivo.

A few trials showed correlation between survival and immunologic response [18, 20, 37]. For example, Kyte et al. [37] showed that DC vaccinated patients survived longer than non-vaccinated when retrospectively compared to a control group of patients receiving standard treatment in the same period at the same hospital. Also, Schadendorf et al. [68] performed a trial that failed to demonstrate DC vaccination superiority to dacarbazine treatment. However, explorative subgroup analyses indicated that vaccination treated HLA-$A2^+$/HLA-$B44^-$ patients survived longer than dacarbazine treated patients, implying a treatment effect in this group. Even though the data lack sufficient statistical strength, they emphasise the importance of careful patient selection for these kinds of trials.

The patients with SD constitute a rather heterogeneous and often poorly defined group; in some trials response is only recognised as SD if the patient was in progression at inclusion while other trials accept sustained SD in this response group. In two trials [53, 54] the SD was included as PD, i.e. no response. Unfortunately, specific information regarding these issues is frequently lacking (Table 2) making it more than difficult to interpret the results.

Response data have been analysed with (clinical response) as well as without (objective response) inclusion of SD patients as described. When we analysed the SD group separately we found that the use of synthetic peptides were associated with a significantly higher response rate which differ from the objective response group, where autologous tumour antigens were superior. Whether this is due to the heterogeneity of the patient group or actually a result of differences in the anti-tumour efficacy of immune responses induced by peptides contra autologous antigen preparations needs to be further clarified. The use of helper antigen or adjuvant and induction of antigen specific T cells were also found to be associated with a higher frequency of SD. These findings underscore that despite the outlined problems SD is an important response parameter in connection

with biologic therapies such as DC vaccines. It is, however, imperative that the criteria for SD responses are well-defined and carefully described in future trials if this response category is to be accepted as a meaningful treatment response.

Perspectives

In the present review we have scrutinised the parameters that influence the efficacy of DC based vaccination against cancer. The laborious and time consuming procedures associated with in vitro propagation of DC, implies that much effort has focused on studying the potential of in vivo targeting of DC, e.g. by administration of antigen in combination with a ligand for receptors expressed by DC [17, 58]. Whether such strategies will be superior awaits further study [76]. Considering more conventional treatment modalities, preliminary data suggests a possible synergistic effect with therapeutic vaccination [3]. Thus, combination of different therapies may be required to obtain better clinical responses with therapeutic DC vaccination. Remarkable results have been achieved in chemo-lymphodepleted melanoma patients, by adoptive transfer of in vitro expanded tumour infiltrating lymphocytes (TIL) in combinations with high dose IL-2 [19]. A widespread use of this approach is, however, hampered by the lack of readily expandable TIL in most cancers. To circumvent this problem, vaccination might be a useful tool for induction of antigen specific T cells which could then be harvested for in vitro expansion and transfer back to the patient upon lymphodepletion [59].

Combining vaccination strategies and chemotherapy might be another option. Preliminary data suggests a potential synergistic effect of anti-cancer vaccines and chemotherapy [24]. Moreover, regulatory T cells (Treg) can be depleted by administration of chemotherapy prior to immunotherapy. For instance, low-dose cyclophosphamide decreases the number and function of Treg cells [43]. Treg play an important role in the maintenance of immune tolerance and may be one of the obstacles of successful tumour immunotherapy. Another promising way of increasing vaccine induced immune response is by antibody mediated blocking of the cytotoxic T lymphocyte associated protein-4 (CTLA-4) which is expressed on T cells and inhibits activation when engaged by CD80 on antigen presenting cells.

In conclusion, therapeutic DC vaccination is able to induce antigen specific immune response in melanoma patients and to cause tumour regression in a subset of the treated patients. However, there is still limited knowledge about the optimal vaccine generation, administration and immune monitoring. Further optimisation of DC vaccination should be accomplished through development of improved methods for DC/antigen preparation and through continued clinical trials with well-defined clinical, biological and immunological endpoints.

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