Therapeutic vaccination with autologous mRNA electroporated dendritic cells (DC) in patients with advanced melanoma

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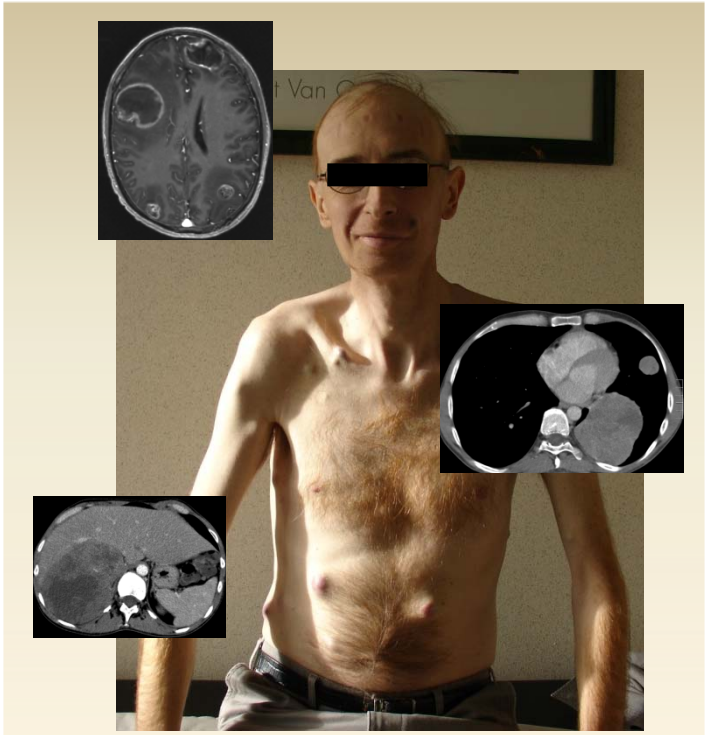
Background on advanced melanoma

- Aggressive cancer with a poor prognosis [1]
  - Meta-analysis 1y OS = 25.5% (95% CI, 23.6-27.4%)
- Highly resistant against cytotoxic agents [2]
  - No randomized trial to improved OS
- Sensitive to small molecule inhibitors in the presence of activating BRAF or cKIT mutations [3]
- Immunogenic cancer [4]
  - Immunoediting
  - Anti-melanoma T-cell response
  - Cancer/germline-, differentiation- & tumor specific Ag's

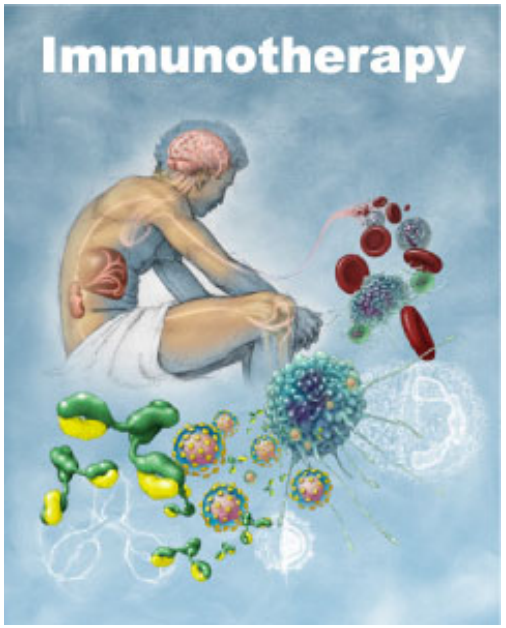


36y old stage IV-M1c
Melanoma patient

1. Korn E L et al. JCO 2008; 2. Tsao H et al. NEJM 2004; 3. Flaherty K et al. NEJM 2010; 4. Boon et al. Annu Rev Immunol. 2006

Immunotherapy

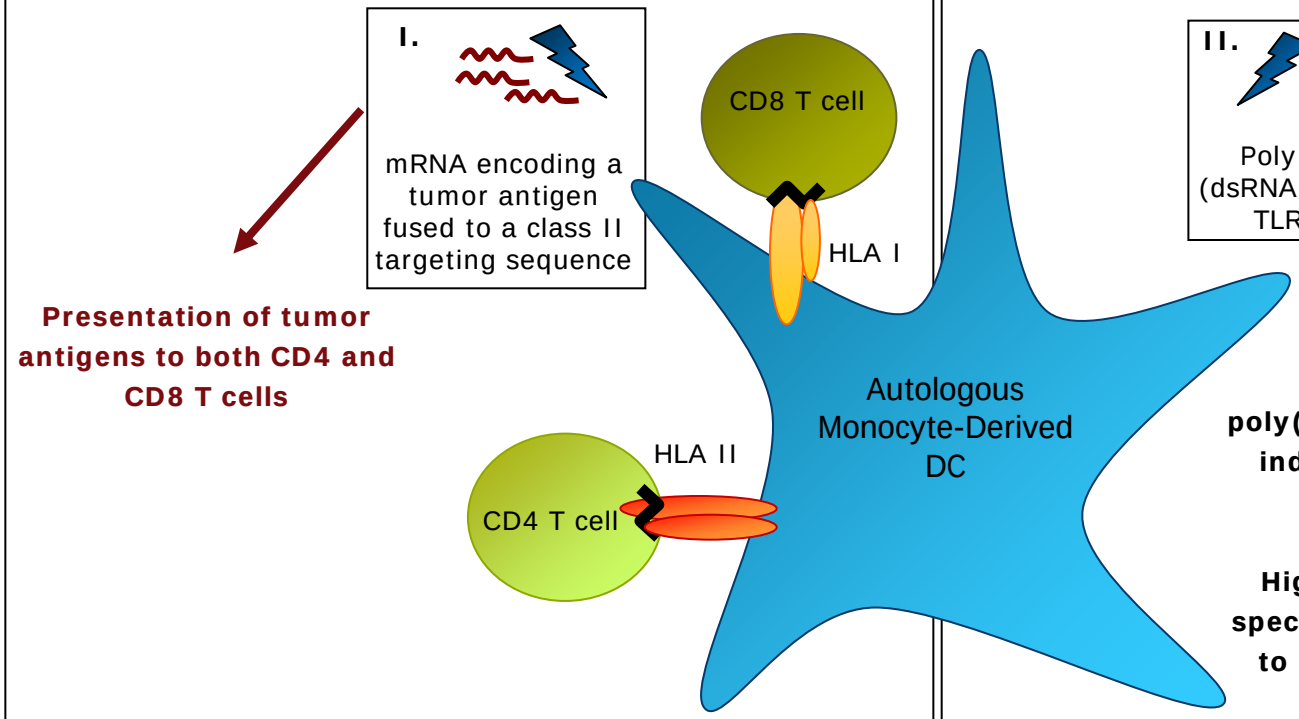
- **Modalities with activity against melanoma**
  - Cytokines (IFNa, Il2, Il21)
  - T-cell co-stimulatory signal receptor targeted mAb's
  - Therapeutic vaccines (peptides, proteins)
  - Autologous cellular immunotherapy
    - Dendritic cell therapy, adoptive T-cell therapy
- **Combinatorial immunotherapy**
  - ↗ : Melacine or Alvac-gp100M + IFNa-2b [1]
  - ↗ : gp100 peptide vaccine + HD IL-2 [2]
  - ↘ : gp100 peptide vaccine + Ipilimumab [3]
- **Efficacy criteria for anti-tumor activity**
  - Immune-related response criteria (irRC) [4]
    - Ipilimumab for advanced melanoma
  - Improved OS without increase in tumor response rate or TTP [5]
    - Sipuleucel-T for castration-resistant prostate cancer



1. Astsaturov Clin Cancer Res, 2003; Mitchell JCO 1994; 2. Schwartzentruber ASCO AM 2009; 3. Hodi NEJM 2010; 4. Wolchock Clin Cancer Res 2009; 5. Kantoff NEJM 2010

PolyI:polyC12U Autologous Dendritic Cells

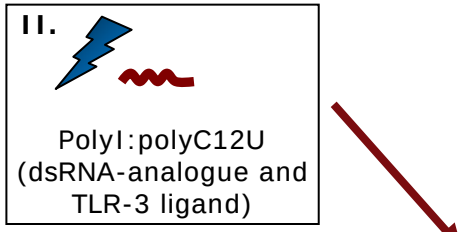
Antigen presentation by DC



Presentation of tumor antigens to both CD4 and CD8 T cells

Bonehill et al. J Immunology 2004

Immunostimulatory capacity



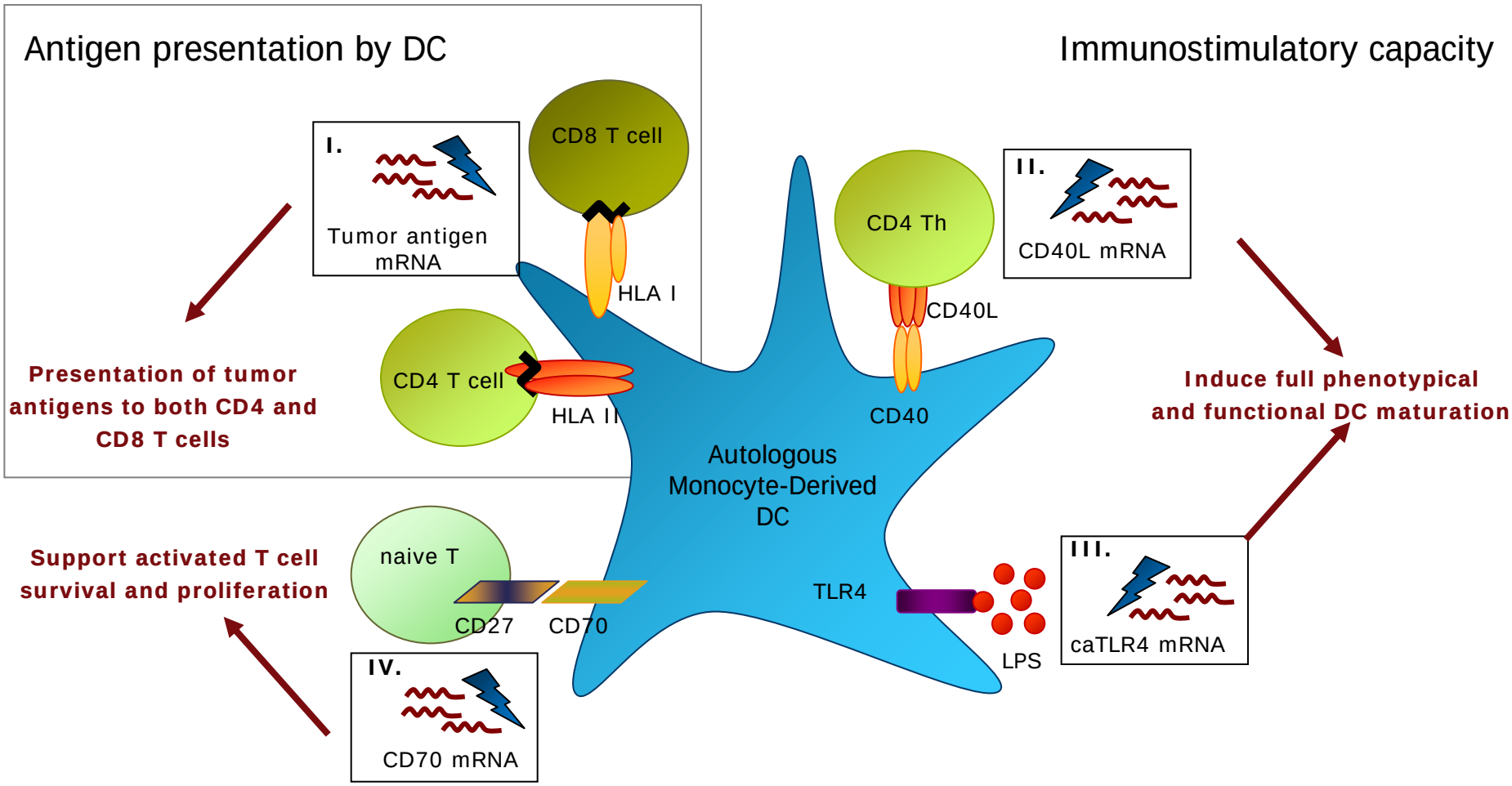
Induce DC maturation

Co-electroporation of DCs with poly(I:C(12)U) + Melan-A/MART-1 mRNA induced strong anti-Melan-A/MART-1 CD8(+) T-cell responses

Higher numbers of Melan-A/MART-1-specific CTLs were obtained as compared to DCs matured with an inflammatory cytokine cocktail

Michiels A et al. Gene Ther 2006

caTLR4, CD70 & CD40L (TriMix) Autologous Dendritic Cells



Bonehill, A et al. Mol Ther, 2008
Bonehill, A et al. Clin Cancer Res. 2009

Institutional clinical trial program on autologous mRNA electroporated DC therapy

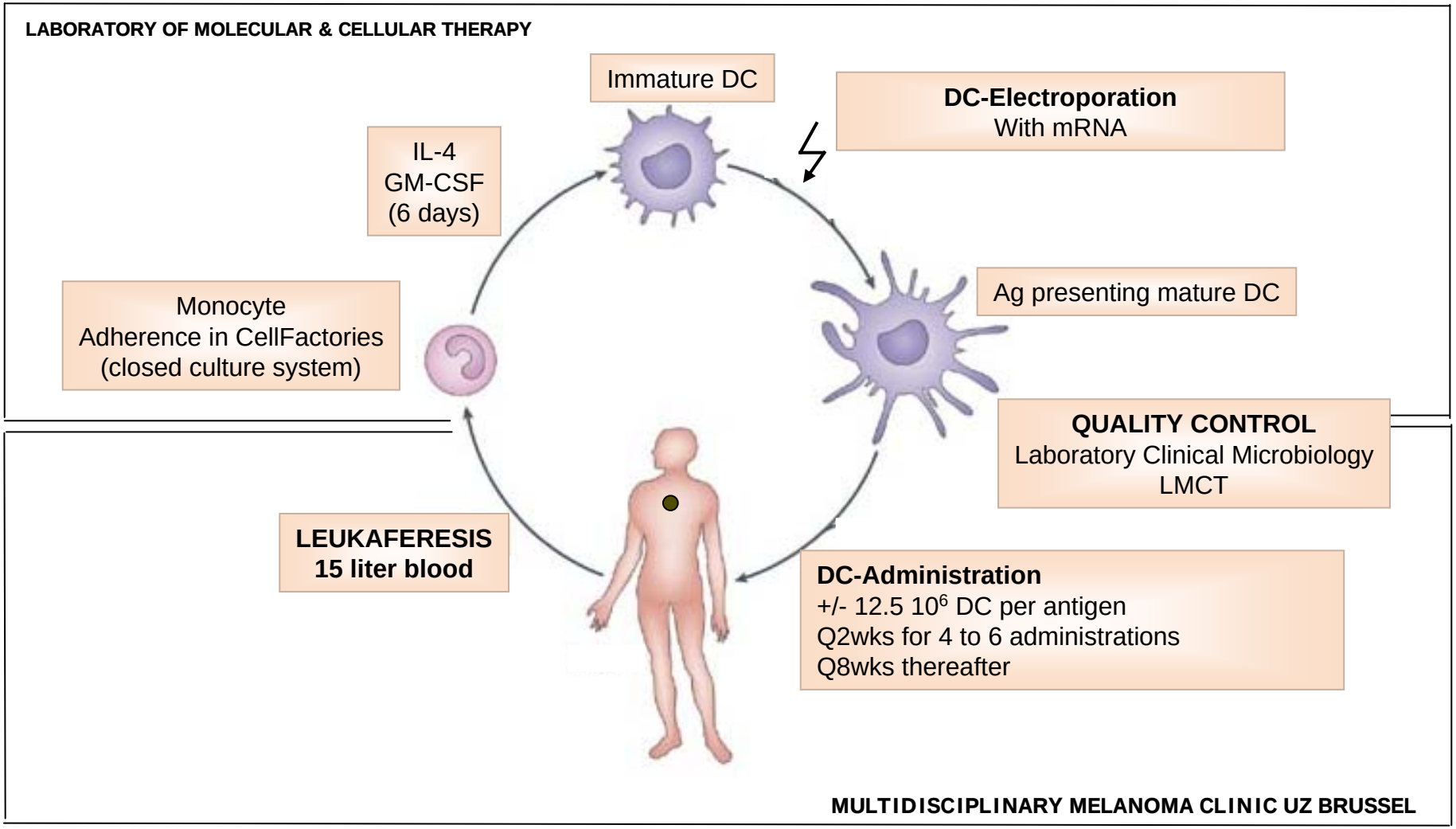
Recruitment period	No. of patients	Autologous DC maturation	Electroporated antigen mRNA		IFNa-2b [5 MIU TIW]
June 2005 Sept 2007	13	Poly I:polyC12U	MAGE.A1-DC.LAMP MAGE.A3-DC.LAMP MAGE.C2-DC.LAMP Tyrosinase-DC.LAMP gp100-DC.LAMP MelanA-DC.LAMP	ID	At PD
	24				Con-comitant
Oct 2007 June 2009	33	CD40L, CD70, caTLR4	MAGE.A3-DC.LAMP MAGE.C2-DC.LAMP Tyrosinase-DC.LAMP gp100-DC.LAMP	ID	From week 8
Dec 2009 Ongoing	3			ID/IV	-
	Total: 73				

Primary endpoint: Feasibility & safety

Secondary endpoints: Anti-tumor response (*signs of activity*)

Immunological response

Treatment Procedure



Patient baseline demographics

		No.	%
No. patients (male/female)		**73 (46/27)**	
Median age (years; range)		**46 (27-75)**	
AJCC stage	**III (recurrent disease)**	30	**41**
	IV (IV-M1a / -M1b / -M1c)	43 (10 / 7 / 26)	**69 (14 / 10 / 36)**
Disease status	**No measurable lesions**	30	**41**
	Measurable lesions	43	**59**
LDH	**≤ ULN**	61	**84**
	1 - 2x ULN	12	**16**
Primary site	**Extremities**	24	**33**
	Trunk	28	**38**
	Head and neck	10	**14**
	Acral	5	**7**
	Unknown	6	**8**
Prior Therapy	**Surgery**	71	**97**
	Chemotherapy	24	**33**
	Radiotherapy	25	**34**
	Immunotherapy	14	**19**

Treatment related adverse events (CTCAEv3.0)

481 therapeutic DC-administrations	No. Patients (%)	
	Grades 1/2	Grades 3/4
DC-related (73 patients)		
Local injection site reactions	73 (100)	0
Fever, myalgia	4 (5.5)	0
Skin depigmentation	13 (17.8)	0
IFNa-2b related (61 patients)		
Constitutional symptoms	56 (91.8)	5/61 (8.2)
Depression	2 (3.3)	0
Bullus lesions acral skin	2 (3.3)	0
Hyperthyroidism	1 (1.6)	0

Immunomonitoring

CD8+ DTH infiltrating lymphocyte (DIL) response				
	Antigens			
	gp100	Tyrosinase	Mage-C2	Mage-A3
Pré-DC administration (n= 10)				
Positive DTH test	0	0	0	0
Post 4x administration DC (n= 21)				
Positive DTH test	1 (4%)	9 (42%)	10 (47%)	7 (33%)
Average $CD8^+CD137^+$ DIL (%)	3.9	7	12.6	13.7
Range $CD8^+CD137^+$ DIL (%)	-	0.9-19.2	2.5-21.9	1.5-34.6

A CD8+ T-cell response was considered positive when both the % of CD137 positive cells exceeded twice the background percentage and the secretion of either IFN-g or TNF-a was 1,5 times elevated compared to background. The percentages shown are after subtraction of the background, being the CD137 expression by DIL in response to autologous EBV-B cells presenting an irrelevant Ag.

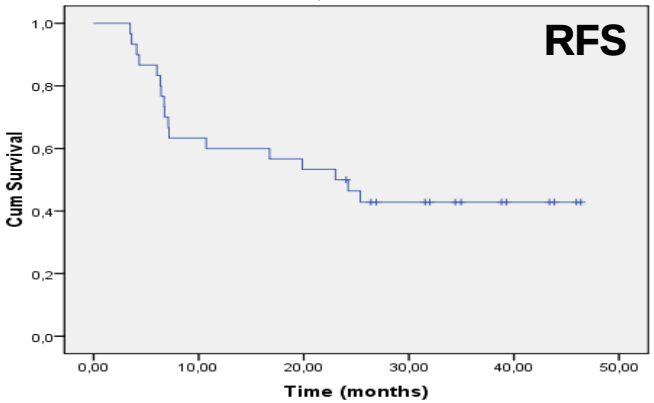
I. Jolanda M. de Vries et al. Journal of Clinical Oncology, Vol 23, No 24 (August 20), 2005: pp. 5779-5787

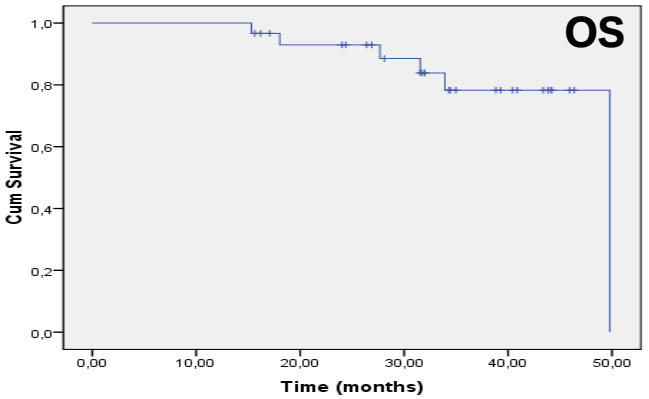
Survival of patients without measurable disease

73 eligible patients

30 patients
No measurable disease

43 patients
Measurable Disease



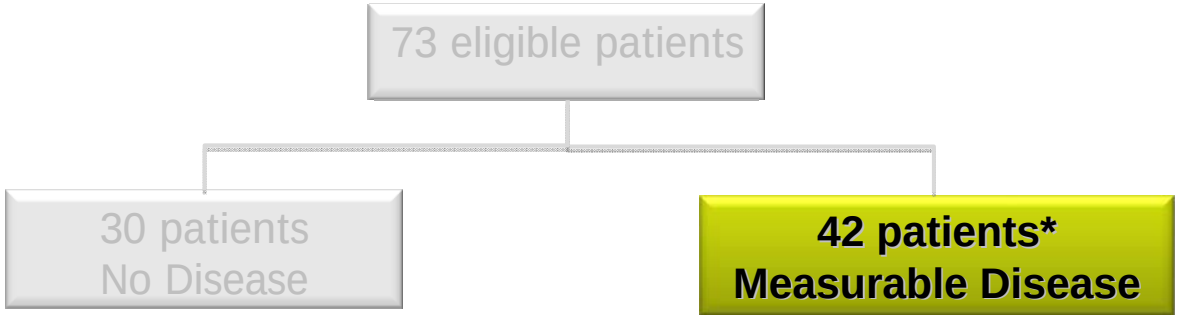


	Study population	Historical controls *
2y RFS% (95% CI)	50% (32-68)	± 45%
2y OS% (95% CI)	92% (83-100)	± 65%

No significant correlation with RFS was found for the baseline co-variates analyzed

*Eggermont et al. The Lancet. 2008; Kirkwood et al. JCO 1996; Balch et al JCO 2001

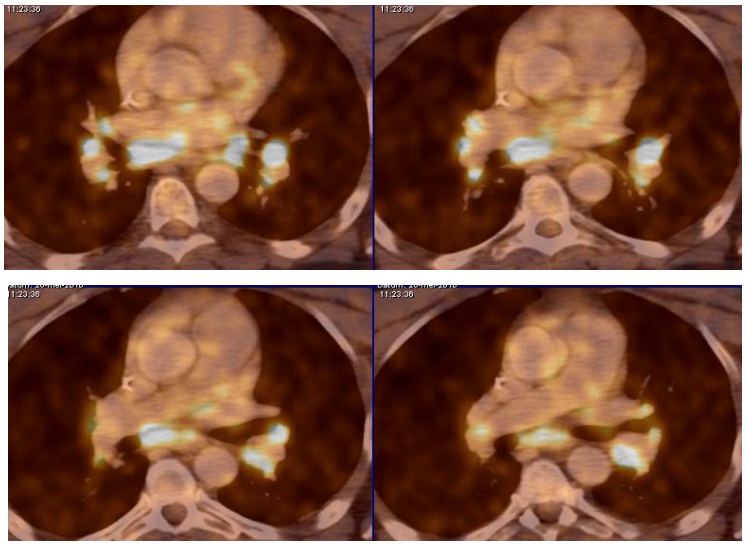
Best objective tumor response

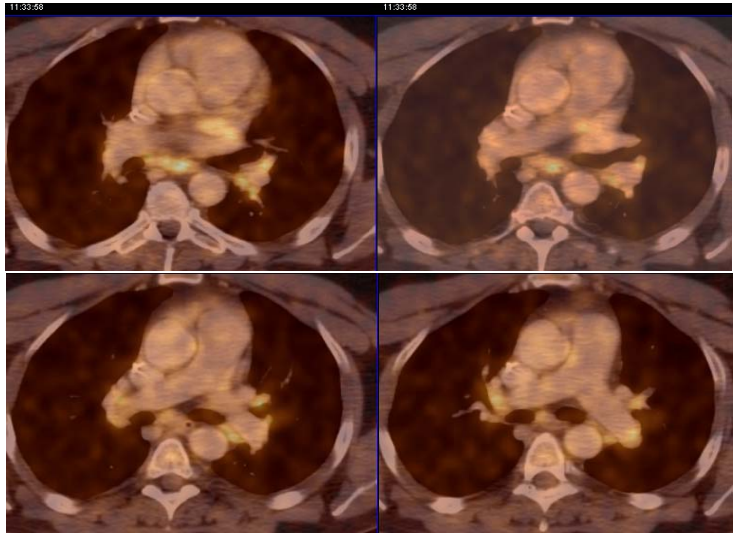


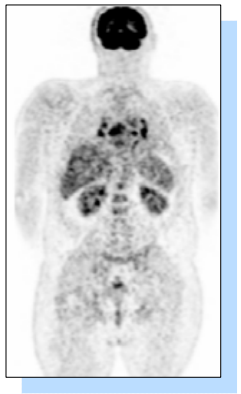
	RECIST (%)
CR	0 (0)
PR	1 (2.4)
SD	17 (40.5)
DCR (CR+PR+SD)	18 (42.9)

* 1 patient not evaluable for response

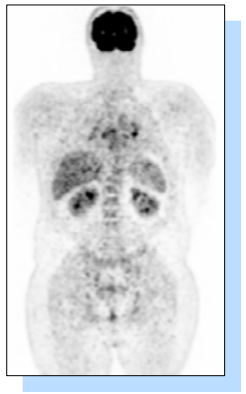
Tumor response: Case Illustration 1







△SPLD CT = - 18%
△SUV-FDG/PET = - 39%

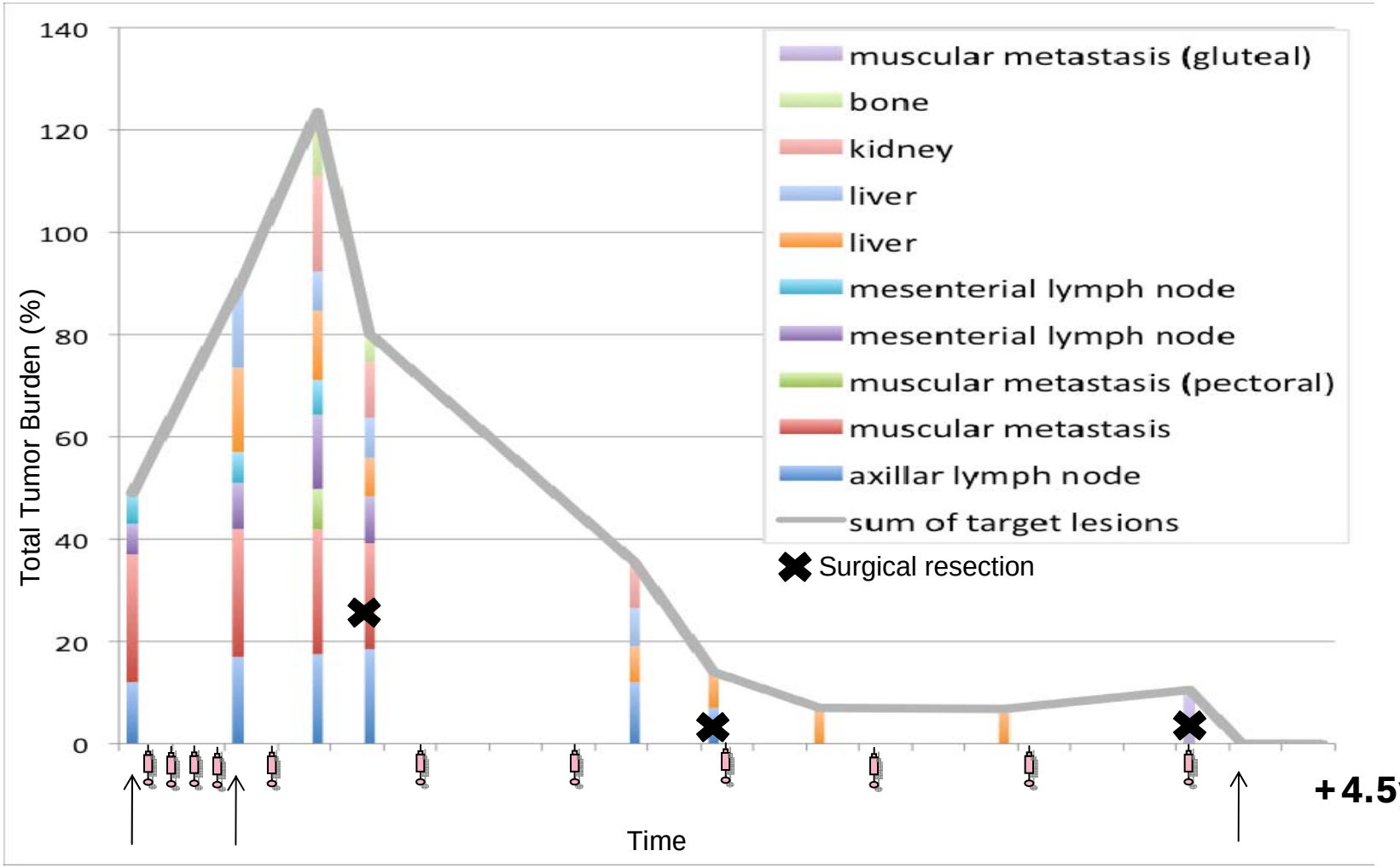


Baseline

Assessment W8 – Confirmed W16

46y male patient, stIV-M1b (nl LDH & CRP), refractory to DTIC

Atypical tumor response: case illustration 2



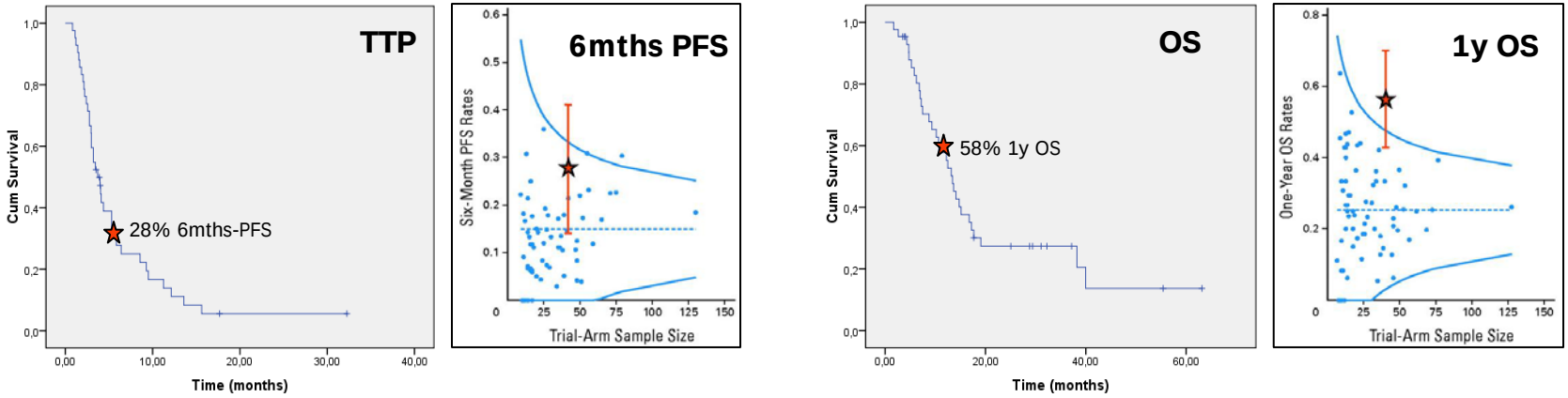
58y female patient, stage IV-M1c, refractory to DTIC

Survival in patients with evaluable disease

73 eligible patients

30 patients
No Disease

43 patients
Measurable Disease



Median follow-up: 33 months (range 3-63)
Median Progression-free survival: 3.7 months (95% CI 2.6-4.7)
Median Overall Survival: 13.4 months (95% CI 11-15)

Korn E L et al. JCO 2008

Univariate analysis of the baseline prognostic markers for survival in patients with measurable disease (n = 42)

Baseline co-variates	Median (95% CI)° (Months)	Log-Rank (p-value)	Hazard Ratio (95% CI)*
Progression-free survival			
Elevated CRP (N/Y)	**4.3 (2.7-5.9) vs. 1.5 (0.9-2.1)**	**<0,001**	**0.18 (0.07-0.47)**
WHO-PS 0 vs. 1-2	**6.3 (2.0-10.6) vs. 2.3 (1.6-3.1)**	**<0,001**	**0.29 (0.14-0.61)**
Elevated LDH (N/Y)	**5.3 (2.7-7.9) vs. 2.3 (1.6-3.1)**	**0.001**	**NSS**
AJCC stage other vs. IV-M1c	**5.6 (0-13.4) vs. 3.2 (2.0–4.3)**	**0,004**	**NSS**
Overall survival			
Elevated LDH (N/Y)	**15.1 (11.1-19.1) vs. 6.9 (5.4-8.5)**	**0.001**	**0.27 (0.12-0.62)**
WHO-PS 0 vs. 1-2	**15.1 (8.7-21.5) vs. 7.2 (6.1-8.3)**	**0,010**	**0.41 (0.19-0.86)**
Elevated CRP (N/Y)	**14.7 (12.0-17.4) vs. 7.2 (4.7-9.6)**	**0,006**	**NSS**
AJCC stage other vs. IV-M1c	**17.6 (12.4-22.8) vs. 10.2 (7-13.3)**	**0,028**	**NSS**

° Determined by Kaplan Meier survival estimates

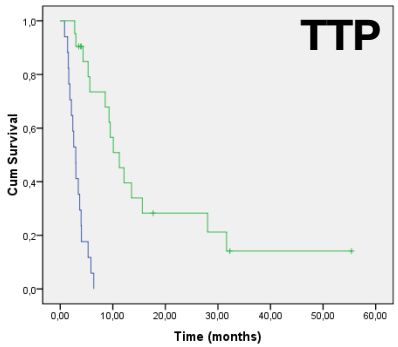
* Determined by Cox forward logistic regression including all co-variables that were significant by Log Rank test in univariate analysis.

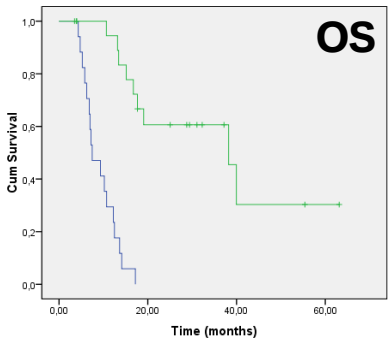
Landmark-analysis of survival from week 8 (post 4x DC-administration)

73 eligible patients

30 patients No Disease

38 Patients Measurable Disease
Alive & Evaluated for Response at 8w





Co-variates (N/Y)		Median (Months; 95% CI)	Log-Rank (p-value)	Hazard Ratio (95% CI)
Disease control by RECIST	PFS	2.9 (2.4-3.4) vs. 9.2 (7.5-11.0)	<0.001	0.14 (0.05-0.36)
	OS	9.3 (3.3-15.3) vs. 38.2 (10.4-65.9)	<0.001	0.22 (0.09-0.55)
Disease control by irRC	PFS	2.9 (2.1-3.7) vs. 11.2 (7.6-14.8)	<0.001	0.09 (0.03-0.26)
	OS	7.4 (4.3-10.6) vs. 38.2 (16.9-59.5)	<0.001	0.08 (0.02-0.23)

Significance was retained in subgroup analysis according to the prognostic baseline co-variates AJCC stage, WHO-PS, LDH and CRP and Cox multivariate analysis

Conclusions

- **In patients with advanced melanoma, cellular immunotherapy with autologous mRNA electroporated dendritic cells combined with IFN-a2b**
  - Feasible, well tolerated, and immunogenic
  - Associated with anti-tumor activity, characterized by atypical tumor response patterns
  - Overall survival compared favorably with historical control data (rather than RFS and PFS, relying on conventional criteria)
- **Further clinical trials are indicated**
  - Randomized, controlled, phase II clinical trial on TriMix-DC + IFNa2b in patients without measurable disease at baseline
  - Two-stage, phase II clinical trial on TriMix-DC + Ipilimumab in patients with measurable disease at baseline

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