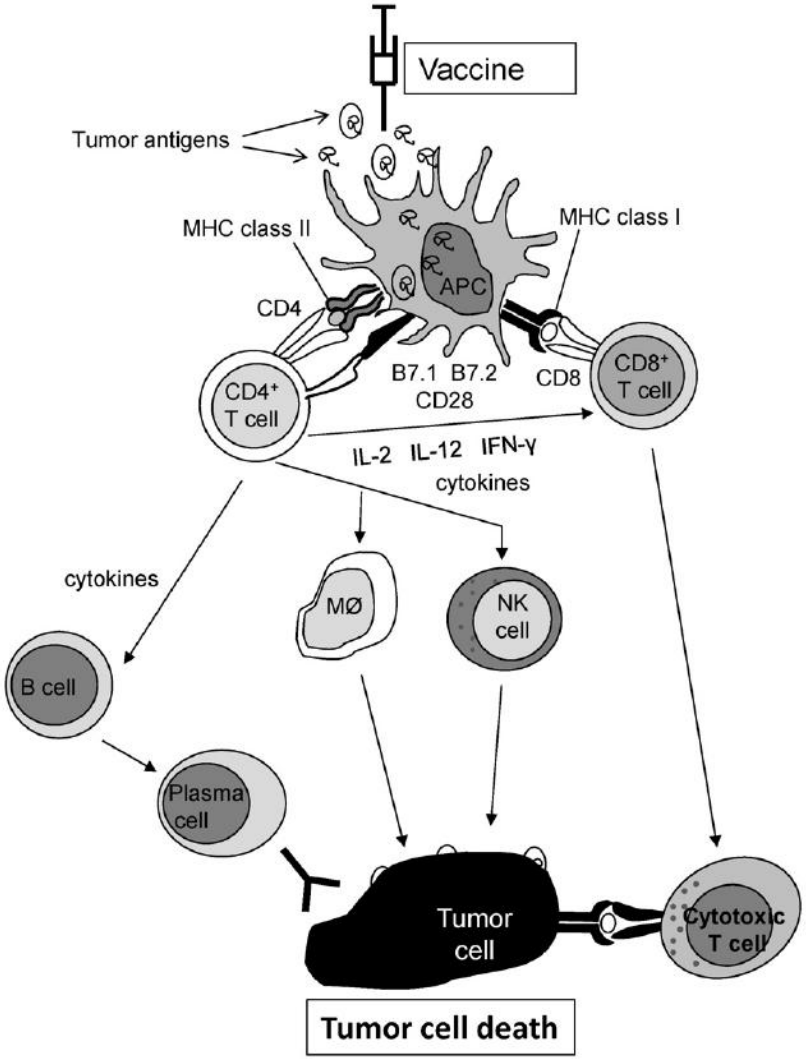
Werkgroep Immunotherapie Nederland
voor Oncologie

Tumorantigens and the immunesystem



Types of tumor antigens

- Antigens encoded by mutated genes, Ras oncogene
- Differentiation antigens, Tyrosinase, PSA, CEA
- Overexpressed or ubiquitously expressed proteins, HER2
- Viral antigens, EBV, HPV
- Tumor-specific shared antigens

Tumor-specific antigens

- Expressed in wide variety of tumortypes
- Frequent: melanoma, lungcarcinoma, sarcoma, bladder carcinoma
- Rarely: brain tumor, renal carcinoma, leukemia
- Normal tissues: testis, placenta --- no expression of MHC class I molecules
- Cancer-testis antigens
- Example: MAGE-A, -B and –C gene families

MAGE antigen expression in tumors

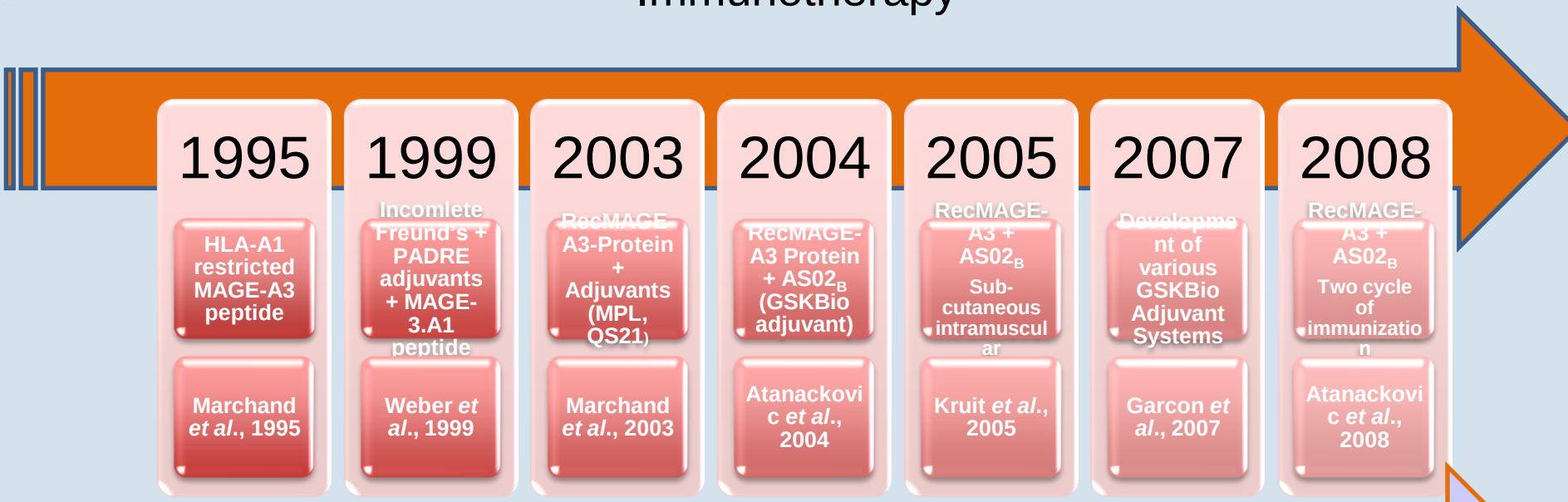
Table 1. Correlation of cancer/testis (CT) protein expression with clinicopathologic parameters and prognosis

Tumor type	Antigen	Association	Reference
Melanoma	MAGE-A1,MAGE-A2, MAGE-A3, MAGE-A4	Tumor thickness and metastasis	(46)
Non-small-cell lung cancer	MAGE-A1,MAGE-A3, MAGE-A4,MAGE-A10, MAGE-C1	Advanced tumor type, nodal and pathologic stages as well as pleural invasion	(39)
Pancreatic cancer	MAGE-A3	Poor survival	(88)
Hepatocellular carcinoma	MAGE-C1	Reduced overall survival	(89)
Multiple myeloma	MAGE-A1,MAGE-A3, MAGE-A4, MAGE-C1	Stage and risk status of disease	(90–92)
Serous ovarian carcinomas	MAGE-A4	Inverse correlation between expression and patient survival	(93)
Melanoma	NY-ESO-1	Thicker primary lesions and a higher frequency of metastatic disease	(94)

MAGE-A3 tumor antigen

- Tumor-specific
- Identified via screening with anti-tumor killer T-cells
- Not expressed in normal cells except testis
- Member of a large family of genes
- Associated with poor prognosis
- Easy to detect by RT-PCR on tumor tissue
- Expressed in various tumor types
  - Lung 35-50%
  - Bladder 30-58%
  - Head & neck 49%
  - Melanoma 36-76%

Evolution of Clinical Trials using MAGE-A3 Tumor Antigen as Target for Immunotherapy



Development of Antigen-Specific Cancer Immunotherapeutics (ASCI)*

** ASCI are investigational compounds*

Initiation of a Phase II study assessing two different MAGE-A3 ASCI formulations in Melanoma NCT00086866 (EORTC16032-18031-GSK249553/008) in 2004

Eerdere MAGE-3 studies (I)

- Studie met MAGE-A3 peptide
- 39 melanoom patienten met stadium III en IV
- MAGE-3A1 peptide, HLA-A1 restrictie
- Subcutane toediening, maandelijkse injectie 3x

- 25 patienten complete behandeling
- In 7 patienten traden responsen op, 3 complete remissies
- Regressie in (sub)cutane en lymfekliermetastasen
- Geen significante toxiciteit
- Geen cytotoxische T-cel response gedetecteerd

Response in patient treated with MAGE-A3 peptide

11-08-2000

10-03-2004

Eerdere MAGE-3 studies (II)

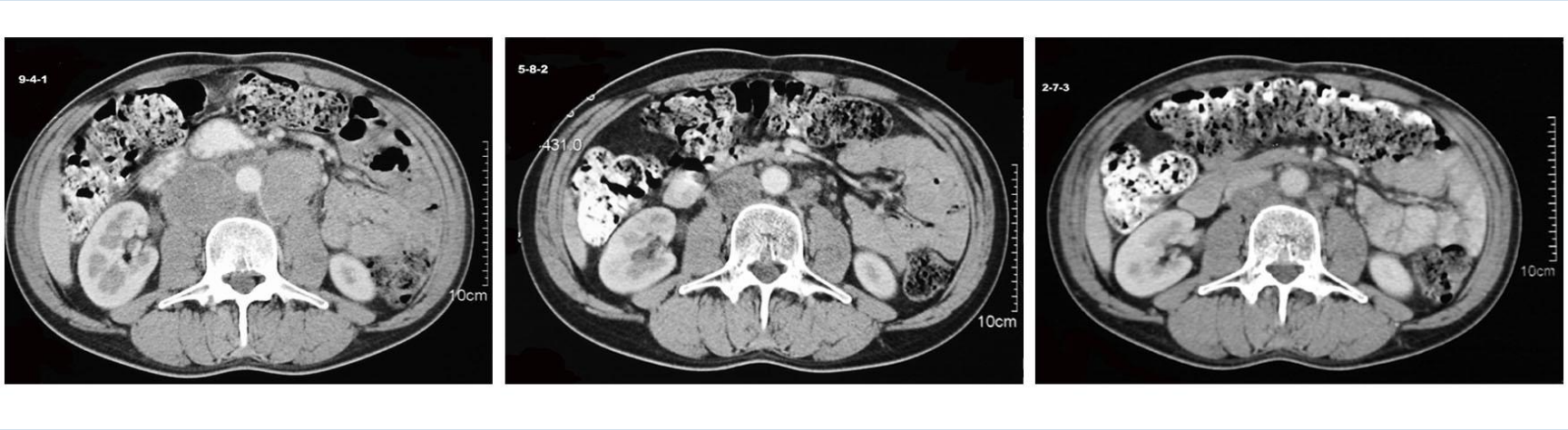
- Fase I/II studie met rec-MAGE-3 proteine
- 32 patienten, gemetastaseerd melanoom, stadium III en IV M1a
- 6 3-weekse toedieningen, intradermaal en subcutaan

- 26 patienten voltooiden behandeling
- In 5 patienten responsen, 1 PR en 4 mixed responsen
- Milde toxiciteit, meest graad I-II
- 1 patient met MAGE-3 CD4 T-cel response
- Geen anti-MAGE-3 antilichaam response

Eerdere MAGE-3 studies (III)

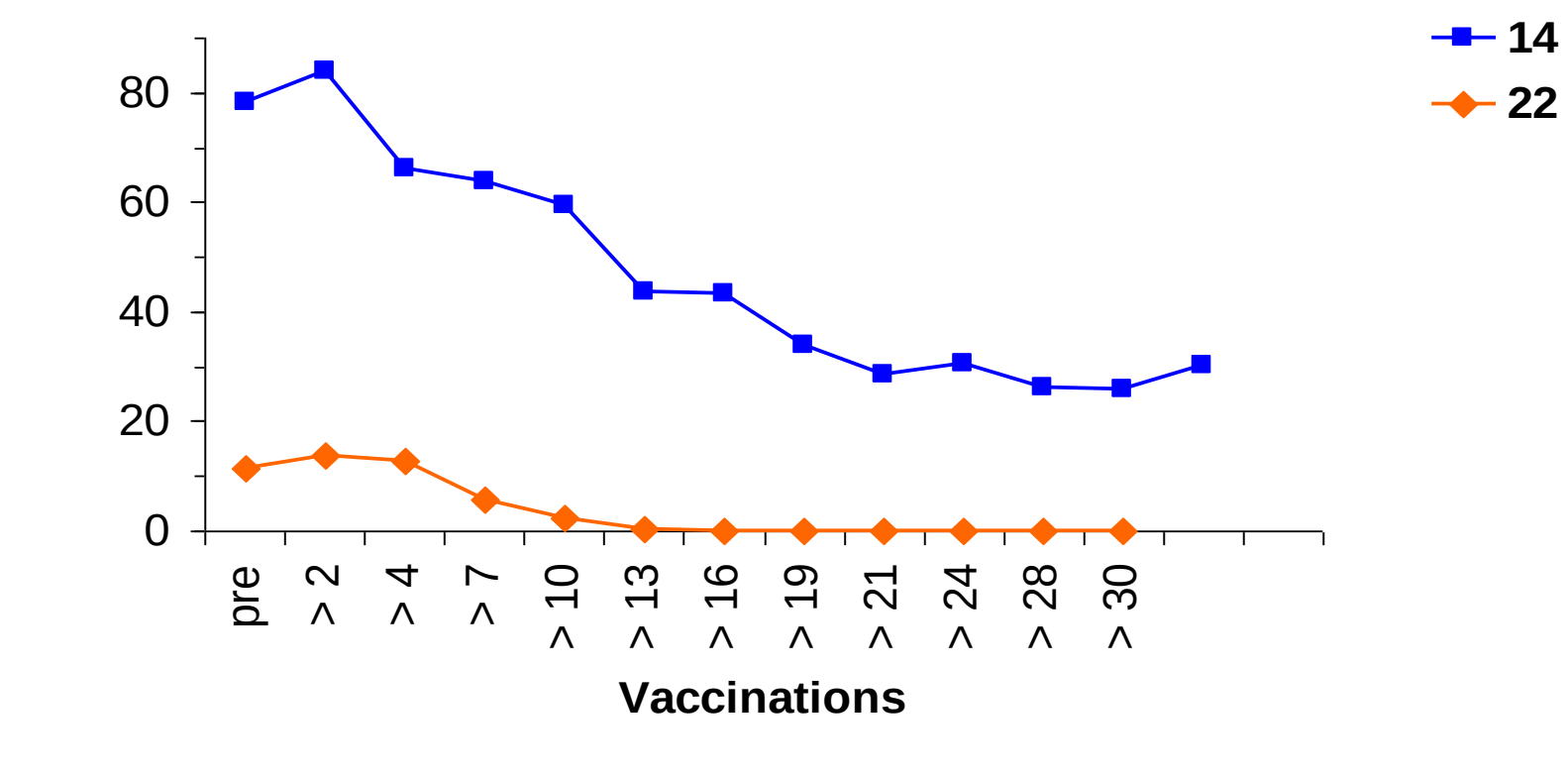
- Fase I/II studie met rec-MAGE-3 proteine en immunologisch adjuvans AS02B
- 57 patienten (49 melanoom, 3 blaas, 2 NSCLC, 2 slokdarmcarc, 1 HHcarc), allen stadium IV
- 4 3-weekse toedieningen intramusculair gevolgd door 2 toedieningen elke 6 weken
- Effectiviteit: 2 PR, 2 mixed responsen en 1 SD
- Toxiciteit: meest graad 1-2
- 6/20 patienten T-cel respons
- 23/24 patienten anti-MAGE-3 antilichaam response

Response in patient treated with MAGE-A3 protein and AS02B



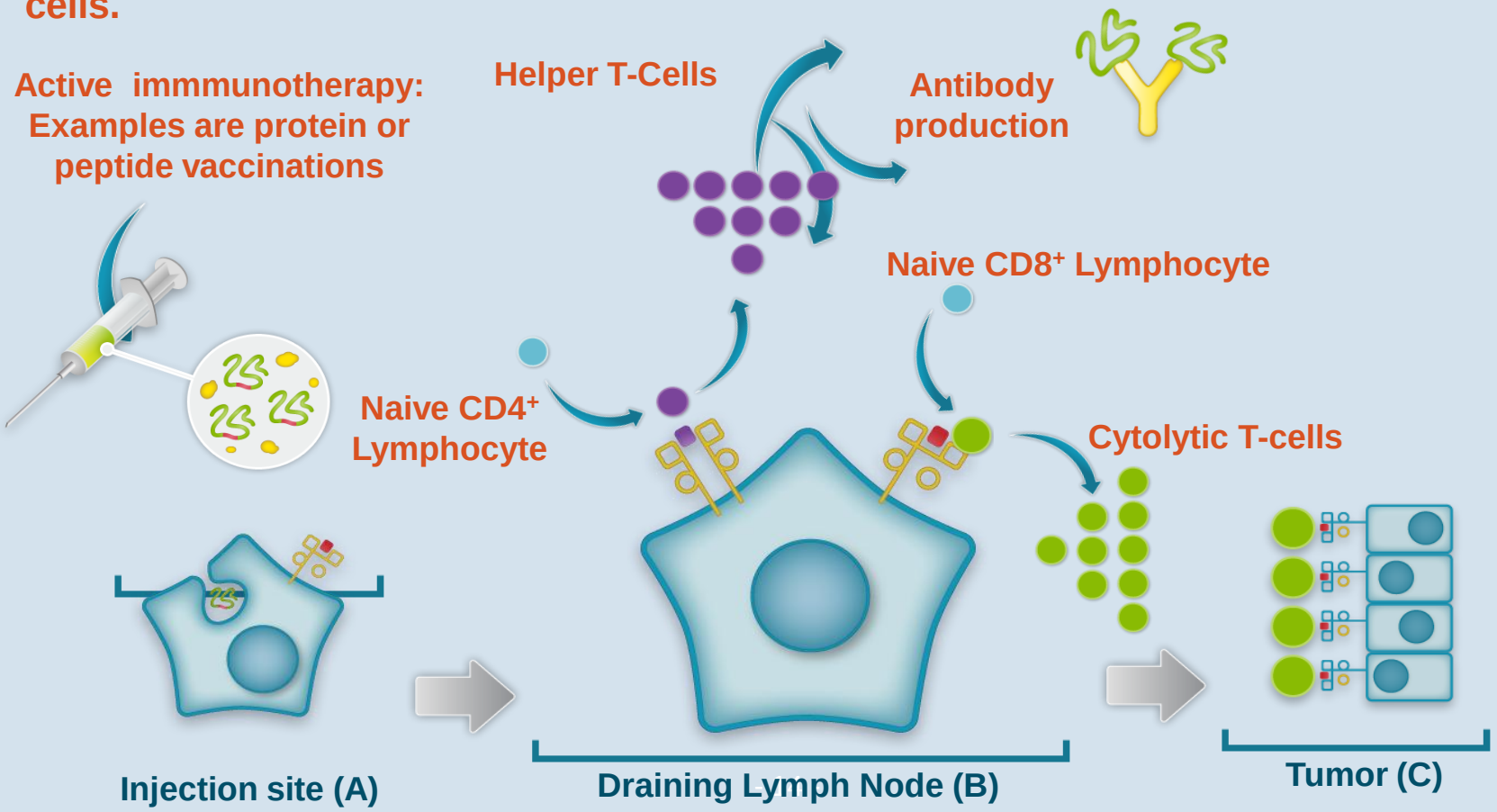
- 09-04-2001 05-08-2002 02-07-2003

Response ontwikkeling in 2 responderende patienten



Active Immunotherapy towards Tumor Antigen: a new Approach to treat Cancer

- **Active immunotherapy aims at teaching the patient's own immune defenses to recognize the tumor cells expressing a tumor antigen and to selectively destroy these cells.**



MAGE-A3 ASCIs Formulations tested in Phase II Study in Melanoma

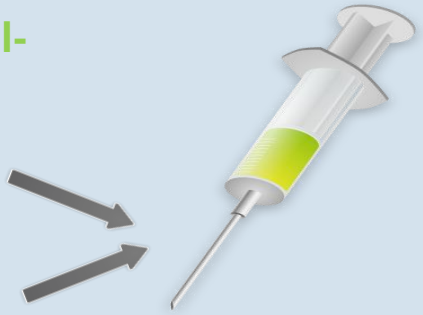
MAGE-A3 ASCI

RecMAGE-A3

+

AS15 OR AS02$_B$

Recombinant MAGE-A3 protein:
Purified fusion protein comprising full-length MAGE-A3
Dose & administration: 300 µg i.m



Immunostimulant (= an Adjuvant System, GSKBio proprietary)

AS15 (MPL, QS21, CpG, liposome-based Adjuvant System)

AS02$_B$ (MPL, QS21, o/w emulsion based Adjuvant System)

MPL: 3-O-desacyl-4'- monophosphoryl lipid A
QS21: (Quillaja Saponaria Molina, fraction 21) Antigenics
CpG: Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs

Phase II in Melanoma - Study Design

- **Open, randomized, Phase II study NCT00086866 (EORTC study 16032-18031 – GSK 249553/008)**

Metastatic Melanoma
- Unresected stage III and Stage IV M1a
- 1st line metastatic treatment
- Progressive disease
- MAGE-A3 (+) tumor

Stratified
- Stage (III in-transit, III, IV M1a)
- Lesion size (< 2cm, > 2 cm)
- Center

R

$N_{planned}$ = 34 → **MAGE-A3 + AS02$_B$**

- Cycle 1: q2w x 6
- Cycle 2: q3w x 6
- Cycle 3: q6w x 4
- Cycle 4: q3m x 4 - q6m x 4
- Total: 4 years

$N_{planned}$ = 34 → **MAGE-A3 + AS15**

TOXICITY

	Arm 1 (AS15)			Arm 2 (AS02B)		
	Grade 1	**Grade 2**	**Grade 3**	**Grade 1**	**Grade 2**	**Grade 3**
Fatigue	**11 (30%)**	**10 (27%)**	**3 (8%)**	**9 (25%)**	**6 (16%)**	
Anorexia	**2 (5%)**	**1 (3%)**	**1 (3%)**	**3 (8%)**		
Fever	**16 (43%)**	**10 (27%)**		**18 (50%)**	**3 (8%)**	
Chills	**9 (24%)**	**2 (6%)**		**4 (11%)**		
Sweating	**3 (8%)**	**1 (3%)**		**1 (3%)**		
Nausea	**8 (22%)**	**3 (8%)**		**8 (22%)**		
Vomiting	**1 (3%)**	**1 (3%)**		**2 (6%)**		
Injection site reaction	**19 (54%)**	**17 (46%)**		**22 (61%)**	**12 (36%)**	**1 (3%)**
Hypopigmentation				**1 (3%)**		
Rash/desquamation	**1 (3%)**			**1 (3%)**		
Hypertension	**2 (6%)**	**1 (3%)**			**3 (8%)**	
Hypotension	**2 (6%)**					
Sinus arrythmia					**1 (3%)**	

Phase II in Melanoma – Safety Results

- 75 patients – 372 MAGE-A3 + $AS02_B$ & 415 MAGE-A3 + AS15 doses administered
- Overall appears to be well tolerated
  - Mostly grade 1 and 2 toxicities
  - No signs of autoimmunity observed
- 3 grade 3 AE possibly related to treatment
  (2 in AS15 arm [Fatigue – DIC] – 1 in $AS02_B$ arm [Tumor Flare])
- No grade 4 AE related to treatment
- No SAE related to treatment
- No withdrawals due to toxicity
- No noticeable difference in toxicity between AS15 and $AS02_B$

Phase II in Melanoma - Demographics

		AS15 (N=36) N(%)	AS02B (N=36) N(%)
Age	< 40 yrs	3 (8.3)	3 (8.3)
	40 - 60 < yrs	8 (22.2)	8 (22.2)
	>= 60 yrs	25 (69.4)	25 (69.4)
	Median (yrs)	69.0	65.0
	Range (yrs)	30.0 – 86.0	29.0 – 86.0
Stage	III-ITMets	10 (27.8)	8 (22.2)
	III-other	15 (41.7)	19 (52.8)
	IV (distant skin only)	11 (30.6)	9 (25.0)
Ulceration	No	14 (38.9)	17 (47.2)
	Yes	22 (61.1)	19 (52.8
Lesion < 2 cm	No	15 (41.7)	10 (27.8)
	Yes	14 (38.9)	20 (55.6)
	unknown	7 (19.4)	6 (16.7)
Adjuvant therapy	No	23 (63.9)	20 (55.6)
	IFN alpha-2b	7 (19.4)	14 (38.9)
	Other	6 (16.7)	2 (5.6)

Out of 165 screened patients, 97 had a MAGE-A3 (+) tumor - 72 eligible patients (75 randomized)

Phase II in Melanoma – Clinical Results

- **Results of the follow-up phase of the study : median follow-up time of 48 months**
- **5 clinical objective responses were observed**

	recMAGE-A3 + AS02$_B$		recMAGE-A3 + AS15	
	N	**Duration**	**N**	**Duration**
CR	**0**		**3**	**11 months 28+ months 55+ months**
PR	**1**	**7 months**	**1**	**6 months**
SD > 16 wk	5		5	
PD*	30		26	
NE	0		1	

•MixR in 3 (AS02$_B$) and 4 (AS15) patients, respectively

•CR: Complete Response, PR: Partial Response, SD: Stable disease, PD: Progressive Disease, NE: Non evaluable

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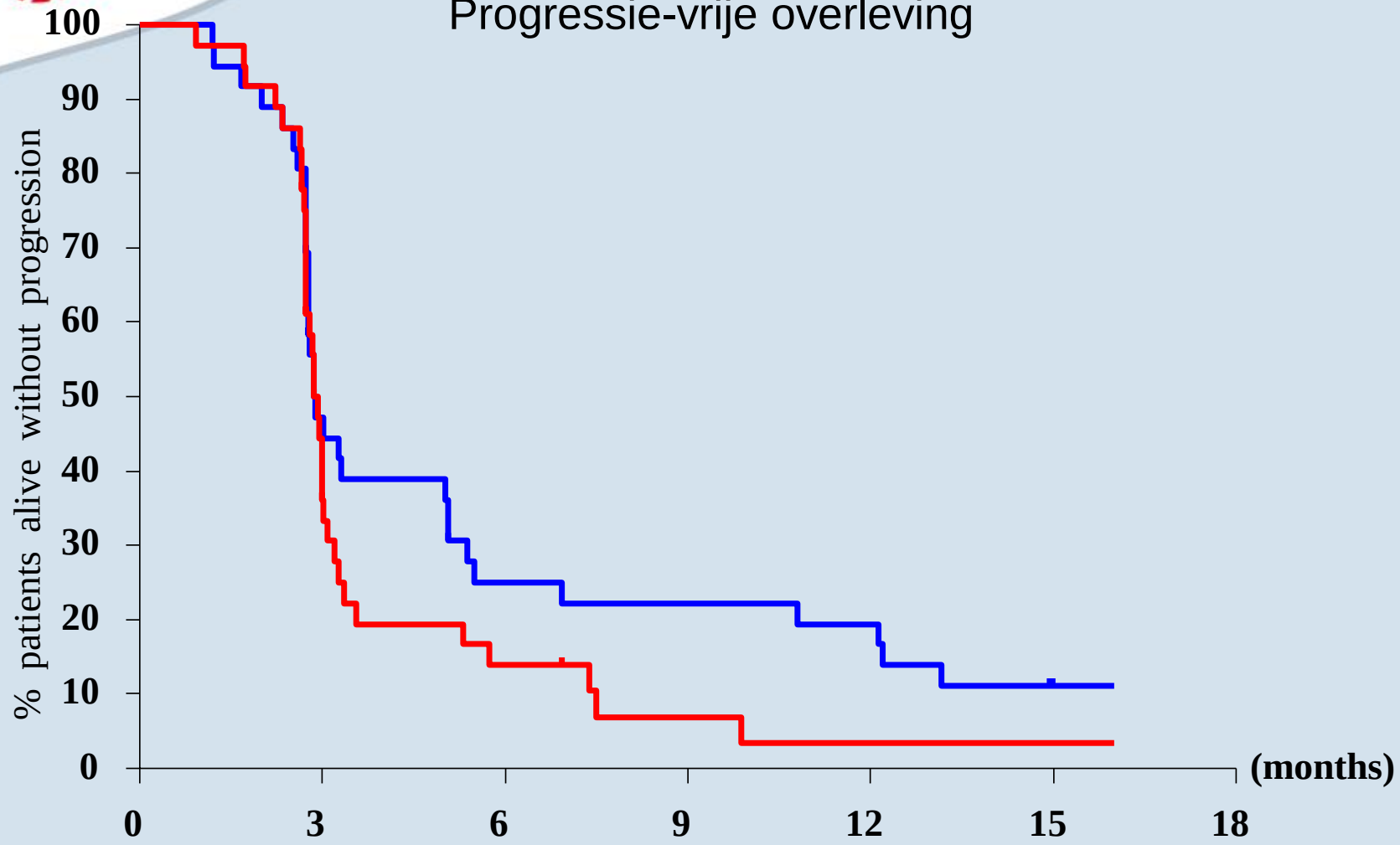
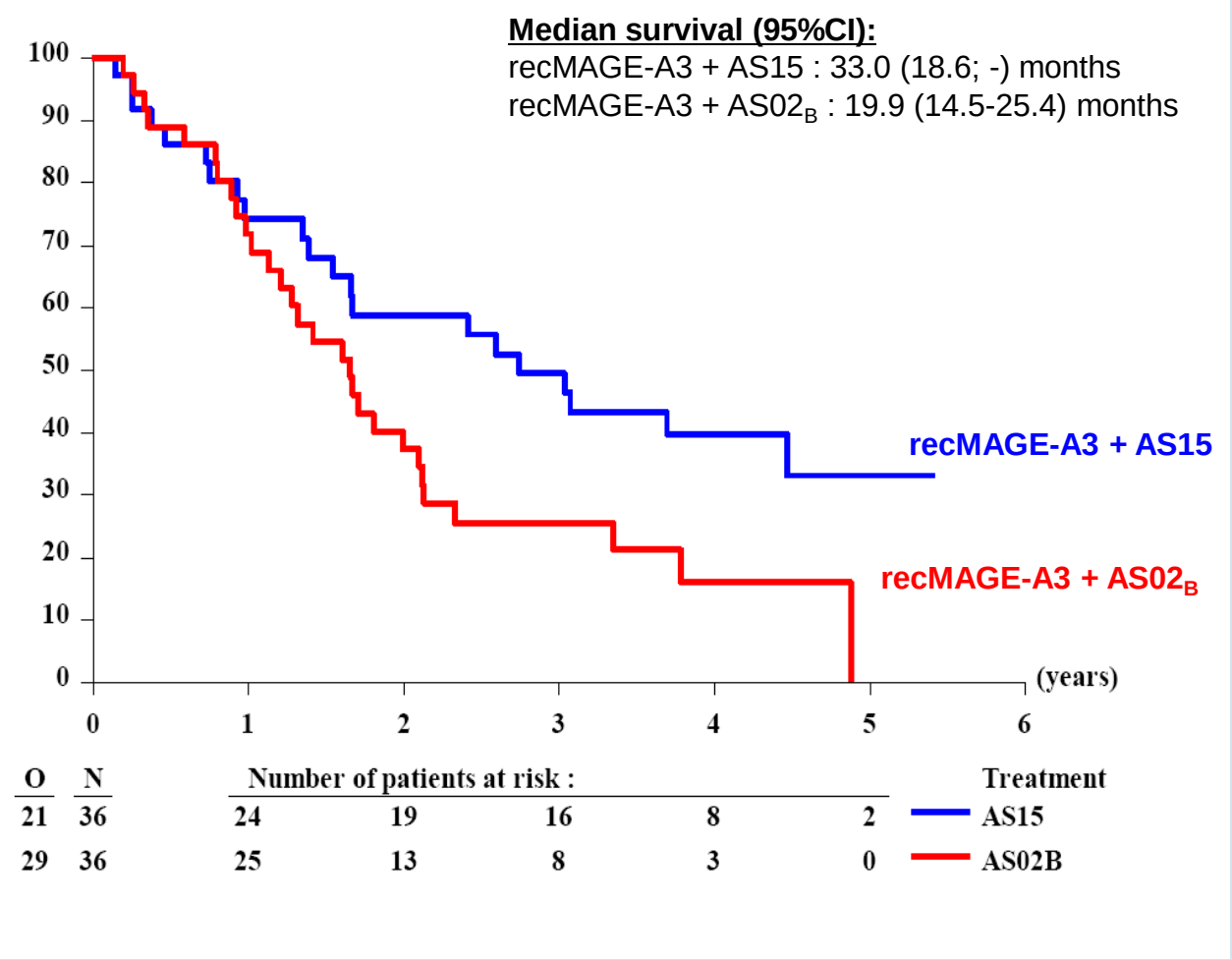


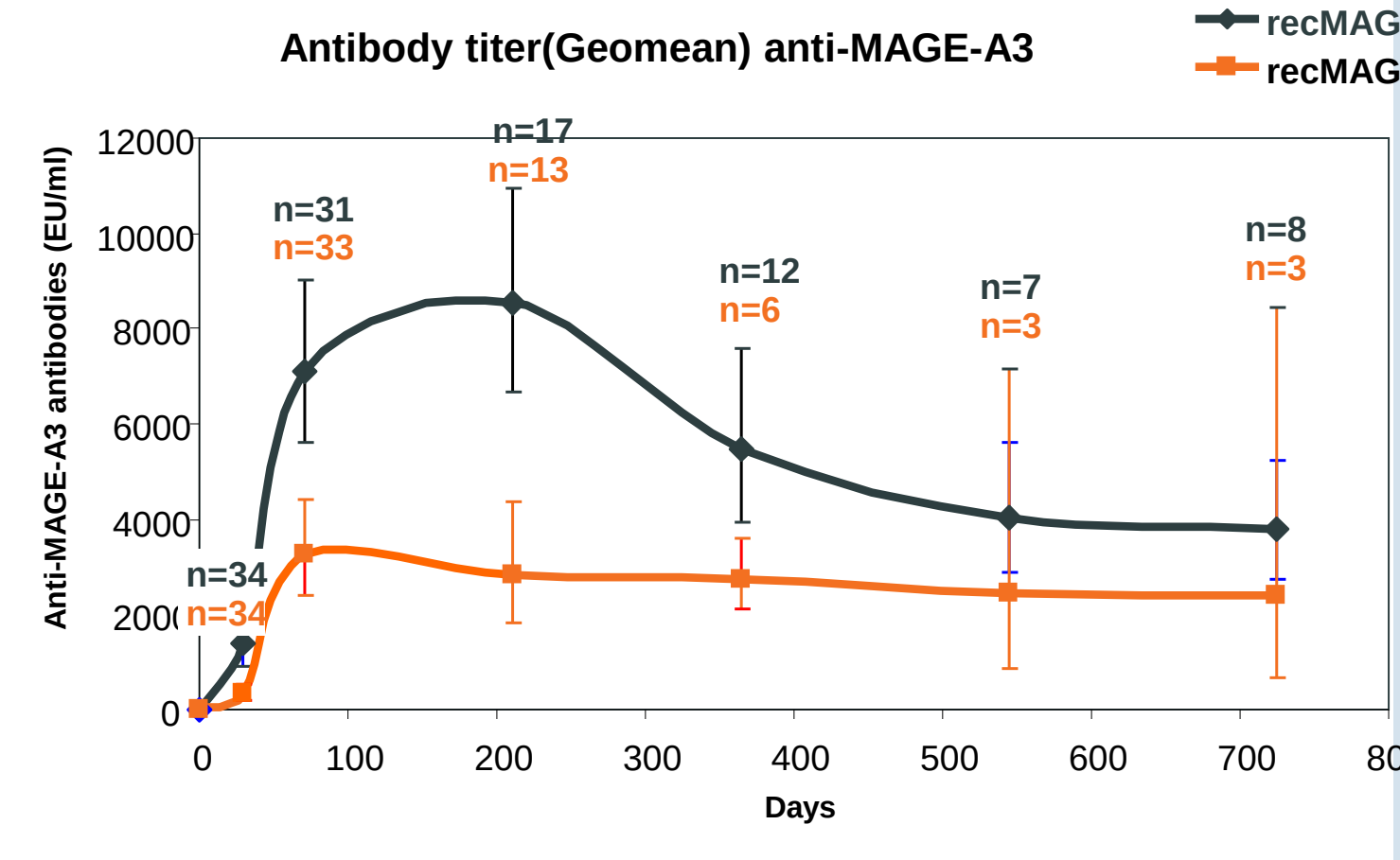
O	N	Number of patients at risk :					Treatment
32	36	17	9	8	7	2	AS15
34	36	13	5	2	1	1	AS02B

Phase II in Melanoma – Overall Survival



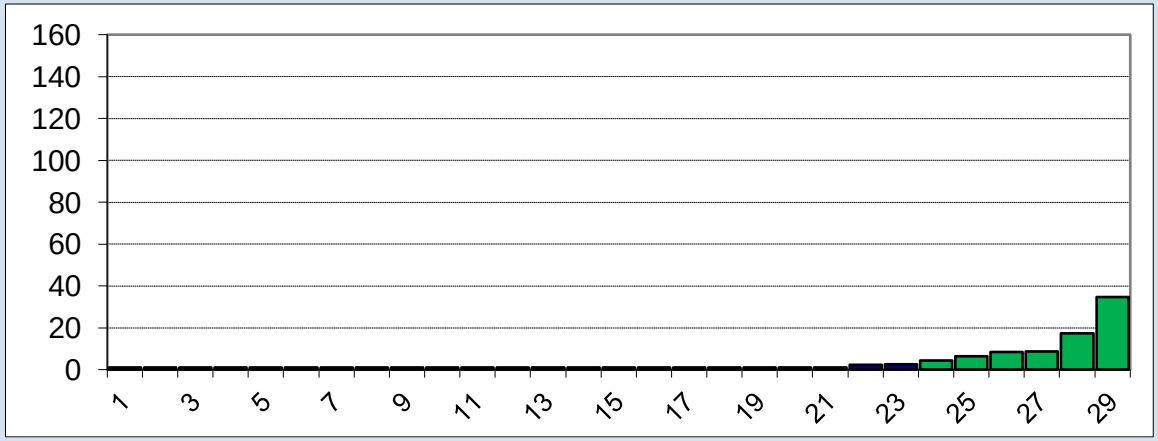
Phase II in Melanoma - Induction of anti-MAGE-A3 antibody

- Immunological Adjuvants Systems are not equivalent



Phase II in Melanoma - Induction of anti-MAGE-A3 CD4+ T-cells

RecMAGE-A3 + AS02$_B$

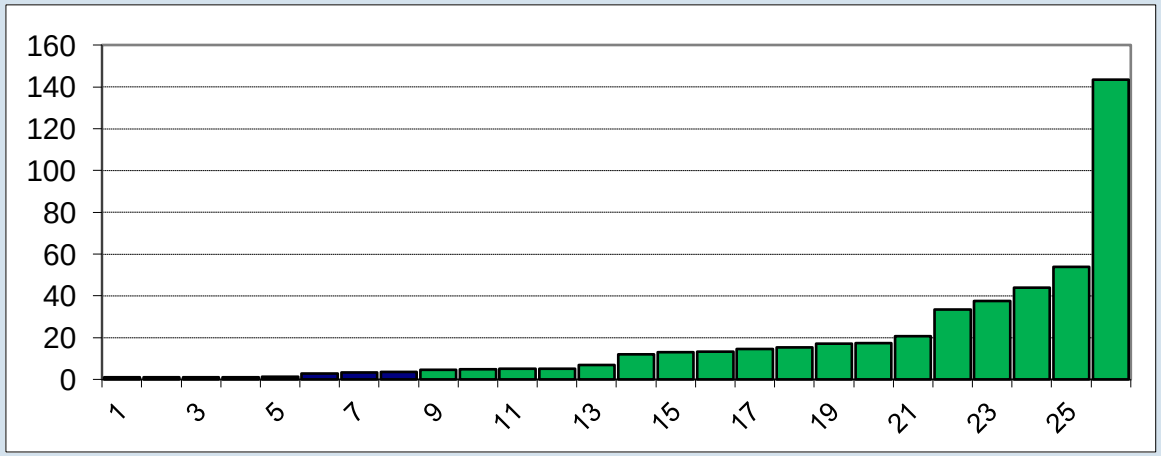


CD4 + T cell Responders*

6/29

21%

RecMAGE-A3 + AS15



18/26

69%

*CD4 + T cell Responders are defined as when ratio>4-fold

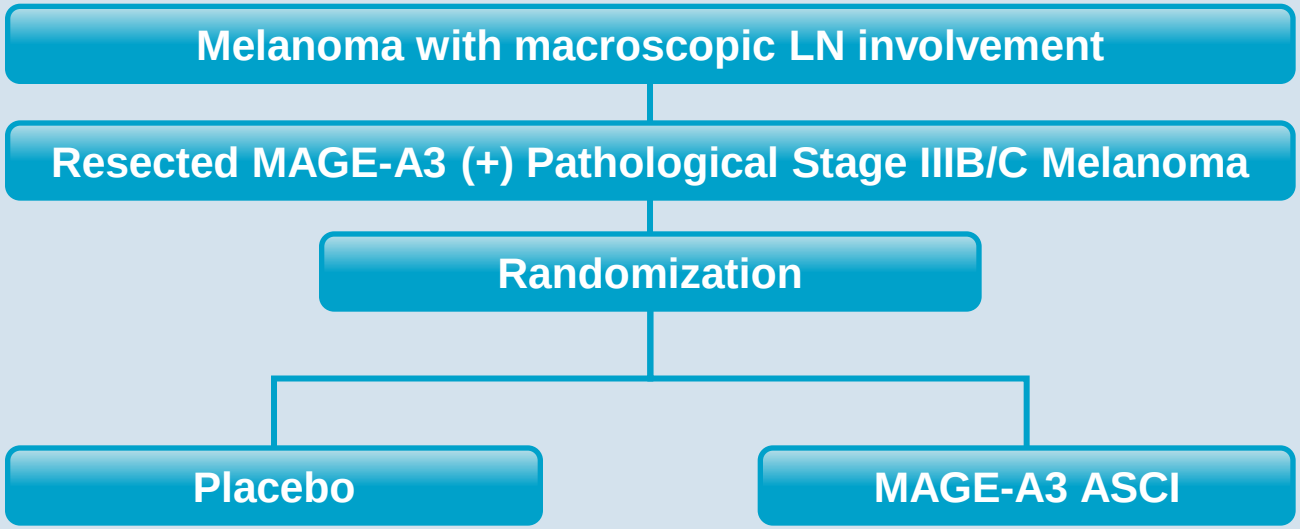
- **Immunological Adjuvants Systems are not equivalent**

Melanoma Phase II - Conclusions

- Both MAGE-A3 ASCI formulations are well tolerated
- MAGE-A3 + AS15 shows clinical activity in metastatic melanoma
  - Long-lasting responses
  - MAGE-A3 + AS15 seems to be more active than MAGE-A3 + $AS02_B$
- MAGE-A3 + AS15 yielded high MAGE-A3-specific antibody titers
- MAGE-A3 + AS15 resulted in significant anti-MAGE-A3 CD4+ T-cell induction
- AS15 is selected for Phase III development

Phase III study - DERMA

ADjuvant immunothERApy with MAGE-A3 in melanomA



- Primary objective
  - Clinical efficacy of MAGE-A3 ASCI in the overall population
  - Primary endpoint: DFS
- Secondary objectives
  - Other clinical indicators of safety and efficacy
  - Translational research: gene expression profile

Event-driven analyses
- Final in 2012 (tentative)