UNIVERSIDAD DE CHILE

Inmunología anti tumoral

ICMB

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GOBIERNO DE CHILE
FONDEF • CONICYT

oncoBIOmed
Biotecnología Celular Oncológica

Tecnofarma

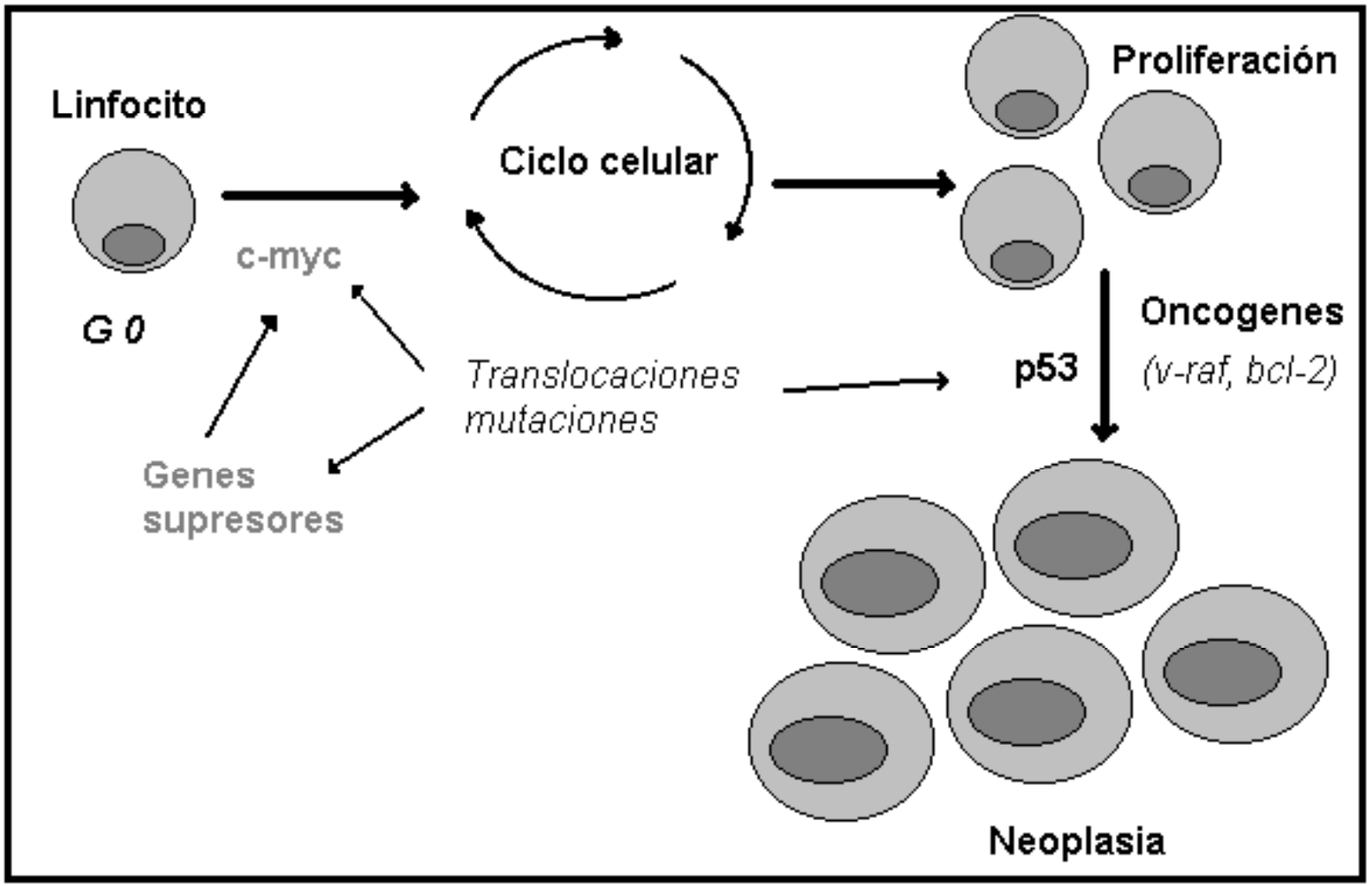
Neoplasia

- Tejido formado por células que proliferan descontroladamente debido a alteraciones en su material genético.
- Las células neoplásicas junto con las células del sistema inmune que las infiltran forman los tumores.
- Las células tumorales capaces de migrar a otros tejidos formando metástasis se denominan tumores malignos.

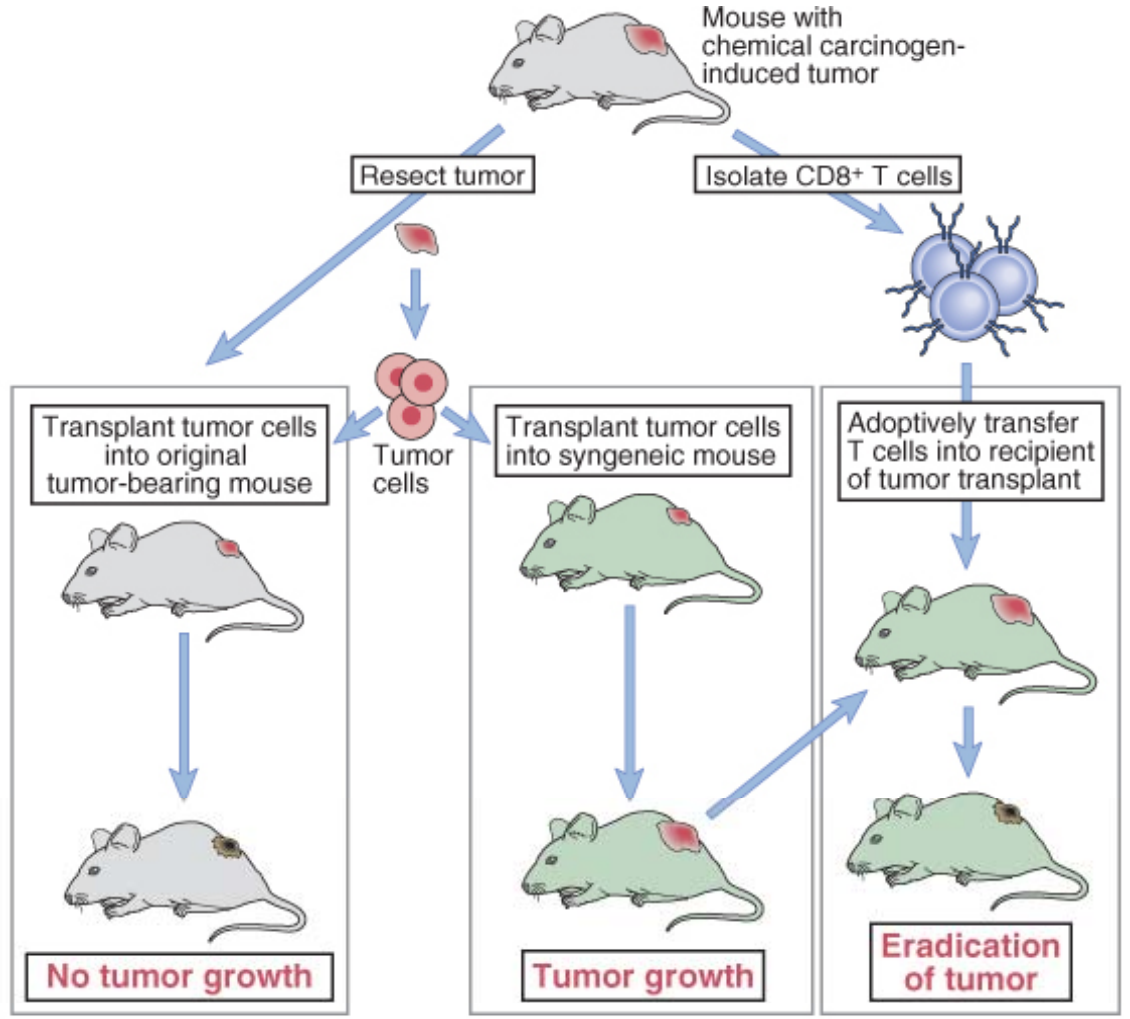
Factores que inciden en la transformación maligna de las células.

- Luz ultravioleta
- Sustancias químicas (tabaco)
- Radioactividad
- Infecciones virales
- Alteraciones congénitas

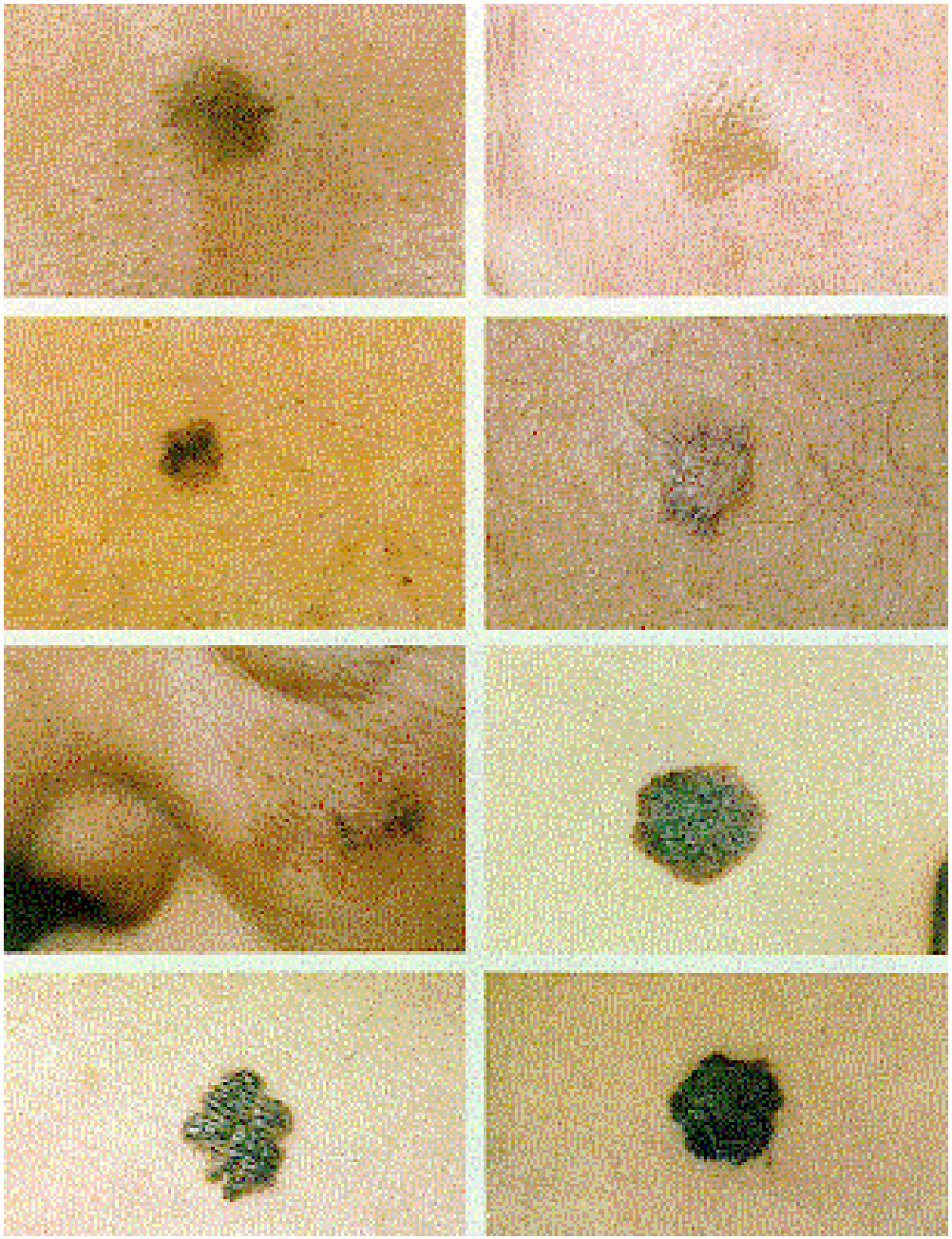
Transformación celular



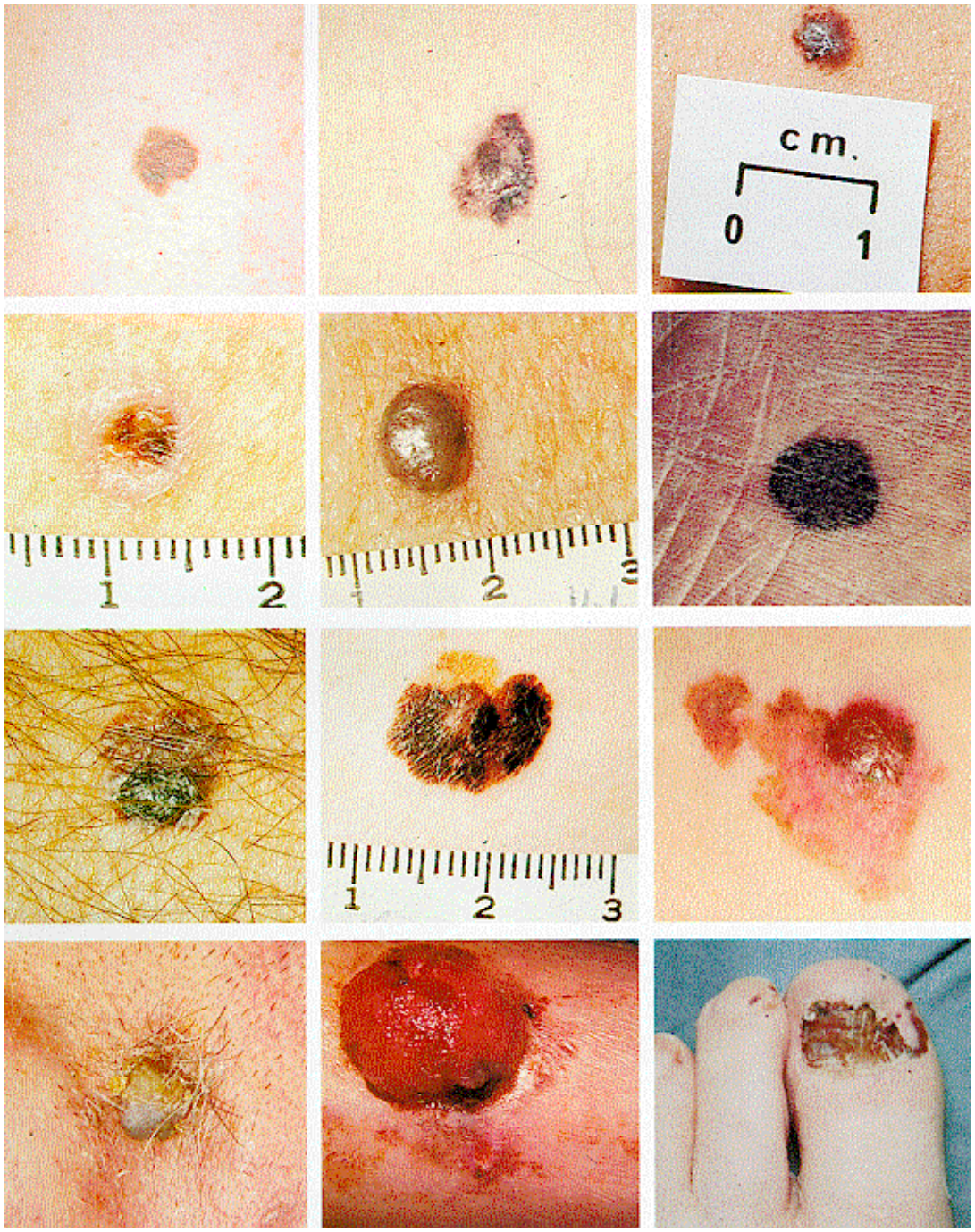
Los tumores pueden ser inmunogénicos



Apariencia del melanoma maligno

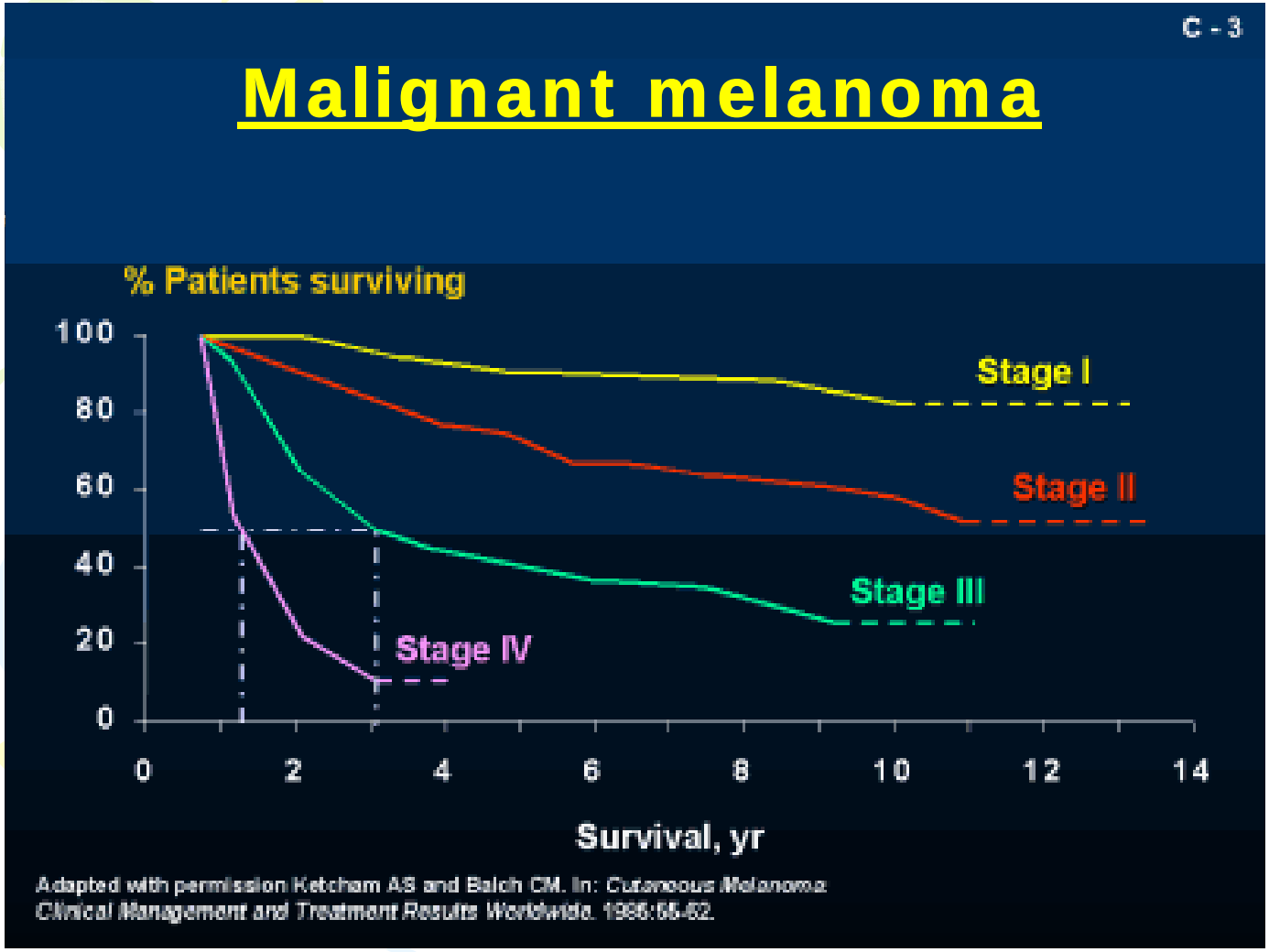


Apariencia del melanoma maligno

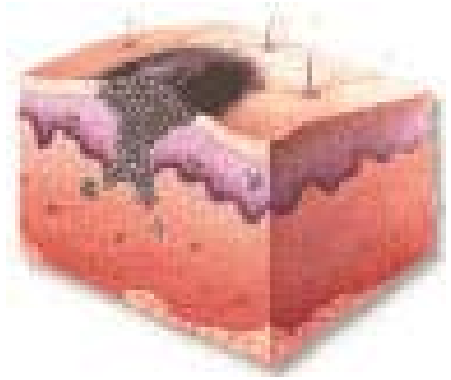


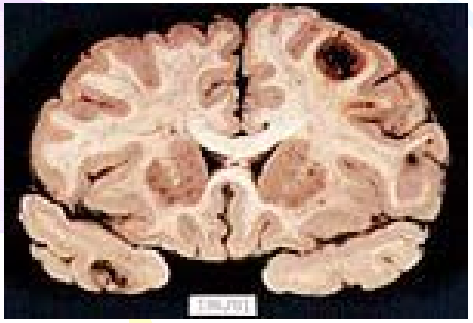
C - 3

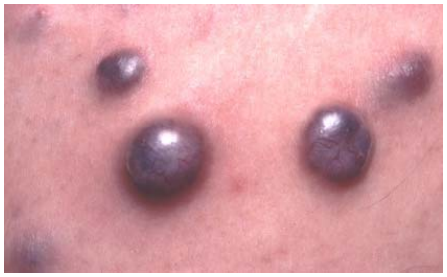
Malignant melanoma

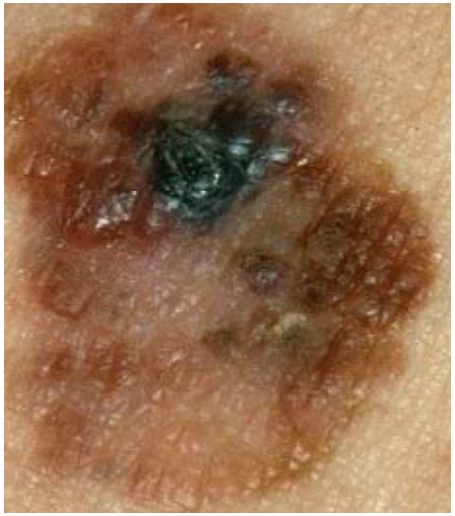


Adapted with permission Ketcham AS and Balch CM. In: *Cutaneous Melanoma: Clinical Management and Treatment Results Worldwide*. 1986:55-62.

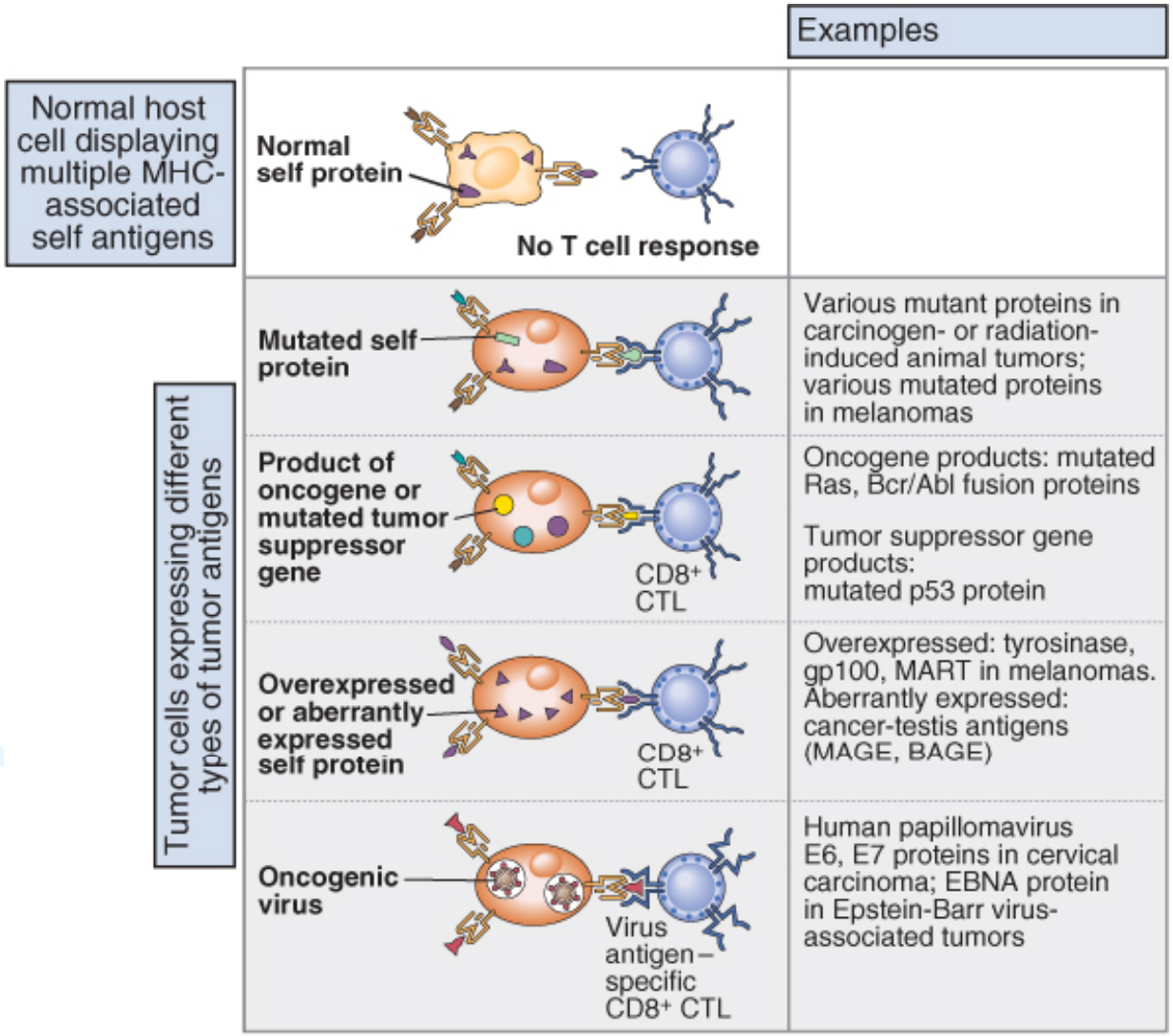








Tipos de antígenos asociados a tumor (TAA)

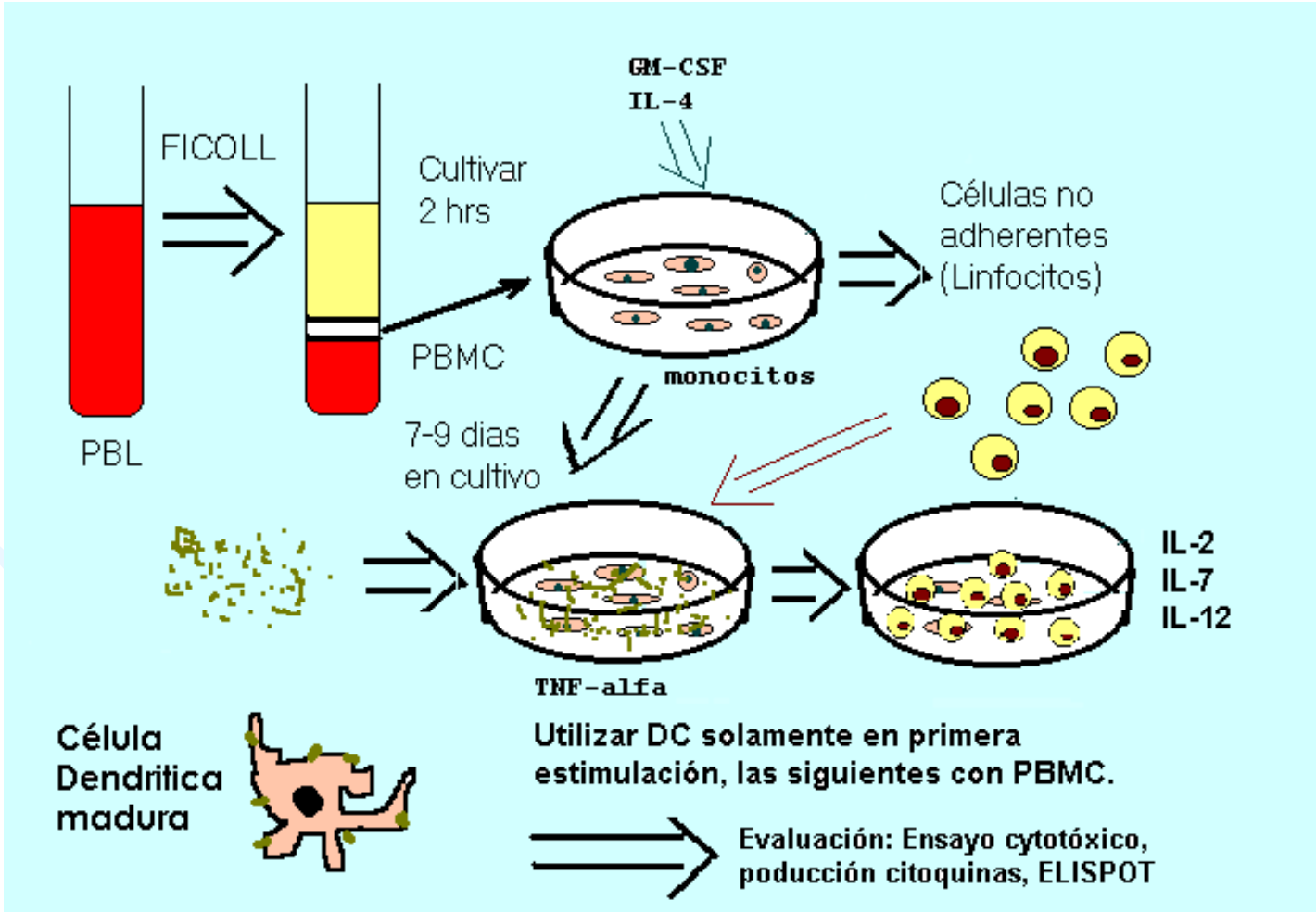


Receptor Melanocortina 1 (MC1R) un antígeno de melanoma.

MC1R es un receptor de 35 kD expresado en células de melanoma, melanocitos, hipófisis y células gonadales.

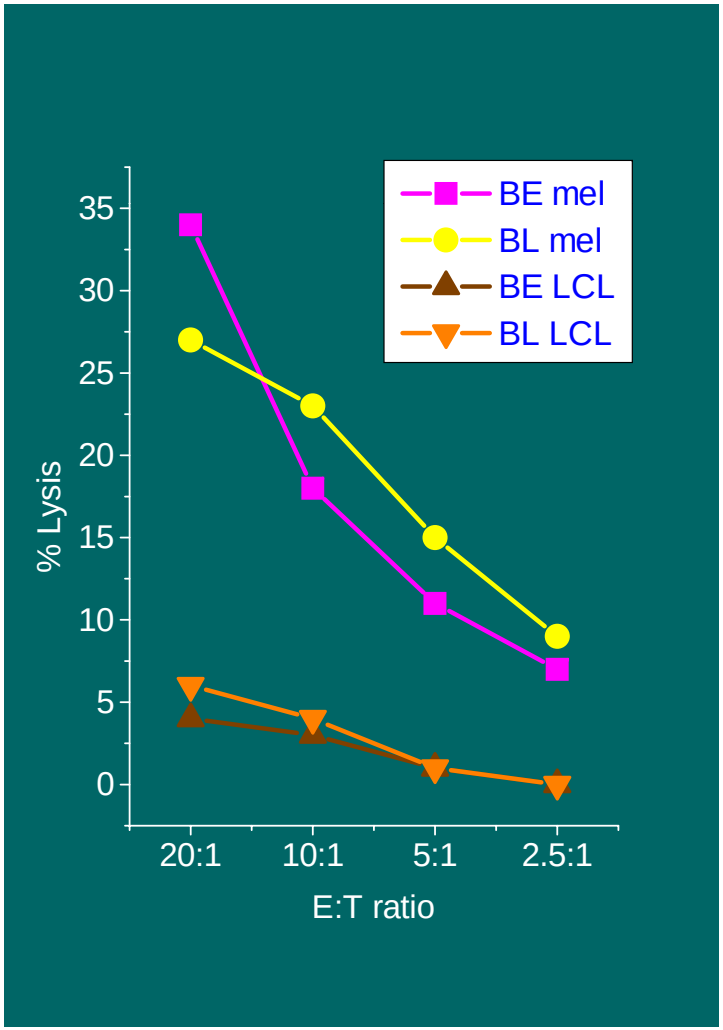
Es el ligando natural de la hormona estimulante de melanocitos alfa (MSH-α), la que junto con estimular la la producción de melanina actúa como neurotrasmisor, estimulante endocrino y modulador de la respuesta inflamatoria.

Inducción *in vitro* de CTL específicas contra péptidos a partir de PBMC.

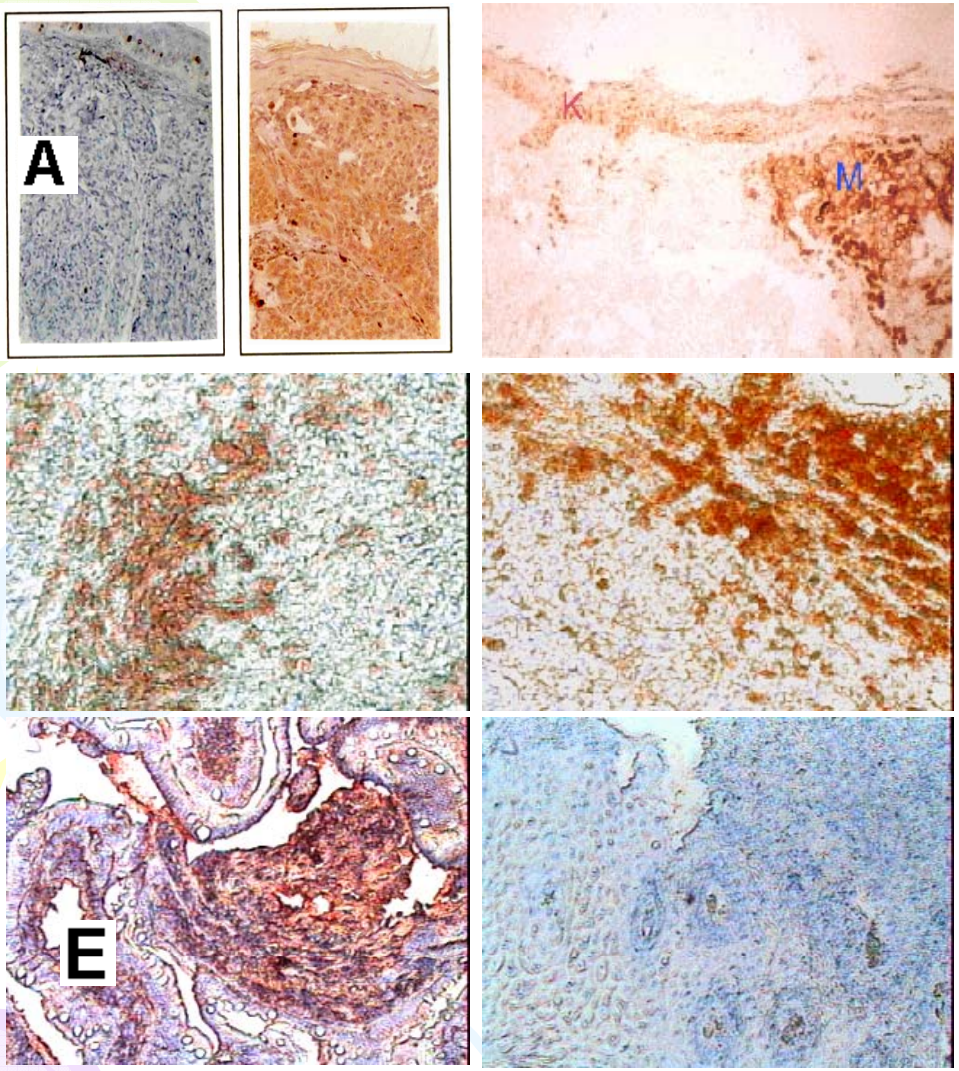


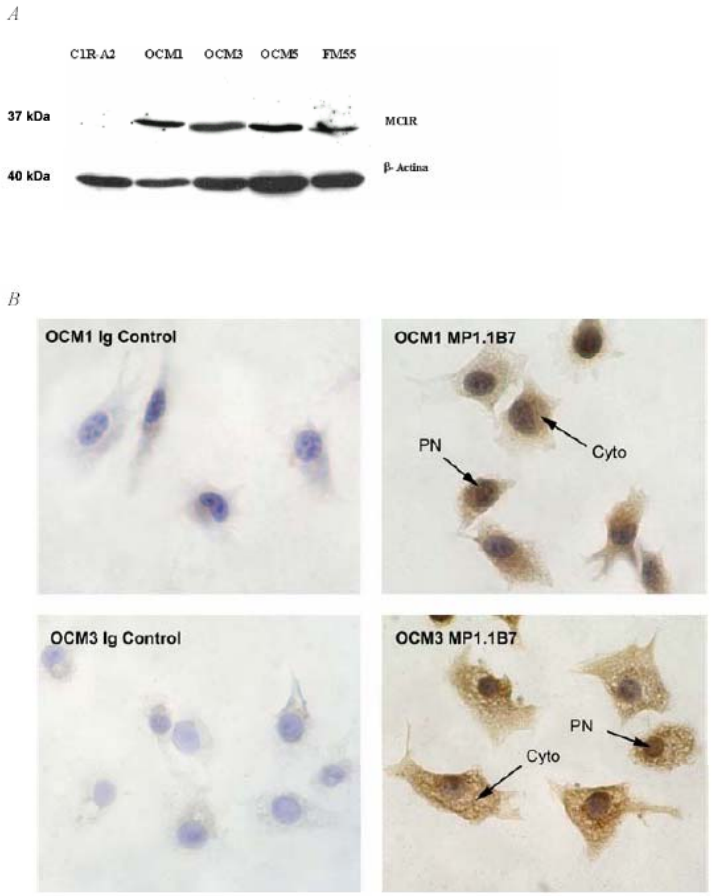
CTL específicas para péptidos derivados de MC1R pueden reconocer melanomas

- CTL inducidas con los péptidos MC1R244 (TILLGIFFL), MC1R283 (FLALIICNA) y MC1R291 (AIIDPLIYA), pueden ser generadas *in vitro.*
- Estas CTL tienen la capacidad de reconocer células de melanoma que expresen HLA-A2.



Inmunohistoquímica de tejido de melanoma





Salazar-Onfray F et al. 2003. *Br. J. Cancer.* **87**:414-422.
López M. et al. 2006. *Invest Ophthalmol Vis Sci. ;48(3):1219-27.*

MECANISMOS DE ESCAPE A LA VIGILANCIA DEL SISTEMA INMUNE

Exposición crónica, deficiente y sin señales de alarma de los antígenos tumorales (tolerancia)

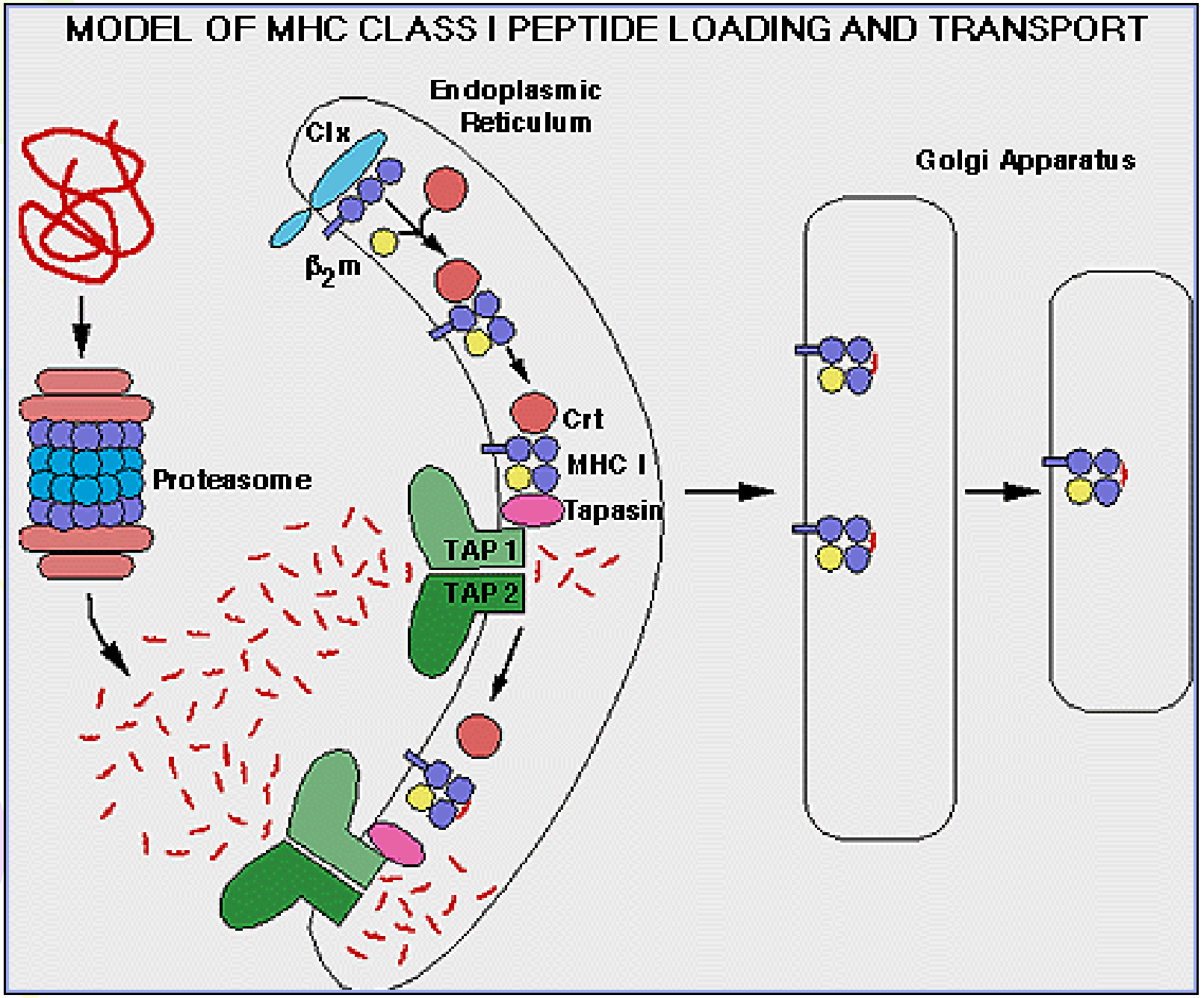
Disminución o pérdida de la expresión de MHC y/o antígenos asociados al tumor.

Disminución o pérdida de la expresión de moléculas co-estimuladoras, en el tumor o en las células dendríticas.

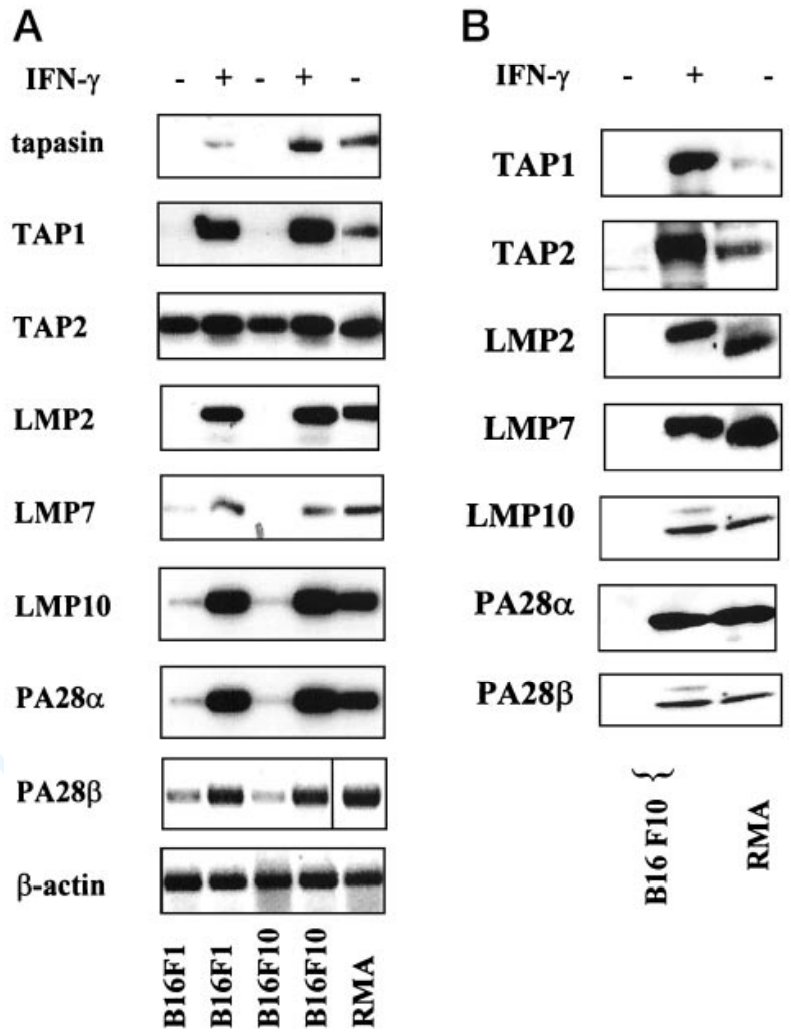
Neutralización de la respuesta inmune a través de la inducción de anergia en los linfocitos T.

Activación, mediada por el tumor, de células T reguladoras o células del sistema inmune que secretan citoquinas inhibidoras.

Secreción de factores inmunosupresivos por los tumores (Prostaglandina E_2, e IL-10).


MODEL OF MHC CLASS I PEPTIDE LOADING AND TRANSPORT
Endoplasmic Reticulum
Golgi Apparatus
Clx
β2m
Crt
MHC I
Tapasin
TAP 1
TAP 2
Proteasome

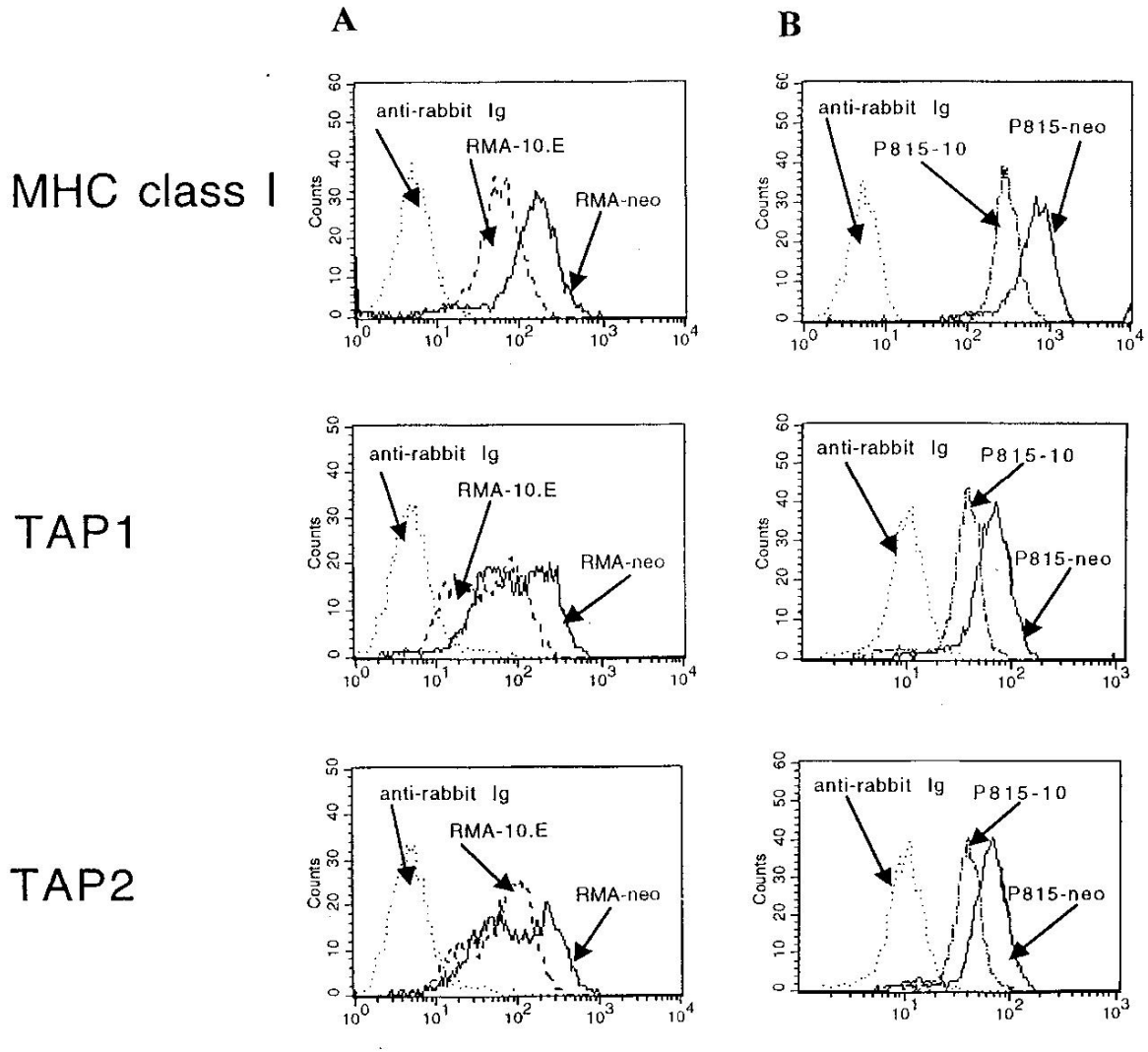
Disminución de moléculas asociadas a la presentación antigénica en clase I



Disminución MHC de clase I

- Deficit en la transcripción o translación de β2 microglobulina
- Defectos en la maquinaria de procesamiento antigénico
- Pérdida de la expresión alélica, (pérdida de DNA genómico
- Disregulación en la expresión de citoquinas
- Disminución en el “repertorio” de HLA en la superficie de la célula tumoral, compromete significativamente la capacidad de reconocimiento del SI

Inhibición de la presentación antigénica en tumores mediada por IL-10



IL-10 inhibe la presentación MHC class I en otros sistemas

- IL-10 dowregulates MHC class I, enhances NK lysis and inhibits tumor metastasis. (Kundu et al. 1997 Cell Immunol. 180:55).
- EBV vIL-10 and hIL-10 downregulates TAP-1 in B lymphocytes (Zeidler et al. 1997. Blood 90:2390).
- IL-10 downregulates MHC Class I and Class II and ICAM-1 in melanomas. (Yue et al. 1997. Int. J.Cancer 71:630)
- Chlamydia pneumoniae induces IL-10 expression and Dowregulates MHC class I expression. (Caspar-Bauguil et al. 2000. J. Inf. Dis. 182:1394).
- Regulatory activity of autocrine IL-10 on dendritic cell functions. (Corinti S et al. J Immunol. 2001. 166(7):4312-8).
- Epstein-Barr virus encoded interleukin-10 inhibits HLA-class I, ICAM-1, and B7 expression on human monocytes: implications for immune evasion by EBV. (Salek-Ardakani S et al.Virology. 2002. 304(2):342-51).
- Orf virus-encoded interleukin-10 inhibits maturation, antigen presentation and migration of murine dendritic cells. (Lateef Z et al. J Gen Virol. 2003. 84(5):1101-9).

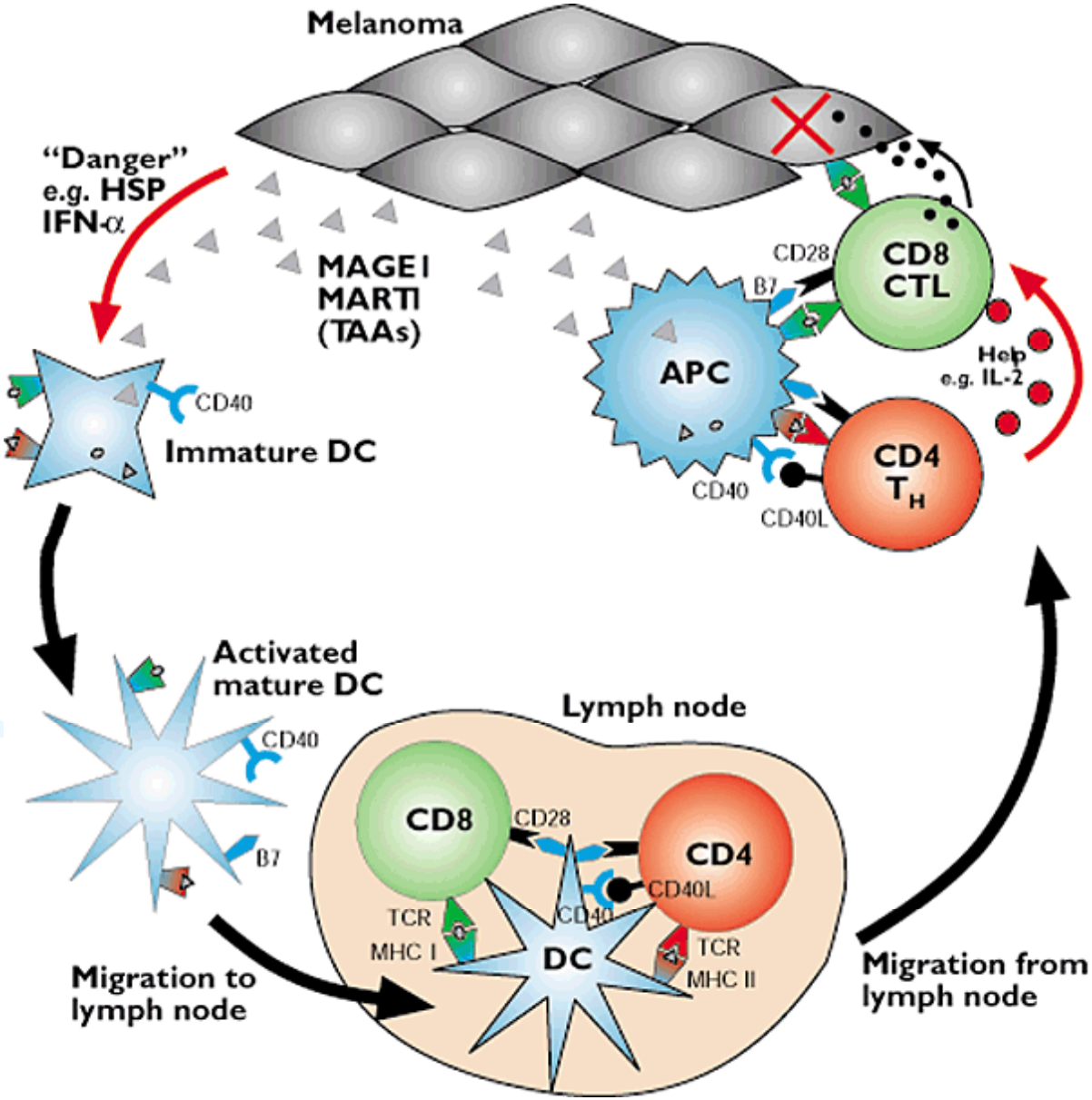
Conclusión Parte II

- IL-10 inhibe la respuesta anti tumoral mediada por CTL y aumenta la sensibilidad de los tumores a la lísis mediada por las células NK.
- Estos cambios están relacionados a la capacidad de IL-10 de inhibir la presentación antigénica MHC clase I en los tumores.
- Este efecto está relacionado a la capacidad de IL-10 de inhibir la expresión de las móleculas TAP.

Conclusiones

- Dar señales de activación adecuadas
- Eliminación o modulación de factores inhibitorios
- Evitar la emergencia de fenotipos inmunoresistentes, con la combinación de varias estrategias de tratamiento

Modelo de activación de la respuesta inmune contra los tumores



Ensayo clínico de fase I para tratamiento de melanoma maligno

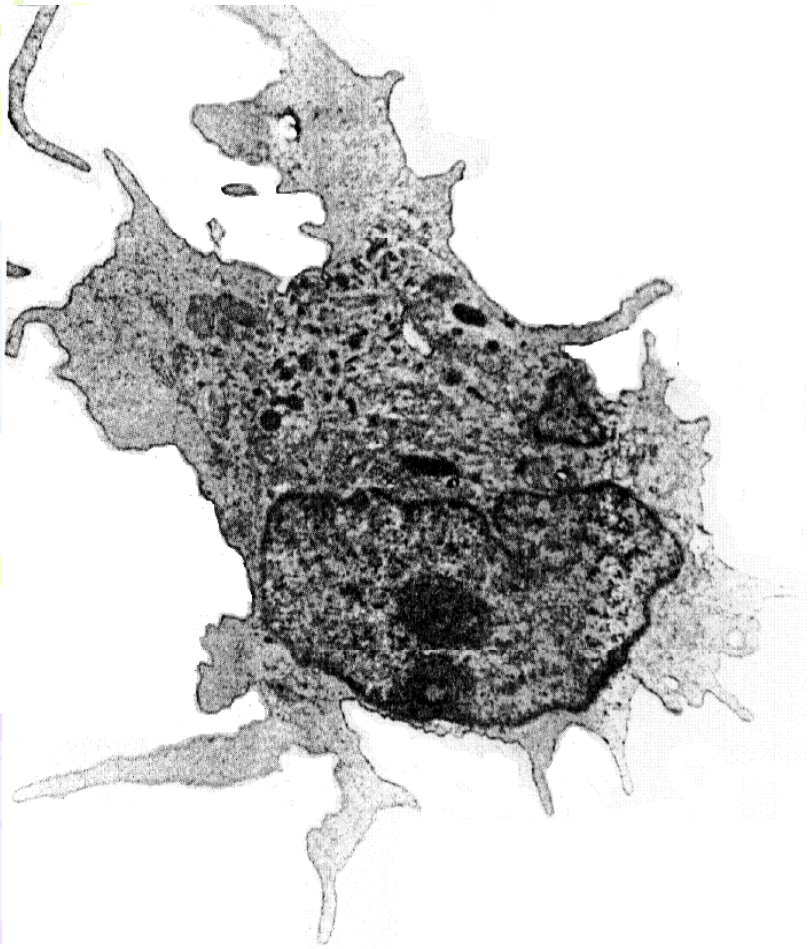
BIOSEGURIDAD

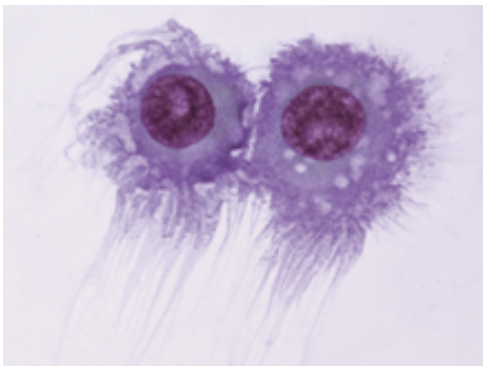
RESPUESTA INMUNOLOGICA

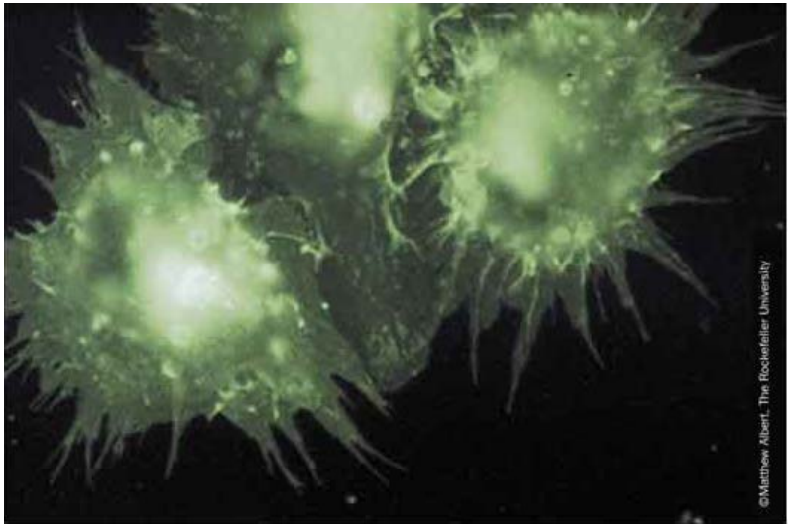
- IN VIVO: DTH
- IN VITRO: ELISPOT .

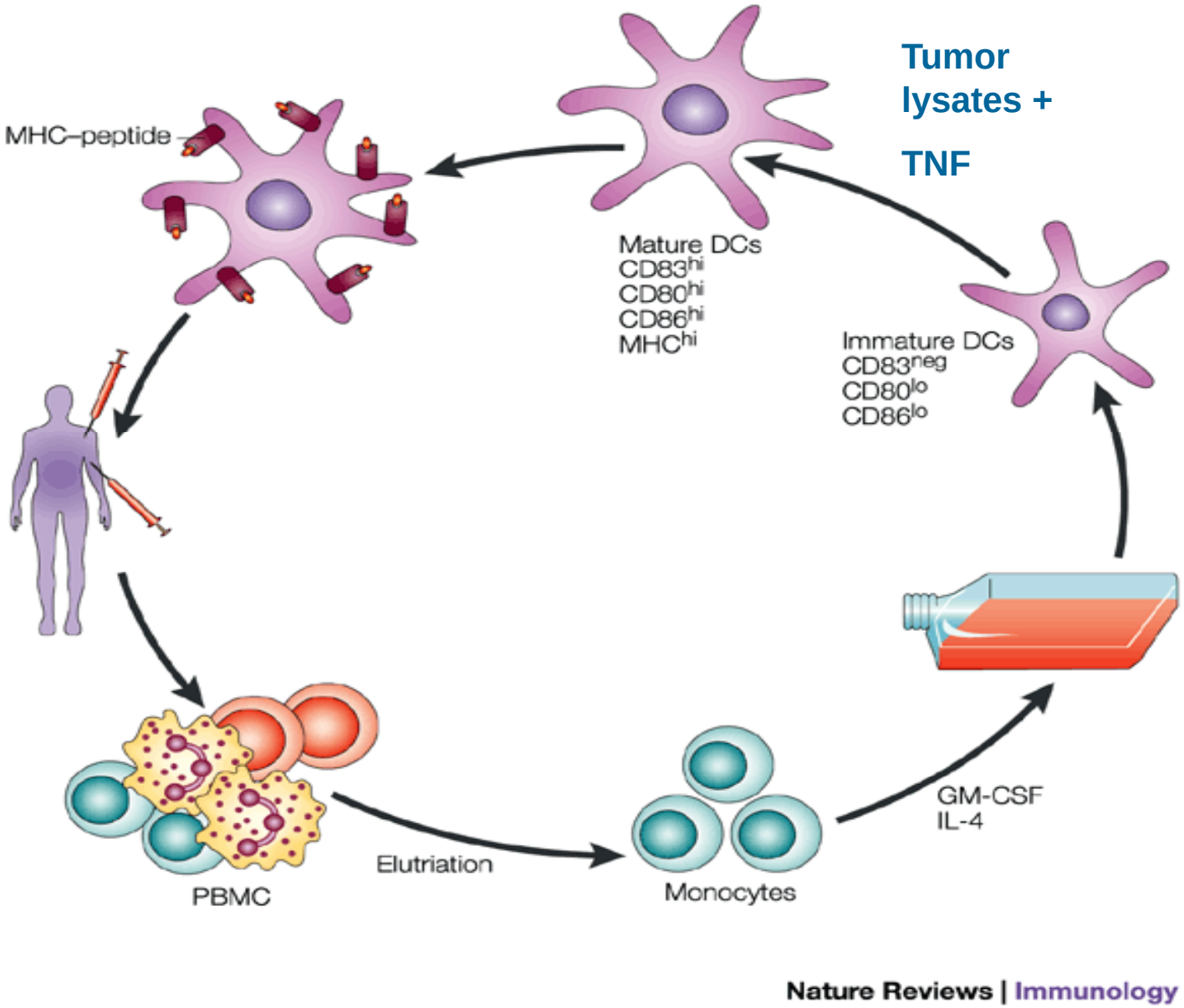
RESPUESTA CLINICA

Célula dendrítica la más potente presentadora de antígenos

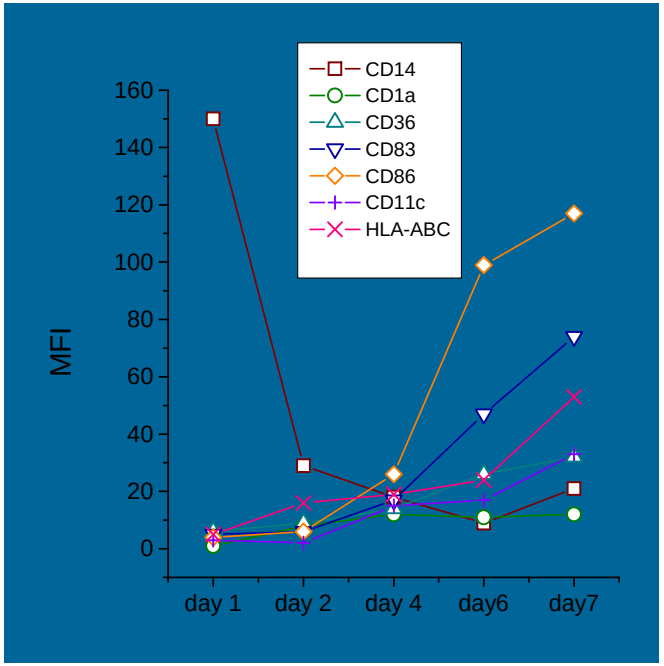


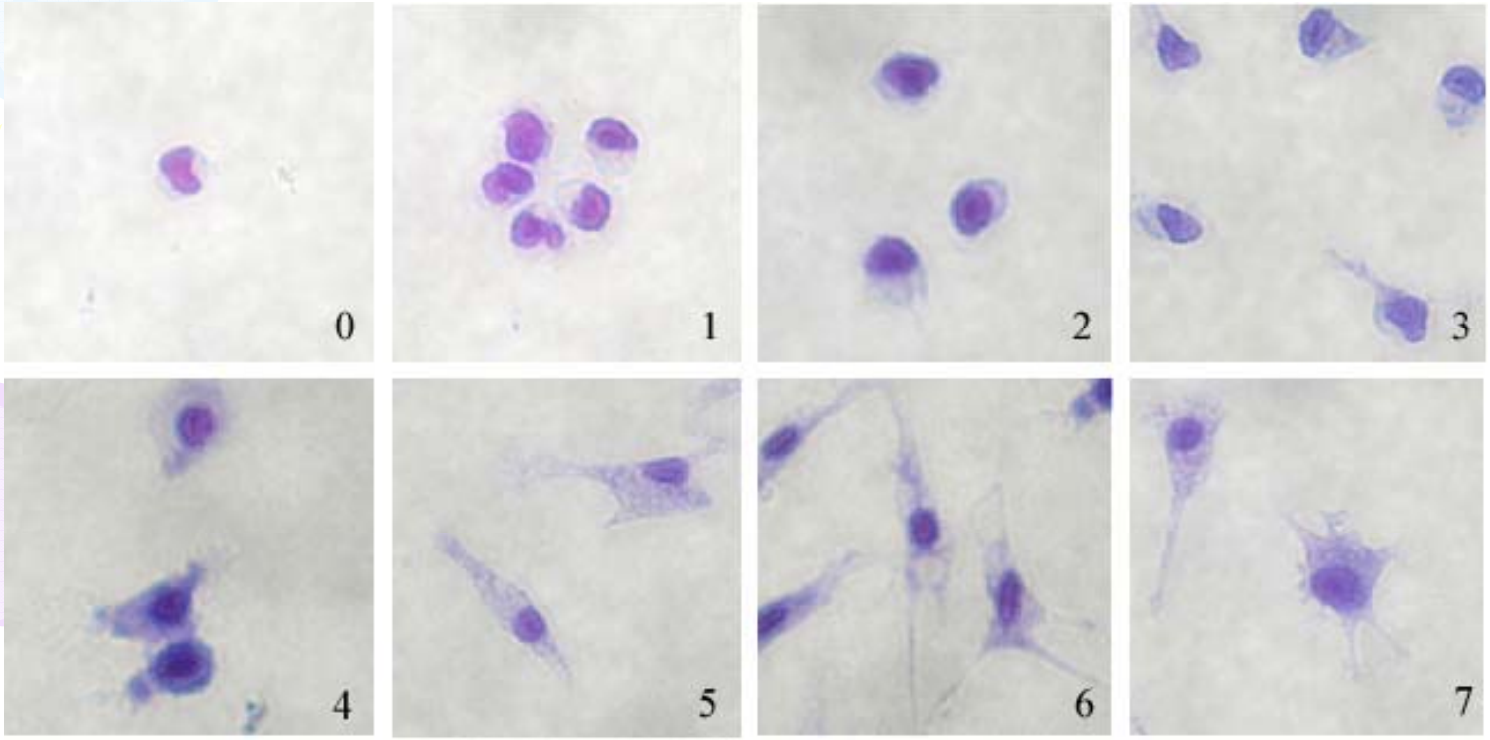





Tumor
lysates +
TNF
MHC–peptide
Mature DCs
CD83hi
CD80hi
CD86hi
MHChi
Immature DCs
CD83neg
CD80lo
CD86lo
GM-CSF
IL-4
Elutriation
PBMC
Monocytes
Nature Reviews | Immunology

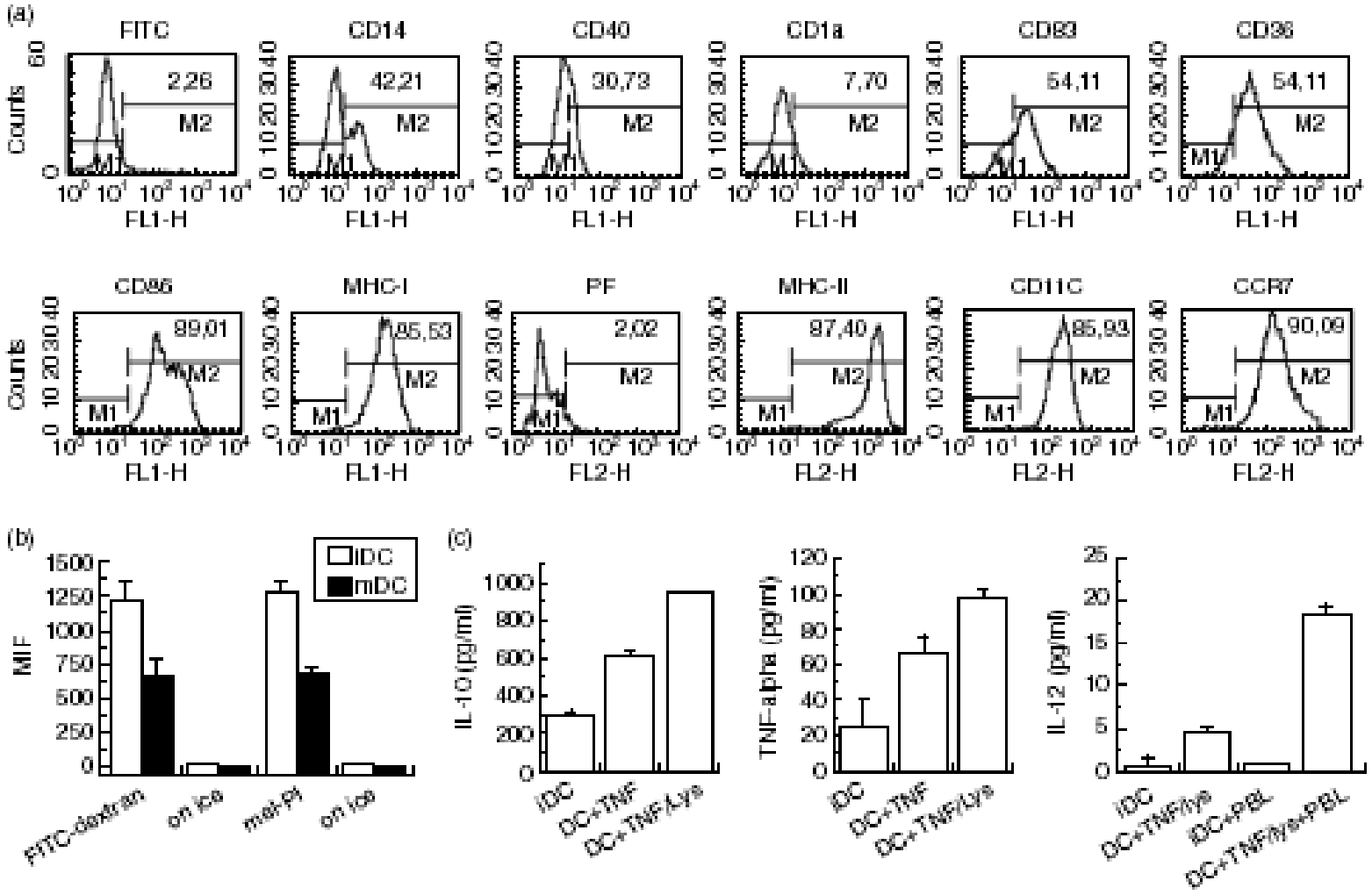
Evolución morfológica y fenotípica de DC producidas *in vitro*



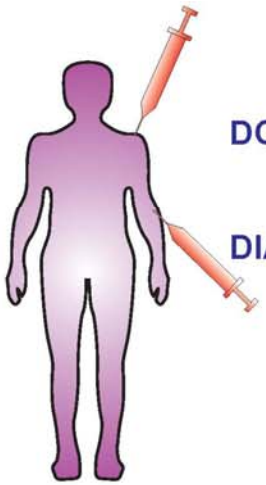


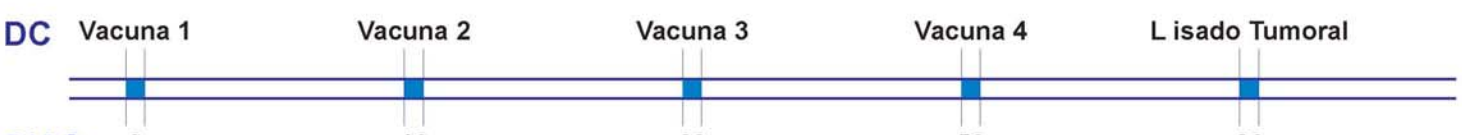
In vitro properties of DCs

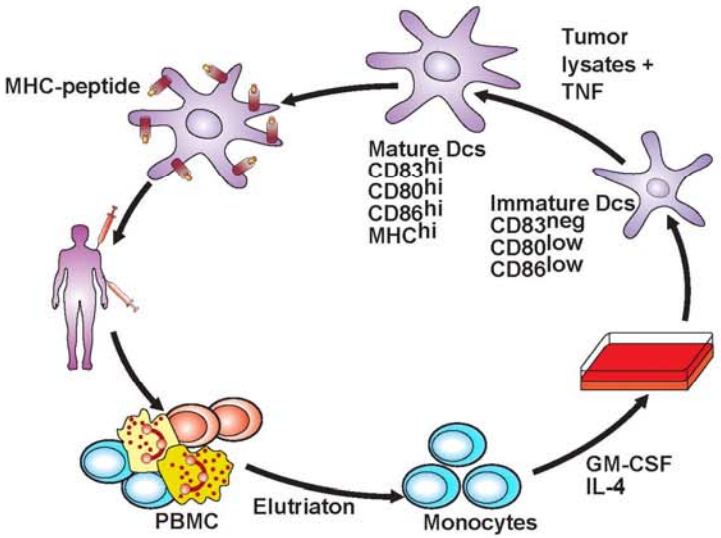
A. Escobar *et al.*



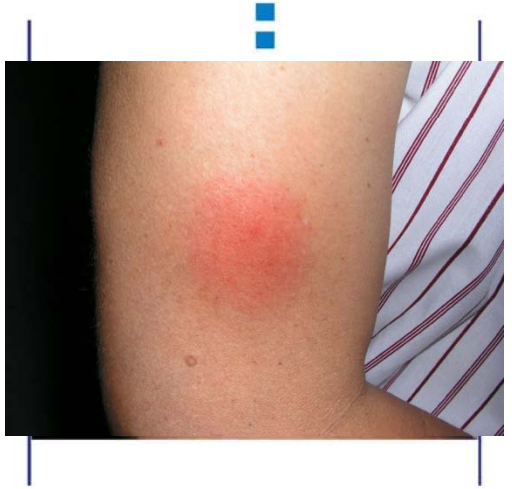
Escobar et al. 2005. Clin. Exp. Immunol. (in press)







Nature Reviews I Immunology

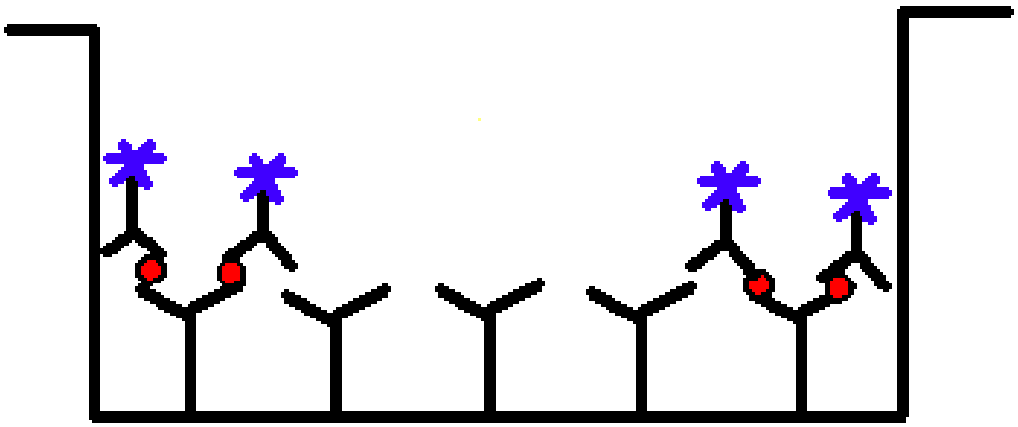


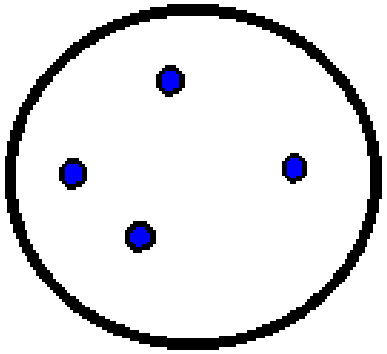
CODIGO	FN	SEXO	DIAGNOSTIC	FECHA DG.	ESO PROTO	CLARK	HLA	TUMOR 1°	METASTASIS	TTO ADIC.	TIPO TAC.	F. ADVERSOS	ESP. ELISPO	TB ELI [...]	B LISADO [...]	ESTABLE [...]	P. POST-TAC. [.....]
42-MT	27/4/50	F	Ca Colon	1/9/05	29/3/06	Nf.corr	.rtipificar	Cólon	ulmón e Hígado	Cirugía+quimi	rDC+mix	na	na realizada	na realizada	na realizada	5	5
28-MT	1/4/61	M	MM etapa III Al	1/9/04	11/4/05	IV, Breslau III	(+)	ntaria pantorr	1 de 10) con ca	Cirugía	DC+KLH	na	na realizada	6 (+)	40 (+)	16	16
29-MT	26/11/33	F	MM etapa IV	1/12/95	5/2005; 09/03	r Melanoma u	(-)	al tarso jo may	Clark IV, metá	Cirugía	DC+KLH; 2°r	na	na realizada	1° ciclo (-)	° ciclo 3mm (-	7	15
30-MT	13/3/39	F	MM etapa IV	1/9/94	11/5/05	IV, Breslau II	(+)	Pantorrilla izq	cidiva en zon	Cirugía, IFN2	DC+KLH	na	na realizada	(-)	(-)	15	15
03-OM	1/6/66	F	MM etapa IV	1/12/02	3/9/03	III	(-)	cánea pámula	y del grupa yu	Cirugía+radia	DC+KLH+IL-2	na	(-)	10 (+)	45 (+)	35	35
CT-014	22/5/41	M	MM etapa IV	21/6/05	6/3/03	ark IV, Breslau	(+)	cuero cabellu	cia+met en tra	Cirugía	DC+KLH+IL-2	na	(+)	5 (+)	12 (+)	45	45
11-MT	9/7/48	F	MM etapa IV	1/12/03	21/7/04	ark IV, Breslau	(+)	tremidad inf	ir y en tránsita	Cirugía	DC+KLH+OHA	na	(-)	3 (-)	6 (+)	23	25
15-MT	7/1/54	M	MM etapa IV	1/6/02	1/9/04	Breslau IV	(+)	Escápula Der	ervical der.; m	Cirugía	DC+KLH	na	na realizada	na realizada	na realizada	0	0
12-MT	15/1/54	F	Muveal etapa	1/8/03	4/8/04	Nf.corr	(+)	Ocular	or orbita; hep	a+radioterap	DC+KLH	na	na realizada	2 (-)	7	6	10
20-MT	3/7/57	M	MM etapa IV	1/9/02	29/9/04	Breslau 1.3mm	(-)	ared abdomin	Pulmon aror	Cirugía	DC+KLH	Cefalea leve	na realizada	3 (-)	5 (+)	24	24
38-MT	11/9/40	M	MM pediculad	1/2/02	22/9/05	Breslau 6.2mr	(+)	ral hemorrág	últiples, cere	Cirugía+radia	DC+KLH	na	na realizada	3 (-)	(-)	0	3
34-MT	29/9/50	M	MM etapa IV	7/3/05	4/7/05	S/R	(-)	cervical ante	Regional or	Cirugía	DC+KLH	na	na realizada	(-)	(-)	0	3
07-OM	30/12/47	F	MM etapa III Al	26/3/02	29/3/04	aria no encon	(+)	Pantorrilla izq	inguinal (+) r	Cirugía	a ciclo DC+KL	na	(+)	10 (+)	18 (+)	30	30
10-OM	3/2/36	M	MM uveal	9/6/99	10/5/04	Nf.corr	(+)	Ojo derecho	, cuero cabell	Cirugía	DC+KLH	Cefalea leve	(-)	(-)	(-)	0	5
27-MT	16/2/63	F	MM etapa III	1/1/03	20/2/05	S/R	(+)	Párpado der.	Cervical	Cirugía	DC+OHAI	flu like leve	(+)	5 (+)	(-)	19	19
CT-001	3/1/60	F	MM etapa IV	3/4/01	20/9/02	IV	(+)	Tórax der.	mamaria y nód	Cirugía	DC+KLH	na	(+)	6 (+)	10 (+)	23	26
24-MT	10/9/23	F	MM etapa IV	1/3/03	19/1/05		(+)	Rodilla izq	nglia r regiona	Radio	Dc+OHAI	desconocimiento	na realizada	na realizada	(+)	21	21
46-MT	1/5/81	F	MM etapa IV	1/5/05	2/10/06	V, Breslau IV	(+)	ova escápula i	, gl axilar or e i	Radio	DC+KLH	na	En vacunación	En vacunación	En vacunación	En vacunación	
19-MT	24/1/51	M	MM etapa IV	1/8/04	12/10/04	Breslau IV	(+)	vaspitz muria	acrural izq. GL	Cirugía	DC+OHAI+IL-2	na	na realizada	7 mm (+)	14 mm (+)	24	24
21-MT	7/5/60	M	MM etapa IV	1/10/04	15/12/04	Breslau III	(-)	men, periom	ranrita baja p	Cirugía	DC+OHAI	na	na realizada	8 mm (+)	16 mm (+)	22	22
CT-22	15/8/33	F	Ca Colon IV	1/7/03	15/12/04	Nf.corr	(+)	Cólon	Hígada	Quimioterapi	DC+OHAI	na	Na realizada	na realizada	na realizada	retirada 03/05	Retirada 03-05
14-MT	17/4/70	M	Ca Colon IV	1/1/03	20/8/04	Nf.corr	(-)	Cólon	'gada, peritan	Cirugía		na	Rip 2 vac	Rip 2 vac	Rip 2 vac	Rip 2° vacuna	0
26-MT/LT	6/2/62	M	a Non Hodgkir	1/6/97	28/1/05	Nf.corr	ND	Naral	cidiva local m	qía+quimia+r	DC+OHAI	na	ND	ND	ND	8	14
32-MT	1/3/38	M	Ca vorical	1/5/03	18/5/05	Nf.corr	ND	Vejiga	eritanea, L3	tamida, MitC,	DC+KLH	na	ND	ND	ND	0	7
37-MT	15/10/32	M	Ca vorical IV	1/9/03	20/9/05	Nf.corr	(-)	Vejiga	Negativa	BCG	DC+KLH	eritema tóra	ND	ND	ND	14	14
36-MT	10/1/58	F	MM etapa IV	14/6/05	20/9/05	Breslau IV	(+)	dorral der	cula, cerebra,	Cirugía	DC+KLH+OHA	eritema local	(-)	9 mm (-)	17 mm (+)	10	13
18-MT	11/4/05	M	MM etapa IV	1/8/04	5/10/04	III, Breslau 3.5	(-)	raor capular	anglia r axilar	Cirugía	DC+OHAI+IL-2	na	ND	8 mm (+)	(-)	7	12
31-MT	5/5/05	M	Ca prartata	25/6/05	11/5/05	Nf.corr	(-)	prártata	pelvia nar	rm, radia, quir	DC+OHAI	na	ND	ND	ND	6	12
33-MT	27/5/46	F	MM etapa IV	24/6/05	28/5/05	ark IV Breslau	ND	pierna der	der, gl r cervic	Cirugía+radia	DC+OHAI	na	ND	9 mm (-)	(-)	7	16
44-MT	?	F	Ca pulmonar	1/5/06	28/7/06	Nf.corr	(+)	pulmón	hepáticar	Radioterapia	DC+KLH	na	ND	ND	ND	0	ra entorcera vacuna
45-MT	2/12/34	F	MM etapa IV	1/3/05	1/9/06	IV, Breslau 3.8	ND	talón der	al der, pierna y	ía+quimia (da	DC+KLH	(ri can tomad	En vacunación	En vacunación	En vacunación	En vacunación	En vacunación
CT-02		M	MM etapa IV			Clark IV, Breslau	(-)	ambra derecha	urir columna v	ugía del prim	DC +OHAL	na	na realizada	na realizada	na realizada	0	0
25-MT	30/4/05	M	MM etapa III Al	2/9/03	28/1/05	III, Breslau 2.5	ND	braza izq	glr axilar or	Cirugía+radia	DC+KLH+OHA	na	ND	10mm	20mm (+)	22	22
13-MT	?	F	MM etapa IV	1/1/04	13/8/04	Clark IV	(+)	uera cabellud	metártarir in	cirugía	DC+KLH+OHA	na	(+)	(-)	7 mm (+)	26	26
40-MT	31/3/53	M	MM etapa IV	1/12/99	10/1/06	Clark II	(-)	pubir	nalar, pulmán	ía+radia+ter	DC+KLH	na	ND	6 mm (+)	3 mm (-)	0	6
35-MT	28/8/63	F	MM etapa IV	1/4/05	20/9/05	Kk III, Breslau	(-)	pie der	, más comprar	Cirugía	DC+KLH	eritema local	ND	(-)	(-)	7	12
17-OM	7/11/64	M	MM etapa IV	1/3/01	15/9/04	V	(-)	malar der	regional or, ar	Cirugía+radia	DC+OHAI+IL-2	na	ND	6 mm (+)	2 mm (-)	7	11
23-MT	23/1/35	F	MM de mucar	1/1/04	16/12/04	? Breslau 1.5	(+)	vulva	guinalor der, p	Cirugía	DC+OHAI	na	ND	6 mm (+)	10 mm (+)	0	5
CT-008	4/2/67	F	MM etapa IV	21/6/05	1/10/02	Breslau IV	(+)	Rodilla izq	pulmonar or m	a+radioterap	DC+KLH	na	(-)	7 mm (+)	(-)	1	ira dp 3°vac 5 meror
06-MT	15/8/58	M	MM etapa III a	28/10/93	14/1/04	? Breslau 3.6	(-)	ambra izquier	nglia r cervica	Cirugía	DC+KLH+IL-2	na	ND	8 mm (+)	8 mm (+)	34	34
08-MT	12/8/83	F	MM etapa III a	26/6/05	21/4/04	Clark III	(+)	pierna izq	glr inguinalor	Cirugía	DC+KLH	a, comp. Erta	(-)	(-)	13 mm (+)	24	n por ser reingresada a lar 45
09-OM	6/5/05	M	MM etapa IV	23/6/05	5/5/04	IV	(-)	malar izq	a, req. Subor	Cirugía	DC+KLH+IL-2	ar leve, baja a	ND	ND	ND	tacala par ráp	aca de protocolo
MT-01	22/4/05	M	MM etapa IV	1/11/02	9/7/03	?	?	retro-orbital	a, cartavorte	Cirugía+radia	DC+KLH	na	na realizada	na realizada	na realizada	0	Rip dp 2°dosir
16-MT	9/5/05	M	MM etapa IV	24/6/05	10/9/04	V	(+)	oja mayor pie	ulmón e Hígado	Cirugía	DC+OHAI	na	na realizada	na realizada	na realizada	0	Rip dp 2°dosir
CT-012	3/5/05	M	otapa IV más I	2/10/02	30/5/03	V	(-)	uera cabellud	Hígada	Cirugía	DC+KLH	na	ND	Se retira	Se retira	Se retira	Se retira
04-OM	6/6/67	M	MM etapa IV	24/9/01	9/10/03	, Breslau 12m	(+)	Cara	ita y cervical	Cirugía	DC+KLH+IL-2	na	(-)	7 mm (+)	15 mm (+)	36	36
CT-010	16/5/05	M	MM etapa IV	20/6/05	7/12/02	Clark IV	(+)	piel pomula de	color, hueso n	Cirugía	DC+KLH	na	(+)	8 mm (+)	10 mm (+)	0	18 meror
CT-009	16/6/78	F	MM etapa III Al	20/5/02	20/9/02	Breslau II	(-)	ambra izquier	y axilar or par	Cirugía	DC+KLH	na	(-)	7 mm (+)	10 mm (+)	48	48
02-MT	27/4/05	F	MM etapa IV	19/6/05	11/8/03	IV, Breslau 2.5	(+)	murla der??	ulmón e Hígado	Cirugía	DC+KLH	na	?	?	?	?	?
CT-003	13/10/67	M	MM etapa IV	1/8/01	20/8/02	ark IV Breslau	HLA-A2 (+)	-ra izquierda	ulmón e Hígado	Cirugía	C+OHAL requ	na	(+)	7mm (+)	7mm (+)	20	26
CT-011	19/4/05	F	MM etapa IV	22/6/05	10/12/02	Breslau IV	HLA-A2 (+)	dorra	axilar or, hígado	Cirugía	DC+KLH	na	(+)	6 mm (+)	(-)	0	7
CT-005	25/4/05	M	MM etapa IV	13/7/99	11/4/02	III, Breslau 4.8	(-)	piel murla izq	guinalor izq, p	Cirugía	DC+OHAL	fiebre	(-)	6 mm (+)	(-)	18	21
05-MT	26/5/05	M	MM etapa IV	1/5/99	17/3/02	Clark IV	(+)	piel abdomen	inguinalor iz	Cirugía	DC+KLH+IL-2	flu like leve	(+)	6 mm (+)	9 mm (+)	22	36
39-MT	29/4/05	M	MM etapa IV	23/6/05	14/11/05	NO disponible	(-)	traauricular	dlor, pulmán, hí	Cirugía	DC+KLH	na	(+)	5mm (+)	10 mm (+)	11	11
CT-012	3/5/05	F	MM etapa IV	6/2/02	27/1/03	III, Breslau 2	(+)	planta pie der	ir inguinalor iz	Cirugía	DC+KLH+IL-2	fiebre leve	(-)	6 mm (+)	(-)	0	11
CT-004	27/12/38	M	MM etapa IV	14/6/05	8/9/03	Clark IV	HLA-A2 (-)	ráxica izquier	múltiples, hígado	Cirugía	DC+OHAL	Na	(-)	7 mm (+)	(-)	18 meror	21 meror
49-MT	15/6/80	F	MM etapa IV	26/6/05	3/9/06	k IV, Breslau	HLA-A2 (+)	Hombra izq	ultiples cru y	Cirugía	DC+KLH	flu like leve	na realizada	3mm (+)	5mm (+)	3	3

Enrolled patients

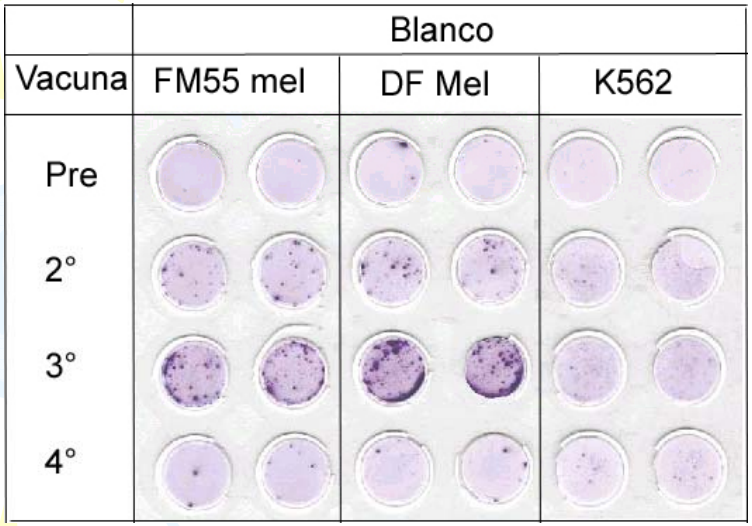
- 41 Stage IV melanoma Patients
- 8 Stage III melanoma Patients
- 12 Patients with other cancer (colon, prostate, lymphoma)

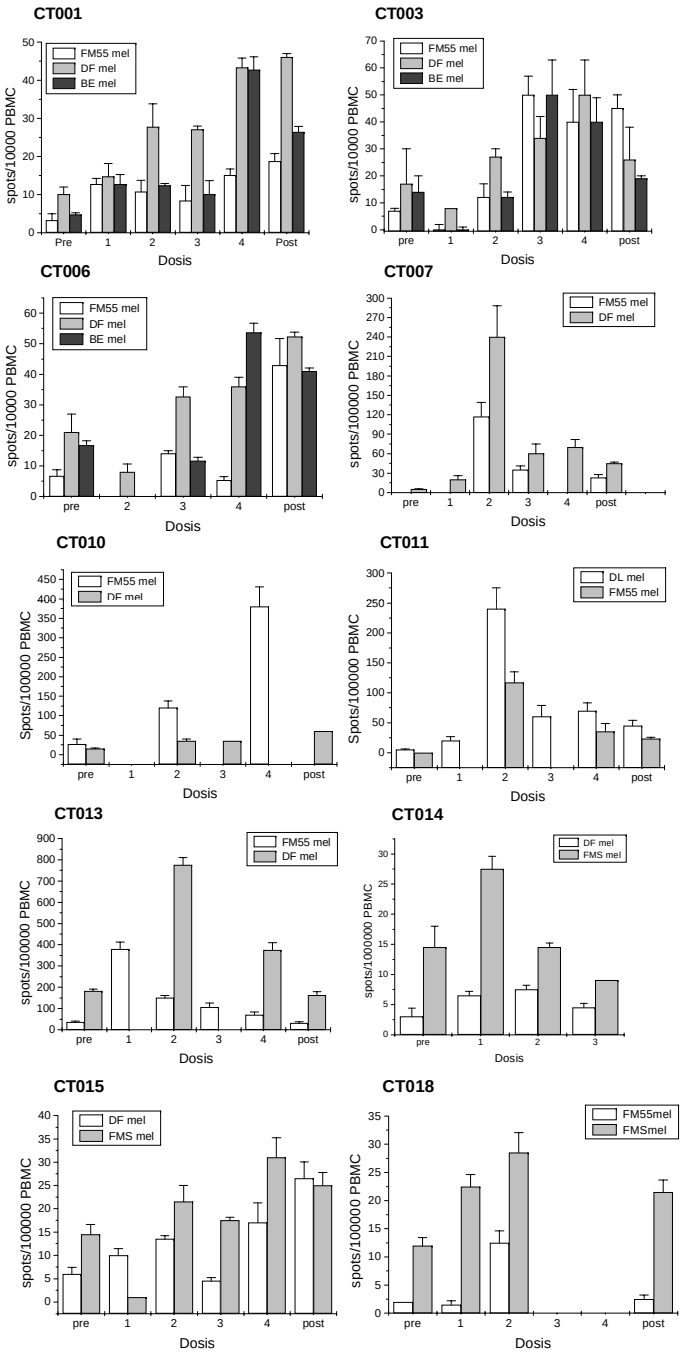
ELISPOT





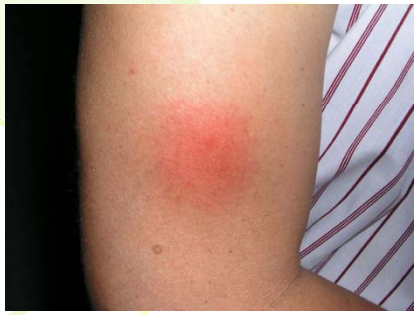
IFN production of vaccinated patients



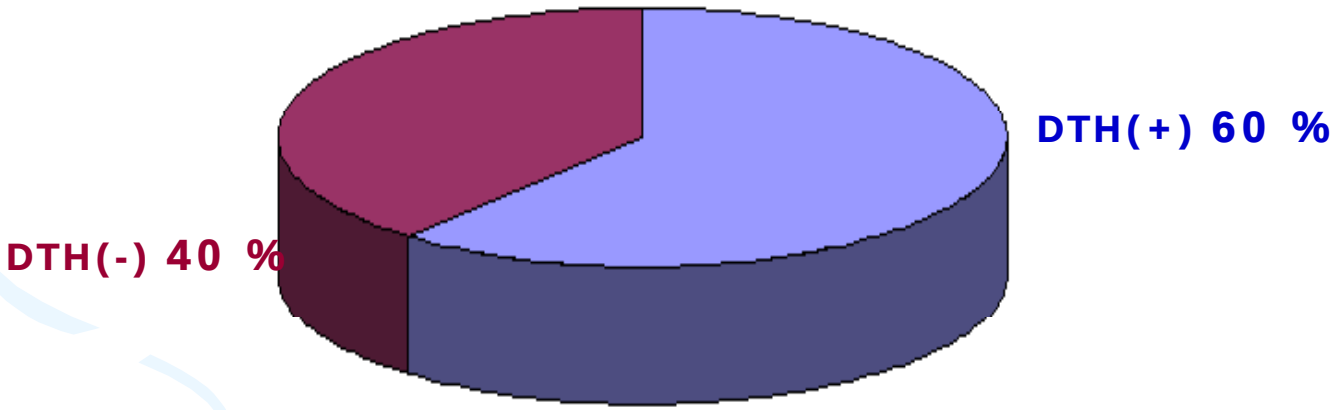


Escobar et al. 2005. Clin. Exp. Immunol. (in press)

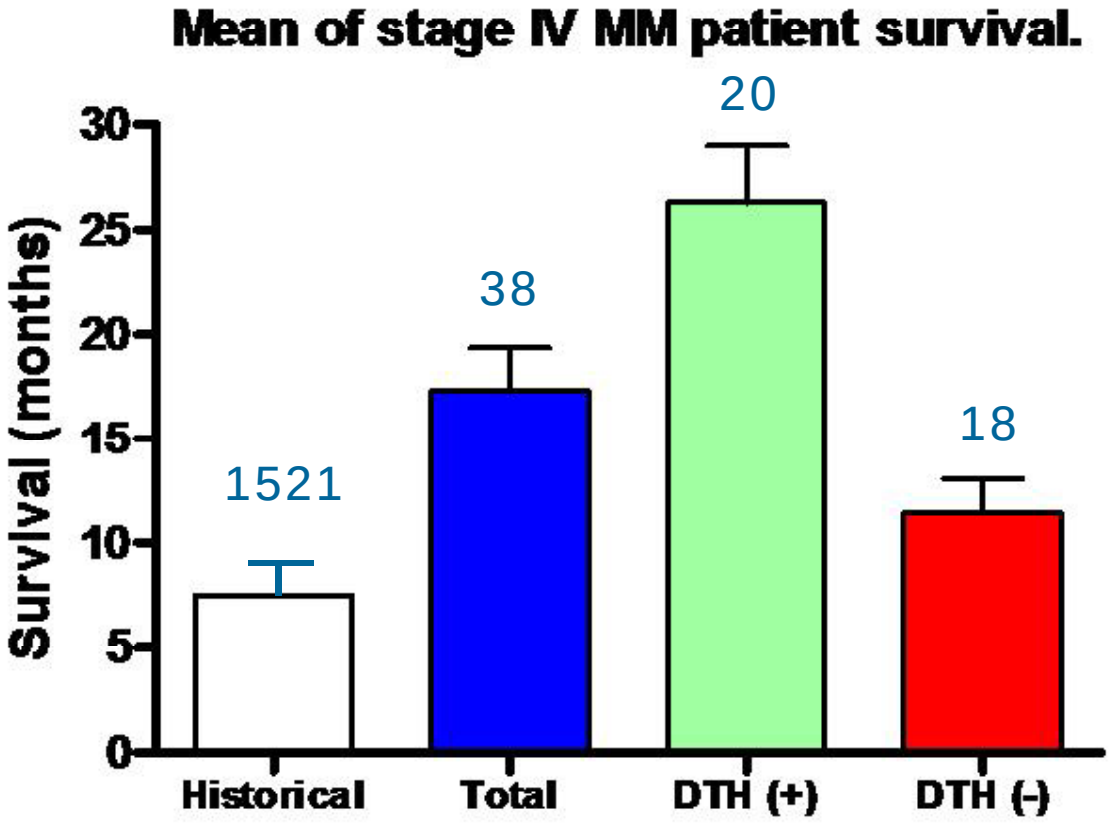
Figure 3



Percentage of DTH + response in vaccinated cancer patients (n=61)



In vivo immunological response correlates with longer patient survival

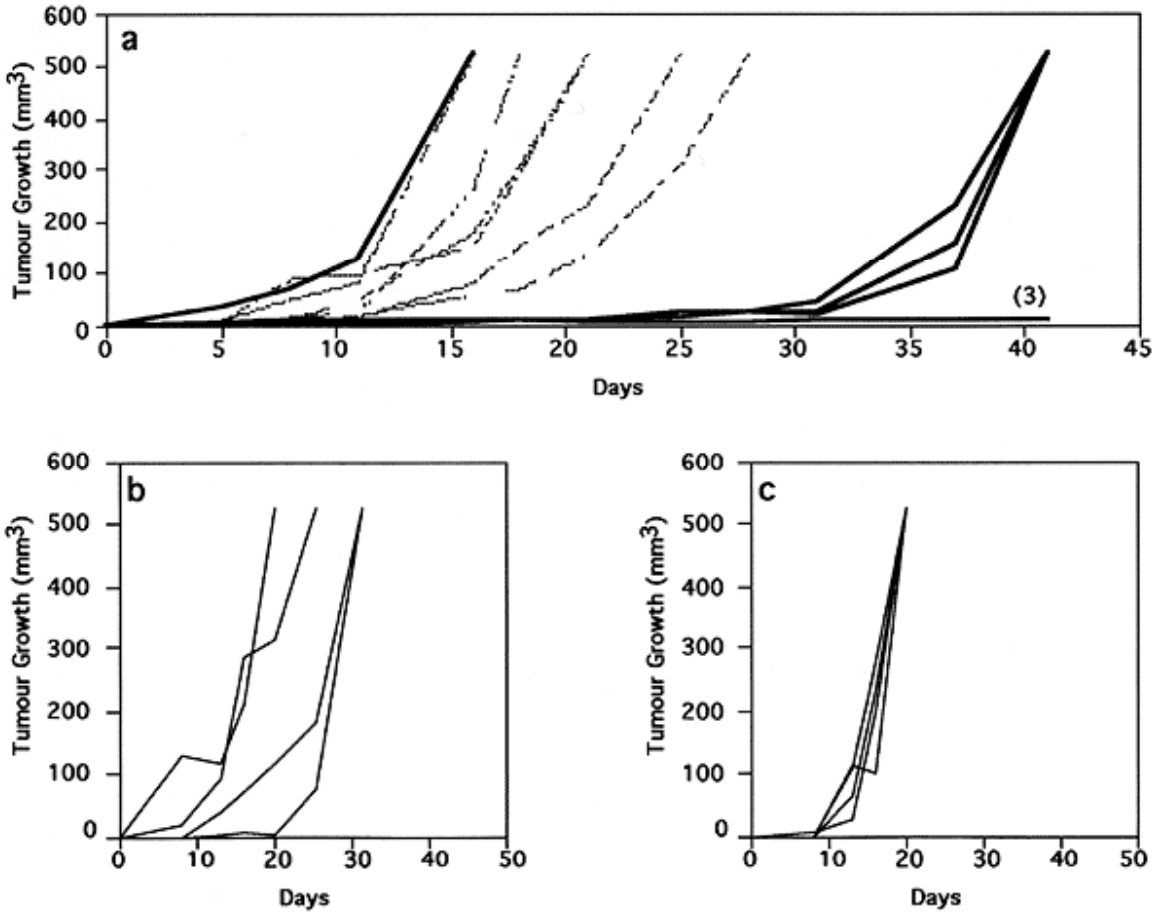


Barth A. et al. Prognostic factors in 1,521 melanoma patients with distant metastases.J Am Coll Surg. 1995 (John Wayne Cancer Institute).

Linfocitos T reguladores
T CD4+ CD25* foxp3+ (Treg)

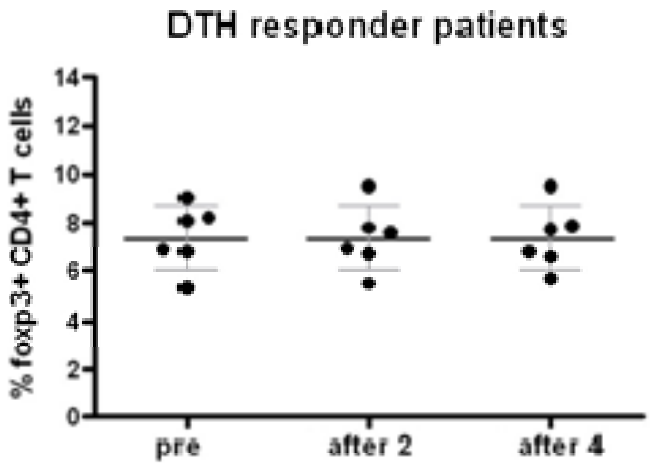
- LT capaces de suprimir la respuesta inmune fueron descritos en 1970
- NKT, LT CD8+ $\gamma\delta$, LTCD4+/CD25+ foxp3+ (Treg)
- LTCD4+/CD25+ foxp3+ *in vitro* inhiben la transcripción de IL2 y la proliferación de otros linfocitos
- LTCD4+/CD25+ foxp3+ expresan CTLA-4
- Existe evidencia de TGFβ unida a membrana linfocitos (TH3)
- Otra subpoblación es altamente productora de IL-10 (TR1)
- Existe evidencia controversial acerca de su origen y su rol

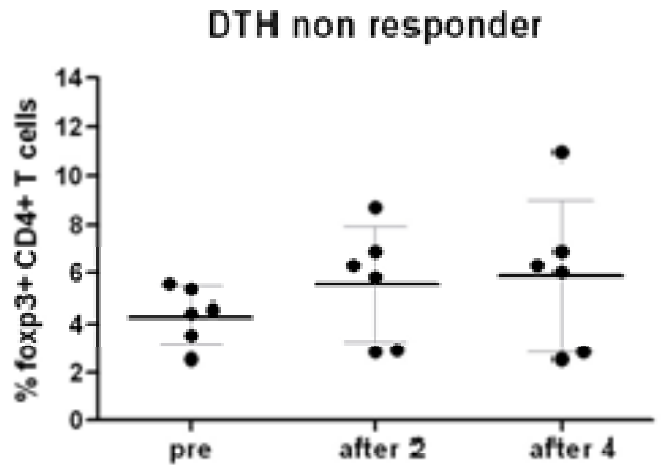
Depleción de células CD25+ con mAb disminuye crecimiento tumoral



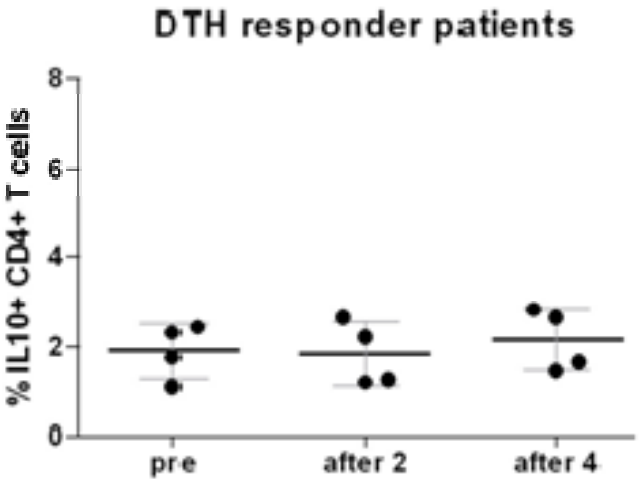
Immunoregulatory responses

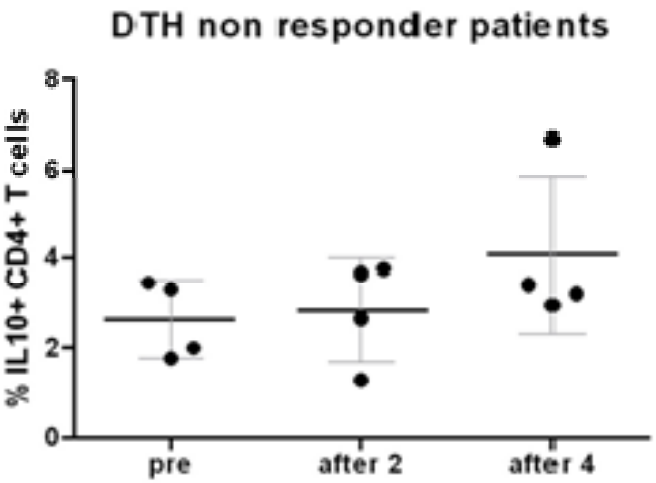
A



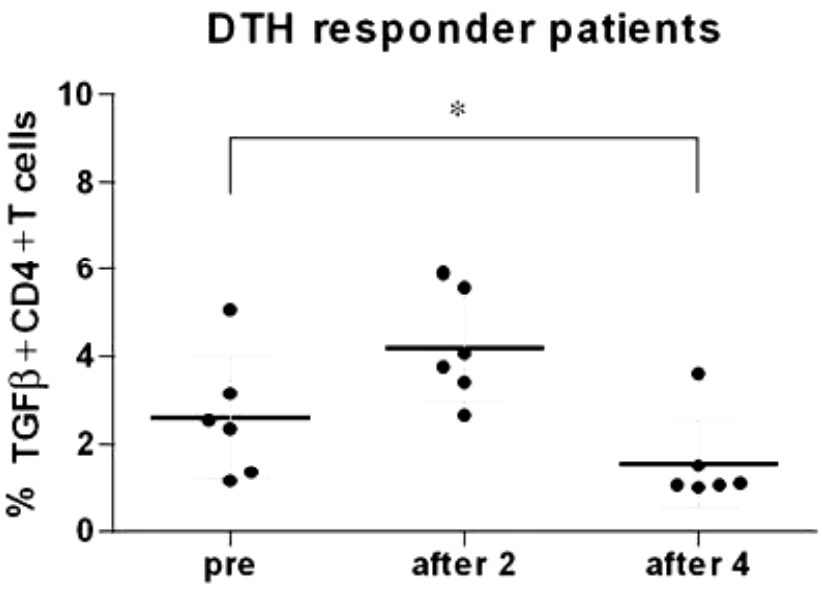


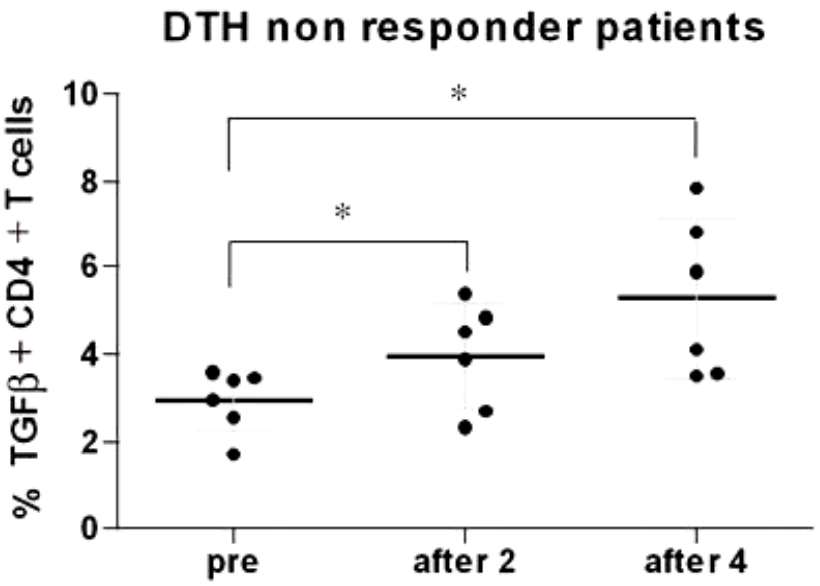
B





TGF-β+ CD4+ T cells percentage are increased in PBMC from DTH- non responder patients





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Dendritic cell immunizations alone or combined with low doses of interleukin-2 induce specific immune responses in melanoma patients

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Summary

Dendritic cell (DC)-based therapy has proved to be effective in patients with a variety of malignancies. However, an optimal immunization protocol using DCs and the best means for delivering antigens has not yet been described. In this study, 20 patients with malignant melanoma in stages III or IV were vaccinated with autologous DCs pulsed with a melanoma cell lysate, alone ($n=13$) or in combination with low doses of subcutaneous (s.c.) interleukin (IL)-2 injections ($n=7$), to assess toxicity, immunological and clinical responses. Monocyte-derived DCs were morphological, phenotypic and functionally characterized *in vitro*. Peripheral blood mononuclear cells (PBMC), harvested from patients either prior to and after the treatment, were analysed using enzyme-linked immunosorbent spot (ELISPOT). After vaccination, 50% of the patients tested (seven of 13) from the first group and (three of seven) from the second, showed an increase in interferon (IFN)-γ production in response to allogeneic melanoma cell lines but not to controls. Four of five tested human leucocyte antigen (HLA)-A2$^+$ patients with anti-melanoma activity also showed specific T cell responses against peptides derived from melanoma-associated antigens. Delayed type IV hypersensitivity reaction (DTH) against melanoma cell lysate was observed in six of 13 patients from the group treated with DC vaccines only and four of seven from the group treated with the combination of DCs and IL-2. Significant correlations were found between DTH-positive responses against tumour lysate and both disease stability and post-vaccination survival on the stage IV patients. There were no toxicities associated with the vaccines or evidence of autoimmunity including vitiligo. Furthermore, no significant enhancement was observed as a result of combining DC vaccination with IL-2. Our data suggest that autologous DCs pulsed with tumour lysate may provide a standardized and widely applicable source of melanoma specific antigens for clinical use. It is safe and causes no significant side effects and has been demonstrated to be partially efficient at triggering effective anti-melanoma immunity.

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Conclusions

- Vaccination with autologous DCs pulsed with allogeneic tumor cell lysate is safe and causes no side effects on patients
- After vaccination, between 50 to 60% of tested patients showed an increase on IFN-γ production or DTH reactions in response to allogeneic melanoma cell lines but not to non-melanoma tumor controls.
- Significant correlations were found between DTH positive responses and disease stability and post vaccination patient survival.
- DTH responder patients showed decreased proportion of TGF-β CD4+ T cells after vaccinations
- At the contrary non responder patients shows an augmented percentage of TGF-β CD4+ cells in PBMC after vaccination

Phase I melanoma trial puts Chile on the map

A Chilean phase I trial with an autologous dendritic cell (DC) vaccine against malignant melanoma has shown the agent to be safe and capable of producing an antitumour immune response. Although similar trials are taking place in the USA (*Proc Am Soc Clin Oncol* 2003; **22:** 178, *J Clin Oncol* 2003; **21:** 4016–26), this is the first immunotherapy study in Chile. The project is being carried out at the University of Chile, Santiago, with support from the Karolinska Institute, Stockholm, Sweden.

Since 2000, 13 patients with stage IV melanoma have been enrolled in the trial. Researchers used leukapheresis to extract peripheral blood and then treated the cells with interleukin 4 and granulocyte-macrophage-stimulating factor to obtain DCs, which were then incubated with a lysate of three allogenic melanoma cell lines and tumour necrosis factor α before being injected back into the patients in four

Courtesy of Flavio Salazar

New immunology laboratory in Santiago, Chile.

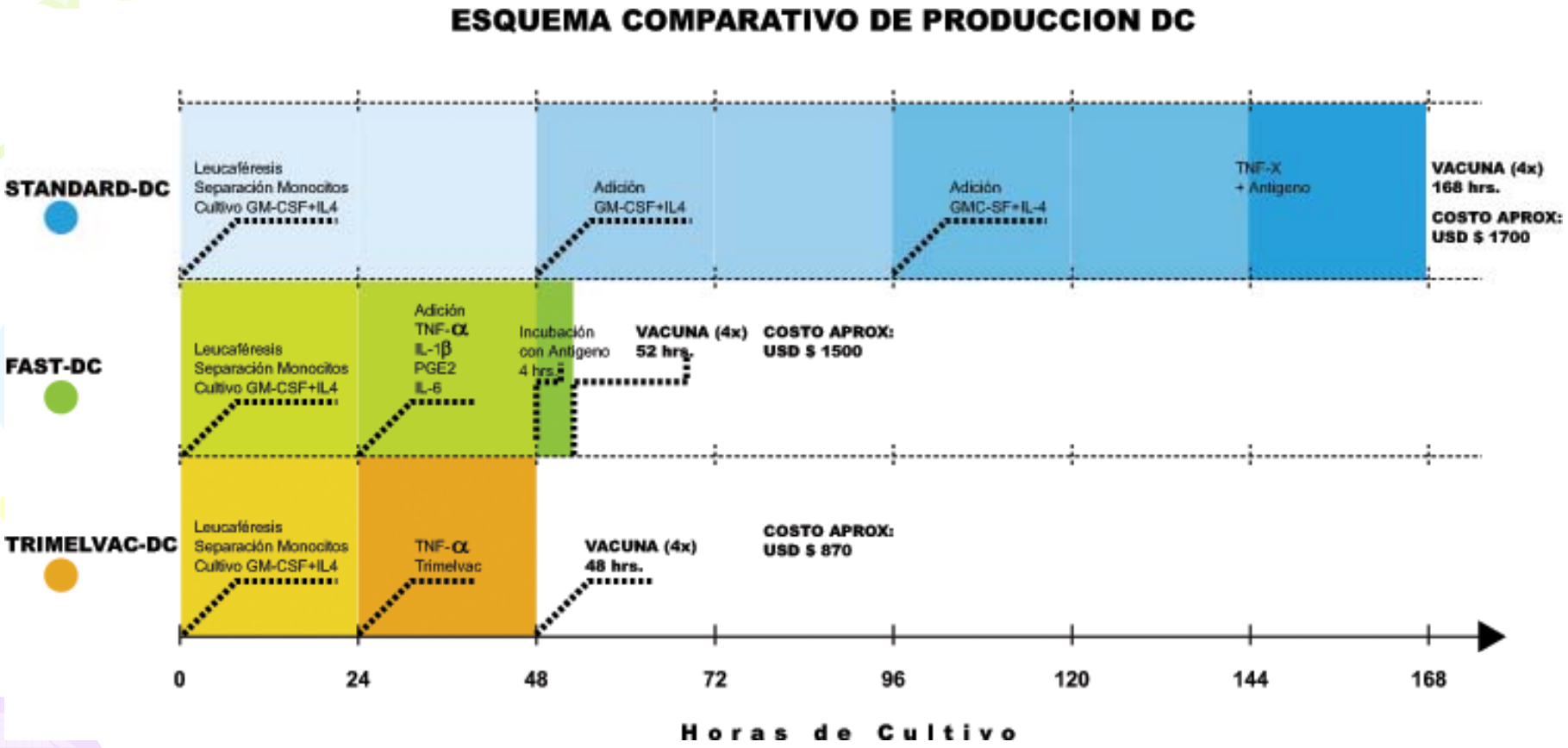
doses over 2 months. No serious toxicity has been detected so far. Although there has been no effect on melanoma progression, analyses have shown the peripheral mononuclear cells of eight patients (62%) produced increased interferon γ in response to allogenic melanoma cells, and six patients (46%) responded positively to delayed-type hypersensitivity tests.

On the basis of these results the researchers were granted state funding to build a laboratory that complied with international standards. It is the first cancer-cell immunotherapy centre in South America. The team are now starting a phase II trial of a regimen that intersperses the vaccine with low doses of interleukin 2. "Interleukin 2 stimulates the propagation of cytotoxic T-lymphocytes in vivo. We are using low doses because it is possible to obtain the same effect as with high doses but without the side-effects", says Flavio Salazar, BioMedical Sciences Institute, University of Chile, Santiago.

Mario Rosemblatt, Life Science Foundation, Santiago, Chile, applauds the initiative: "It is admirable that there are people prepared to use their knowledge of basic research for the benefit of Chilean patients. This project is very important because, despite having the necessary capability, very few people are conducting pioneering medicine in Chile".

Claudia Orellana

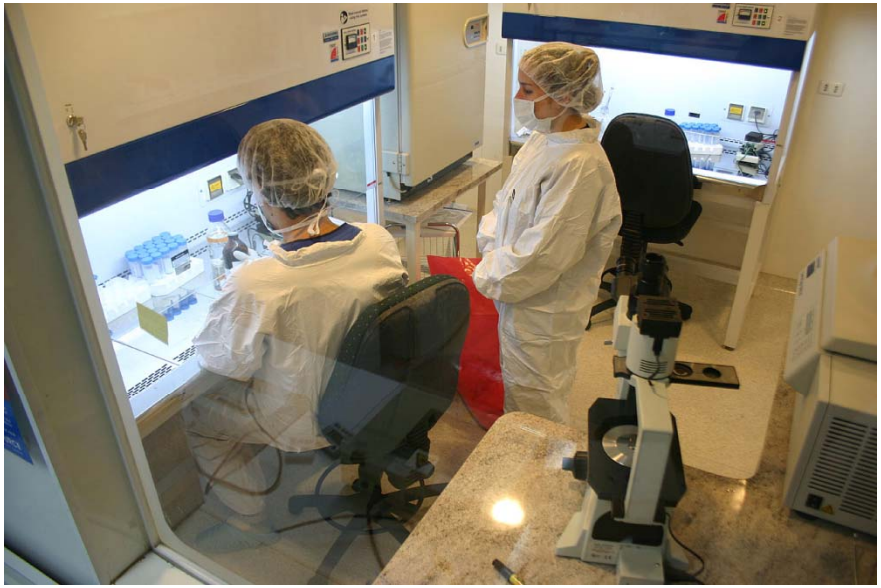
Procedimiento optimizado



Laboratorio GMP

Equipamiento Moderno

Laboratorio GMP



Interacción básico-clínica

Trabajo en equipo