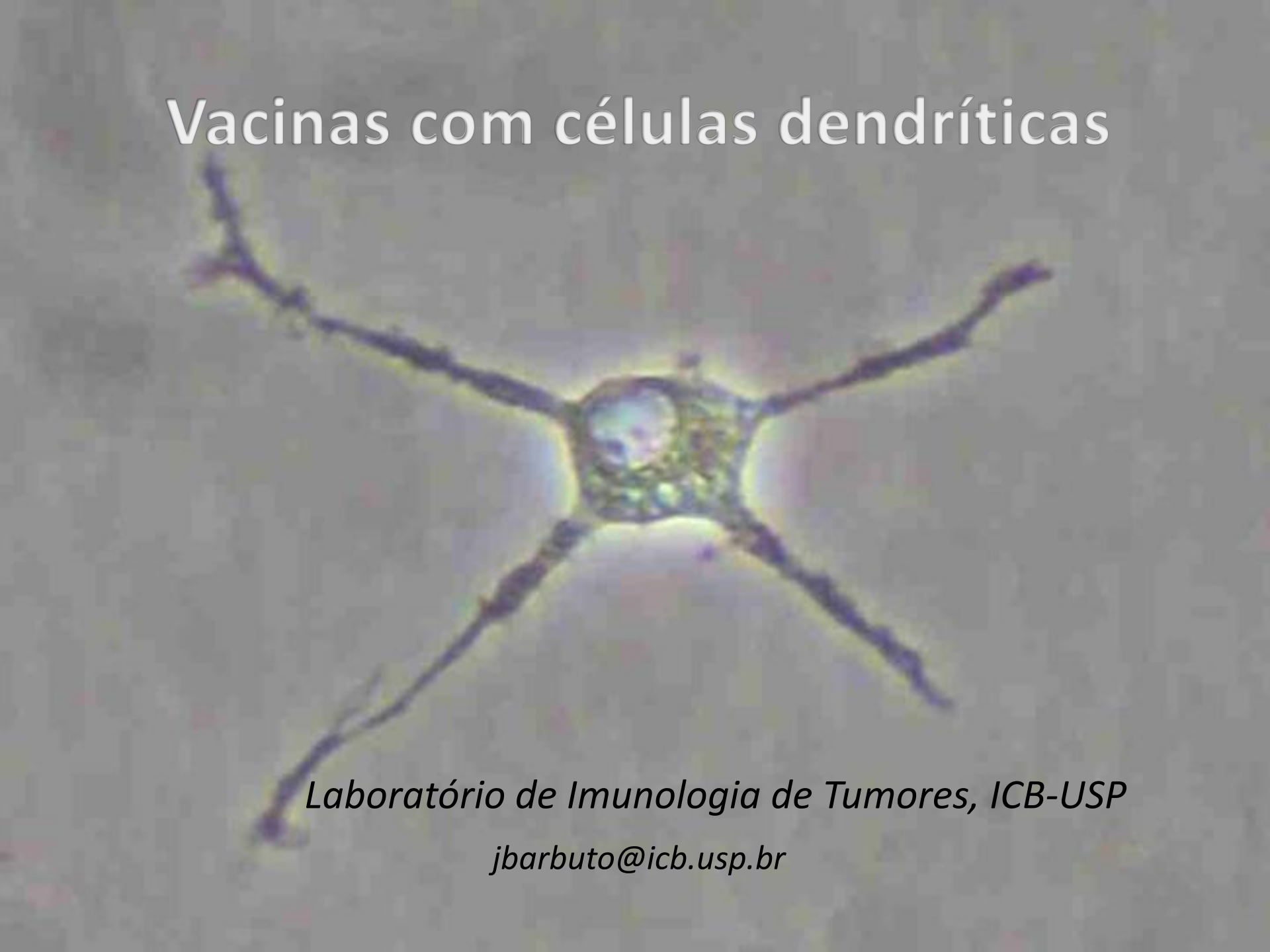
Vacinas com células dendríticas



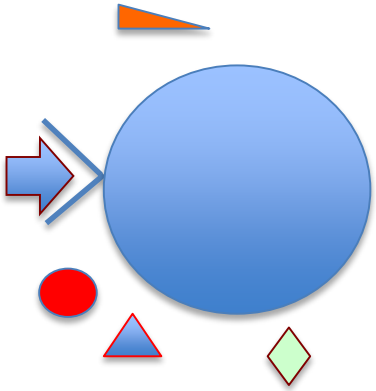
Laboratório de Imunologia de Tumores, ICB-USP

jbarbuto@icb.usp.br

O Sistema Imune:

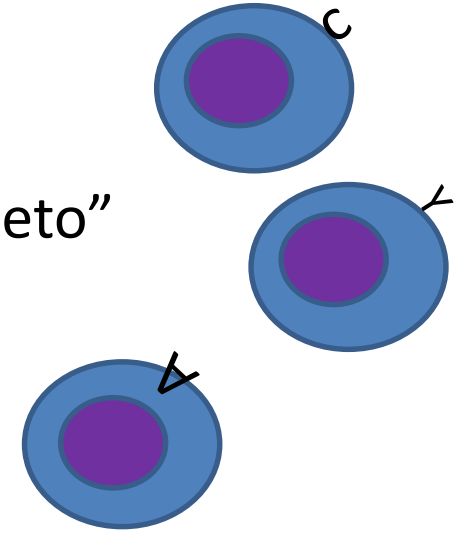
O sistema imune não é um dragão (ainda que guiado pela princesa...)

Ele é “dócil” e sua tendência natural é a tolerância!!



As células só são capazes de responder ao que é “predito” por seus receptores!

Sinais “imprevistos” passariam desapercebidos, mas…



O Sistema Imune gera um repertório “completo” de receptores distribuídos clonalmente nos linfócitos T e B, que reconhecem “tudo”
- os <u>antígenos</u>

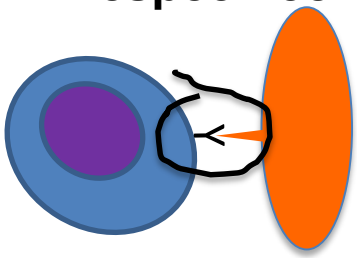
O Sistema Imune é, portanto, um *“tradutor universal”*

Conseqüências do reconhecimento de um antígeno

Tolerância imunológica → **Memória**

Aceitação do Ag

Reconhecimento específico



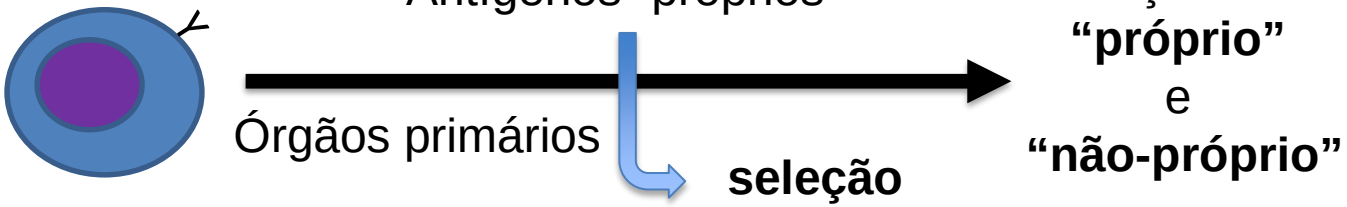
Questão essencial na Imunologia

Eliminação do Ag

"Resposta imune" → **Memória**

Decisão entre tolerância e “resposta”:

Função do Repertório

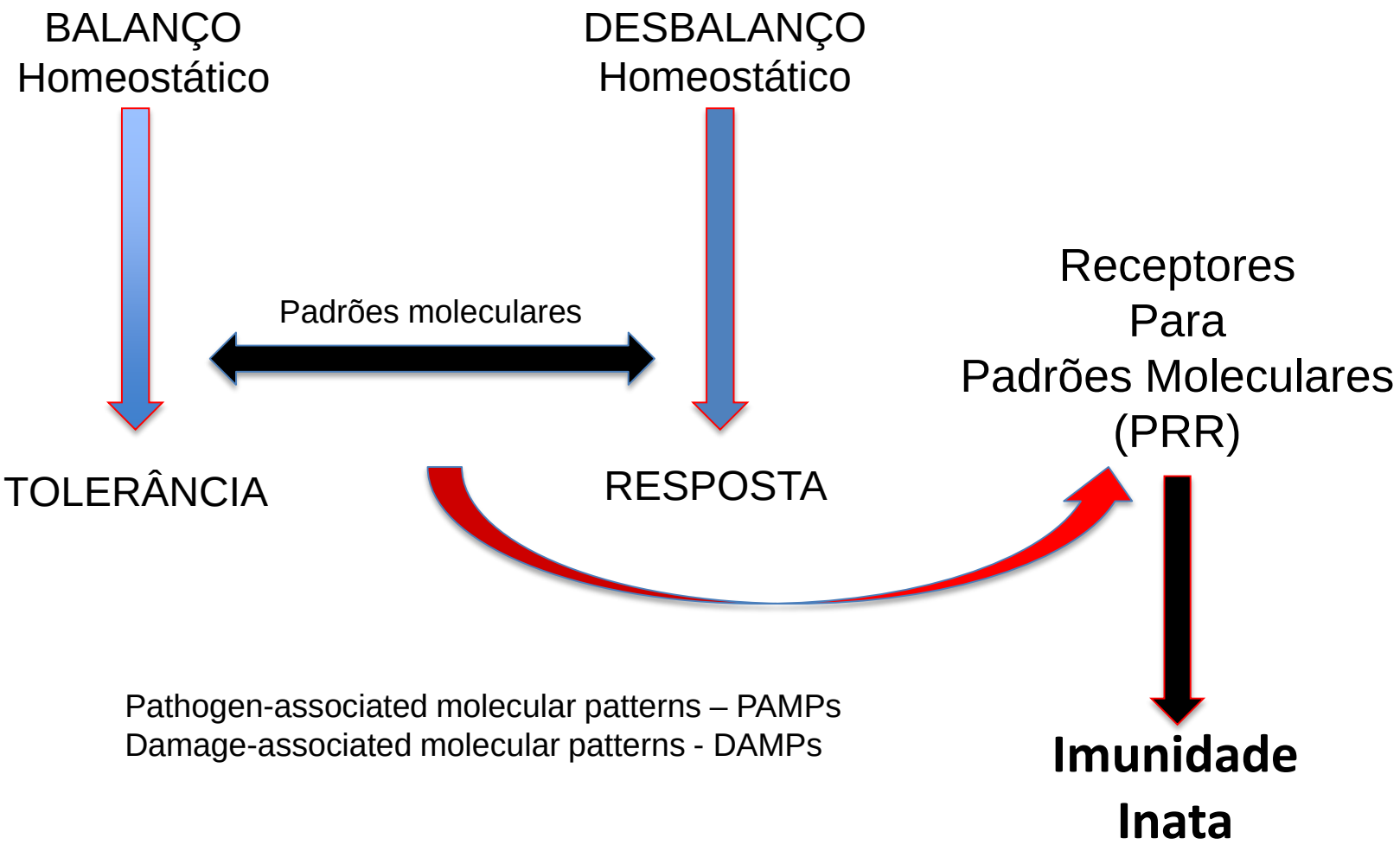


Repertório **completo**
Gerado aleatoriamente
Em linfócitos T e B

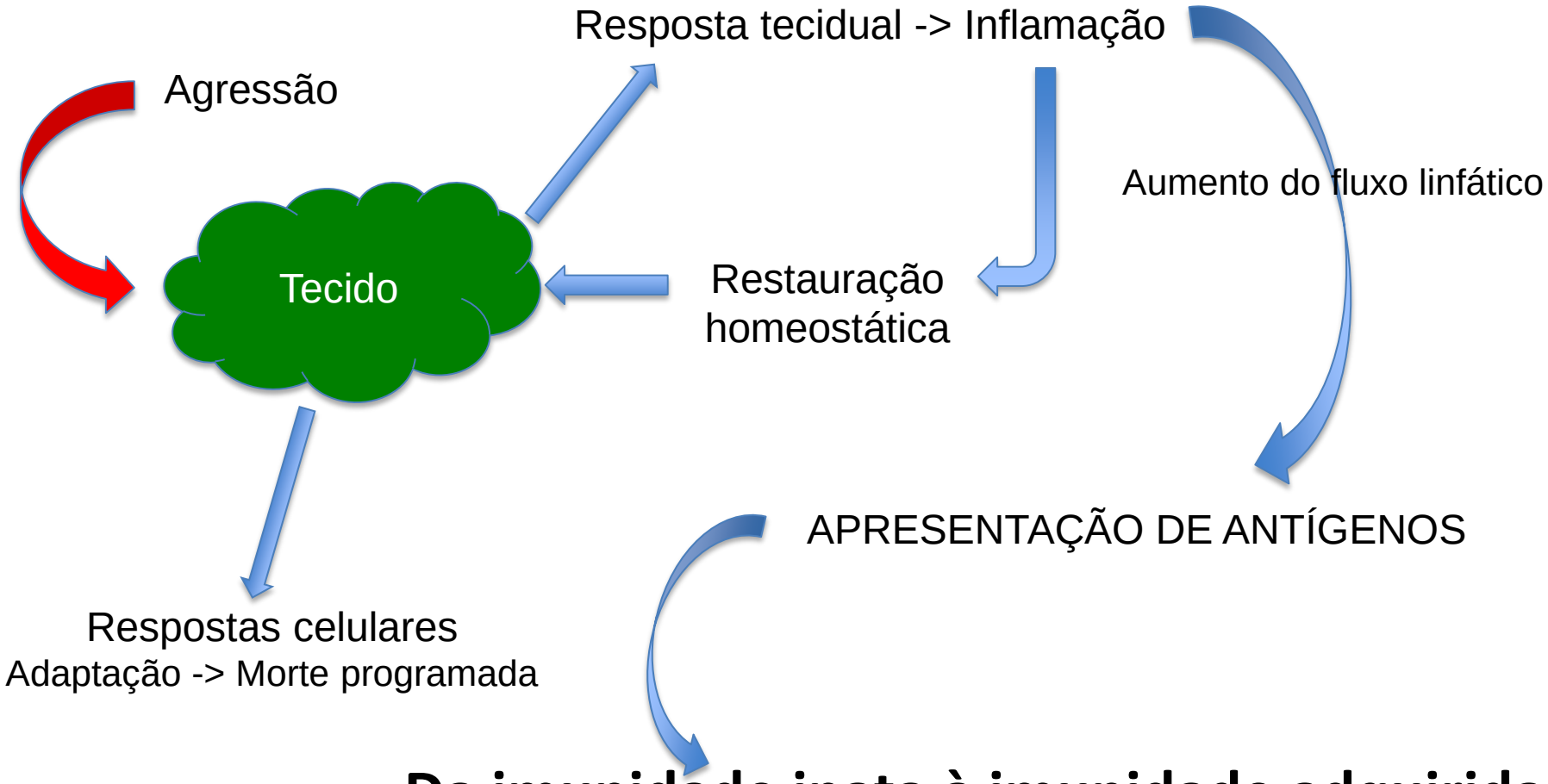
MAS:
E a microbiota normal?
E os antígenos alimentares?
E as doenças auto-imunes?

- não é apenas uma questão de repertório!

Decisão entre tolerância e resposta:
É também uma questão de (micro)ambiente!

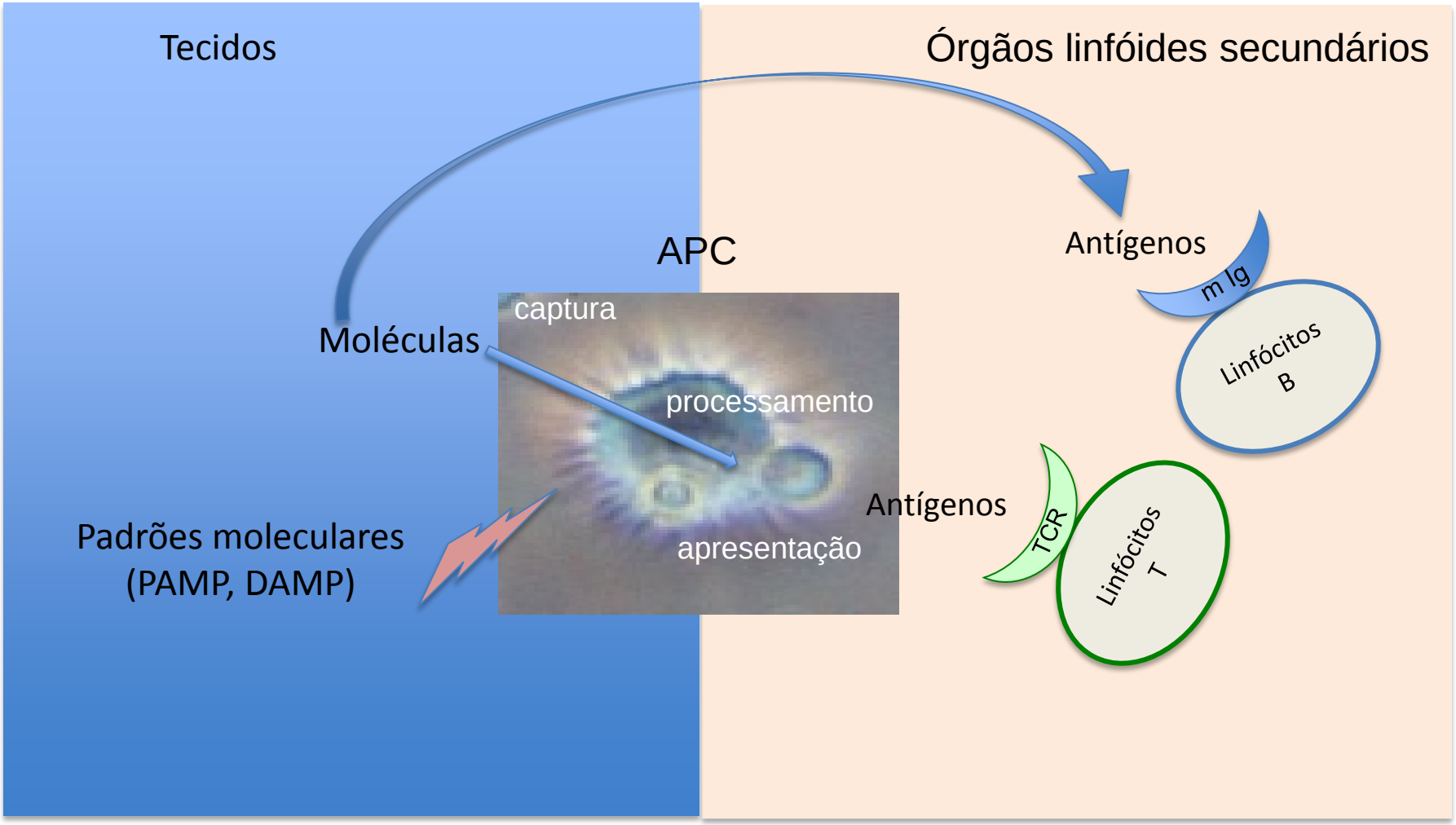


Os PRR desencadeiam vários fenômenos, mas, essencialmente: **Inflamação**

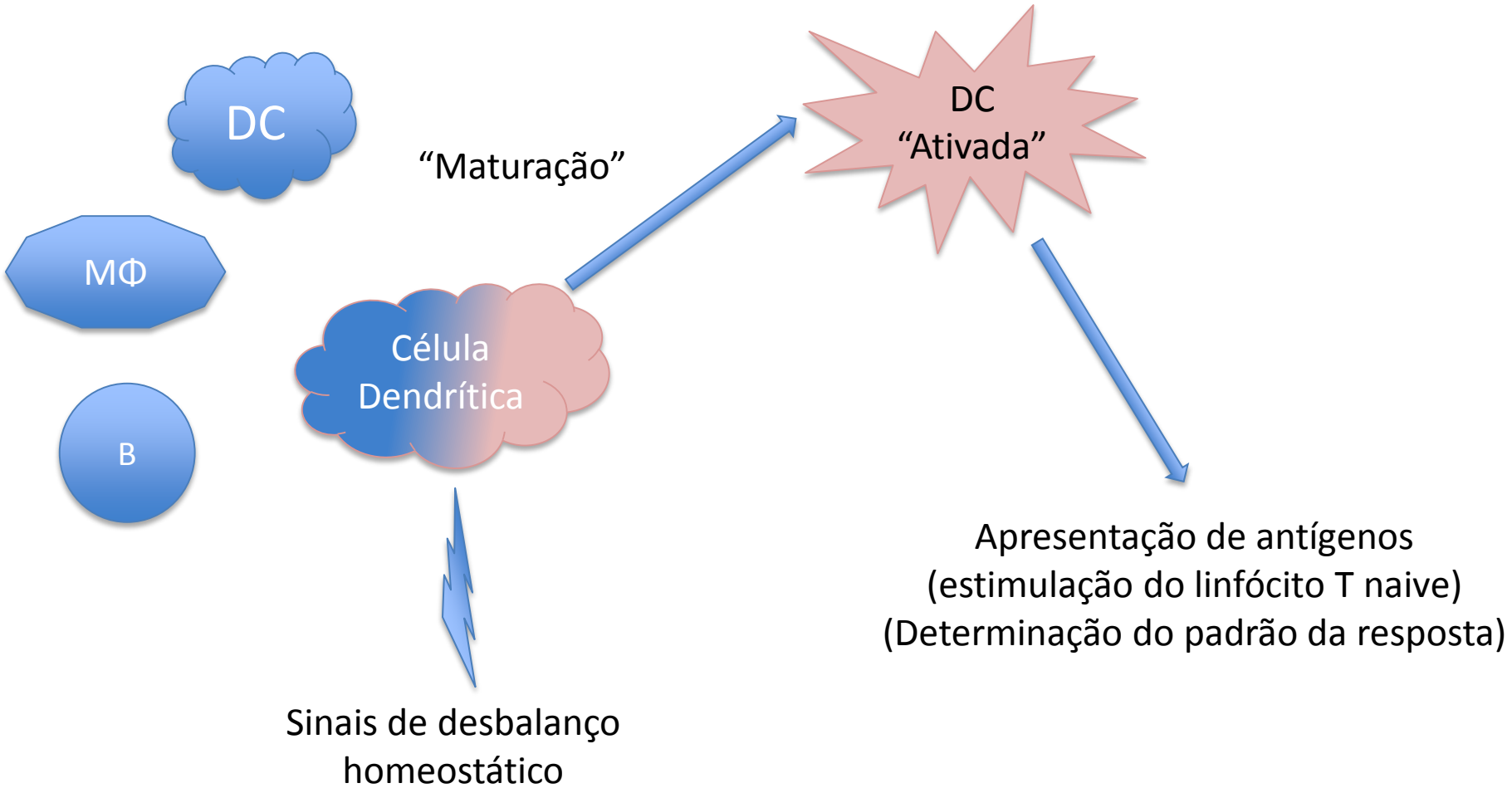


Da imunidade inata à imunidade adquirida

Apresentação de antígenos: ponte entre imunidade inata e adquirida



Células apresentadoras de antígenos (APC)



IDENTIFICATION OF A NOVEL CELL TYPE IN PERIPHERAL LYMPHOID ORGANS OF MICE

I. Morphology, Quantitation, Tissue Distribution*

By RALPH M. STEINMAN‡ and ZANVIL A. COHN

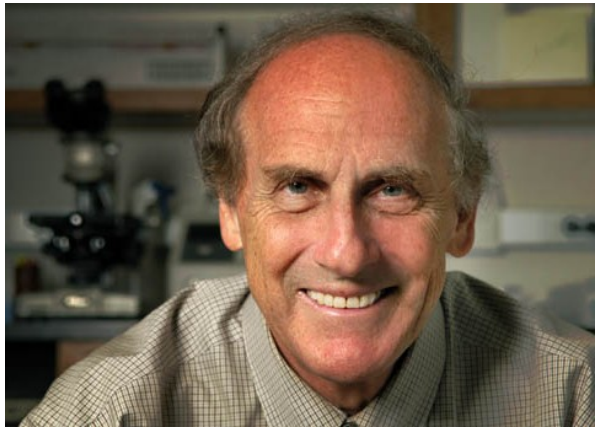
(*From The Rockefeller University, New York 10021*)

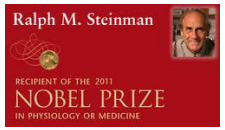
(Received for publication 19 January 1973)

During the course of observations on the cells of mouse spleen that adhere to glass and plastic surfaces, it was clear that this population was quite heterogeneous. In addition to mononuclear phagocytes, granulocytes, and lymphocytes, we noticed a large stellate cell with distinct properties from the former cell types. In this paper, we describe the morphology, quantitation, and tissue distribution of this novel cell as identified in vitro. In following papers, we will further characterize it with respect to its functional properties in vitro, as well as its localization and properties *in situ*.

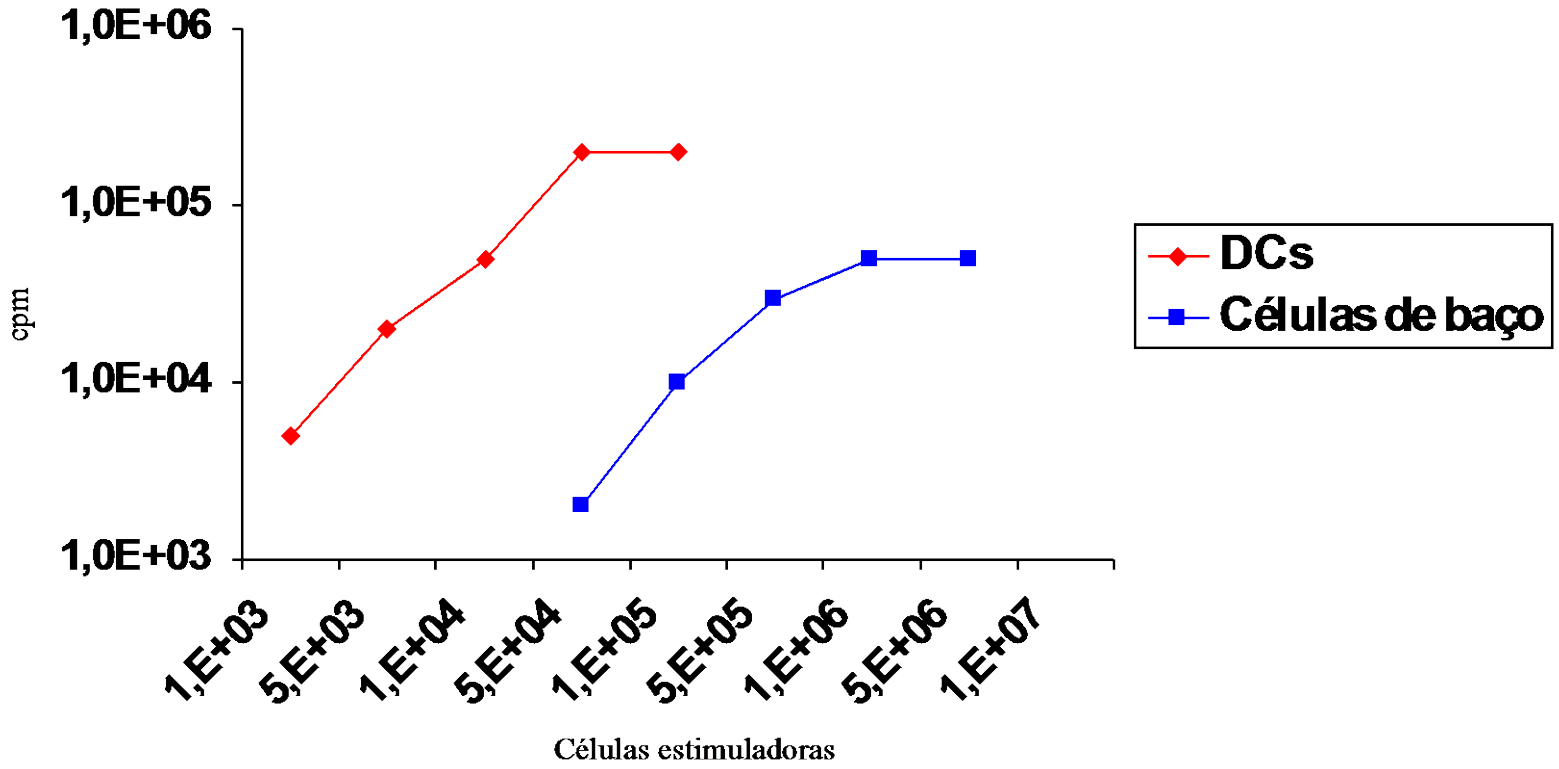
SUMMARY

A novel cell type has been identified in adherent cell populations prepared from mouse peripheral lymphoid organs (spleen, lymph node, Peyer's patch). Though present in small numbers (0.1–1.6% of the total nucleated cells) the cells have distinct morphological features. The nucleus is large, refractile, contorted in shape, and contains small nucleoli (usually two). The abundant cytoplasm is arranged in processes of varying length and width and contains many large spherical mitochondria. In the living state, the cells undergo characteristic movements, and unlike macrophages, do not appear to engage in active endocytosis. The term, dendritic cell, is proposed for this novel cell type.





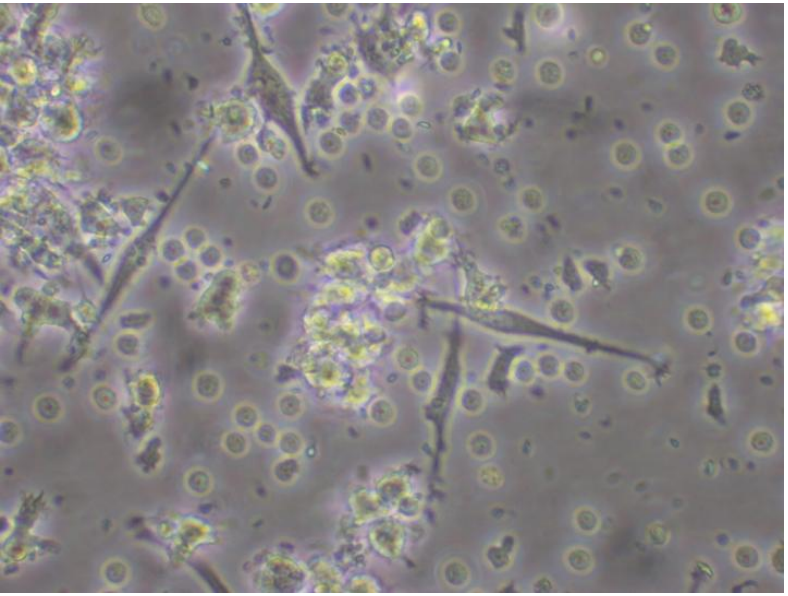
Células dendríticas são APCs “únicas”



Steinman & Witmer, 1978

Ativação/Maturação das DCs

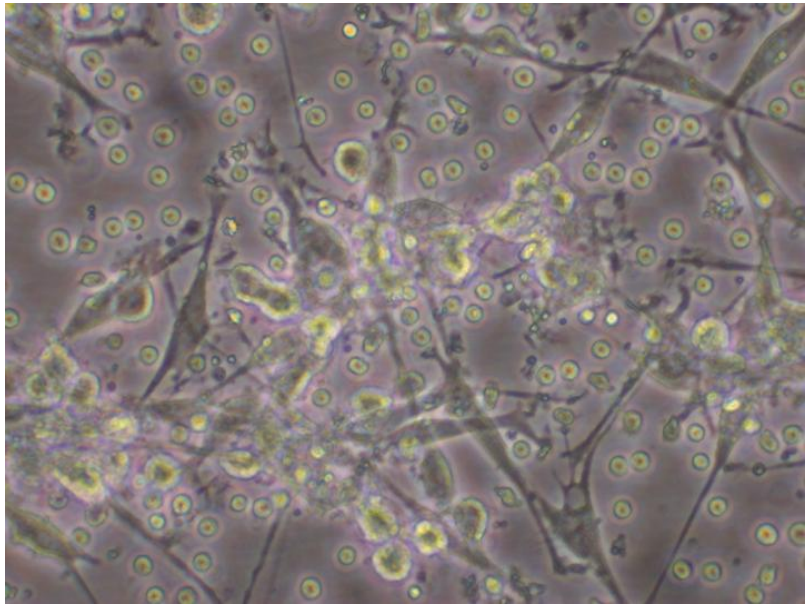
iDCs



Alta endocitose
Alta síntese/baixa densidade de HLA
Baixa capacidade co-estimuladora
CCR1, 5 e 6 expressos

“desequilíbrio”

mDCs

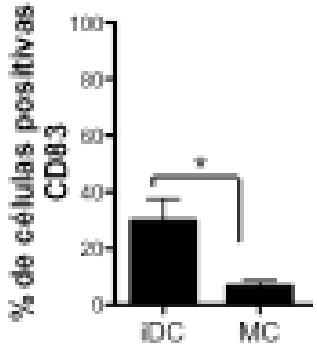


Baixa endocitose
Baixa síntese/alta densidade de HLA
Alta capacidade co-estimuladora
CCR7 expresso

Tecidos periféricos → *MIP-3b/SLC* → **Órgãos linfóides (DC interdigitante)**

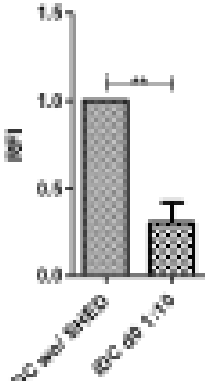
Interações com células do microambiente modificam as DCs

Mastócitos



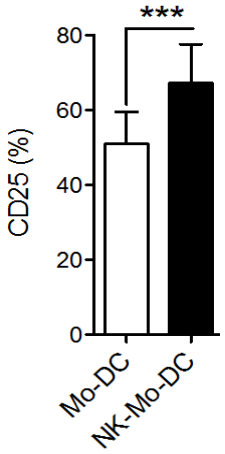
Expressão de CD83

Células-tronco mesenquimais



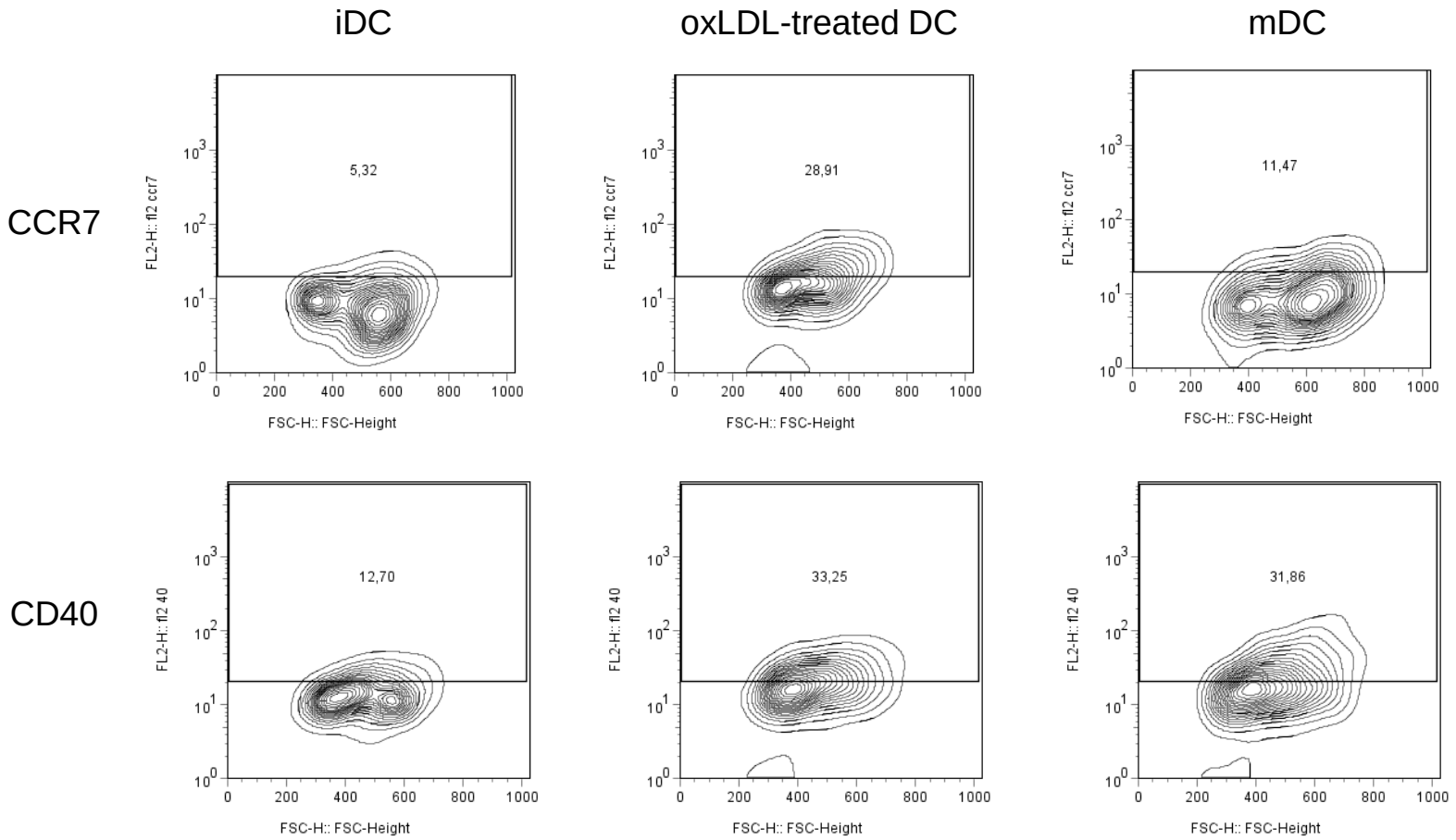
Expressão de BDCA-1

Células NK



Ativação de Linfócitos T

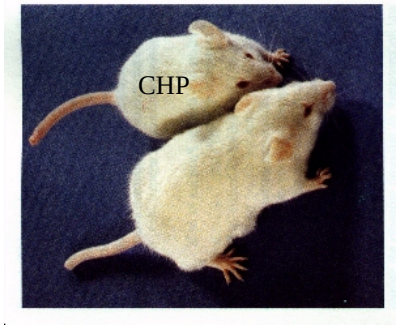
DCs são sensíveis a diferentes sinais de "desequilíbrio"

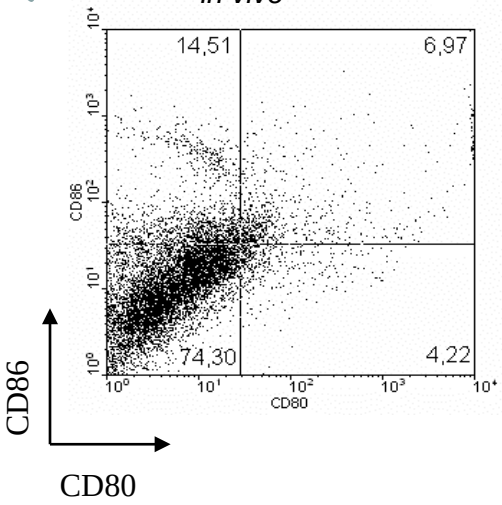


Cohabitation with a B16F10 melanoma-bearer cage mate influences behavior and dendritic cell phenotype in mice

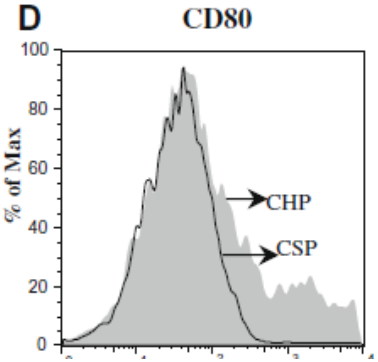
M.Y. Tomiyoshi [a], M. Sakai [b], R.B. Baleeiro [a], D. Stankevicius [b], C.O. Massoco [b], J. Palermo-Neto [b], J.A.M. Barbuto [a,*]

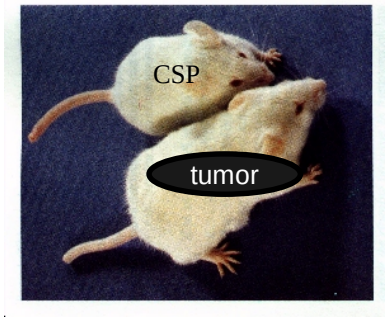
DCs "residentes"
in vivo

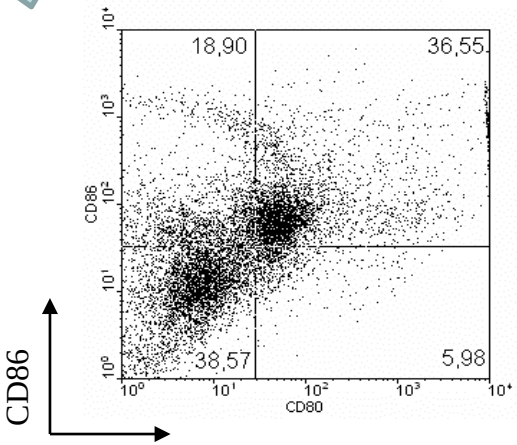


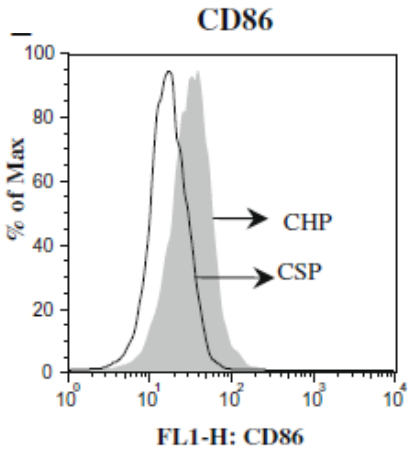


DCs "novas"
in vitro



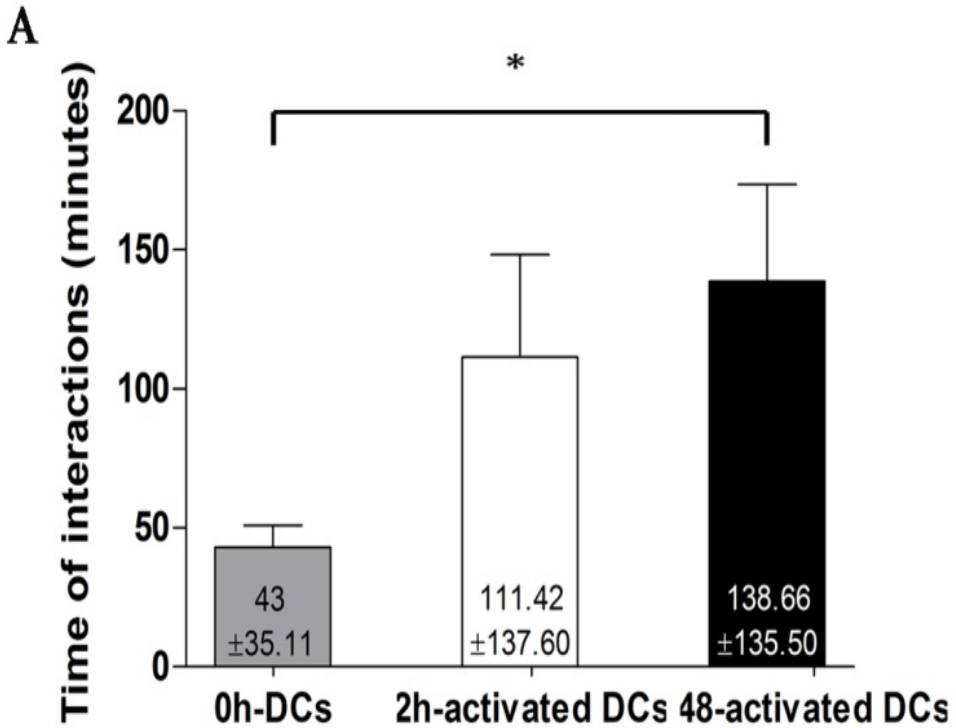






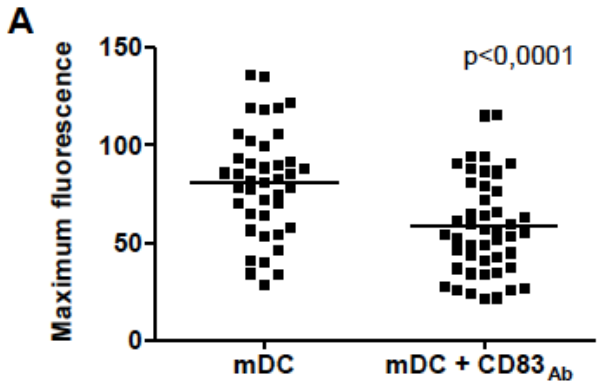
Brain, Behavior, and Immunity 23 (2009) 558–567

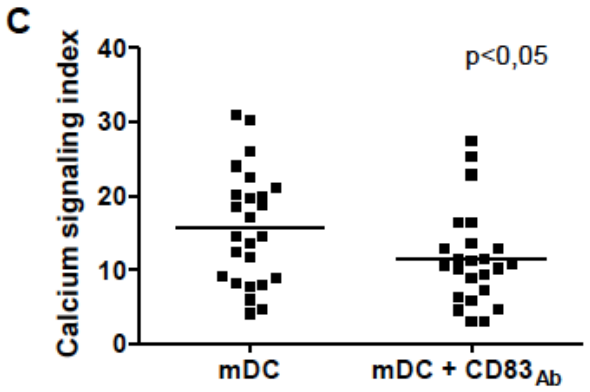
Ativação das DCs permite que interajam mais longamente com os linfócitos T

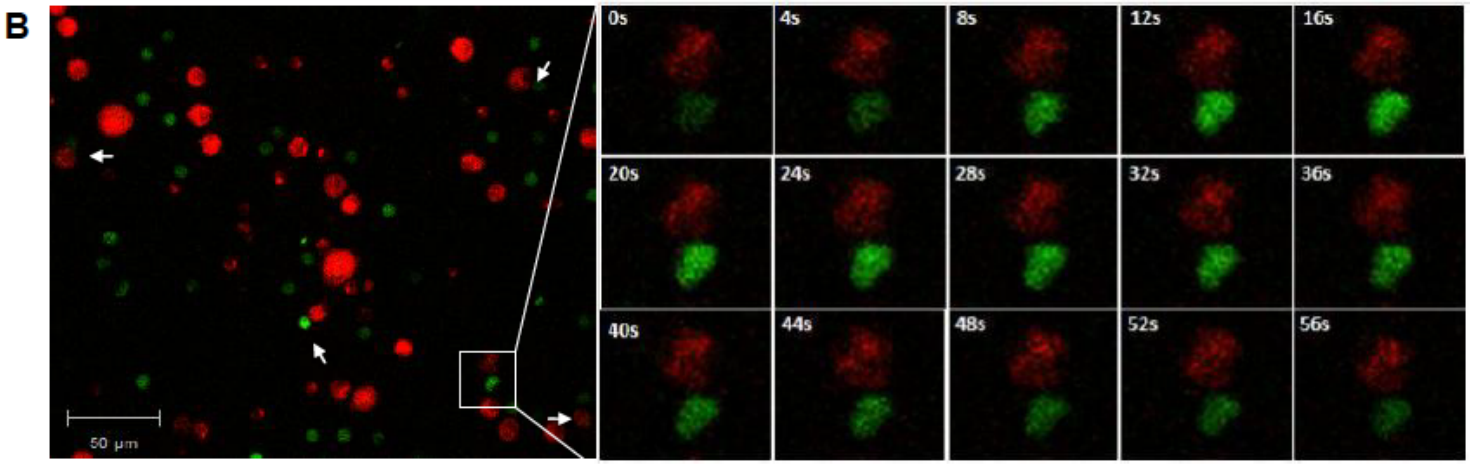


Karen Steponavicius-Cruza, Vanessa Morais Freitas, José Alexandre M. Barbuto. Dendritic cells and T lymphocytes interactions in a novel 3D system. 3rd International Conference on Tissue Engineering. Procedia Engineering (2013)

Ativação das DCs permite que sinalizem mais eficientemente para os linfócitos T







Pinho MP, Migliori IK, Flatow EA, Barbuto JA. Dendritic cell membrane CD83 enhances immune responses by boosting intracellular calcium release in T lymphocytes. J Leukoc Biol. 2014

Estado funcional das DCs no câncer?

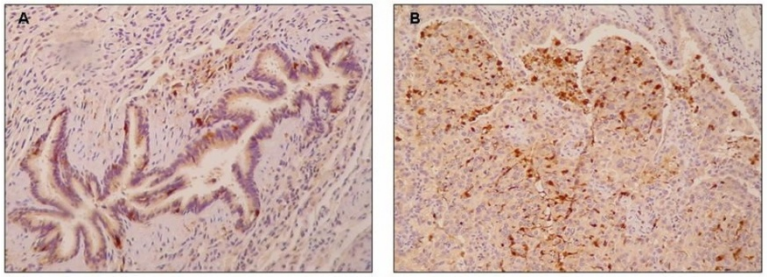
- Balanço entre macrófagos e DCs no tumor?
- Estado de ativação/maturação das DCs?

Baleeiro RB, Anselmo LB, Soares FA, Pinto CA, Ramos O, Gross JL, Haddad F, Younes RN, Tomiyoshi MY, Bergami-Santos PC, Barbuto JA. High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-alpha in lung cancer. Cancer Immunol Immunother. 2008;57(9):1335-45

Nos tumores, DCs são disfuncionais

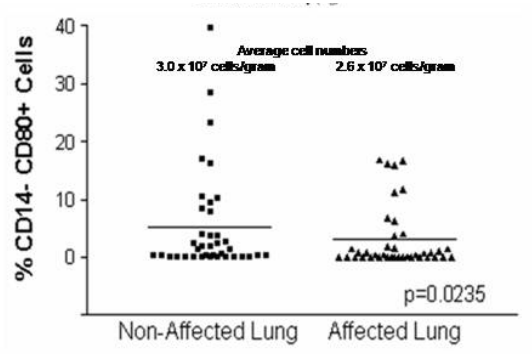
- Distribuição

Lung Tumor



(S100+ cells)

- Ativação



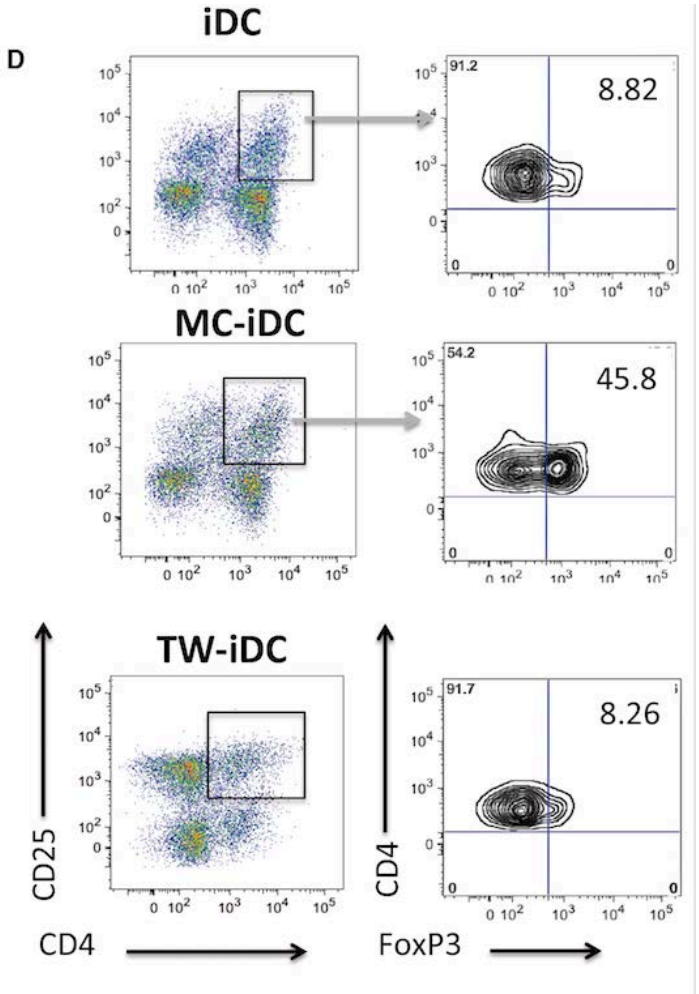
- "Subtipos"

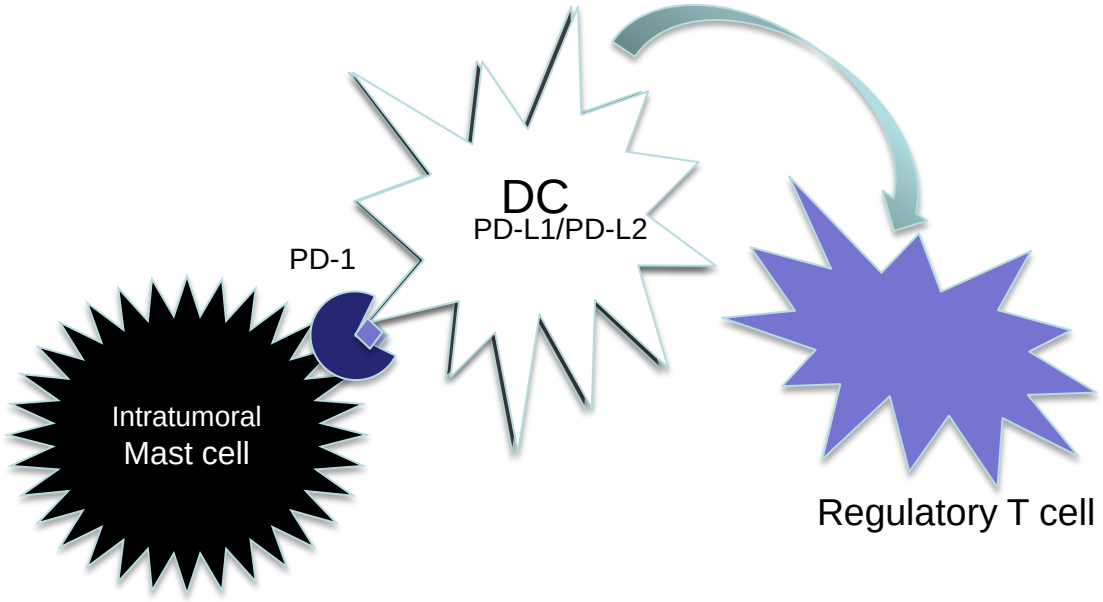
	Lung	Tumor	P
CD1a	6.2	39.3	0.0371
S-100	27.5	59.3	0.0177
CD68	172.4	298.8	0.0108
Mast cells	0.5	2.9	0.0522

Baleeiro, R. B. et al. (2008). High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-alpha in lung cancer. *Cancer Immunology, Immunotherapy : CII*, *57*(9), 1335–1345.

Vias para disfunção das DCs

Mastócitos – desviando a forma de apresentação de Ag





Rodrigues CP et al..Front Immunol. 2016 Jan 25;7:9.

- SE :
  - DCs são fundamentais para a indução de respostas imunes,
  - E estão alteradas nos tumores;

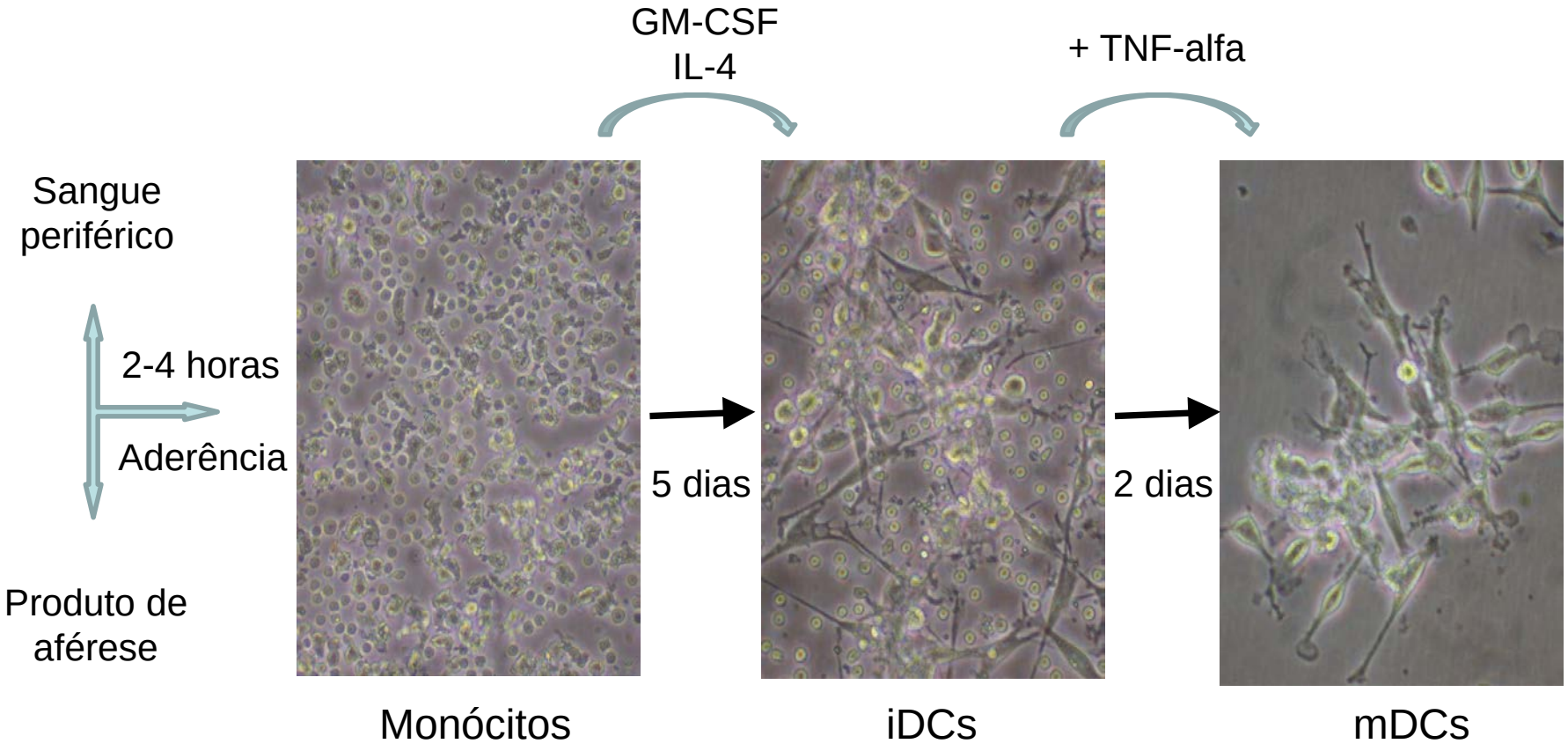
- ENTÃO:

  DCs “funcionais” seriam um instrumento poderoso para intervenção em portadores de câncer!

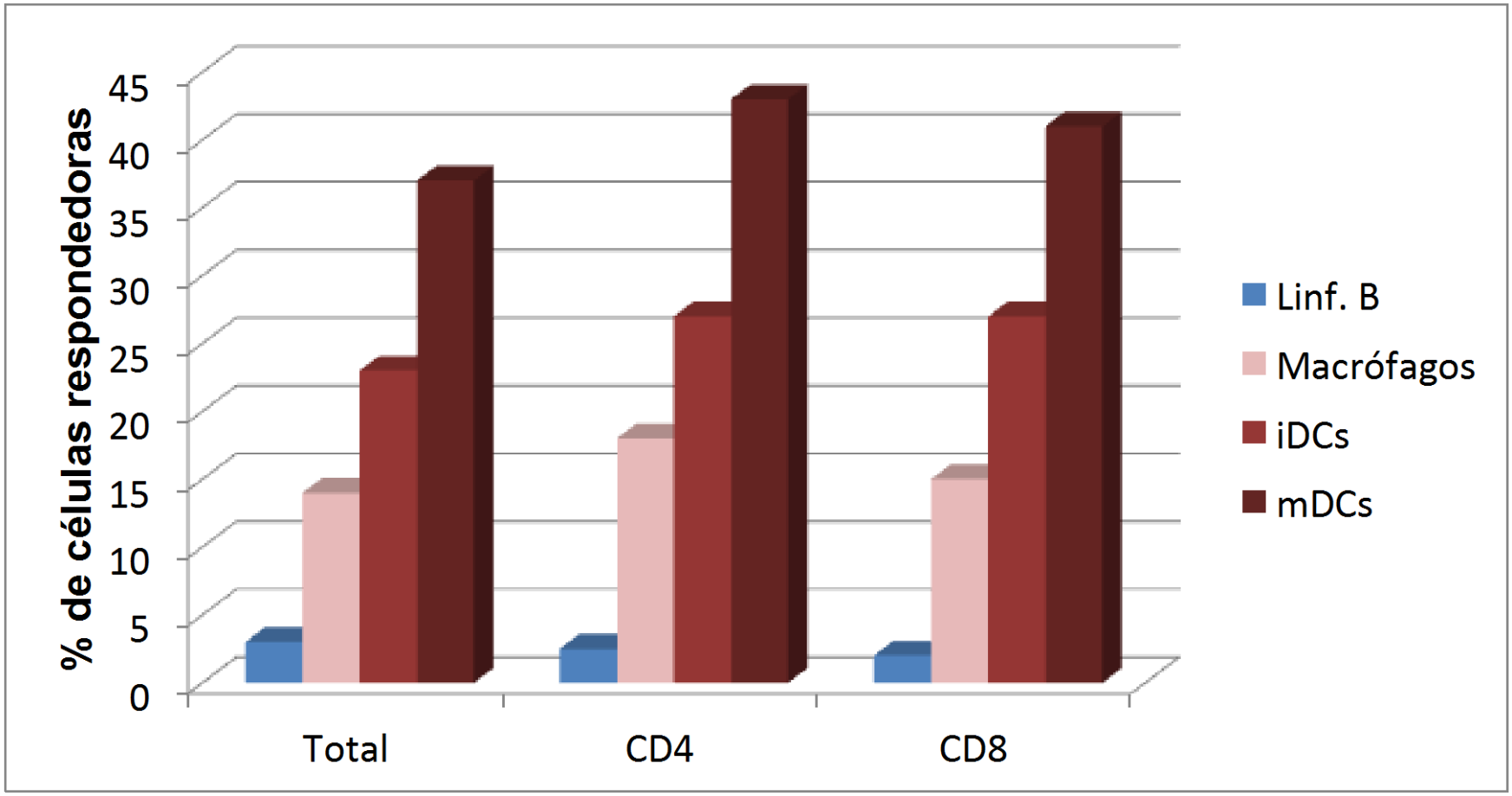
Vacina contra o câncer

Como conseguir DCs para uso clínico?

Diferenciação de DCs in vitro

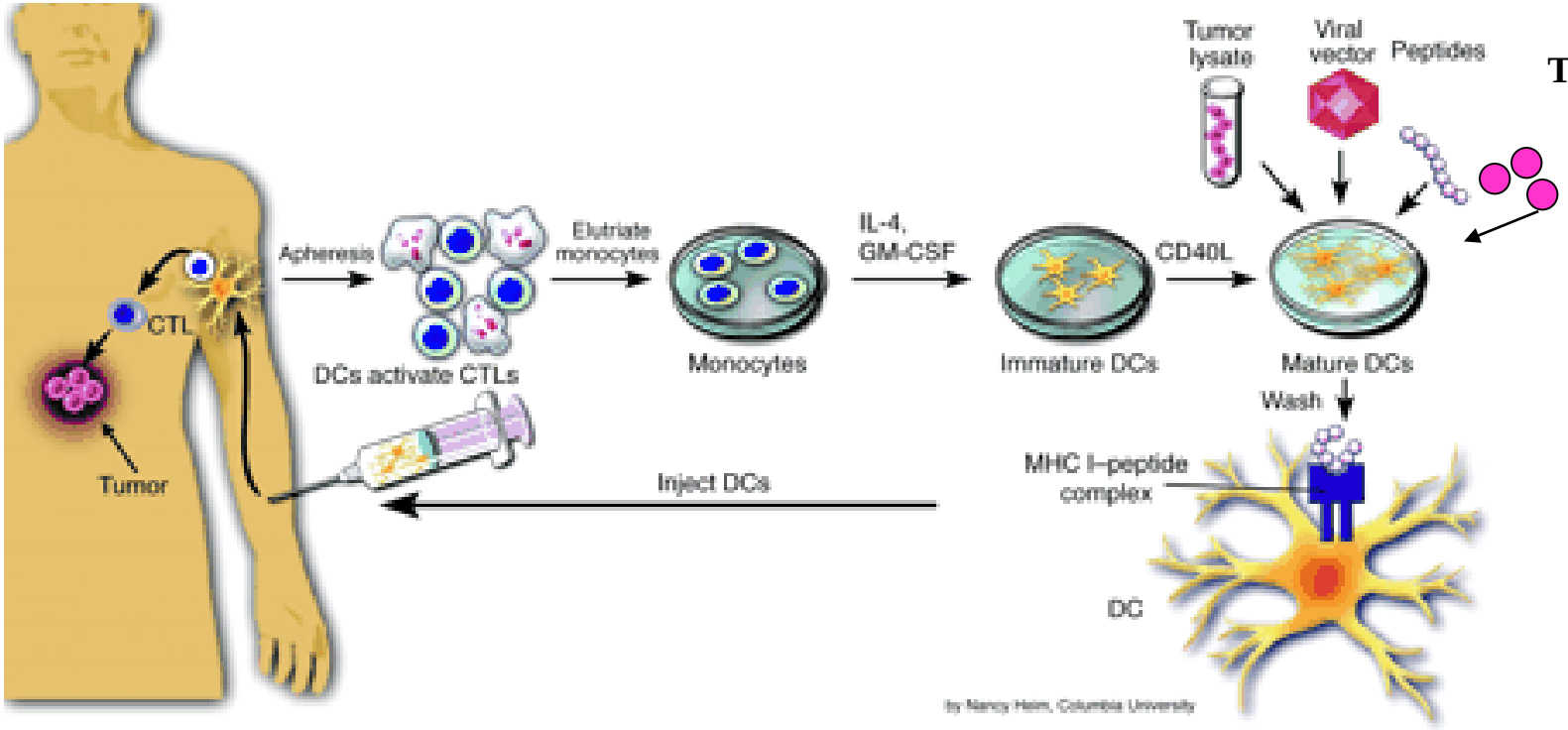


Maturação das DCs *in vitro também* aumenta seu poder linfoestimulador



Chin L S. Avaliação da ativação linfocitária por diferentes combinações de células apresentadoras de antígenos (ICB-USP, 2010).

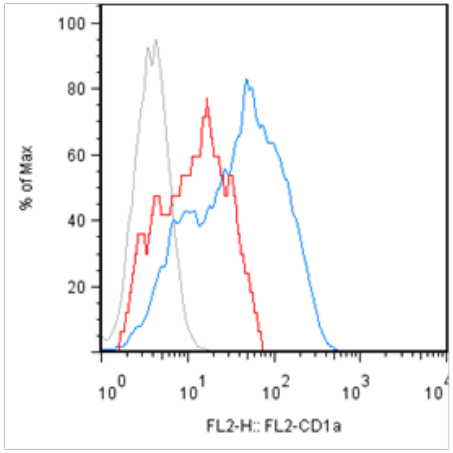
Estratégias de vacinação baseadas em DCs

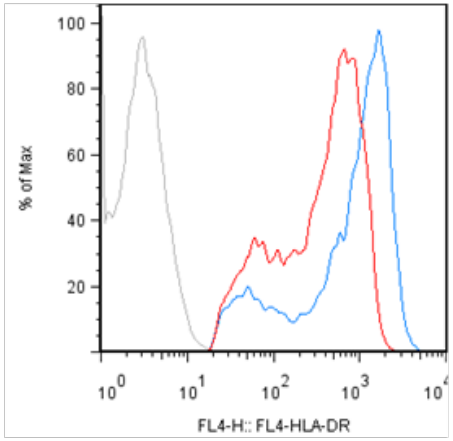


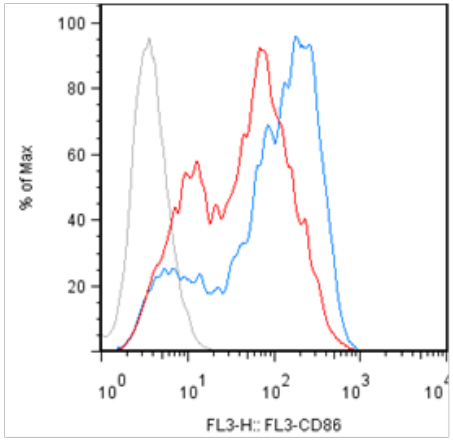
Berzofsky et al., 2004 (modificado)

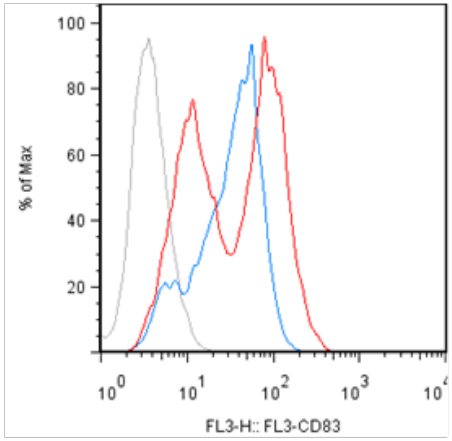
DCs derivadas de monócitos (Mo-DCs) são heterogêneas

Efeitos do meio de cultura





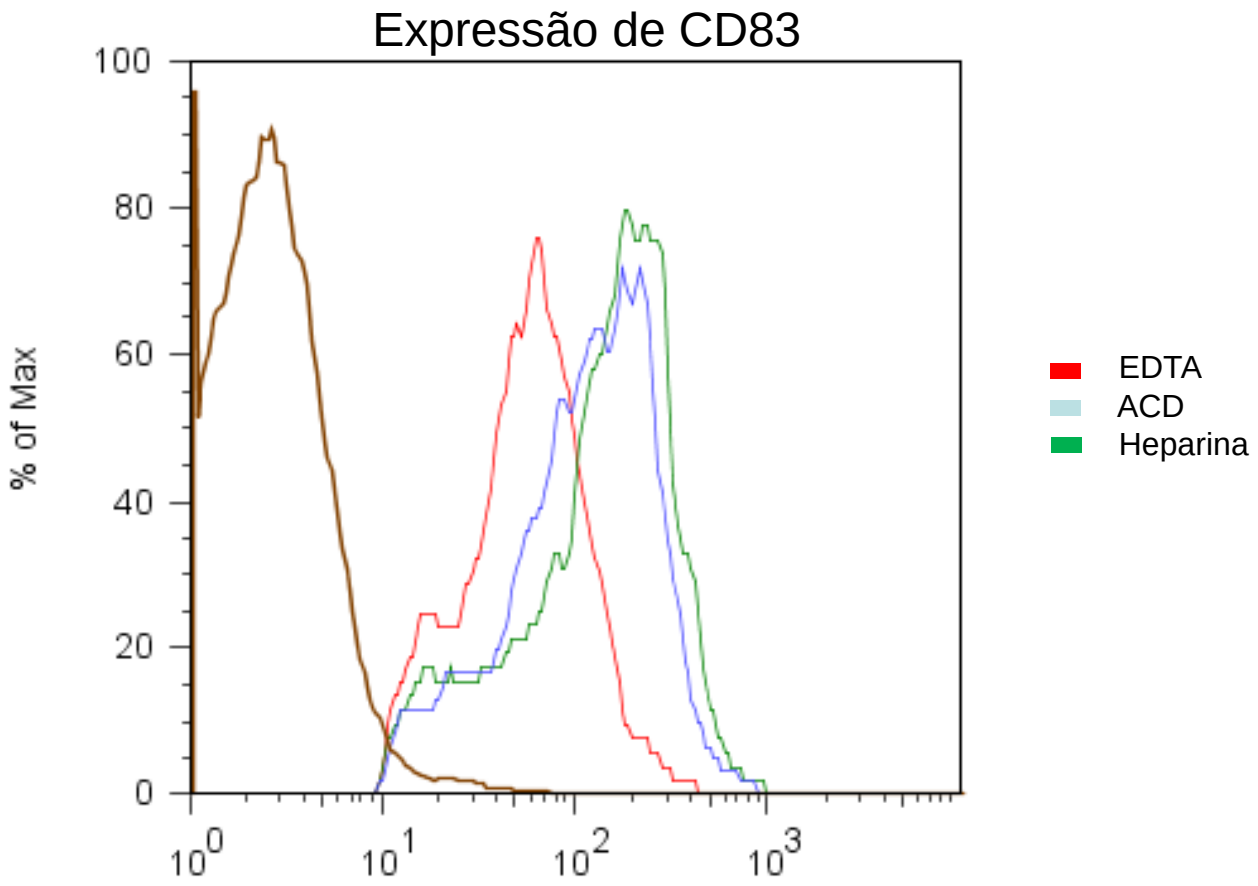




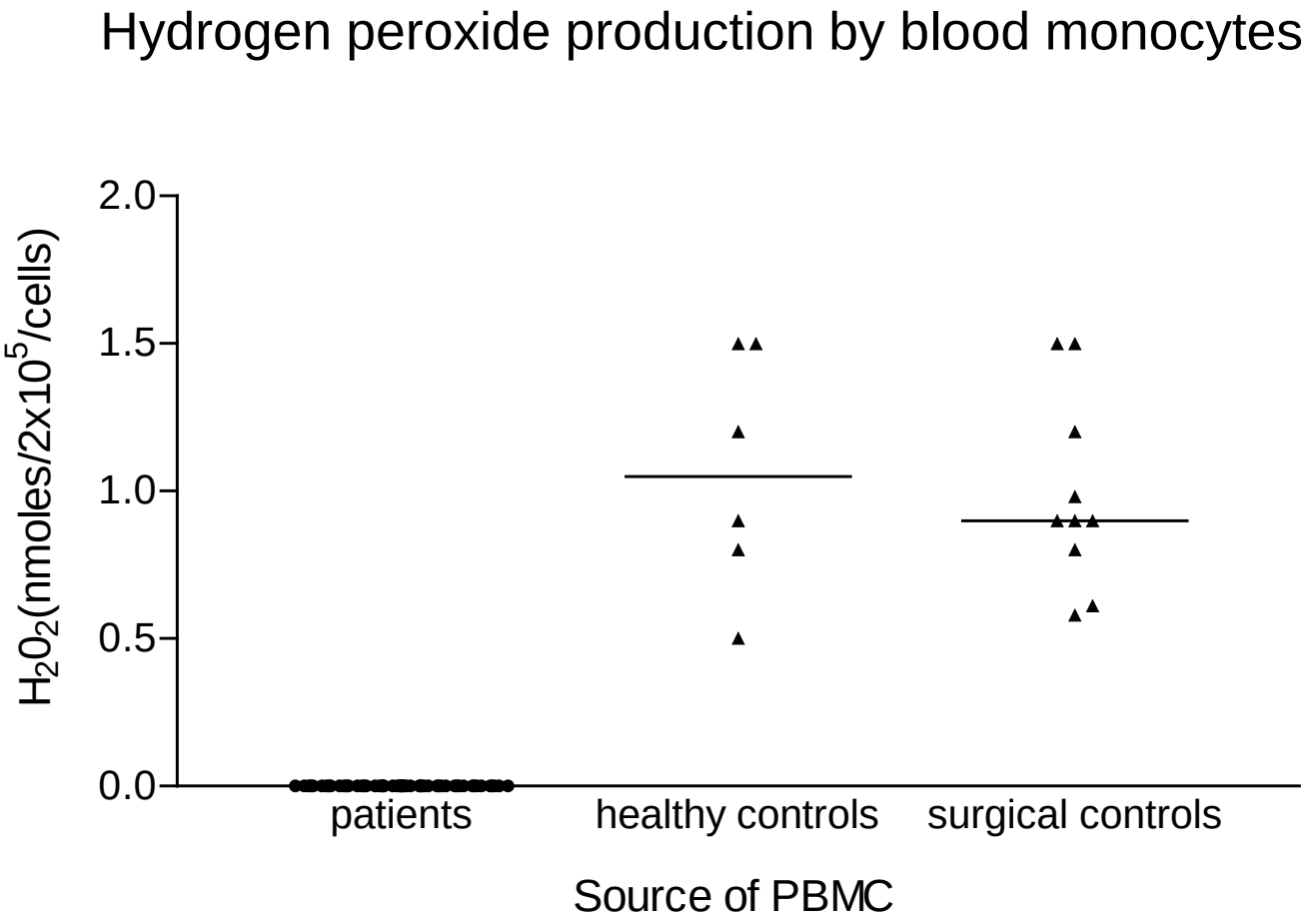
Meio AIM-V
Meio RPMI-1640 +10% SFB

Mo-DCs são heterogêneas

Efeitos do anticoagulante



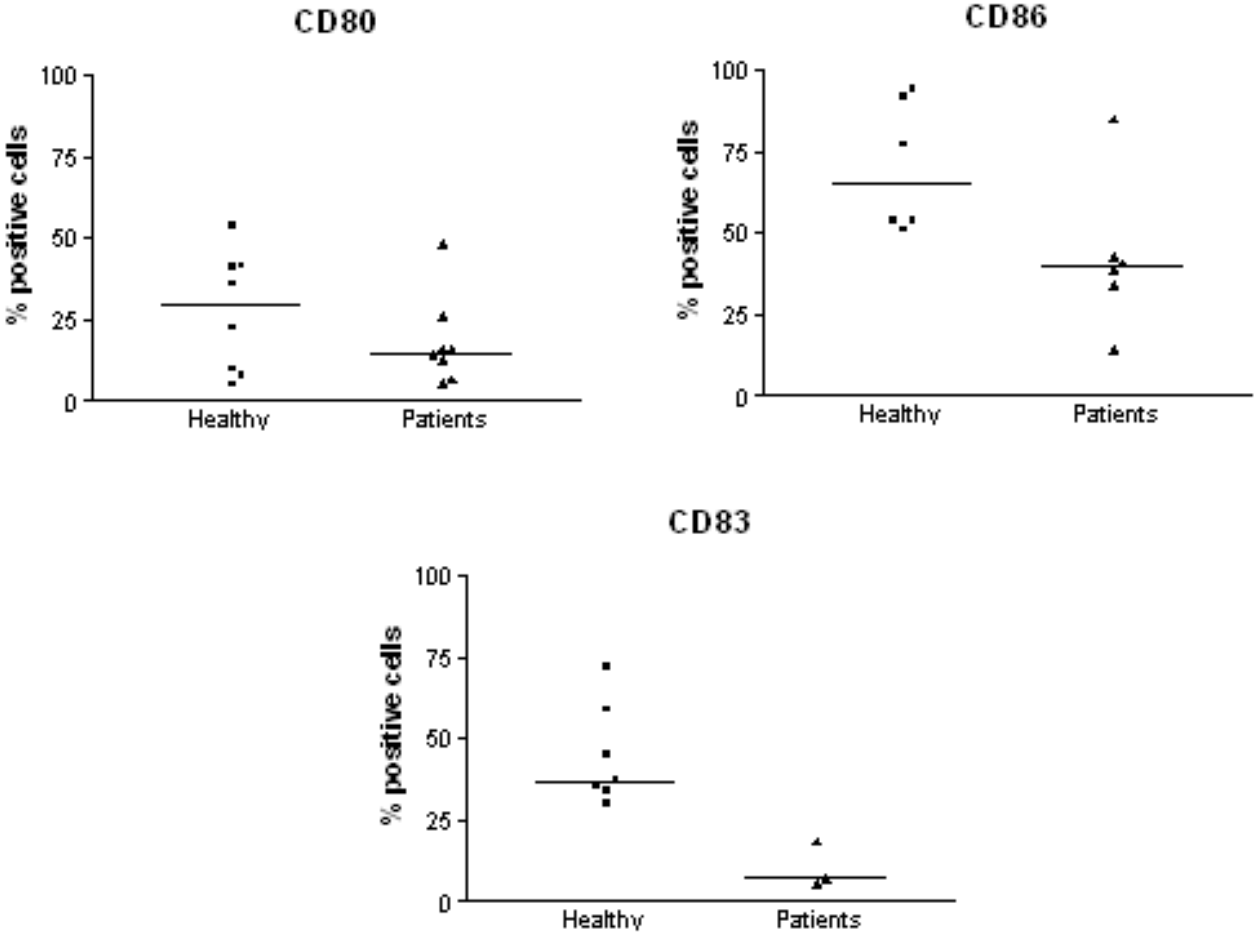
Monócitos de pacientes com câncer são “deficientes”



Treglia-Dal Lago M et al. ***Pancreatology****. 2006;6(4):273-8.*

Mo-DCs de pacientes com câncer apresentam distúrbios fenotípicos

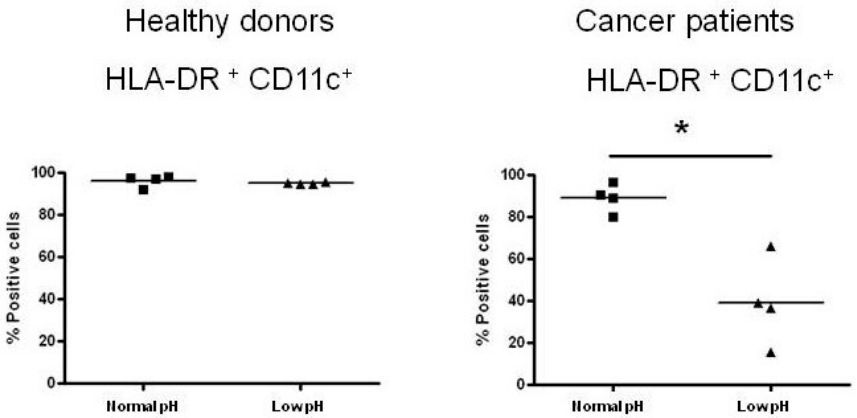
Pacientes com carcinoma renal e melanoma



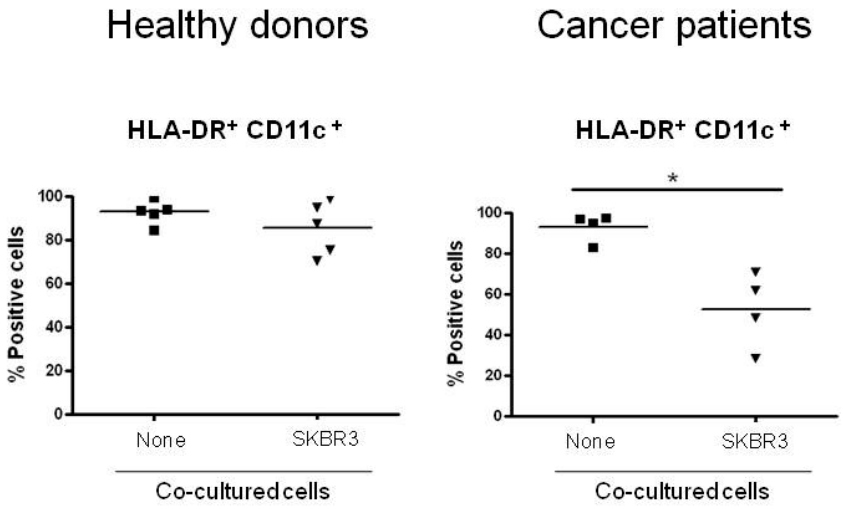
Neves AR, Ensina LF, Anselmo LB, Leite KR, Buzaid AC, Câmara-Lopes LH, Barbuto JA. Dendritic cells derived from metastatic cancer patients vaccinated with allogeneic dendritic cell-autologous tumor cell hybrids express more CD86 and induce higher levels of interferon-gamma in mixed lymphocyte reactions. Cancer Immunol Immunother. 2005;54(1):61-6

Mo-DC de pacientes com câncer sofrem mais em condições “adversas” do microambiente

Baixo pH



Presença de células tumorais



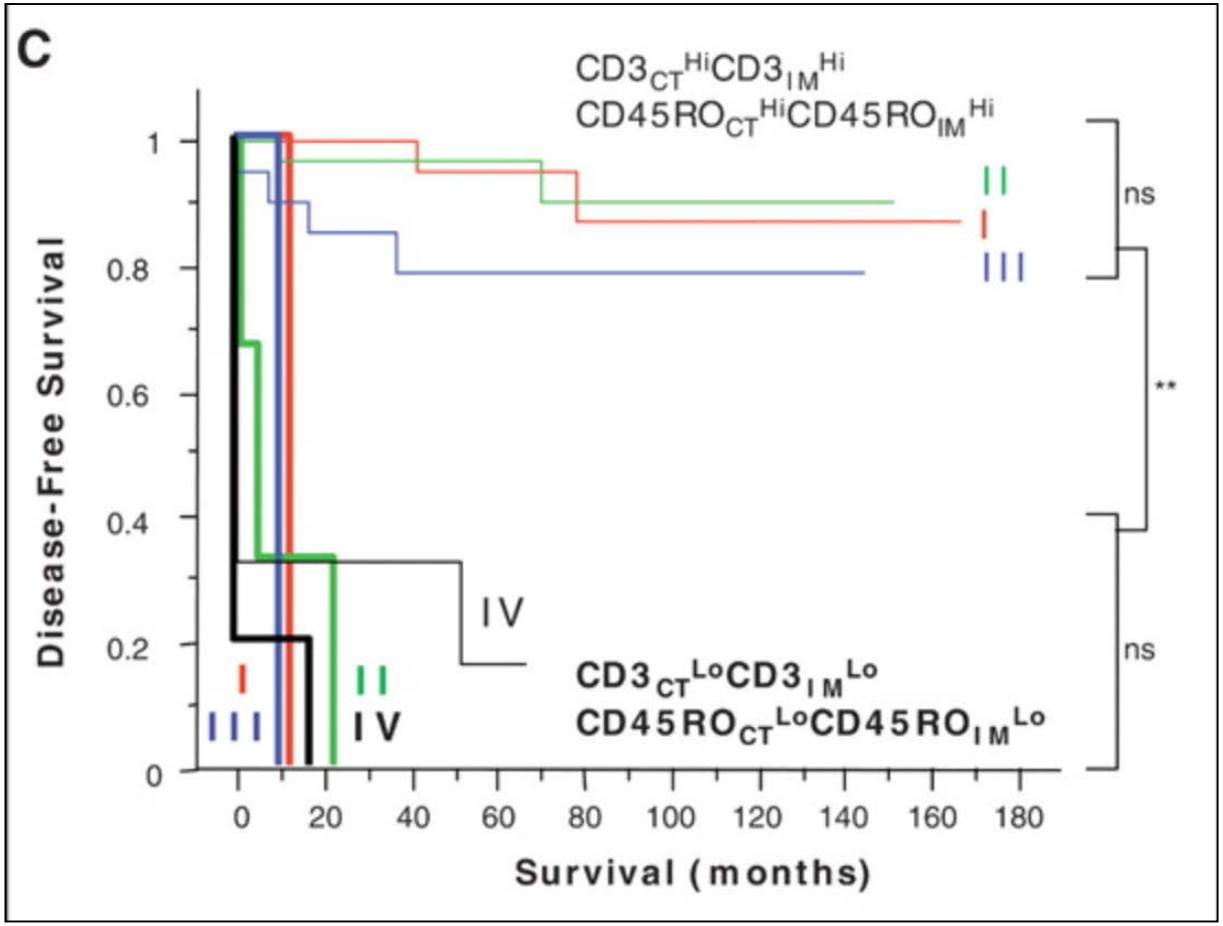
Functional deficits in monocytes and monocyte-derived dendritic cells of breast cancer patients
[1]A.P.S Azevedo, [2]F. Laginha , [1]G.G Romagnoli, [3]F.R.F. Nascimento, [1]P.C. Bergami-Santos, [4]M.A. Nagai and [1]J.A.M. Barbuto. (submetido)

Are dysfunctional monocyte-derived dendritic cells in cancer an explanation for cancer vaccine failures?

"...what one should urgently seek is the means to bypass this functional deviation and obtain mature and functionally effective monocyte-derived dendritic cells from cancer patients, thus achieving the expected functional response of the patients' immune system."

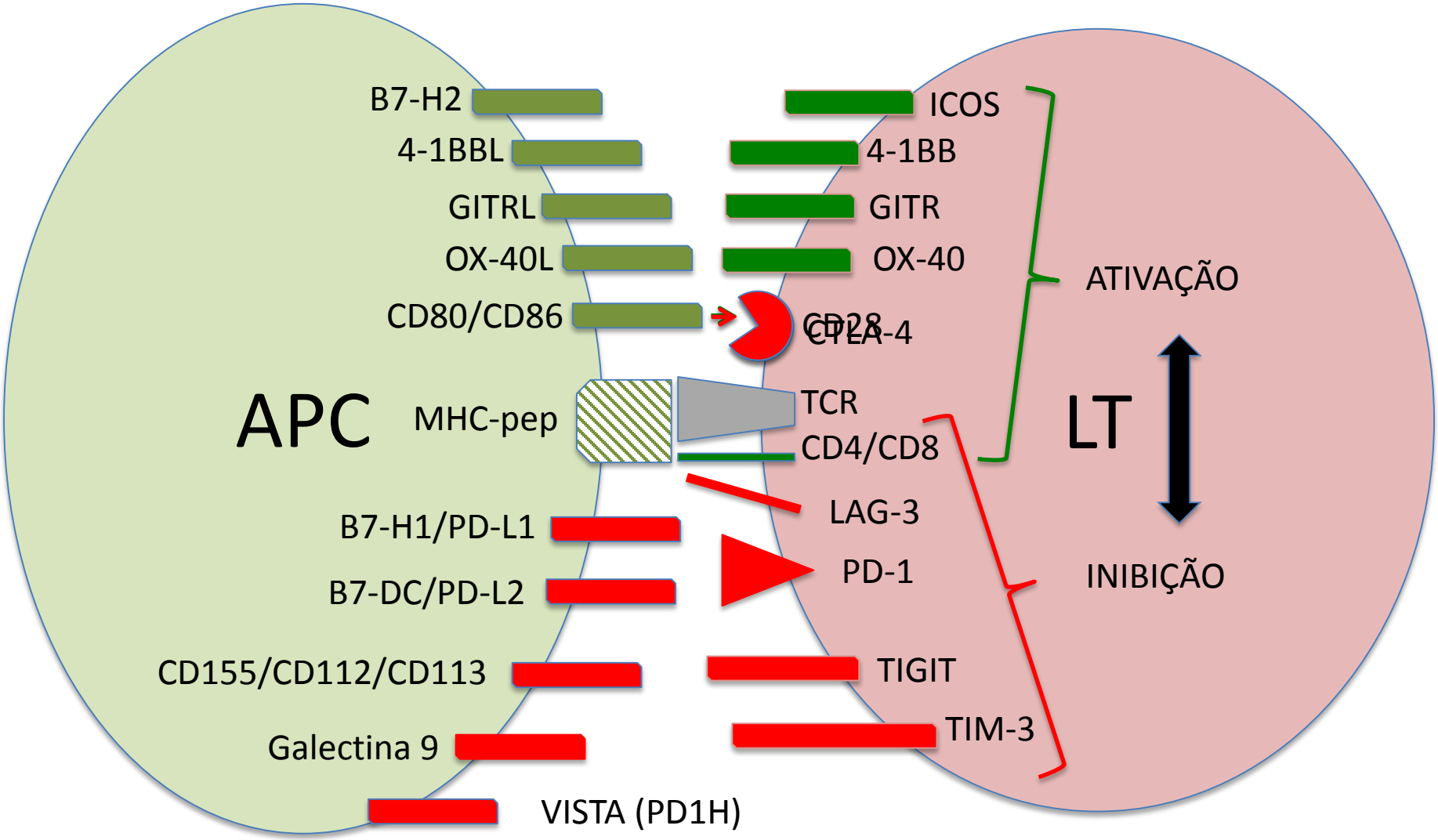
Immunotherapy (2013) **5**(2), 105–107

Por outro lado, os dados clínicos mostram que o sistema imune influencia a evolução da doença



Jérôme Galon et al. Science 2006;313:1960-1964

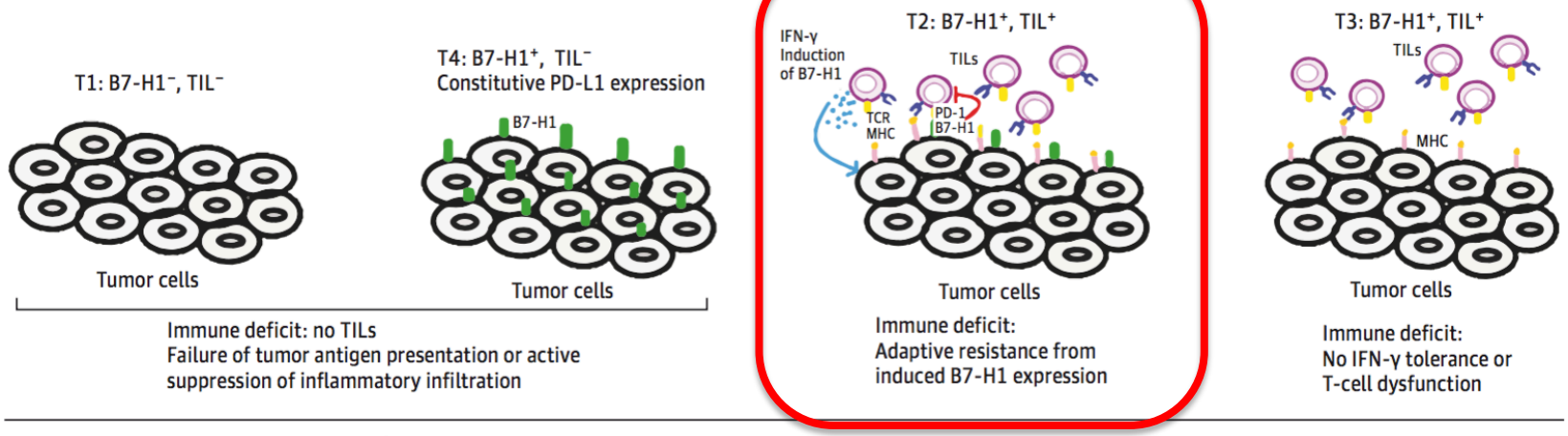
Explorando os *checkpoints* do sistema imune:



Imunoterapia às “avessas”

Mas somente “desregular” o sistema pode não ser suficiente...

Figure. Tumor Immunity in MicroEnvironment (TIME) Classification



B7-H1 indicates B7 homolog 1; IFN, interferon; MHC indicates major histocompatibility complex; PD-1, programmed cell death 1; T1-T4, tumor subtypes; TIL, tumor-infiltrating lymphocyte.

Zhang, Y. and L. Chen. 2016. “Classification of Advanced Human Cancers Based on Tumor Immunity in the MicroEnvironment (TIME) for Cancer Immunotherapy..” *JAMA oncology*.

To treat cancer we need a

TEAM

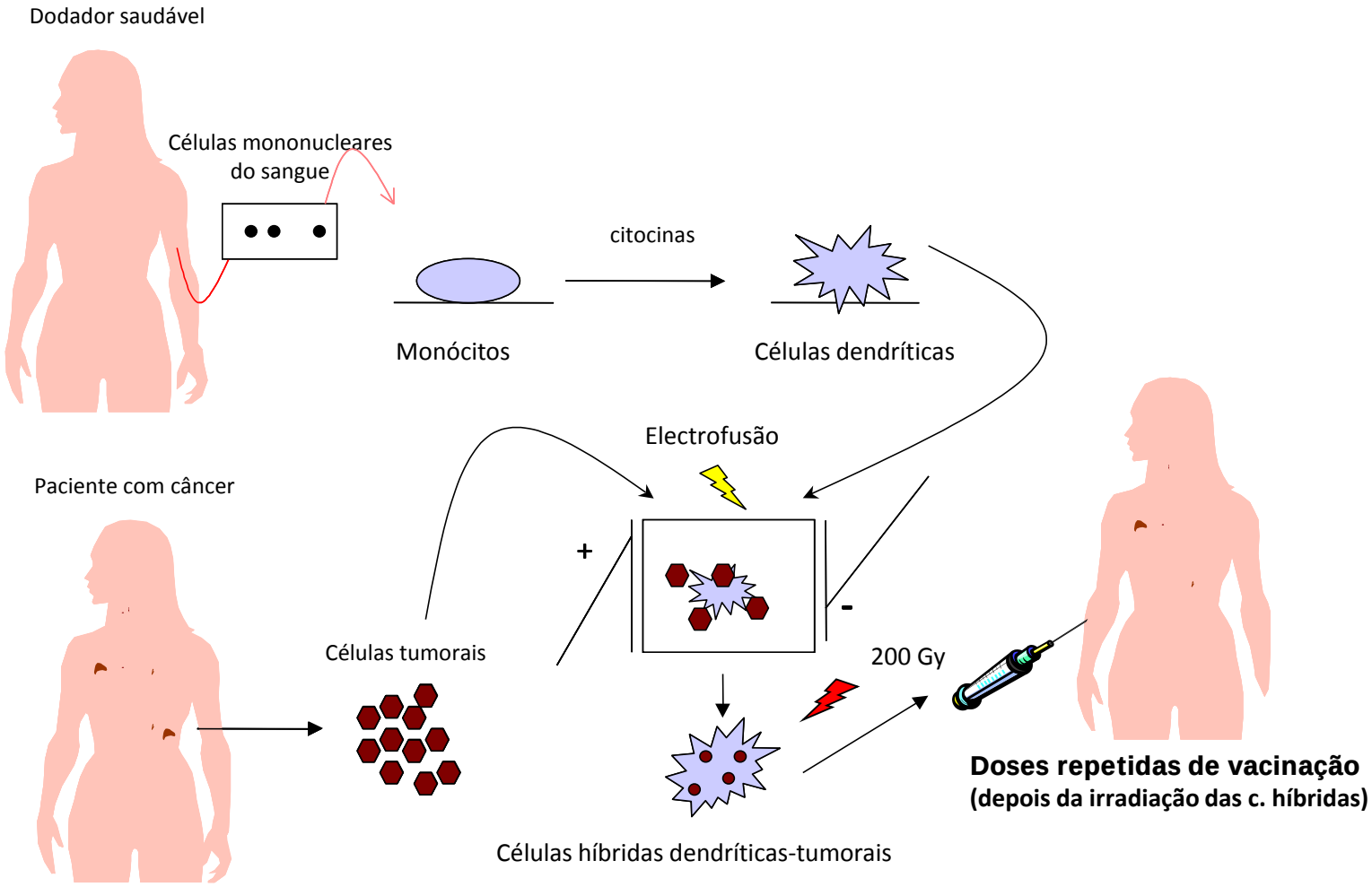
Tumor **E**nvironment **A**ctive **M**odulation

A célula dendrítica

Um sensor muito sensível do microambiente
E o “portal” da imunidade adquirida

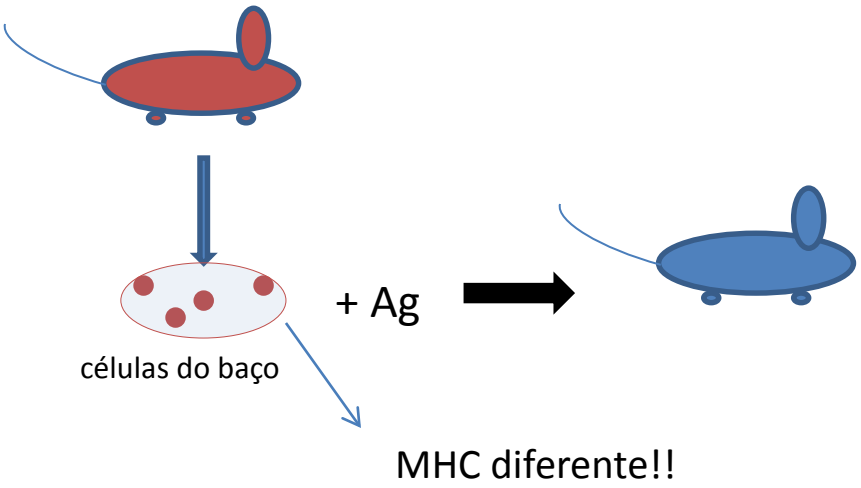
Mas, e os “defeitos” das DCs dos pacientes??

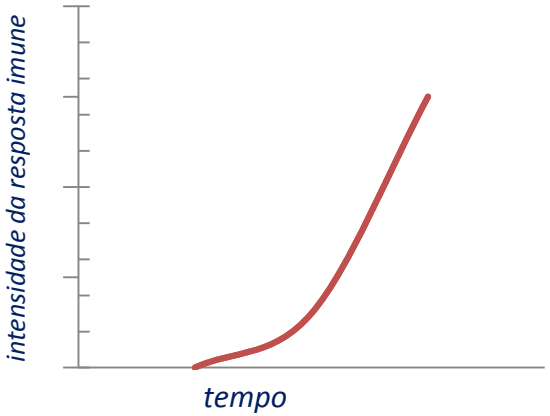
Um a estratégia para contornar o defeito e ganhar um "bônus":

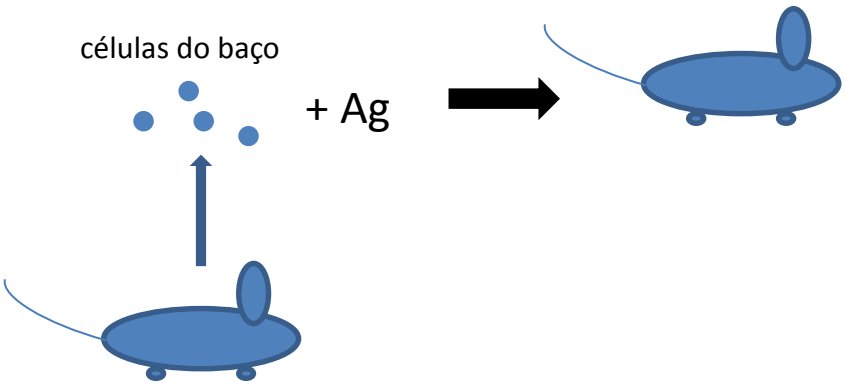


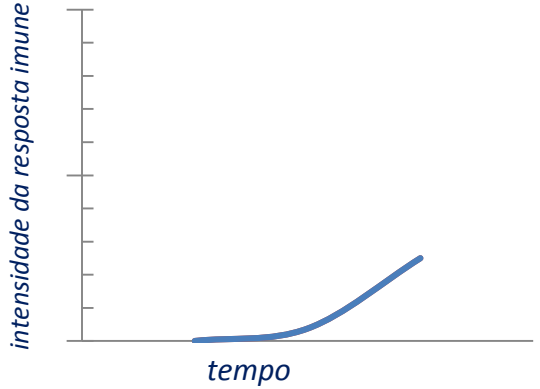
Barbuto JAM, Ensina LFC, Neves AR, Bergami-Santos PC, Leite KRM, et al. 2004. *Cancer Immunol. Immunother.* 53(12):1111–18

O bônus: o efeito alogenêico



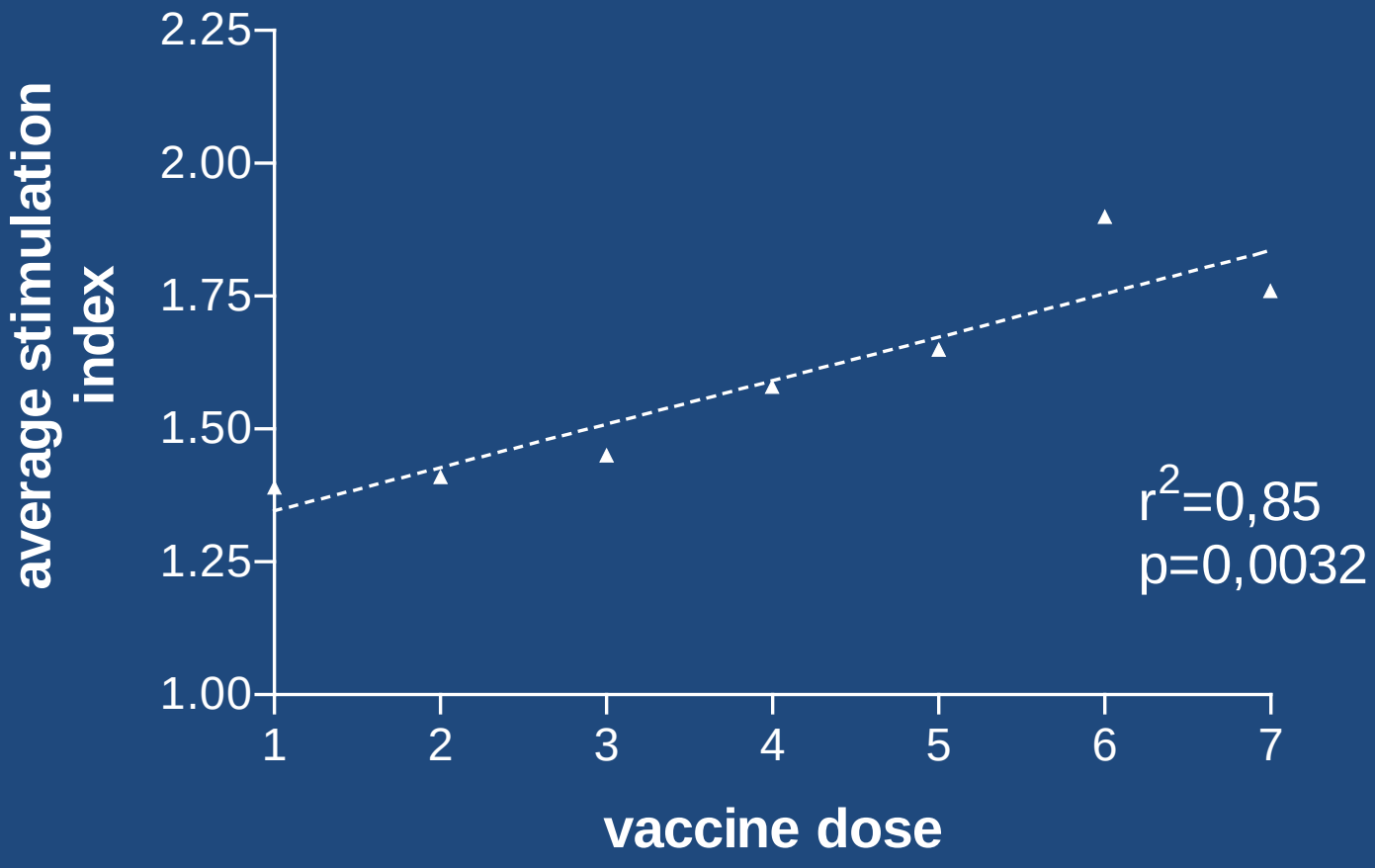






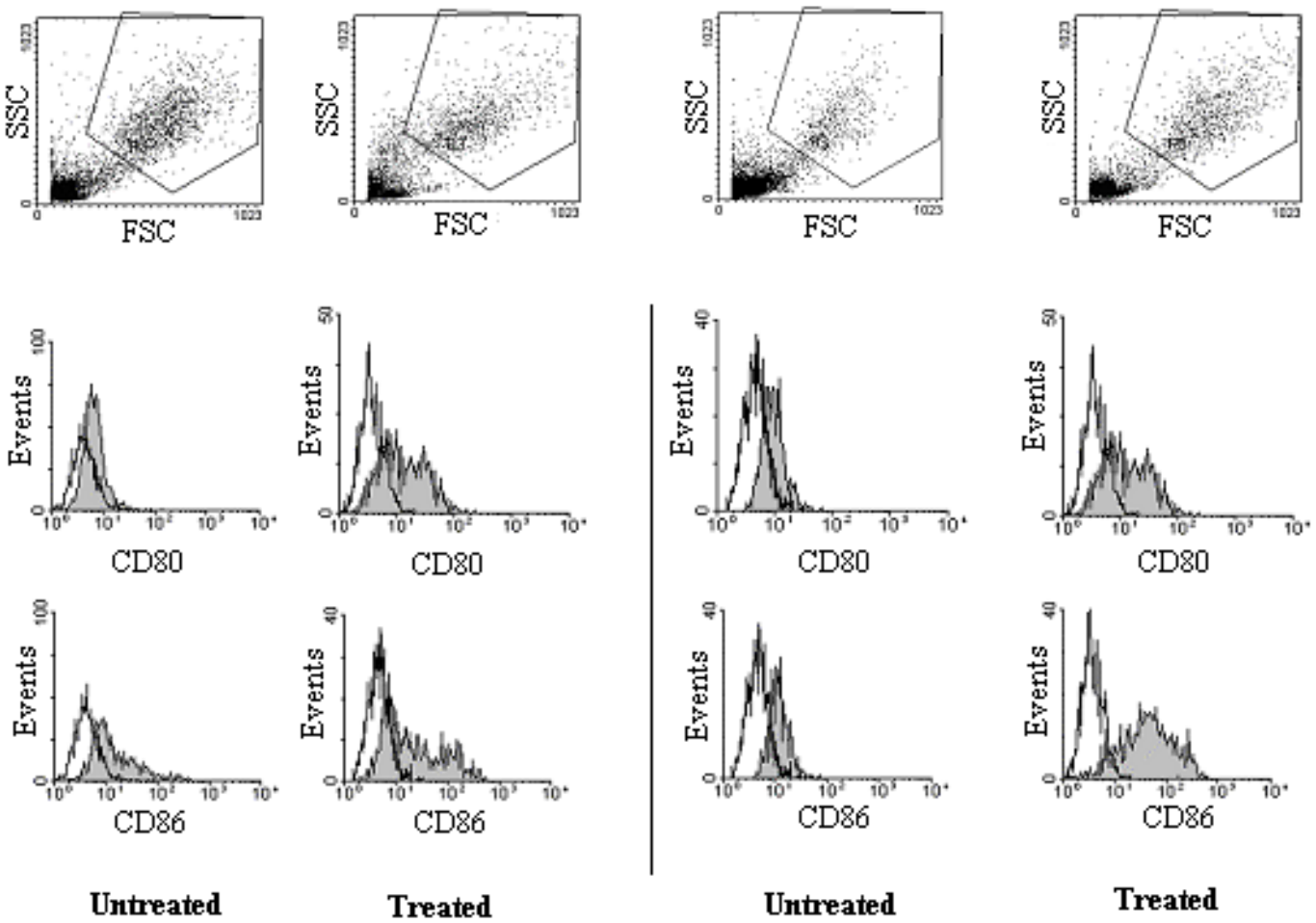
Recuperação imune nos pacientes

Resposta proliferativa de linfócitos ao tumor autólogo



Cancer Immunol Immunother 53:1111-8, 2004

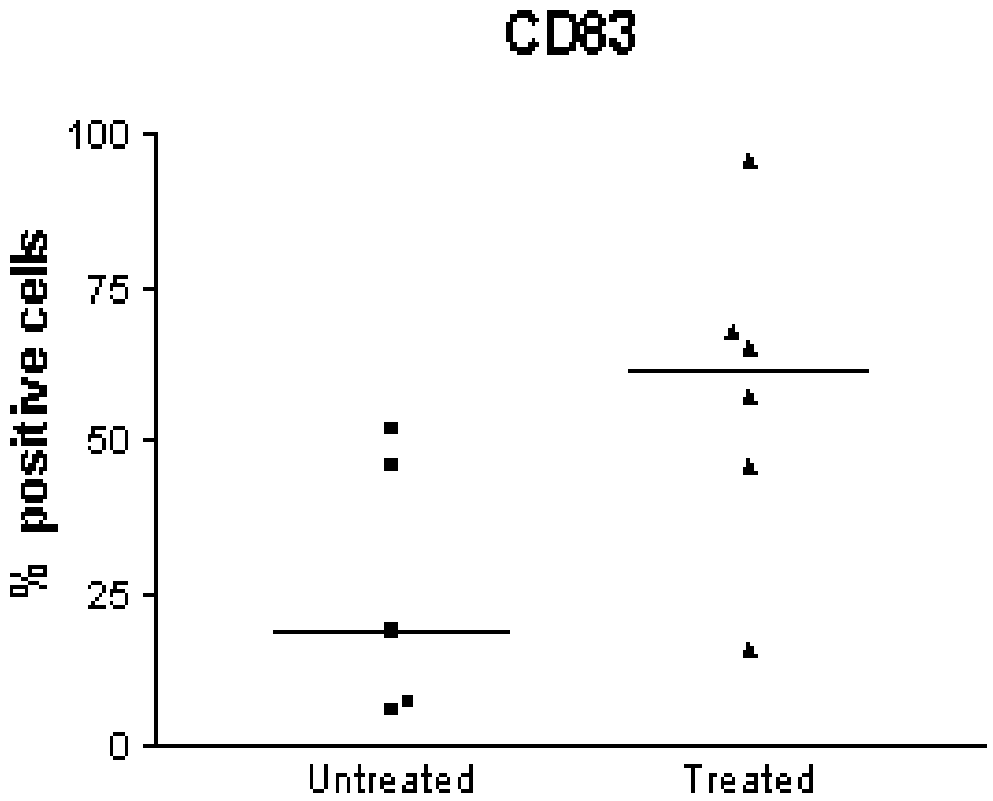
Recuperação imune nos pacientes
Diferenciação de DC a partir do sangue



Cancer Immunol Immunother 54:61-6, 2005

Recuperação imune nos pacientes

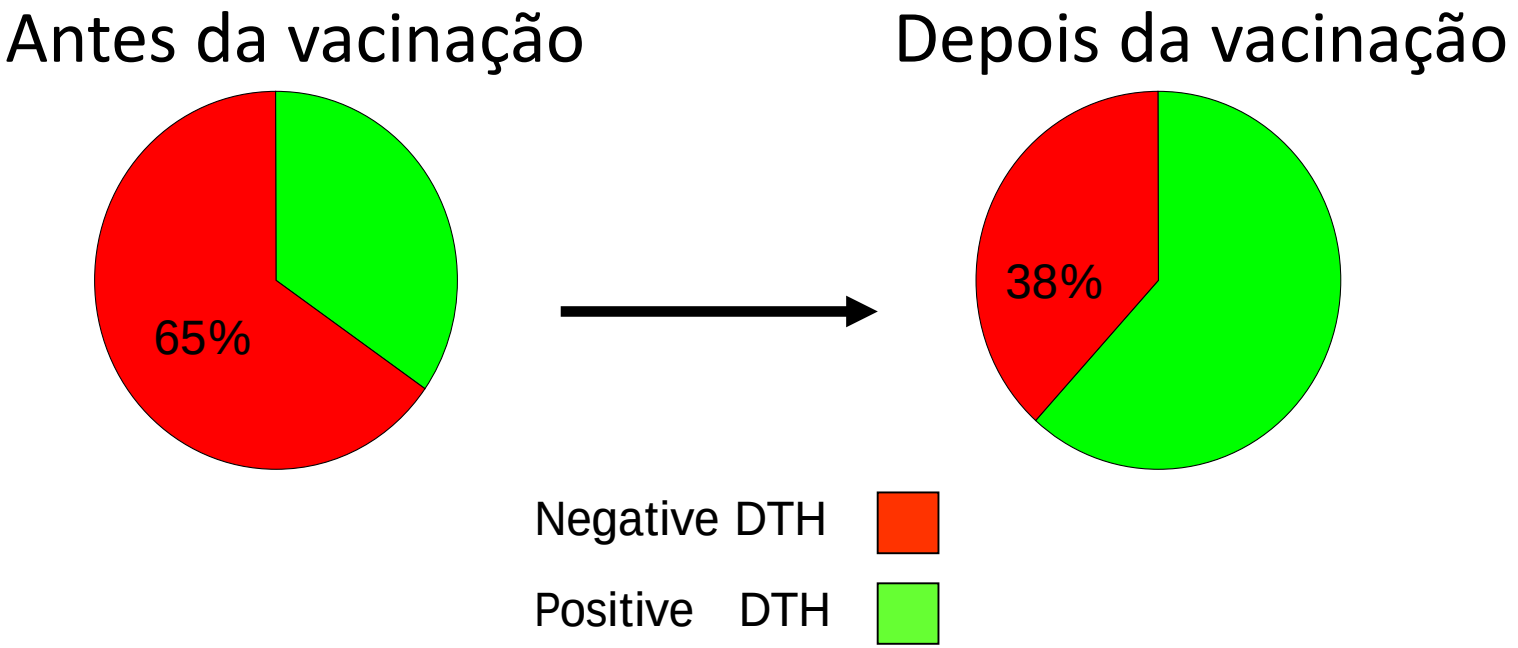
Ativação *in vitro de* DC



Cancer Immunol Immunother 54:61-6, 2005

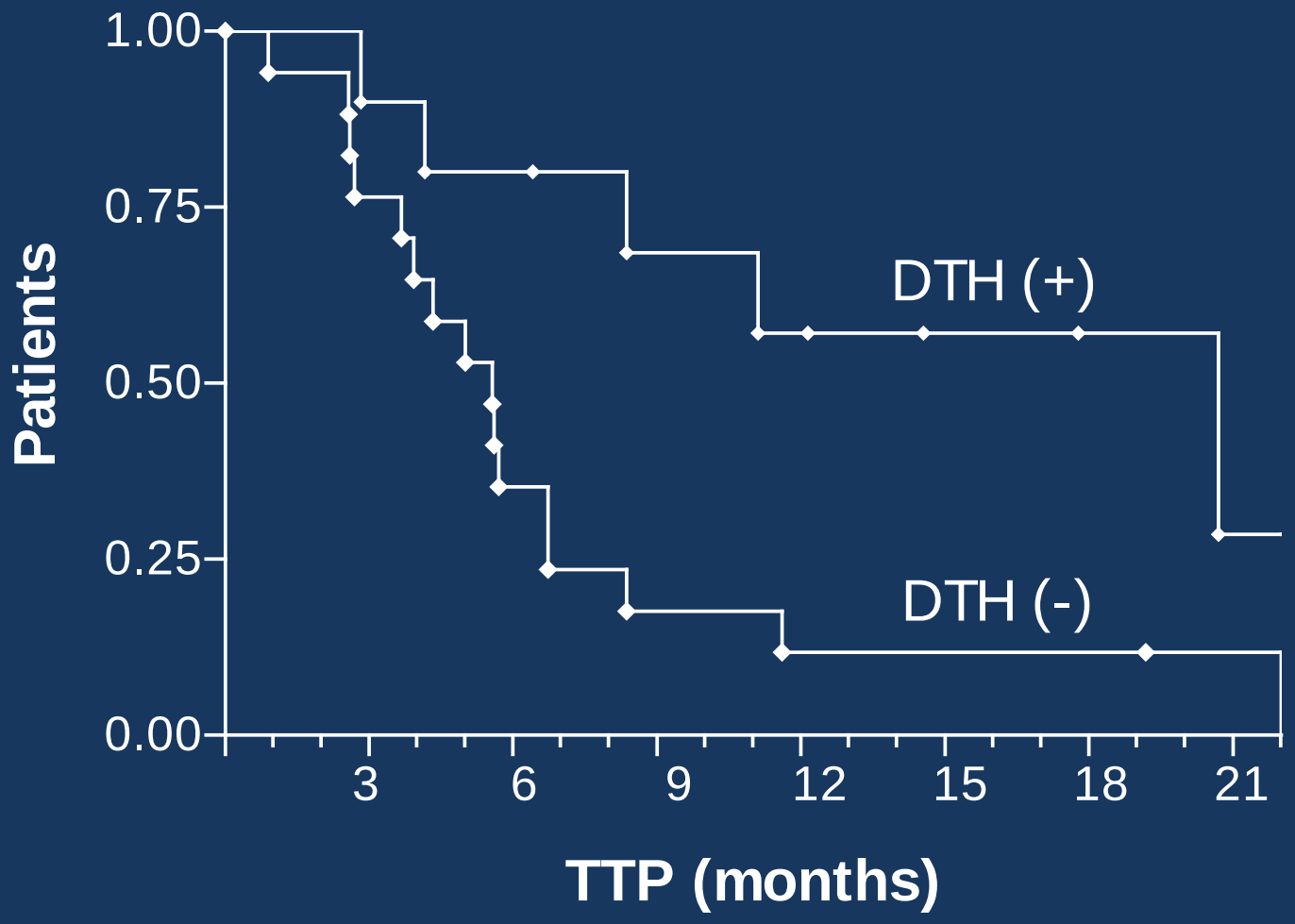
Recuperação imune nos pacientes

Reações de hipersensibilidade tardia cutânea



PPD
Trycophitin
Histoplasmin
Candidin

Reações de HT e *Time to Progression* (TTP)



Avaliação clínica

	Melanoma		Renal Carcinoma	
	n	TTP (months)	n	TTP (months)
MR	0	-	3	6,7 (5 to 21)
SD	8	11,1 (4 to 17+)	14	8,4 (3,7 to 19+)
DP	5	2,3 (1,2 to 2,6)	5	1,1 (0,9-1,1)
Overall		6,7		6,7

MR – *measurable* response; SD – stable disease; DP – disease progression

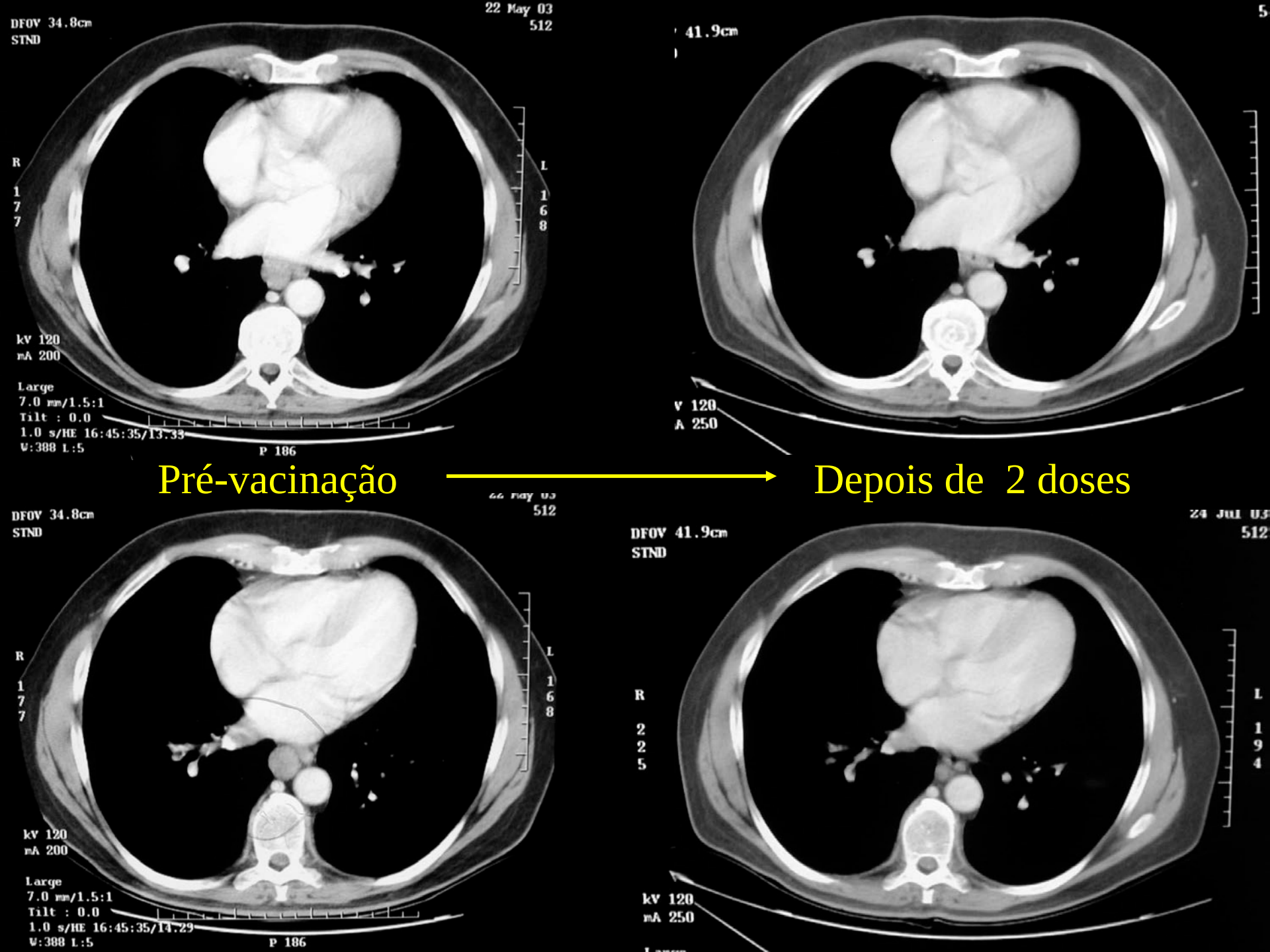
Cancer Immunol Immunother (2004) 53: 1111–1118
DOI 10.1007/s00262-004-0551-7

ORIGINAL ARTICLE

Jose Alexandre M. Barbuto · Luis F. C. Ensina
Andreia R. Neves · Patrícia C. Bergami-Santos
Katia R. M. Leite · Ricardo Marques · Frederico Costa
Siderleny C. Martins · Luiz H. Camara-Lopes
Antonio C. Buzaid

Dendritic cell–tumor cell hybrid vaccination for metastatic cancer

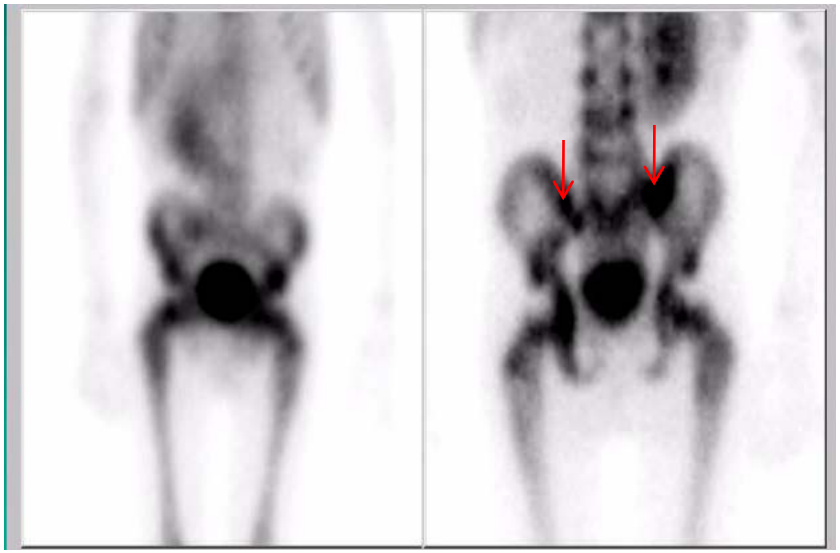
Abstract Dendritic cells are the most potent antigen-presenting cells, and the possibility of their use for cancer vaccination has renewed the interest in this therapeutic modality. Nevertheless, the ideal immunization protocol with these cells has not been described yet. In this paper we describe the preliminary results of a protocol using autologous tumor and allogeneic dendritic hybrid cell vaccination every 6 weeks, for metastatic melanoma and renal cell carcinoma (RCC) patients. Thirty-five patients were enrolled between March 2001 and March 2003. Though all patients included presented with large tumor burdens and progressive diseases, 71% of them experienced stability after vaccination, with durations up to 19 months. Among RCC patients 3/22 (14%) presented objective responses. The median time to progression was 4 months for melanoma and 5.7 months for RCC patients; no significant untoward effects were noted. Furthermore, immune function, as evaluated by cutaneous delayed-type hypersensitivity reactions to recall antigens and by peripheral blood proliferative responses to tumor-specific and nonspecific stimuli, presented a clear tendency to recover in vaccinated patients. These data indicate that dendritic cell–tumor cell hybrid vaccination affects the natural history of advanced cancer and provide support for its study in less advanced patients, who should, more likely, benefit even more from this approach.


DFOV 34.8cm
STND
22 May 03
512
R
L
kV 120
mA 200
Large
7.0 mm/1.5:1
Tilt : 0.0
1.0 s/HE 16:45:35/13.33
W:388 L:5
P 186
Pré-vacinação
Depois de 2 doses
DFOV 34.8cm
STND
512
kV 120
mA 200
Large
7.0 mm/1.5:1
Tilt : 0.0
1.0 s/HE 16:45:35/14.29
W:388 L:5
P 186
DFOV 41.9cm
STND
24 JUL 03
512
R
L
kV 120
mA 250

E no neuroblastoma?

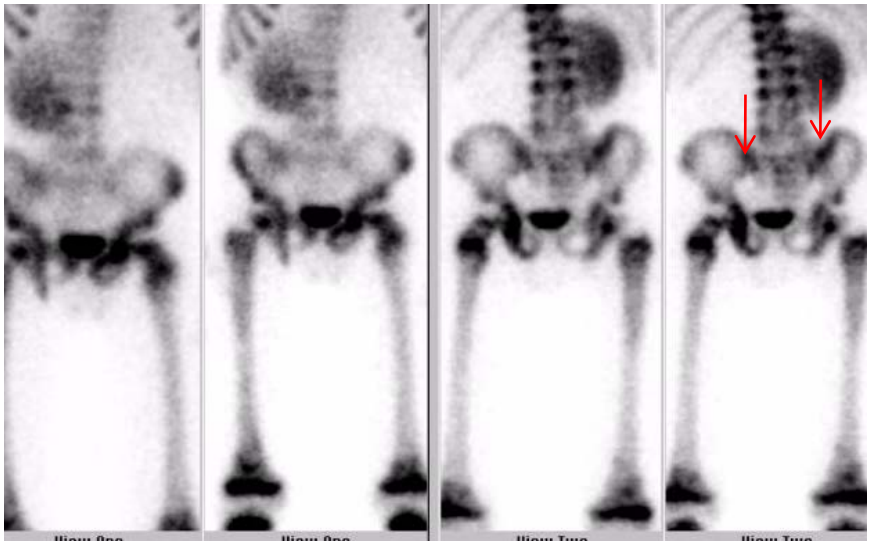
Lesões ósseas

Pré-vacinação



LDH >2000

Pós-vacinação

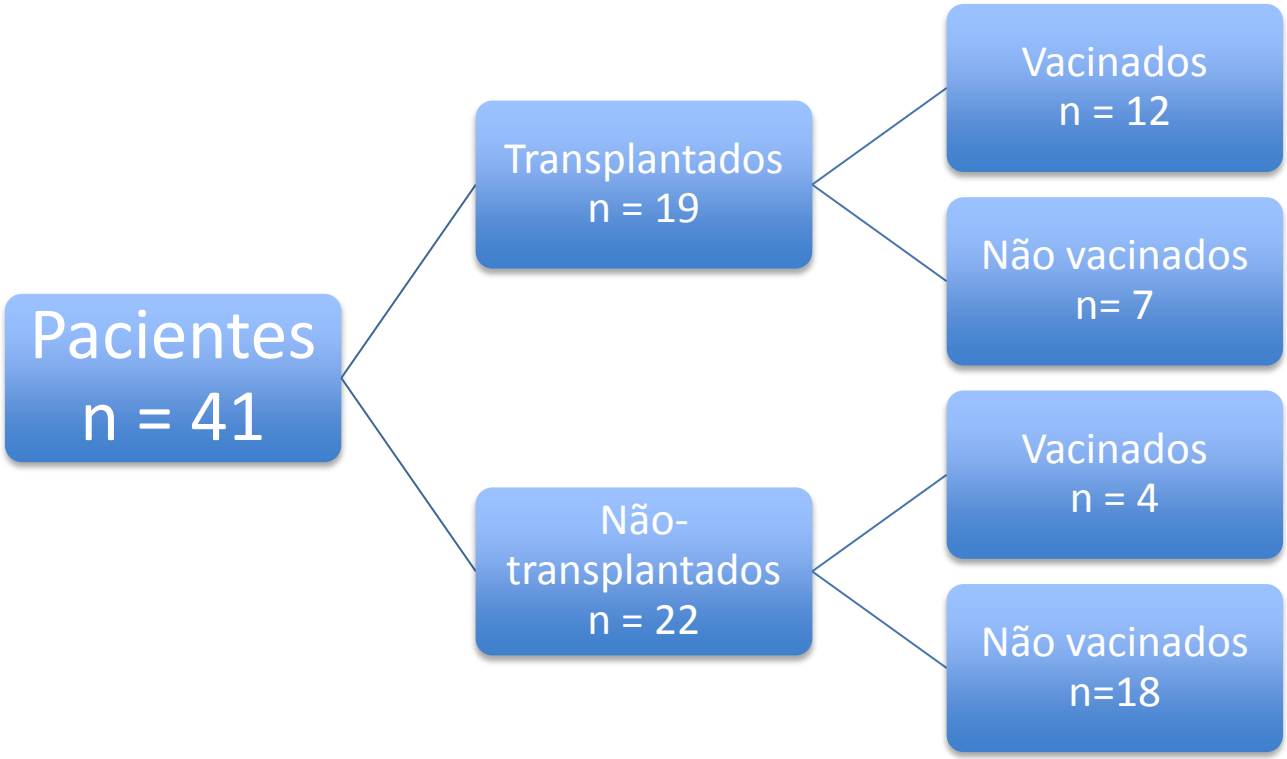


LDH < 500

Response of advanced refractory neuroblastoma to dendritic-tumor cell hybrid vaccination

Estudo corrente: Vacina com DCs no neuroblastoma

(resumo de inclusões)



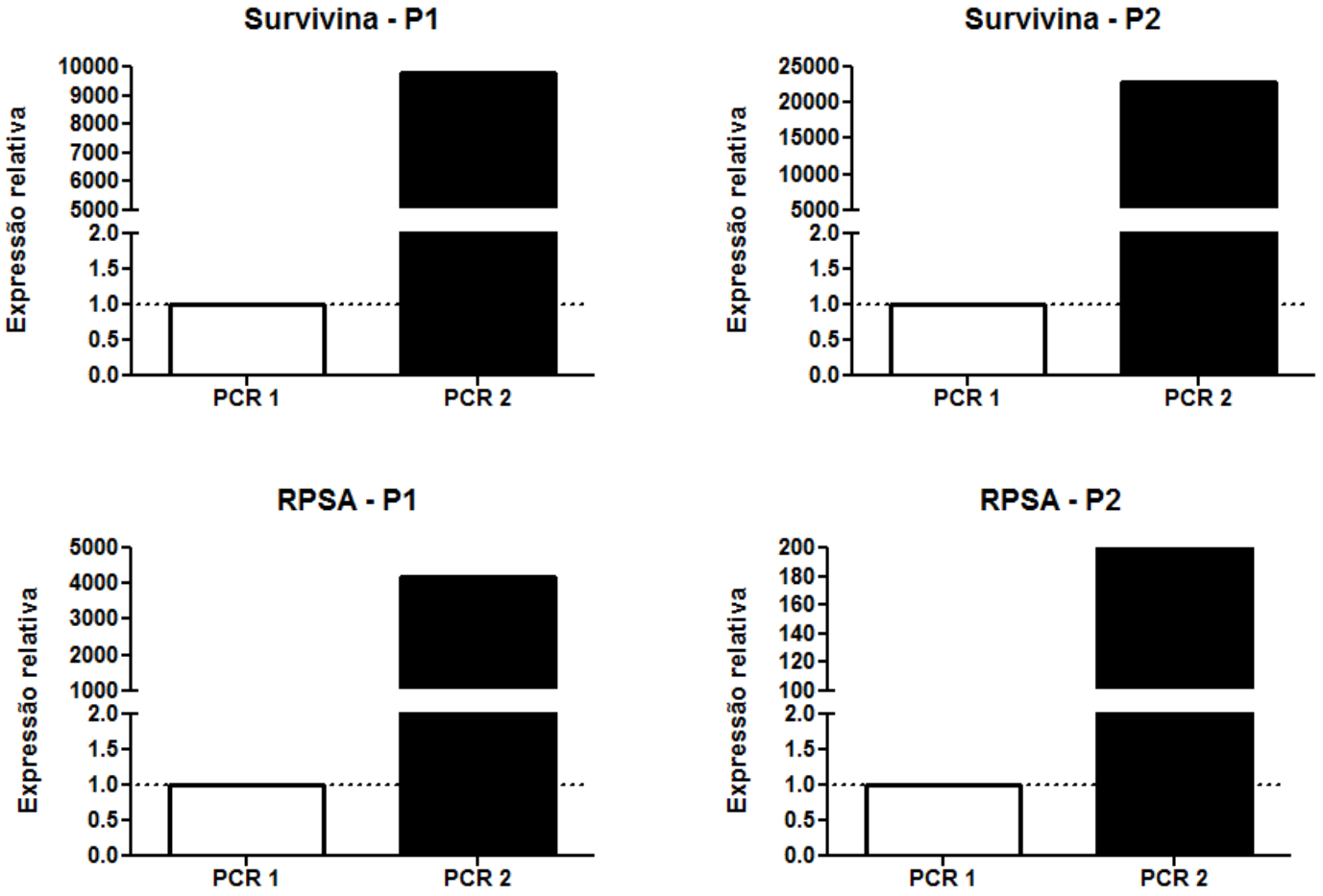
Dendritic cell-based tumor vaccines:

We are not yet there, but we are on the way!

E daqui, para onde??

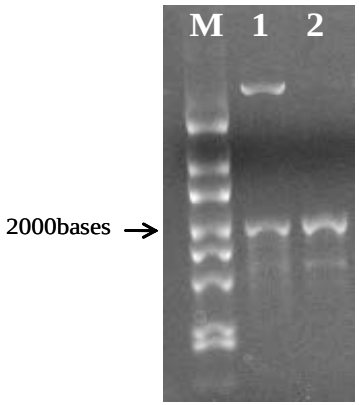
- “Entrega” de antígenos para as DC?
- “Melhorar” o fenótipo/função das DC?

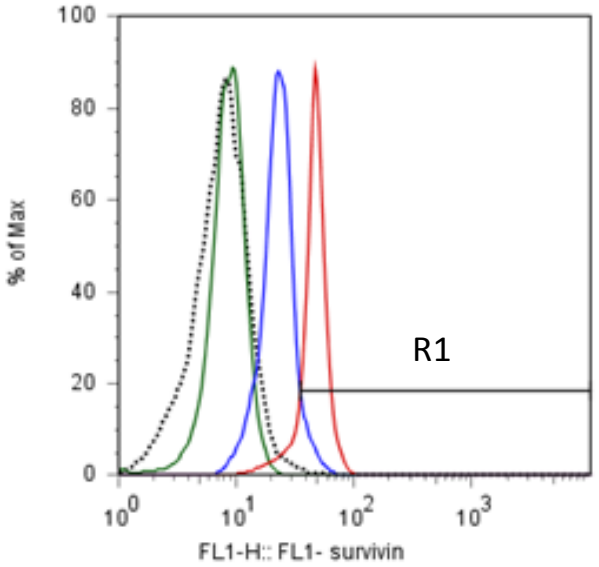
Tumor Ags-coding mRNA may be expanded *in vitro* by total RNA amplification



Toniolo et al.,, 2010.

In vitro transcribed survivin mRNA transfected into DCs



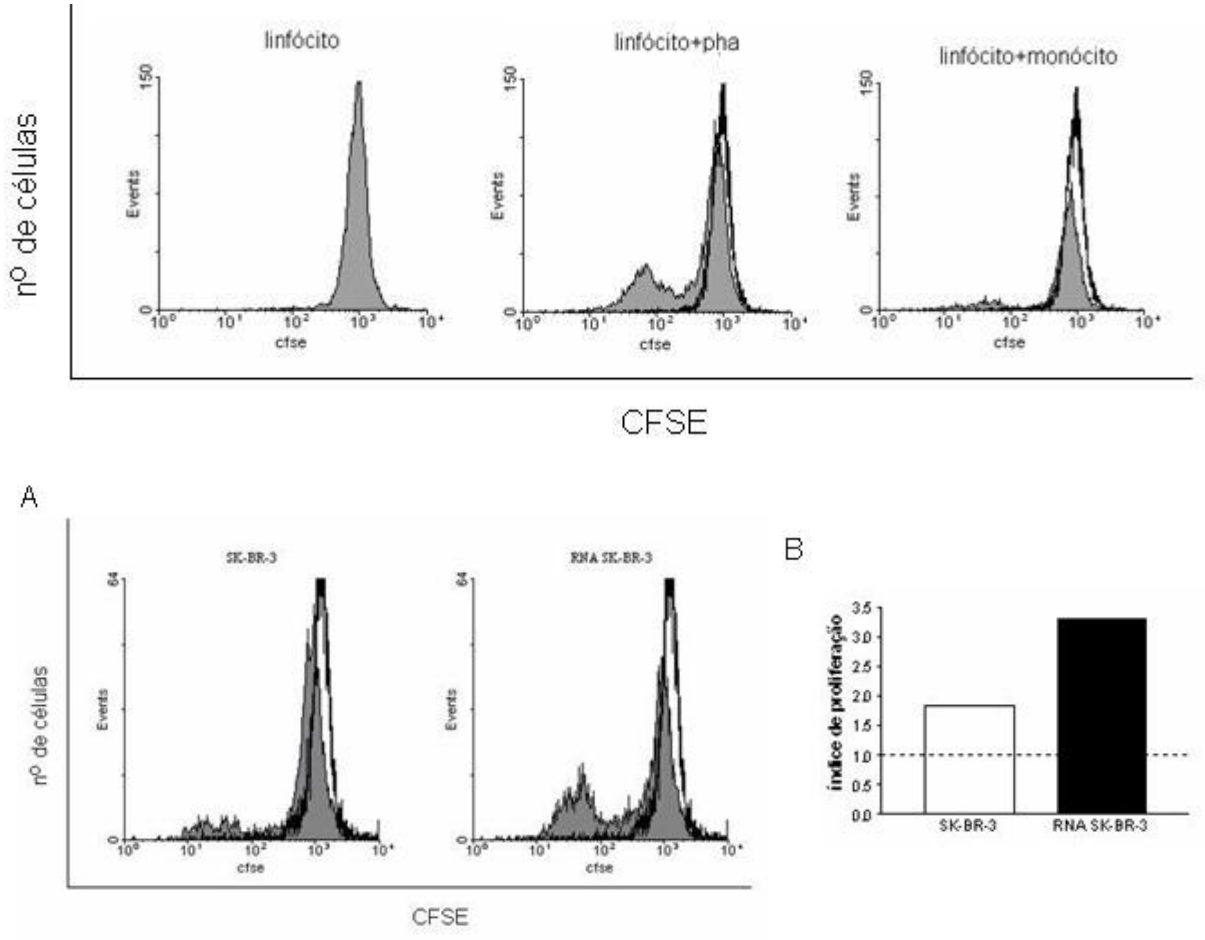


Lane M: RNA molecular weight marker
Lane 1: unclean mRNA
Lane 2: clean mRNA

- Untreated DCs
- DCs treated with transfection agent
- Endogenous survivin expression
- mRNA transfected DCs

Toniolo et al., 2010.

Tumor RNA-transfected cells:
Targets for lymphocyte responses



Menezes et al., ICI - 2007.

Muitos caminhos, a construir e a percorrer!