Universidade Federal de Pelotas
Centro de Desenvolvimento Tecnológico
Curso de Graduação em Biotecnologia
Disciplina de Engenharia Tecidual

Aplicações do Cultivo Celular no Desenvolvimento de Vacinas

Carlos Eduardo Cunha

Gustavo Moreira

Sumário

- Histórico (Vacina da Poliomielite)
- Vacina para Raiva
- Vacina para Influenza
- Vacinas baseadas em Células Dendríticas
- Vacina para Febre Amarela
- Vacinas para Câncer
- Conclusão

Histórico

- Início do séc. XIX – Primeiros Cultivos
- 1940-1950 – Avanços Importantes
  - Vírus da Poliomielite

John Franklin Enders

Thomas Huckle Weller

Frederick Chapman Robbins

Histórico

- 1952 – Primeira Vacina em Cultivo Celular

Jonas Salk

Propagação do Vírus da Poliomielite Humana em células Vero

- 1954 – Prêmio Nobel para o grupo de Enders

Histórico

- 1953-1955 – Teste da Vacina Salk

- Maior experimento da história (2 milhões de crianças)

2010, 85, 309–320 No. 32

World Health Organization

Organisation mondiale de la Santé

Weekly epidemiological record
Relevé épidémiologique hebdomadaire

6 AUGUST 2010, 85th YEAR / 6 AOÛT 2010, 85e ANNÉE
No. 32, 2010, 85, 309–320
http://www.who.int/wer

Vacinas para raiva

Posição da Organização Mundial da Saúde

Aspectos Gerais

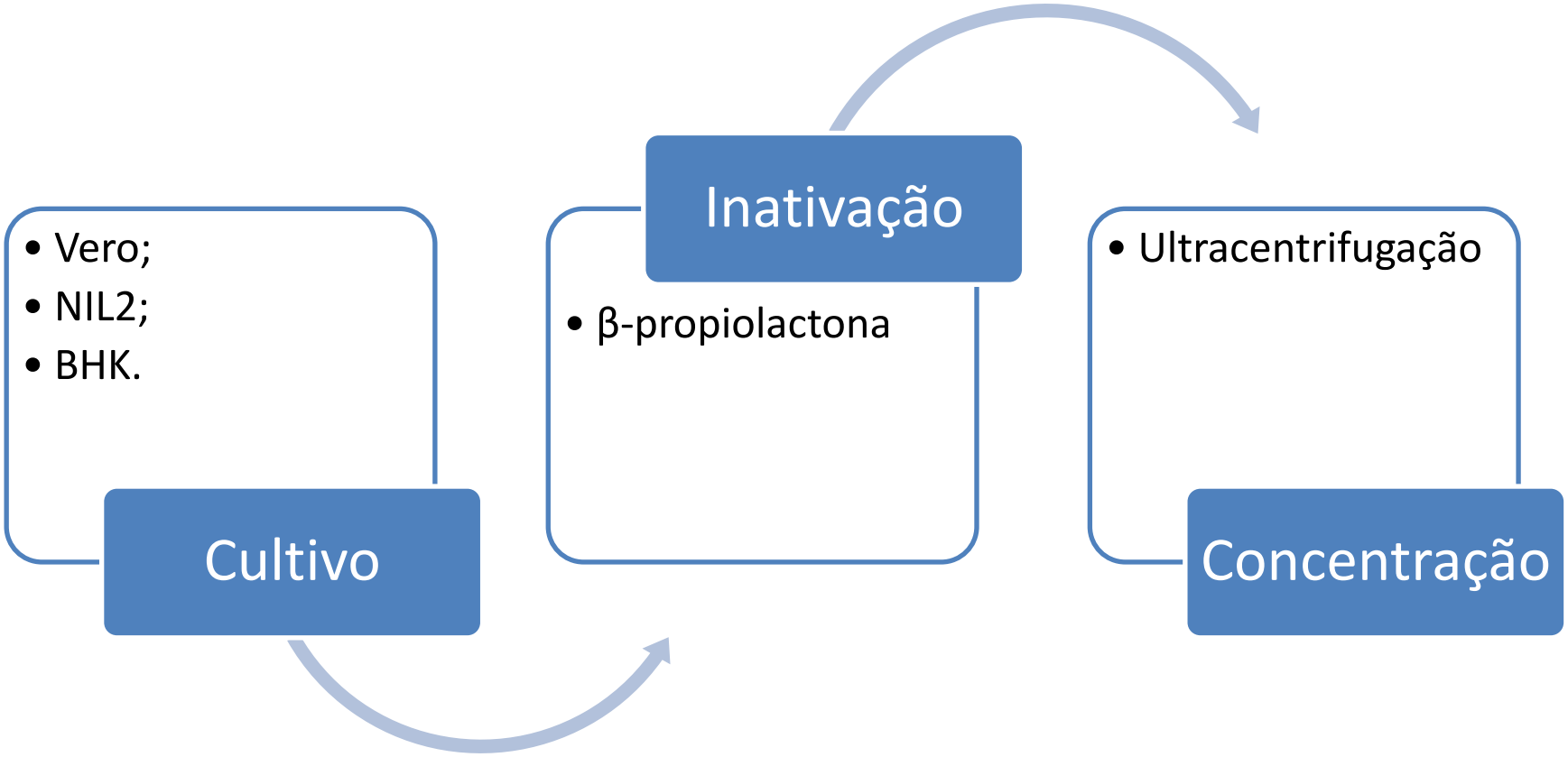
- **Agente etiológico**: Vírus de RNA envelopado da família Rhabdoviridae;
- **Transmissão**: mordidas de carnívoros e morcegos;
- Pode infectar qualquer mamífero, causando encefalite. Inevitavelmente fatal;
- É a principal doença zoonótica.

Vacina & Cultivo Celular

- Primeiramente em cultura de tecido nervoso:
  - Baixa imunogenicidade e alto nível de reações adversas.

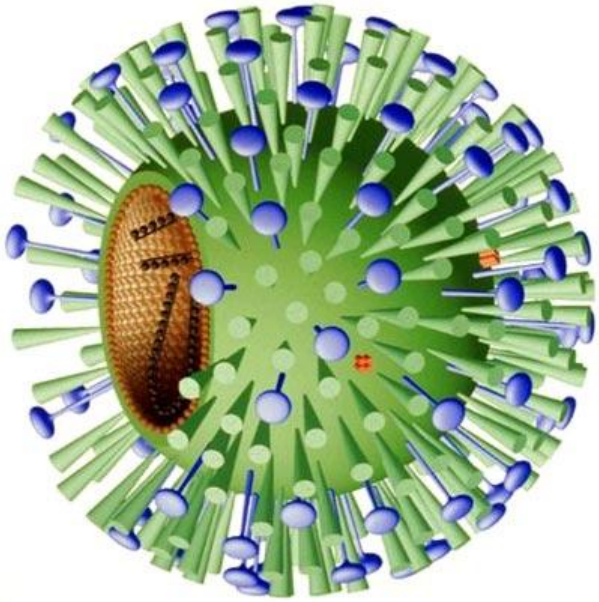
- Vírus produzidas em cultivo celular (Vero, NIL2 ou BHK) e ovos embrionados seguido de inativação por β-propiolactona;

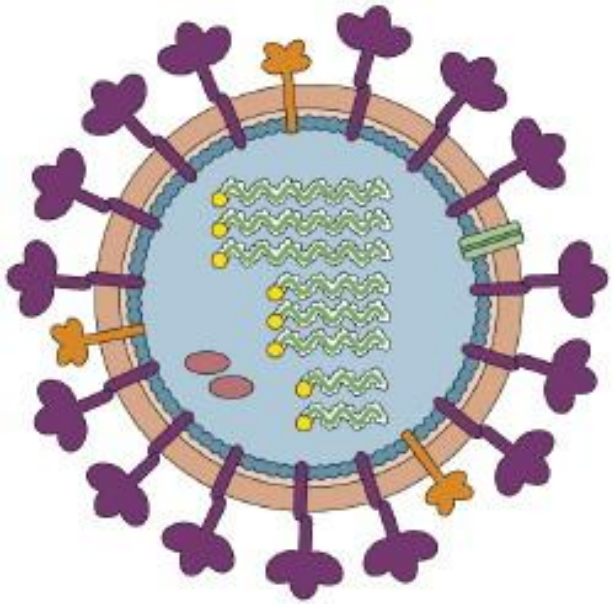
Vacina & Cultivo Celular



Influenza

- OMS
  - Vacina trivalente anual

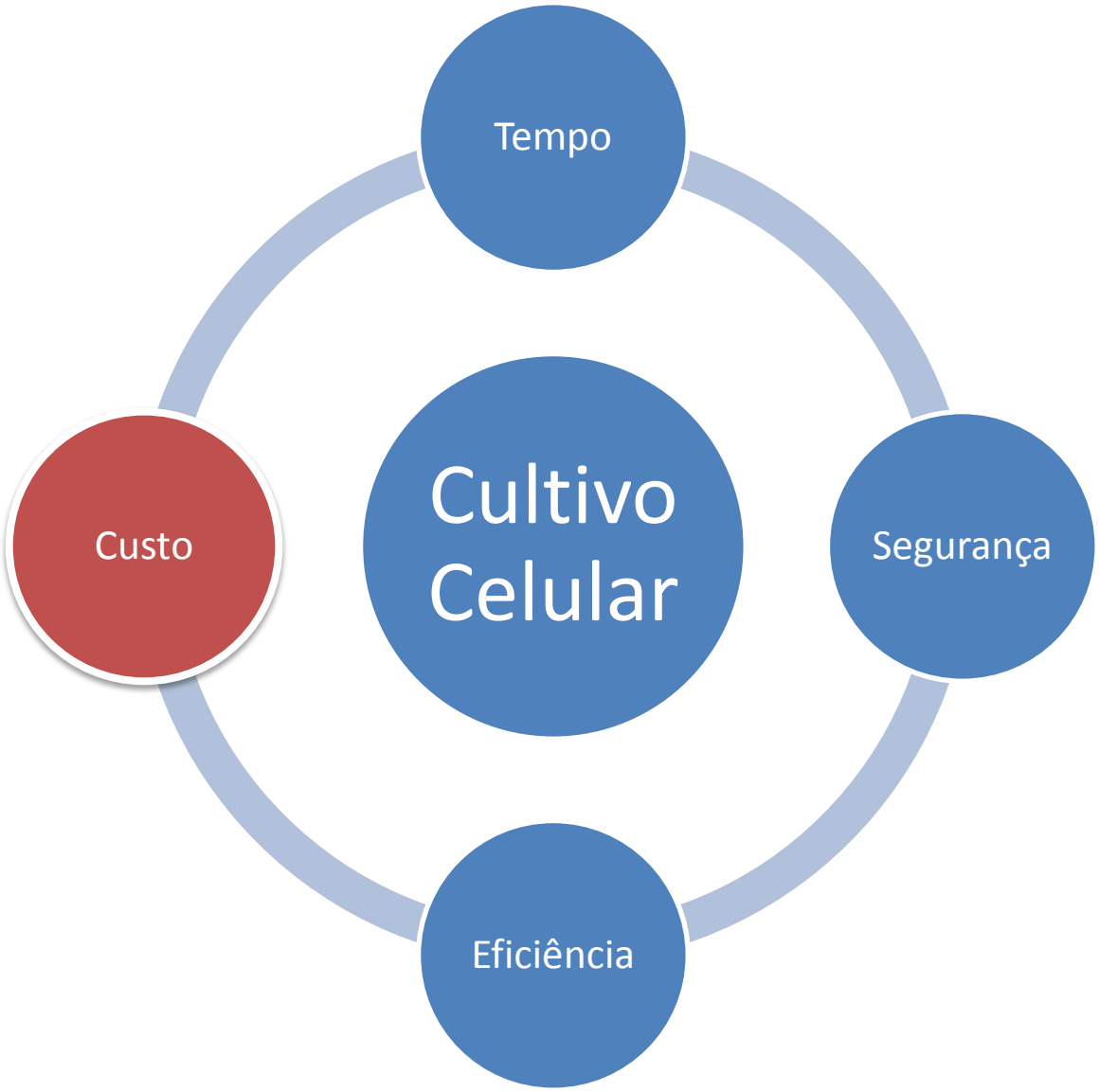




Influenza

- Produção Atual
  - Ovos
  - 6-12 meses
  - 1 ou 2 ovos por dose
  - Contra indicações
  - Contaminação
  - Eficiência duvidosa

Influenza



Influenza

- Avanços no Cultivo Celular

MEDIA RELEASE • COMMUNIQUE AUX MEDIAS • MEDIENMITTEILUNG

Optaflu®, the Novartis cell culture-derived influenza vaccine, receives positive opinion supporting European Union regulatory approval

- *Novartis leading the introduction of influenza cell culture manufacturing – the first major innovation in influenza vaccine manufacturing in more than 50 years*
- *Optaflu® to help meet growing need for seasonal influenza vaccines, production technology has potential for quick scale-up in case of an influenza pandemic*
- *Novartis proprietary cell culture technology offers possibility to obtain a better matched vaccine with circulating viruses than currently available technology*

Basel, April 27, 2007 – Novartis has received a positive opinion supporting European Union approval of its cell culture-derived seasonal influenza vaccine Optaflu®, which is aiming to become the first influenza vaccine to utilize a mammalian cell line, rather than chicken eggs, for antigen production.

NOVARTIS

Influenza

- Avanços no Cultivo Celular

Novartis MF59® adjuvanted cell culture-based vaccine shows strong immune response in A(H1N1) clinical trials

- First pilot trial of investigational A(H1N1) vaccine with 100 subjects indicates strong, potentially protective, immune response in 80% of subjects after one dose, more than 90% after two doses.
- MF59® adjuvanted cell culture-based A(H1N1) vaccine was well tolerated, pain at the injection site the most frequent adverse event.
- Larger pivotal trials with both cell culture and traditional egg based vaccines under way to include more than 6000 adults and children.

Basel, September 3 2009 - A pilot trial of Novartis adjuvanted cell culture-based A(H1N1) vaccine[1] indicates that the "swine flu" vaccine elicited a strong immune response and was well tolerated. The trial was run by the UK's University of Leicester and University Hospitals of Leicester. The vaccine, to be called Celtura®, was tested with 100 healthy volunteers, aged between 18 and 50.

- 100 pessoas
- Europa, 2009
- Eficiência:

<u>80% 1ª dose</u>

<u>90% 2ª dose</u>

Influenza

- Avanços no Cultivo Celular

Novartis inaugurates large-scale US based cell-culture influenza vaccine manufacturing facility

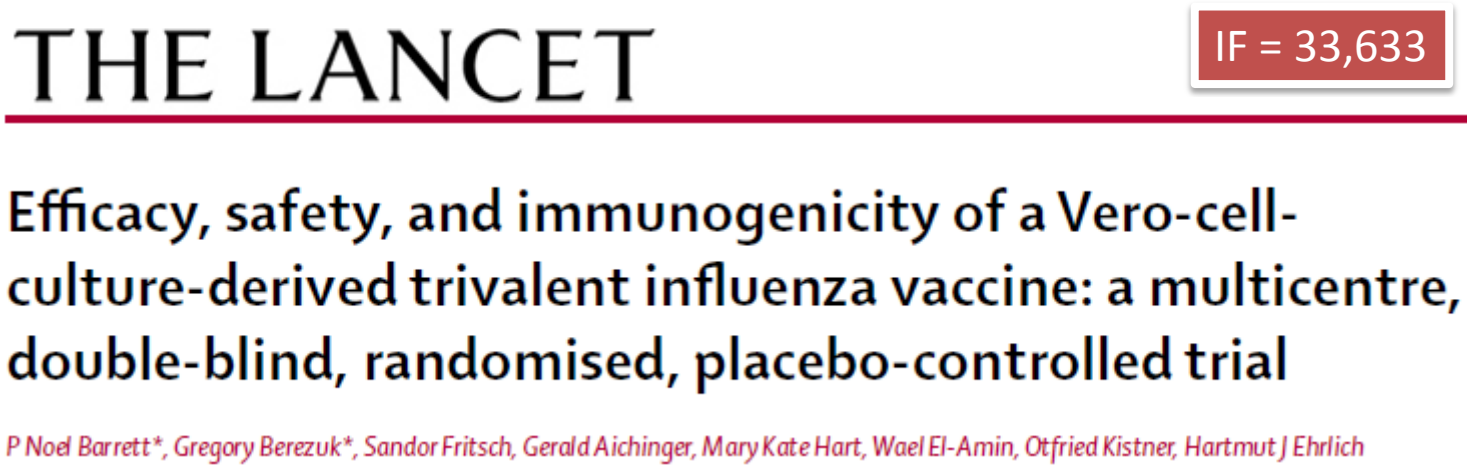
- Total investment of nearly USD 1 billion through a partnership between Novartis and the US Department of Health and Human Services
- Inauguration marks important milestone in using modern biotechnologies for flu vaccine production to replace the 50 year-old egg-based process
- Facility designed to supply 150 million doses of pandemic vaccine within 6 months of influenza pandemic declaration; facility ready to respond to a pandemic as early as 2011 if licensed in an emergency

Basel, November 24, 2009 - Today, Novartis officially inaugurated the US's first ever large-scale flu cell culture vaccine and adjuvant manufacturing facility in Holly Springs, North Carolina. The facility is a result of a partnership between Novartis and the US Department of Health and Human Services (HHS). It is the first of its kind in the United States and highlights an important milestone in efforts to improve influenza vaccine manufacturing technology in the US and enhance domestic pandemic preparedness.

- US$ 1 bilhão
- Produção para 2013

Influenza

- Avanços no Cultivo Celular
  - 2011



Influenza

- Avanços no Cultivo Celular
  - 2008-2009
    - A/H1N1
    - A/H3N2
    - B

Influenza

- Avanços no Cultivo Celular
  - Cultivo em células Vero
  - 7250 pessoas (3626 vacinadas)
  - Eficiência: **78,5%** (contra 73% da já utilizada)
  - **Tempo de produção**: 10 semanas menor

Mais tempo para OMS

Influenza

- Avanços no Cultivo Celular
  - 2011

Vaccine

IF = 3,572

Statistical optimization of influenza H1N1 production from batch cultures of suspension Vero cells (sVero)

Cristian Paillet[c], Guillermina Forno[a,c,*], Nicolas Soldano[c], Ricardo Kratje[a,b], Marina Etcheverrigaray[a,b]

- Produção 3 vezes maior de vírus

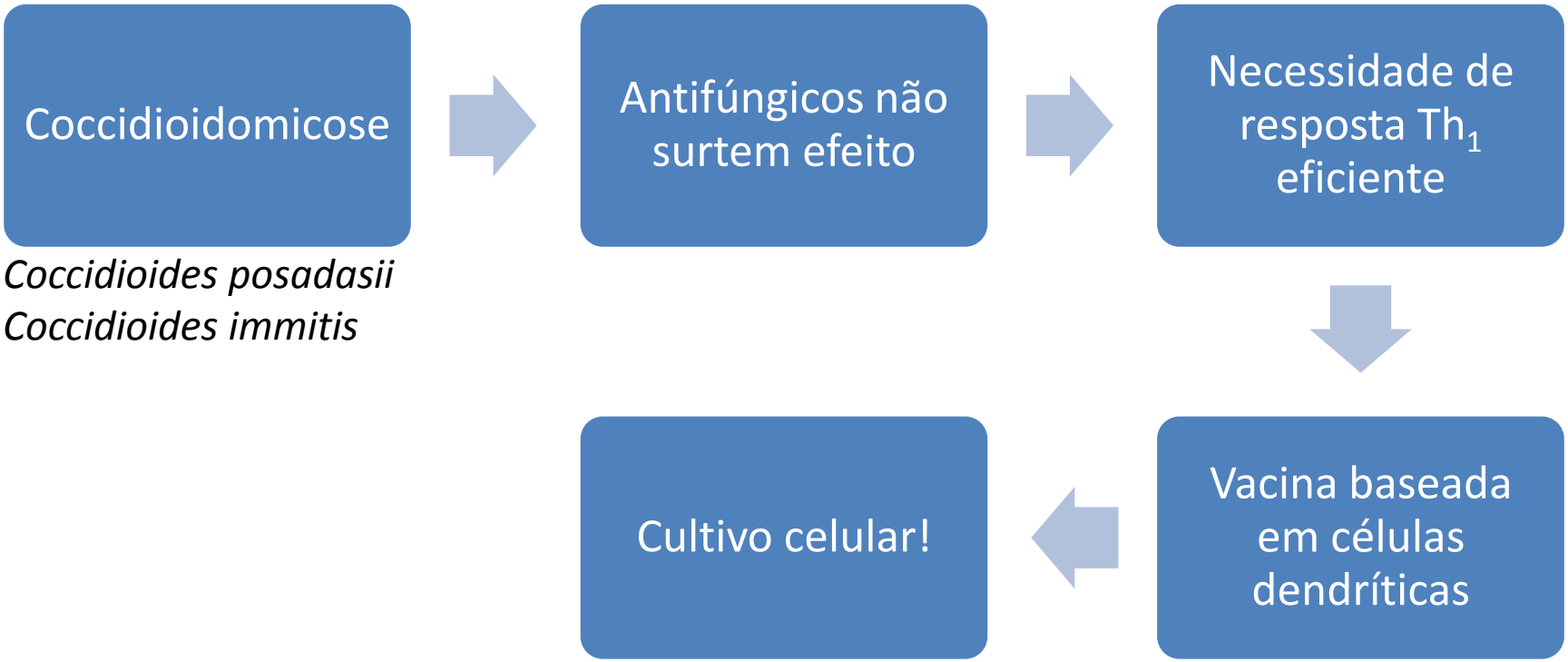
Vilekar *et al. BMC Immunology* 2010, **11**:60
http://www.biomedcentral.com/1471-2172/11/60

BMC Immunology

Vacinas baseadas em células dendríticas

In vivo trafficking and immunostimulatory potential of an intranasally-administered primary dendritic cell-based vaccine

Aspectos Gerais



Coccidioides posadasii
Coccidioides immitis

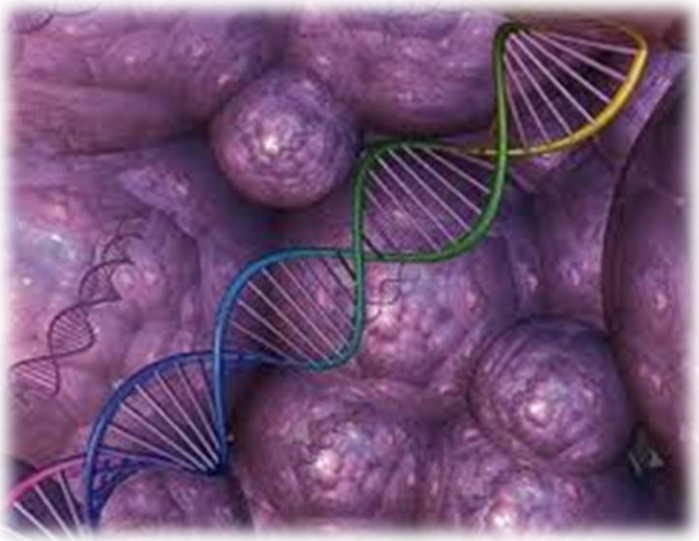
Cultivo Celular

- Isolamento de DCs a partir da medula óssea;
- 37 °C em 5% CO_2: coletas aos 2 e 4 dias de cultivo;
- RPMI 1640:
  - Antibióticos;
  - 10% SFB;
  - IL4 e GM-CSF;
  - Aminoácidos não-essenciais de MEM.

Cultivo Celular & Transfecção

- Incubação das DCs em DMEM a 37 °C em atmosfera de 5% CO_2;
  - Lavagem com o mesmo meio previamente.

- Reagente TransIT-TKO;
  - 2 µg de cada plasmídeo.



Co-cultivo

- DCs:
  - Transfectadas e não-transfectadas;
- Linfócitos (células T) do baço;
- 1:64; 24h;
- Marcação com anticorpos para verificar ativação das células T *in vitro* por citometria de fluxo.

Resultados

- DCs transfectadas expressaram o antígeno Ag2/PRA e ativaram células T;
- Produção de células T de memória e IFN-γ;
- DCs administradas de forma intranasal migraram para sangue, pulmão e timo.

Resultados

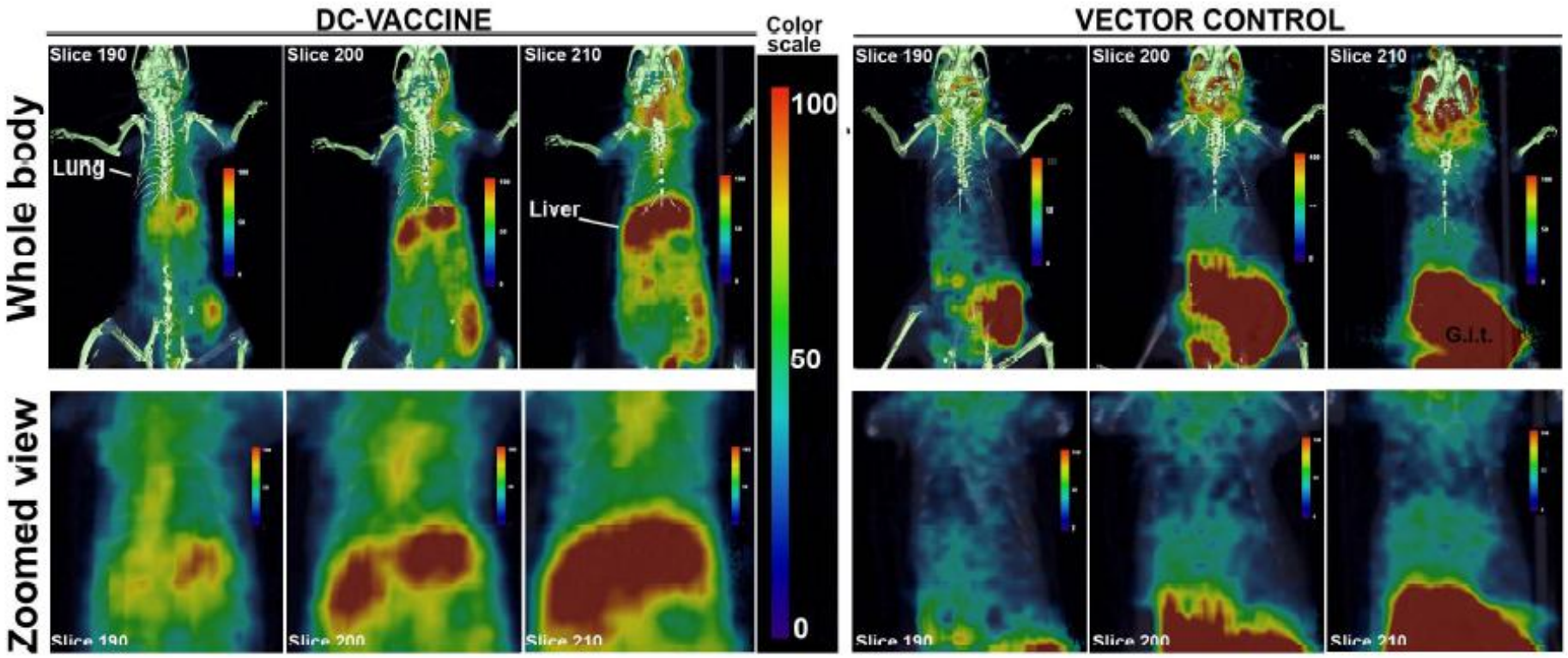


Figure 10 Fused positron emission tomography (PET) and computed tomography (CT) images of BALB/c mice intranasally instilled with homologous primary DCs expressing HSV1-TK. The primary DCs were either co-transfected with pVR1012-Ag2/PRA-cDNA and pVR1012-TK (labeled as DC-vaccine), or with vector plasmid DNA (labeled as Vector Control). The PET-CT imaging was performed after 7 days of cells administration. About 2 h prior to imaging, ^{18}F-FIAU (a substrate for HSV1-TK) was intravenously injected. Images are from one representative mouse from each group. Six mice were included per group in three different experiments. In the second panels of each group, the whole body images were zoomed to get closer view of ^{18}F-FIAU accumulation (DC-migration) in thoracic area. A significant accumulation of ^{18}F-FIAU was observed in lung and liver of mouse injected with DCs expressing TK. Overall, the mice receiving DC-vaccine retained more ^{18}F-FIAU radioactivity than the Control mice. G.i.t (gastrointestinal tract).

Febre Amarela

- 2011

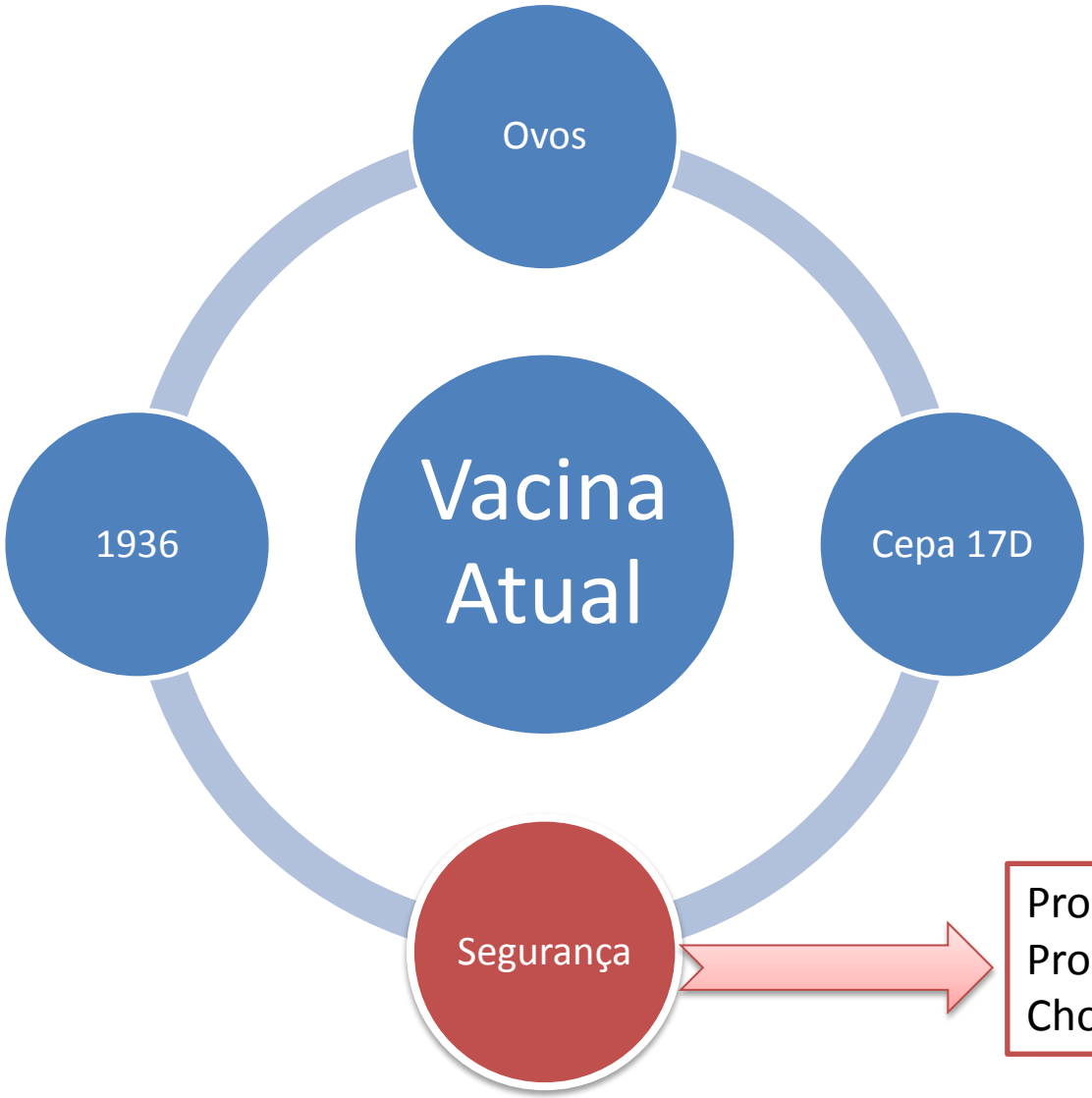
The NEW ENGLAND JOURNAL of MEDICINE

An Inactivated Cell-Culture Vaccine against Yellow Fever

Thomas P. Monath, M.D., Elizabeth Fowler, Ph.D., Casey T. Johnson, D.O., John Balser, Ph.D., Merribeth J. Morin, Ph.D., Maggie Sisti, B.S., and Dennis W. Trent, Ph.D.

IF = 53,484

Febre Amarela



Problemas Viscerais → 1:250.000
Problemas Neurais → 1:125.000
Choques Anafiláticos → 1: 55.600

Febre Amarela

- Avanços no Cultivo Celular
  - ➢XRX-001
    - Cepa 17D em células Vero
    - Suspensão com microesferas
    - 60 pessoas (24 dose baixa e 24 dose alta)
    - Segura
    - **Eficiência**: 13% e 46% 1ª dose
      88% e 100% 2ª dose

Febre Amarela

- Avanços no Cultivo Celular
  - Vacina contra JEV → 3 doses
  - Estudos para diminuir doses
  - Vacina atual protege por toda a vida

Wen *et al. Journal of Biomedical Science* 2011, **18**:44
http://www.jbiomedsci.com/content/18/1/44

NSC
The cost of publication in *Journal of Biomedical Science* is borne by the National Science Council, Taiwan

JOURNAL OF BIOMEDICAL SCIENCE

Vacinas para câncer

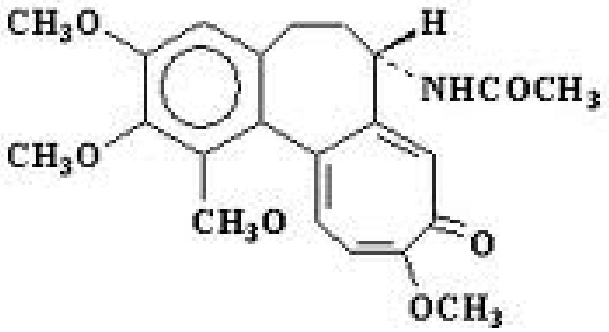
Specific microtubule-depolymerizing agents
augment efficacy of dendritic cell-based cancer vaccines

Aspectos Gerais

- Padrões moleculares associados a dano:
  - Morte celular imunogênica;
  - Estimulam fagocitose, maturação e apresentação de antígenos de DCs;

- Agentes despolimerizantes específicos para microtúbulos:
  - Atividade anti-câncer;
  - Ativação da DCs.

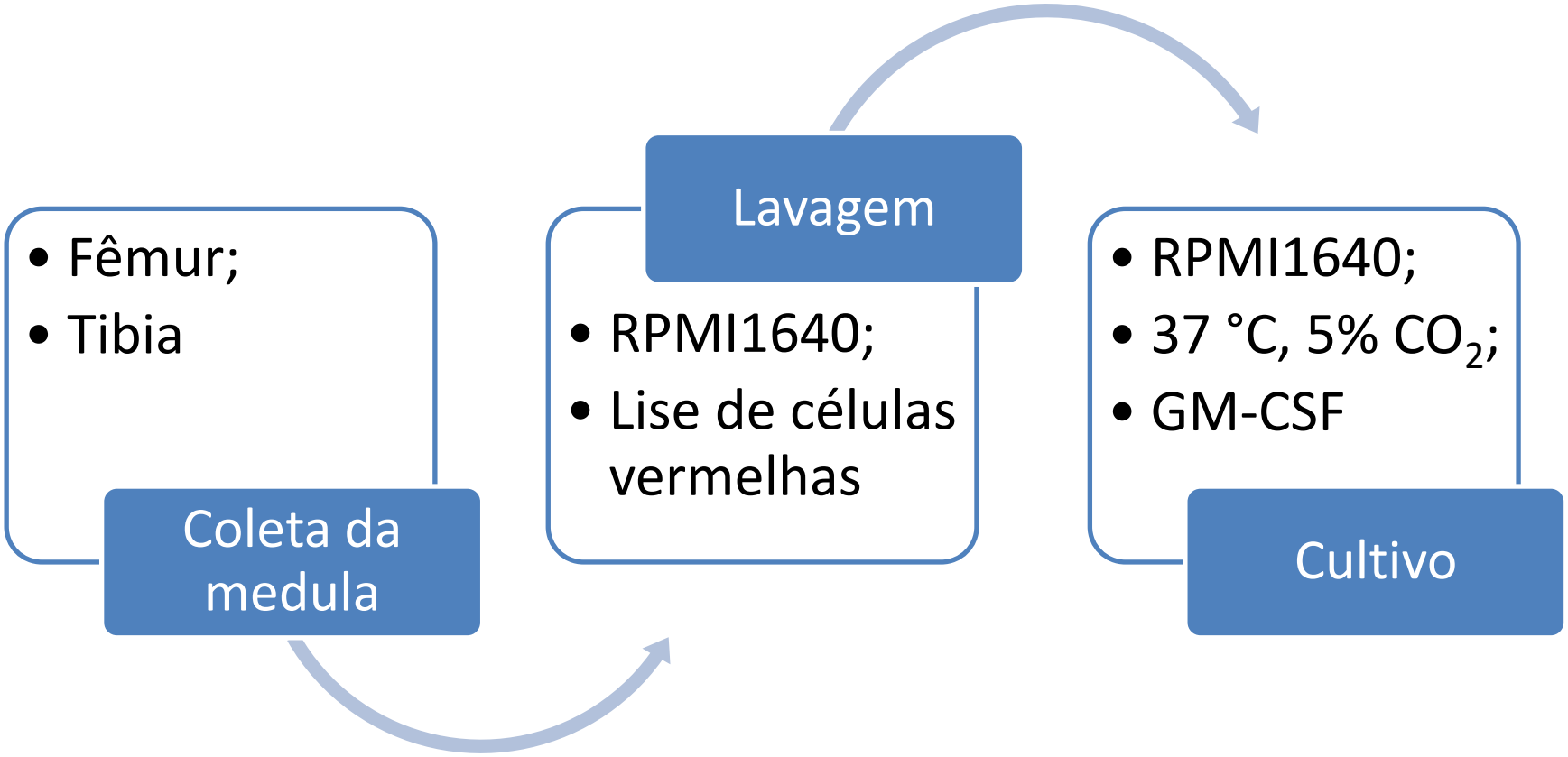
Colchicine
$C_{22}H_{25}NO_6$



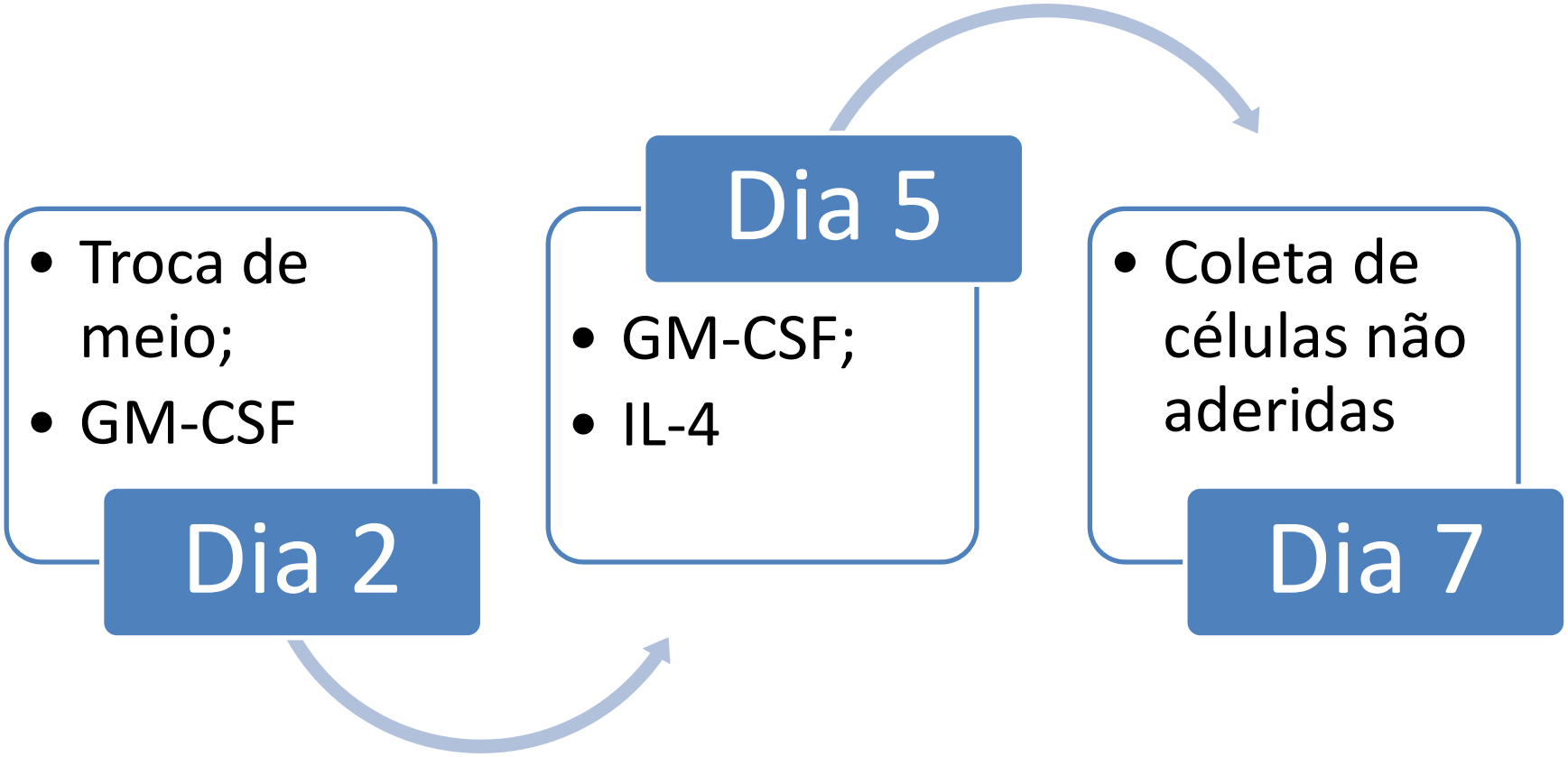
Cultivo Celular

- B16F10 (melanoma de camundongo):
  - DMEM + 10% SFB, atbs e 2 mM L-glutamina;
- BMDCs:
  - RPMI1640 + 10% SFB, atbs e 2 mM L-glutamina.
- 37 °C, 5% CO_2, placas de 96 poços.

Isolamento de BMDCs – Dia 0

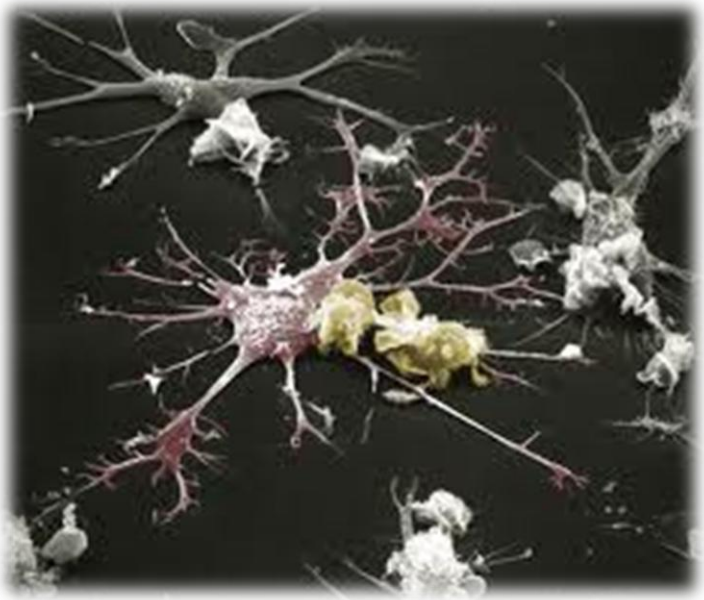


Isolamento de BMDCs



Resultados

- Vacinas baseadas em DC:
  - Estimularam a ativação de linfócitos T citotóxicos;
  - Aumentaram taxas de sobrevivência em camundongos.



Conclusão

- Cultivo celular é uma boa opção para produção de vacinas contra doenças virais;

- Extrema importância para o desenvolvimento de vacinas baseadas em células homólogas;

- Bom custo-benefício no caso de pandemia/epidemia.

谢谢!

Obrigado!