Desenvolvimento de vacinas contra o HIV-1

Daniela Santoro Rosa

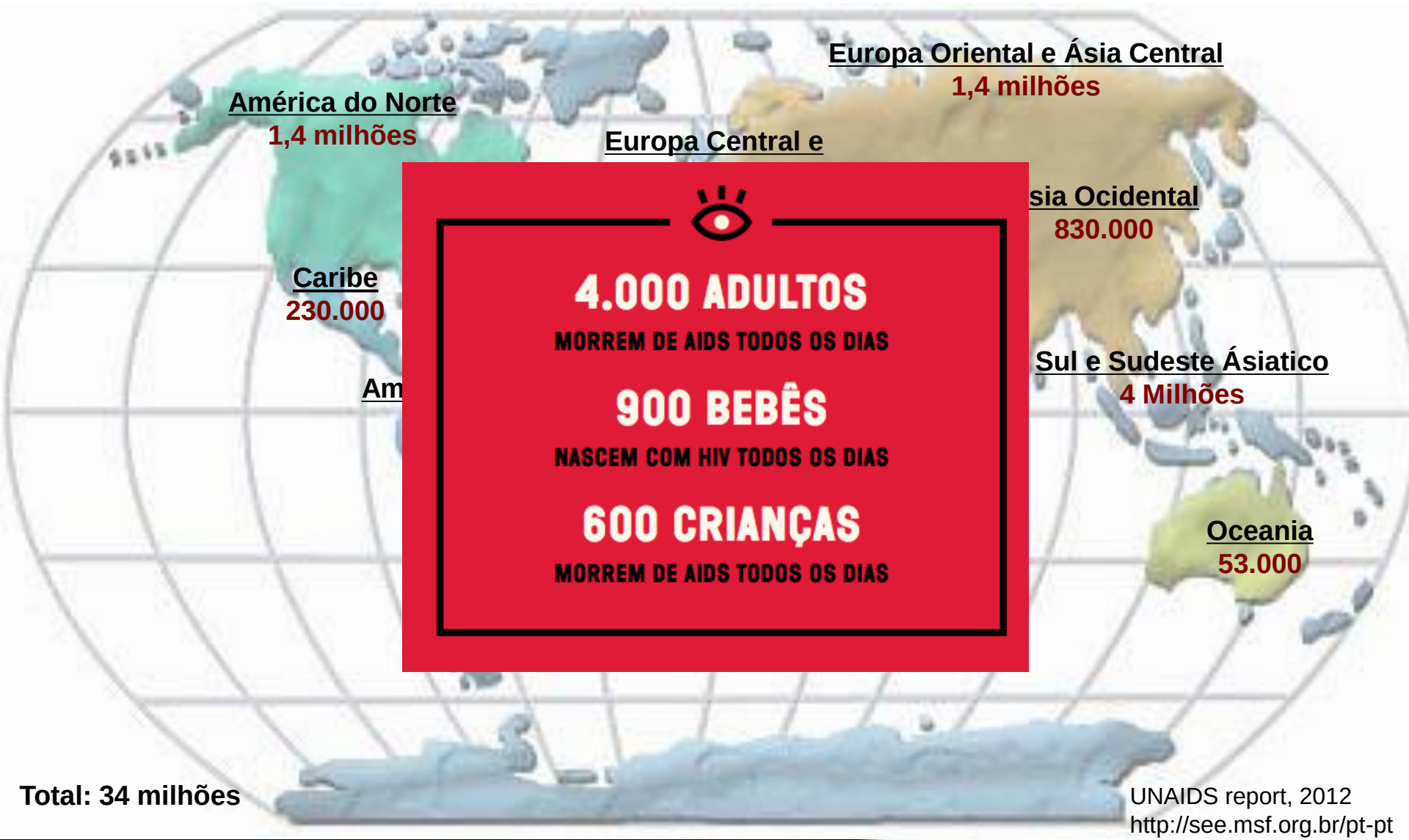
Laboratório de Vacinas Experimentais (LaVEx)

Disciplina de Imunologia

Universidade Federal de São Paulo- UNIFESP

Simpósio sobre produção de vacinas no Brasil- ABC Dez 2013

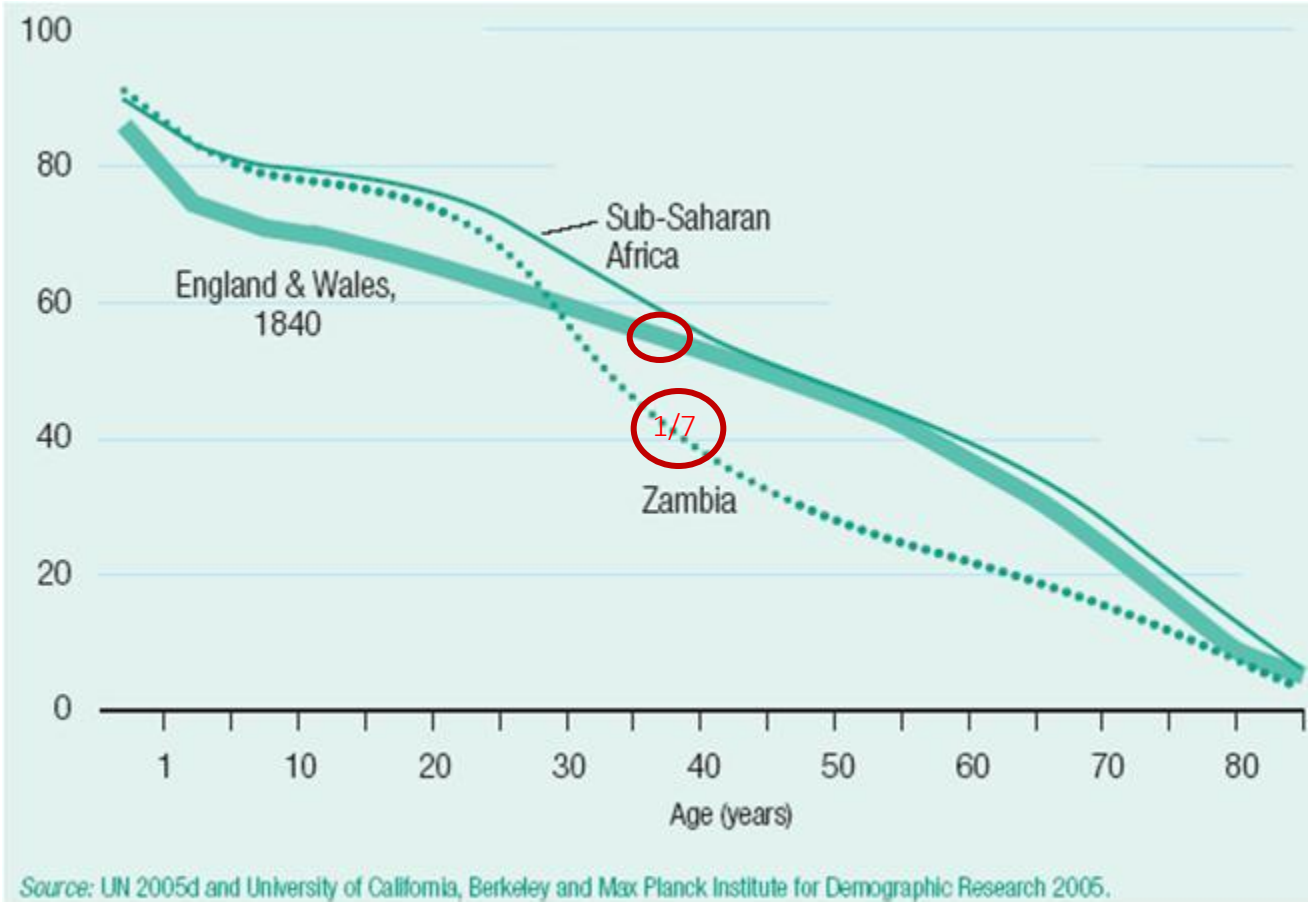
Epidemiologia

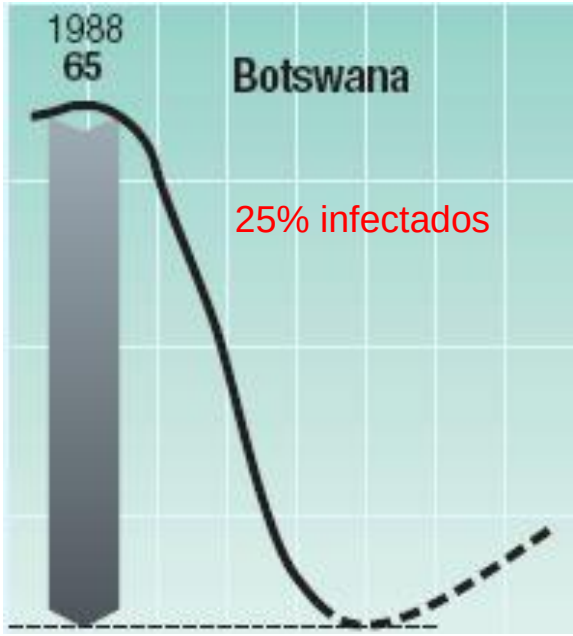


Total: 34 milhões

UNAIDS report, 2012
http://see.msf.org.br/pt-pt

Impacto da infecção pelo HIV na expectativa de vida

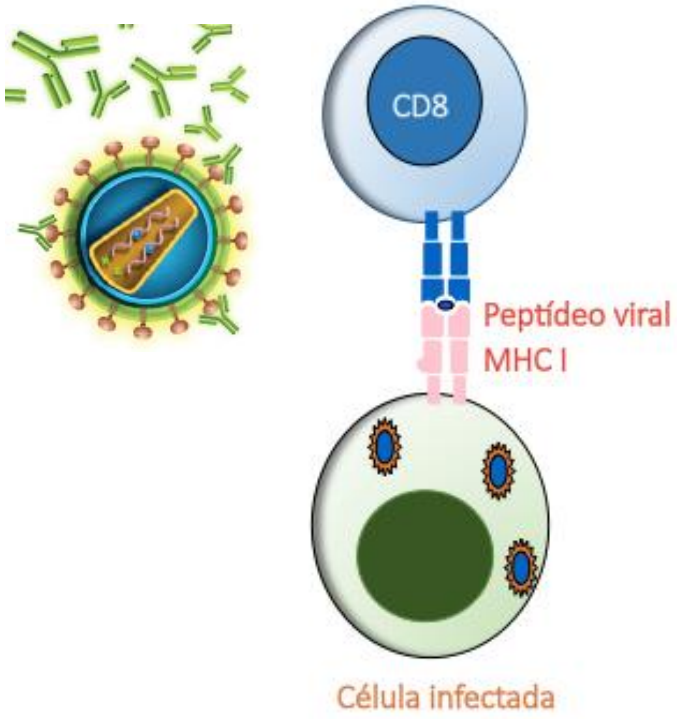


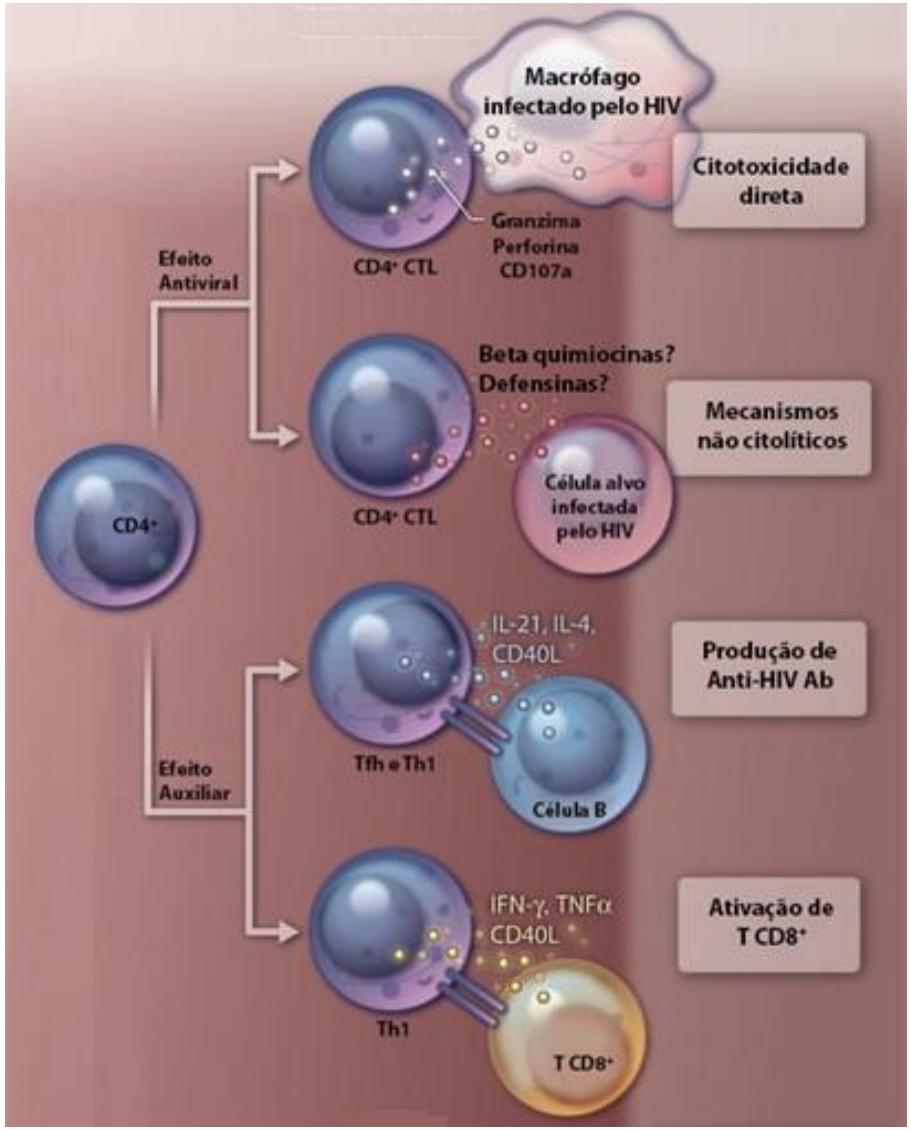


39
2010-2015

United Nations Department of Economic and Social Affairs

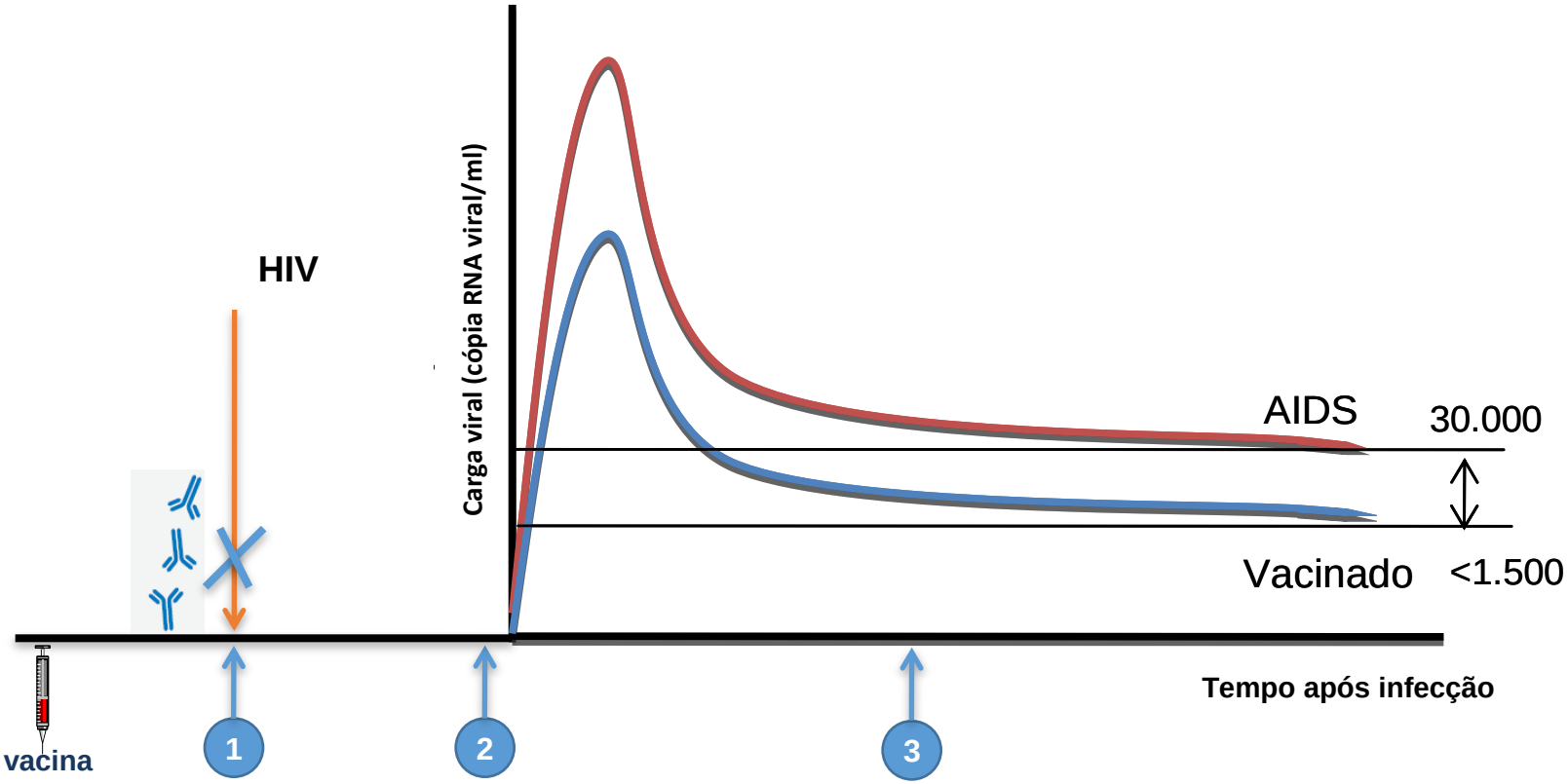
Aspectos na imunidade protetora contra o HIV





Klatt and Silvestre, 2012.

Como deve ser uma vacina contra o HIV?



Imunidade estéril

Prevenir infecção

Imunidade parcial

Diminuir viremia
transmissão
Parar a progressão

Imunidade parcial

Terapêutica

Diminuir viremia
transmissão
Parar a progressão

Rosa *et al.*, 2008

Até hoje...

35 ensaios clínicos realizados

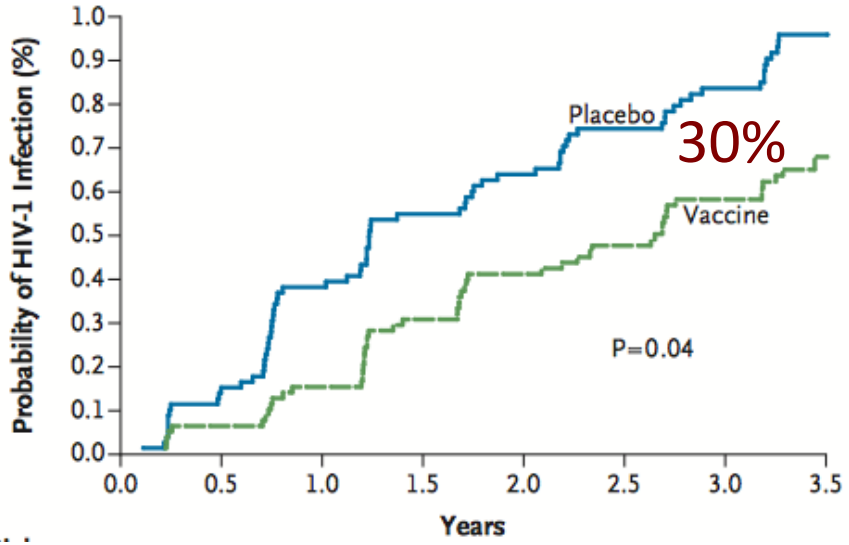
5 ensaios clínicos de eficácia (IIb/III)

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 3, 2009 VOL. 361 NO. 23

Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand

Supachai Rerks-Ngarm, M.D., Punnee Pitisuttithum, M.D., D.T.M.H., Sorachai Nitayaphan, M.D., Ph.D., Jaranit Kaewkungwal, Ph.D., Joseph Chiu, M.D., Robert Paris, M.D., Nakorn Premsri, M.D., Chawetsan Namwat, M.D., Mark de Souza, Ph.D., Elizabeth Adams, M.D., Michael Benenson, M.D., Sanjay Gurunathan, M.D., Jim Tartaglia, Ph.D., John G. McNeil, M.D., Donald P. Francis, M.D., D.Sc., Donald Stablein, Ph.D., Deborah L. Birx, M.D., Supamit Chunsuttiwat, M.D., Chirasak Khamboonruang, M.D., Prasert Thongcharoen, M.D., Ph.D., Merlin L. Robb, M.D., Nelson L. Michael, M.D., Ph.D., Prayura Kunasol, M.D., and Jerome H. Kim, M.D., for the MOPH–TAVEG Investigators*



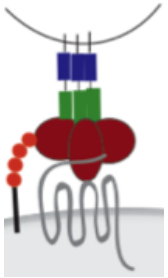
- ✓ 40% respostas de LTCD4⁺ HIV-específicos
- ✓ Sem resposta de CD8⁺ HIV-específicos
- ✓ Anticorpos pouco neutralizantes porém com capacidade de se ligarem ao vírus (ligadores)

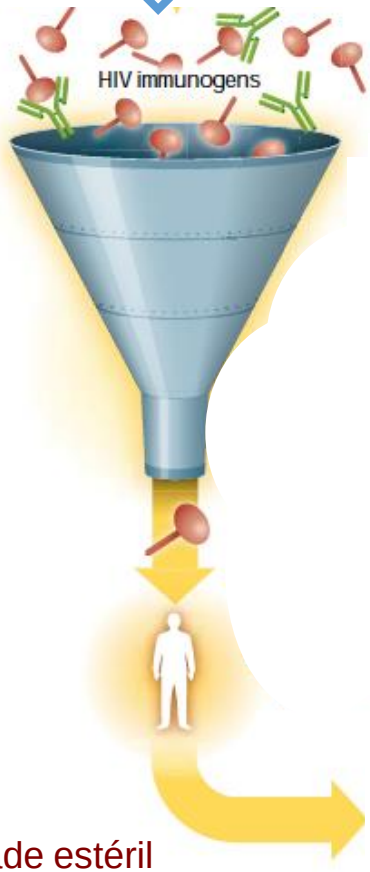
Montefiori *et al.*, 2013

Dificuldades encontradas

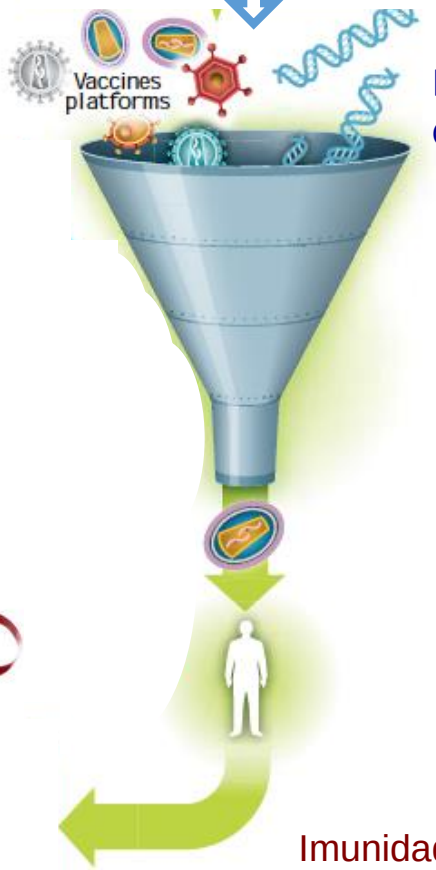
Produção de anticorpos

monômeros





Respostas de células T



LT $CD8^+$

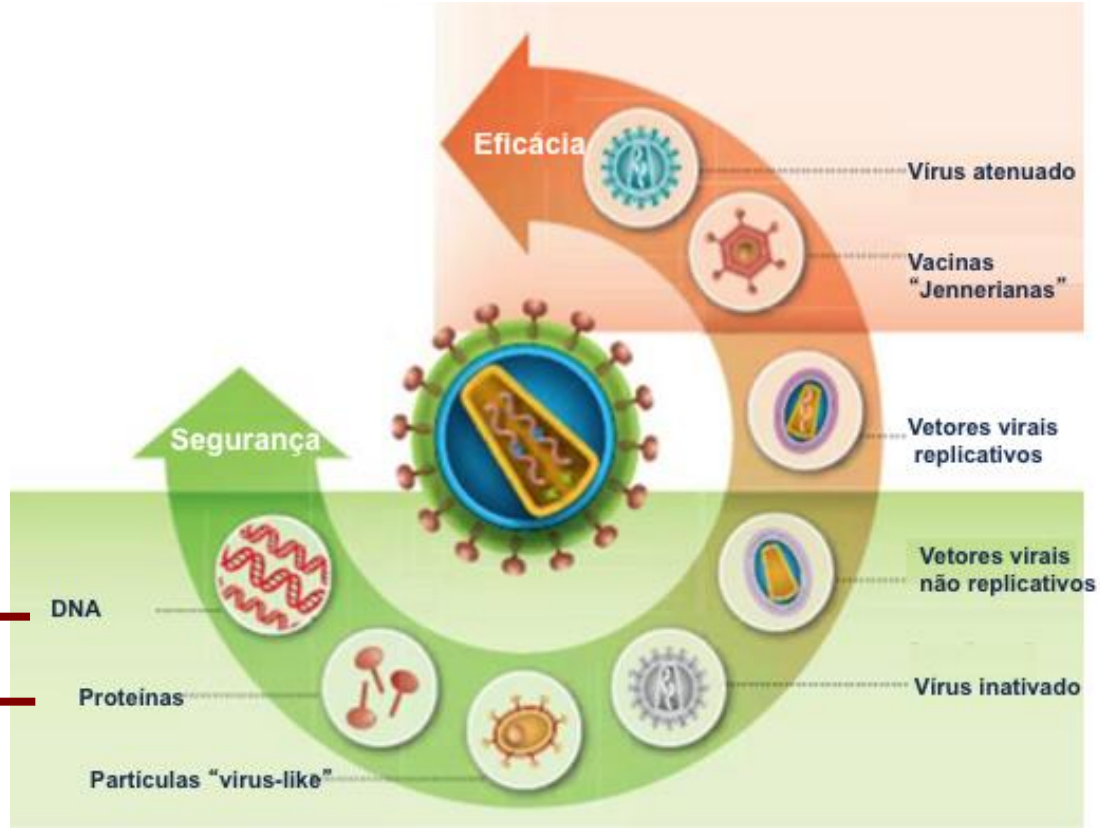
Genes ou proteínas inteiras

Imunidade estéril

Imunidade parcial

Novas estratégias para o desenvolvimento de imunógenos

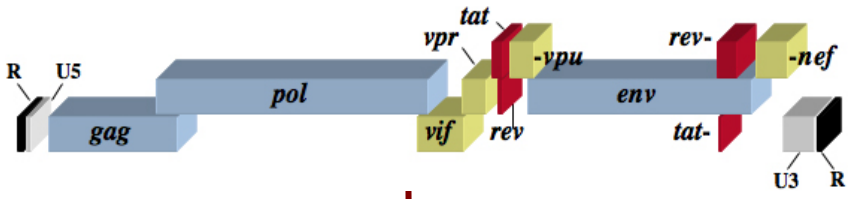
Plataformas para desenvolvimento de vacinas



HIVBr18 ← DNA

gp140 ← Proteínas

Vetores virais não replicativos → Ad5HIVBr18



TEPITOPE

Identificação de 18 epítopos promíscuos

Ensaio de ligação à moléculas de HLA DR humanas

Reconhecimento por CMSP de pacientes HIV^{+}

Desenho da vacina

Avaliação da imunogenicidade

Fonseca et al. AIDS 2006

Ligação à moléculas de HLA DR humanas (17)

Peptide	Binding/IC_{50} (nM)																	HLA-DR binding	
	DRB1*0 1:01	DRB1*0 3:01	DRB1*0 4:01	DRB1*0 4:05	DRB1*0 7:01	DRB1*0 8:02	DRB1*0 9:01	DRB1*1 0:01	DRB1*1 1:01	DRB1*1 2:01	DRB1*1 3:02	DRB1*1 5:01	DRB1*1 6:02	DRB3*0 1:01	DRB3*0 2:02	DRB4*0 1:01	DRB5*0 1:01	#bound molecules	(%)
p17(73-89)	68	1916	444	271	1082	52	207	87	338	216	2748	488	69	479	4825	337	4585	12	70,6
p24(33-45)	7,1	-	3,9	24	2808	1,3	32	6,5	121	290	27413	117	7,6	7247	7956	175	295	12	70,6
p24(131-150)	4,5	88	7,4	105	85	4,1	14	3,9	16	25	8,4	5,1	4,0	263	37	14	38	17	100,0
p6(32-46)	2,3	1171	3,1	18	69	5,1	21	2,2	31	9,2	3551	57	10	2480	722	74	45	14	82,4
pol(63-77)	22	10459	124	224	2859	16	135	74	202	183	9629	98	74	1152	2469	22	37	12	70,6
pol(136-150)	37	1535	2187	4513	6709	284	2094	871	705	3181	26	70	608	2008	8882	495	1019	8	47,1
pol(785-799)	17	-	196	6242	208	435	550	59	2378	4137	10080	262	976	4538	27248	778	3321	9	52,9
gp41(261-276)	308	3959	1298	575	231	158	473	83	577	754	3537	725	1221	669	7787	1207	596	11	64,7
gp160(19-31)	5241	-	9577	701	5661	877	6294	318	9668	5675	-	5352	5239	2012	-	4319	20992	3	17,6
gp160(174-185)	15	9088	63	19	26	124	17	3,5	1072	75	135	3256	113	14	290	1257	1110	12	70,6
gp160(188-201)	59	7309	329	169	432	188	291	63	3532	1650	109	800	54	466	440	5422	200	13	76,5
gp160(481-498)	2,6	599	789	468	19	13	18	2,6	175	70	92	5,9	53	1506	905	28	310	16	94,1
ver(11-27)	28	229	6,8	54	2484	25	233	19	500	1,0	7796	79	529	574	172	0,59	286	16	94,1
vpr(58-72)	1556	23324	453	440	28413	544	4555	87	9754	462	-	391	2111	4812	21526	199	4836	7	41,2
vpr(65-82)	1383	361	228	66	5574	79	579	58	2622	2386	3772	272	671	17564	2414	51	5246	9	52,9
vif(144-158)	0,39	15181	3,9	3,1	16	235	14	1,2	51	95	15323	8,1	5,8	839	409	199	3,9	15	88,2
nef(180-194)	89	5,5	111	89	157	593	187	44	60	1248	709	141	110	6,5	18	4013	7,5	15	88,2
vpu(6-20)	266	506	590	815	4100	335	881	18	2212	1078	-	1332	5338	603	1461	10334	137	9	52,9
																		DR binding to >50% peptides	
#peptides bound to HLA-DR	15	6	15	16	9	18	15	18	11	11	6	15	14	9	8	5	11	13/17	76%

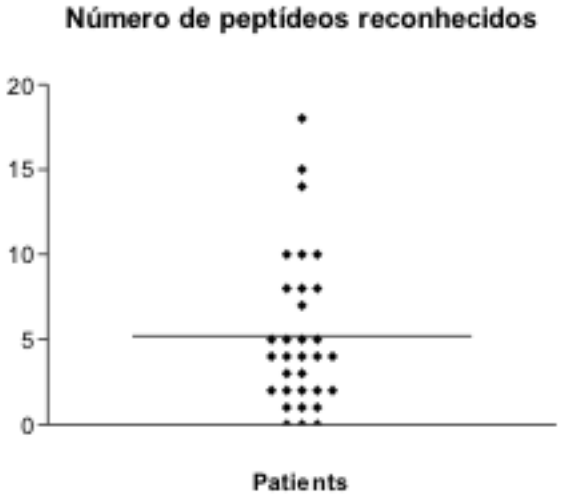
Cada peptídeo se ligou a várias moléculas de HLA DR

Reconhecimento por CMSP de pacientes HIV+

- ✓ ELISPOT: CMSP de 90% dos pacientes HIV+ reconheceram pelo menos um peptídeo
- ✓ Em média reconhecimento de 5 peptídeos
- ✓ Respostas de CD4+ e CD8+

Identification of novel consensus CD4 T-cell epitopes from clade B HIV-1 whole genome that are frequently recognized by HIV-1 infected patients

Simone G. Fonseca[a,b,c], Adriana Coutinho-Silva[a,b,c], Luiz Augusto M. Fonseca[a,c], Aluisio C. Segurado[c,d], Sandra L. Moraes[a,c,e], Hélcio Rodrigues[b], Juergen Hammer[f], Esper G. Kallás[c,g], John Sidney[h], Alessandro Sette[h], Jorge Kalil[a,b,c] and Edecio Cunha-Neto[a,b,c]



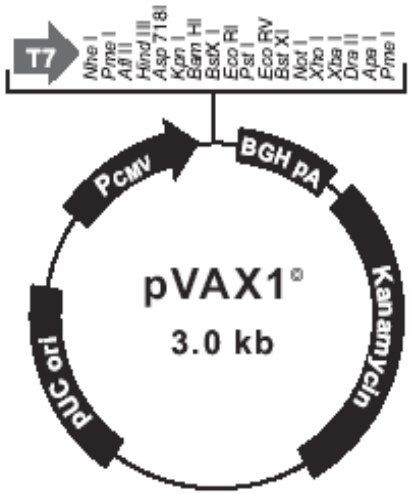
Desenho da vacina

p17 (73-89)	p24 (33-45)	p24 (131-150)	p6 (32-46)	pol (63-77)	pol (136-150)	pol (785-799)	gp41 (261-276)	gp160 (19-31)
gp160 (174-185)	gp160 (188-201)	gp160 (481-498)	rev (11-27)	vpr (58-72)	vpr (65-82)	vif (144-158)	vpu (6-20)	nef (180-194)

Espaçadores GPGPG

Epitopos Novos

Otimização de códons



gene multiepitópico :

HIVBr18

(1110pb)

Determinação da Imunogenicidade

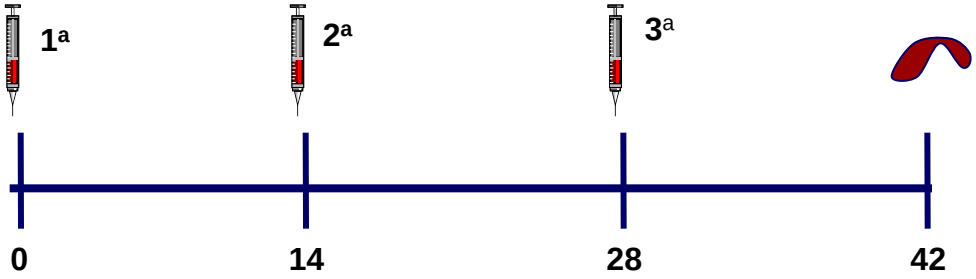
I – Magnitude

II – Longevidade

III – Amplitude

IV- Cobertura (camundongos transgênicos para moléculas de HLA classe II humanas)

Imunização



BALB/c
DR2
DR4
DQ6
DQ8

100ug DNA/dose: HIVBr18 ou pVAX 1

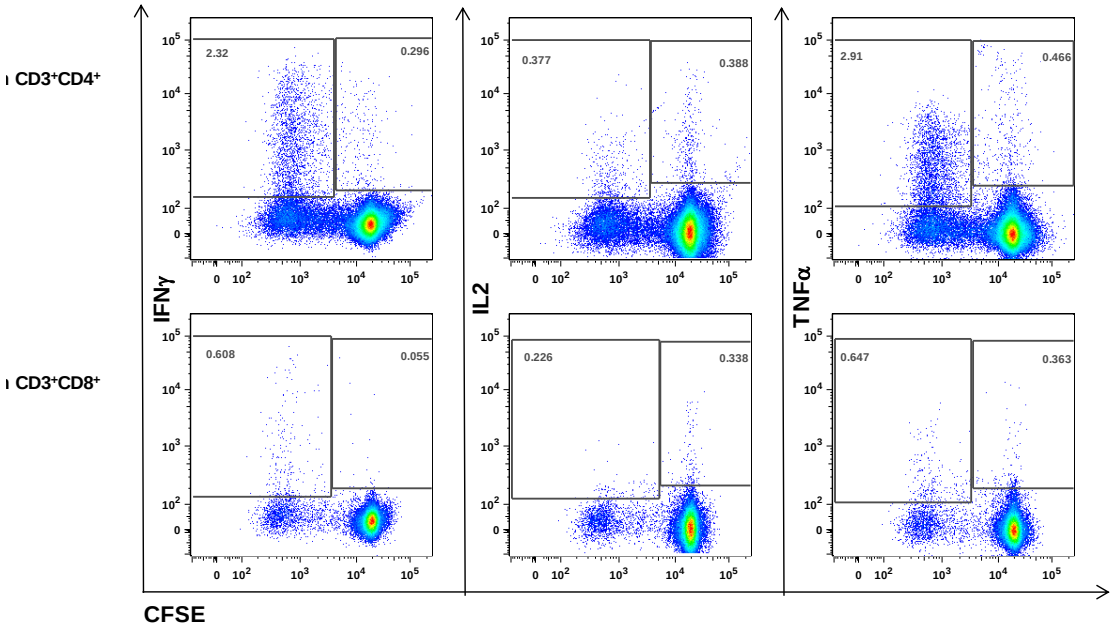
Imunogenicidade HIVBR18

OPEN ACCESS Freely available online

PLOS one

A DNA Vaccine Encoding Multiple HIV CD4 Epitopes Elicits Vigorous Polyfunctional, Long-Lived CD4$^+$ and CD8$^+$ T Cell Responses

Daniela Santoro Rosa[1,3,4☯], Susan Pereira Ribeiro[1,3☯], Rafael Ribeiro Almeida[1], Eliane Conti Mairena[2,3], Edilberto Postól[2,3], Jorge Kalil[1,2,3], Edecio Cunha-Neto[1,2,3*]



Cobertura HIVBR18

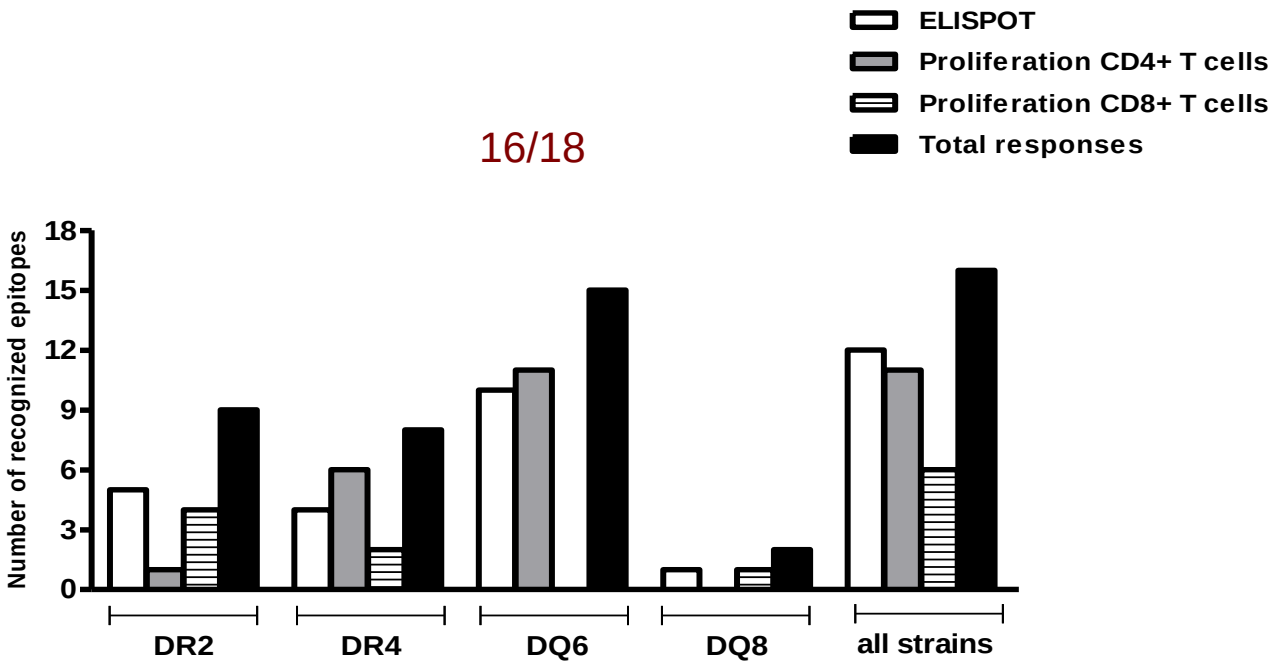
OPEN ACCESS Freely available online

PLoS one

A Vaccine Encoding Conserved Promiscuous HIV CD4 Epitopes Induces Broad T Cell Responses in Mice Transgenic to Multiple Common HLA Class II Molecules

Susan Pereira Ribeiro[1,3☯], Daniela Santoro Rosa[1,3☯], Simone Gonçalves Fonseca[1,2], Eliane Conti Mairena[2,3], Edilberto Postól[2,3], Sergio Costa Oliveira[4], Luiza Guilherme[2,3], Jorge Kalil[1,2,3], Edecio Cunha-Neto[1,2,3*]

16/18



Vaccines & Vaccination

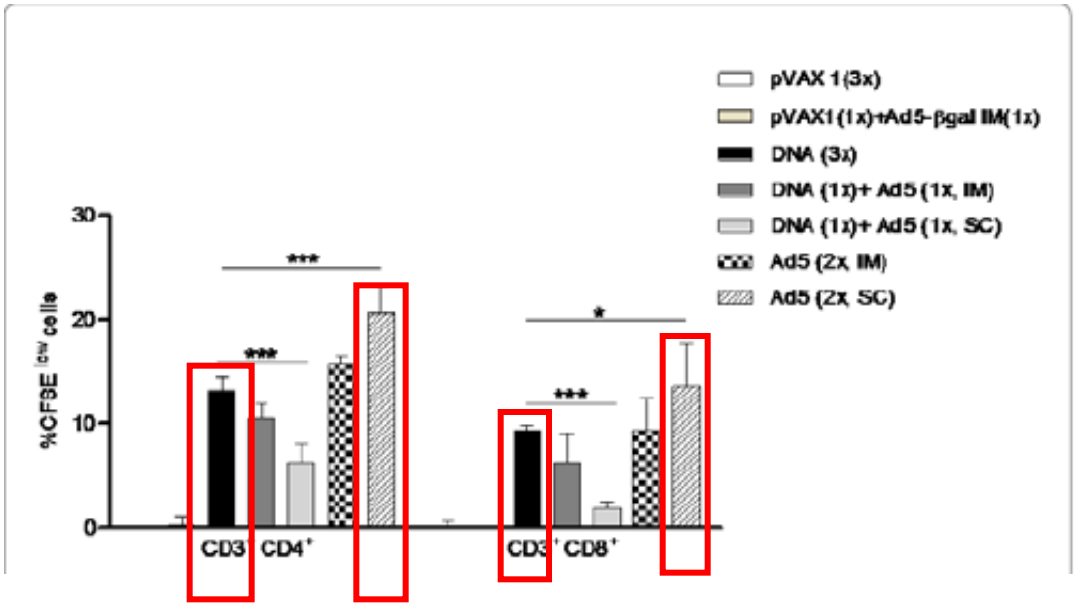
Rosa et al. J Vaccines Vaccin 2011, 2:4
http://dx.doi.org/10.4172/2157-7560.1000124

Research Article Open Access

A Recombinant Adenovirus Encoding Multiple HIV-1 Epitopes Induces Stronger CD4+ T cell Responses than a DNA Vaccine in Mice

Daniela Santoro Rosa[1,2,4], Susan Pereira Ribeiro[1,2], Rafael Ribeiro Almeida[1], Eliane Conti Mairena[2,3], Jorge Kalil[1,2,3] and Edecio Cunha-Neto[1,2,3]*

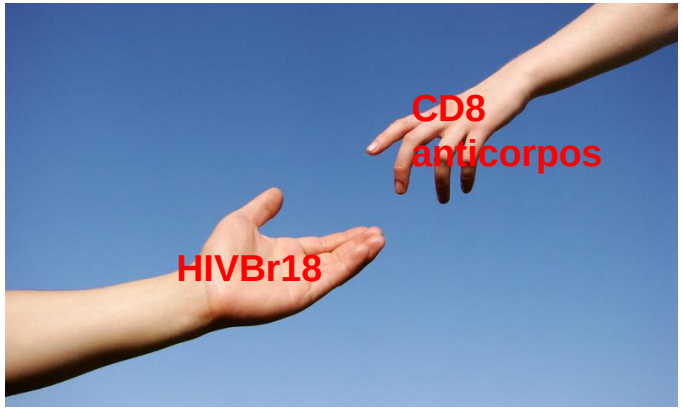
[1]Laboratory of Clinical Immunology and Allergy-LIM60, Division of Clinical Immunology and Allergy, Department of Medicine, Brazil
[2]Institute for Investigation in Immunology-INCT, Sao Paulo, Brazil
[3]Heart Institute (InCor), University of Sao Paulo School of Medicine, Sao Paulo, Brazil
[4]Division of Immunology-Federal University of Sao Paulo-UNIFESP, Sao Paulo, Brazil



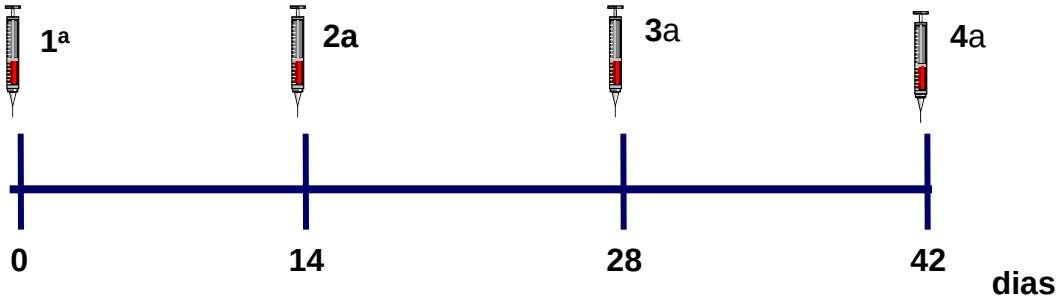
Imunização com HIVBr18 induz

Respostas contra múltiplos epítopos no contexto de diferentes moléculas de HLA classe II

Respostas Th1, polifuncionais e de longa duração



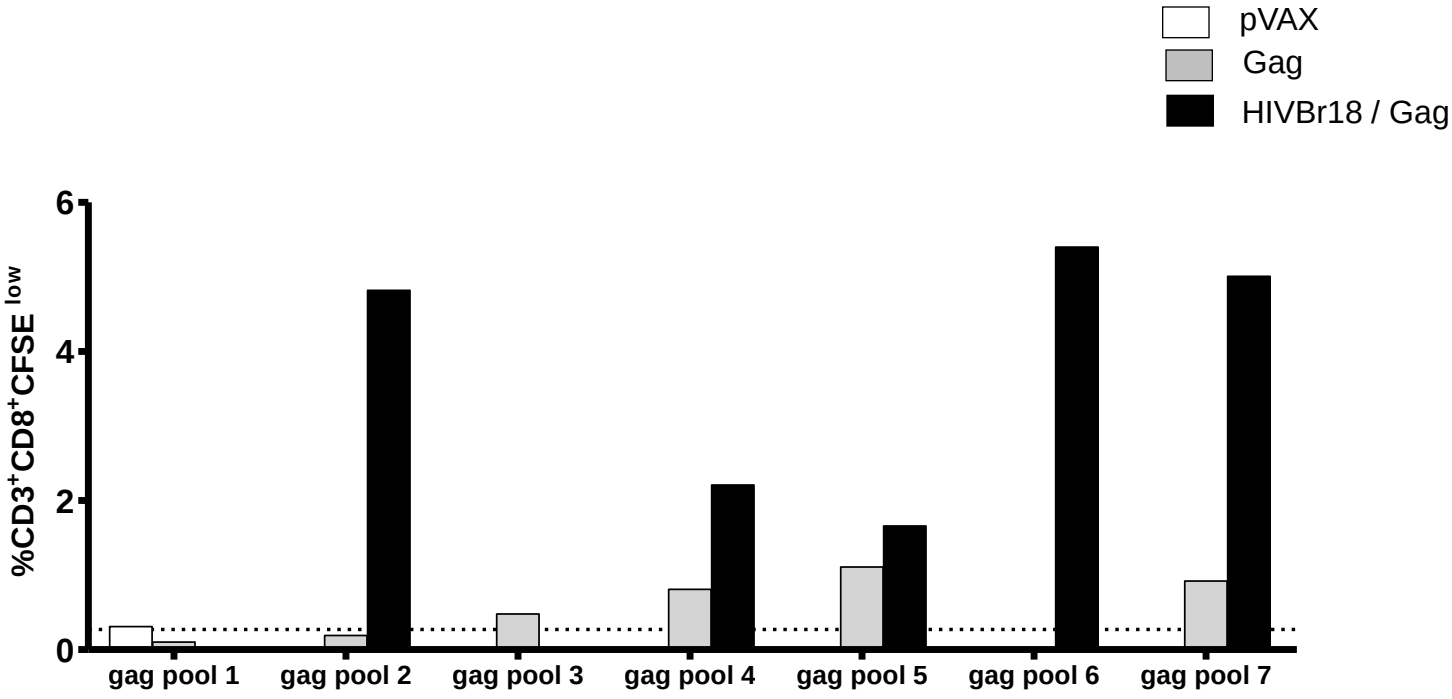
Efeito da imunização inicial com HIVBr18 na resposta de CD8



100ug DNA/dose

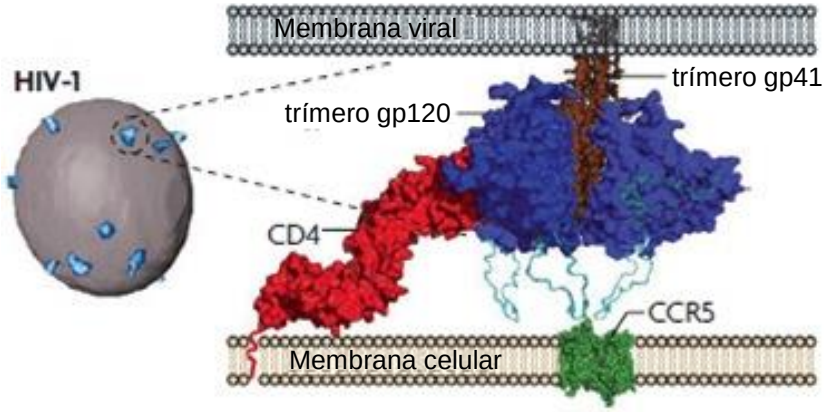
4x Gag

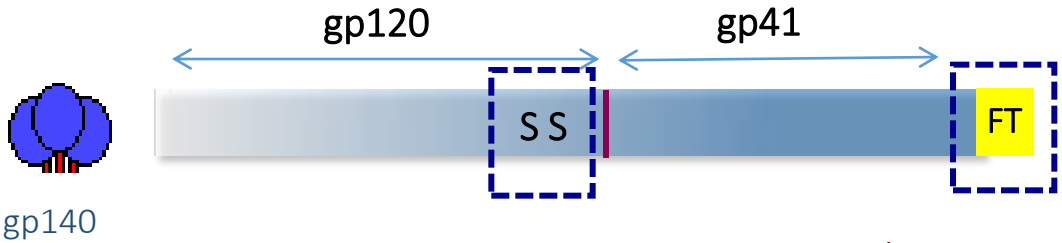
2x HIVBr18
2x Gag



Aumento magnitude/amplitude

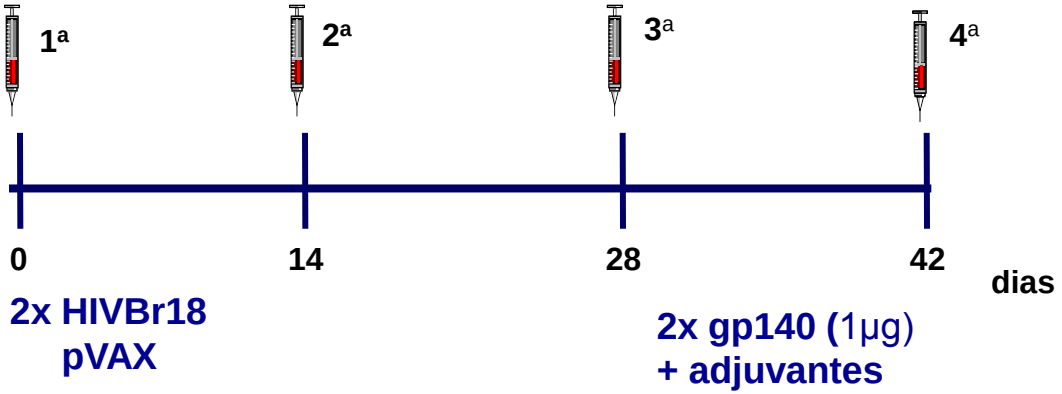
Efeito da imunização inicial com HIVBr18 na resposta humoral





Plasmídeo gentilmente cedido por **Michel C. Nussenzweig**

Efeito da imunização inicial com HIVBr18 na resposta humoral



Adjuvante completo de Freund → "padrão ouro"

Hidróxido de alumínio – Alum → adjuvante mais utilizado em vacinas

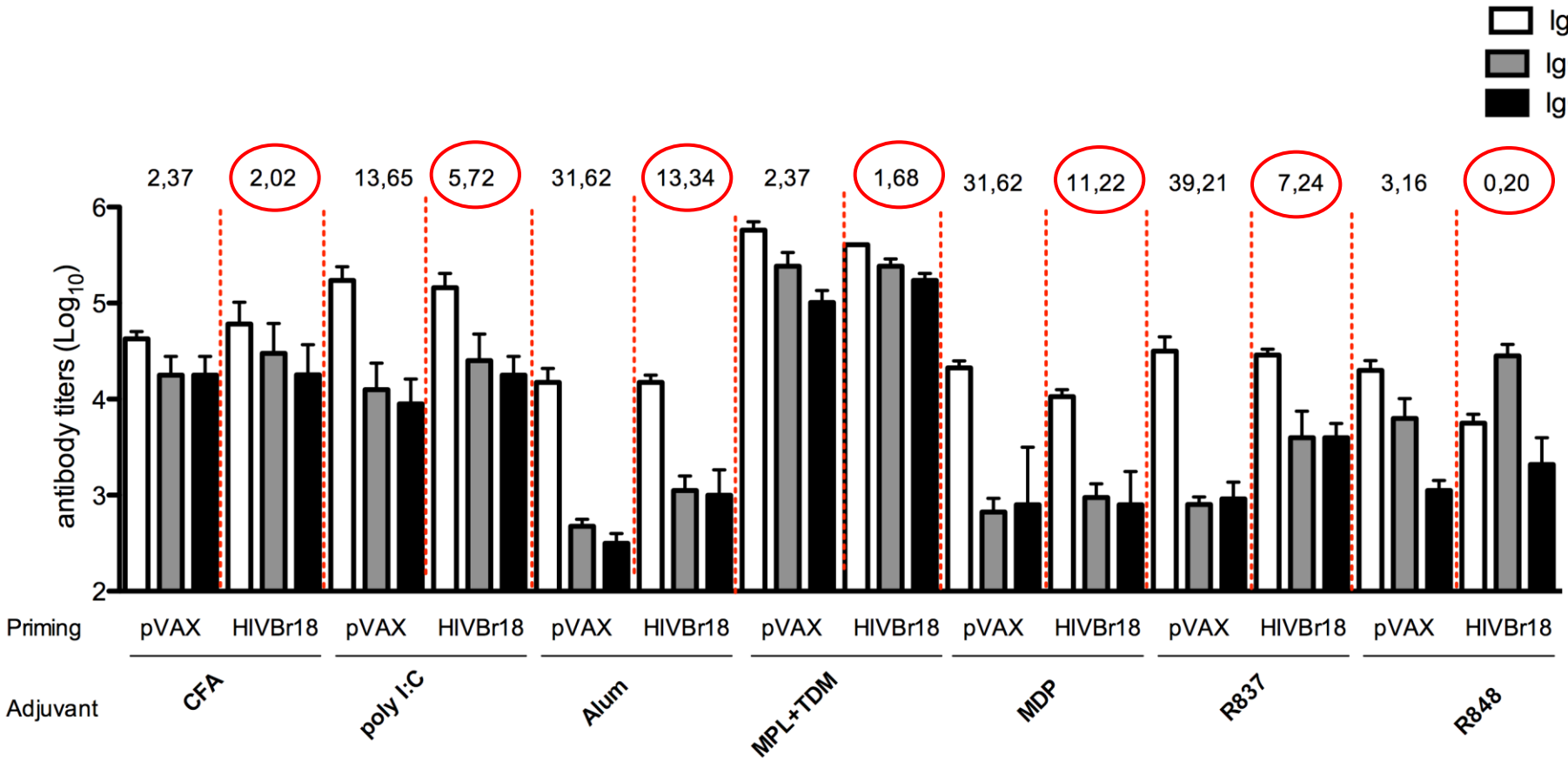
Agonistas de TLR:

poly IC → TLR3
MPL/TDM → TLR4
R837-imiquimod →
R848- resiquimod
CpG ODN → TLR9

Agonistas de NLR:

Muramil dipeptídeo (MDP

Subclasses de IgG



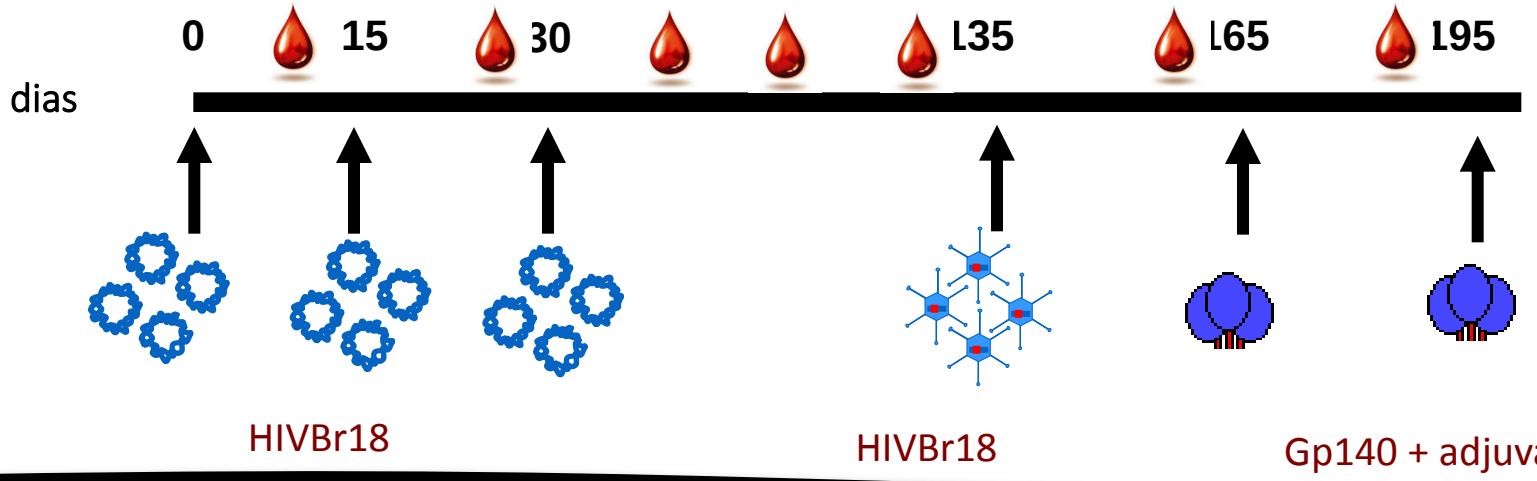
HIVBr18: Diminuição IgG1/IgG2a
Sem alteração magnitude.

Experimento piloto em Rhesus

INSTITUTO BUTANTAN

Análises:

-Peso
-Temperatura
-Hemograma
-Resposta humoral
-Resposta celular



Agradecimentos

MEDICINA USP

LIM-60

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Drª Susan Ribeiro
Rafael Almeida Ribeiro

INCOR HCFMUSP
CIÊNCIA E HUMANISMO

Prof. Dr. Jorge Kalil
Eliane Conti Mairena

UNIFESP
UNIVERSIDADE FEDERAL DE SÃO PAULO

Juliana de Souza Apostólico

INSTITUTO BUTANTAN

Vania Mattaraia
Elizabeth Ghiuro

La Jolla Institute
FOR ALLERGY AND IMMUNOLOGY

Alex Sette
John Sidney

NIH National Institute of Allergy and Infectious Diseases
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Mario Roererer
Srinivas Rao

Apoio financeiro

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FAPESP

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico