CTC
Centro de Terapia Celular
Center for Cell-Based Therapy

FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
SCIENTIA TERMINUM AMOVERE

Developing CART-cells Therapies in Brazil

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Cellicon Valley

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USP
Universidade de São Paulo

Potential Conflict of Interest

Consultant / Advisory Board	Novartis	BMS	Janssen	Libbs	Pfizer	Amgen
Grant / Research Support	CNPq		ASH		DECIT/MS	
Honoraria / Speaker's Bureau			Janssen			
Patent / Stock / Royalties	-					
Affiliation to any Board of Directors or Council	SUPERA Parque					
Off-label medications use discussing	-					

The views expressed are my own and do not represent the opinion of The Clinical Hospital and The Center for Cell Therapy of FMRP-USP

How to develop a CAR T-cell platform in low-middle income countries?

- Restricted budget
- Restricted human resources to develop new technologies
- Delay to define regulatory aspects of advanced cell therapy
- Establish new GMP facilities
- Generation of vectors in GMP conditions
- Translational research
- Clinical units and clinicians with the expertise need to

The CAR T-cell in Brazil: Initiatives

- **INCA:**
  - Martin Bonamino: pre-clinical study with sleeping beauty transposon vectors
- **Boldrini:**
  - Pedro Campos Lima: pre-clinical studies with CD19
- **Celluris+Eretz Bio (Einstein):**
  - Pre-clinical studies with CAR anti-CD123 e -CD33
- **FMRP-USP - Center for cell-based Therapy (Ribeirão Preto)**
  - Brazilian CAR T Platform
- **Prodigy: Einstein, USP-SP, PUC-UFPR, Sírio Libanês**

The CAR T-cell in Brazil: Initiatives

Human Gene Therapy

DOI: 10.1089/hum.2018.218

1

CAR T cells generated using *Sleeping Beauty* transposon vectors and expanded with an EBV-transformed lymphoblastoid cell line (LCL) display antitumor activity *in vitro* and *in vivo*.

Leonardo Chicaybam*1,2, Luiza Abdo*1, Mayra Carneiro*1, Bárbara Peixoto3, Mariana Viegas1, Priscila de Sousa1, Márcia C. Fornazin4, Maria C. Spago4, Angelo Brunelli Albertoni Laranjeira4, Pedro O. de Campos-Lima5, Alexandre Nowill4, Luciana Rodrigues Carvalho Barros1, Martín H. Bonamino1,2#

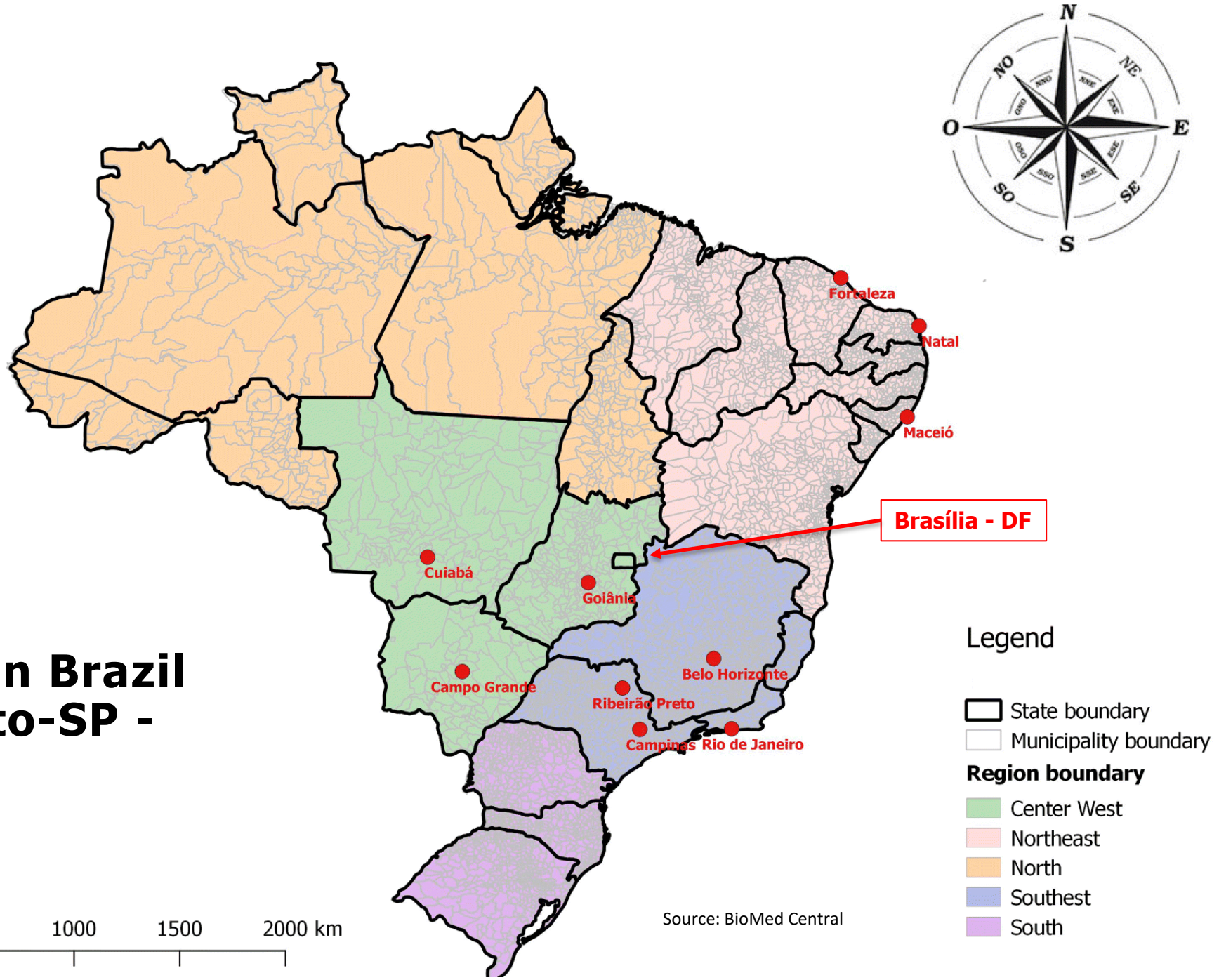
Slide courtesy Martin H Bonamino

The CAR T-cell in Brazil

- Brazilian CAR T Platform Ribeirão Preto-SP -

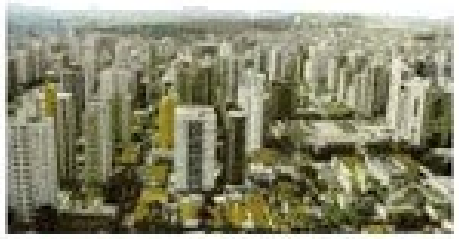
- **Principle: 100% Brazilian technology**
  - Design and validate new lentiviral vectors (new clones) for clinical purposes
  - Develop the platform for pre-clinical studies
  - New translational research lab focused on advanced cell therapy
  - Manufacture and scale lentiviral vectors up in GMP conditions
  - Manufacture CAR T-cells in an affordable cost to low-middle income countries
  - Establish collaborations and launch clinical trials

The CAR T-cell in Brazil
- Ribeirão Preto-SP -



Source: BioMed Central

The CAR T-cell in Brazil - Ribeirão Preto-SP -



Downtown Ribeirão Preto

Campus of the University of São Paulo at Ribeirão Preto with the city of Ribeirão Preto behind it. Notice the campus lake and the Clinics Hospital (the large and rectangular building).

Source: sisne.org

Brazilian CAR T Platform - Ribeirão Preto-SP

- New scFv clones -

Figure 1. QC SDS-PAGE [CD123]

Lane 1: Marker

Lane 2: CD123

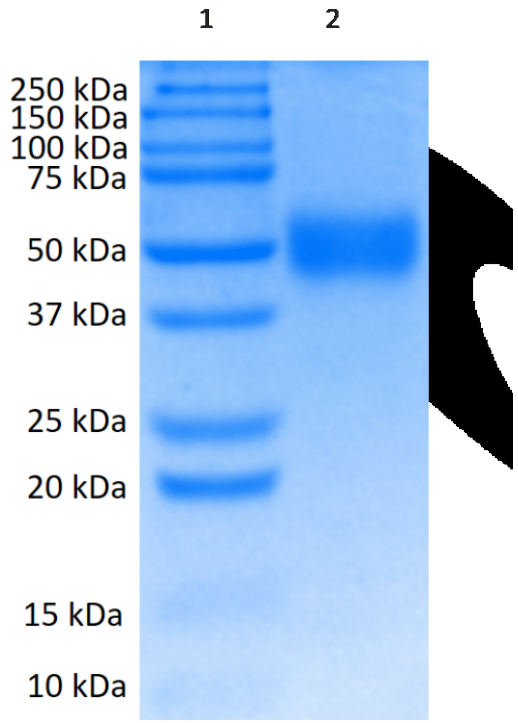


Table 5. QC soluble ELISA of the positive clones [CD123]

Clones	Induce Temp.	Coating: CD123	Coating: BSA	No coating
10	30°C	2.0026	0.0641	0.0553
	37°C	2.5711	0.0654	0.0439
13	30°C	2.1305	0.0640	0.0631
	37°C	2.3632	0.0652	0.0599
	37°C	1.0574	0.0600	0.0478
26	30°C	2.7119	0.0646	0.0436
	37°C	2.1732	0.0654	0.0453
	37°C	1.7632	0.0659	0.0609
TG1	30°C	0.0643	0.0645	0.0450
PBS	-	0.0545	0.0570	0.0578

Unpublished data [confidential]

Brazilian CAR T Platform - Ribeirão Preto-SP
- Virus production and processing -

Transfection

Producer cell line

Transfection reagents

Medium composition

Clarification

Filtration to remove debris

Tangential Flow Filtration

DNAse treatment

Purification Concentration

Pore size

Flow rate

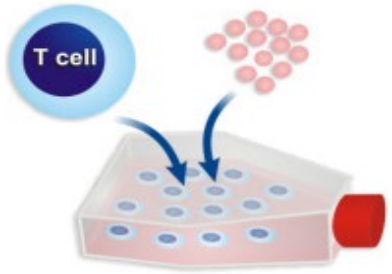
Saturation

Chromatography for purification (?)

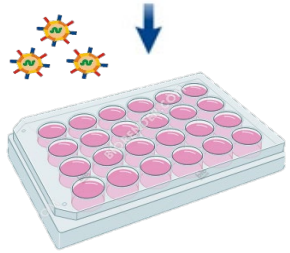
Filter sterilization

Experimental design

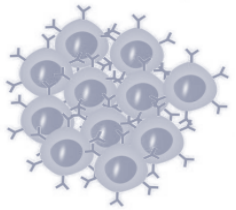
Leukapheresis



T-cells selection and activation

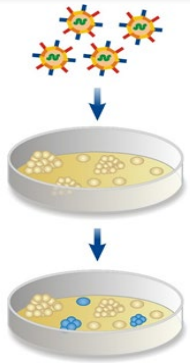


Genetic Modification

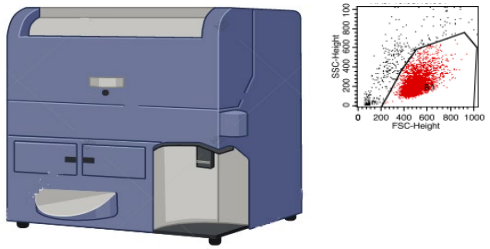


Cell expansion

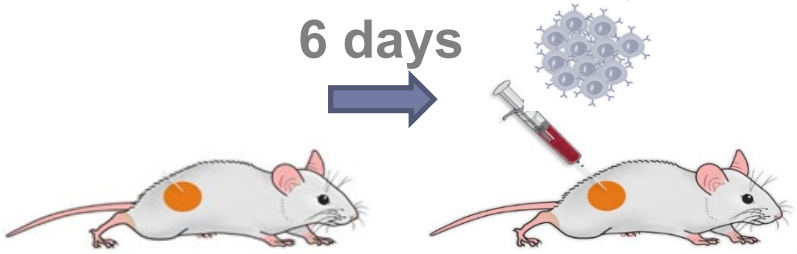
Quality Control



Generation of RAJI-Luciferase cells



Flow cytometry



Tumor cell transplantation

Injection of CAR-T cells

IVIS analysis

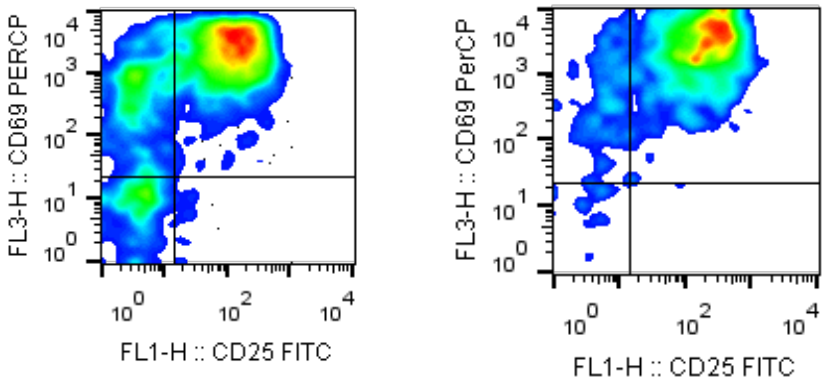
Lentivirus production - GMP

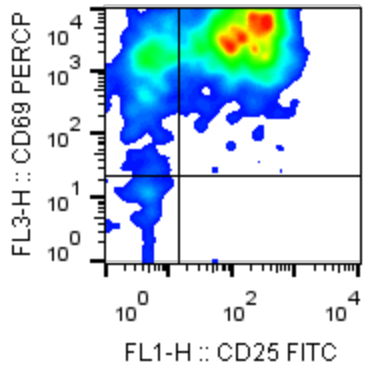
Parameter	Acceptance criteria	Batch#1	Batch#2	Batch#3
Lentiviral Titer (Infecting Units/mL)		$6.9x10^7$	$7.7x10^7$	$1.0x10^8$
Microbiologic	negative	negative	negative	negative
Endotoxin	<5.0 EU/mL	0.728	0.853	0.57
Mycoplasma	<1.0	0.21	0.25	0.61

CAR19 T cells generation and expansion – healthy donors

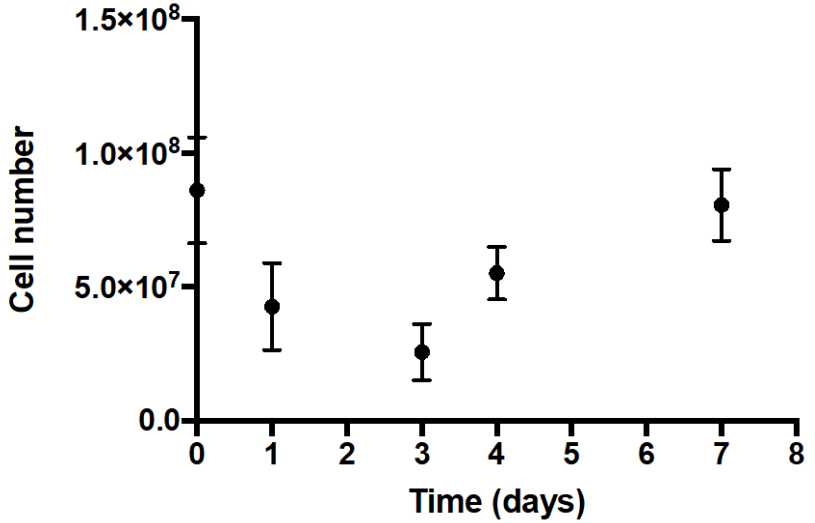
- Cell therapy laboratory – GMP (n=3, three donors)

Cell activation

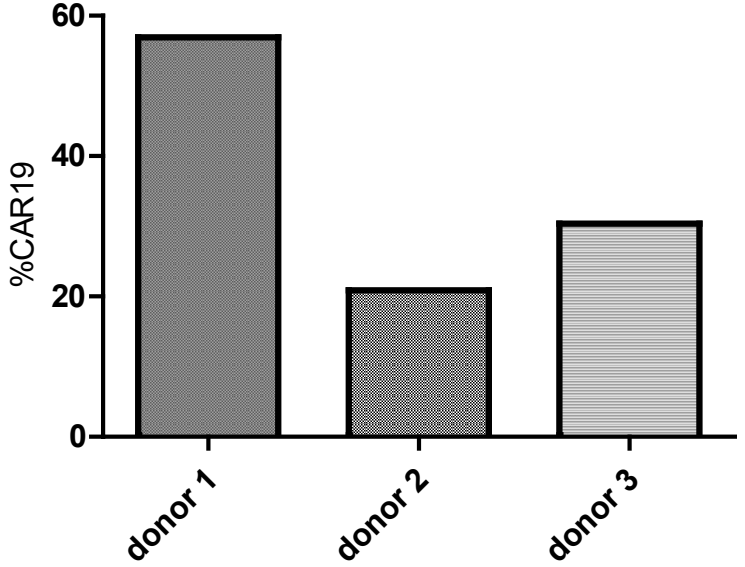




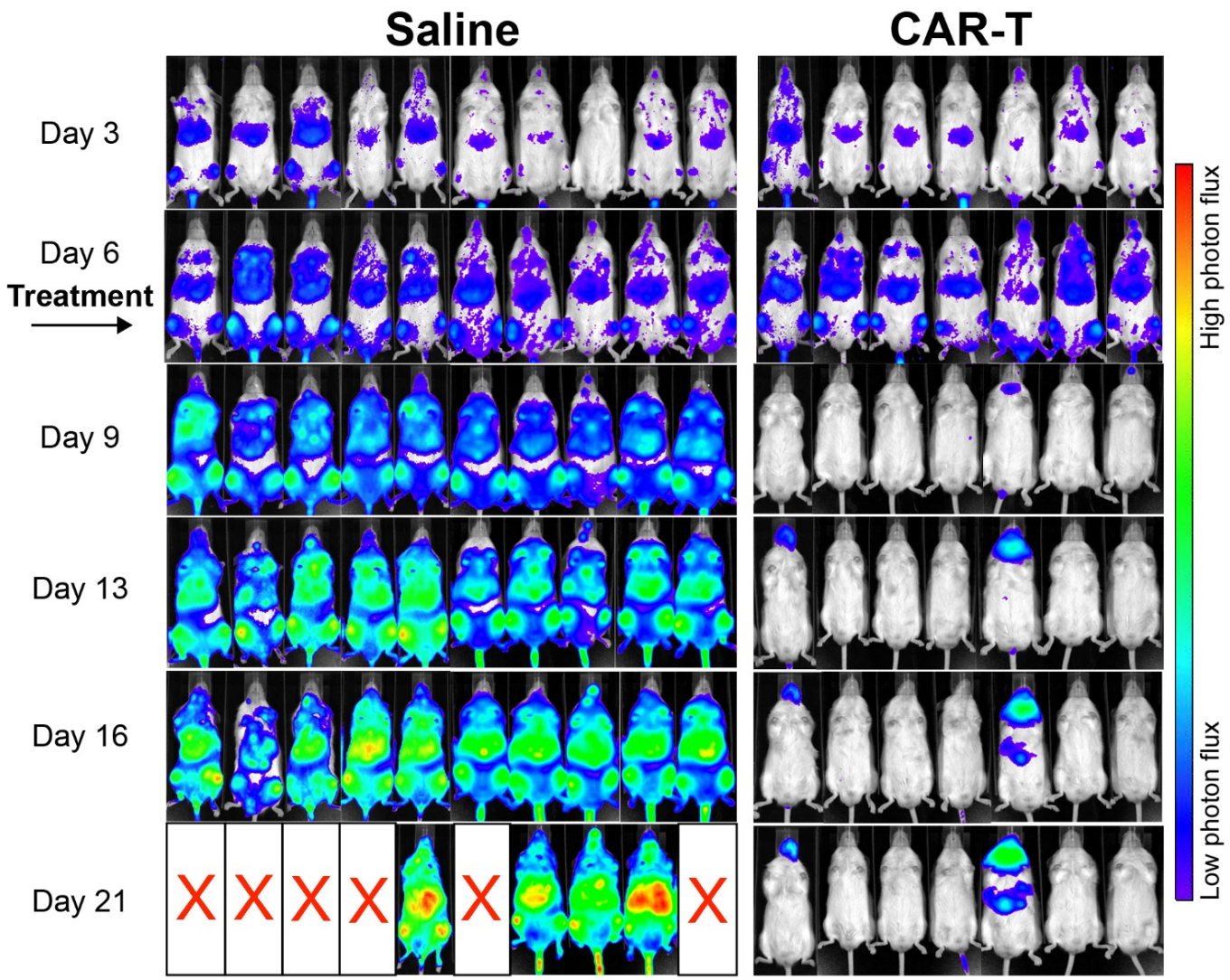
Cell expansion

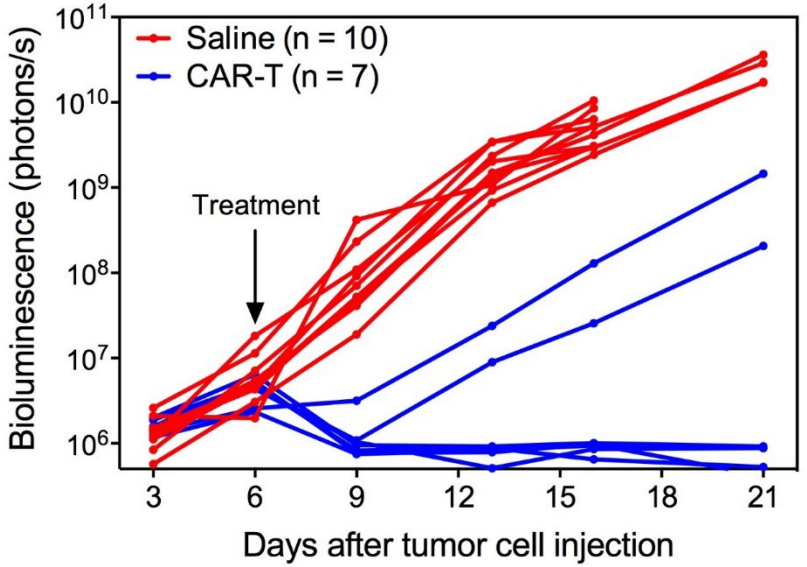


CAR19 expression



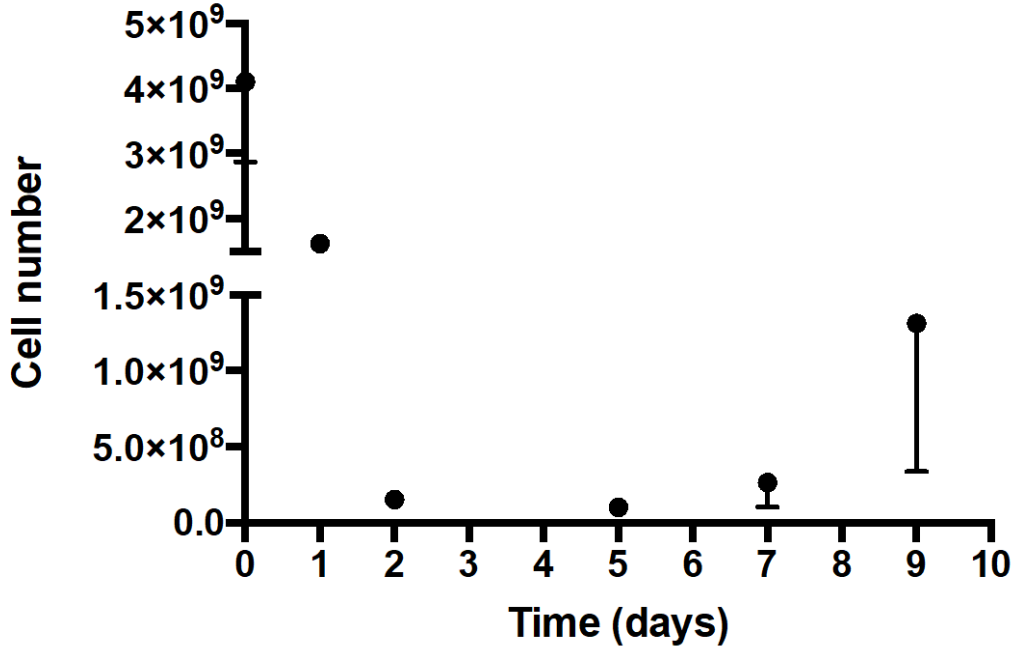
Anti-tumoral efficacy of CAR19 T cells in animal model

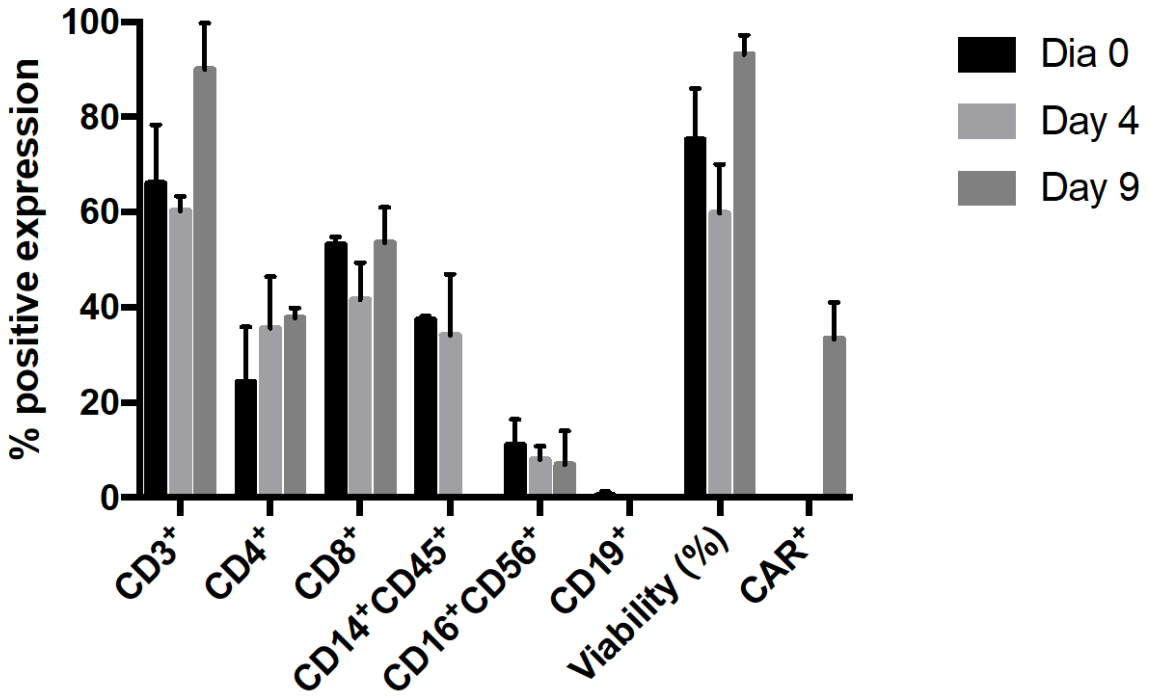




CAR19 T cells – Lymphoma patients

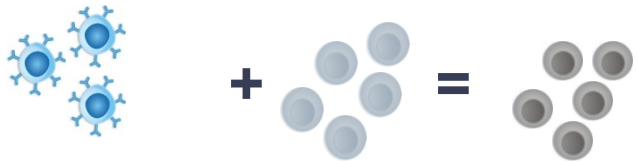
- Cell therapy laboratory – GMP (n=2, two independent donors)

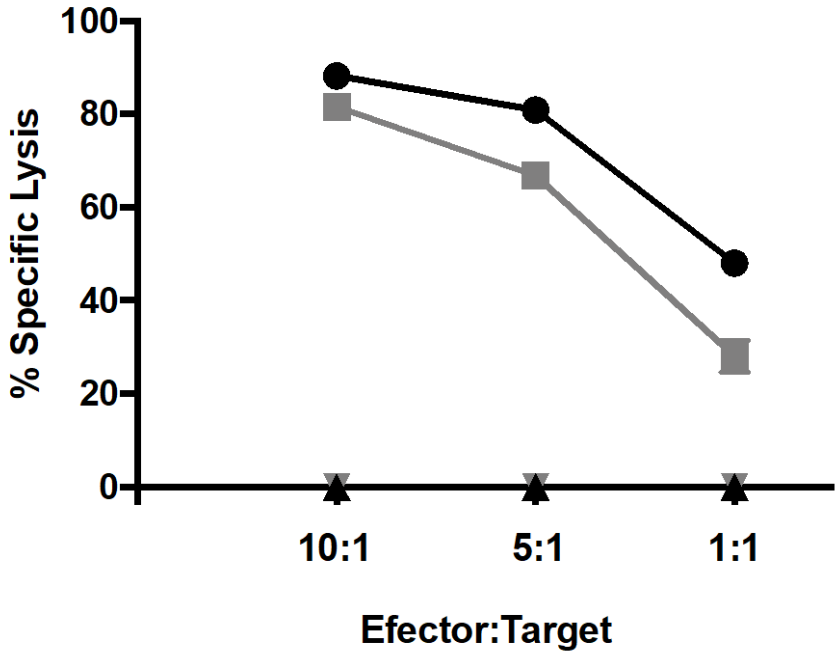


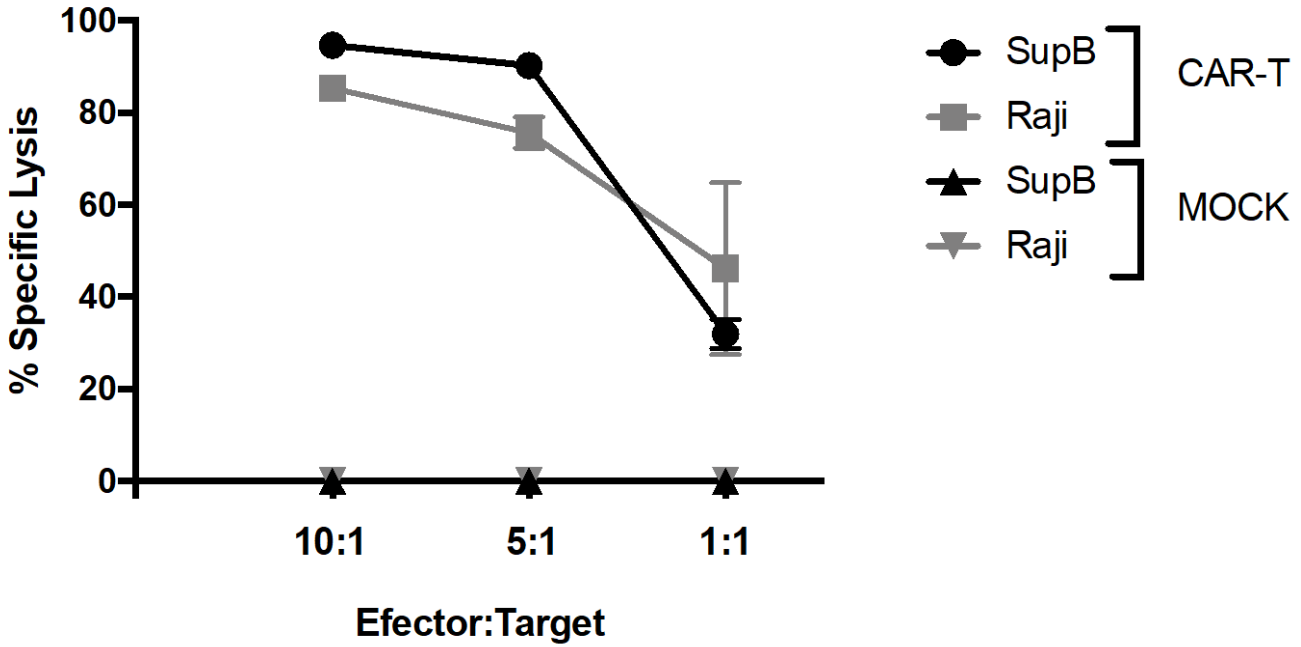


CAR19 T cells – Lymphoma patients

- *In vitro* cytotoxicity

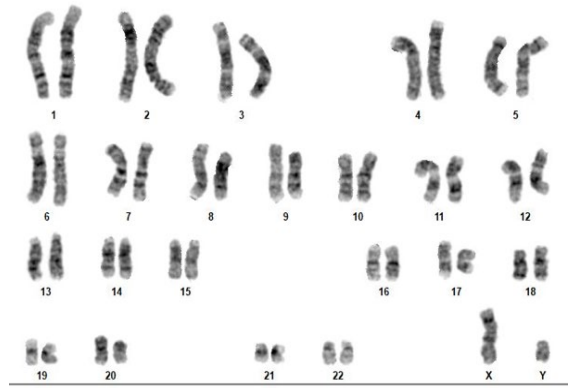




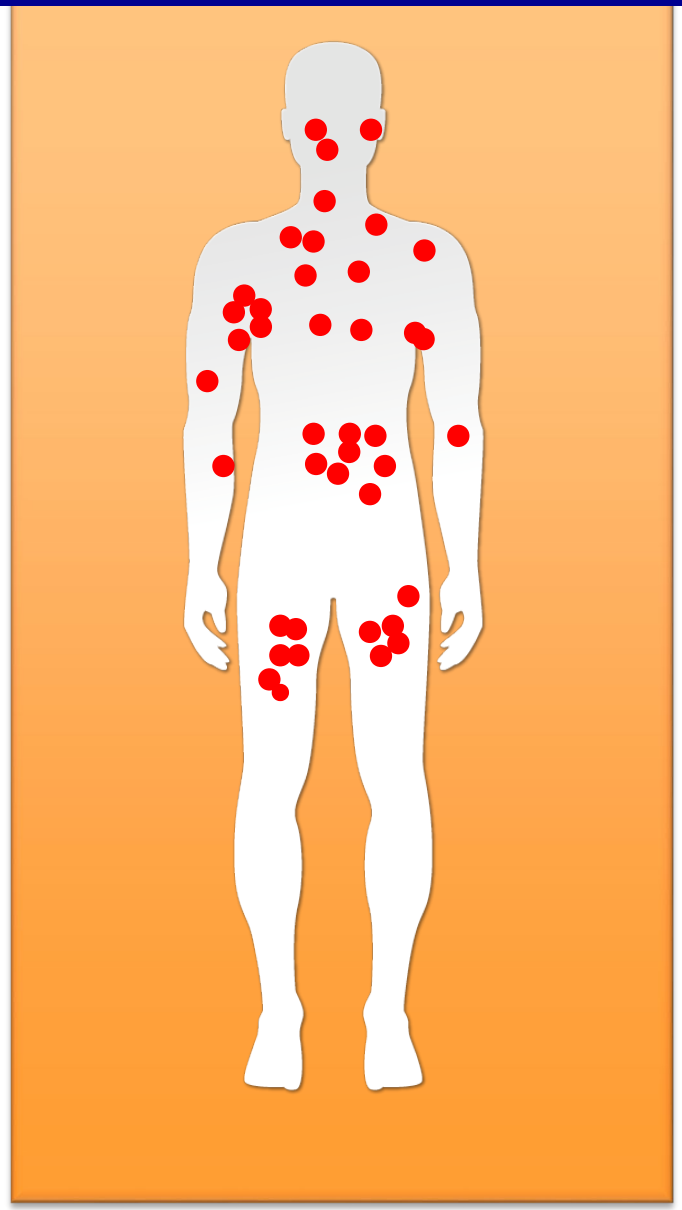


CAR19 T cells – Lymphoma patients

	Patient 1
Microbiologic	Negative
Endotoxin (<5.0 EU/mL)	0.77 EU/mL
Mycoplasm	Negative
%CD3+ cells ≥ 80%	95.9%
Cytogenetic	OK
Transduction efficiency	27.9%



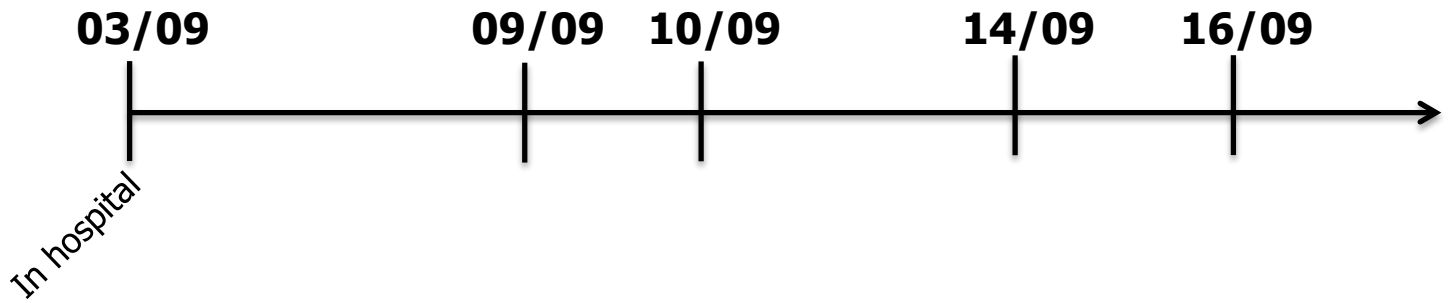
The 1st Brazilian CAR T Cell Recipient



63y, male, diagnosed in 09/14/2017 with Non-Hodgkin Diffuse Large B-cell Lymphoma, non-GCB (Hans Algorithm), Double-Expressor, stage IIB and R-IPI score ?.

- 1st treatment (set./2017) ➔ 8 R-CHOP + Radiotherapy
  No response ➔ bone marrow biopsy confirmed
- 2nd treatment ➔ R-GDP + autologous
  Progression of disease after 2 cycles
- 3rd treatment ➔ R-ICE
  Progression of disease after 4 cycles
  Lymphocytes collection by apheresis ➔ CAR T cell
- 4th treatment ➔ Polatuzumab + bendamustine + rituximab
  Progression of disease after 3 cycles

The 1st Brazilian CAR T Cell Recipient



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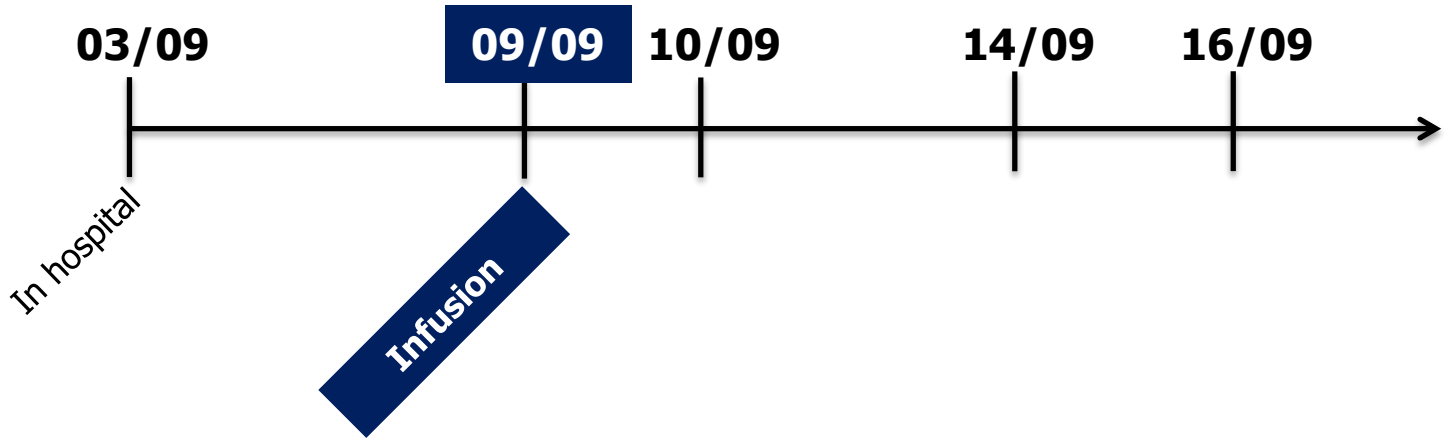

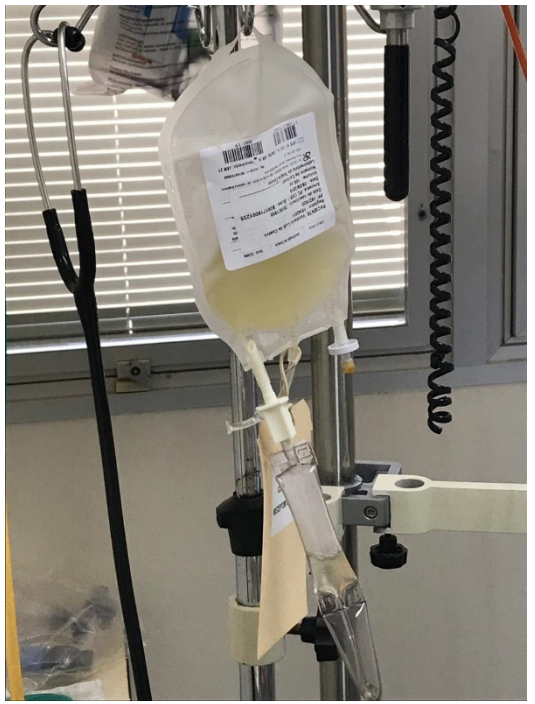

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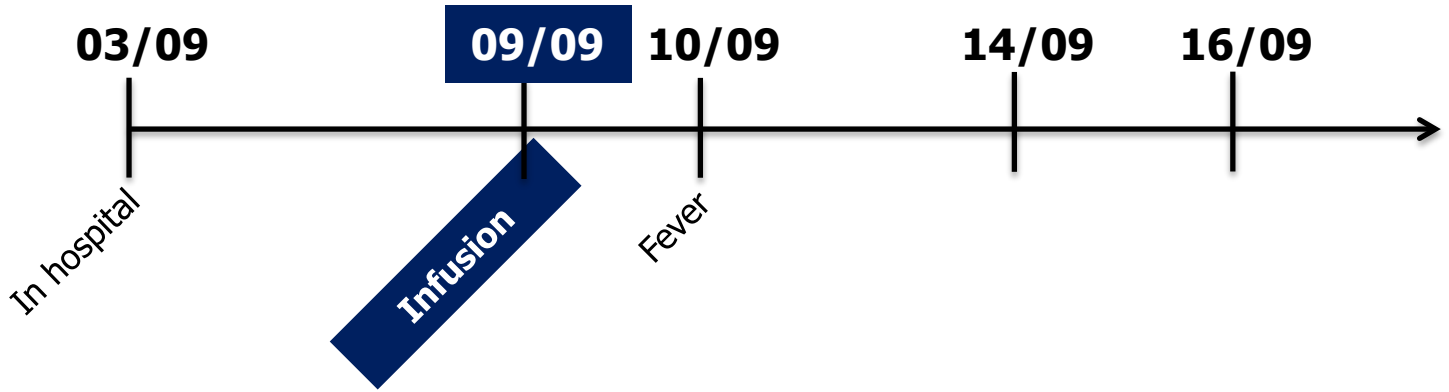

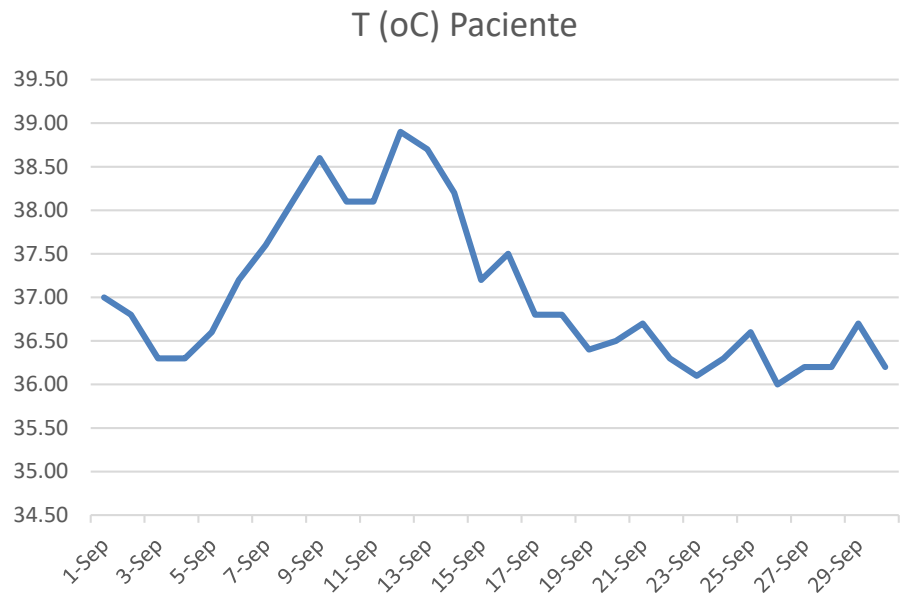

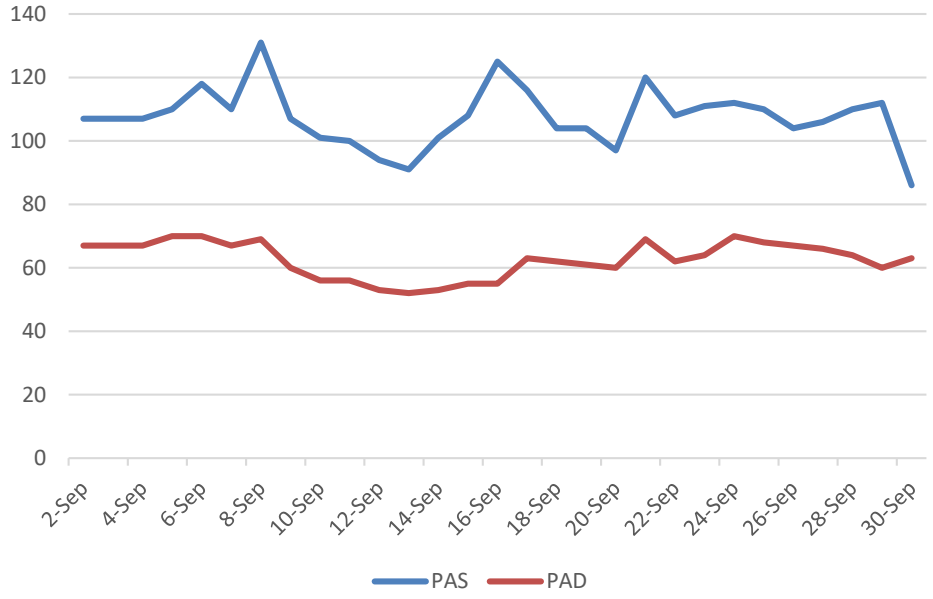

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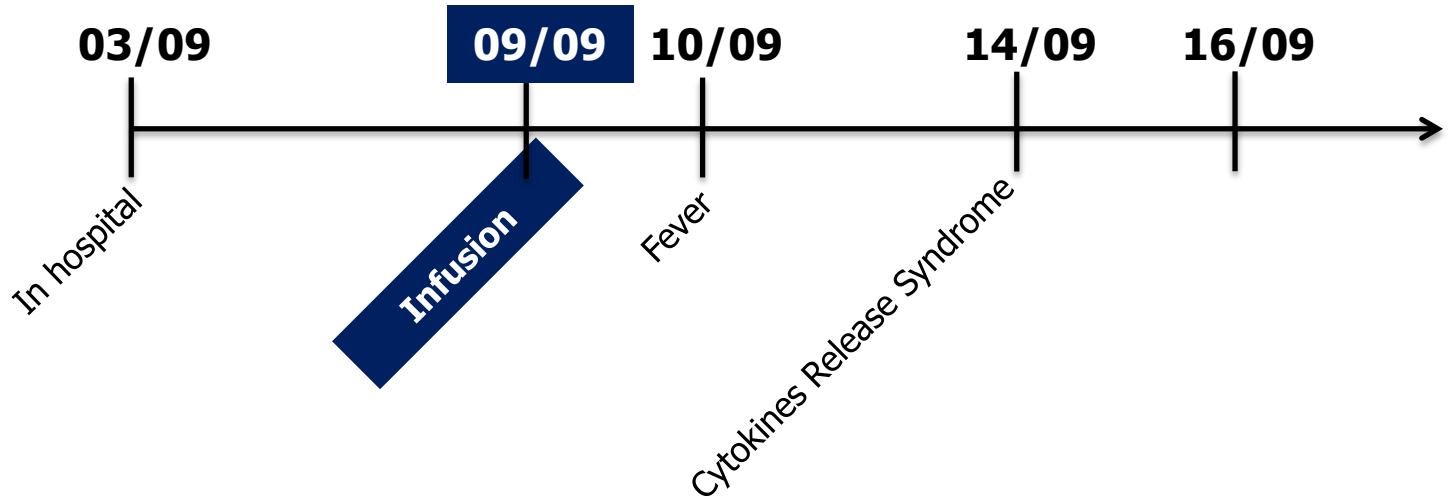
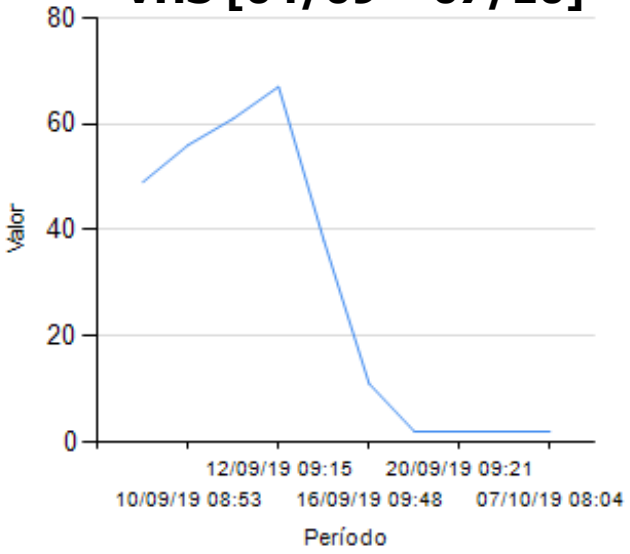
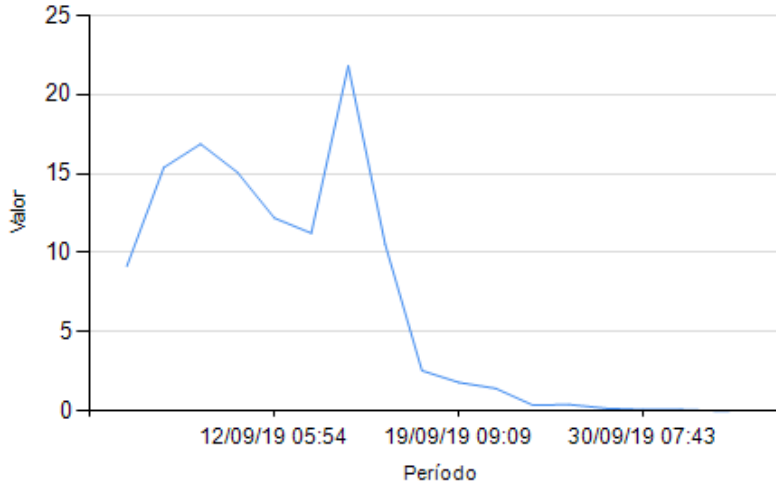


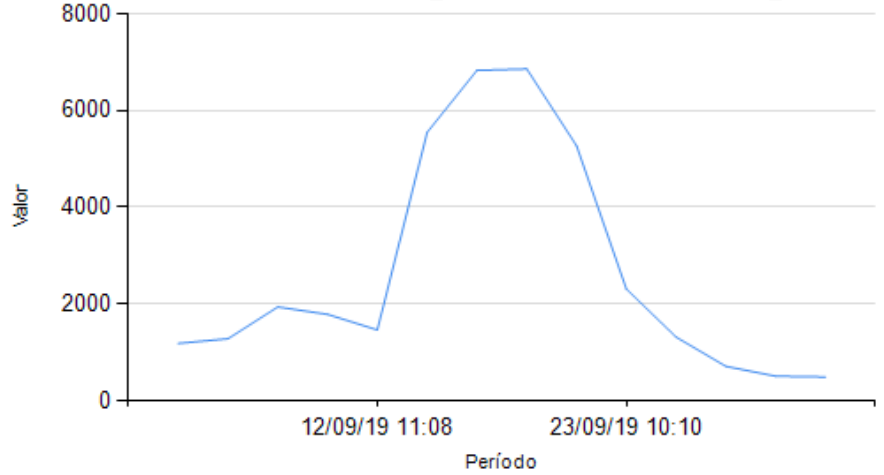
VHS [04/09 – 07/10]



PCR [04/09 – 07/10]



Ferritin [04/09 – 07/10]



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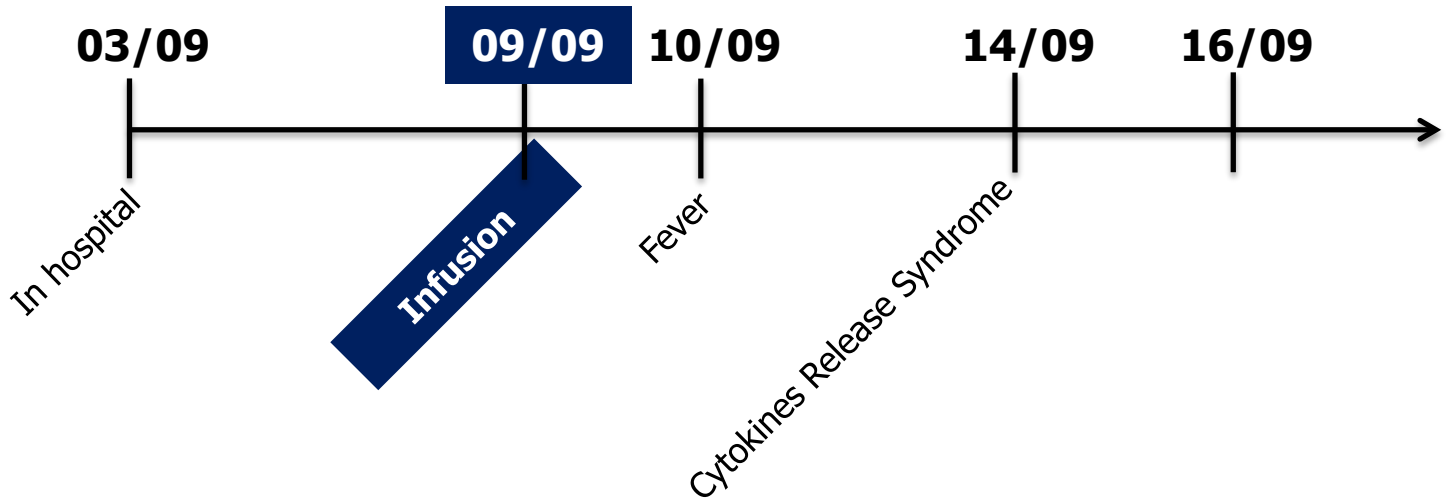
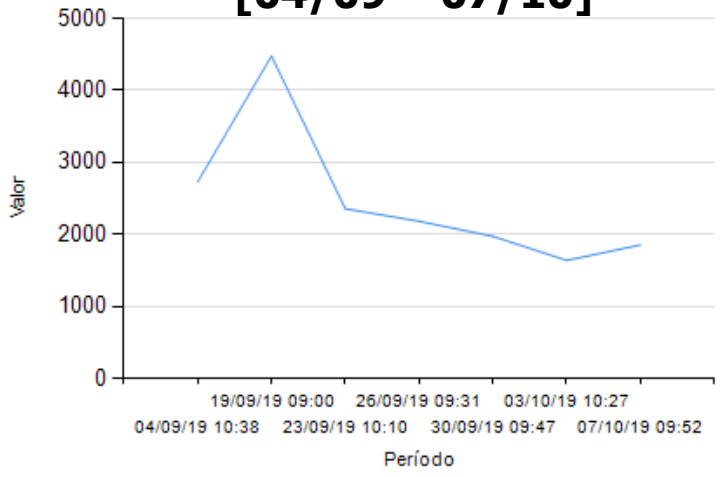
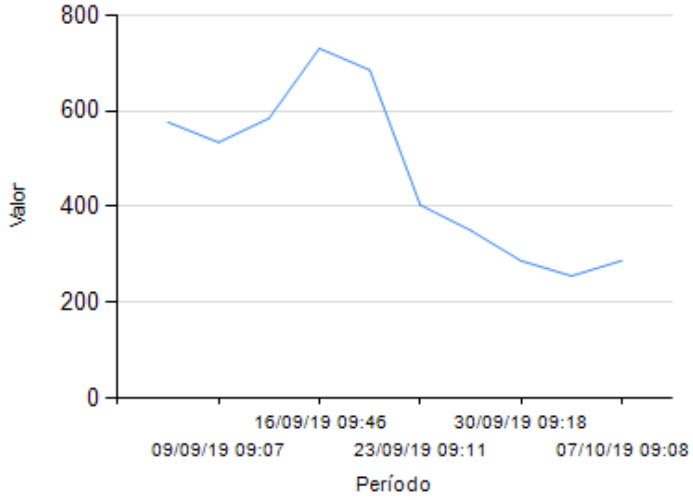


Beta 2 Microglobulin [04/09 – 07/10]



LDH [04/09 – 07/10]



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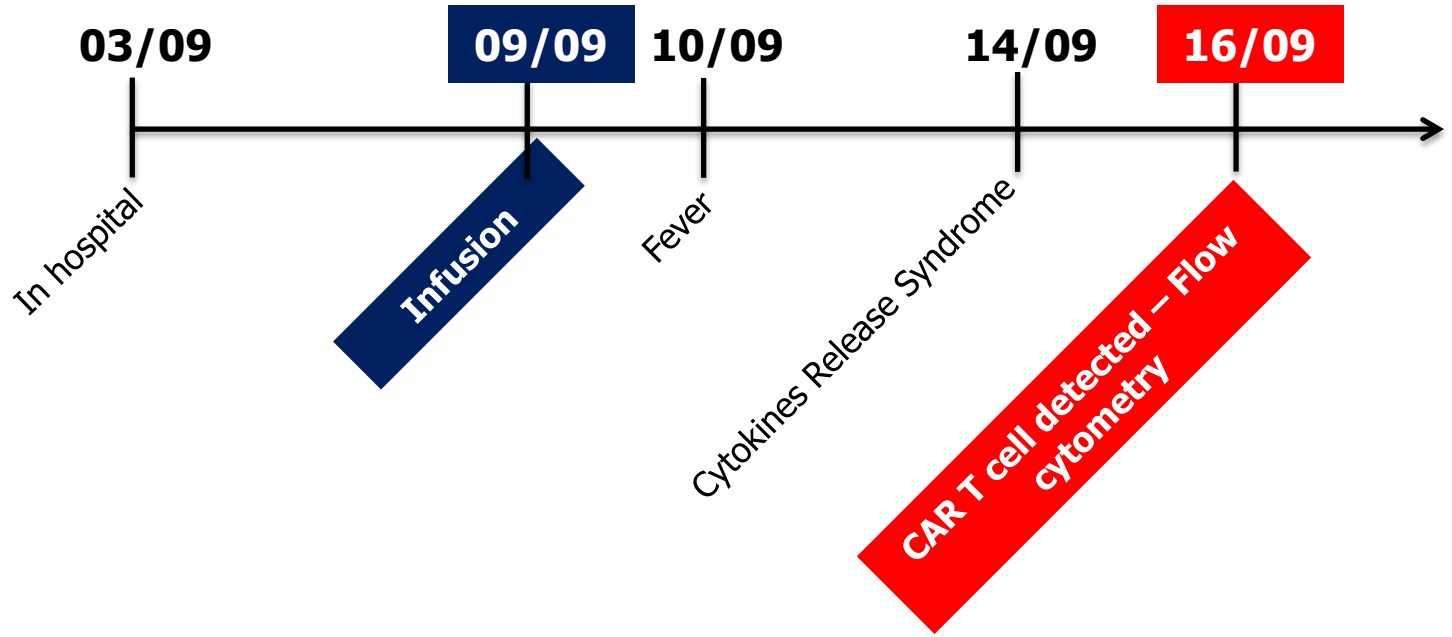
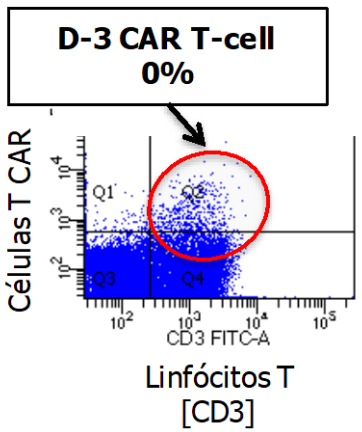
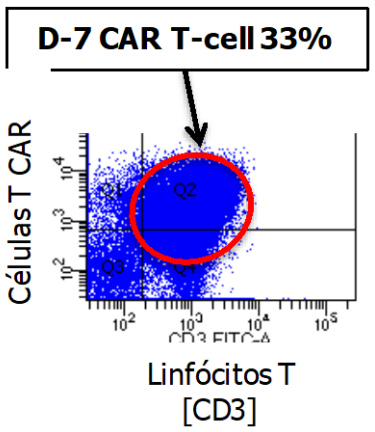
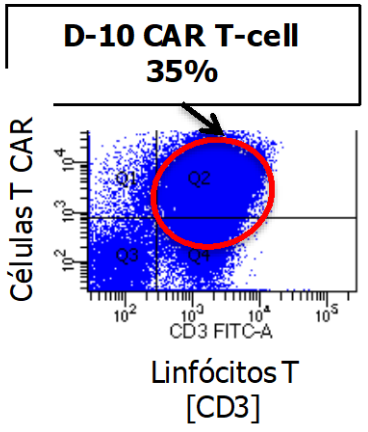
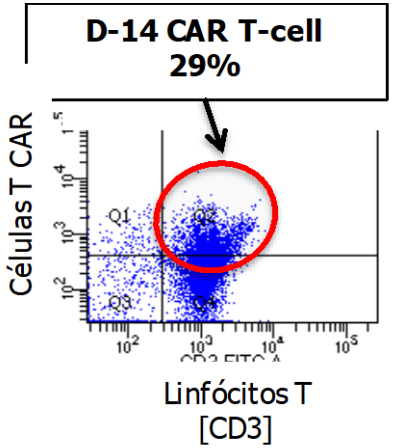
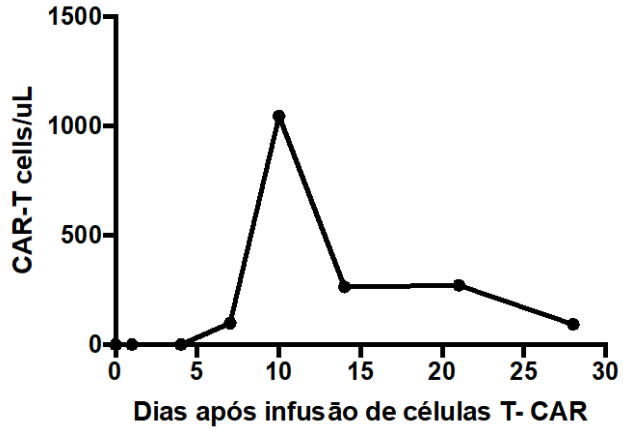




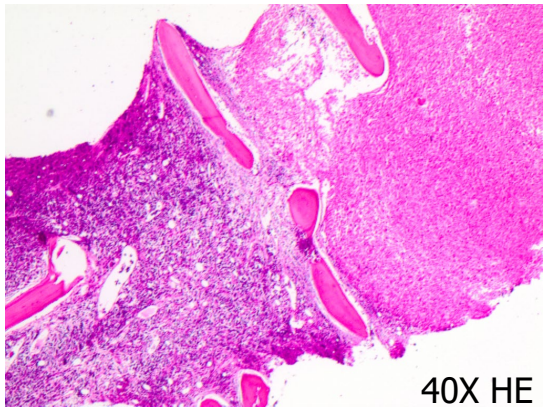




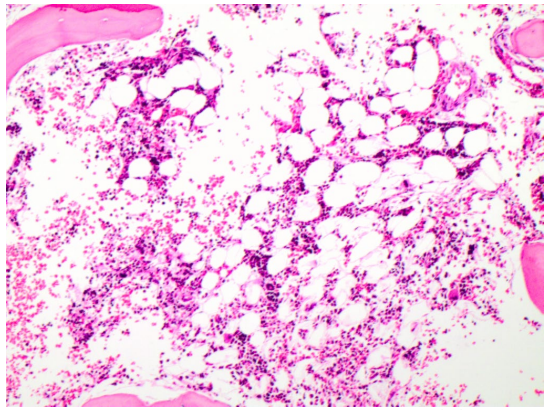




The 1st Brazilian CAR T Cell Recipient



40X HE



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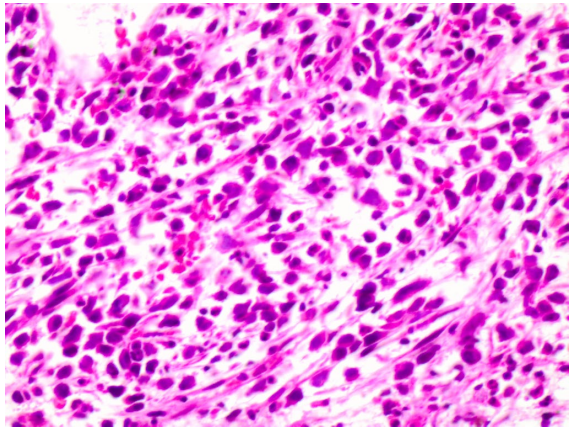
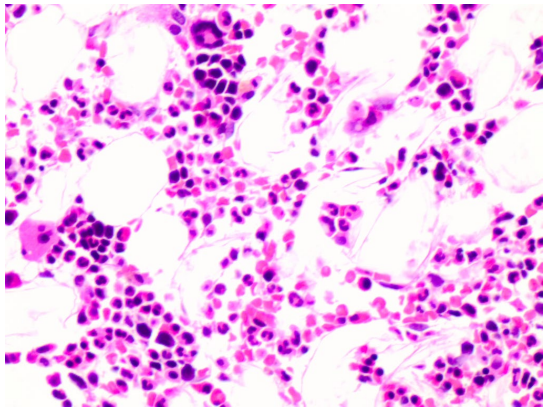


400X HE

BEFORE

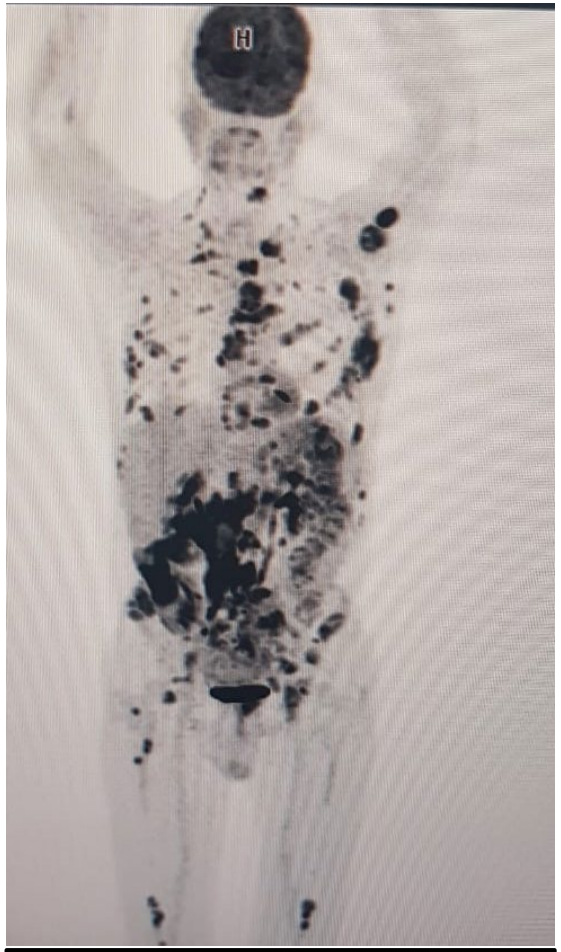


400X HE

AFTER

EARLY BONE MARROW BIOPSY (D+30)

The 1st Brazilian CAR T Cell Recipient



BEFORE

AFTER

EARLY PET-CET (D+30)

Getting CAR T-cell Therapy into Clinical Trials in Brazil
- Challenges and Opportunities -

- Restricted budget
- Restricted human resources to develop new technologies
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Getting CAR T-cell Therapy into Clinical Trials in Brazil
- Challenges and Opportunities -

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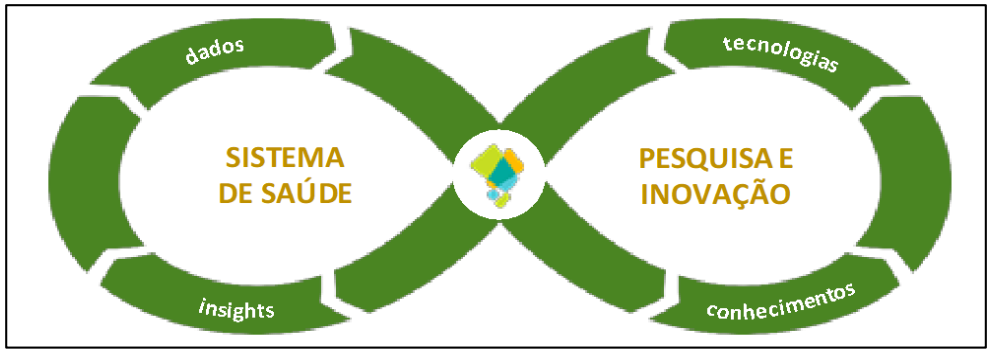
GENOMAS BRASIL
Programa Nacional de Genômica e Saúde de Precisão



SAÚDE DE PRECISÃO

SAÚDE DE PRECISÃO 1.0 - DIAGNÓSTICA

FINALIDADE

diagnóstico

CARACTERÍSTICAS

utiliza informações ômicas multidimensionais, sobretudo a genômica, aliados a dados clínicos e de estilo de vida, para obter um diagnóstico mais preciso e precoce

BENEFÍCIOS

- maior acurácia e precisão no diagnóstico
- capacidade de predizer a susceptibilidade de desenvolver doenças, permitindo a tomada de medidas curativas ou preventivas em tempo oportuno
- capacidade de predizer a resposta do indivíduo a uma determinada terapêutica, tornando a intervenção mais segura e eficaz
- evita desperdícios com condutas clínicas não eficazes

FRONTIERA DO CONHECIMENTO E TECNOLÓGICO

- tecnologias biocomputacionais capazes de processarem grande volume de dados
- utilização de biossensores e wearables para geração de dados de saúde e de estilo de vida em complementariedade as informações do perfil genômico

SAÚDE DE PRECISÃO 2.0 - DISRUPTIVA

FINALIDADE

terapêutica

CARACTERÍSTICAS

utiliza tecnologias genômicas para customizar o tratamento, empregando ferramentas de edição genômica e produtos de terapias avançadas (terapia gênica, terapia celular e engenharia tecidual)

BENEFÍCIOS

- Personalização do tratamento com base na variabilidade individual genômica
- aumento da eficácia terapêutica
- perspectiva de cura

FRONTIERA DO CONHECIMENTO E TECNOLÓGICO

- aprimoramento tecnológico e a automação dos processos de fabricação em escala industrial
- aprimoramento na tecnologia de testagem da qualidade do produto terapêutico final
- desenho de estudos clínicos

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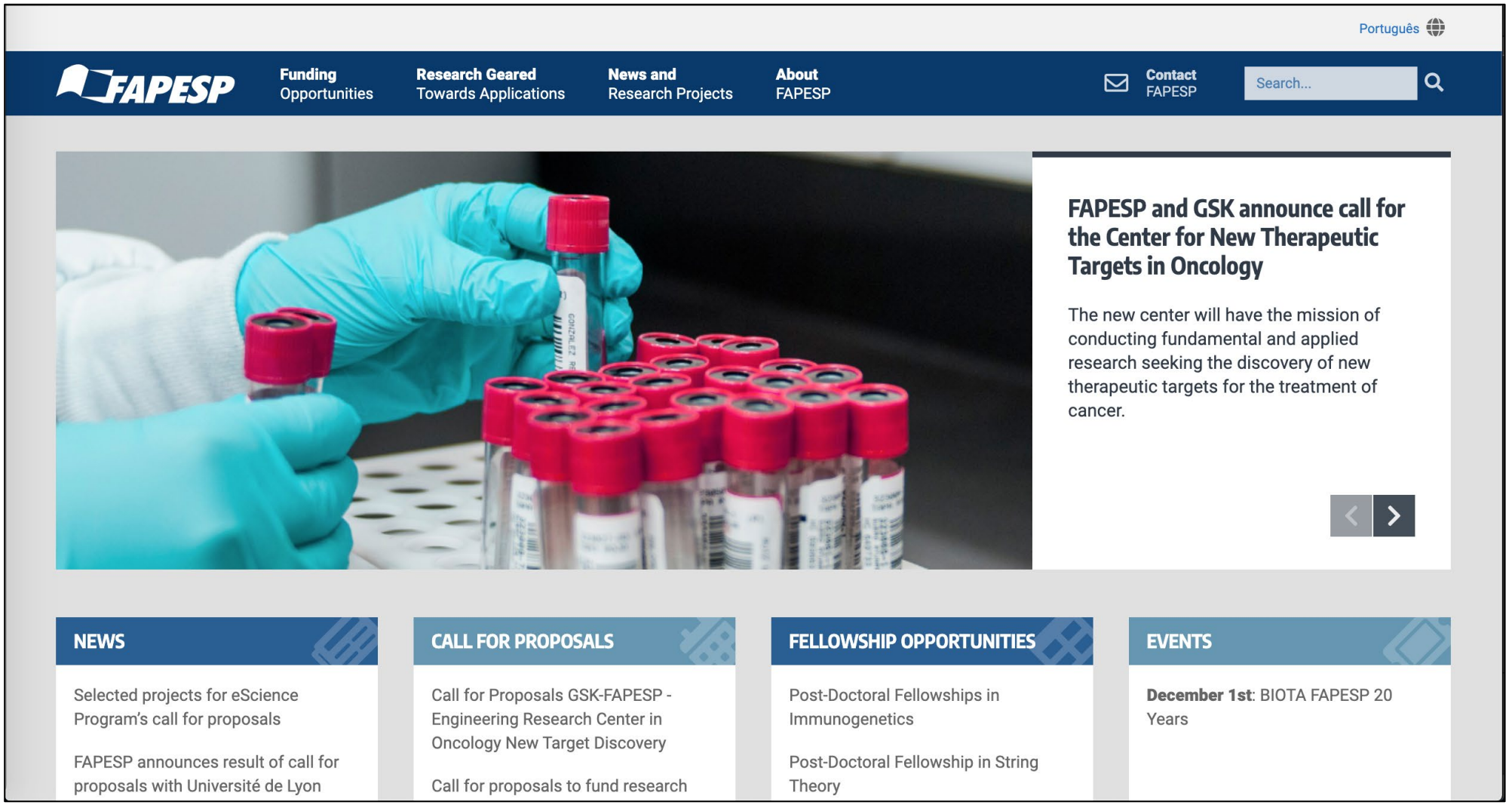

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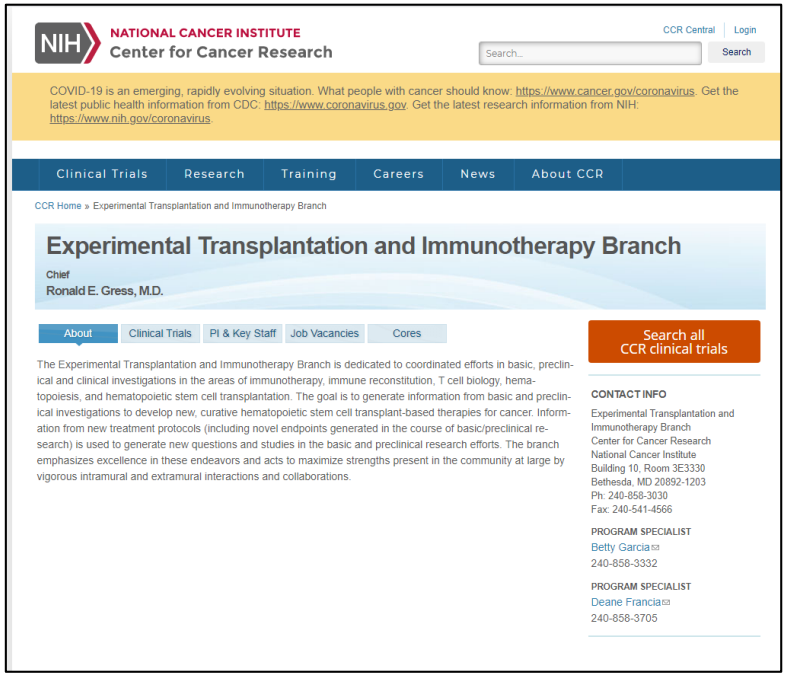
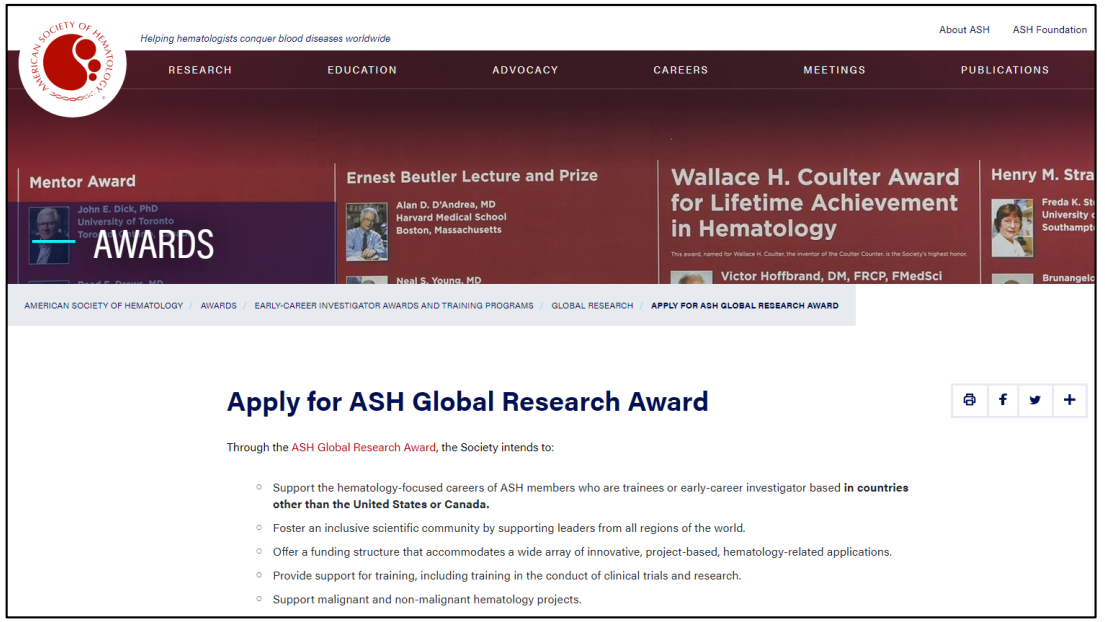
International collaboration – research and manufacturing





ASH Global Research Award
CTC - Ribeirão Preto & ETIB - NCI/NIH

Principal Investigator:

Renato Guerino-Cunha, M.D. - Ph.D.

Local Mentor:

Prof. Dr. **Eduardo Magalhães Rego**, M.D. - Ph.D.
Professor Titular FMRP-USP

Global Mentor:

Dr. **Ronald Gress**, M.D.
Experimental Transplantation and Immunotheray Branch - NCI/NIH
Clinical Center Research (CCR) - NCI/NIH

Prêmio: US$ 150.000,00 → 100% destinado à pesquisa

Instituição Sede: CTC - Ribeirão Preto

American Society of Hematology
Helping hematologists conquer blood diseases worldwide

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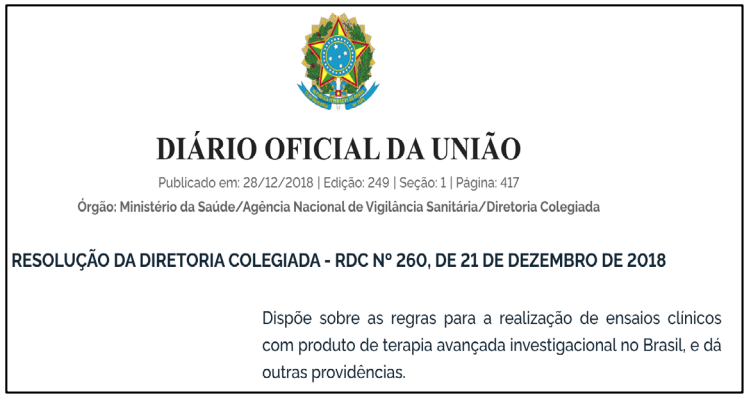

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Dispõe sobre o registro de produto de terapia avançada e dá outras providências.

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RENETA

Quem Somos | Informações sobre PTA | Documentos da Rede | Contato

NOSSA EQUIPE

A RENETA é formada por profissionais com expertise em produtos de terapia avançada pertencentes a prestigiadas instituições de ensino, pesquisa e assistência à saúde brasileiras.

SAIBA MAIS SOBRE A NOSSA EQUIPE

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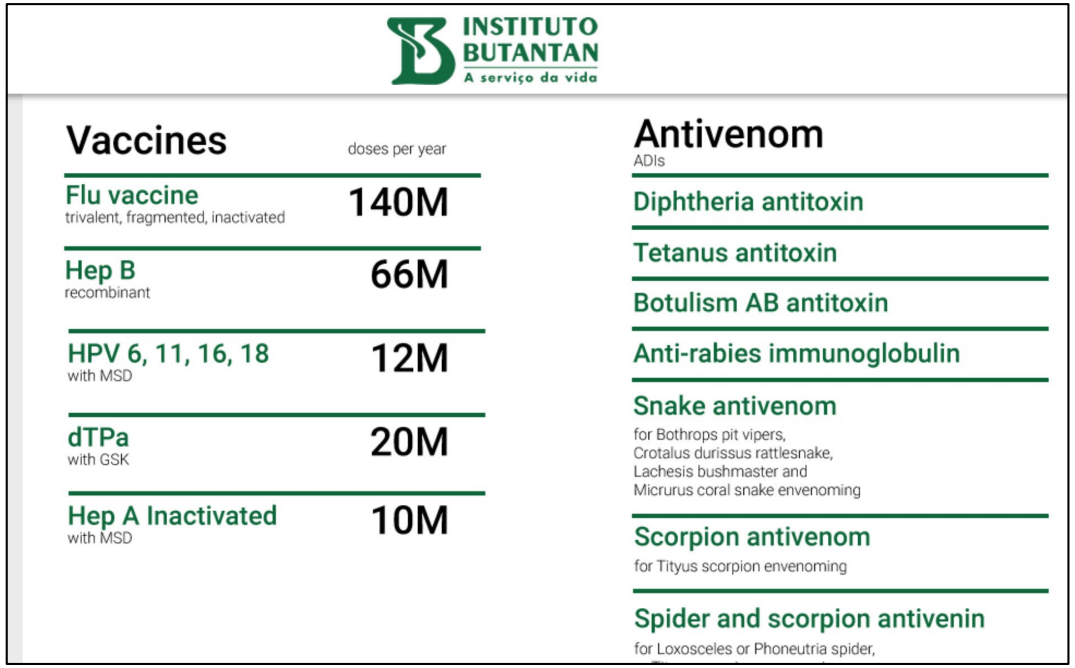

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Acknowledgements

Thanks!

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DrRenatoCunha