

Efecto de los oligonucleótidos antisentido sobre la expresión específica de los genes de células mononucleares/linfocitos en sangre periférica “in vitro”



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VNIVERSITAT
D VALÈNCIA

Índice

- Introducción
 - FoxP3
 - ASOs
- Hipótesis
- Objetivos
- Metodología
- Aplicaciones posibles en hematología

Introducción: sistema inmune vs cáncer

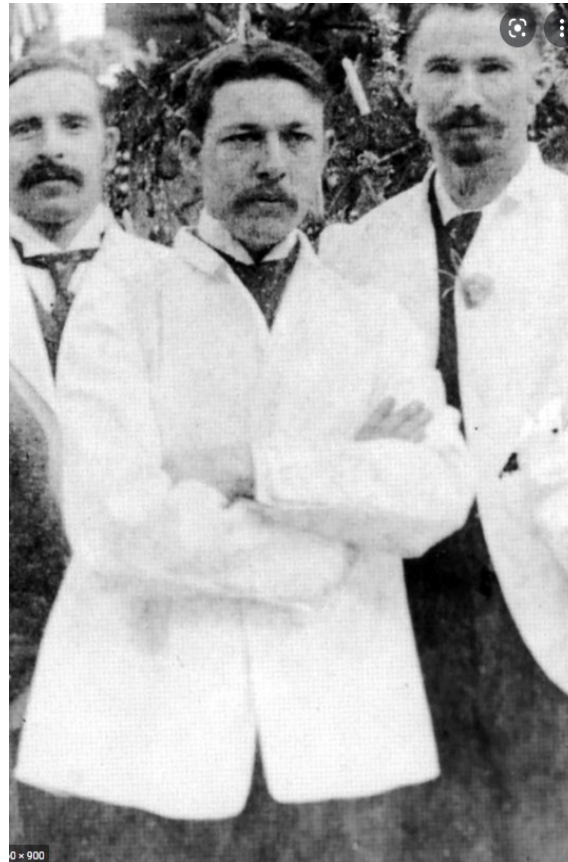
Erysipelas of the leg



Erysipelas of the lower leg. The rash is intensely red, sharply demarcated, swollen, and indurated.

Reproduced with permission from: Berg D, Worzala K. *Atlas of Adult Physical Diagnosis*. Philadelphia: Lippincott Williams & Wilkins, 2006. Copyright © 2006 Lippincott Williams & Wilkins.

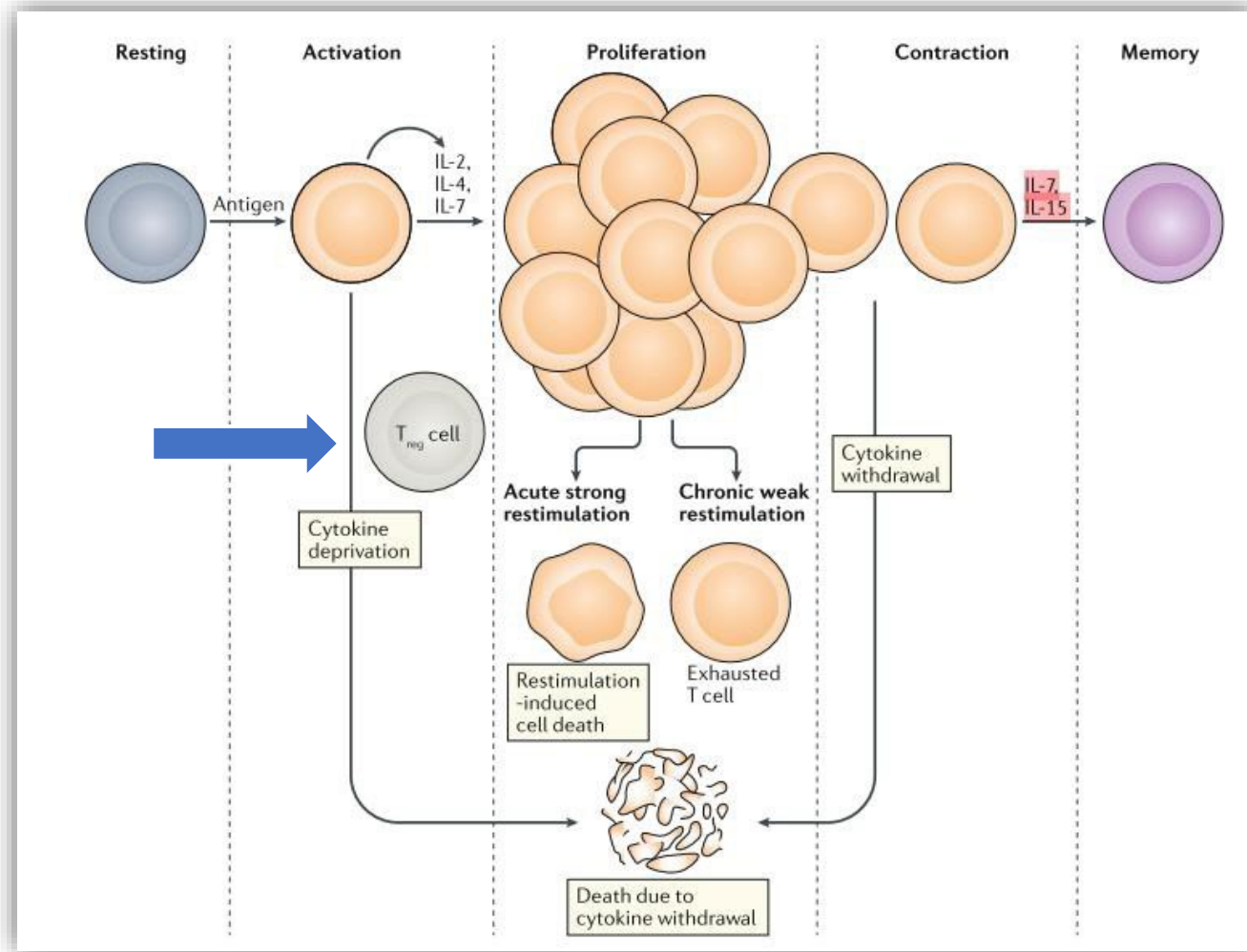
UpToDate®



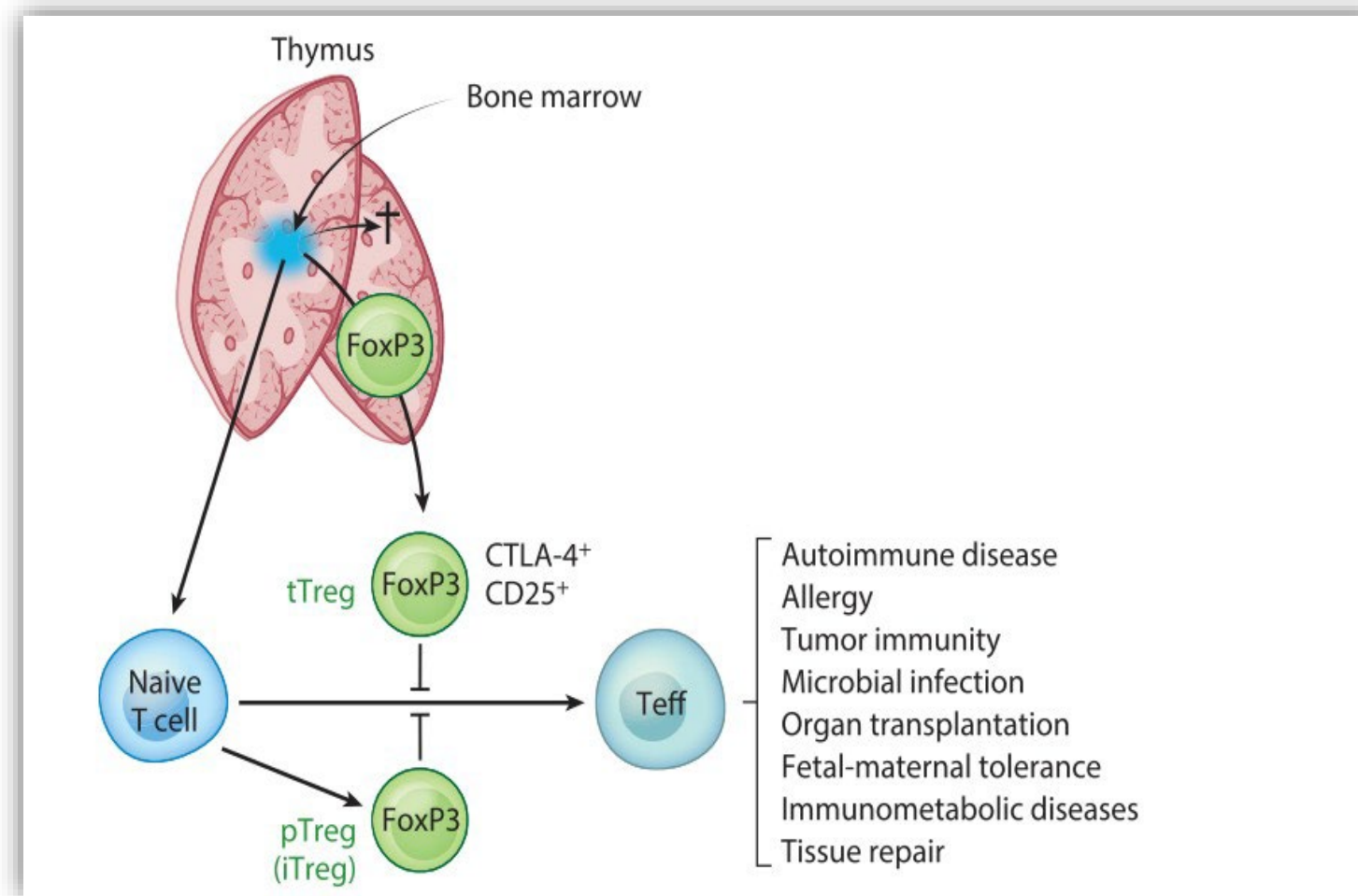
Frank Macfarlane Burnet



Introducción: linfocitos Treg

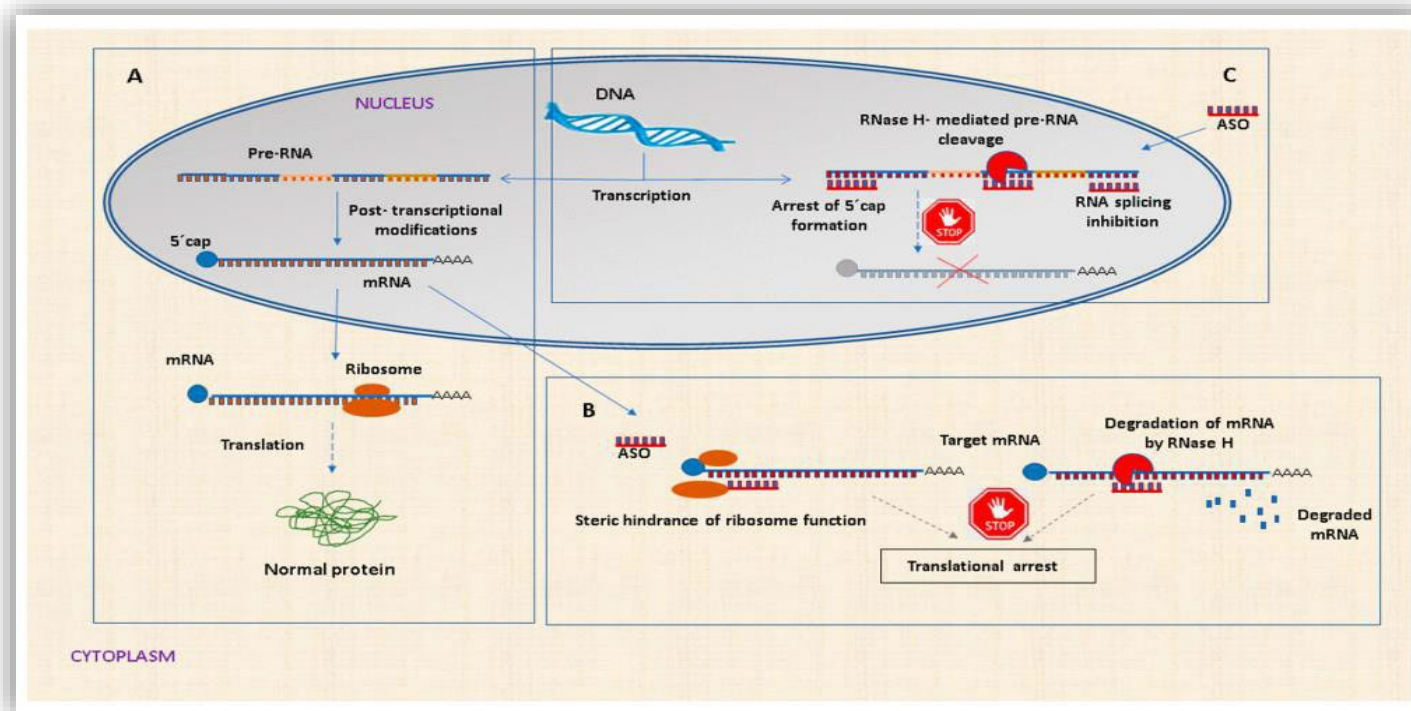


Introducción: FoxP3



Introducción: ASOs

Los ASO (oligonucleótido anti sentido) son nucleótidos sintéticos monocatenarios cortos de ADN o ARN que son complementarios a un objetivo de ARNm. Están diseñados para silenciar genes específicos al interferir con el proceso de traducción del ARNm, bloqueando así la síntesis de proteínas. (Bennett, et al 2017).



Introducción: ASOs

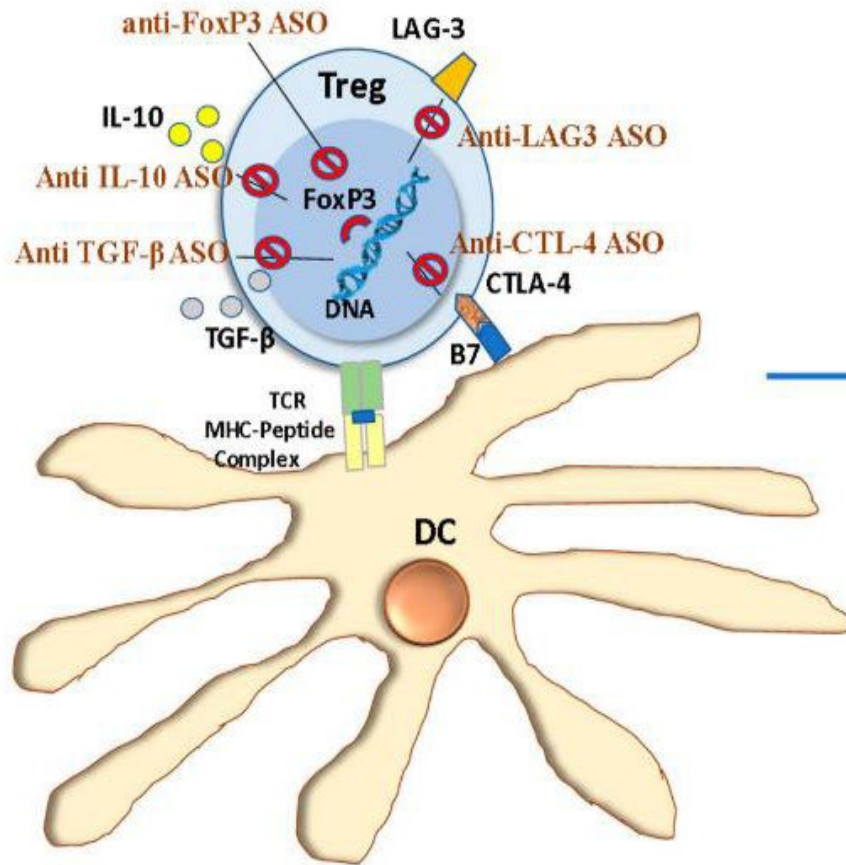
Table 1 | FDA-approved oligonucleotide therapeutics

| Name (market name), company | Target (indication) | Organ (ROA) | Chemistry (modality) | FDA approval | Comments |
|--|--|----------------------|---|----------------|--|
| Fomivirsen (Vitravene), Ionis Pharma Novartis | CMV UL123 (cytomegalovirus retinitis) | Eye (IVI) | 21mer PS DNA (first-generation ASO) | August 1998 | First approved nucleic acid drug Local delivery Withdrawn from use owing to reduced clinical need |
| Pegaptanib (Macugen), NeXstar Pharma Eyeteck Pharma | VEGF-165 (neovascular age-related macular degeneration) | Eye (IVI) | 27mer 2'-F/2'-OME pegylated (aptamer) | December 2004 | First approved aptamer drug Local delivery Limited commercial success due to competition |
| Mipomersen (Kynamro), Ionis Pharma Genzyme Kastle Tx | APOB (homozygous familial hypercholesterolaemia) | Liver (SQ) | 20mer PS 2'-MOE (gapmer ASO) | January 2013 | Rejected by EMA owing to safety Limited commercial success due to competition |
| Defibrotide (Defitelio), Jazz Pharma | NA (hepatic veno-occlusive disease) | Liver (IV) | Mixture of PO ssDNA and dsDNA | March 2016 | Unique sequence-independent mechanism of action |
| Eteplirsen (Exondys 51), Sarepta Tx | DMD exon 51 (Duchenne muscular dystrophy) | Skeletal muscle (IV) | 30mer PMO (steric block ASO) | September 2016 | Systemic delivery to non-hepatic tissue Low efficacy |
| Nusinersen (Spinraza), Ionis Pharma Biogen | SMN2 exon 7 (spinal muscular atrophy) | Spinal cord (IT) | 18mer PS 2'-MOE (steric block ASO) | December 2016 | Local delivery |
| Patisiran (Onpattro), Alnylam Pharma | TTR (hereditary transthyretin amyloidosis, polyneuropathy) | Liver (IV) | 19 + 2mer 2'-OME modified (siRNA LNP formulation) | August 2018 | First approved RNAi drug Nanoparticle delivery system Requires co-treatment with steroids and antihistamines |
| Inotersen (Tegsedi), Ionis Pharma Akcea Pharam | TTR (hereditary transthyretin amyloidosis, polyneuropathy) | Liver (SQ) | 20mer PS 2'-MOE (gapmer ASO) | October 2018 | Same gapmer ASO platform as mipomersen |
| Givosiran (Givlaari), Alnylam Pharma | ALAS1 (acute hepatic porphyria) | Liver (SQ) | 21/23mer Dicer substrate siRNA (GalNAc conjugate) | November 2019 | Enhanced stability chemistry Hepatocyte-targeting bio-conjugate |
| Golodirsen (Vyondys 53), Sarepta Tx | DMD exon 53 (Duchenne muscular dystrophy) | Skeletal muscle (IV) | 25mer PMO (steric block ASO) | December 2019 | Same PMO chemistry platform as eteplirsen |

Introducción: ASOs

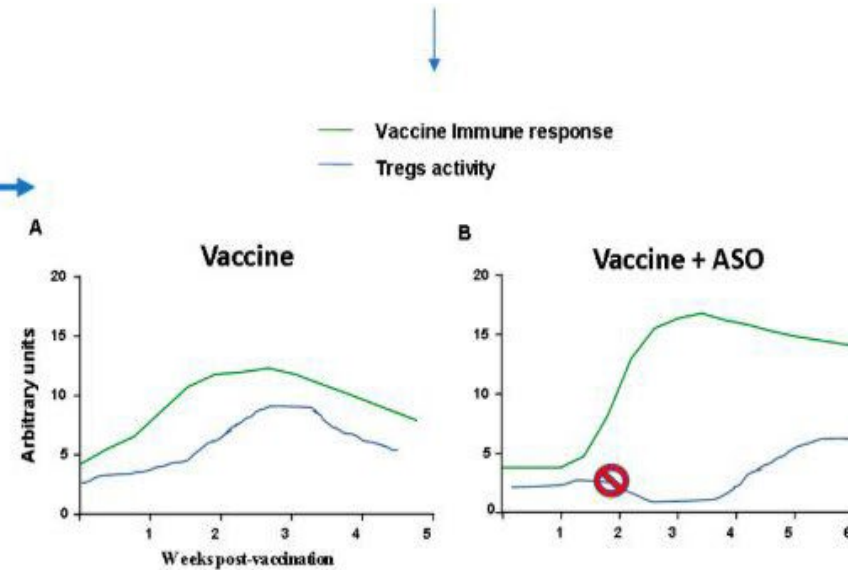
Transcription factors and cytokines

Immune checkpoints



Adjuvant effect of ASOs blocking Tregs immunosuppression

- Transient reduction of immunosuppressive Tregs function in the phase of immune induction
- Enhanced vaccine immunogenicity is expected



1

Metodología: supervivencia DMEM vs RPMI

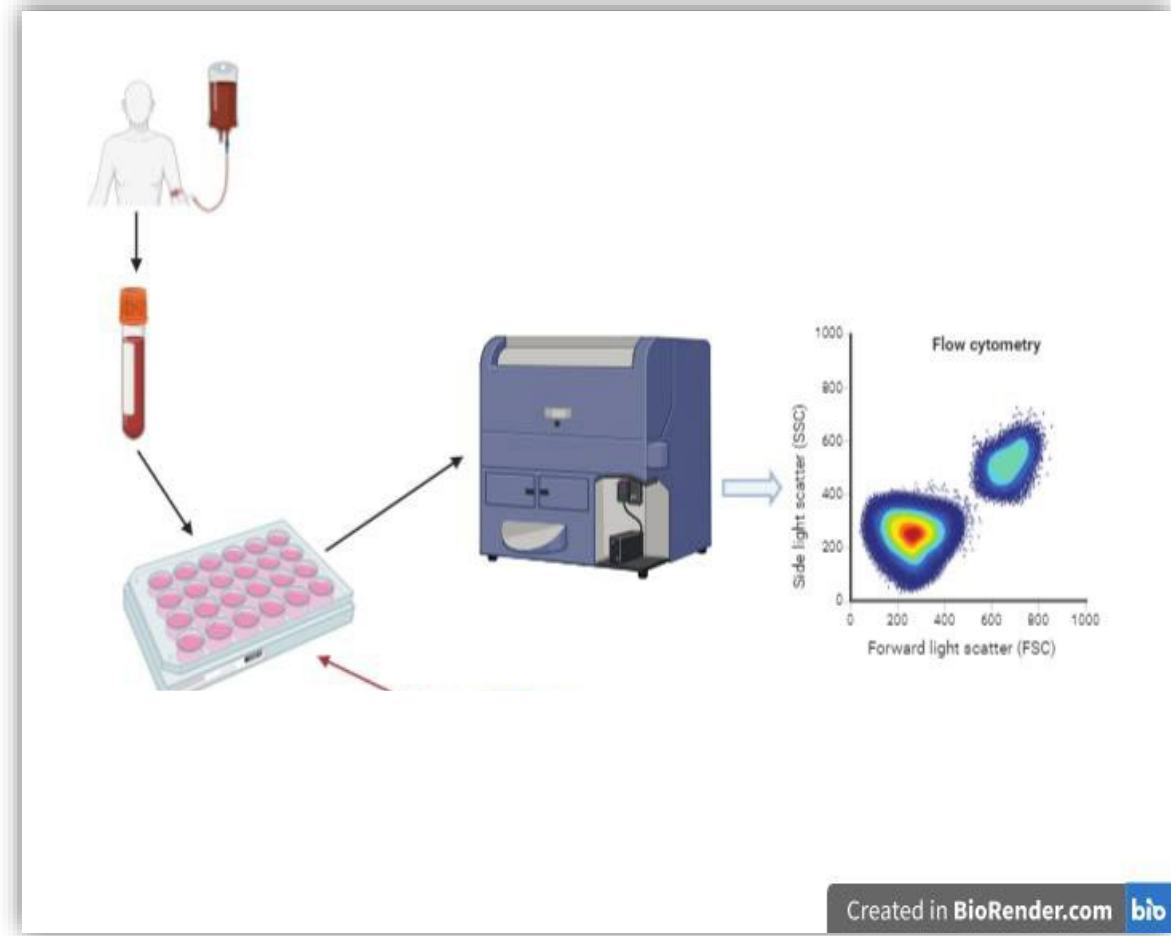
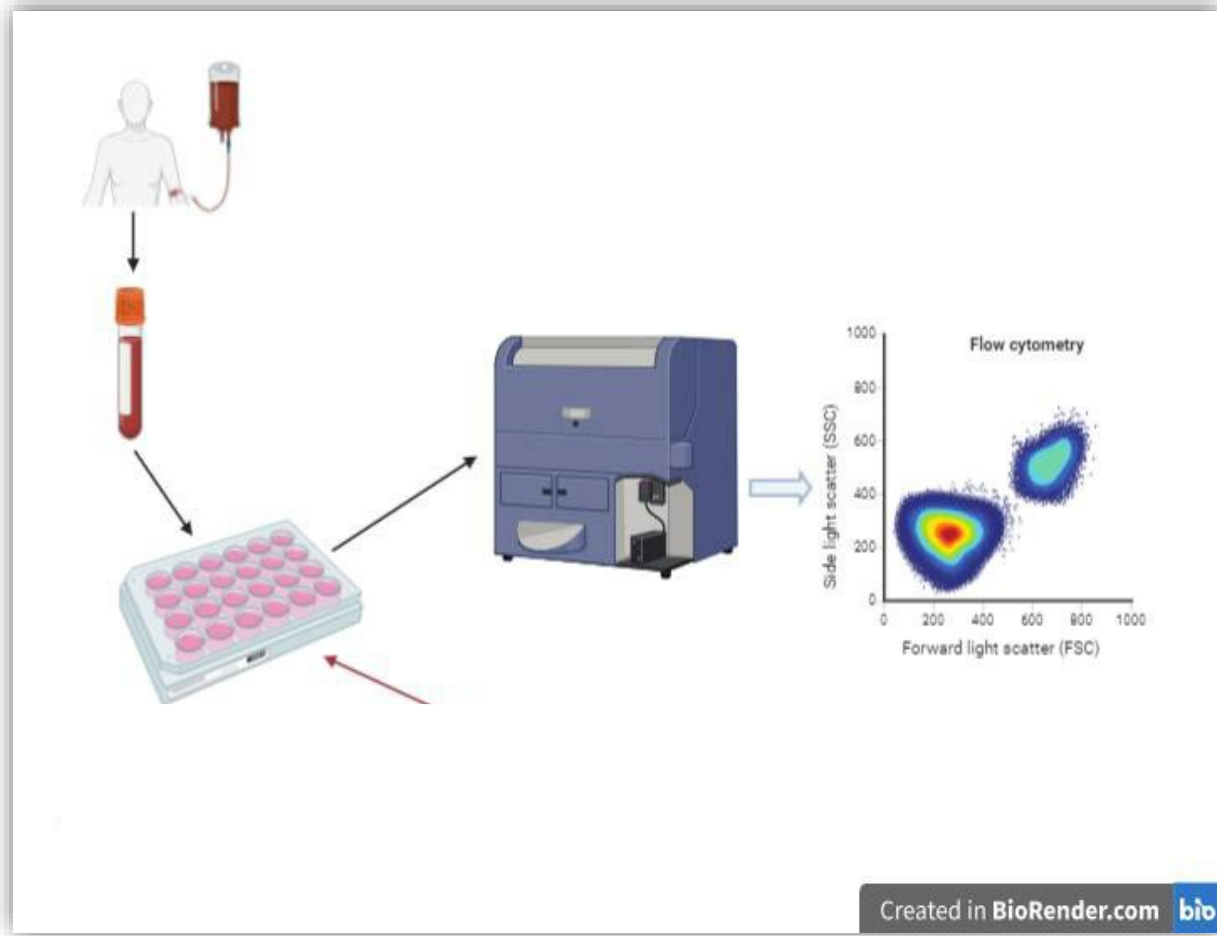
| FITC | PE | PercP Cy5.5 | PE Cy7 | V-450 | V-500 | APC | APC Cy7 |
|------|------|-------------|--------|----------|-------|-----|---------|
| CD8 | CD56 | 7-AAD | CD19 | CD4/CD20 | CD45 | CD3 | CD38 |

Metodología: supervivencia DMEM vs RPMI

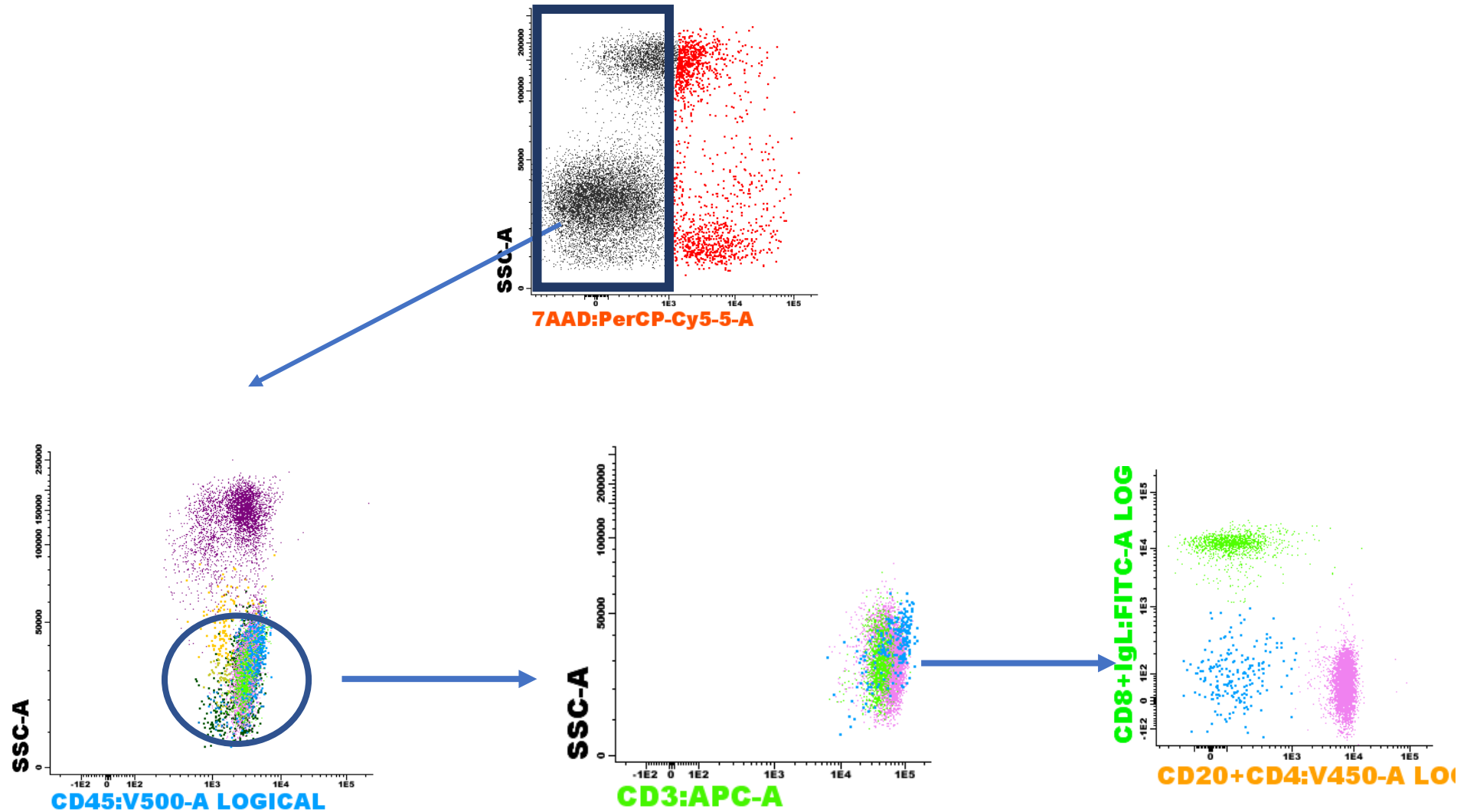
RPMI

1h, 24h, 48h, 72h, 96h, 7 días

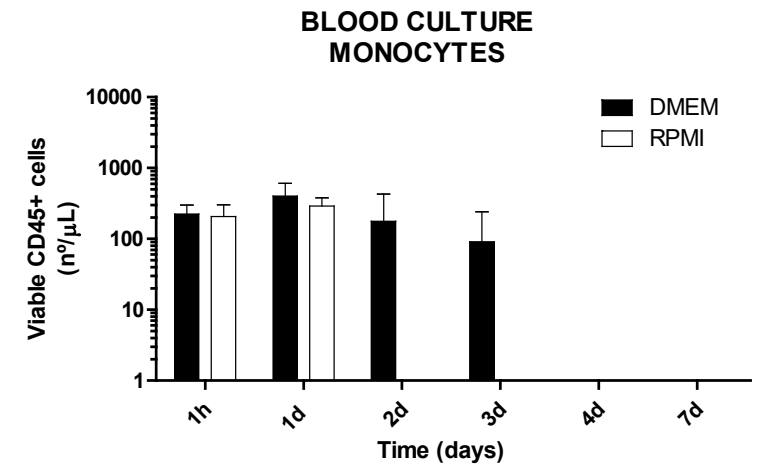
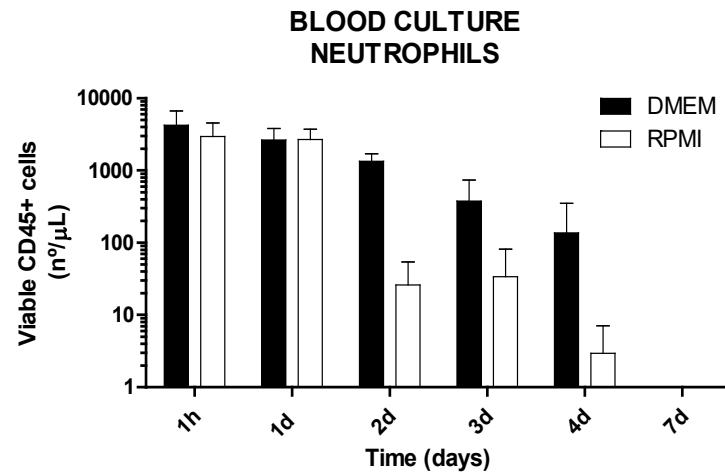
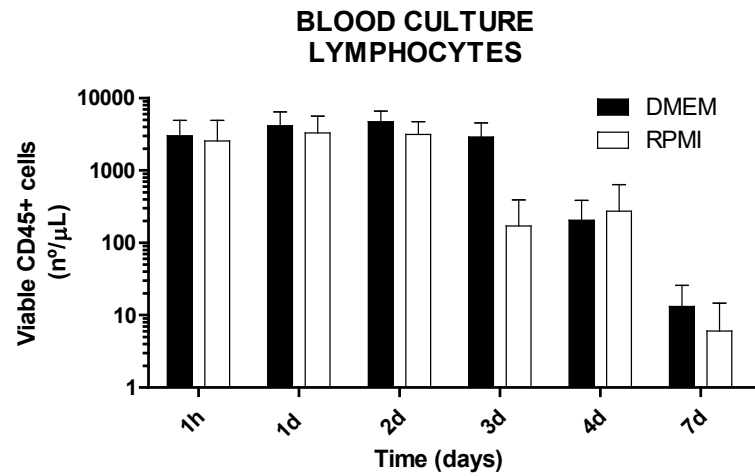
DMEM



Metodología: supervivencia DMEM vs RPMI



Metodología: supervivencia DMEM vs RPMI

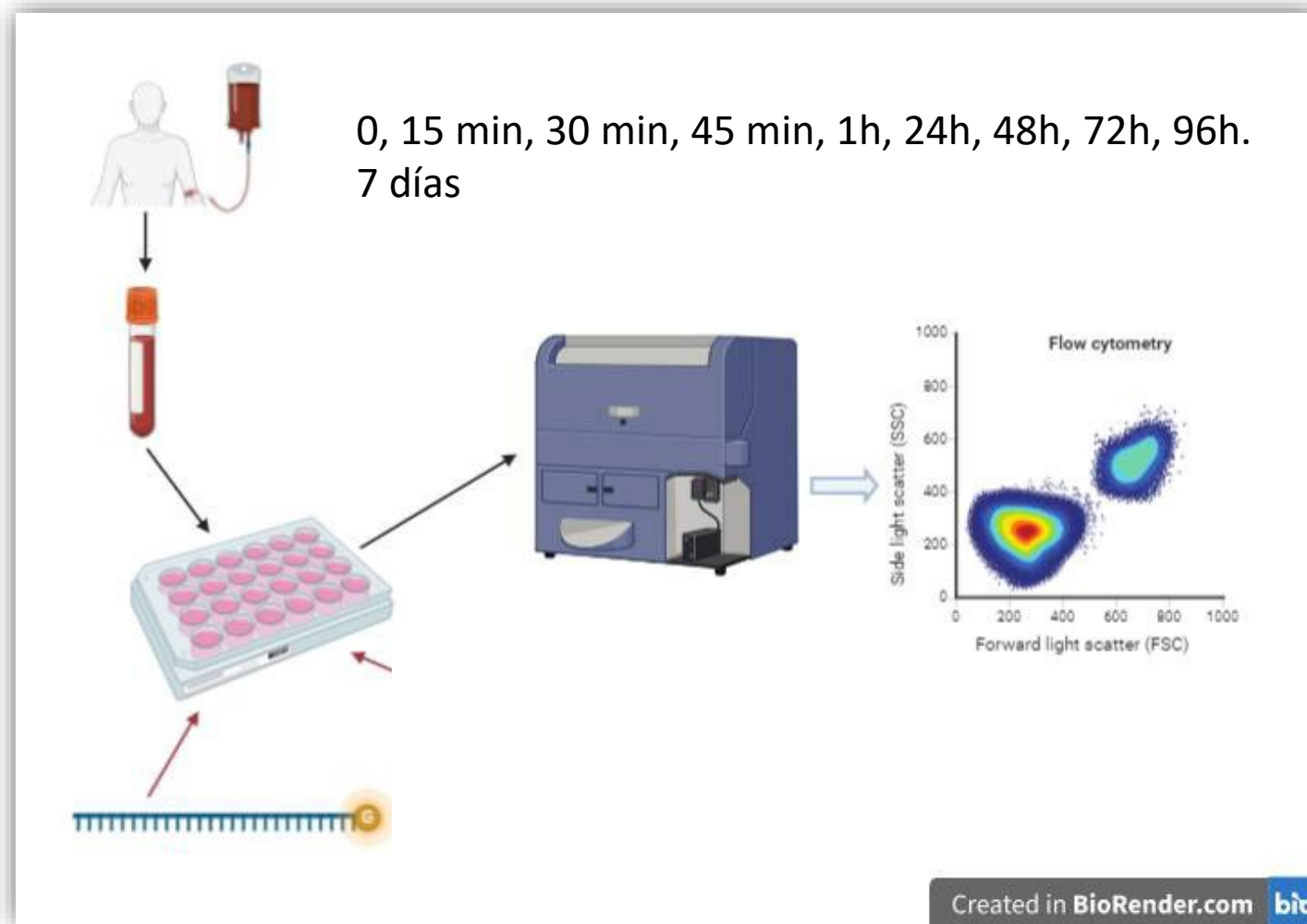


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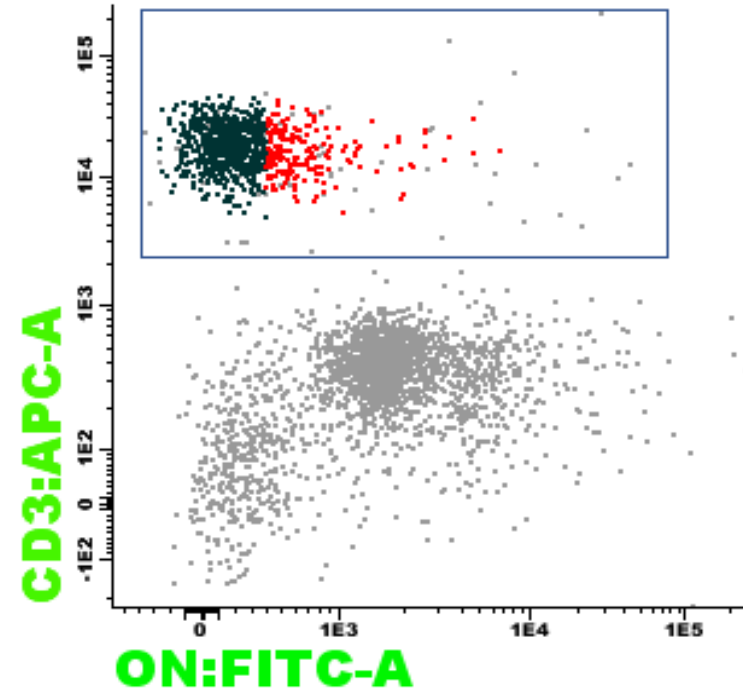
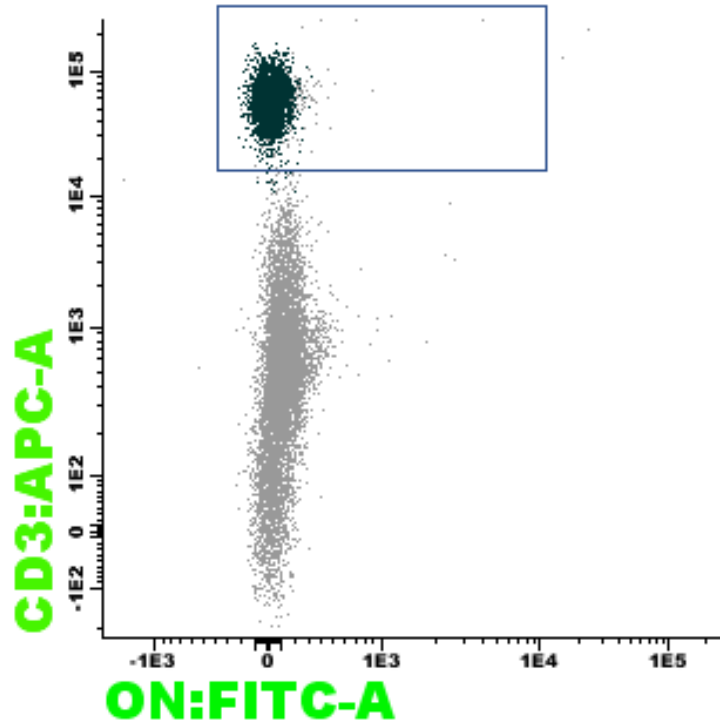
Metodología: entrada del ASOs

| FITC | PE | PercP Cy5.5 | PE Cy7 | V-450 | V-500 | APC | APC Cy7 |
|------|------|-------------|--------|----------|-------|-----|---------|
| ON | CD56 | 7-AAD | CD8 | CD4/CD20 | CD45 | CD3 | CD19 |

Metodología

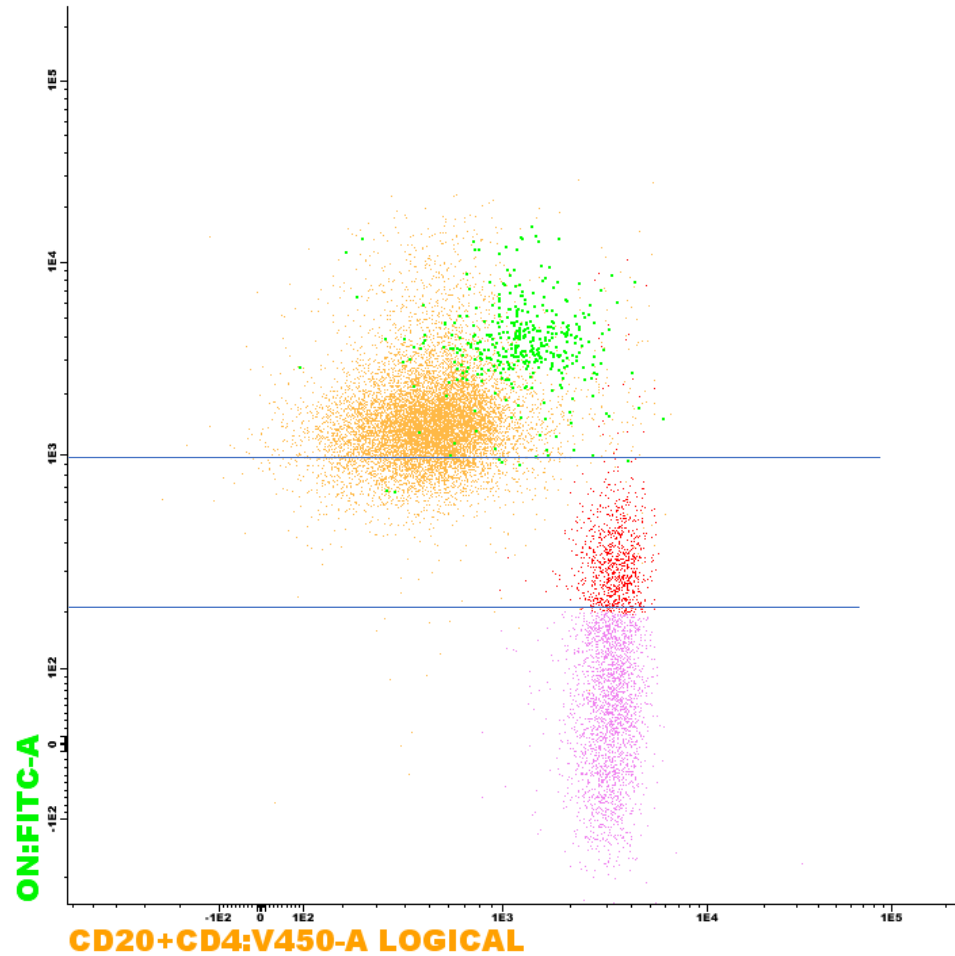


Metodología: entrada del ASOs

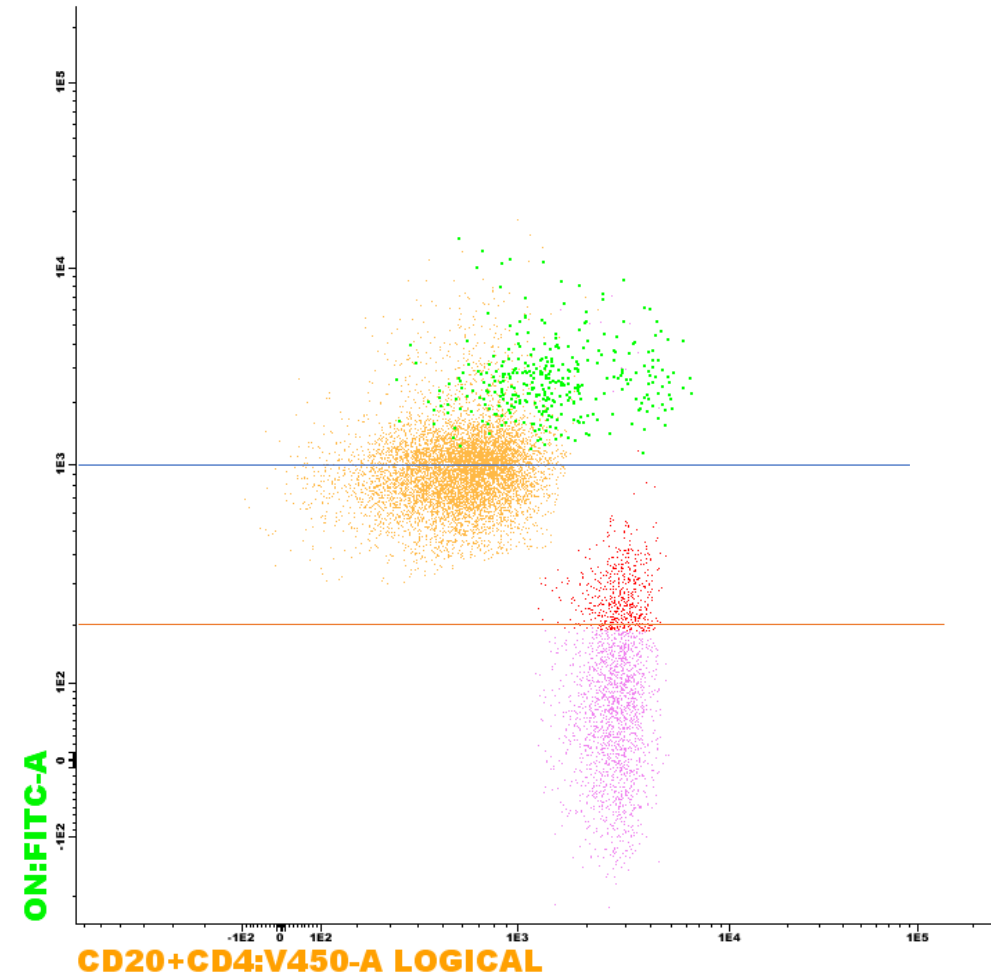


Metodología: entrada del ASOs

SIN APANTALLAMIENTO



APANTALLAMIENTO



Metodología: entrada del ASOs

| ON- | Lymphocytes | | Neutrophiles | | Monocytes | |
|------|-------------|-------|--------------|-------|-----------|------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 75.93 | 40.02 | 51.58 | 57.50 | 1.89 | 0.94 |
| 15 m | 105.18 | 69.69 | 7.16 | 8.34 | 1.06 | 1.87 |
| 30 m | 80.50 | 38.95 | 2.44 | 2.85 | 0.14 | 0.28 |
| 45 m | 66.18 | 28.18 | 1.24 | 2.08 | 0.00 | 0.00 |
| 60 m | 39.37 | 13.68 | 0.41 | 0.78 | 0.05 | 0.10 |
| 24 h | 23.38 | 20.55 | 0.04 | 0.08 | 0.00 | 0.00 |
| 48 h | 26.70 | 23.99 | 0.00 | 0.00 | 0.00 | 0.00 |
| 72 h | 14.07 | 10.72 | 0.00 | 0.00 | 0.00 | 0.00 |
| 96 h | 18.30 | 21.75 | 0.00 | 0.00 | 0.00 | 0.00 |
| 7 d | 3.38 | 3.91 | 0.00 | 0.00 | 0.00 | 0.00 |

| ON- | T cells | | B cells | | NK cells | |
|------|---------|-------|---------|-------|----------|-------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 51.53 | 24.25 | 10.33 | 7.32 | 13.67 | 8.56 |
| 15 m | 73.34 | 44.39 | 13.32 | 10.92 | 18.60 | 14.75 |
| 30 m | 58.90 | 23.97 | 8.41 | 6.36 | 13.37 | 8.90 |
| 45 m | 49.05 | 17.74 | 6.38 | 4.34 | 10.94 | 6.36 |
| 60 m | 37.46 | 5.07 | 3.83 | 2.44 | 6.10 | 3.10 |
| 24 h | 18.46 | 15.47 | 3.16 | 3.23 | 1.83 | 2.02 |
| 48 h | 22.50 | 19.48 | 2.99 | 3.18 | 2.26 | 3.03 |
| 72 h | 13.23 | 10.35 | 0.45 | 0.53 | 0.84 | 1.01 |
| 96 h | 16.46 | 20.80 | 0.22 | 0.43 | 0.43 | 0.86 |
| 7 d | 3.23 | 4.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| ON- | CD4+ T cells | | CD8+ T cells | | CD4-CD8- T cells | |
|------|--------------|-------|--------------|-------|------------------|------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 36.07 | 19.54 | 11.93 | 4.80 | 3.51 | 1.25 |
| 15 m | 49.00 | 31.16 | 20.97 | 12.71 | 3.36 | 3.24 |
| 30 m | 39.95 | 19.04 | 15.47 | 4.55 | 3.45 | 1.14 |
| 45 m | 33.14 | 14.03 | 13.08 | 3.57 | 2.89 | 1.50 |
| 60 m | 26.25 | 5.61 | 9.51 | 2.64 | 1.72 | 0.47 |
| 24 h | 13.10 | 11.65 | 4.65 | 3.45 | 0.57 | 0.54 |
| 48 h | 15.42 | 14.08 | 6.07 | 4.64 | 0.60 | 0.52 |
| 72 h | 10.63 | 9.92 | 2.19 | 2.58 | 0.47 | 0.61 |
| 96 h | 11.95 | 13.64 | 5.73 | 6.77 | 0.43 | 0.86 |
| 7 d | 2.18 | 2.98 | 0.93 | 0.94 | 0.13 | 0.15 |

| ON+ | Lymphocytes | | Neutrophiles | | Monocytes | |
|------|-------------|-------|--------------|-------|-----------|-------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 7.86 | 7.13 | 93.13 | 62.77 | 10.50 | 6.50 |
| 15 m | 39.32 | 23.47 | 183.54 | 75.35 | 22.30 | 13.98 |
| 30 m | 42.59 | 24.28 | 172.56 | 73.61 | 15.96 | 9.97 |
| 45 m | 46.38 | 19.16 | 166.69 | 35.81 | 17.65 | 8.51 |
| 60 m | 45.52 | 14.17 | 130.28 | 20.84 | 14.02 | 5.74 |
| 24 h | 27.29 | 20.03 | 52.96 | 22.72 | 3.14 | 2.23 |
| 48 h | 33.79 | 29.94 | 43.43 | 46.27 | 0.94 | 1.25 |
| 72 h | 35.97 | 15.66 | 33.78 | 38.40 | 0.00 | 0.00 |
| 96 h | 27.16 | 18.81 | 7.06 | 2.12 | 0.00 | 0.00 |
| 7 d | 18.27 | 7.07 | 0.00 | 0.00 | 0.00 | 0.00 |

| ON+ | T cells | | B cells | | NK cells | |
|------|---------|-------|---------|------|----------|-------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 8.12 | 4.43 | 1.80 | 1.08 | 1.43 | 0.80 |
| 15 m | 26.37 | 14.38 | 8.51 | 7.01 | 4.70 | 2.85 |
| 30 m | 29.61 | 17.16 | 7.19 | 3.97 | 7.09 | 4.88 |
| 45 m | 29.87 | 11.35 | 8.42 | 3.52 | 8.09 | 4.64 |
| 60 m | 29.94 | 8.11 | 7.38 | 2.75 | 6.15 | 4.88 |
| 24 h | 16.08 | 8.72 | 3.60 | 3.65 | 7.57 | 7.87 |
| 48 h | 19.56 | 16.94 | 3.69 | 3.69 | 10.04 | 11.18 |
| 72 h | 27.85 | 12.55 | 2.52 | 2.10 | 6.86 | 3.31 |
| 96 h | 22.72 | 14.60 | 1.77 | 1.54 | 4.75 | 5.43 |
| 7 d | 17.72 | 6.16 | 0.12 | 0.24 | 0.44 | 0.71 |

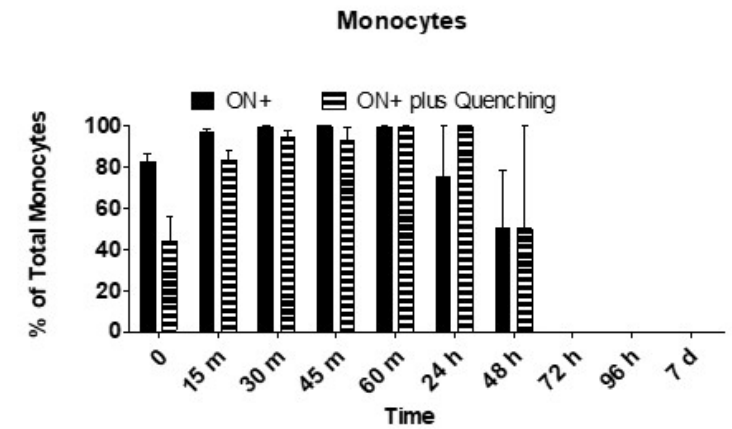
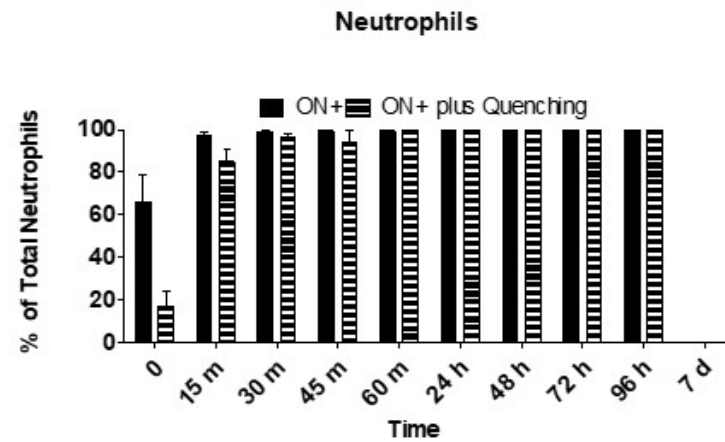
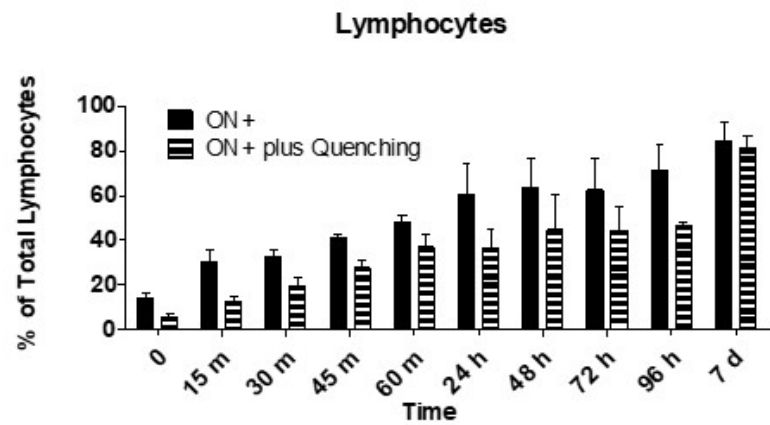
| ON+ | CD4+ T cells | | CD8+ T cells | | CD4-CD8- T cells | |
|------|--------------|-------|--------------|------|------------------|------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 5.17 | 3.23 | 2.39 | 1.24 | 0.56 | 0.39 |
| 15 m | 18.07 | 10.96 | 7.14 | 4.07 | 1.15 | 0.96 |
| 30 m | 19.79 | 12.50 | 6.61 | 3.18 | 1.78 | 0.41 |
| 45 m | 19.48 | 10.37 | 8.28 | 2.18 | 2.27 | 0.55 |
| 60 m | 18.89 | 7.12 | 8.43 | 1.67 | 2.64 | 1.05 |
| 24 h | 9.92 | 7.49 | 4.23 | 1.81 | 1.93 | 0.70 |
| 48 h | 14.74 | 13.13 | 4.38 | 2.69 | 2.76 | 2.28 |
| 72 h | 19.34 | 10.43 | 8.99 | 7.12 | 1.19 | 1.52 |
| 96 h | 16.13 | 11.30 | 5.82 | 3.01 | 2.05 | 1.33 |
| 7 d | 12.80 | 5.33 | 4.04 | 2.17 | 0.88 | 0.73 |

| ON+/Q | Lymphocytes | | Neutrophiles | | Monocytes | |
|-------|-------------|--------|--------------|--------|-----------|-------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 47.44 | 26.79 | 33.50 | 23.11 | 7.99 | 6.55 |
| 15 m | 170.35 | 98.82 | 139.75 | 79.88 | 18.59 | 14.90 |
| 30 m | 231.13 | 168.53 | 175.59 | 110.83 | 24.97 | 30.07 |
| 45 m | 262.29 | 166.86 | 198.16 | 108.45 | 32.74 | 40.20 |
| 60 m | 156.96 | 38.35 | 110.90 | 24.16 | 14.95 | 9.42 |
| 24 h | 74.35 | 25.85 | 41.22 | 10.62 | 6.55 | 3.67 |
| 48 h | 57.24 | 8.07 | 34.14 | 0.16 | 1.37 | 1.94 |
| 72 h | 23.21 | 4.30 | 9.73 | 1.51 | 0.00 | 0.00 |
| 96 h | 25.77 | 13.44 | 5.26 | 2.76 | 0.00 | 0.00 |
| 7 d | 14.99 | 2.95 | 0.00 | 0.00 | 0.00 | 0.00 |

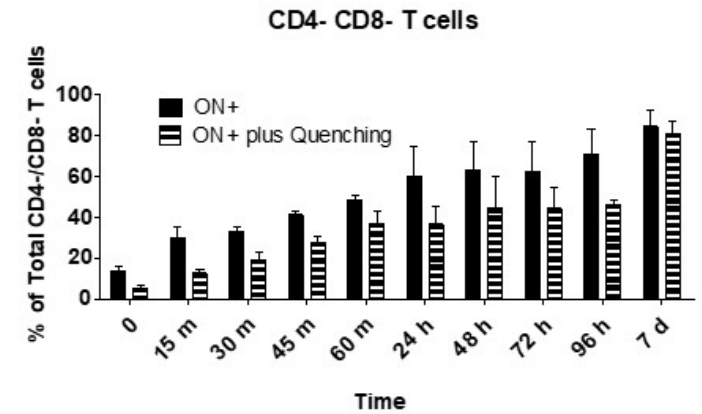
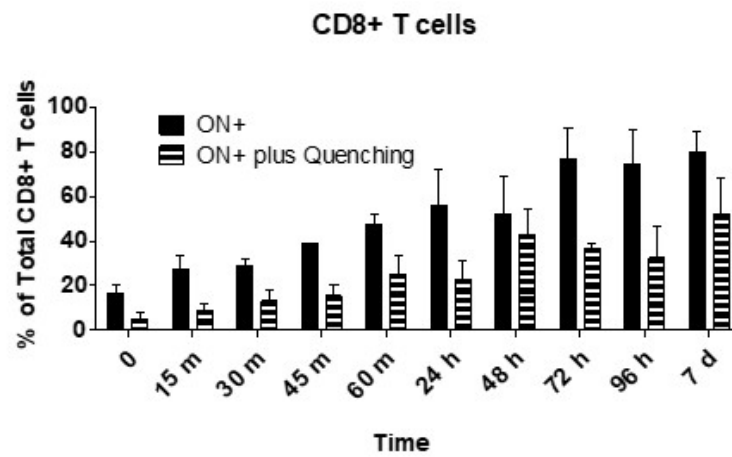
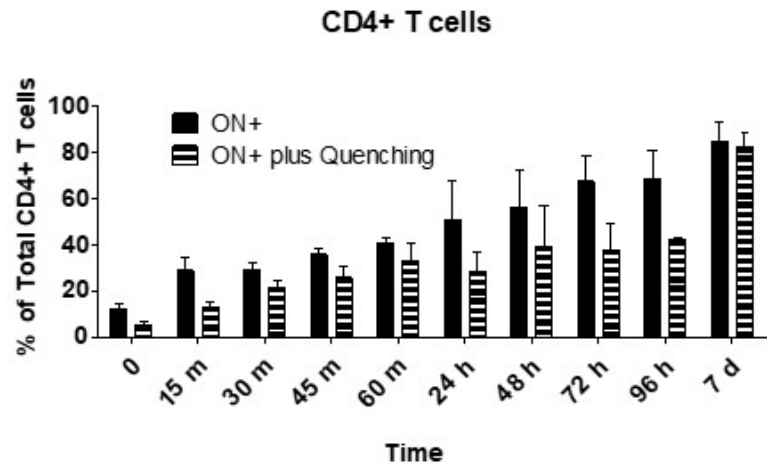
| ON+/Q | T cells | | B cells | | NK cells | |
|-------|---------|-------|---------|------|----------|------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 4.89 | 3.06 | 0.32 | 0.26 | 0.71 | 0.46 |
| 15 m | 10.09 | 4.39 | 0.98 | 0.72 | 1.40 | 0.98 |
| 30 m | 24.19 | 23.75 | 3.34 | 4.17 | 4.56 | 3.68 |
| 45 m | 15.72 | 15.94 | 3.37 | 3.01 | 6.00 | 4.55 |
| 60 m | 22.03 | 8.08 | 5.27 | 7.22 | 3.90 | 2.35 |
| 24 h | 17.77 | 13.65 | 3.44 | 4.48 | 5.52 | 6.15 |
| 48 h | 17.33 | 5.80 | 0.83 | 0.47 | 3.58 | 0.96 |
| 72 h | 9.75 | 4.96 | 1.07 | 0.74 | 2.81 | 1.39 |
| 96 h | 17.71 | 8.98 | 0.20 | 0.35 | 2.84 | 2.46 |
| 7 d | 14.99 | 2.95 | 0.00 | 0.00 | 0.00 | 0.00 |

| ON+/Q | CD4+ T cells | | CD8+ T cells | | CD4-CD8- T cells | |
|-------|--------------|-------|--------------|------|------------------|------|
| Time | Average | SD | Average | SD | Average | SD |
| 0 | 2.67 | 1.74 | 1.53 | 1.35 | 0.70 | 0.78 |
| 15 m | 7.23 | 5.40 | 2.31 | 0.83 | 1.18 | 1.06 |
| 30 m | 16.84 | 19.94 | 4.81 | 3.95 | 2.52 | 1.08 |
| 45 m | 13.03 | 8.50 | 6.01 | 4.66 | 2.66 | 1.82 |
| 60 m | 12.65 | 6.09 | 6.40 | 2.16 | 3.00 | 0.98 |
| 24 h | 9.61 | 8.90 | 5.39 | 3.40 | 2.83 | 1.64 |
| 48 h | 8.96 | 3.69 | 6.08 | 1.22 | 2.29 | 0.88 |
| 72 h | 5.26 | 3.67 | 3.68 | 1.69 | 0.82 | 0.40 |
| 96 h | 12.85 | 8.12 | 3.86 | 1.47 | 1.18 | 1.08 |
| 7 d | 9.95 | 3.29 | 3.31 | 0.80 | 1.72 | 1.84 |

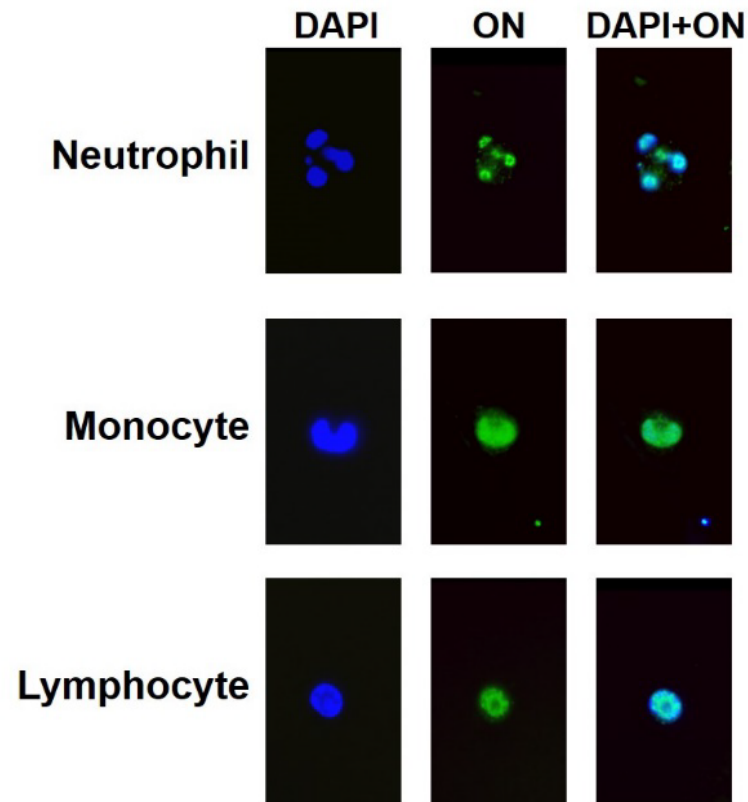
Metodología: entrada del ASOs



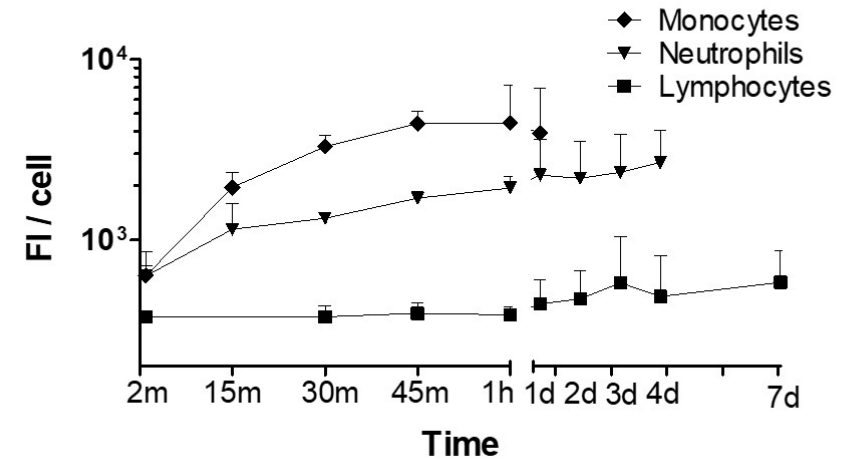
Metodología: entrada del ASOs



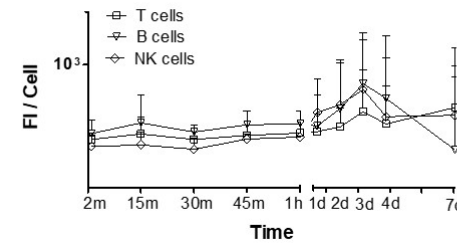
Metodología: entrada del ASOs



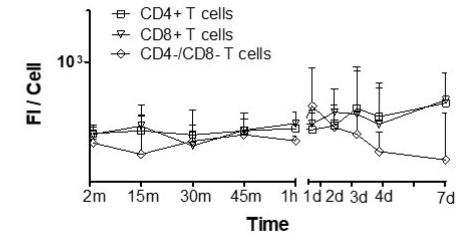
Leukocytes Kinetics Fluorescence Intensity



Lymphocytes Kinetics Fluorescence Intensity



T Lymphocytes Kinetics Fluorescence Intensity



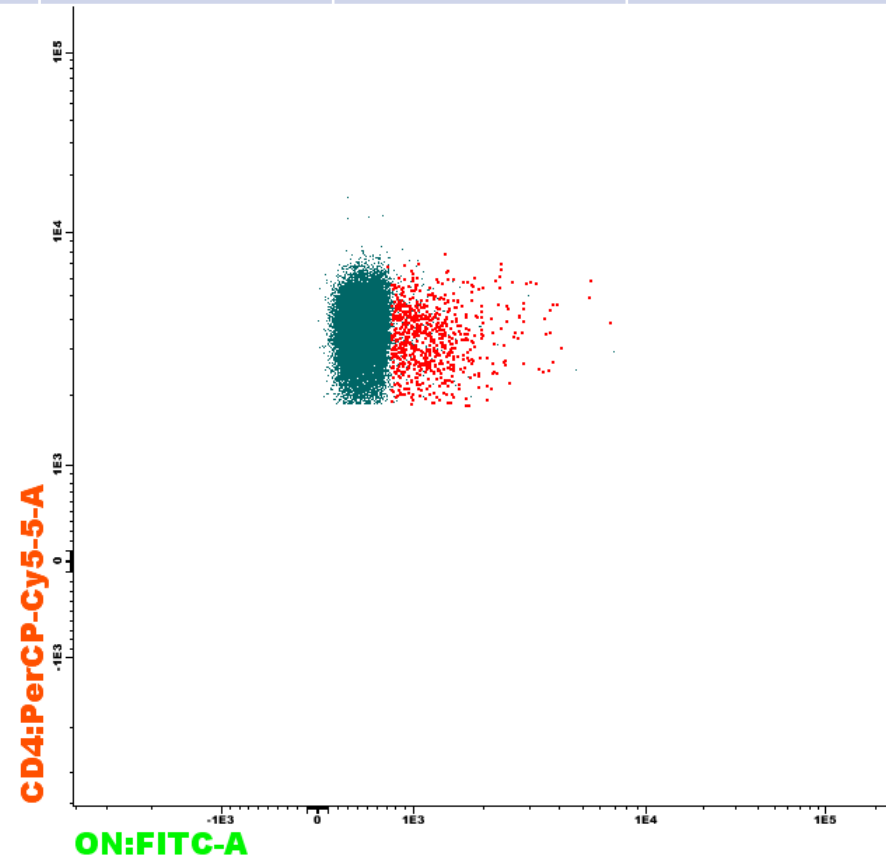
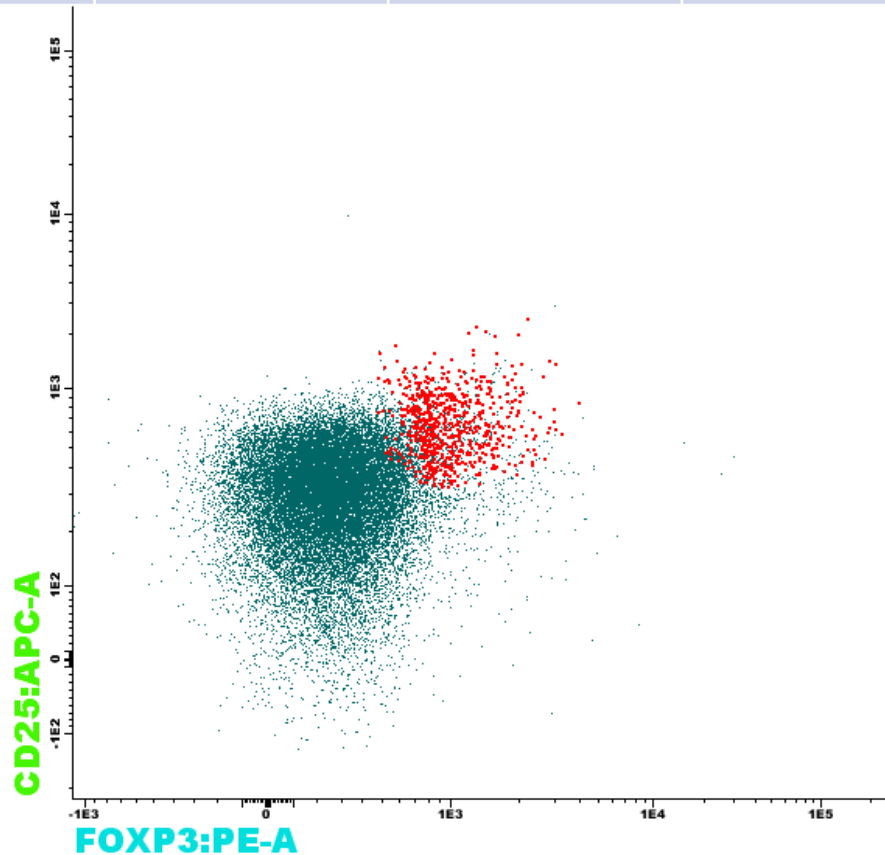
3

Metodología: Treg

| FITC | PE | PercP Cy5.5 | PE Cy7 | V-450 | BV-510 | APC | APC H7 |
|------|-------|-------------|--------|-------|------------|------|--------|
| | FOXP3 | CD4 | CD8 | | VIABILIDAD | CD25 | CD3 |

Metodología: Treg

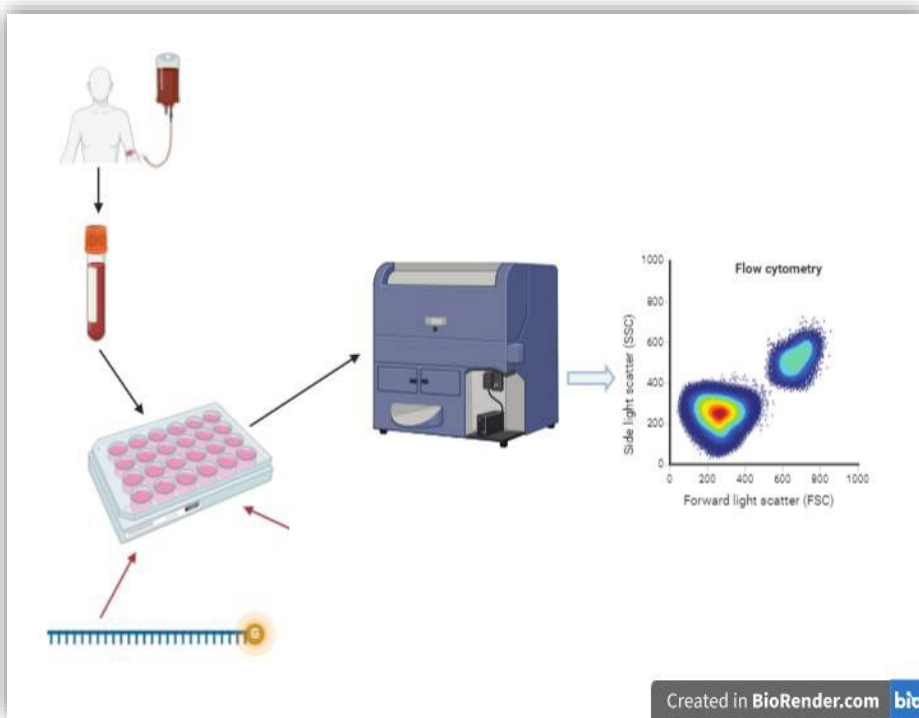
| FITC | PE | PercP Cy5.5 | PE Cy7 | V-450 | BV-510 | APC | APC H7 |
|------|-------|-------------|--------|-------|------------|------|--------|
| ON | FOXP3 | CD4 | CD8 | | VIABILIDAD | CD25 | CD3 |



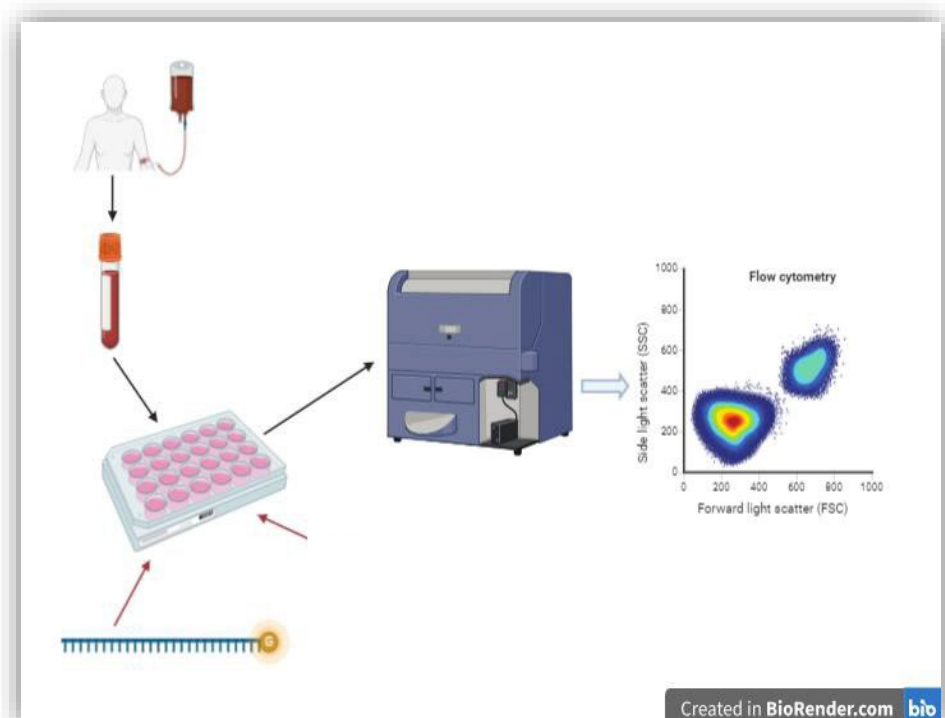
Metodología: Treg

1h, 48h, 96h. 7 días

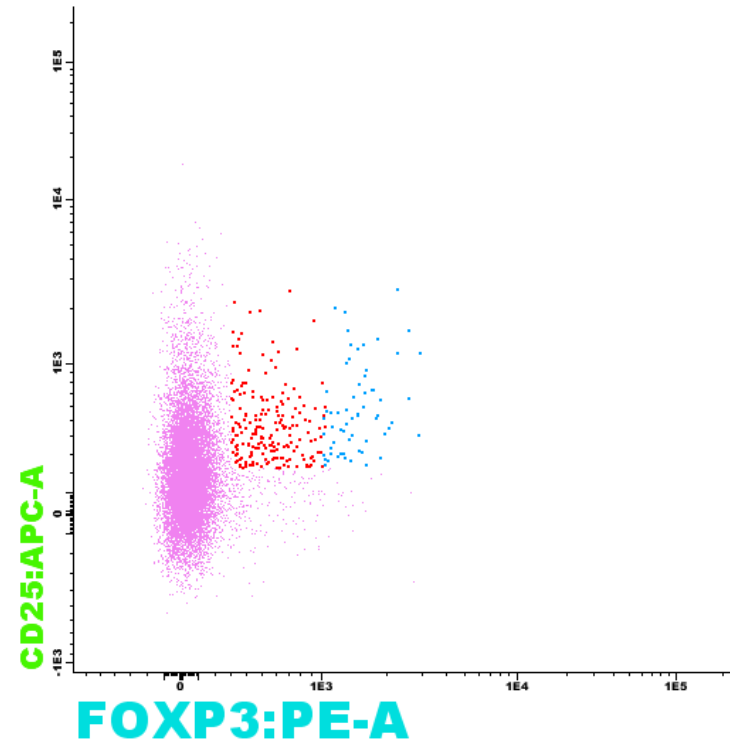
ASOs



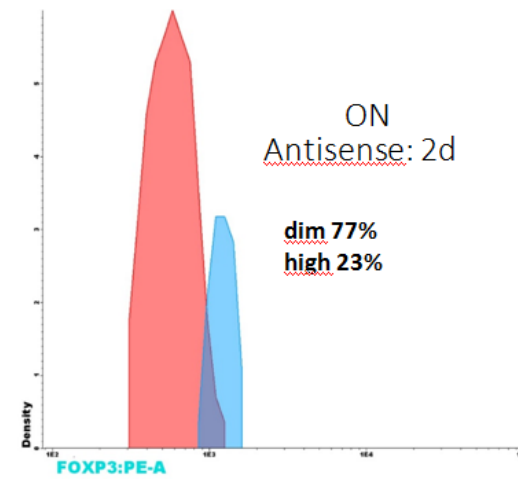
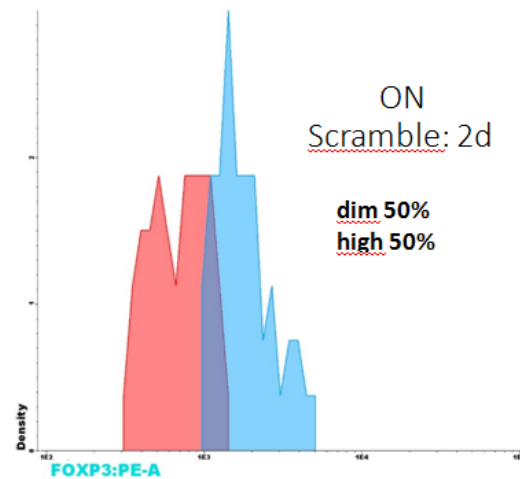
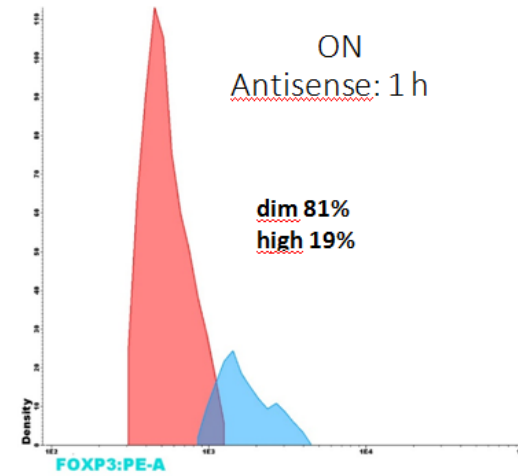
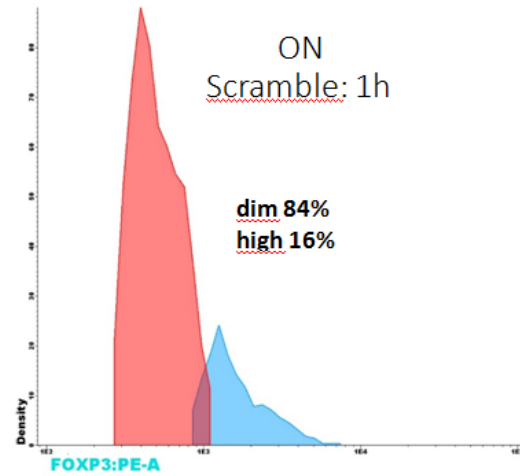
SCRAMBLED



Metodología: Treg



Metodología: Treg



Metodología

- Realizar resultados del silenciamiento.
- Panel con CTLA-4 y PD-1.
- Experimentos con células tumorales: paneles de activación y paneles con el resto de poblaciones de linfocitos T.

| FLUOROCROMO | FITC | PE | PER-CP Cy5.5 | PE-Cy7 | V-450 | V-500 | APC | APC-Cy7 |
|-------------|---------------|---------------|--------------|--------------|-------|--------------|---------------|---------|
| ANTICUERPO | CD294 (CRTH2) | CD183 (CXCR3) | CD4 | CD8 | | ¿Viabilidad? | CCD196 (CCR6) | CD3 |
| PROPOSITO | TH2 | TH1 | T-Helper | T-Citotóxica | | | TH17 | T-Cel |
| | 561659 | | | | | | | |

| FLUOROCROMO | FITC | PE | PerCP-Cy5.5 | PE-Cy7 | BV-421 | BV-510 | APC | APC-Cy7 |
|----------------|--------------|--------------------------------------|-------------|--------------|--------------|--------------|-------------|---------|
| ANTICUERPO | <u>IFN-γ</u> | IL-4 Solo este fluorocromo | CD4 | <u>TNF-α</u> | IL-10 | IL-17 | CD25 | |
| REFERENCIAS | 340449 | 340456 | 566923 | 557647 | 564053 | 563295 | | |
| OTRAS OPCIONES | <i>IL-1</i> | <i>IL-1</i> | | | <i>IL-6</i> | | | |

Aplicaciones en hematología

- Construcción de paneles de anticuerpos que pueden ser útiles en determinados casos.

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An HK2 antisense oligonucleotide induces synthetic lethality in HK1-HK2+ multiple myeloma

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STAT3 antisense oligonucleotide AZD9150 in a subset of patients with heavily pretreated lymphoma: results of a phase 1b trial

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ORIGINAL ARTICLE

Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy

K.J. Pasi, S. Rangarajan, P. Georgiev, T. Mant, M.D. Creagh, T. Lissitchkov, D. Bevan, S. Austin, C.R. Hay, I. Hegemann, R. Kazmi, P. Chowdary, L. Gercheva-Kyuchukova, V. Mamonov, M. Timofeeva, C.-H. Soh, P. Garg, A. Vaishnav, A. Akinc, B. Sørensen, and M.V. Ragni

ABSTRACT

BACKGROUND

Current hemophilia treatment involves frequent intravenous infusions of clotting factors, which is associated with variable hemostatic protection, a high treatment burden, and a risk of the development of inhibitory alloantibodies. Fitusiran, an investigational RNA interference (RNAi) therapy that targets antithrombin (encoded by SERPINC1), is in development to address these and other limitations.

MUCHAS GRACIAS.