Inmunoterapia en infección fúngica

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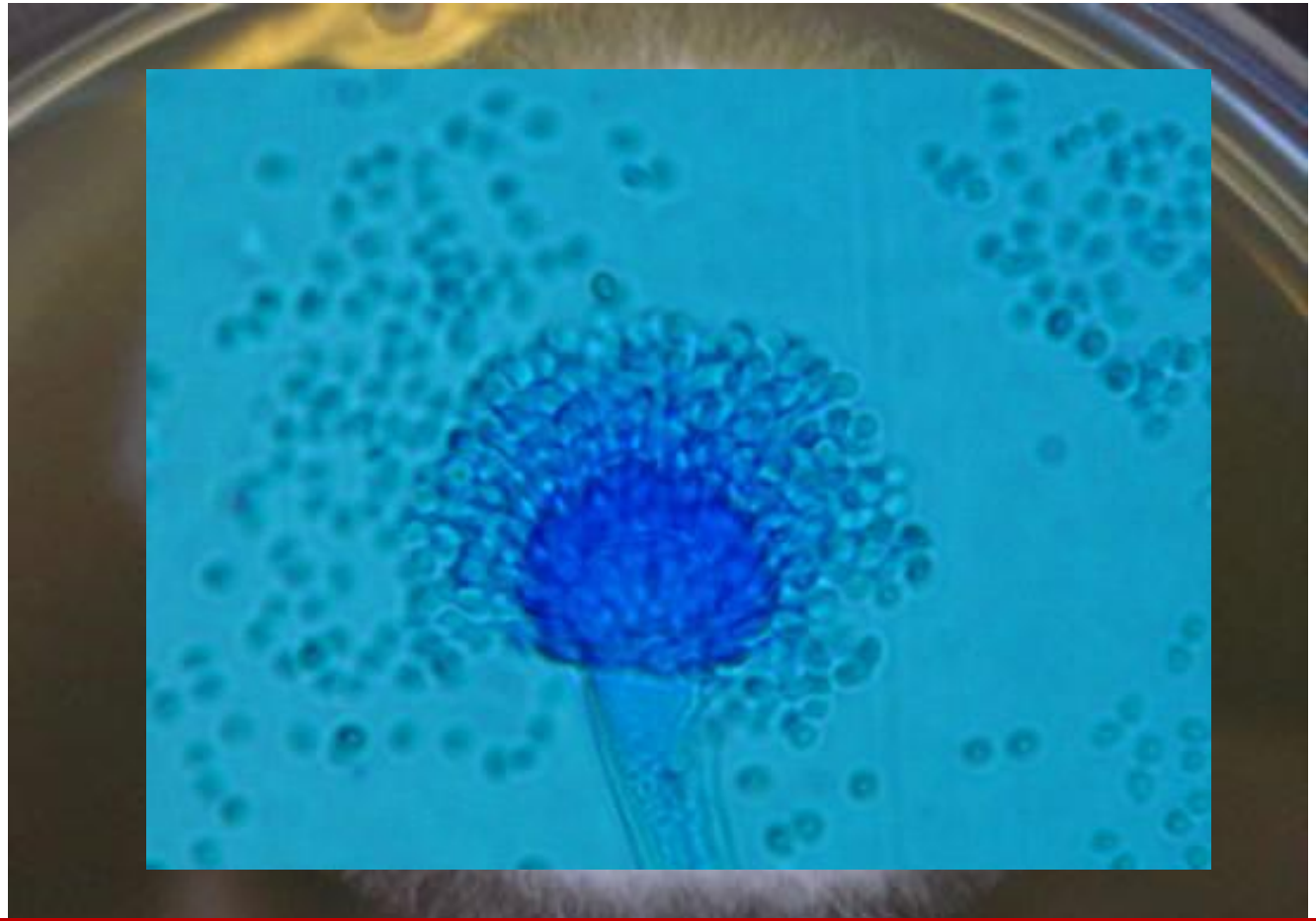
Fundació Lluita contra les Infeccions

- **Problemática actual de la infección fúngica invasora**

1. Nuevos huéspedes susceptibles a presentar infección fúngica
2. Hay un aumento de las IFIs causadas por hongos raros y multirresistentes asociados a gran mortalidad (IFI de brecha)
3. Los antifúngicos actuales están asociados a importantes toxicidades, costes, e interacciones farmacológicas
4. La eficacia clínica de los antifúngicos es subóptima o incluso nula si no se recupera la inmunidad

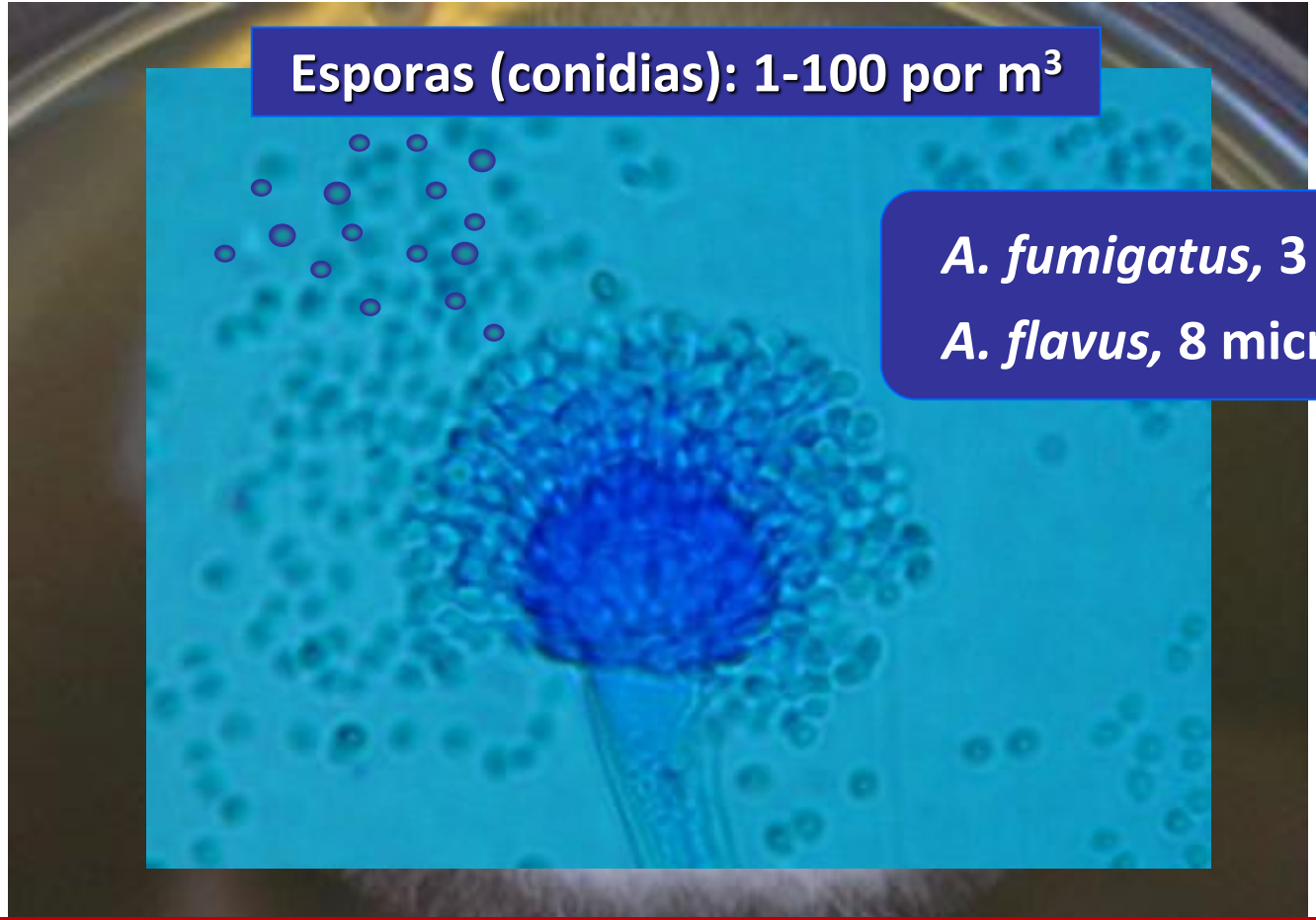
- **Respuesta inmunológica en la infección fúngica**

Aspergillus spp.



Ubícuo (suelo, polvo, agua, materia orgánica en descomposición…)

Aspergillus spp.



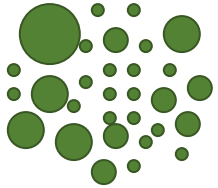
Esporas (conidias): 1-100 por m^3

A. fumigatus, 3 micras

A. flavus, 8 micras

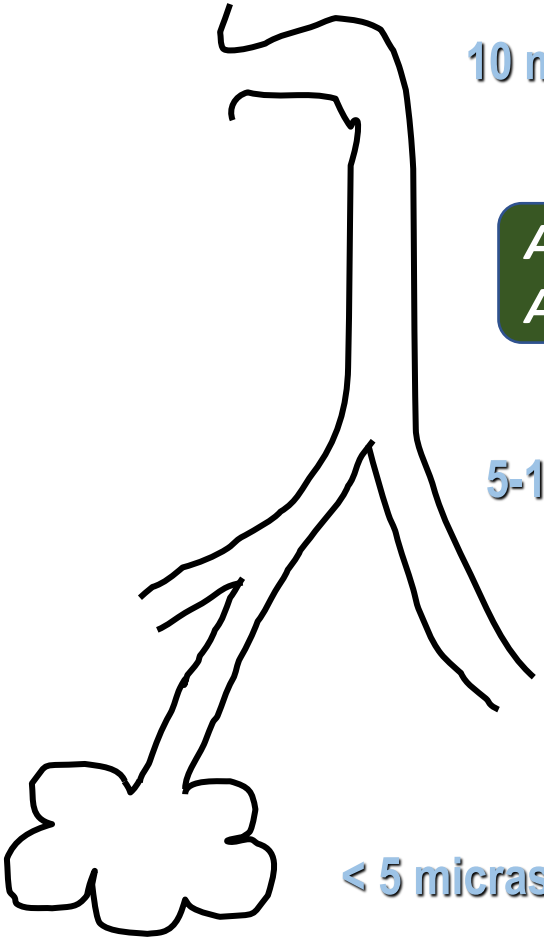
Ubícuo (suelo, polvo, agua, materia orgánica en descomposición…)

Esporas (conidias) ambientales



≥ 10 micras

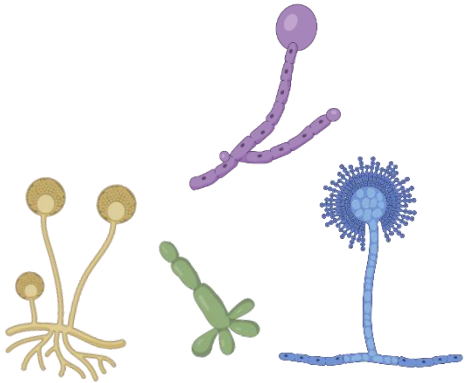
1 m

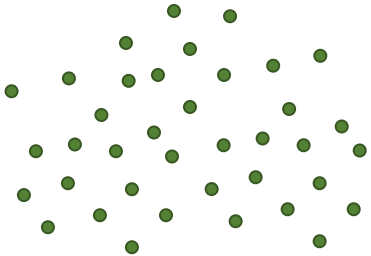


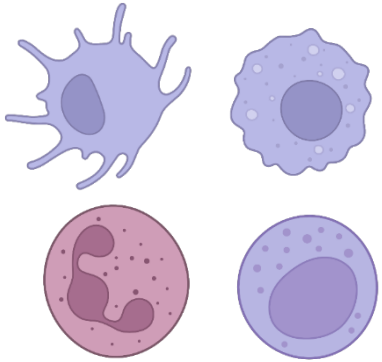
A. flavus
A. niger

A. fumigatus

Innate immunity







Pathogen Associated Molecular Patterns: β-glucan, zymosan, chitin, LPS, mannan…

PAMPs

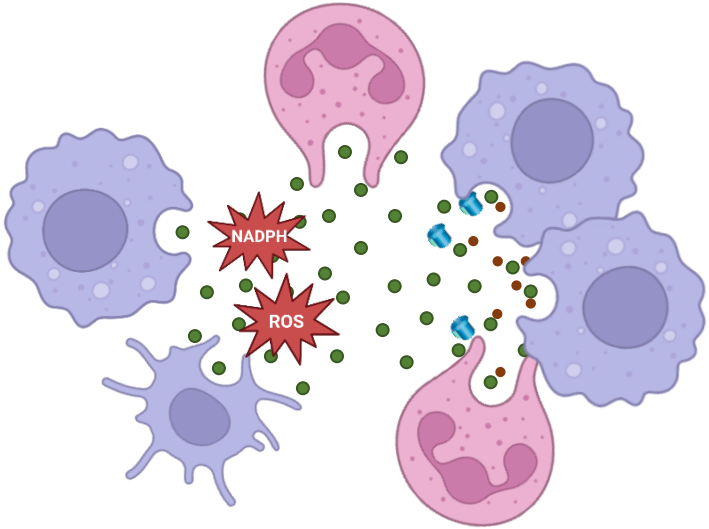
PRRs

Pattern Recognition Receptors: Toll like receptors (TLRs), C-type lectin receptors (CLRs), Nod-like receptors (NLRs), Mannose binding receptors (MBRs)…

Innate immunity

PAMPs — PRRs

Phagocytosis + Release of antimicrobial components



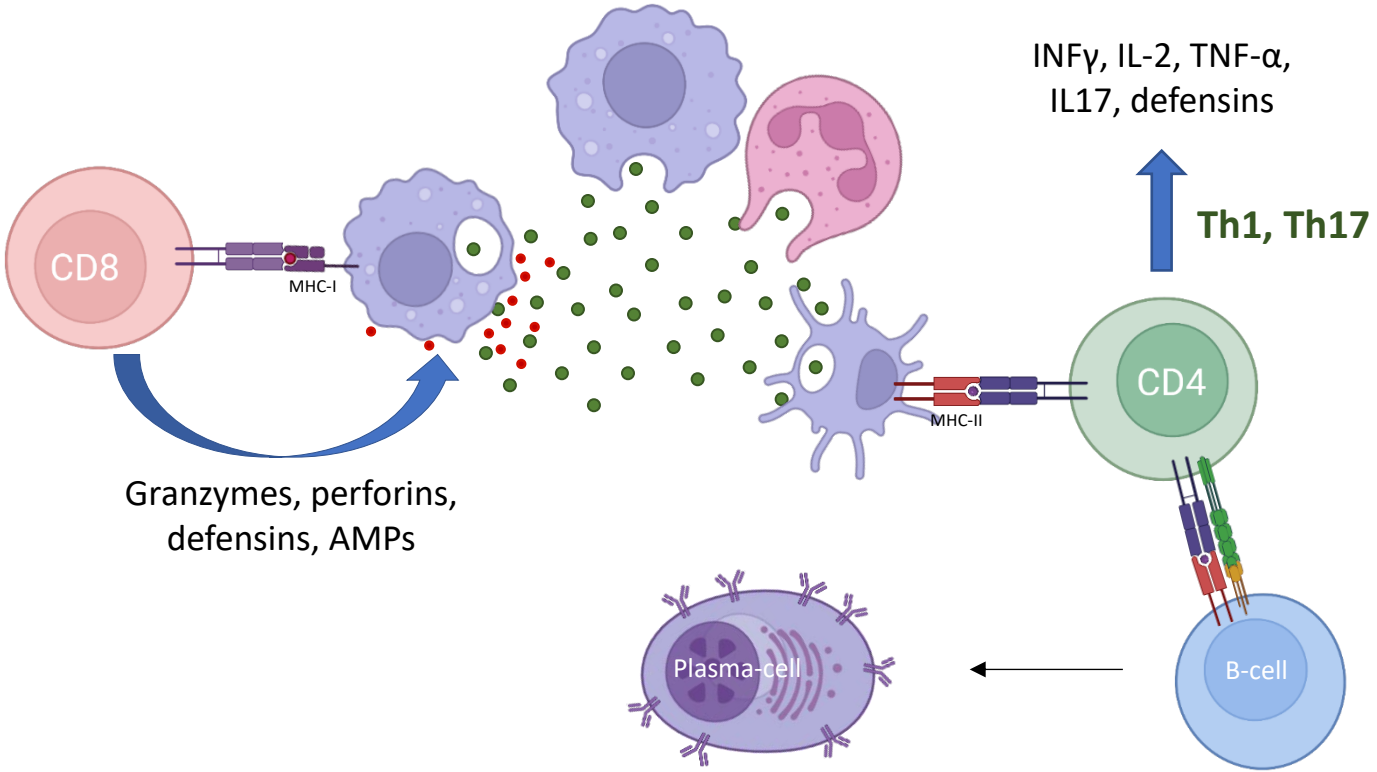
Complement system, antifungal peptides, collectins defensins

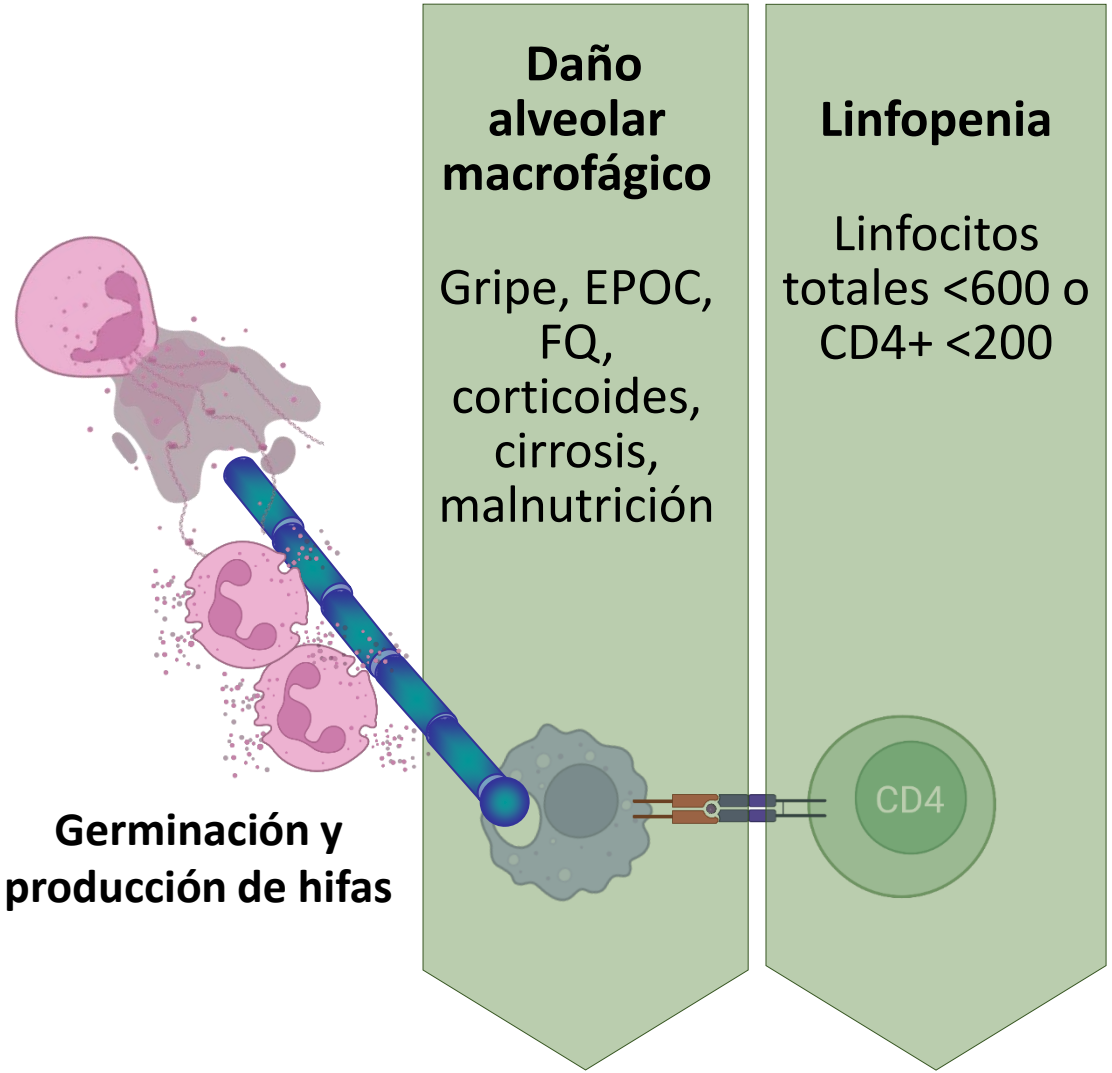
↓

Opsono-phagocytosis

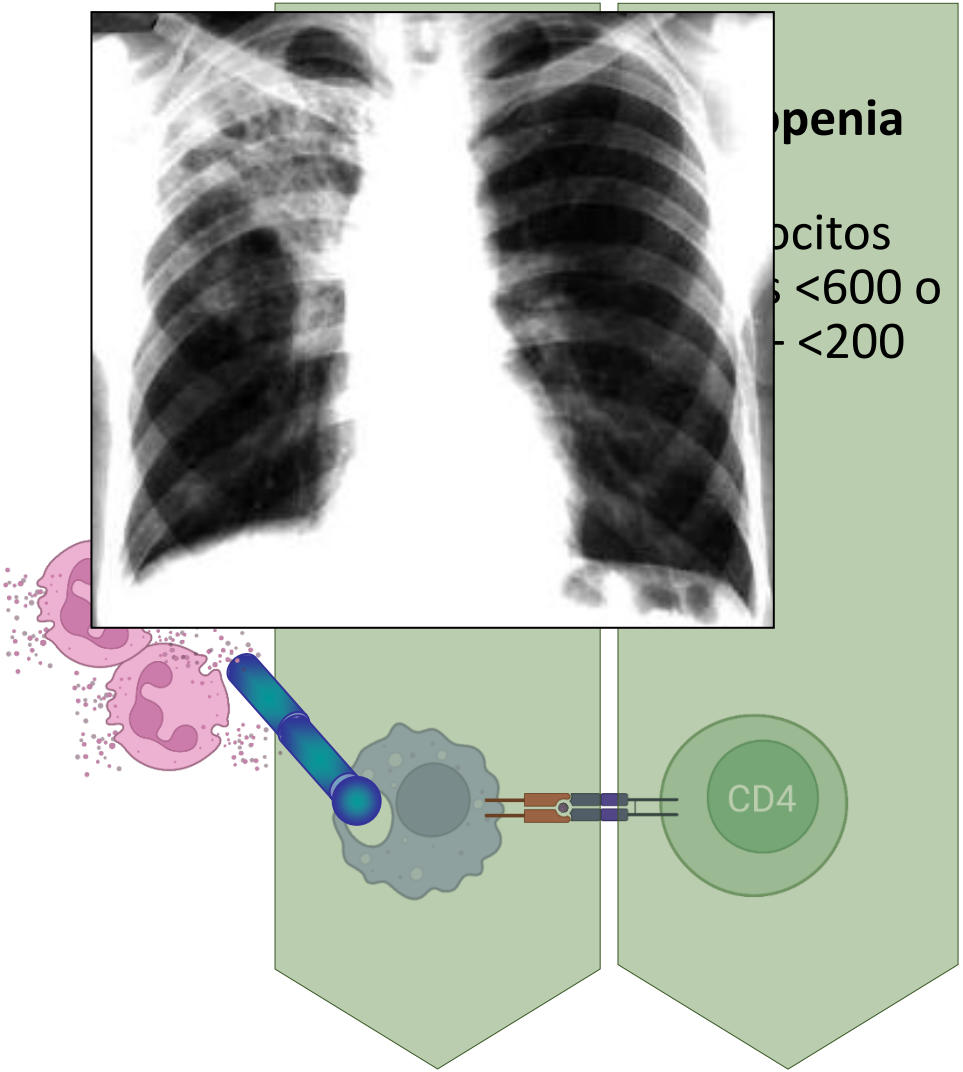
Humoral component

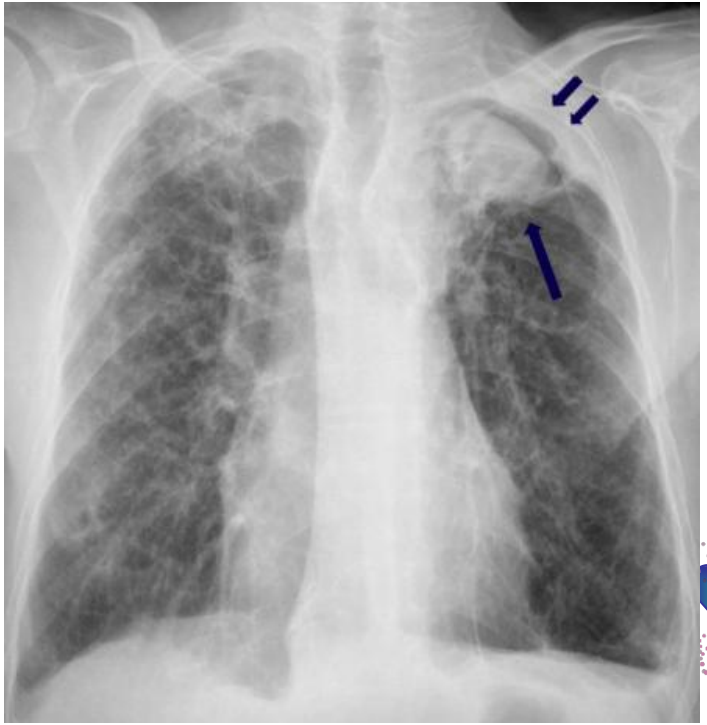
Adaptative immunity

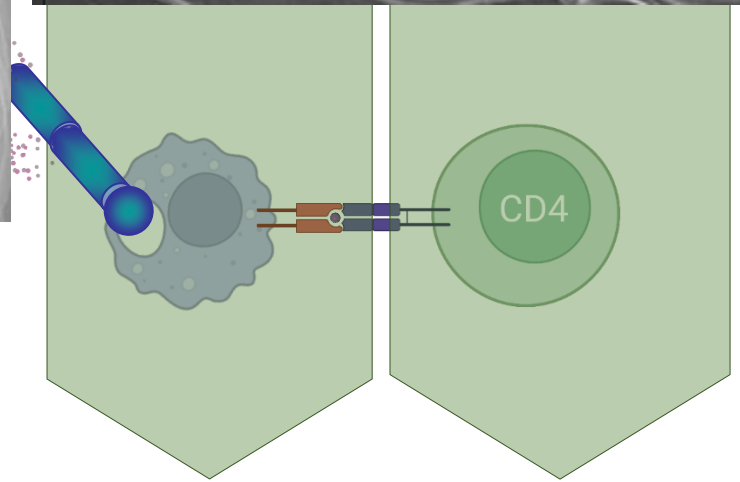



Daño alveolar macrofágico
Gripe, EPOC, FQ, corticoides, cirrosis, malnutrición
Linfopenia
Linfocitos totales <600 o CD4+ <200
CD4
Germinación y producción de hifas

Aspergilosis pulmonar crónica







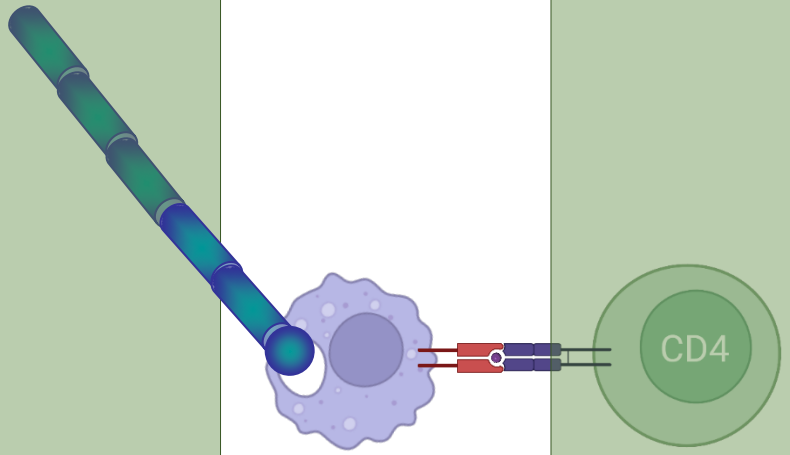
Aspergiloma

Neutropenia

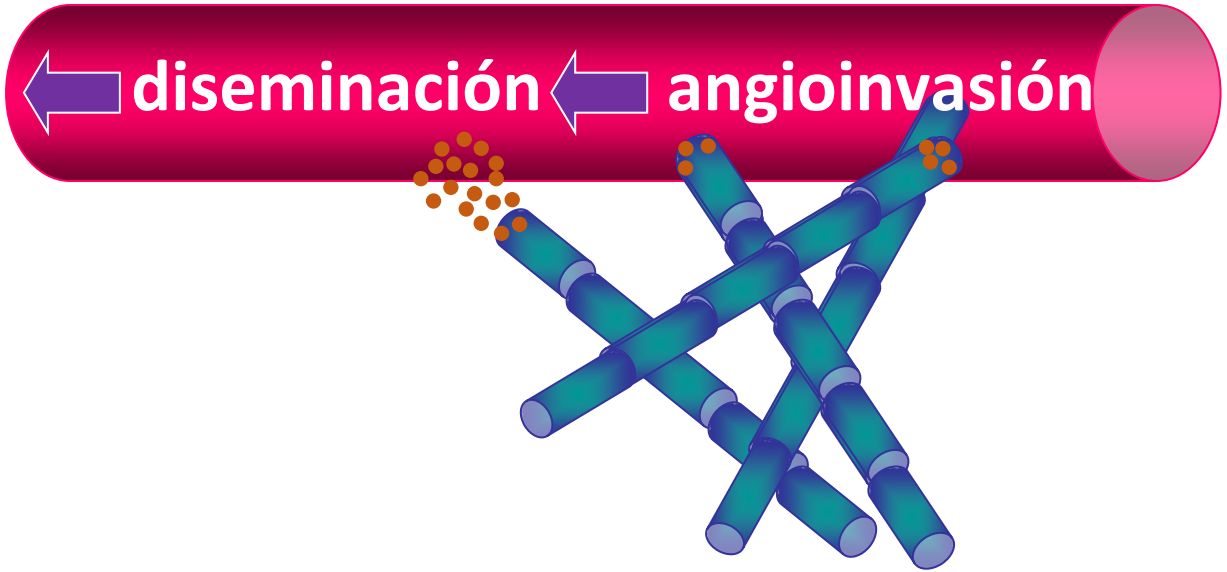
Enfermedad granulomatosa crónica

Linfopenia

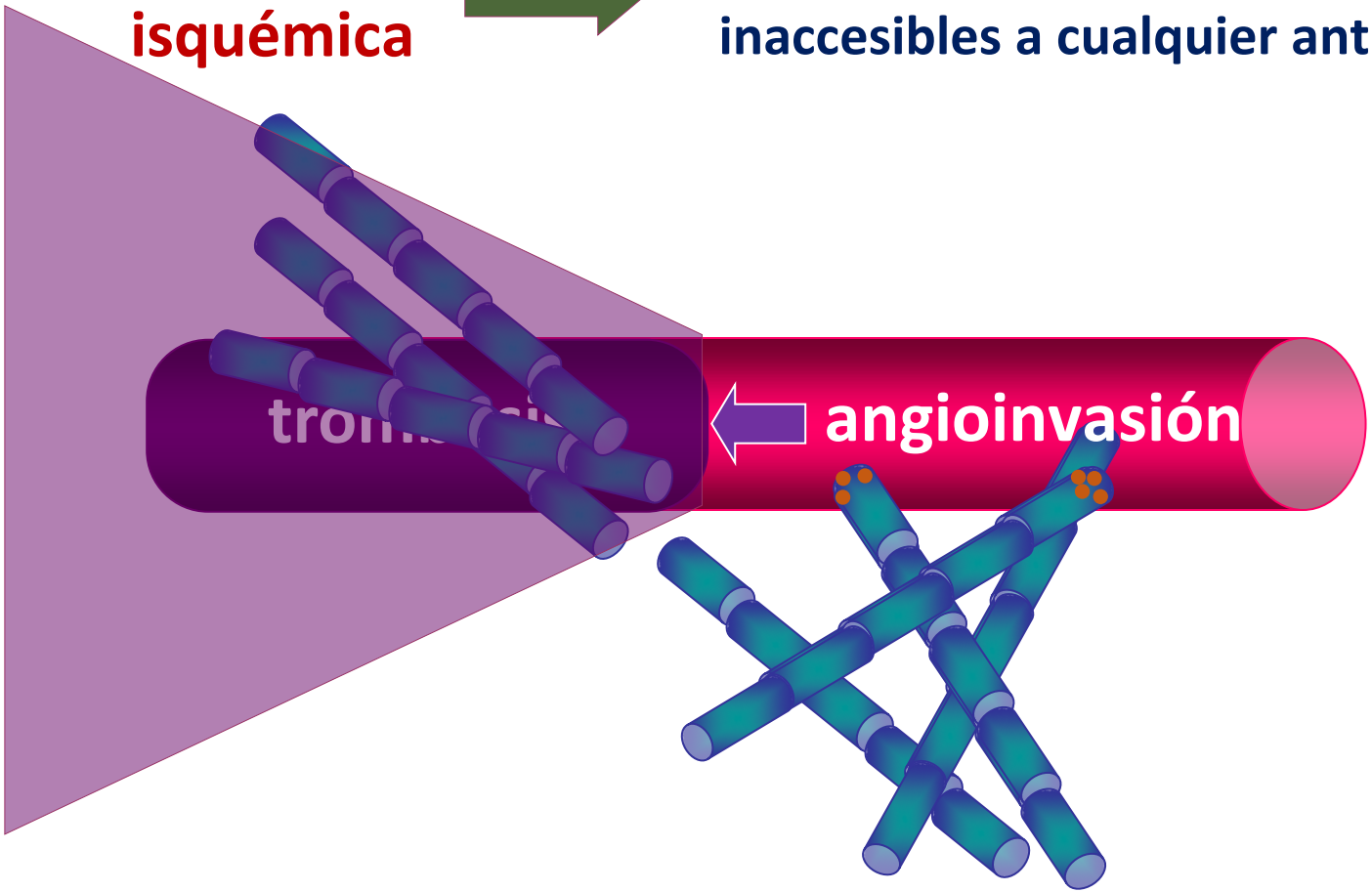
Linfocitos totales <600 o CD4+ <200

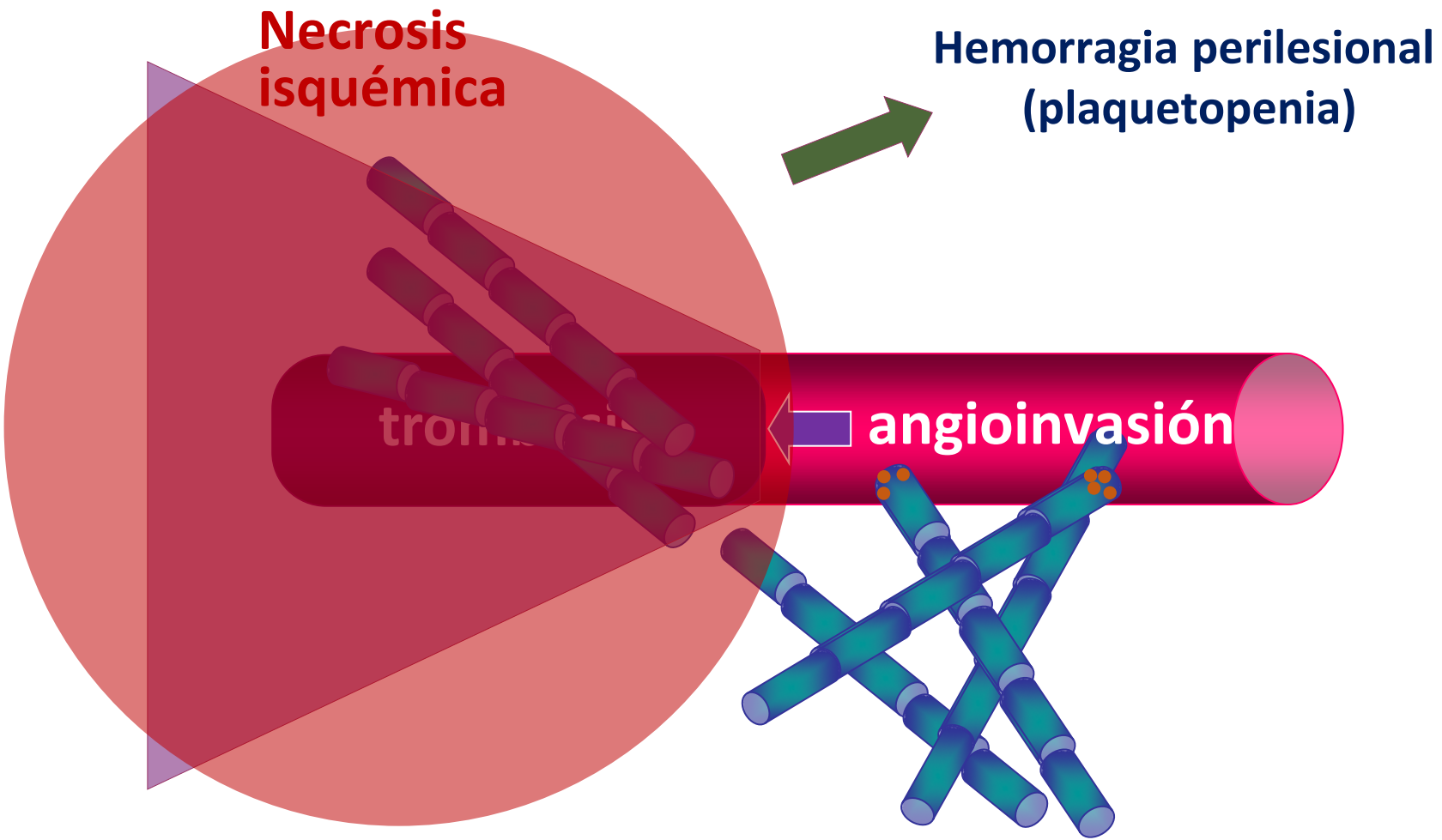


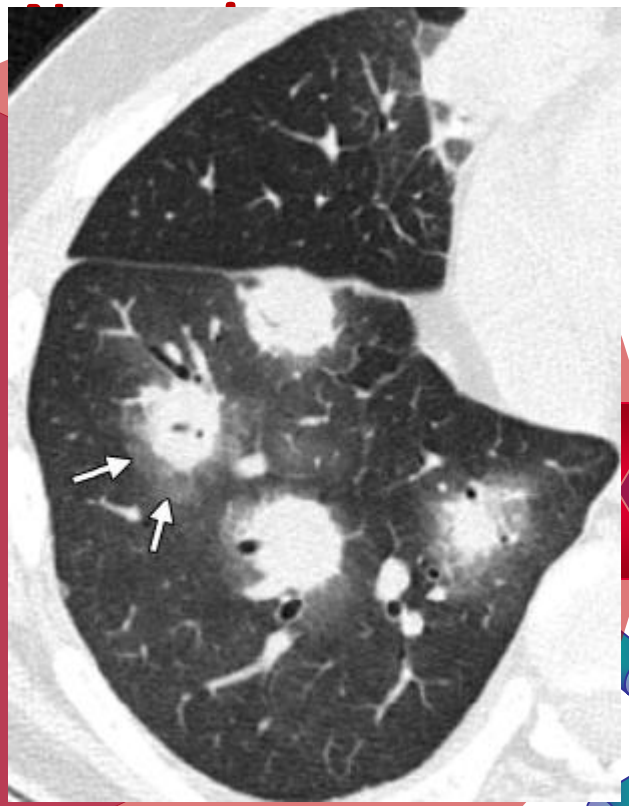
Liberación de enzimas proteolíticas capaces de degradar cualquier material orgánico (celulosa, quitina, queratina, lignina…)



Necrosis isquémica → **Desarrollo de espacios avasculares inaccesibles a cualquier antifúngico**




Necrosis isquémica
Hemorragia perilesional (plaquetopenia)
angioinvasión



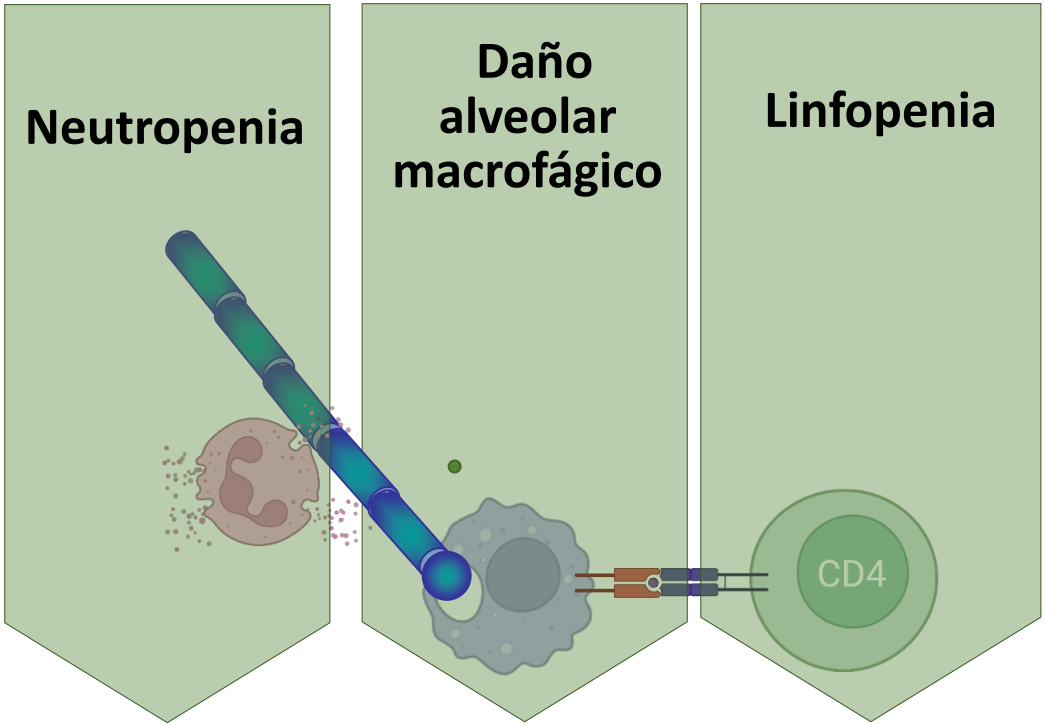
Aspergilosis pulmonar invasiva

Con angioinvasión

- nódulos-masas con halo
- signo de la media luna
- diseminación sistémica
- **AGA en suero ≥ 0.5…**

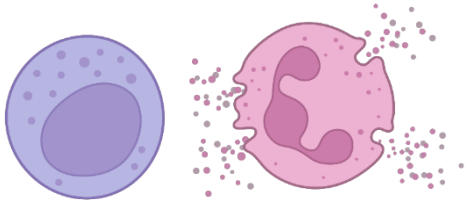
Sin angioinvasión

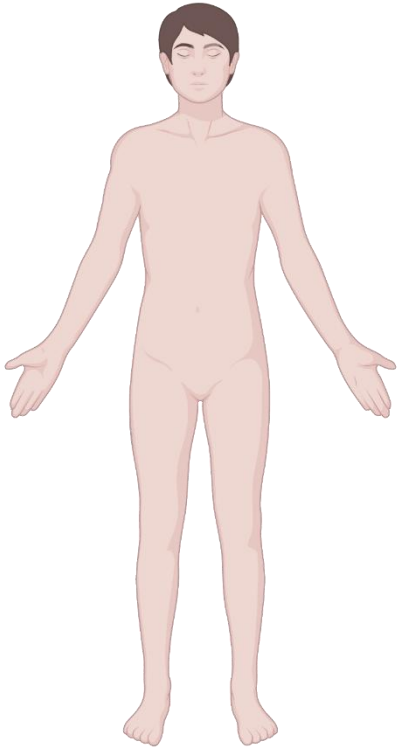
- cavitación/fibrosis crónica
- IgG (precipitinas) frente a *Aspergillus*
- **AGA en BAL ≥1**



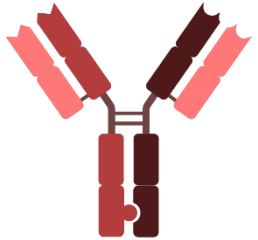
- **Inmunoterapia en infección fúngica**

Non-specific Immune stimulation



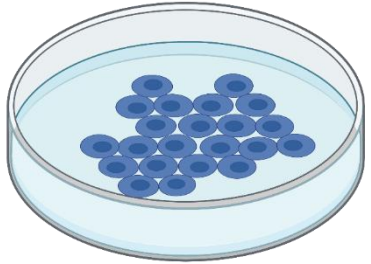


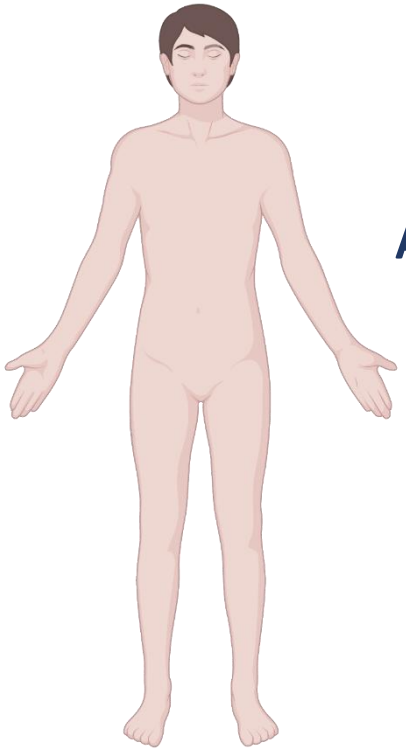
Monoclonal antibodies



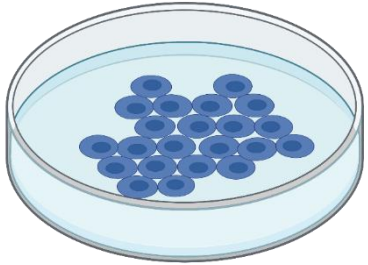
Vaccination strategies

Adoptive cell transfer





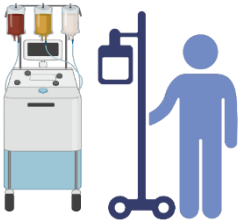
Adoptive cell transfer



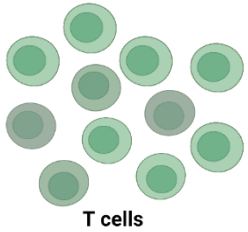
SPECIFIC T-CELL INFUSION

CHIMERIC ANTIGEN RECEPTOR THERAPY

ADOPTIVE CELL TRANSFER

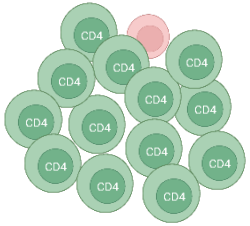


1. Apheresis



2. T-cell collection

Asp specific CD4-cells (1:200)

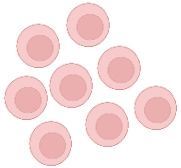


3. CD8 and Treg depletion

Lysate

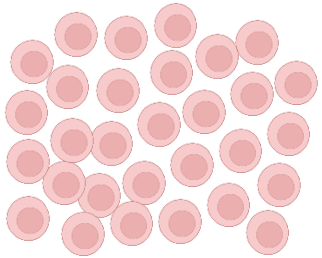
4. Fungal antigen presentation

Based on Th1 response

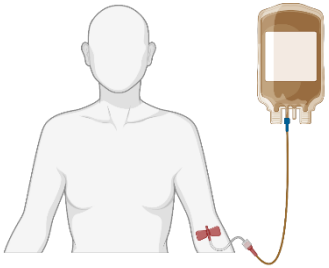


5. Selection of fungal specific T-cells

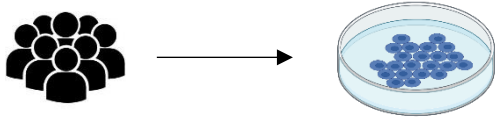
Through cytokine stimulus



6. *Ex-vivo* expansion

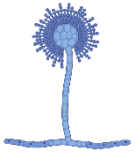


7. Patient infusion



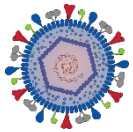
91 haplo-HSCT recipients

Aspergillus and CMV specific T-cells generation



23 invasive aspergillosis

- 10 adoptive therapy + L-AMB
- 13 L-AMB (controls)



68 CMV reactivation

- 27 adoptive therapy + GCV
- 41 GCV (controls)

Perruccio et al. *Blood.* 2015; 15;106:4397-406

Controls

Table 2. Invasive aspergillosis in 13 patients who did not receive *Aspergillus*-specific T-cell therapy

Patient no.	Invasive aspergillosis	Galactomannan antigenemia at diagnosis, ng/mL*	Galactomannan antigenemia under amphotericin therapy,† ng/mL	Outcome (time after diagnosis, wk)
1	P: mult/bilat	4.5 ± 0.7	3.1 ± 0.3	X Death (5)
2	P: monolat	3.5 ± 0.6	3.6 ± 0.6	X Death (7)
3	P: mult/bilat	1.9 ± 0.1	1.4 ± 0.1	X Death (4)
4	P: mult/bilat + pl eff	4.1 ± 0.2	3.3 ± 0.5	Resolution (17)
5	P: mult/bilat	3.1 ± 0.1	1.5 ± 0.06	Resolution (4)
6	P: mult/bilat	1.8 ± 0.4	2.6 ± 0.6	Resolution (4)
7	P: mult/bilat	4.7 ± 0.4	2.7 ± 1.3	X Death (5)
8	P: mult/bilat	2.8 ± 0.3	2.3 ± 0.8	X Death (4)
9	P: monolat	2.9 ± 0.3	3.0 ± 0.15	Resolution (4)
10	P: monolat + pl eff	3.9 ± 0.3	3.3 ± 0.7	Resolution (5)
11	P: monolat + pl eff	1.8 ± 0.4	2.9 ± 0.5	X Death (4)
12	P: mult/bilat	2.9 ± 0.2	3.3 ± 0.6	Resolution (5)
13	P: monolat + pl eff	3.2 ± 0.2	2.8 ± 0.3	Resolution (6)

ACT

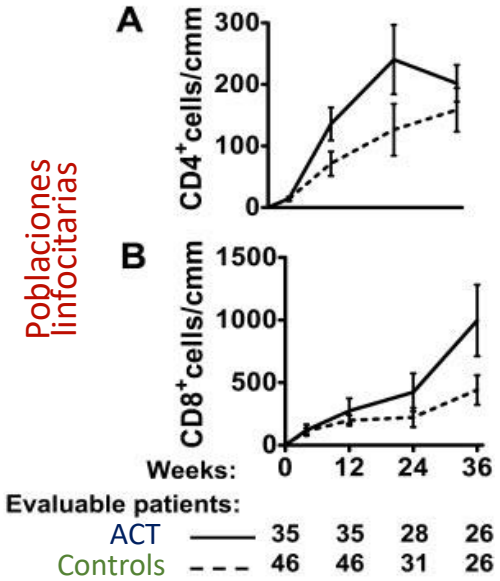
Table 3. *Aspergillus*-specific immune reconstitution and outcome of invasive aspergillosis in 10 patients who received *Aspergillus*-specific T-cell therapy

Patient no.	Invasive aspergillosis before cell therapy	Galactomannan antigenemia before T-cell therapy,* ng/mL	Cell therapy, day after transplantation	*Aspergillus*-specific $CD4^+$ reconstitution, day after cell therapy	Galactomannan antigenemia after cell therapy,† ng/mL	Outcome (time after diagnosis, wk)
1	P: mult/bilat + pl eff + CNS	3.4 ± 0.6	37	21	0.8 ± 0.1	X Death (4)
2	P: mult/bilat + sin	4.2 ± 0.4	23	21	0.3 ± 0.1	Resolution (4)
3	P: mult/bilat + pl eff	1.6 ± 0.5	32	21	0.4 ± 0.1	Resolution (5)
4	P: mult/bilat + pl eff	2.0 ± 0.6	32	21	0.2 ± 0.0	Resolution (12)
5	P: monolat + sin	1.9 ± 0.7	25	21	0.4 ± 0.1	Resolution (14)
6	P: mult/bilat	2.8 ± 0.3	17	21	0.6 ± 0.5	Resolution (5)
7	P: mult bilat + pl eff + sin	2.1 ± 0.5	17	21	0.8 ± 0.1	Resolution (10)
8	P: mult/bilat	2.2 ± 0.1	21	21	0.7 ± 0.2	Resolution (6)
9	P: monolat	3.1 ± 0.2	21	21	0.6 ± 0.2	Resolution (7)
10	P: mult/bilat	3.1 ± 0.3	21	21	0.4 ± 0.3	Resolution (8)

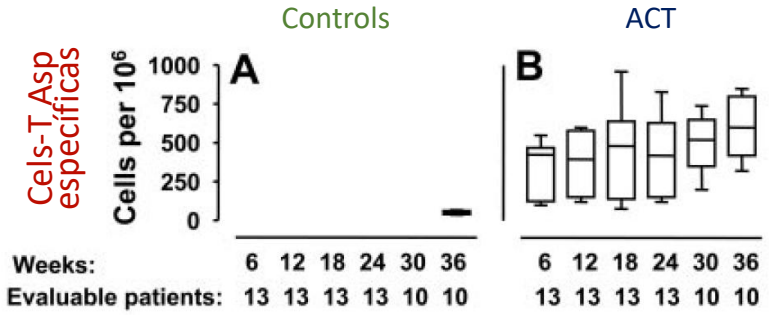
- Mortalidad: 46% vs 10%
- Negativización más rápida del AGA
- ↑INF-γ y ↓IL-10

Perruccio et al. *Blood.* 2015; 15;106:4397-406

Poblaciones linfocitarias



Cels-T Asp específicas



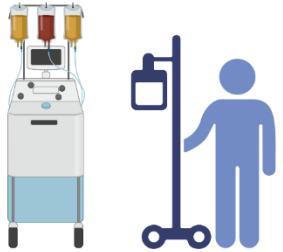
- La recuperación de las células T fue clara y duradera (*in-vivo* expansión)
- Inmunidad T específica

Se han desarrollado nuevos métodos para la obtención más rápida de cels T específicas

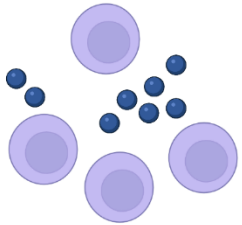
Proceso costoso, relativamente largo, y HLA dependiente

Perruccio et al. *Blood.* 2015; 15;106:4397-406

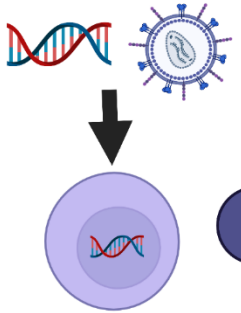
CHIMERIC ANTIGEN RECEPTOR THERPAY



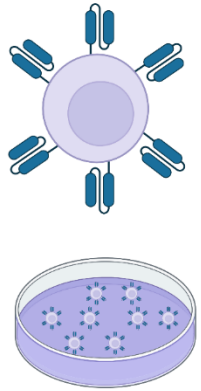
1 T-cells are collected by leukapheresis from the patient's blood



2 T-cells are enriched and activated

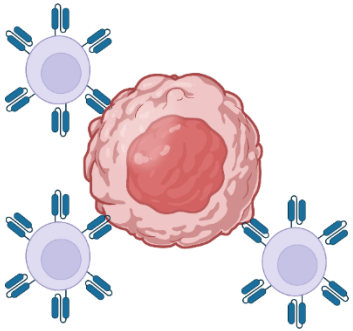


3 A gene containing the new chimeric antigen receptor is inserted in T cells via lentivirus transduction



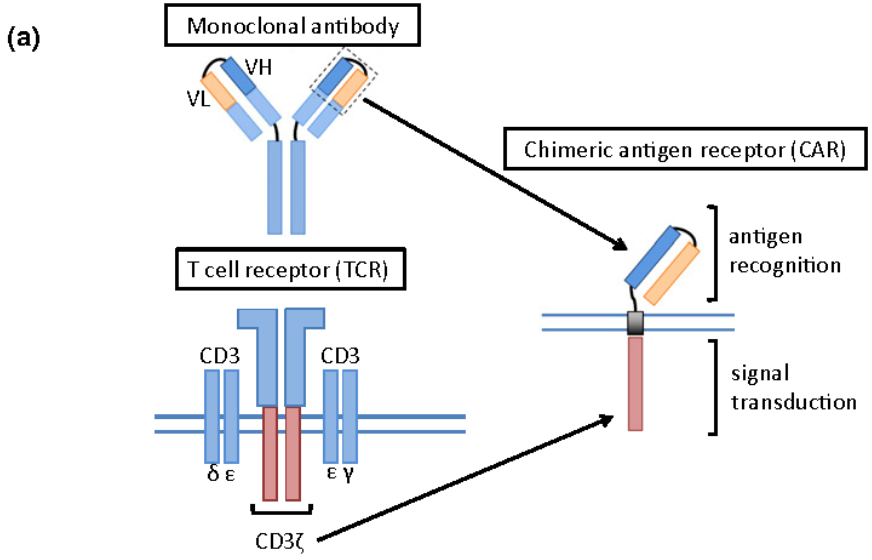
4 The resultant CAR-expressing T cells are expanded through culture

5 The final product is infused intravenously



6 CAR-T cells attack cancer cells

Puerta-Alcalde et al. Infectious Complications in Biologic and Targeted Therapies. 1st Ed. 2022

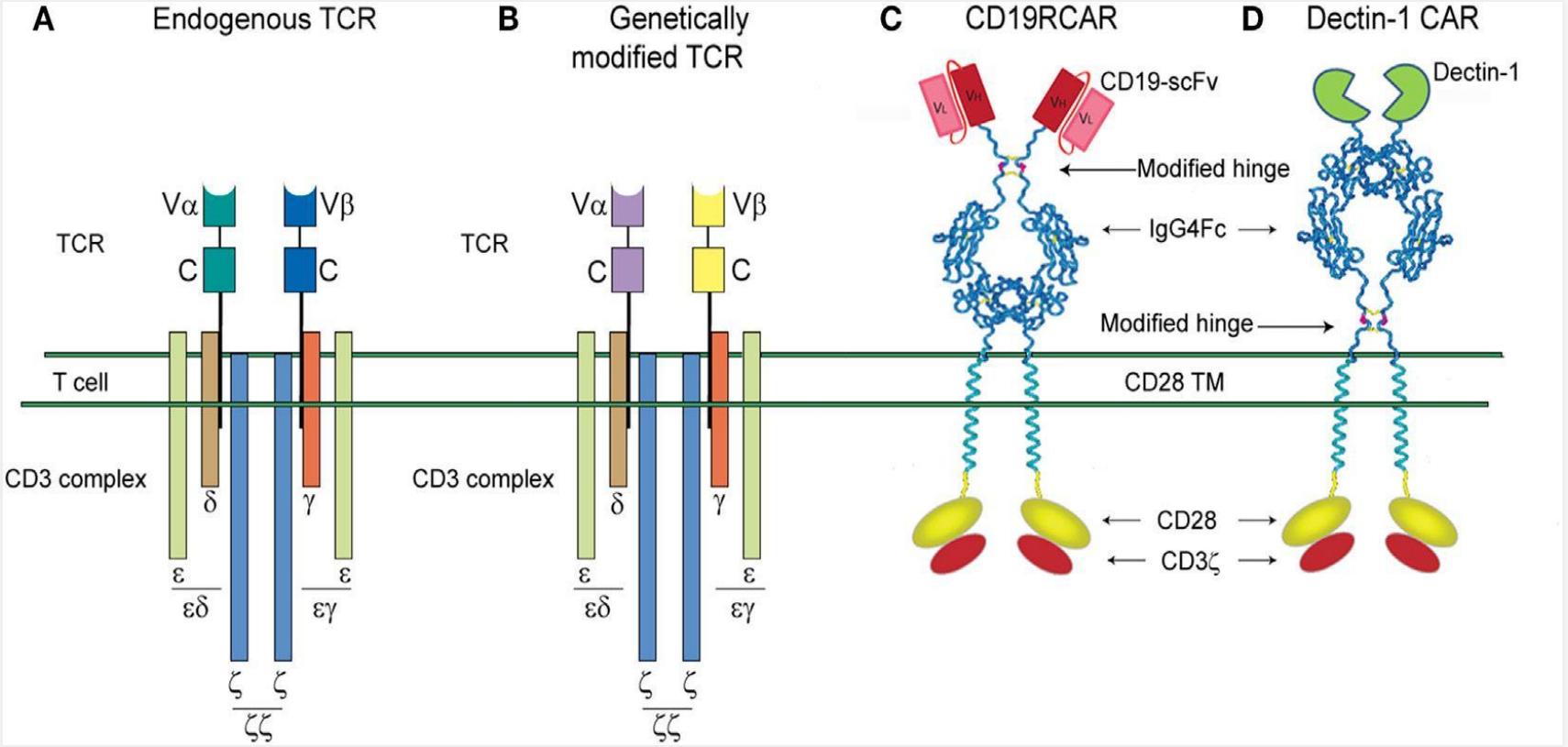


No restringido por el HLA

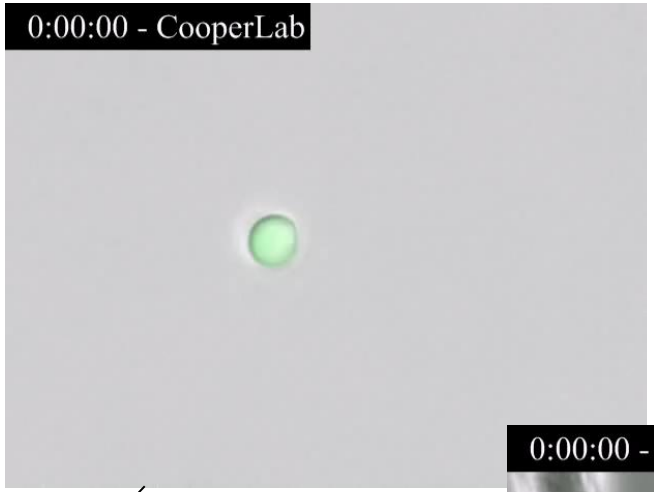
Las células diana tienen poca capacidad de inmunoedición para evadir la detección

El CAR se puede dirigir contra múltiples antígenos

Makita et al. *Cancer Sci.* 2017; 108:1109-18

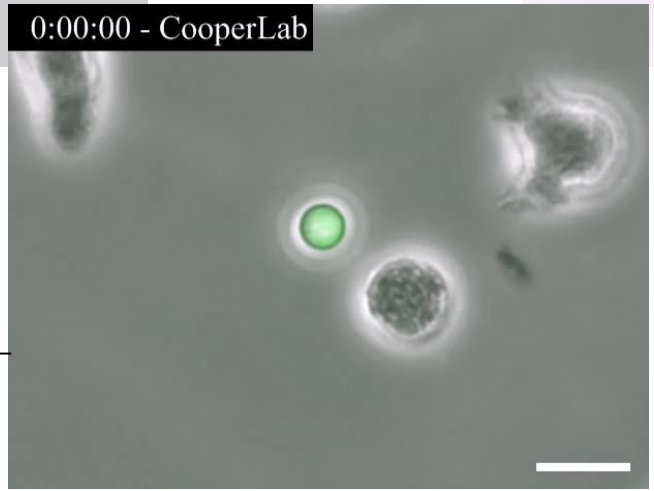


Kumaresan et al. *Front Immunol*. 2018; 8:1939



Controls

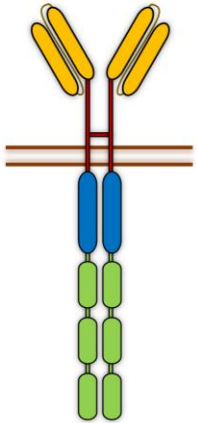
CD19 CAR-T



D-CAR

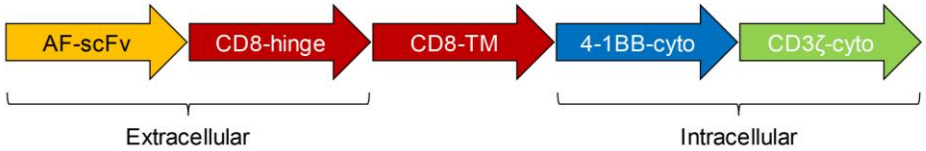
Kumaresan et al. *Front Immunol*. 2018; 8:1939

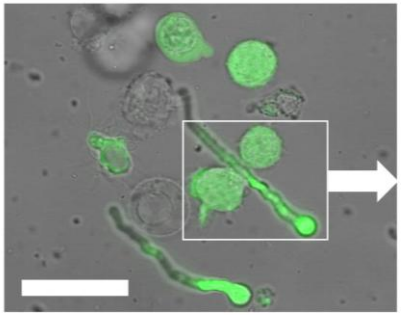
mAb AF-269-5: IgM-isotype antibody recognizing *Aspergillus* hyphae

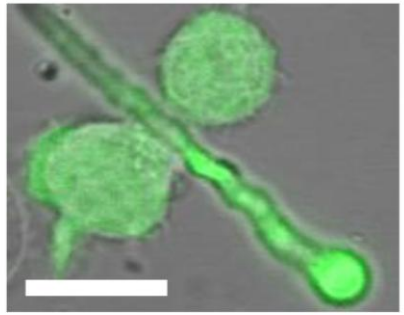


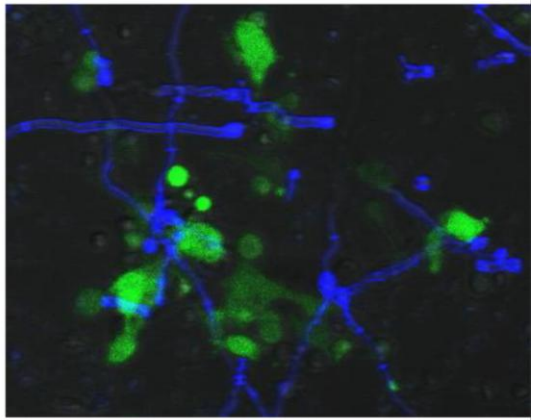
Receptor: AF-scFv
Hinge: CD8
Transmembrane domain: CD8
Co-stimulatory domain: 4-1BB
Activation domain: CD3ζ

Extracellular
Intracellular

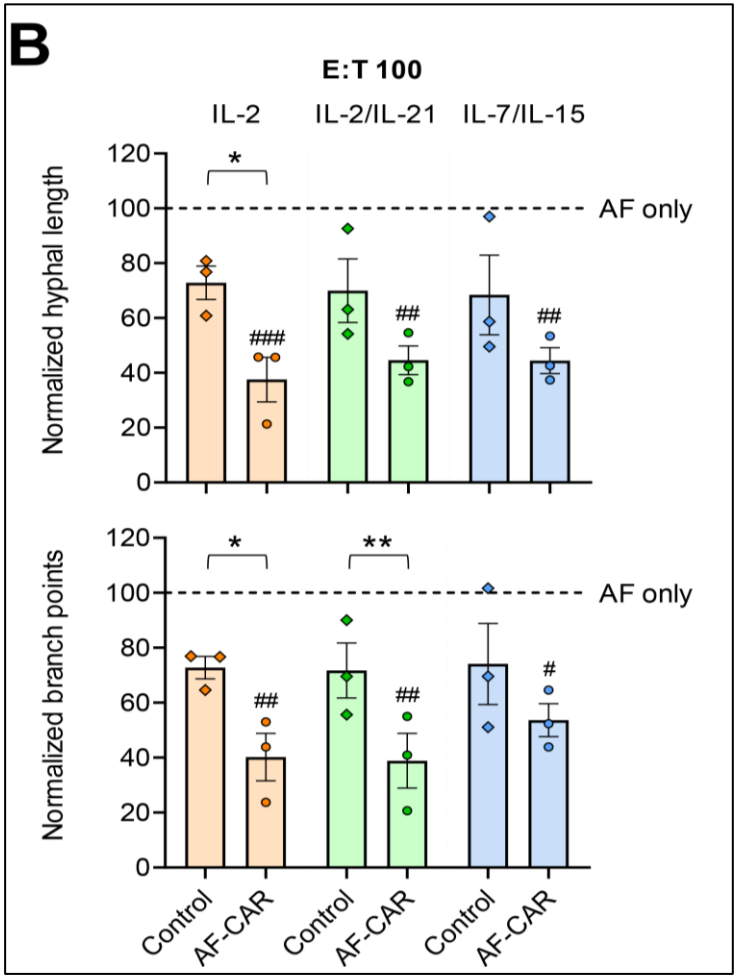


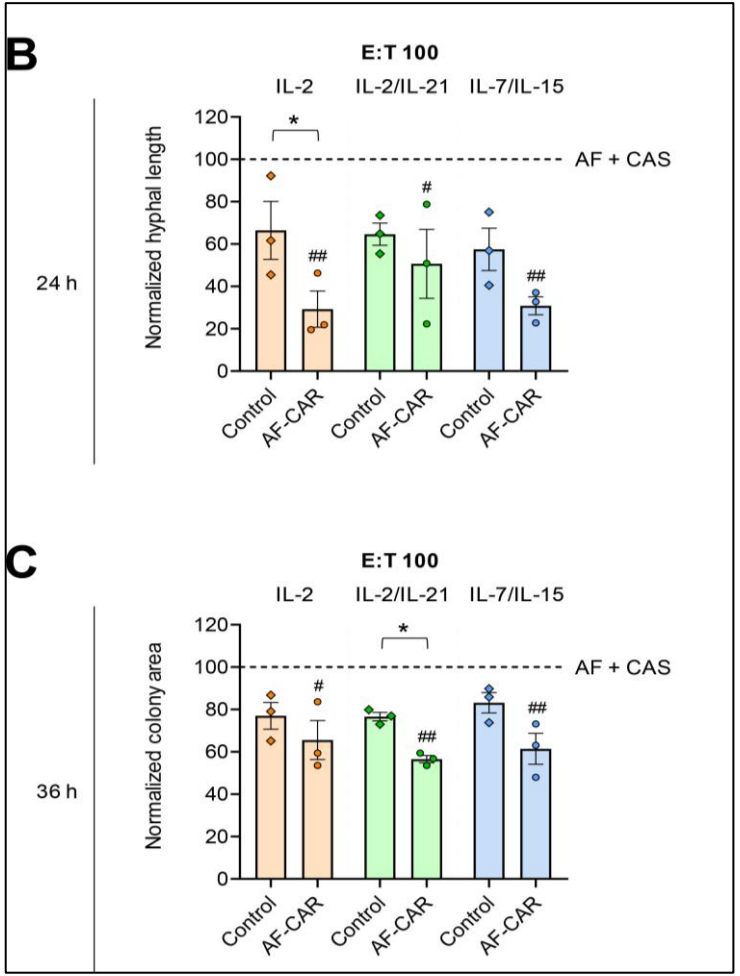




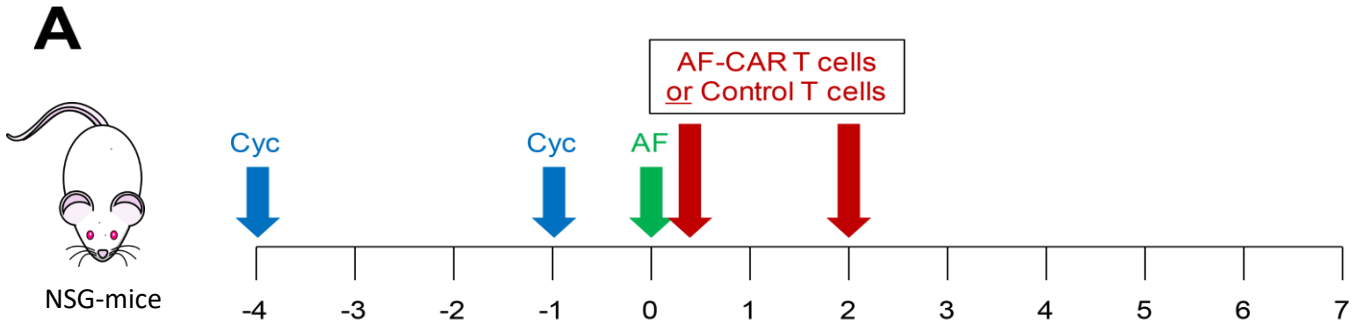


Kumaresan et al. *mBio*. 2024;15(4):e0341323.





Kumaresan et al. *mBio*. 2024;15(4):e0341323.

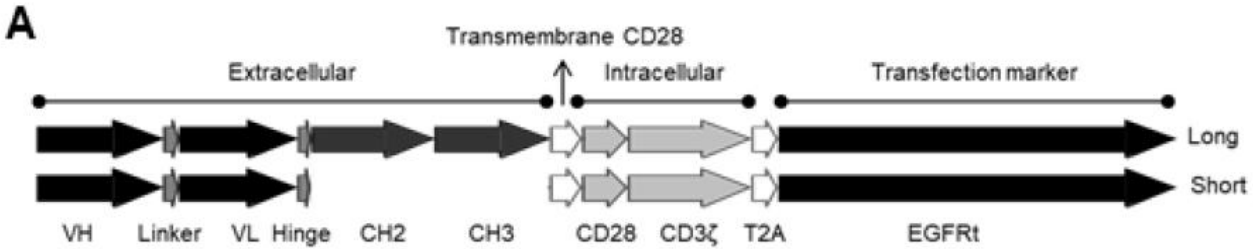


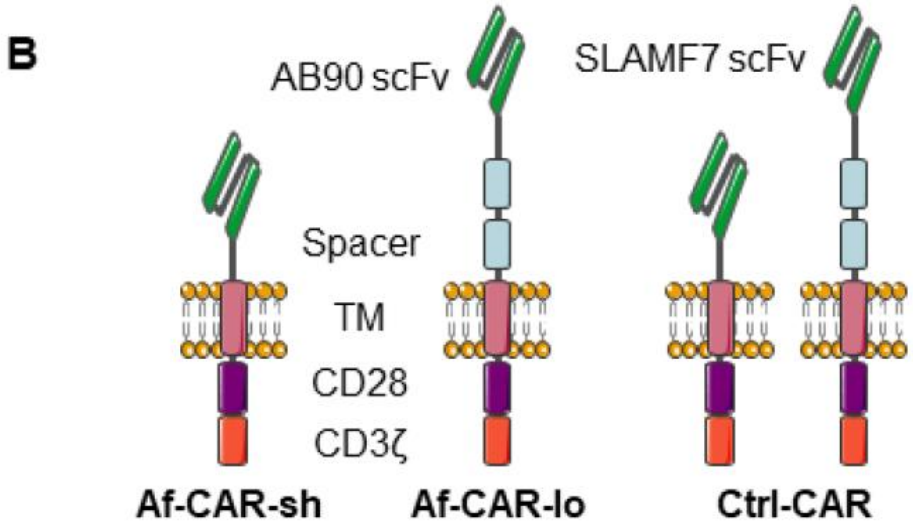
Mortality:
11/12 vs 6/12

↓

5/6 stable disease

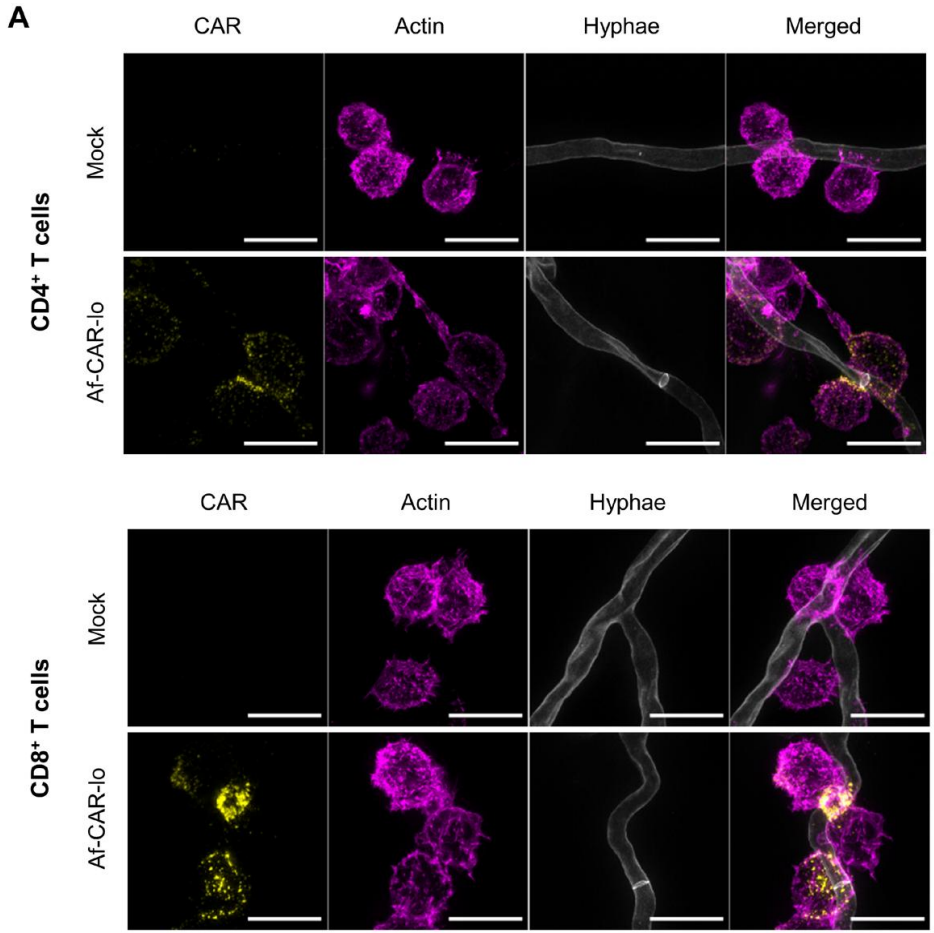
Kumaresan et al. *mBio*. 2024; 15(4):e0341323.

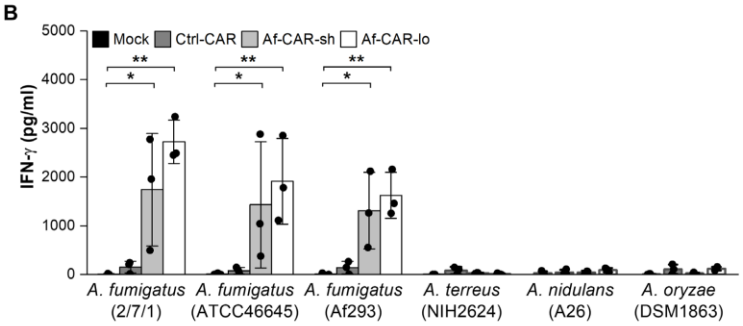


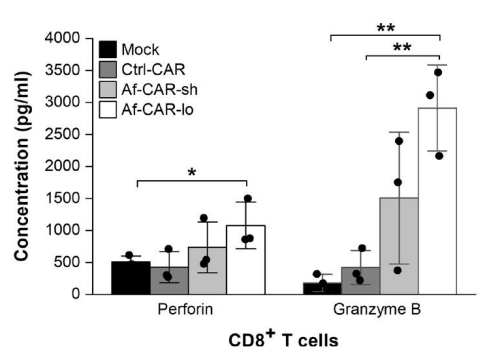


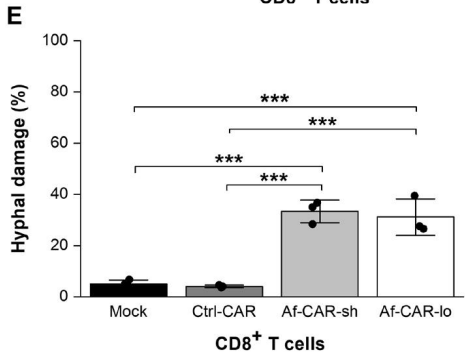
mAb AB90-E8 specifically targets *A. fumigatus* strains, with no reactivity for other *Aspergillus* or fungal species

Seif et al. *Sci Transl Med.* 2022.28;14(664):eabh1209









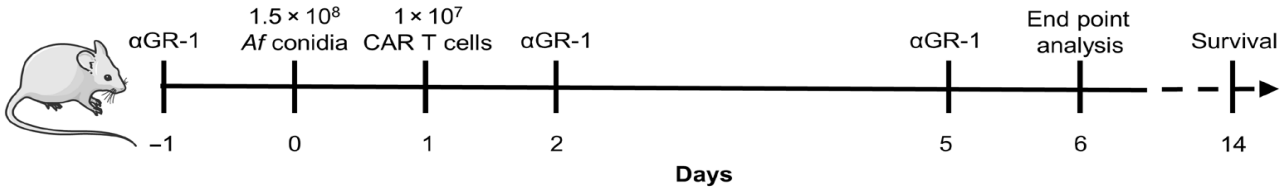
9h-assay

Seif et al. *Sci Transl Med.* 2022.28;14(664):eabh1209

Af-CAR AB90-E8

Neutropenic mouse model

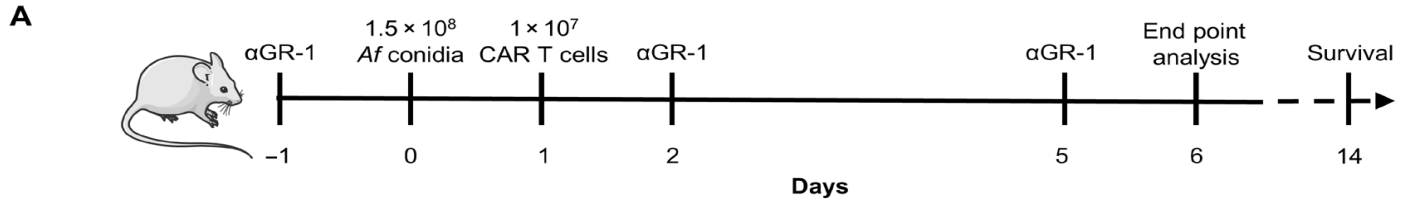
A

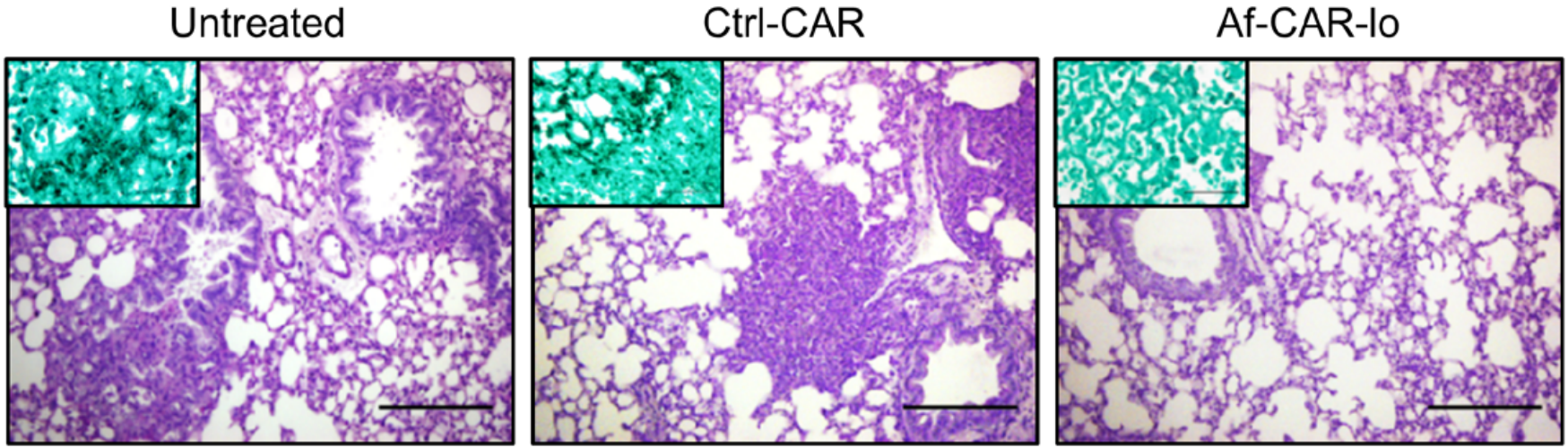


Increased survival
retained
ly weight

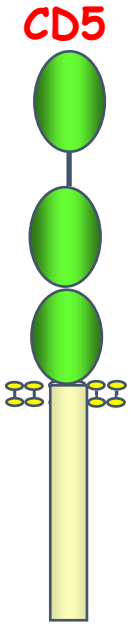
Seif et al. *Sci Transl Med.* 2022.28;14(664):eabh1209

Neutropenic mouse model





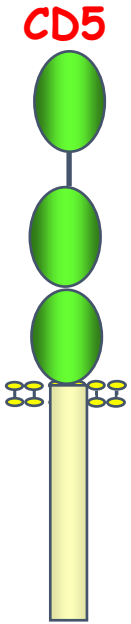
Seif et al. *Sci Transl Med.* 2022.28;14(664):eabh1209



PRR tipo Scavenger Receptor

Expresado en Linfos-T, Treg, algunos Linfos-B, y células dendríticas

Uno de sus principales ligandos es el β-glucano



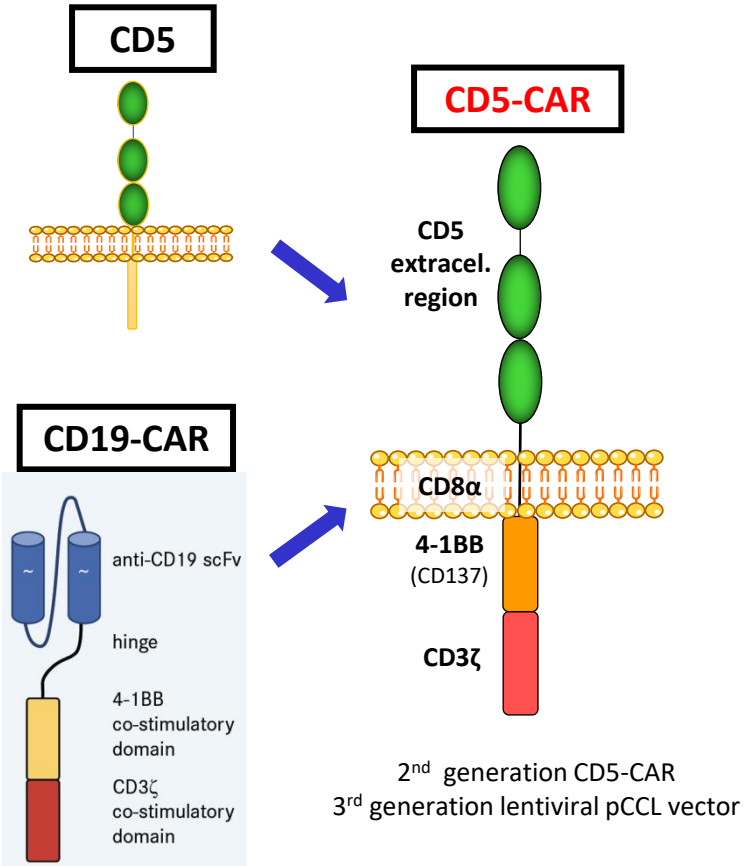
i) La forma soluble de CD5 humano (**shCD5**) aglutina especies fúngicas a través del **reconocimiento del β-glucano**

ii) La **Kd** de interacción shCD5-β-glucano es similar a la reportada para Dectina-1-β-glucano

iii) La unión del CD5 a la membrana interactúa con el β-glucano e induce la **señalización intracelular**, y la liberación de **citoquinas**

iv) Los ratones **deficientes en CD5** son más susceptibles a las **IFIs**

v) La infusión de shCD5 **reduce la mortalidad** de los ratones sometidos a la inducción de zymosan, así como de *C. albicans* y *C. neoformans*



2nd generation CD5-CAR
3rd generation lentiviral pCCL vector

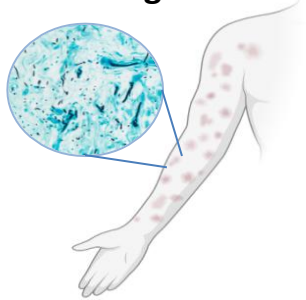
¿Y por qué NKs?

1. No presentan aloreactividad (no EICR)
2. Gran capacidad citotóxica (perforinas, gramzimas), apoptosis
3. Opsonización y liberación de citoquinas: INF-γ, TNF-α, GM-CSF…
4. Eficaz en modelos animales
5. Capacidad de memoria inmunológica

FIRST-IN-HUMAN USE

Acute myeloid leukemia (AML)

Disseminated fungal infection

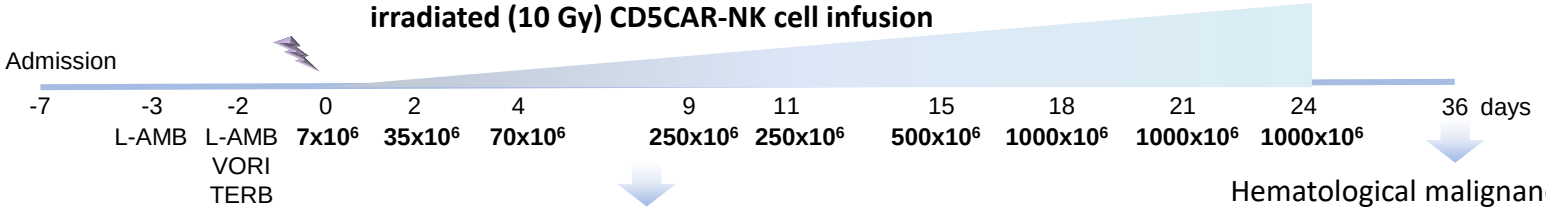


- **Multidrug-resistant *Fusarium petroliphilum*** unresponsive to antifungal treatment (liposomal amphotericin B + voriconazole + terbinafine)

Compassionate use is approved for CD5CAR-NK92 cells treatment:
First-time-in-human use

AEMPS

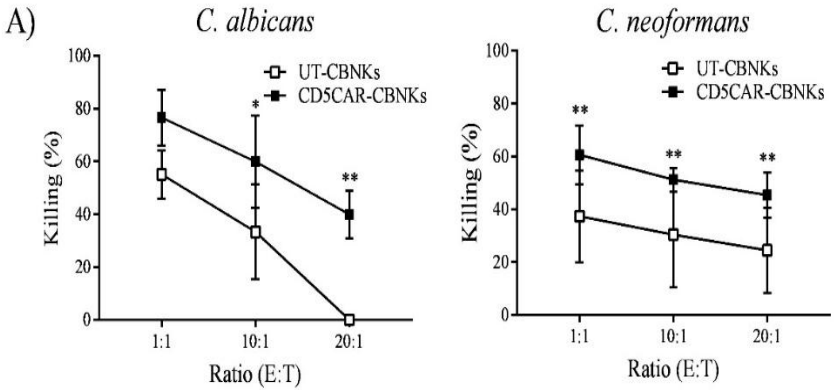
irradiated (10 Gy) CD5CAR-NK cell infusion

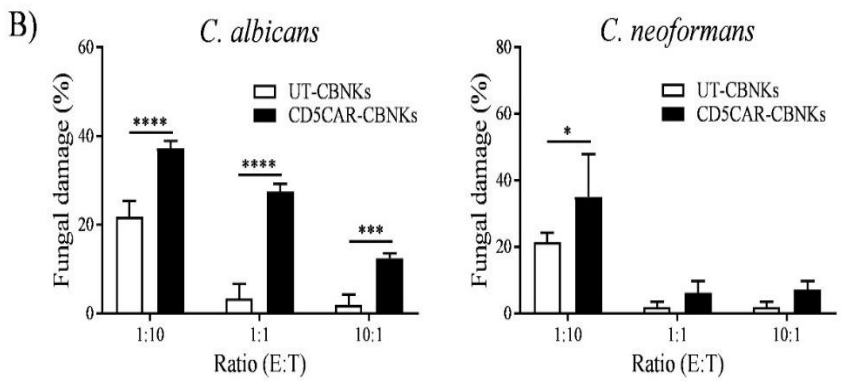


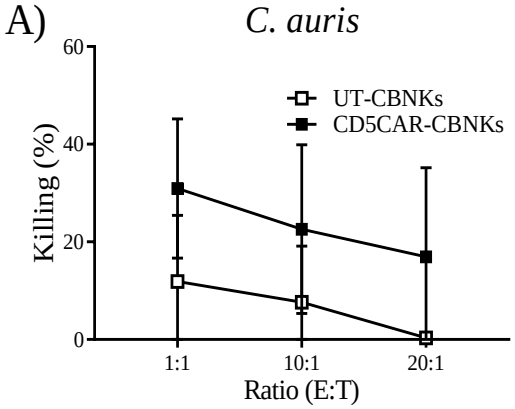
Hematological malignancy progresses
Patient's comfort is prioritized and finally died

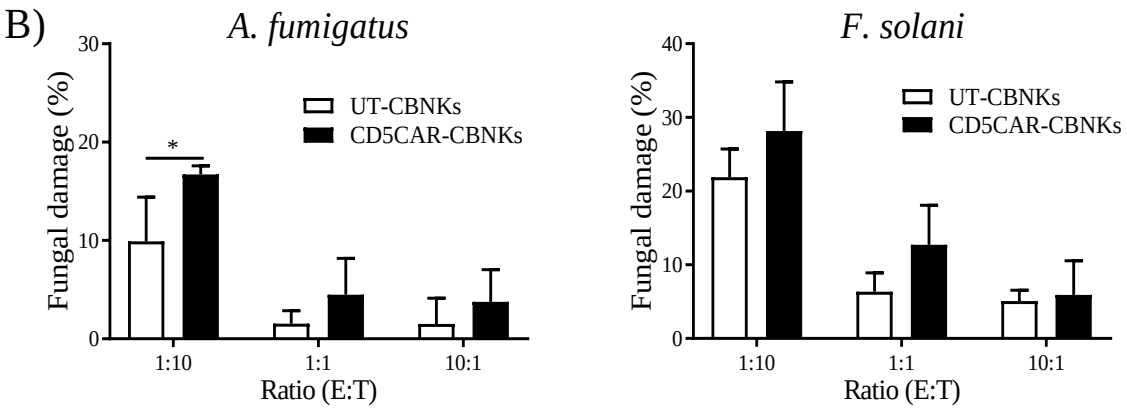
Clinical efficacy assessment:

- Infusions well tolerated; no cytokine release syndrome
- Persistent fever and cough
- Chest CT scan showed a dissociated evolution
- **No appearance of new skin lesions and improvement of some of them**

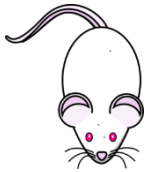






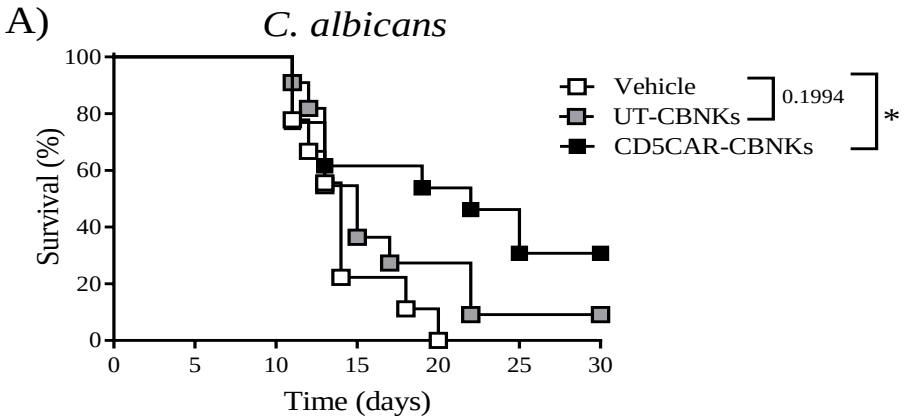


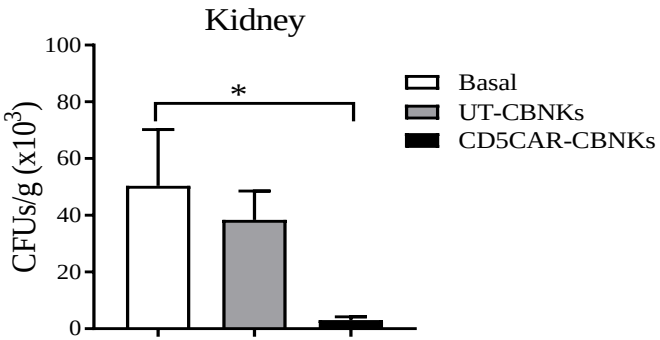
Velasco de-Andres et al. *Under review*



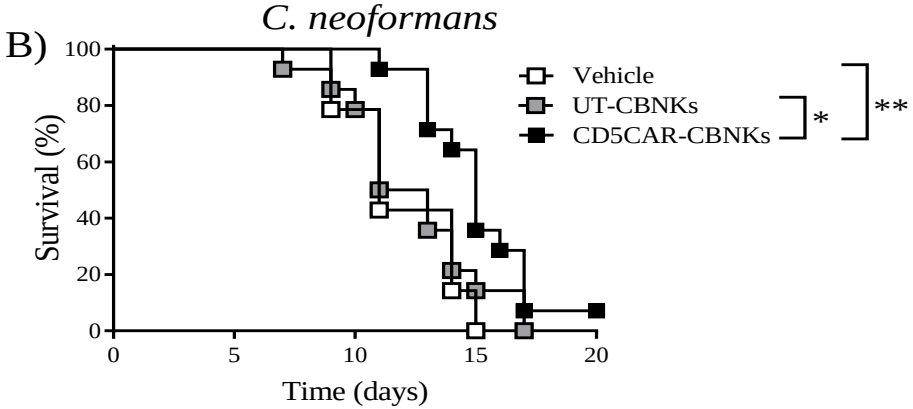
NSG-mice

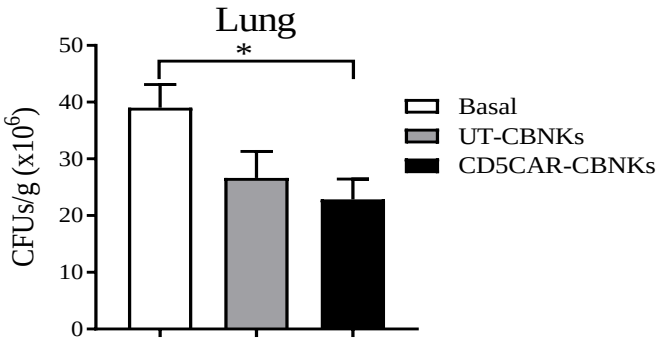
A)





B)





CD5-CAR was associated with reduced fungal burden, increased survival of mice, and increased markers of cytotoxicity and INF-γ

Velasco de-Andres et al.
Under review

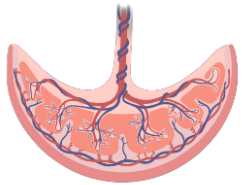
PICI

PROYECTO INVESTIGACIÓN CLÍNICA INDEPENDIENTE

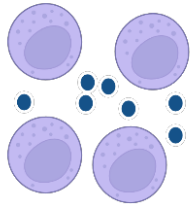
Células CD5CAR-NK de producción propia para el tratamiento de la enfermedad fúngica invasiva refractaria: ensayo clínico de fase I.

CD5CAR-NK PRODUCTION

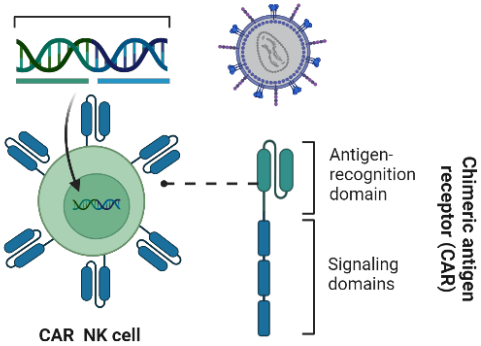
PRODUCCIÓN TERAPIA CD5CAR-NK



1 NK-cells are obtained from donor cord blood

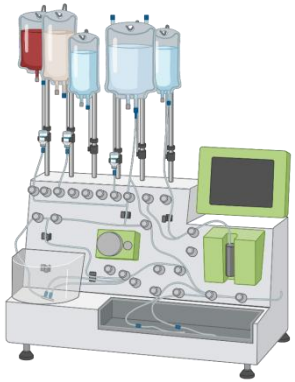


2 NK-cells are enriched and activated



3 A gene containing the CD5-CAR is inserted into the cell via lentivirus transduction

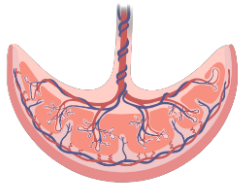
CliniMACS Prodigy. Milteny Biotec.



4 GMP expansion of NK-cells and CD5-CAR transduction

CD5CAR-NK PRODUCTION

PRODUCCIÓN TERAPIA CD5CAR-NK



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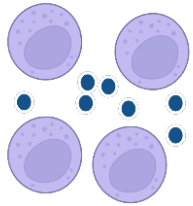
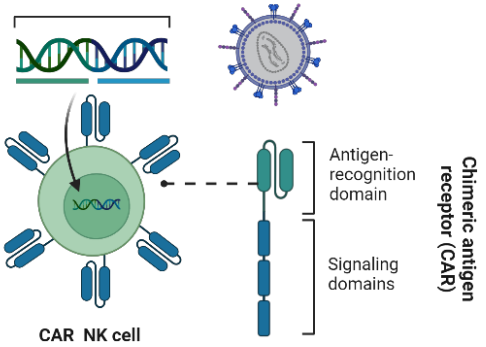


2 NK-cells are enriched and activated



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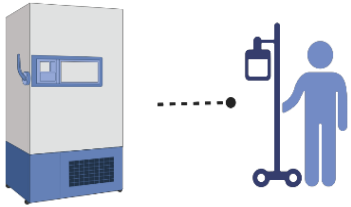
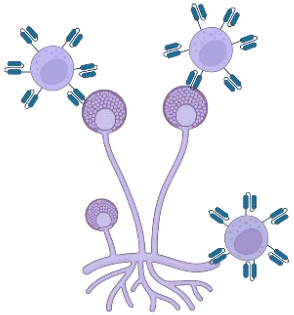
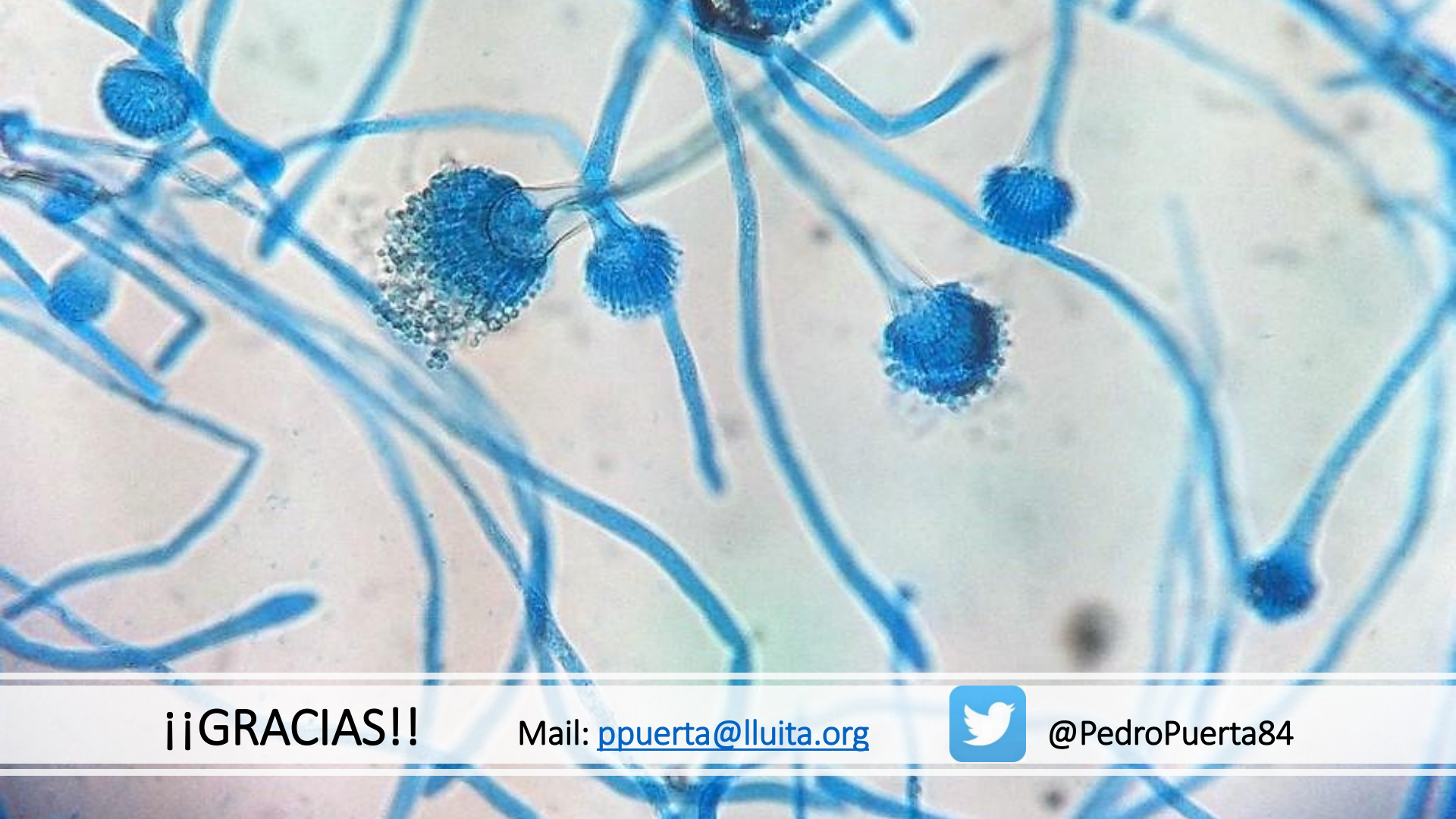
4 GMP expansion of NK-cells and CD5-CAR transduction



5 CAR cells are frozen and thawed when the fungal infection is diagnosed: *off-the-shelf therapy*



6 CAR-NK cells attack fungal cells



¡¡GRACIAS!!

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@PedroPuerta84