

# Place des CAR/CAAR-T cells

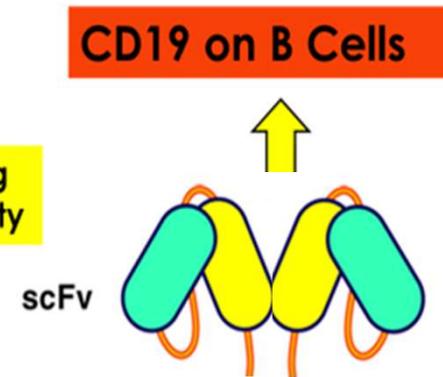
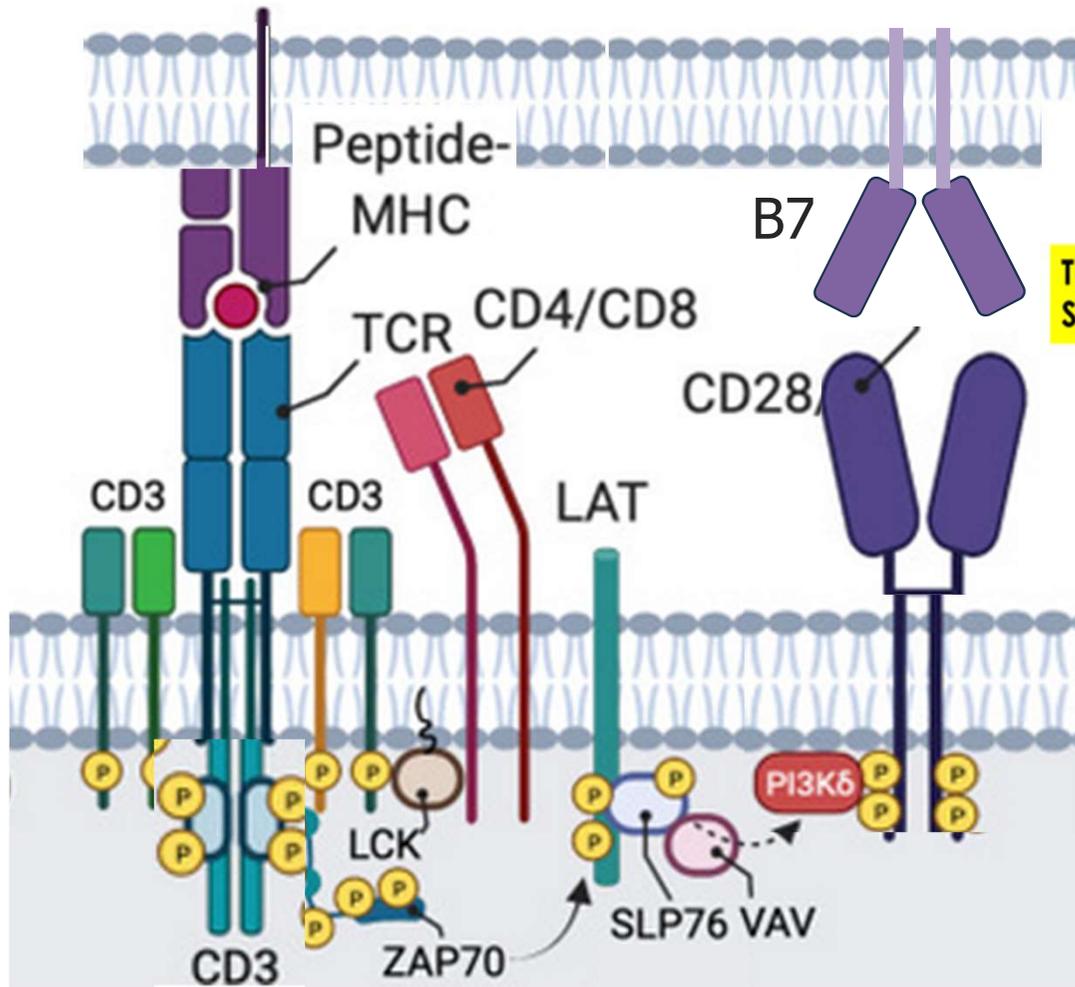
## Applications cliniques

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Hôpital Cochin, AP-HP.CUP, Paris



# T Cell vs. Chimeric Antigen Receptors

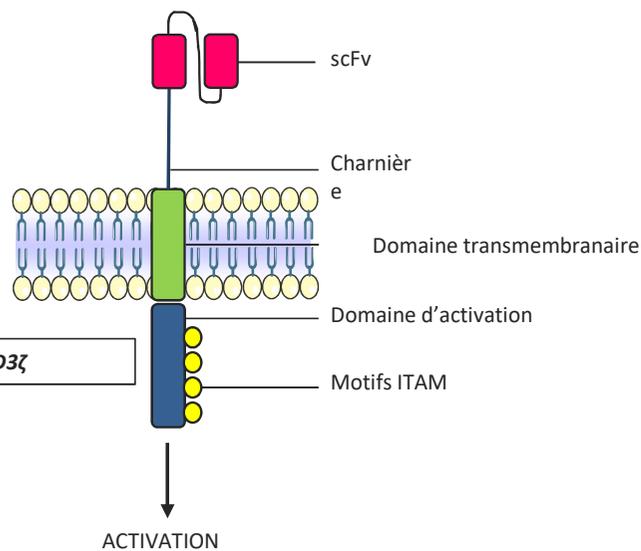


- Direct (MHC-independent) Ag Binding
- Combined Signals 1+2
- Extended Efficacy (effector and memory)

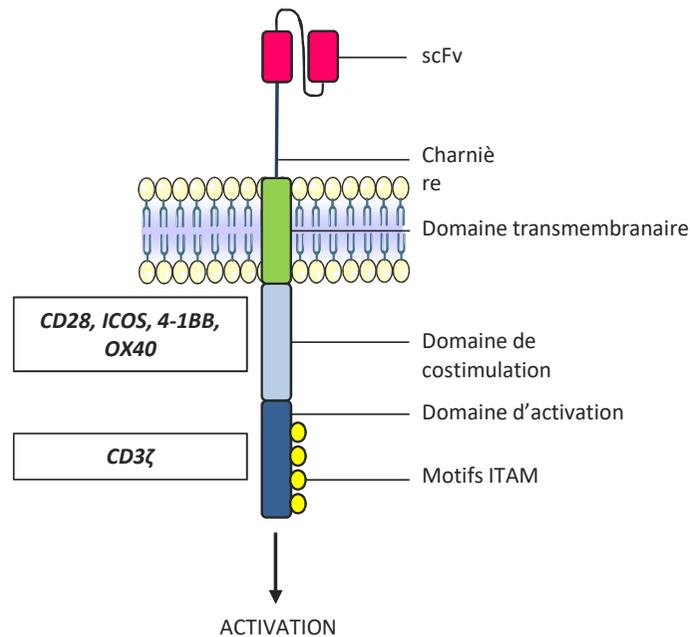
# Cellules CAR-T: nouvelle technologie de thérapie génique et cellulaire

- Modifications du domaine intracellulaire définissant plusieurs générations de cellules CAR-T

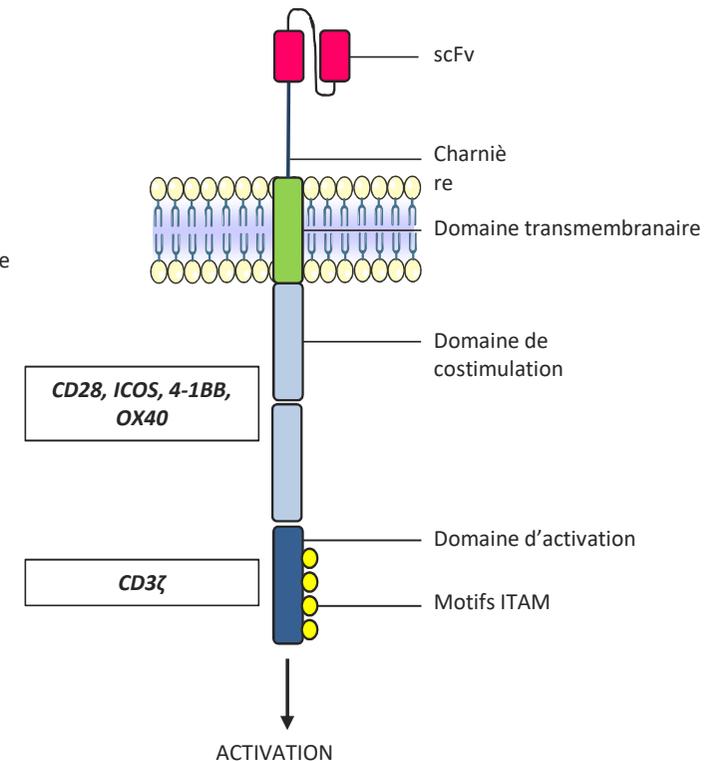
## 1<sup>ère</sup> génération



## 2<sup>nde</sup> génération



## 3<sup>ème</sup> génération



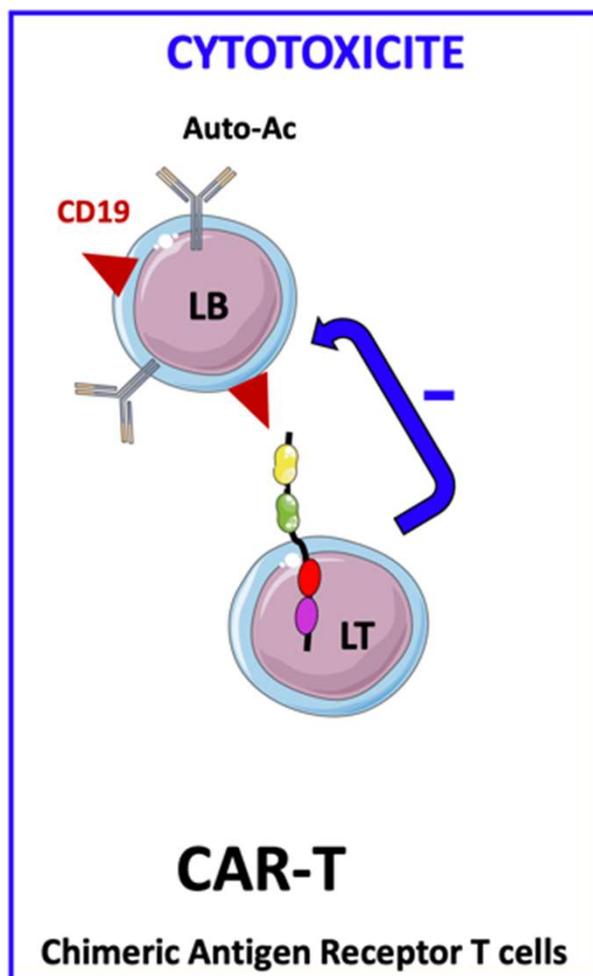
*Adapté de Orvain et al, Arthritis Rheumatol 2021*

# Cellules CAR-T autorisées par la FDA

Médicament	Compagnie	Antigène cible	Domaines de Costimulation Activation	Indication	Date d'approbation
Tisagenlecleucel (Kymriah)	Novartis Pharmaceuticals	CD19	4-1BB CD3 $\zeta$	Adultes avec une leucémie aiguë lymphoblastique	30 août 2017
Axicabtagene ciloleucel (Yescarta)	Gilead	CD19	CD28 CD3 $\zeta$	Adultes avec un lymphome B à grandes cellules réfractaire ou en rechute	18 octobre 2017
Brexucabtagene autoleucel (Tecartus)	Gilead	CD19	CD28 CD3 $\zeta$	Adultes avec un lymphome du manteau réfractaire ou en rechute	24 juin 2020
Lisocabtagene maraleucel (Breyanzi)	Bristol Myers Squibb	CD19	4-1BB CD3 $\zeta$	Adultes avec un lymphome B à grandes cellules réfractaire ou en rechute	5 février 2021

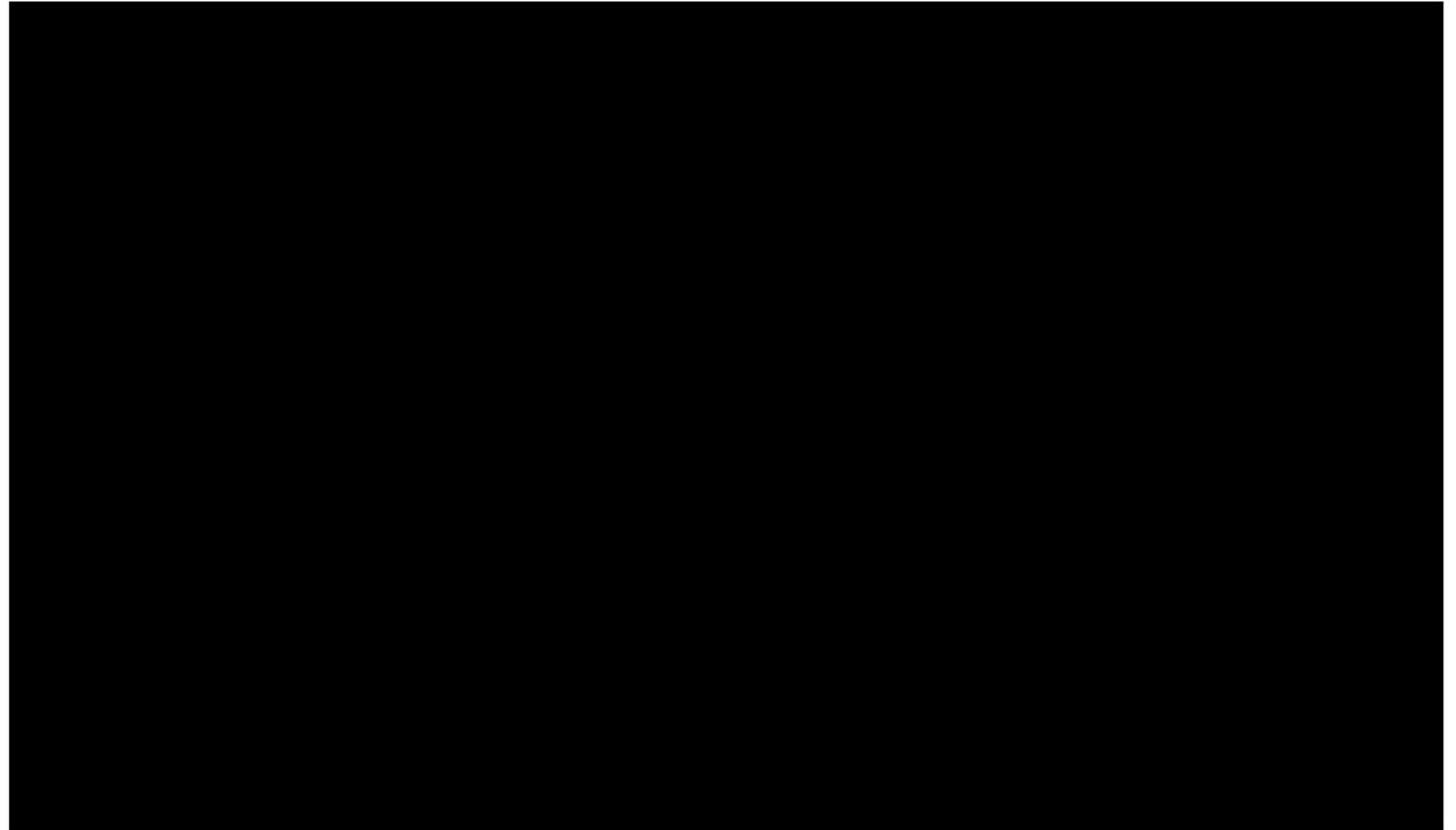
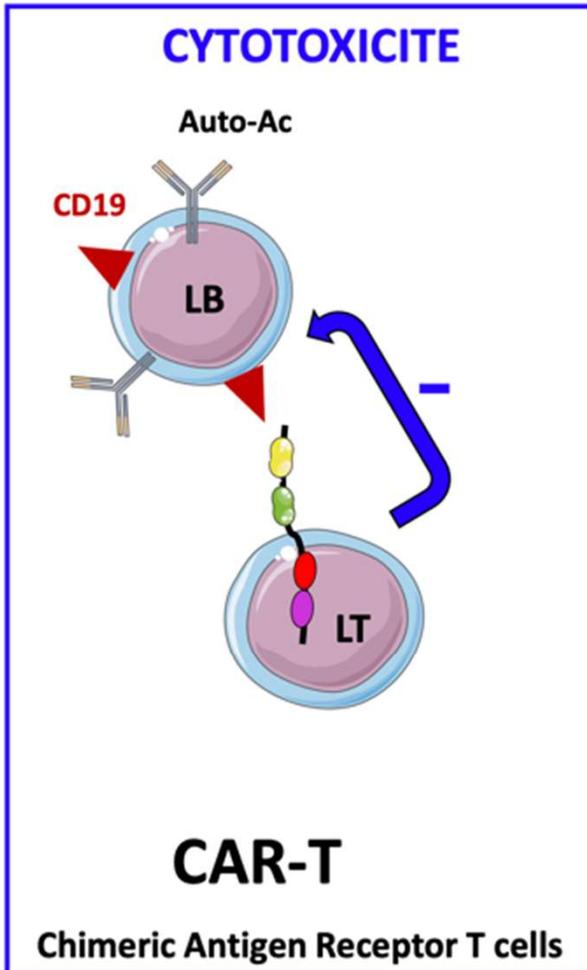
*Adapté de Orvain et al, Arthritis Rheumatol 2021*

## Cellule CAR-T utilisée en hématologie: CART-T anti- CD19



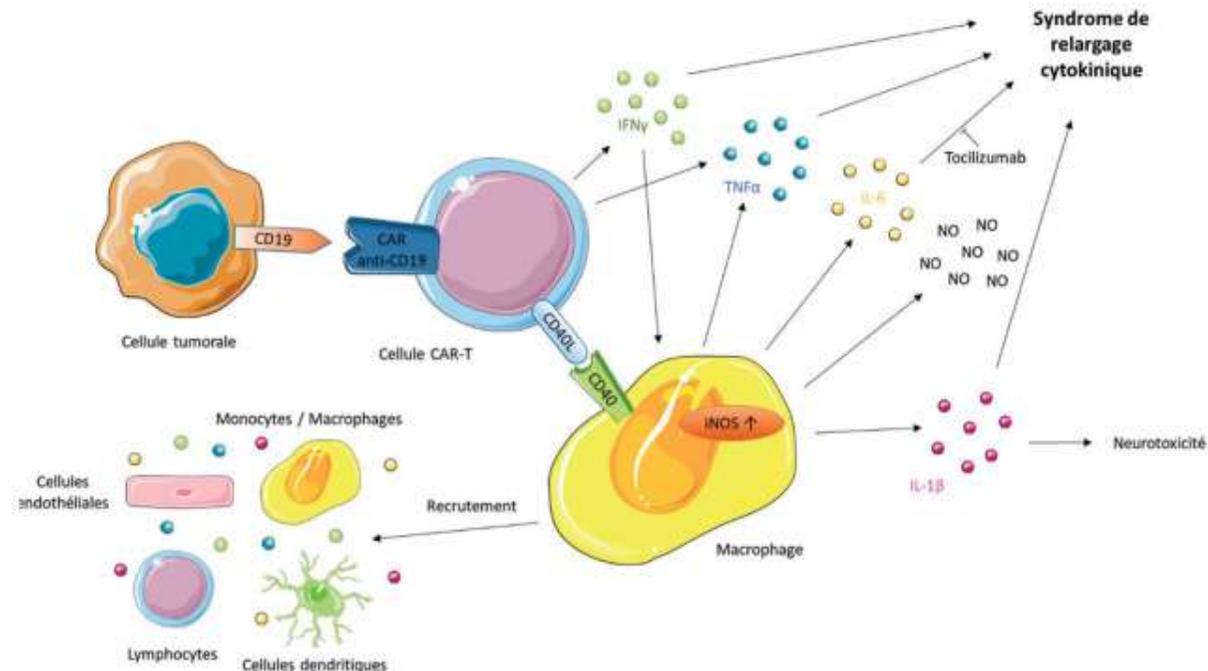
- **Traitement des hémopathies lymphoïde**
  - > Lymphome B à grandes cellules
  - > Leucémies aiguës lymphoblastiques
- **Effet lympho-déplétant plus large et profond que celui obtenu avec les Ac monoclonaux anti-CD20**

# How work CAR-T cells?

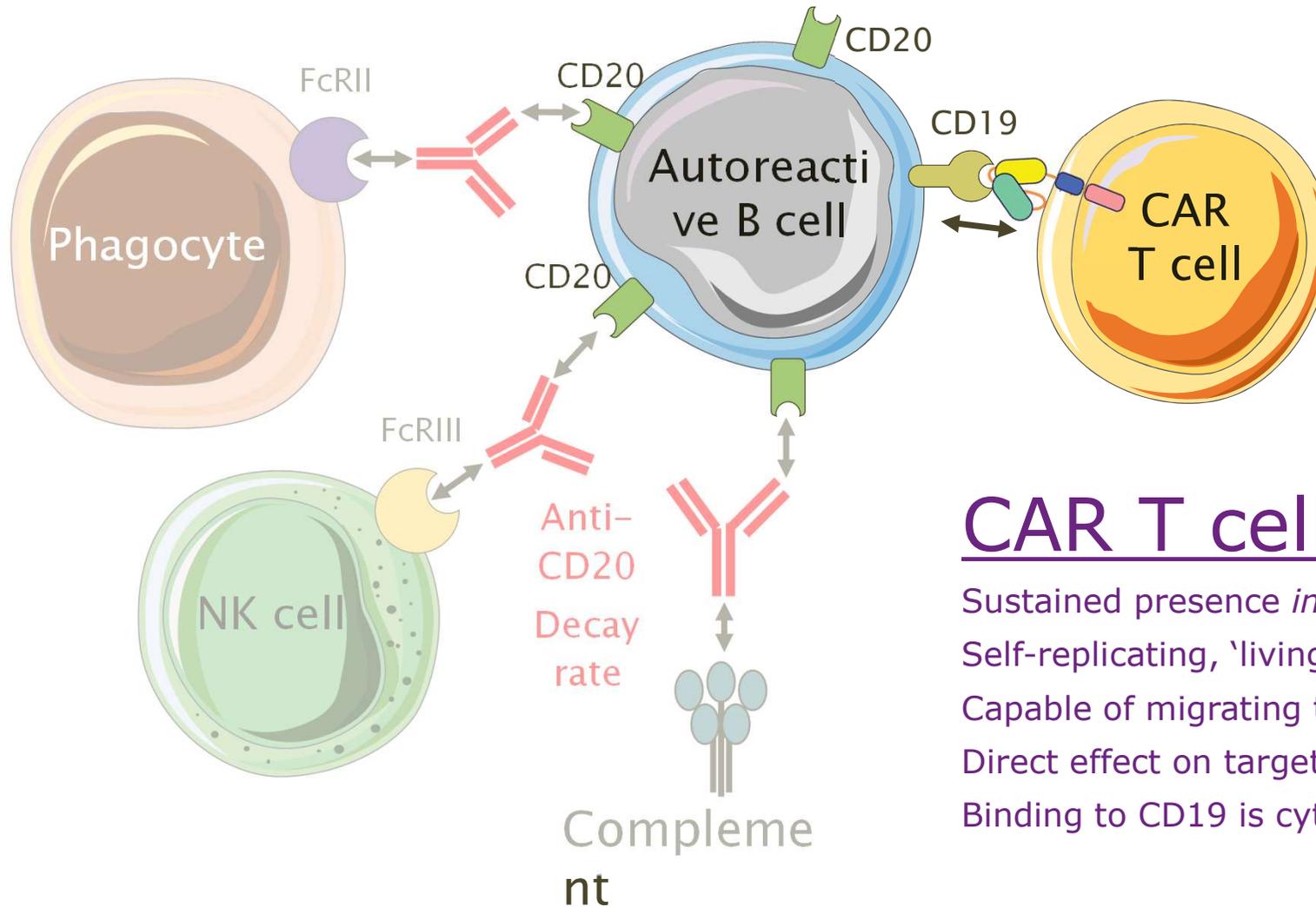


# Limites d'utilisation des cellules CAR-T anti-CD19

- **Syndrome de relargage cytokinique**
  - 54 à 91% des patients traités par des cellules CAR-T CD19
  - Fièvre, hypotension, insuffisance respiratoire pouvant conduire à une défaillance multi-viscérale
- **Neurotoxicité**



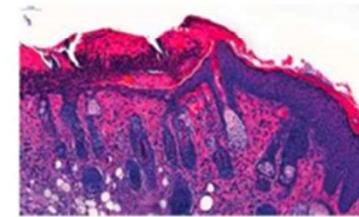
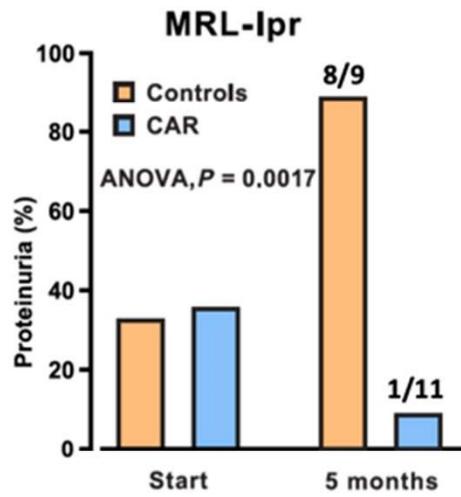
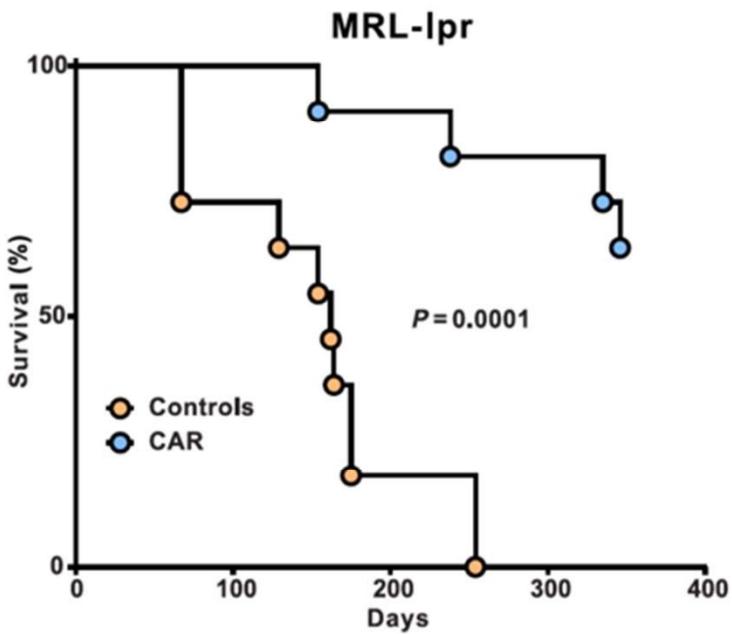
# Approaches to B cell Depletion



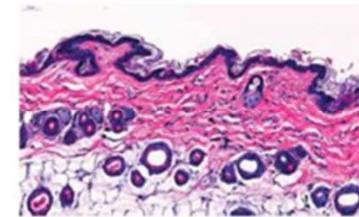
## CAR T cells:

- Sustained presence *in vivo*
- Self-replicating, 'living drug'
- Capable of migrating to tissues
- Direct effect on target B cells
- Binding to CD19 is cytotoxic

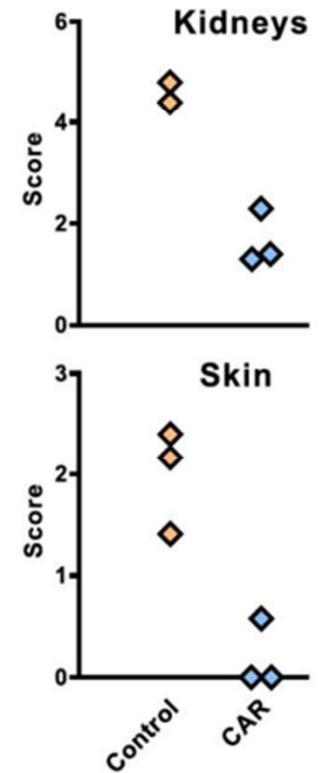
**Amélioration de la survie et du phénotype lupique des souris MRL-lpr traitées par CAR-T anti-CD19**



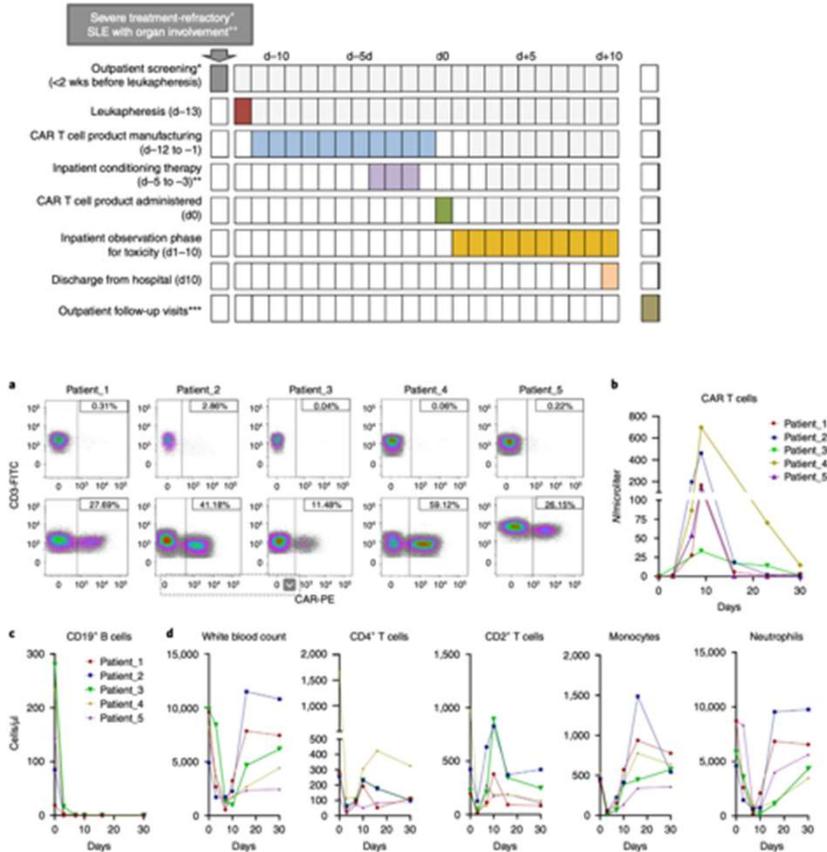
Control



CAR



# Premières expériences d'utilisation des CAR-T anti-CD19 dans le lupus



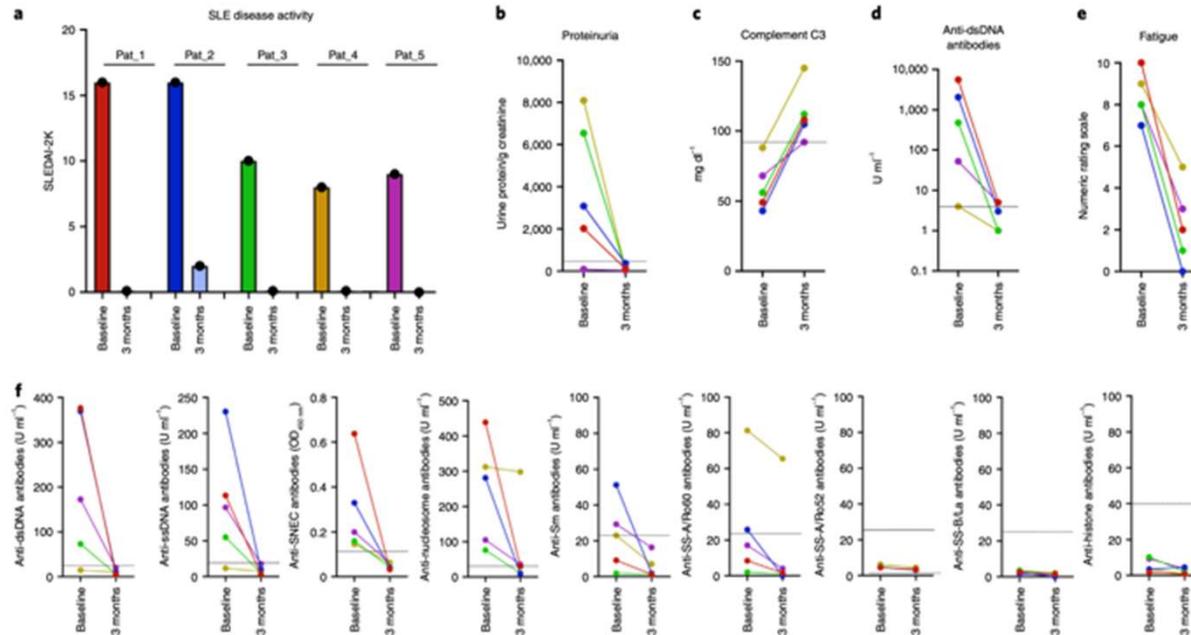
**Fig. 2 | CAR T cell expansion in vivo and depletion of B cells.** **a**, Dot plots showing CD3<sup>+</sup> T cells (y axis) and CAR T cells (x axis) at day +1 and day +9 after CAR T cell administration. CAR T cells expanded in all five patients with SLE from day 1 to day 9. **b**, Circulating CAR T cell numbers in the five patients with SLE within the first 30 d after treatment (N=5). **c**, Circulating B cell numbers in the five patients with SLE within the first 30 d after treatment (N=5). **d**, Numbers of circulating total white blood cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, monocytes and neutrophils in the five patients with SLE within the first 30 d after treatment (N=5).

**Table 1 | Patient characteristics at baseline**

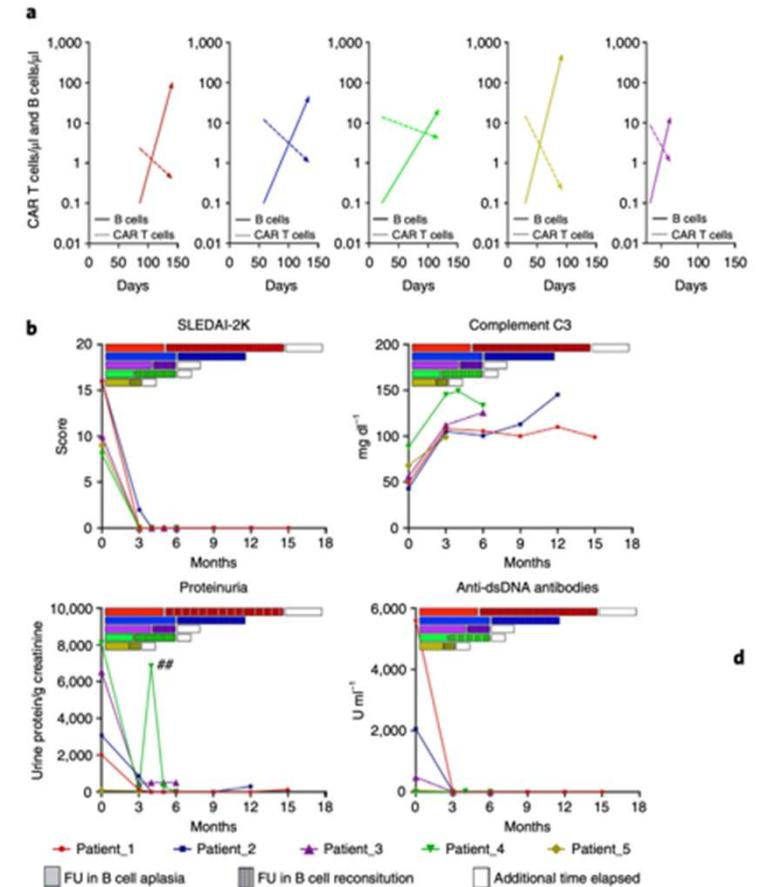
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
<b>Demographics</b>					
Age (years)	22	23	22	24	18
Sex (female/male)	F	M	F	F	F
Disease duration (years)	4	1	6	9	3
Disease activity SLEDAI-2K (score)	16	16	10	8	9
<b>Laboratory values</b>					
Baseline hemoglobin (g dl <sup>-1</sup> )	10.0	14.60	9.60	13.10	12.20
Baseline white blood cells (N μl <sup>-1</sup> )	8.69	5.36	5.85	3.88	7.25
Baseline lymphocytes (N μl <sup>-1</sup> )	0.7	1.2	1.4	1.4	1.4
Baseline platelets (N μl <sup>-1</sup> )	279	188	198	398	278
Baseline C3 (mg dl <sup>-1</sup> )	49	43	56	88	68
Baseline anti-dsDNA (U ml <sup>-1</sup> )	5,600	2,060	479	4	52
Baseline ANA (titer)	1:10,000	1:3,200	1:10,000	1:3,200	1:1,000
Proteinuria (mg per 24 h)	2,015	3,080	6,539	8,096	88
Other autoantibodies	NUC, Sm	NUC, Sm Ro60	NUC, PCNA	NUC, Sm, Ro60	NUC, Sm, Ku
<b>Organ involvement</b>					
Skin (presence/absence)	+	+	+	+	+
Kidney (presence/absence)	+ (stage III)	+ (stage III)	+ (stage IV)	+ (stage III/V)	+ (stage III/V)
Joints (presence/absence)	-	+	+	+	+
Lungs (presence/absence)	+	-	+	+/-	-
Heart (presence/absence)	+	-	-	+	-
Other (presence/absence)	HEM	-	SER	MYO	HEM
<b>Treatments</b>					
Glucocorticoid pulses (yes/no)	+	+	+	+	+
Hydroxychloroquine (yes/no)	+	+	+	+	+
MMF (yes/no)	+	+	+	+	+
Azathioprine (yes/no)	-	-	-	+	+
Cyclophosphamide (yes/no)	+	+	+	-	-
Rituximab (yes/no)	+	-	-	-	-
Belimumab (yes/no)	+	+	+	+	+
Other (yes/no)	TAC	-	-	MTX, LEF	-

ANA, antinuclear antibody; C3, complement factor C3; HEM, hematologic abnormalities of SLE; LEF, leflunomide; MTX, methotrexate; MYO, myositis; NUC, anti-nucleosome antibodies; PCNA, proliferating cell nuclear antigen; SER, serositis; TAC, tacrolimus.

# Efficacité clinique

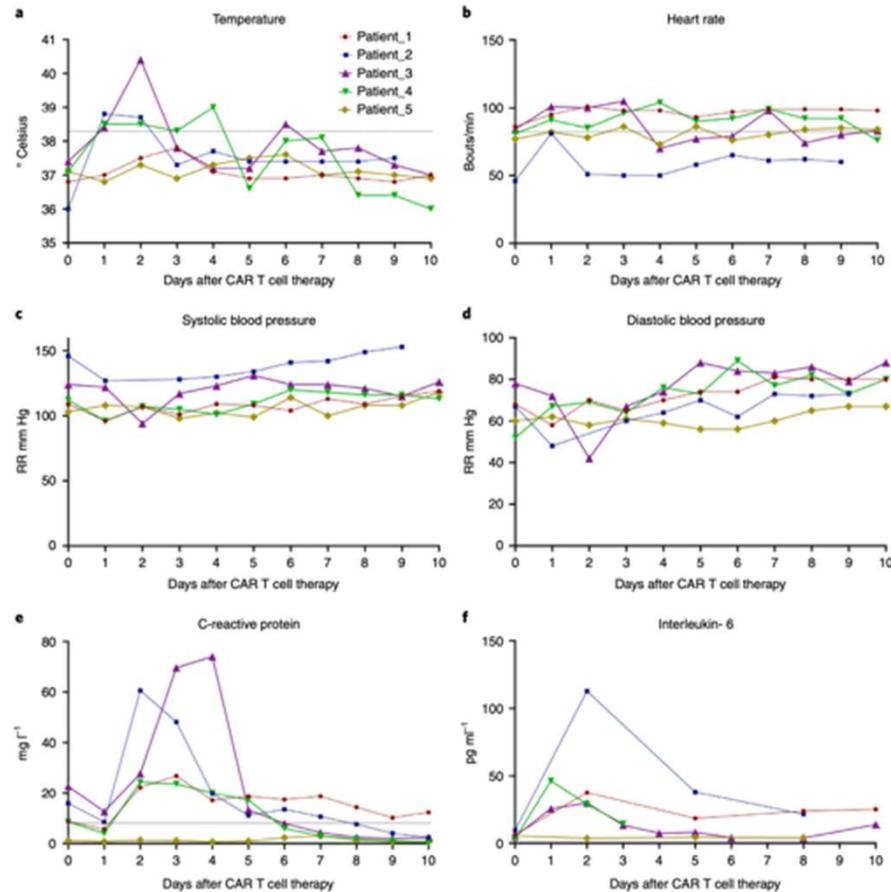


**Fig. 3 | Effects of CAR T cell treatment on the activity of systemic lupus erythematosus. a**, SLEDAI-2K scores at baseline and 3 months after CAR T cell administration (N=5). **b**, Proteinuria at baseline and 3 months after CAR T cell administration (N=5). **c**, Complement factor C3 levels at baseline and 3 months after CAR T cell administration (N=5). **d**, Anti-dsDNA antibodies assessed by radioimmunoassay at baseline and 3 months after CAR T cell administration (N=5). **e**, Fatigue measured by numerical rating scale (0-10) at baseline and 3 months after CAR T cell administration (N=5). **f**, ELISA-based quantification of antibodies against double stranded (ds) DNA, single stranded (ss) DNA, secondary necrotic cells (SNECs), nucleosomes, Smith (Sm) antigen, Sjogren's syndrome (SS)-A/Ro60, SS-A/Ro52 and SS-B/La antigens and histones at baseline and 3 months after CAR T cell administration (N=5).



Mackensen et al, Nature Med 2022

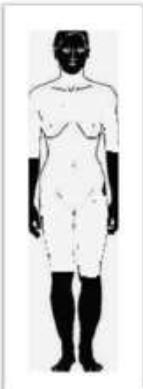
# Tolérance de la procédure



**Fig. 5 | Safety of CAR T cell treatment in systemic lupus erythematosus. a-f,** Body temperature (a), heart rate (b), systolic blood pressure (c), diastolic blood pressure (d), serum levels of C-reactive protein (e) and IL-6 (f) during the first 10 d after CAR T cell administration (all N=5); no IL-6 measurements are depicted in patient 4 after receiving anti-IL-6R infusion (tocilizumab) as the measurement of IL-6 is not reliable (false high) during detection of neutralizing antibodies.

# Sclérodermie systémique (SSc)

## Fibrosis



ScS cutanée limitée



ScS cutanée diffuse

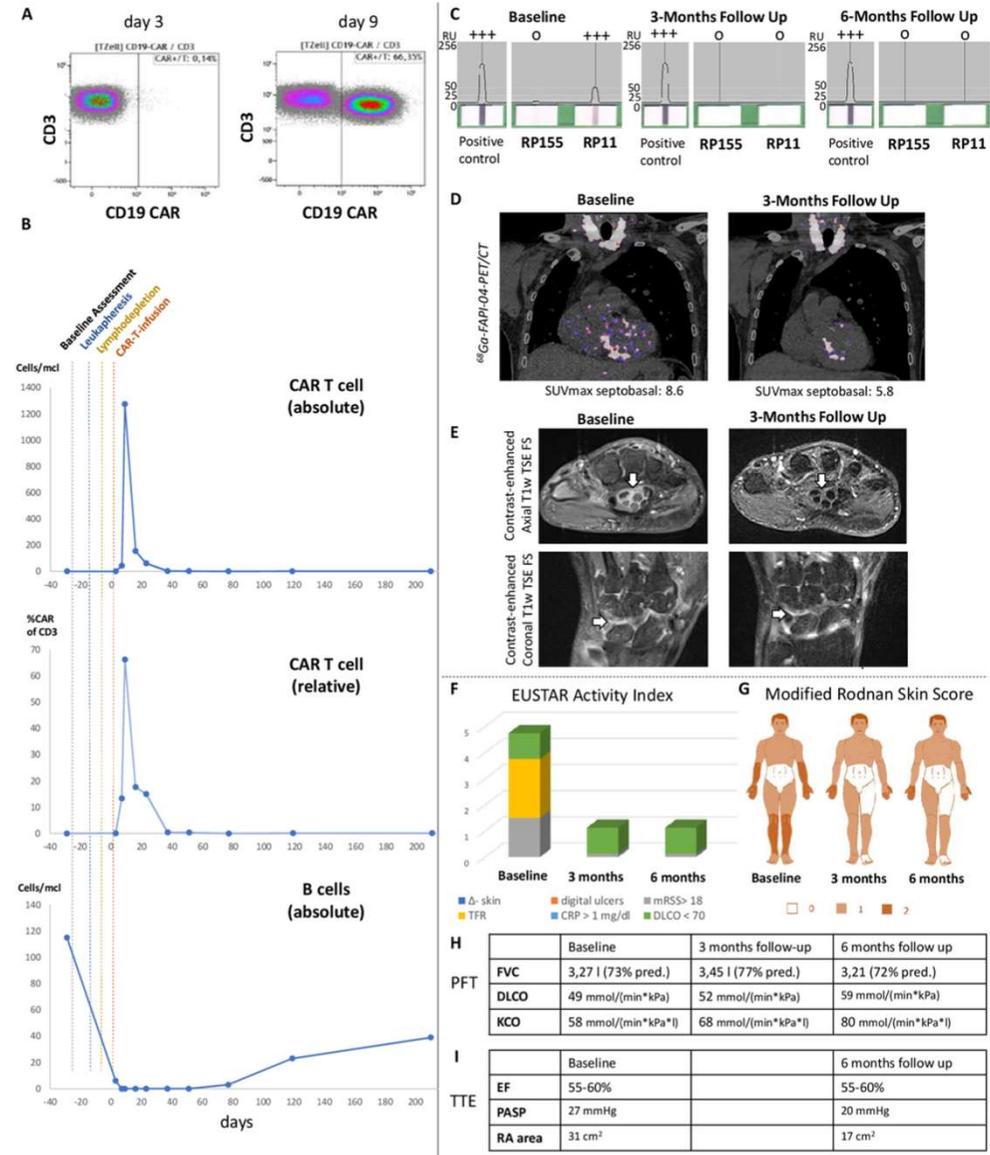


## Generalized micro-angiopathy



# CD19-targeted CAR-T cells in SSc

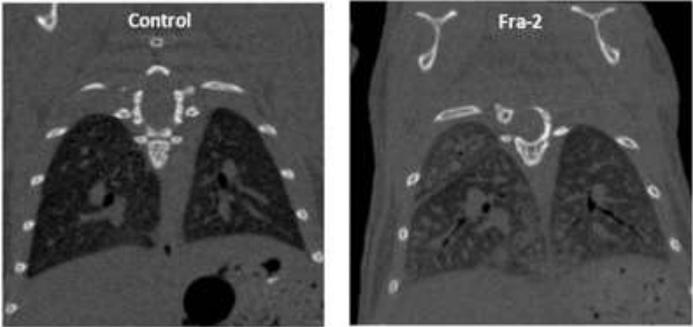
- ✓ 60 y.o man
- ✓ Early diffuse cutaneous SSc
- ✓ Positive anti-RNA polymerase III Ab
- ✓ Diffuse myocardial fibrosis
- ✓ Mild lung fibrosis
- ✓ Postcapillary PAH
- ✓ Raynaud phenomenon
- ✓ Arthritis
- ✓ Failure of MTX and MMF



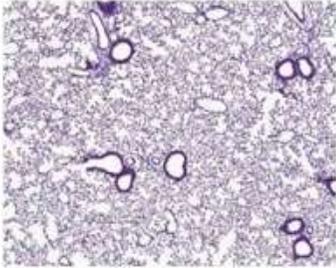
Bergmann et al, Ann Rheum Dis 2023

# Souris transgénique Fra-2

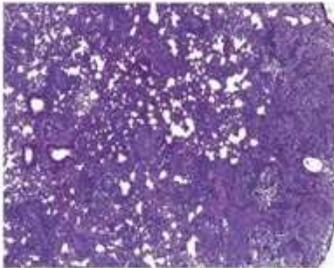
## A Fibrose pulmonaire



**B C57BL/6**

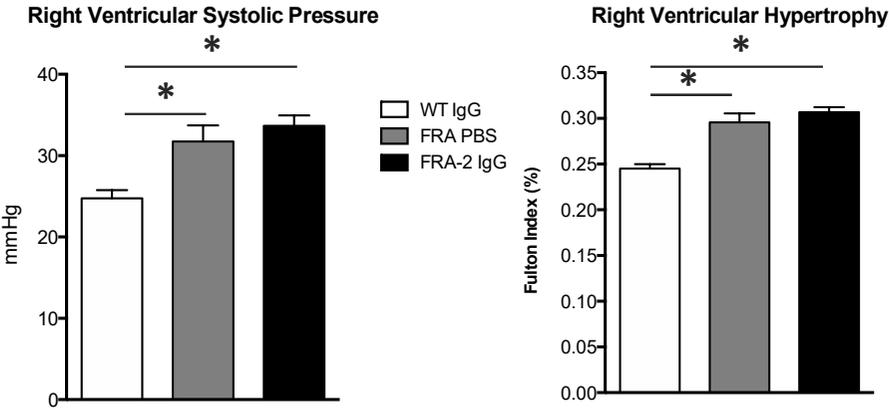


**Fra-2**



## C Hypertension pulmonaire

**C**

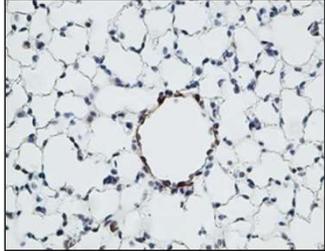
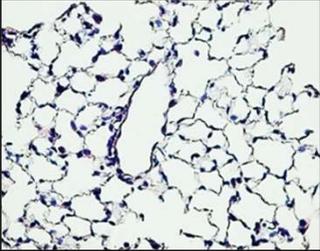


**D**

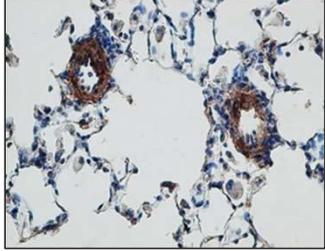
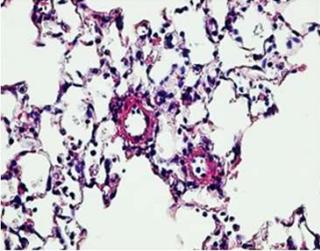
**H&E**

**ASMA**

**C57BL/6**



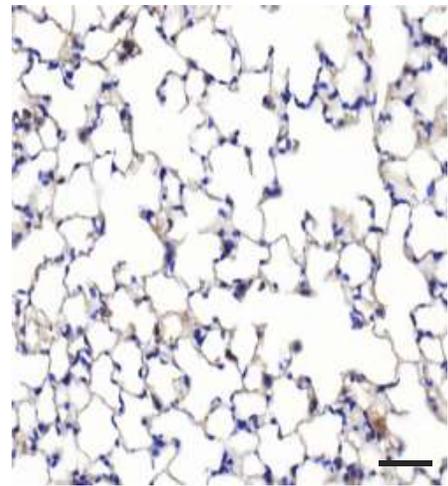
**Fra-2**



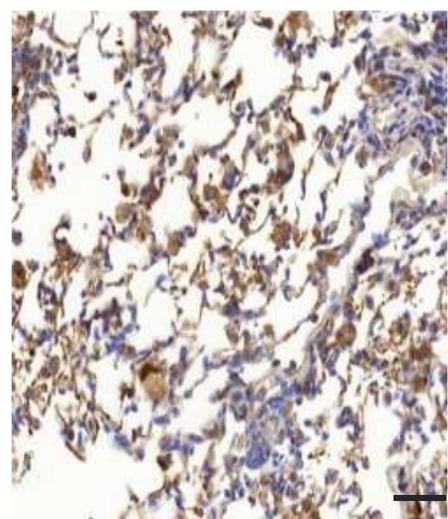
*Avouac et al, Arthritis Rheumatol 2016 & Ann Rheum Dis 2017*  
*Elhai et al, PNAS 2016*

# Déplétion des lymphocytes B sous anti-CD20 et anti-CD20 + CAR-T CD19

CD22

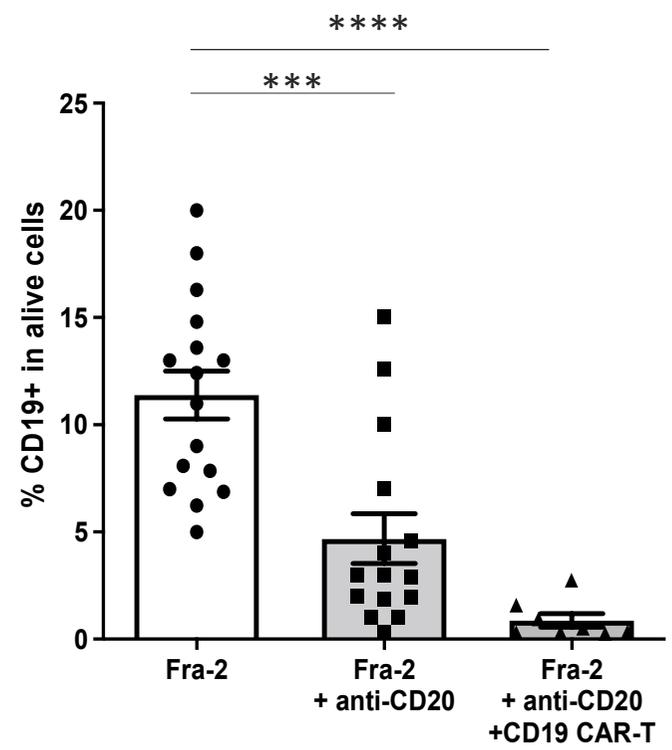


C57BL/6

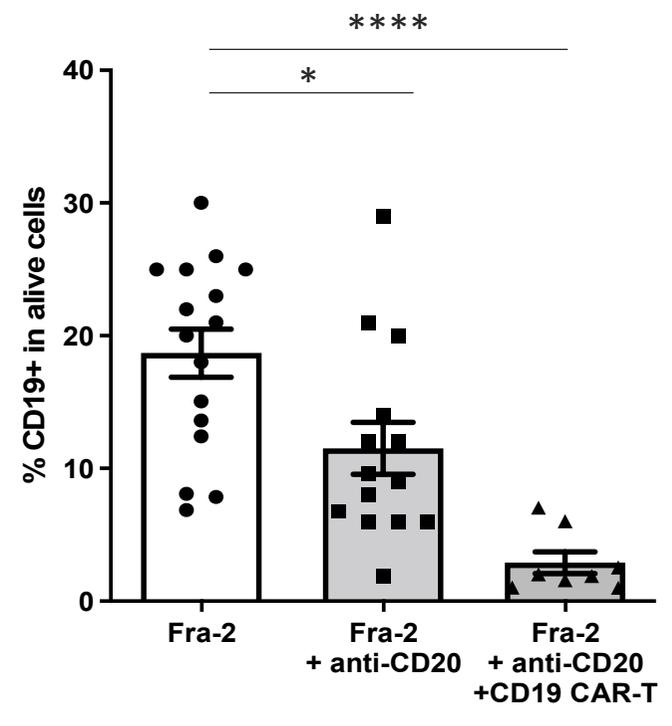


Fra-2

SANG

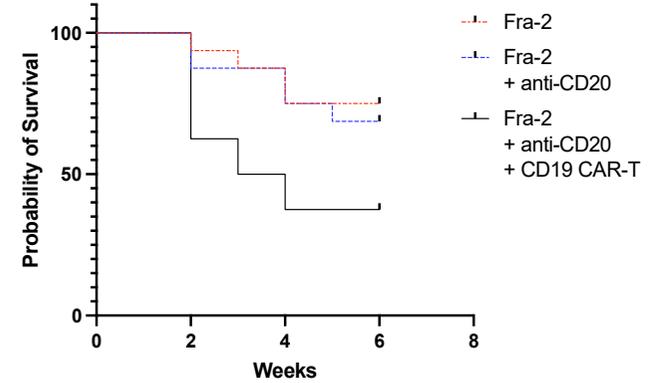
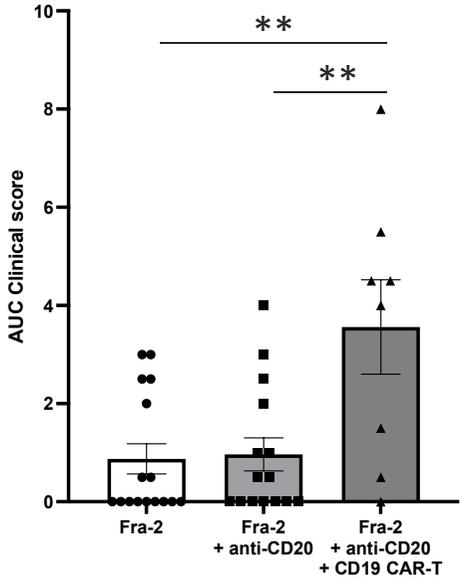
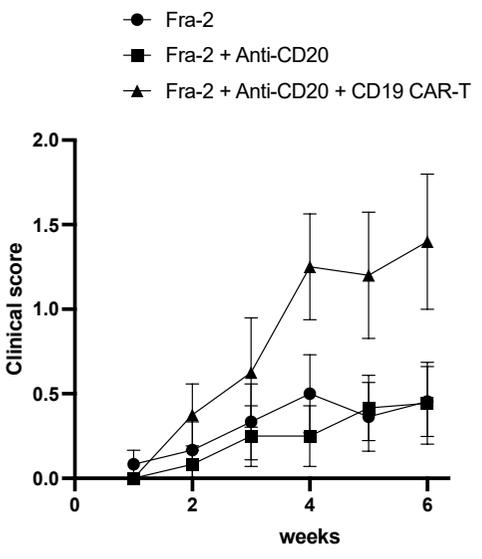
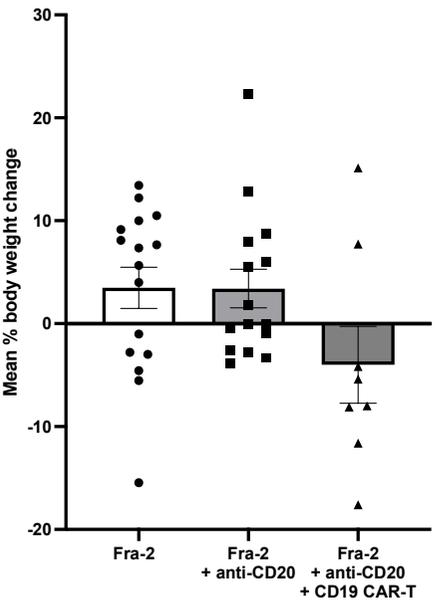


POUMON



\* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  determined by one-way analysis of variance with Tukey's post hoc test

# Les cellules CAR-T CD19 induisent une aggravation du score Clinique et une surmortalité chez la souris Fra-2

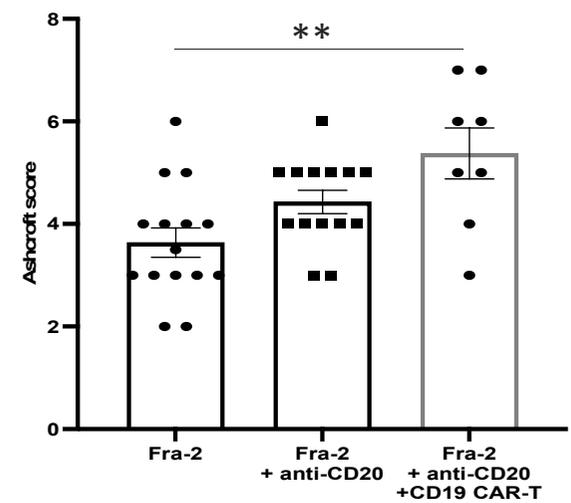
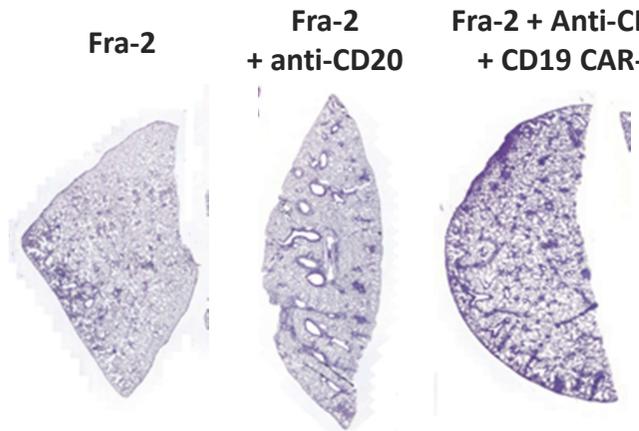
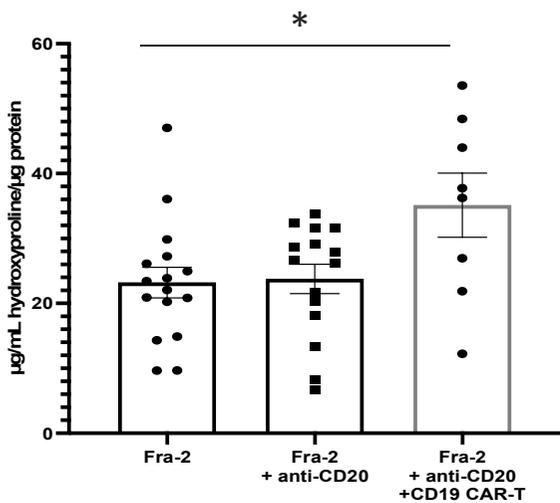
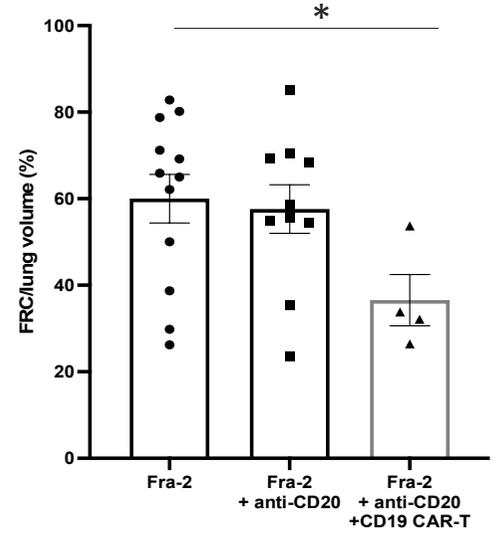
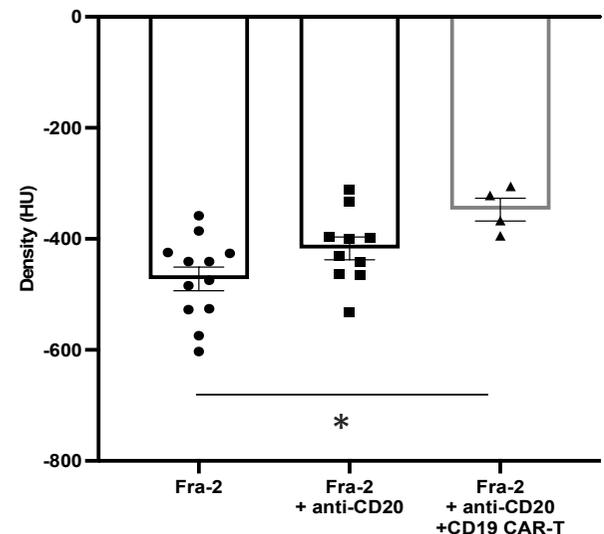
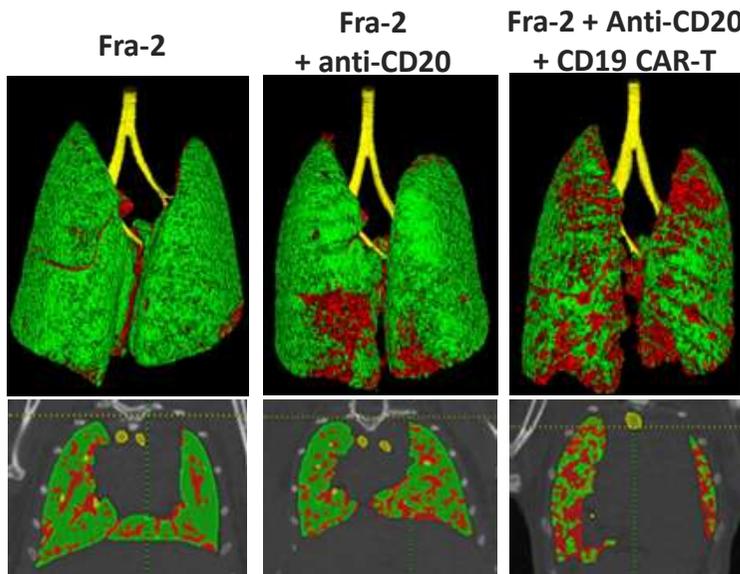


Weeks	0	2	3	4	5	6
Fra-2	16	16	15	14	14	12
Fra-2+anti-CD20	15	15	15	13	11	10
Fra-2 + anti-CD20 + CD19 CAR-T	8	8	5	4	4	3

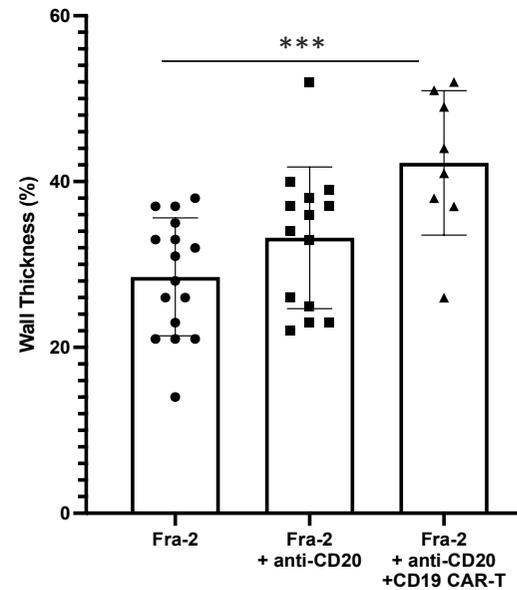
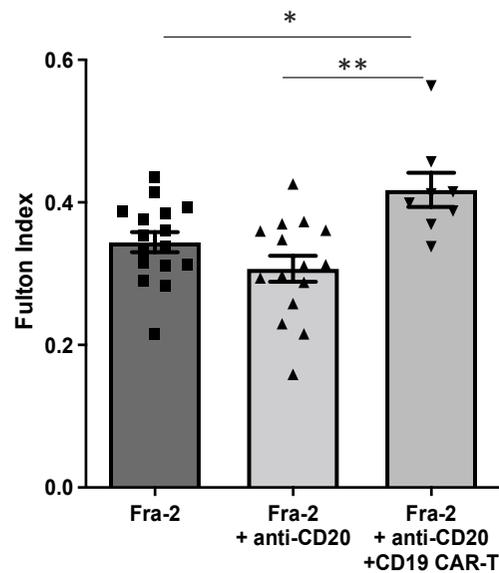
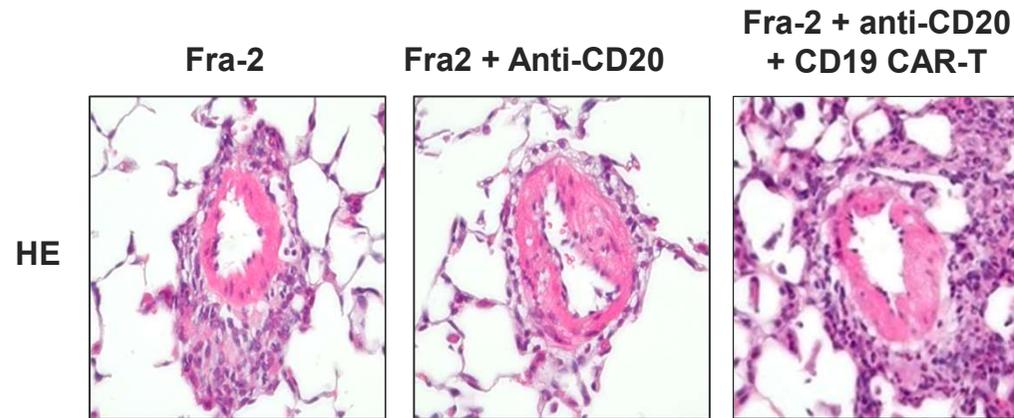
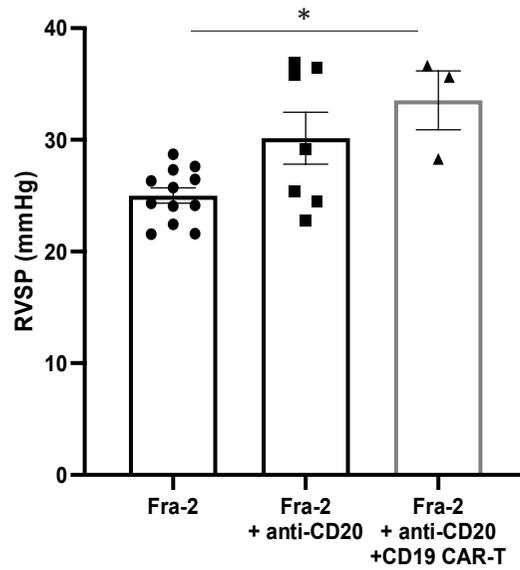
**Avouac et al, Arthritis Rheumatol 2023  
Collaboration with Philippe Bousso, Institut Pasteur**

\*\*p<0.01, determined by one-way analysis of variance with Tukey's post hoc test

# Les cellules CAR-T CD19 induisent une aggravation de la PID chez les souris Fra-2

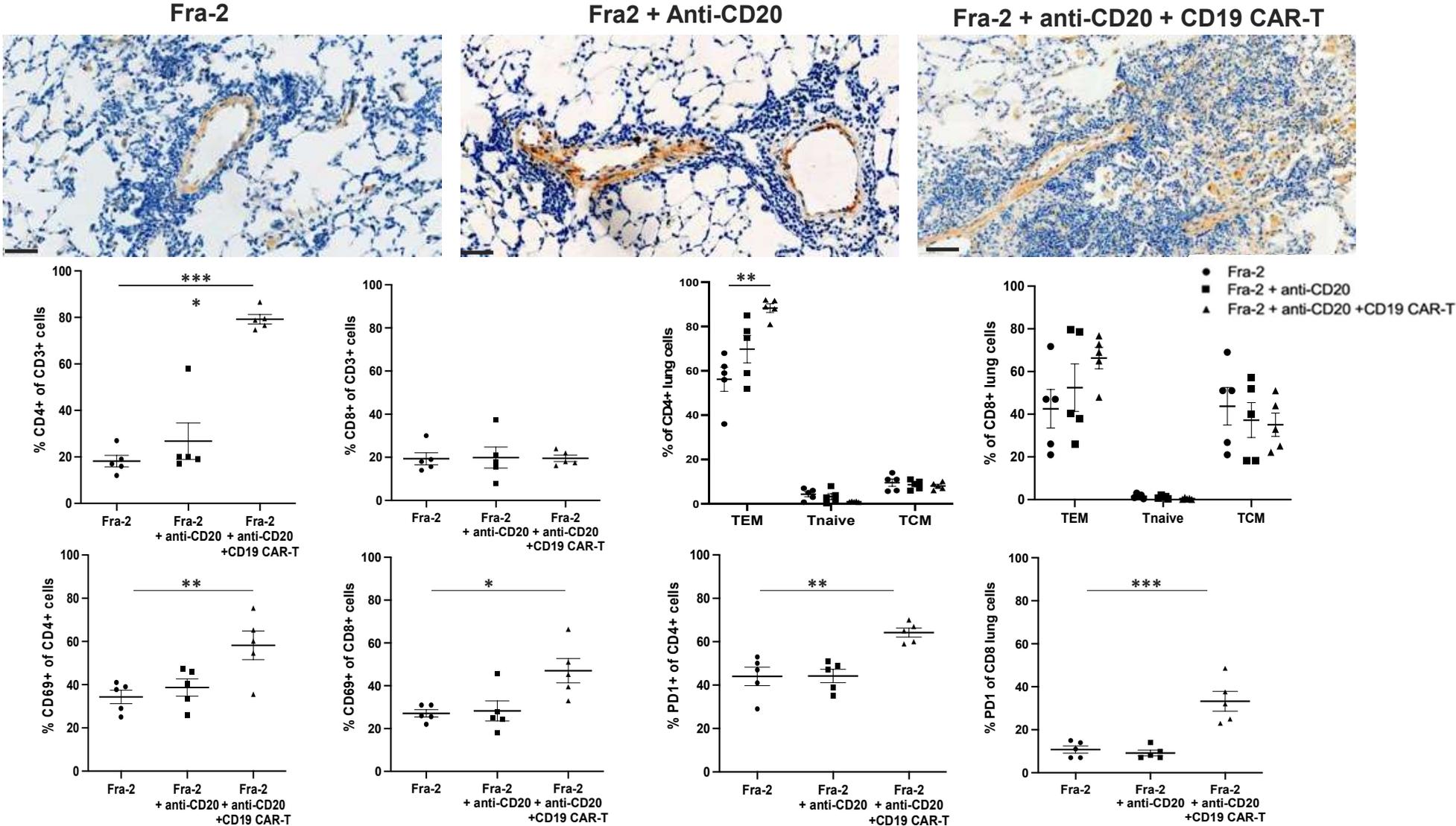


# Les CAR-T CD19 induisent une aggravation de l'hypertension pulmonaire chez la souris Fra-2



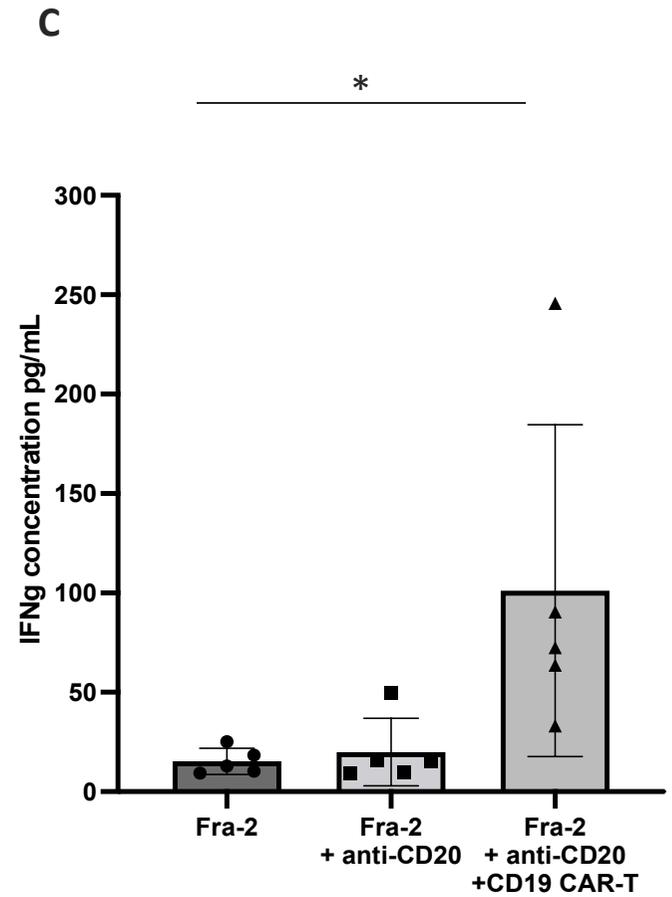
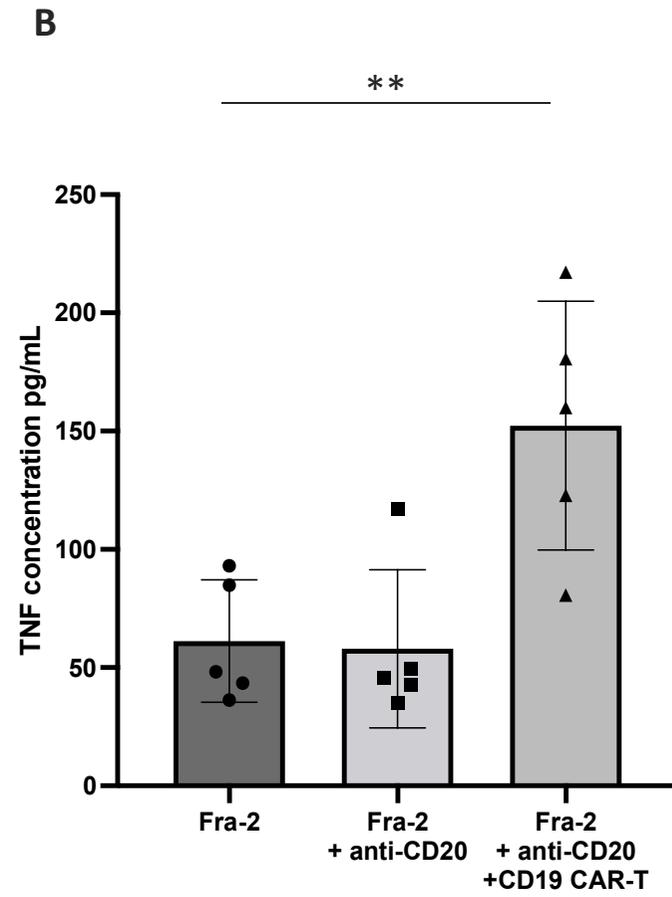
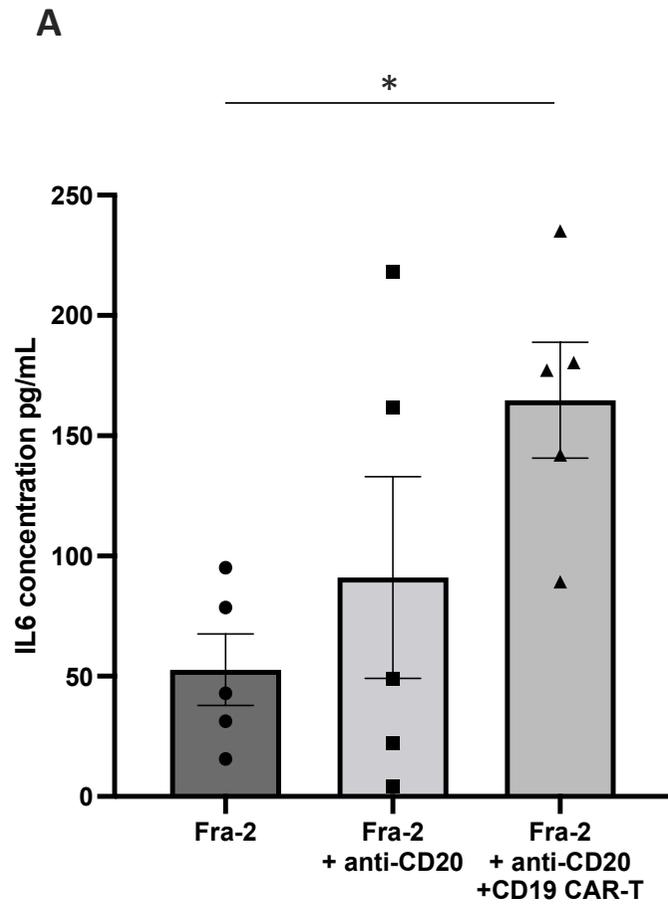
\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,  
determined by one-way analysis of  
variance with Tukey's post hoc test

# Les CAR-T CD19 s'accumulent dans le poumon et augmentent la réponse T



**Avouac et al, Arthritis Rheumatol 2023**

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, determined by one-way analysis of variance with Tukey's post hoc test



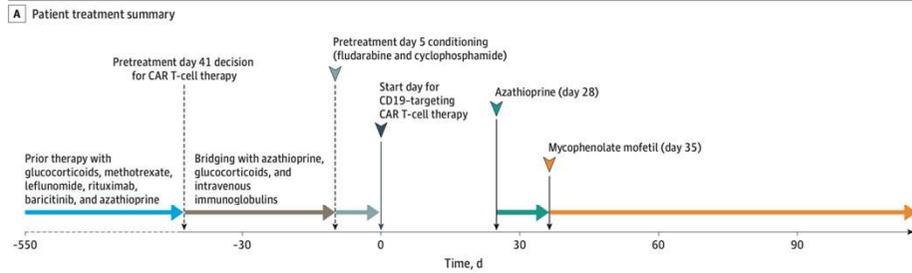
## Synthèse de ces résultats

- **Les thérapies anti-B n'ont pas été efficaces chez les souris transgéniques Fra2.**
- **L'inflammation pulmonaire exacerbée des souris Fra2 a stimulé**
  1. **l'accumulation et l'expansion des cellules CAR-T CD19**
  2. **l'activation des lymphocytes T et une inflammation systémique**
  3. **l'aggravation de la maladie.**
- **Nos données incitent à la prudence lors de la future utilisation des cellules CAR-T CD19 dans la PID des connectivites**

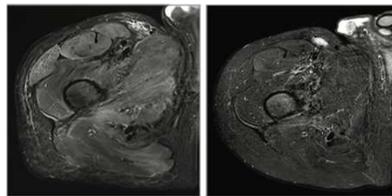
# CD19-targeted CAR-T cells in myositis

- ✓ Homme de 41 ans
- ✓ SAS, anti-JO1 +

Figure 1. Patient's Clinical Details and Treatment History

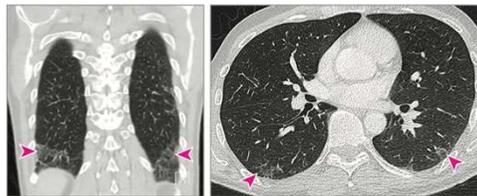


**B Magnetic resonance imaging of right thigh muscle**

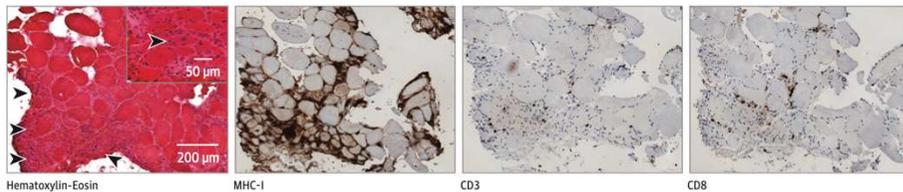


30 Days before CD 19-targeting CAR T cells  
120 Days after CD 19-targeting CAR T cells

**C Pretreatment computed tomography of lungs**

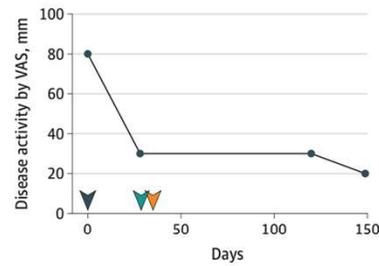


**D Pretreatment histopathology of right sartorius muscle**

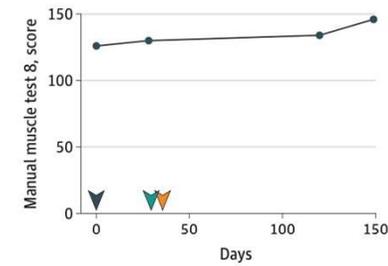


Hematoxylin-Eosin MHC-1 CD3 CD8

**E Physician Global Assessment**



**F Manual muscle test 8**



**G Pulmonary function test**

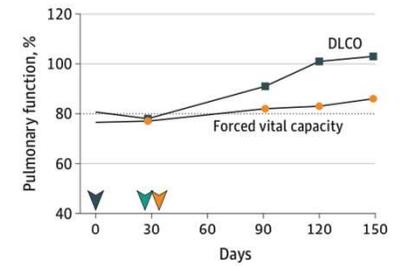
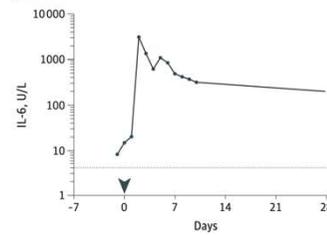
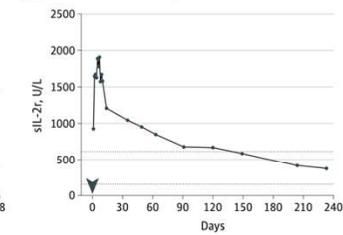


Figure 2. Quantification of CD19-Targeting CAR T Cells and Early Immunologic Responses

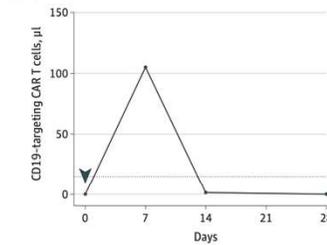
**A Interleukin-6**



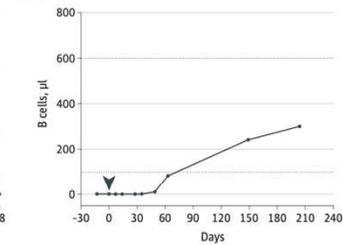
**B Soluble interleukin-2 receptor**



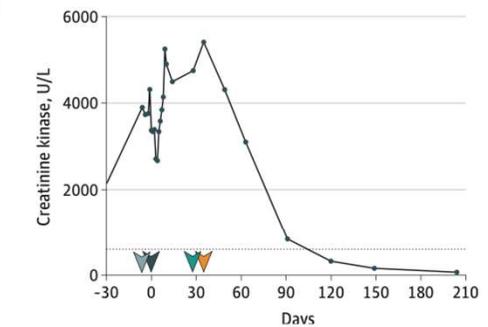
**C CD19-targeting CAR T cells**



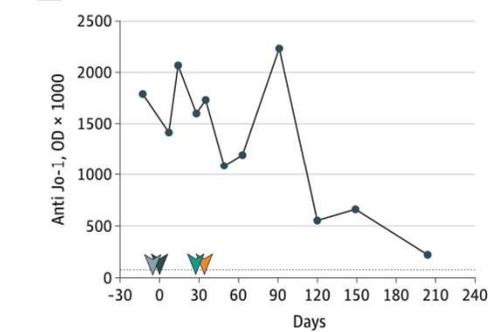
**D B cells**



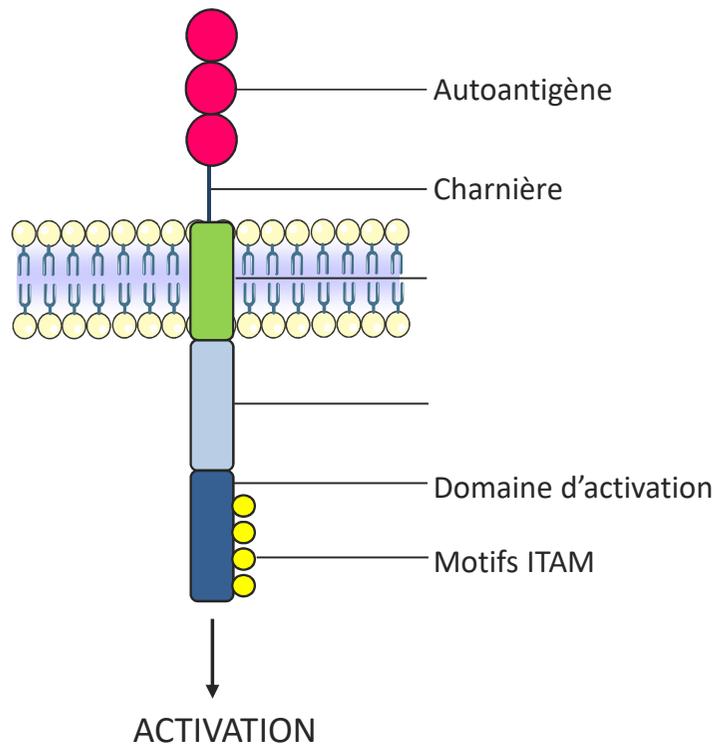
**C Creatinine kinase**



**B Anti Jo-1 autoantibodies in peripheral blood**

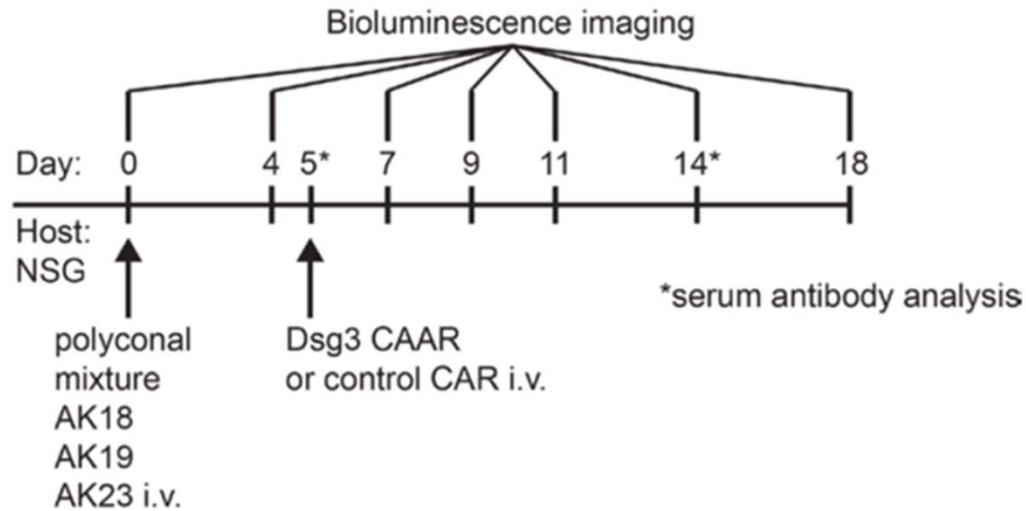


# Cellules CAAR-T (chimeric autoantibody receptor )

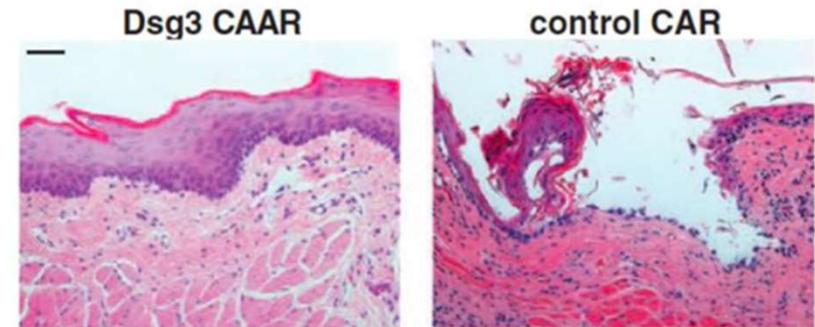
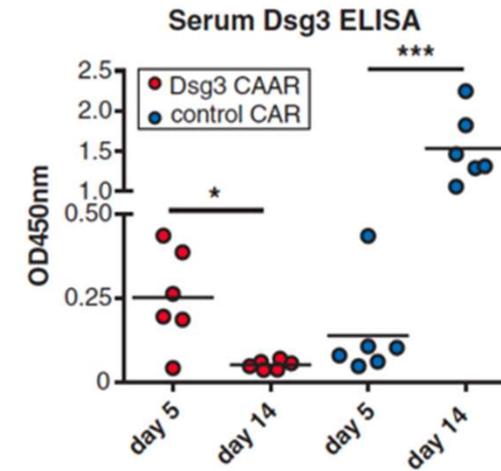


- Les cellules CAAR-T expriment un autoantigène extracellulaire reconnu par le BCR
- La reconnaissance d'autoantigène par les lymphocytes B autoréactifs conduit à l'activation du CAAR et à la lyse spécifique des cellules B pathogènes
- Pas d'utilisation chez l'homme – expérience préclinique
- Difficulté: nécessité de connaître la structure moléculaire de l'autoantigène ciblé

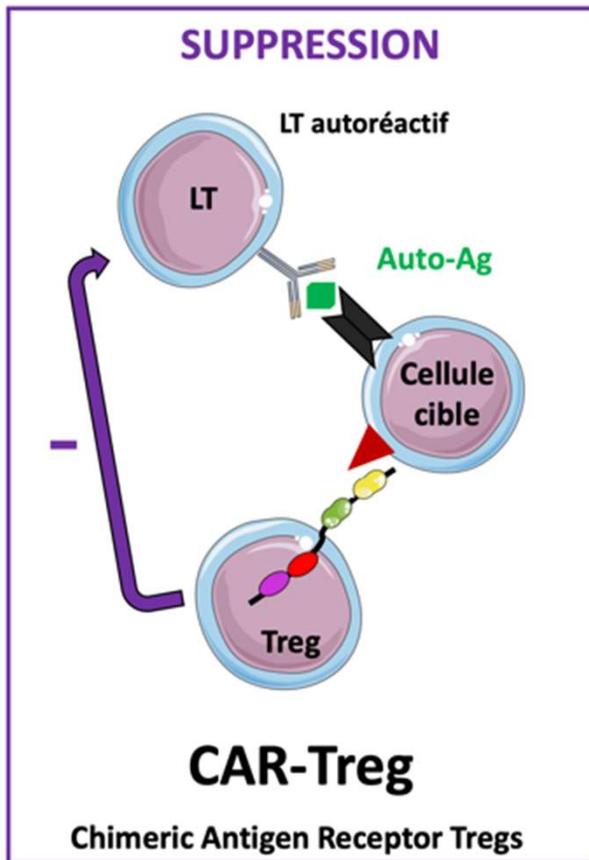
Mélange de 3 hybridomes producteurs d'Ac anti-Dsg3,  
injectés par voie i.v.



Dsg3 CAAR-T contrôle les hydridomes, **réduit le titre d'Ac, et prévient le décollement cutané**



# Cellules CAR-Treg

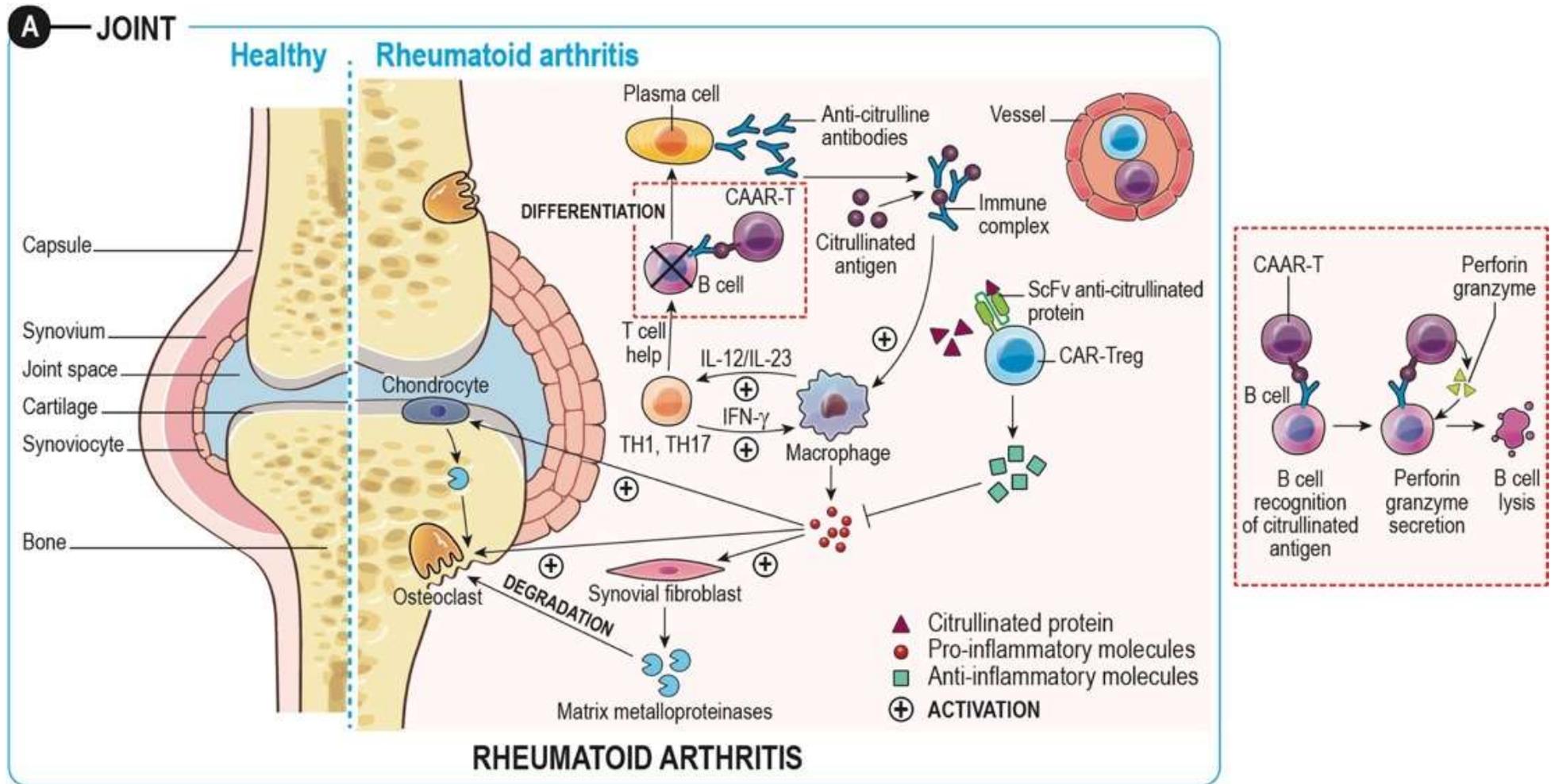


- Transfert de Treg dans le diabète de type 1 ou la GVH
- Limite: absence de spécificité: effets « off target »
  - > Réduction des défenses anti-infectieuses et anti-tumorales
- CAR-Treg ciblage d'un antigène spécifique
  - > Accumulation sur le site de l'antigène cible
  - > Meilleure capacité de suppression des réponses effectrices

# Utilisation préclinique des cellules CAR-T

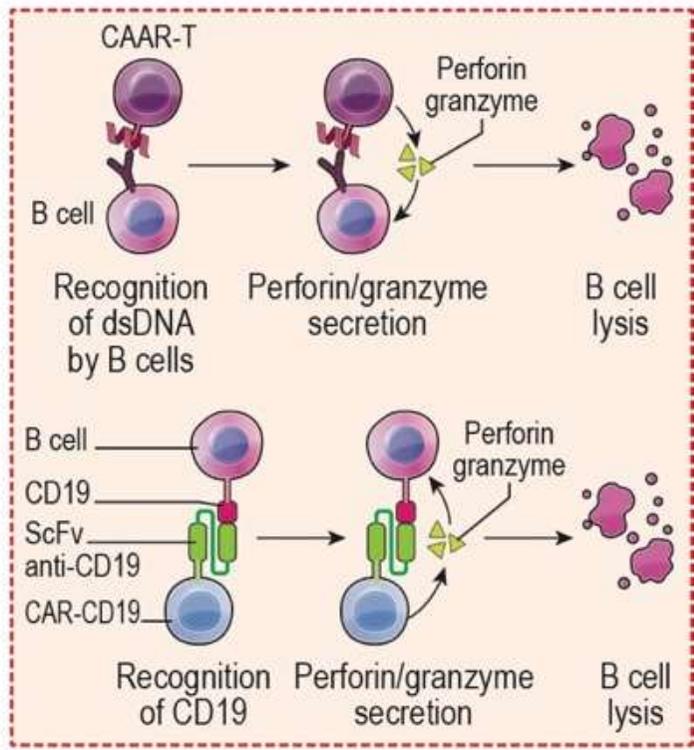
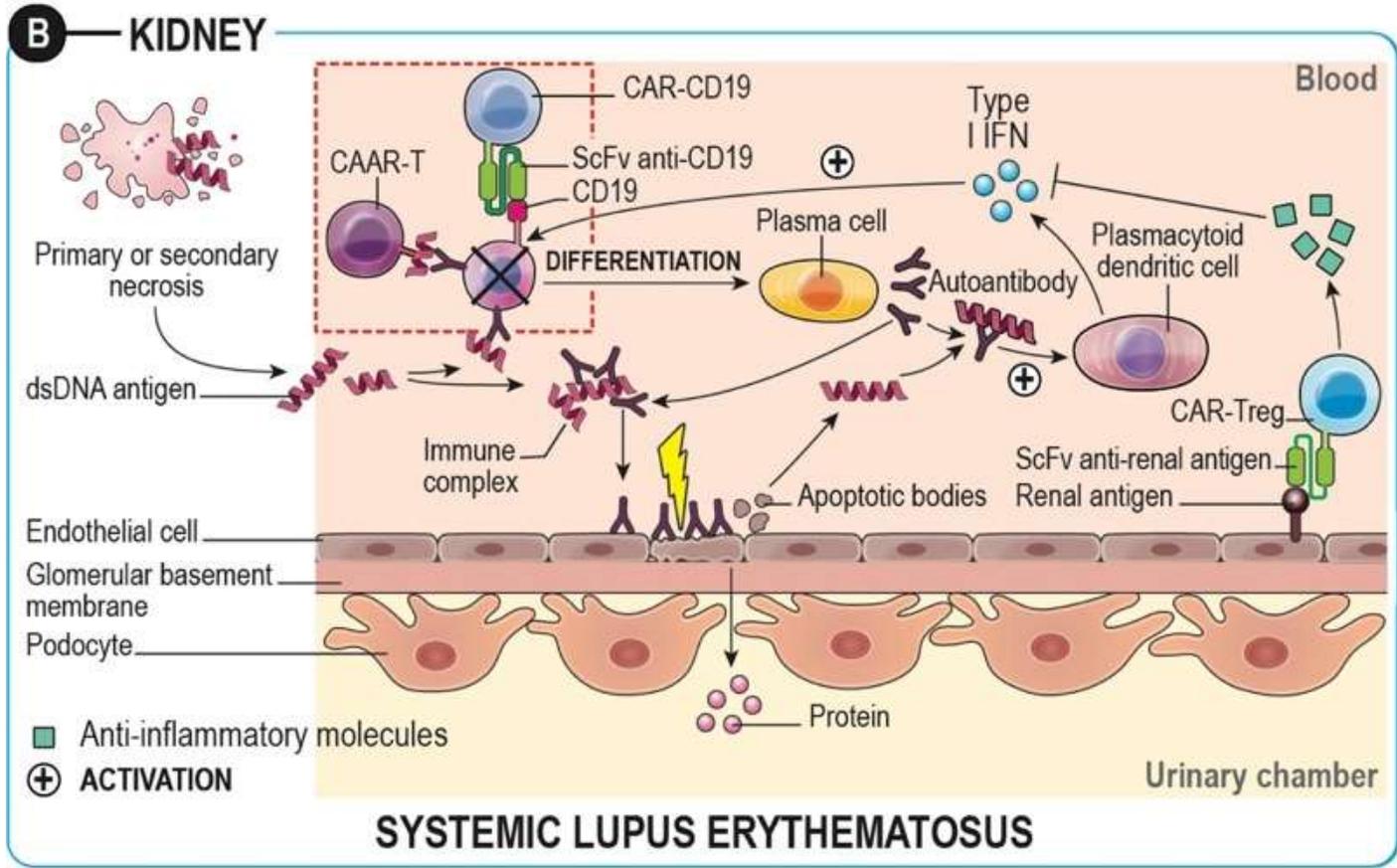
	maladie	Cible antigénique	Modèle murin	Domaines de Costimulation/activation	Critères de jugement	Références
<b>Cellules CAR-T</b>						
	Diabète de type 1	CMH I-A <sup>b7</sup> -B:9-23(R3)	NOD	CD28/CD3 $\zeta$ or CD28/CD137/CD3 $\zeta$	Retarde le développement du diabète	Zhang et al
	Lupus systémique	CD19	(NZBxNZW) F1 or MRL <sup>fas/fas</sup>	CD28/CD3 $\zeta$	Déplétion B persistante, Diminution de la production d'anticorps et allongement de la survie	Kansal et al
<b>Cellules CAAR-T</b>						
	Pemphigus vulgaire	Dsg3	PV hybridoma (NOD-scid-gamma)	CD137/CD3 $\zeta$	Délétion spécifique des lymphocytes B Dsg3 set diminution des autoanticorps anti-Dsg3	Ellebrecht et al
<b>CAR-Treg</b>						
	Sclérose en plaques	MOG	Encéphalite autoimmune expérimentale (EAE)	CD28/CD3 $\zeta$	Migration des CAR-Tregs dans le cerveau Amélioration des symptômes et réduction de la production cérébrale de cytokines pro-inflammatoires	Fransson et al
	Colite	ACE	Colite induite par le TNB	CD28/FcR $\gamma$	Migration des CEA-CAR Treg dans la muqueuse colique. Amélioration des symptômes et de la survie	Elinav et al
	Colite	ACE	CEABAC Ou transfert de cellules ACE/CD4+	CD28/CD3 $\zeta$	Accumulation des CAR-Tregs dans le colon. Régression des signes de colite	Blat et al
	Cancer colo-rectal	ACE	Colite induite par le DSS	CD28/CD3 $\zeta$	Diminution de la croissance tumorale	Blat et al
	Diabète de type 1	Insuline	NOD	CD28/CD3 $\zeta$	Absence de prévention du développement du diabète	Tenspolde M et al

# Perspectives d'utilisation des cellules CAR-T dans la PR

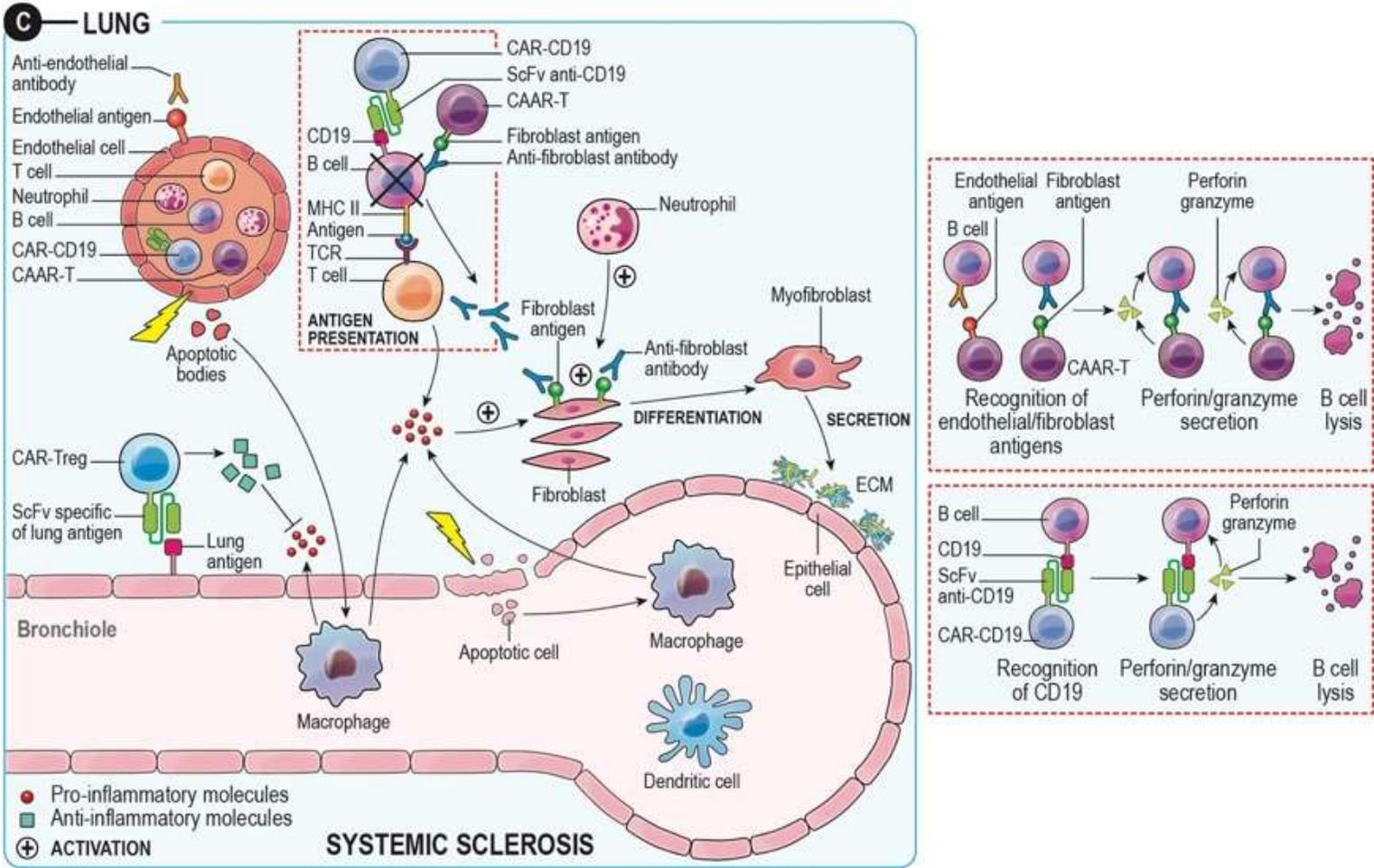


*Orvain et al, Arthritis Rheumatol 2021*

# Perspectives d'utilisation des cellules CAR-T dans le lupus systémique



# Perspectives d'utilisation des cellules CAR-T dans la sclérodémie systémique



Orvain et al, Arthritis Rheumatol 2021

# Conclusion

- **Cellules CAR-T: efficacité significative dans les hémopathies B, conduisant à la réflexion de leur potentielle utilisation dans les maladies auto-immunes**
- **A court terme: utilisation des cellules CAR-T CD19 dans le lupus. Puis SSc et myopathies inflammatoires. Prudence avec la PID**
- **La conception de cellules CAAR-T dirigées contre des antigènes ciblés par des auto-anticorps est une approche prometteuse pour supprimer les lymphocytes B pathogènes dans la PR, le lupus systémique et la SSc**
- **La conception des CAR-Treg pourrait permettre leur adressage dans les tissus lésés**
  - Identifier les antigènes exprimés par les tissus affectés
  - veiller à améliorer la stabilité des CAR-Treg pour éviter toute inversion de leur plasticité
- **Les limites potentielles de l'utilisation de la thérapie cellulaire CAR-T sont**
  - le syndrome de relargage de cytokines
  - leur coût
- **Potentiel nouvel arsenal thérapeutique pour les maladies auto-immunes rhumatismales ne bénéficiant pas traitement curatif à ce jour**

