# Myelin Oligodendrocyte Glycoprotein (MOG)-CAR-Tregs A novel approach to treat multiple sclerosis

Poster P475

Jihane Frikeche [1], Xavier Mouska [2], Damien Treguer [2], Emma Proïcs[2], Alexandra Menardi [2], Papababacar Fall [2], Joanne Salhab[2], Sandrine Rouquier[1], Yue Cui[1], Tobias Abel[1], Jung Hua Yeh [2], Marie Dittmer[3], Denise C Fitzgerald[3], Julie Gertner-Dardenne[1], Maurus de la Rosa[1] and Céline Dumont[2]

[1] Research, Sangamo Therapeutics, Valbonne, France. [2] Preclinical Pharmacology and Safety, Sangamo Therapeutics, Valbonne, France. [3] Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Northern Ireland, UK.

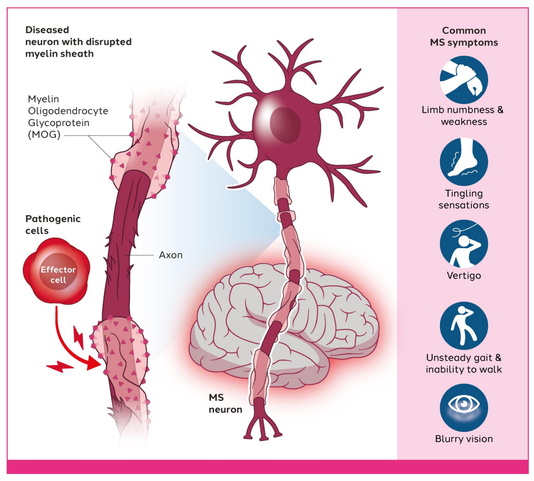
## Introduction

### Multiple sclerosis & Myelin oligodendrocyte glycoprotein (MOG)

Multiple sclerosis (MS) is an autoimmune disease affecting the central nervous system (CNS), in which the immune system attacks the myelin sheath resulting in different symptoms (see below). There is a high medical need for new treatments with improved efficacy and safety profile.

MOG is a promising tissue-restricted target for MS:

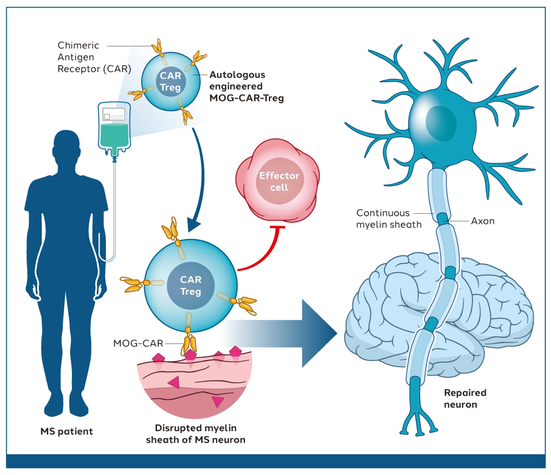
- MOG is expressed on the surface of myelin sheath in the CNS
- Several immuno-dominant epitopes from MOG have been described in MS[3]




### MOG-CAR Treg concept: restoring immune homeostasis in the CNS

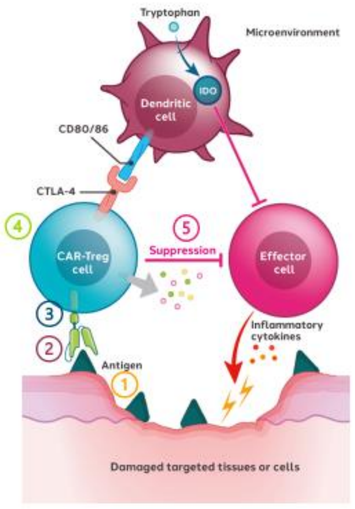
Chimeric Antigen Receptor (CAR)- Regulatory T cells (Tregs) in MS:

- Tregs are a subset of immune cells with immunomodulatory functions
- Treg dysfunction is believed to play a key role in the pathogenesis of MS[1,2]
- Polyclonal Tregs showed a good safety profile but weak efficacy in clinical trials
- Introduction of a CAR targeting MOG in the CNS is a strategy to potentially increase Treg potency




### Our objectives

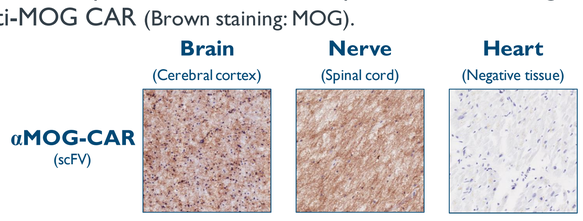
1. **Targeted Antigen**
   - Antigen distribution
   - Colocalized with inflammation
2. **CAR scFv**
   - High target specificity and suitable affinity
   - Low tonic signaling
   - Low immunogenicity
3. **CAR signaling domain**
   - Treg activation & expansion signal
   - Tregs survival and persistence
4. **Biomarkers**
   - Gene signature
   - Pathway/Liquid biomarkers
   - Treg efficacy and patient selection
5. **In vitro functionality**
   - CAR-mediated activation
   - CAR-mediated suppressive function (Tconv & DC suppression)
   - CAR Treg stability and persistence
6. **In vivo efficacy**
   - Reduction of clinical score
   - Reduction of inflammatory signs




## Results

### 1 The MOG antigen is specifically expressed in the CNS

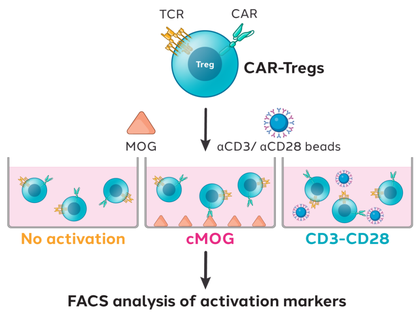
Histology analysis on human brain/spinal cord confirms the presence of the target and binding specificity of Sangamo's anti-MOG CAR (Brown staining: MOG).

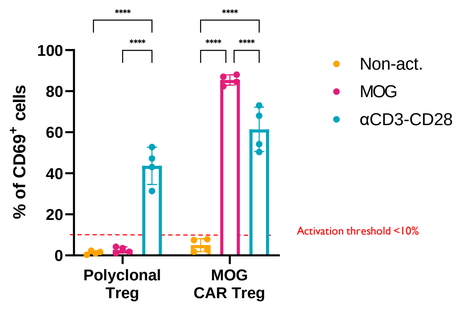



### 2 In vitro selection of an scFv with the lowest background of activation

Our lead candidate is a 2nd generation CAR, composed of an anti-MOG scFv screened from a large human library (~5K). Selection criteria: (i) binding to MOG, (ii) lowest tonic signaling, (iii) highest signal-to-noise ratio.

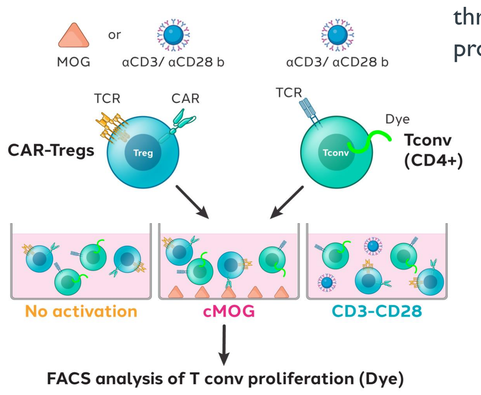
**Activation assay**



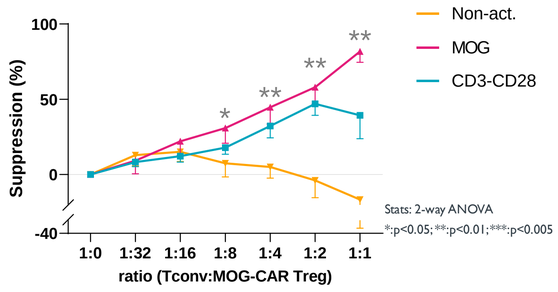




### 3 In vitro MOG-CAR mediated suppressive activity

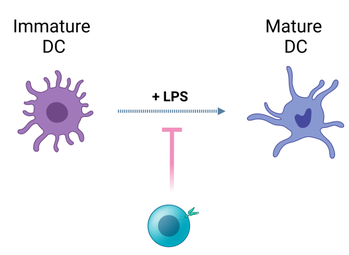
**Suppression assay**

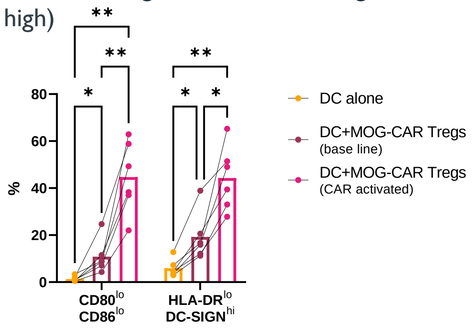



MOG-CAR Tregs are efficiently and significantly activated through their CAR and can suppress CD4 conventional T cell proliferation and CD8 T cells (*data not shown*) in vitro.




In coculture with dendritic cells (DC), activated MOG-CAR Tregs can induce tolerogenic DC (CD80low/ CD86low and HLA-DRlow/DC-SIGN high)

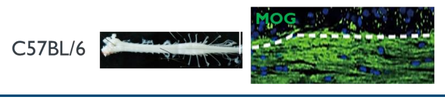


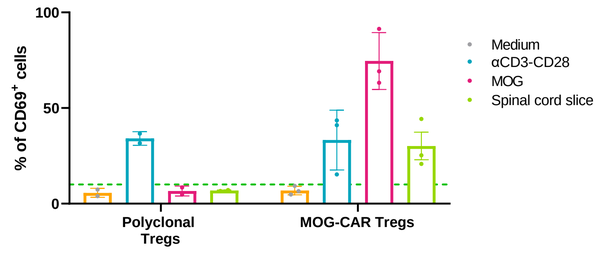




### 4 MOG-CAR Tregs are activated in an ex vivo target engagement assay

In ex vivo culture with mouse spinal cord, human MOG-CAR Tregs can be activated through their target engagement. Activation marker (CD69) increases after 24h of culture.

Longitudinal spinal cord slice co-culture system

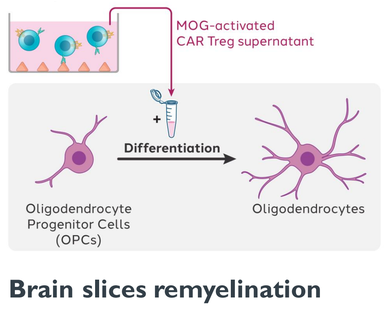


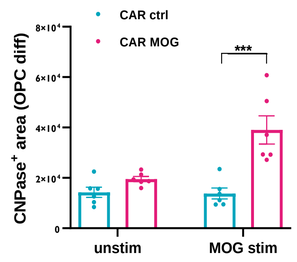




### 5 Factor secreted by activated MOG-CAR Tregs enhance OPC differentiation and remyelination in vitro

**OPC differentiation**

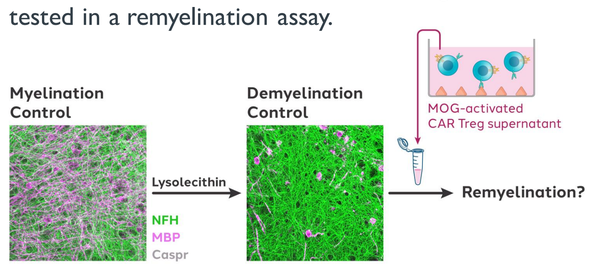
Conditioned media from mouse CAR Tregs stimulated or not through the CAR (MOG) were tested in a pure OPC culture model.

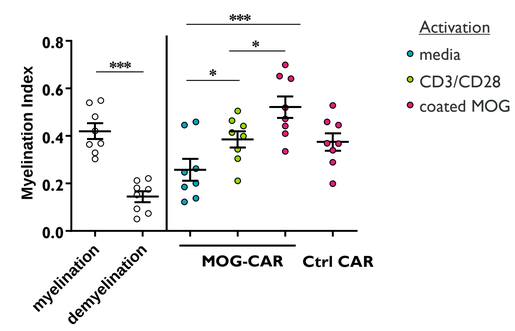



**Brain slices remyelination**

After demyelination of brain slices using lysolecithin, conditioned media from mouse CAR Tregs were tested in a remyelination assay.



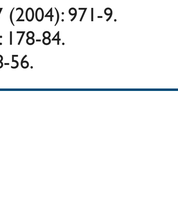

### 6 Mouse MOG-CAR Tregs are efficacious in an adoptive EAE transfer model

We demonstrated the functionality of MOG-CAR Treg cells in an adoptive transfer EAE in C57Bl/6 mice. Mice treated with MOG-CAR Tregs showed a lower clinical score and a reduction of pathogenic cells compared to control mice.

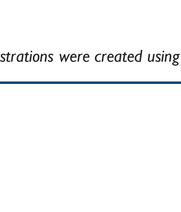
**MOG-CAR Tregs reduce EAE disease score**

| | | | | |
|---|---|---|---|---|
| Suppression saline vs MOG-CAR | 71% | 53% | 56% | 54% |
| Suppression saline vs ctrl CAR | 33% | 30% | 9% | 9% |


**MOG-CAR Tregs reduce the infiltration of CD4 pathogenic cells in the CNS**




**MOG-CAR Tregs reduce pathogenic cells activity**




## Conclusion

Autologous MOG-CAR Tregs provide a potentially long-lasting treatment option to address the underlying cause of the disease:

- CAR Treg binding to MOG triggers CNS-specific activation, retention, and proliferation of Tregs in the inflamed CNS
- MOG-CAR Tregs can promote OPC differentiation and remyelination
- Activated MOG-CAR Tregs suppress pathogenic inflammatory cells in the CNS and ameliorated disease score in EAE

## References

1. Viglietta *et al. The Journal of experimental medicine* vol. 199,7 (2004): 971-9.
2. Kumar *et al. Journal of neuroimmunology* vol. 180,1-2 (2006): 178-84.
3. Weissert et al. *Journal of immunology* vol. 169,1 (2002): 548-56.

*Some of the illustrations were created using Biorender.com*

**Presented at the ESGCT 30th Annual Congress, 2023**