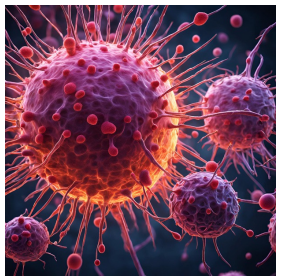
# Efficacité anti-tumorale d'une stratégie CAR-T cell anti-GD2 optimisée dans l'environnement immunosuppresseur du glioblastome

Dr Loïc Reppel (IMoPA – Nancy – UMR 7365 CNRS-UL)

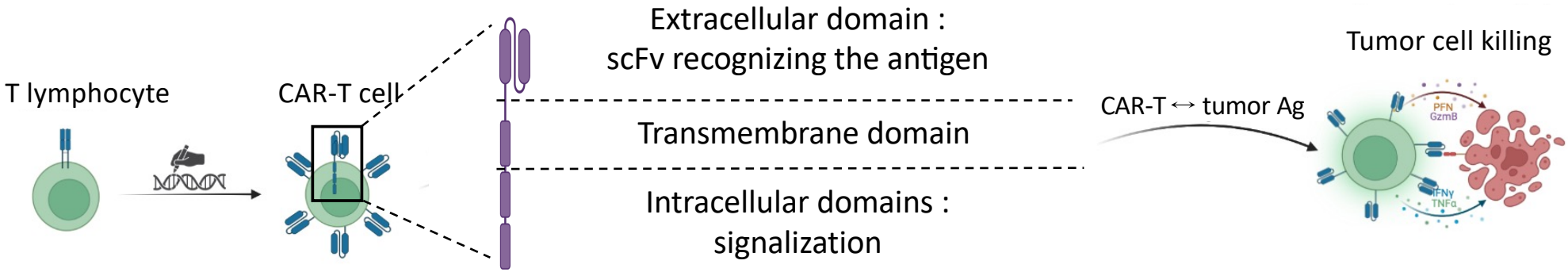
**Premières journées de recherche en Immuno-Oncologie**

**13 et 14 mars 2025 Agora Hautepierre CHU Strasbourg**



## CAR-T cells as antitumor therapeutic strategy

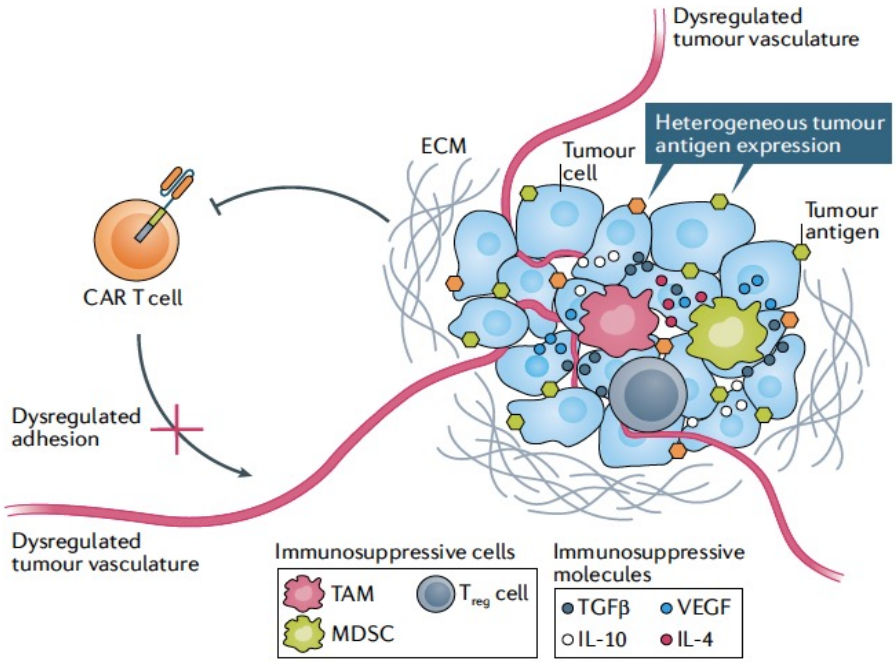
⊕ **Significant success in hematologic malignancies**




**Limited potential in solid tumors…**

*Sterner and Sterner. 2021*

# CAR-T cells in solid tumors



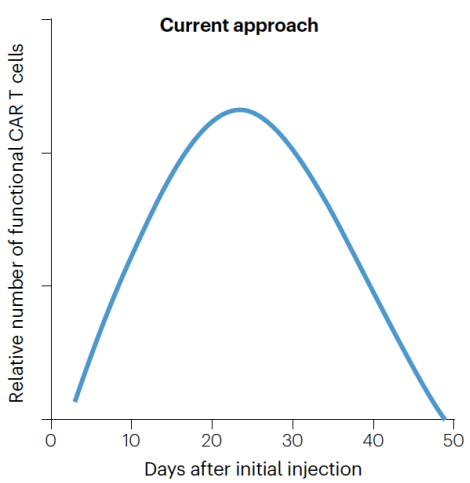

**Overview of the barriers to CAR-T cells in solid tumors**

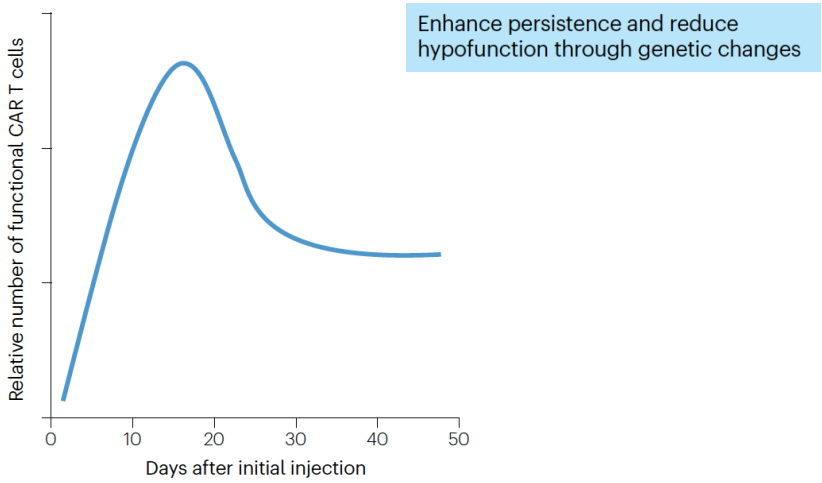
**Challenges to overcome :**

- **Paucity of specific target antigens**
- **Heterogeneity of antigen expression**
- **Physical Barriers/poor trafficking**
- **Immunosuppressive tumor microenvironment (TME)**
- **Short persistence and loss of effector function (exhaustion)**

*Hou AJ , Nat rev, 2021; Albelda SM, Nat rev, 2024*

# CAR-T cells in solid tumors



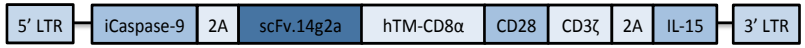

Limited time window in which adequate numbers of functional CAT-T cells are present within tumors

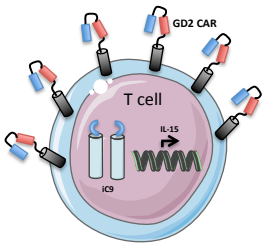
**Transient effect**

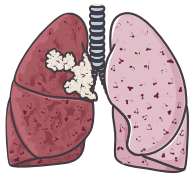
**Improvement of the activity of CAR-T cells in patients**

*Albelda SM, Nat rev, 2024*

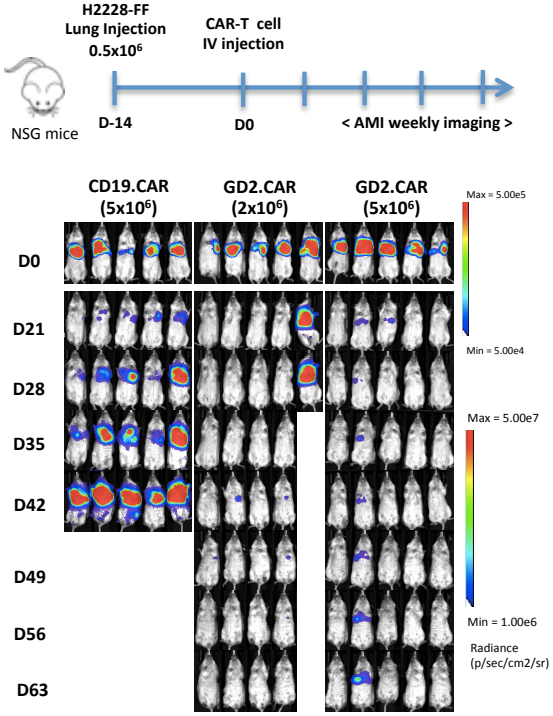
# Optimized GD2 CAR-T cells in solid tumor



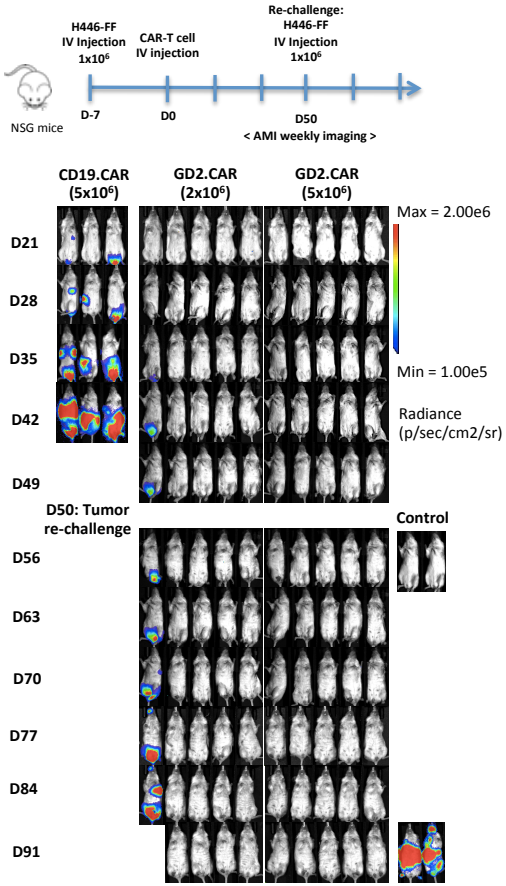



**NSCLC orthotopic model**




**SCLC metastatic model**




*Reppel L, J Immunother Cancer, 2022*

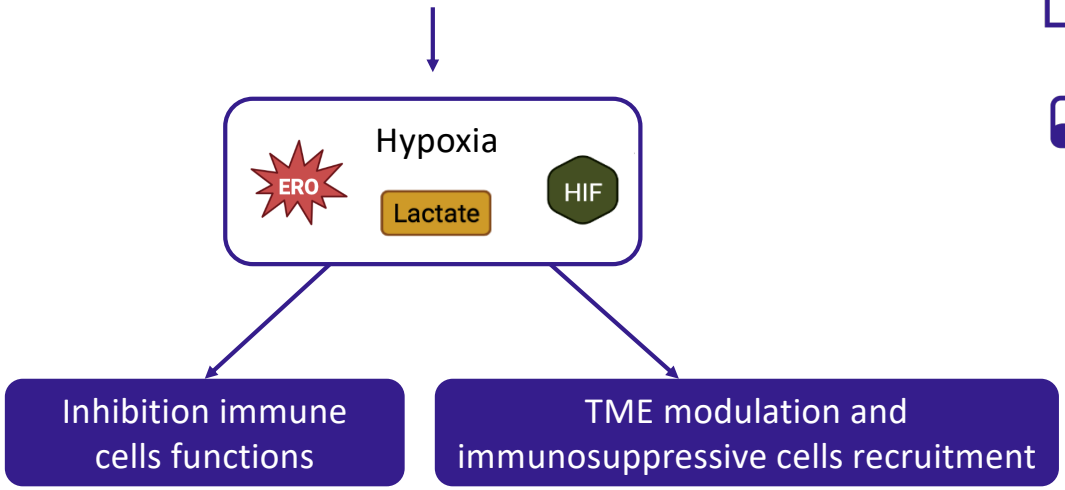
# Glioblastomas or grade IV gliomas and their microenvironment

5 year post-diagnostic survival < 5%

Current therapy > 90% recurrence

## *Metabolic remodelling and hypoxia*

Tumor and ME growth
Angiogenesis limited

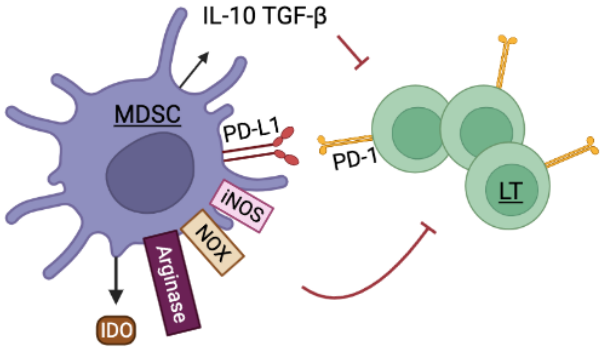



## *MDSCs and immunosuppressive mechanisms*

(CD33+ CD11b+ **HLA-DR$^{low}$**)

**Monocytic** (CD14+), **granulocytic** (CD15+), « **early** »

x12 GBM patients peripheral blood

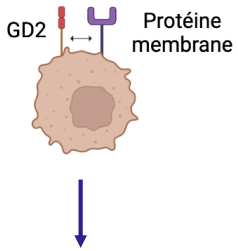



*Di Ianni and al, 2021 ; Grabowski and al, 2020 ; De Leo and al, 2020.*

# GD2 disialoganglioside as a glioblastoma target

## *GD2 disialoganglioside*

Membrane glycosphingolipid




Proliferation, migration, apoptose resistance

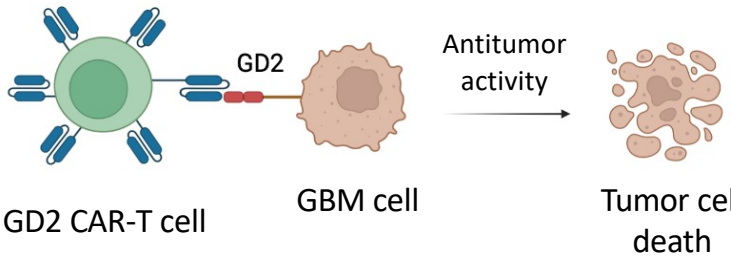
**GBM associated Ag (80%)** → Poor prognosis

**Gangliosides drive the tumor infiltration and function of myeloid-derived suppressor cells**

**Assefa Wondimu[1,*], Yihui Liu[1,3,*], Su Yan[1], Daniel Bobb[1], Jennifer S.Y. Ma[2], Lina Chakrabarti[2], Saša Radoja[2,3], and Stephan Ladisch[1,3,4]**

## *GD2 CAR-T cells*

*Demonstrated by Prapa M. (2021) in vitro and in vivo :*

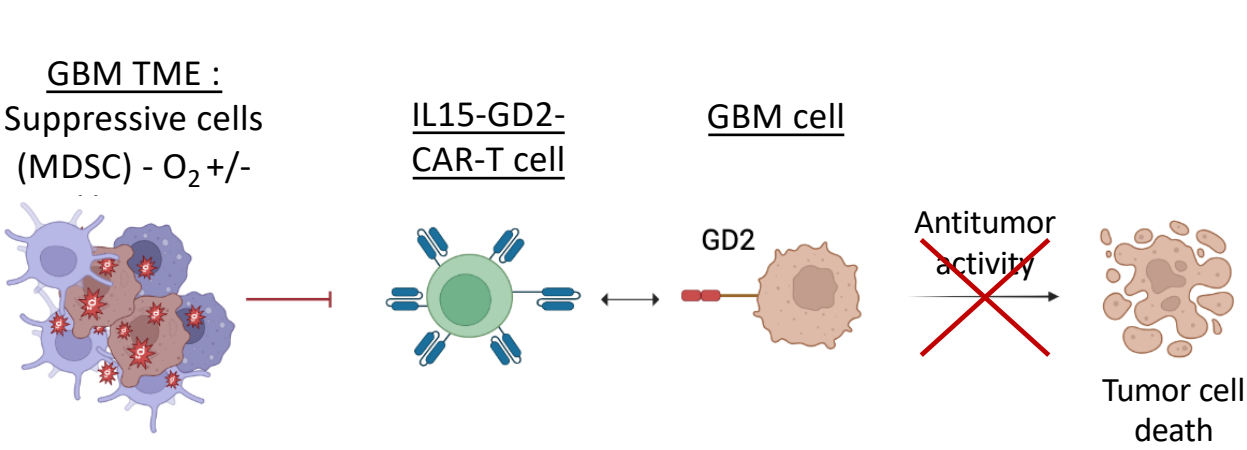


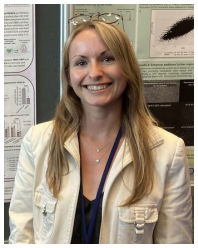

→ **Post-treatment recurrence *in vivo***

IL-15 transgen inclusion in CAR construction : → **Recurrence control *in vivo***

Immunodepressed models : immunosuppressive TME impact on CAR-T cell efficacy poorly studied

*Nazha and al, 2020 ; Prapa and al, 2021 ; Gargett and al, 2022 ; Zhang and al, 2023*



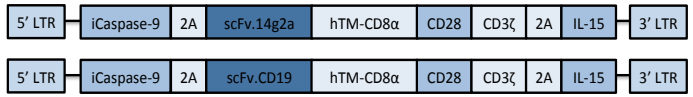



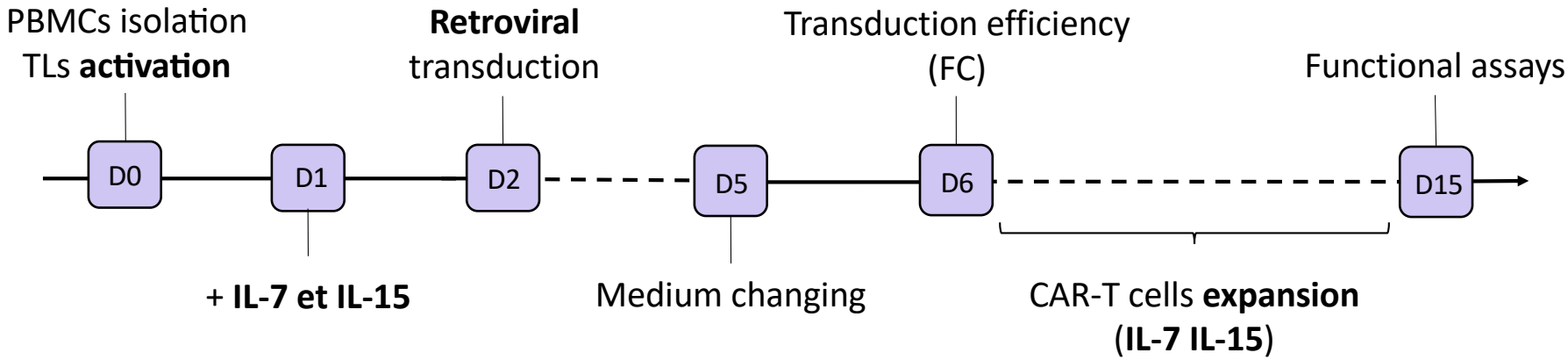
C. Lamorlette
PhD student

1. *In vitro* antitumor efficiency of IL15-GD2-CAR-T cells in GBM model
2. *In vitro* impact of MDSC on CAR-T cell efficiency in GBM model
3. TME modulation by MDSC targeting to improve CAR-T cell efficiency

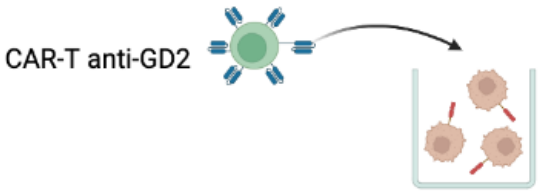
Development of a therapeutic strategy combining GD2 CAR-T cells and anti-TME agent in order to improve CAR-T cell antitumor efficacy durability

## Experimental strategy : generation of CD19 control and GD2 CAR-T cells




**Functional tests** of CAR-T cells on **GBM** models **:**




| GD2+ cell lines | T98G - Ln229 |
|---|---|
| GD2- cell line | U87 |

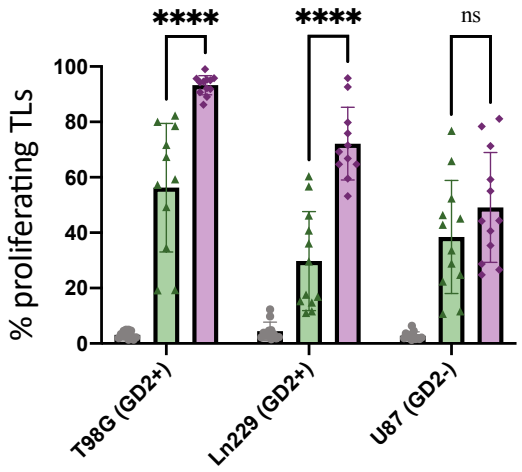
- **D15-H6** **CAR-T cells CD107a** degranulation assay
- **D16** **IL-2 and IFN-γ quantification** in cocultures
- **D19** CAR-T cells p**roliferation** analysis
- **D20** **Residual tumor cells** percentage analysis

# CAR-T cell proliferation and cytotoxicity after cocultures

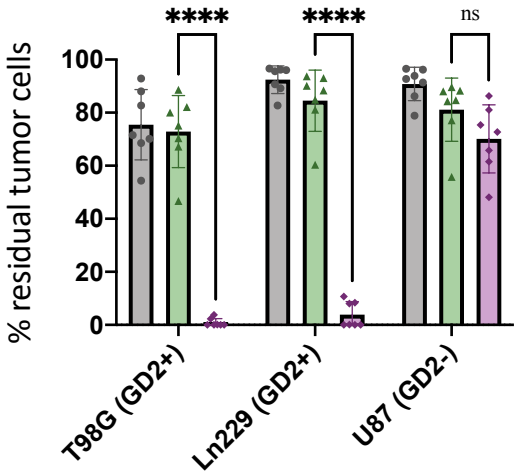
***Transduction efficiency ~ 70%***

No transduced T cells
CD19.CAR
GD2.CAR

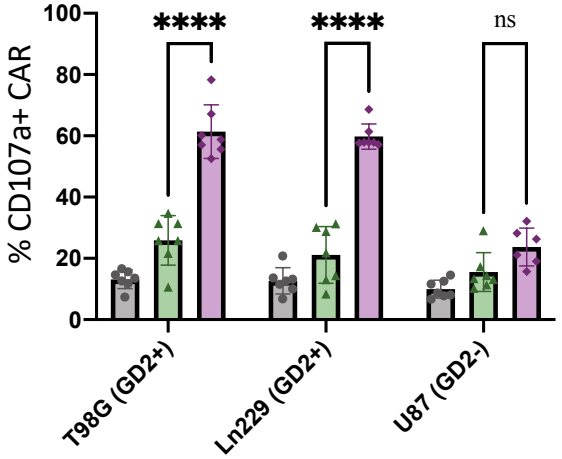
***% Proliferating CAR-T cells (1:1)***




***% Residual tumor cells (1:5)***



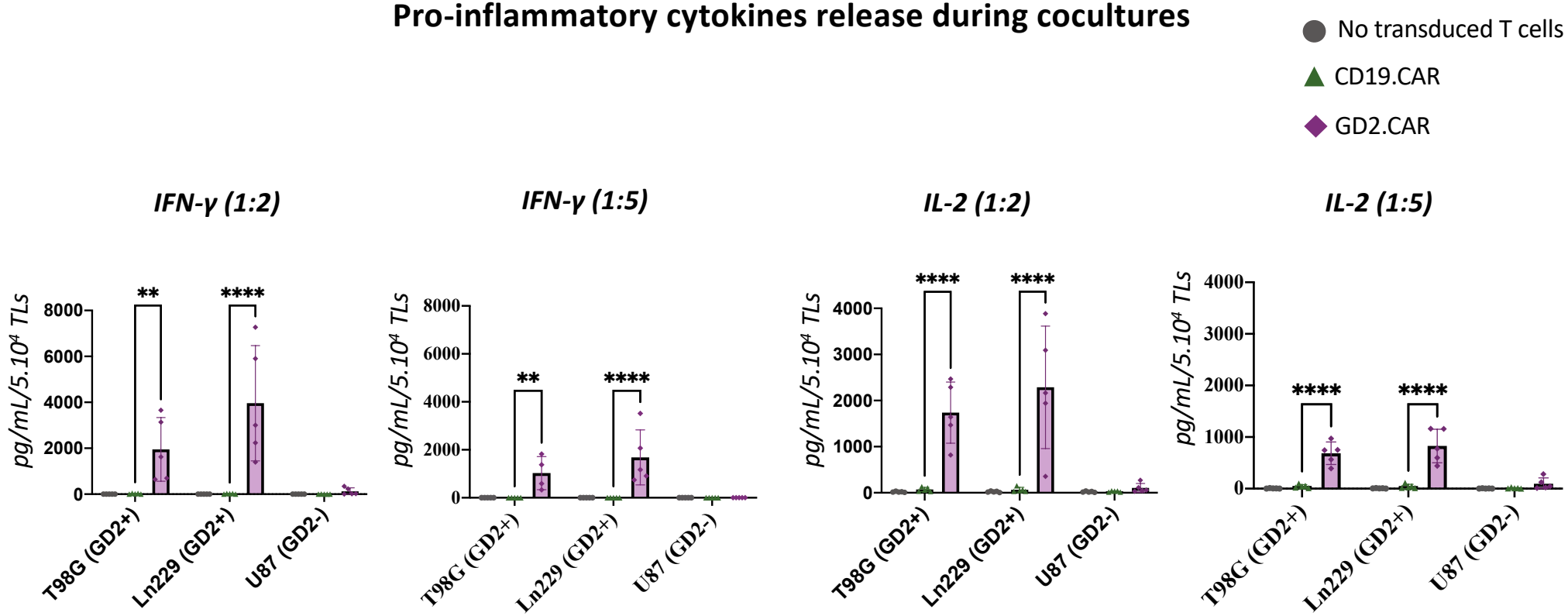

***% CAR-T cells degranulation (1:1)***




Specific antitumor activity of GD2 CAR-T cells

*GBM : glioblastoma ; TLs : T lymphocytes ; Ratio (E:T) : Effector:Target*

## Pro-inflammatory cytokines release during cocultures

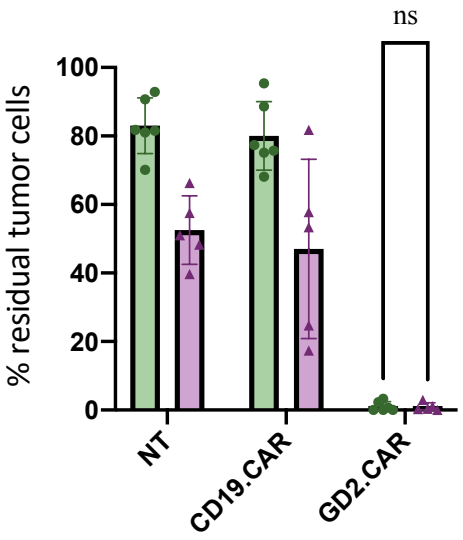



Specificity of CAR-T cell cytotoxic activity is confirmed by pro-inflammatory cytokines release

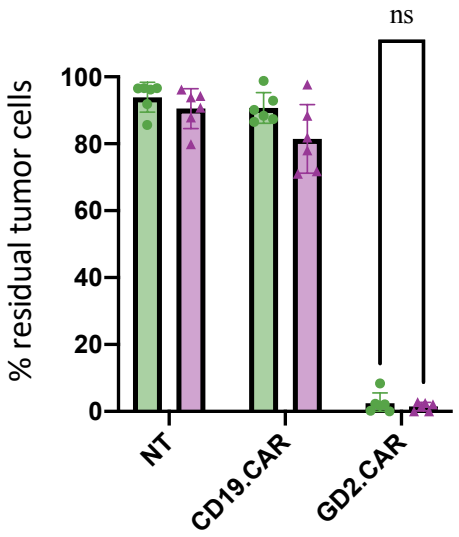
## Impact of hypoxia (2% $O_2$) on CAR-T cell cytotoxicity (1:5)

Normoxia
Hypoxia

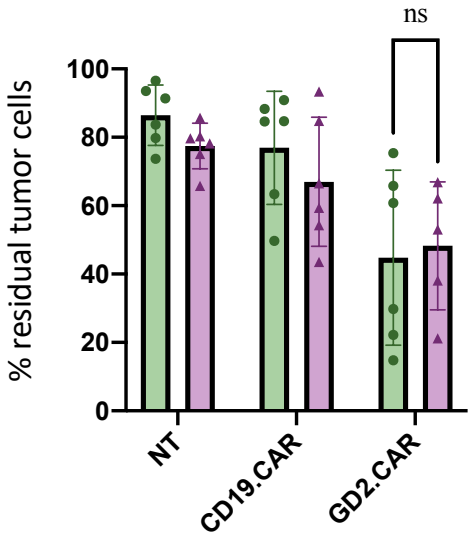
**CAR-T cells and T98G (GD2+)**




**CAR-T cells and Ln229 (GD2+)**




**CAR-T cells and U87 (GD2-)**



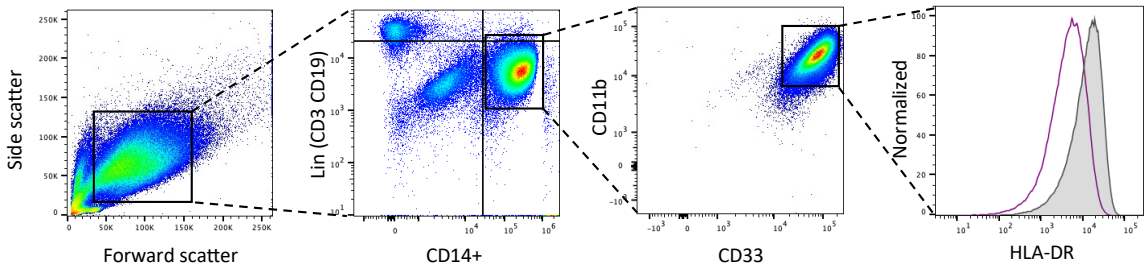

No impact of hypoxia on cytotoxic activity of CAR-T cells

*TLs : T lymphocytes*

# Generation of GBM induced m-MDSCs

***Phenotype analysis of CD14+/CD33+/CD11b+ cells***

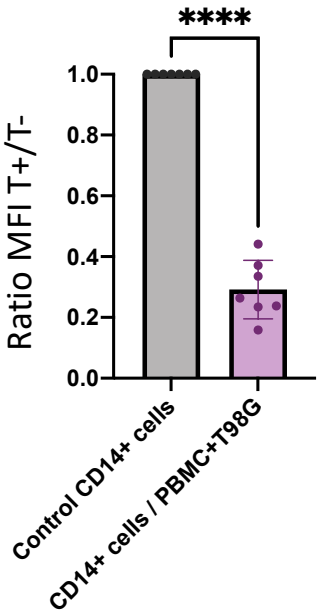
*CD14+ sorting purity : 70-80%*




Controls CD14+ cells

(PBMC+T98G) CD14+

**HLA-DR MFI ratio**



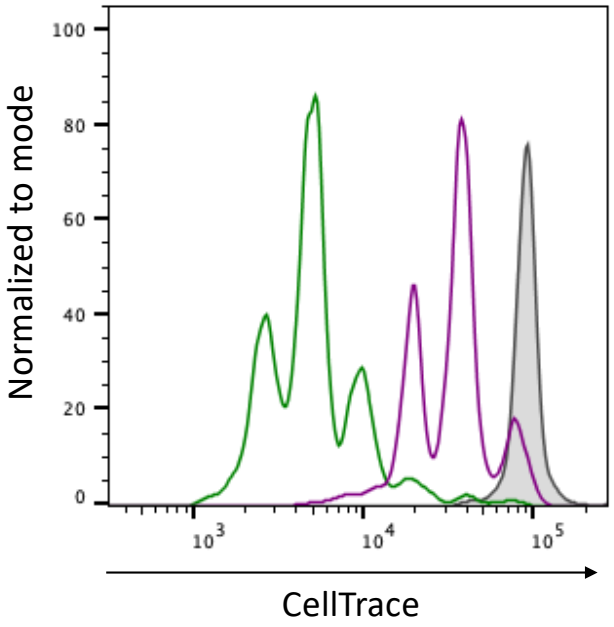

CD14+/CD33+/CD11b+ : ↘ MFI HLA-DR after 7 days cocultures between PBMCs and T98G cell line

*GBM : glioblastoma ; MDSCs : myeloid derived supressor cells ; TL : T lymphocyte*

# Generation of GBM induced m-MDSCs

***Immunosuppressive activity of CD14+ cells : TLs proliferation index after cocultures with CD14+ cells***

*Flow cytometry analysis for one representing experience*

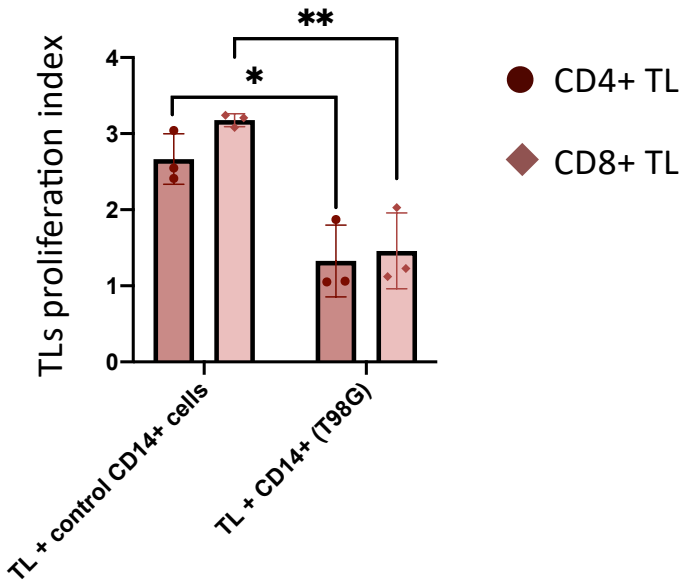



Non stimulated T cells

Stimulated T cells
+ control CD14+ cells

Stimulated T cells
+ CD14+ / PBMCs+T98G

**TL proliferation index**
**after cocultures with CD14+ cells**



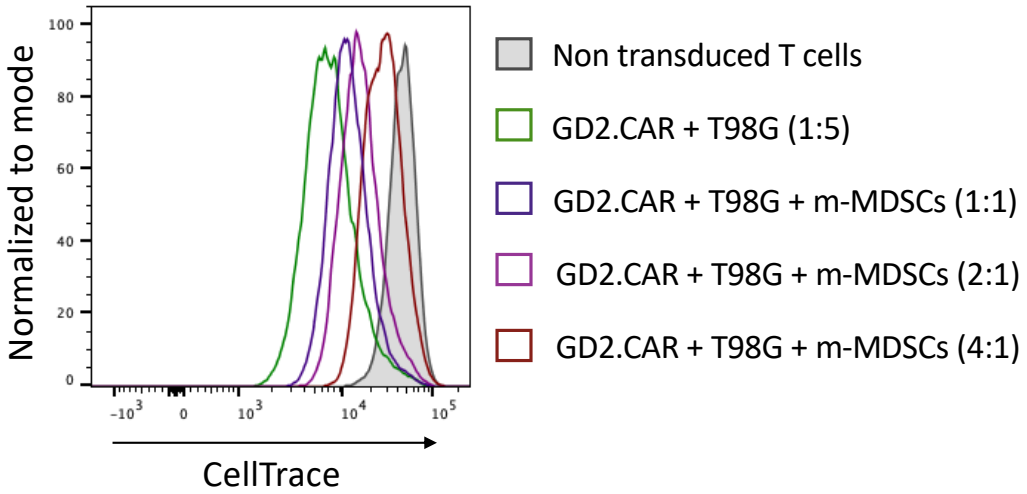

CD14+ cells from cocultures PBMC/T98G reduced proliferation of T cells

*GBM : glioblastoma ; MDSCs : myeloid derived supressor cells ; TL : T lymphocyte ; FC : flow cytometry*

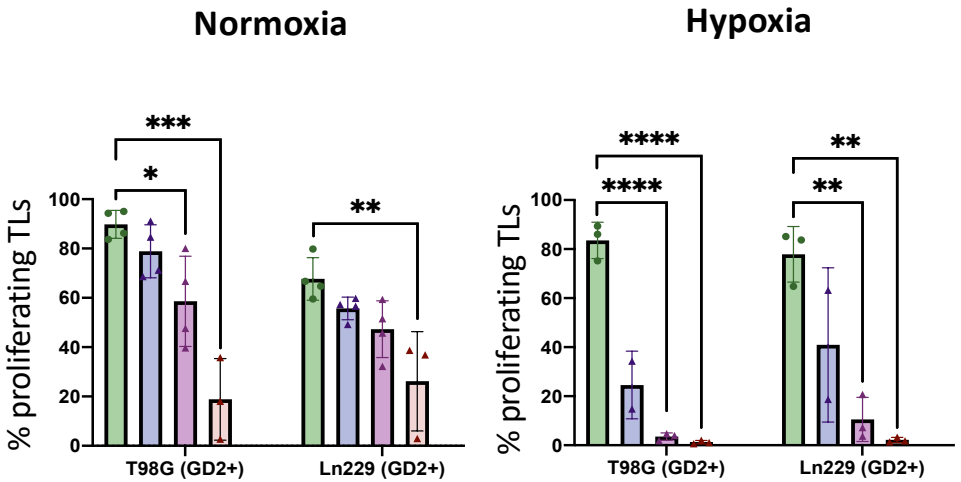
# Generation of GBM induced m-MDSCs

***Immunosuppressive activity of CD14+ cells cocultured with CAR-T cells***

*Flow cytometry analysis for one representing experience*



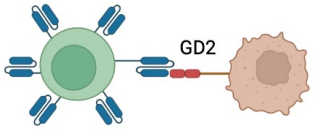

GD2.CAR seuls
GD2.CAR + CD14+ 1:1
GD2.CAR + CD14+ 2:1
GD2.CAR + CD14+ 4:1



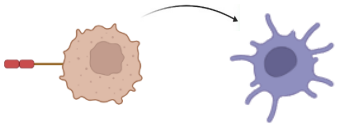

GBM induced m-MDSCs can reduce GD2 CAR-T cells proliferation in our GBM *in vitro* model

*GBM : glioblastoma ; m-MDSCs : monocyte-like myeloid derived supressor cells ; GD2.CAR : GD2 CAR-T cells ;*

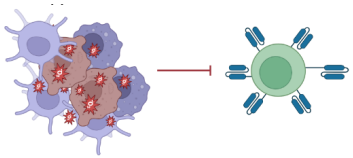
## Conclusions




GD2 CAR-T cells exert a **specific** and **efficient** cytototoxic activity against GD2+ GBM tumor cells *in vitro* in both normoxia and hypoxia condition

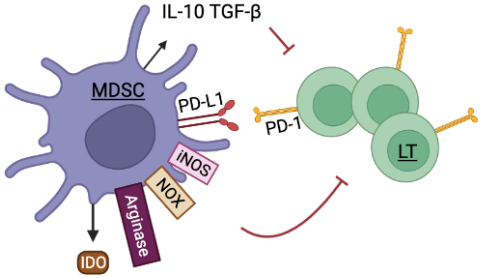


**T98G** GBM cell line can **induce m-MDSCs** *in vitro*



T98G induced **m-MDSCs inhibit** CAR-T cells **proliferation** and are **potentialized in hypoxia**

## Perspectives




MDSCs characterization : mechanism of action **to target m-MDSC**

3D models (spheroids)

Clinical study: patient samples

# Merci pour votre attention

Marie-Thérèse Rubio
David Moulin

**Cindy Lamorlette**
José-Arthur Guimaraes
Maud D'aveni
Laura Boulangé
Danièle Bensoussan

Sophie Pinel
Cédric Boura
Alicia Chateau
Tristan Briot

Gianpietro Dotti
Barbara Savoldo
Rania Tsahouridis

Dr Loïc Reppel (IMoPA – Nancy – UMR 7365 CNRS-UL)

**Premières journées de recherche en Immuno-Oncologie**

**13 et 14 mars 2025**