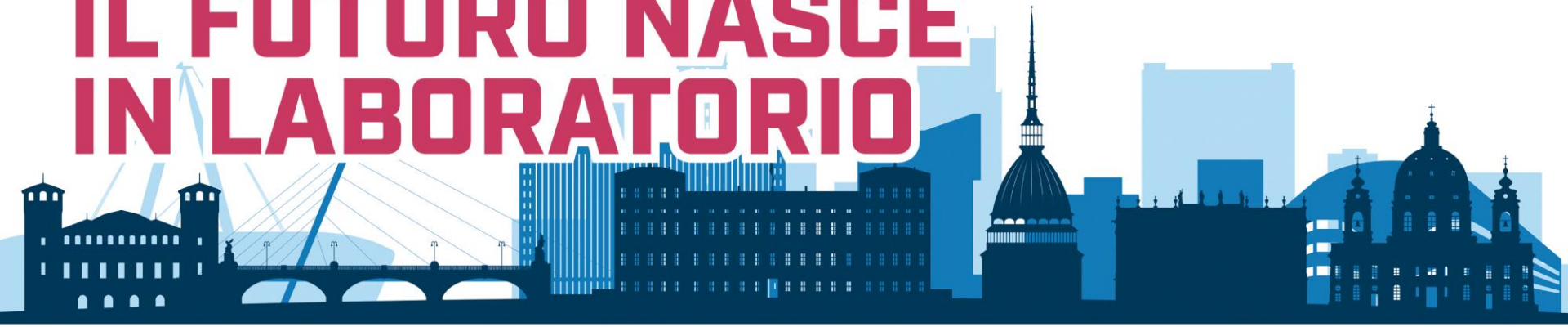
REGIONALE SIES – Delegazione Piemonte

# YOUNG SCIENCE FORUM: IL FUTURO NASCE IN LABORATORIO



## Setup of an in-house small-scale anti-CD19 CAR T-cell manufacturing process: immunophenotypical characterization and functional assessments

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TORINO, ACCADEMIA DI MEDICINA | 4-5 GIUGNO 2026

## Disclosures of Valentina Griggio

| Company name | Research support | Employee | Consultant | Stockholder | Speakers bureau | Advisory board | Other |
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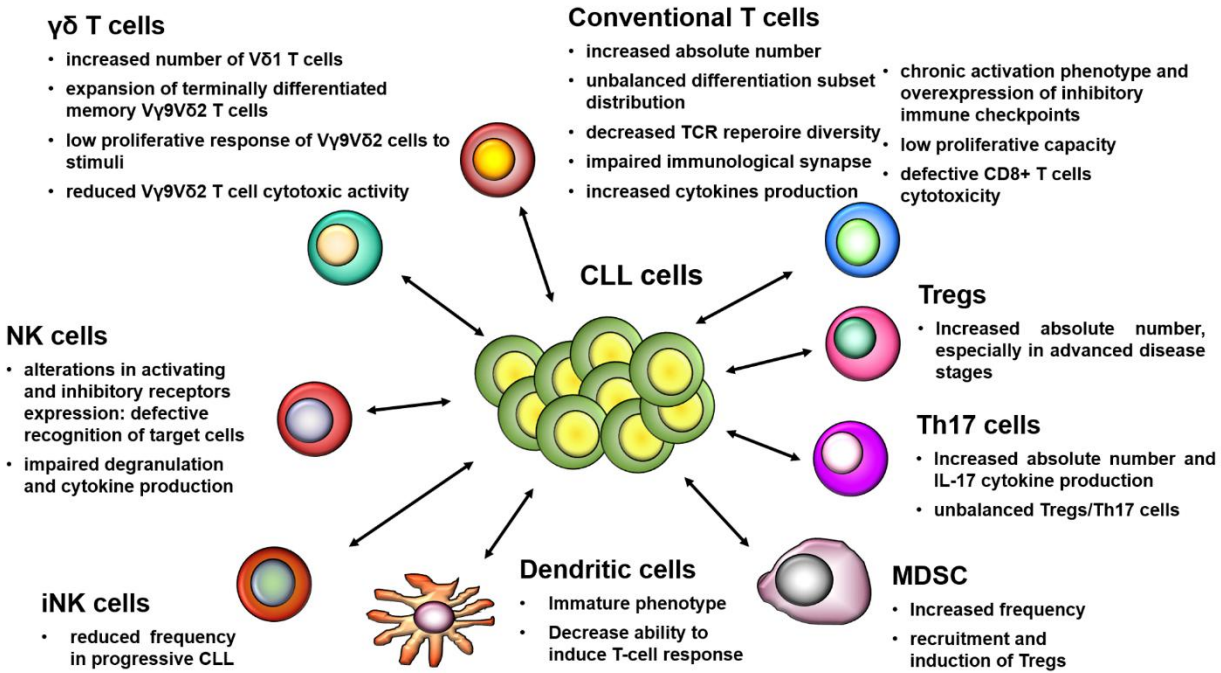
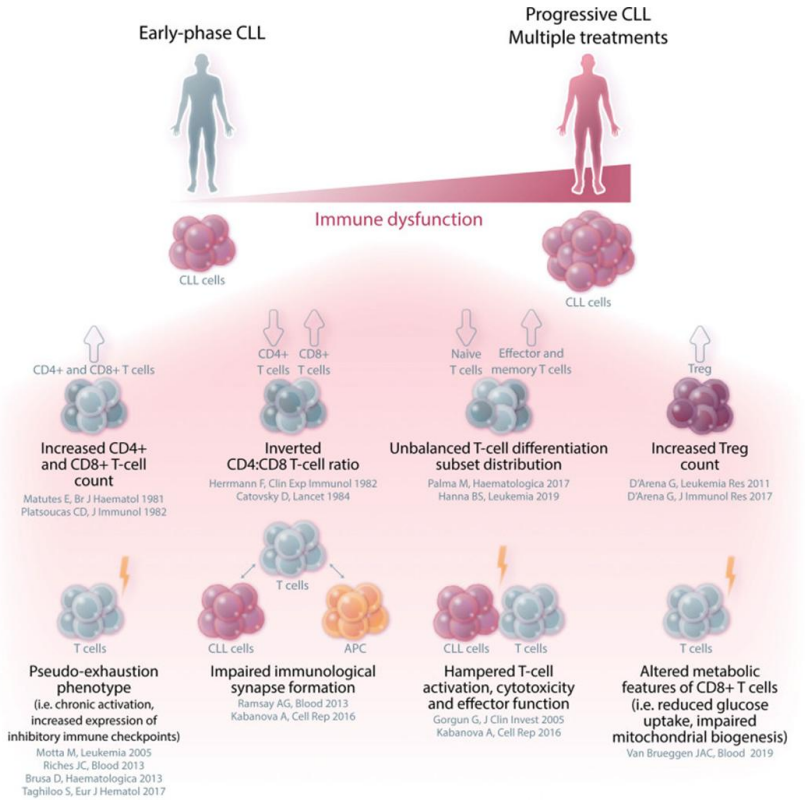
No conflict of interest to disclose




The complex interactions between leukemic cells, microenvironment and immune system in CLL

*Griggio V et al. Frontiers in Immunology (2020)*




*Vitale et al. Hemasphere (2023)*

T-cell compartment, in particular, is characterized by multiple phenotypical and functional alterations

The clinical correlates related to immune dysfunction in CLL

- infections
- autoimmune phenomena
- second primary malignancies
- poor response to vaccines
- suboptimal efficacy of immunotherapy

While **CD19 CAR T-cell therapy** has revolutionized the treatment of B-cell malignancies, its efficacy in CLL remains suboptimal:

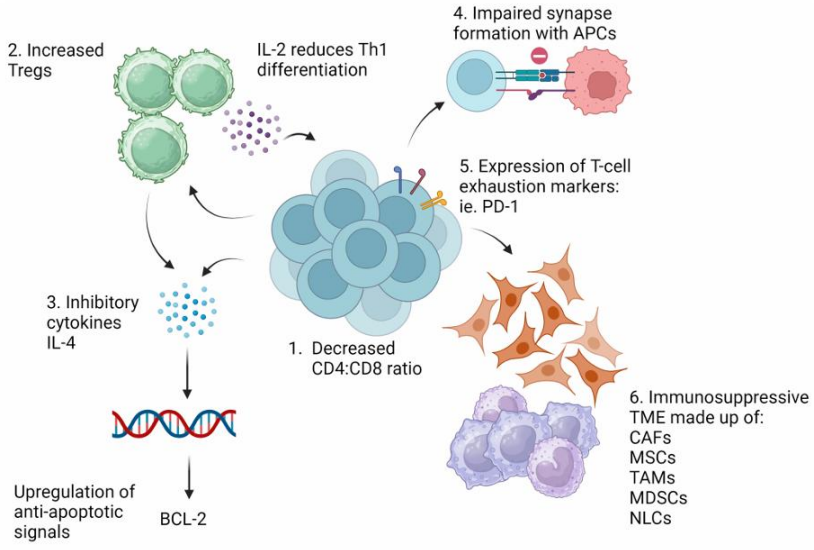
Clinical trial involving limited number of partecipants with CLL

Less deep and durable responses compared with other B-cell malignancies

| Product Administered | Product Specifics | Patients Evaluable for Response, Number | Lymphodepleting Chemotherapy | ORR | CR Rate | Median DOR | Median PFS | Median OS | Safety | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Axi-cel (KTE-C19) + IV IL-2 | Second generation | CLL = 4 | Flu/Cy | 75% (3/4) | 25% (1/4) | 7 months | NA | NA | Grade ≥3 AEs 100% (4/4) | 67 |
| Axi-cel (KTE-C19) | Second generation | CLL = 4<br>RS = 1 | Flu/Cy | CLL: 100% (4/4)<br>RS: 100% (1/1) | CLL: 75% (3/4)<br>RS: 0% (0/1) | CLL: 14 months<br>RS: 1 months | NA | NA | CLL: grade ≥3 AEs 50% (2/4)<br>RS: grade ≥3 AEs 100% (1/1) | 68 |
| Brexu-cel (KTE-X19) | Second generation; depletion of circulating CD19-expressing malignant cells | CLL =15 | Flu/Cy | 47% (7/15) | 13% (2/15) | NA | NA | NA | Grade ≥3 AEs 100% (15/15)<br>CRS 80% (12/15), grade ≥3 CRS 7% (1/15)<br>NE 73% (11/15), grade ≥3 NE 20% (3/15) | 70 |
| Tisa-cel (CTL019) | Second generation | CLL = 3 | Different regimens (bendamustine ± rituximab, pentostatin/Cy) | 100% (3/3) | 33% (1/3) | 10 months | NA | NA | NA | 71 |
| Tisa-cel (CTL019) | Second generation | CLL = 14[#] | Different regimens (Flu/Cy, bendamustine, pentostatin/Cy) | 57% (8/14) | 29% (4/14) | CR patients, 40 months<br>PR patients, 7 months | 7 months | 29 months | CRS 63% (9/14), grade ≥3 CRS 43% (6/14)<br>NE 36% (5/14), grade ≥3 NE 7% (1/14) | 72 |
| Tisa-cel (CTL019) | Second generation | CLL = 32 ($5 \times 10^8$ CAR T cells, HD = 19; $5 \times 10^7$ CAR T cells, LD = 13) | Different regimens (bendamustine, Flu/Cy, pentostatin/Cy, oxaliplatin/Flu/cytarabine/ofatumumab, gemcitabine/oxaliplatin) | HD: 53% (10/19)<br>LD: 31% (4/13) | HD: 37% (7/19)<br>LD: 15% (2/13) | NA | 1 months[a] | 64 months[a] | CRS 63% (24/38), grade ≥3 CRS 24% (9/38)[a]<br>Grade≥3 NE 8% (3/38)[a] | 73 |
| MSKCC CD19 CAR T cells | Second generation | CLL = 8 | None or Cy | 0% | 0% | NA | NA | NA | Grade ≥3 AEs 50% (4/8) | 75 |
| MSKCC CD19 CAR T cells | Second generation | CLL = 16[#] | None or different regimens (Cy, bendamustine, Flu/Cy) | 38% (6/16) | 19% (3/16) | NA | 3 months | 17 months | CRS 100% (16/16), grade≥3 CRS 12% (2/16)<br>NE 38% (6/16), grade ≥3 NE 6% (1/16) | 76 |
| JCAR014 | Second generation; 1:1 ratio of CD4+:CD8+ CAR T cells; ex vivo stimulation with antigen-presenting cells | CLL = 18<br>RS = 5 | Cy, Flu, or Flu/Cy | CLL: 72% (13/18)<br>RS: 60% (3/5) | CLL: 11% (2/18)<br>RS: 40% (2/5) | NA | 8.5 months[a] | NR | CLL: CRS 95% (18/19), grade ≥3 CRS 10% (2/19), NE 37% (7/19), grade ≥3 NE 26% (5/19)<br>RS: CRS 40% (2/5), grade ≥3 CRS 0. NE 20% (1/5), grade ≥3 NE 20% (1/5) | 86 |
| Liso-cel (JCAR017) | Second generation; 1:1 ratio of CD4+:CD8+ CAR T cells; removal of non-T-cell impurities before activation and transduction; ex vivo stimulation with cytokines | CLL = 96 | Flu/Cy | 48% (46/96) | 18% (17/96) (CR/CRi) | 35 months | 18 months | NA | CRS 85% (99/117), grade ≥3 CRS 10% (10/117)<br>NE 45% (53/117), grade ≥3 NE 19% (22/117) | 89 |
| CD19 CAR T cells (Baylor College of Medicine) | Third generation with 2 costimulatory domains (CD28 and 4-1BB) | CLL = 2 | Flu/Cy | 50% (1/2) | 0% | NA | 13 months | >40 months | CRS 2% (3/15), grade ≥3 CRS 7% (1/15)[a]<br>NE grade ≥3 13% (2/15)[a] | 91 |
| CD19 CAR T cells (Heidelberg University Hospital) | Third generation with 2 costimulatory domains (CD28 and 4-1BB) | CLL = 4 | Flu/Cy | 43%[c] | 29%[c] | NA | NA | NA | CRS grade ≥3 0.7% (2/27)[a]<br>NE grade ≥3 0%[a] | 97 |
| MSKCC CD19 CAR T cells | Fourth generation with 2 signaling domains (CD28 and CD3ζ) and 4-1BB ligand expression | CLL = 9<br>RS = 3 | Flu/Cy or Cy alone | CLL: 44% (4/9)<br>RS: 67% (2/3) | CLL: 33% (3/9)<br>RS: 67% (2/3) | NA | NA | NA | CRS 39% (11/28), grade ≥3 CRS 3% (1/28)[a]<br>CRS 39% (11/28), grade ≥3 CRS 10% (3/28)[a] | 98 |
| JCAR014 and ibrutinib | Second generation | CLL = 18 | Flu/Cy | 83% (15/18) | 22% (4/18, all CRi) | NA | NA (1-year PFS, 38%) | NA (1-year OS, 64%) | CRS 74% (14/19), grade ≥3 CRS 0%<br>NE 26% (5/19), all grade 3 | 112 |
| CTL119 and ibrutinib | Second generation | CLL = 16 | Flu/Cy or bendamustine | 69% (11/16) | 44% (7/16) | NA | NR (48-months PFS, 70%) | NR (48-months OS, 84%) | CRS 95% (18/19), grade ≥3 CRS 11% (2/19)<br>NE 26% (5/19), grade ≥3 NE 5% (1/19) | 113 |
| Liso-cel (JCAR017) and ibrutinib | Second generation | CLL = 19 | NA | 95% (19/19) | 47% (9/19) CR/CRi | NA | NA | NA | CRS 74% (14/9), grade ≥3 CRS 5% (1/19)<br>NE 32% (6/19), grade ≥3 NE 16% (3/19) | 114 |

*Vitale C et al., Haemasphere (2023)*

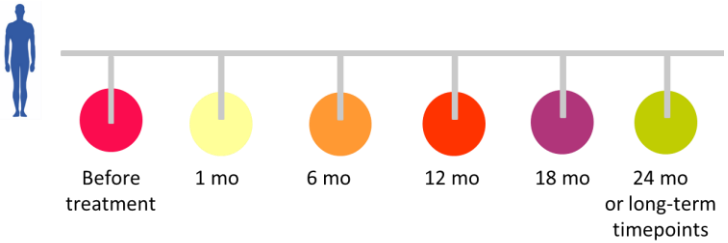
One of the major determinants of CAR T-cell therapy limited efficiency is T-cell exhaustion and a tumor-supportive TME




Therefore, a deeper understanding of these mechanisms is essential to improve CAR T-cell manufacturing and therapeutic outcomes

*Borogovac A et al., Cancer Drug Resist (2023)*

One of our laboratory's main research interest is the study of the **immunomodulatory properties** of targeted therapies. Besides their direct anti-tumor activity, targeted agents such as **ibrutinib** or **venetoclax** have shown positive immune changes




- Immunophenotype
- Functional assays
- Correlation with prognostic factors, disease and response variables

641. CLL: BIOLOGY AND PATHOPHYSIOLOGY, EXCLUDING THERAPY: POSTER III | NOVEMBER 29, 2018

**Ibrutinib Treatment Mitigates Phenotypic Alterations of Non-Neoplastic Immune Cell Compartments in Chronic Lymphocytic Leukemia**

Valentina Griggio, Candida Vitale, MD, Maria Todaro, PhD, Francesca Romana Mauro, Chiara Salvetti, MD, Daniela Pietrasanta, Iolanda Donatella Vincelli, MD, Lydia Scarfo, MD, Giovanni Del Poeta, MD, Gianluca Gaidano, MD PhD, Valter Gattei, MD, Robin Foà, MD, Mario Boccadoro, MD, Marta Coscia, MD

Check for updates

*Blood* (2018) 132 (Supplement 1): 4412.

https://doi.org/10.1182/blood-2018-99-116068

641. CHRONIC LYMPHOCYTIC LEUKEMIA: BASIC AND TRANSLATIONAL | NOVEMBER 3, 2025

**Venetoclax-based therapy remodels T-cell and NK-cell immunity in patients with chronic lymphocytic leukemia**

Giorgia Mancin, Valentina Griggio, Rebecca Jones, Giulia Bondielli, Andrea Visentin, Riccardo Moia, Anna Maria Frustaci, Alessandro Bosi, Marzia Varettoni, Francesca Perutelli, Maria Chiara Montalbano, Gioacchino Catania, Paolo Sportoletti, Luca Laurenti, Francesca Mauro, Lorella Orsucci, Luca Arcaini, Francesco Passamonti, Marina Deodato, Gianluca Gaidano, Livio Trentin, Benedetto Bruno, Candida Vitale, Marta Coscia

Check for updates

*Blood* (2025) 146 (Supplement 1): 5657.

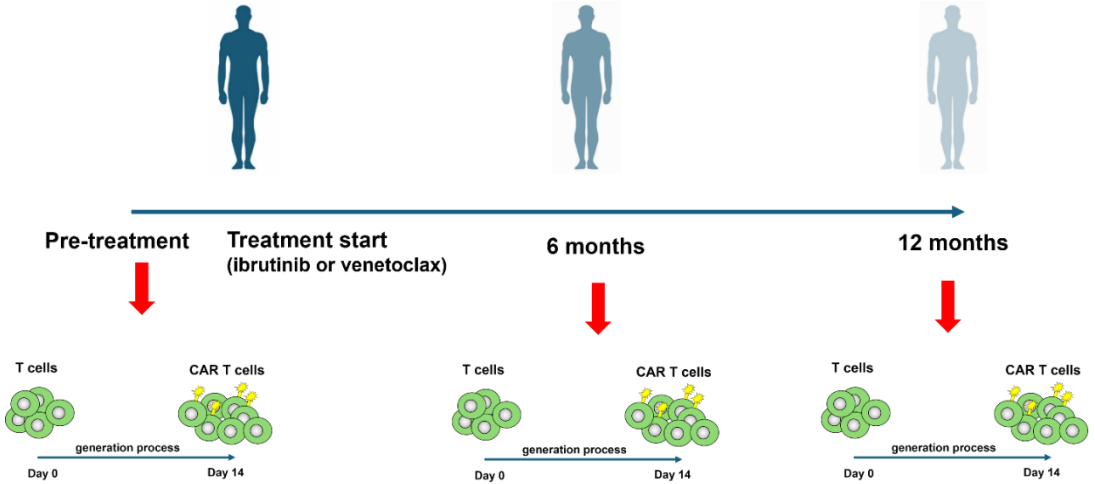
https://doi.org/10.1182/blood-2025-5657

Immune changes are possibly linked to off-tumor and off-target effects and could also be associated to the reduction of tumor burden

The **GIMEMA CLL2020** study aims to evaluate, in CLL patients, the impact of treatment with targeted agents (i.e. **ibrutinib** or **venetoclax**) on T cells and on patient-derived **anti-CD19 CAR T** cells in terms of:

- manufacturing efficiency
- immunophenotypical profile
- functional properties

Samples were collected before the start of treatment and after 6 and 12 months of therapy with ibrutinib (n=7) or venetoclax (n=8)




The functionality and persistence of infused CAR T cells are strictly connected to the composition and the fitness of both the starting T-cell source and the final CAR T-cell product

## CD19 CAR T-cell generation workflow

### Starting material – day 0

PBMC usually contain a large number of leukemic B cells

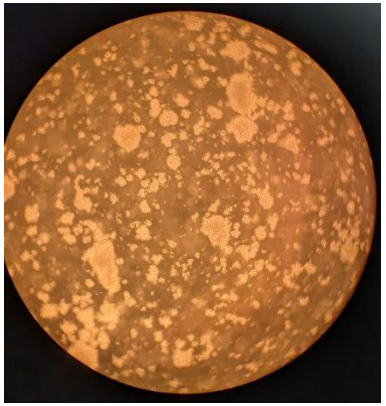
→ B-CELL DEPLETION (positive selection of leukemic cells)

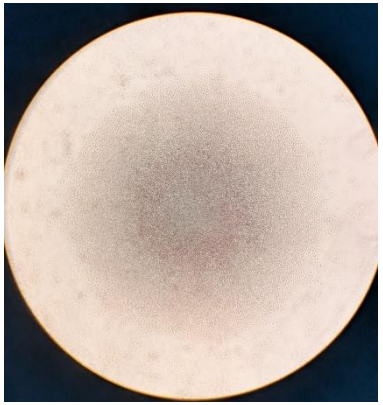
→ T-CELL ENRICHMENT (negative selection of T cells)

## CD19 CAR T-cell generation workflow

### T-cell activation – day 0

Delayed activation or fail to activate when working with thawed PBMC





→ T CELLS ARE ACTIVATED USING A COLLOIDAL MATRIX CONTAINING αCD3 and αCD28 agonists

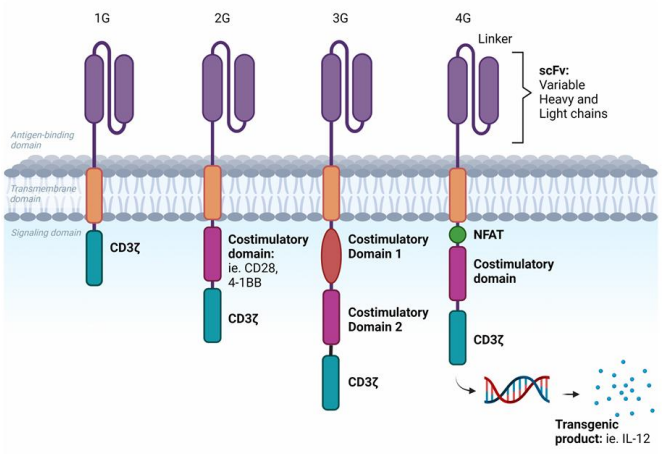
→ Early T-cell activation is evaluated by the appearance of characteristic cellular clusters under light microscopy. When sufficient material is available, activation status is further characterized by flow cytometry through the expression of **CD69** and **CD25**

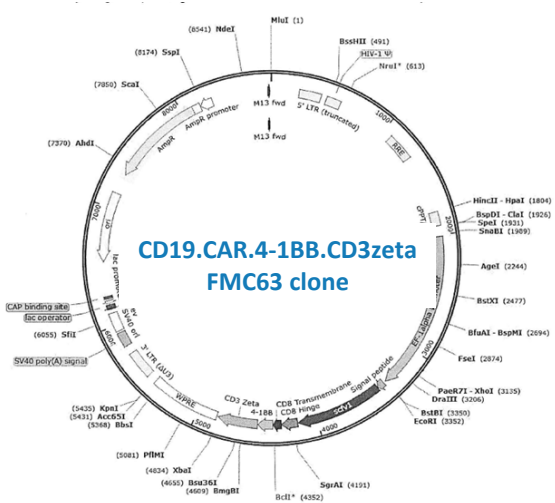
## CD19 CAR T-cell generation workflow

# T-cell lentiviral transduction – day 1-3

Target antigen: **CD19**

### Second generation CAR T cells



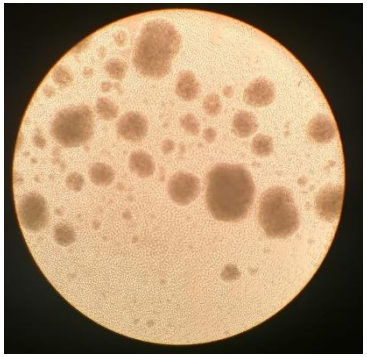




### CAR expression

Anti-idiotype antibodies specifically recognize the variable scFv region of a CAR

*Borogovac A et al., Cancer Drug Resist (2023)*

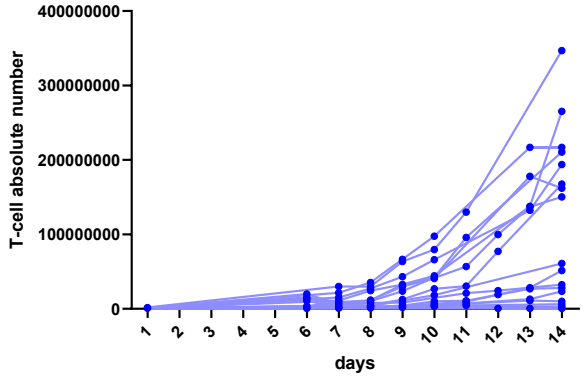
Our **CD19 CAR T-cell generation workflow**



## CAR T-cell expansion – approx 9 days

IL-7/IL-15-driven expansion depends on:

- T-cell differentiation status
- T-cell exhaustion
- metabolic fitness




T-cell proliferative ability can vary substantially among CLL patients depending on disease-related immune dysfunction and baseline T-cell composition

→ CAR T CELLS ARE CULTURED IN THE PRESENCE OF IL-7 AND IL-15

## CD19 CAR T-cell immunophenotypical characterization

**Immune determinants** of response to CAR T-cell therapy

Favourable

- Memory-like T-cell phenotype
- High ex vivo proliferative potential
- IL-6/STAT3 pathway activity

Unfavourable

- Higher PD-1, TIM-3 and LAG-3 expression
- Terminal differentiation

*Fraietta JA et al., Nature Medicine (2018)*

**Lineage composition**
**CD3, CD4, CD8**

**T-cell differentiation subsets**
**CCR7, CD45RA, CD95**

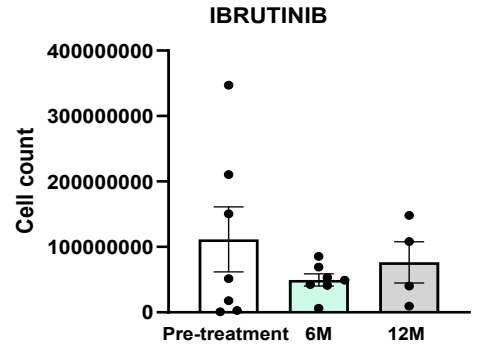
**Activation markers**
**CD25, CD69, HLA-DR**

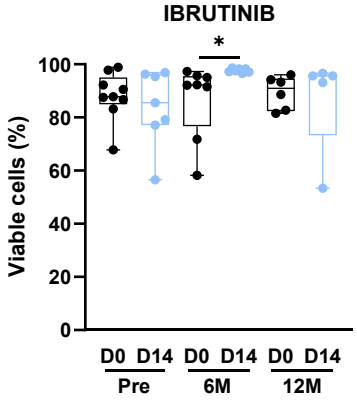
**T helper subsets**
**CCR6, CXCR3**

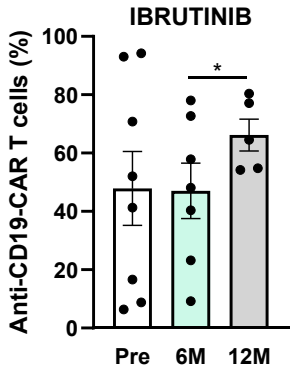
**Immune checkpoint**
**PD-1, TIM-3, CTLA-4, TIGIT, BTLA**

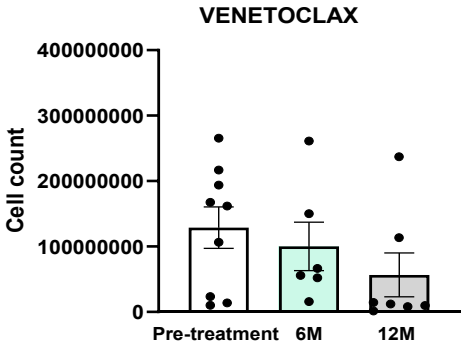
**Migration markers**
**CD62L, CXCR3**

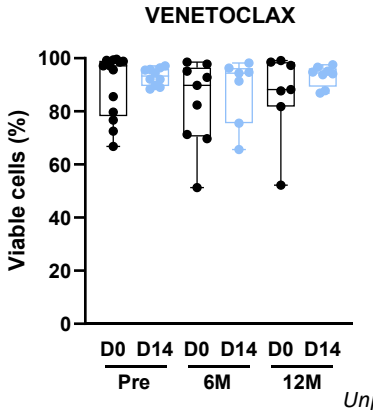
# Generation process efficiency: expansion ability, viability and CAR transduction efficacy

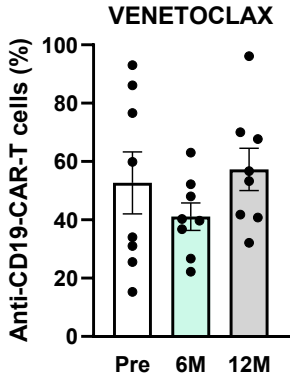


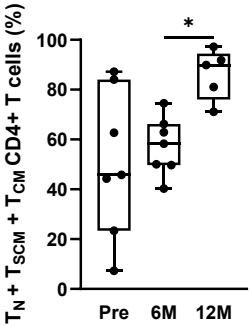



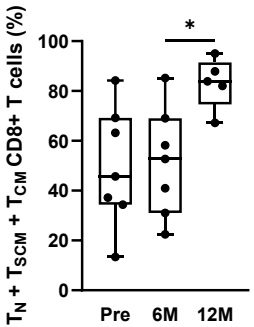




*Unpublished data, international workshop on CLL (iwCLL) Congress 2025 (poster #1902)*

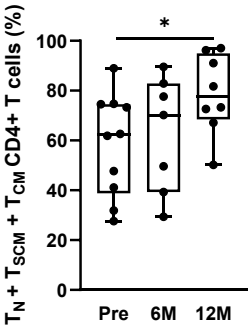
# Targeted therapy induces changes in the subpopulation frequency of CAR T cells

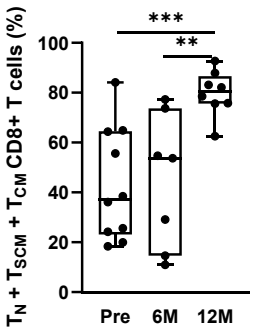
## IBRUTINIB



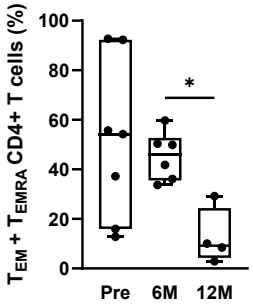

## VENETOCLAX

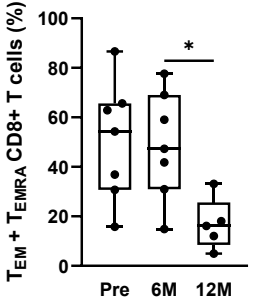



↑ Less differentiated T-cell subsets

## IBRUTINIB

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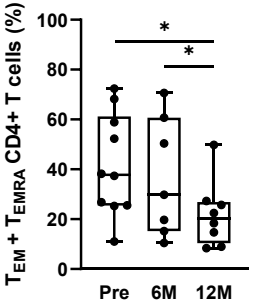
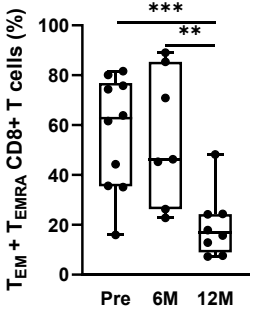
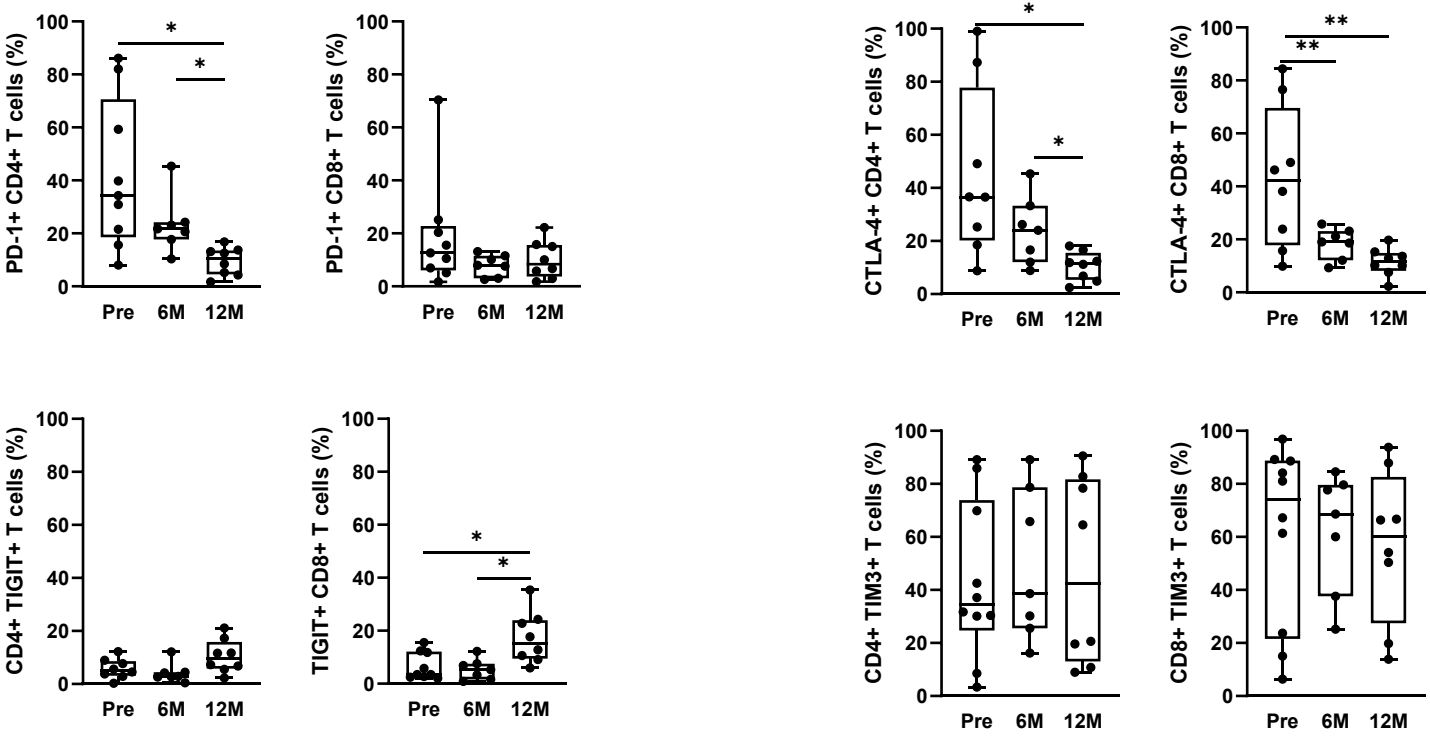



↓ More differentiated T-cell subsets

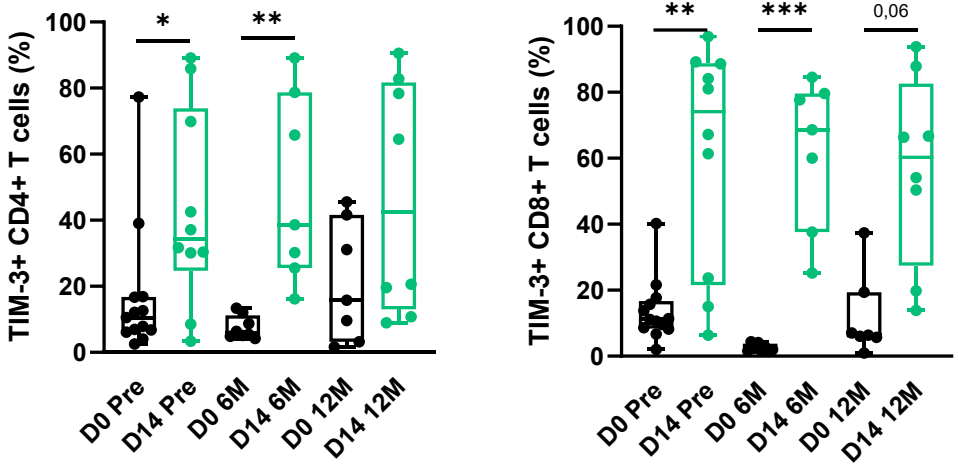
*Unpublished data, international workshop on CLL (iwCLL) Congress 2025 (poster #1902)*

# Treatment with venetoclax modulates the expression of immune checkpoint molecules




*Unpublished data, international workshop on CLL (iwCLL) Congress 2025 (poster #1902)*

## TIM-3 expression increases during *ex vivo* CAR T-cell expansion




Prolonged *ex vivo* culture can alter T-cell fitness through progressive acquisition of exhaustion-associated phenotypes

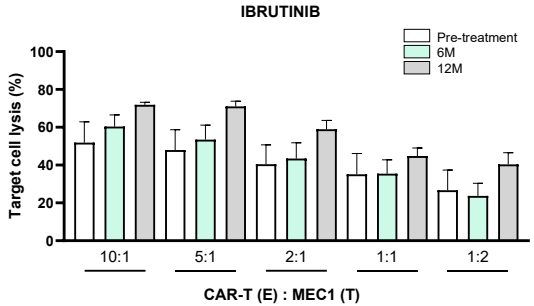
*Unpublished data, international workshop on CLL (iwCLL) Congress 2025 (poster #1902)*

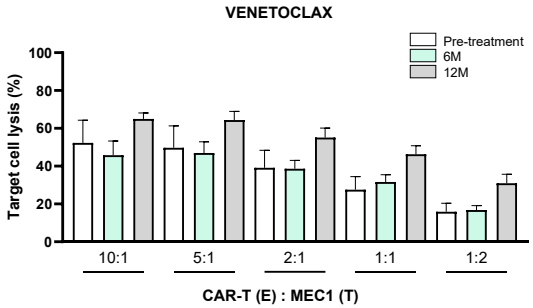
## CD19 CAR T-cell functional assessments

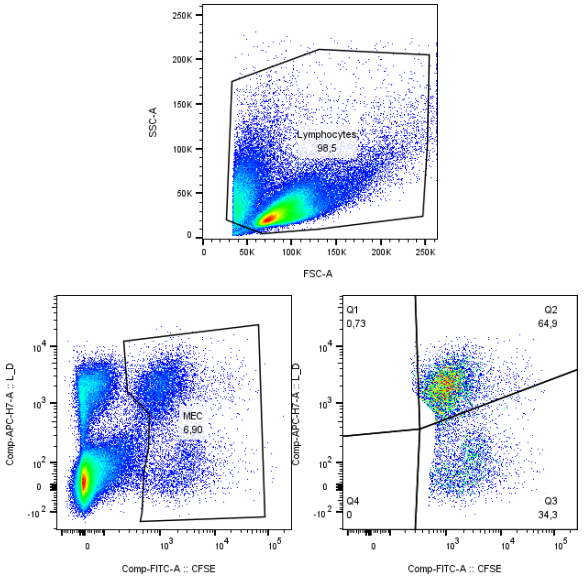
- **Target cell killing**
- **Proliferation ability**
- **Cytokine production (i.e. IFNγ, TNFα)**
- **Degranulation assay**

Autologous CLL cells are not always available for functional assays

## CAR T cells efficiently eliminate CD19+ target cells




Conventional 2D coculture assays do not fully recapitulate the CLL tumor microenvironment

*Unpublished data, international workshop on CLL (iwCLL) Congress 2025 (poster #1902)*

## Future applications and optimization

- An in-house small-scale manufacturing platform was successfully established for the generation and characterization of **CLL patient-derived CD19 CAR T cells**
- This platform provides a versatile tool to investigate the impact of **patient-, disease- and treatment-related variables** on CAR T-cell manufacturing and fitness, enabling direct comparisons between starting T cells and final CAR T-cell products
- The workflow can be further **optimized**, particularly by reducing manufacturing time and implementing more physiologically relevant functional models
- The platform may also serve as a preclinical system to evaluate **immunomodulatory agents and manufacturing strategies** aimed at improving CAR T-cell quality in CLL

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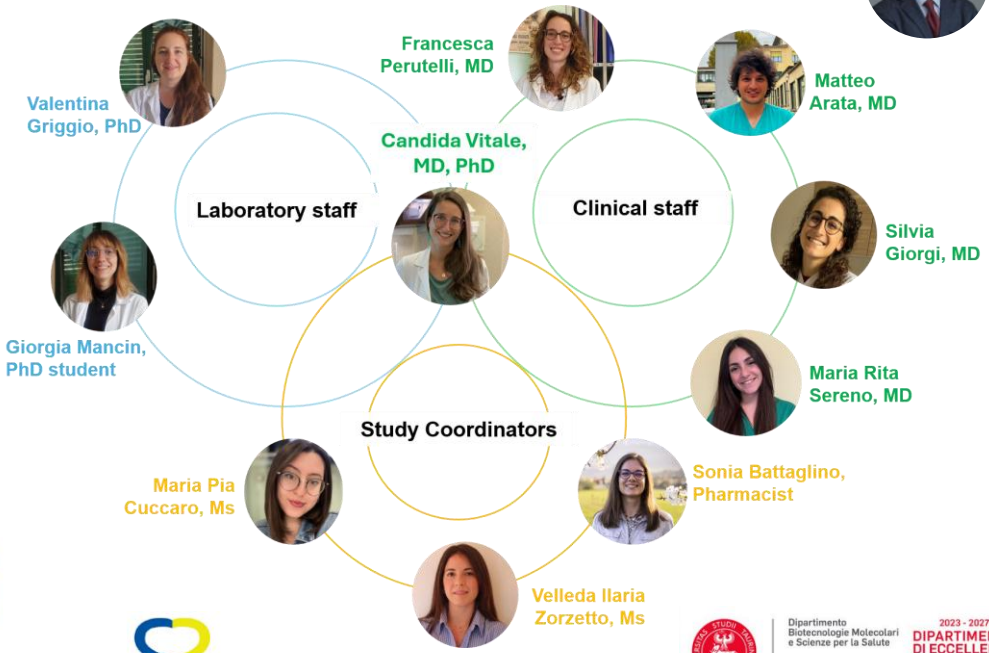


## Acknowledgements


**Prof. Marta Coscia**

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA

**Alfonso Piciocchi**
**Valeria Sargentini**

fondazione GIMEMA onlus
per la promozione e lo sviluppo della ricerca scientifica sulle malattie ematologiche. FRANCO MANDELLI

**Riccardo Moia**
**Prof. Gianluca Gaidano**

UPO
UNIVERSITÀ DEL PIEMONTE ORIENTALE

**Alessandro Bosi**
**Prof. Francesco Passamonti**

Fondazione IRCCS Ca' Granda
Ospedale Maggiore Policlinico

**Lydia Scarfò**
**Prof. Paolo Ghia**

OSPEDALE SAN RAFFAELE

**Prof. Robin Foà**

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## Thank you!

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2023 - 2027 DIPARTIMENTO DI ECCELLENZA