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CAR T-cells in B cell lymphomas and leukemias

Cancer Crosslinks
Gunilla Enblad
2016-10-19

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Outline

- Clinical background
- What is a CAR cell?
- Clinical results
- Uppsala experiences
- Future

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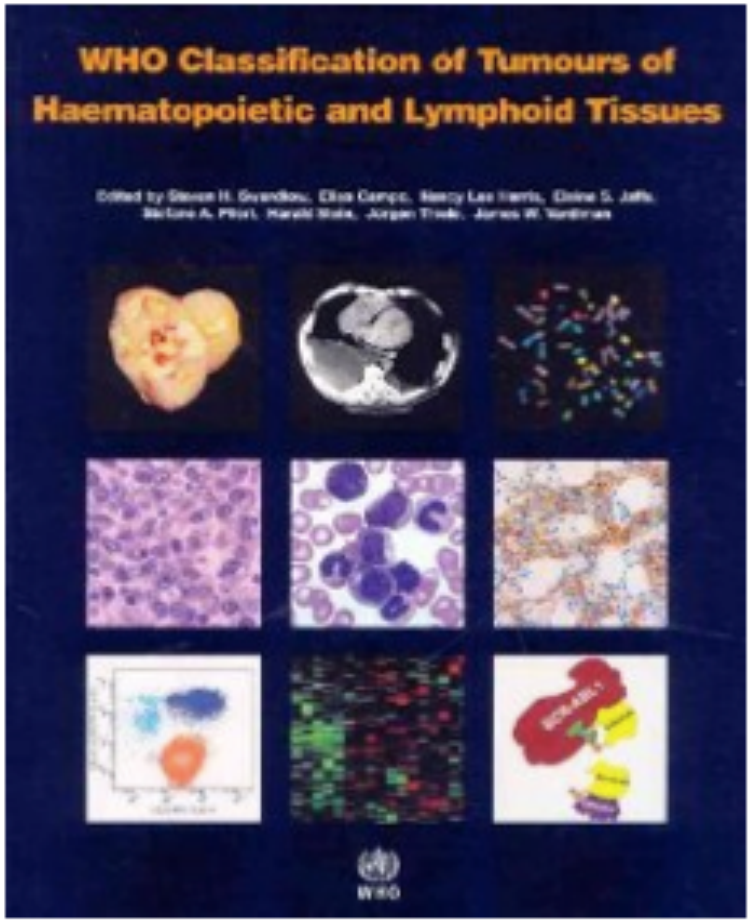
Background

Lymphomas in Sweden

- B-cell lymphoma 1700/year
- T-cell lymphoma 150/year
- Hodgkin lymphoma 170/ year

Leukaemia

- Acute lymphoblastic 100/ year
- Acute myeloid 350/year



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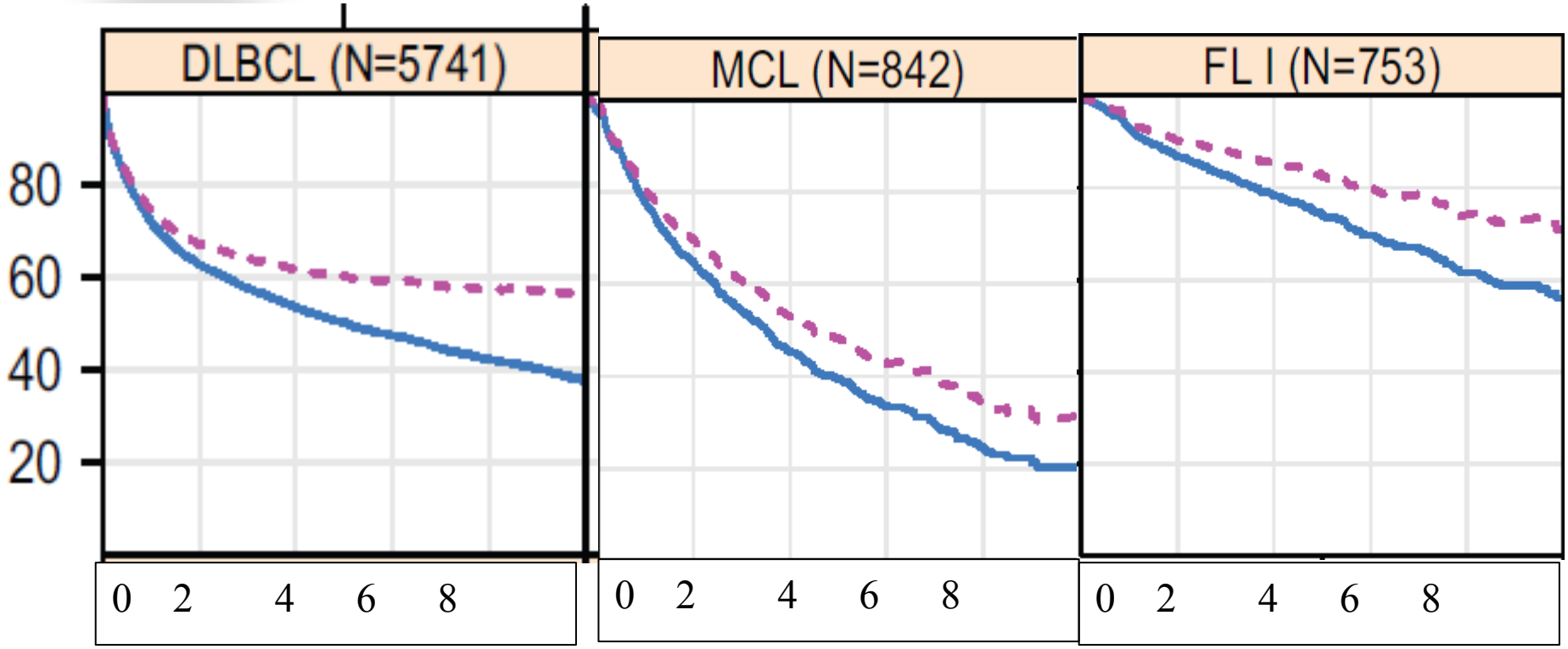
Do we need new treatments?



Despite progress in treatment many patients still die of their disease

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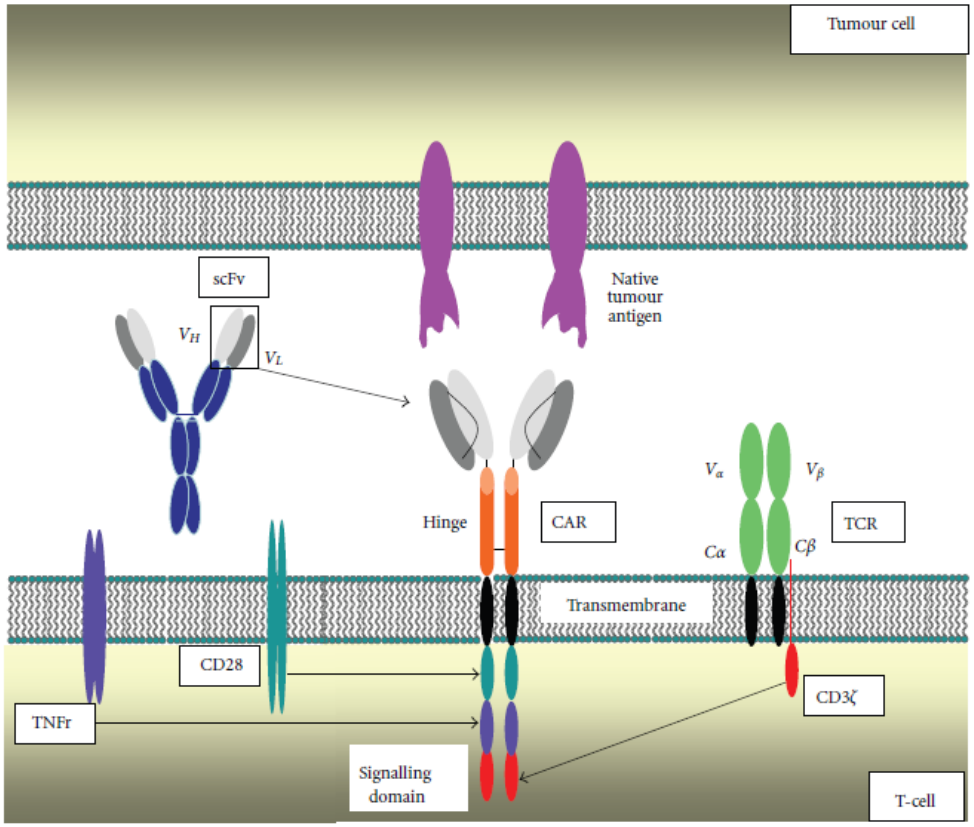
What is a CAR-cell?

- CAR-cells are autologous T-cells
- Genetically modified with a transferred antibody domain (scFV) fused to parts of the T-cell receptor (Z-chain)
- The construct is delivered to the T-cells in the laboratory via a retrovirus vector



Maher J. ISRN Oncology 2012:1-23

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CAR T-cells targets

- Most studies have been performed on tumours expressing CD19
- Expressed on B-cell lymphomas and leukemias
- And normal B-cells
- But not on hematopoietic stem cells or any other cells in the body
- Low likelihood of off-target effects

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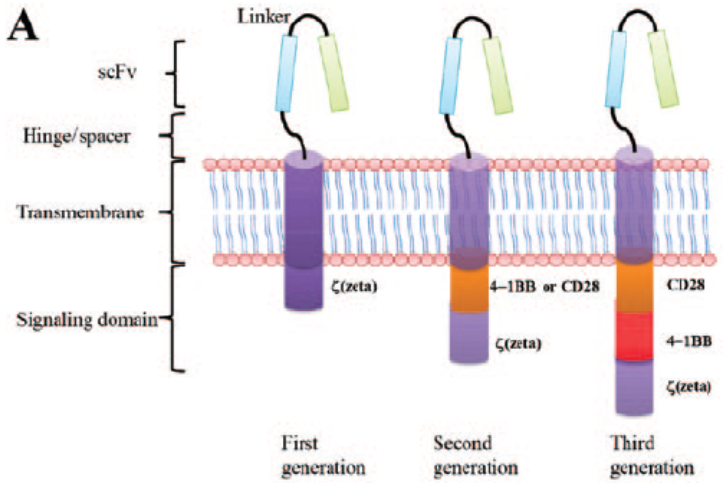
The history of CAR-cells

- 1:st generation
  - No co-stimulatory molecules, rapid clearance from the circulation, small clinical effects
- 2:nd generation
  - Co-stimulatory molecules added (CD28, 4-1BB (CD137)
  - Addition of lymphodepletion
  - Clinical effects
- 3:rd generation
  - CD28 and 4-1BB
  - On-going studies, better *in vitro*- but *in vivo*?



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The tumour micro-environment

- The tumour micro environment is often immune inhibitory
  - Regulatory T-cells, myeloid derived suppressor cells, some tumour associated macrophages
- Lymphodepletion necessary
  - Often cyclophosphamide and fludarabine

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CAR T-cells in lymphoma and leukemia

Case report Porter et al. NEJM 2011.

- Patient with CLL
- Diagnosis 1996, requiring treatment 2002
- Treated with rituximab, fludarabine, bendamustine, alemtuzumab
- Chemotherapy refractory, p53 mutated. Bone marrow engagement and lymph nodes
- Pre-treatment with pentostatin 4 mg /m^2 and cyclophosphamide 600 mg /m^2 dag -4
- 2nd generations CAR 19 T-cell (4-1BB) July 2010

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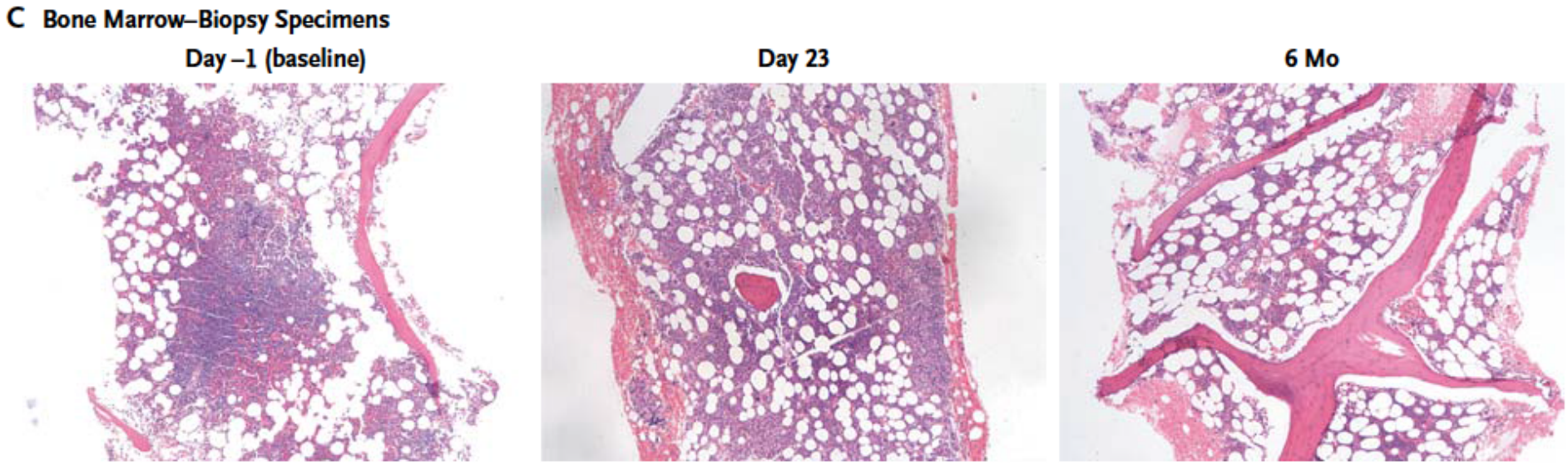
CAR T-cells in lymphoma and leukemia

Case report Porter et al. NEJM 2011 forts

- d 14 fever and chills- Cytokine release syndrome
- d 22 tumor lysis syndrome, fluids rasburicase
- d 23 normal bone marrow
- d 28 no palpable lymph nodes



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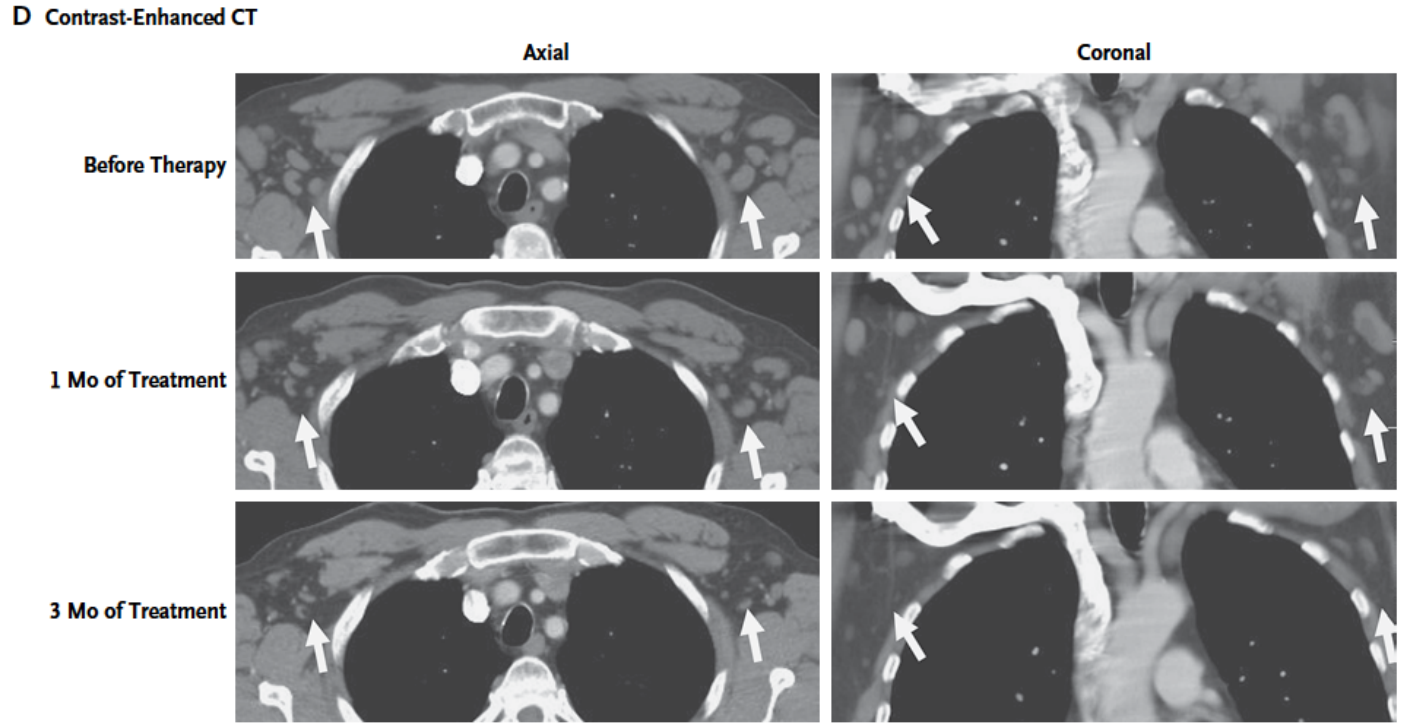
CAR T-cells in lymphoma and leukemia

Case report Porter et al. NEJM 2011 forts

Molecular remission October 2014!



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Korchenderfer et al. JCO 2014

- 2nd generation CAR (CD28)
- 15 patients with B-cell lymphoma
  - 9 DLBCL (8 refractory)
  - 6 indolent
- Pre-treatment with
  - Cyklophosphamide 60-120 mg/kg d-5
  - Fludarabine 25 mg/m^2 d -5---1
  - One toxic death, one lost to follow up

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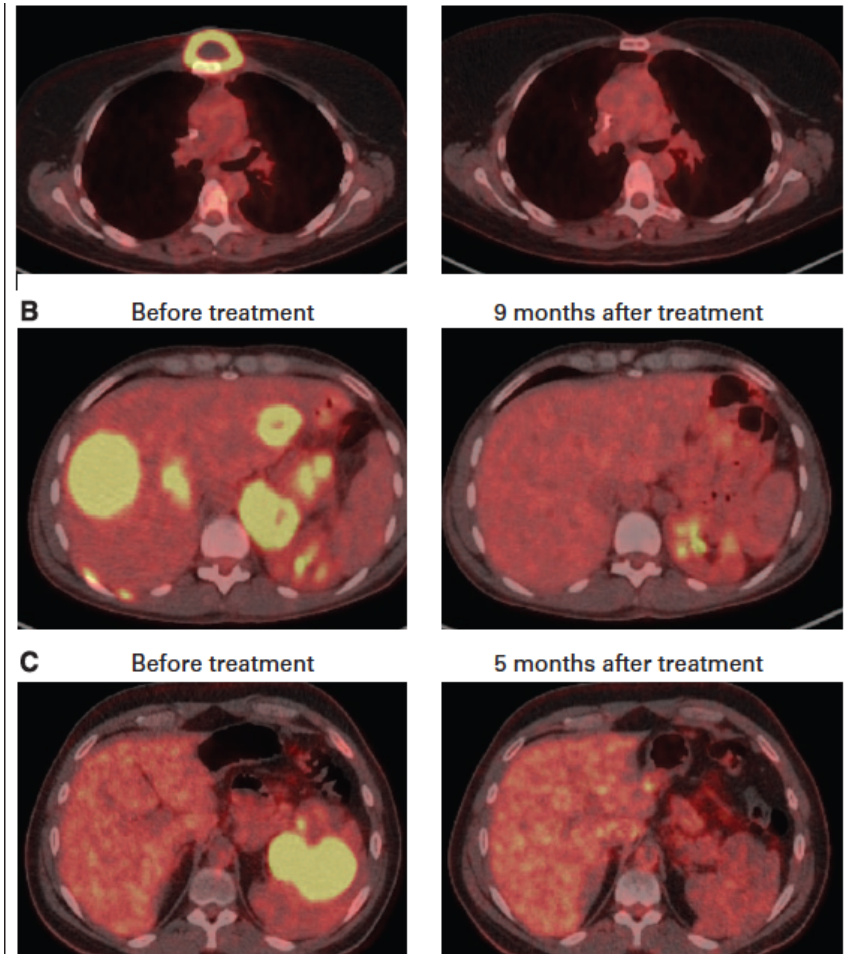
Korchenderfer et al. JCO 2014

- DLBCL: 4 CR, 2 PR, 1 SD
- Indolent: 4 CR, 2 PR
- In total 9 responses ongoing 6-23+ months



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Maude et al. NEJM 2014

- 30 patients with ALL, 25 children, 5 adult
  - 26 B-ALL in 1st to 4th relapse
  - 3 B-ALL refractory
  - 1 T-ALL with CD19 expression
  - 18 previous allogeneic transplantation

- Pre-treatment with different chemotherapy, most often with
  - Cyklophosphamide 600 mg/m^2
  - Fludarabine 30 mg/m^2 d 1-3

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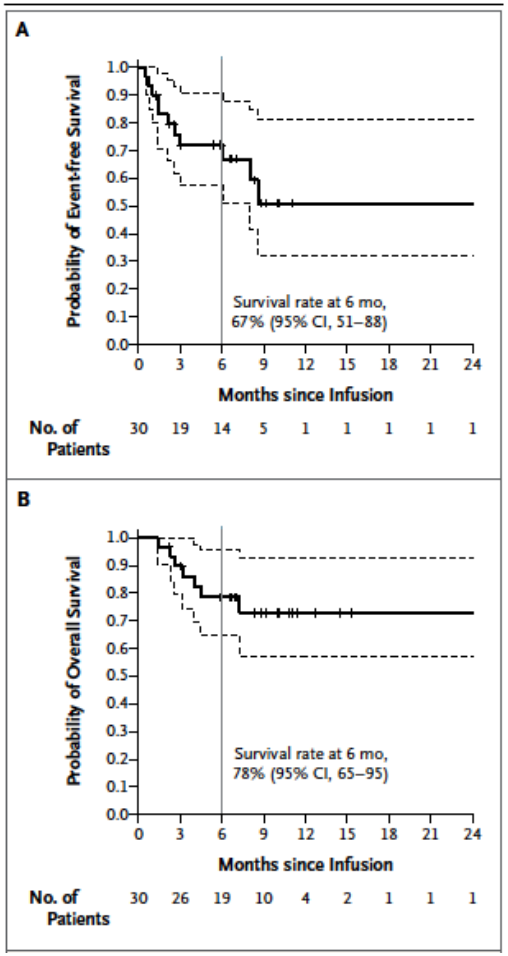
Maude et al. NEJM 2014

- CR 27/30 1 month
- Molecular CR 22/27
- 2/ 2 CR with CNS eng
- 19/27 CCR
  - 3 allogeneic transplant
  - 1 DLI
  - 15 no more treatment



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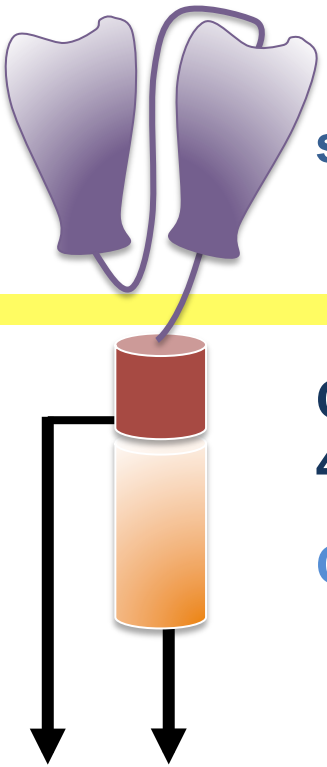
Toxicity of CAR-T cells

- Tumor lysis syndrome
- Cytokine release syndrome
  - Fever, chills, hypotension
  - Might require intensive care unit
  - Treated with Tocilizumab (anti IL6R)
  - Can be fatal
- CNS toxicity
  - Encephalopathy, self-limiting
  - Brain edema- three reported cases fatal
- B-cell aplasia

Clinical Success of CAR T Cell Therapy

2nd Generation (2G)



scFv – Ab

CD28 or 41BB

CD3-ζ

Antigen Recognition
Costimulation

- Remarkable effect in ALL (60-80% CRs across different trials at U-PENN, MSKCC, NIH)

- Lymphoma more resistant
  a) physical barriers (stroma, endothelium)
  b) immunosuppression

CRs are possible if high dose preconditioning

60-120 mg/kg cyclophosphamide
25mg/m2 fludarabine 3-5x
(Kochenderfer et al JCO 2015)

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Summary of Clinical Results

- >100 published patients
- Lymph depleting pre-treatment necessary
- Fludarabine seems important
- ≥2nd generation CAR T-cells are needed
- Long-lasting complete remissions can be achieved
- Effect seems better in ALL than in lymphoma

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Summary of Clinical Results

- No clear dose response relationship between the number of injected CAR T cells and effect
- Weak relationship between tumour burden and response
- Strong relationship between tumour burden and toxicity
- Cytokine release syndrome and neurological toxicity can be serious or even fatal

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CD19-TARGETING 3RD GENERATION CAR T CELLS FOR REFRACTORY B CELL LYMPHOMA OR LEUKEMIA – A PHASE I/IIa TRIAL

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Phase I/IIa Clinical Trial for CD19+ B Cell Leukemia and Lymphoma

Aim

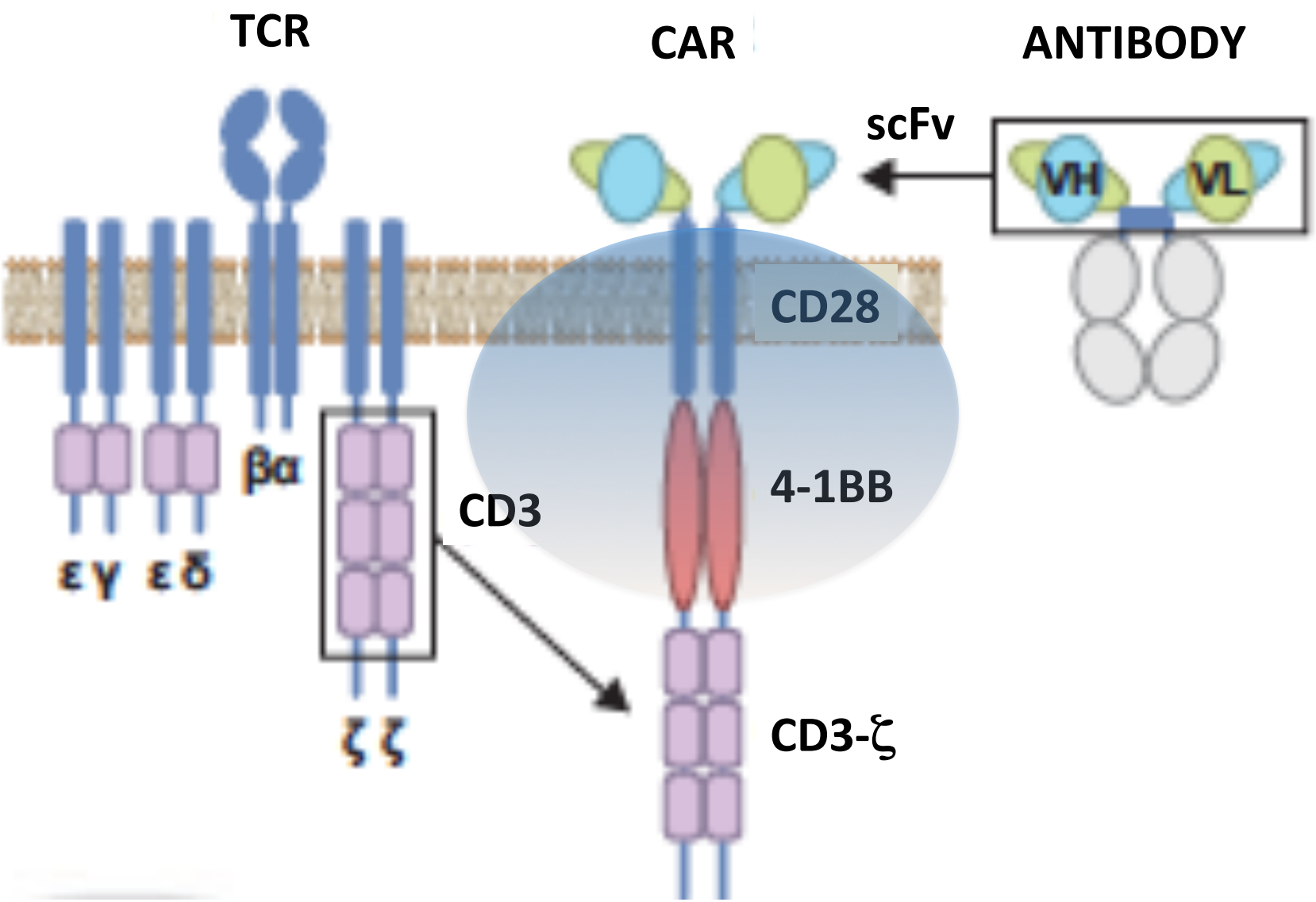
The study aimed to evaluate the feasibility of CAR T cells in patients with refractory CD19+ B cell lymphoma or leukemia by studying the tolerance, toxicity, biological effects and anti-tumor responses post treatment.

Prof Gunilla Enblad
Principal Investigator

Asc Prof Hans Hagberg
Co-Investigator

Prof Angelica Loskog
Sponsor

Dr Hannah Karlsson
Trial Manager


TCR
CAR
ANTIBODY
scFv
VH
VL
CD28
4-1BB
CD3
CD3-ζ
βα
εγ εδ
ζ ζ

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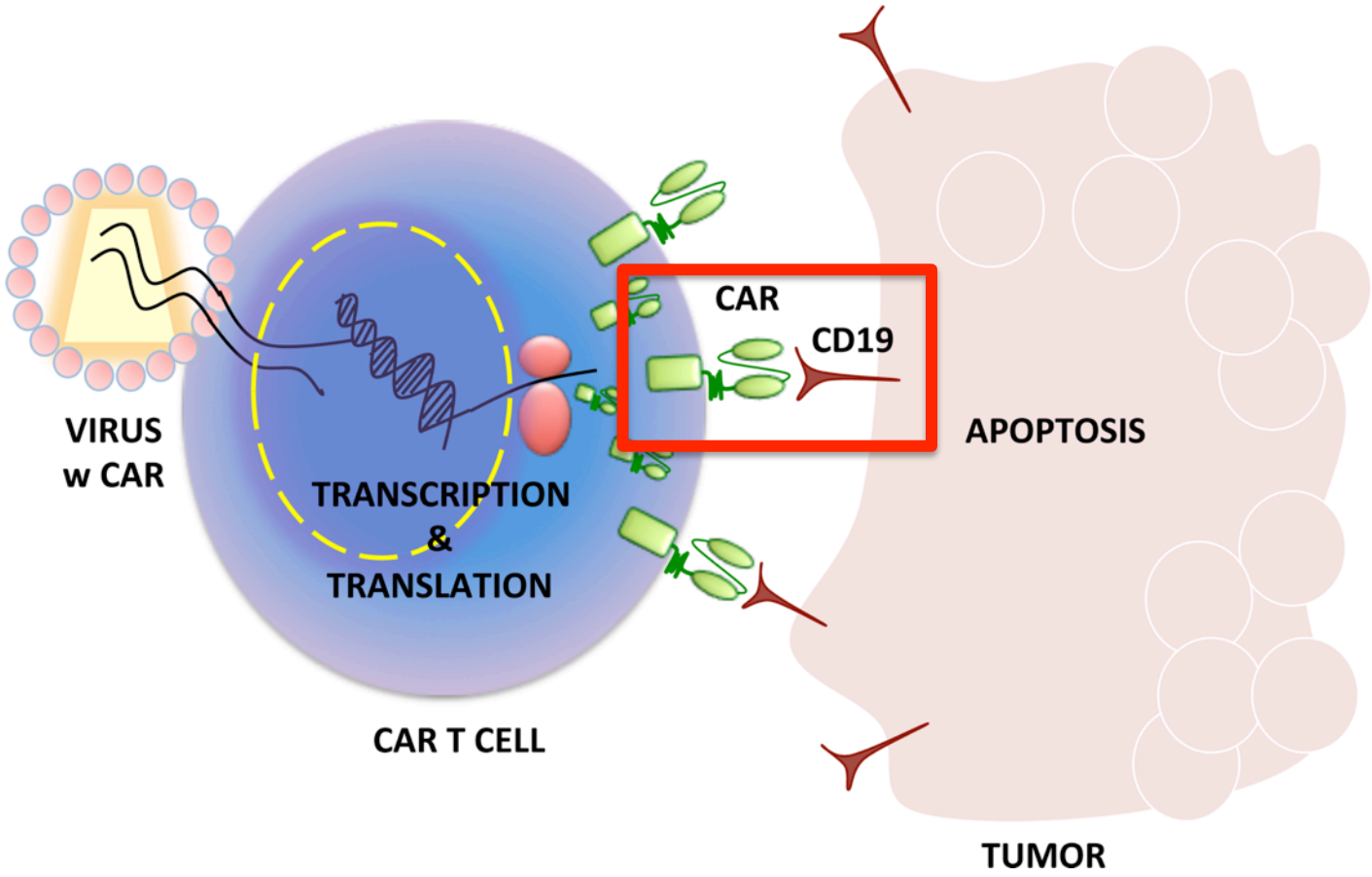
Genetic Engineering of CAR T Cells

MLV-based retrovirus

Baylor College of Medicine



CD19+ B cell leukemia/lymphoma

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Inclusion Criteria

- Relapsed or refractory CD19+ B-cell lymphoma or leukemia
  - After autologous or allogeneic transplant or not eligible for transplant

- Measurable disease.
- Performance status ECOG 0-2.
- ≥18 years old.
- Fertile females/males must consent to use contraceptives during participation of the trial.
- Adequate bone marrow, renal, hepatic and cardiovascular function.
- Signed informed consent.

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Clinical Grade (GMP) CARs

1) Production of gene vehicle (MLV-3G CAR)

Center for Cell and Gene Therapy

Baylor College of Medicine

The viral vector was produced and tested för sterility, identity and function.

2) Manufacture of 3G CAR T cells

Vecura GMP Facility

Karolinska Hospital

One batch per patient was produced and tested for sterility, identity and CAR expression.

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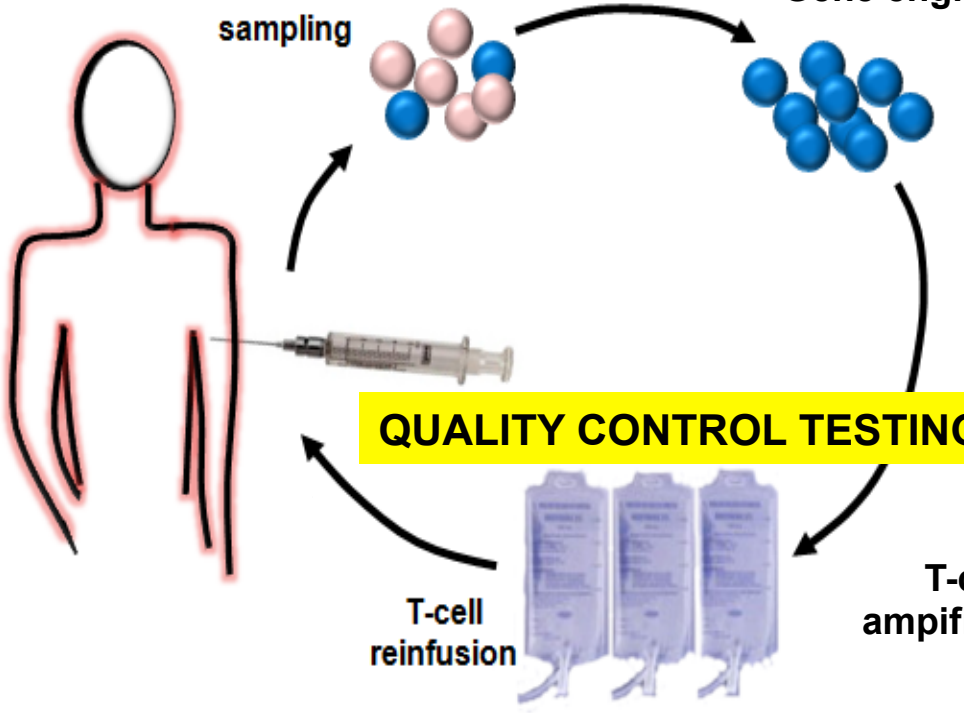
CAR T Cell Batch Manufacture: 6-18 days

Center for Cell and Gene Therap
Baylor College of Medicine

Dept of Oncology
Uppsala University Hospital

T-cell sampling

Gene engineering: 3rd CAR T cells



Vecura
Karolinska University Hospi

QUALITY CONTROL TESTING: 14-21 days

T-cell reinfusion

T-cell ampification

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Procedure

- Inclusion
- Blood sample for T-cell preparation (30 ml) and biobank
- Biopsy for CD19 expression and biobank
- Manufacturing minimum 5 weeks
- Pre-treatment 1-2 months clinicians choice in order to control disease
  - After the first 4 patients all patients also received preconditioning with Cyclophosphamide 500 mg/m^2 d -3 and Fludarabine 25 mg/m^2 d-3--1

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Procedure

- CAR T-cells was given as an intravenous injection
- Premedication with clemastine
- Monitored 2-24 h
- Bi-weekly blood chemistry for 3 weeks
- Weekly blood chemistry for week 4-6
- CT-scan after 3,6 9 and 12 months

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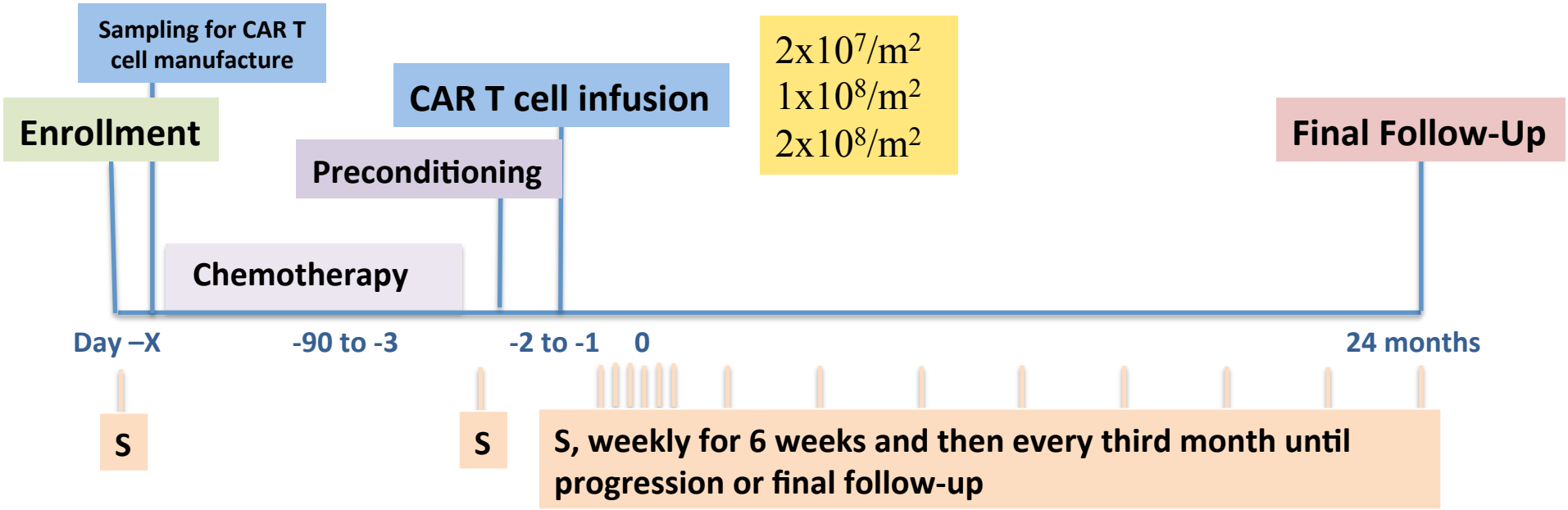
Treatment Schedule

Cohort 1: without preconditioning (4 patients)

Cohort 2: with preconditiong: cyclophosphamide 500mg/m2, 3x fludarabine 25mg/m2) (11 patients)



S = Samples for toxicity, efficacy and research

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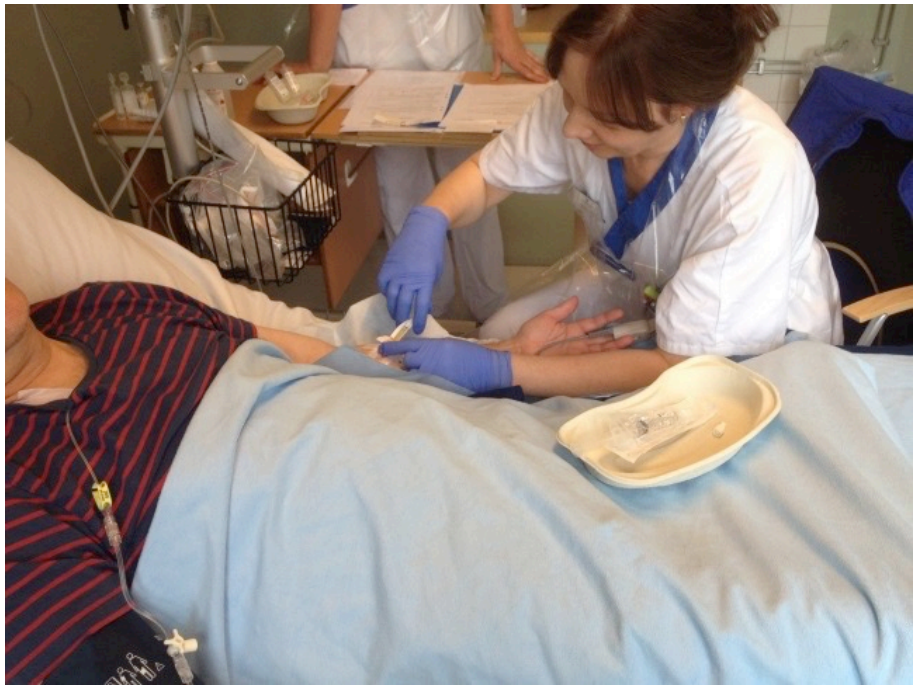
Enrollment & CAR Batch Production

- 19 patients have accepted inclusion
- 18 CAR batches (1 failure)
- 15 have been treated (3 died prior to infusion)



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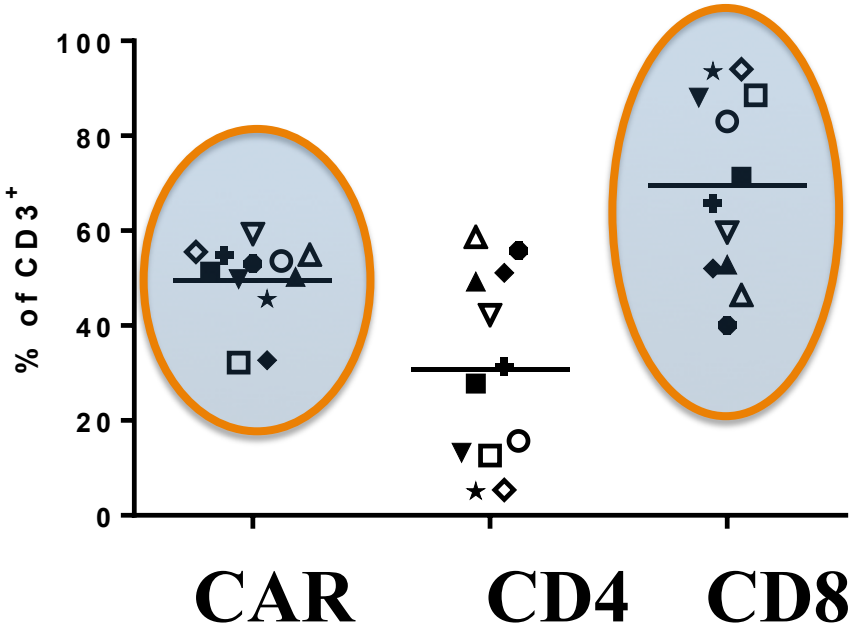
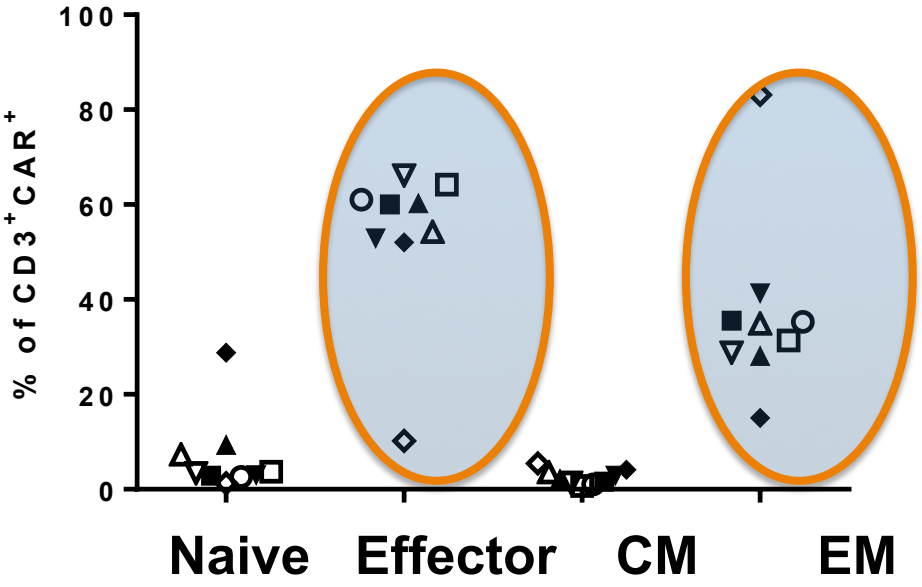
CAR Batches: Phenotype





Effector T cells ($CD45RA^{+}CCR7^{-}$)
Naive T cells ($CD45RA^{+}CCR7^{+}$)
Central Memory (CM) T cells ($CD45RA^{-}CCR7^{+}$)
Effector Memory (EM) T cells ($CD45RA^{-}CCR7^{-}$)

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The Patients

- **15 patients (7 male, 8 female), Age 24-71 years**
  - **DLBCL 6 (3 FL tr)**
  - **CLL 2**
  - **MCL 2**
  - **ALL 4**
  - **FL tr Burkitt 1**

- **All end stage patients with short expected survival**

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Adverse Events

Cytokine Release Syndrome (CRS)

Most patients had mild flu like symptoms only 3 were serious

1 of 3 received tocilizumab (aIL6R ab) to resolve the CRS
all 3 required hospitalization

CNS toxicity

Many patients had mild symptoms that can be signs of CNS toxicity
only 2 were serious and required hospitalization

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Results

- CR in 4/11 patients with lymphoma
  - 1 CLL
  - 2 DLBCL
  - 1 FL tr to DLBCL
- CR in 2/4 patients with ALL
  - 1 Relapse after allogeneic trasnplantation
  - 1 Relapse refractory to chemotherapy

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Summary of results

- 6/15 CR
- Median 5 months (3-18+)
- All but one patient have relapsed (CLL)
  - Remarkably good effect of additional treatment in 4 patients (2 DLBCL- gemcitabine, 1 CLL- ibrutinib, 1 MCL Flu/cy +RT)
- One ALL relapse CD19 negative

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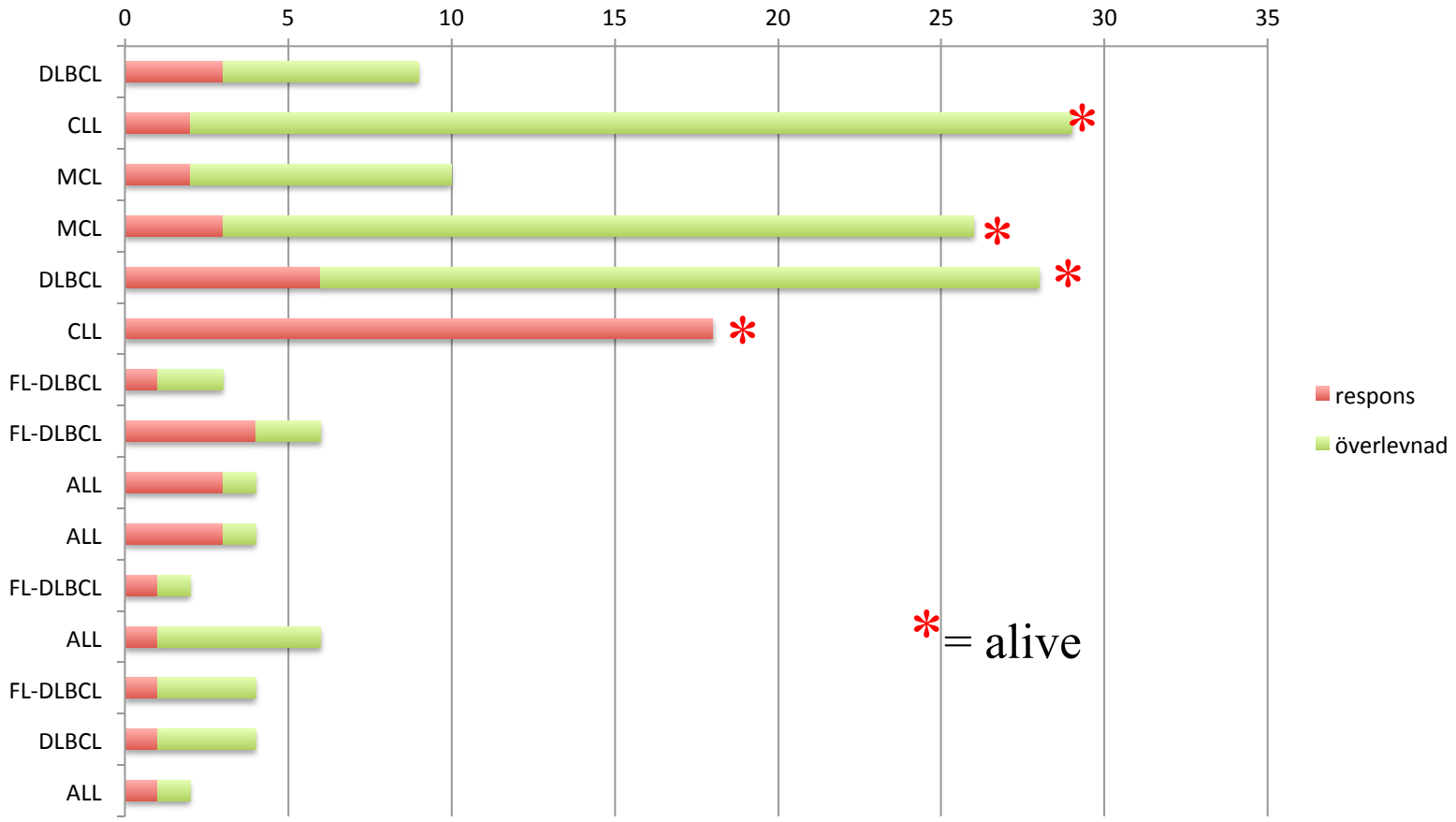
Results



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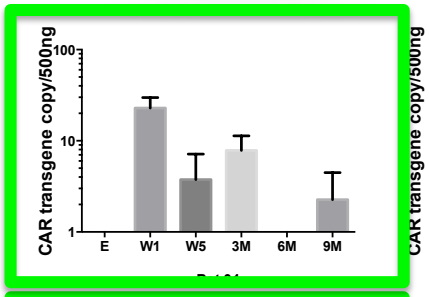
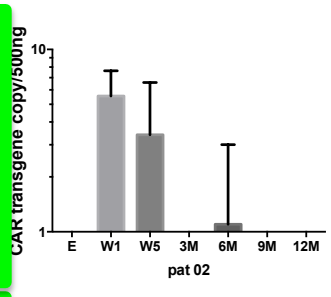
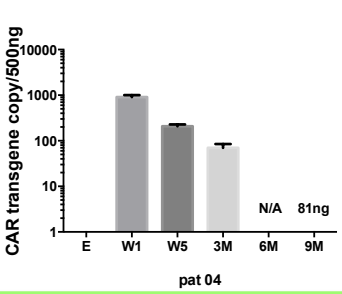
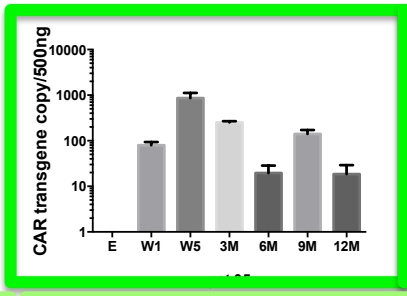
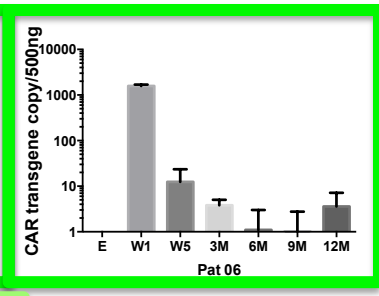
CAR T Cell Survival Post Infusion

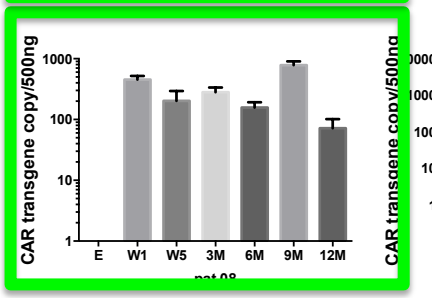


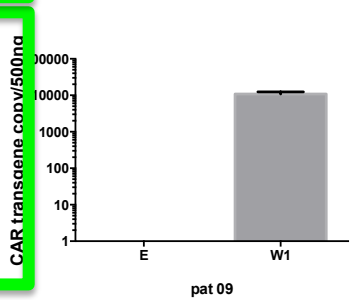


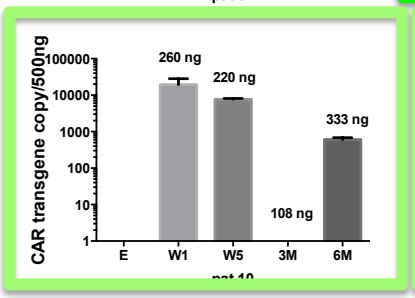


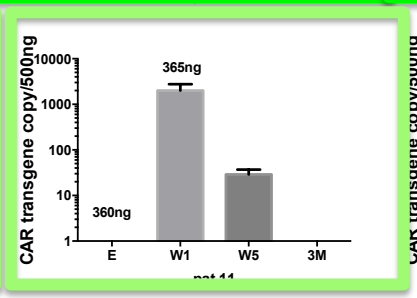


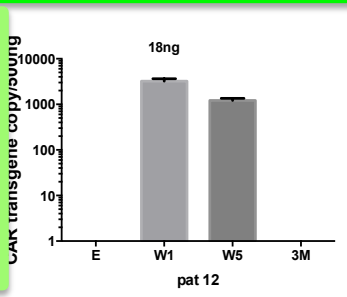


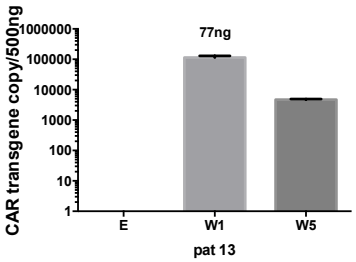


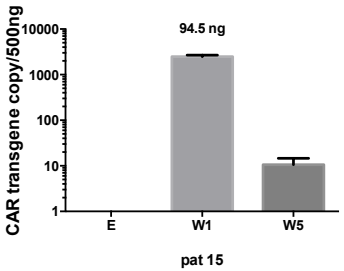


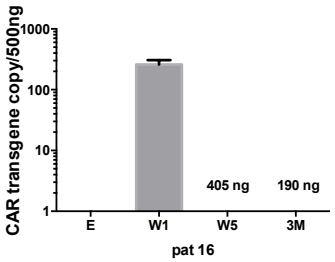


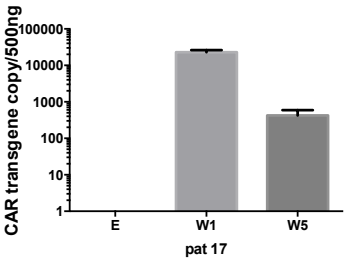












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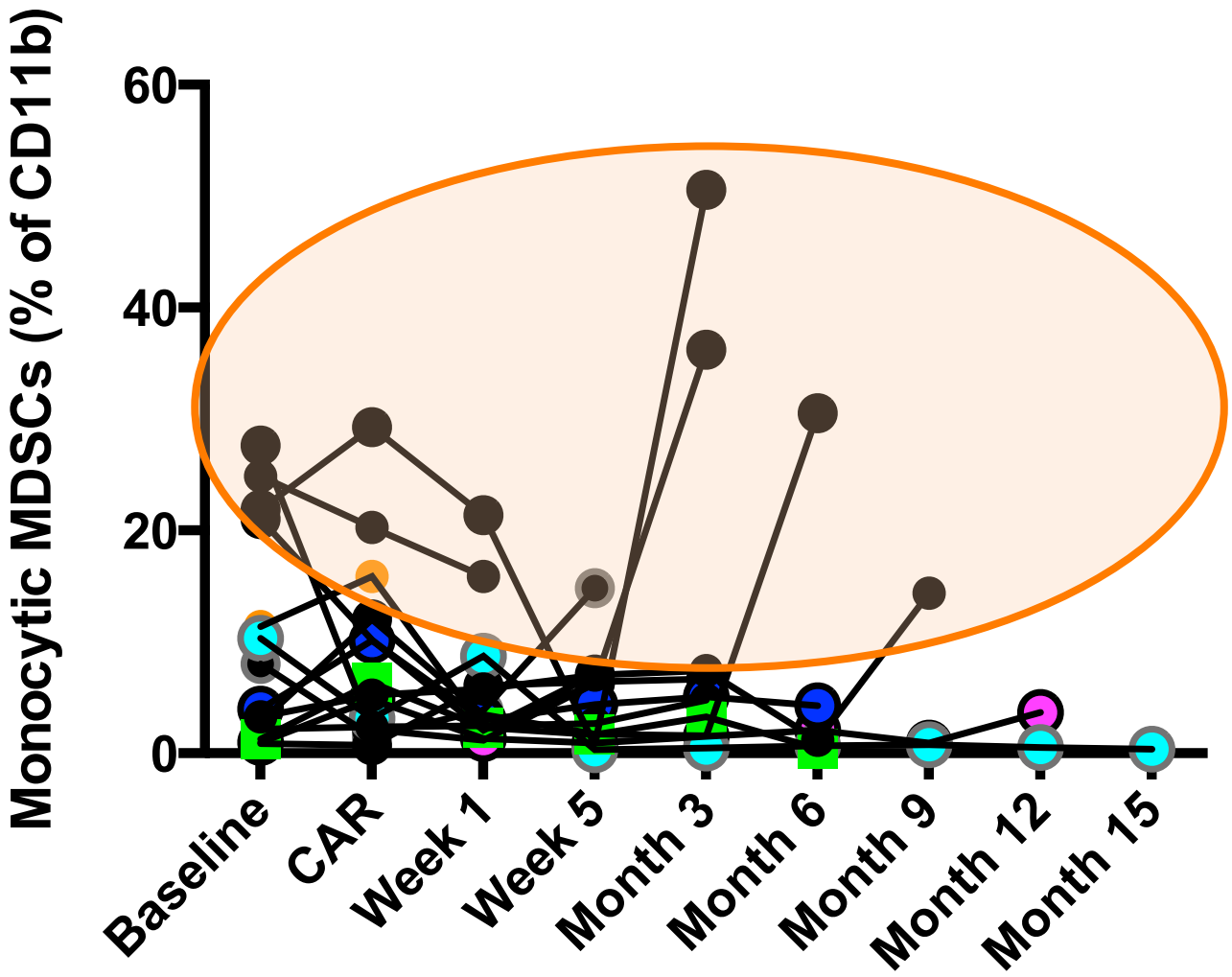
Monocytic MDSCs Are Present in Low Level In Responding Patients



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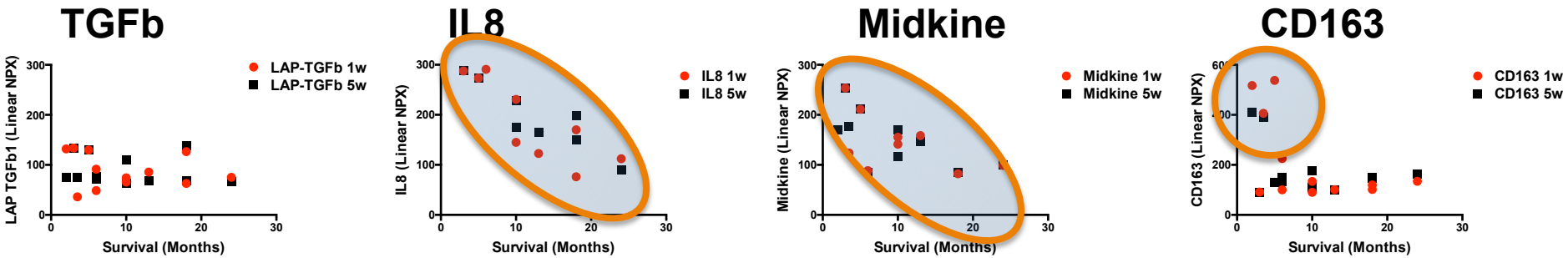
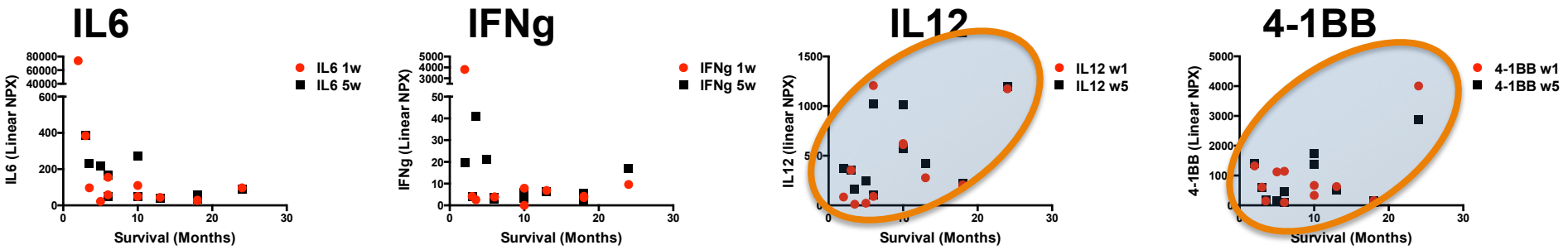
High IL8 Level Correlates To Poor Survival

ProSeek Proteomics (233 analytes)

Suppressive Milieu



Stimulating Milieu



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Trial Conclusions

Clinical Responses

- 15 patients were safely treated with 3G CAR T cells
- 4/15 patients are still alive

Biomarker analysis

- CAR T cells have so far been detected >12 months post infusion
- Immune profile in blood is an important indicator of response
- Myeloid suppressors seem to hamper CAR function

Next trial...

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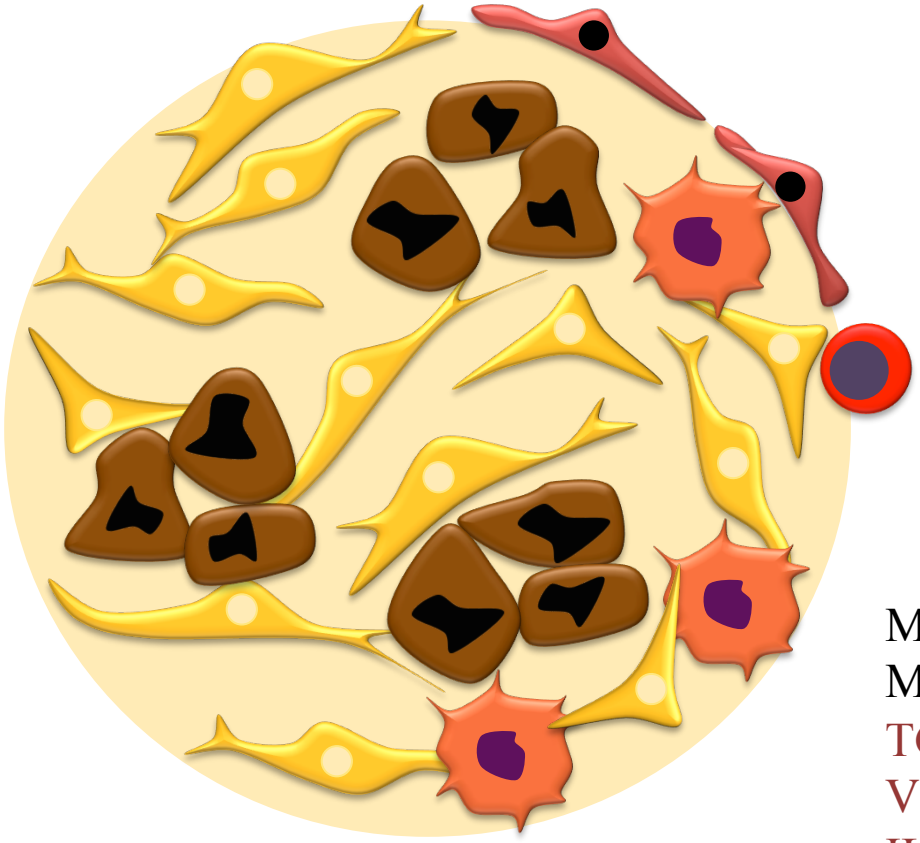
Gemcitabine Chemotherapy Reduces Myeloid Cells



Gemcitabine

M2 macrophages
MDSCs

TGFb	IL10	MPO	FGF2
VEGF	IL1b	PGE2	MMPs
IL6	CCL2	Arginase I	
iNOS			
CXCL12	CXCL15	IL8	

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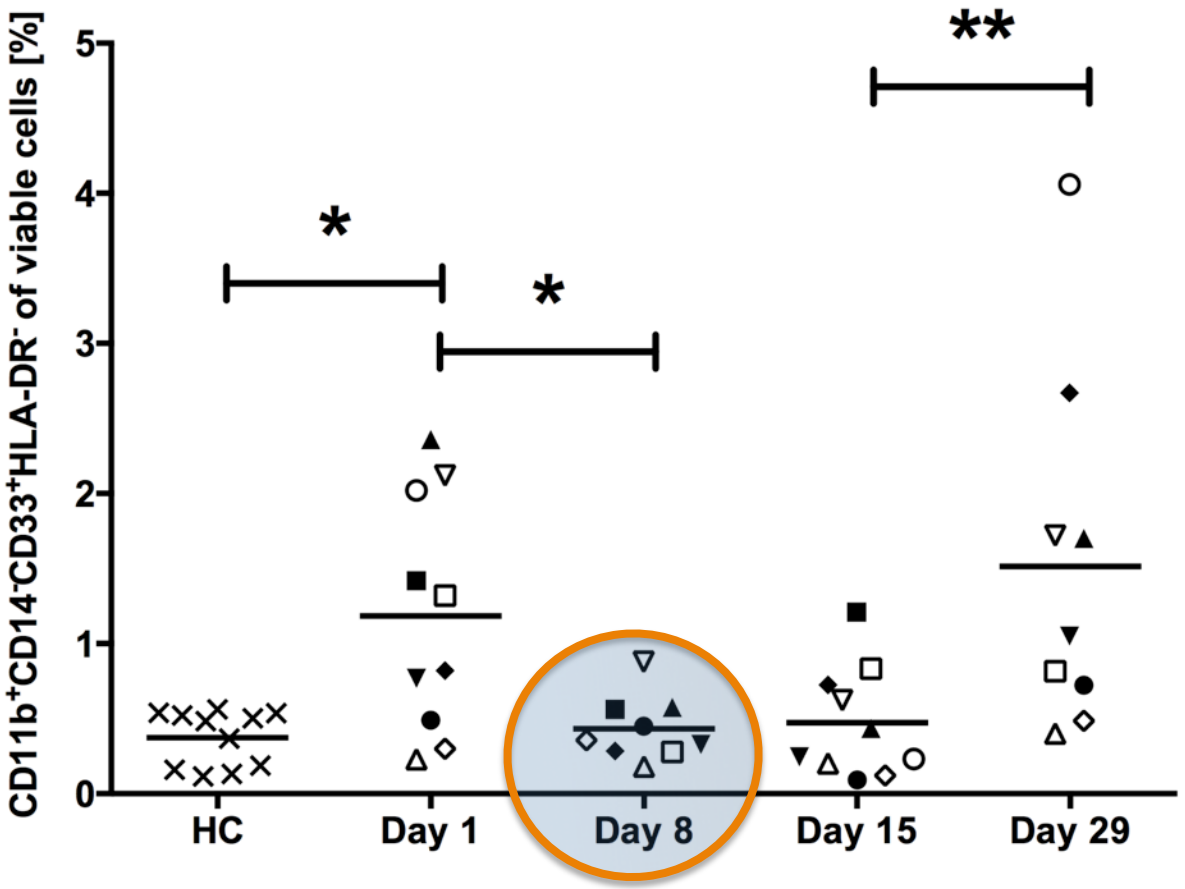
Gemcitabine Reduces MDSCs In Patients with Pancreatic Cancer



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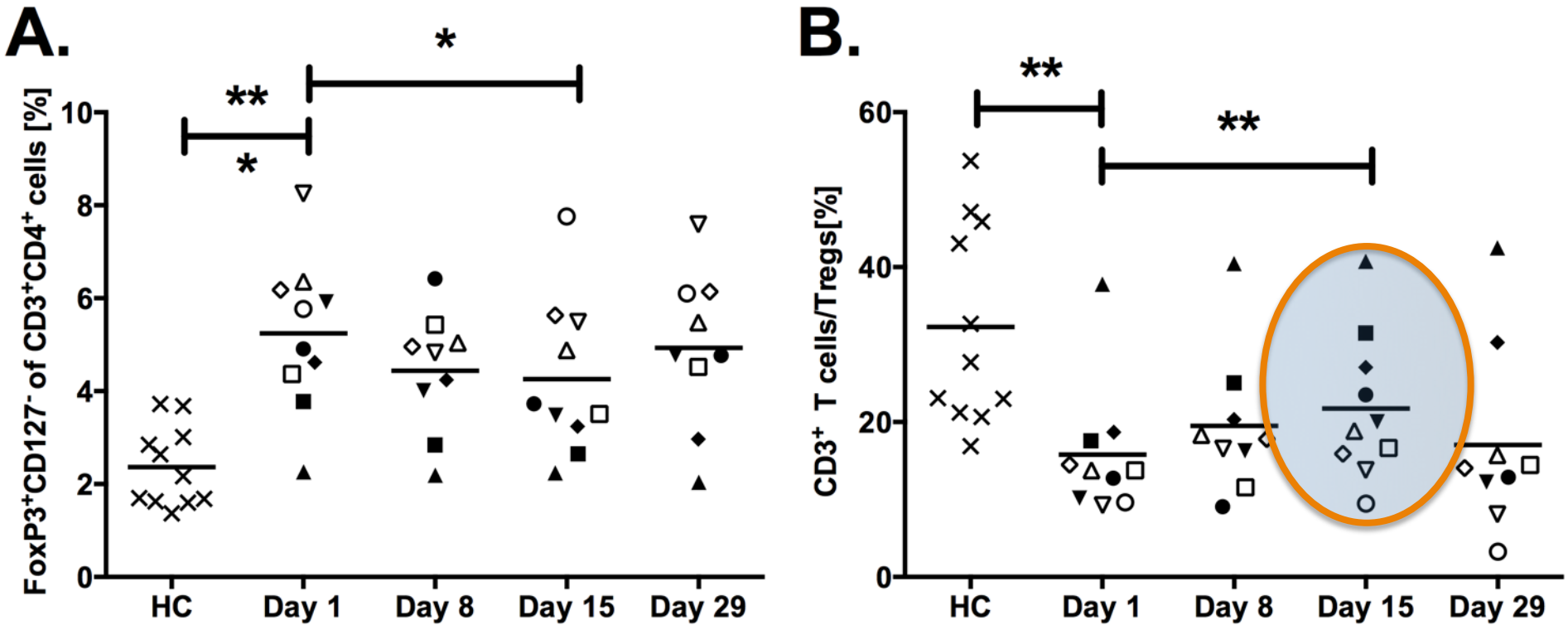
Gemcitabine Restores T effector/Treg Balance



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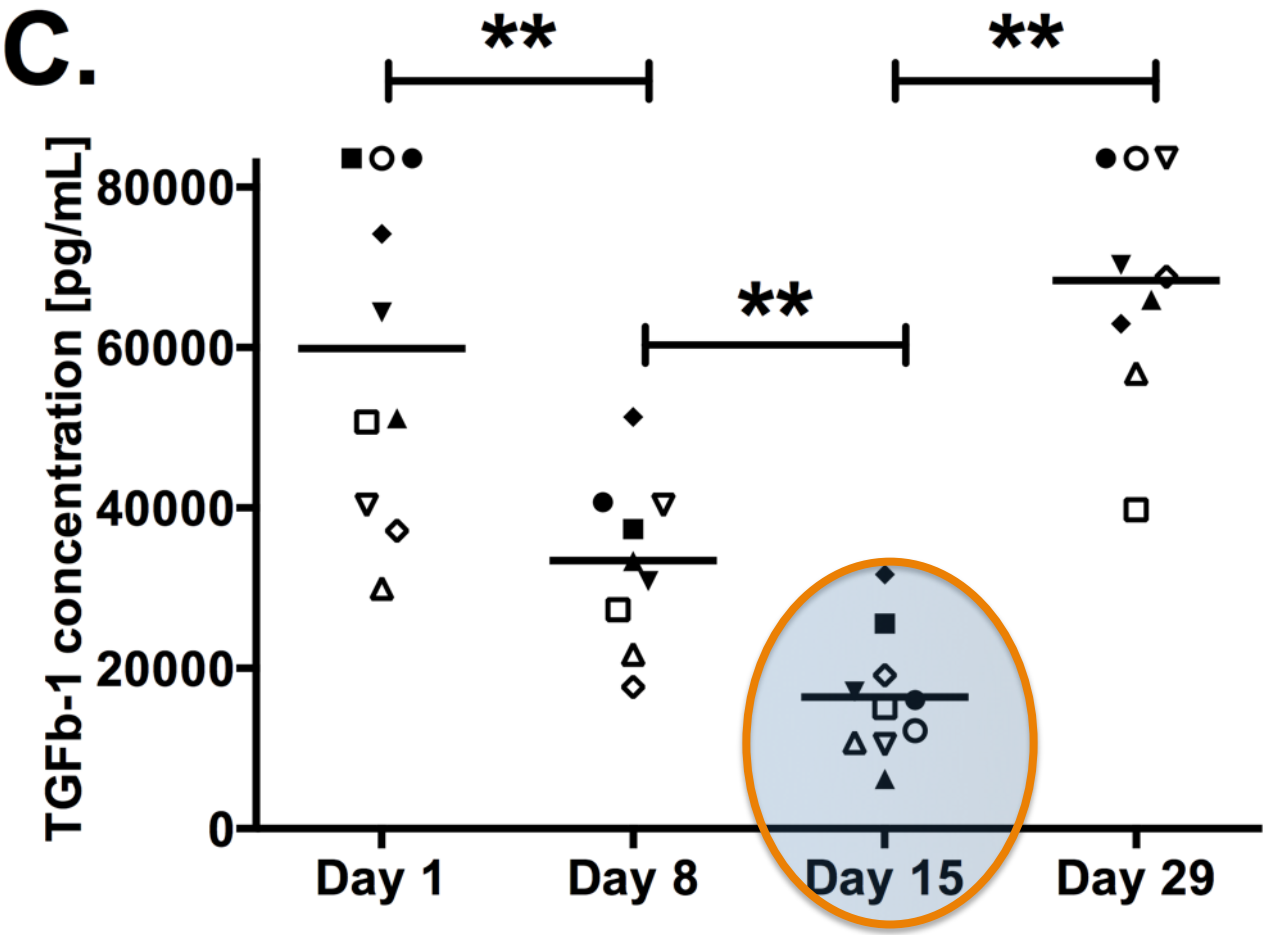
Gemcitabine Reduces TGFbeta



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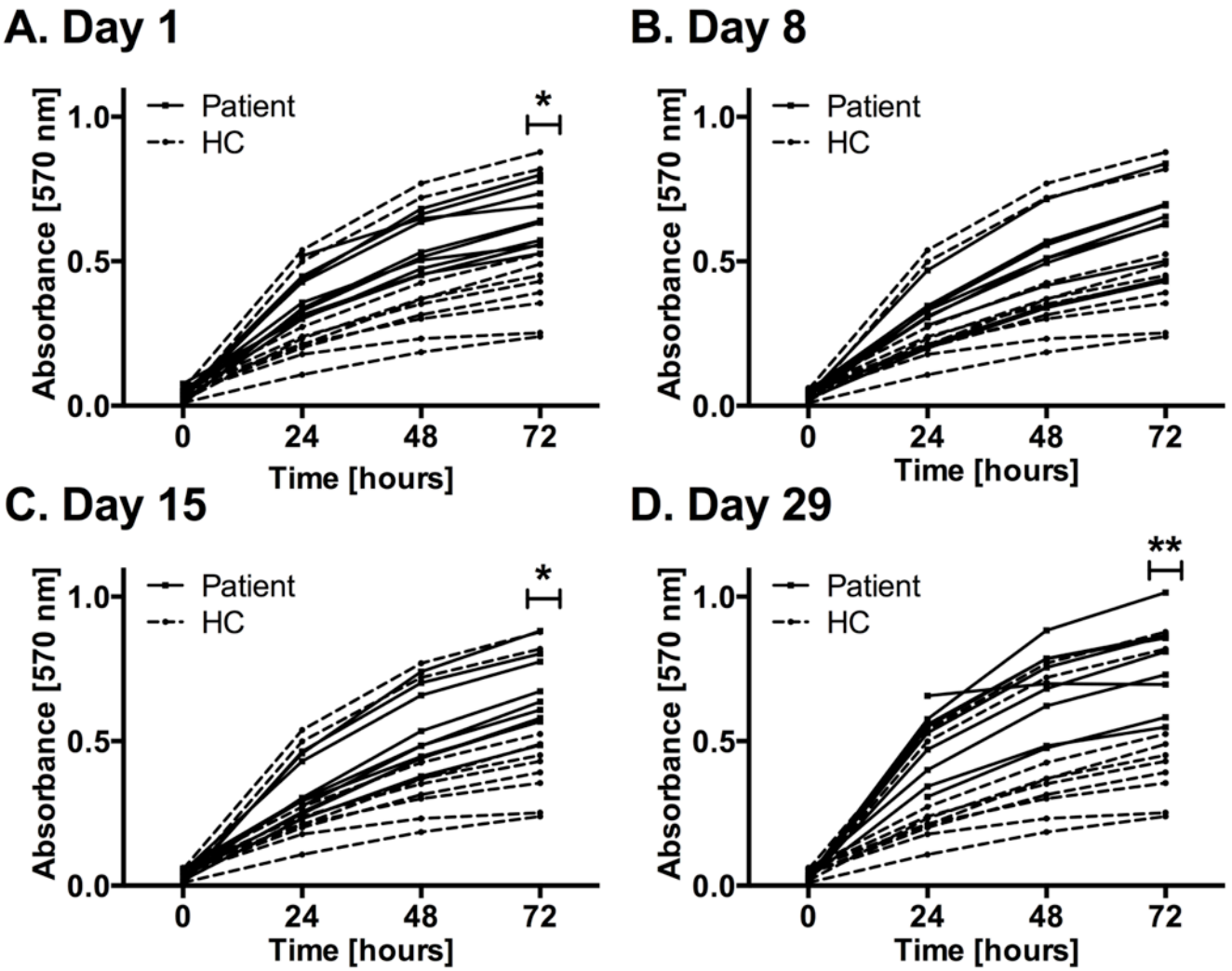
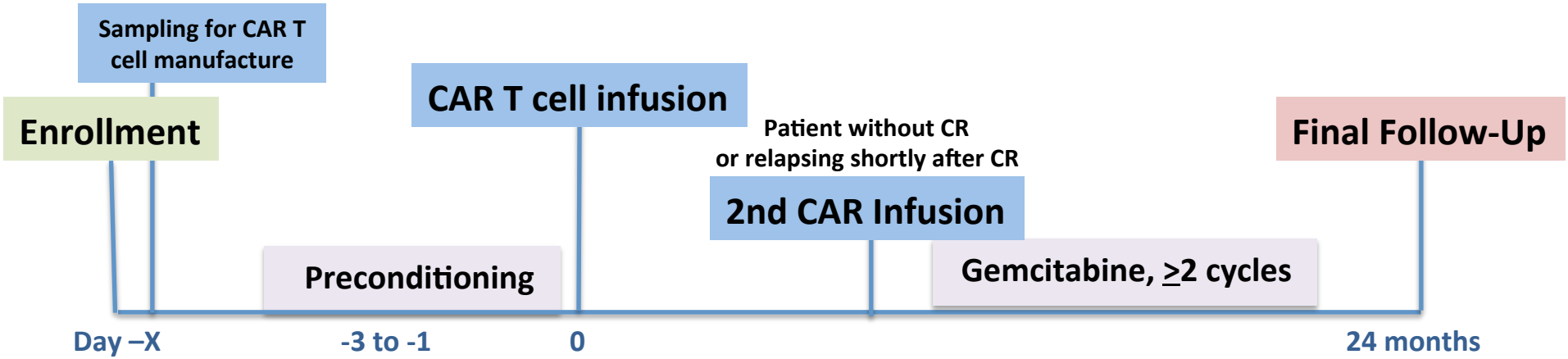
Gemcitabine Does Not Affect T Cell Proliferatio



New Clinical Protocol: 30 patients, beginning Q1 2017



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Conclusions

- CAR T-cells is a powerful new immunotherapy
- Can probably cure terminally ill patients
- Better in ALL than in lymphoma
- When interpreting results consider
  - Differences in patient selection
  - Differencs in preconditioning
  - Differences in CAR T cells

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Conclusions

Issues to be solved in future studies

- How to get the CAR cells to act in the lymphoma tissue?
  - Immunosuppressive milieu?
  - Physical barriers?
- Lack of persistence of CAR T cells?
- Timing of the treatment?
- Combination with other drugs? Repeated treatments?

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