CAR T-cells in B cell lymphomas and leukemias

Cancer Crosslinks
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Outline

• Clinical background
• What is a CAR cell?
• Clinical results
• Uppsala experiences
• Future
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
Do we need new treatments?

Despite progress in treatment many patients still die of their disease.
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
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
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?
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
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 /m² and cyclophosphamide 600 mg /m² dag -4
- 2nd generations CAR 19 T-cell (4-1BB) July 2010
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
CAR T-cells in lymphoma and leukemia

Case report Porter et al. NEJM 2011 forts
Molecular remission October 2014!
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/m² d -5---1
  - One toxic death, one lost to follow up
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
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/m²
  – Fludarabine 30 mg/m² d 1-3
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
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)
Summary of Clinical Results

• >100 published patients
• Lymph depleting pre-treatment necessary
• Fludarabine seems important
• >2\textsuperscript{nd} generation CAR T-cells are needed
• Long-lasting complete remissions can be achieved
• Effect seems better in ALL than in lymphoma
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
CD19-TARGETING 3RD GENERATION CAR T CELLS FOR REFRACTORY B CELL LYMPHOMA OR LEUKEMIA – A PHASE I/IIa TRIAL
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.
Genetic Engineering of CAR T Cells

MLV-based retrovirus

Baylor College of Medicine

VIRUS w CAR

TRANScription & translation

CAR T CELL

CAR

CD19

CD19+ B cell leukemia/lymphoma

APOPTOSIS

TUMOR
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.
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 for 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.
CAR T Cell Batch Manufacture: 6-18 days

Gene engineering: 3rd CAR T cells

Dept of Oncology
Uppsala University Hospital

Vecura
Karolinska University Hospital

QUALITY CONTROL TESTING: 14-21 days
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/m² d -3 and Fludarabine 25 mg/m² d-3--1
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
Treatment Schedule

Cohort 1: without preconditioning (4 patients)

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

Sampling for CAR T cell manufacture

Enrollment

Chemotherapy

Preconditioning

CAR T cell infusion

Final Follow-Up

S, weekly for 6 weeks and then every third month until progression or final follow-up

S = Samples for toxicity, efficacy and research
Enrollment & CAR Batch Production

- 19 patients have accepted inclusion
- 18 CAR batches (1 failure)
- 15 have been treated (3 died prior to infusion)
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⁻)
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
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
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 transplantation
  – 1 Relapse refractory to chemotherapy
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
Results

Months

* = alive
CAR T Cell Survival Post Infusion

- Pat 01: CAR transgene copy/500ng
- Pat 02: CAR transgene copy/500ng
- Pat 04: CAR transgene copy/500ng
- Pat 05: CAR transgene copy/500ng
- Pat 06: CAR transgene copy/500ng
- Pat 08: CAR transgene copy/500ng
- Pat 09: CAR transgene copy/500ng
- Pat 10: CAR transgene copy/500ng
- Pat 11: CAR transgene copy/500ng
- Pat 12: CAR transgene copy/500ng
- Pat 13: CAR transgene copy/500ng
- Pat 15: CAR transgene copy/500ng
- Pat 16: CAR transgene copy/500ng
- Pat 17: CAR transgene copy/500ng
Monocytic MDSCs Are Present in Low Level In Responding Patients
High IL8 Level Correlates To Poor Survival
ProSeek Proteomics (233 analytes)

Suppressive Milieu

TGFb
LAP-TGFb 1w  
LAP-TGFb 5w

IL 8
LAP-TGFb 1w  
LAP-TGFb 5w

Midkine
Midkine 1w  
Midkine 5w

CD163
CD163 1w  
CD163 5w

Stimulating Milieu

IL6
IL6 1w  
IL6 5w

IFNg
IFNg 1w  
IFNg 5w

IL12
IL12 w1  
IL12 w5

4-1BB
4-1BB w1  
4-1BB w5

High IL8 Level Correlates To Poor Survival
ProSeek Proteomics (233 analytes)
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...
Gemcitabine Chemotherapy Reduces Myeloid Cells

- M2 macrophages
- MDSCs
- TGFβ, IL10, MPO, FGF2
- VEGF, IL1b, PGE2, MMPs
- IL6, CCL2, Arginase I
- iNOS, CXCL12, CXCL15, IL8
Gemcitabine Reduces MDSCs In Patients with Pancreatic Cancer
Gemcitabine Restores T effector/Treg Balance

A. FoxP3+CD127- of CD3+CD4+ cells [%]

B. CD3+ T cells/Tregs [%]
Gemcitabine Reduces TGFbeta
Gemcitabine Does Not Affect T Cell Proliferation

A. Day 1

B. Day 8

C. Day 15

D. Day 29
New Clinical Protocol: 30 patients, beginning Q1 2017

Sampling for CAR T cell manufacture

Enrollment

Preconditioning

Day -X

-3 to -1

0

CAR T cell infusion

Patient without CR or relapsing shortly after CR

2nd CAR Infusion

Gemcitabine, ≥2 cycles

24 months

Final Follow-Up

Prof Gunilla Enblad
Principal Investigator

Asc Prof Hans Hagberg
Co-Investigator

Prof Magnus Essand
Sponsor

Dr Tanja Lövgren
Trial Manager
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
  – Differences in preconditioning
  – Differences in CAR T cells
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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