Allo-CAR-T cells

18th International Myeloma Workshop
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Vienna
Disclosure

Honoraria and consultancy/advisory role with Takeda, Amgen, Celgene, Janssen, and Bristol-Myers Squib

Research funding from Takeda, Amgen, Janssen, and Novartis;
Reprogramming T-cells to treat cancer

- **Autologous** CAR T-cells approved for MM, ALL, lymphoma treatment
- **Allogenic** CAR T-cells just entered phase I/II of clinical trials
**Autologous CAR-T cells**

- Leukapheresis
- 4-5 weeks
- CAR T-cells expansion

**Allogenic CAR-T cells**

- CAR introduction
- CAR T-cells
-輸入

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Autologous CAR-T cells

Allogenic CAR-T cells from haploidentical donors

Leukapheresis

CAR introduction

4-5 weeks

CAR T-cells expansion
<table>
<thead>
<tr>
<th>Autologous CAR T-cells</th>
<th>Allogenic CAR T-cells</th>
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<tr>
<td><strong>Pros:</strong></td>
<td><strong>Pros:</strong></td>
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<tr>
<td>- no GvH</td>
<td>- off the shelf</td>
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<tr>
<td>- few genetic manipulation</td>
<td>- available for multiple patients</td>
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<tr>
<td>- approved (some)</td>
<td>- available several times for one patient if needed</td>
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<tr>
<td><strong>Cons:</strong></td>
<td><strong>Cons:</strong></td>
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<tr>
<td>- Complicated logistic</td>
<td>- potential GvH</td>
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<tr>
<td>- limited availability</td>
<td>- Potential higher risk of infection</td>
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<tr>
<td>- limited numbers of application</td>
<td>- not yet approved</td>
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<tr>
<td>- long production time</td>
<td>- Not yet proved safety &amp; efficacy</td>
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<tr>
<td>- expensive</td>
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The main issue why allo CAR T cells are not yet widely used is the HLA incompatibility.
How to make allogenic (CAR) T-cell?

Genetic manipulation
- Deletion of TCR
- Deletion of MHC I
- Introduction of CAR
- Introduction of suicide gene

Tissue compatibility
How to make allogenic (CAR) T-cell?

**Genetic manipulation**
- Deletion of TCR
- Deletion of MHCI

**Tissue compatibility**

- CRISPR/Cas9
  - Ren et al. 2017
  - Georgiadis et al. 2018
  - Ngoc Duong et al. 2019
  - Liu et al. 2017
  - Zhang et al. 2017

- Base Editing (modified CRISPR/Cas9):
  - Weber et al. 2019

In last decade we could observe the rise of new tools, that allow us precise gene modification, such as Talen Nucleases or CRIPSR/Cas9. We can introduce point mutation that will introduce premature stop codon or disrupt protein splicing, which results in knock-out of the protein.
Chimeric Antigen Receptor introduction: targeting T-cell towards tumor

Methods of introducing CAR
- Lentiviral vector
  Kalos et al 2012
  Priceman et al 2018
- AAV Vector
  Mosti et al 2021
- Gammaretorviral vector
  Pampush et al 2020

Most people use viral vectors for integration of CAR (lenti or retro vectors) or AAV for long expression.
Four generations of "classical" CARs

Paubelle et al. 2018

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and many more new CARs...coming

Jan et al 2021
Juillerat et al 2019
Sahillioglu et al 2021
Tahmasebi et al. 2020

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Currently there are many different universal CAR types. And this year we had more than 4 completely new designs of CARs presented. Each CAR type is built in order to increase efficiency, safety or accuracy of the therapy.
Suicide gene insertion
to ensure cell therapy safety

Delivery of suicide gene
• Lentiviral vector
  Budde et al 2013
  Duong et al 2019
• Retroviral vector
  Straathof et al 2005
Suicide genes
immediate removal of CAR T-cells

- **enzyme induced apoptosis** (like iCaspase9)
- **Extracellular example**
  
  **CD20** - antigen from B-cells that trigger cell death upon stimulation with antibody eg Rituximab (Allo 715 has a CAR with rituxamab recognition domain – so this is suicide gene and CAR in one)

- **Toxic drug**
  
  **HSV-TK** – Herpes simplex virus thymidine kinase – creates toxic product in presence of acyclovir or ganciclovir

- **iCas9** - Inducible Caspase 9 – triggers apoptosis after stimulation with AP1903 (FDA approved drug)
Clinical trials with allo-CAR-T
## Clinical trials with allo-CAR-T

<table>
<thead>
<tr>
<th>Allo CAR-T</th>
<th>n</th>
<th>Diagnose</th>
<th>ORR</th>
<th>CR/≥VGPR</th>
<th>Toxicity (CRS/GvHD)</th>
<th>status study</th>
<th>published</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALLO-501 ALLO-647</td>
<td>19</td>
<td>RR LBCL/FL</td>
<td>63%</td>
<td>37%/-</td>
<td>32%/0%</td>
<td>recruiting</td>
<td>ASCO 2020 abstract 8002</td>
</tr>
<tr>
<td>ALLO-715 ALLO-647</td>
<td>31</td>
<td>RR MM</td>
<td>55%</td>
<td>-/32%</td>
<td>45%/0%</td>
<td>recruiting</td>
<td>ASH 2020 abstract 129</td>
</tr>
<tr>
<td>ALLO-605 ALLO-647</td>
<td></td>
<td>RR MM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>recruiting</td>
<td></td>
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<tr>
<td>P-BCMA-ALLO1</td>
<td></td>
<td>RR MM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>recruiting</td>
<td></td>
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<tr>
<td>Anti-CD19</td>
<td></td>
<td>R/R B cell malignancies</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>recruiting</td>
<td></td>
</tr>
</tbody>
</table>

ASCO 2020, abstr. 8002; ASH 2020, abstr. 129; 
https://clinicaltrials.gov/ct2/show/NCT04516551 
https://clinicaltrials.gov/ct2/show/NCT05000450 
https://clinicaltrials.gov/ct2/show/NCT04960579
Allo-501 (lymphoma)/Allo-715 (MM), Allo-647 CAR

Talens are delivered with electroporation
- TALEN-mediated TRAC KO eliminates TCRα expression to minimize risk of GvHD
- TALEN-mediated CD52 KO allows selective lymphodepletion with ALLO-647 that is anti-CD52 MoAb

Delivery of CAR and Rituximab recognition domain by Lentiviral vector

https://www.allogene.com/allocar-t-therapy
ALPHA Study: phase 1, open-label, multicenter dose escalation study in R/R Non-Hodgkin Lymphoma (ALLO-501 and ALLO-647)

Eligibility Criteria
- ECOG 0 or 1
- At least 2 prior lines of therapy, including an anti-CD20 monoclonal antibody
- Prior autologous CAR T allowed if tumor remains CD19+

R/R LBCL/FL

Lymphodepletion regimens
LD 1: Fludarabine 30mg/m2 + Cyclophosphamide 300mg/m2 + ALLO-647 13mg/d x 3 days
LD2/LD3: Fludarabine 30mg/m2 + Cyclophosphamide 300mg/m2 + ALLO-647 30mg/d x 3 days
**ALPHA Study:** phase 1, open-label, multicenter dose escalation study in R/R Non-Hodgkin Lymphoma (ALLO-501 and ALLO-647)

**Endpoints**

**Primary**
- Safety and dose-limiting toxicity of ALLO-647/Flu/Cy followed by ALLO-501

**Secondary**
- Overall response rate
- ALLO-501 cell kinetics
- ALLO-647 pharmacokinetics

**AE of Interest**

<table>
<thead>
<tr>
<th>AE of Interest</th>
<th>Grade 1 n (%)</th>
<th>Grade 2 n (%)</th>
<th>Grade 3 n (%)</th>
<th>Grade 4 n (%)</th>
<th>Grade 5 n (%)</th>
<th>All grades n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine release syndrom</td>
<td>2 (9%)</td>
<td>4 (18%)</td>
<td>1 (5%)</td>
<td>-</td>
<td>-</td>
<td>7 (32%)</td>
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<tr>
<td>ICANS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>GvHD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Infection</td>
<td>5 (23%)</td>
<td>4 (18%)</td>
<td>2 (9%)</td>
<td>-</td>
<td>-</td>
<td>11 (50%)</td>
</tr>
<tr>
<td>Infusion reaction</td>
<td>1(5%)</td>
<td>9 (41%)</td>
<td>1 (5%)</td>
<td>-</td>
<td>-</td>
<td>11(50%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>-</td>
<td>1 (5%)</td>
<td>7 (32%)</td>
<td>7 (32%)</td>
<td>-</td>
<td>15 (68%)</td>
</tr>
</tbody>
</table>

ALLO-501 and ALLO-647 have a manageable safety profile.
**ALPHA Study:** phase 1, open-label, multicenter dose escalation study in R/R Non-Hodgkin Lymphoma (ALLO-501 and ALLO-647)

### Conclusions
- ORR observed in 12/19 (63%) patients with 37% CR
- 9 of the 12 (75%) responding patients remain in response as of the data cutoff
- Higher dose ALLO-647 appear to associate with deeper responses (50% CR)

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UNIVERSAL Study: phase 1, open-label, multicenter dose escalation study in R/R Multiple Myeloma (ALLO-715 and ALLO-647)

Eligibility Criteria
• ECOG 0 or 1
• adults with R/R MM
• ≥ 3 previous therapies (including IMiD, PI, anti-CD38), refractory to last therapy

Endpoints
Primary
• safety and tolerability
Secondary
• lymphodepletion regimen and recommended ALLO-715 phase II dose
• anti-tumor activity (ORR, DoR, PFS, MRD)
• ALLO-715 cellular kinetics
• ALLO-647 pharmacokinetics

Day -5
Lymphodepletion
FCA or CA Regimen
Fludarabine 30 mg/m²/day x 3 days
Cyclophosphamide 300 mg/m²/day x 3 days
ALLO-647 13-30 mg x 3 days

Day 0
Treatment
Single ALLO-715 Infusion on Day 0
40, 160, 320, 480 x 10^6 CAR+ T-cells

Follow-up

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**UNIVERSAL Study:**° phase 1, open-label, multicenter dose escalation study in R/R Multiple Myeloma (ALLO-715 and ALLO-647)

- Manageable safety profile
- No GvHD or ICANS
- 45% experienced grade 1/2 CRS; low use of tocilizumab (19%) and steroids (10%), 23% experienced grade 1/2 infusion reaction to ALLO-647
- Serious AEs (grade ≥3) in 19%
  - 5 (16%) grade ≥ 3 infection
  - 1 grade 5 event related to progressive myeloma in CA cohort

<table>
<thead>
<tr>
<th>AE of Interest, n %</th>
<th>Safety Population (N = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRS</td>
<td>14 (45)</td>
</tr>
<tr>
<td>- Grade 1</td>
<td>5 (16)</td>
</tr>
<tr>
<td>- Grade 2</td>
<td>9 (29)</td>
</tr>
<tr>
<td>- Grade ≥ 3</td>
<td>0</td>
</tr>
<tr>
<td>Infection (bacterial, fungal, viral)</td>
<td>13 (42)</td>
</tr>
<tr>
<td>- Grade 1</td>
<td>2 (7)</td>
</tr>
<tr>
<td>- Grade 2</td>
<td>6 (19)</td>
</tr>
<tr>
<td>- Grade 3</td>
<td>4 (13)</td>
</tr>
<tr>
<td>- Grade 5</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Infusion reaction to ALLO-647</td>
<td>7 (23)</td>
</tr>
<tr>
<td>- Grade 1</td>
<td>4 (13)</td>
</tr>
<tr>
<td>- Grade 2</td>
<td>3 (10)</td>
</tr>
</tbody>
</table>

AE, adverse event; CA, cyclophosphamide/ALLO-647; CRS, cytokine-release syndrome; GVHD, graft-vs-host disease; ICANS, immune effector cell–associated neurotoxicity syndrome

ASH 2020, abstr. 129;
UNIVERSAL Study: a phase 1, open-label, multicenter dose escalation study in R/R Multiple Myeloma (ALLO-715 and ALLO-647)

Conclusions
- ALLO-715 and ALLO-647 have a manageable safety profile
- Dose-dependent activity
  - 60% of patients in FCA plus 320 x 10^6 dose of ALLO-715 cohort achieved response;
  - 40% achieved ≥ VGPR
  - 5/6 patients assessed with ≥ VGPR had negative MRD status

*Clinical response evaluation based on IMWG response criteria. ≥ VGPR defined as sCR, CR or VGPR.

Median follow-up 3.8 months
Anti-CD19 Allo-CAR-T Cells from haplo-ID donors:\(^1\) phase 1, uncontrolled, multicenter study in R/R B cell Malignancies after HSCT

Adult patient with R/R B-cell malignancies after HSCT

Lymphodepletion regimens
- Fludarabin (30mg/m\(^2\)/d) and Cyclophosphamide (300mg/m\(^2\)/d) for 3 days
- OR
- Bendamustine (90mg/m\(^2\)/d) for 2 days

Treatment
- anti-CD19 allo-CAR-T cells from haplo-ID donor in a 3+3 dose-escalation strategy, 3\times10^6 /kg
- 6\times10^6 /kg
- 1\times10^7 /kg

Primary endpoint
- safety profile, toxicity, efficacy 4 weeks after infusion, the long-term efficiency 2 years after infusion

Estimated primary completion date – December 2021

1 [https://clinicaltrials.gov/ct2/show/NCT04516551](https://clinicaltrials.gov/ct2/show/NCT04516551)
P-BCMA-ALLO1 Allogeneic CAR-T Cells therapy in R/R Multiple Myeloma: phase 1, single group, open-label multicenter study

- Adult patient with R/R MM ≥ 3 previous therapies with a proteasome inhibitor, immunomodulatory agent (IMiD), and anti-CD38 therapy

- Allogeneic BCMA-targeted chimeric antigen receptor CAR T-cell

- Endpoints: safety, anti-myeloma effect of P-BCMA-ALLO 1 (ORR)

Patients enrolled from October, 2021, estimated primary completion date February, 2023
Estimated enrollment 40 participants

1 https://clinicaltrials.gov/ct2/show/NCT04960579
Our experience with allogenic CAR T-cells

- Development of triple knock-out in primary T-cells from healthy donors
- Combination with standard CAR
- iCaspase9 system as suicide gene for safety management
Conclusion

In last decade we could observe the rise of new tool, that allow us precise gene modification

With recent breaktroughs in synthetic biology, who knows what kind of tools will be used for gene modification of T-cells in future

Allogenic CAR T-cells just entered phase I/II of clinical trials

Based on first data (No GvHD; limited CRS; ORR 60% in MM) Allo-CAR T remaining to be perspective.

We just need more data
<table>
<thead>
<tr>
<th>Cell therapy team</th>
<th>Genomic and bioinformatics team</th>
<th>BioBank</th>
<th>Cell and molecular biology team</th>
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<tbody>
<tr>
<td>Juli Rodríguez Bago</td>
<td>Tereza Ševčíková</td>
<td>Petra Vrublová</td>
<td>Matouš Hrdinka</td>
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<td>Piotr Celichowski</td>
<td>David Žihala</td>
<td>Lucie Broskevičová</td>
<td>Michal Řurech</td>
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<td>Benjamin Motais</td>
<td>Anjana Anilkumar Sithara</td>
<td>Romana Lesniáková</td>
<td>Alexander Vdovin</td>
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<td>Sandra Charvátová</td>
<td>Martina Zátopková</td>
<td>Eva Panáčová</td>
<td>Sofiya Lezhava</td>
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<td>Dominka Vrlová</td>
<td>Nela Trífajová</td>
<td>Marcello Turi</td>
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<td>Administration</td>
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<td>Dhwani Radhakrishnan</td>
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<td>Lenka Mršťáková</td>
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<td>Hana Šahinbegovič</td>
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<td>Markéta Dorociaková</td>
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<td>Renata Šnaurová</td>
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<td>Hematopoietic stem cell laboratory</td>
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<td>Nikola Garbová</td>
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<td>Zdenek Kořítek</td>
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<td>Jana Neuwirthová</td>
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<td>Lukáš Grebeníček</td>
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<td>Ondřej Venglář</td>
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<td>Department of Hematooncology</td>
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