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Allo-CAR-T cells

18th International
Myeloma Workshop
September 8-11, 2021
Vienna

www.fno.cz

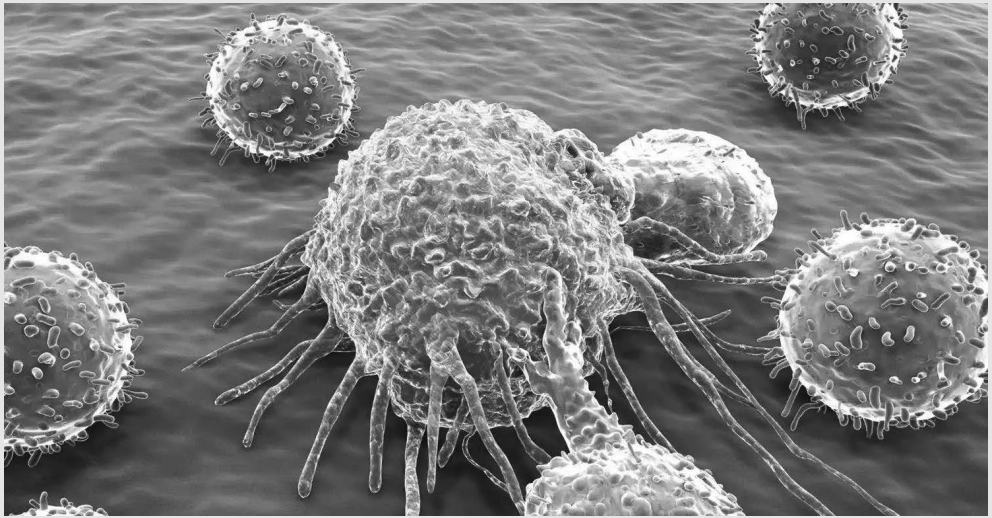
Disclosure

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

Research funding from Takeda, Amgen, Janssen, and Novartis;

Reprograming T-cells to treat cancer

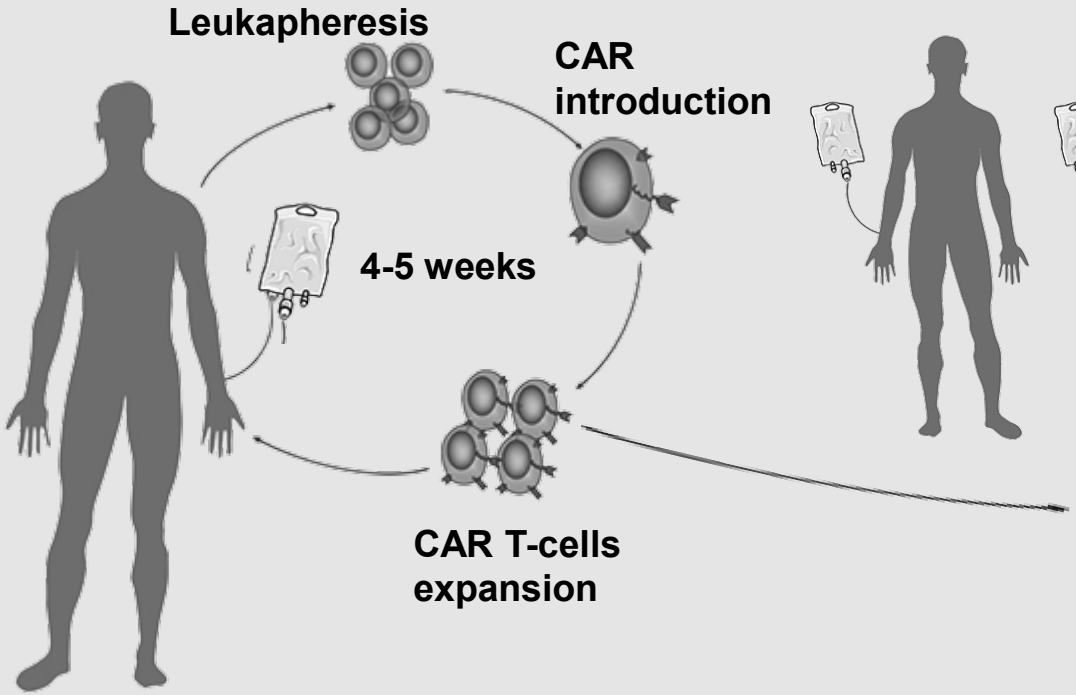
- **Autologous** CAR T-cells approved for MM, ALL, lymphoma treatment
- **Allogenic** CAR T-cells just entered phase I/II of clinical trials

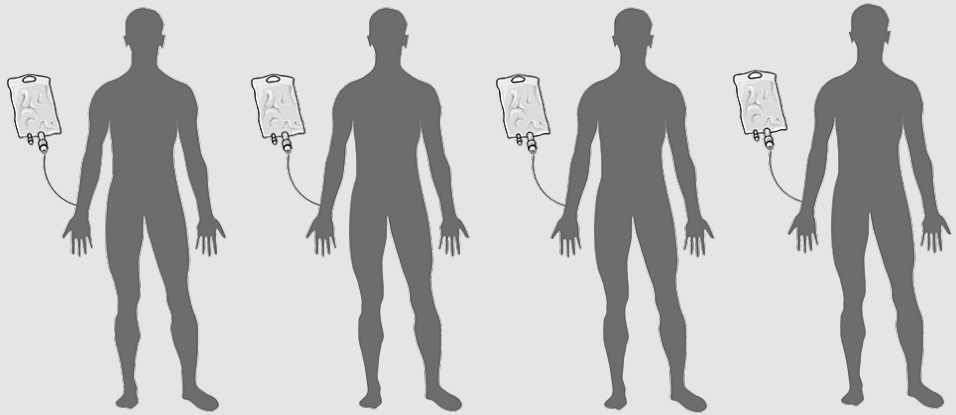


Juan Gartner/ Getty Images

Autologous CAR-T cells

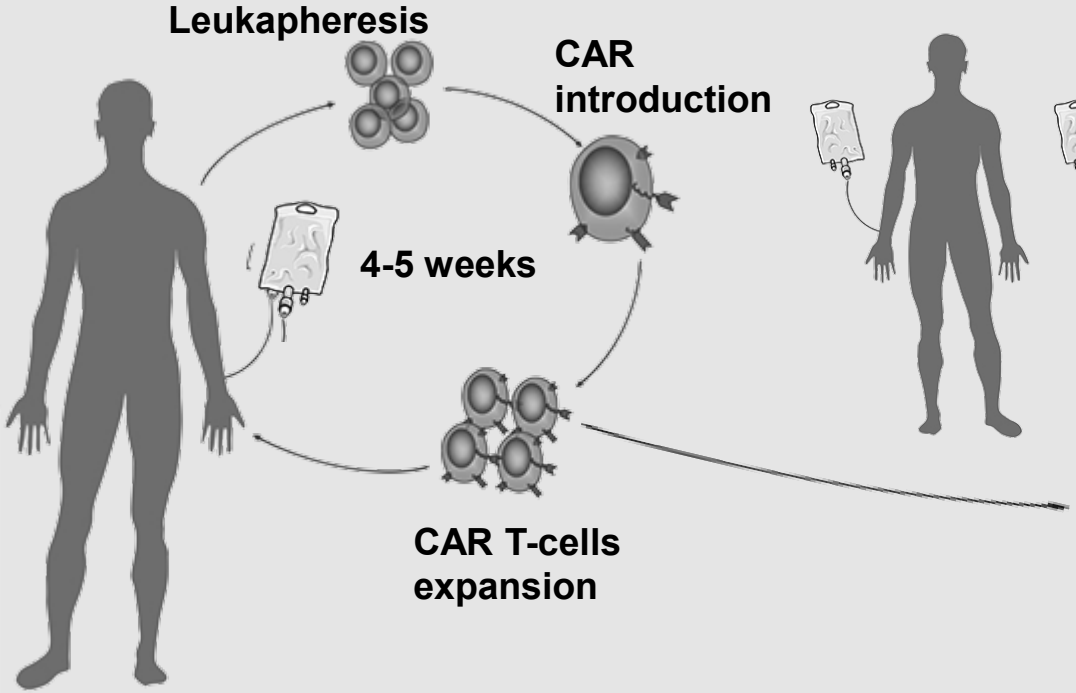
Allogenic CAR-T cells



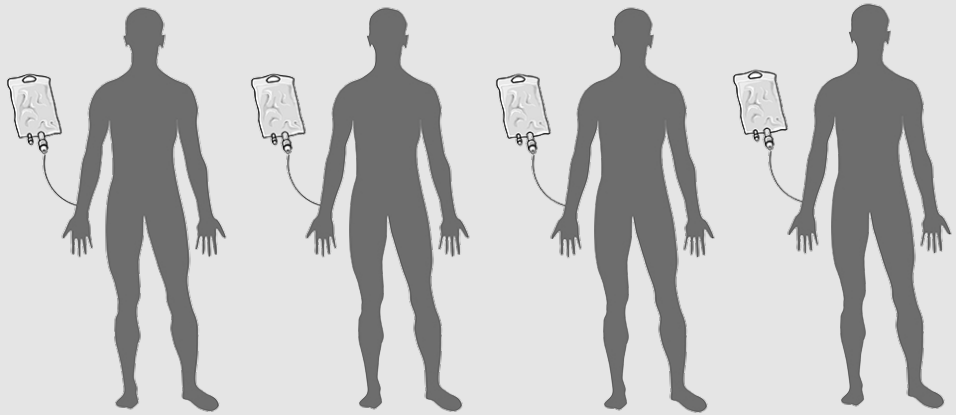


Autologous CAR-T cells

Allogenic CAR-T cells from haploidentical donors



Allogenic CAR-T cells



Autologous CAR T-cells

Pros:

- no GvH
- few genetic manipulation
- approved (some)

Cons:

- Complicated logistic
- limited availability
- limited numbers of aplication
- long production time
- expensive

Allogenic CAR T-cells

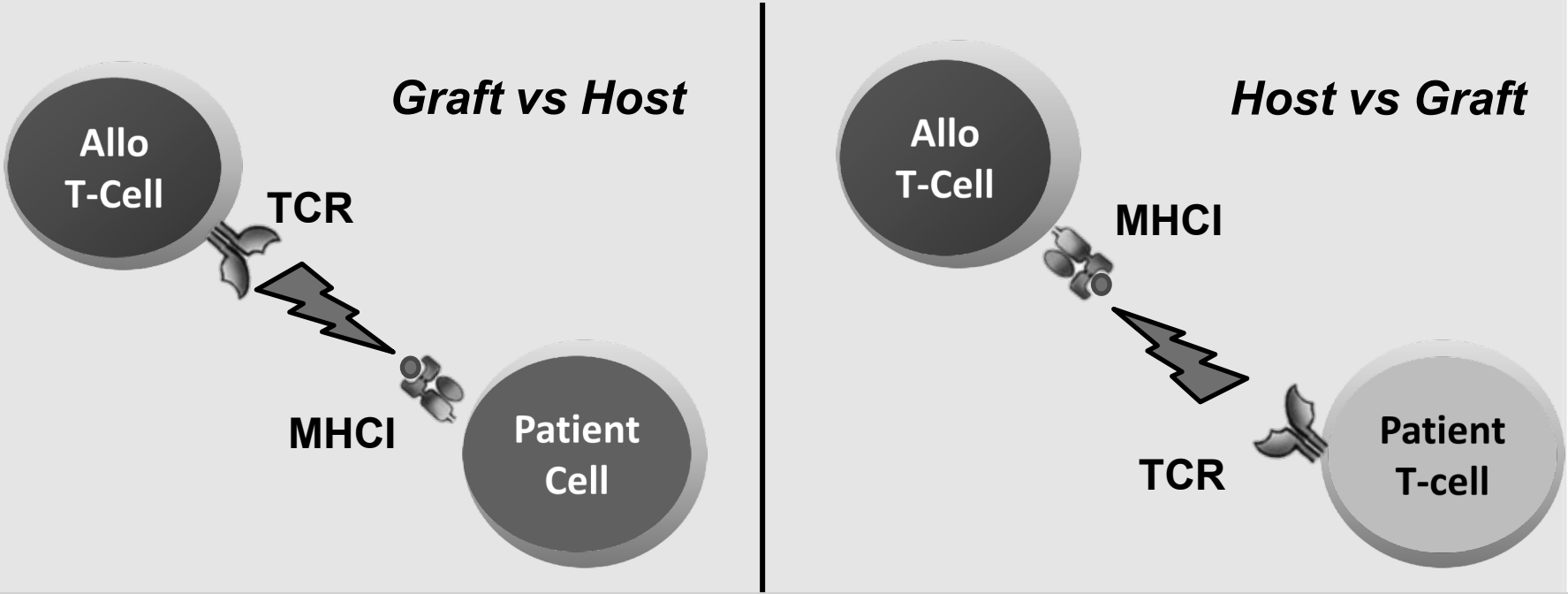
Pros:

- off the shelf
- available for multiple patients
- available several times for one patient if needed

Cons:

- potential GvH
- Potential higher risk of infection
- not yet approved
- Not yet proved safety & efficacy

Allogenic T-cells: tissue incompatibility



The main issue why allo CAR T cells are not yet widely used is the HLA incompatibility

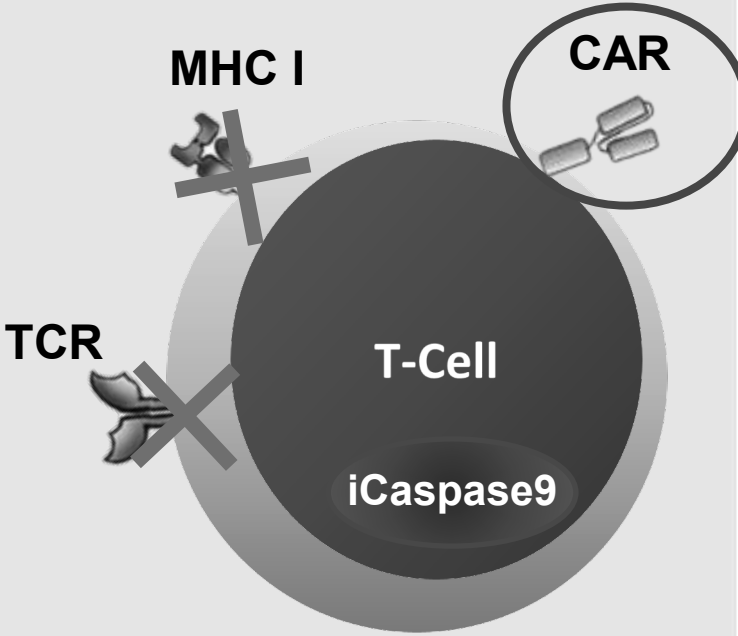
How to make allogenic (CAR) T-cell ?

Genetic manipulation

- Deletion of TCR
- Deletion of MHCI

} **Tissue compatibility**

- Introduction of CAR
- Introduction of sucide gene



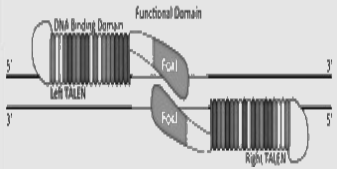
How to make allogenic (CAR) T-cell ?

Genetic manipulation

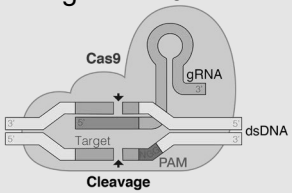
- Deletion of TCR
- Deletion of MHCI

} **Tissue compatibility**

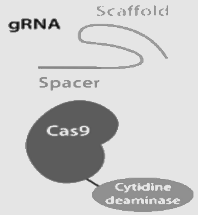
Talen or Zinc finger Nucleases
Poirot et al 2015
Torikai et al 2012
Torikai et al 2013

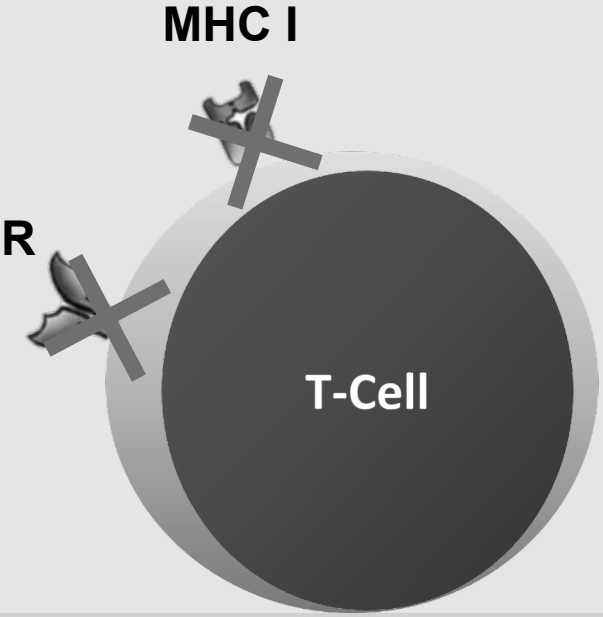


CRISPR/Cas9
Ren et al. 2017
Georgiadis et al. 2018
Ngoc Duong et al. 2019
Liu et al. 2017
Zhang et al. 2017



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

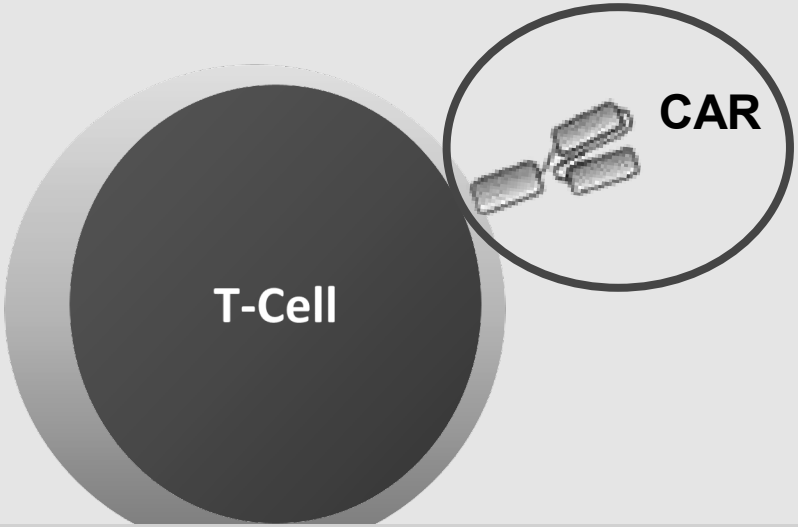




In last decade we could observe the rise of new tools, that allow us precise gene modification, such as Talen Nucleases or CRIPSR/Cas9. We can introduce point mutation that will introduce premature stop codon or disrupt protein splicing, which results in knock-out of the protein.

Chimeric Antigen Receptor introduction:

targeting T-cell towards tumor

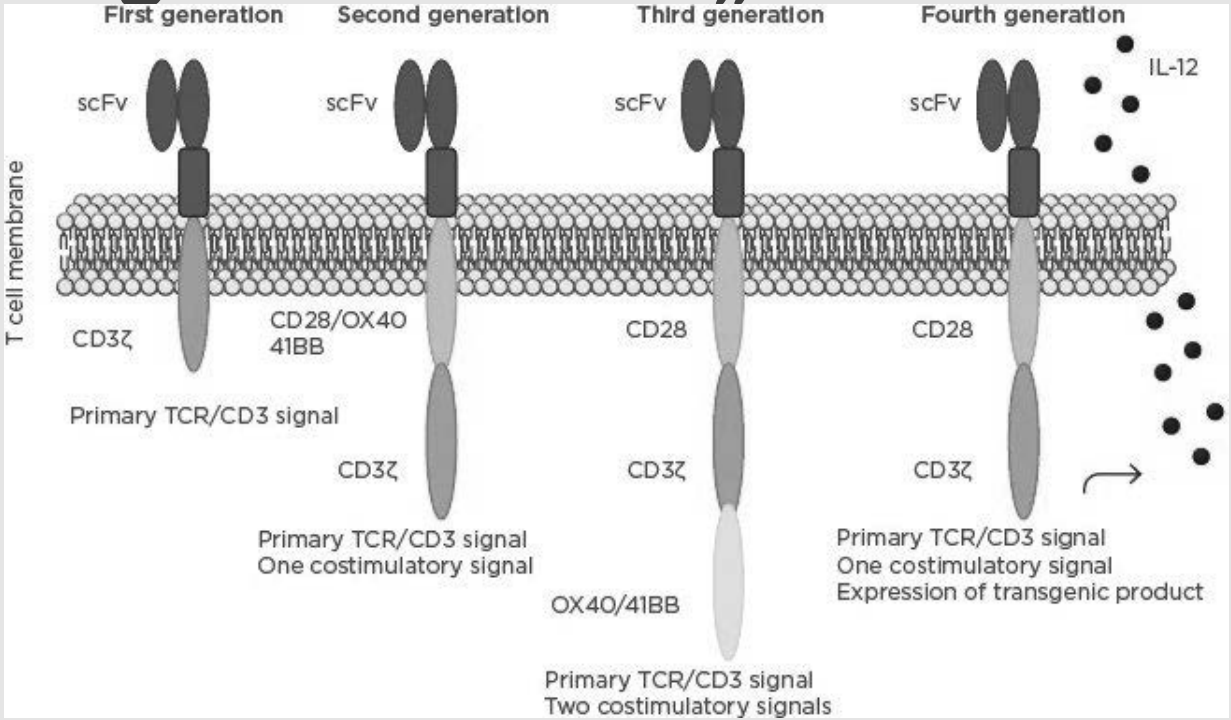


Methods of introducing CAR

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

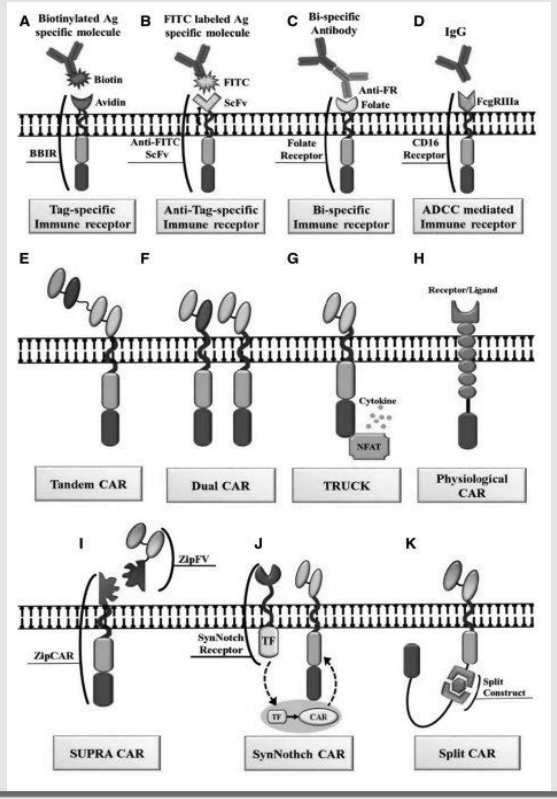
Most people use viral vectors for integration of CAR (lenti or retro vectors) or AAV for long expression.

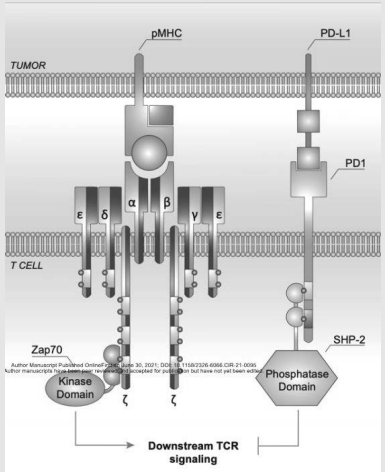
Four generations of „clasical" CARs

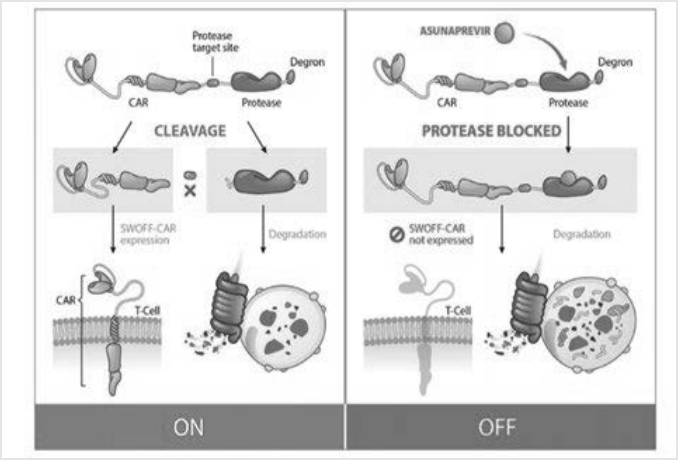


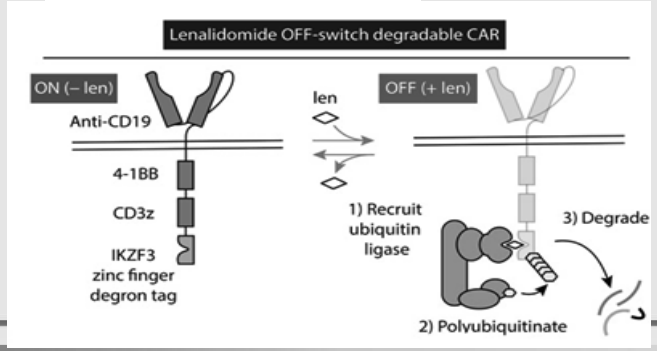
Paubelle et al. 2018

and many more new CARs...coming









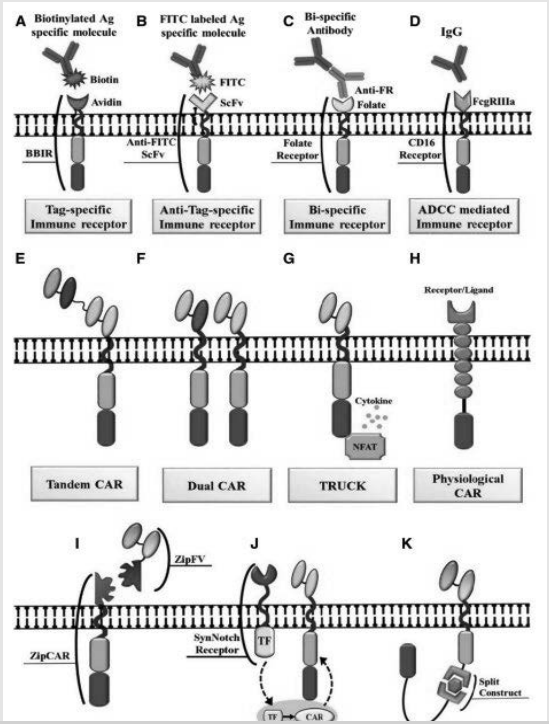
Jan et al 2021

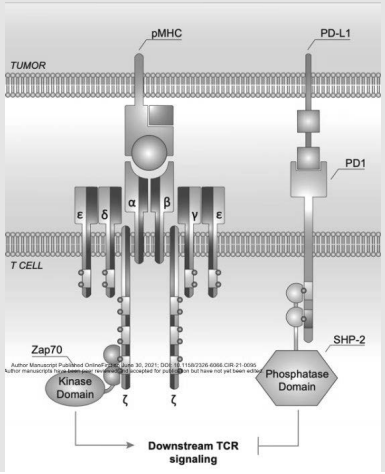
Juillerat et al 2019

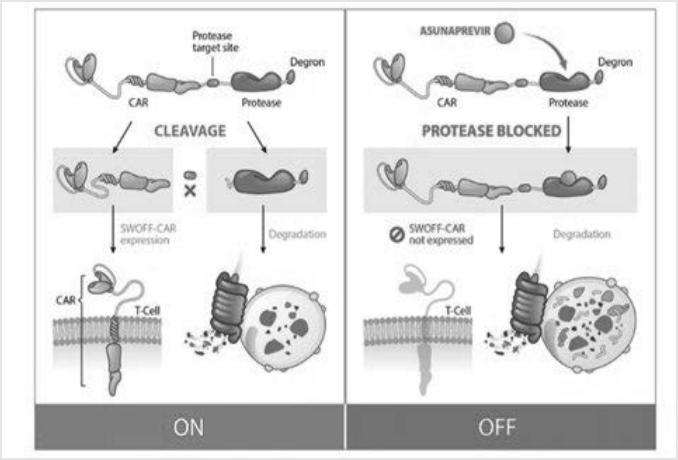
Sahillioglu et al 2021

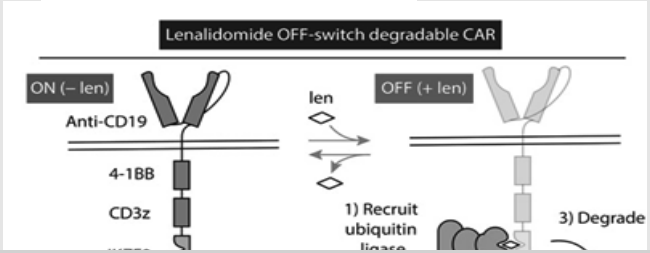
Tahmasebi et al. 2020

and many more new CARs...coming









Jan et al 2021

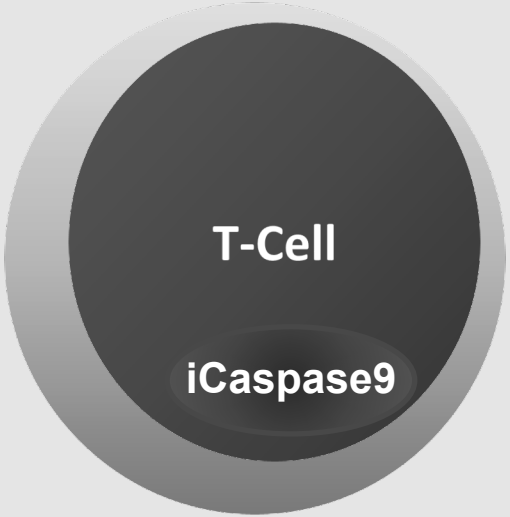
Juillerat et al 2019

Sahillioglu et al 2021

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

Suicide gene insertion

to ensure cell therapy safety

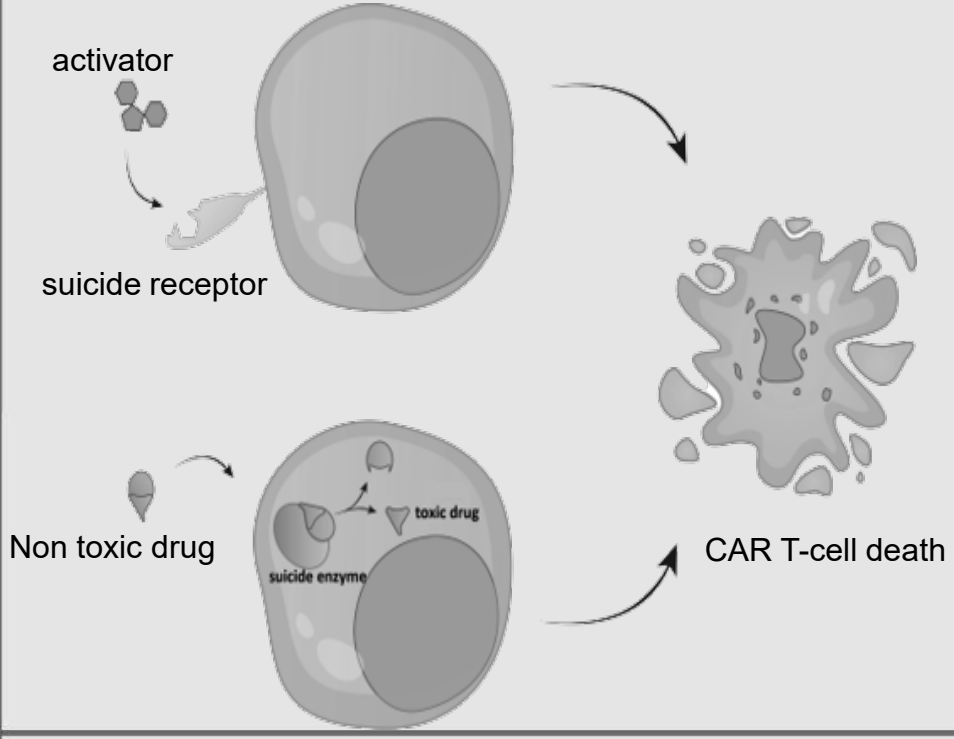


Delivery of suicide gene

- 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 uppon 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

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

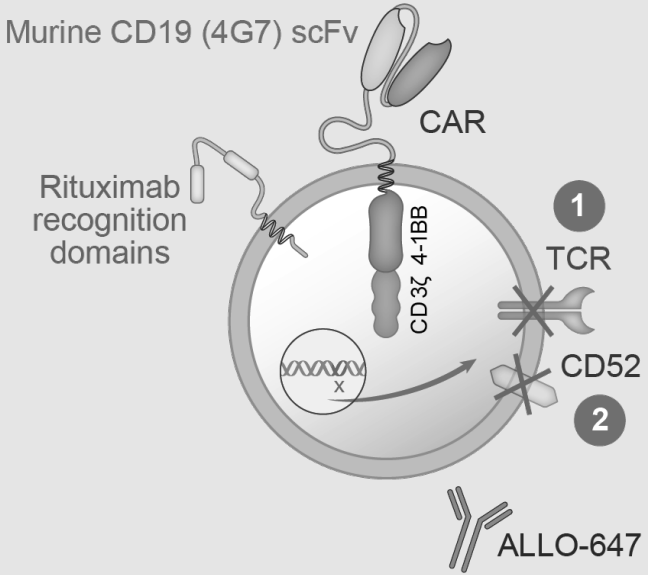
ASCO 2020, abstr. 8002;ASH 2020, abstr. 129;
https://clinicaltrials.gov/ct2/show/NCT04516551
https://clinicaltrials.gov/ct2/show/NCT05000450
https://clinicaltrials.gov/ct2/show/NCT04960579

Allo-501(lymphoma)/Allo-715 (MM), Allo-647 CAR

Talens are delivered with electoporation

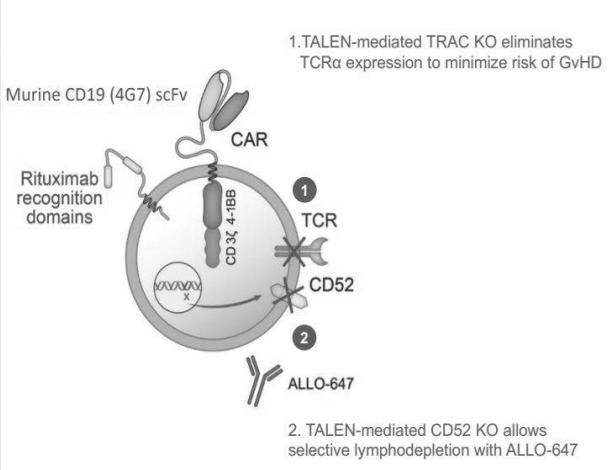
- TALEN-mediated **TRAC KO** eliminates TCRα expression to minimize risk of GvHD
- TALEN-mediated **CD52 KO** allows selective lymphodepletion with ALLO-647 that is anti-CD52 MoAb

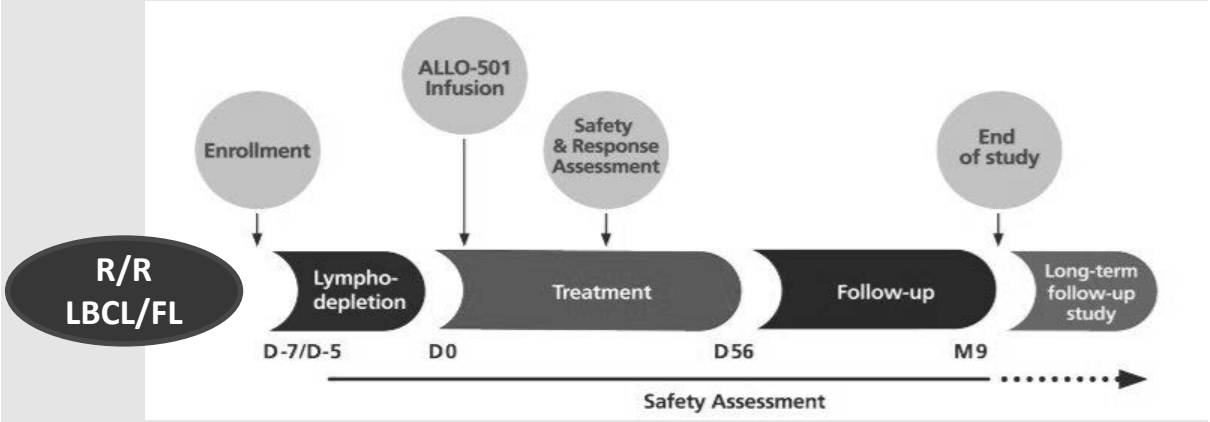
Delivery of **CAR** and **Rituximab recognition domain** by Lentiviral vector



https://www.allogene.com/allocar-t-therapy

ALPHA Study:[1] 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+

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

	DL1	DL2	DL3
Cell Dose ALLO-501	40 x 10^6CAR$^+$ T cells	120 x 10^6CAR$^+$ T cells	360 x 10^6CAR$^+$ T cells

ASCO 2020, abstr. 8002;

ALPHA Study:[1] 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 pharmacokin

ALLO-501 and ALLO-647 have a manageable safety profile

AE of Interest	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Grade 5 n (%)	All grades n (%)
Cytokine release syndrom	2 (9%)	4 (18%)	1 (5%)	-	-	7 (32%)
ICANS	-	-	-	-	-	-
GvHD	-	-	-	-	-	-
Infection	5 (23%)	4 (18%)	2 (9%)	-	-	11 (50%)
Infusion reaction	1(5%)	9 (41%)	1 (5%)	-	-	11(50%)
Neutropenia	-	1 (5%)	7 (32%)	7 (32%)	-	15 (68%)

ASCO 2020, abstr. 8002;

ALPHA Study:[1] phase 1, open-label, multicenter dose escalation study in R/R Non-Hodgkin Lymphoma (ALLO-501 and ALLO-647)

Cell Dose and LD regimen	39mg ALLO-647			ALL 39mg ALLO-647 (N = 11)	90mg ALLO-647		All 90mg ALLO-647 (N=8)	All Patients (N=19) Rate (95%CI)
	40 x 10^6 CAR^+ cells (N=4)	120 x 10^6 CAR^+ cells (N=4)	360 x 10^6 CAR^+ cells (N=3)		120 x 10^6 CAR^+ cells (N=6)	360 x 10^6 CAR^+ cells (N=2)		
ORR, n (%)	3 (75%)	3 (75%)	1 (33%)	7 (64%)	4 (67%)	1 (50%)	5 (63%)	12/19 (63%) (38%, 84%)
CR , n (%)	1 (25%)	1 (25%)	1 (33%)	3 (27%)	4 (67%)	0 (0%)	4 (50%)	7/19 (37%) (16%, 62%)

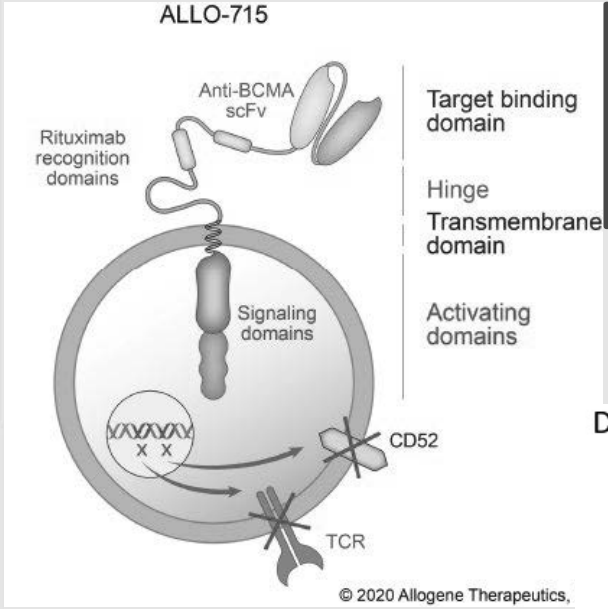
Median follow-up 3.8 months

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)**

ASCO 2020, abstr. 8002;

UNIVERSAL Study:[1] 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*

ASH 2020, abstr. 129;

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

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

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

- **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**

ASH 2020, abstr. 129;

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

Cell Dose and LD Regimen	FCA Cohort						CA Cohort	
ALLO-715	40	160	320	320	320	480	160	320
ALLO-647	Low (n = 3)	Low (n = 4)	Low (n = 6)	High (n = 4)	All (n = 10)	Low (n = 3)	Low (n = 3)	Low (n = 3)
ORR, n (%)	--	2 (50)	3 (50)	3 (75)	6 (60)	1 (33)	--	2 (67)
≥ VGPR, n (%)	--	1 (25)	3 (50)	1 (25)	4 (40)	--	--	1 (33)

*Clinical response evaluation based on IMWG response criteria.[2] ≥ VGPR defined as sCR, CR or VGPR

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

Median follow-up 3.8 months

ASH 2020, abstr. 129;

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/m2/d) and Cyclophosphamide (300mg/m2/d) for 3 days
OR
Bendamustine (90mg/m2/d) for 2 days

Treatment

anti-CD19 allo-CAR-T cells from haplo-ID donor in a 3+3 dose-escalation strategy, 3×10^6 /kg 6×10^6 /kg 1×10^7/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 htt ps://clinicaltrials.gov/ct2/show/NCT04516551

P-BCMA-ALLO1 Allogeneic CAR-T Cells therapy in R/R Multiple Myeloma:[1] 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

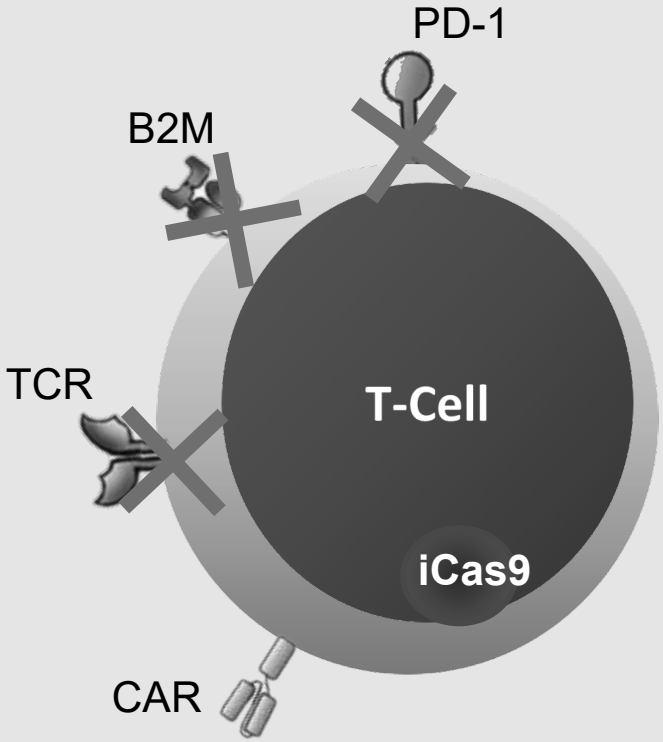
safety, anti-myeloma effect of P-BCMA-ALLO 1 (ORR)

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

1 https://clinicaltrials.gov/ct2/show/NCT04960579

Our expirience 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 managment



Conlusion

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

Blood Cancer Research Group
www.brcg.cz

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- Zuzana Walek

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- Marcello Turi
- Dhwani Radhakrishnan
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- Jana Neuwirthová