T cell immunotherapy of Cancer

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Objectives

1. Review the structure of CAR and TCR and their recognition of tumor antigens and signaling mechanisms.

2. Describe strategies to modify recombinant receptors to increase their function.

3. Discuss strategies involved in target selection.

4. Review published clinical experience efficacy and toxicity.

Rationale for T cell immunotherapy

1. Specificity- TCR
2. Mechanisms- direct cytotoxicity, cytokines, chemokines
3. Systemic trafficking
4. Memory response
5. Ability to numerically expand T cells *in vitro*.

Difficulties in clinical use of T cell therapy

1. Low frequency of T cells for each specificity < 10^{-4}
2. Thymic selection, tolerance, and low avidity for self-antigens
3. Immunosuppressive tumor environment and PD-1L, Treg, MDSC
4. Poor survival of activated T cells *in vivo*
5. On target toxicity against normal tissue, off target toxicity

Early Milestones in T cell Adoptive Cell Therapy

- TIL (Tumor Infiltrating Lymphocytes) for melanoma. Rosenberg 1988. Use of intensive non-myeloablative lymphodepletion (2005). Myeloablative conditioning (2008)

  Complete Responses in patients with bulky disease, sustained=cure

- DLI (Donor Lymphocyte Infusion) post-allogeneic BMT for leukemia. Kolb 1988, Slavin 1988

- Anti-CMV T cell clones post-BMT. Riddell *et al* 1992

- CAR- chimeric antigen receptor. Single chain variable Ab (scFv). Eshar 1993

- Gene-modified virus-specific T cells for EBV lymphoproliferative disease Rooney *et al*. 1995

Transgenic T cells for Cancer Therapy

- Two major strategies: CAR (chimeric antigen receptor) or high-affinity TCR (T-cell receptor)
- The CAR molecule consists of an extracellular single-chain antibody fragment (scFv) linked to intracellular signaling components. Recognizes a cell-surface target.
- The TCR associates with natural CD3 and signals like endogenous TCR. Recognizes peptide/MHC molecules.
- The receptor is transferred to a population of host T cells (CD8 and CD4) by lentivirus or retrovirus vector.
- The recombinant receptor re-directs the specificity toward a tumor-specific or tumor-associated antigens.

Structure of a CAR receptor

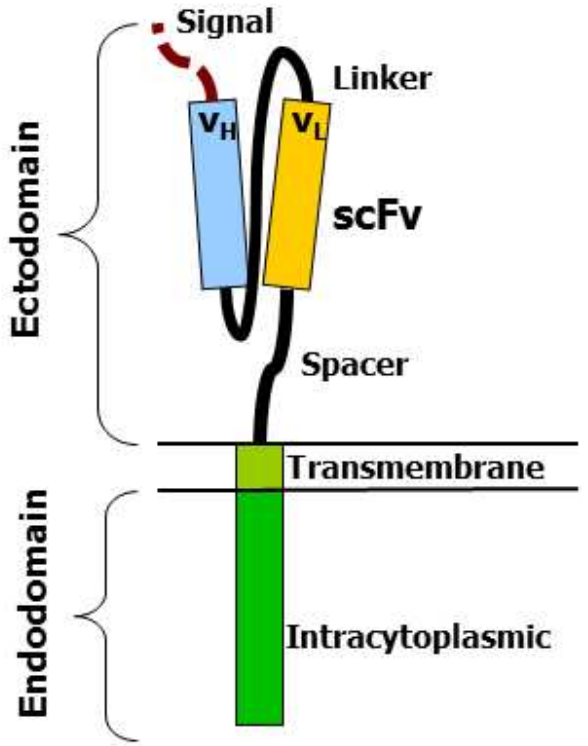
The scFv has very high binding affinity and specificity of mAb

The spacer is from CD8.

Transmembrane and proximal signaling are from CD3-ζ or FcεR

The cytoplasmic signaling domain is enhanced with tandem ITAM domains from CD28, CD137 (4-1BB), CD134 (OX40).

Activate: PI3K, PLCγ, MAPK, NF-κB



Factors affecting performance of CAR T cells

First generation= CD3-ζ, **-** limited proliferation and persistence *in vivo*. Minimal clinical activity in multiple trials (exception was anti-GD2 in NBL 3/19 CR). Induction of anergy due to lack of co-stimulatory signals. Generation of HAMA responses.

Second generation= CD28-CD3-ζ, Promising responses with anti-CD19 or CD20 in hematologic malignancy. Proliferation and long-term persistence. Depletion of B cells impairs HAMA response. Persistent re-stimulation by emerging B cells.

Third generation= CD28-CD3-ζ-CD137

Expression of CAR in EBV-reactive T cells. Provision of antigen stimulation through the native TCR and co-stimulation by EBV expressing B cells. Resulted in longer persistence than non-EBV reactive T cells expressing the CAR when transfused together.

Variable Anti-CD19 CAR Strategies in Five Different Clinical Trials All Produced Responses

scFv mAb	linker	ITAM signaling	Gene transfer	Prep	IL-2
FMC63	CD8-α	CD137/CD3-ζ	Lentivirus	Yes	No
FMC63	CD28	CD28/CD3-ζ	Retrovirus	Yes	Yes
SJJ25C1	CD28	CD28/CD3-ζ	Retrovirus	Yes	No
FMC63	IgG_1 hinge and CH2/3	CD28/CD3-ζ vs. CD3-ζ	Retrovirus	No	No
FMC63	IgG_4 hinge	CD3-ζ	Electroporation	Yes	Yes

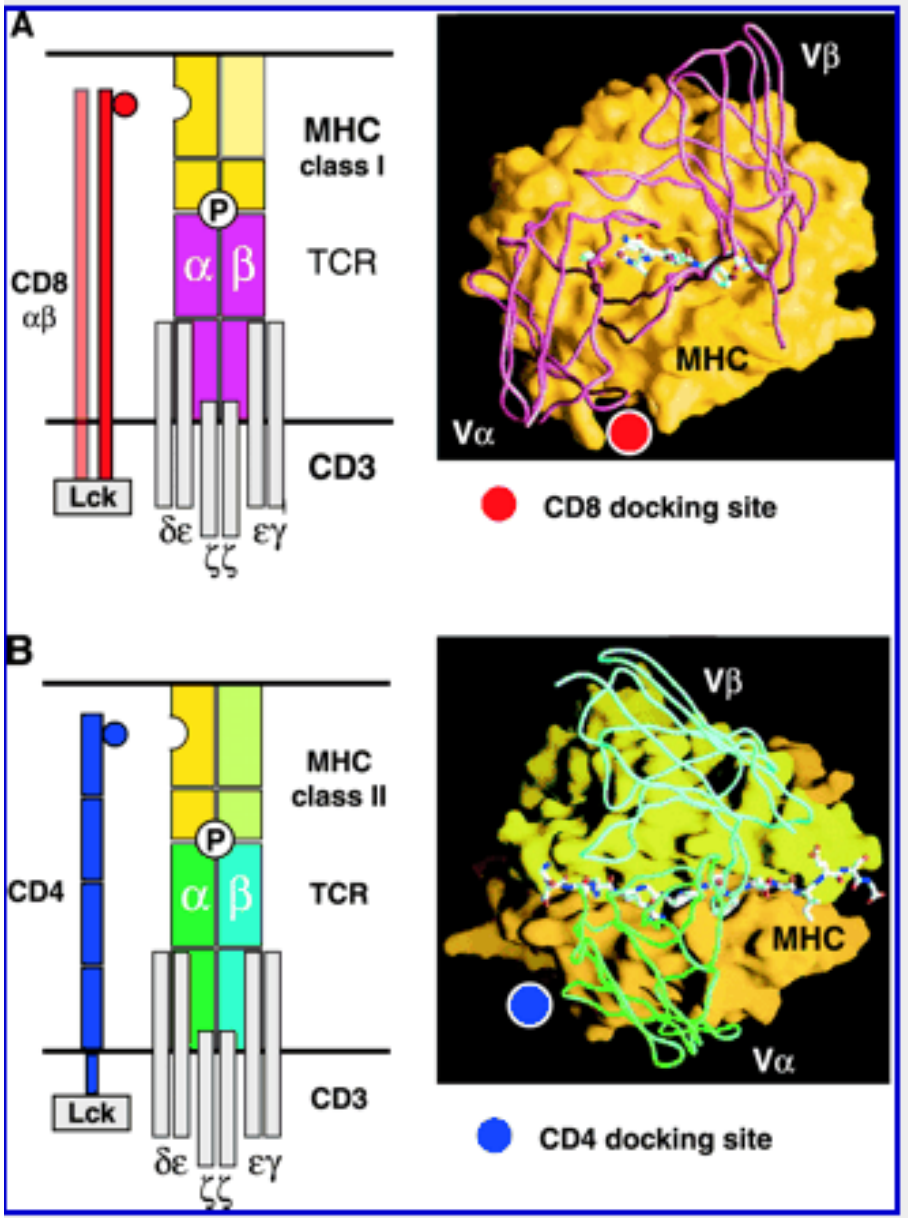
Published Clinical Studies of CAR T-cell Therapy

Disease	Target	CAR structure	Responses /# treated	Author/Journal	Yr
CLL	CD19	CD137-CD3-ζ	1/1* Tumor Lysis	Porter NEJM	2011
CLL, preB-ALL	CD19	CD28-CD3-ζ	3 SD for 6 m/8	Brentjens Blood	2011
CLL, Lymphoma	CD19	CD137-CD3-ζ	1CR,5PR,1SD/7	Kochenderfer Blood	2012
ALL	CD19	CD137-CD3-ζ	2/2	Grupp NEJM	2013
NHL	CD20	CD137-CD28-CD3-ζ	1 PR, 2 NED/3	Till Blood	2012
AML	Lewis Y	CD28-CD3-ζ	none/4	Ritchie Mol Ther.	2013
RCC	CAIX	CD3-ζ	none/11	Lamers Mol Ther.	2013
Neuroblastoma	CD171 (NCAM)	CD3-ζ	1PR/6	Park Mol Ther.	2007
Neuroblastoma	GD2	CD3-ζ	3CR/19	Louis Mol Ther.	2011
Colorectal	ERBB2	CD28-CD137-CD3-ζ	0/1* Toxicity	Morgan Mol Ther	2010
Colorectal	TAG72	CD3-ζ	1SD/16	Warren CanGeneTher	1998
Colorectal, Breast	CEA	CD3-ζ	2 minor resp/7	Ma CCBRM	2002

Structure of the TCR MHC-peptide complex

MHC-I binds 9-11 AA peptide Fragments that are processed by the proteasome, shuttled into the ER by TAP.
The TCR Vα and Vβ have AA residues that bind to MHC or the peptide.
Site-directed mutation of contact residues can increase the affinity of binding.

MHC-II binds longer peptides 15 AA but the core contact region is the central 9 AA. The configuration of the Vα and Vβ in relation to peptide is different.



Factors affecting the performance of TCR-transgenic T cells

Insufficient avidity of endogenous TCR against normal proteins. Site directed mutation in CDR3 to generate high-affinity variants. Phage display mutant libraries. Derivation of T cells in HLA-transgenic mice.

Mis-pairing of transgenic TCR α and β chains with the endogenous TCR chains. Introduction of cysteines to form a second disulfide bond, or knob/hole.

Unequal production of TCR α and β chains from the vector. Use of cleavable linker sequence.

Insufficient CD3 to form complete TCR complexes. Inhibit endogenous TCR with siRNA or zinc finger nucleases. Include CD3 molecules in vector.

Inability to rapidly inactivate T cells. Addition of HSV-TK, Inducible caspase 9, CD20 gene (Rituximab)

Published Clinical Trials of TCR transgenic T cells

Disease	Target	Responses/#treated	Author/Journal	Year
Melanoma	MART1	1 PR/15	Duval Clin.Can.Res.	2006
	MART1	4OR/31	Morgan Science	2006
	MART1	6PR/20*Toxicity	Johnson Blood	2009
Melanoma	GP100	1CR, 2PR/16	Johnson Blood	2009
Mel/Esophag Synovial Sarcoma	MAGE-A3 (A02)	1CR, 4PR/9* Toxicity	Morgan J.Immunother	2013
Melanoma/myeloma	MAGE-A3 (A01)	0/2* Toxicity	Linette Blood	2013
Melanoma	p53	no data/14	Davis Clin.Can.Res.	2010
Melanoma	NY-ESO-1	2CR, 7PR/17	Robbins J.Clin.Oncol.	2011
Myeloma	NY-ESO-1	3CR, 7PR/11	Rapoport ASH abstract	2010
Colorectal	CEA	1 PR/3* Toxicity	Parkhurst Mol Ther.	2011

Unanticipated off-target Toxicity by MAGE-A3 TCR

1. Linette *et al* constructed a high-affinity HLA-A01 restricted MAGE-A3 TCR.
   TCR was initially derived from an HLA-A01 melanoma patient- then affinity enhanced.
   Preclinical testing did not reveal any toxicity against normal tissue.

Patient #1- 63 yo Melanoma lymphodepleted with cytoxan 60 mg/kg x 2, 10^{10} T cells.
Developed fever/neutropenia 2 days later then cardiac failure 4 days after T cell infusion.
Attributed to fever/cytokines.
Patient #2- 57 yo Myeloma melphalan 200 mg/m2-ASCT. 2.4 x 10^{9} T cells. *C diff.*
2 days after ACT fever, next day pericardial effusion, death 5 days after ACT.
Pathology showed lymphocyte infiltration of heart and myonecrosis.
Investigation showed no MAGE-A3 in heart. Cross-reactivity to epitope from titin.

2. Morgan *et al* used high-affinity HLA-0201 restricted anti-MAGE-A3 epitope which also present in MAGE-A9 and one AA conservative difference in MAGE-A12.
Patients received lymphodepletion cytoxan 60 mg/kg x 2 and fludarabine 25 mg/m2 x 5 days and 6 or 8 x 10^{10} cells. Three of 9 patients developed neurologic symptoms, seizures, MRI changes within 5 days of ACT. Autopsy of 2 patients showed T cell infiltrate and leucoencephalopathy. Gene expression analysis showed low levels of MAGE-A12 in brain tissue.

Unanticipated On-target Toxicity

1. Anti-Carbonic anhydrase IX CAR cells (CD3ζ) for treatment of RCC showed grade 2-4 hepatobiliary toxicity, even at the lowest dose of 0.2×10^9 cells. Liver biopsy showed T cell infiltrate,Lamers 2013.

2. Anti-CEA high-affinity TCR transgenic T cells caused severe transient colitis in all 3 patients that was categorized as a dose-limiting toxicity. Expression of CEA on colon epithelial cells was observed. Parkhurst 2010.

3.Anti-melanocyte toxicity, vitiligo, uveitis, hearing changes were observed with anti-MART-1 or GP-100 TCR. These effects were associated with the higher-affinity TCR and responded to corticosteroids. Johnson 2009

Response-associated toxicity

1. Tumor-lysis syndrome. Patient treated with CD19-CAR T cells (1.4 x 10^7 cells). On day 14 days fever, chills. Day 22 he developed tumor lysis syndrome. Day 28 CR.
   Proliferation of T CAR T cells (1000-fold) to 20% of circulating lymphocytes and high levels of IFN-γ, IL-6, CXCL9, CXCL10 on Day 23 resolving by day 31.

2. Cytokine toxicity. A 39 yr old melanoma patient received 10^{10} anti-HER2 CAR (CD228-CD137-CD3ζ) T cells after extensive lymphodepletion (cytoxan 60 mg/kg x 2 days and fludarabine 25 mg/m2 x 5 days). 15 minutes after infusion she developed respiratory distress and required intubation. Levels of IFN-γ, IL-6, GM-CSF, TNF-α. T cells were mainly present in lung and lymph nodes not tumor. HER2 is expressed at low levels in pulmonary endothelium.

3. Cytokine vs. Tumor lysis. A 69 yr old CLL patient with extensive bulky disease received 3 x 10^7 /kg anti-CD19 CAR (CD28-CD3ζ) after cytoxan 1.5 gm/m^2. Fever, dyspnea, hypotension developed 20 hrs later and death 44 hrs post ACT. Serum K+, Phos, Creat, and uric acid were rapidly increasing.

Conclusions

1. T cell therapy is able to induce durable CRs in heavily pretreated patients with bulky disease. Specificity of a population of peripheral T cells can be modified by gene transfer of CAR or TCR molecules.

2. CAR molecules have high affinity (nM) for targets on the cell surface including; CD19, CD20, GD2, CAIX, FR, and ERBB2. Greatest success in CLL, ALL and lymphoma. Various signaling domains, preparative regimens, cell dose and disease targets are in active development.

3. TCR targeting peptide epitopes derived from frequently expressed cancer-testis antigens NY-ESO-1 or MAGEA3 are effective in melanoma, synovial sarcoma, myeloma. Absence of target gene in normal tissue is important.

Audience Questions

1. Which of the following signaling domains has not been used in CAR T cells?

A. TCR intracellular domain
B. CD137 (4-1BB)
C. CD28
D. CD3ζ

Audience Questions

2. What is the most important parameter in determining clinical response to T cell immunotherapy?

A. Cell dose
B. Amount of Tumor
C. Transient toxicity from cytokine release
D. Affinity of the receptor

Audience Questions

3. CAR T cell therapy has induced CR in which of the following diseases?

A.CD19 positive CLL
B.CAIX positive Renal Cell Carcinoma
C.GD2 positive Neuroblastoma
D.CEA positive Colorectal Carcinoma
E.A and C