Highlights from IMW 2021

1-2 febbraio 2022
Bologna
Royal Hotel Carlton

Alessandro Rambaldi

Meccanismi di Resistenza

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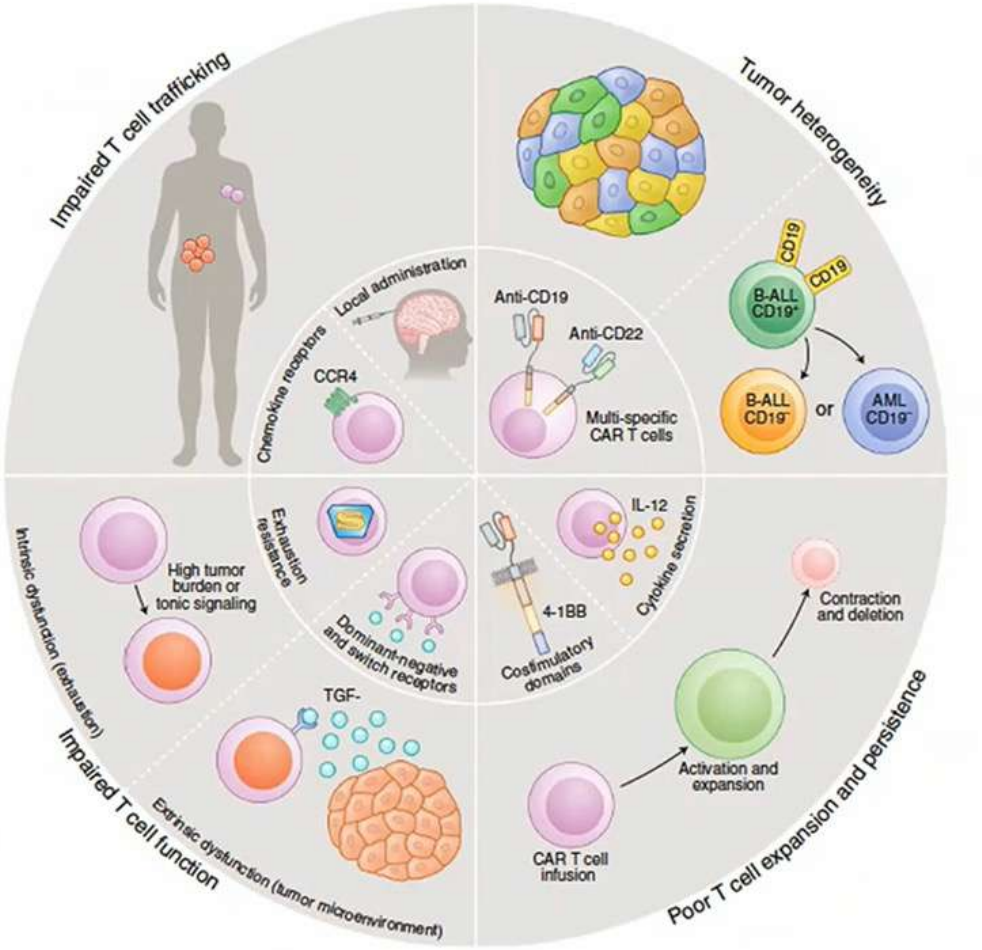
CAR-T & Bispecific Antibodies: mechanisms of resistance

Yi Lin, MD PhD
Associate professor of Medicine
Division of Hematology
Mayo Clinic

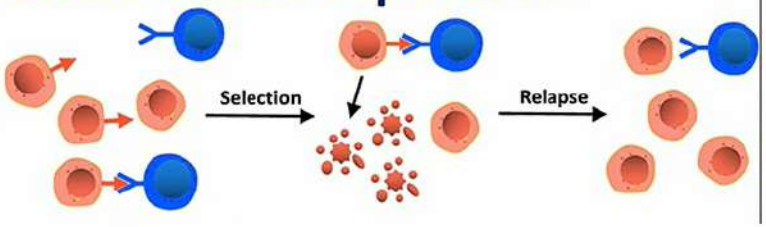
Mechanisms of resistance



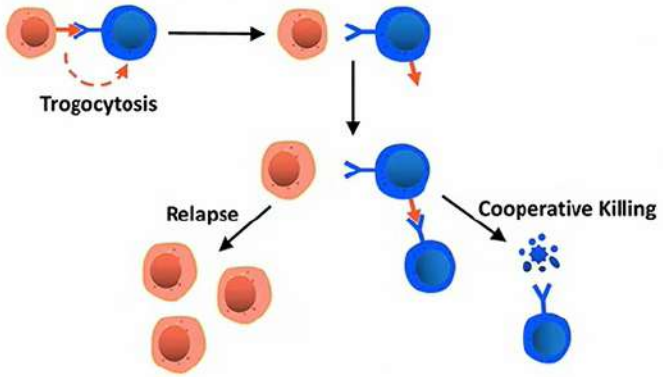
Majzner & Mackall. Nat Med 2019.

Changes in target antigen expression on tumor cells

Clonal selective pressure

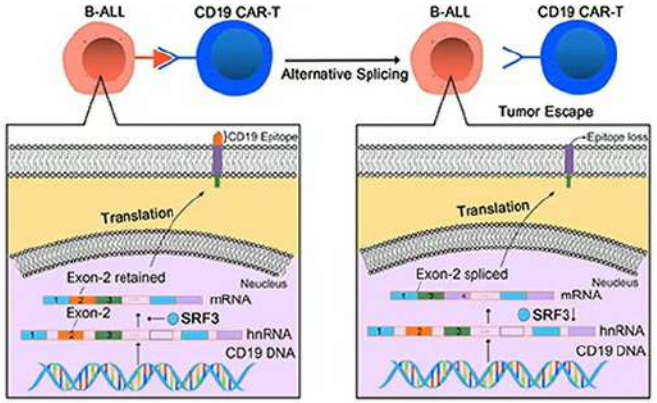


Trogocytosis

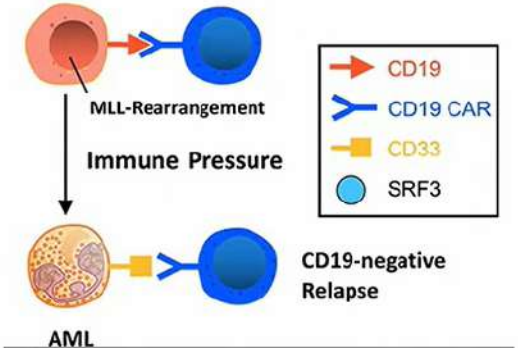


Majzner & Mackall. Nat Med 2019. Xu et al Front Immun 2019.

Splice Variant



Lineage Switch



Expression of CAR targets at relapse

Disease	CAR Target Antigen Present @ Relapse	CAR Target Antigen Negative Relapse		
		Loss of Expression	Splice Variant	Lineage Switch
B-ALL CD19-CD28 CAR[1,2] CD19-41BB CAR[3,4,5]	++ +	+ ++	Reported[1,4]	Myeloid[5, 6]
B-NHL[7]	++	+	Reported	Unknown
MM[9,10,11, 12]	++	+ (bi-allelic deletion)	Unknown	Unknown

Heterozygous deletion is increased from NDMM to RRMM.
This could increase to risk of antigen loss from bi-allelic deletion with immunotherapy.

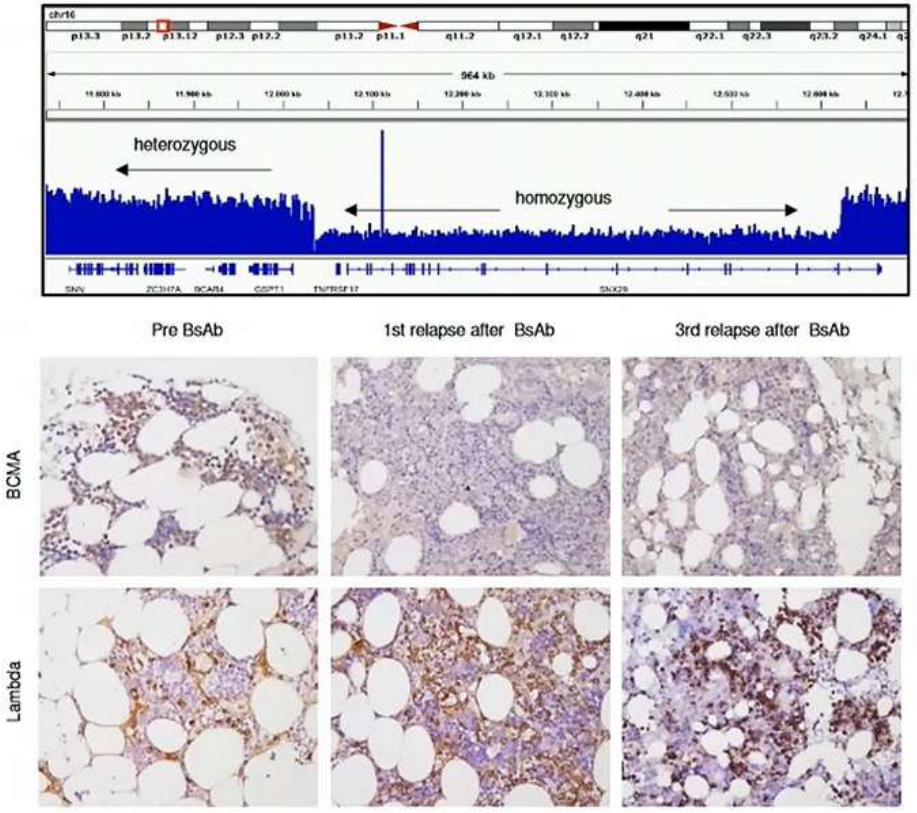
1. Lee et al. Lancet 2015. 2. Park et al. NEJM 2018. 3. Maude et al. NEJM 2014. 4. Maude et al. NEJM 2018. 5. Gardner et al. Blood 2017. 6.Jacoby et al. Nat Commun 2016. 7. Neelapu et al. ASH 2019 abstr 203. 9. Cohen et al. JCI 2019. 10. Munshi et al. NEJM 2021. 11. Samur et al. Nat Comm 2021. 12. Da Via et al. Nat Comm 2021.

Yi Lin. IMW 2021

Bi-allelic BCMA loss also reported in resistance to bispecific antibody

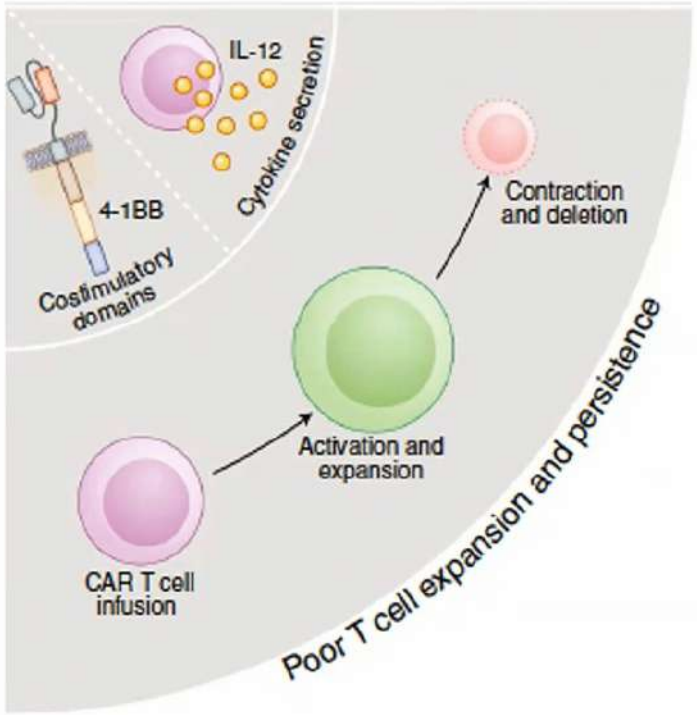
56M penta-refractory MM treated with AMG420

- Pre-treatment - BCMA expression detected by IHC, heterozygous del 16p
- Relapse 6 mo – clonal biallelic deletion, loss of BCMA expression by IHC
- KRd – 20 mo, BCMA expression loss was persistent
- Belantamab – primary refractory



Truger et al. Blood Adv 2021

CAR T Expansion & Persistence



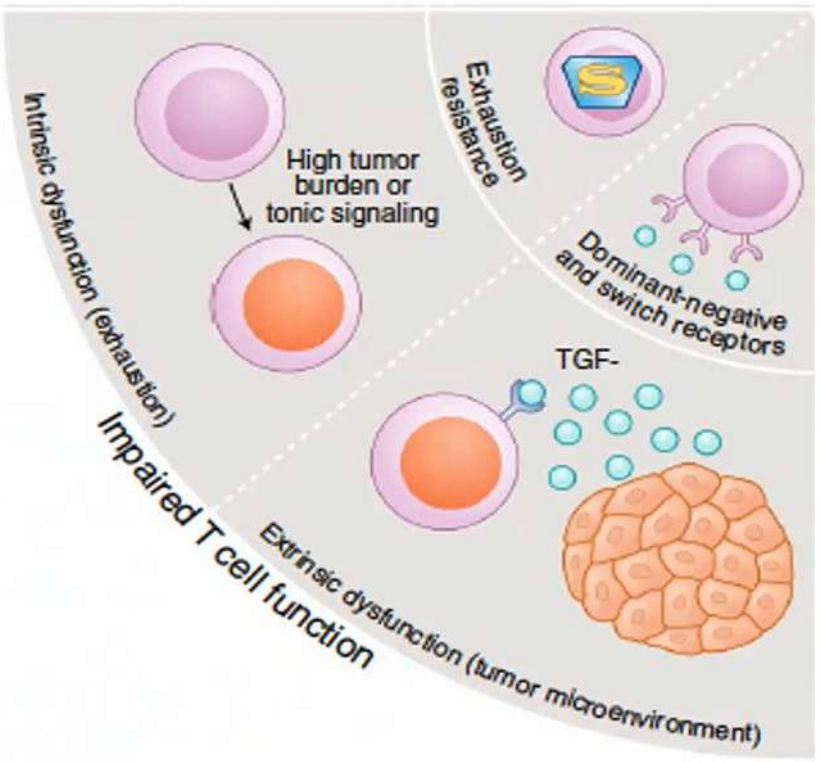
CAR T detection is limited by the threshold of detection by flow cytometry and sequencing

Majzner & Mackall. Nat Med 2019.

- CAR T expansion correlates with clinical response in B-ALL[1,2,3,4], NHL[5, 6], & MM[7,8,9,10,11]
- CAR T persistence to maintain disease control
  - Necessary in ped B-ALL[1,2,3]
  - Not necessary in B-NHL[5,6]
  - Data evolving in myeloma, suggest relevant[7,8,9,10,11]

1. Lee et al. Lancet 2015. 2. Park et al. NEJM 2018. 3. Jacoby et al AJH 2018. 4. Maude et al NEJM 2018. 5. Schuster et al NEJM 2019. 6. Locke Lancet Oncol 2019. 7. Brudno Blood 2018. 8. Cohen et al JCI 2019. 9. Raje NEJM 2019. 10. Munshi et al. NEJM 2021. 11. Berdeja et al. Lancet 2021.

CAR-T Dysfunction

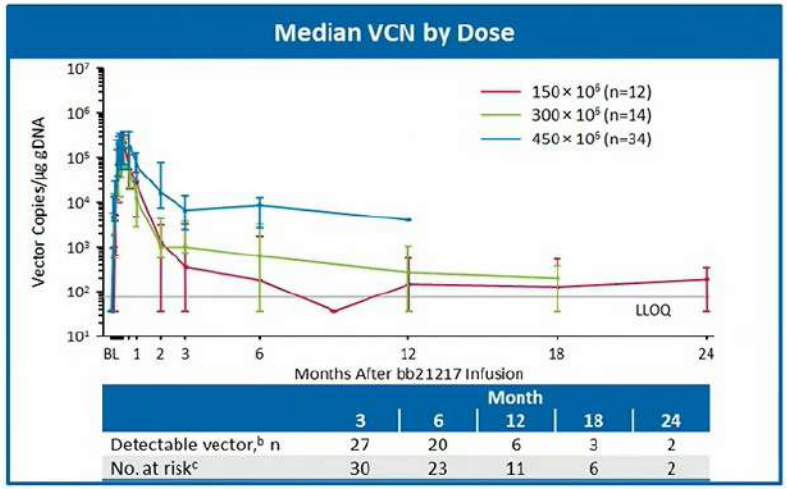


CAR T cell features correlated with clinical response

- CD8+CD45RO-CD27+ in collected cells (CLL[1], MM[2,3])
- Polyfunctional cytokine productions[4]
- TET2 (Tet methylcytosine dioxygenase 2) loss of function[5]
  - CAR integration at this gene resulted in loss of function
  - Clonal expansion of CD8 T with Tcm features

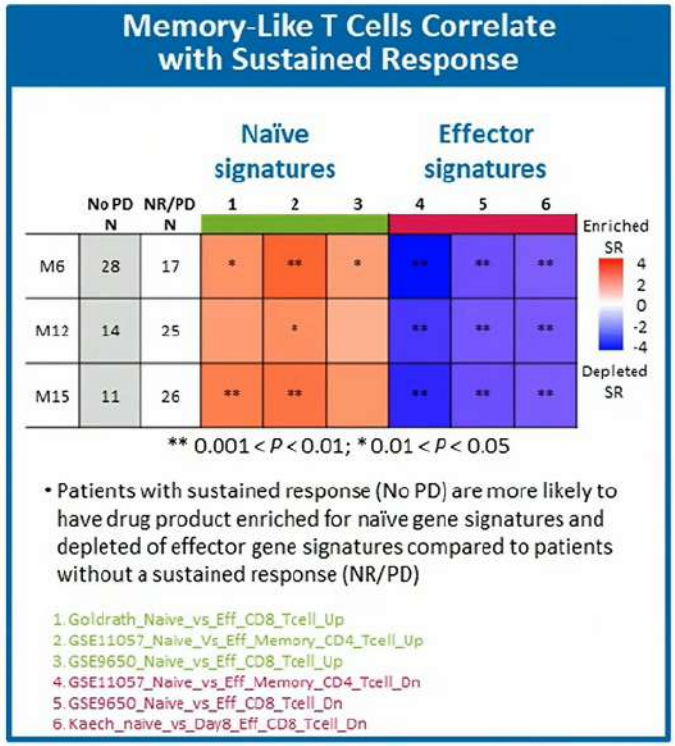
1. Fraietta et al Nat Med 2018. 2. Cohen et al. JCI 2019. 3. Garfall et al. Bld Adv 2019. 4. Rossi et al Blood 2018. 5. Fraietta et al Nature 2018.

CAR-T with more naïve, memory like phenotype associated with persistence and response



bb21217 (CRB-402)

- Detectable up to 1 year at the highest dose
- mDOR 17.5 mo



Alsina et al. ASH 2020, abstr 130.

Patients resistant to CAR-T cells & bi-specific antibodies have exhausted phenotype

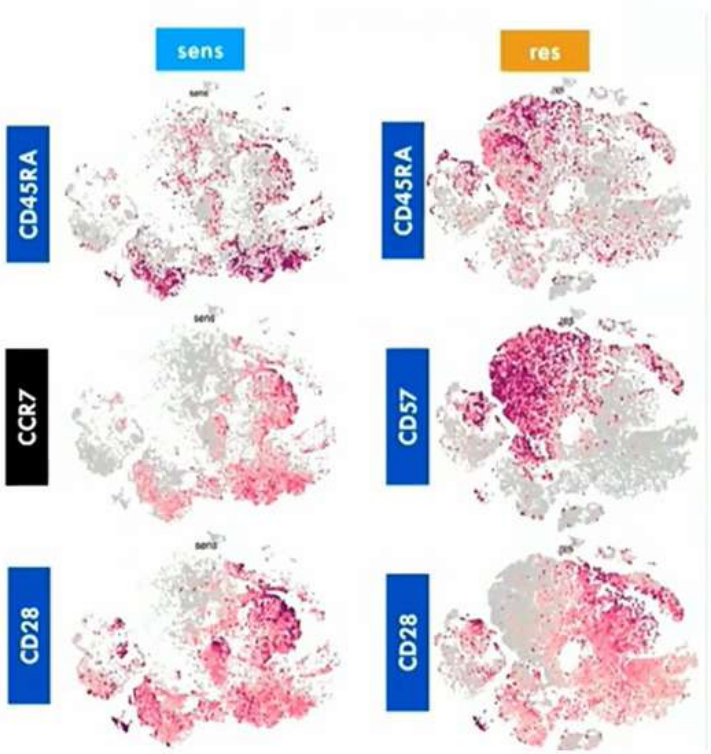
- CITE-seq of serial BM and PB samples at pre- and post treatment and relapse (n = 12)
- Patients resistant to therapy have T cells with terminal exhaustion and senescent profile

Top features Up in S vs R

Feature ID	Gene	Log2 FC	P-Value
CD45RO	PTPRC	1.3	0
CD62L	SELL	1.1	0
CD127	IL7R	0.9	0
CD28	CD28	0.8	0
CD4	CD4	0.8	0
CD27	CD27	0.5	3.46E-238
CD197	CCR7	0.4	1.36E-139

Top features Up in R vs S

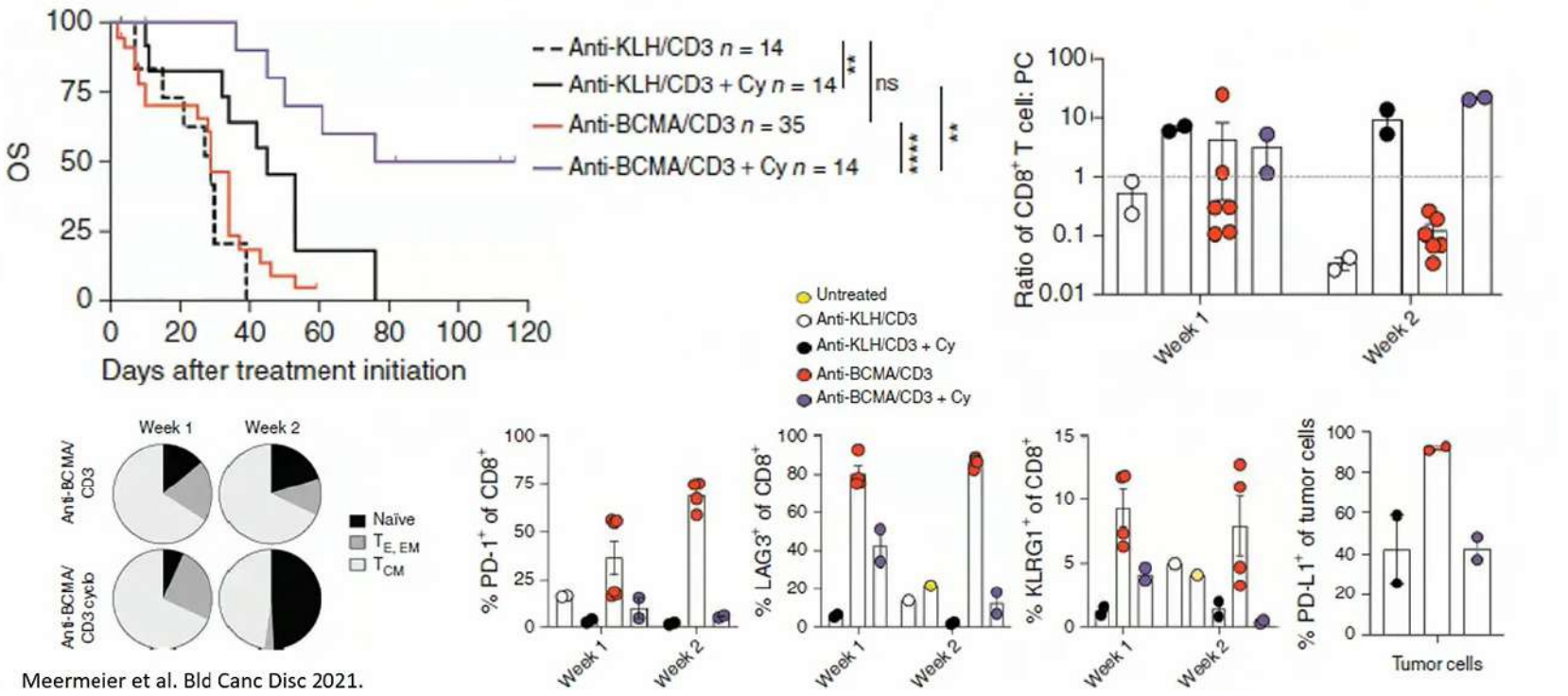
Feature ID	Gene	Log2 FC	P-Value
CD366	HAVCR2	8.9	0
CD57	B3GAT1	8.9	0
CD8	CD8	1.3	0



Leblay et al. ASH 2020, abstr 719.

Role of chemotherapy to enhance T cell activities to bispecific antibodies

Cyclophosphamide enhance bispecific antibody activities by improving T cell persistence and phenotype in murine model.



Meermeier et al. Bld Canc Disc 2021.

Resistance to retreatment & sequencing

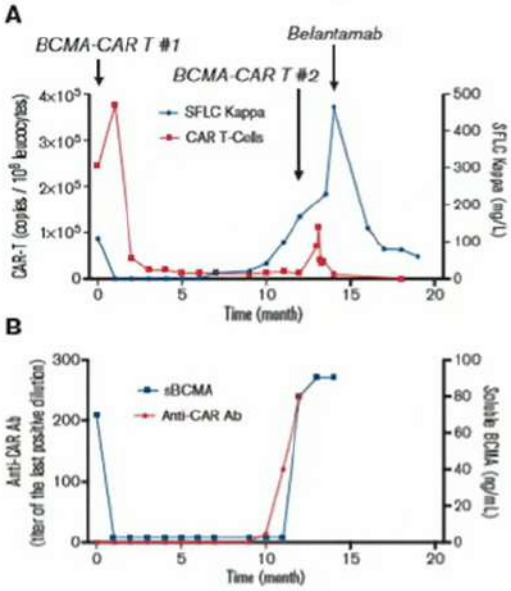
Ide-cel (KarMMa-1 Study) [1]

- ADA became detectable at month 3 and increased over time
- Retreatment response were only seen in those without ADA (n=28)
- Likely not the only mechanism of resistance – mPFS of retreatment 1 mo

Retreatment Response	ADA – Positive (n = 16)	ADA – negative (n = 12)
Yes	0	6 (50%)
No	16 (100%)	6 (50%)

Novel CAR design to reduce immunogenicity

- CT053 studies in China with 2 years follow-up (n=24, phase I)[2]
  - Median CART persistence 172 days, detectable up to 1 year
  - No ADA detected



- Case reports of BCMA ADC working after BCMA CART[3,4]
- Emerging data on efficacy of bispecific and CAR-T in sequence

1. Munshi et al. NEJM 2021. 2. Hao et al. ASH 2020, abstr 136.
3. Cohen et al. Bld Adv 2019. 4. Gazeau et al. Bld Adv 2021.

Resistance mechanisms by timeline

Mechanism of Resistance	Primary Refractory	Early Relapse	Late Relapse
Tumor Ag target change	Not likely	Less likely	More likely
CAR T expansion	Yes	Possible	Possible
CAR T persistence	No	Possible Disease dependent	Possible Disease dependent
CAR T/T cell dysfunction	Yes	Yes	Yes
ADA immunity	Not likely	Likely	More Likely
Immune suppression	Yes	Yes	Yes

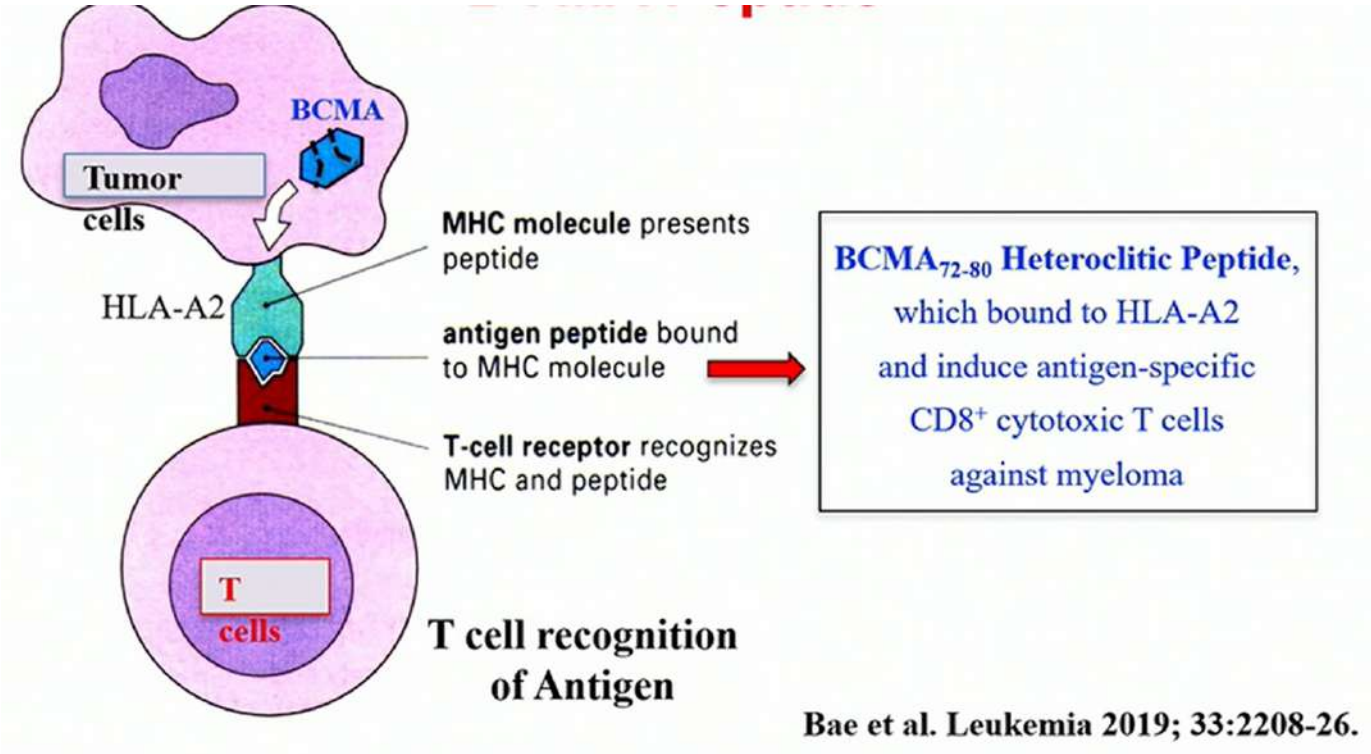
Yi Lin. IMW 2021

CAR T-Cell Therapies in Multiple Myeloma: What Challenges Must Be Overcome?

- First, centralized manufacturing of autologous CAR T cells limits access to this treatment modality. On average, 10% to 20% of the patients are unable to receive the CAR T cells because of disease progression or other complications during manufacturing time
- A second challenge to improving response to CAR T-cell therapy is intrinsic fitness of the T cells. Having T cells with more naive, stem cell–like memory phenotypes in the collected cells is associated with clinical response. Different manufacturing strategies can skew autologous CAR T cells toward this phenotype.
- A third challenge is the immunogenicity that can develop against the CAR. Because the extracellular part of the CAR uses a portion of antibody construct to bypass major histocompatibility complex presentation for tumor antigen recognition, antidrug antibodies (ADAs) against the CAR can develop
- Finally, additional antigen targets beyond BCMA will be important

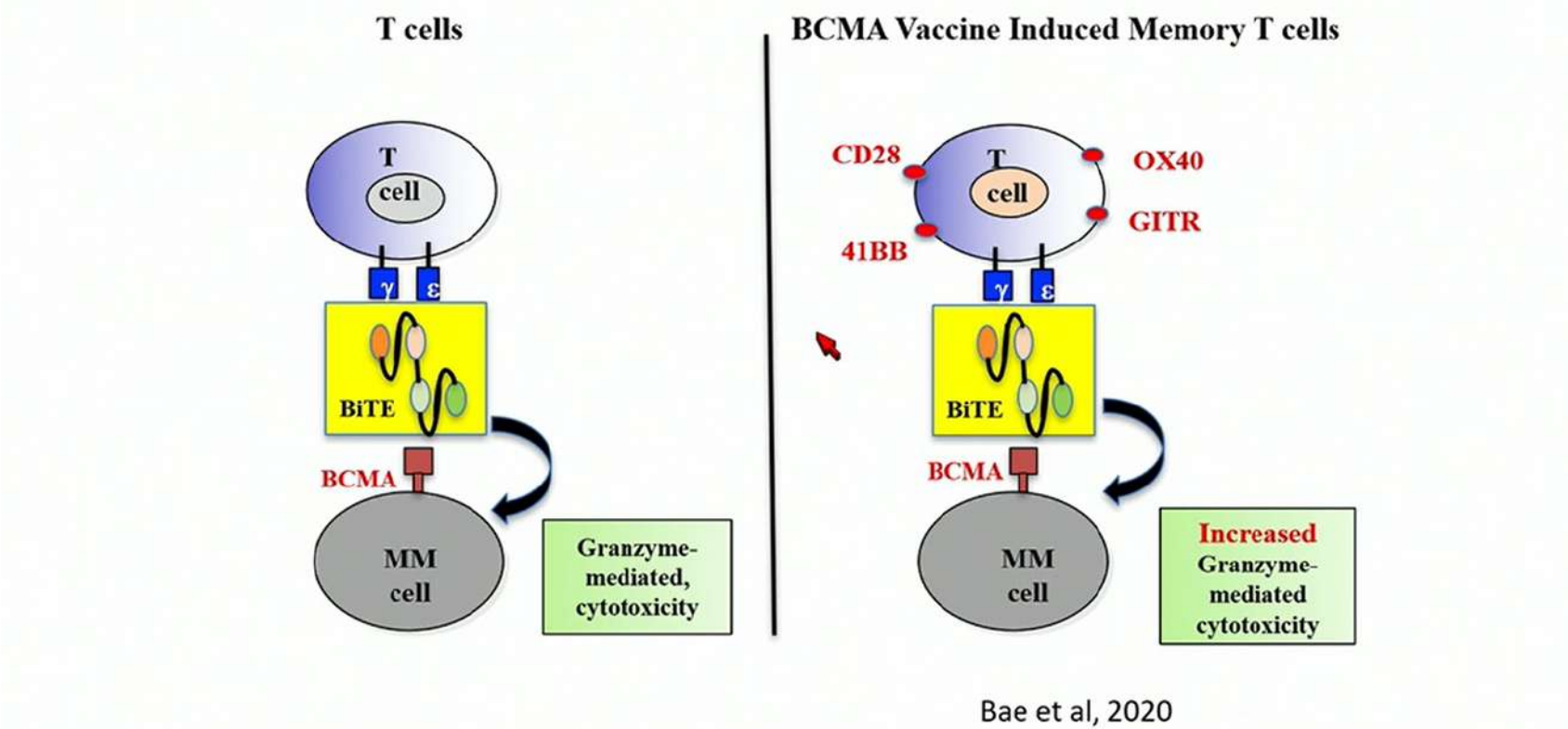
Yi Lin, MD, PhD, ASCO Daily News, November 18, 2021

Incorporating vaccination into BiTE treatment Paradigm: HLA-A2-specific BCMA peptide



Anderson K. IMW 2021

Combination BCMA peptide nanoparticle vaccine and BCMA BiTE to enhance engagement and anti-myeloma activity of memory CTL



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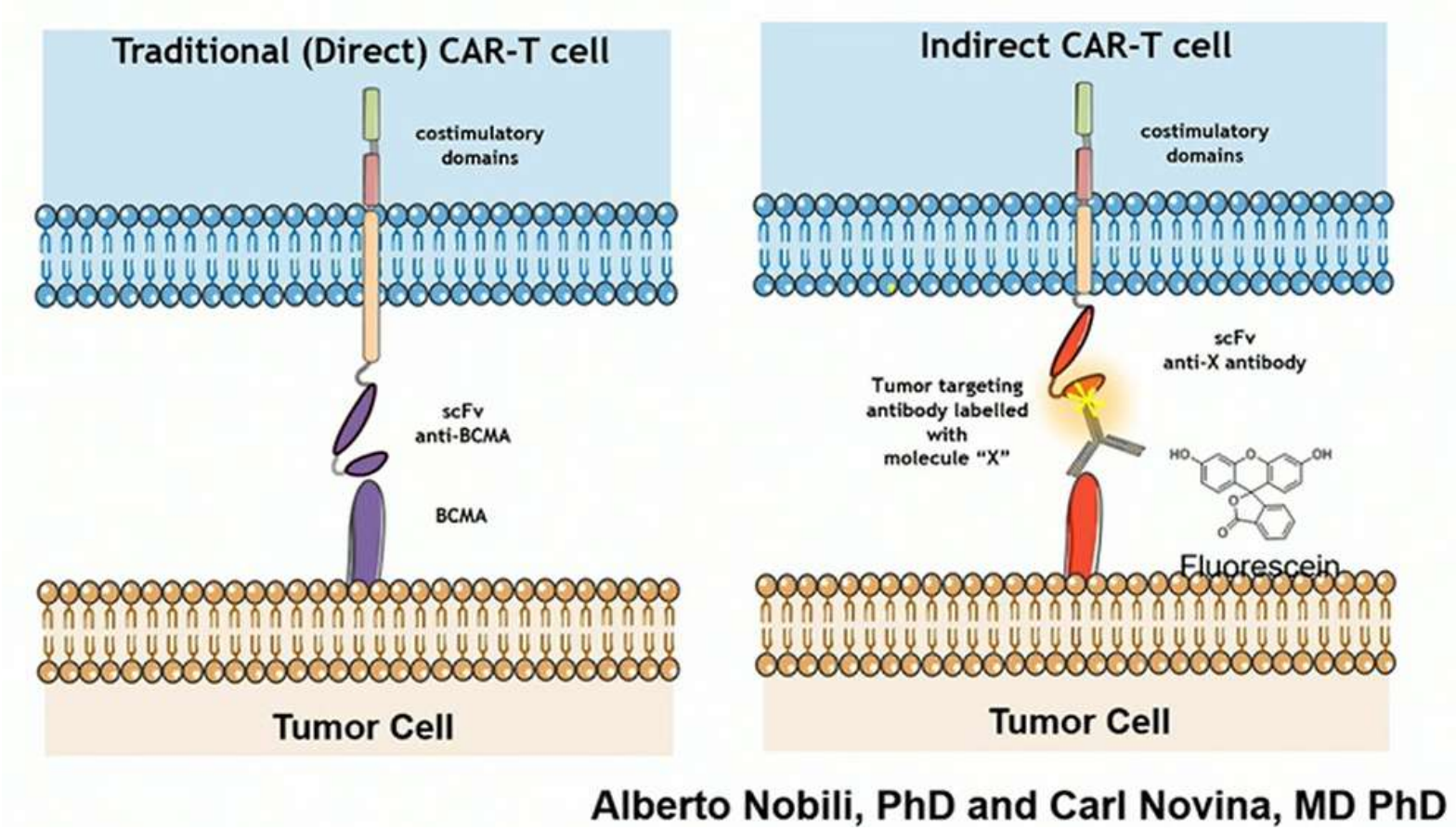
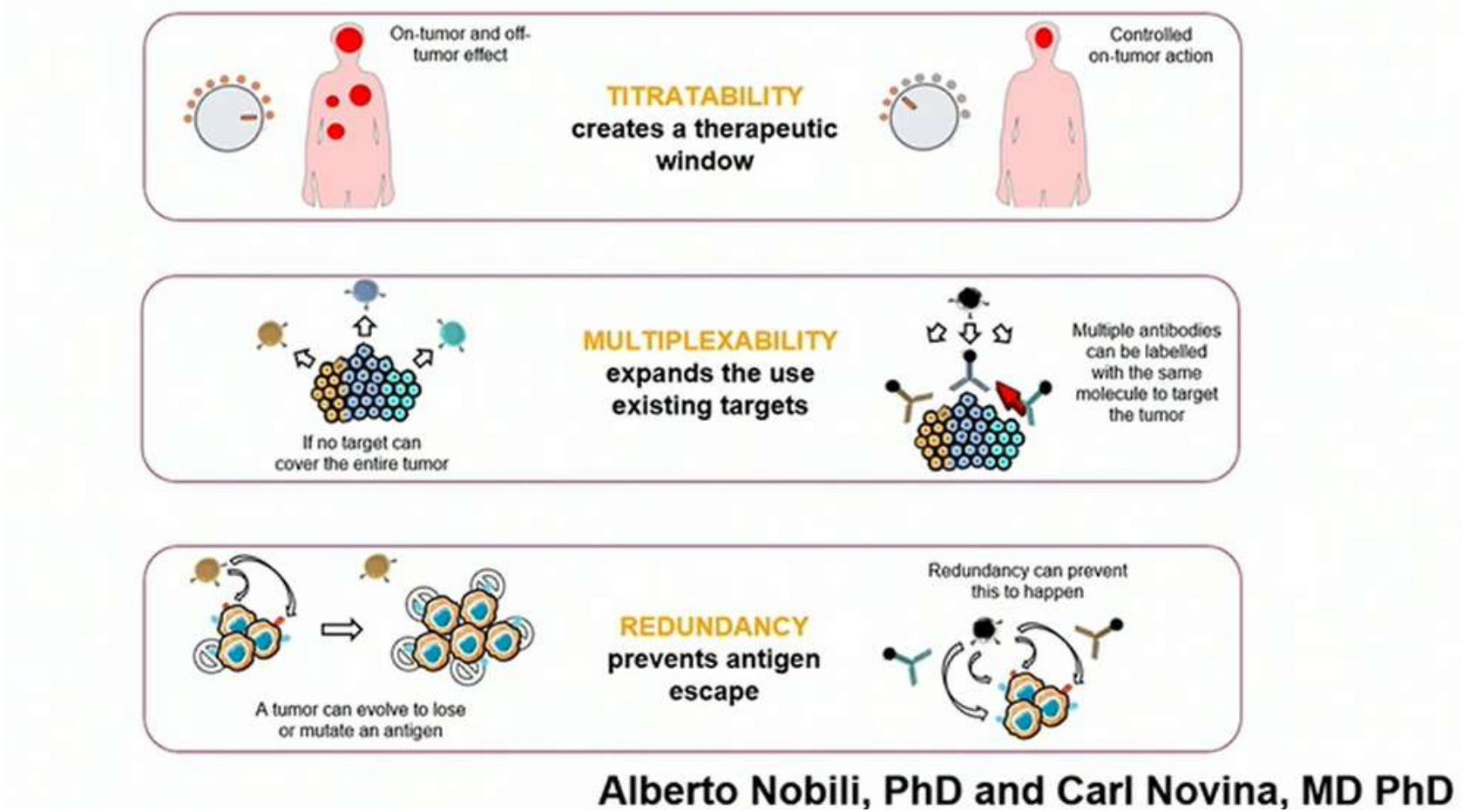
BAT-CAR: Binary Activated T Cell with Chimeric Antigen Receptor



Anderson K. IMW 2021

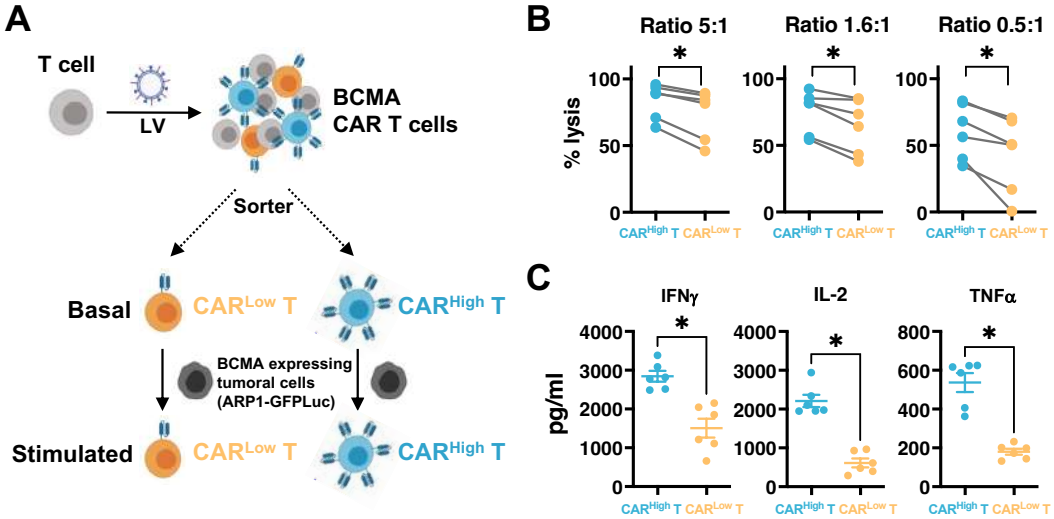
BAT-CARs target limitations of CAR-T cells



Anderson K. IMW 2021

CAR density influences CAR-T cells efficacy

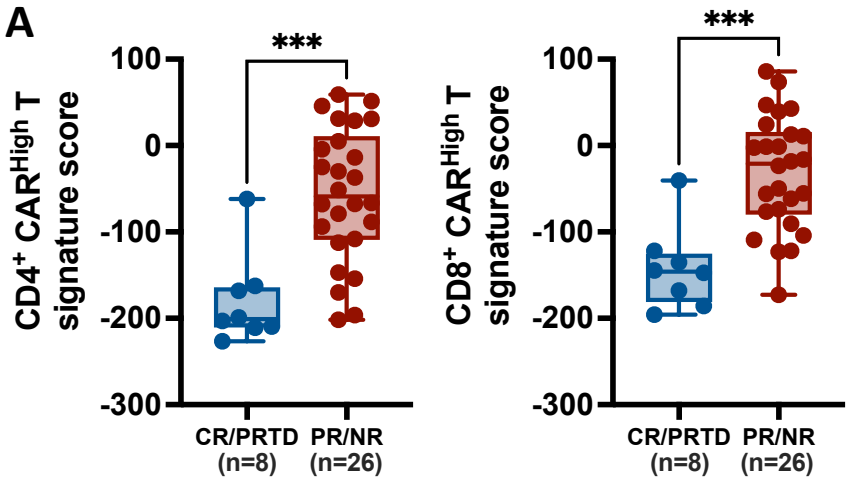
- Functional and transcriptional studies demonstrate that CAR T cells with high expression of the CAR construct show an increased tonic signaling with upregulation of exhaustion markers, increased *in vitro* cytotoxicity



Paula Rodriguez-Marquez, Blood in press

CAR density influences CAR-T cells efficacy

- However, CAR T cells with high expression of the CAR construct show a decrease in *in vivo* BM infiltration
- Patients treated with CAR T cell products enriched in CAR ^{High} T cells show a significantly worse clinical response



Paula Rodriguez-Marquez, Blood in press

Future Directions

- Novel cellular sources
- Novel targets in the tumor cells and the BM microenvironment (CD73)
- Novel approaches to overcome resistance like mRNA CARs and BAT CARs