Donor-derived CD19-specific CAR^{+} T-cell therapy after haploidentical hematopoietic stem-cell transplantation

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Hyatt Orlando International Airport Hotel

December 3, 2015 (afternoon)

ZIOPHARM Oncology

Some of the technology described was advanced through research conducted at the MD Anderson Cancer Center by Laurence Cooper, M.D., Ph.D. Both MD Anderson Cancer Center and Dr. Cooper have a financial interest in ZIOPHARM Oncology, Inc., and Intrexon Corporation. On May 7, 2015, Dr. Cooper was appointed as the Chief Executive Officer at ZIOPHARM. Dr. Cooper is now a Visiting Scientist at MD Anderson.

Because MD Anderson is committed to the protection of human subjects and the effective management of its financial conflicts of interest in relation to its research activities, MD Anderson has implemented an Institutional Conflict of Interest Management and Monitoring Plan (Plan) to manage and monitor the conflict of interest with respect to MD Anderson' s conduct of this research.

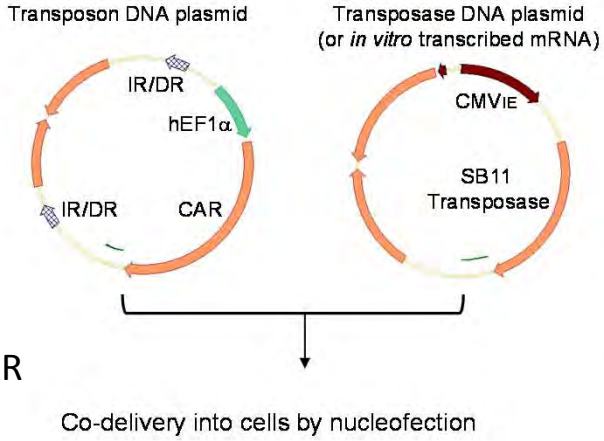
Trial design

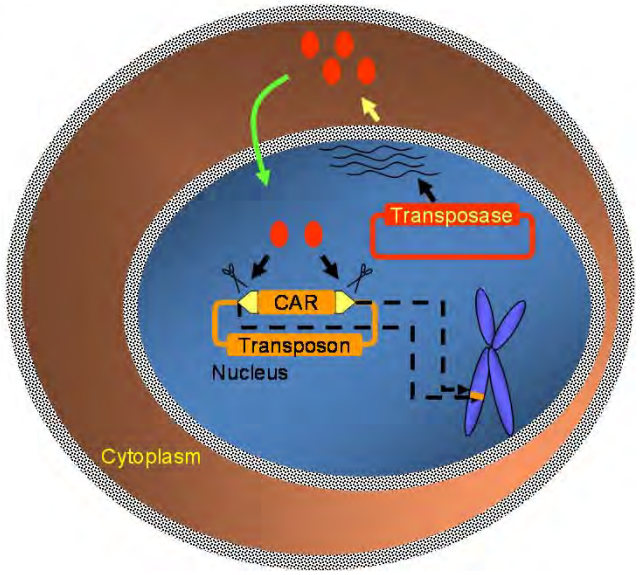
- Determine the safety and MTD of infusions of donor-derived genetically modified T cells for patients with B-cell-derived lymphoid malignancies after haploidentical hematopoietic stem-cell transplantation (HSCT)
  - Conditioning regimen – Fludarabine/ Melphalan and 2GyTBI
  - GVHD prophylaxis – Post-transplant Cy, tacrolimus and MMF
  - T cells infused 42 to 84 days after infusion of peripheral blood stem cells (PBSC)
- Non-viral gene transfer using the *Sleeping Beauty* system to express second generation CD19-specific CAR (signaling through CD28 and CD3-ζ with IgG4-based extracellular stalk)
- 8 patients infused
- T-cell dosing (based on recipient body surface area)
  - Starting dose $10^6/m^2$
  - Current dose $10^8/m^2$

Data based on work previously undertaken at MDACC

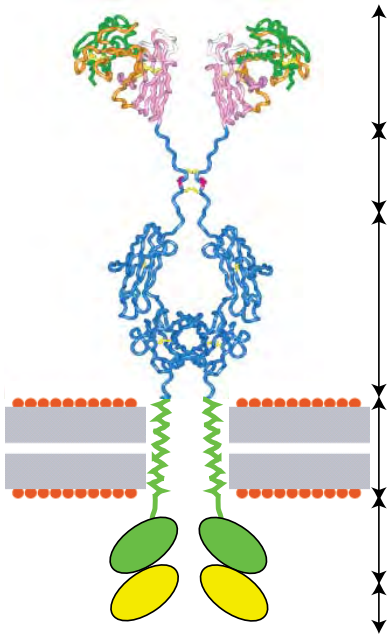
Redirect T-cell specificity to CD19 using *Sleeping Beauty* system

Singh H & Cooper LJ. Immunol Rev. 2014





2nd generation CD19-specific CAR (**CD19RCD28**)



scFv

Modified hinge

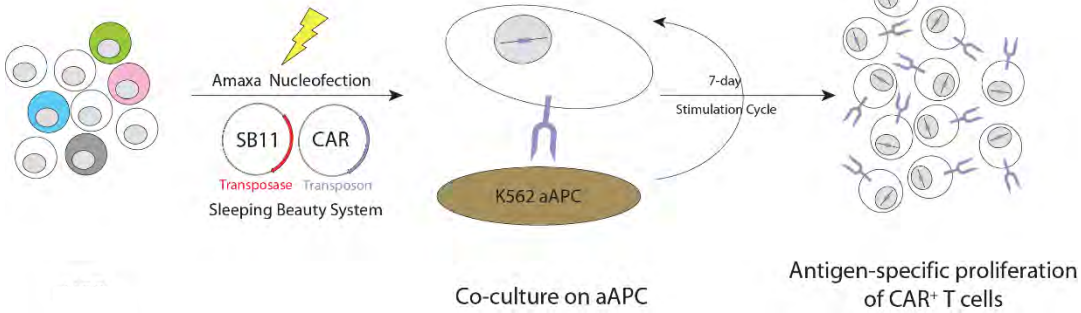
IgG_4Fc

CD28TM

CD28 ⟶ Signal 2

CD3ζ ⟶ Signal 1

Shown as a homo-dimer



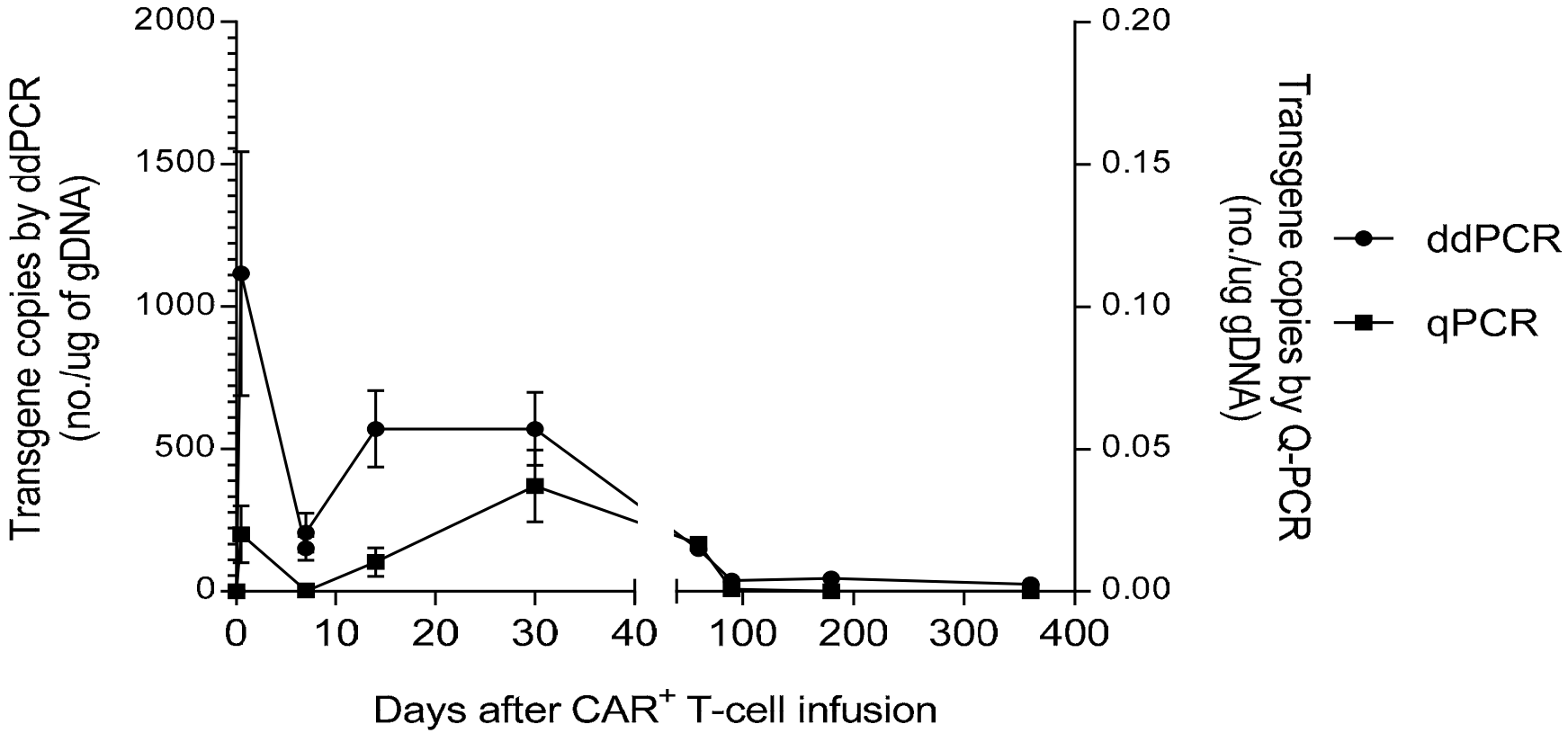
Data based on work previously undertaken at MDACC

Patient characteristics at infusion of haploidentical genetically modified T cells

UPN	Age	Histology	Stage at HSCT	Prior lines therapy	Prep. regimen	T-cell Dose Level	T-cell Dose Level (per m^2)	Wt (kg)	BSA (m^2)	Total T cells ($x10^8$)	% CAR^+	WBC at infusion (k/uL)	ALC at infusion (k/uL)
P580	31	B-ALL	Refractory, MRD^{neg}	4, allo-HSCTx2	FM	A	10^6	82	2.03	0.02	70.4	5.8	696
P513	25	B-ALL	Refractory, MRD^{neg}	4, auto-HSCT	FM	A	10^6	61	1.74	0.02	93.3	3.5	1295
P732	36	B-ALL	CR2, MRD^{pos}	2	FM	B	10^7	81	2.04	0.2	67.8	3.8	190
P671	52	Follicular	Transformed DLBL, PET^{neg}	4	FC-TBI2	B	10^7	91	2.2	0.21	95.2	6.1	366
P723	23	B-ALL	PIF in CR, MRD^{neg}	2	FM	B	10^7	93	2.04	0.21	58.8	2.8	756
P771	46	B-ALL	CR1, MRD^{neg}	1	FM-TBI2	C	5×10^7	86	1.92	0.96	56.8	6.2	496
P783	21	B-ALL	PIF, MRD^{pos}	3	MTF	D	10^8	107	2.27	1.14	90.3	4	120
P788	37	B-ALL	Refractory, MRD^{neg}	4, allo-HSCT	FM-TBI2	D	10^8	64	1.74	1.14	83.8	2.9	522

Data based on work previously undertaken at MDACC

Persistence of haploidentical genetically modified T cells



Data based on work previously undertaken at MDACC

Average time of T-cell persistence within autologous and allogeneic recipients after infusion determined by ddPCR

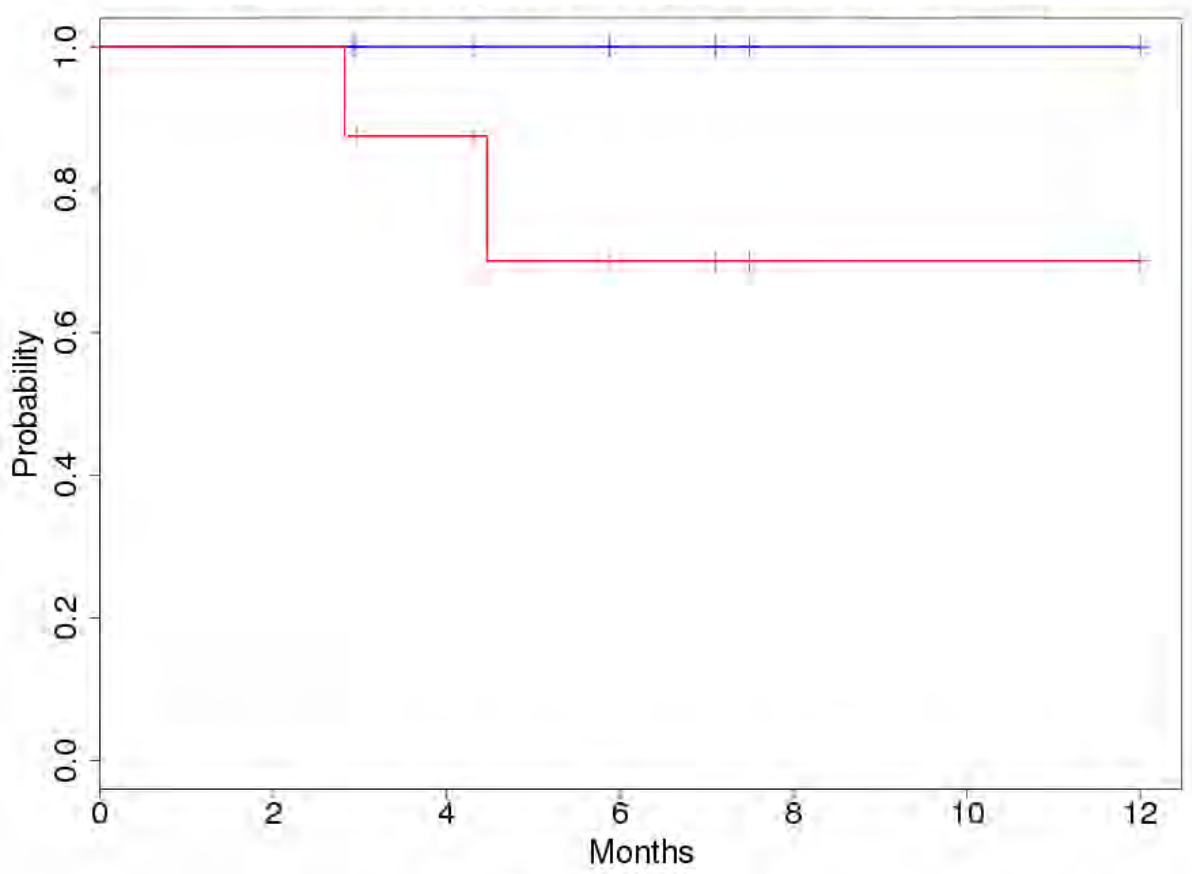
T-cell Dose Level and recipients (allo versus auto)	Number of patients infused	Average time (days) transgene detected	Maximum time (days) transgene detected
MSD (allo)	10	52	90
Haplo (allo)	8	54	180
All Auto patients	7	201	360
All Allo patients	19	51	180

MSD = matched-sibling donor

Data based on work previously undertaken at MDACC

PFS and OS after haploidentical HSCT

N=8 pts treated and 6 alive in CR



All patients tolerated the infusions with no significant increase in incidence of GVHD

Data based on work previously undertaken at MDACC

Patient outcomes following infusion of haploidentical genetically modified T cells

UPN	Age	Histology	Stage at HSCT	Prior lines therapy	Prep. regimen	T-cell Dose Level	T-cell Dose Level (per m^2)	Relapse or progression after T-cell infusion	Disease status after T-cell infusion	Response duration (days) at last follow up	Toxicity
P580	31	B-ALL	Refractory, MRD ^{neg}	4, allo-HSCTx2	FM	A	10^6	No	CCR	544	None
P513	25	B-ALL	Refractory, MRD ^{neg}	4, auto-HSCT	FM	A	10^6	No	CCR	216	aGVHD skin
P732	36	B-ALL	CR2, MRD ^{pos}	2	FM	B	10^7	Yes	Alive	136	None
P671	52	Follicular	Transformed DLBL, PET^{neg}	4	FC-TBI2	B	10^7	No	CCR	402	None
P723	23	B-ALL	PIF in CR, MRD ^{neg}	2	FM	B	10^7	No	CCR	179	cGVHD skin
P771	46	B-ALL	CR1, MRD ^{neg}	1	FM-TBI2	C	5×10^7	No	CCR	117	None
P783	21	B-ALL	PIF, MRD ^{pos}	3	MTF	D	10^8	Yes	Alive	86	None
P788	37	B-ALL	Refractory, MRD ^{neg}	4, allo-HSCT	FM-TBI2	D	10^8	No	CCR	81	None

Data based on work previously undertaken at MDACC

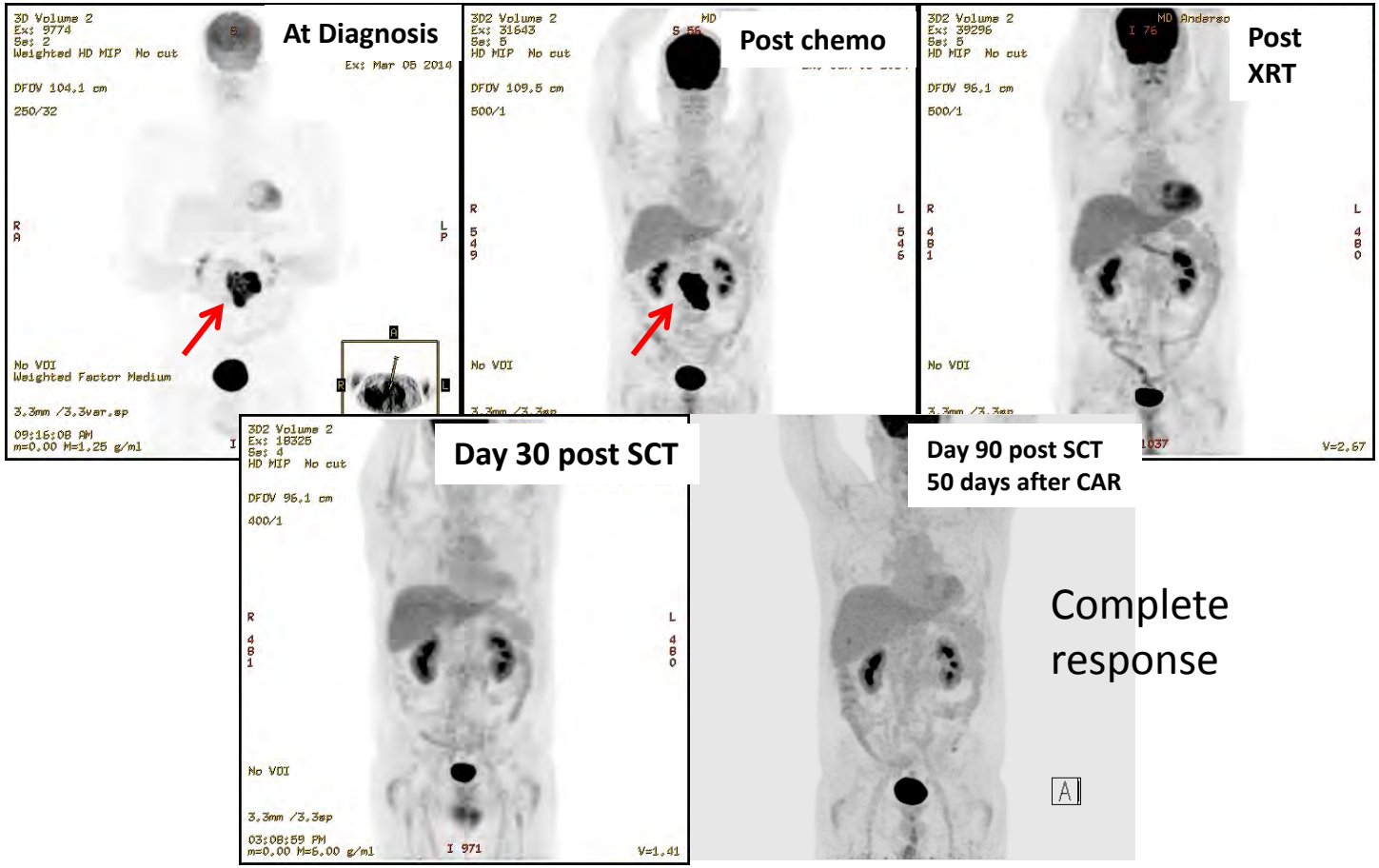
Donor-derived CD19-specific CAR$^+$ T cells infused for primary refractory DLBCL

- 52 yo man (UPN P671) with history of FL transformed to DLBCL in 2013
- Treatment prior to haplo-HSCT and donor-derived CD19-specific T cells
  - R-CHOP x 6 followed by progression
  - R-ICR x 2 followed by progression
  - R-DHAP x 2 followed by progression
  - R-hyperCytoxan & XRT with partial response
- At haplo-HSCT and donor-derived CD19-specific CAR$^+$ T cells in 2014
  - Refractory to chemotherapy
  - Bulky disease in the mesenteric area
- Haplo-HSCT from son after Flu/Cy/TBI
- Donor-derived $10^7/m^2$ T cells infused 45 days after peripheral blood stem cells
- No aGVHD or cGVHD
- In CR at 6 months – off immunosuppression with 100% donor chimerism

Data based on work previously undertaken at MDACC

Haplo-HSCT and donor-derived CAR^{+} T cells for primary refractory bulky DLBCL

Initial intra-abdominal mass size 7x16x15 cm



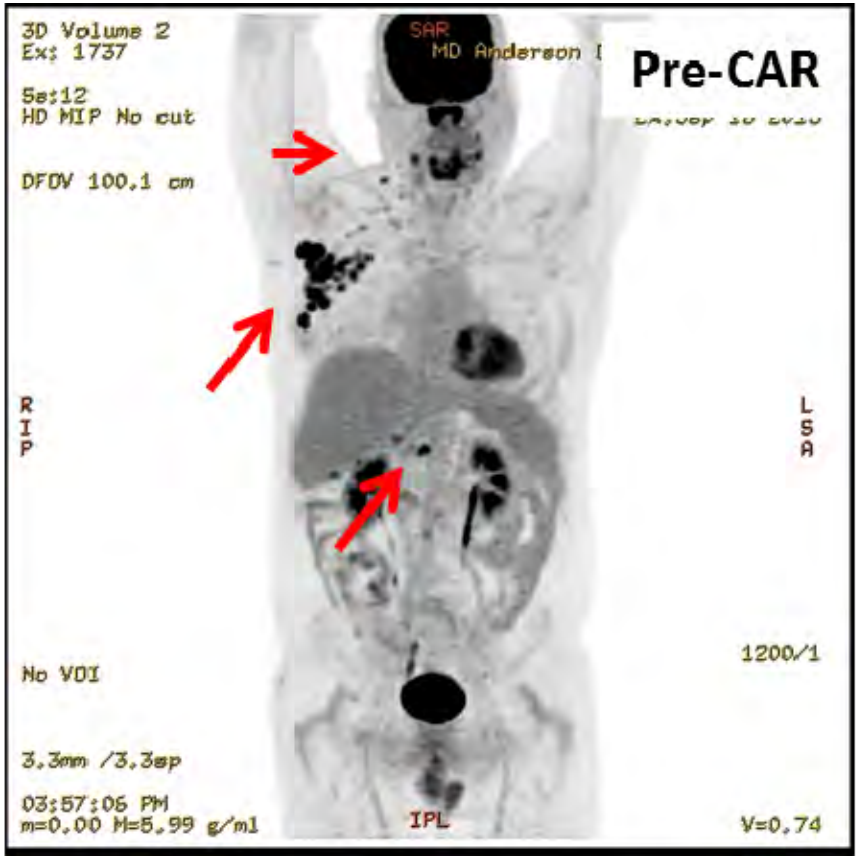
Data based on work previously undertaken at MDACC

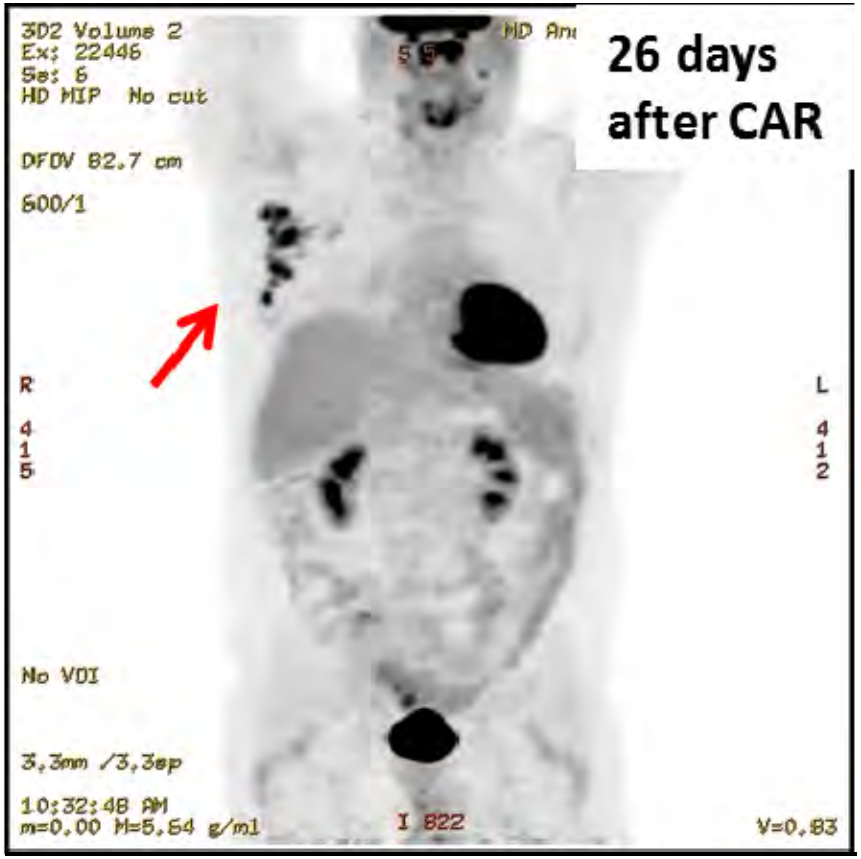
Second infusion of haplo CD19-specific CAR$^+$ T cells

- UPN P671 then relapsed in 2015
  - PET/CT – multiple LN – R axilla, L axillar, neck, abdomen
  - Biopsy R axilla showed recurrent CD19$^+$ FL
- Re-infusion of CAR$^+$ T cells $5x10^7/m^2$ in 2015 without lymphodepleting chemotherapy
- Repeat PET scan one month after T-cell infusion clearance of disease from all sites except R axilla
  - Improvement in SUV uptake in the R axilla from 13.0 to 7.9 SUV
- Repeat R axilla LN FNA and core biopsy 2 months after T-cell infusion
  - No evidence of lymphoma

Data based on work previously undertaken at MDACC

Disease relapse at day +402 (left) and after repeat infusion of CAR+ T cells (right)

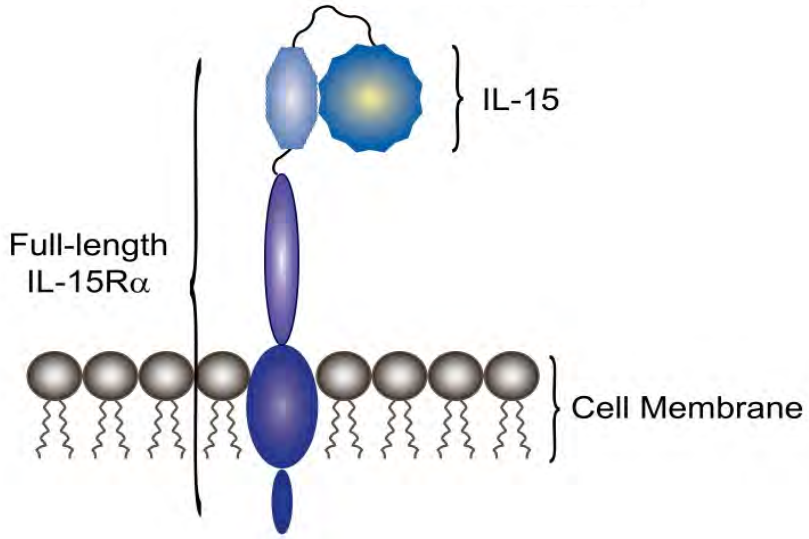




Data based on work previously undertaken at MDACC

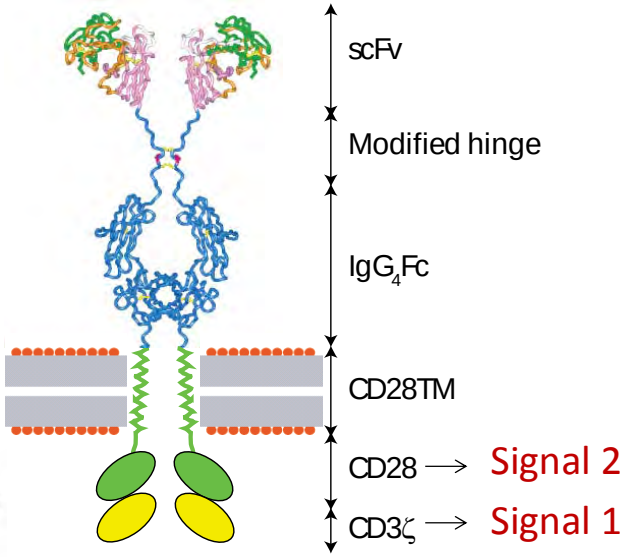
Next-generation technology
Improving therapeutic potential of CAR^{+} T cells by co-signaling through cytokine receptor

Membrane-bound IL15 (mIL15)



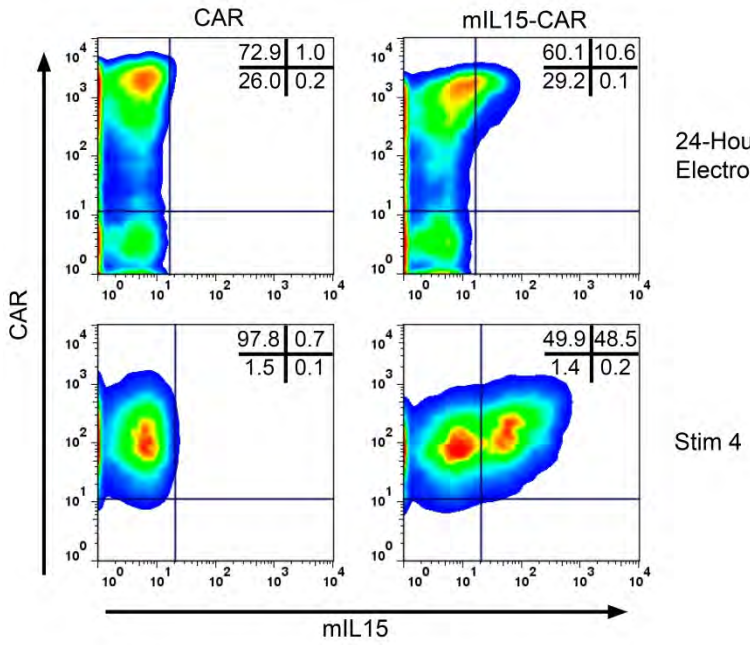
Provide “signal 3”

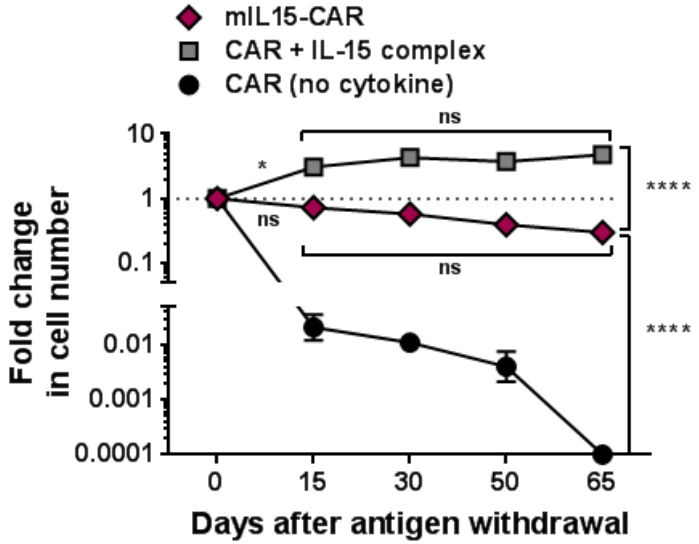
2nd generation CD19-specific CAR
(**CD19RCD28**)

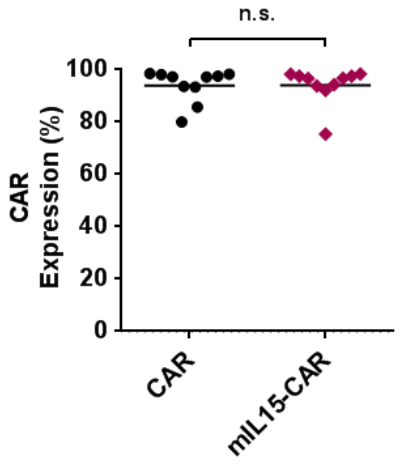


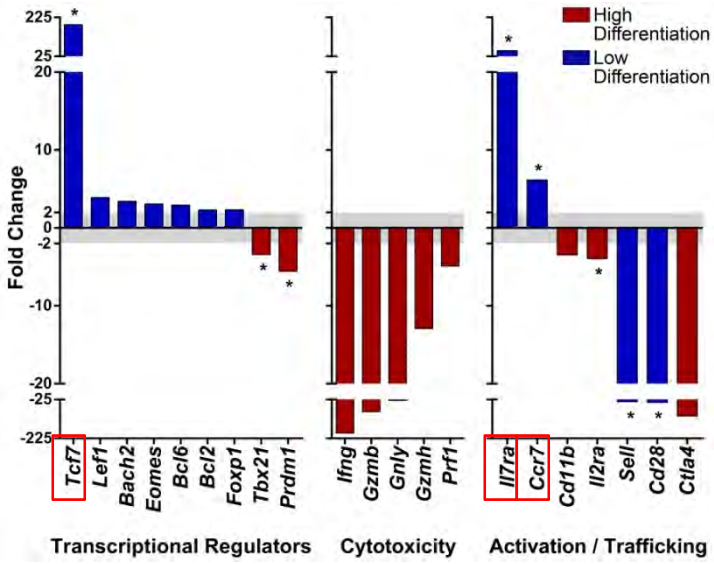
Data based on work previously undertaken at MDACC

Stable co-expression of mIL15 & CAR





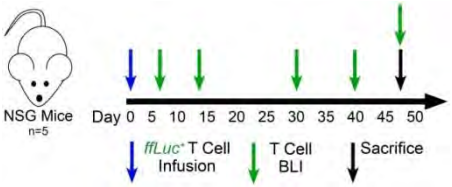




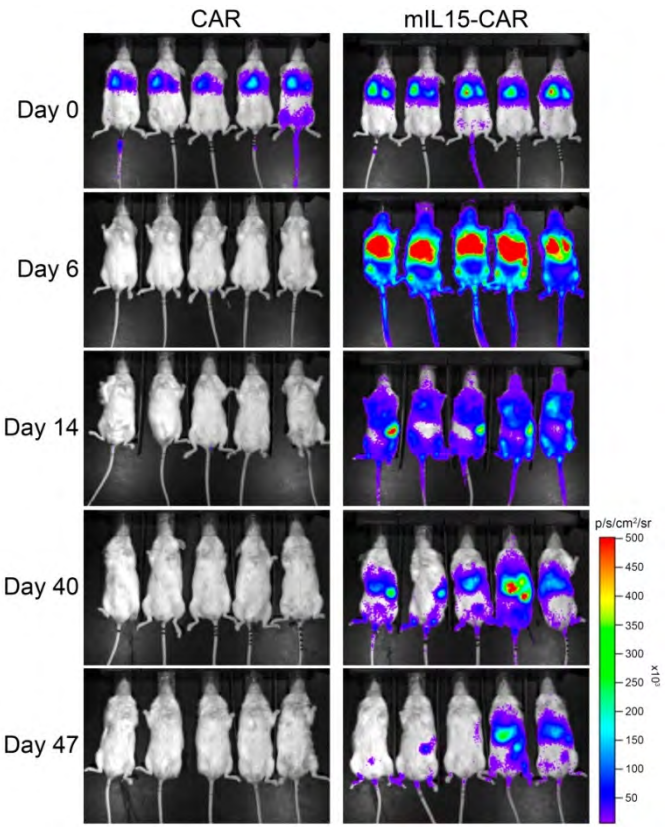
*Protein expression validated by flow cytometry

Data based on work previously undertaken at MDACC

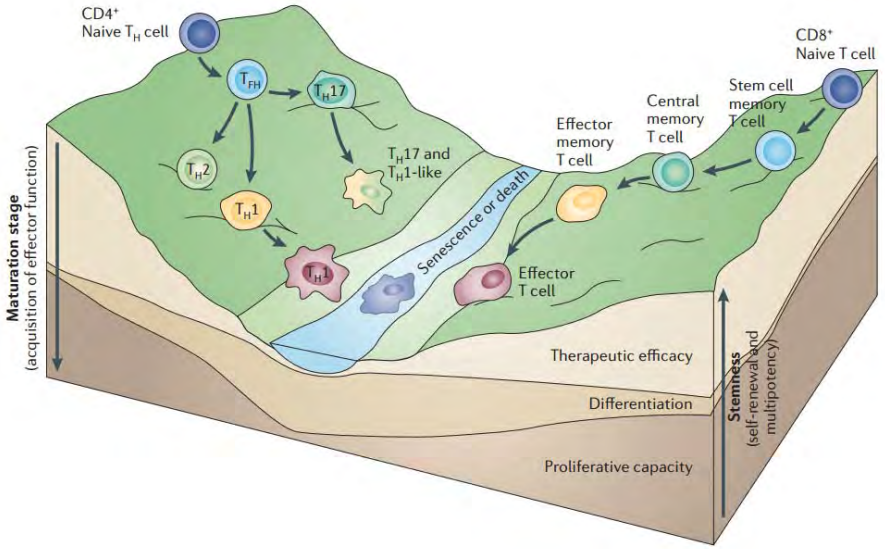
mIL15^{+}CAR^{+} T cells persist in the absence of CAR activation and have T_{SCM}-like phenotype



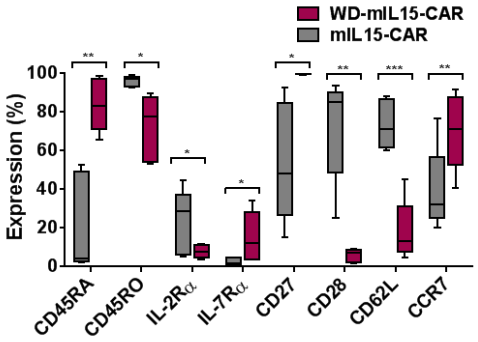
T-cell BLI

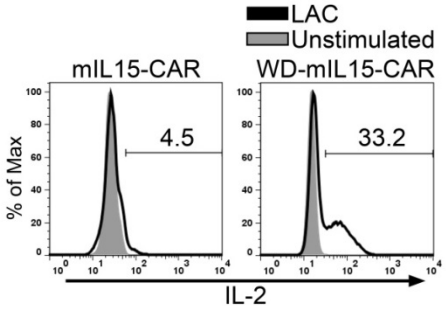


Persisting T-cell Phenotype (*in vitro*)



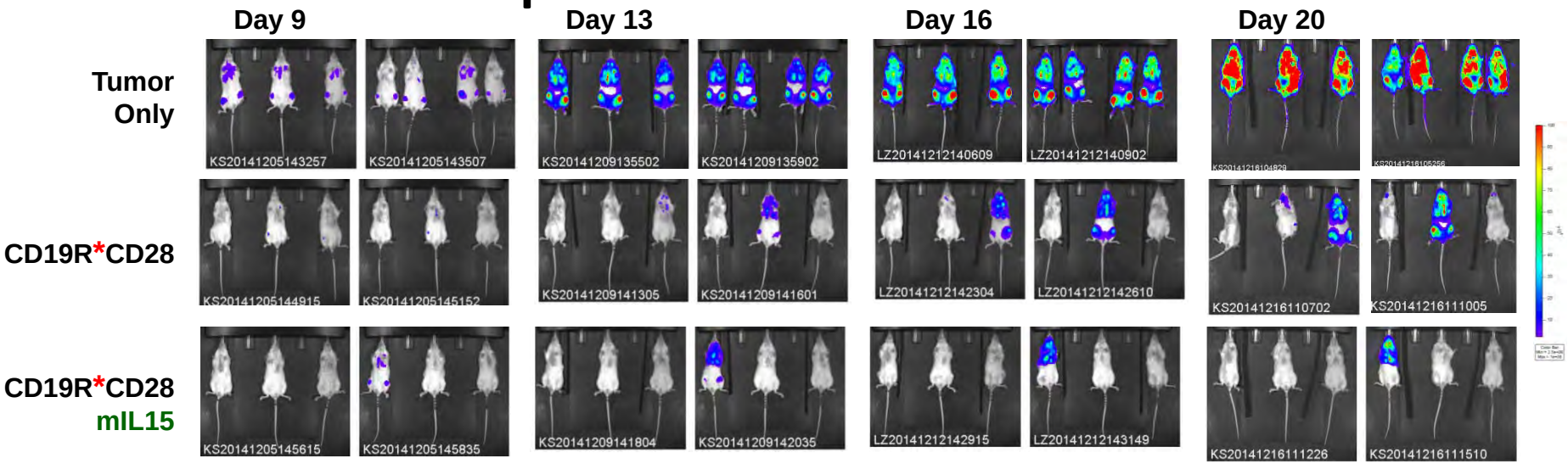
Gattinoni, Klebanoff, Restifo 2012 Nat Rev Cancer 12: 671-684.

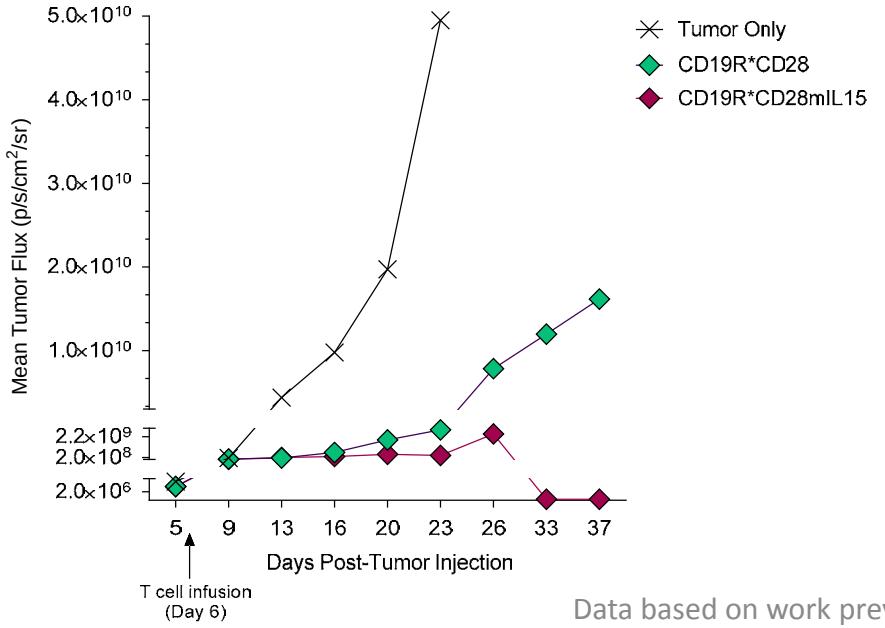




Data based on work previously undertaken at MDACC

Superior *in vivo* activity of CAR co-expressed with mIL15





Data based on work previously undertaken at MDACC

Conclusions

- *Sleeping Beauty* system can be used to generate CAR^+ T cells to target $CD19^+$ malignancies after HSCT
- Infused haplo-identical CAR^+ T cells persist after infusion
- Evidence of anti-tumor effects
  - 75% CR at median 5.2 month follow up
- No increase in GVHD despite up to $10^8/m^2$ of donor-derived T cells being infused
  - Supports further development of off-the-shelf CAR^+ T-cell therapy
- Next-generation *Sleeping Beauty* trial safe-to-proceed (IND 16474, clinical trial.gov NCT02529813) at MDACC

Additional information presented by Dr. Kebriaei at 5:15 pm on December 7, 2015, ASH abstract 862, Session 723

Acknowledgments

- MDACC SCTCT
  - Partow Kebriaei
  - Stefan Ciurea
- Ziopharm Oncology
- Intrexon