

CHOC
UCI Health

Regulatory T Cells in GVL and GVHD Post-Allogenic Stem Cell Transplant for High-Risk Acute Leukemia.

11-08-2024

Rishi Chavan MD



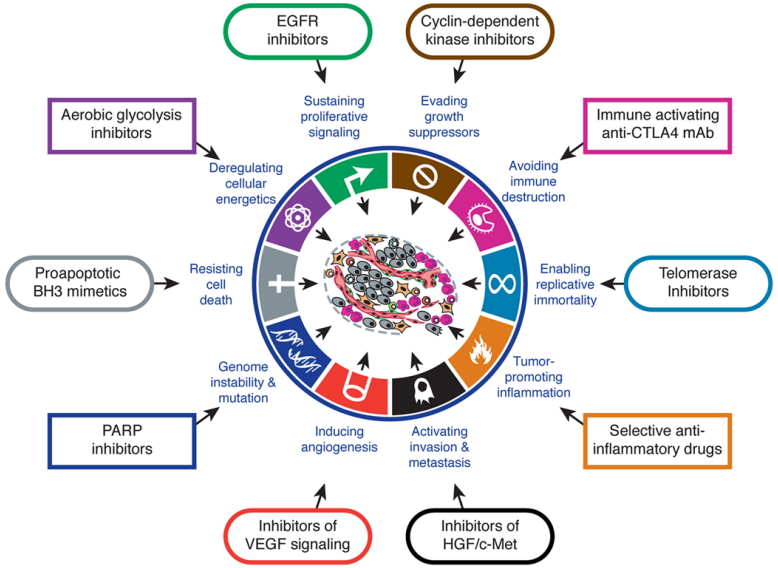
Disclosures

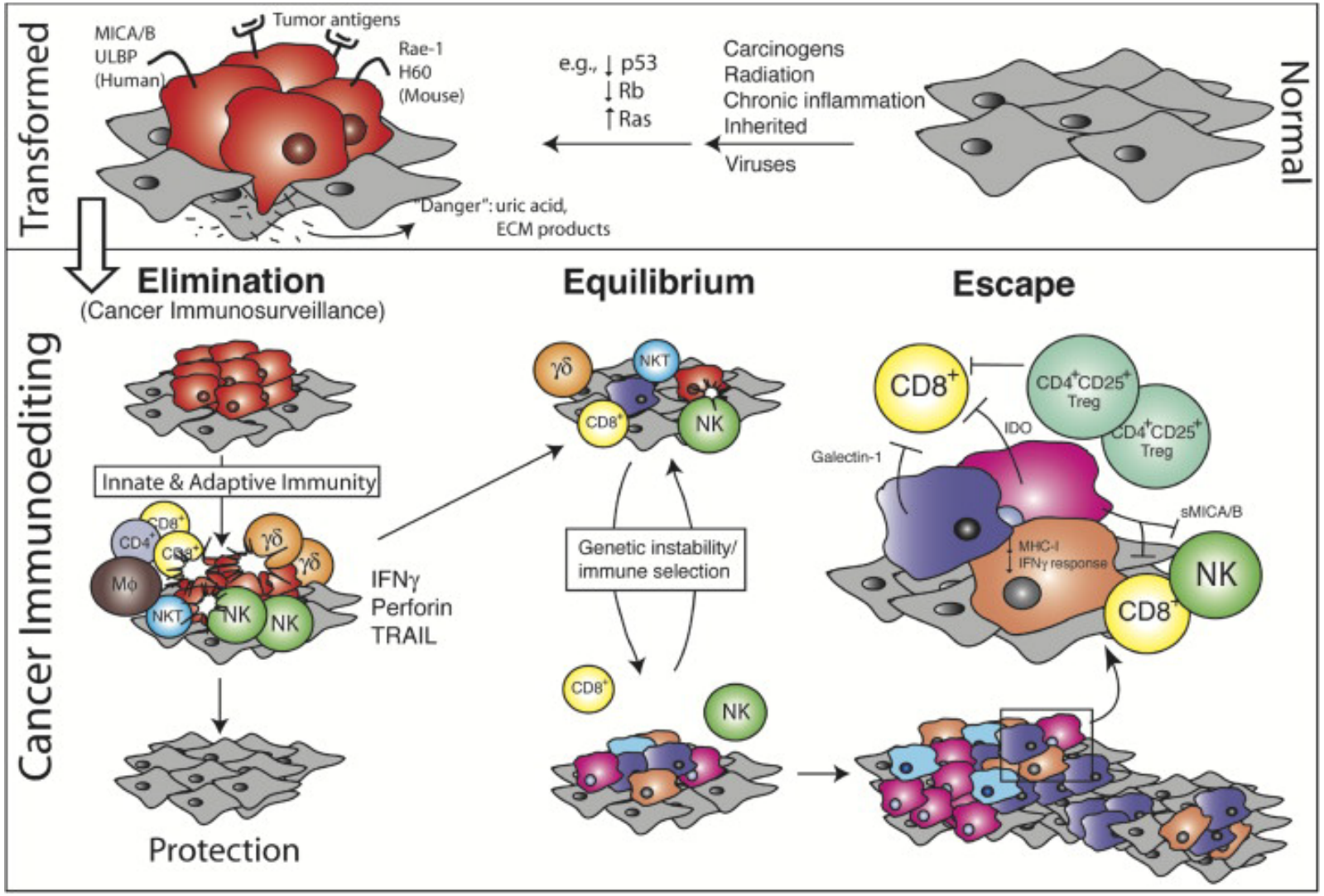
- None

Learning Objectives

- Why certain patients may respond to cancer immunotherapies while others may not.
- The story of Tregs.
- Explore the clinical possibilities of Treg surveillance and optimization in treating cancer and other auto immune conditions.

Is cancer a failure of our Immune system?

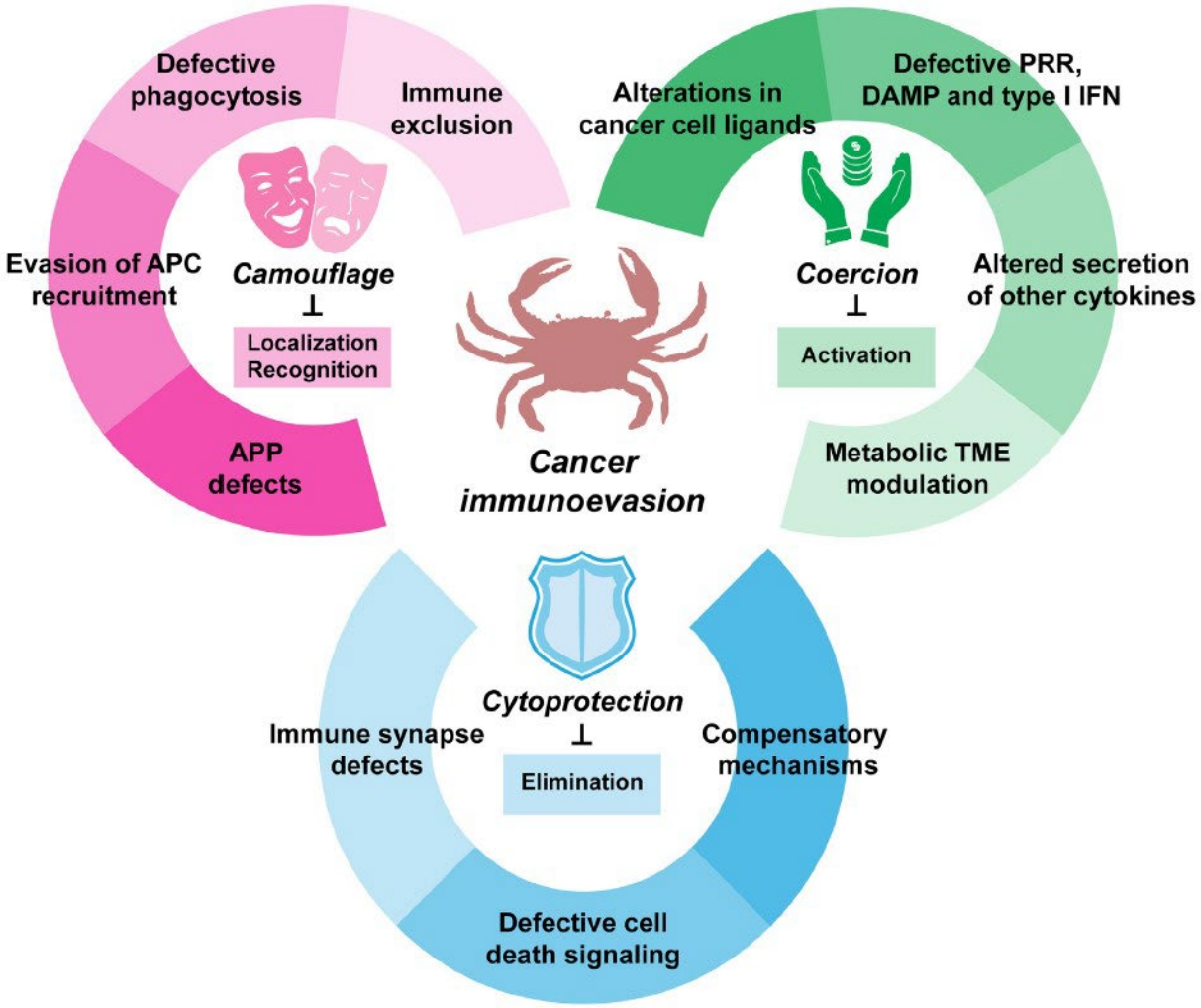




Douglas Hanahan, Robert A. Weinberg et al. **Hallmarks of Cancer: The Next Generation**, Cell 2011

Dunn G et al. **The Immunobiology of Cancer Immunosurveillance and Immunoediting** *Immunity, 2004*

Is cancer a failure of our Immune system?



Three Es model, the host immune system

- eliminates malignant cell precursors and
- contains microscopic neoplasms in a dynamic equilibrium,
- preventing cancer outgrowth until neoplastic cells acquire genetic or epigenetic alterations that enable immune escape.

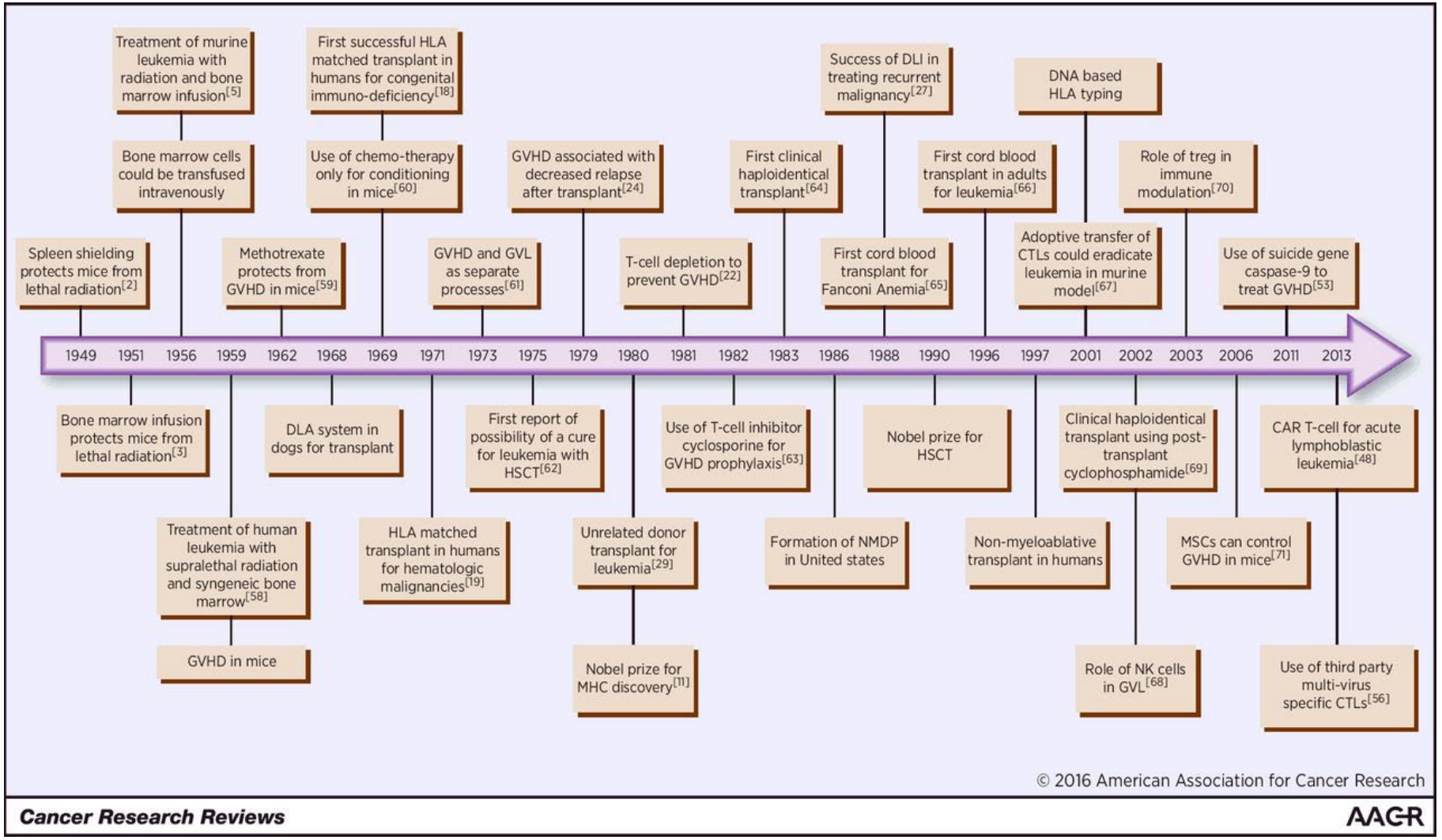
This immune evasive phenotype originates from various mechanisms that can be classified under a novel "three Cs" conceptual framework:

(1) camouflage, which hides cancer cells from immune recognition,
(2) coercion, which directly or indirectly interferes with immune effector cells,
(3) cytoprotection, which shields malignant cells from immune cytotoxicity.

Blocking the ability of neoplastic cells to evade the host immune system is crucial for increasing the efficacy of modern immunotherapy and conventional therapeutic strategies that ultimately activate anticancer immunosurveillance.

Galassi et al., ***The hallmarks of cancer immune evasion***, Cancer Cell (2024)

Allogenic SCT → Autologous CAR T cells → *Allogenic (Donor derived) CAR T cells*



What we:

Want

- Sustained GVL

Don't want

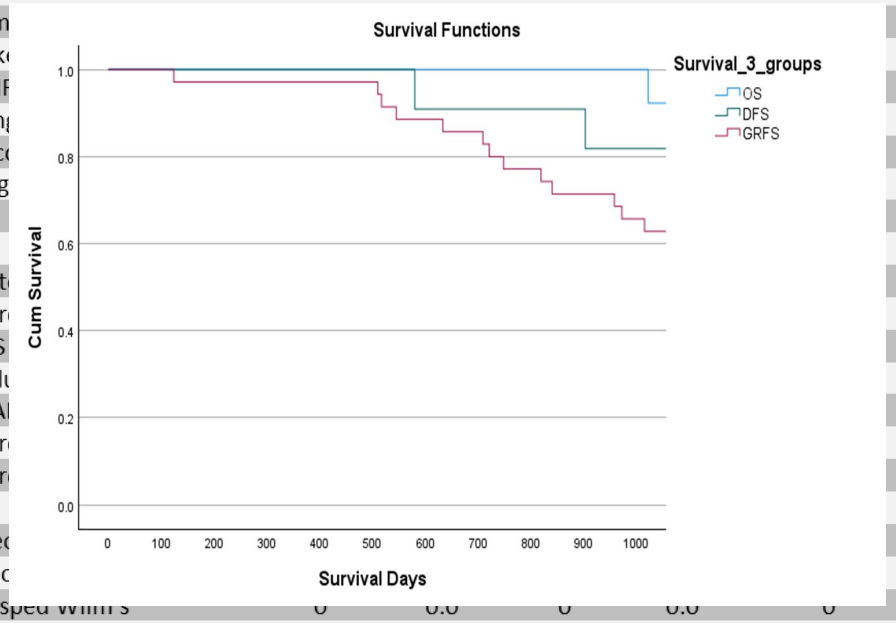
- Severe GVHD
- Infections
- Organ toxicity

Cancer Res. 2016;76(22):6445-6451.
doi:10.1158/0008-5472.CAN-16-1311

CHOC Stem cell transplant and cellular therapy program 2019-2023

Demographics

	ALLO (N=30)		ALLO-HID (N=45)		ALLO-HID Boost (N=2)		AUTO (N=41)		Auto Boost (N=8)		CAR-T (N=20)		ALLO-Unrelated (N=9)		UCBT (N=1)	
CY	N	%;range	N	%;range	N	%;range	N	%;range	N	%;range	N	%;range	N	%;range	N	%;range
2019	6	20.0	12	26.7	0	0	6	14.6	1	12.5	0	0	3	33.3	1	100
2020	5	16.7	6	13.3	0	0	8	19.5	2	25	3					
2021	6	20.0	9	20.0	1	50	6	14.6	1	12.5	5					
2022	8	26.7	6	13.3	0	0	17	41.5	2	25	9					
2023	5	16.7	12	26.7	1	50	4	9.8	2	25	3					
Age	7.7	0.09-21	7.7	0.64-26	15	2.2-17.5	4	0.42-23	4	1.8-17	15					
ALL	7	23.3	17	37.8	1	50	0	0.0	0	0	20					
AML	5	16.7	13	28.9	1	50	0	0.0	0	0	0					
ATRT	0	0.0	0	0.0	0	0	7	17.1	1	12.5	0					
Beta Thalassemia	0	0.0	1	2.2	0	0	0	0.0	0	0	0					
Bone Marrow Failure	0	0.0	1	2.2	0	0	0	0.0	0	0	0					
CGD	1	3.3	0	0.0	0	0	0	0.0	0	0	0					
Diam																
Dysk																
ETMF																
Ewing																
Fanc																
Gang																
HD																
HLH																
Hunt																
Marr																
MDS																
Medu																
MPAI																
Neur																
Neur																
NHL																
Oste																
Pinec																
Relapsed Wilm's																
SAA	5	16.7	1	2.2	0	0										
SCIDS	3	10.0	0	0.0	0	0										
Sickle Cell Disease	1	3.3	1	2.2	0	0										
Wiskott-Aldrich	1	3.3	0	0.0	0	0	0	0.0	0	0	0	0	0	0.0	0	0



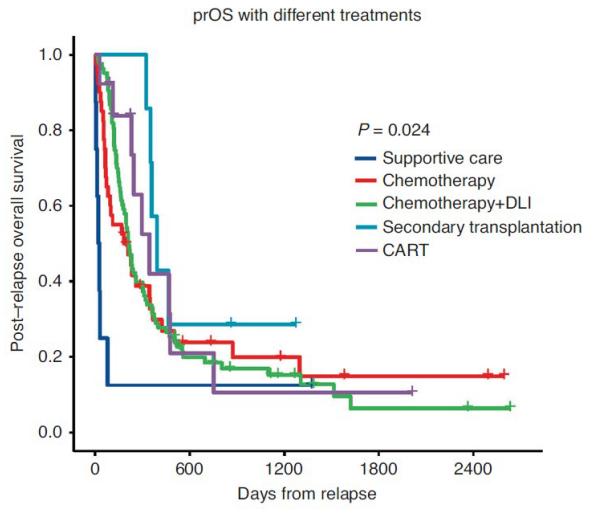
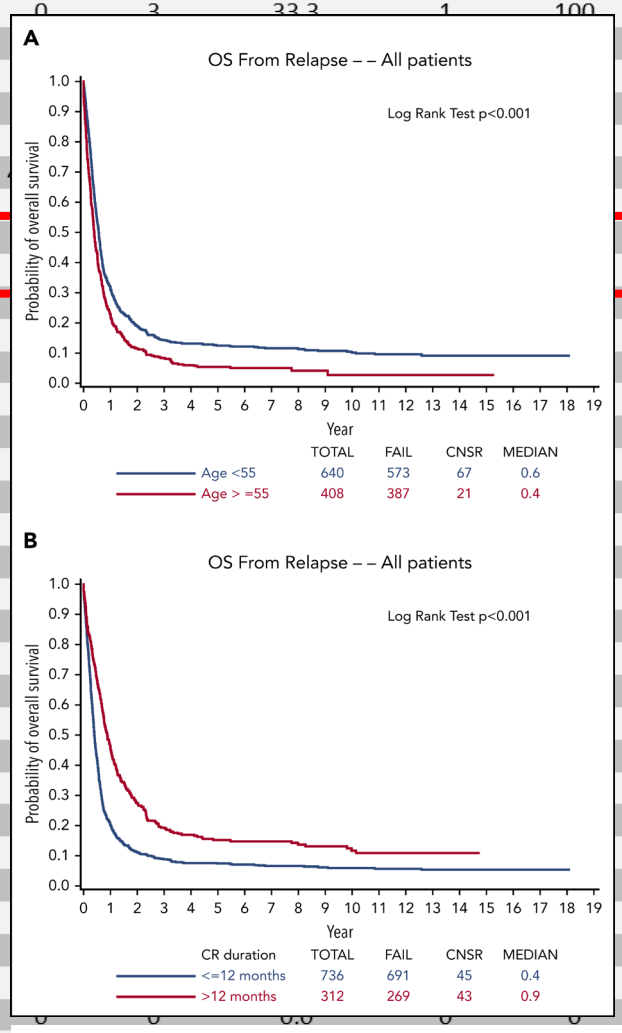


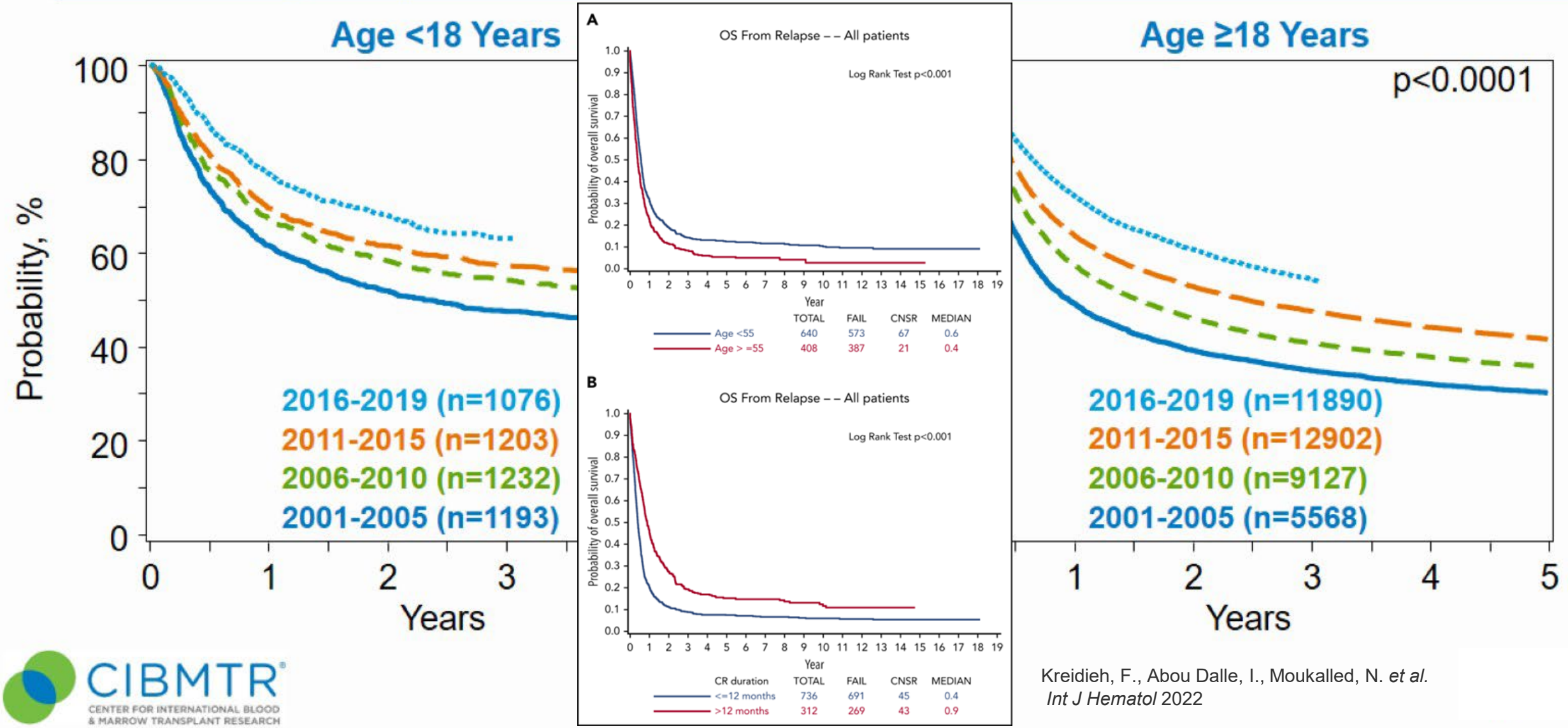
Fig. 4 Post-relapse OS with different treatments. prOS post-relapse overall survival, DLI donor lymphocyte infusion, CART chimeric antigen receptor T cells.

Gao, Y., Wu, H., Shi, Z. *et al. Bone Marrow Transplant* 2023



Kreidieh, F., Abou Dalle, I., Moukalled, N. *et al. Int J Hematol* 2022

Trends in Survival after Allogeneic HCTs for Acute Myelogenous Leukemia (AML), in the US, 2001-2019



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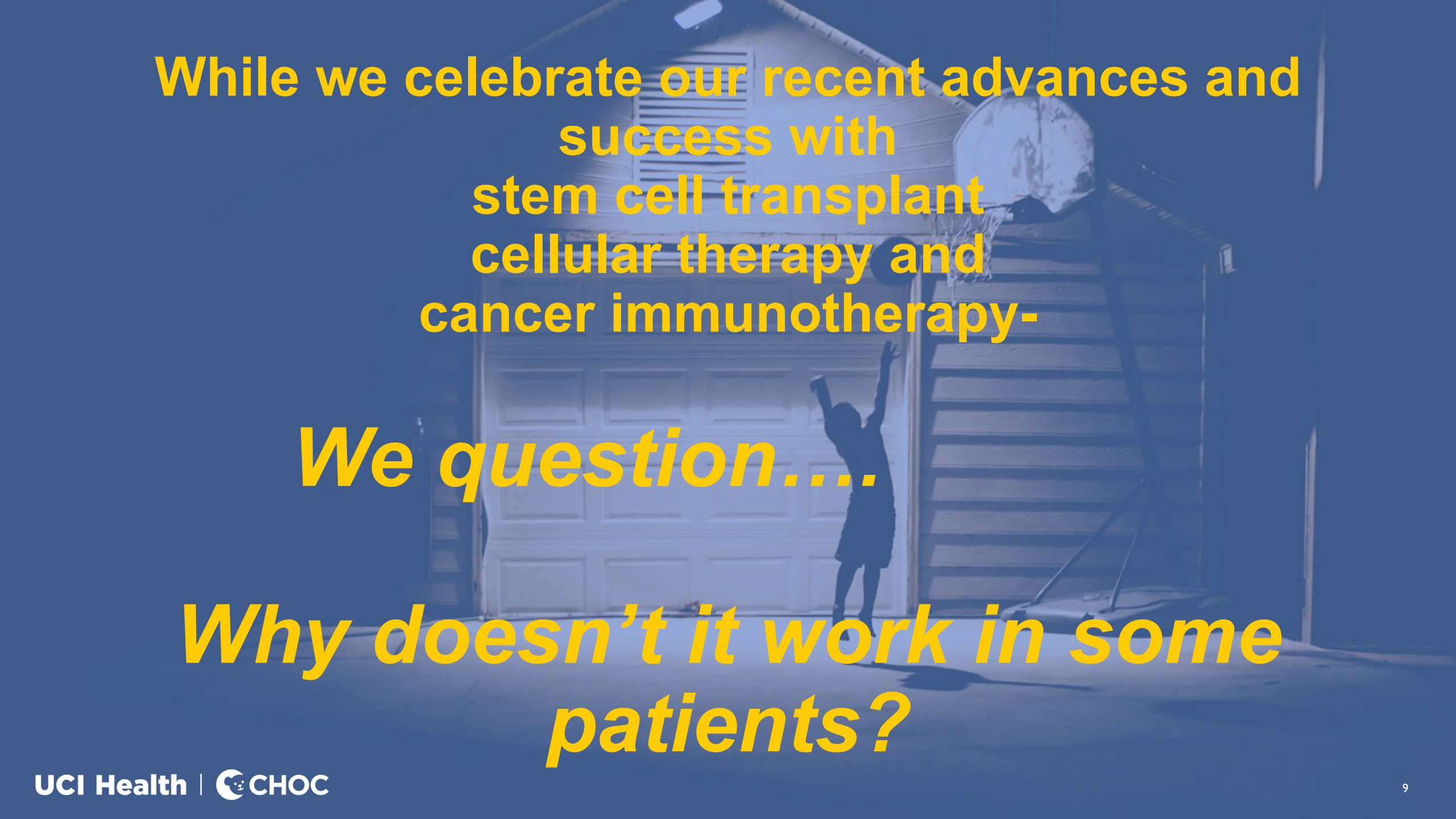
Kreidieh, F., Abou Dalle, I., Moukalled, N. *et al.* *Int J Hematol* 2022



While we celebrate our recent advances and success with stem cell transplant cellular therapy and cancer immunotherapy-

We question….

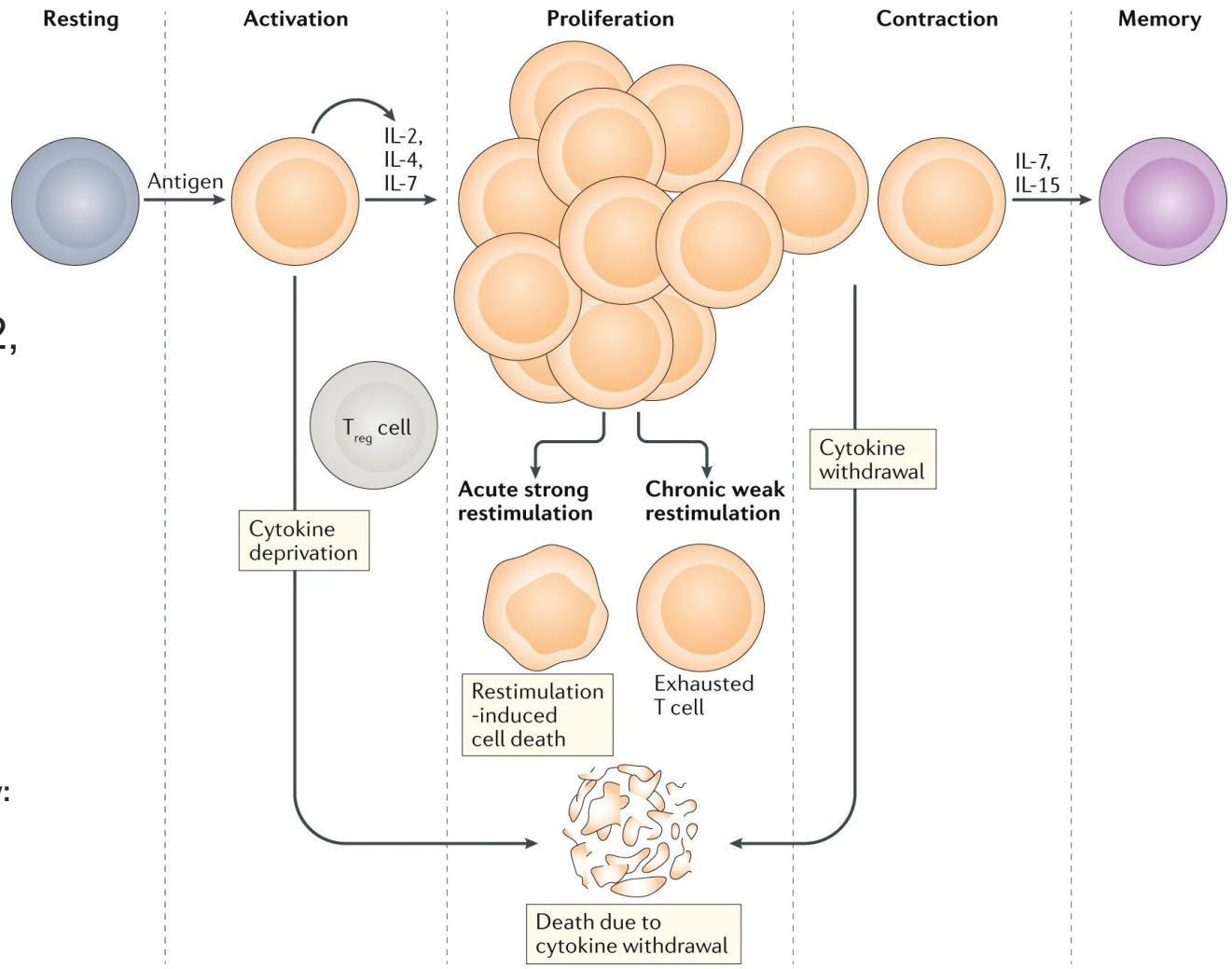
Why doesn't it work in some patients?

T cell primer

Resting T cells become activated after stimulation by cognate antigen in the context of an antigen-presenting cell and co-stimulatory signals.

Activated T cells produce and consume proliferative/survival cytokines, for example, IL-2, IL-4 and IL-7, and begin to expand in number.

If $CD4^+CD25^+$ regulatory T (T_{reg}) cells are present, they can deprive the cycling T cells of proliferative/survival cytokines, especially IL-2, causing them to undergo apoptosis.

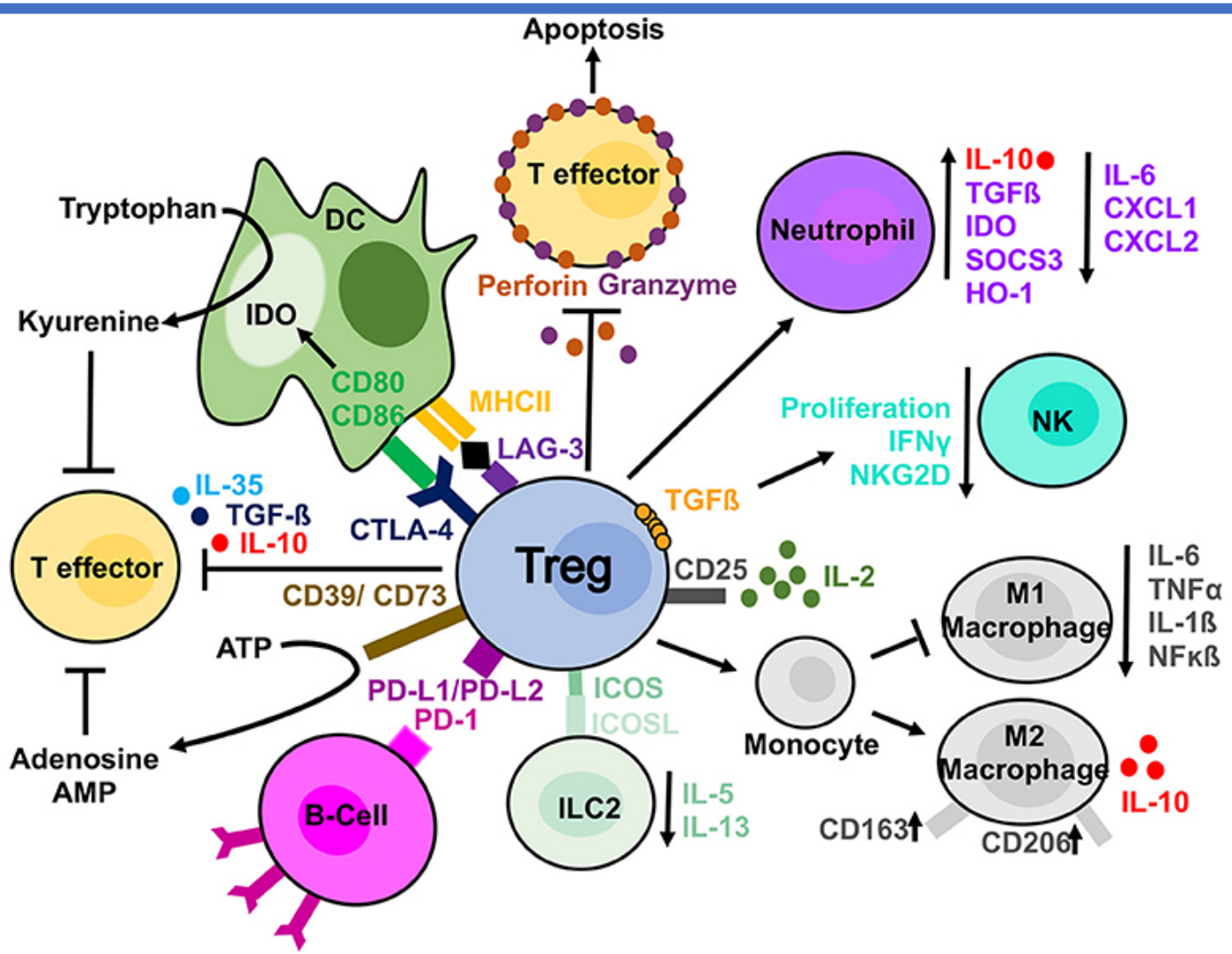


Waldman, A.D., Fritz, J.M. & Lenardo, M.J. **A guide to cancer immunotherapy: from T cell basic science to clinical practice.** *Nat Rev Immunol* **20**, 651–668 (2020).

Currently we have 15 patients and 14 related donors enrolled

Patient / Recipient				Donor			Transplant					GVHD			
Patient ID	Age	Gender	Diagnosis (R/R Leukemia)	Age (yrs)	Gender	Relation to patient	HLA match	Stem cell source	Conditioning Regimen	GvHD prophylaxis	Day of ANC engraft-ment	GVHD	Day of GVHD start	Relapse	Day of Significant infection (+)
CHOC_001	18 y.o	F	VHR B Cell ALL	20	M	Brother	Haplo	Bone Marrow	CY / TBI	ptCY, Tacro, MMF	19	No		No	61
CHOC_002	13 months	F	AML	41	M	Father	Haplo	Bone Marrow	BU/FLU/CY	ptCY, Tacro, MMF	14	Grade 1 (skin)	78	No	11
CHOC_003	19 y.o	F	AML	21	F	Unrelated	MUD 9/10	Peripheral Blood	BU/FLU/CY	ptCY, Tacro, MMF	16	No		No	104
CHOC_004	15 y.o	F	VHR B Cell ALL	19	M	Brother	Haplo	Bone Marrow	CY / TBI	ptCY, Tacro, MMF	17	Grade 1 (skin)	16	No	25
CHOC_005	5 y.o	F	AML	45	M	Father	Haplo	Bone Marrow	Ritux/BU/FLU/CY	ptCY, Tacro, MMF	13	Grade 2 (skin), Grade 3 (liver)	85 / 90	No	10 / 139
CHOC_006	11 y.o	M	VHR B Cell ALL	42	M	Father	Haplo	Peripheral Blood	TestB / CY / TBI	ptCY, Tacro, MMF	14	Grade 3 (skin)	130	No	10
CHOC_007	17 y.o	M	VHR B Cell ALL	43	M	Father	Haplo	Bone Marrow	BU/FLU/TT	ptCY, Tacro, MMF	No	No		Yes	21
CHOC_008	17 y.o	F	AML	21	M	Brother	MRD	Bone Marrow	BU/CY	MiniMTX	13	Grade 1 (skin, possible liver)	24	No	55
CHOC_009	7 y.o	M	Relapsed ALL	21	M	Half-Brother	Haplo	Bone Marrow	cXRT/CY/TBI	ptCY, Tacro, MMF	14	No		No	8
CHOC_010	10 y.o	M	Relapsed ALL	44	M	Father	Haplo	Bone Marrow	TestB / CY / TBI	ptCY, Tacro, MMF	16	No		No	44
CHOC_011	19 y.o	M	AML	30	F	Sister	MRD	Bone Marrow	BU/CY	MiniMTX	17	Grade 1 (liver)	22	No	46
CHOC_012	11y.o	M	VHR B Cell ALL	60	M	Father	Haplo	Peripheral Blood	TestB / CY / TBI	ptCY, Tacro, MMF	15	Grade 1 (skin)	31	No	NA
CHOC_013	18 y.o	M	T cell ALL	40	F	Mother	Haplo	Peripheral Blood	TestB / CY / TBI	ptCY, Tacro, MMF	24	Grade 4 (liver)	68	No	19
CHOC_014	12 y.o	F	VHR B Cell ALL	21	M	Brother	MRD	Bone Marrow	CY / TBI	MiniMTX, CSA, MMF	18	No		No	NA
CHOC_015	17 y.o	M	AML	22	F	Half-Sister	Haplo	Bone Marrow	BU/FLU/CY	ptCY, Tacro, MMF	18	No		No	NA

Regulatory T cells (Treg) primer



Regulatory T (Treg) cells are a subset of CD4+ T cells with immunosuppressive effects through various cellular and humoral mechanisms:

- cytotoxic T lymphocyte antigen 4 (CTLA-4)-mediated suppression of antigen-presenting cells,
- consumption of IL-2 and
- production of immune inhibitory cytokines (IL-10, IL-35, and TGFβ) and
- via molecules like (perforin and granzyme), which damage target cell membrane leading to apoptosis.
- Tregs can sequester, by the high expression of CD25, IL-2 from the microenvironment reducing effector T cells proliferation
- IL-2 starvation reduces NKs from proliferating and exhibiting effector functions as well.
- NKs can be directly affected by Tregs in a membrane bound TGF-ß dependent manner.
- Tregs have a direct effect on B-cells via PDL1/PD-1 interaction and DCs via both CTLA-4 and LAG-3.

Clinical trials to translate research for better patient care

Bedside → Bench

- Tregs:Tcons:NK cells, their respective cytokines and gut microbiome as **biomarkers** in
  - Allogenic SCT
  - CAR-T cell response
  - Inflammatory Bowel Disease
  - Multiple Sclerosis
  - ITP

Bench → Bedside

- Modulating Tregs:Tcon:NK cells and gut microbiome supported by cytokines
  - Sustained GVL in Allogenic stem cell transplant
  - CAR-T cell persistence
  - Transplant for IBD treatment
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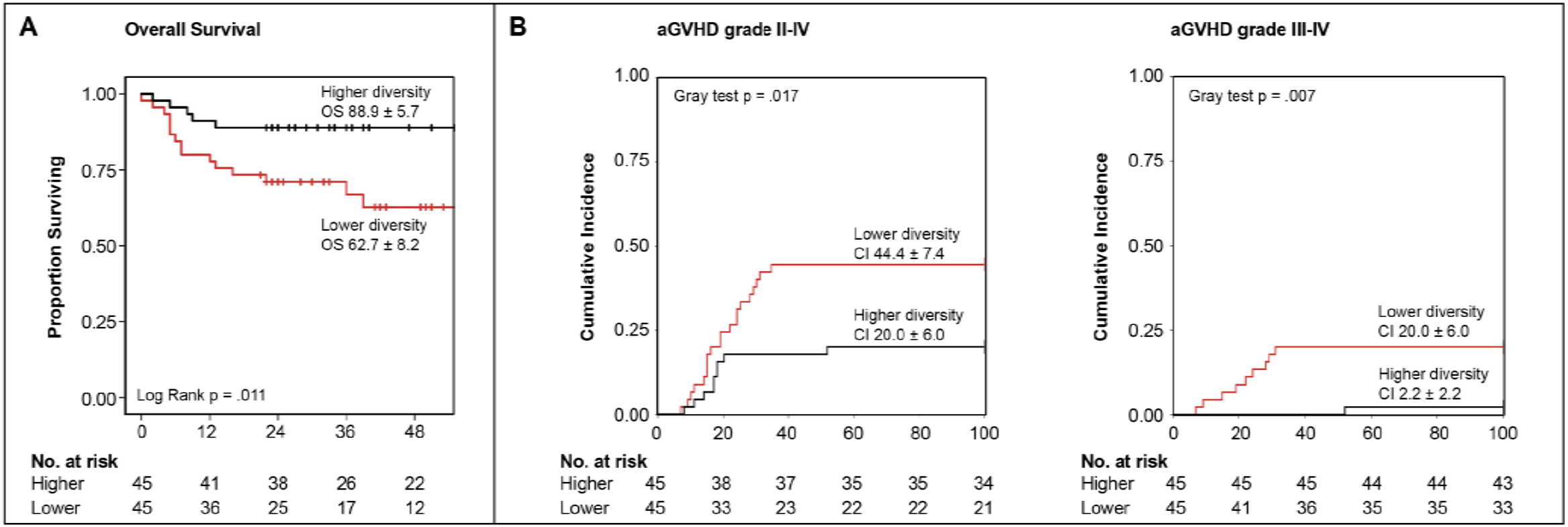
Gut microbiota diversity before ALLO HSCT as predictor of mortality in children

Figure 2



Masetti et al Blood 2023

Role of Regulatory T Cells in Predicting Outcomes of HSCT and CAR-T cells

1. Sequential time points for testing Treg percentages for host immune profiling

2. Monitor engraftment, GVHD, disease progression, infections and organ toxicity

7-14 days prior to stem cell transplant (SCT)

Day 0 SCT (on donated product, not recipient)

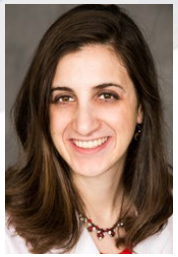
After transplant at Day 30, Day 60, Day 100, and Day 180, Day 270

One year post allogeneic hematopoietic SCT

CHOC Research
GO BEYOND

Keri Zabokrtsky
Nerida Guerrero

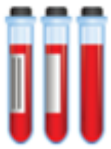
Clinical Co-investigators
- **Dr Van Huynh**
- **Dr Carol Lin**
- **Dr Jamie Frediani**
- **Dr Ivan Kirov**



Flowcytometry protocol

Caitlin Calhoun
BSc, PharmD, RPh
Faith Kim Bsc.

1.) Collect 2-4 ml blood into a purple top collection tube (EDTA)



Storage Protocol:

- Room temp, use immediately, or as soon as possible same day
- Should not exceed more than 30 minutes room temperature for plasma extraction

3.) CD4⁺/CD25⁺/CD127^{dim}/CD56/CD8/L/D

- Cells to analyze: 1.0x10^6
- Custom **MASTERMIX (REA Ab):**
  - CD4 (VioGreen)-V2
  - CD25 (VioBRIGHT 515)-B1
  - CD127(PE)-B2
  - CD56 (PE-Vio770)-B4
  - CD8 (APC Vio770)-R2
  - Viobility 405/452-V1

5.) Analysis of CD45/ CD4⁺/CD25⁺/CD127^{dim}/FoxP3/CD56/ CD8/L/D

- Identify lymphocytes via FSC/SSC
- Identify Live Cells
- Treg identification with CD4/FSC ->CD127(x)/CD25(y)->CD4(y) and FoxP3(x)

1. Collection → 2. Isolation → 3.Cell Surface Staining → 4.Intracellular staining → 5.Flow Analysis → 6.Compiled Data Analysis

2.) StraightFrom Whole Blood CD3+ Microbeads

- Rapid positive selection of CD3+ cells directly from whole blood or marrow.

4.) FoxP3

- Fixation and Permeabilization of surface-stained cells.
- Intracellular staining with Anti-FoxP3 (APC) – R1

5.) Compiled Data Analysis

- Clinical Relevance/Implications after analysis of compiled data
- Figure generation for progress reports, presentations and publications

2.Separation → 3. Storage Bank → 4. Cytokine testing

2.) Plasma separation

- Centrifugation

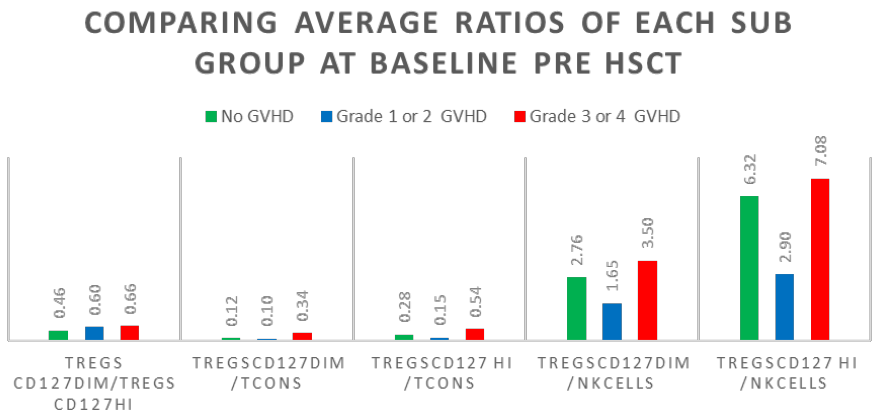
3.) Storage

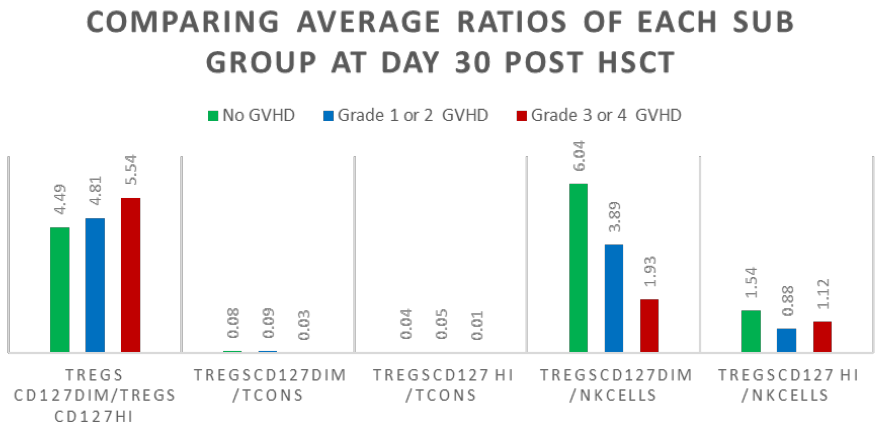
- -80°C up to 6 months

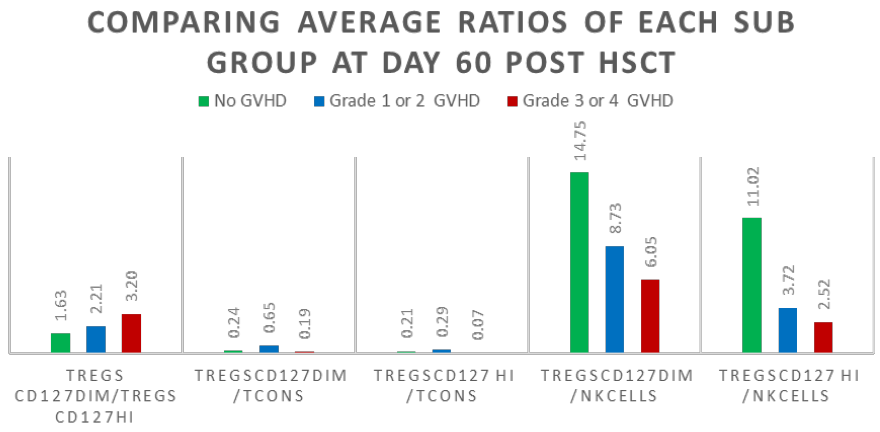
4.) Cytokine Testing

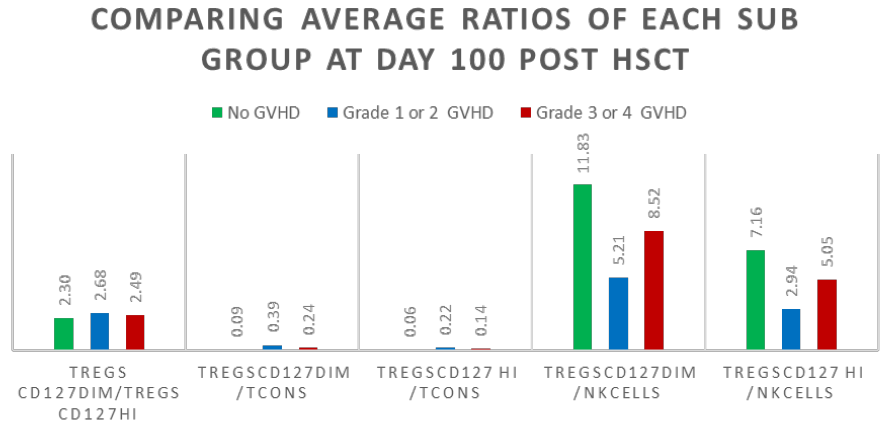
- 7 time points
- Cytokines: IL-2, IL-6, IL-7, IL-10, IL-15, IL-35, INFγ, TNF-a, CTLA-4, TGF-B
- NK/T Kit: GM-CSF, Granzyme B, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17A, IL-21, MCP-1 (CCL2), Perforin, and TNF-α

Preliminary data - Patient CHOC 001 – CHOC015









Preliminary clinical analysis of the first 15 samples up to Day 100

Patients/recipients

- Age:13 months to 19 years,
- 8 male and 7 females
- 6 AML and 9 ALL.

GVHD subgroups:

- 7 with no GVHD
- 5 with Grade 1 or 2 GVHD
- 3 with Grade 3 or 4 GVHD.

Relapse free survival : 13 surviving

- 1 death - early relapse
- 1 death – infections, toxicities

Donors: 14 related, 1 unrelated,

- 19 to 44 years, 11 male and 4 female

Are Tregs the bad guys or a good guys?

Sustained GVL – CAR T cell expansion and persistence

- Duell J, Topp MS et al. Frequency of regulatory T cells determines the outcome of the T-cell-engaging antibody blinatumomab in patients with B-precursor ALL. *Leukemia. 2017*
- Good, Z., Spiegel, J.Y., Sahaf, B. et al. Post-infusion CAR T_{Reg} cells identify patients resistant to CD19-CAR therapy. *Nat Med 2022*.
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Acute and chronic GVHD

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- Soares MV,, Lacerda JF. Naive and Stem Cell Memory T Cell Subset Recovery Reveals Opposing Reconstitution Patterns in CD4 and CD8 T Cells in Chronic Graft vs. Host Disease. *Front Immunol. 2019*
- Gooptu M, Cutler CS. Effect of Sirolimus on Immune Reconstitution Following Myeloablative Allogeneic Stem Cell Transplantation: An Ancillary Analysis of a Randomized Controlled Trial Comparing Tacrolimus/Sirolimus and Tacrolimus/Methotrexate (Blood and Marrow Transplant Clinical Trials Network/BMT CTN 0402). *Biol Blood Marrow Transplant. 2019*

Right proportion Treg:Tcon:NKcell at right time

Clinical trials to translate research for better patient care

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THANKS !!

- Keri Z, Caitlin C, Faith K, Charrissa C, Sahar N, Nerida G, Kathy D, Dorian C, Kristen K, Yostina A, Regina T, Hiba K, Leia R, Phuong D, Sasha P, Brent D, Terence S and other members of the research institute
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