RT with CAR-T: Bridging, Priming, Debulking, Consolidation or All the Above

Bouthaina Dabaja, MD

University of Texas MD Anderson Cancer Center

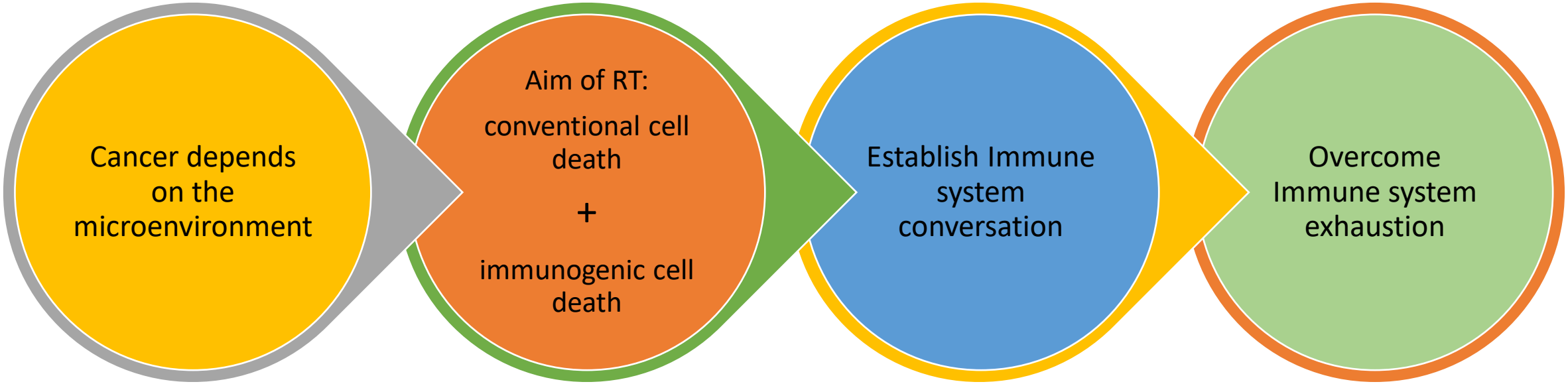
Disclosures

Research supports

- Seattle Genetics
- Kite

- Advisory to ONO-Pharma

The new paradigm of Cancer Treatment



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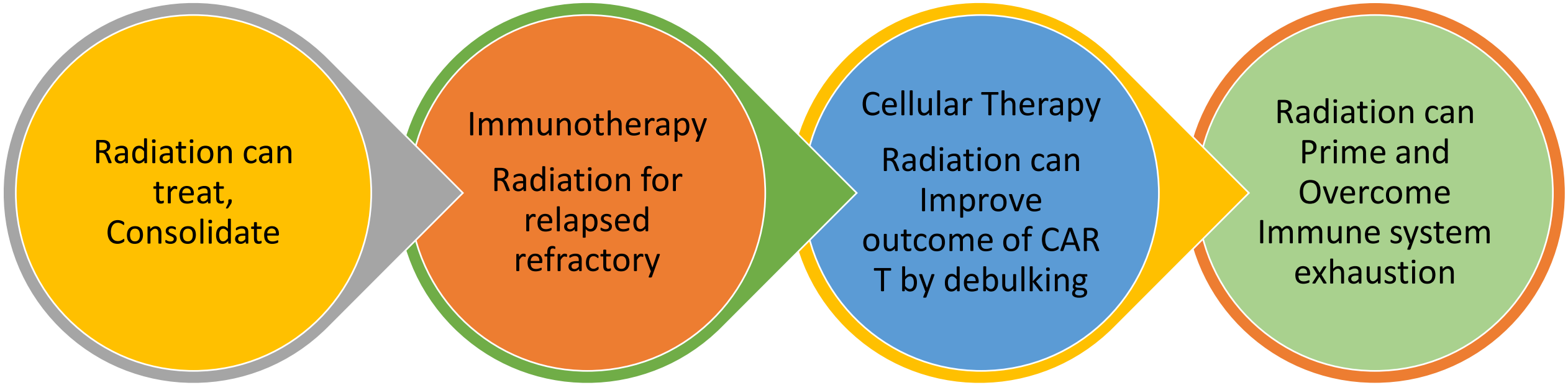
Timeline of RT role



Traditional role

2010-2012

Overall Better survival

2010-2017

Buy time while Manufacturing

2017-2022

Future until bi-specifics take over

2022-2030

Conduct translation studies on the effect/ timing of radiation rather than a binary response / no response

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Objectives of my talk

Landscape of Cellular and Immunotherapy

Early evidence for the clinical benefit of radiation

What we know about priming with radiation

The current ongoing research and future direction

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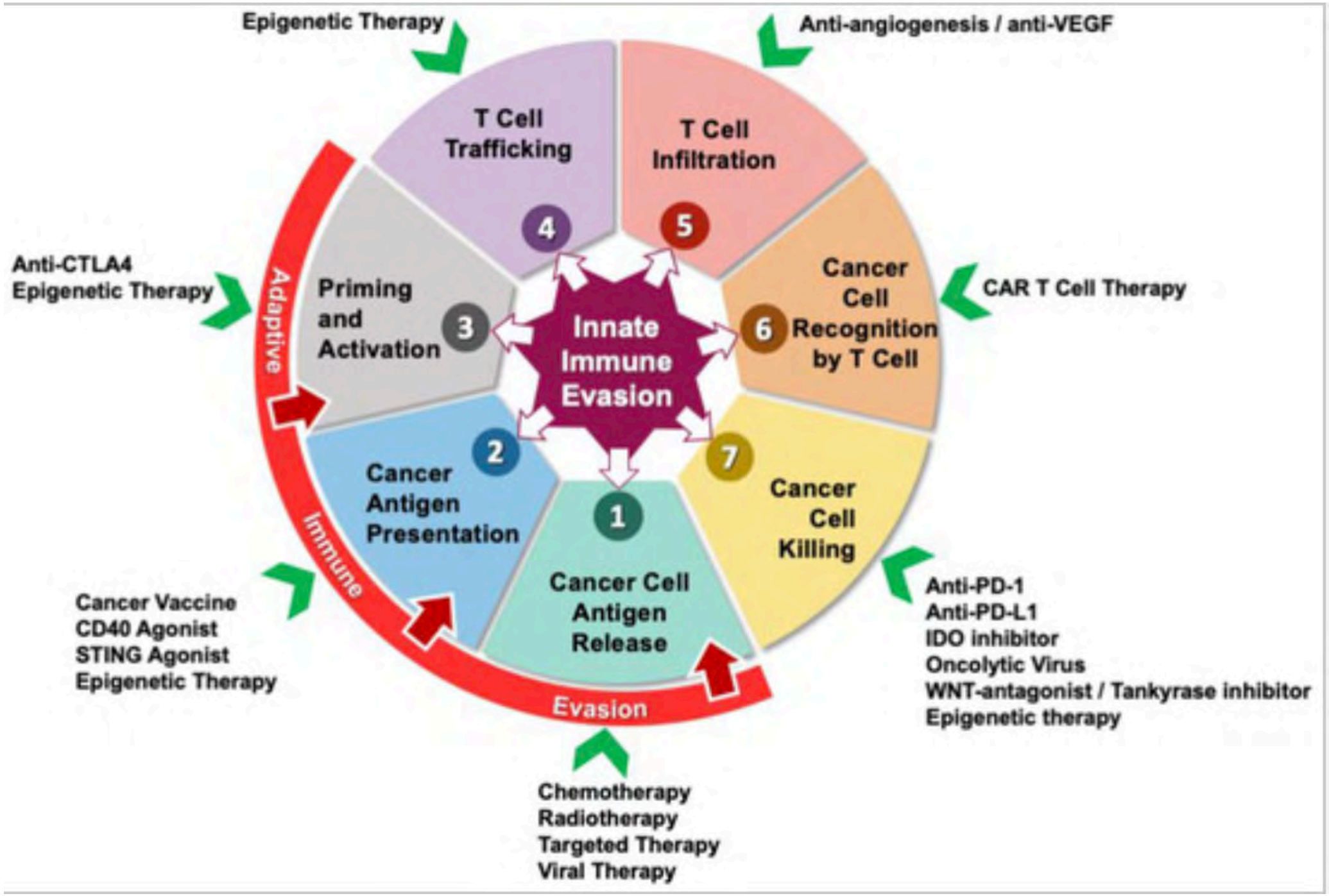
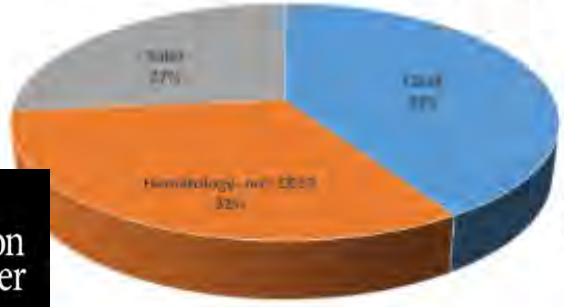


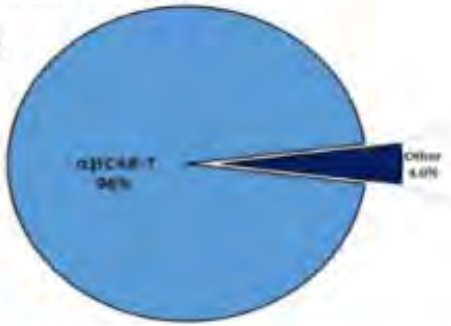
FDA-Approved CAR T-Cell Therapies

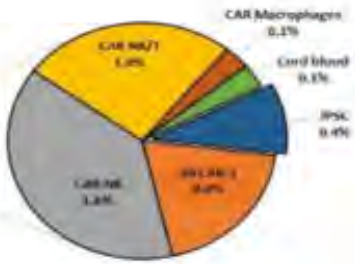
Generic Name	Brand Name	Target Antigen	Targeted Disease	Patient Population	CAR structure
Tisagenlecleucel	Kymriah	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL	BBz, lenti
			B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL	
Axicabtagene ciloleucel	Yescarta	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL	28z, γ-retro
			Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma	
Brexucabtagene autoleucel	Tecartus	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL	28z, γ-retro
			B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL	
Lisocabtagene maraleucel	Breyanzi	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL	BBz, lenti
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma	BBz, lenti
Ciltacabtagene autoleucel	Carvykti	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma	BBz, lenti

NCI- https://www.cancer.gov/about-cancer/treatment/research/car-t-cells



CD19 CAR trials account for 41% of 730 CART trials listed at clinicaltrials.gov (March 2022)
Globerson-Levin, Eur J Immunol, 2021





Courtesy of Fred Hutch

MUCH OF THIS SUCCESS IS DUE TO CELLULAR IMMUNOTHERAPIES

KEY DRIVERS OF MARKET GROWTH:

- Development of advanced cell-based therapies
  - 1,483 clinical trials in 2020
- Rising prevalence of cancer
  - 19.2M new cases in 2020, projected 24.5M by 2030
- Increasing R&D investment by key companies
- Increasing number of product approvals
- Growing adoption of cell-based immunotherapies

FRED HUTCH

GENE & CELL THERAPY IS "BOOMING"

- In 2020, **$19.9 billion in financing**

"By 2025, we predict that the FDA will be approving 10 to 20 cell and gene therapy products a year based on an assessment of the current pipeline and the clinical success rates of these products."[4] **Dr. Scott Gottlieb**
FDA Commissioner, 2017 - 2019

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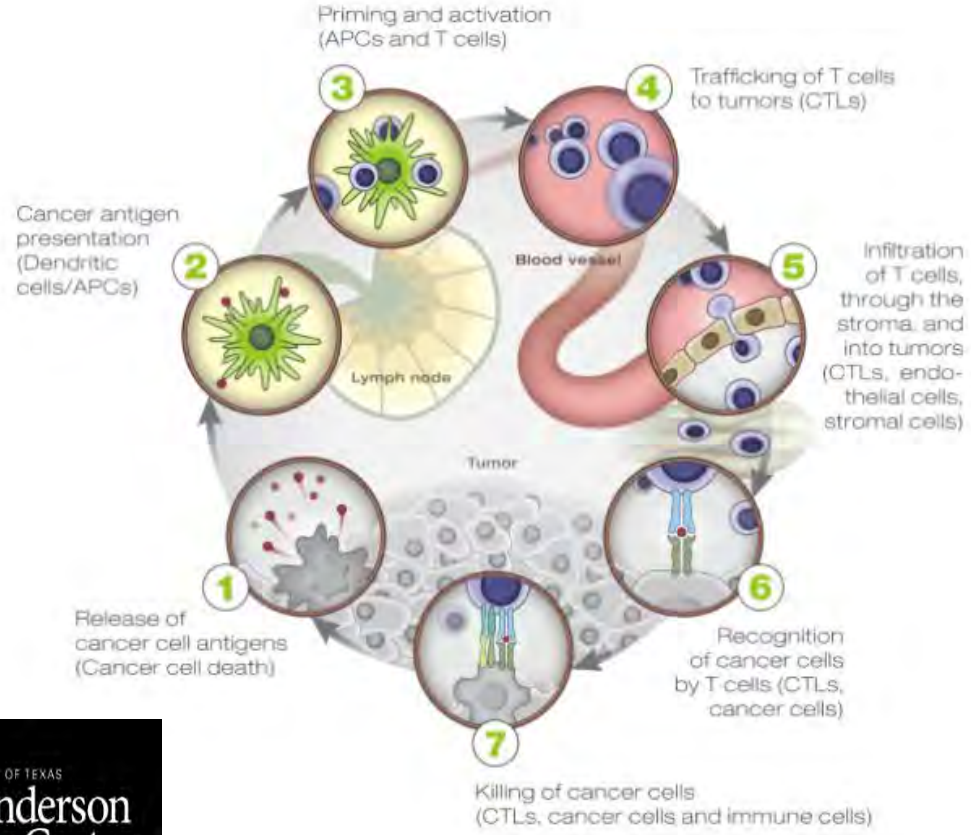
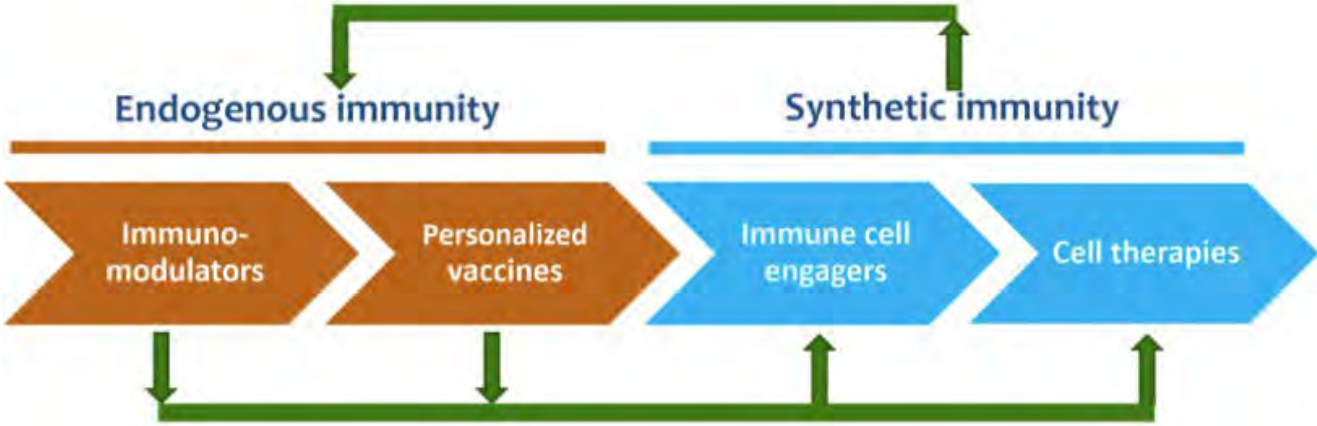
The future: deploying multiple modalities to address both the intrinsic and extrinsic challenges to T cell* function

* or other effector cell





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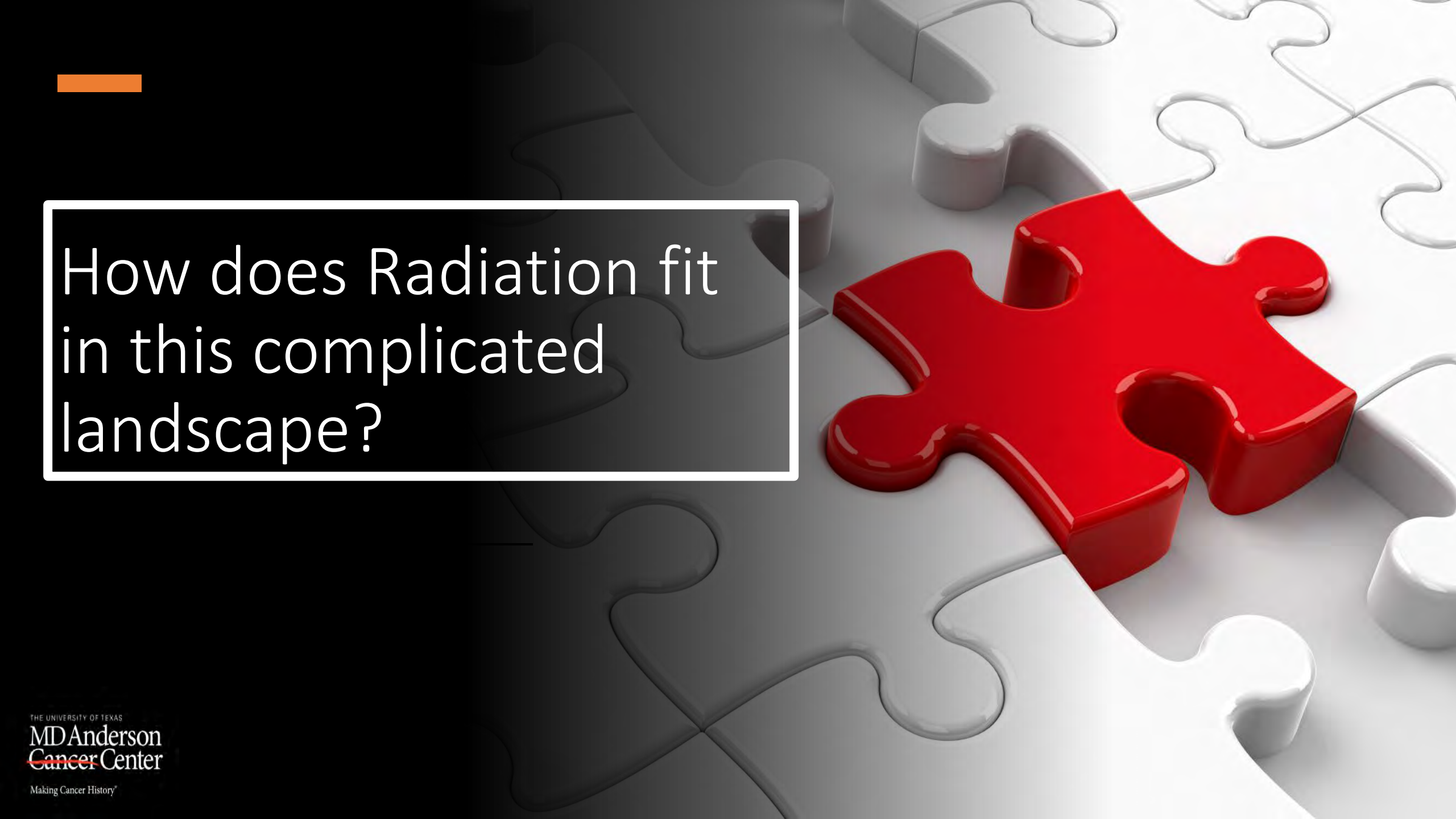
How does Radiation fit in this complicated landscape?



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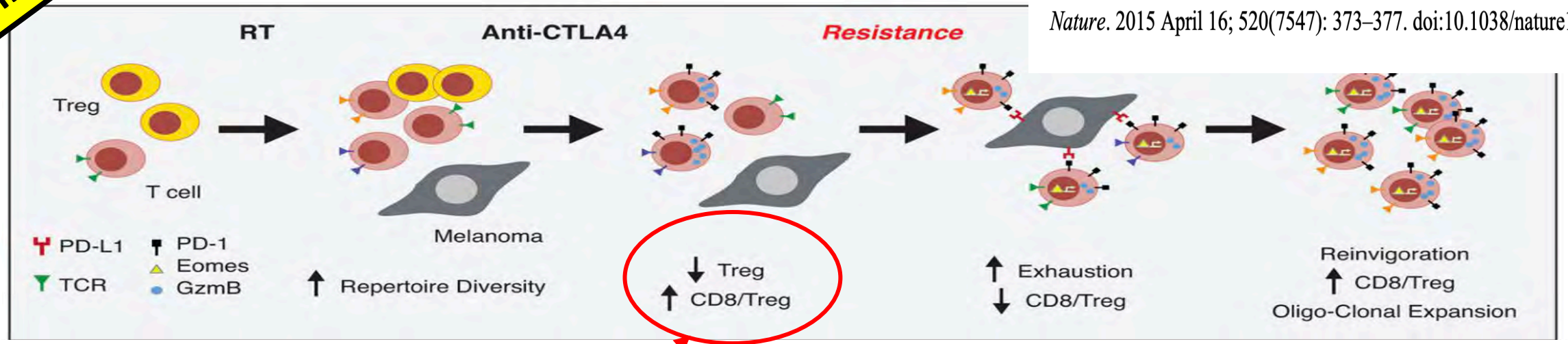
Priming

Combining radiation and anti-CTLA4 or anti-PD1 immunotherapy for the treatment of melanoma.



Nature. 2015 April 16; 520(7547): 373–377. doi:10.1038/nature14292.

the University of Pennsylvania group demonstrated the importance of timing and sequence

After the initial response of combining anti-CTLA4 antibodies with radiation---------

Resistance developed by upregulation of PDL1 leading to T-cell exhaustion_____

Resistance was reversed by adding PDL1 blockade.

Priming

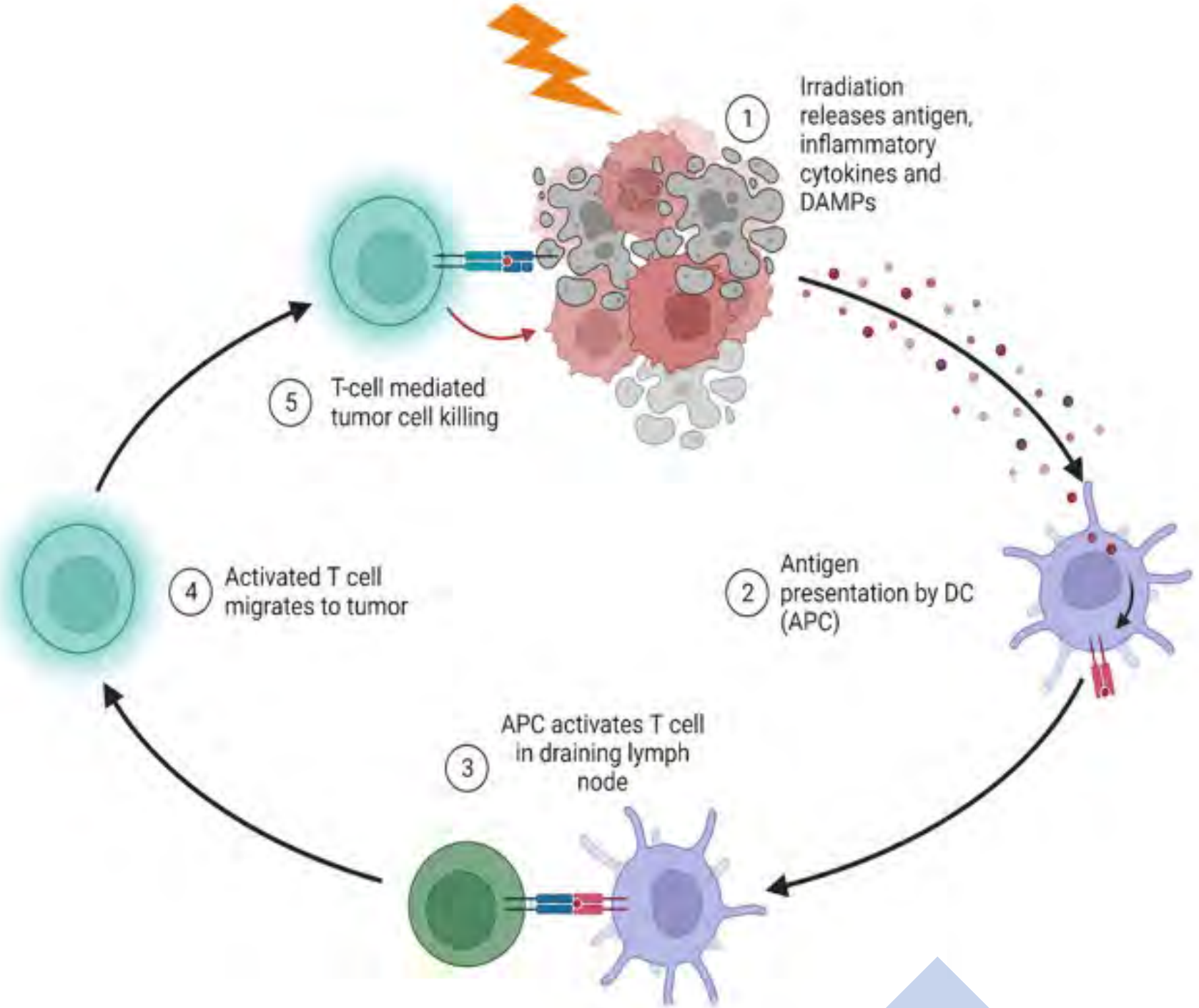
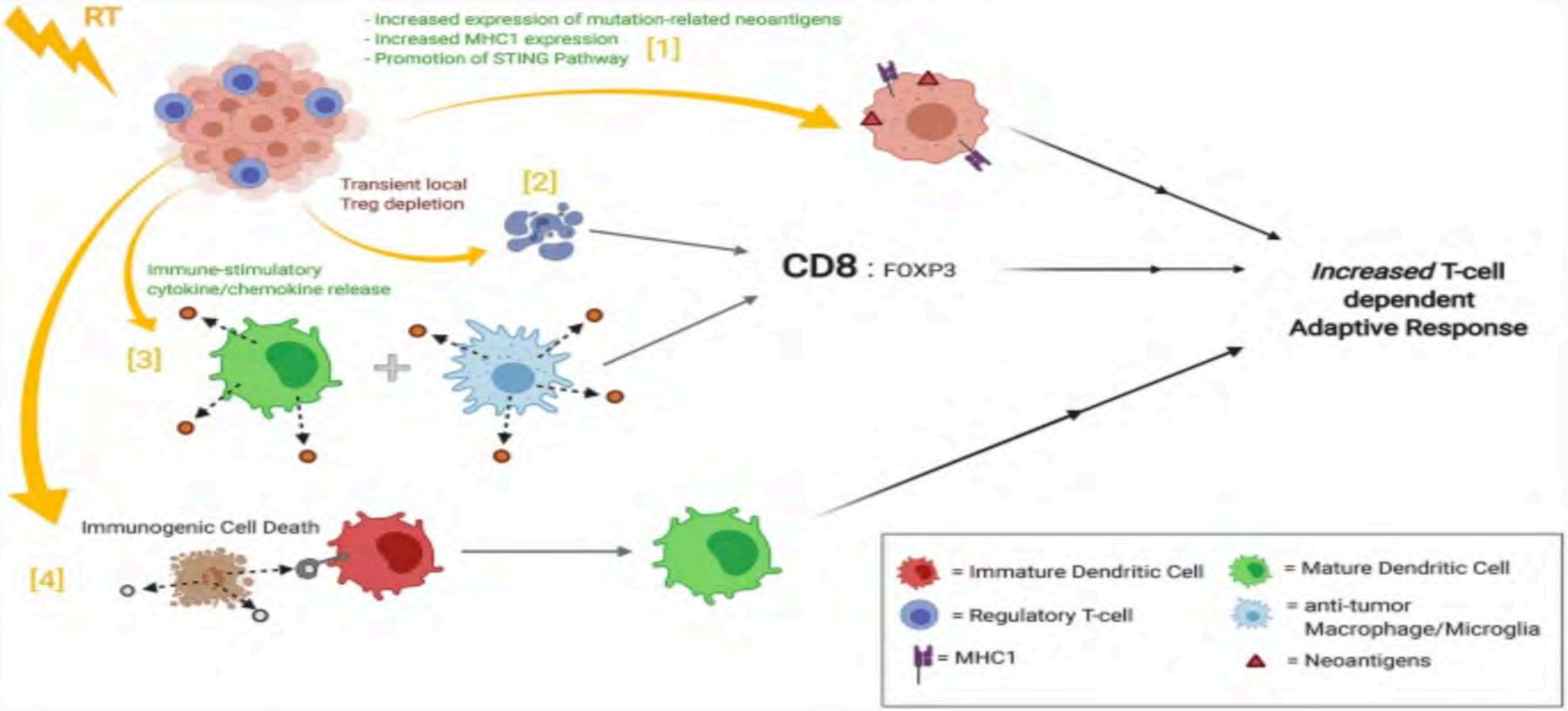


Fig. 1



Strategies that improve antigen presentation:

- Induce epitope spreading,
- Perpetuate existing antitumor T cell responses
- Combat tumor antigen escape.

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NeuroMolecular Medicine **volume 24**, pages3–7 (2022)

What is the Available evidence?

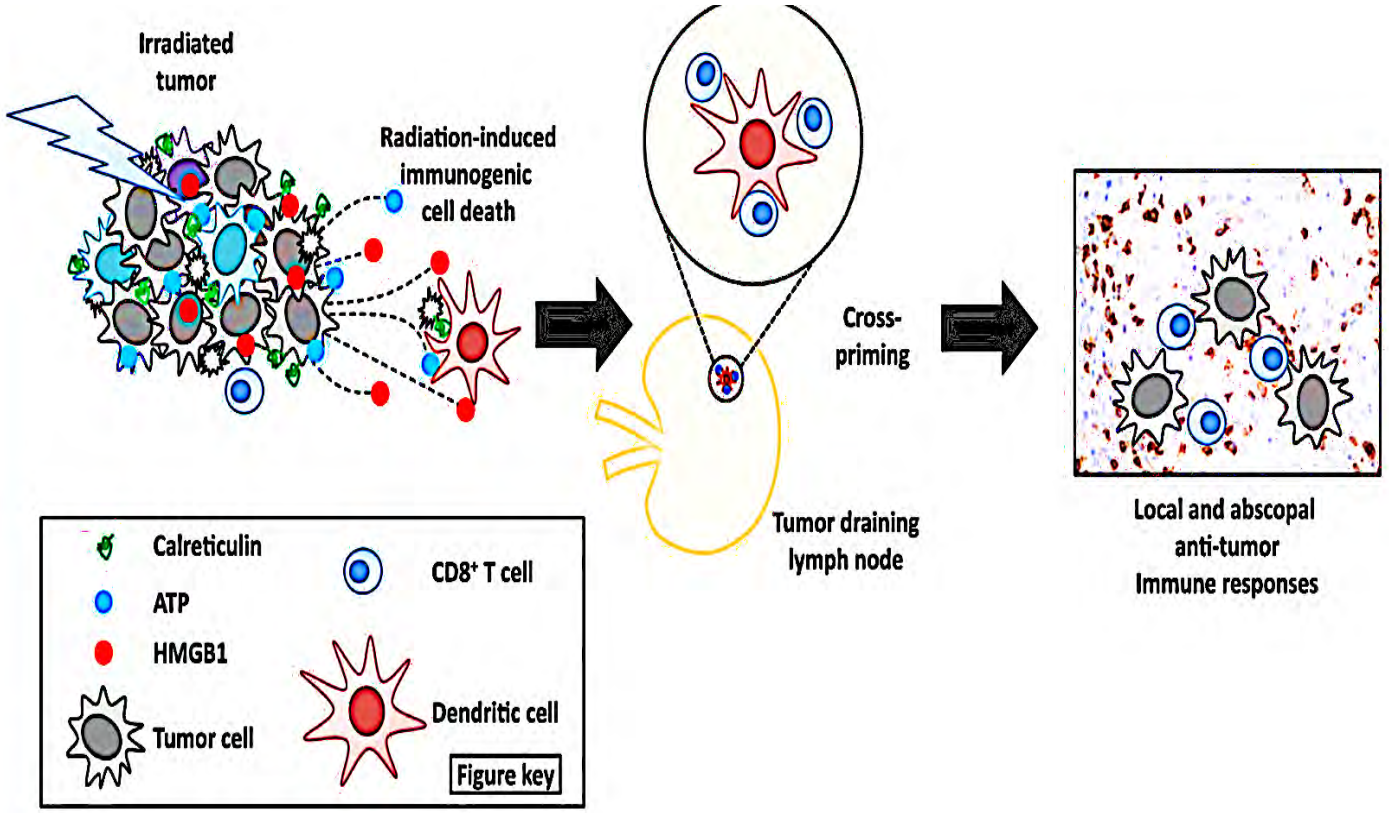
Radiotherapy and Immunogenic Cell Death

Encouse B. Golden, MD, PhD,* and Lionel Apetoh, PhD†,‡,§

Seminars in RADIATION ONCOLOGY

Immunogenic cell death= Priming

The dispersion of radiotherapy-induced immune-stimulating tumor antigens released from dying tumor cells into the surrounding milieu



T cell surface translocation DC the “eat me signal”

CD 47 DC (“do not eat me signal”) expressed by tumors

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Supportive evidence

What is the Priming Dose of Radiation?

Low-Dose Irradiation Programs Macrophage Differentiation to an iNOS$^+$/M1 Phenotype that Orchestrates Effective T Cell Immunotherapy

Felix Klug, Hridayesh Prakash, Peter E. Huber, Tobias Seibel, Noemi Bender, Niels Halama, Christina Pfirschke, Ralf Holger Voss, Carmen Timke, Ludmila Umansky, Kay Klapproth, Knut Schäkel, Natalio Garbi, Dirk Jäger, Jürgen Weitz, Hubertus Schmitz-Winnenthal, Günter J. Hämmerling, and Philipp Beckhove

Cell PRESS

- normalization of aberrant vasculature
- recruitment of tumor-specific T cells in human pancreatic carcinomas
- T-cell-mediated tumor rejection

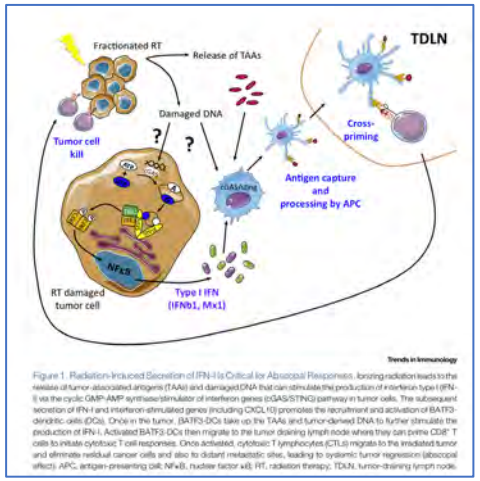
  prolonged survival in otherwise immune refractory spontaneous and xeno-transplant mouse tumor models.

Trends in Immunology
CellPress REVIEWS

Review

Immunological Mechanisms Responsible for Radiation-Induced Abscopal Effect

María E. Rodríguez-Ruiz, Claire Vanpouille-Box, Ignacio Melero, Silvia Chiara Formenti, and Sandra Demaria



Ionizing Radiation has multiple immune-modulatory effects:

Production of IFN-b, DCs infiltrating the tumor
promoting the cross-presentation of tumor antigens to CD8 T cells

T cell infiltration following radiation

- IFN-stimulated genes that encode the chemokines
  - CCL5, CXCL16 (C-X-C motif chemokine ligand 16), CXCL10

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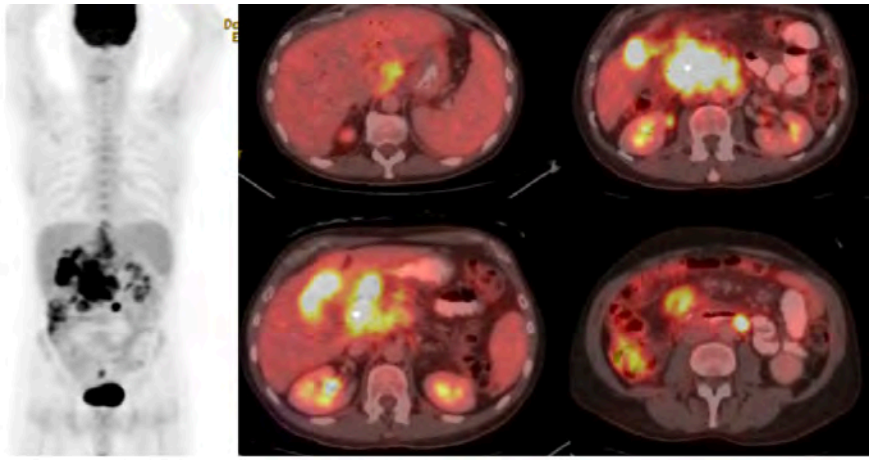
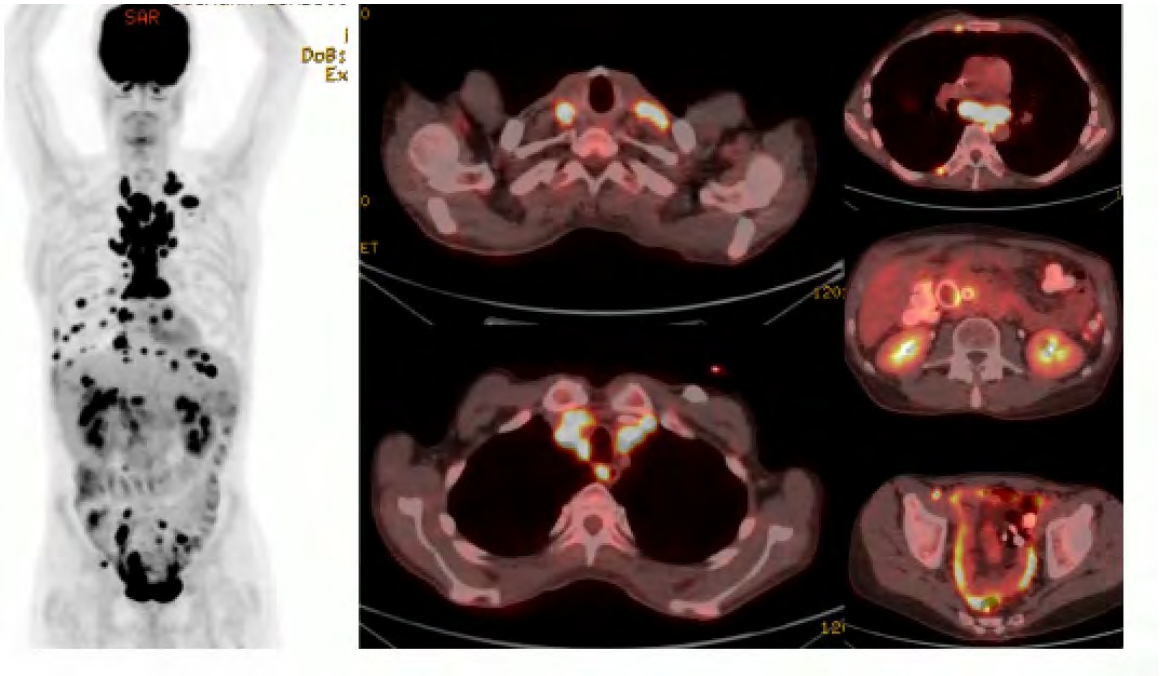
How does this translate clinically?

Are we sure that the immunological changes equate a clinical benefit ?

An example of what we see in clinic
Confirming that radiation does stimulate the immune system



Before Radiation



Day 7 form start of RT: flare of nodes outside of RT

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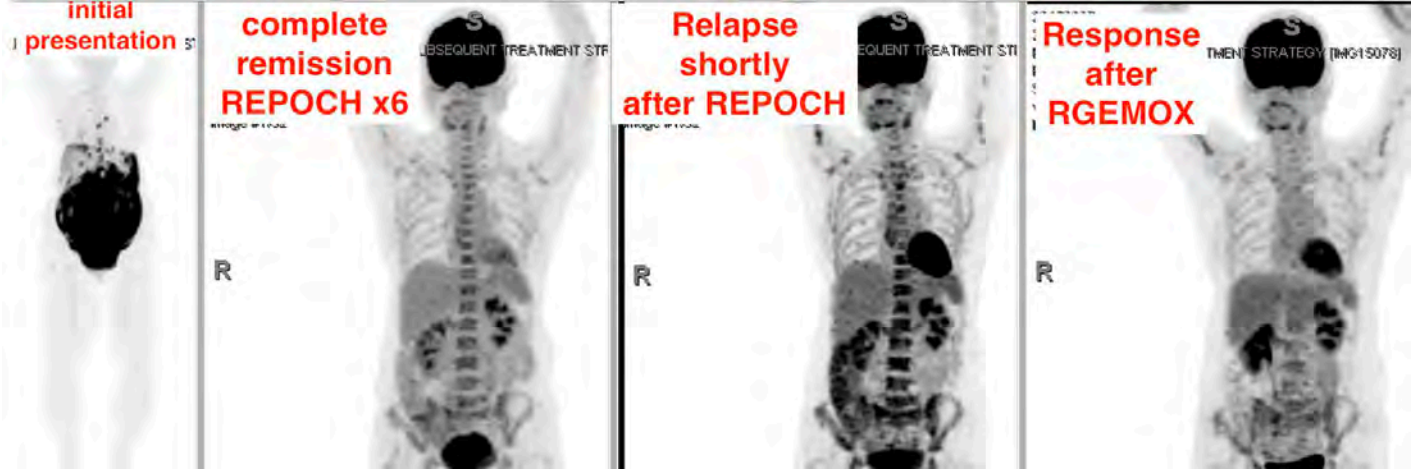
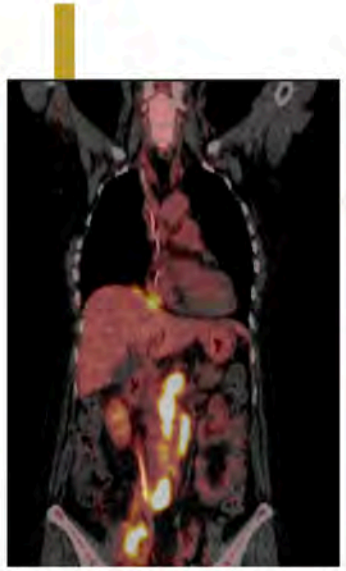
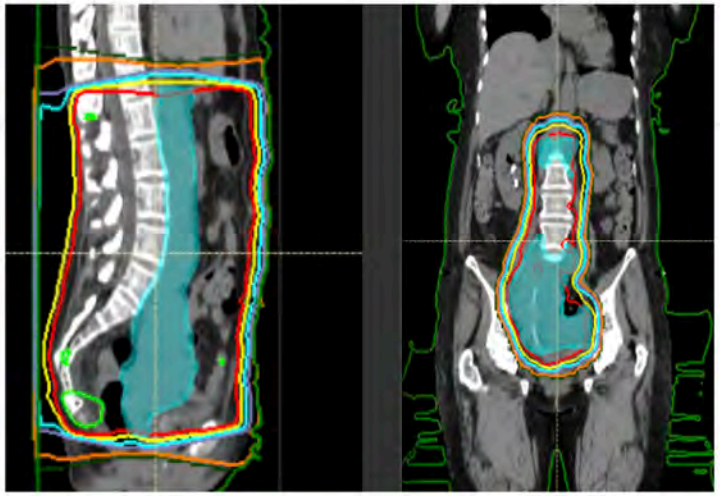
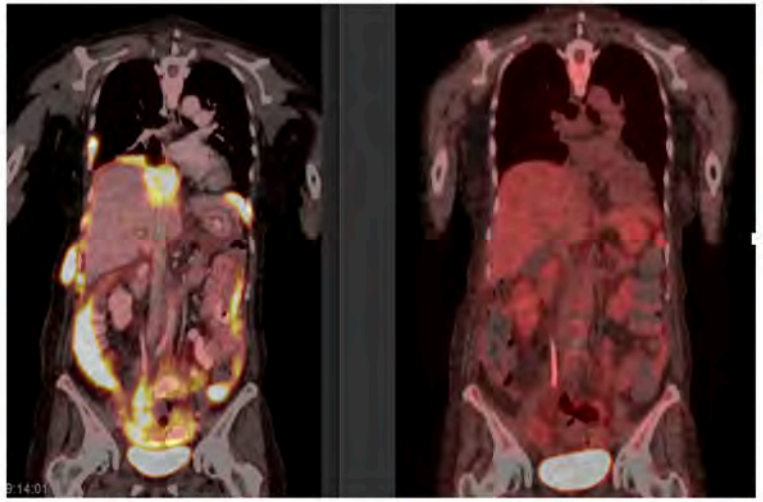




Presentation to RO mass along the PA area to pelvis



Field of radiation treating all but the diaphragmatic lesion



PET done before proceeding to CAR T Infusion suspected relapse everywhere except in the Irradiated field

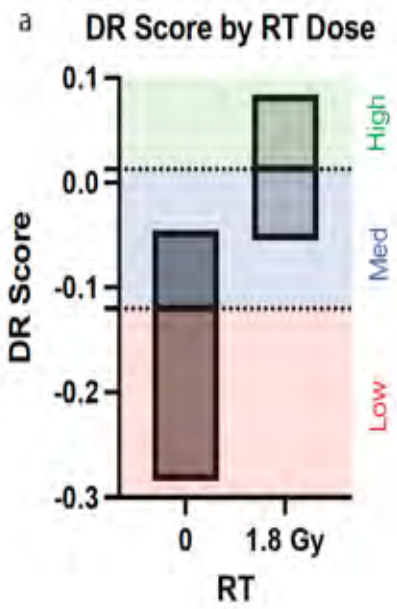
Proceeded to Car T and achieved a durable complete remission

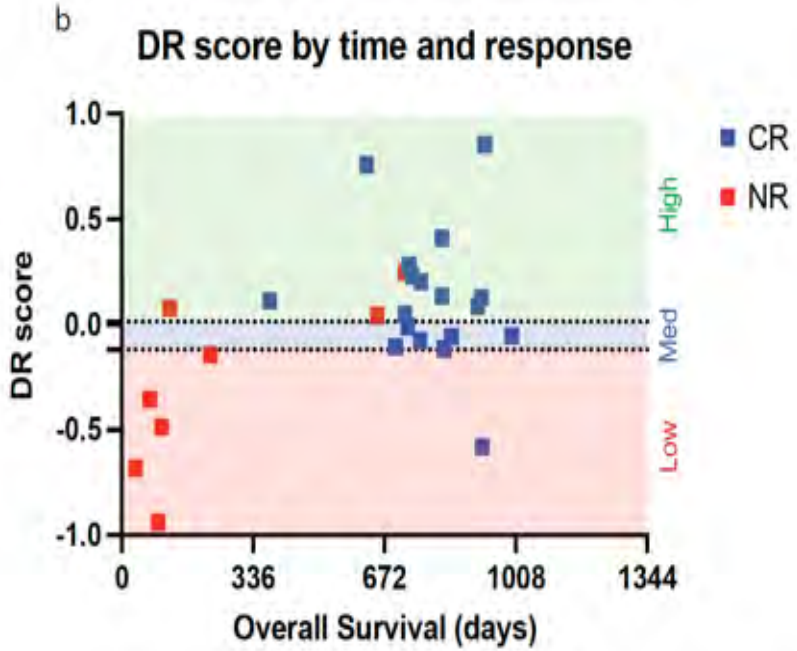
Exploring low-dose Radiation as low as 1.8 Gy

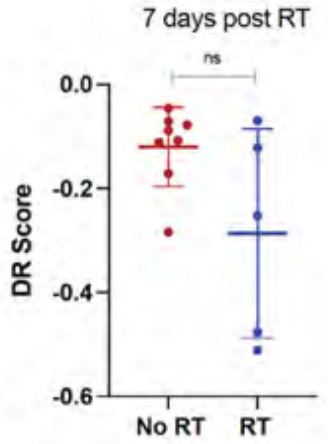
RESEARCH ARTICLE | APRIL 24, 2023

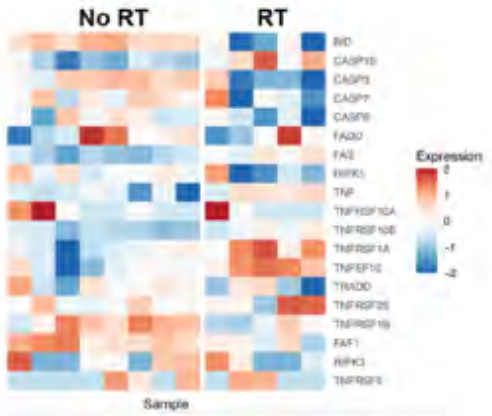
Intrinsic tumor resistance to CAR T cells is a dynamic transcriptional state that is exploitable with low-dose radiation

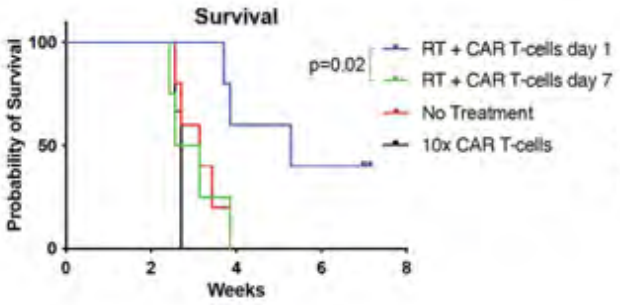
Alexander B. Kim, Ssu-Yu Chou, Solomon Kang, Eric Kwon, Matthew Inkman, Jeffrey Szymanski, Neal Andruska, Cian Colgan, Jin Zhang, Joanna C Yang, Nathan Singh, Carl DeSelm

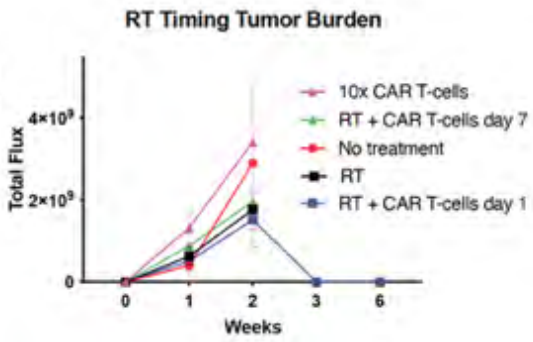












- LD-TBI plus CAR T cells on day 0 exhibited superior tumor control and OS (median: 110 days) as compared to all other groups.
- considerable expansion of circulating T cells

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CDD press

www.nature.com/cddis

ARTICLE OPEN

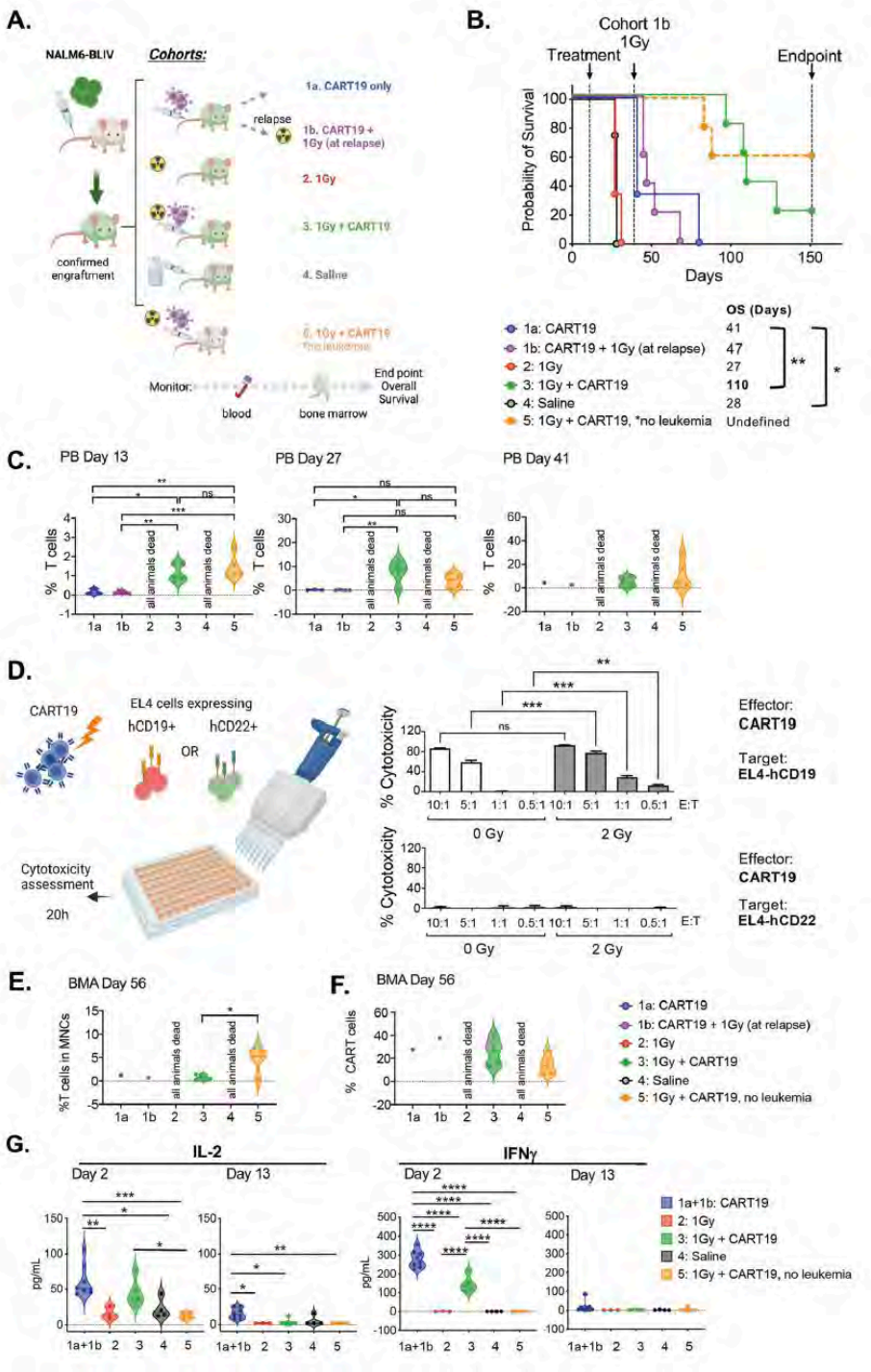
Check for updates

Radiation therapy improves CAR T cell activity in acute lymphoblastic leukemia

Mayumi Sugita[1,8], Takahiro Yamazaki[2,8], Mohammad Alhomoud[1], Jérémie Martinet[1,3,4], Jean-Baptiste Latouche[3,4], Encouse Golden[2], Olivier Boyer[3,4], Koen Van Besien[1], Silvia C. Formenti[2,5], Lorenzo Galluzzi[2,5,6,9]✉ and Monica L. Guzman[1,5,6,7,9]✉

Low-dose total body irradiation (LD-TBI) superior expansion of CAR T cells in vivo.

RT to elicit death receptor (DR) expression by malignant cells enabling some degree of CAR-independent tumor killing



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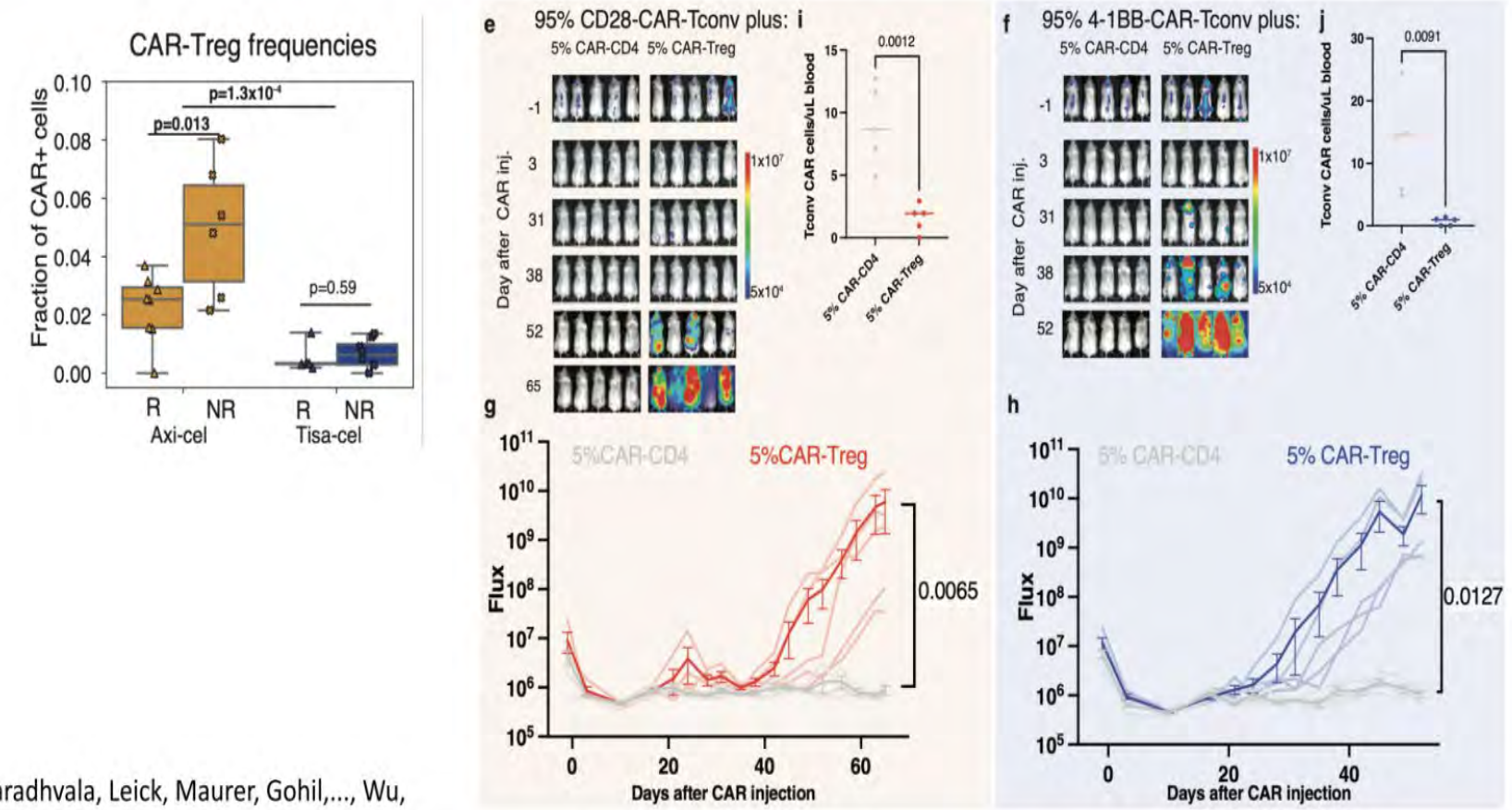
Priming

CAR-Tregs may have a role in depressing responses



Haradhvala, Leick, Maurer, Gohil,..., Wu, Getz & Maus *Nature Medicine 2022*

Elevations in CAR-T regulatory cells among non-responders to Axi-cel

- capable of suppressing conventional CAR-T cell expansion
- driving late relapses in an in vivo model.

The capacity for even small increases in CAR-T regulatory cells to drive relapse.

RESEARCH ARTICLE Open Access

Ionizing radiation modulates the phenotype and function of human CD4+ induced regulatory T cells

Samantha S. Beauford, Anita Kumari and Charlie Garnett-Benson*

Created an Opportunity for Radiation

Conclusions: Our findings demonstrate that while human T_{REG} cells are more resistant to radiation-induced death, treatment causes downregulation of Foxp3 expression, as well as modulation in the expression of T_{REG} signature molecules associated with suppressive activity. Functionally, irradiated TGF-β1-induced T_{REGS} were less effective at inhibiting CD8+ T cell proliferation. These data suggest that doses of radiotherapy in the hypofractionated range could be utilized to effectively target and reduce T_{REG} activity, particularly when used in combination with cancer immunotherapies.

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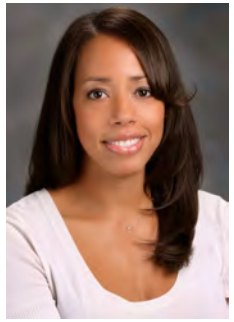
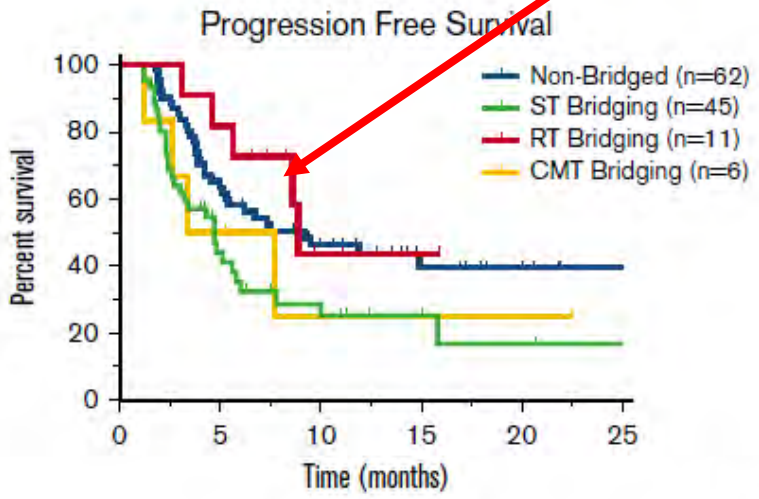
Does the preclinical data translate
into a clinical benefit?

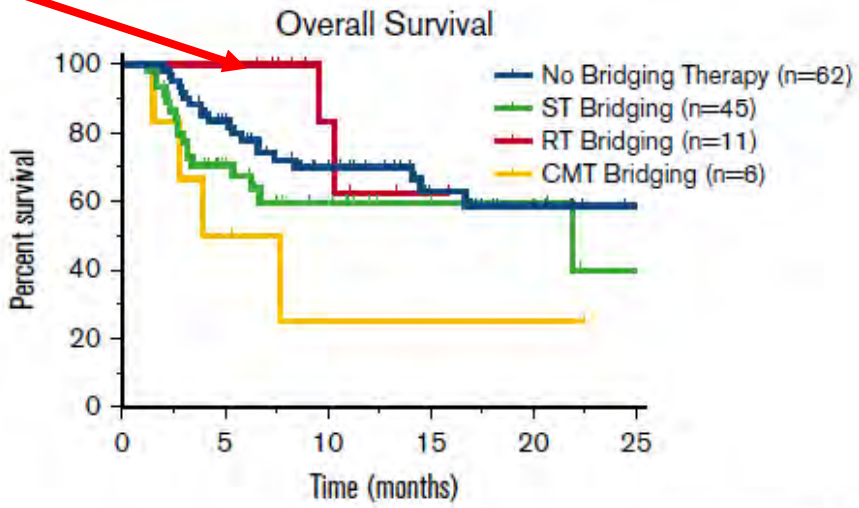
Radiation priming is effective





No Bridging	62	37	22	11	5	1
ST Bridging	45	16	8	4	3	1
RT Bridging	11	10	3	2	0	0
CMT Bridging	6	4	2	2	1	0

No bridging vs ST p=0.01
No bridging vs RT p=0.52
No bridging vs CMT p=0.36
ST vs RT p=0.05
ST vs CMT p=0.78
RT vs CMT p=0.17



No Bridging	62	48	30	16	7	2
ST Bridging	45	26	12	5	5	2
RT Bridging	11	11	5	2	1	0
CMT Bridging	6	4	2	2	2	0

No bridging vs ST p=0.17
No bridging vs RT p=0.40
No bridging vs CMT p=0.03
ST vs RT p=0.15
ST vs CMT p=0.30
RT vs CMT p=0.02

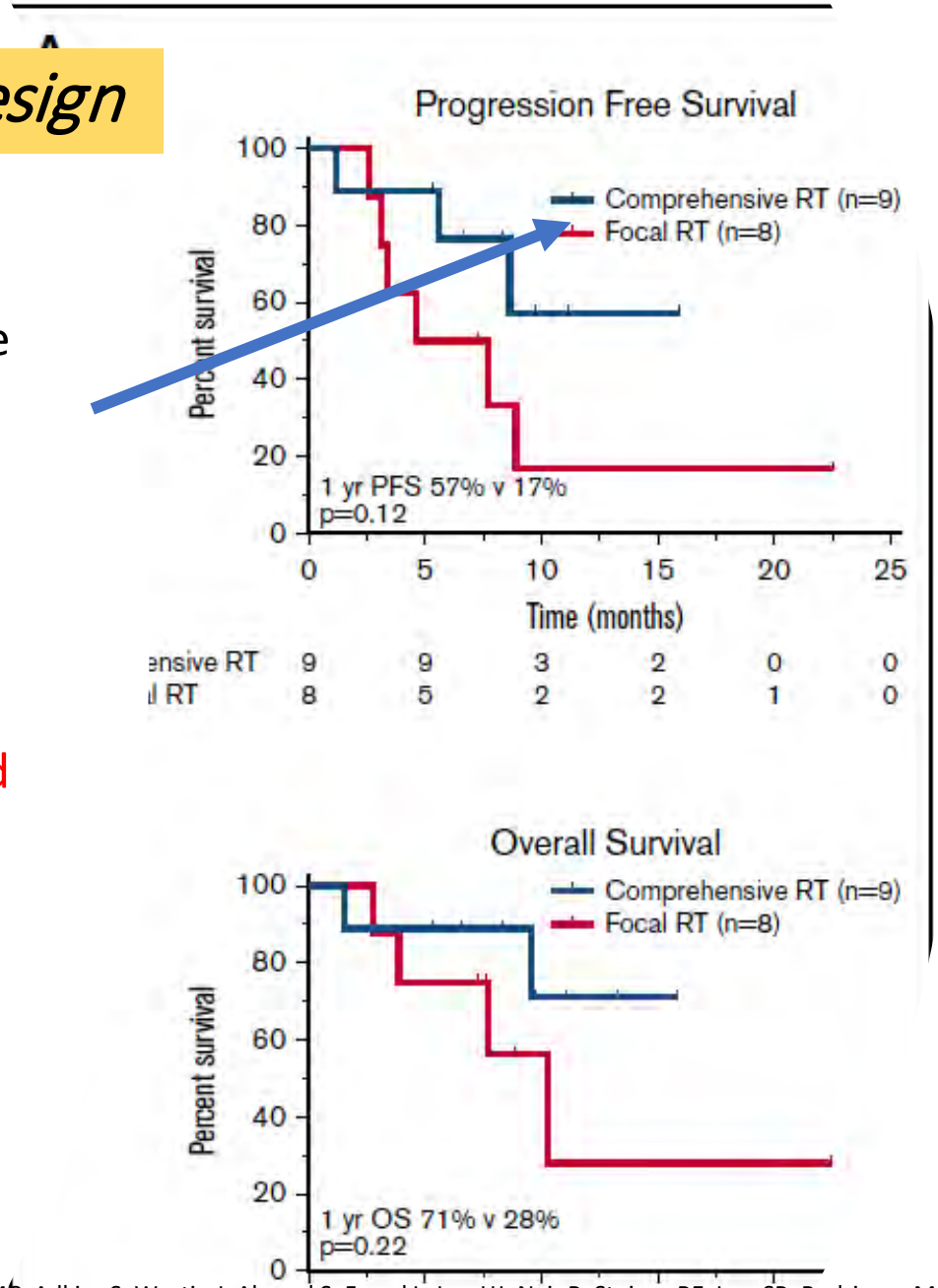
- Compared to patients that did not receive BT
  - 1-year PFS 44% for no BT vs 25% for ST cohort (p=0.01)
- Patients treated with CMT had a poor outcome
  - 1-year OS 25% and median OS of 3.9 months

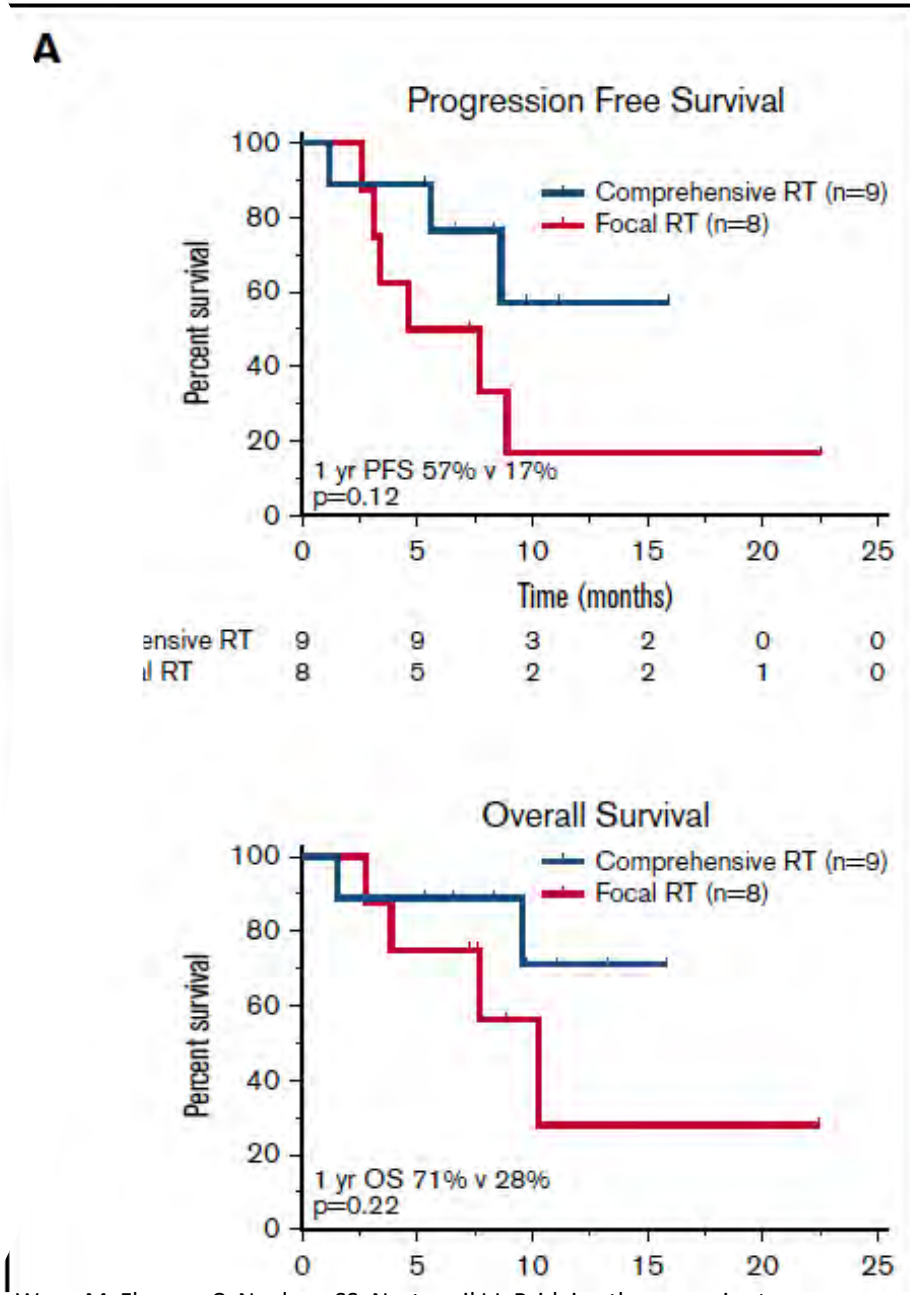
Pinnix CC, Gunther JR, Dabaja BS, Strati P, Fang P, Hawkins MC, Adkins S, Westin J, Ahmed S, Fayad L, Lee HJ, Nair R, Steiner RE, Iyer SP, Rodriguez MA, Wang M, Flowers C, Neelapu SS, Nastoupil LJ. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. Blood Adv. 2020 Jul 14;4(13):2871-2883; PMID: 32589728;

Impact of RT Field Design

- Nine patients comprehensive RT field (CMT, n=2; RT, n=7)

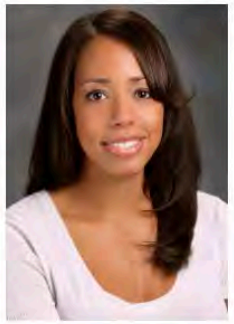
- 8 patients received focal RT fields (CMT, n=4; RT, n=4)
  - 6 progressed or relapsed
  - 3 at sites not included in the RT field





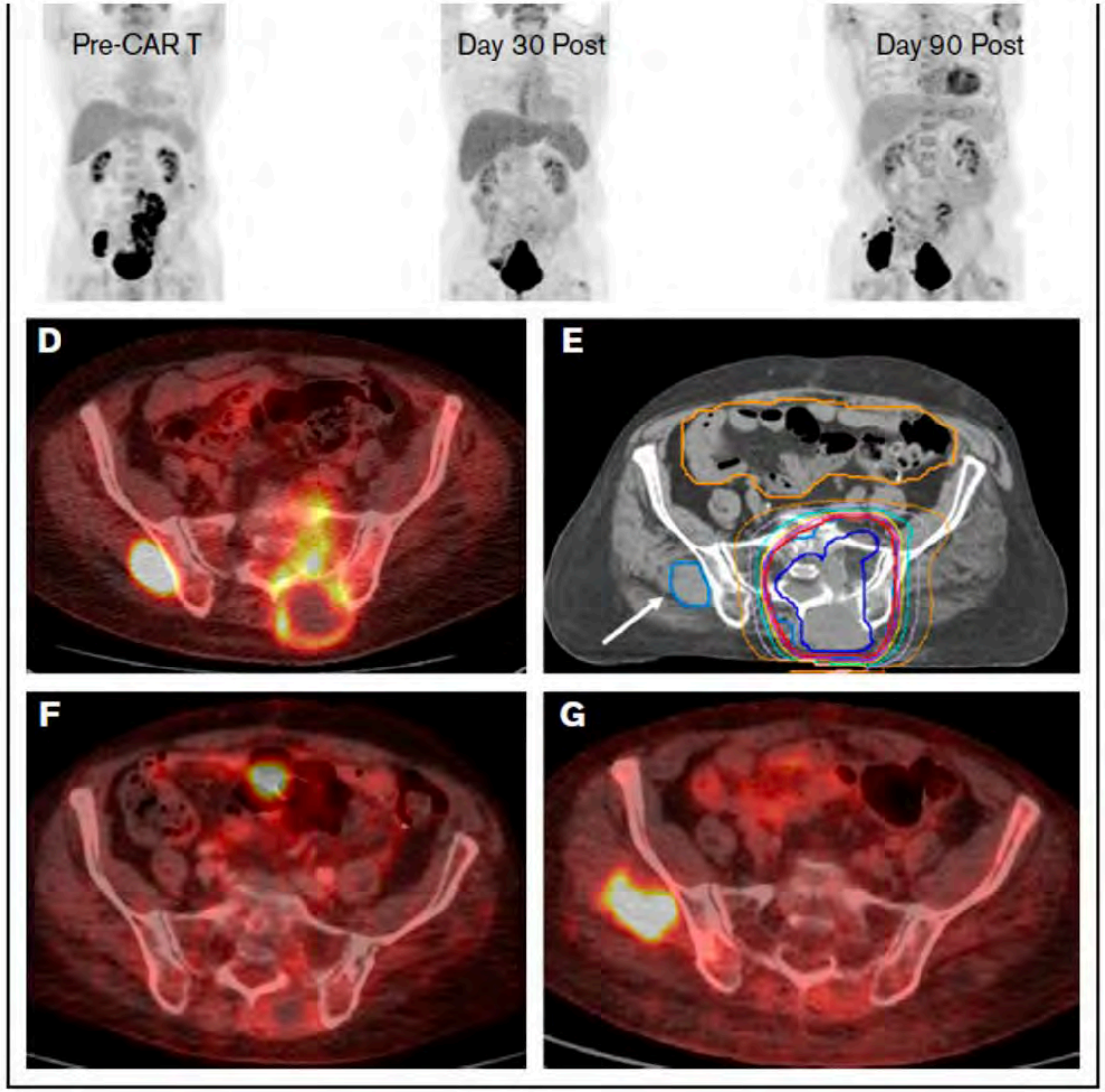
Pinnix CC, Gunther JR, Dabaja BS, Strati P, Fang P, Hawkins MC, Adkins S, Westin J, Ahmed S, Fayad L, Lee HJ, Nair R, Steiner RE, Iyer SP, Rodriguez MA, Wang M, Flowers C, Neelapu SS, Nastoupil LJ. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. Blood Adv. 2020 Jul 14;4(13):2871-2883; PMID: 32589728;

Importance of RT Field Size for Bridging Therapy?



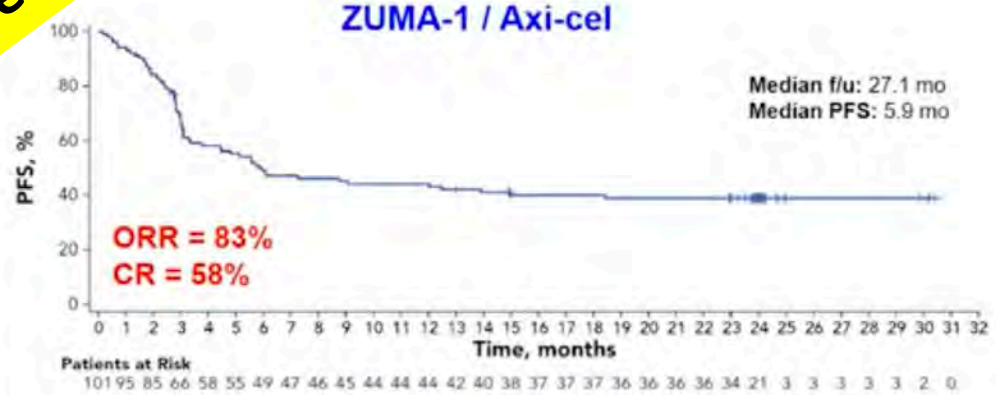
- Stage IV primary refractory HGBL-DH (MYC/BCL2) after DA R-EPOCH
- R-DHAP → progression
- P/w neurologic symptoms
- CAR-T with RT bridging

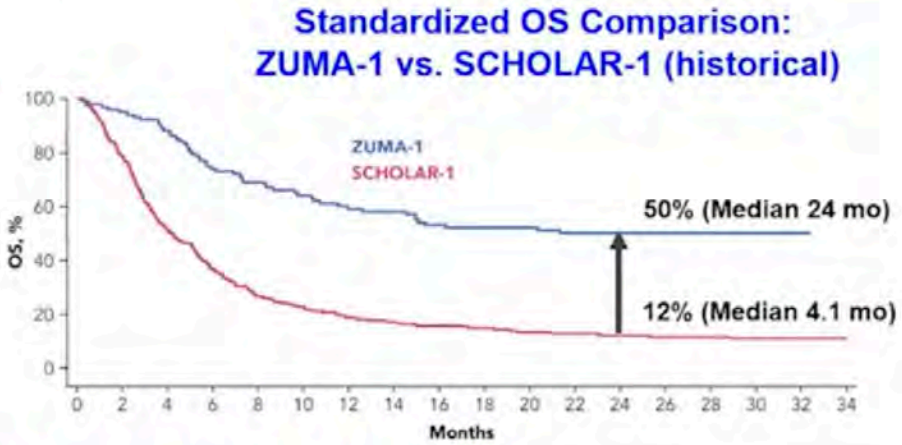
- Relapsed in un-irradiated site

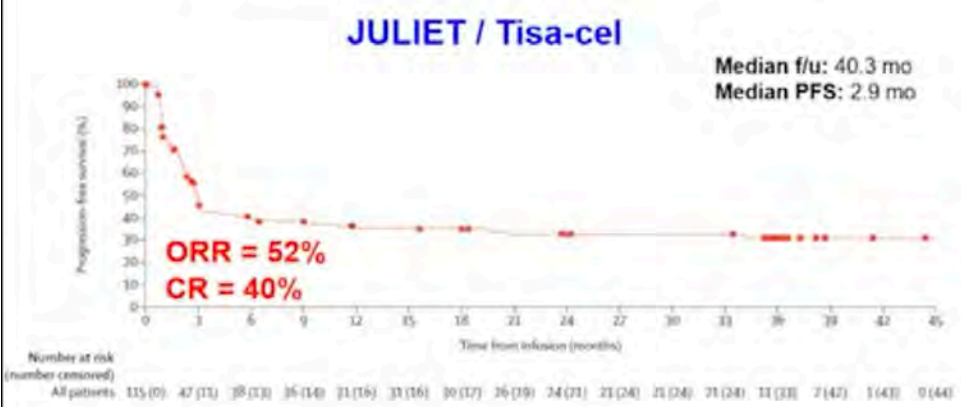


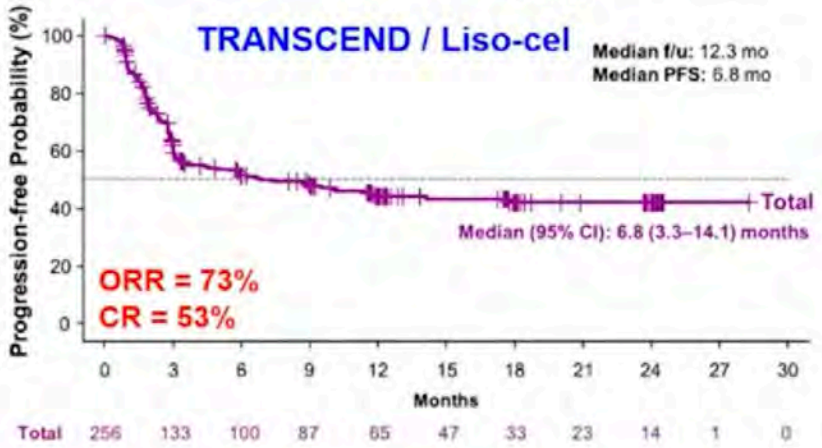
Avoiding further Chemotherapy

CD19 CART in ≥3rd line LBCL









Neelapu et al. *N Eng J Med* 2017; Locke et al. *Lancet Oncol* 2019
Schuster et al. *N Eng J Med* 2019; Schuster et al. *Lancet Oncol* 2021
Abramson et al. *Lancet* 2020; Neelapu et al. *Blood Adv* 2021

1. Better than standard of care
2. Those who get CR it is durable

Adopted from Dr. Sattva Neelapu

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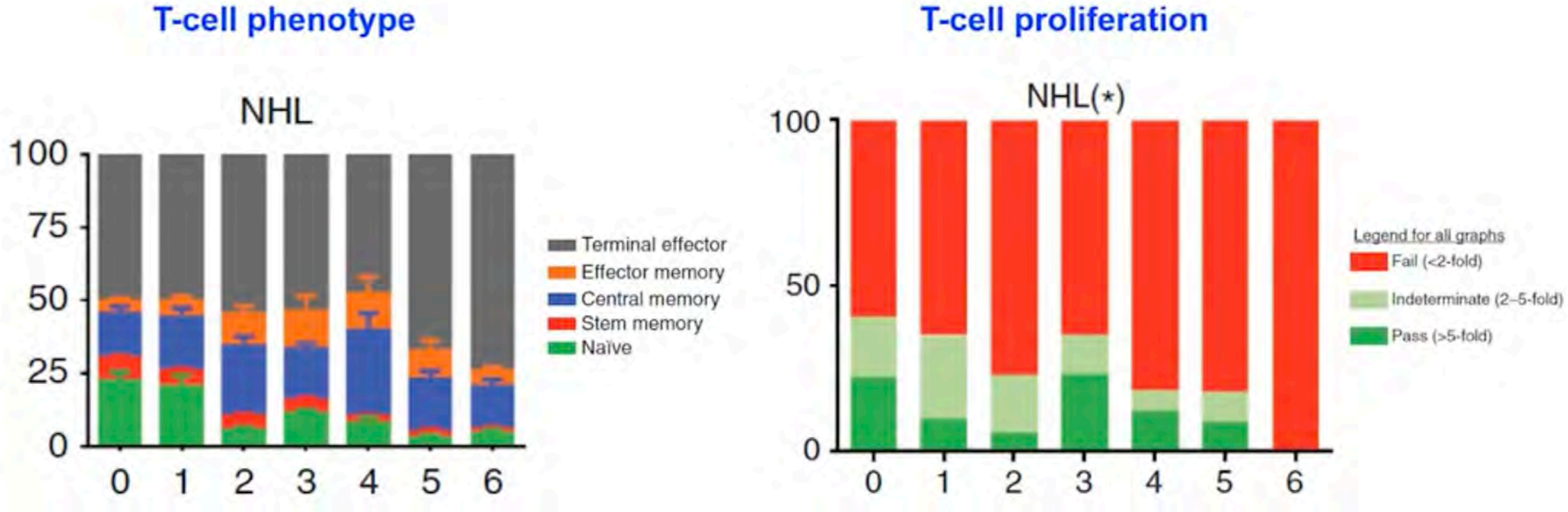
Avoiding Chemotherapy

Chemotherapy impairs immune cell phenotype and function



Naïve and terminal effector are impaired by chemotherapy

Das et al. *Cancer Discov* 2019; 9(4): 492-499

Adopted from Dr. Sattva Neelapu

ZUMA 7 moving CAR T to second line

Avoiding Chemotherapy

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Survival with Axicabtagene Ciloleucel in Large B-Cell Lymphoma

J.R. Westin, O.O. Oluwole, M.J. Kersten, D.B. Miklos, M.-A. Perales, A. Ghobadi, A.P. Rapoport, A. Sureda, C.A. Jacobson, U. Farooq, T. van Meerten, M. Ulrickson, M. Elsawy, L.A. Leslie, S. Chaganti, M. Dickinson, K. Dorritie, P.M. Reagan, J. McGuirk, K.W. Song, P.A. Riedell, M.C. Minnema, Y. Yang, S. Vardhanabhuti, S. Filosto, P. Cheng, S.A. Shahani, M. Schupp, C. To, and F.L. Locke, for the ZUMA-7 Investigators and Kite Members*

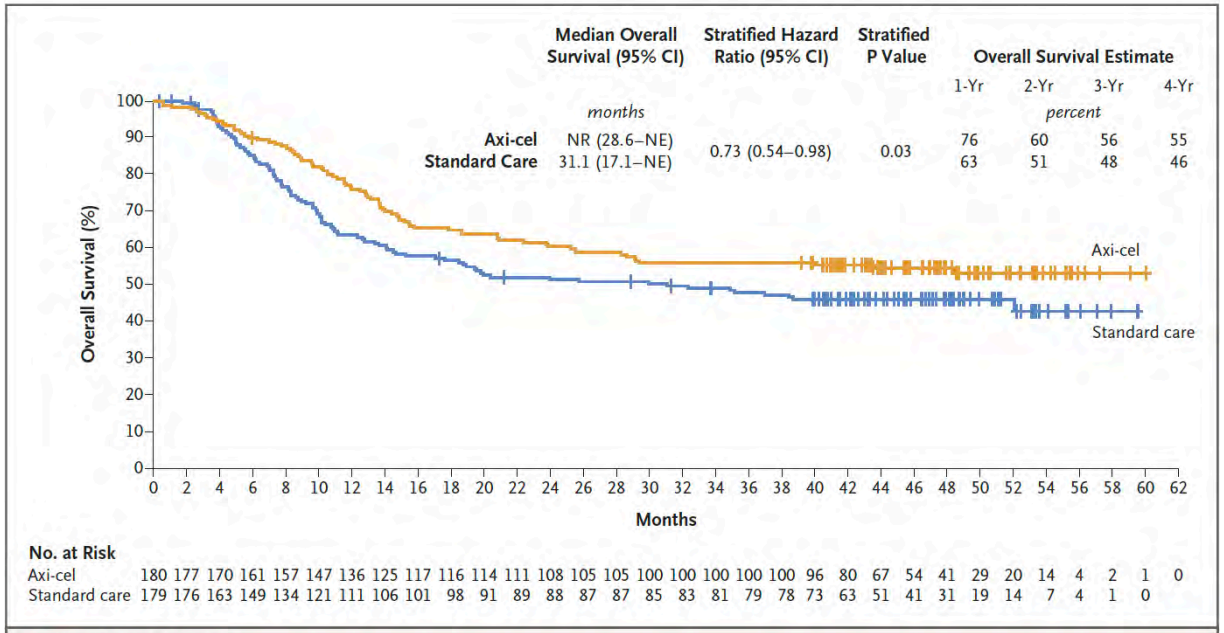
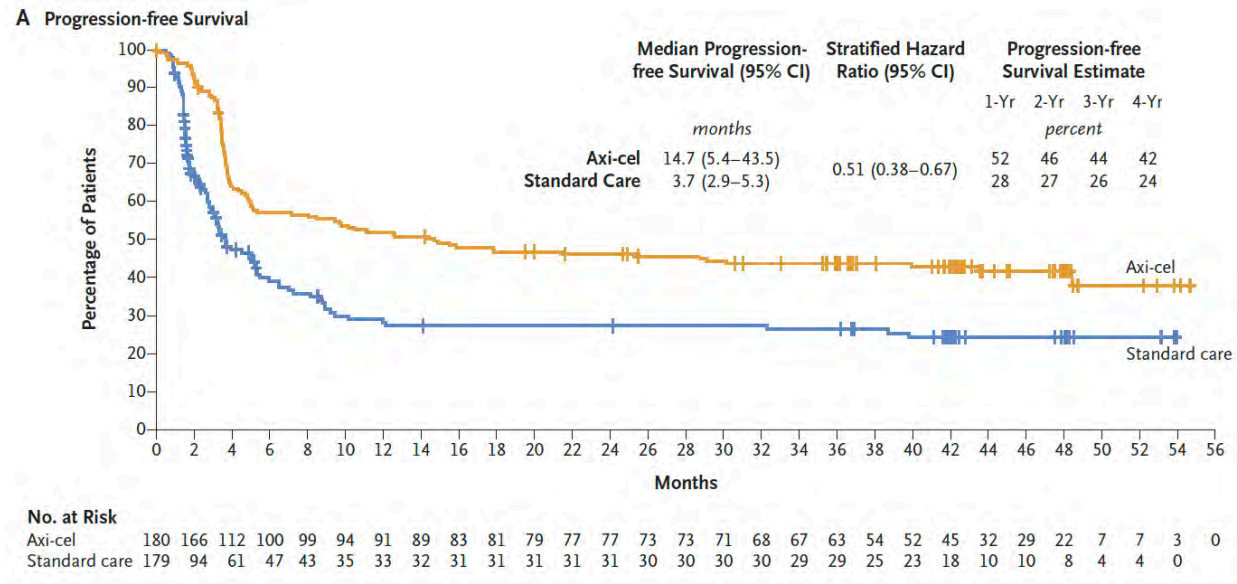


Figure 1. Overall Survival.



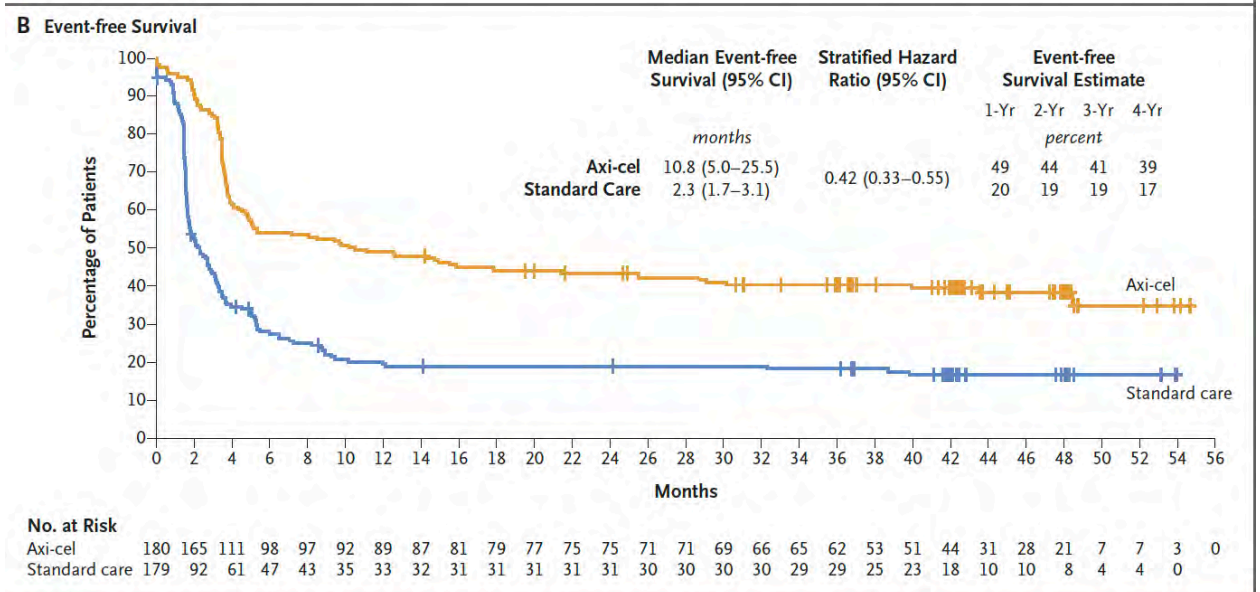
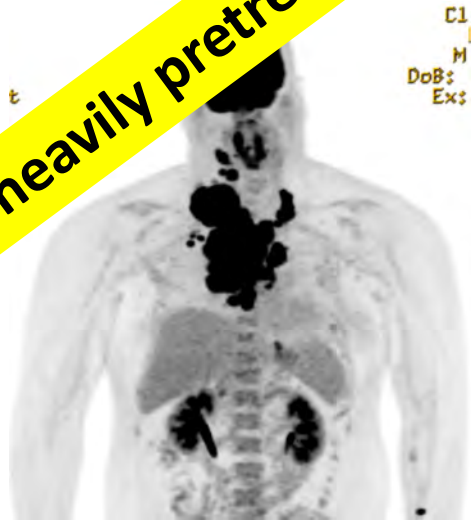
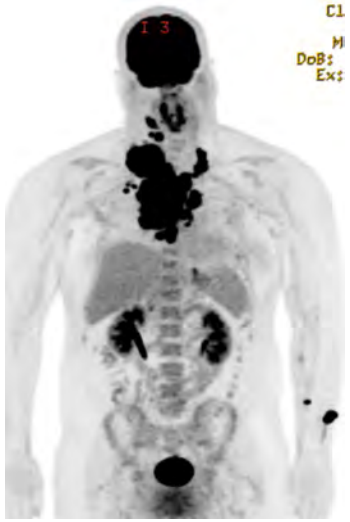


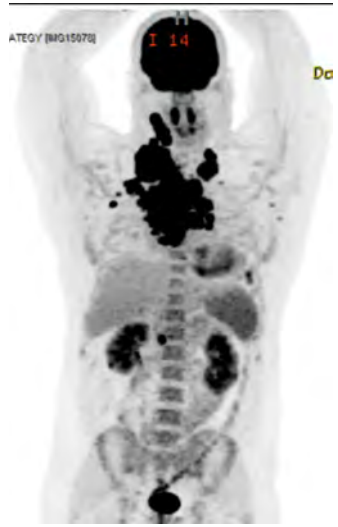
Figure 2. Progression-free Survival and Event-free Survival, as Assessed by the Investigator.

Primary Mediastinal Lymphoma

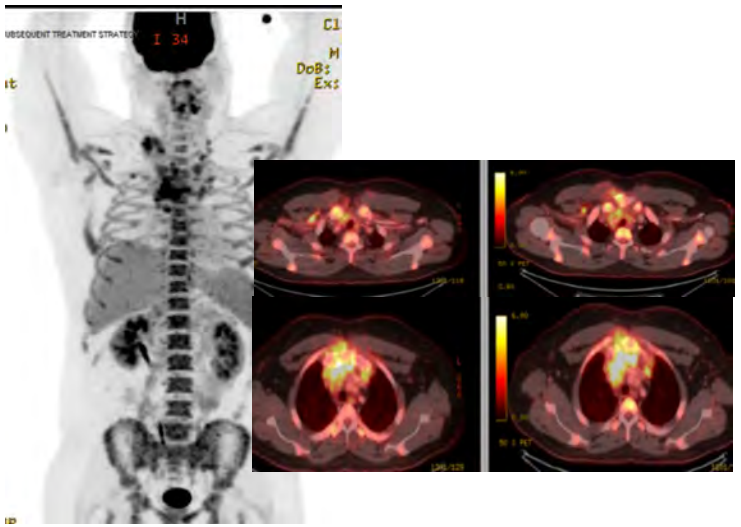
Example of heavily pretreated

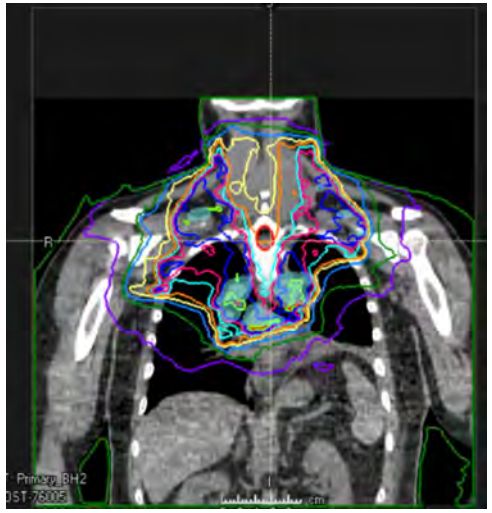






BV-Nivo on 2020-0686 PACIFIC → Progression → DA-REPOCH x 4





Relapse ----- Hypercytoxan → Radiation to 30 Gy → Post CAR T 10 months in CR

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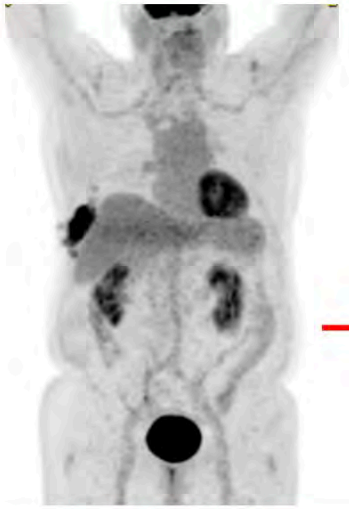
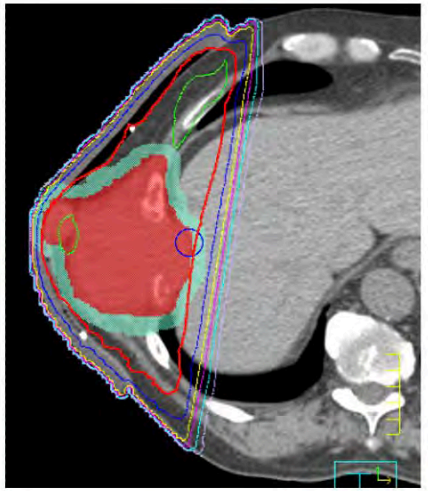
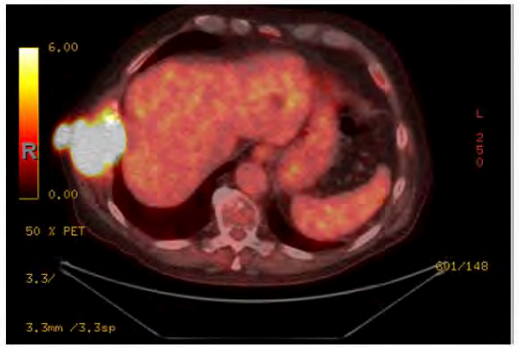
Radiation a better Alternative to further chemotherapy

Refractory after 6 RCHOP R-DHAP x 1 → initial response but rapid progression

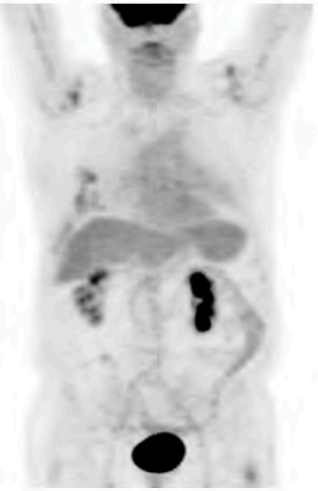
Pre-RT/Pre-CART

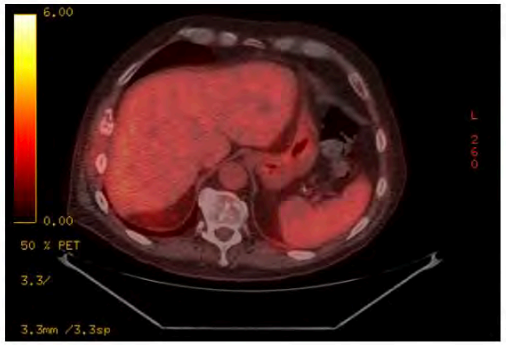






Post 30-day PET-CT: CR with 5PS of 3





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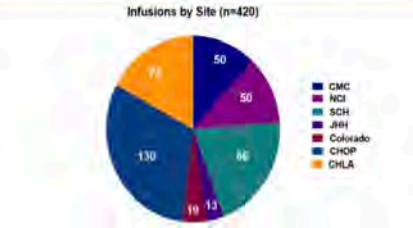
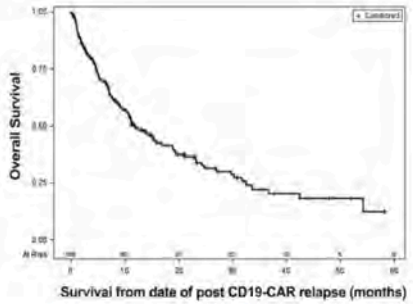
Predicting relapse post CAR T
An opportunity to improve the outcome

Outcomes for post-CD19 CAR Relapse are Poor

- Retrospective, multicenter study of 420 children and young adults receiving CD19 CAR T-cells
  - 166 (39.5%) with relapse
- Median overall survival (OS):
  - 11.9 months (95% CI: 9.0-17.9 mo)
- 12 month OS: 49.4%
- Salvage options, limited
  - Particularly for CD19^{neg} relapse

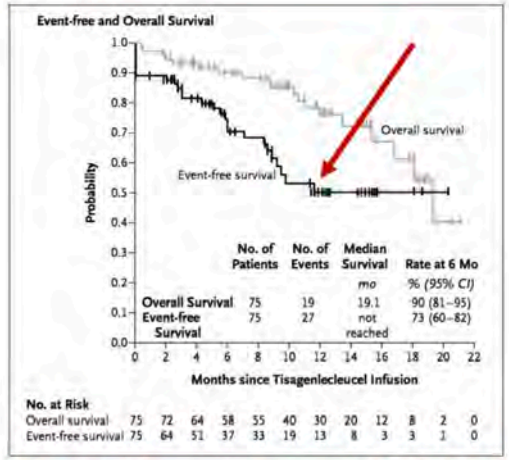




Lamble A/Shah NN, Blood Adv, 2023

CD19 CAR T-cells for Long Term Cure

- Need for CAR T-cell persistence and subsequent relapse immunophenotypes differ between B-ALL and mature B-cell lymphomas
- Treatment paradigm for relapsed/refractory B-ALL (pre-CD19 CAR T-cell era):
  - Achieve remission→ AlloHSCT
- A fraction of patients will be able to achieve long-term cure with CD19 CAR T-cells alone
  - Avoiding additional short and long-term toxicities from additional chemotherapy and HSCT



Can we improve upon 50% EFS?

Maude SL, et al. NEJM 2018

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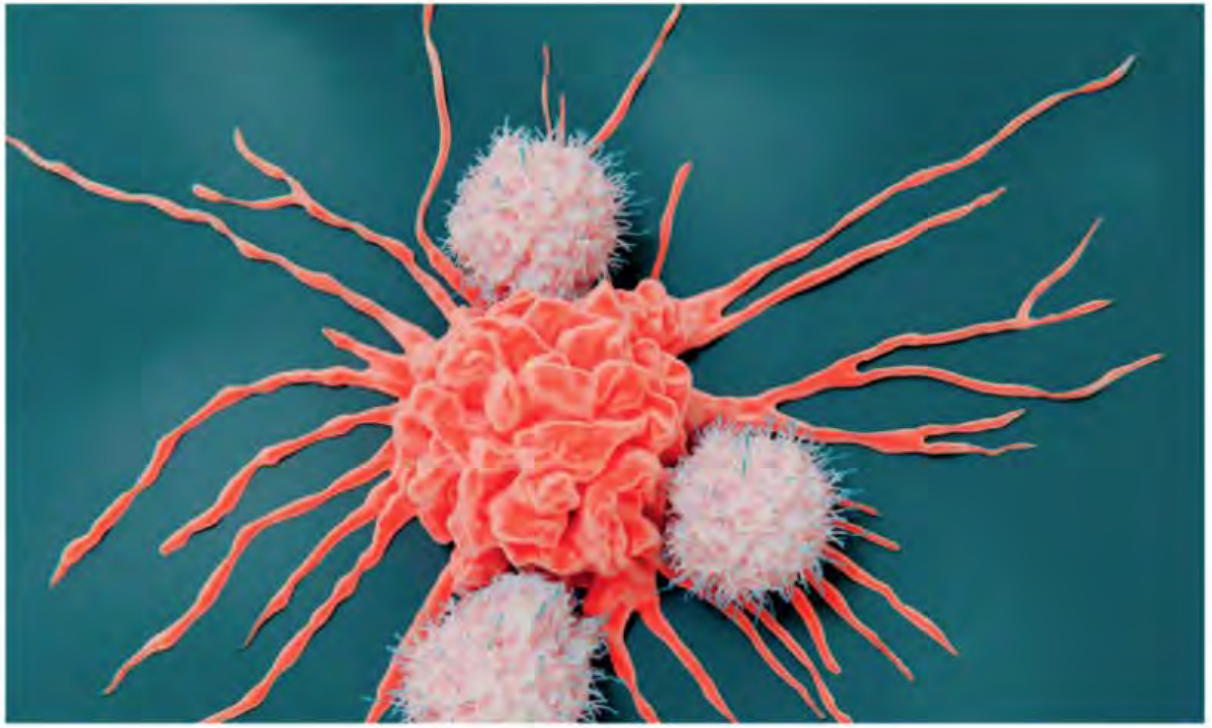
Industry appetite for natural killer cells intensifies

Natural killer cells are attractive as cancer immunotherapy agents because – unlike T cells – they evade immune rejection and do not induce cytokine storms. But capturing their activity in effective therapies remains a work in progress.

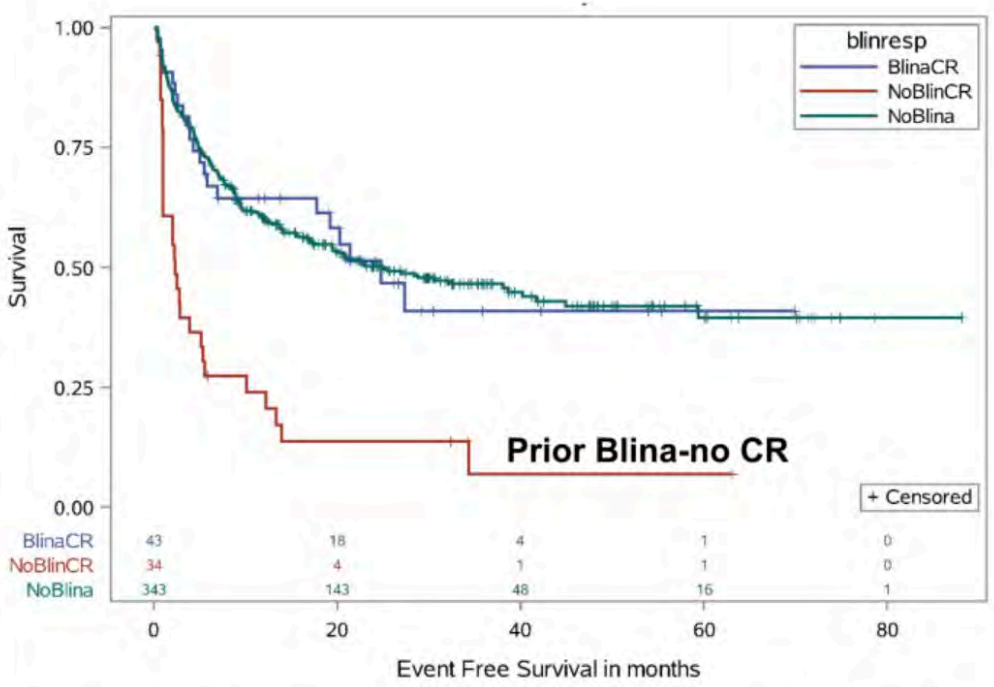


By Cormac Sheridan

Interest in natural killer (NK) cells has escalated as large players drum up collaborations to bring NK-driven programs and tools into their portfolios. In December, Sanofi deepened its commitment to antibody-based NK cell engagers, expanding an ongoing partnership with Marseille-based Innate Pharma. A few months earlier, Sanofi

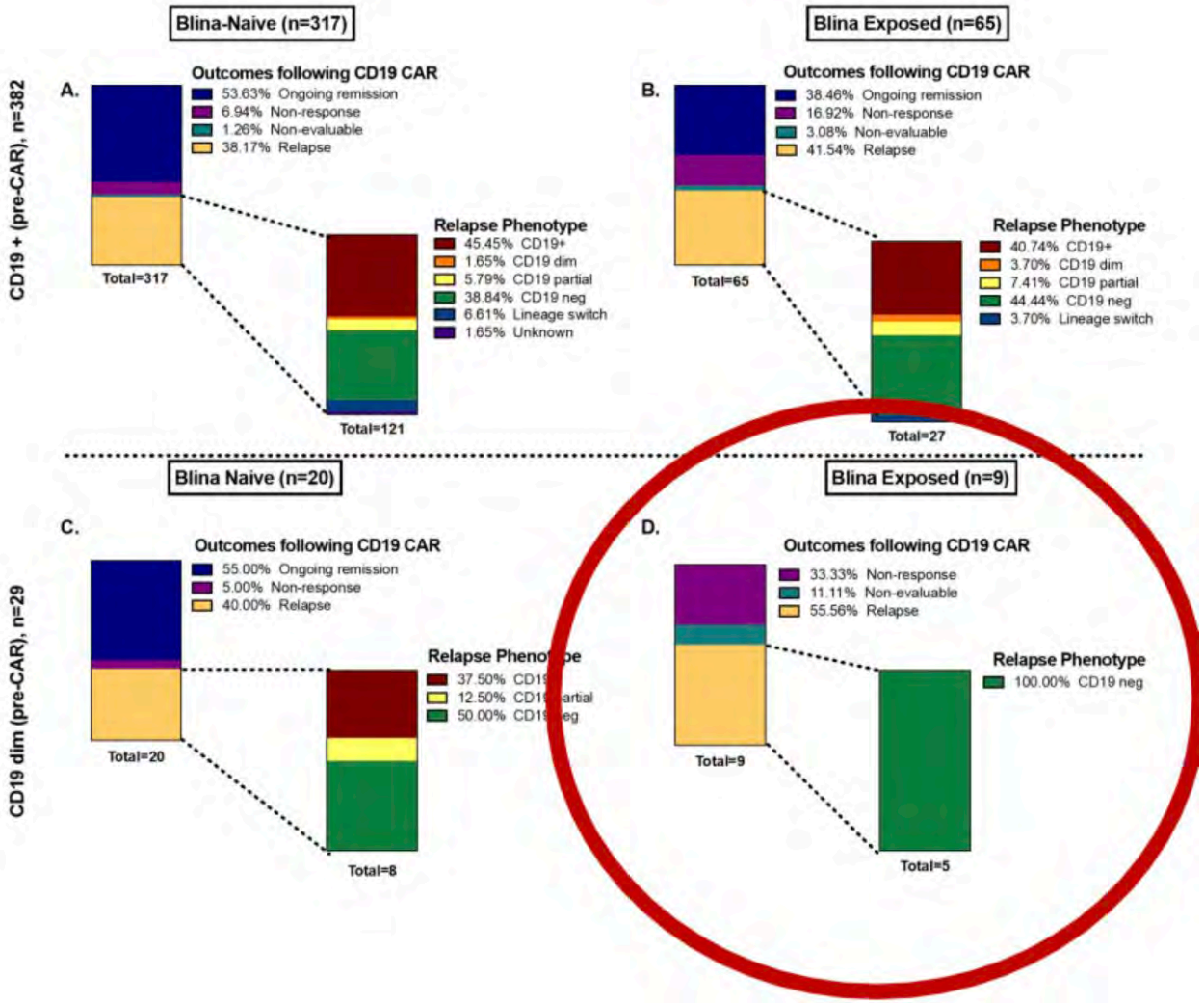
Predicting relapse post CAR T
An opportunity to improve the outcome

Pre CAR T-cell Targeted Therapy May Influence Relapse Phenotype



Development of CD19dim disease pre-CAR may influence post CAR T-cell relapse phenotype.

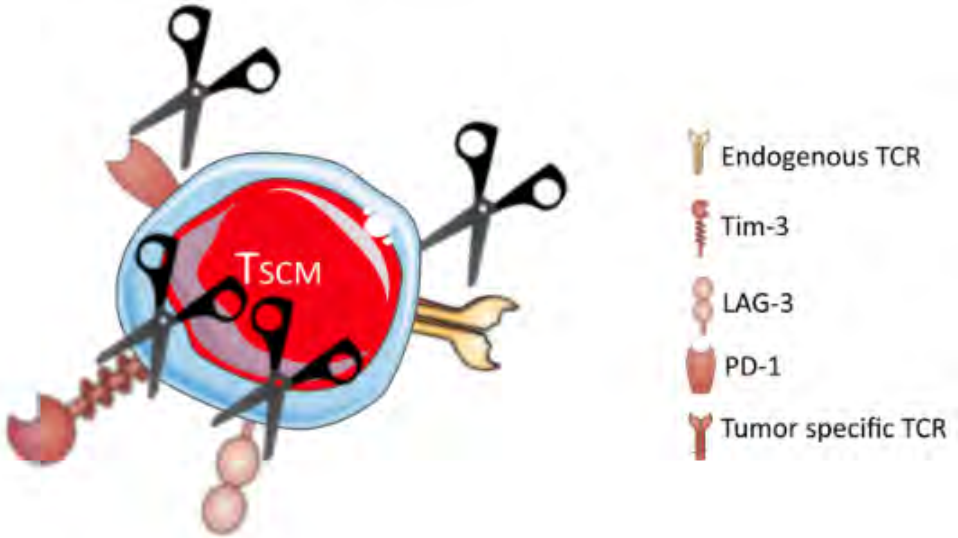
FURTHER STUDY is needed



Myers R/Shah NN, JCO, 2021

Adoptive T-cell therapy for cancer: Overcoming T cell exhaustion with genome editing

To **design** and **develop** a panel of **CRISPR/Cas9** molecules targeting genes encoding **inhibitory receptors** in order to enhance the anti-tumor activity of **TCR-edited memory stem T cells**



How do we pivot our research to account for the immune conversation?

When is the best time to introduce radiation

Never before pheresis? And why

After pheresis and before infusion?

How about after infusion?

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Radiation A Priming Mechanism Prior to Adoptive Cell Therapy in CNS And Systemic Acute Leukemia

Specific Aim 2: Identify changes in circulating T-cell phenotypes and functions before and after CSI or TBI as conditioning for CAR-T cell therapy, and after CAR T

Specific Aim 1: Identify changes in circulating inflammatory and immunosuppressive cytokines before at specified time points in both blood and CSF (for those with CNS disease) (before radiation, after radiation and before CAR T, day +7 and +14 of CAR T)

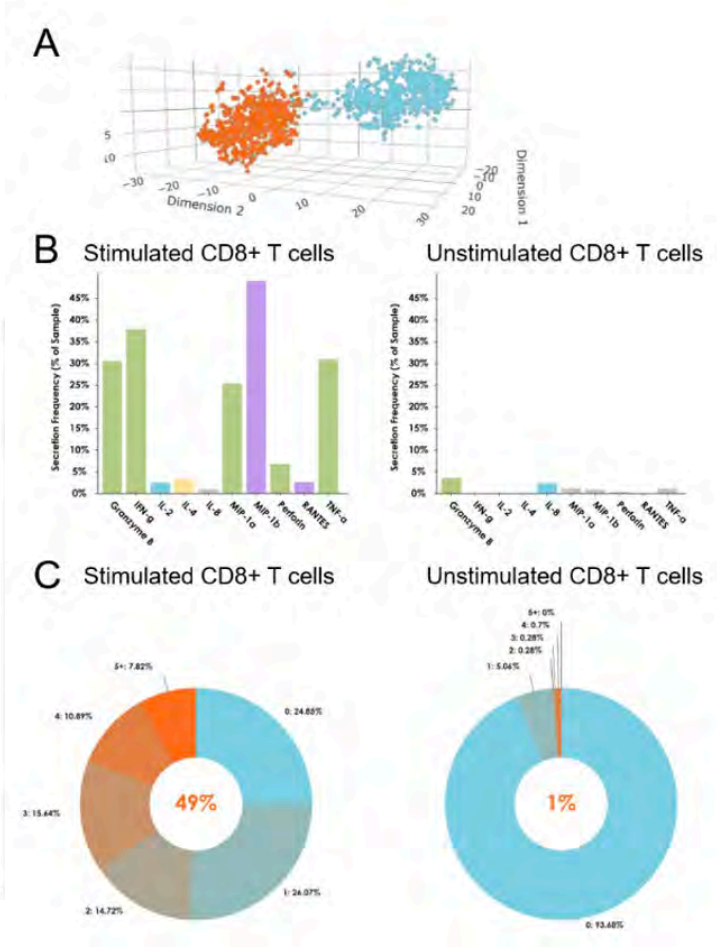


Figure. Multiplexed immune phenotypic analysis of peripheral blood CD8⁺ T cells. CD8⁺ T cells were selected from human peripheral blood mononuclear cells and assayed using the Isolyte adaptive immune response panel. **(A)** tSNE plot between unstimulated (orange) and stimulated (blue) CD8⁺ T cells shows distinct phenotypes. **(B)** Cytokines upregulated in stimulated CD8⁺ T cells (left panel) compared to unstimulated CD8⁺ T cells (right panel). **(C)** Stimulation

Phase II Study of CAR-T with Bridging RT in Relapsed/Refractory Indolent Non-Hodgkin Lymphoma

Susan Wu

Screen/consent relapsed/refractory iNHL

Near comprehensive RT* (4Gy)

Bridging steroids at MD discretion

Apheresis → Axi-cel Manufacture → Flu/Cy conditioning → CART Infusion Day 0

Blood

Pre RT
T-cell flow

Pre-LD chemo
T-cell flow

D0, 2, 4, 7, 14
Cytokine 3 panel (SOC)

D0, 7, 14, 30
T-cell flow

Biopsy

Pre RT biopsy
scRNASeq +scTcRseq
+ scBCRseq +
CosMx

Additional biomarker collection at
3, 6, 9, 12, 18, 24 months

Relapse biopsy*
scRNASeq + scTcRseq
scBCRseq
CosMx

Other

Apheresis product
scRNASeq +scTcRseq

Infusion Product
ssRNASeq +TcRSeq

*If sample is clinically indicated

Primary Endpoint:
- AE: Grade 3+ CRS

Secondary Endpoints:
- ORR, CRR, DOR, PFS, MRD
- In-RT field disease control
- Additional adverse events (G1+, G3+)
- Cytokine profile
- T-cell expansion, persistence, immunophenotype

Phase II Study of Salvage Radiation Treatment (RT) after B-cell Maturation Antigen (BCMA) Chimeric Antigen Receptor T-cell Therapy (CAR-T) for Relapsed Refractory Multiple Myeloma

Penny Fang

CART Infusion → Day 20+ PET or other imaging → Inclusion Criteria:
- R/R multiple myeloma
- Active disease on day 20+ PET or other imaging scan after SOC CAR-T infusion

→ Informed Consent → Radiation Treatment

Study Endpoints:
- Overall response rate
- Duration of response among responders
- Progression free survival
- Safety
- Complete response rate
- Overall survival
- Local control within RT field

Biomarker Collection:
- Optional/Archived biopsy tissue
- Blood collection
- Optional stool collection

Before RT → RT → Last week of RT → ~ 2 weeks post-RT → Disease Progression

Umbrella Protocol: 2020-1150 Outcomes after Chimeric Antigen Receptor Therapy (CAR-T) and Radiation Therapy (RT) for Hematologic Malignancies Primary Objective:

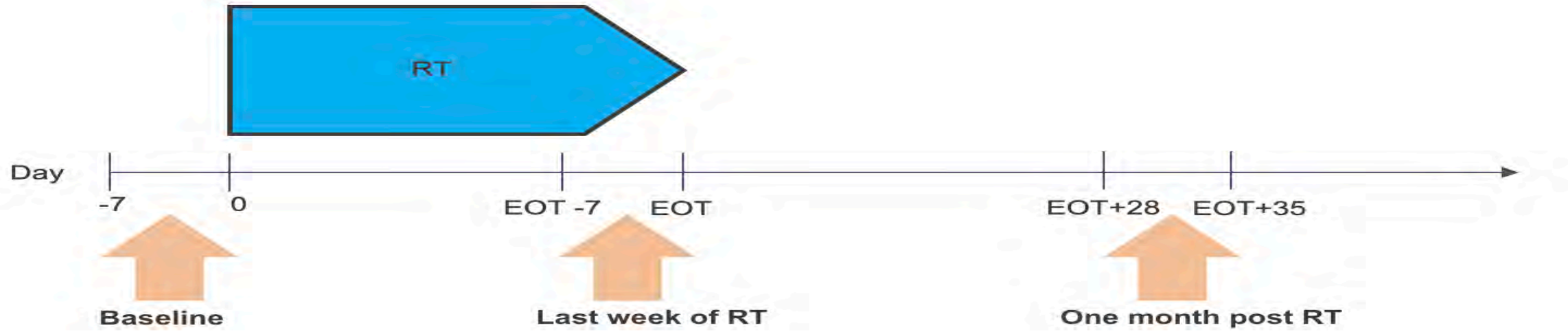
- Study clinical outcomes of patients with hematologic malignancies receiving standard-of-care chimeric antigen receptor therapy (CAR-T) and radiation therapy (RT)

Secondary Objectives:

1. To study patient-specific factors and treatment-related factors and outcomes

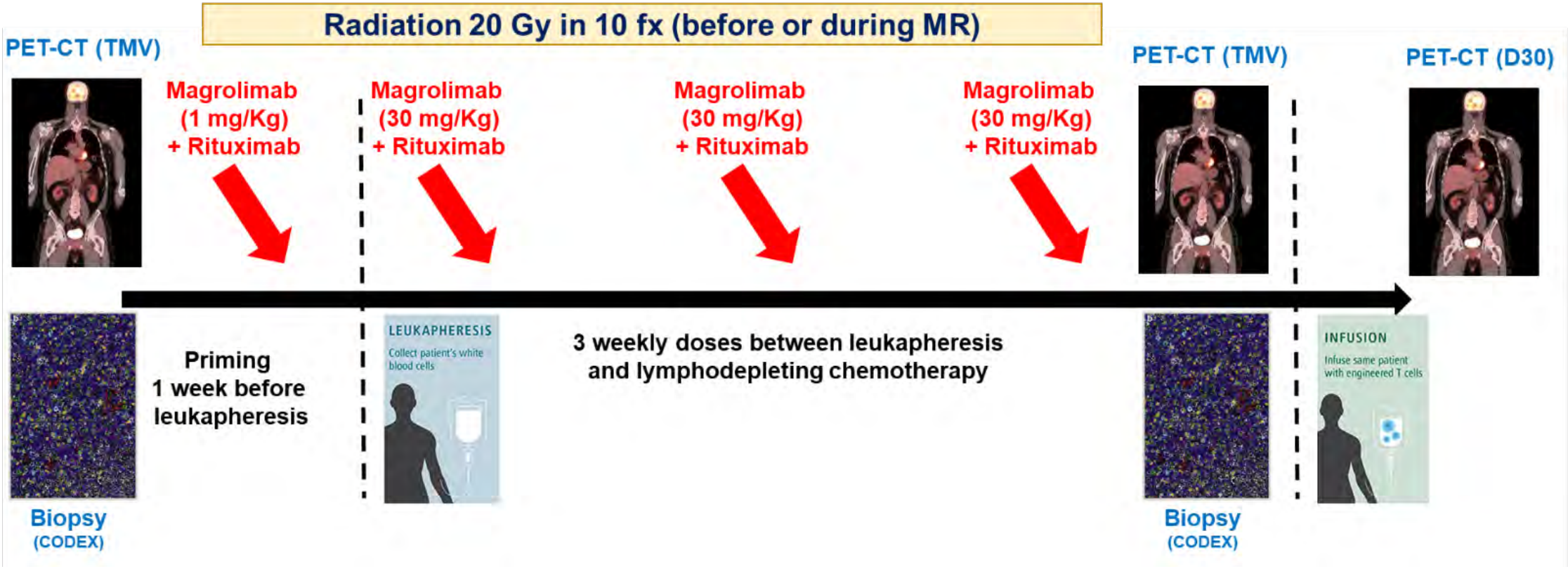
2. To study the relationship between radiation dose, target, technique, and timing with respect to CAR-T and clinical outcomes in patients

3. To study the relationship between patient-specific factors and treatment-related factors and treatment toxicity



A Phase II Study of Magrolimab, Rituximab and Radiation as Bridging Strategy Before CAR T-Cell Therapy in Patients with Relapsed or Refractory Large B-cell Lymphoma

Penny Fang



Radiation and Immunological changes in Chloroma (Extramedullary AML)

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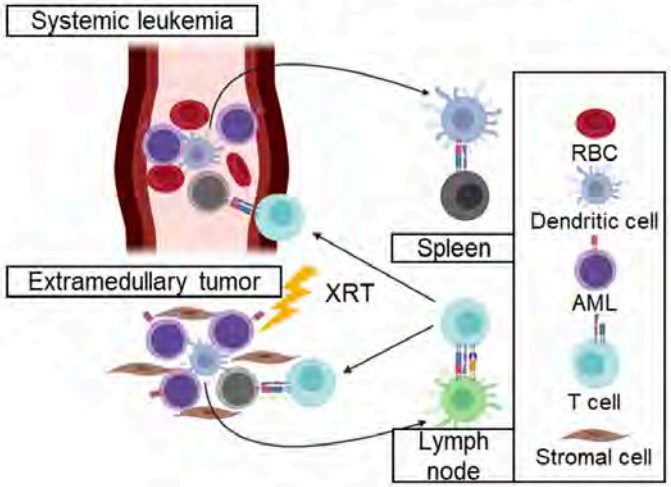
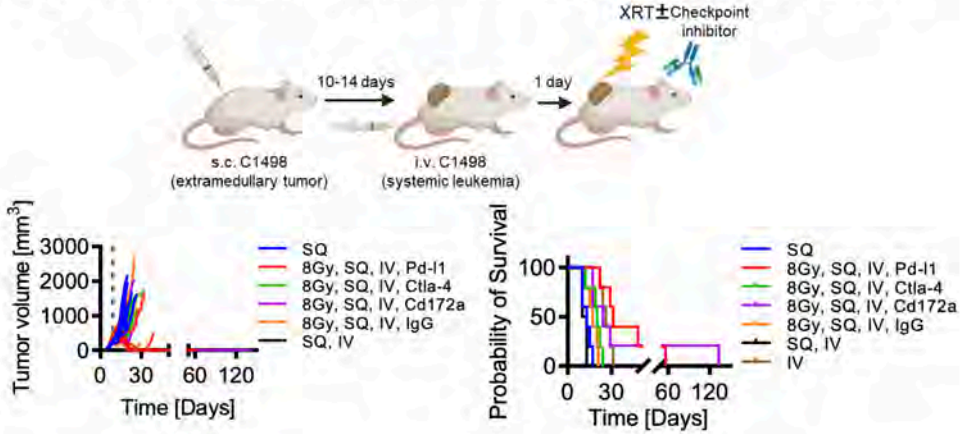
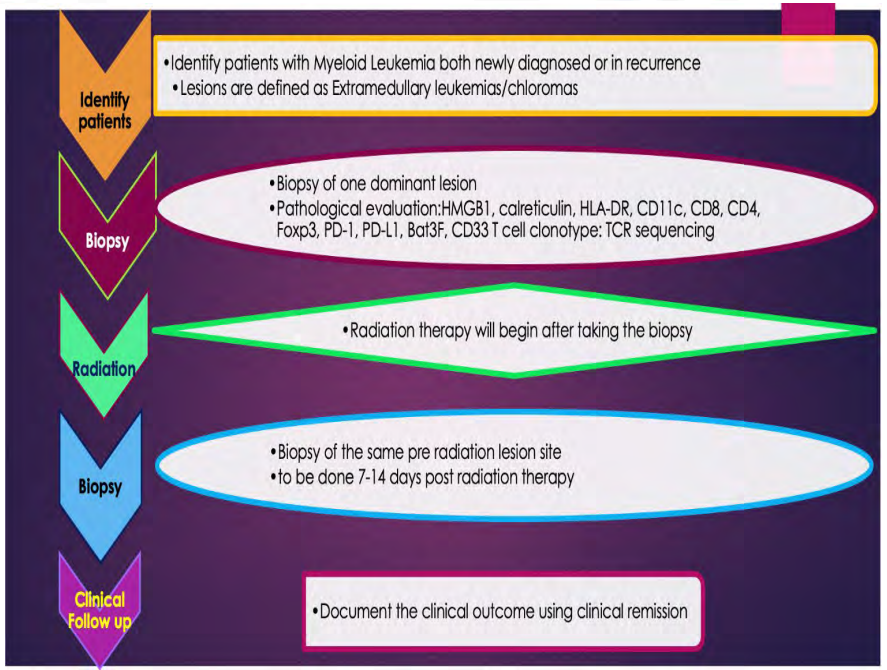
Immune differences between extramedullary and systemic leukemias



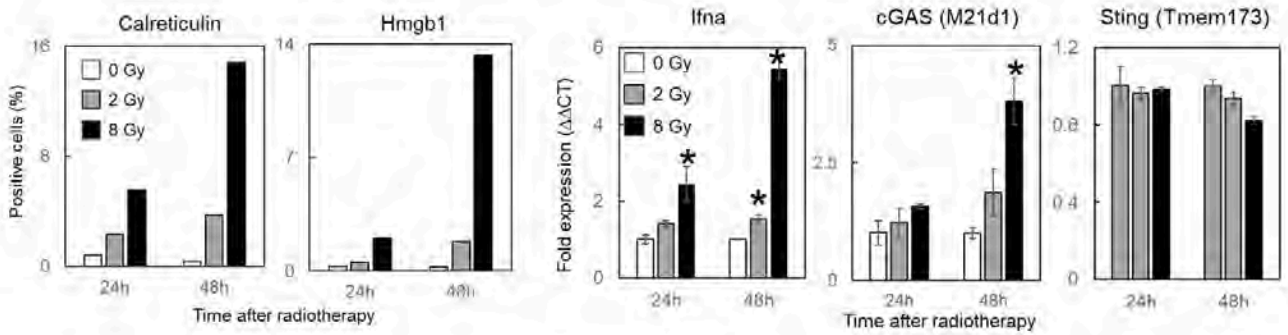
Radiotherapy with anti-PD-1 or anti-CD47 potentially improves survival in mice with systemic leukemias



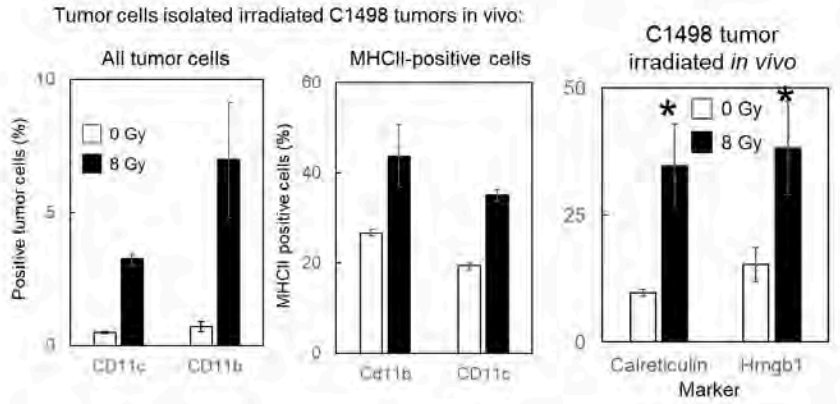


Radiation and Immunological changes in Chloroma (Extramedullary AML)

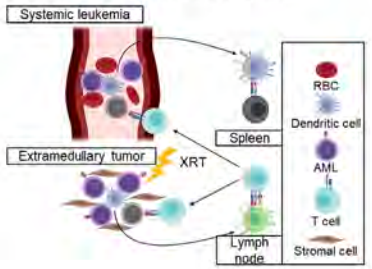
Radiotherapy increases DAMP and cGAS-STING-IFN pathway expression *in vitro*

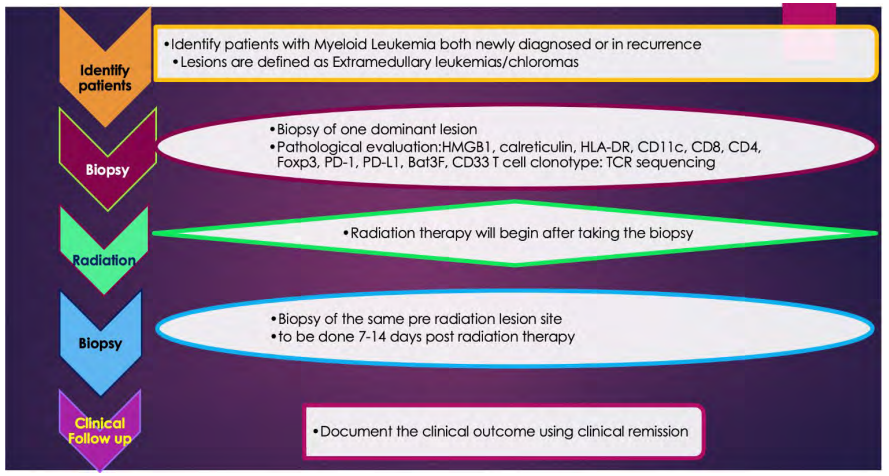


Radiotherapy increases macrophage infiltration and DAMP expression *in vivo*



Immune differences between extramedullary and systemic leukemias





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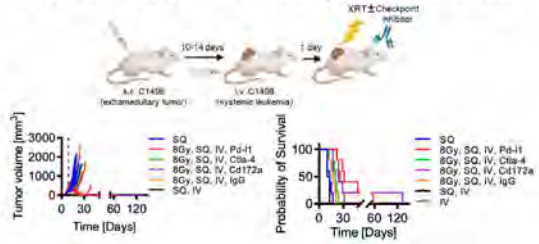


We have a lot of work to do

The answer is through Translational Studies

Mechanism of Radiation priming

Correct timing to introduce Radiation

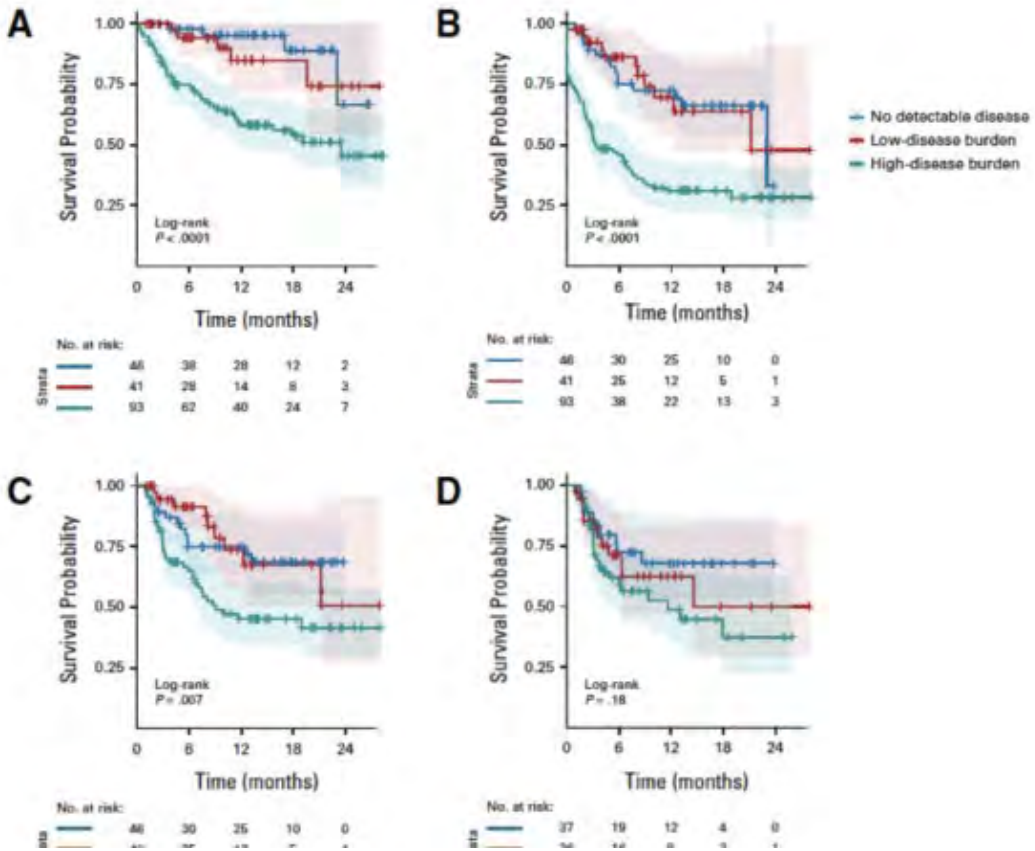
How does Radiation affect the CAR T cell therapy persistence fitness

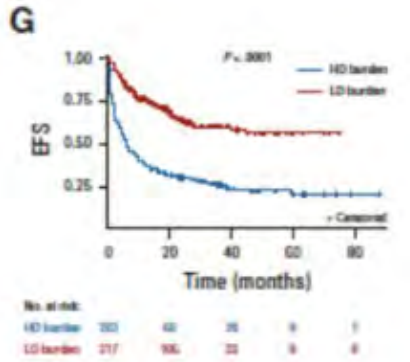
How does it affect the outcome

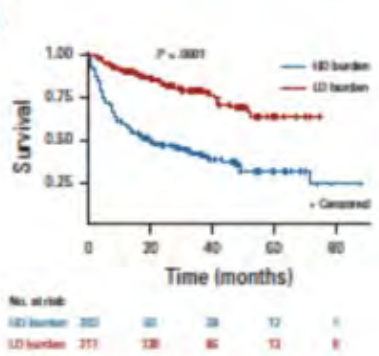
How to use the aforementioned as a steppingstone towards bi-specifics

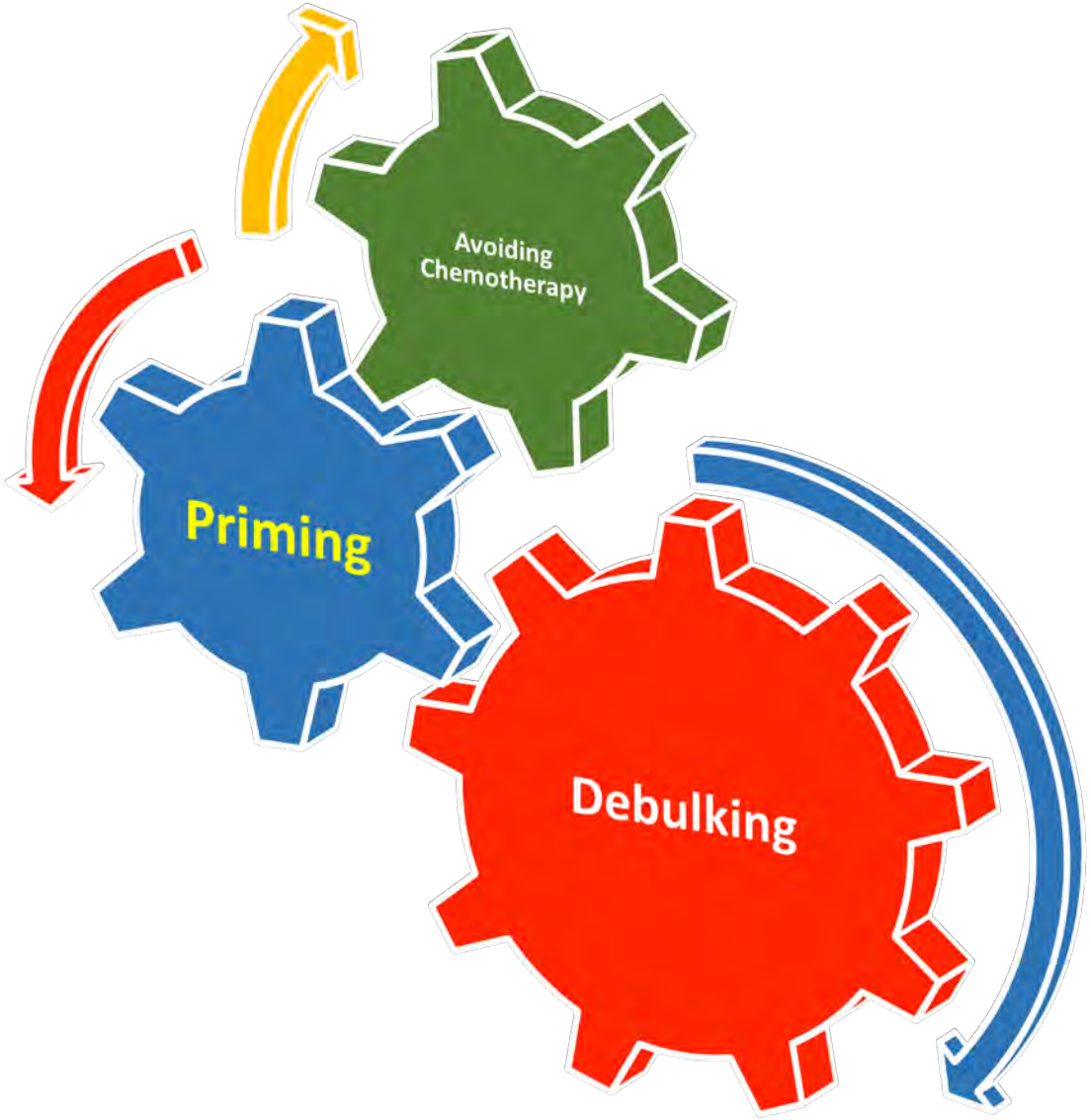
Debulking

Disease Burden Impacts Response and Toxicity

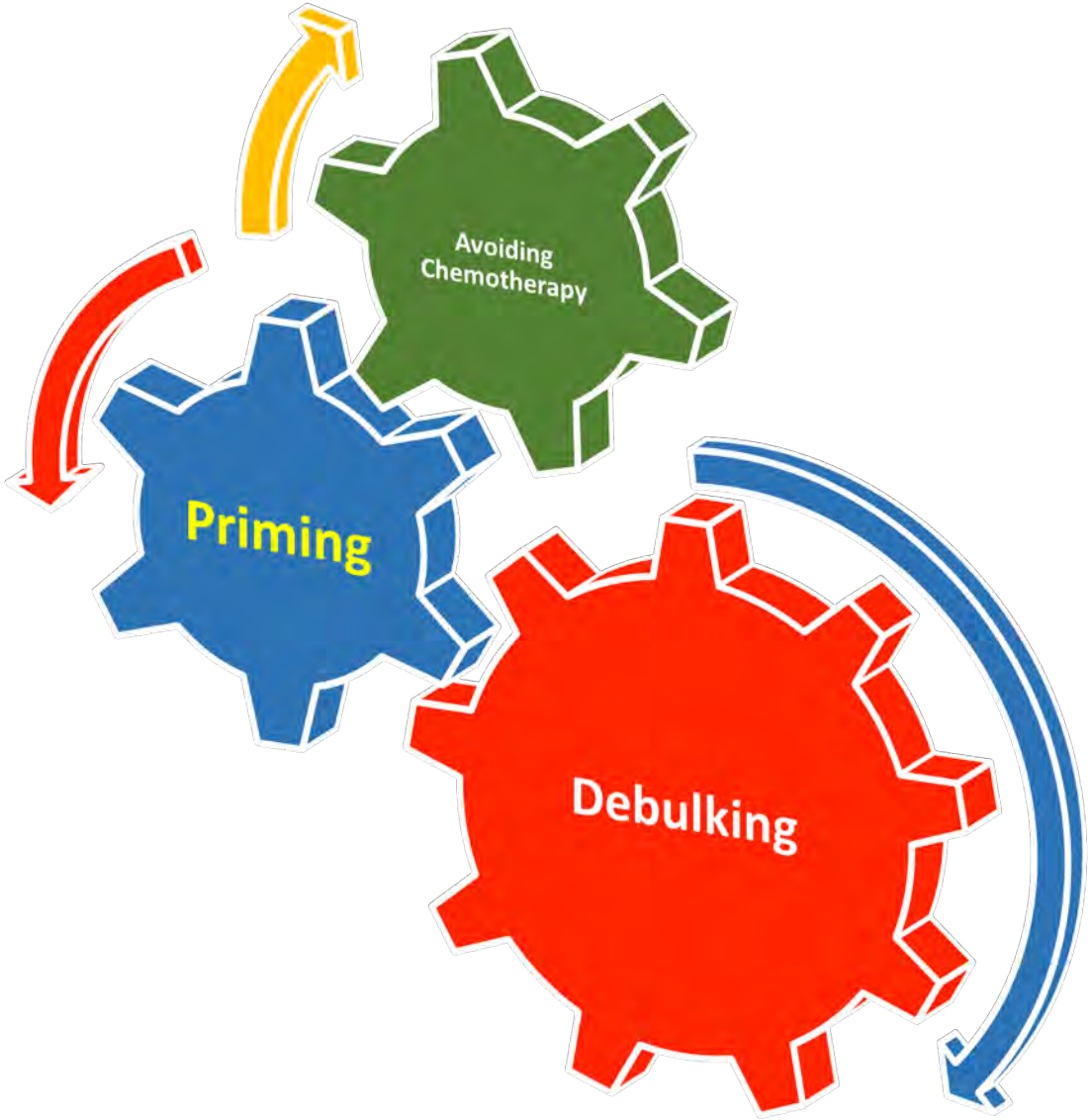






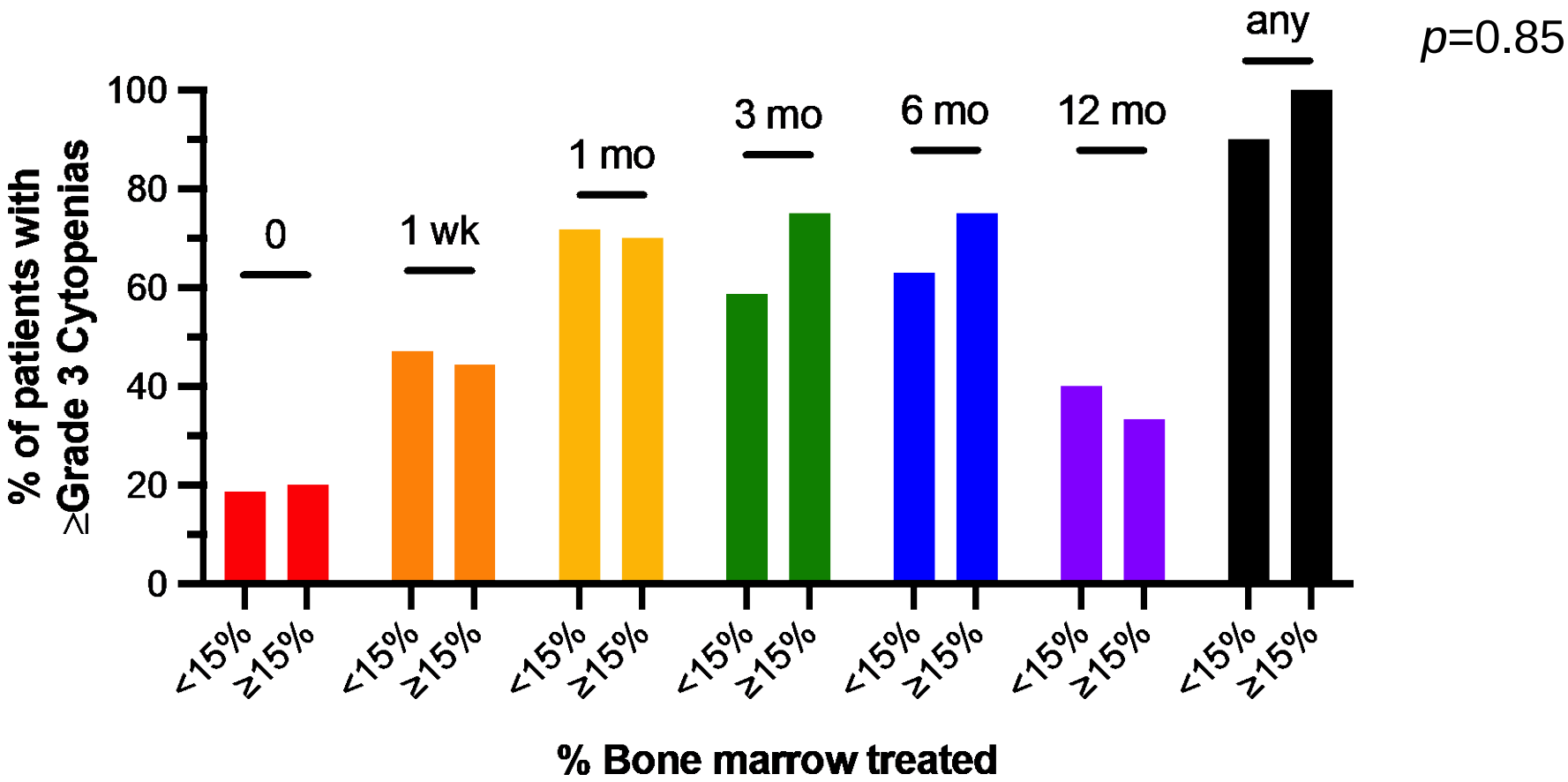

Avoiding
Chemotherapy
Priming
Debulking

Priming



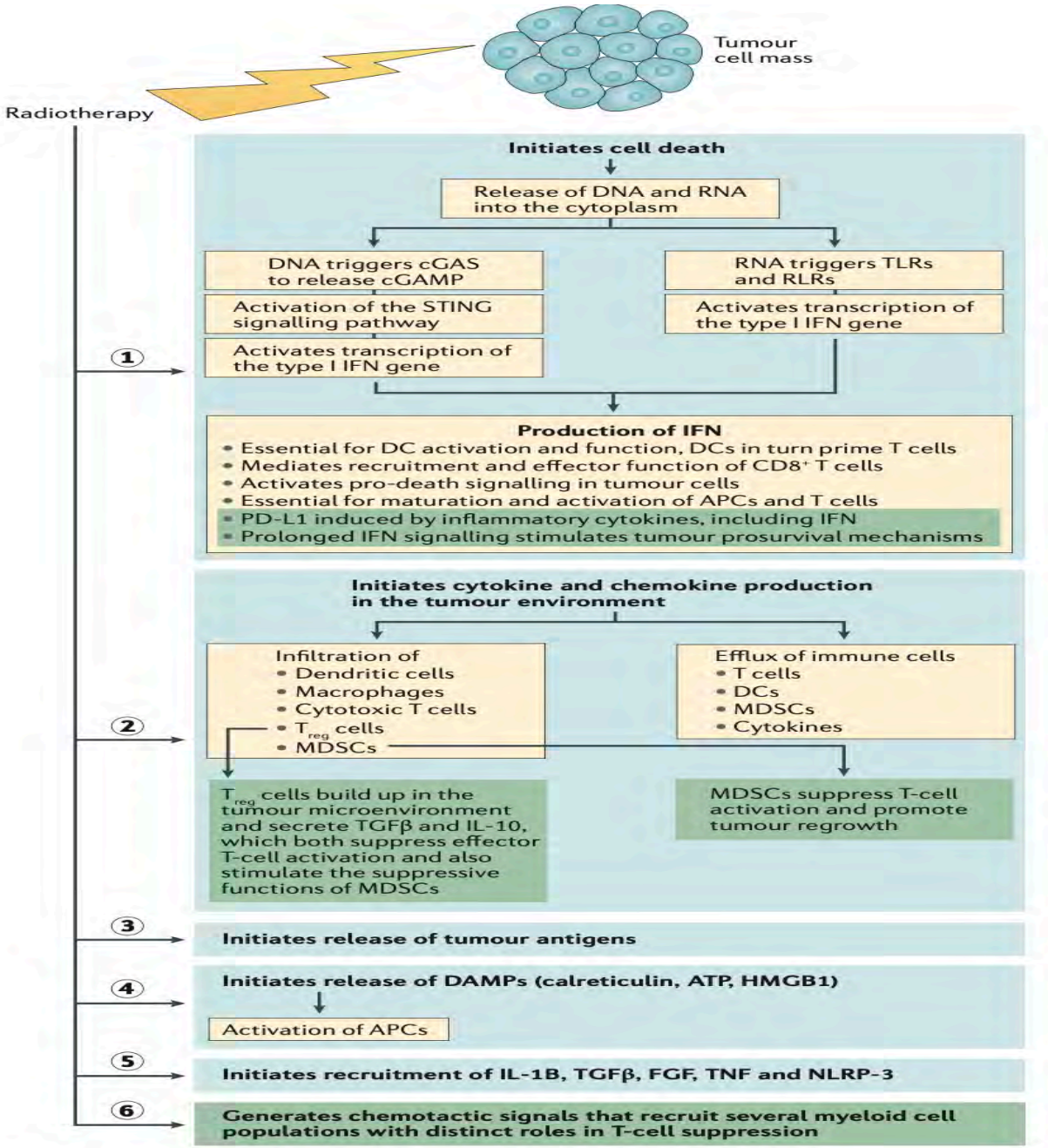
≥15% BM treated with bridging RT does not correlate with ≥Grade 3 cytopenias at any timepoint

Gohar Manzar



Radiotherapy and immunotherapy: a beneficial liaison?

Ralph R. Weichselbaum[1], Hua Liang[1], Liufu Deng[1] and Yang-Xin Fu[2]



RESEARCH ARTICLE Open Access

Ionizing radiation modulates the phenotype and function of human CD4+ induced regulatory T cells

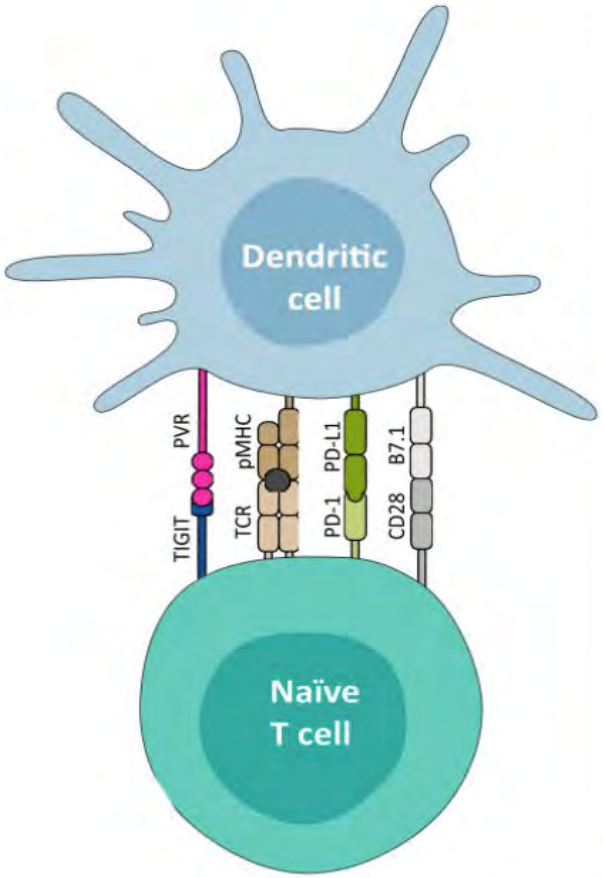
Samantha S. Beauford, Anita Kumari and Charlie Garnett-Benson*

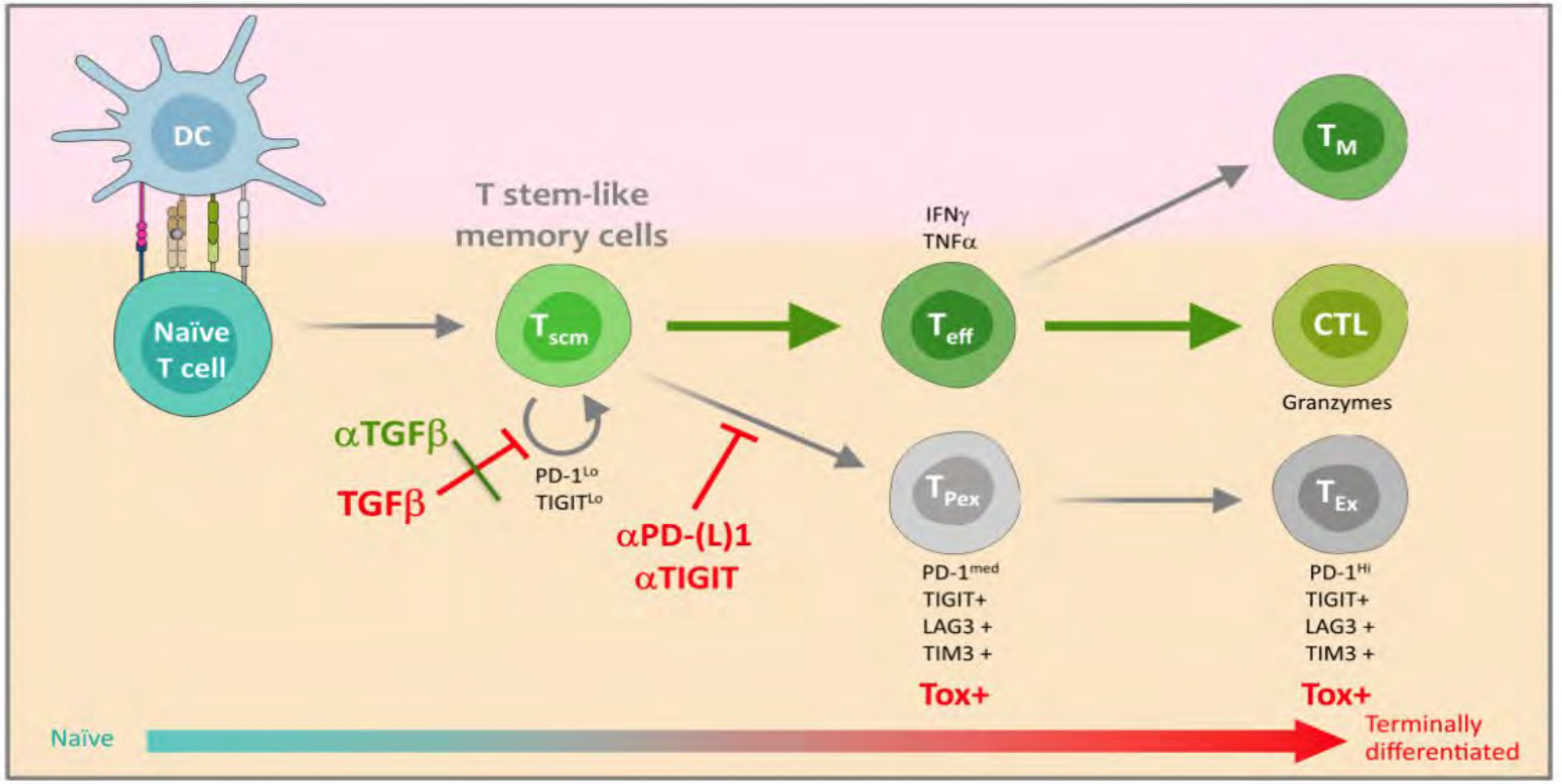
Conclusions: Our findings demonstrate that while human T_{REG} cells are more resistant to radiation-induced death, treatment causes downregulation of Foxp3 expression, as well as modulation in the expression of T_{REG} signature molecules associated with suppressive activity. Functionally, irradiated TGF-β1-induced T_{REGS} were less effective at inhibiting CD8+ T cell proliferation. These data suggest that doses of radiotherapy in the hypofractionated range could be utilized to effectively target and reduce T_{REG} activity, particularly when used in combination with cancer immunotherapies.

Opportunity for Radiation

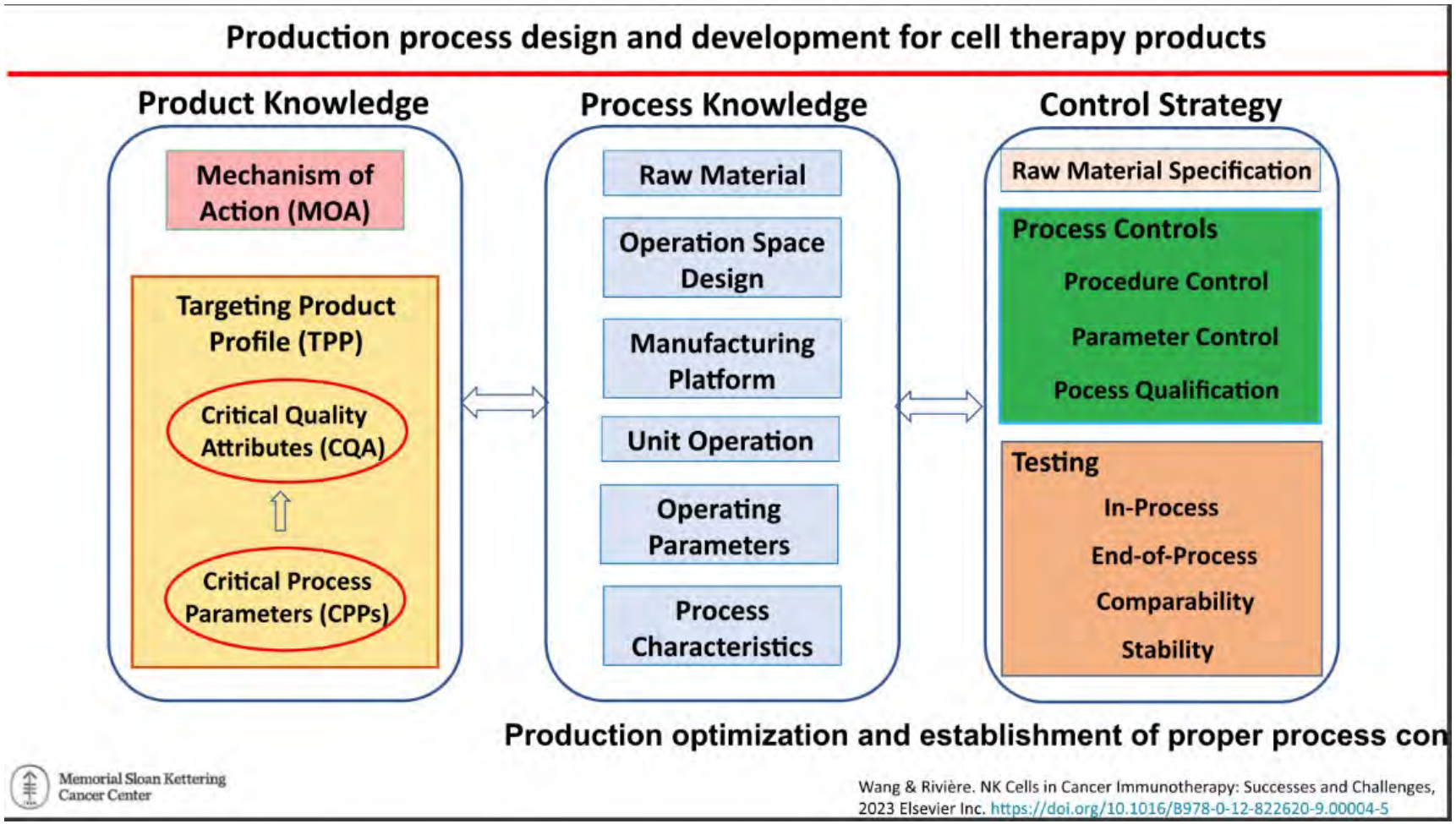
Combined PD-L1/PD-1 and TIGIT blockade re-directs the differentiation of activated T cells to T_{eff}/T_{mem} rather than T_{ex}

TGFβ blockade may expand Tscm compartment, further enhancing differentiation of Teff/Tm cells



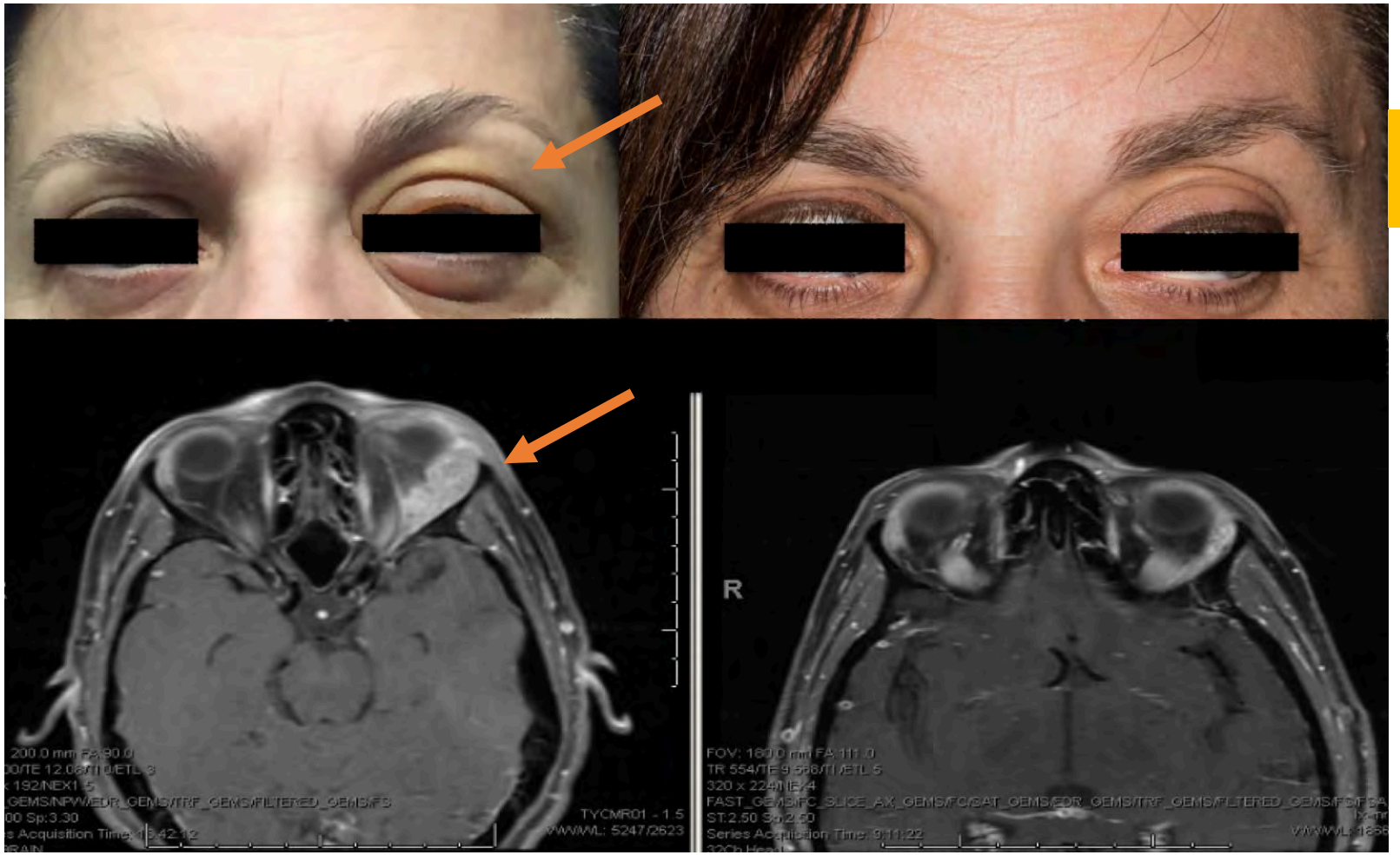


Castiglioni et al (2023) in revision
Hu et al (2022) JEM
Ma et al (2022) JEM


Production process design and development for cell therapy products
Product Knowledge
Mechanism of Action (MOA)
Targeting Product Profile (TPP)
Critical Quality Attributes (CQA)
Critical Process Parameters (CPPs)
Process Knowledge
Raw Material
Operation Space Design
Manufacturing Platform
Unit Operation
Operating Parameters
Process Characteristics
Control Strategy
Raw Material Specification
Process Controls
Procedure Control
Parameter Control
Pocess Qualification
Testing
In-Process
End-of-Process
Comparability
Stability
Production optimization and establishment of proper process con
Memorial Sloan Kettering Cancer Center
Wang & Rivière. NK Cells in Cancer Immunotherapy: Successes and Challenges, 2023 Elsevier Inc. https://doi.org/10.1016/B978-0-12-822620-9.00004-5

As opposed to solid tumors
Activating the immune microenvironment has been clinically successful
Boom Boom= 2 Gy x 2

MALT of the eye



50 MONTHS
POST 4 GY

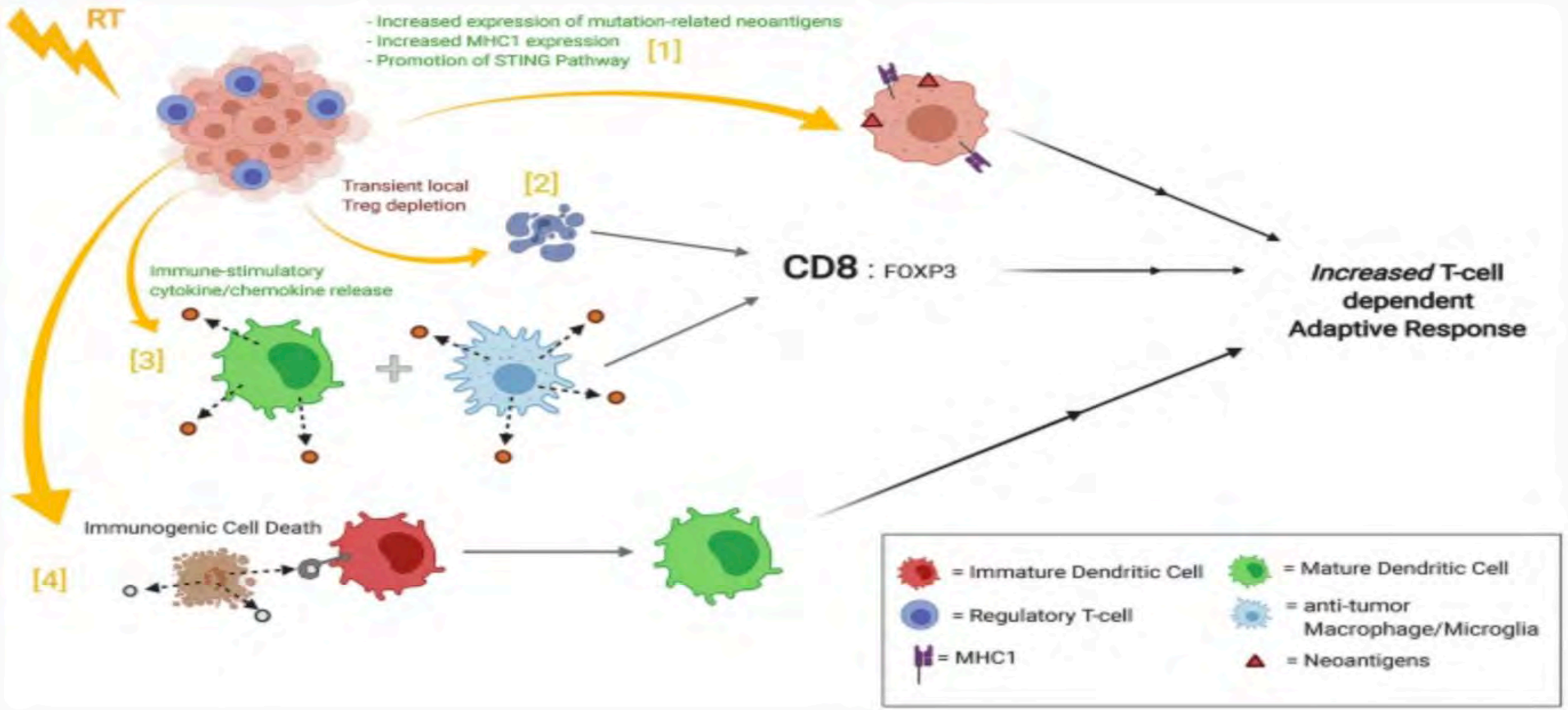
Rapid manufacturing (24-48 hrs)

Protocol	Target	Indication	Process	Length	Cell dose (CAR+ cells)	Response	CRS ICANs		
NCT04638270	CD19	ALL	Fast CART	1 day	5 x 10 4 CAR+ T/kg (n=3) 1 x 10 5 CAR+ T/kg (n=4) 1.5 x 10 5 CAR+ T/kg (n=3)	100% CR 90% MRD-	90%	Zhang et al	ASH 2019
NCT04129099	CD19/CD22	ALL	Fast CART Gracell	1 day	6.0 ×10 4 /kg (n=2) 1.0-1.5 ×10 5/kg (n=7) 2.25 ×10 5/kg (n=1)	90% CR	60%	Yang et al	ASH 2020
NCT04318327 **PHE885**	BCMA (41BBz)	Myeloma	T- Charge™ Novartis	< 2 days	2.5 × 10 6 (n=4) 5 × 10 6 (n=10) 14.3 × 10 6 (n=1)	33% sCR, 93% ORR		Sperling et al	ASH 2021
NCT05172596 **PHE885** **Phase II**	BCMA (41BBz)	Myeloma	T- Charge™ Novartis	< 2 days	5.0×106	N/A		Munshi et al	EHA, 2022
NCT03960840 **YTB323**	CD19 (41BBz)	DLBCL	T- Charge™ Novartis	2 days	1-2.5×10 6 (n=4) 5-12.5×10 6 (n=10) 25-40×10 6 (n=1)	73% (3 mo)	27%	Flinn et al	ASH 2021
					1-2.5×10 6 (n=4) 5-12.5×10 6 (n=28) 25×10 6 (n=7) 40×10 6 (n=6)	@DL2, 63% (12/19, 3 mo), 69% (11/16, 6 mo)	33% 11%	Barba et al.	ASH 2022

Rapid manufacturing of non-activated potent CAR T cells. Milone et al. Nat Biomed engineering. 2022

24 hrs, Serum Starvation, IL-7/IL-15, Deoxynucleosides

Fig. 1

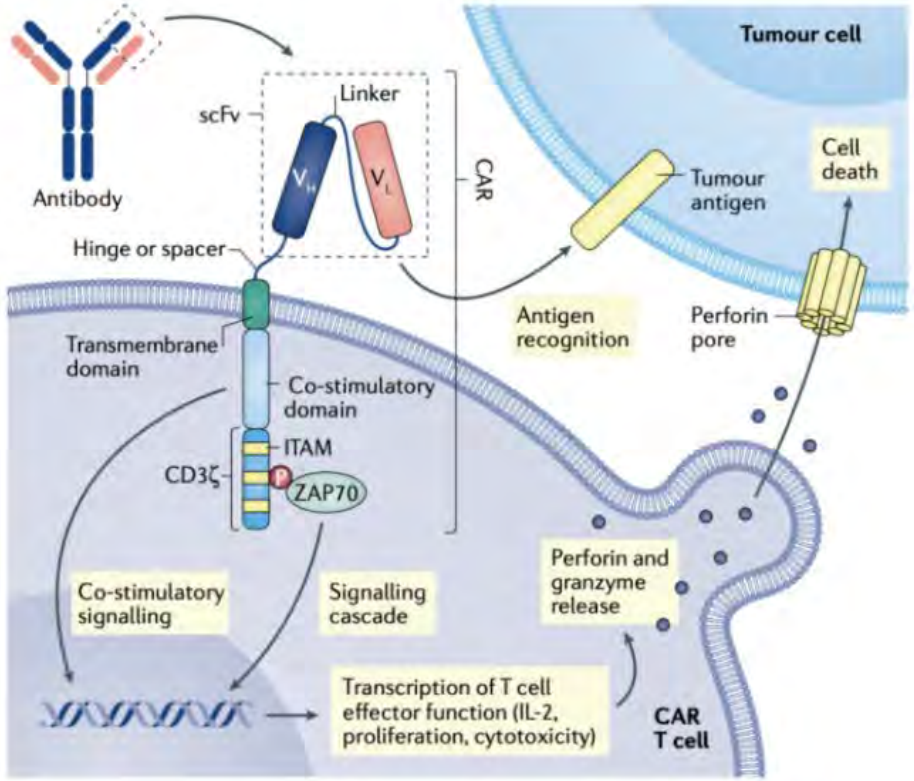


Strategies that improve antigen presentation:

- Induce epitope spreading,
- Perpetuate existing antitumor T cell responses
- Combat tumor antigen escape.

NeuroMolecular Medicine **volume 24**, pages3–7 (2022)

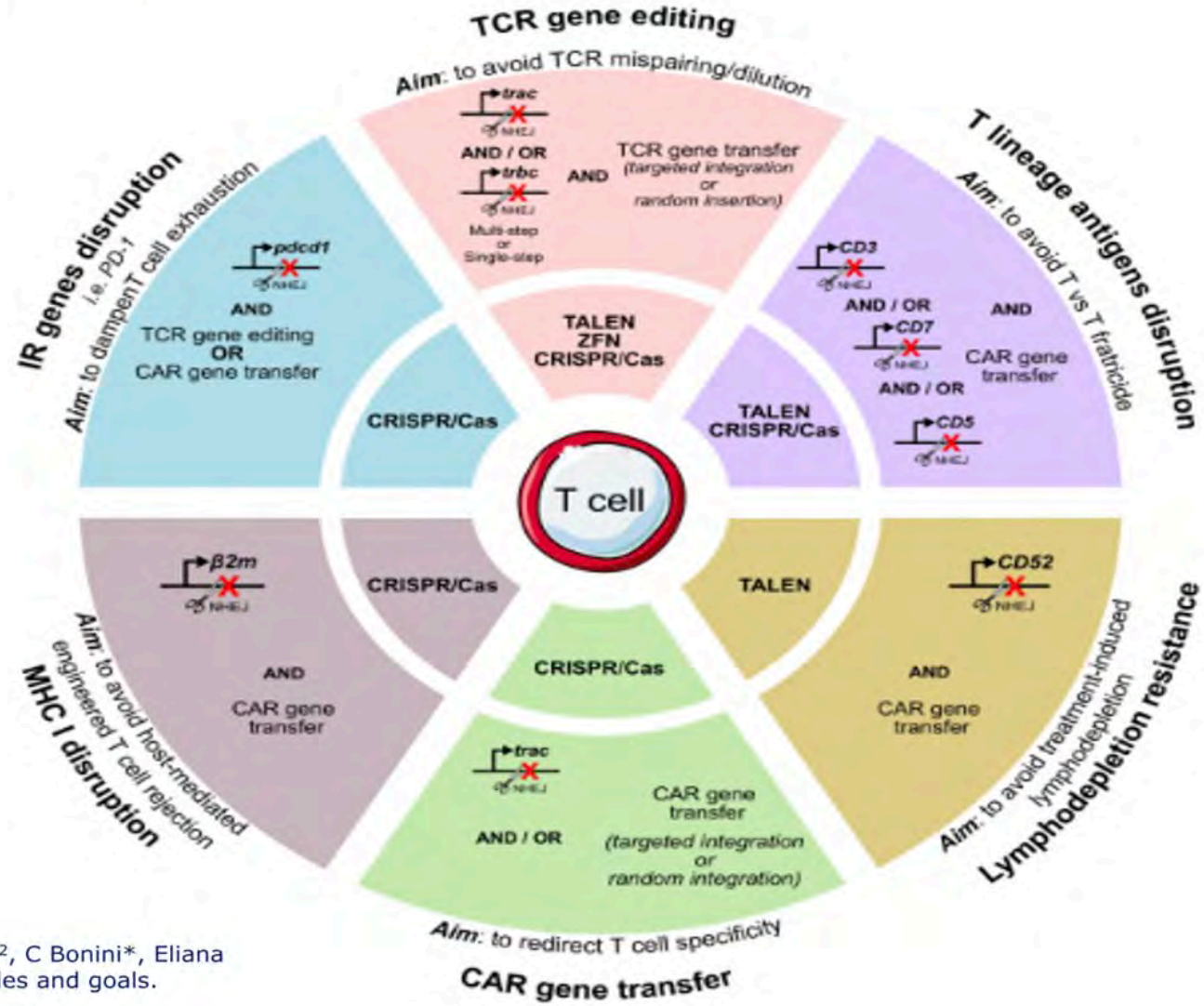
What are the important mechanisms behind CAR T cells?



Nature Reviews Cancer, Larson & Maus 2021

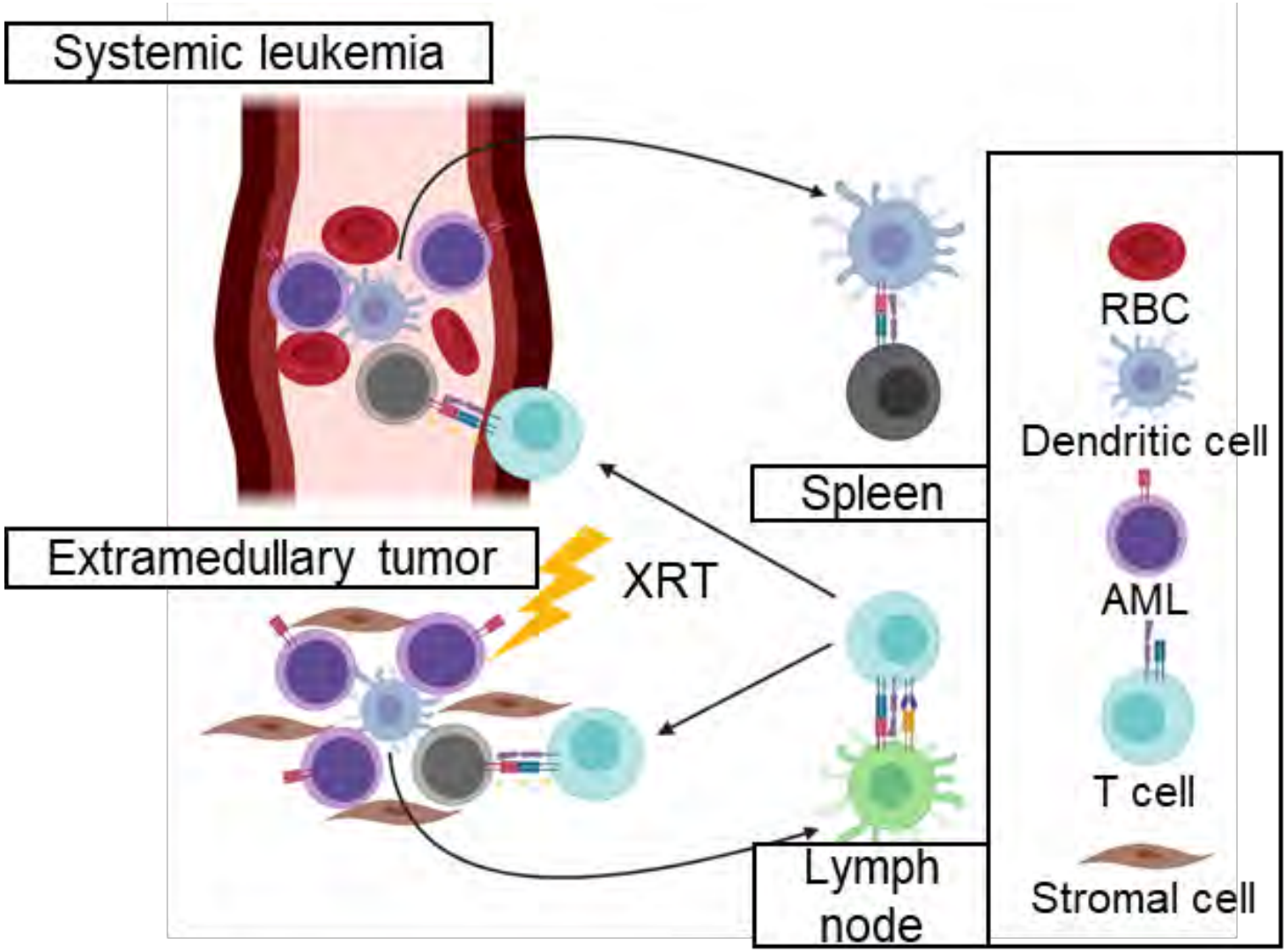
- Induce tumor lysis / trigger apoptosis
- Produce cytokines
- Recruit bystanders
- Proliferate
- Persist / form memory

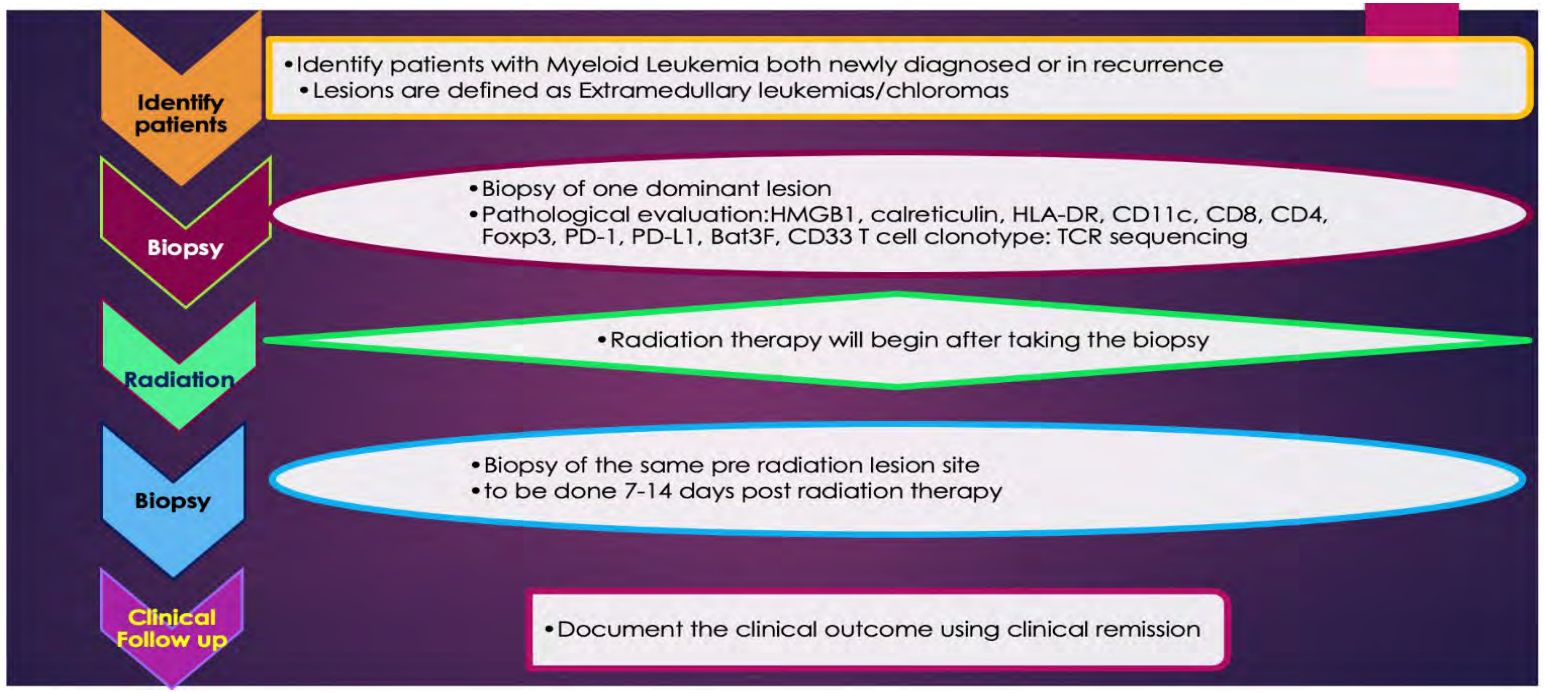
Genome editing at service of ACT



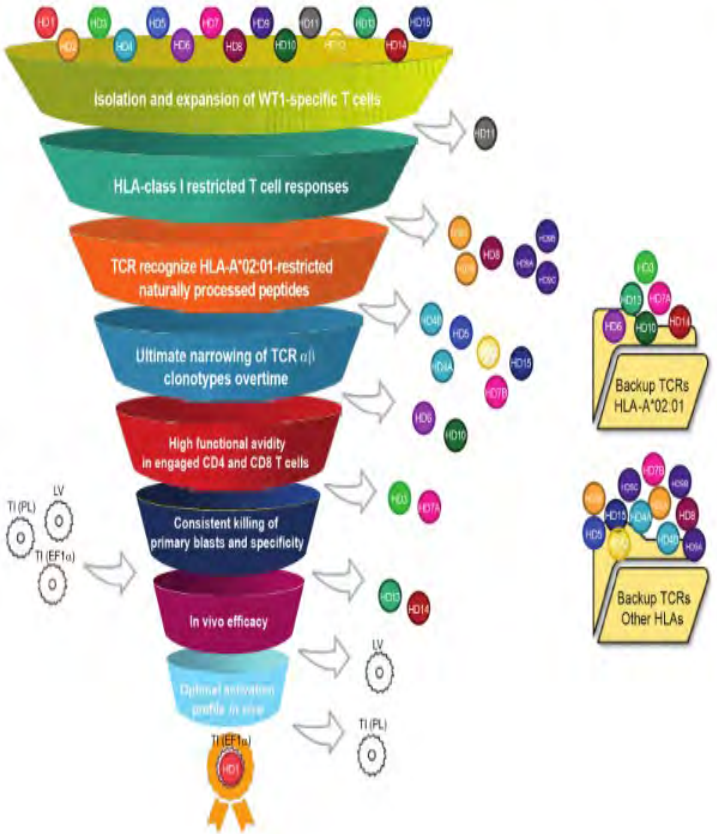
F Manfredi, BC Cianciotti, A Potenza, E Tassi, M Noviello, A Biondi, F Ciceri[1,2], C Bonini*, Eliana Ruggiero* TCR redirected T cells for cancer treatment: achievements, hurdles and goals. **Frontiers in Immunol**. *2021*

Immune differences between extramedullary and systemic leukemias




Identify patients
•Identify patients with Myeloid Leukemia both newly diagnosed or in recurrence
•Lesions are defined as Extramedullary leukemias/chloromas
Biopsy
•Biopsy of one dominant lesion
•Pathological evaluation:HMGB1, calreticulin, HLA-DR, CD11c, CD8, CD4, Foxp3, PD-1, PD-L1, Bat3F, CD33 T cell clonotype: TCR sequencing
Radiation
•Radiation therapy will begin after taking the biopsy
Biopsy
•Biopsy of the same pre radiation lesion site
•to be done 7-14 days post radiation therapy
Clinical Follow up
•Document the clinical outcome using clinical remission

Funnel approach drives the selection of the relevant features for the generation of a highly functional TCR T cell product

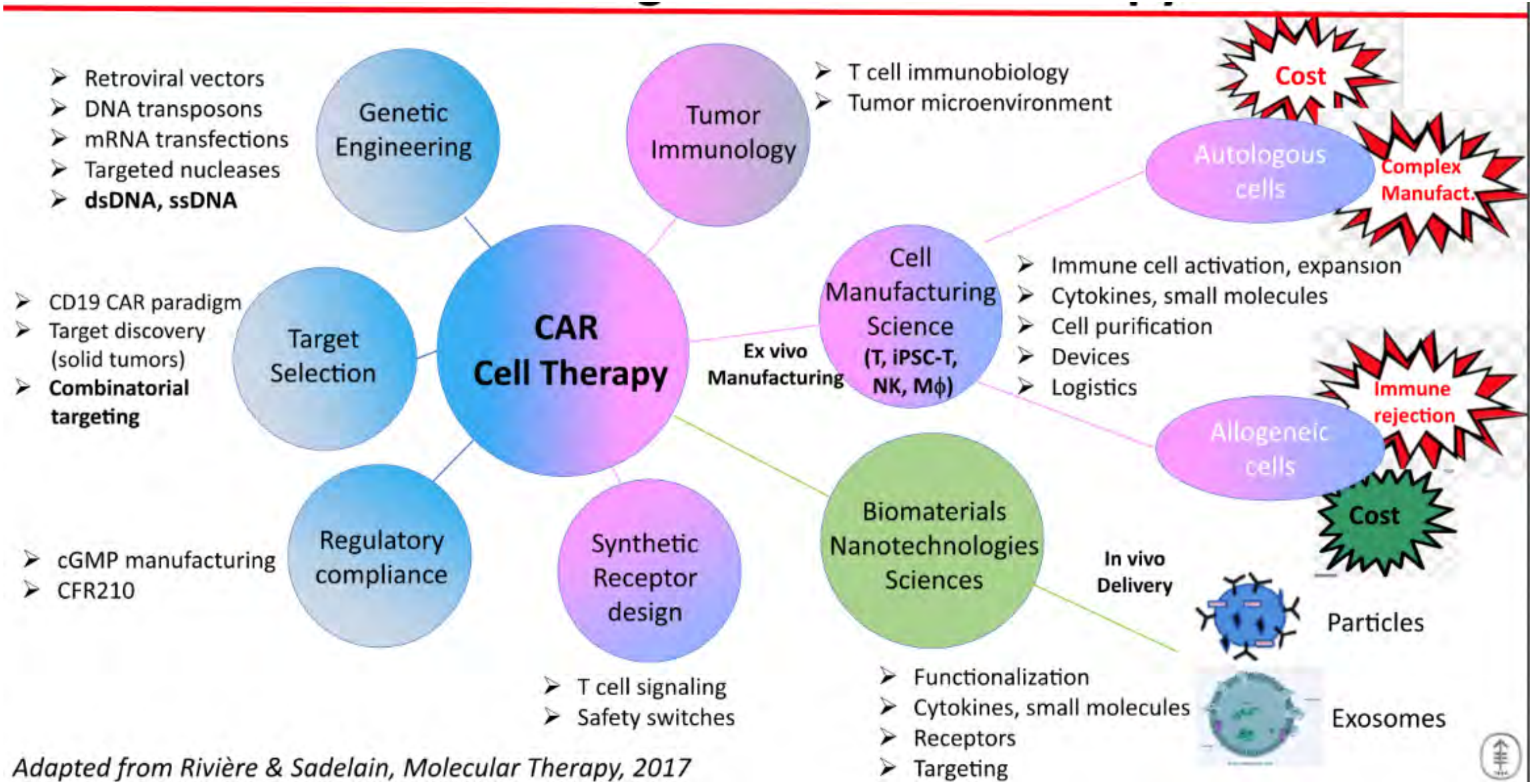


September 16th 2021

US **FDA** acceptance of investigational new drug application **NTLA5001**, CRISPR/Cas-engineered TCR-T cell candidate for **acute myeloid leukemia**

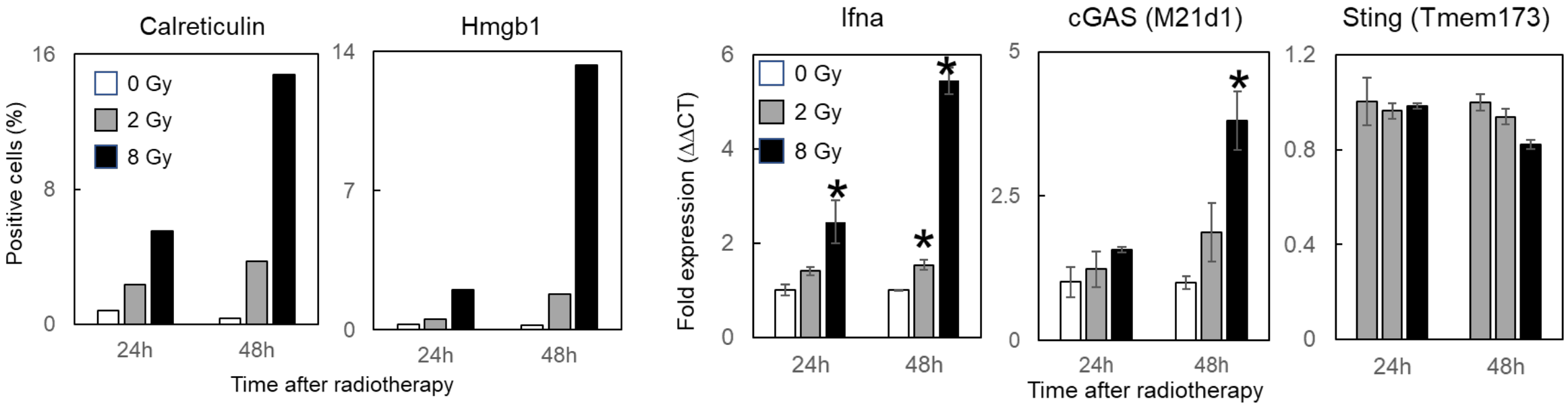
- Pipeline for the isolation of tumor-specific TCRs from healthy donors and cancer patients' circulating T cells
- Simultaneous editing of endogenous TCR α and β chain genes using CRISPR/Cas9 technology (efficiency >90%)
- Transduction of T-cells with lentiviral vectors encoding WT1-specific TCRs (efficiency >95% of $CD8^+$ T cells)
- Targeted TCR KI in TRAC and simultaneous TRBC KO (efficiency 70-80%)
- TCR edited T-cells specifically and efficiently kill primary $WT1^+$ leukemic blasts in vitro and in vivo

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

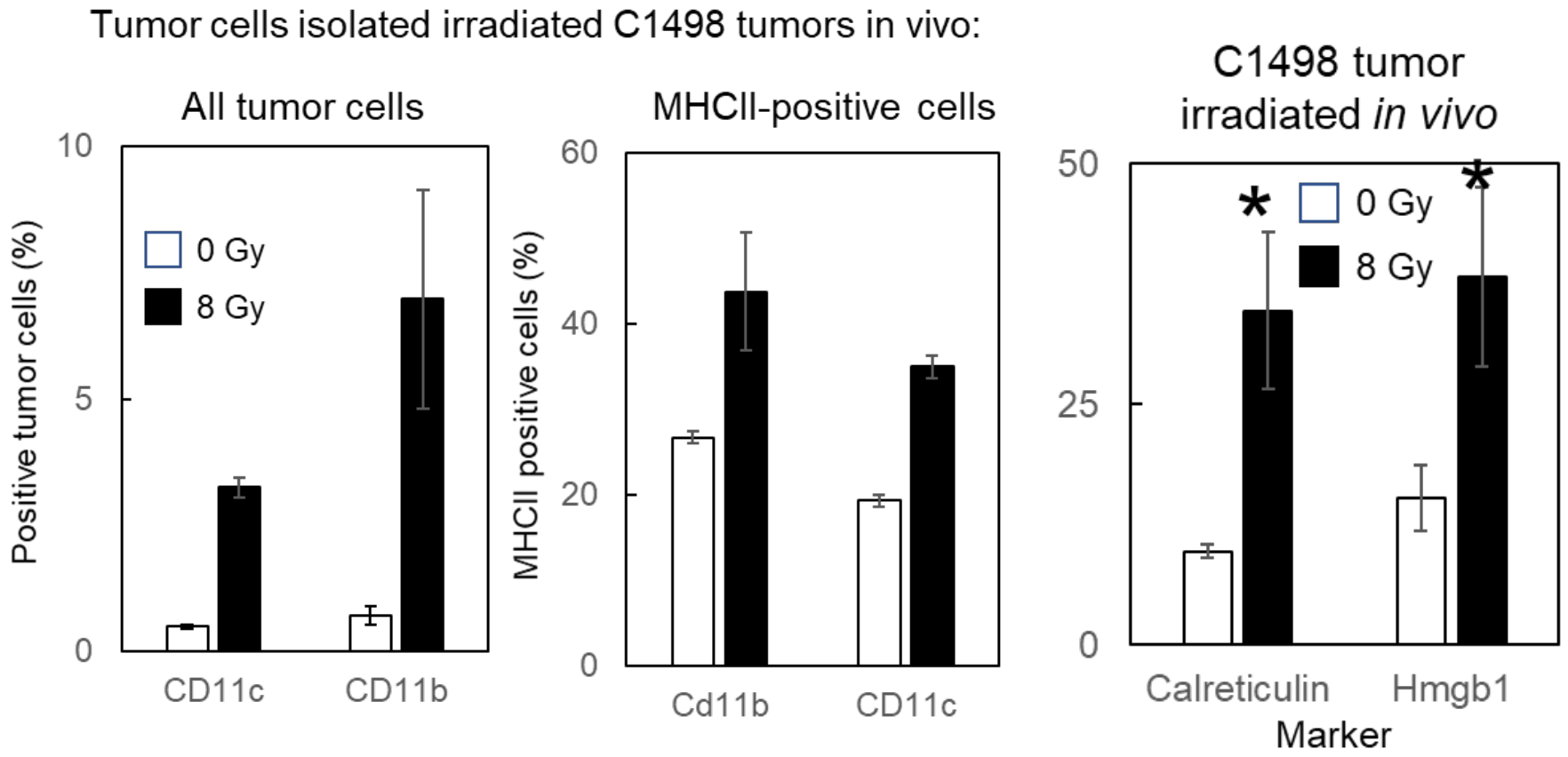


Adapted from Rivière & Sadelain, Molecular Therapy, 2017

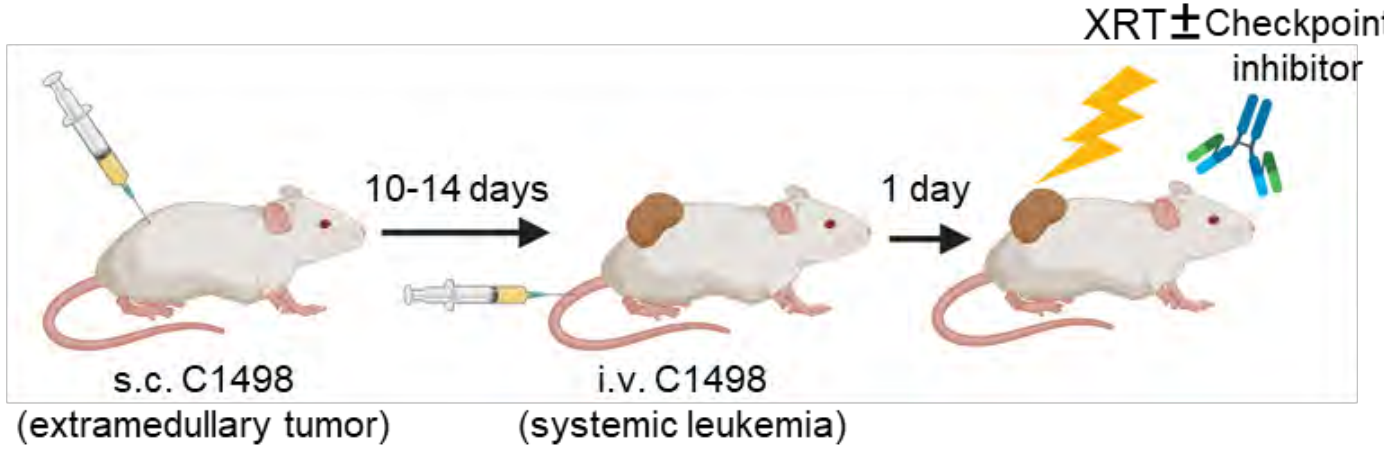
Radiotherapy increases DAMP and cGAS-STING-IFN pathway expression *in vitro*

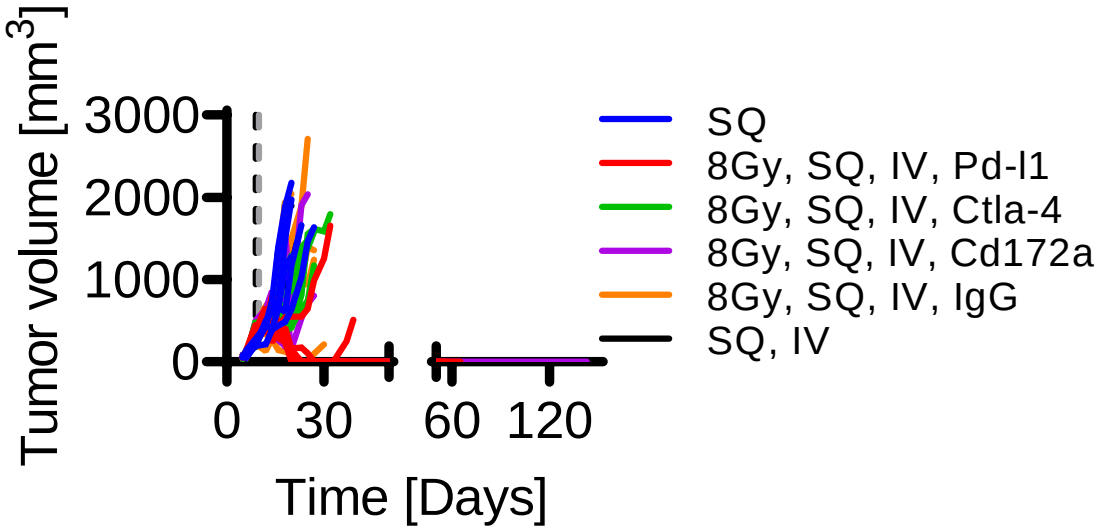


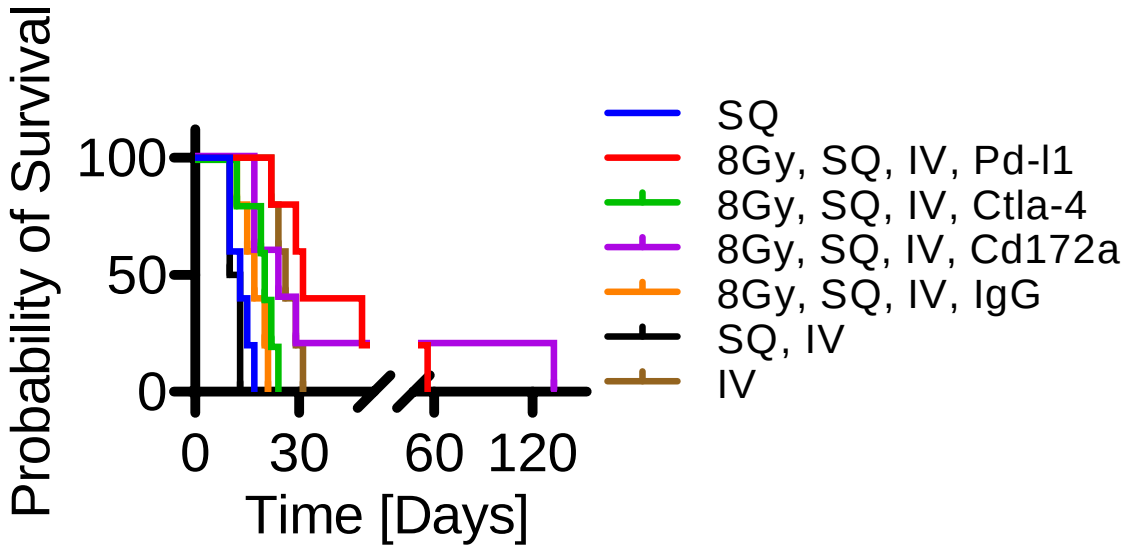
Radiotherapy increases macrophage infiltration and DAMP expression *in vivo*



Radiotherapy with anti-PD-1 or anti-CD47 potentially improves survival in mice with systemic leukemias







ACT with engineered T cells
Challenges and Opportunities

Increasing function and persistence of T cells

Bondanza et al, Blood 2006
Kaneko et al, Blood, 2009
Cieri et al, Blood, 2013
Oliveira et al, Science Transl Med 2015
WO 2007017915
WO 2021219758

Wendell A.L. & June C.H., *Cell, 2016*

Redirecting T cell specificity (CAR & TCR)

Casucci et al, Blood 2013
Norelli et al, Nat Med 2018
Greco et al, Sci Transl Med 2022
Provasi, Genovese et al Nat Med 2012
Mastaglio et al, Blood 2017
Ruggiero et al, Sci Transl Med 2022
US P.A.N. 12/927,292
Patent N. 9937207

Remodeling of microenvironment Identifying suppression mechanisms (checkpoint inhibitors)

Noviello, Manfredi et al Nat Comm 2019
Toffalori et al, Nat Med 2019

Increasing the safety profile of T cells (suicide genes)

Bonini et al, Science 1997
Bonini et al, Nat. Med. 2003
Recchia et al, PNAS 2006
Ciceri et al, Blood 2007

- Radiating a tumor initiates cancer cell death through immunogenic cell death markers, and chemokines in the tumor microenvironment leading to among others maturation of dendritic cells and macrophages, which in turn activates tumor-specific CD 8+ T cells that infiltrate the tumor. Antigens released from dying cancer cells facilitate the cross-presentation of tumor antigens. While activating the adaptive and innate immune response, radiation can also stimulate the differentiation of T regulatory via TGF and interleukin 10, inhibiting activated T cells that promote tumor progression.
-
- Now that we know that radiation holds promise for its ability to positively modulate the immune system let us look at the current clinical evidence and how to best incorporate it.
-