

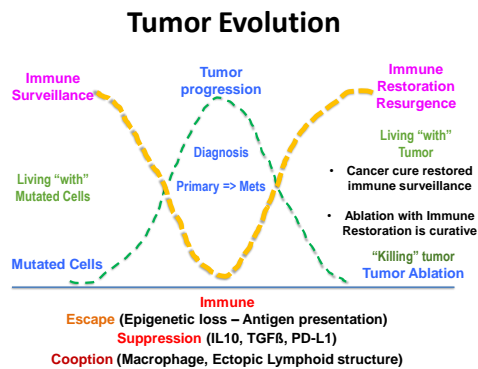
Immune Priming with Ultrasound

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Conflicts and Grants

- NIH (R01 EB009040)
- NCI SBIR grant with CellDex Therapeutics, Inc.
- Project Energy with Johnson & Johnson

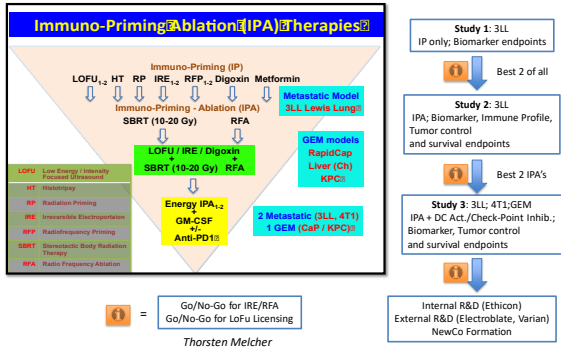


Focal Oncology Clinical Adaptive Learning (FOCAL) Cancer Clinic Network

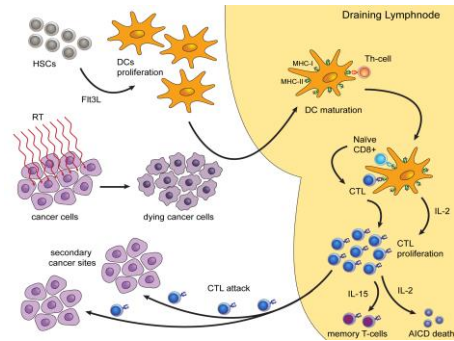
1. **Ablative Therapies for local control** induces anti-tumoral immunity, which in turn helps local control.
2. **Ablative Therapies for systemic immunity:**
Immune Priming Ablation (IPA) for In Situ Tumor Vaccines
 - a. UPR => ER stress => Antigen Processing / Presentation
 - b. "Eat Me" and DAMP signals
 - c. Reversal of tolerance
 - d. Antigen Presentation (neo-antigens & cryptic antigens)

"Focal Therapy for Systemic Cure"

Project ENERGY.01: Proposed Study Design



Autologous *in situ* tumor vaccines



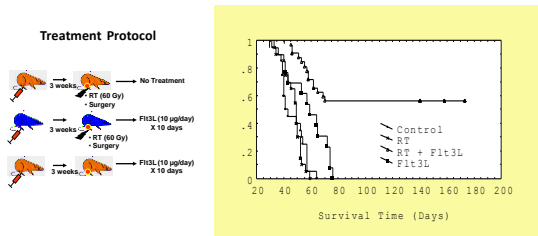
CANCER RESEARCH 91:6024-6032, December 15, 1993

Advances in Brief

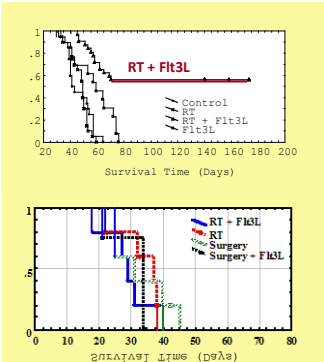
Flt3-Ligand Administration after Radiation Therapy Prolongs Survival in a Murine Model of Metastatic Lung Cancer

Prabir K. Chakravarty, Alan Alfieri, Elaine K. Thomas, Vivek Beri, Kathryn E. Tanaka, Bhadrarasin Vikram, and Chandan Guha¹

Departments of Radiation Oncology (P.K.C., A.A., V.B., B.V., C.G.) and Pathology (K.E.T., E.E.T.), Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York 10461; Roth-Kessel Medical Center, New York, New York 10007 (A.A.); and Immune Corporation, Seattle, Washington 98101 (E.E.T., E.E.T.)



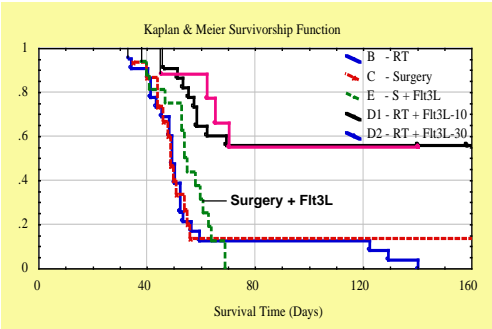
RT + Flt3L Improves Survival of Tumor-bearing C57Bl/6 Mice



C57Bl/6 mice
(RT+Flt3L - 55% cured)

Immunodeficient
Nude mice
(RT+Flt3L - 0% cured)

Systemic Effects of Primary Tumor Irradiation



FLT3 Ligand Immunotherapy and Stereotactic
Radiotherapy for Advanced Non-small Cell Lung
Cancer

- SBRT will be administered during the first week of study therapy.
- A single pulmonary lesion that measures at least 1 cm in greatest dimension will be treated.
- Daily subcutaneous injections of CDX-301 (75 µg/kg) will be administered for 5 days, beginning on the first day of SBRT.

	Pre-rx	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Weeks 16, 24, 32
Stereotactic Radiotherapy (SBRT)		XXX								
FLT3 Ligand Therapy		XXXXX								
History and Physical Examination	X	X	X	X	X		X		X	X
Blood Tests: CBC, CMP	X	X	X	X	X		X		X	X
Whole body PET/CT	X								X	X
Immune Correlates	X		X		X				X	

Sample size: 29 patients

4.3 Primary Endpoint

- The primary endpoint is progression-free survival rate at four months (PFS4), defined as the rate estimate of the percentage of patients who are alive and progression-free at 16 weeks (~4 months) after initiation of study therapy.



FLT3 Ligand Immunotherapy and Stereotactic Radiotherapy for Advanced Non-small Cell Lung Cancer

Nitin Ohri

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Stereotactic Radiotherapy (SBRT)	X X X									
FLT3 Ligand Therapy		XXXXX								
History and Physical Examination	X	X	X	X	X		X		X	X
Blood Tests: CBC, CMP	X	X	X	X	X		X		X	X
Whole body PET/CT	X								X	X
Immune Correlates	X		X		X			X		

Sample size: 29 patients

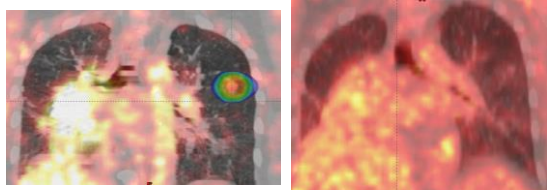
Ablative SBRT dose fractionation

- 34 Gy x 1 Fx
- 18 Gy x 3 Fx
- 10 Gy x 5 Fx

Study Subjects

#	Demographics	Disease Burden	Prior Treatment(s)	SBRT	Clinical Course
1	73 year-old Korean male	Right lung squamous cell carcinoma with multiple lung masses and bilateral mediastinal adenopathy	Carboplatin + gemcitabine	50 Gy in 5 fractions to RLL mass	Progression at 6 weeks, death at 4 months
2	55 year-old Hispanic female	Right lung adenocarcinoma, with bilateral lung nodules	Chemoradiotherapy for localized disease, nivolumab for metastatic disease	34 Gy in 1 fraction to LUL nodule	Partial response at 2 months, stable at 4 months
3	80 year-old Caucasian female	Right lung squamous cell carcinoma with spine and pelvic metastases	Chemoradiotherapy for localized disease, carboplatin + gemcitabine for metastatic disease, nivolumab	50 Gy in 5 fractions to right lung mass	Partial response at 2 months
4	73 year-old Caucasian female	Right lung adenocarcinoma with mediastinal adenopathy and liver metastasis	Carboplatin/ + pemetrexed, maintenance pemetrexed	50 Gy in 5 fractions to RLL mass	PET/CT on 4/27

Patient 2

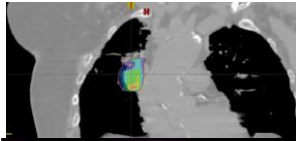


Right lung mass reduced from 5.0 cm (maximum SUV 10.7) to 2.1 cm (maximum SUV 6.5) on first post-treatment PET/CT

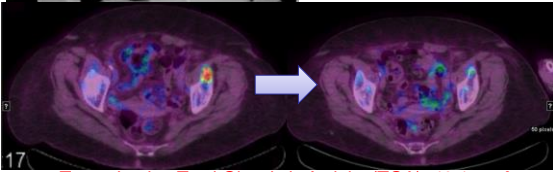
Target Lesion Total Glycolytic Activity (TGA): 1.0 cc → 0.3 cc

Other lesions' TGA: 44.7 cc → 4.5 cc

Patient 3

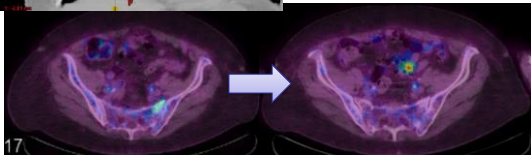
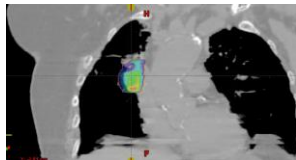


Improvement or
resolution of most of the
osseous foci



Target Lesion Total Glycolytic Activity (TGA): 49.1 cc →
6.2 cc

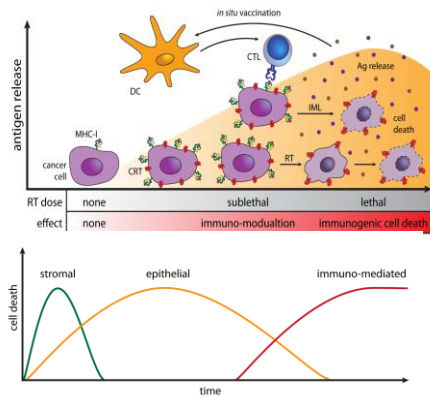
Patient 3



Target Lesion Total Glycolytic Activity (TGA): 49.1 cc →
6.2 cc

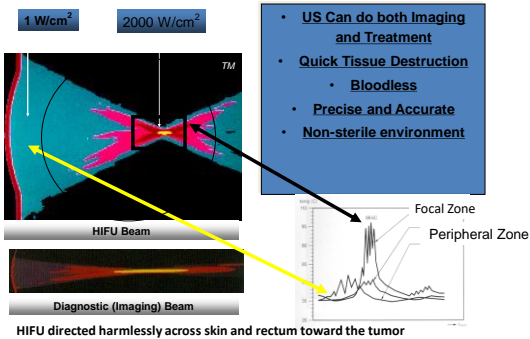
Energy activated *in situ* Tumor Vaccines POC Studies

Energy Immunotherapy	Tumor Models	End Points	Limitation
Single Fraction SFRT 25-60 Gy	<ul style="list-style-type: none"> Flt3L + / - CD40L Lewis Lung 3LL in C57/B16 BN1LNE Liver (HCC) in Balb/c 	<ol style="list-style-type: none"> 1. Primary Tumor Growth 2. Metastases 3. Survival 4. Immune assays 	<ul style="list-style-type: none"> • Murine Ectopic Transplantation Models • RT Dose • Fractionation • RT to Draining Lymph Node
20 Gy	TLR9 agonist	PTG, Mets, Survival	
10 Gy	Listeria-PSA ADXS31-142	PTG and Immune Assays	<ul style="list-style-type: none"> • Break Tolerance to self antigens
20Gy x 3	PD1-Fc	PTG, Mets, Survival	<ul style="list-style-type: none"> • Small Animal Treatment
LOFU	HIFU	PTG, Immune Assays	
LOFU	SBRT (10 Gy x 3)	PTG, Mets, Survival Immune Assays	<ul style="list-style-type: none"> • Lack of Immune Surveillance and carcinogenic environment



Acoustic priming

ULTRASOUND -- ADVANTAGES



Therapeutic Ultrasound as an autologous *in situ* tumor vaccine

- HIFU = High Intensity Focused Ultrasound
- MRgFUS = MR-guided Focused Ultrasound
- TULSA = Transurethral ultrasound ablation
- LOFU = Low intensity (energy) focused ultrasound (LOFU coined by Guha group)
- SST = Sonic Stress Therapy
- APT – Acoustic Priming Therapy

LOFU parameters

	Condition #1	Condition #2	Condition #3	Condition #4	Condition #5
Duty Cycle (%)	1	25	50	75	100
Power (W)	32	16	8	4	2
Time (ms)	1000	625	1250	2500	5000
Thermal Energy (J)	0.32	2.5	5	7.5	10
Peak Negative Pressure (MPa)	8.14	6.08	4.58	3.34	2.46

Thermal Energy

Mechanical Energy

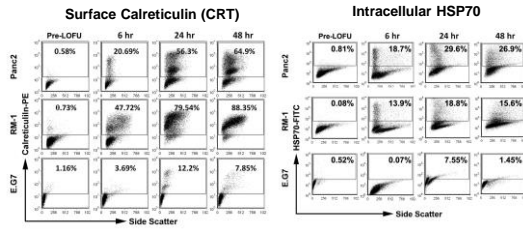
The “sonic stress” of LOFU

Gene function	Genes affected by LOFU treatment
1. Protein Folding	DNAJB1, HSPH1, HSPA1, HSPB1, HSPD1, HSPA4L, CRYAB, HSPA6, HSPA7, HSP90AA1, HSP90AA4P, DNAJA4, FKBP4, LGSN, PTGES3
2. Cell cycle regulation	IER5, JUN, CACYBP, GPRCSA, RRAD, WEE2
3. Cytokines	IL8
4. Receptors	CSF2RB, IL7R, NPR1, RXFP2, FLT4, ITGA2
5. Cytoskeleton integrity	FAM101B, TCP1
6. Transcriptions	ATF3, ANKRD1, EYA4, KAT2A
7. Transporters	SLC22A2, SLC22A16, RHAG
8. Apoptosis regulation	NLR4, ANGPTL4, BAG3
9. Peptidase	NAALADL1, MEP1A, PLOD2

Gene	Control	Treated
CSF2RB	1.000	0.800
DNAJB1	1.000	4.500
HSPB1	1.000	4.500
HSPF1	1.000	1.800
HSPB1	1.000	3.000
HSPD1	1.000	2.500
HSPA4L	1.000	2.200
HSPA6	1.000	10.000
HSPA7	1.000	10.000
HSP90AA1	1.000	1.800
HSP90AA4P	1.000	1.800
HSP90AA4	1.000	4.000
DNAJA4	1.000	7.800
ANKRD1	1.800	2.000
IL8	1.000	2.500
JUN	1.000	2.800
ATF3	1.000	2.800

Figure 2 consists of two panels. The top panel is a bar graph showing the mRNA fold change for Hsp1a1 (132.37) and Hsp1b (80.77). The y-axis is labeled 'mRNA Fold Change' and ranges from -20.00 to 140.00. The bottom panel is a line graph showing the mRNA fold increase over time (0 to 30 hours) for Hsp1b, Hsp1b1-1, Hsp1b1-2, Hsp1b1, Hsp1b1-1, Hsp90a1, and Dnaajb5. The y-axis is labeled 'mRNA fold increase' and ranges from 0 to 80. The x-axis is labeled 'Time post-LOU (h)' and ranges from 0 to 30. Hsp1b shows the highest fold increase, peaking at 5-10 hours. Hsp1b1-1, Hsp1b1-2, Hsp1b1, and Hsp1b1-1 show lower fold increases, peaking at 5-10 hours. Hsp90a1 and Dnaajb5 show very low fold increases.

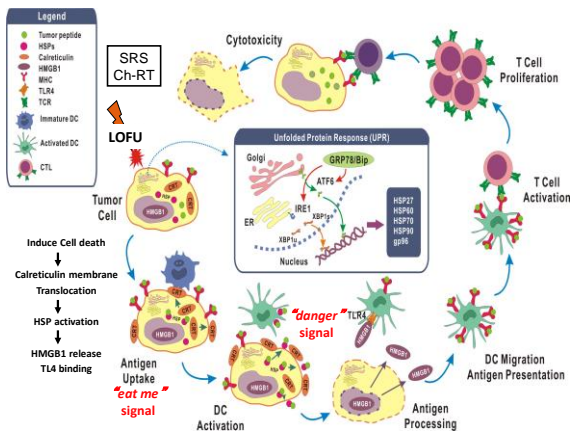
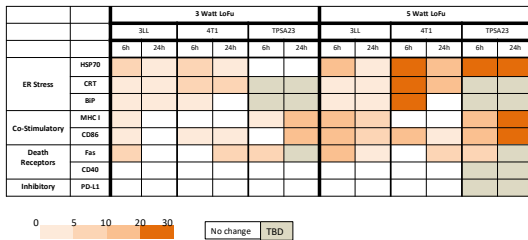
Immunomodulation of tumor cells



In vitro Effect of LOFU on Cell Surface Markers

Summary of in vitro data under JJI-ENERGY

- LoFu is non-ablative
- LoFu induces "sonic stress" on cancer cells
- Sonic stress signature is consistent across cell types and indicative of immune stimulation



The diagram illustrates the T cell response to an antigen. It shows an 'Antigen presenting cell' (APC) taking up an 'Antigen' and presenting it to a 'Dendritic cell' (DC) via 'Antigen uptake'. The DC then undergoes 'Dendritic cell maturation' and presents the antigen to a 'T cell' via 'MHC'. The T cell then interacts with a 'Cytotoxic T cell' (CTL) and a 'Regulatory T cell' (Treg). The CTL is labeled 'Effector T cell response' and the Treg is labeled 'Immunosuppression'. The diagram also shows 'T cell activation and effector' and 'T cell response'.

The diagram illustrates the two-step process of T cell activation and the resulting phenotypes. It is divided into two main horizontal sections: 'T cell Activation' (top) and 'T cell Energy' (bottom).

T cell Activation:

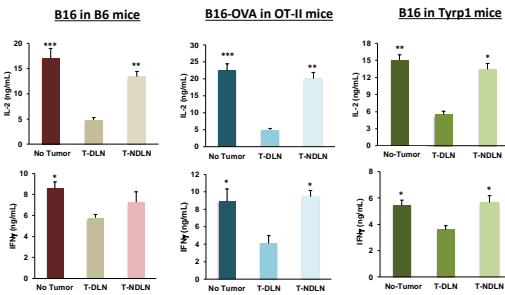
- CD4⁺ T cell:** Represented by a grey sphere.
- Full stimulation:** Indicated by 'CD3 CD28' and a yellow starburst icon. This leads to 'Cytokine production' and 'Cell Proliferation', represented by three blue spheres.
- Re-stimulation:** Indicated by a yellow starburst icon. This leads to 'Cytokine production' and 'Cell Proliferation', represented by three blue spheres.

T cell Energy:

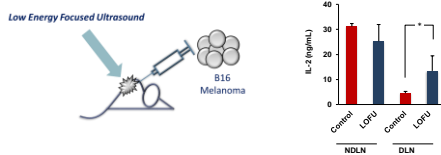
- CD4⁺ T cell:** Represented by a grey sphere.
- Anergic stimulus:** Indicated by a blue starburst icon. This leads to a 'Hyporesponsive phenotype', represented by a purple sphere.
- Re-stimulation:** Indicated by a yellow starburst icon. This leads to a 'Hyporesponsive phenotype', represented by a purple sphere.

The diagram illustrates two distinct signaling pathways for T cell activation. On the left, 'Full Activation' is shown where both CD28 and TCR receptors are engaged. CD28 signaling through the PI3K pathway leads to the activation of MAP kinases, which in turn activate NF- κB . TCR signaling through the Ca^{2+} pathway leads to the activation of Calcineurin (CaN). Both NF- κB and CaN activate NFAT, which then forms a complex with AP-1 and NFAT to initiate the transcription of 'Activation-induced genes'. On the right, 'Anergic Stimulus' is shown where only the TCR is engaged. This leads to a weak or dysregulated Ca^{2+} pathway, resulting in insufficient CaN and NFAT activation. Consequently, the transcription of 'Anergy-inducing genes' is initiated, leading to the expression of co-inhibitory receptors like VISTA.

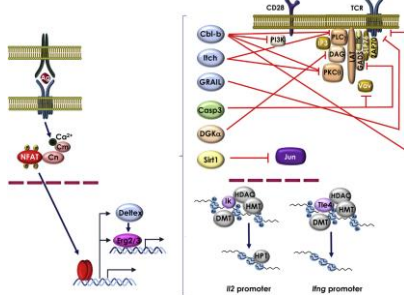
B16 tumors induced CD4⁺ T cell anergy



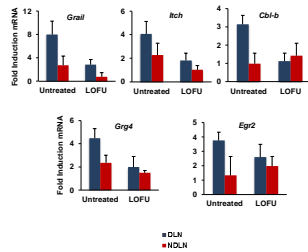
LOFU prevents B16-induced CD4⁺ T cell anergy



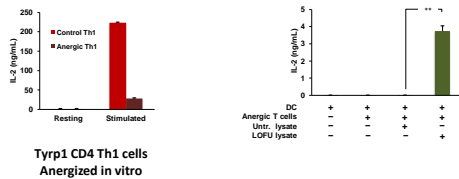
T cell anergy: transcriptional program



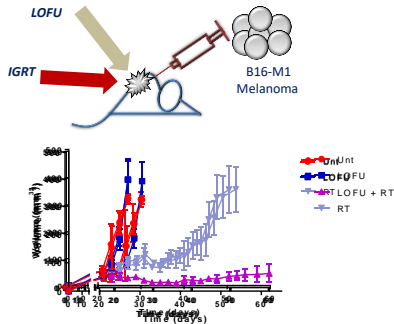
LOFU prevents B16-induced CD4+ T cell anergy



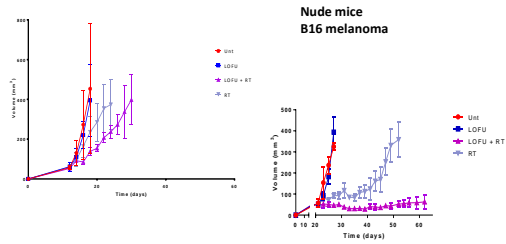
LOFU can reverse established T cell anergy



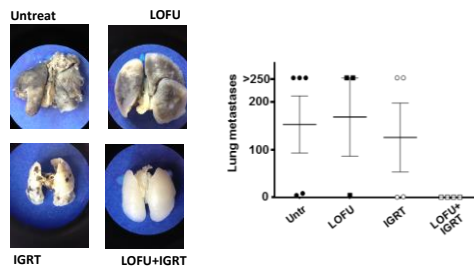
LOFU as immune adjuvant for IGRT



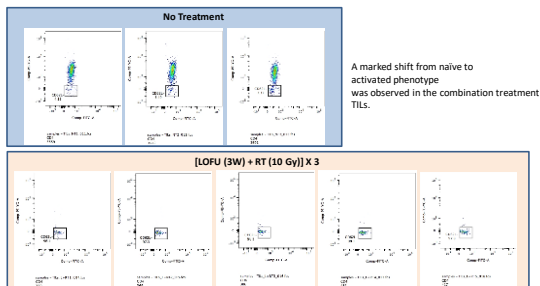
LOFU as adjuvant for IGRT requires T cells

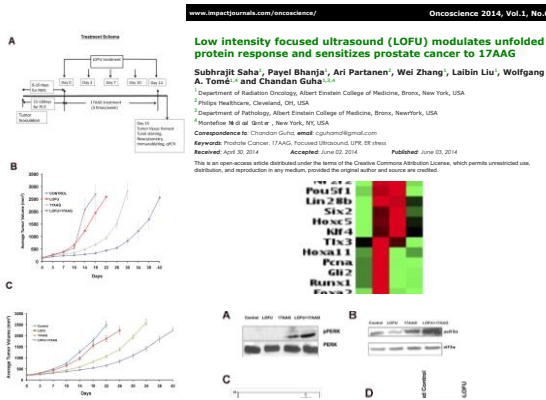
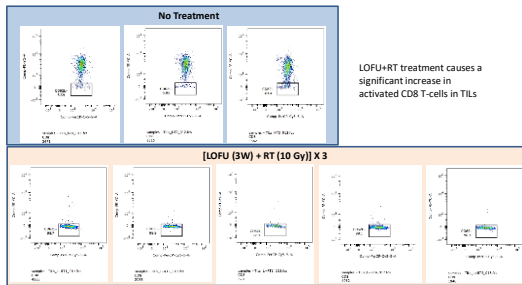


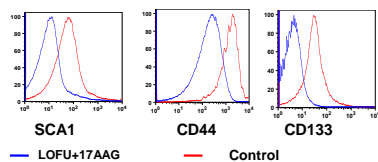
LOFU as adjuvant to potentiate effect of RT and improve control of distal metastases



CD62L^{VE}/CD4^{VE} population in Tumor infiltrating lymphocytes (TILs)

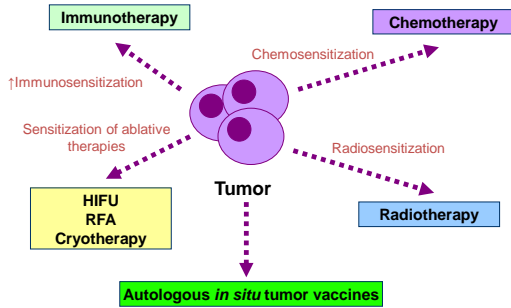






Did the number of cells go down or expression?

Acoustic Priming



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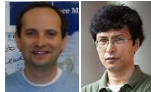
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Immune Therapeutics

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Thank you!



Montefiore
Inspired Medicine



Institute for Onco-Physics
Albert Einstein College of Medicine