Montefiore
Inspired Medicine

Institute for Onco-Physics
Albert Einstein College of Medicine

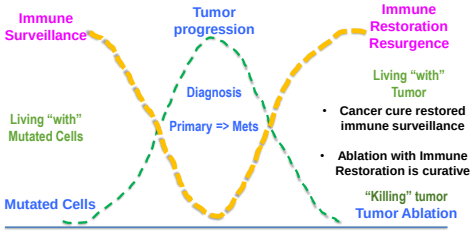
Immune Priming with Ultrasound

Chandan Guha, MBBS, PhD

Professor and Vice Chair, Radiation Oncology
Professor, Urology and Pathology
Director, Einstein Institute of Onco-Physics
Montefiore Medical Center
Albert Einstein College of Medicine, Bronx, NY
cguhamd@gmail.com

Conflicts and Grants
- NIH (R01 EB009040)
- NCI SBIR grant with Celldex Therapeutics, Inc.
- Project Energy with Johnson & Johnson

Tumor Evolution



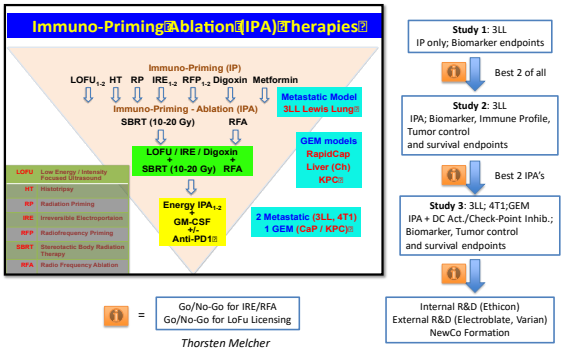
Escape (Epigenetic loss – Antigen presentation)
Suppression (IL10, TGFß, PD-L1)
Cooption (Macrophage, Ectopic Lymphoid structure)

*F*ocal *O*ncology *C*linical *A*daptive *L*earning (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)**

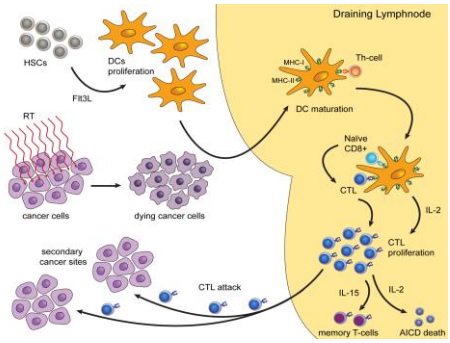
"Focal Therapy for Systemic Cure"

Project ENERGY.01: Proposed Study Design



Thorsten Melcher

Autologous *in situ* tumor vaccines



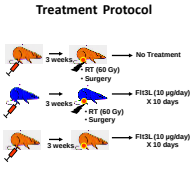
[CANCER RESEARCH 59, 6028–6032, December 15, 1999]

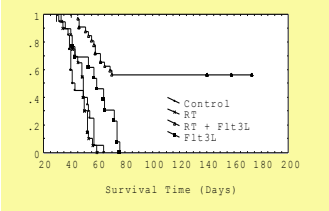
Advances in Brief

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

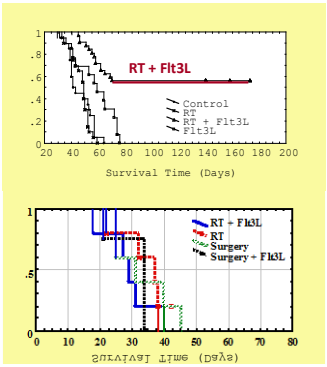
Prabir K. Chakravarty, Alan Alfieri, Elaine K. Thomas, Vivek Beri, Kathryn E. Tanaka, Bhadrasain Vikram, and Chandan Guha[1]

Departments of Radiation Oncology [P. K. C., A. A., V. B., B. V., C. G.] and Pathology [E. K. T., K. E. T.], Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York 10461; Beth Israel Medical Center, New York, New York 10003 [A. A.]; and Immunex Corporation, Seattle, Washington 98101 [E. K. T., K. E. T.]





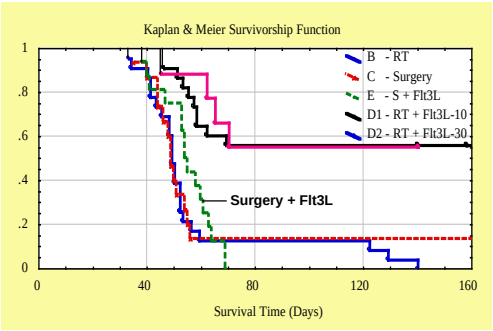
RT + Flt3L Improves Survival of Tumor-bearing C57Bl/6 Mice



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)		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

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

- 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)		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

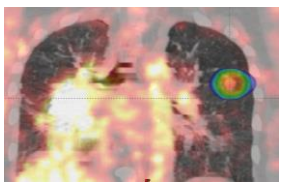
Ablative SBRT dose fractionation

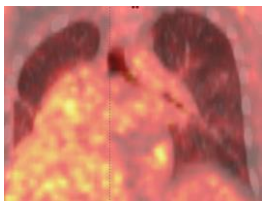
- 34 Gy x 1 Fx
- 18 Gy x 3 Fx
- 10 Gy x 5 Fx

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



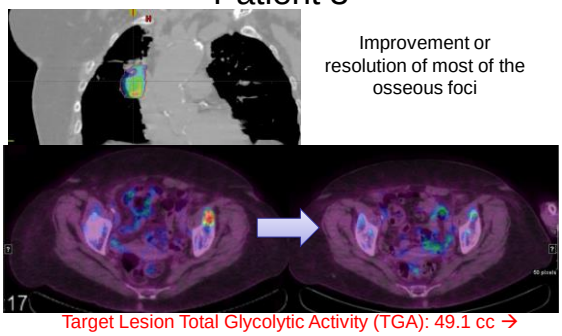


Right lung mass reduced from 5.0 cm (maximum SUV 10.7) to 2.1 cm (maximum SUV 6.5) on first post-treatment PET/CT

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

Other lesions' TGA: 44.7 cc → 4.5 cc

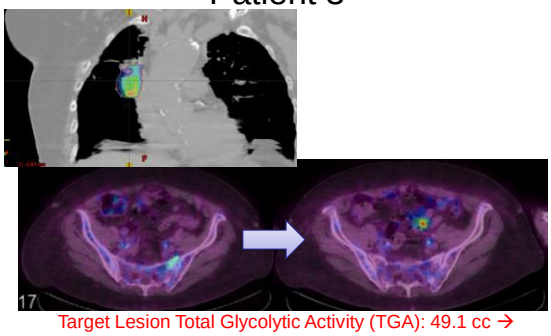
Patient 3



Improvement or resolution of most of the osseous foci

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

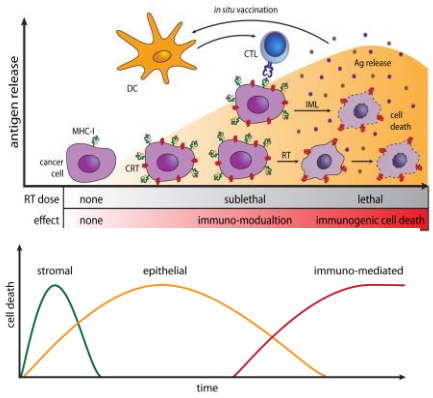
Patient 3



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

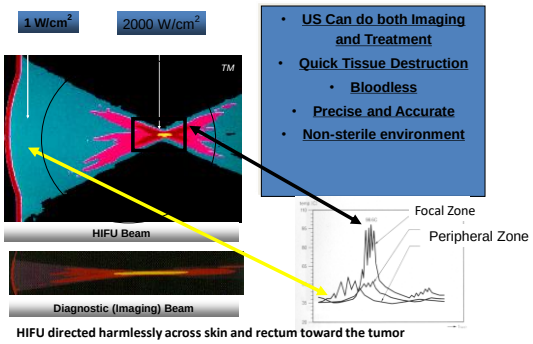
Energy activated *in situ* Tumor Vaccines POC Studies

Energy Immunotherapy		Tumor Models	End Points	Limitation
Single Fraction SFRT 25-60 Gy	Flt3L + / - CD40L	• Lewis Lung 3LL in C57/Bl6 • BN1LNE Liver (HCC) in Balb/c	1. Primary Tumor Growth 2. Metastases 3. Survival 4. Immune assays	• Murine Ectopic Transplantation Models • RT Dose • Fractionation • RT to Draining Lymph Node • Break Tolerance to self antigens • Small Animal Treatment • Lack of Immune Surveillance and carcinogenic environment
20 Gy	TLR9 agonist	• Lewis Lung 3LL in C57/Bl6	PTG, Mets, Survival	
10 Gy	Listeria-PSA ADXS31-142	• TRAMPC1 TPSA23 Prostate Cancer	PTG and Immune Assays	
20Gy x 3	PD1-Fc	• Lewis Lung 3LL in C57/Bl6	PTG, Mets, Survival	
LOFU	HIFU	• TPSA23 Prostate	PTG, Immune Assays	
LOFU	SBRT (10 Gy x 3)	• B16 Melanoma	PTG, Mets, Survival Immune Assays	



Acoustic priming

ULTRASOUND -- ADVANTAGES



- US Can do both Imaging and Treatment
- Quick Tissue Destruction
- Bloodless
- Precise and Accurate
- Non-sterile environment

HIFU directed harmlessly across skin and rectum toward the tumor

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

The "sonic stress" of LOFU

Gene function	Genes affected by LOFU treatment
1. **Protein Folding**	DNAJB1, HSPH1, HSPE1, HSPB1, HSPD1, HSPA4L, CRYAB, HSPA6, HSPA7, HSP90AA1, HSP90AA4P, DNAJA4, FKBP4, LGSN, PTGES3
2. Cell cycle regulation	IER5, JUN,CACYBP, GPRC5A, 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	NLRC4, ANGPTL4, BAG3
9. Peptidase	NAALADL1, MEP1A, PLOD2

Gene Ontology

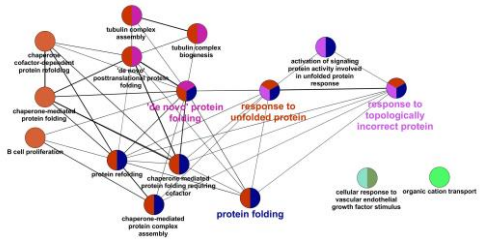


Figure 2 Gene ontology GoTerm network. After LOFU treatment, the gene response showed extensive upregulation of genes that are related to unfolded protein.

Gene Expression with qRT-PCR

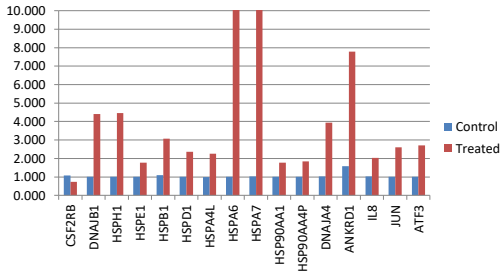
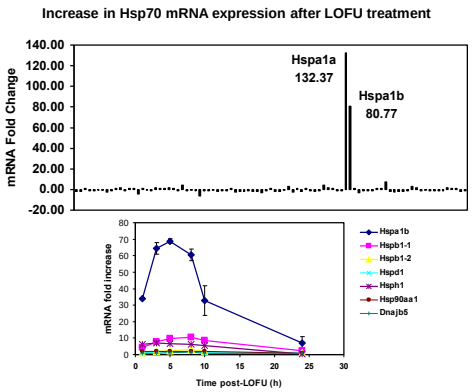
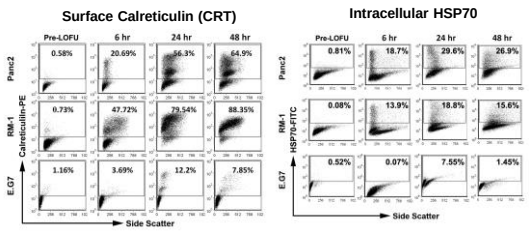


Figure 4 qRT-PCR of select genes from the RNA sequencing. Genes were selected from pathways highlighted in the KEGG pathway analysis and validated using qRT-PCR. The data correlated well with the RNA sequencing results. HSPA6 and HSPA7 were highly regulated to 200+ and 25+ fold respectively.



Immunomodulation of tumor cells



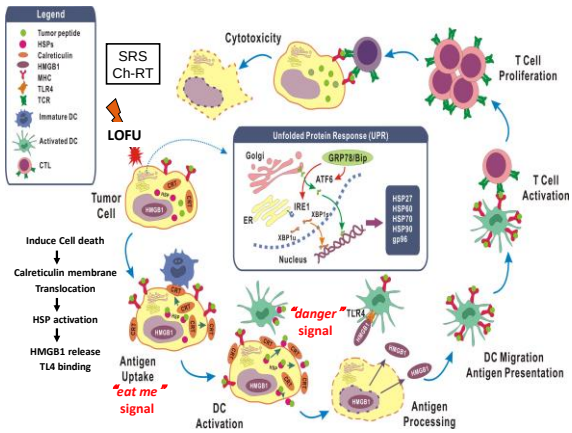
In vitro Effect of LOFU on Cell Surface Markers

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

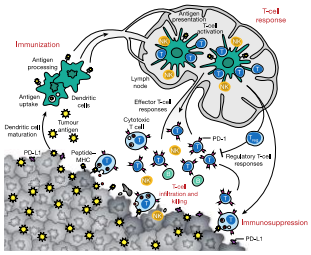
		3 Watt LoFu						5 Watt LoFu					
		3LL		4T1		TPSA23		3LL		4T1		TPSA23	
		6h	24h	6h	24h	6h	24h	6h	24h	6h	24h	6h	24h
ER Stress	HSP70												
	CRT												
	BIP												
Co-Stimulatory	MHC I												
	CD86												
Death Receptors	Fas												
	CD40												
Inhibitory	PD-L1												

0 5 10 20 30 No change TBD

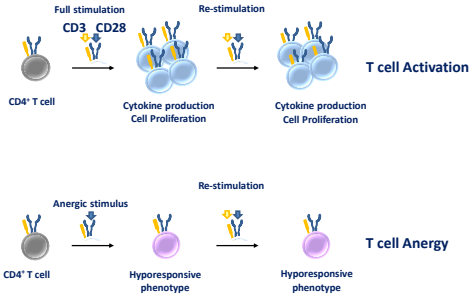


Treat the tumor draining lymph node (TDLN – immune privilege site)

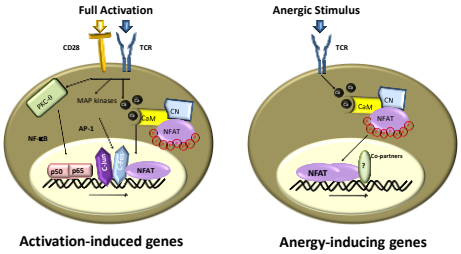
- Reprogram tolerogenic DCs (IDO inhibitors)
- Inhibit regulatory T cells (Treg)
- Reverse T-cell anergy in TDLN – LOFU primary tumor



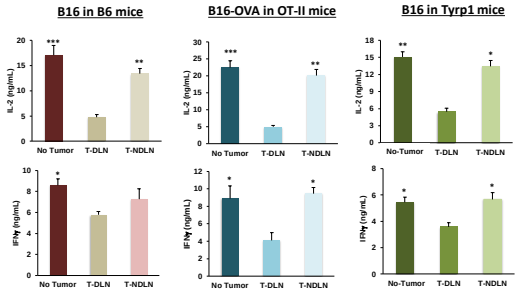
T cell activation vs. T cell anergy



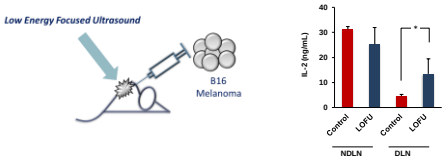
T cell Activation vs. T cell Anergy



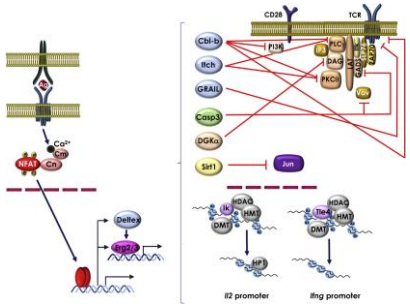
B16 tumors induced CD4+ T cell anergy



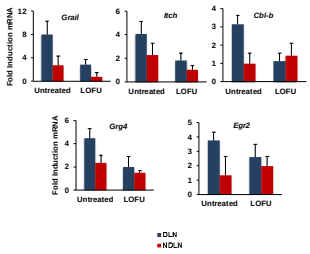
LOFU prevents B16-induced CD4+ T cell anergy



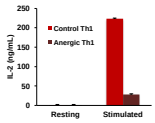
T cell anergy: transcriptional program



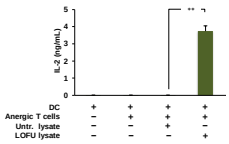
LOFU prevents B16-induced CD4+ T cell anergy



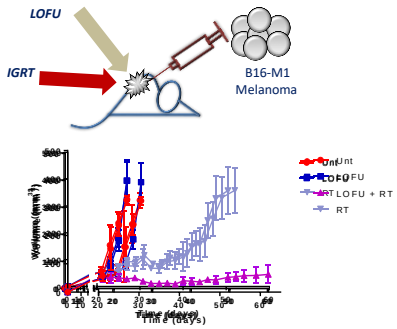
LOFU can reverse established T cell anergy



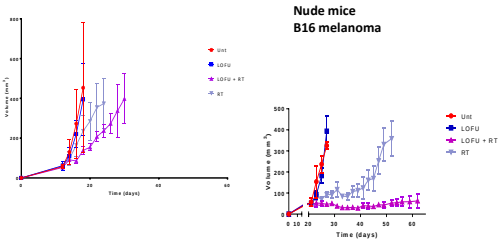
Tyrp1 CD4 Th1 cells
Anergized in vitro



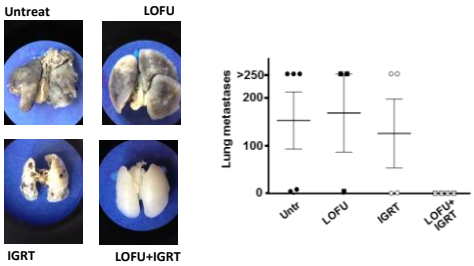
LOFU as immune adjuvant for IGRT



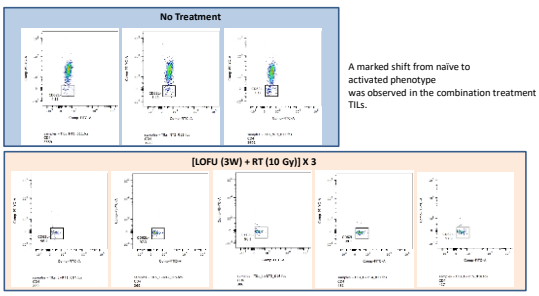
LOFU as adjuvant for IGRT requires T cells



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

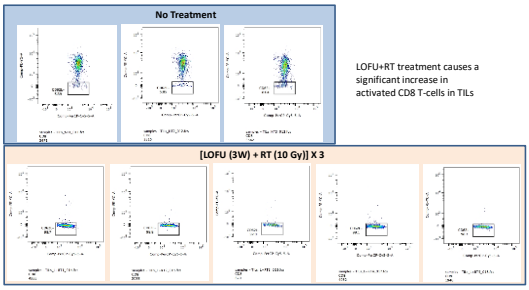


$CD62L^{-VE}/CD4^{+VE}$ population in Tumor infiltrating lymphocytes (TILs)



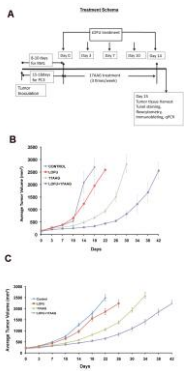
A marked shift from naïve to activated phenotype was observed in the combination treatment TILs.

CD62L^{-VE}/CD8^{+VE} in Tumor infiltrating lymphocytes (TILs)



LOFU+RT treatment causes a significant increase in activated CD8 T-cells in TILs

www.impactjournals.com/oncoscience/ Oncoscience 2014, Vol.1, No.6



Low intensity focused ultrasound (LOFU) modulates unfolded protein response and sensitizes prostate cancer to 17AAG

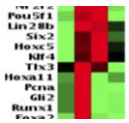
Subhrajit Saha[1], Payel Bhanja[1], Ari Partanen[2], Wei Zhang[3], Laibin Liu[1], Wolfgang A. Tomé[1,4] and Chandan Guha[1,3,4]

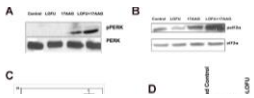
[1] Department of Radiation Oncology, Albert Einstein College of Medicine, Bronx, New York, USA
[2] Philips Healthcare, Cleveland, OH, USA
[3] Department of Pathology, Albert Einstein College of Medicine, Bronx, NewYork, USA
[4] Montefiore Medical Center , New York, NY, USA

Correspondence to: Chandan Guha, **email**: cguhamail@gmail.com

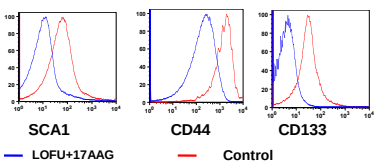
Keywords: Prostate Cancer, 17AAG, Focused Ultrasound, LIFE, ER stress

Received: April 30, 2014 **Accepted**: June 02, 2014 **Published**: June 03, 2014



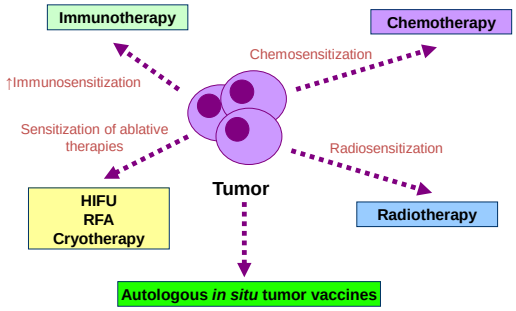


LOFU+17AAG reduces the expression of prostate cancer stem cell marker



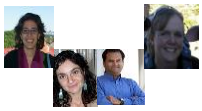
Did the number of cells go down or expression?

Acoustic Priming



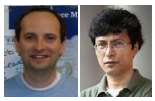
The FOCAL Team

Guha Laboratory
Students.:
Lorenzo Agoni
Karin Skalina
Talicia Savage
Justin Tang
Research Associates:
Huagang Zhang
Associates:
Indranil Basu
Lisa Scandiuzzi



Clinical Trials
Rafi Kabariti
Nitin Ohri
Madhur Garg

Immune Tolerance
Sanmay Bandyopadhyay
Fernando Macian



Medical Physics
Assoc.: Patrik Brodin
Director: Wolfgang Tome

Immune Therapeutics
Steve Almo

Thank you!

Montefiore
Inspired Medicine

Institute for Onco-Physics
Albert Einstein College of Medicine