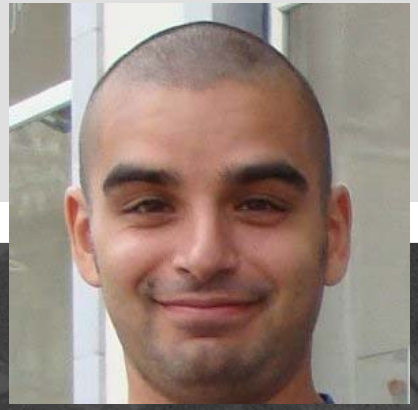
Microfluidics™

ALBANY COLLEGE OF PHARMACY AND HEALTH SCIENCES

WEBINAR: MICROFLUIDIZER: A POTENTIAL TOOL FOR PROCESS DEVELOPMENT TO MANUFACTURE NANOTETRAC FOR PHARMACEUTICAL APPLICATIONS



Steven Mesite
Dir. Inside Sales and Apps.
+1-781-708-0304
smesite@idexcorp.com

Dhruba J. Bharali, Ph.D.
Assistant Dir. of Nanotechnology
+1-518-694-7561
Dhruba.Bharali@acphs.edu

QUADRO FITZPATRICK MATCON ISO 9001:2015 IDEX MATERIAL PROCESSING

COMPANY PROFILE

- Microfluidics was founded in 1982 to produce **high shear** fluid processors using **interaction chamber** technology.
- Headquartered outside of Boston, MA with localized support in **47 countries**. Over **4000** processors sold to **2000** companies.
- Acquired by **IDEX** Corporation (NYSE: IEX) and grouped with **Quadro Engnineering, Fitzpatrick** and **Matcon** to form the **Materials Processing Technologies** Platform.
- Microfluidizer Processors are used for **R+D** and **manufacturing** of active pharmaceutical ingredients, vaccines, inkjet inks, coatings, nutraceuticals and cosmetics.
- Microfluidics has vast **applications** and **machine design** experience.
- **Our customer's success is our success.**

WHAT WE DO BEST

- Nanoemulsions
- Cell disruption
- Polymer nanoparticles
- Liposomes
- Particle size reduction
- Deagglomeration
- MW weight reducution

Customer Testimonial

"The overall satisfaction which we experienced with our laboratory model Microfluidizer processor eliminated the need to consider other equipment when it was time to scale up to production capabilities."

Amylin Pharmaceuticals

M-110P "plug n' play" benchtop lab model

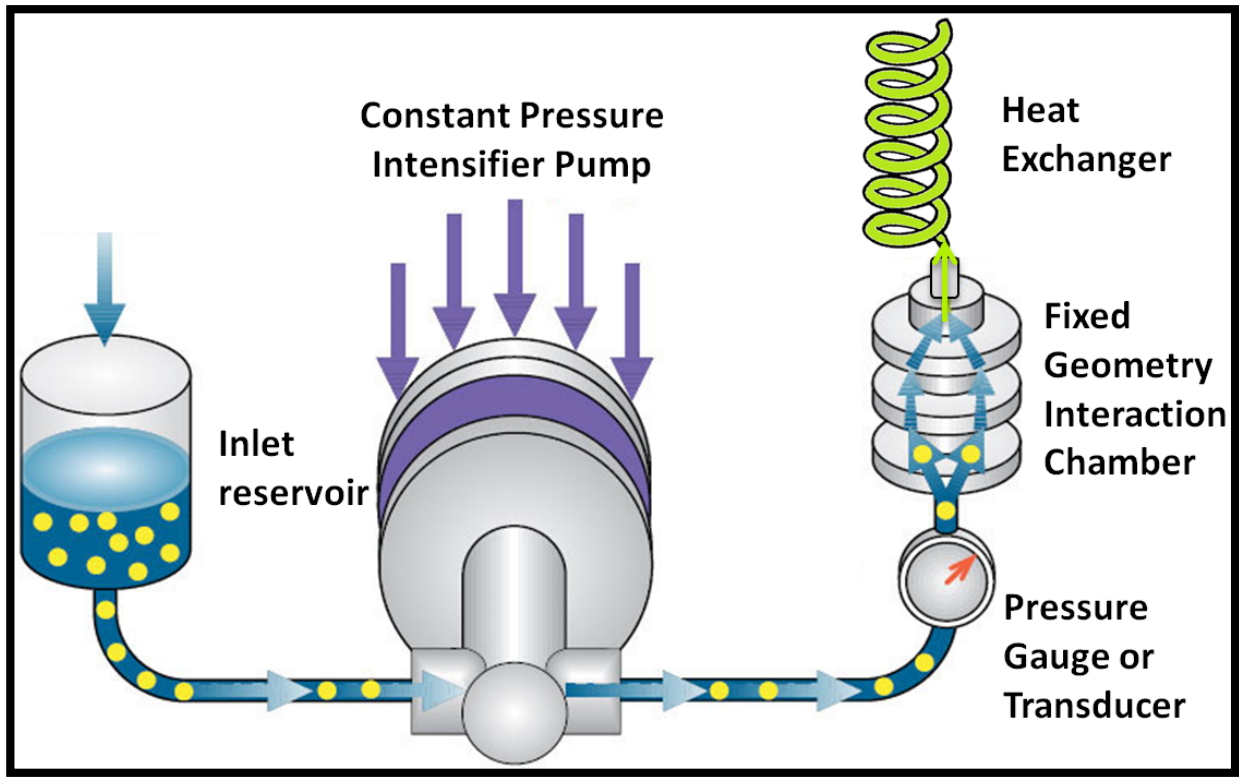
M-110EH-30 pilot scale processor

M-700 series production machine

Fixed-geometry interaction chambers

Microfluidics™

MICROFLUIDIZER SCHEMATIC

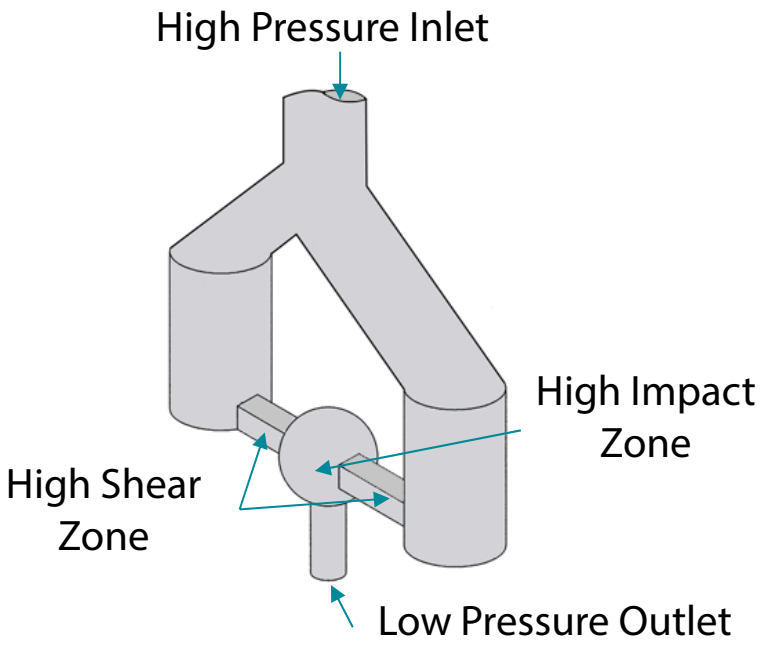


- Continuous Processing
- Can process tricky materials with:
  - High solid content
  - High viscosities
- Can work with a wide range of temperatures
- Cooling occurs quickly

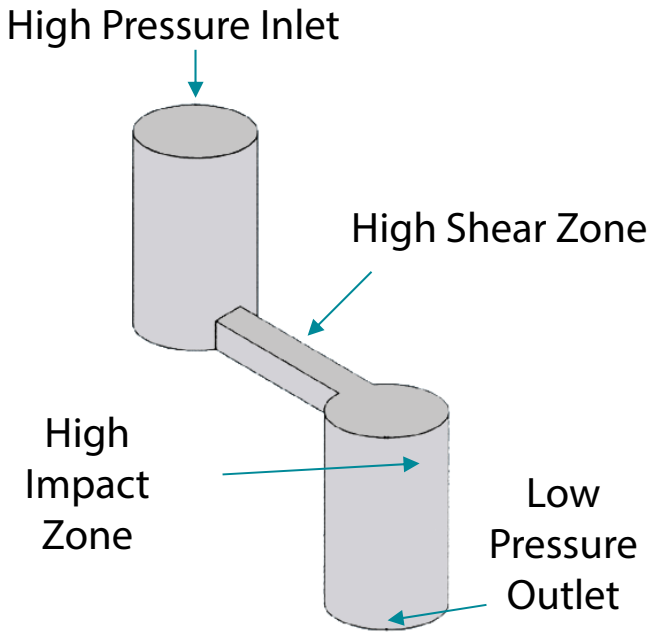
FIXED GEOMETRY INTERACTION CHAMBERS

- **Consistent processing –** Fixed geometry with no moving parts
- **Long-wearing –** Made from diamond or ceramic materials
- **Ease of maintenance –** Clean-in-place and steam-in-place
- **Many options available** – Variable shape and size

"Y"-Type Chamber



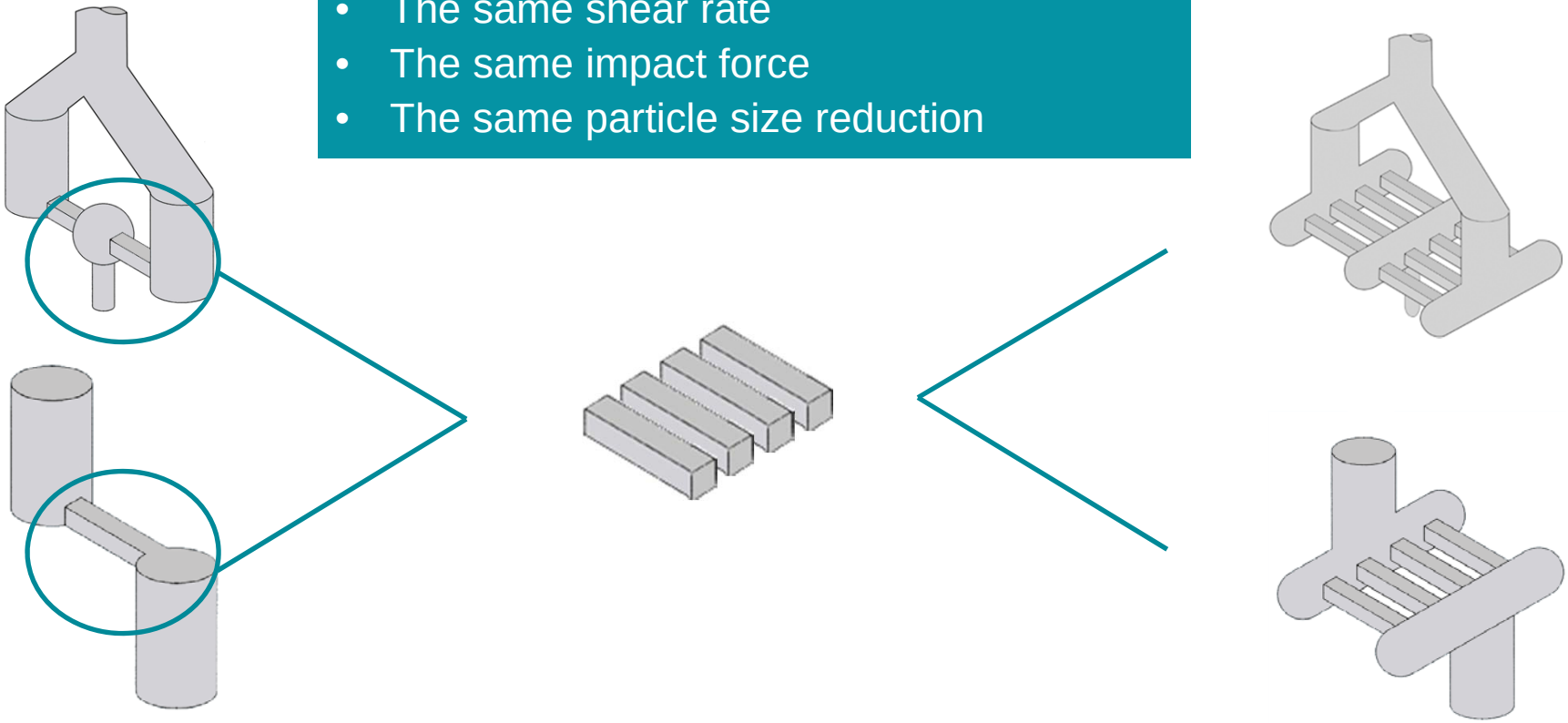
"Z"-Type Chamber



SCALE UP

Identical shaped microchannels ensure:

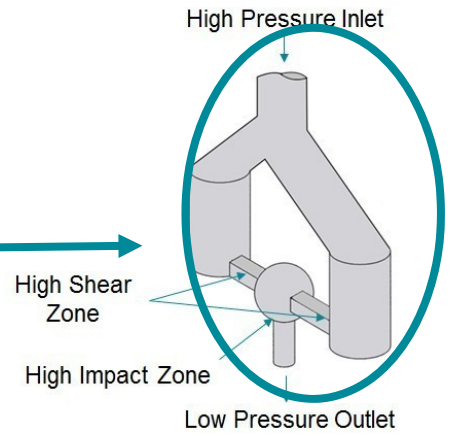
- The same shear rate
- The same impact force
- The same particle size reduction



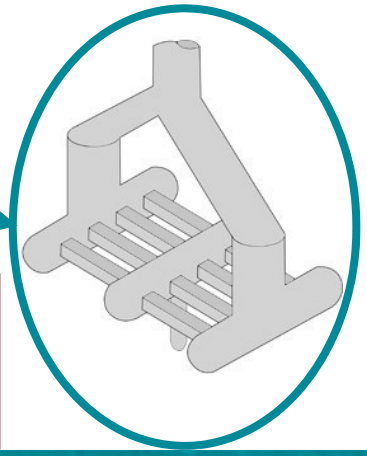
BENEFITS OF MICROFLUIDIZER PROCESSORS

- **How Microfluidics Technology is Unique**
  - > Constant Pressure Processing
  - > High Potential Processing Pressures
  - > Fixed Geometry Interaction Chambers
  - > Multi-Slotted Interaction Chambers

- **Resulting Benefits**
  - > Very small particle size potential
  - > Very consistent processing resulting in very narrow particle size distributions
  - > Guaranteed scale-up from lab scale to production scale



LV1
1 mL hold up volume



M7250-20 Pharmaceutical/ Constant Pressure/SIP
Can process 8 Lpm at 1300 bar

DIVERSE PRODUCT PORTFOLIO

Whatever your batch size, utility, and regulatory requirements, we have a model to suit your needs.

Microfluidizer: A potential tool for process development to manufacture Nanotetrac for Pharmaceutical Applications

Dhruba J. Bharali, Ph.D.

Assistant Director of Nanotechnology
The Pharmaceutical Research Institute
Albany College of Pharmacy and Health Sciences
1 Discovery Drive, Rensselaer, New York-12144
E-mail: Dhruba.Bharali@acphs.edu

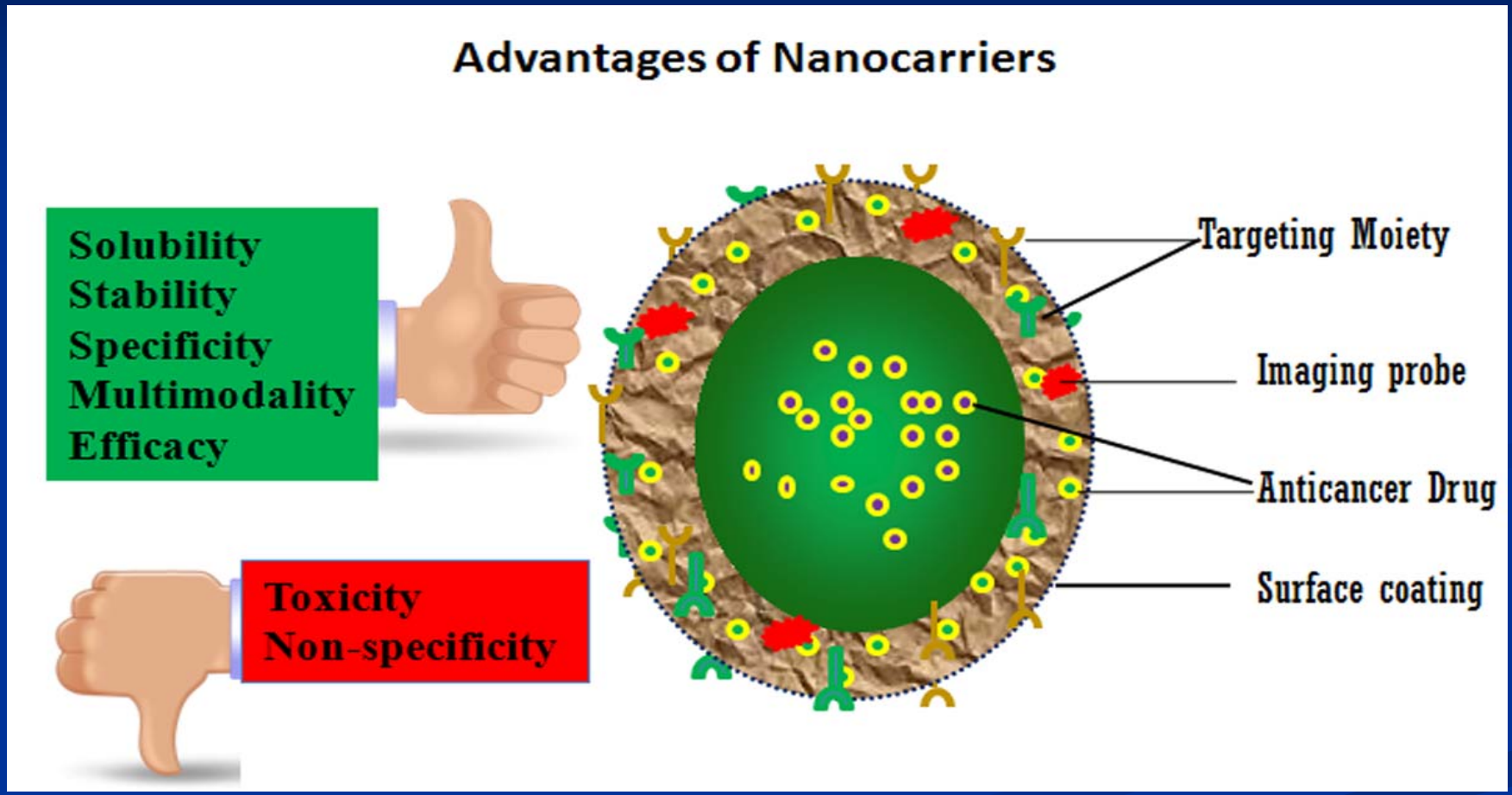
Pharmaceutical Research Institute
ALBANY COLLEGE OF PHARMACY AND HEALTH SCIENCES

EST. 1881
Albany College of Pharmacy
AND HEALTH SCIENCES

Outline of the Presentation

- Introduction to nanotechnology/Nanomedicine
- Nano-conjugated angiogenesis inhibitor
- Scale up of the nanofomulations
- In vitro efficacy study
- In vivo efficacy study
- Summary

Why Nano?



Dhruba J. Bharali, Imtiaz A. Siddiqui, Vaqar M. Adhami, Jean Christopher Chamcheu, Hasan Mukhtar, and Shaker A. Mousa
Nanoparticle Delivery of Natural Products in the Prevention and Treatment of Cancers: Current Status and Future Prospects, ***Cancers*** 2011, 3, 4024-4045

THE CONCEPT:

Thyroid Hormone

Major Advantage:

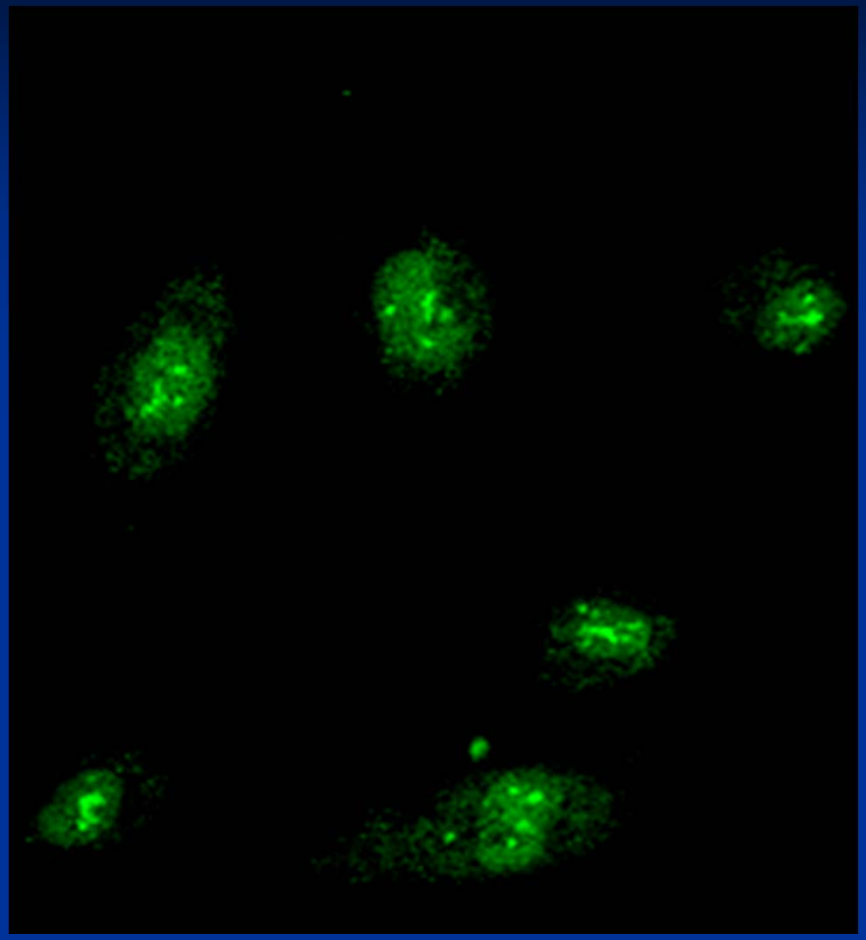
In cancer Patients Lowering thyroid hormone L -T3 (or perhaps the use of thyroid antagonist) improve survival rate (breast cancer, lung, glioblastoma & many others)

Major Disadvantage:

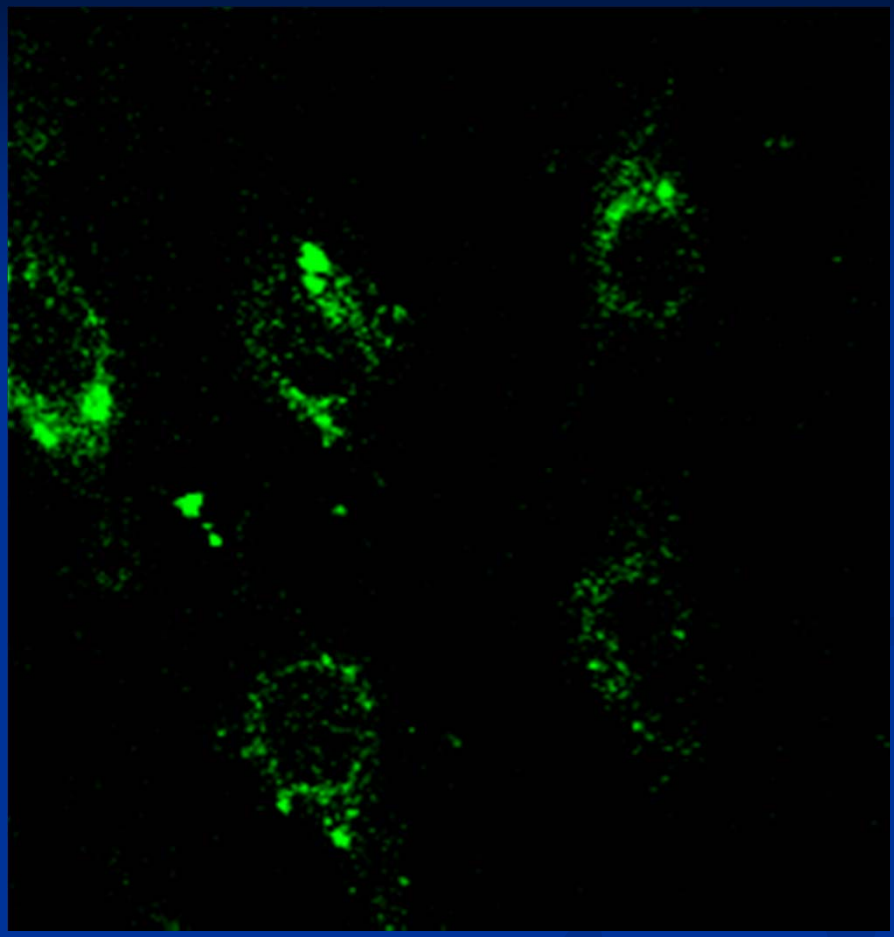
Can exert genomic effects due to its nuclear binding capabilities

Hypothesis: Nanoparticles can restricts it from entering nucleus, while retaining anticancer activities

Blocking Thyroid hormone from entering nucleus



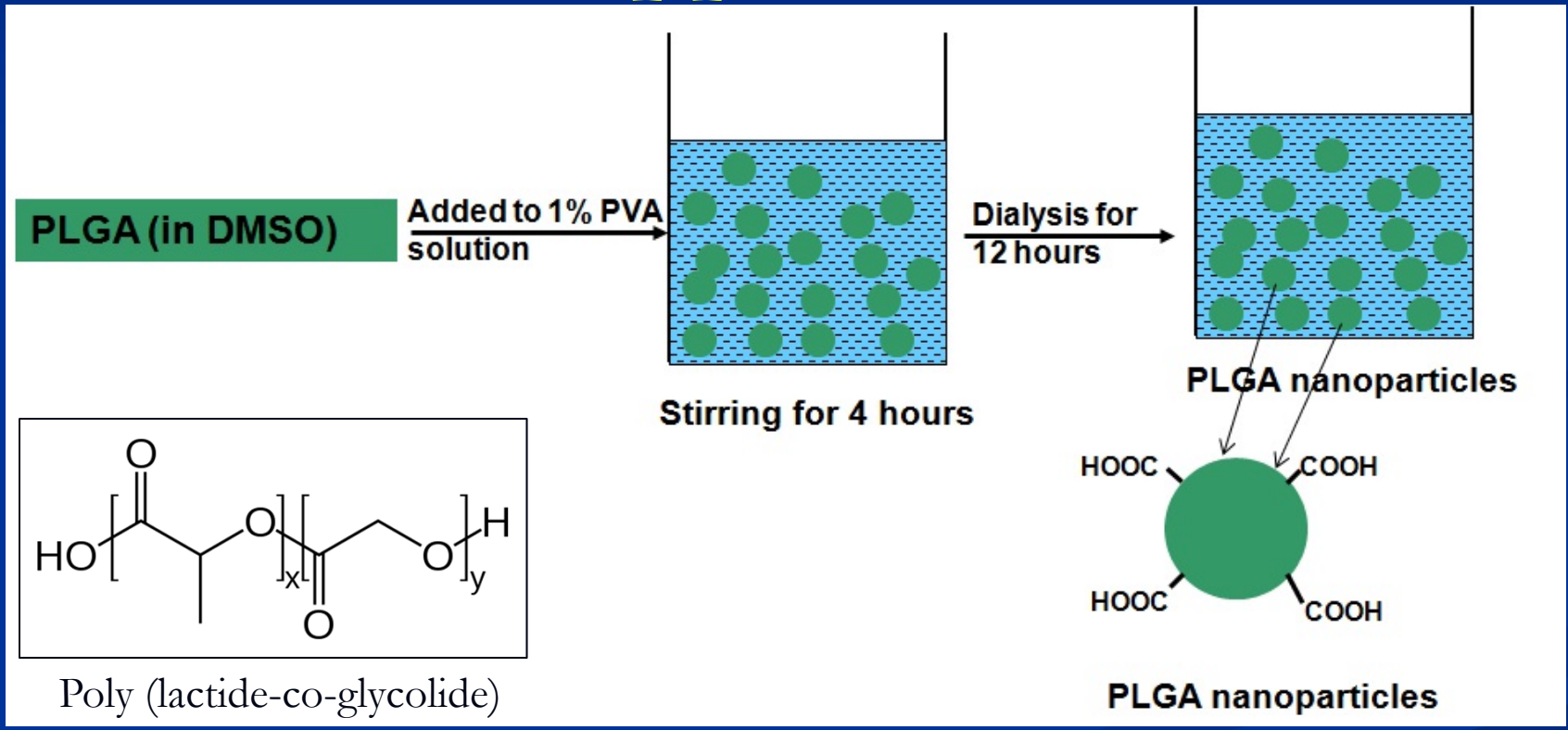
Confocal Imaging of Alexa flour labeled Thyroid Hormone (HDMEC cell)



Confocal imaging of Alexa flour labeled Thyroid Hormone conjugated nanoparticles (HDMEC cell)

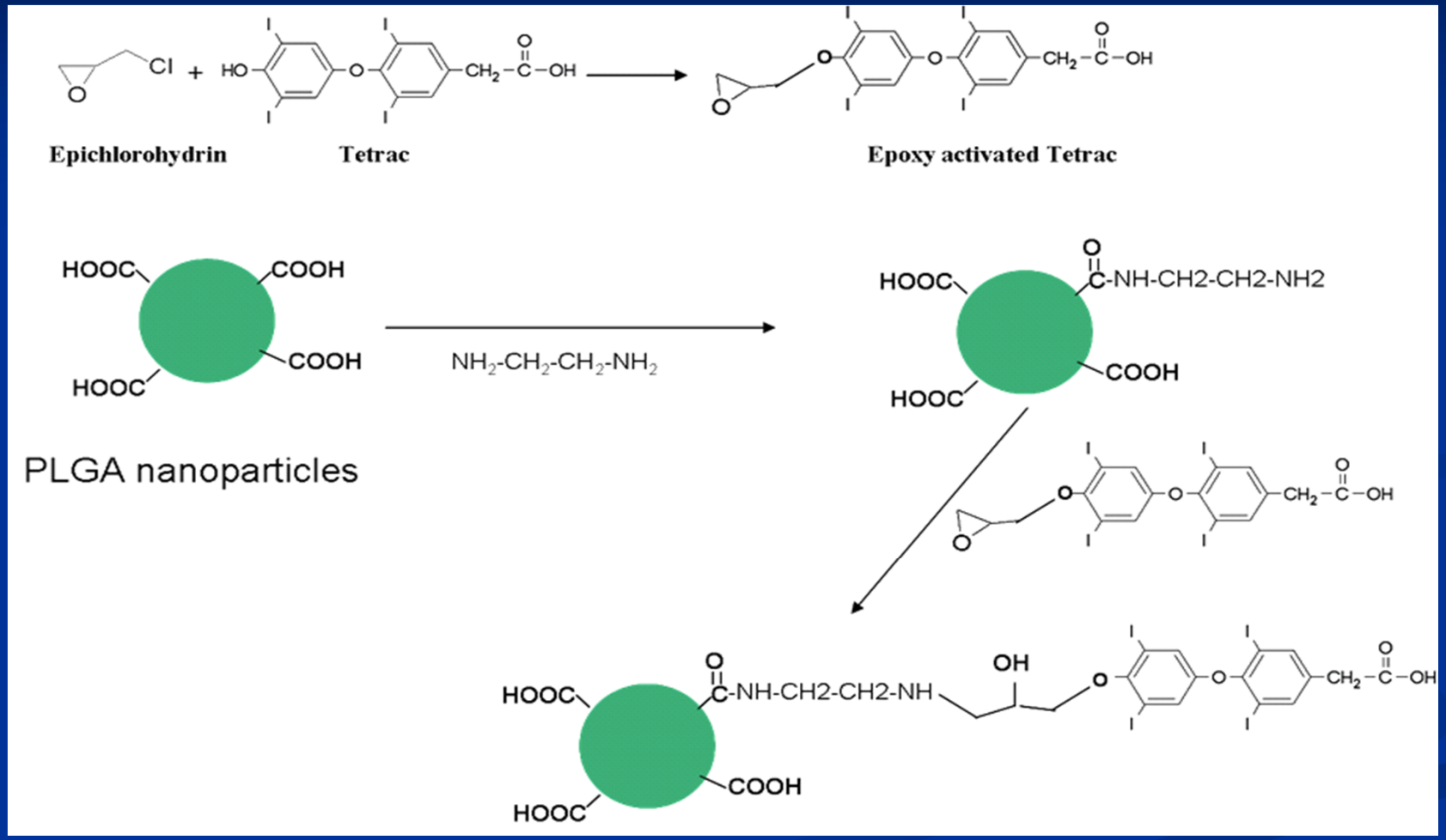
Bharali, D. J., Yalcin, M., Davis, P. J., and Mousa, S. A. (2013) Tetraiodothyroacetic acid-conjugated PLGA nanoparticles: a nanomedicine approach to treat drug-resistant breast cancer, *Nanomedicine 8*, 1943-1954

Synthesis of PLGA Nanoparticles: The Conventional Approach



Schematic Diagram showing the synthesis of PLGA nanoparticles

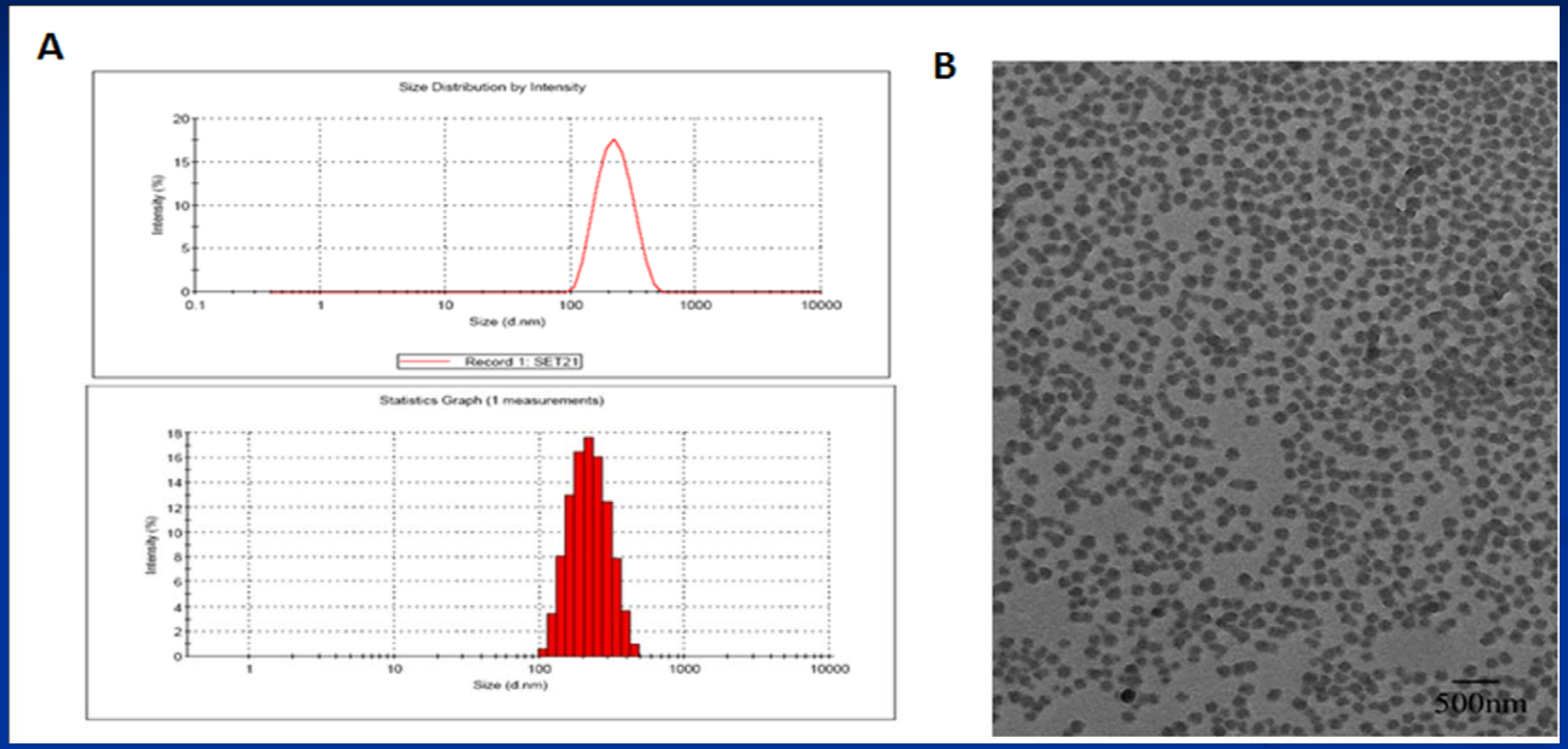
Thyroid hormone Conjugated Nanoparticles for Cancer treatment



Chemical reactions showing the Conjugation of Tetrac to PLGA nanoparticle

Yalcin M, Bharali DJ, Lansing L, Dyskin E, Mousa SS, Hercbergs A, Davis FB, Davis PJ, Mousa SA.. *Anticancer Res.* 2009 Oct;29(10):3825-31

Thyroid hormone Conjugated Nanoparticles for Cancer treatment



Analysis of T-PLGA-NPs by A) dynamic light scattering and B) TEM (Transmission Electron Microscope)

Dhruba J Bharali, Murat Yalcin, Paul J Davis PJ, Mousa SA.. Nanomedicine (Lond). 2013 Dec;8(12):1943-54.

CANCER TREATMENT

a) Non-small cell lung cancer cells
b) Human follicular thyroid cell carcinoma
c) Medullary carcinoma of the thyroid
d) Renal cell carcinoma
e) Pancreatic cancer
f) Prostate cancer
g) Drug resistant breast cancer cell (MCF7-Dx)

PUBLCATIONS:

1. Dhruba J Bharali, Murat Yalcin, Paul J Davis PJ, Mousa SA. Tetraiodothyroacetic acid-conjugated PLGA nanoparticles: a nanomedicine approach to treat drug-resistant breast cancer. Nanomedicine (Lond). 2013 Dec;8(12):1943-54.]
2. Murat Yalcin, Lin HY, Sudha T, Dhruba J Bharali, Meng R, Tang HY, Davis FB, Stain SC, Davis PJ, Mousa SA., Horm Cancer. 2013 4(3)
3. Mousa SA, Yalcin M, Bharali DJ, Meng R, Tang HY, Lin HY, Davis FB, Davis PJ.. Lung Cancer. 2012 Apr;76(1):39-45.
4. Yalcin M, Bharali DJ, Dyskin E, Dier E, Lansing L, Mousa SS, Davis FB, Davis PJ, Mousa SA. Thyroid. 2010 Mar;20(3):281-6.
5. Yalcin M, Dyskin E, Lansing L, Bharali DJ, Mousa SS, Bridoux A, Hercbergs AH, Lin HY, Davis FB, Glinsky GV, Glinskii A, Ma J, Davis PJ, Mousa SA.. J Clin Endocrinol Metab. 2010Apr;95(4):1972-80.
6. Yalcin M, Bharali DJ, Lansing L, Dyskin E, Mousa SS, Hercbergs A, Davis FB, Davis PJ, Mousa SA.. Anticancer Res. 2009 Oct;29(10):3825-31.

NOW WHAT ??

Scale up of the nanoformulations for pre-clinical / clinical Studies/manufacturing ??

Let's synthesize nanoparticles containing 100 g Tetrac equivalent

For 100g T-eq.
We need a Volume
of 6666L

Volume 10 L
T-eq.= 150 mg

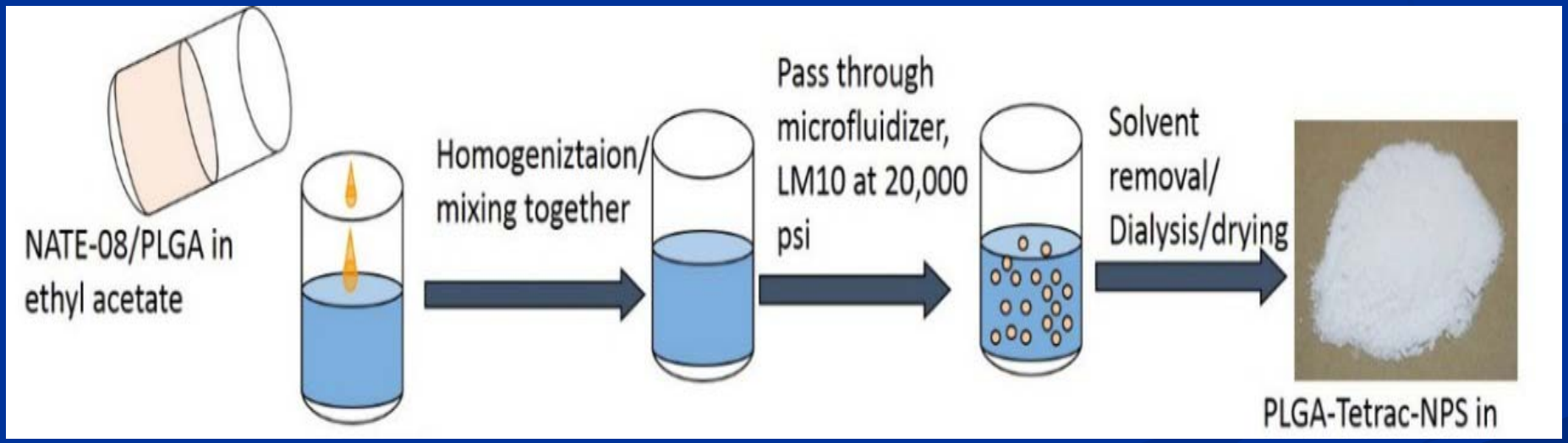
Volume 0.5 L
T-eq.= 7.5 mg

Volume 10ml
T-eq.= .15 mg

BACK TO WORK AGAIN!!!!

THE MACHINE USED to SYNTHESIS NANOPARTCLES

Development of Technique to Synthesis of Tetrac conjugated PLGA Nanoparticles (NDAT) in large scale.



THE COMPARISON

Original Methods Need a volume **around 6666L**

NEW Methods Need a volume around 85L

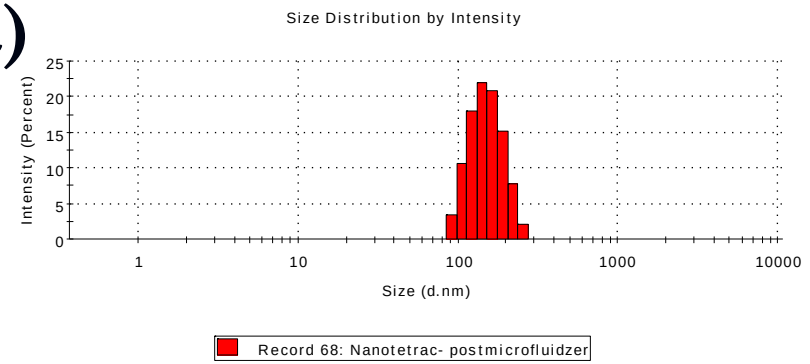
Characterization of Nanotetrac prepared by using Microfluidizer

Technique Use to Characterize Nanotetarc

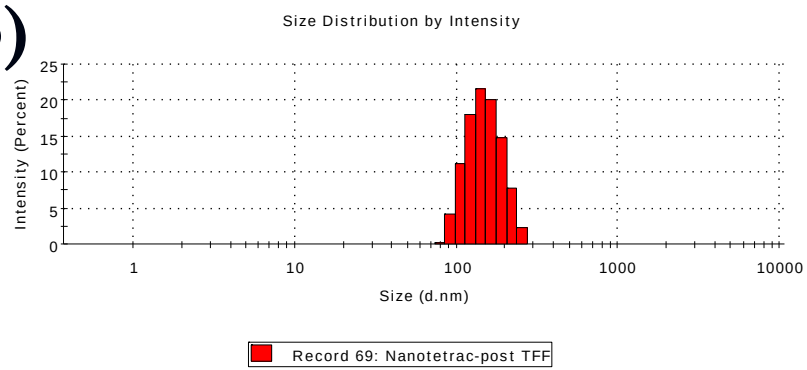
1. Analysis of Tetrac equivalent by UV-spectrophotometer
2. Size analysis by TEM
3. In vitro cellular efficacy
4. In vivo efficacy animal tumor model
5. IVIS Imaging
6. LC/MS

Size Measurement of Nanotetrac

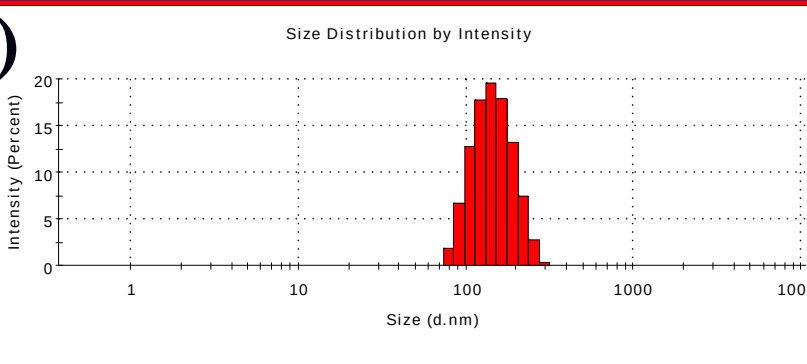
a)



b)

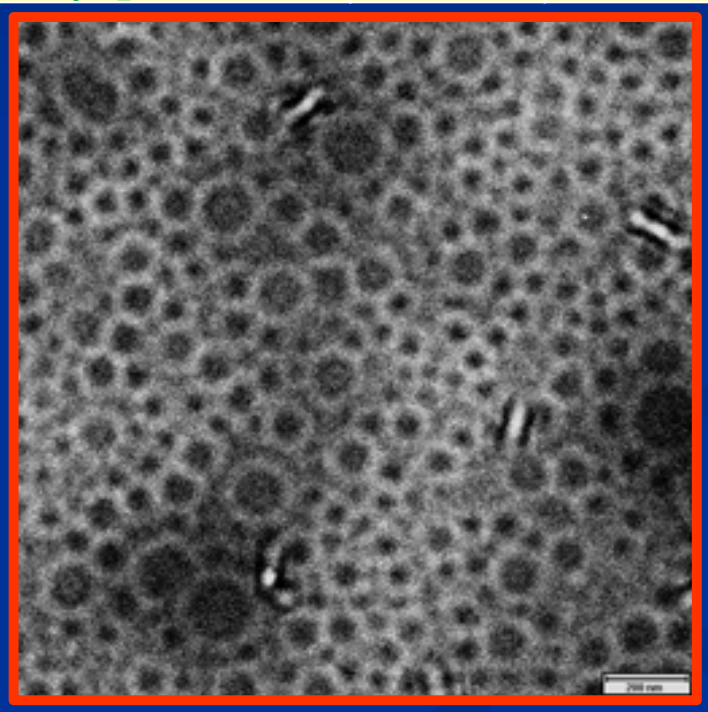


c)



Size measurement by DLS

NanoTetrac	Z-Ave (d.nm)	PdI
a)Post microfluidzer	144.5	0.046
b)Post TFF	143.9	0.036
c)Post lyophilization	138.8	0.052



TEM of Nanotetrac

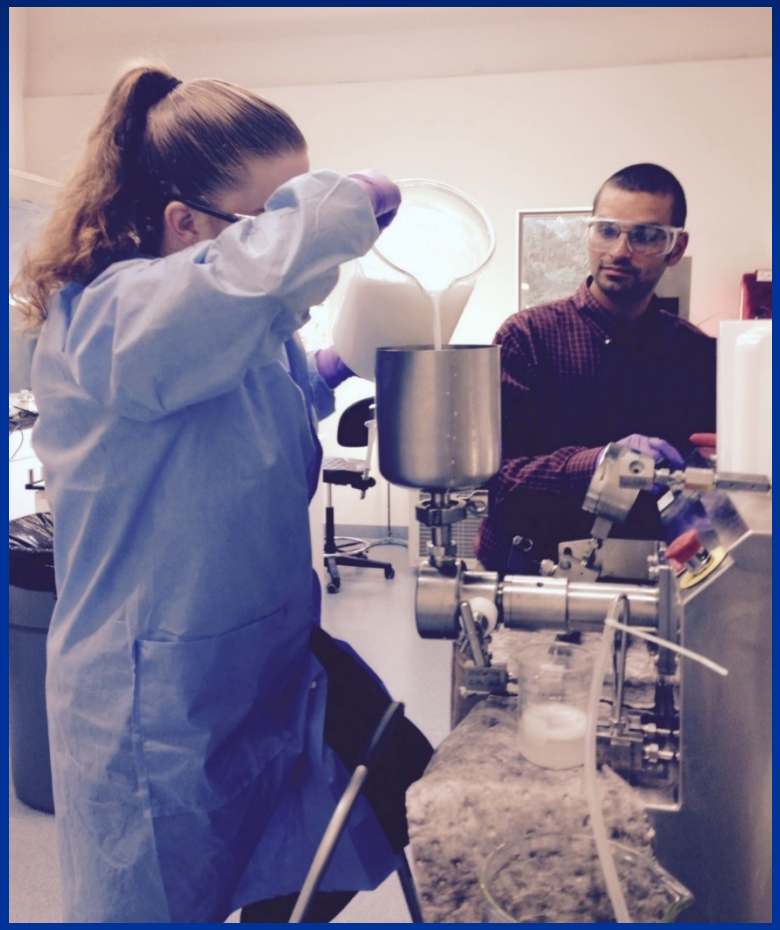
T Sudha, DJ Bharali, M Yalcin, NHE Darwish, M Debreli-Coskun, Q Lin, K Keating, PJ Davis, SA Mousa Manuscript submitted to Nanomedicine 2016

A Trip to Microfluidics Laboratory at Boston, MA

Trip1:
Proof of concept of feasibility of synthesis of Nanotetrac particles using LM10 Microfluidizer

Trip 2:
Feasibility of scalability of the process (using different type of Microfluidizer)

Synthesis of nanoparticles at microfluidics facility using Microfluidizer (Model M-110EH-30K)



Different parameters that effect the Synthesis of Nanotetrac

- Number of Pass
- Pressure
- Type of chamber

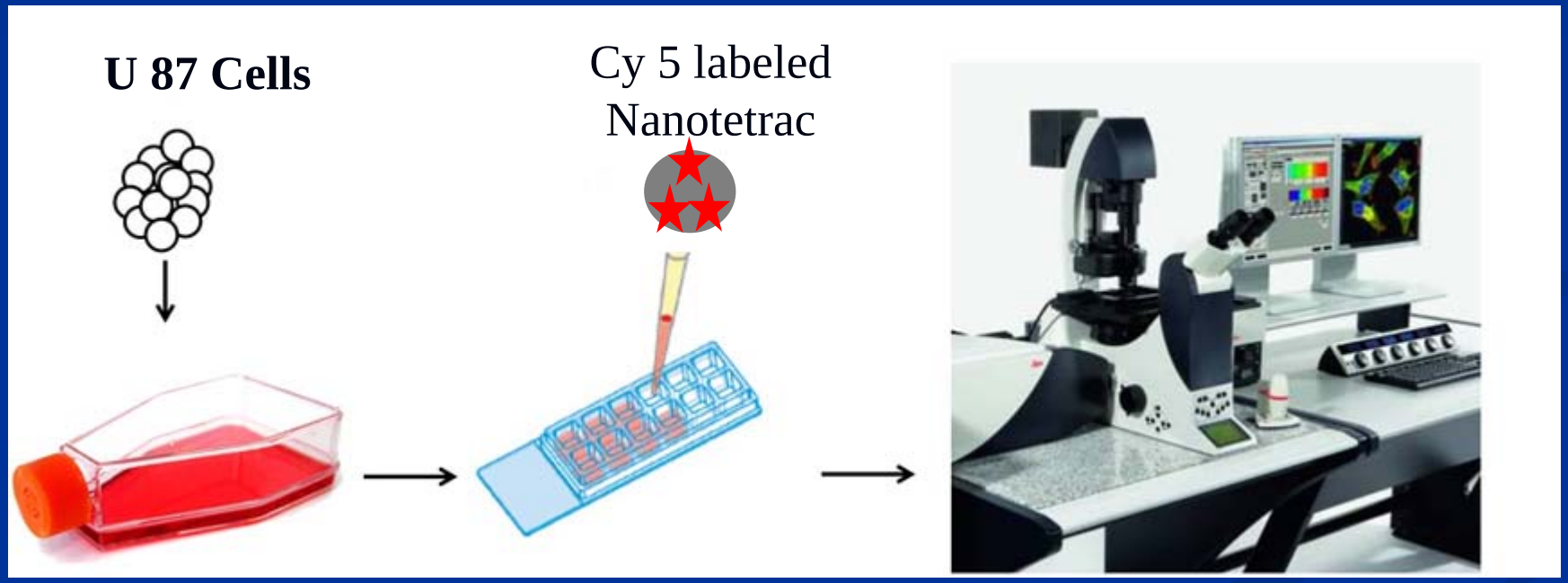
Synthesis of Nanotetrac using in different Type of Microfluidizer

Amount of Starting materials	Total liquid volume (batch size)	Type Microfluidizer used	Size in (nm)
1g	30ml	LM10	~150
2g	60ml	LM10	~150
5g	150ml	LM10/M-110EH-30K	~150
10g	300ml	LM10/M-110EH-30	~150
20g	600ml	LM10	~150
100g	3L	LM10/M-110EH-30	~150

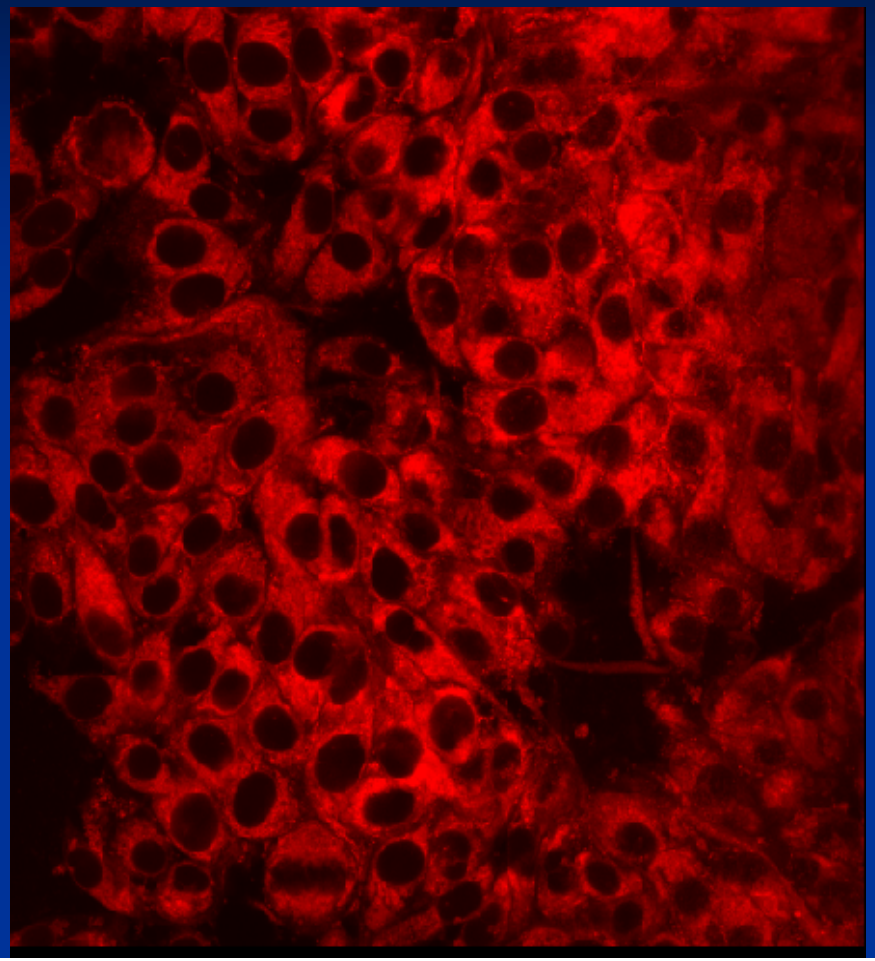
Nanotetrac has been designated by FDA as Orphan Drug Designation for the following cancer

1. Glioblastoma
2. Pancreatic Cancer

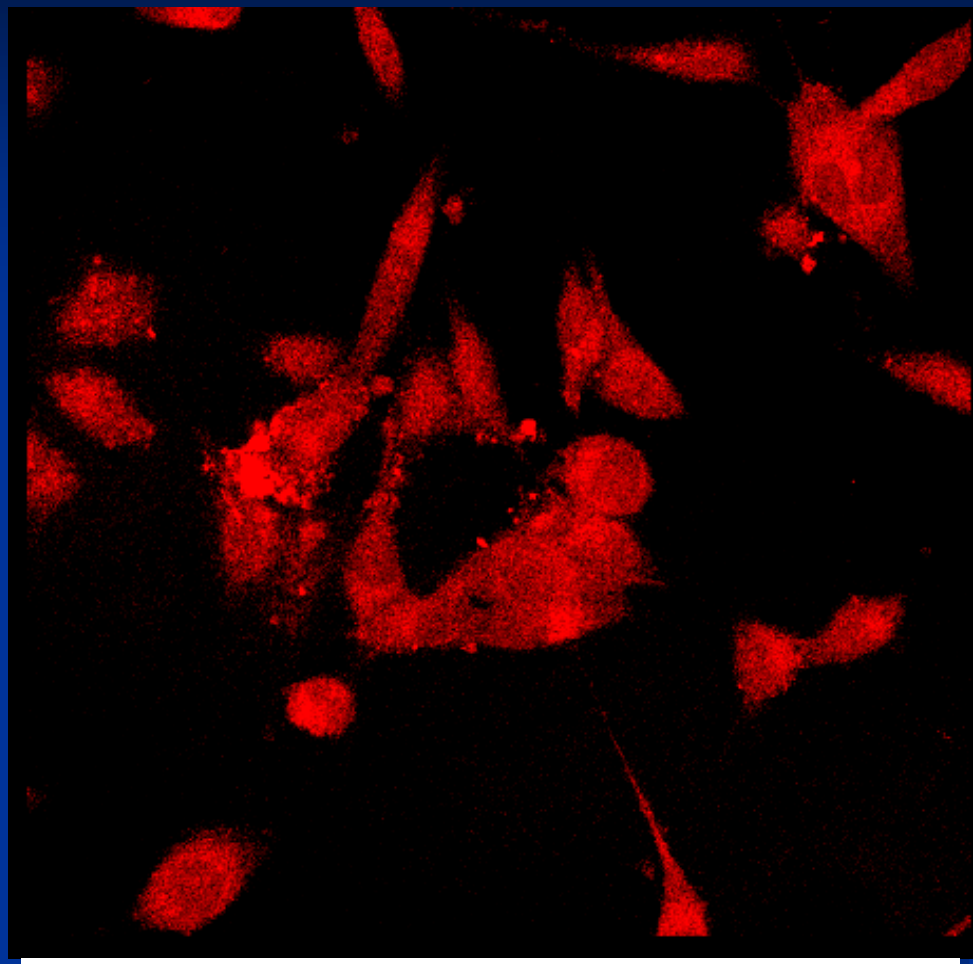
In vitro uptake study of the dye labeled Nanotetrac



Proof of our concept: Blocking Tetrac from entering to the nucleus



Nanotetrac Labled with Cy5



Tetrac Labeled with Cy5

Figure: Confocal Imaging showing the uptake of Tetrac and Naotetrac Labeled Cy5 in U87 cells

Activity of Nanotetrac Against Preclinical Models of Glioblastoma

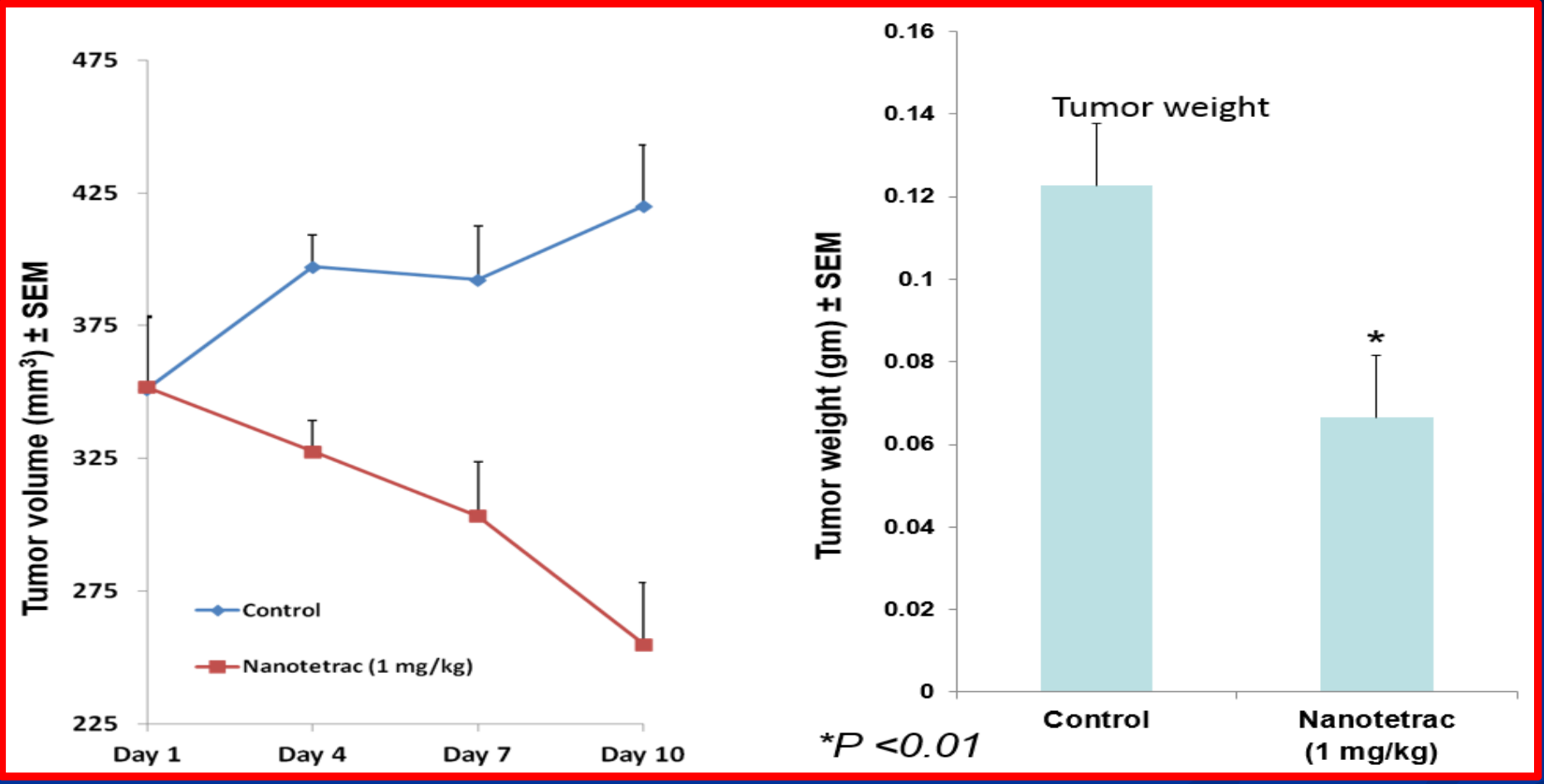
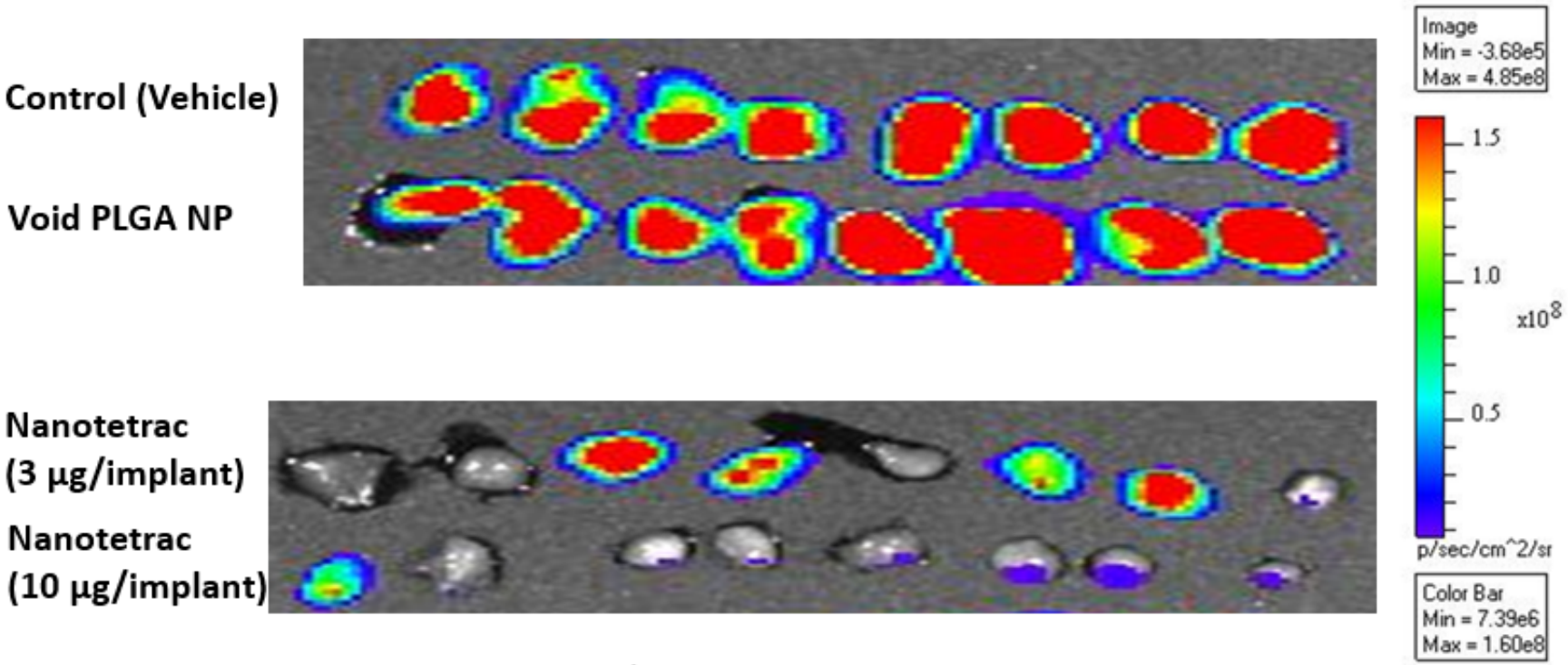


Figure: Effect of daily s.c. Nanotetrac on human U87MG glioblastoma xenograft volume and weight after 10 days of treatment.

T Sudha, DJ Bharali, M Yalcin, NHE Darwish, M Debreli-Coskun, Q Lin, K Keating, PJ Davis, SA Mousa, 2017 Manuscript under preparation

Effect of single dose Nanotetrac vs. tetrac on human U87MG-luc xenograft luminescent signals of implants in nude mice after 16 days.



Nanotetrac at 10 mg/implant approximates 10^{-7} M tissue tetrac equivalent drug concentration.

Induction of necrosis by daily Nanotetrac dose

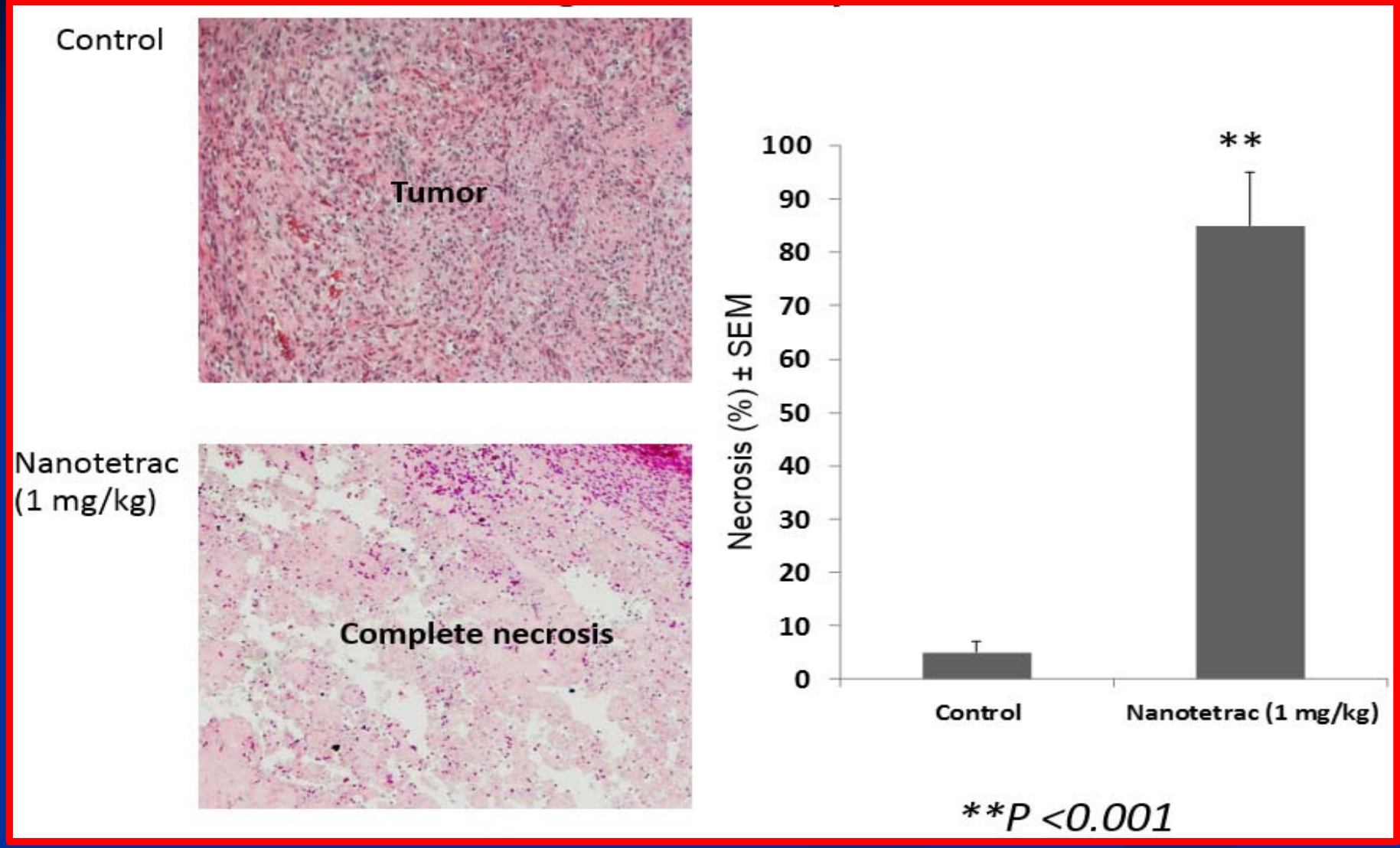
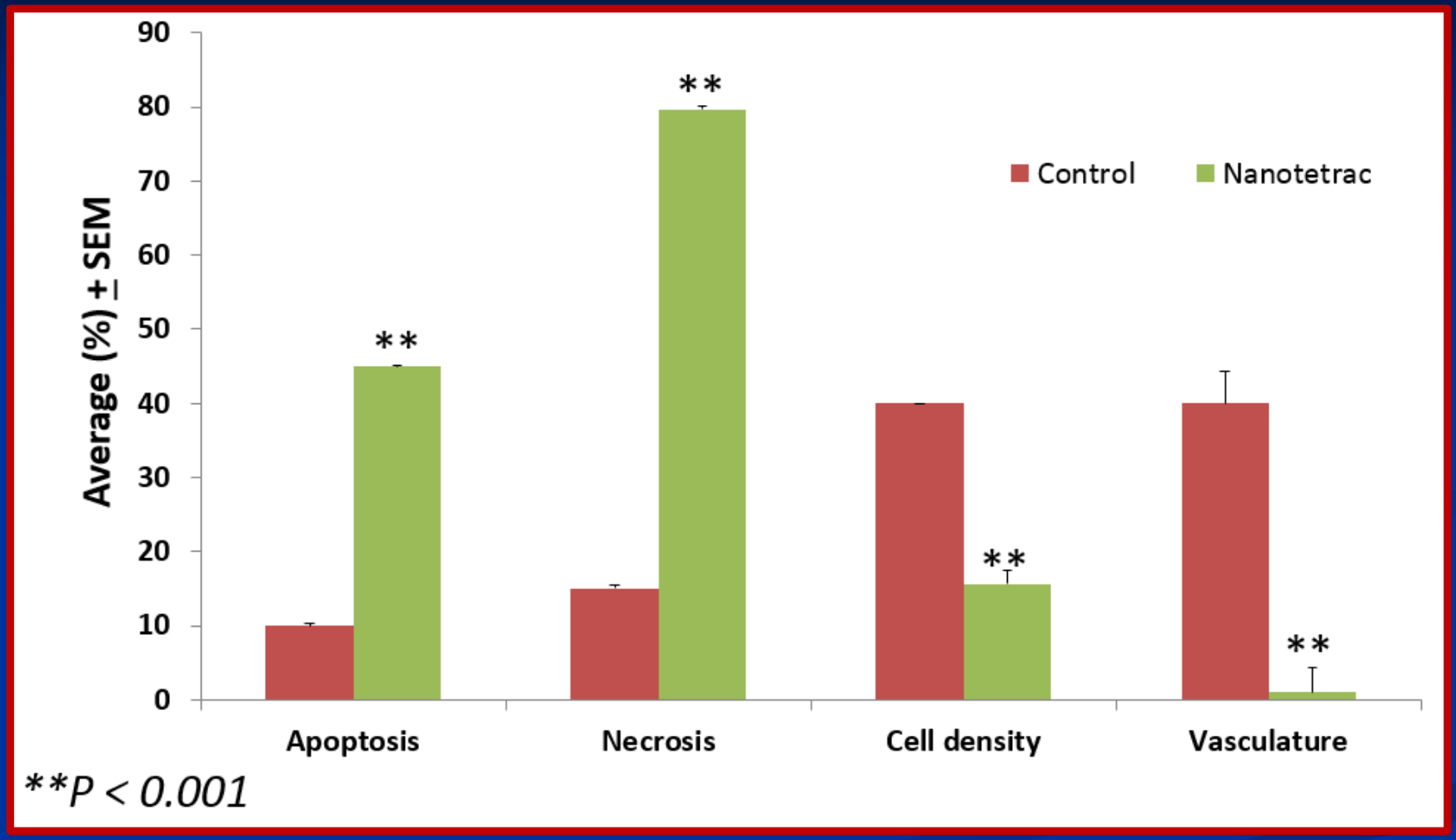


Figure: Induction of necrosis by daily s.c. Nanotetrac in U87MG glioblastoma xenografts x10 days

Effect of Nanotetrac on apotosis and necrosis



Daily s.c. Nanotetrac treatment increases apoptosis and necrosis and decreases cell density and vasculature in U87 xenografts.

Effect of Nanotetrac on Pancreatic Cancer mice tumor model

Control (PBS)

Nanotetrac (0.1 mg/kg)

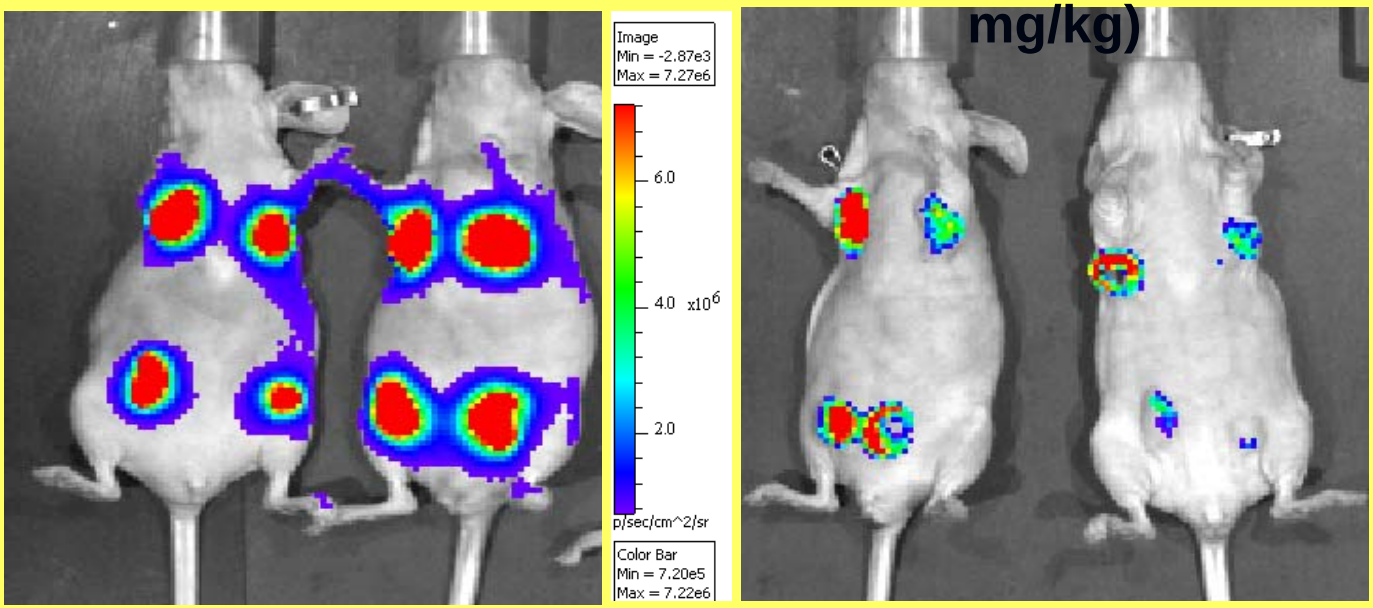


Figure: Intra-tumor treatment effect on Pancreatic xenograft (MPanc96-luc) after 3 injections *IVIS images*

NEXT STEP ???

TECHNOLOGY TRANSFER TO A GMP Facility for manufacturing is

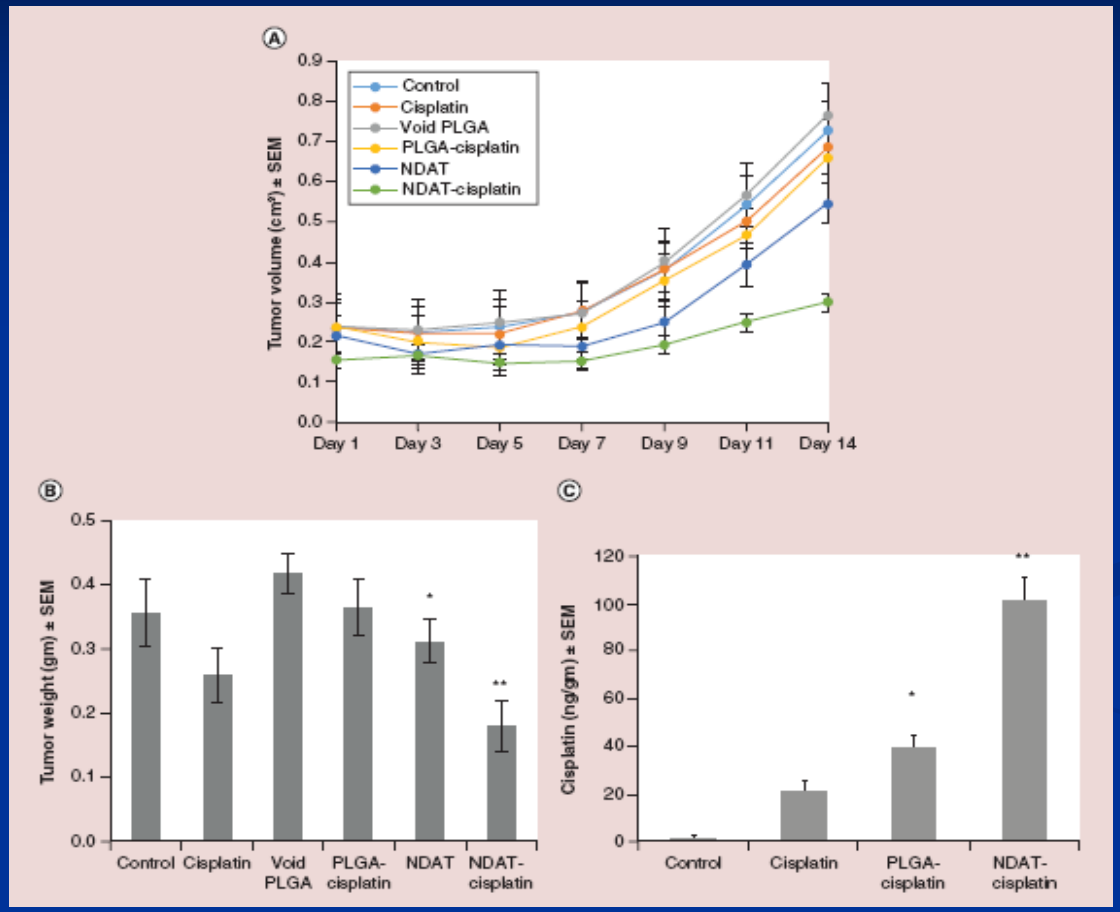
IN PROGRESS!!!

Targeted delivery of chemotherapeutic agents to solid tumors via systemic Nano-diamino-tetrac

1. Cisplatin
2. Doxorubicin
3. Paclitaxel
4. Temozolomide

T. Sudha, D. J. Bharali, N. H. Darwish, M. Debreli-Coskun, Q. Lin, P. J. Davis, et al., Targeted delivery of chemotherapeutic agents to solid tumors via systemic Nano-diamino-tetrac. *Cancer research.* 2016;76:2166-2166

Effect of NDAT (nanotetratc) encapsulating cisplatin in Bladder Cancer



Thangirala Sudha, **Dhruba J. Bharali**, Murat Yalcin, Noureldien H. E. Darwish, Melis Debreli Coskun, Kelly A. Keating, Hung-Yun Lin, Paul J. Davis, Shaker A. Mousa Targeted delivery of cisplatin to tumor xenografts via the nanoparticle component of nano-diamino-tetrac", Nanomedicine ***(Lond)*** **2017** Feb;12(3):195-205

Effects on tumors of urinary bladder 253JBV cancer cell xenografts of daily s.c. administration of control (PBS), cisplatin, void PLGA, PLGA-cisplatin (1 mg/kg b.w. cisplatin adsorbed to PLGA nanoparticles, without tetrac), low dose NDAT (0.3 mg/kg b.w. tetrac equivalent, with empty payload compartment), and NDAT (0.3 mg/kg b.w. tetrac equivalent)-cisplatin (1 mg/kg b.w.). (A) Tumor volumes. Volumes were estimated from caliper measurements. (B) Tumor weights. (C) Cisplatin uptake by bladder tumors in response to administration of control (PBS), cisplatin, PLGA-cisplatin, and NDAT-cisplatin measured with LC-MS/MS. NDAT-cisplatin resulted in tumor drug content 5-fold that of cisplatin alone and 2.5-fold that of PLGA-cisplatin.

Summary

1. **A method for the large scale of synthesis of Tetrac-conjugated nanoparticles (nanotetrac) was developed using Microfluidizer.**
2. **This method is in the process of Tech transfer to contract development and manufacturing organization (CDMO).**
3. **In Mice tumor (glioblastoma, pancreatic cancer) model Nanotetrac effectively reduced tumor volume/weight and widely induced necrosis and apoptosis.**

Acknowledgement

1. Shaker A Mousa, Ph. D, MBA, FACC, FACB
2. Paul Davis, MD
3. Sudha Thangirala, Ph. D
4. Murat Yalcin, Ph. D

THANK YOU