Ultrasound enabled BBB disruption

Ultrasound-mediated BBB permeabilization in primates –
a disruptive approach to blood brain barrier disruption

Preliminary report

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Ultrasound enabled BBB disruption

AGENDA

- The Problem
  - Treatment of brain disease
  - No good alternatives
- Treatment hypothesis
  - Clinical
  - Technical
- Evidence
  - Preclinical
  - Clinical
- Results
  - Exvivo
  - In-vivo
- Summary

No Good Treatment Options for Brain Tumors

Doctors unscrewed the steel ring attached to Amy Cohen Soscia's scalp after a radiosurgery procedure at Brigham & Women's Hospital in Boston last month. (Wendy Maeda/ Globe Staff)

A new peril for breast cancer survivors

Drugs seen ineffective in fighting brain tumors

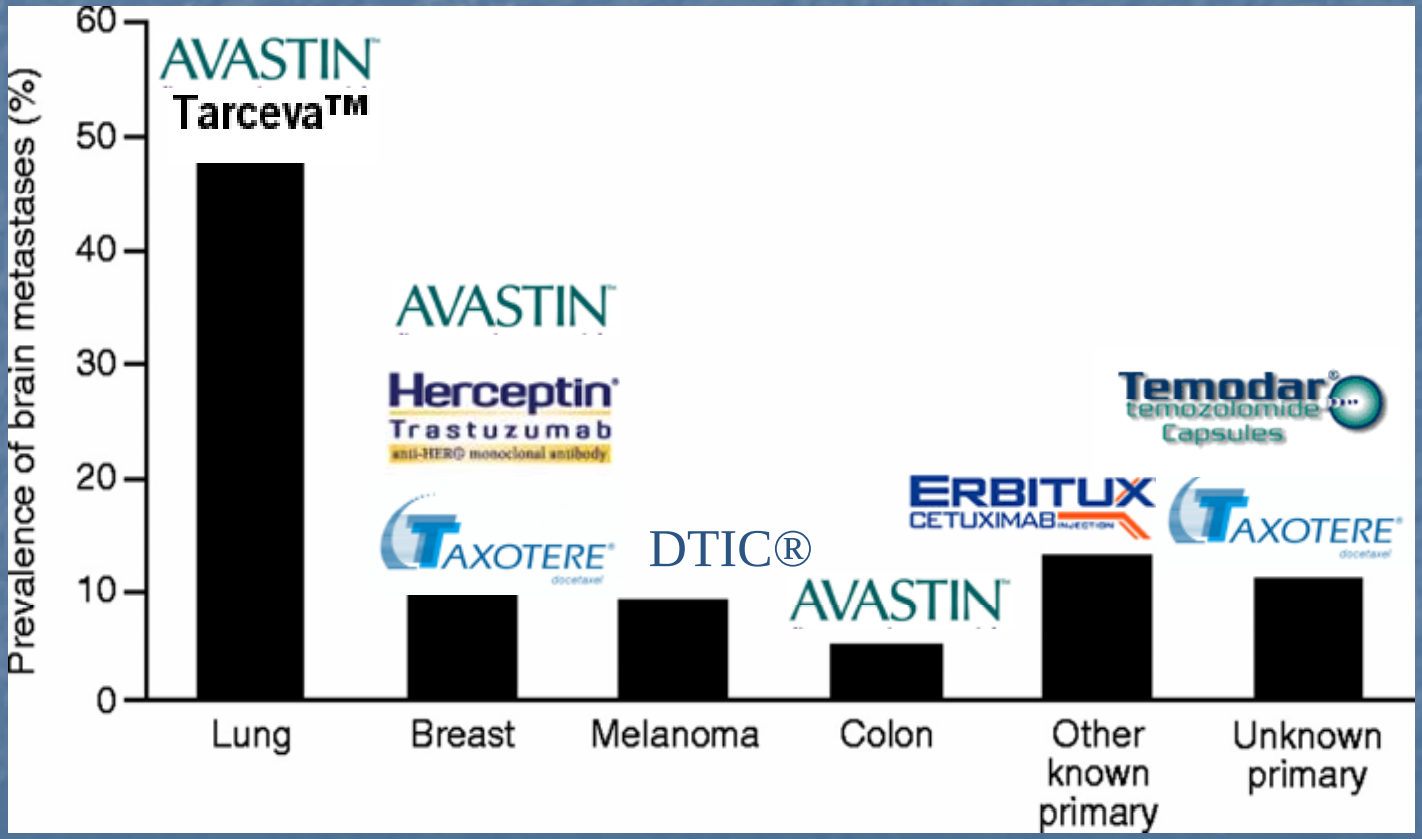
BBB blocks delivery

"A major challenge for treatment of most brain disorders is overcoming the difficulty of delivering therapeutic agents to specific regions of the brain. In its neuro-protective role, the blood-brain barrier (BBB) functions to hinder the delivery of many potentially important diagnostic and therapeutic agents to the brain. Therapeutic molecules and genes that might otherwise be effective in diagnosis and therapy do not cross the BBB into the brain in adequate amounts." *

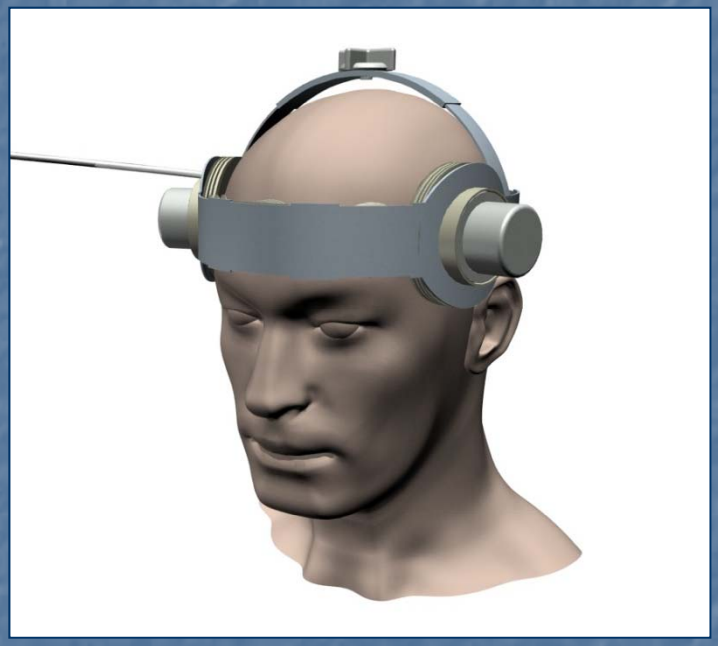
* Dr. Thomas Jacobs - National Institute for Neurological Disease and Stroke

There are many therapeutics

But most are ineffective for treatment of brain tumors



Solution- US enabled BBB disruption



Low Cost, EZ to use Ultrasound

↓

Safely Opens Blood Brain Barrier

↓

Increased Drug Passage

↓

Improved Treatment of Brain Disease

Treatment Hypothesis- clinical

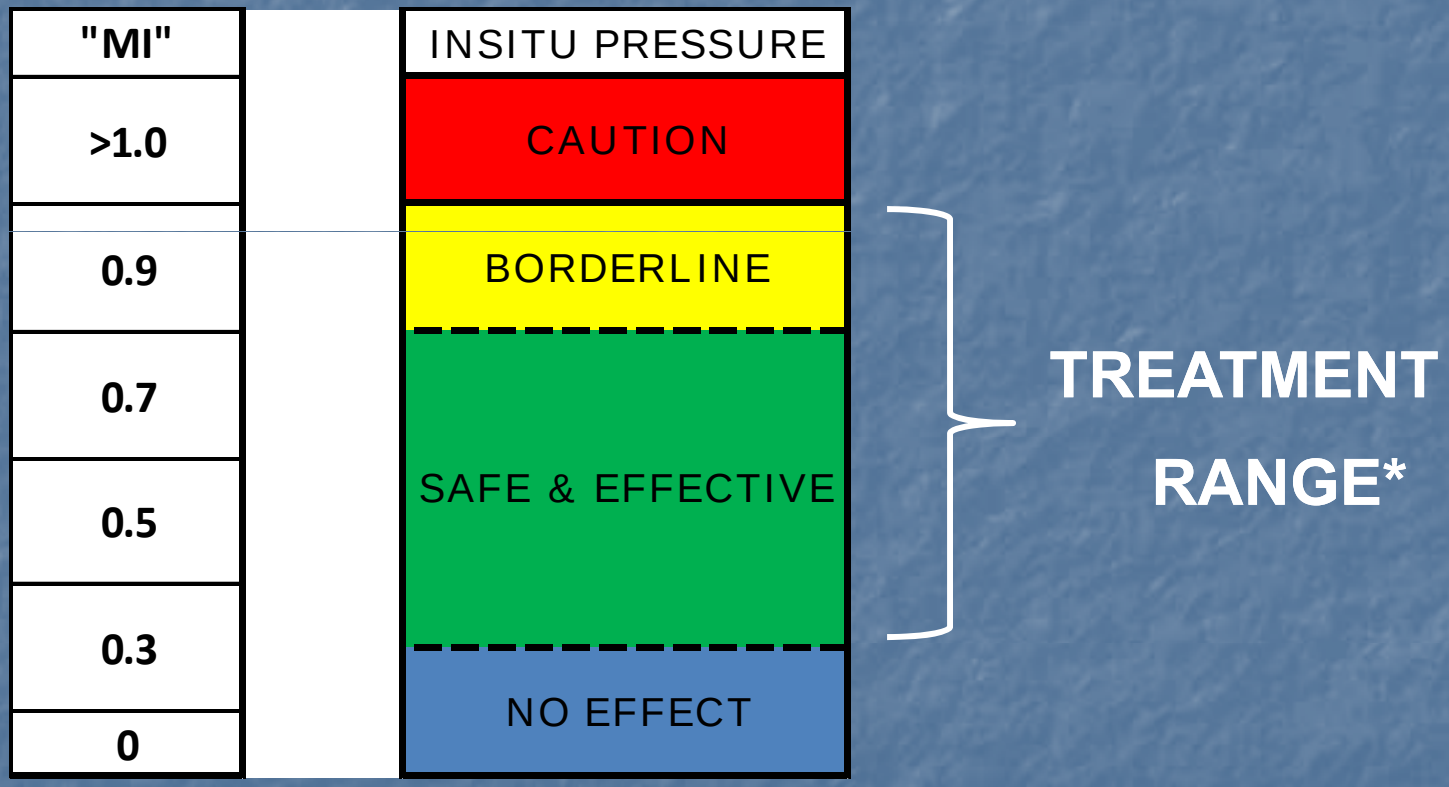
Ultrasound can deliver drugs to treat brain cancer

"The treatment hypothesis is that non-invasive ultrasound exposure of the penumbra around the brain tumor will penetrate the BBB, and deliver therapeutics to healthy tissue. The rationale is that malignant brain tumors are difficult to treat medically, partly because the cells that have migrated away from the bulk tumor, and cannot be reached by most therapeutics or removed surgically. This treatment method has potential applications for CNS mets (central nervous system metastasis) and glioblastoma multiform."*

***Dr Lauren Aubrey, Director of Research MSKCC**

Treatment Hypothesis- technical

300 Khz US alone @ 0.2-1.0 "MI" is safe & effective*



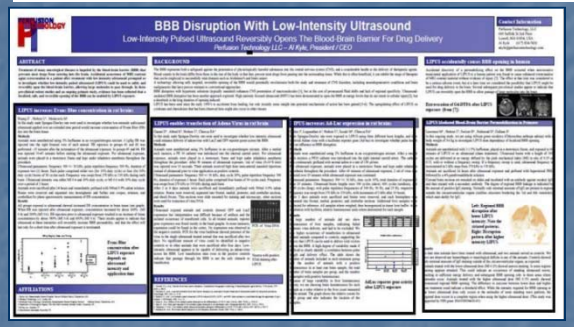
***Ultrasound alone means no gas bubbles, no MRI**

"MI" = P ÷√f, P = in-situ peak rarefaction acoustic pressure not derated

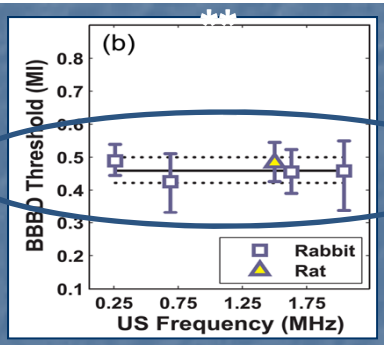
Preclinical Evidence

Multiple Studies suggest a treatment range

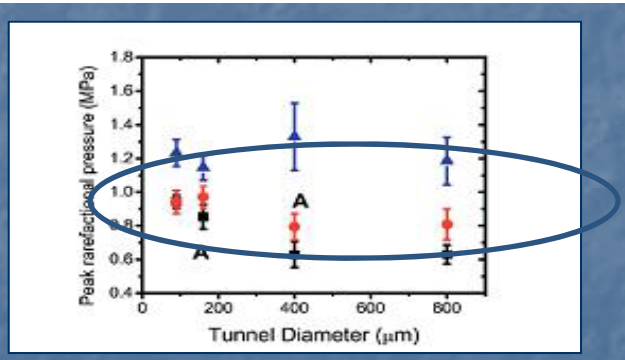
Effect of US Alone MI= .25-.6*



Effect of US + Gas bubbles MI= .46



Safety of US Alone + Gas bubbles MI= 0.8-1.2**



* BBB Disruption with Low Frequency Ultrasound, Kyle UIA 2007

** BBB Disruption appears to be characterized by the MI, McDannold UMB 2008

*** Cavitation Threshold of Microbubbles, Hynynen et al UMB 2006

Clinical Evidence – 300 KHz caused bleeds

Investigators suspect cavitation or BBB disruption

"The study was prematurely stopped because 5/12 from the tPA only group, but 13/14 patients with tPA (tissue plasminogen activator- a clot busting drug) + US showed signs of bleeding in MRI. "

At three months, neither morbidity nor treatment-related mortality nor recanalization rates differed between the two groups (treated with ultrasound + tPA vs. tPA alone). "

"The mechanical index was <0.2 …. emitted in a pulsed fashion with a 5% duty cycle."

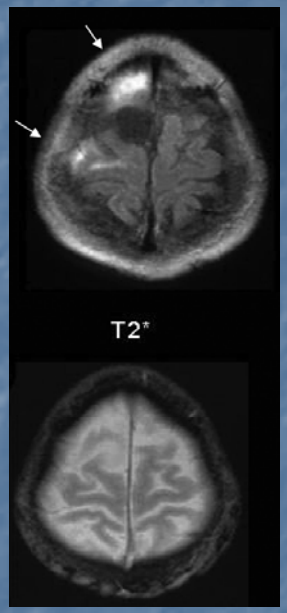
"Suspected reasons (for the high bleed rate) were mechanical action (cavitation), vasodilatation and opening of the BBB." *

*TRUMBI, Daffertshofer et al, Journal Stroke 2007

Clinical Evidence – 300 Khz delivered Gd

*Investigators suspect BBB disruption**

<1 Hour after US exposure



Evidence of Gd delivery

12 hours after US exposure

Gd absorbed, normal image

*BBB Disruption by Low Frequency US , Reinhard et al Journal Stroke 2006

**Gd is gadolinium, an MRI contrast agent

Opinion

Opinion suggests BBB disruption+ tPA caused bleeds- not cavitation

- "The observed bleeding rate with low frequency sono-thrombolysis might be attributable to **primary BBB disruption**." *
- "The assumption that low-frequency **ultrasound might cause BBB disruption** and thus increase the risk of ICH (intracerebral hemorrhage)-particularly in the presence of tPA- appears convincing and is corroborated by our rat experiments" **
- "Ultrasound could…induce BBB opening in areas remote from the brain infarction so that tPA could diffuse in the brain parenchyma. As tPA is a know neurotoxic agent, this **might also explain the occurrence of secondary hemorrhages in TRUMBI."** ***

* BBB Disruption by Low Frequency US , Reinhard et al Journal Stroke 2006

** Ltr to the editor of Stroke, Gerreits et al Journal Stroke 2007

*** Simulation of intracranial acoustic fields Fink et al, UMB 2009

Ex-vivo Acoustics

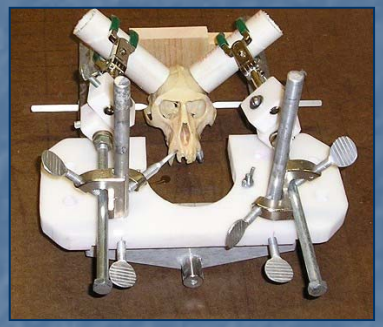
Goal: rule out the possibility of mechanical effects including hotspots by mapping acoustic pressure in two monkey skulls.

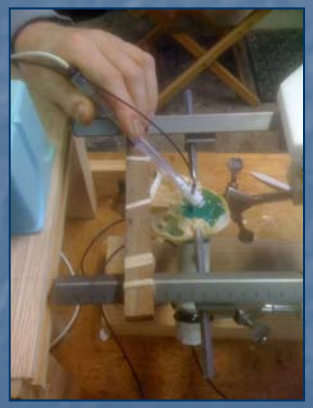
Method:

- Expose monkey skulls to 300 khz
- Measure insitu acoustic pressure
- Register location with XYZ position locator system.

Ex-vivo methods

A total of 445 acoustic pressure samples in two skulls



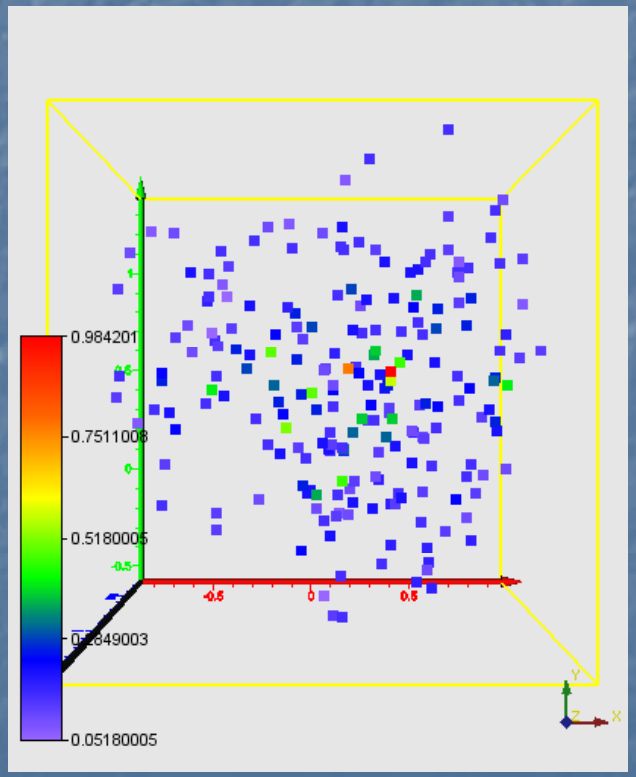


Exposed Skulls to 300 Khz **Hydrophone probing In-situ** **XYZ locator**

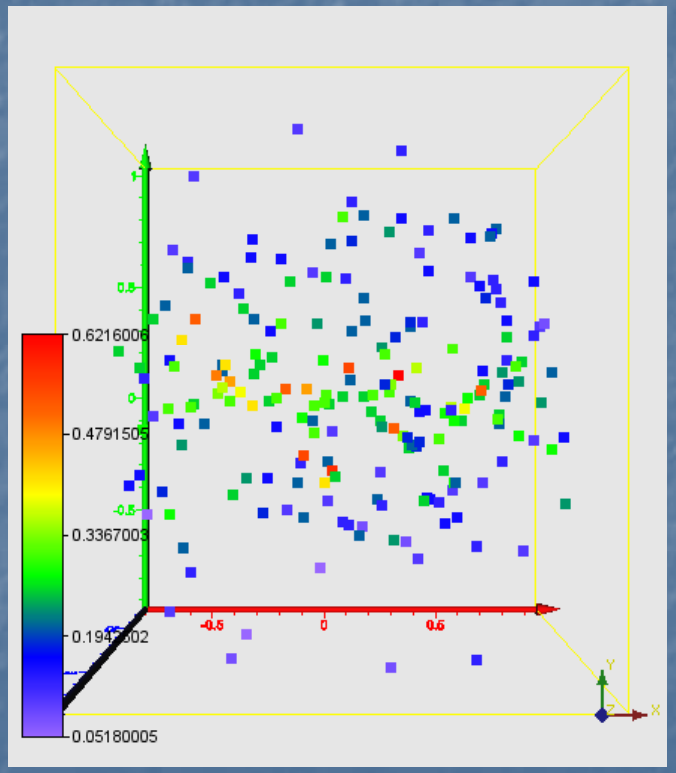
Ex Vivo Results I

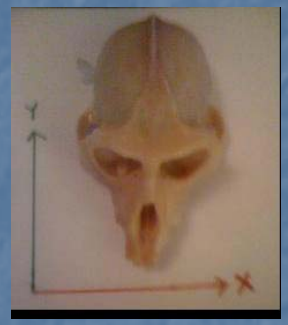
Highest Acoustic Pressure is in center of skull

"Whitetooth"



"Blacktooth"

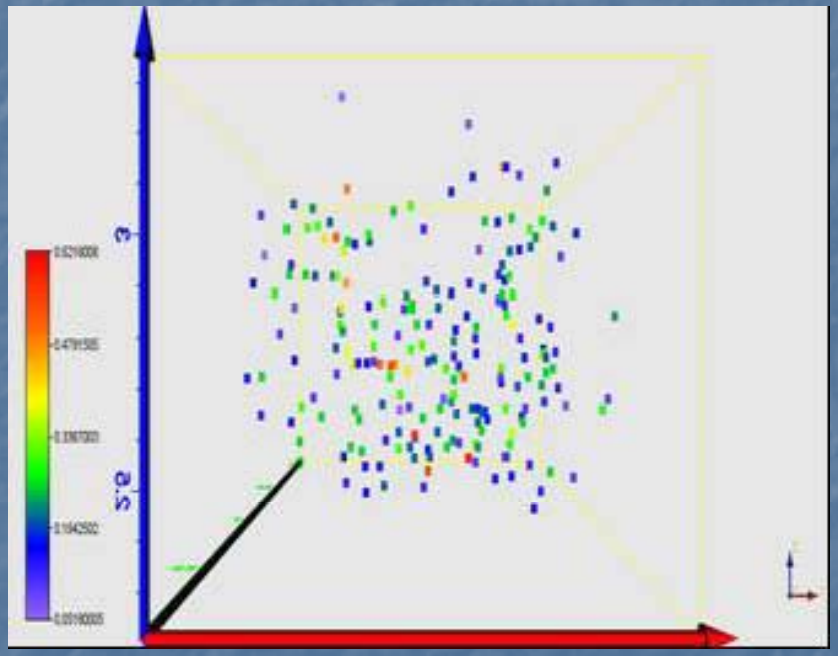


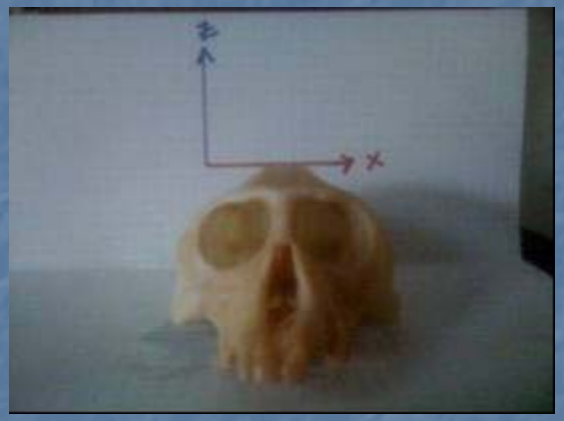


Ex Vivo Results II

Acoustic Pressure distributed throughout the skull

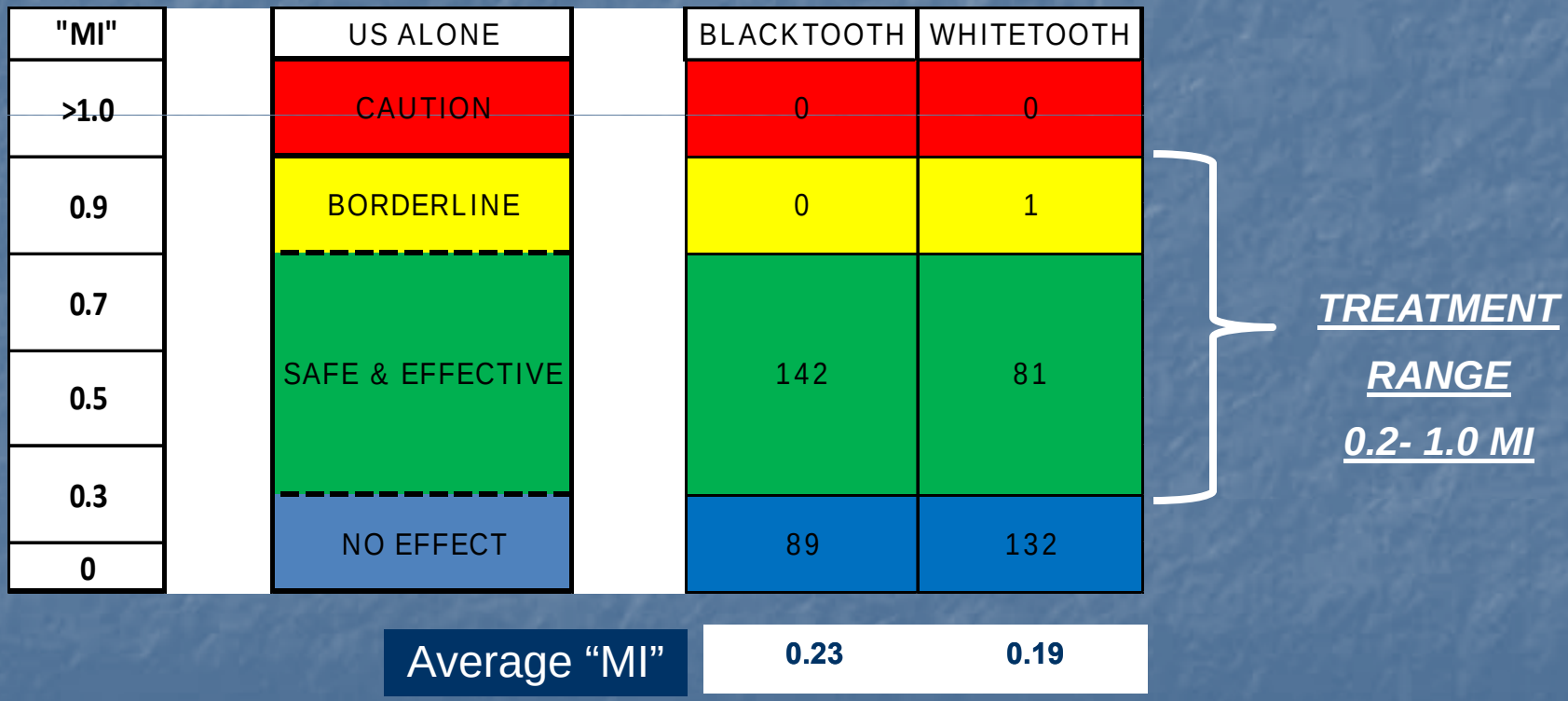
“Blacktooth”





Ex Vivo Results III

All samples of Acoustic Pressure are in the treatment range



In-vivo Histology

Goal: to provide substantial and compelling preclinical evidence that 300 kHz ultrasound effectively, safely and reversibly opens the BBB in NHP.

Specific Aims:

1) quantify the efficacy of US by examining uptake of markers with different molecular weights;
2) determine the kinetics of 300 kHz ultrasound mediated BBB permeabilization and investigate possible mechanisms of the effect;
3) demonstrate that 300 kHz ultrasound can safely deliver clinically relevant macromolecules to the brain parenchyma

In Vivo Results I

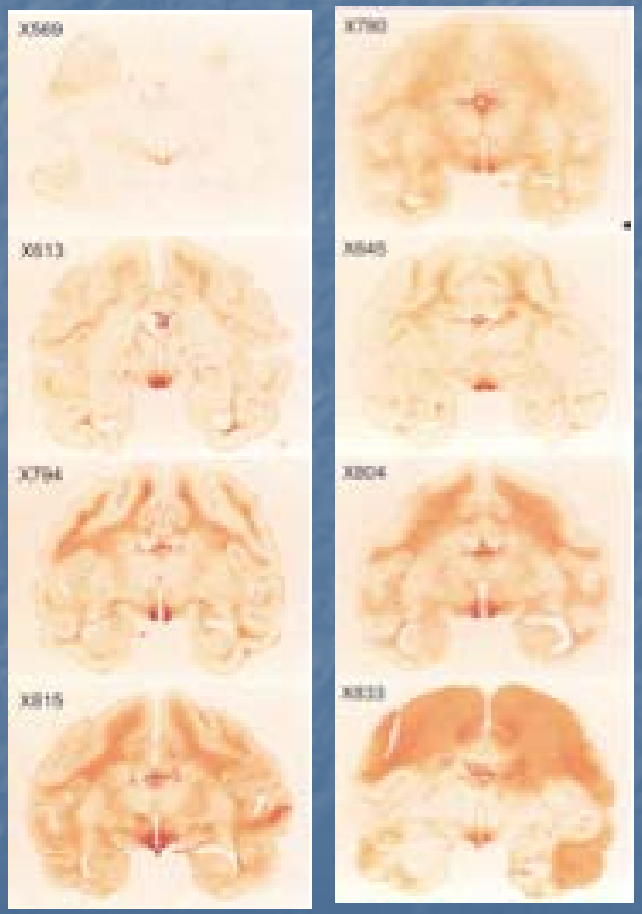
IgG* marker demonstrated Dose Ranging

Control

MI 0.25

MI 0.25
(without FS)

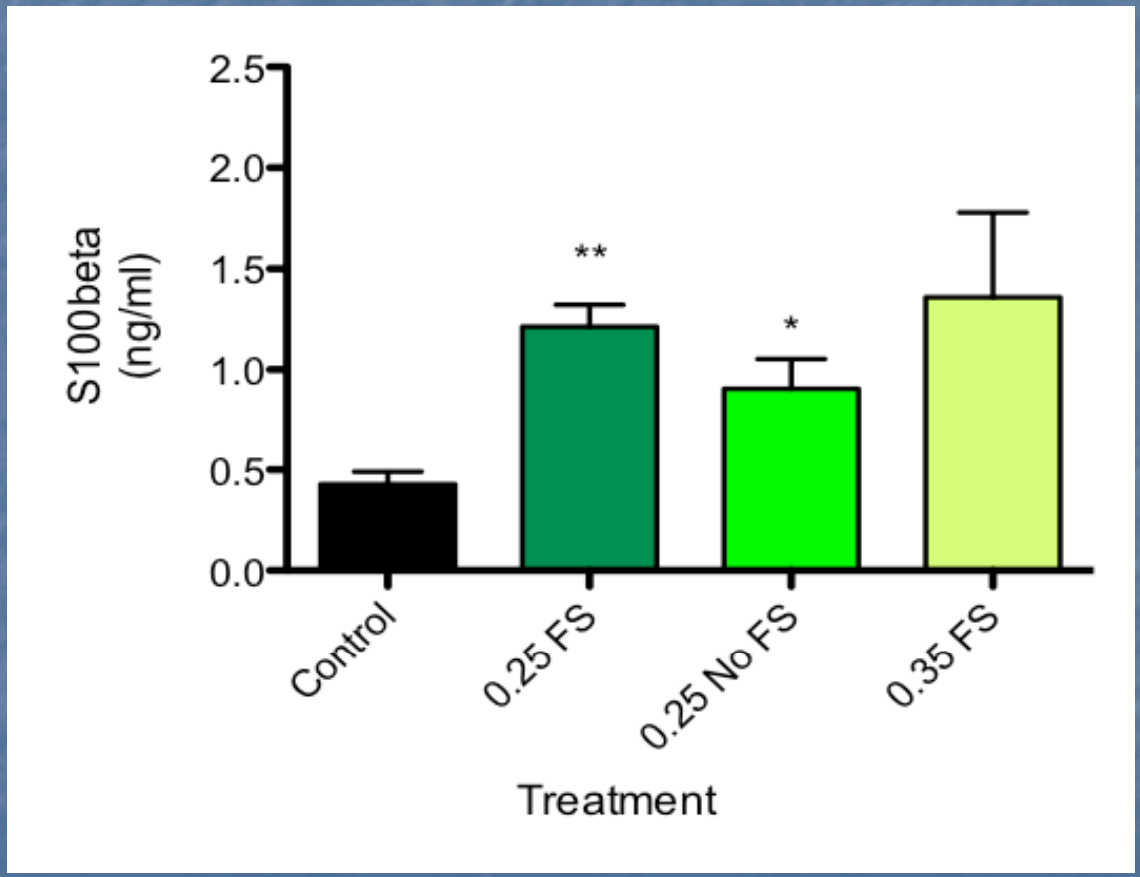
MI 0.35



*IgG is Immuno gamma globulin, a native protein marker for BBB

In Vivo Results II

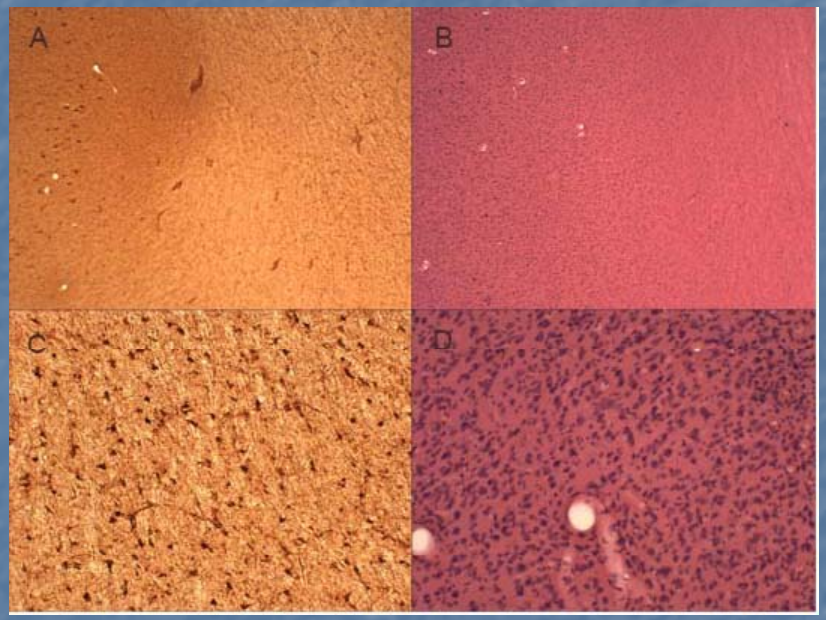
S100beta* marker showed leakage OUT OF the BBB



*S100beta is a biomarker of BBB permeability

In Vivo Results III

H & E* staining showed no evidence of bleeding



10x

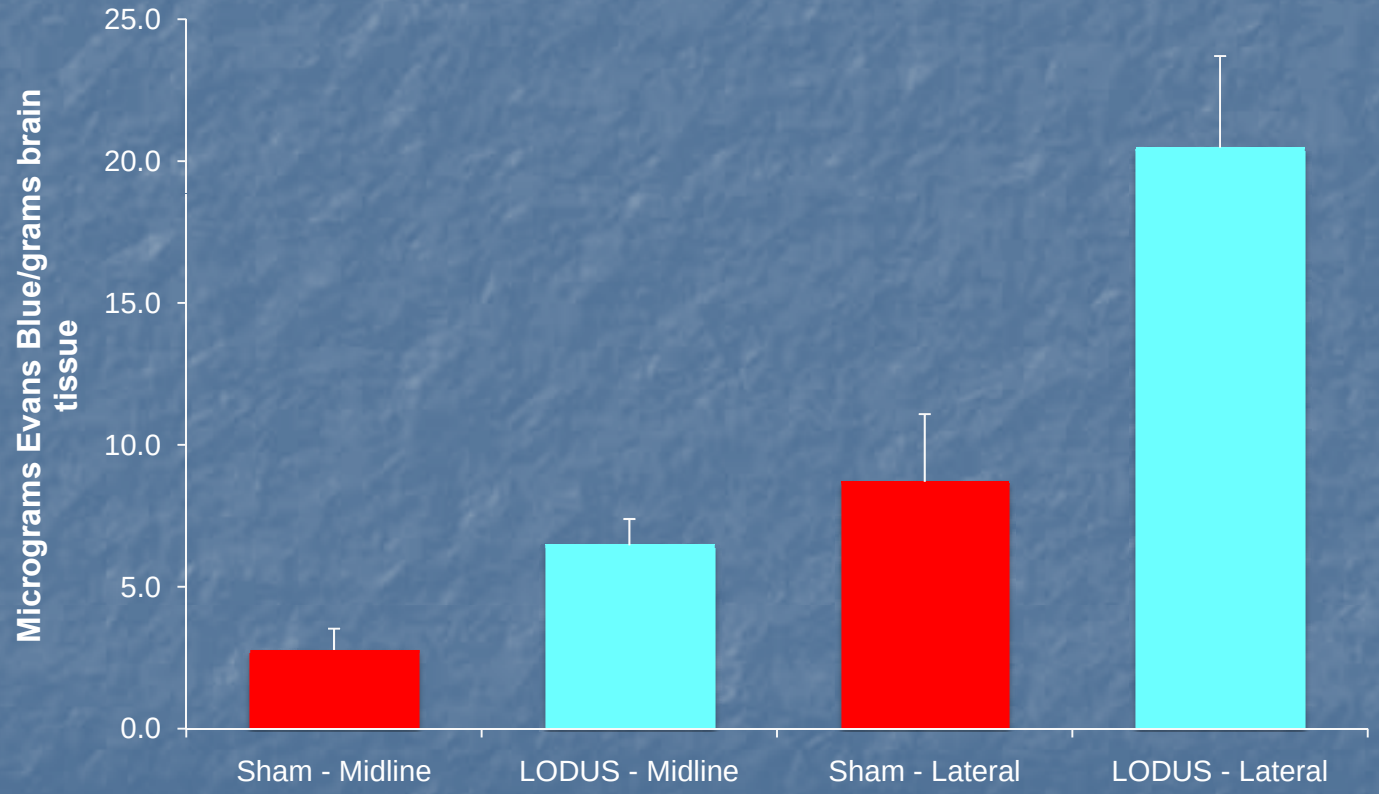
100x

IgG H&E

*H&E is Hematoxylin and Eosin, a common stain to detect bleeding

In Vivo Results IV

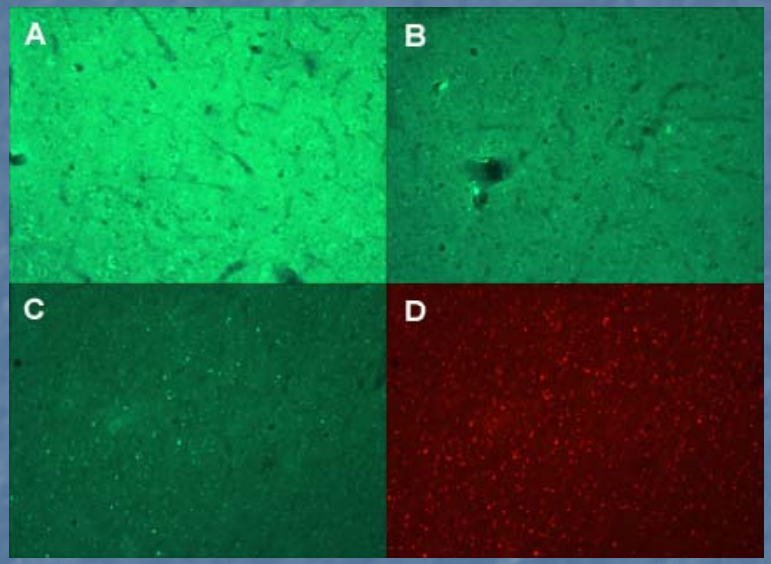
Evans Blue* shows significant delivery in groups of 21 samples



***Evans Blue is a large molecule marker with molecular weight of 59 KD**

In Vivo Results V

Dextran* Markers were confounded by background noise



*** Dextran is a common marker with varying molecular weight**

Summary

Hypothesis

1. 300 KHz US alone disrupts the BBB with no bubbles, MRI or focusing
2. 300 KHz US alone may be useful for treatment of regional brain disease
3. There is a treatment range that is both safe & effective

Evidence

1. Preclinical & Clinical results show BBB disruption with US alone
2. Opinion suggests that TRUMBI bleeds were caused by BBB disruption- not by cavitation

Experiment Results

1. Acoustic pressure in ex vivo skulls ~ 0.2 “MI”, no “hotspots”> 1.0 “MI”
2. Preliminary in vivo primate delivery results are encouraging
3. More work is needed to confirm safety in repeat dosing scenario and quantify delivery of macromolecules of therapeutic interest