



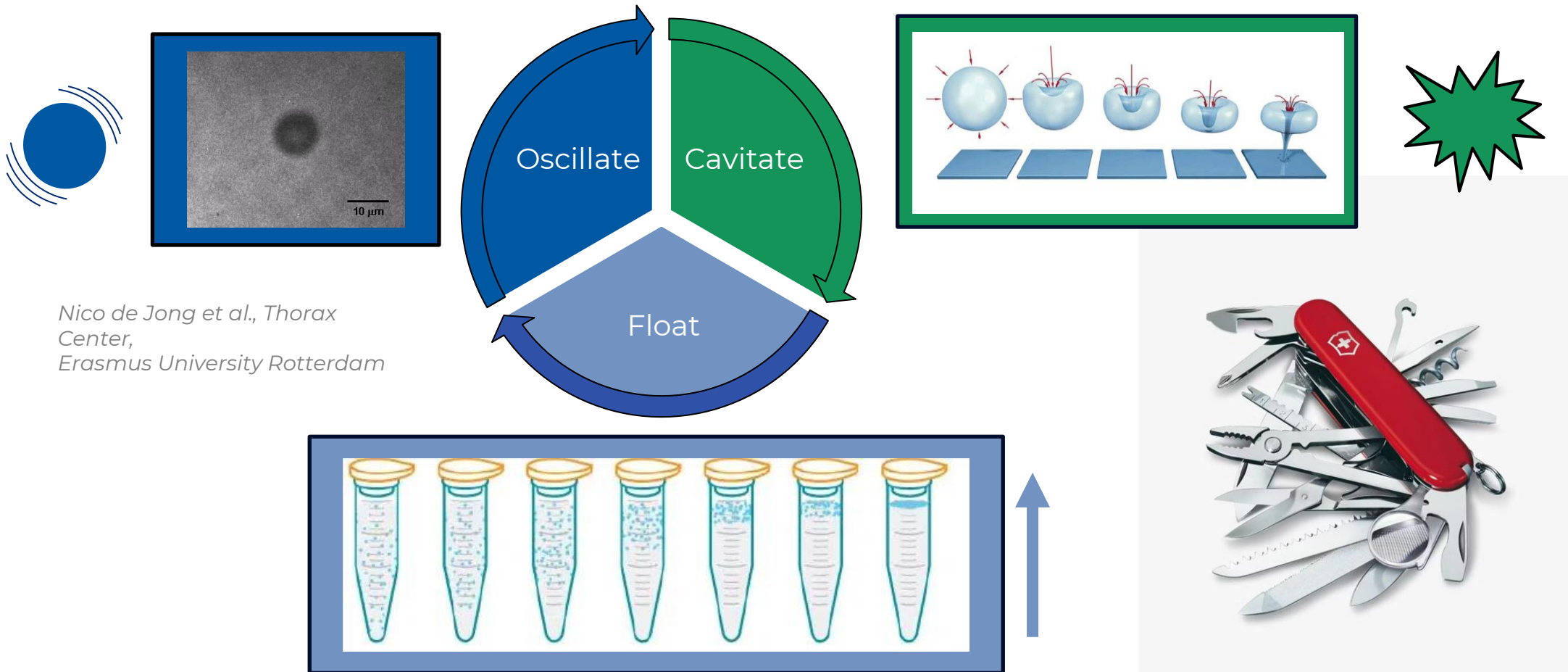
LIFE FROM INSIDE

LIPID-SHELLED MICROBUBBLES AND NANODROPLETS FOR DIAGNOSTIC AND THERAPY APPLICATIONS

SAMIR CHERKAOU

CRS 2025 Annual Meeting
Philadelphia – July 14 -18, 2025

Lipid-shelled microbubble: Diagnostic and Therapy applications



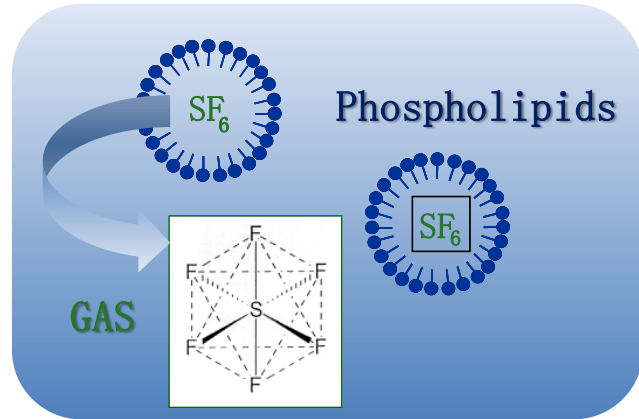


Gas filled microbubbles for CEUS

SONOVue[®]
Sulphur Hexafluoride



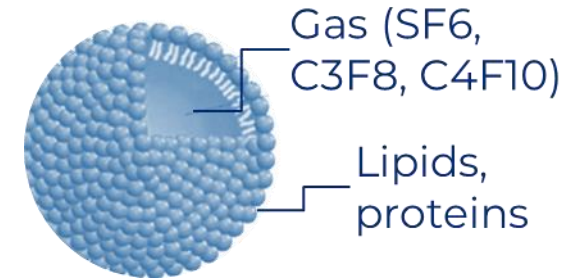
Microbubbles structure



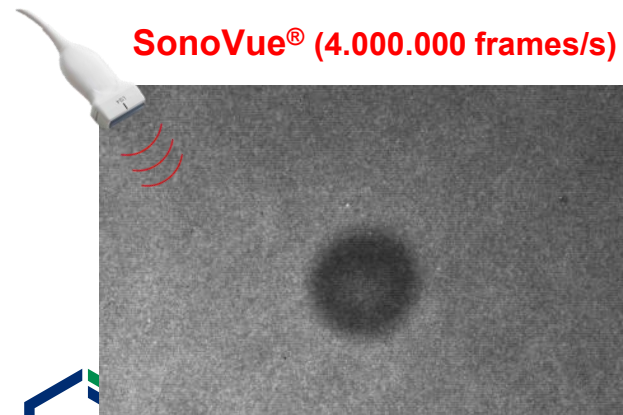
❑ Gas stabilized by a lipid shell

- DSPC
- DPPG
- Palmitic acid
- PEG 4000

❑ Diameter < 8 µm (smaller than RBC)



Marketed CEUS agents	Producer
1- SonoVue/Lumason	Bracco Suisse SA
2- Definity/Luminity	Lantheus Medical Imaging
3- Optison	GE Healthcare
4- Sonazoid	GE Healthcare



- Highly compressible
- Oscillate in response to the incoming ultrasound wave

*Nico de Jong et al., Thorax Center,
Erasmus University Rotterdam*



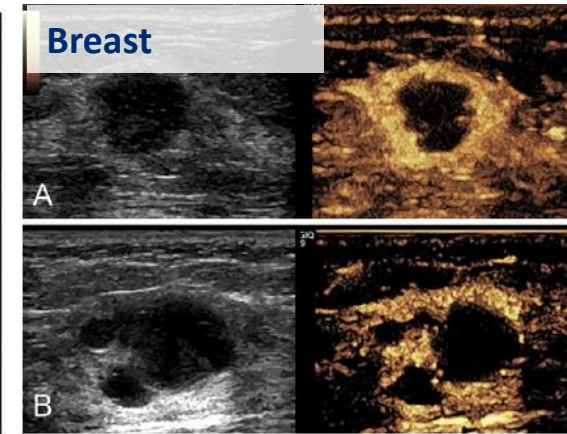
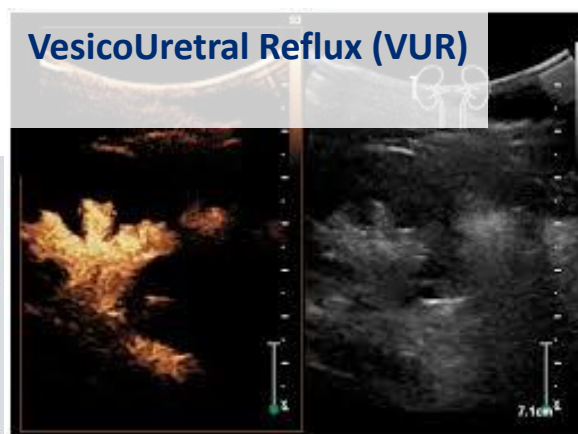
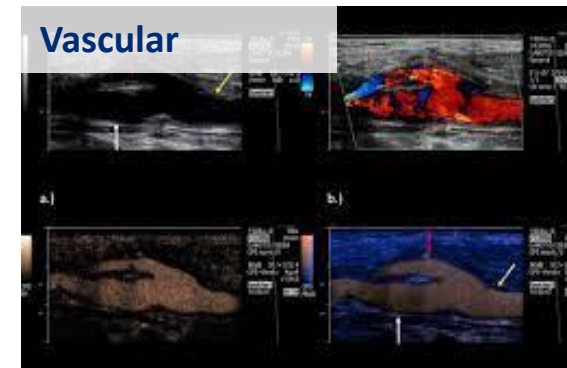
CEUS: Contrast Enhanced UltraSound

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Authorized clinical use of microbubbles for contrast-enhanced ultrasound (CEUS)

Enhance ultrasound signal of the blood visualizing macrocirculation and microcirculation

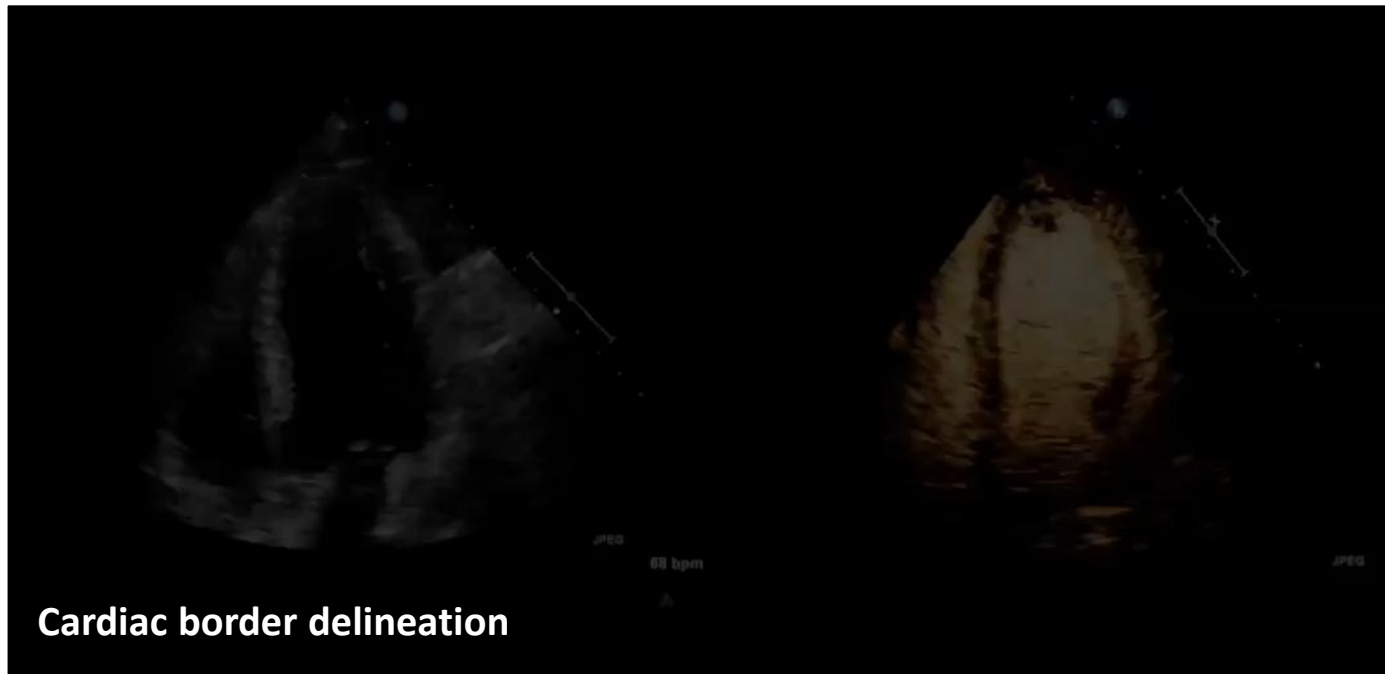
	 BRACCO LIFE FROM INSIDE	 Lantheus Holdings	
Liver Imaging ▶	✓		✓
Breast Imaging ▶	✓		✓
Vascular Imaging ▶	✓		
Cardiac Imaging ▶	✓	✓	✓
VUR Imaging ▶	✓		



Strictly confidential

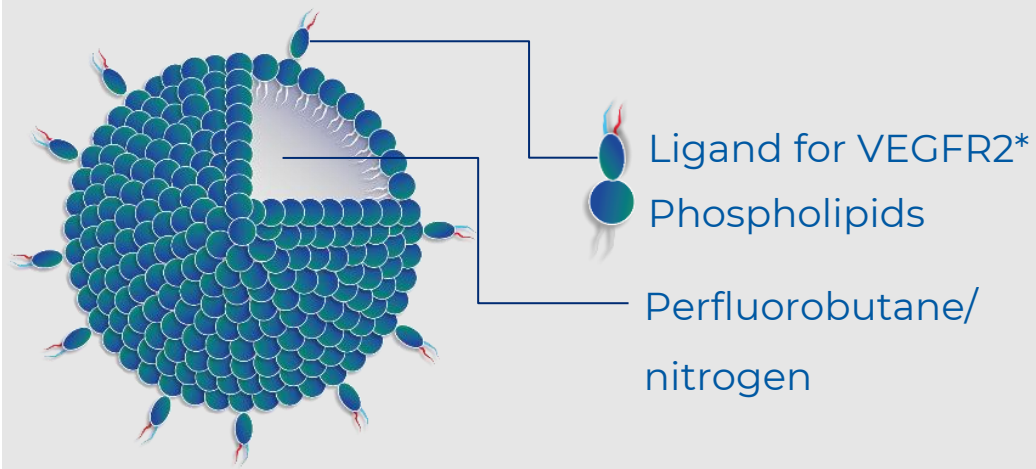
Authorized clinical use of microbubbles for contrast-enhanced ultrasound (CEUS)

SonoVue® was first approved in the EU in 2001 and is currently registered in 44 countries



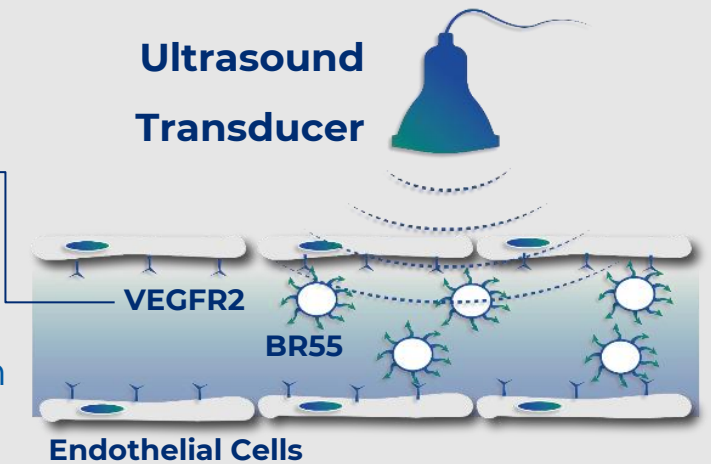
BR55, new contrast agent for UltraSound Molecular Imaging (USMI) of angiogenesis

SCHEMATIC REPRESENTATION OF BR55 MICROBUBBLE

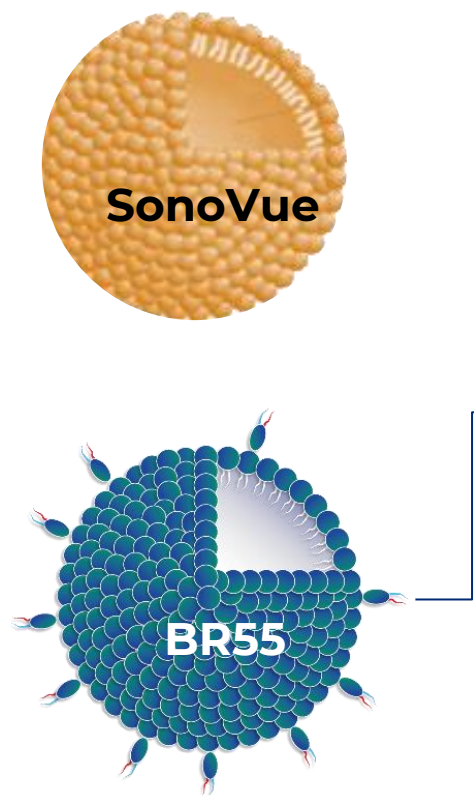


MECHANISM OF ACTION

Marker of angiogenesis:
Involved in pathological conditions (i.e. tumor progression and chronic inflammation)



BRU-2248: a complex PEGylated lipopeptide for VEGFR2 targeting with 260 synthesis steps



BRU-2248

- ❑ Chemical Formula: $C_{385}H_{599}N_{60}O_{131}PS_4$
- ❑ Molecular Weight: 8323.43 g/mol
- ❑ Solid Phase Peptide Synthesis

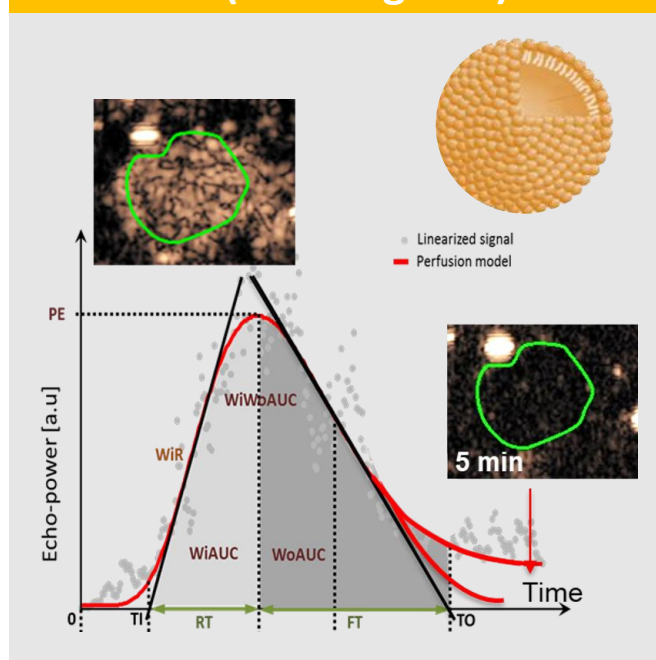
Lipidic portion
(DSPE*)

Heterodimer peptide:
2x cyclic peptides of 23 residues each

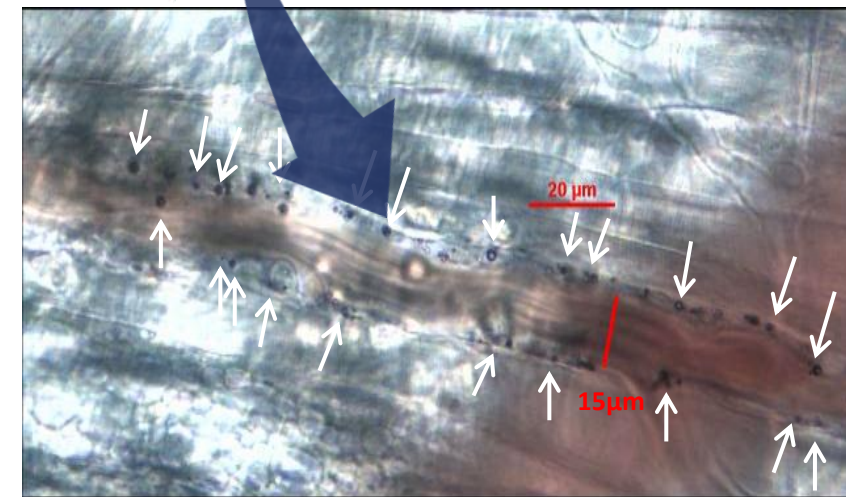
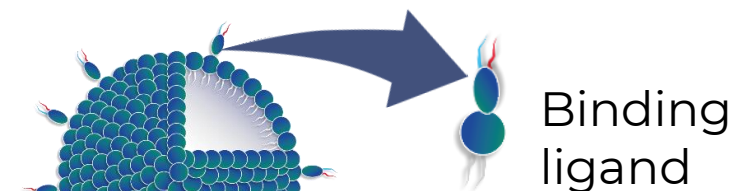
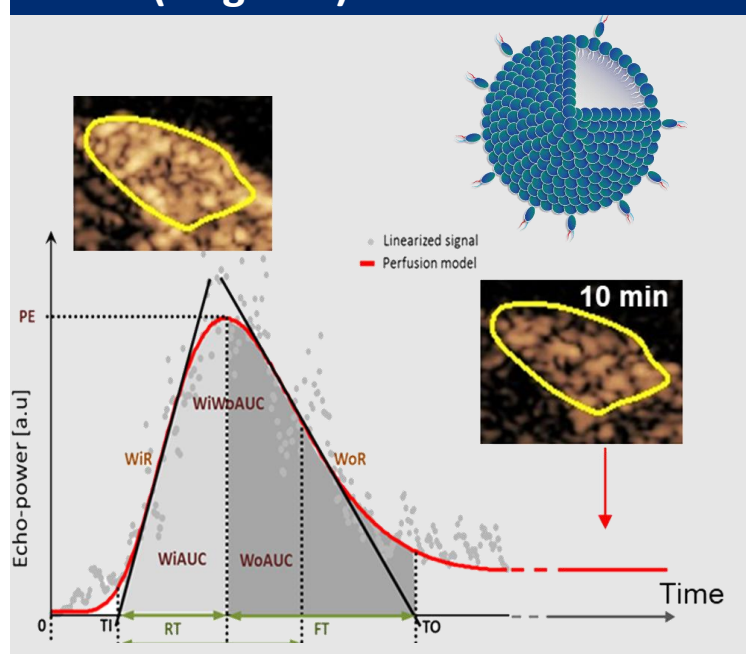
Polyethylene glycol 2000
(PEG2000)

Targeted microbubbles offer insights into both perfusion and molecular characteristics

SonoVue (non targeted)

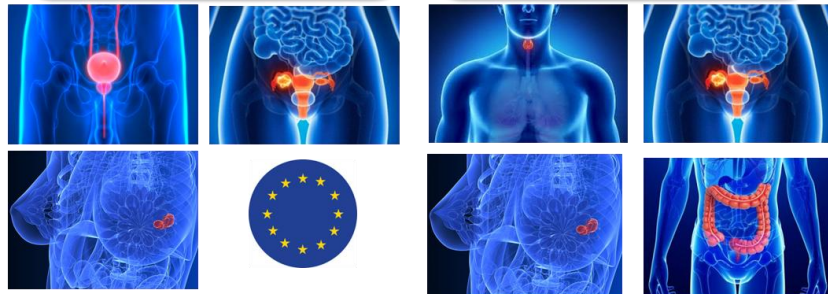
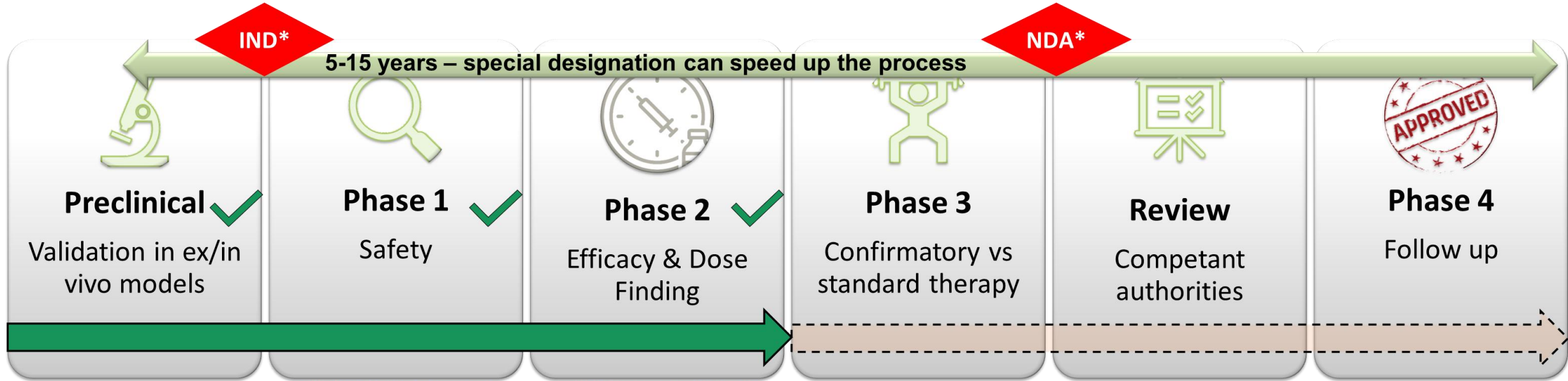


BR55 (targeted)



Clinical development phases:

Phase 2b completed, heading to Phase 3



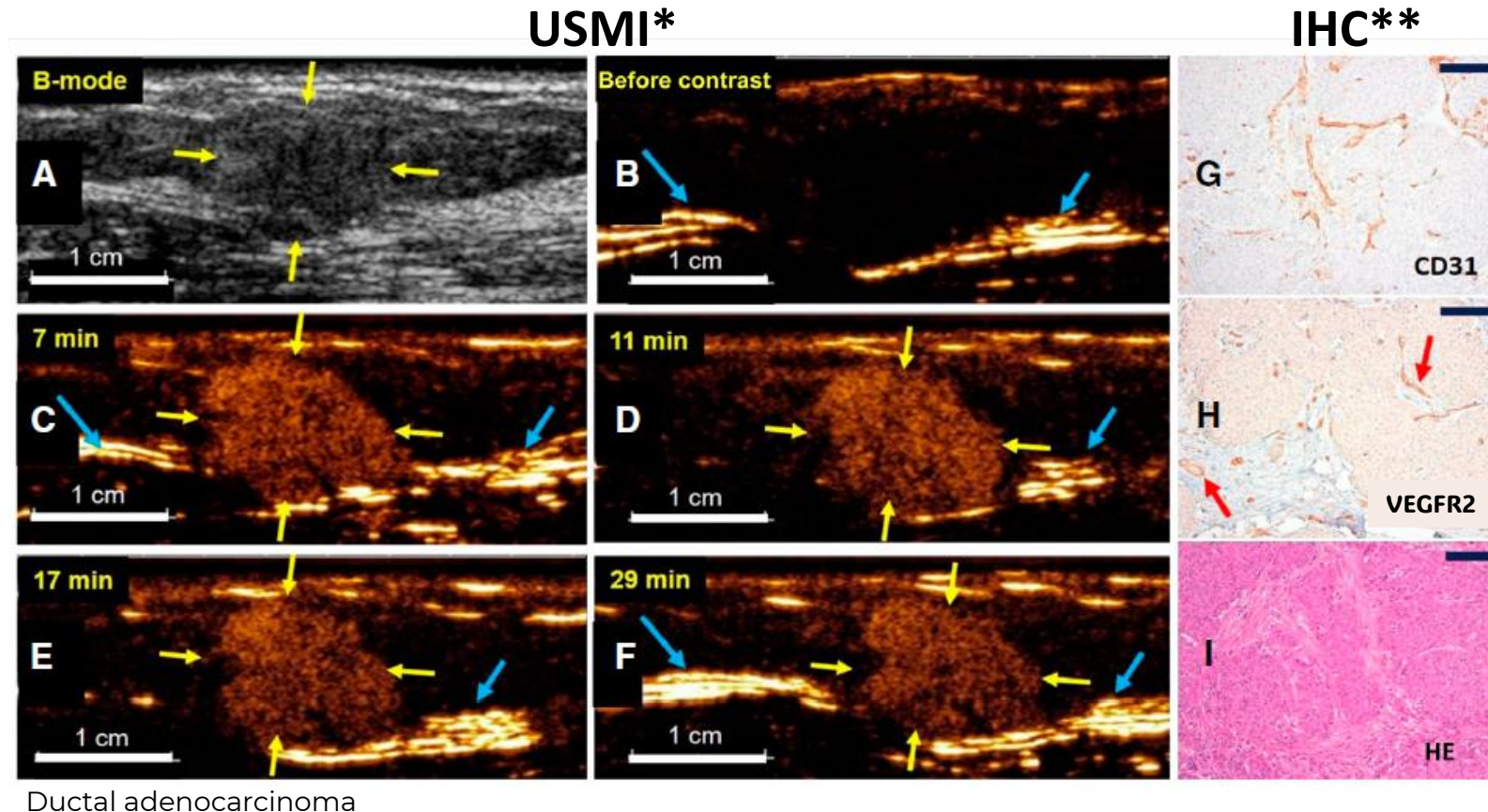
PC – 13 patients, OC:
24 pts, BC: 21 pts



250 pts in total



BR55 signal correlates well with VEGFR2 expression on IHC in breast cancer patients



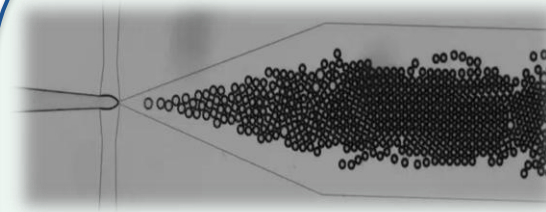
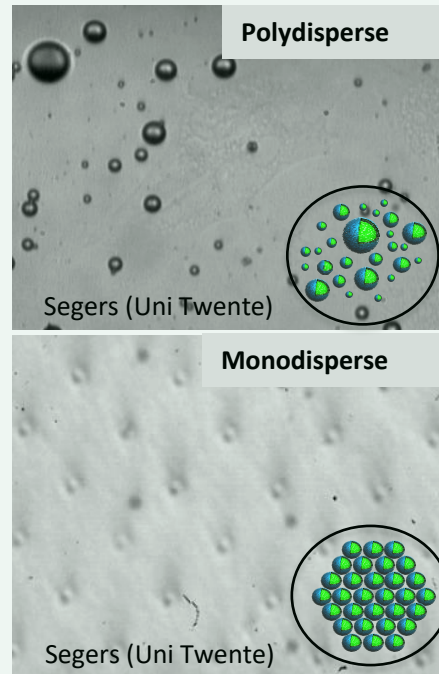
Ductal adenocarcinoma

*VEGFR2: Vascular Endothelial Growth Factor Receptor 2, *USMI: Ultrasound Molecular Imaging, **IHC: Immunohistochemistry

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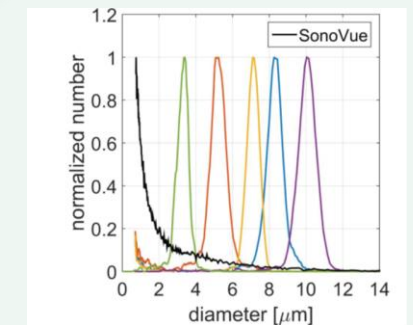
Monodisperse vs polydisperse bubbles

Monosize microbubble using microfluidic technology



Shell composition :
 DSPC/DPPE-PEG5000 (9/1) @50°C
Production rate :
 200-500x10⁶ MSB/mL & 9 mL of product/h

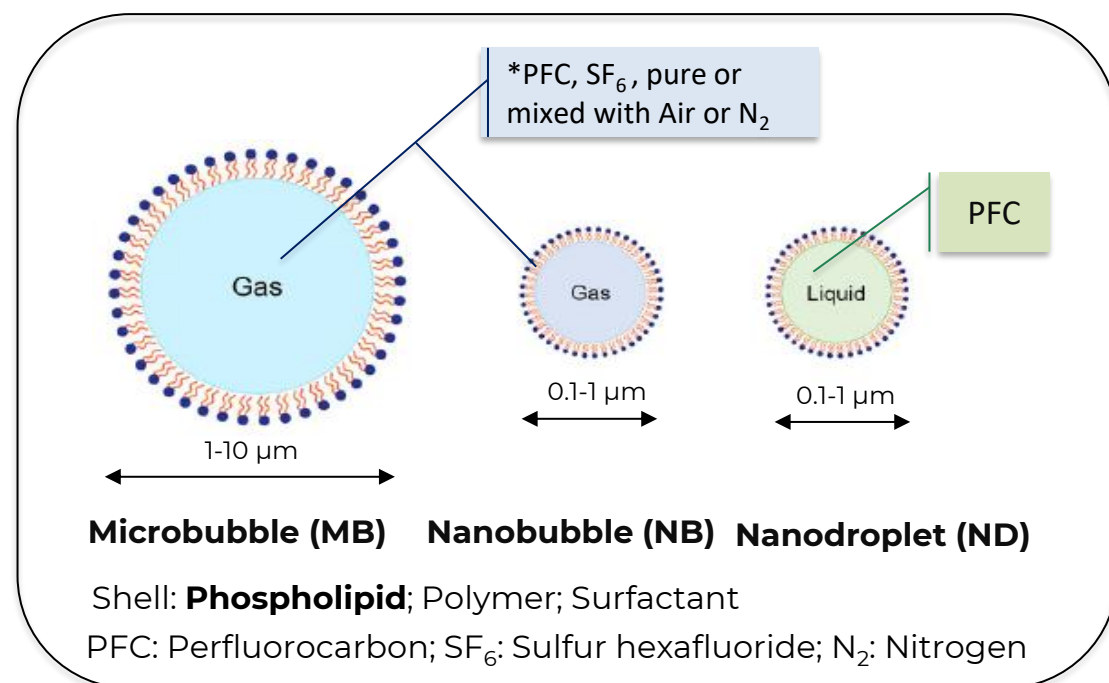
$$d_b \propto D \left(\frac{Q_g}{Q_l} \right)^{0.4}$$



- **Monodisperse microbubbles** as optimal **cavitation nucleus** for US applications
- **15-40 times higher acoustic efficiency** compared to regular polydisperse MB

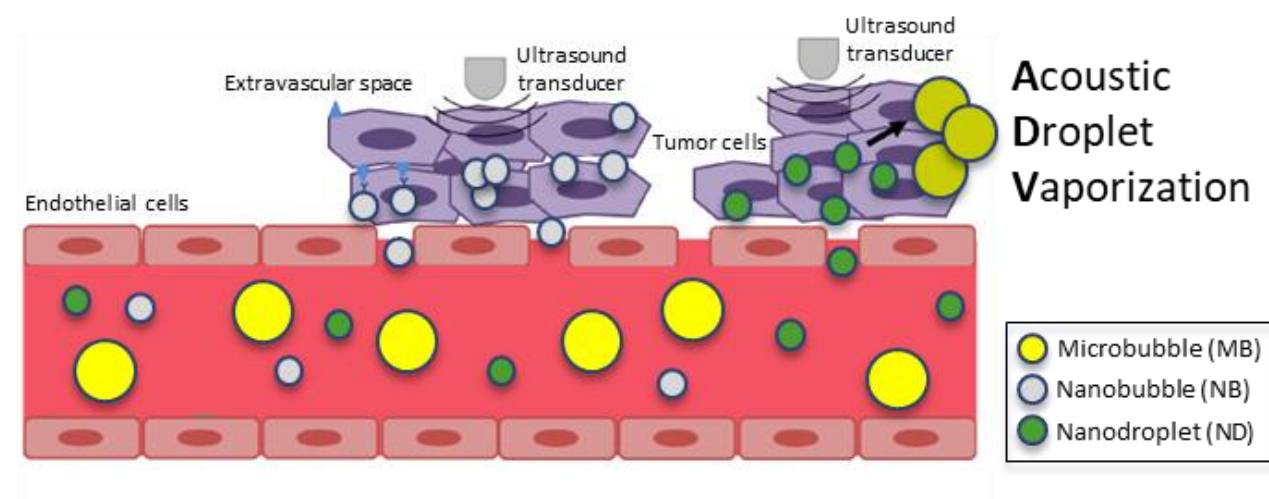
NDs: New frontiers beyond vessels

Nano/micron-sized US responsive agents



Main advantages over microbubbles

- **Extravasation** and **tumor accumulation** via either EPR* effect (NB/ND)
- Improved **stability** and **longer residence time** (ND)
- **Selectively activated**, both spatially & temporally (ND)
 → Acoustic Droplet Vaporization Shift "On/Off"



Ultrasound Contrast Agent - MBs

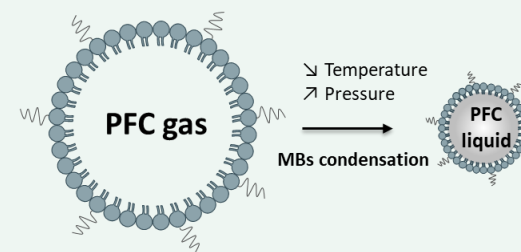
- Short in vivo circulation time due to gas core
- Remain only in the blood circulation due to large size

* EPR Enhanced Permeability Retention

Nanodroplets production

Microbubbles condensation procedure

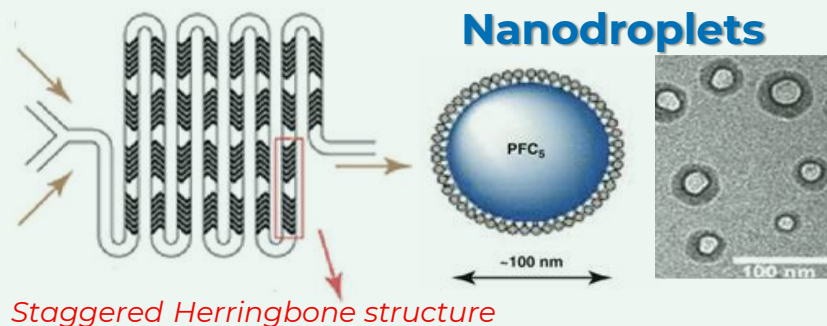
- ✓ Use of **highly volatile** compounds : C_4F_{10} (b.p. $-2^{\circ}C$) or C_3F_8 (b.p. $-36^{\circ}C$)
- ✓ Ability to **modify and manipulate** microbubbles prior to condensation
- ✗ NDs size/distribution depends on **initial MBs**
- ✗ **Low NDs concentration**



Microfluidic technology (NanoAssemblR b

Organic phase :
 - Shell molecules
 - PFC in EtOH

Aqueous phase



NanoAssemblR™
Instrument



Microfluidic
cartridge

❖ Process parameters

- Total Flow Rate (TFR)
- Flow Rate Ratio (FRR)

❖ Formulation parameters

- Encapsulating shell
- Perfluorocarbon core

Targeted size
 $< 400 \text{ nm}$ & $PDI \leq 0.2$



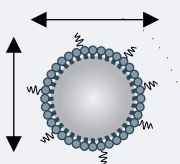
Formulation of various types of PFC nanodroplets in terms of composition and size

Strictly confidential

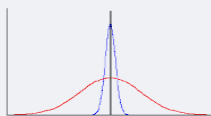
PFC-NDs characterization

PHYSICOCHEMICAL

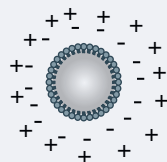
✓ Size



✓ Polydispersity (PDI)



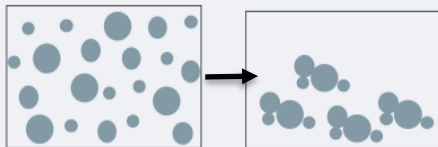
✓ Zeta Potential



✓ Concentration

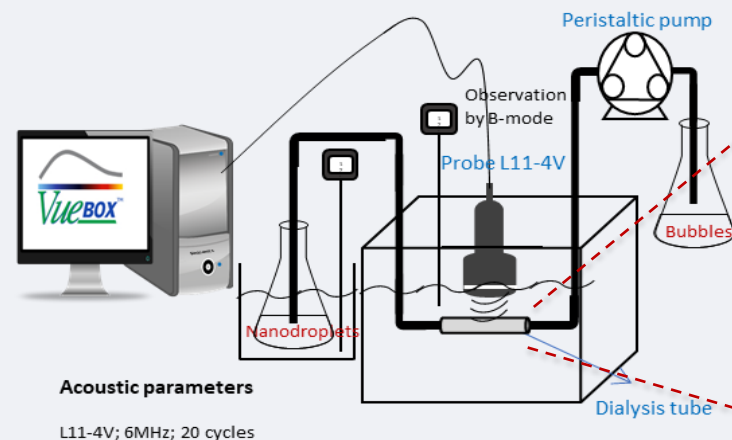


✓ Stability (storage @ 4°C)

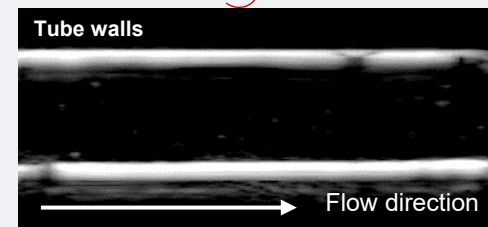
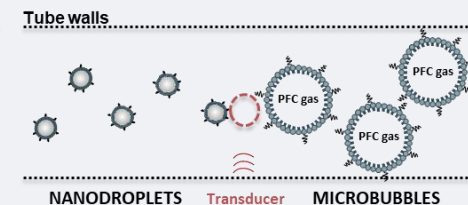


ACOUSTICAL

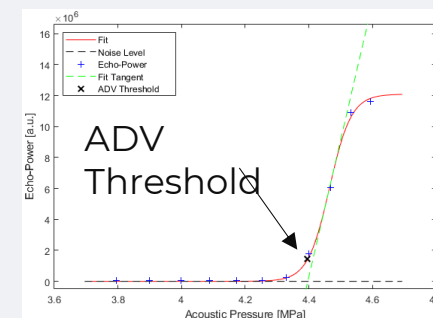
Home-made ADV setup



- An ultrasound probe (6 MHz) is used to activate and image the NDs vaporization for a real-time imaging
- The acoustic pressure increase every 10 seconds until the observation of the nanodroplets vaporization into MBs
- The VueBox™ software is used to measure the echopower values from B-mode imaging videos and thus to determine the ADV threshold



Increase of the echogenicity with the MBs production



VueBox™
Matlab fit

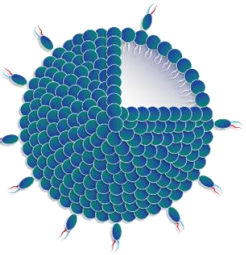
Melich et al. 2020

Strictly confidential

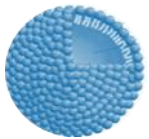
Main Takeaways



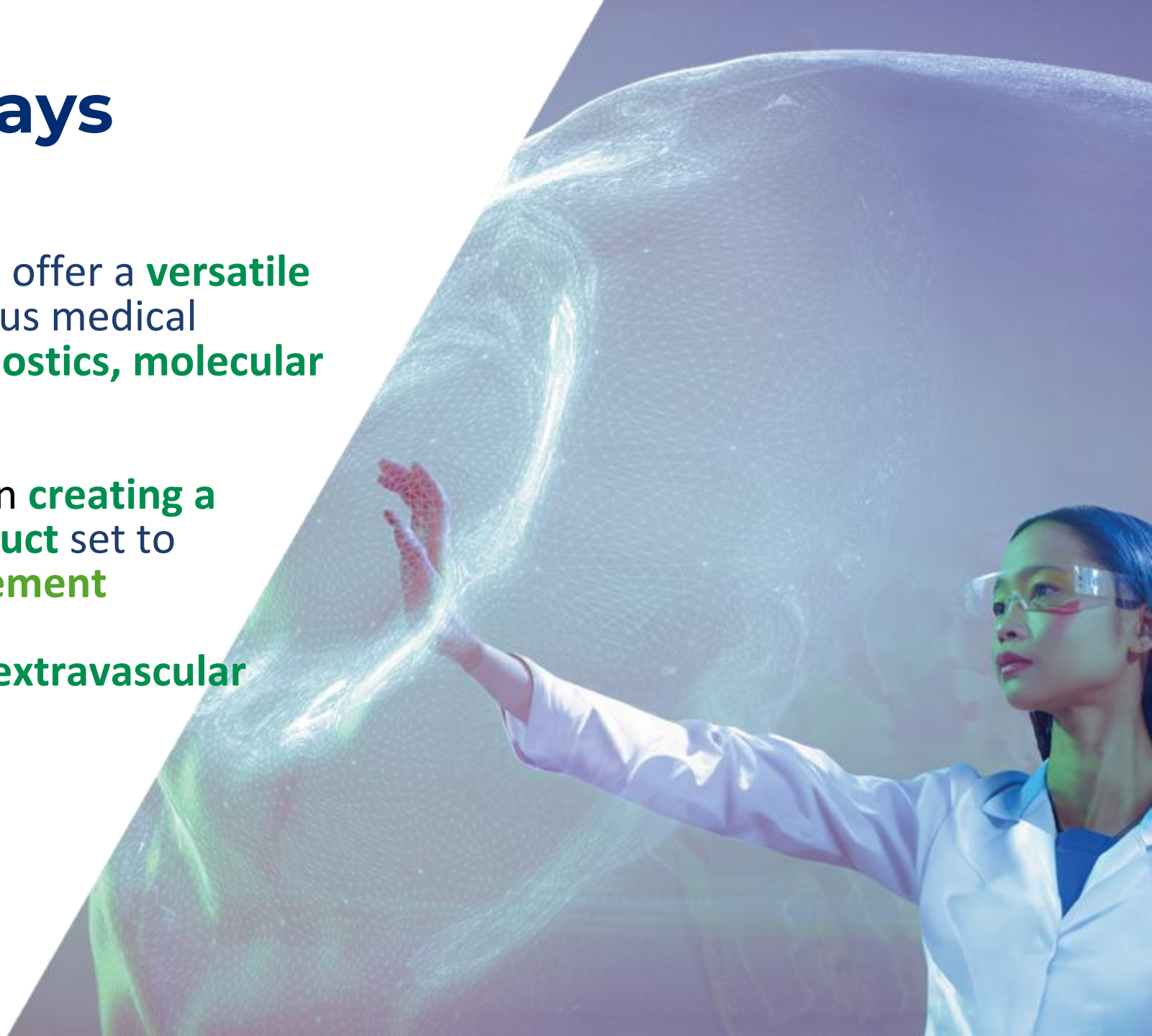
Lipid shelled microbubbles offer a **versatile platform** suitable for various medical applications, such as **diagnostics, molecular imaging and therapy**



Bracco is leading the way in **creating a groundbreaking new product** set to **transform patient management**



New opportunities for **US extravascular theranostic** applications



Acknowledgements

Bracco Suisse, Geneva site, Switzerland

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