FIGHTING INFECTIOUS DISEASES FOR SEVEN DECADES

THE PUBLIC HEALTH RESEARCH INSTITUTE
at the International Center of Public Health

RUTGERS
New Jersey Medical School

MALDI-MS Imaging of Targeted Therapies in Cellular and Necrotic Tissues

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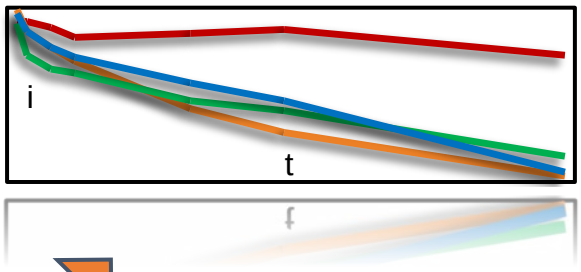
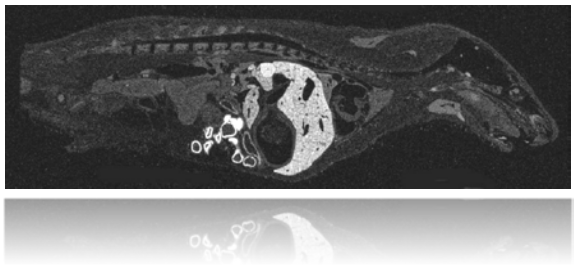
Traditional methods for measuring compound distribution

PK/Metabolism



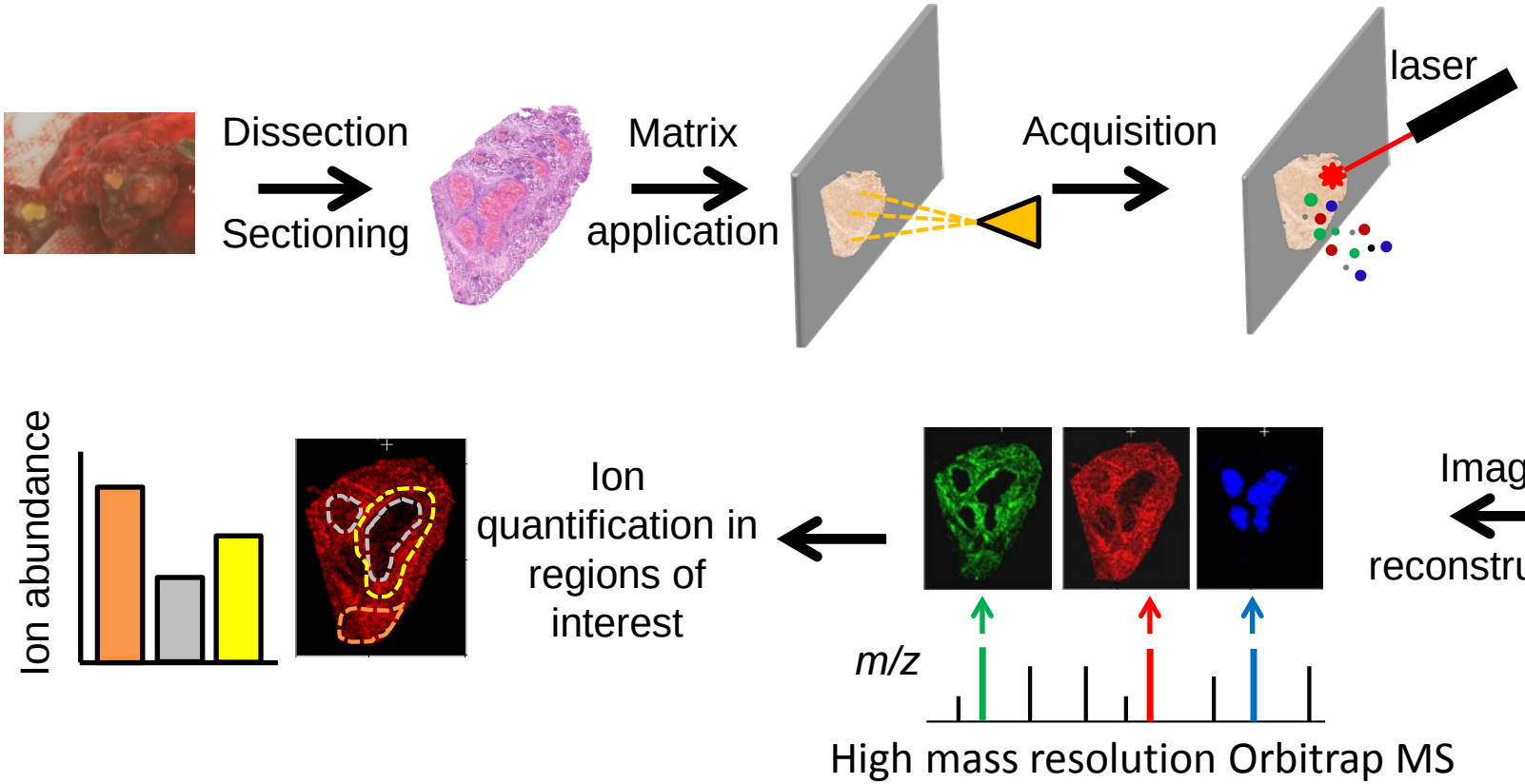
- dissection of animal
- limited spatial resolution
- only parts of animal

Distribution (QWBA)



- label required
- total radioactivity
- not specific

MALDI-mass spectrometry imaging



- Set up inside the BSL3
- No labeling required; can image lipids, drugs/metabolites in the same section

Key factors affecting image quality

Co-crystallization of matrix/analyte/internal standard?

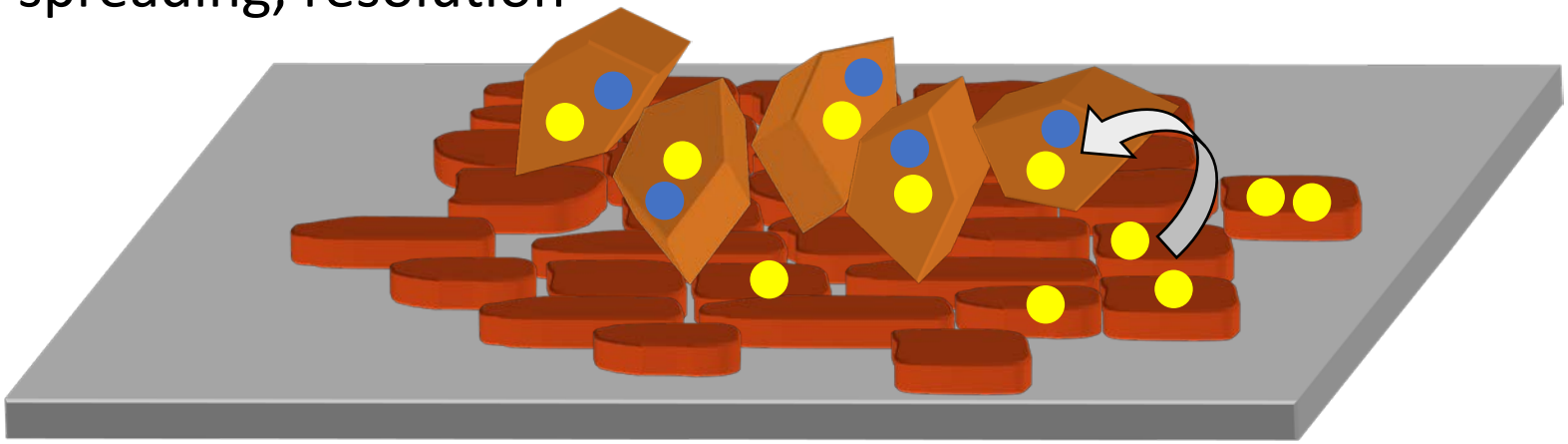
⇨solvation of analyte

⇨spreading, resolution

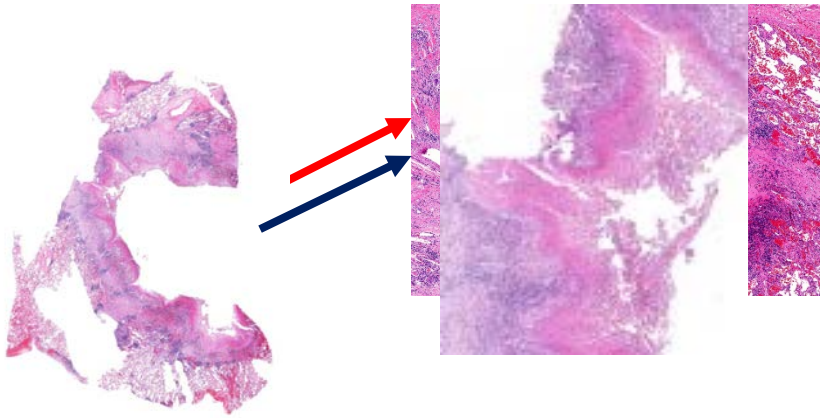
Crystal size

⇨ sensitivity

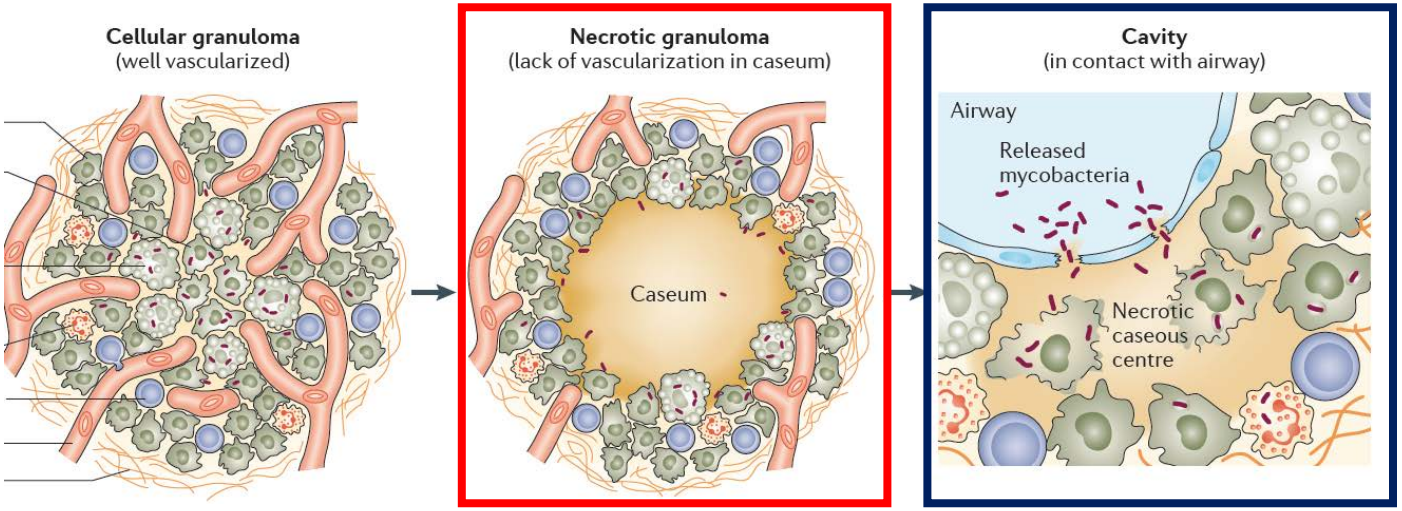
⇨ image resolution



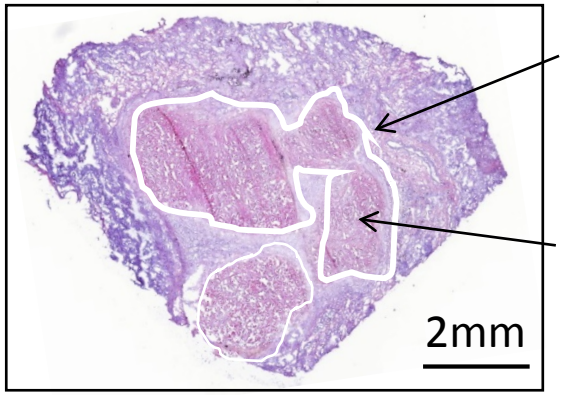
The need to assess lesion drug penetration



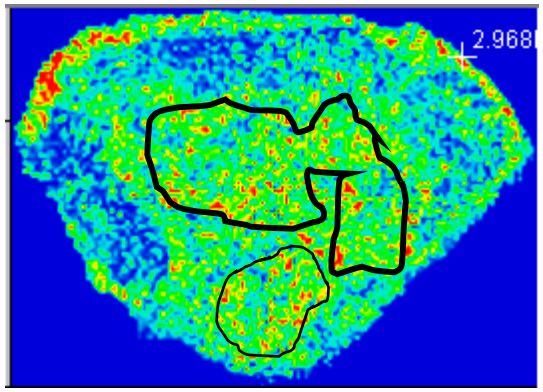
- Existing drug regimens were developed before standard PK measurements existed
- Need to optimize dosing regimens of existing TB drugs (concentrations and combinations)
- Use detailed lesion penetration information for developing novel anti-TB compounds



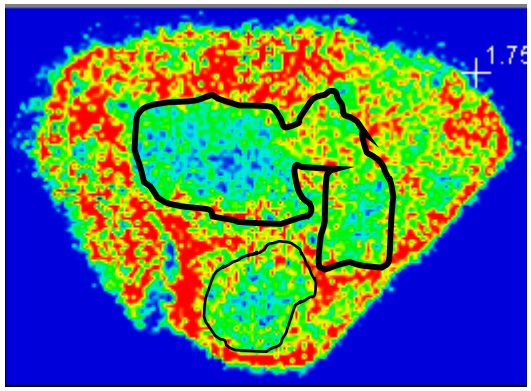
Heterogeneous drug distribution into lesions



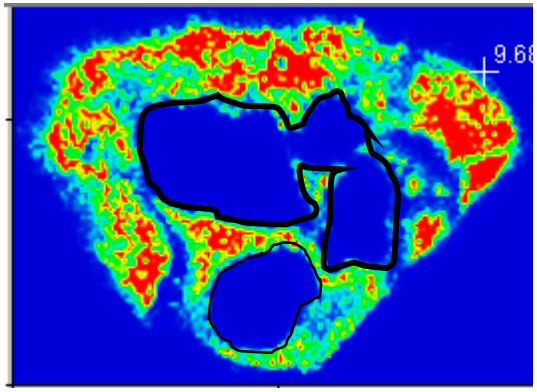
Pyrazinamide



Moxifloxacin



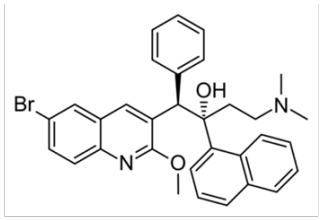
Clofazimine



Limit of detection

Prideaux *et al.,* 2015. Nat Med. Sep 7. doi: 10.1038/nm.3937

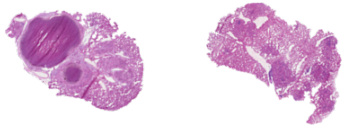
Spatio-temporal drug and metabolite imaging

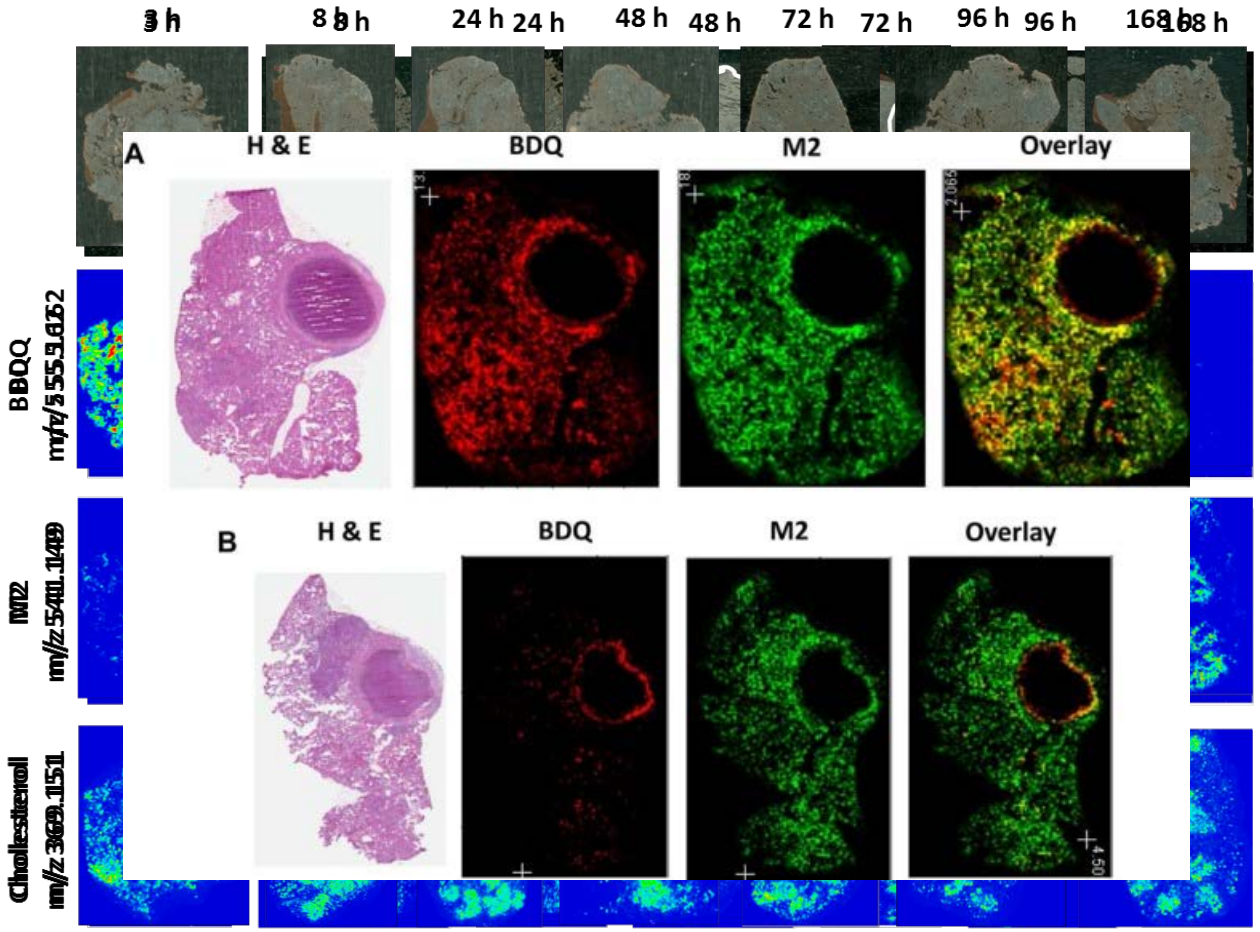


Single-dose

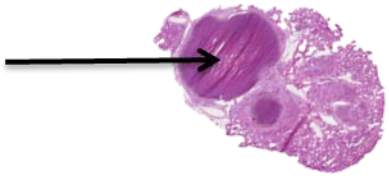
25 mg/kg

0-168h





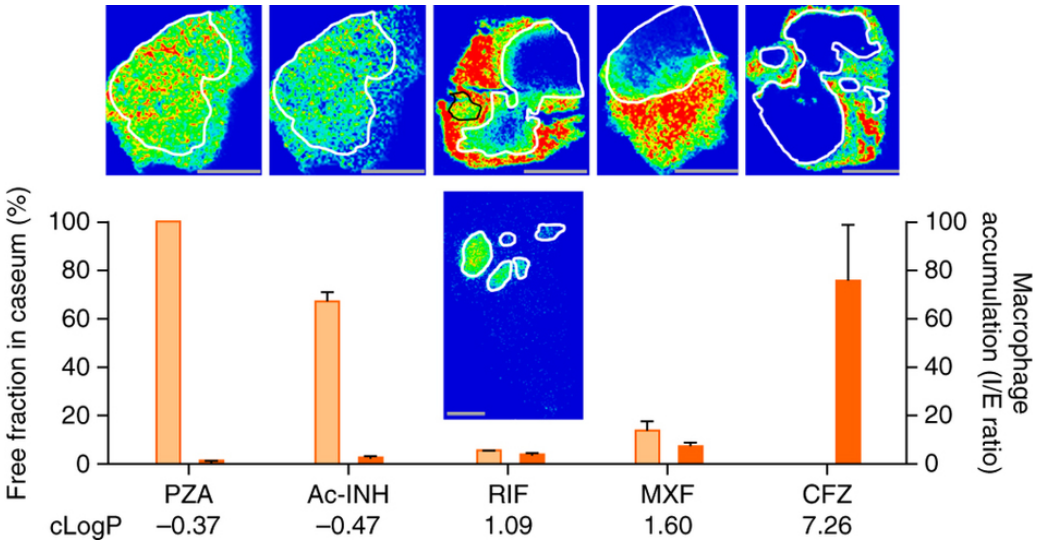
Does BDQ penetrate into caseum?



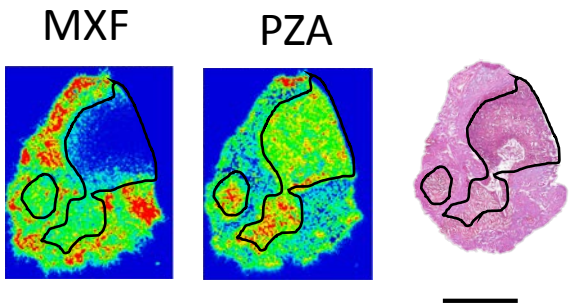
Irwin *et al.* ACS Infect Dis. 2016 Apr 8;2(4):251-267

Factors affecting penetration into necrotic tissue

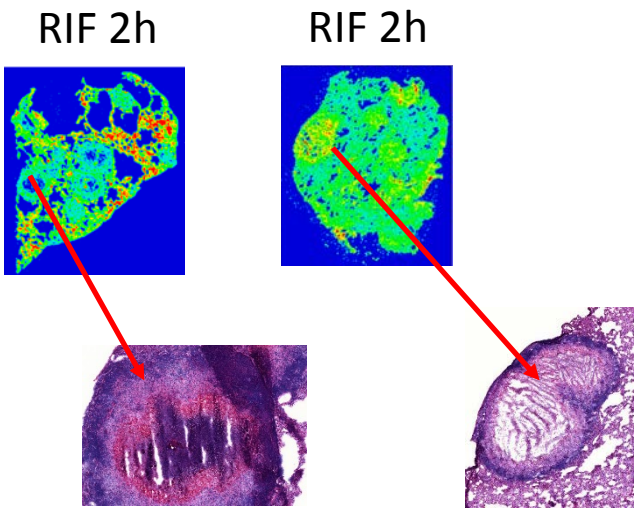
Caseum binding and active macrophage uptake



Lesion (caseum) size

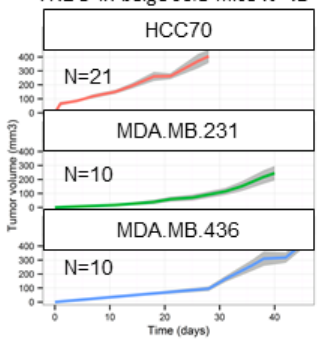


Caseum cellularity



Heterogeneous drug penetrance of veliparib and carboplatin measured in triple negative breast tumors

a) Mammary fat pad implant
TNBC in beige SCID mice **N=41**



b) Dose administration

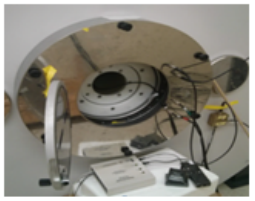
Placebo:	**N=6**
C/placebo:	**N=9**
C/V20mg/kg:	**N=12**
C/V60mg/kg:	**N=12**

c) Blood sampling day 2, **N=15**

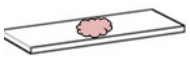
d) Pilot DCE-MRI
C/V60mg/kg: **N=2**

e) Tissue resection day 3
3 hours post 9 doses V+
24 hours post C
N=39

DCE-MRI pilot



e) Tissue resection day 1,
1.5 hours post V/C single dose **N=2**



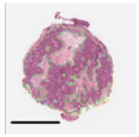
f) 12 µm section mounted on glass plates

g) 12 µm section for DNA extraction

h) 5 or 12 µm section mounted on stainless steel plate

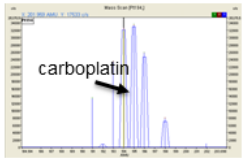
i) Grounding tissue for LC-MS analysis

Cell viability:
H&E stain



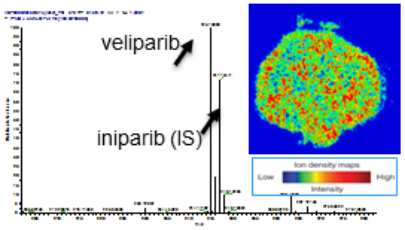
N_{tumors}=50
(all animals)

Carboplatin adducts:
ICP-MS



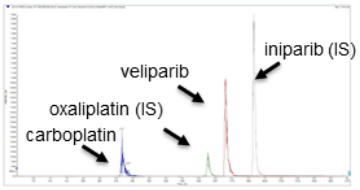
N_{tumors}=13

Veliparib:
MALDI



N_{tumors}=18
N_{muscle} =18

Veliparib (+carboplatin):
LC-MS

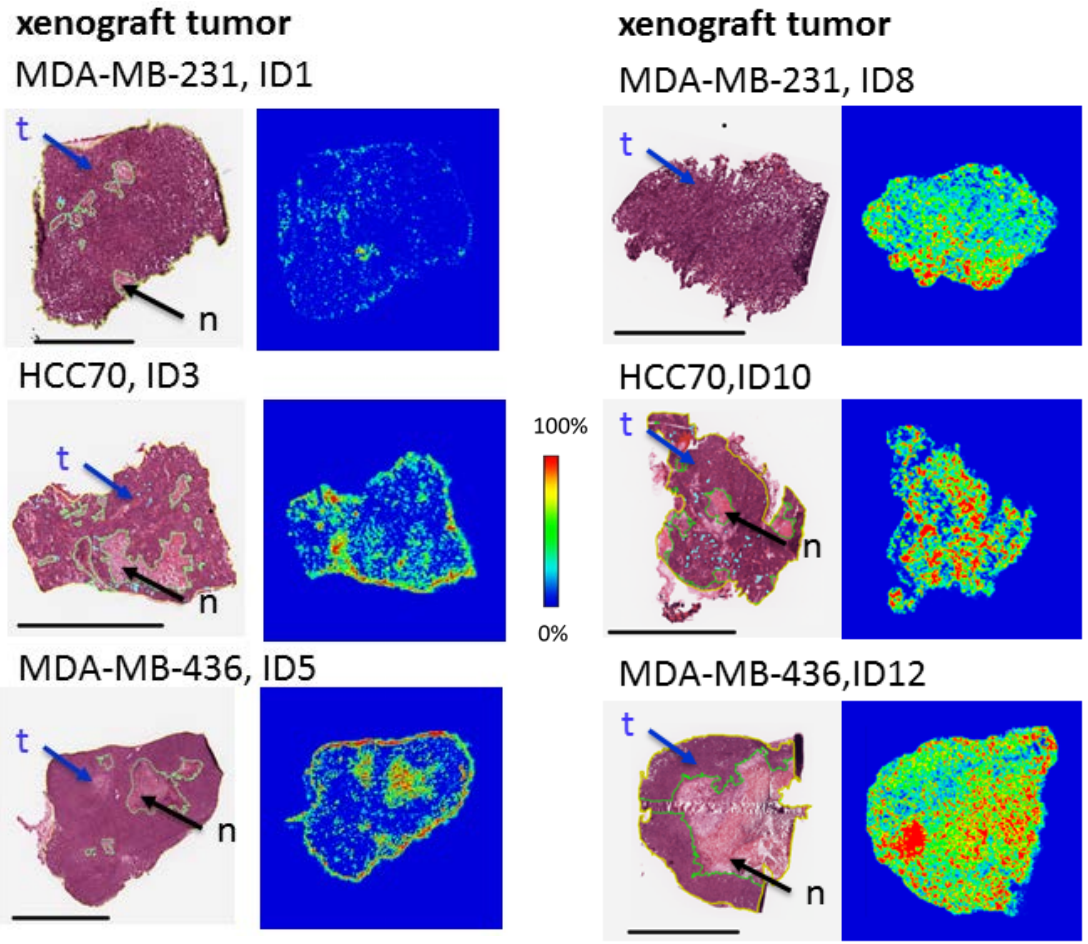


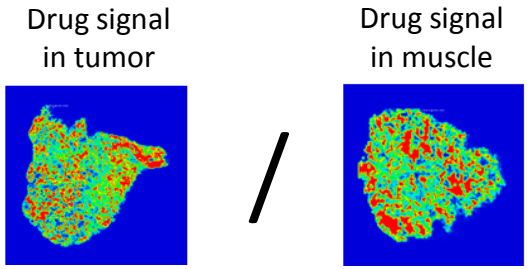
N_{tumors}=36
N_{muscle} =11
N_{liver} =12

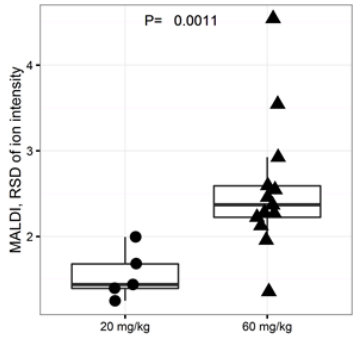
Verliparib distribution within tumors

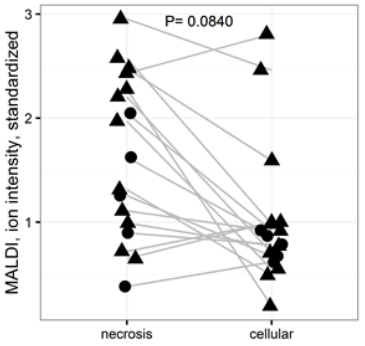
20 mg/kg Veliparib

60 mg/kg Veliparib





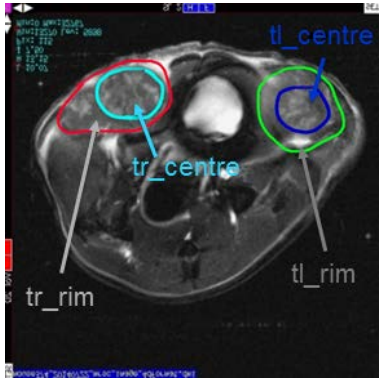




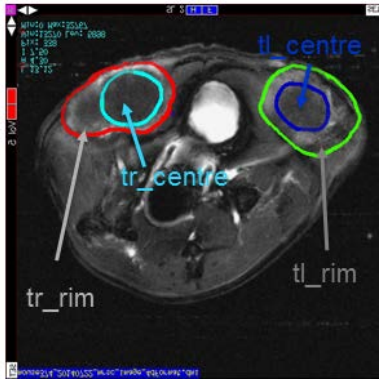
Verliparib distribution within tumors (2)

MRI of contrast agent

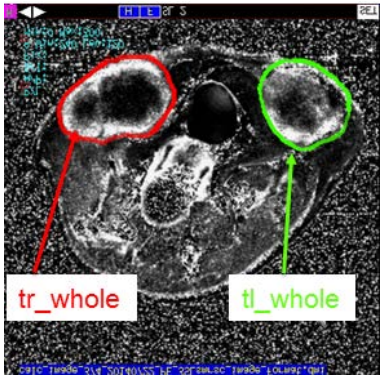
10 minutes

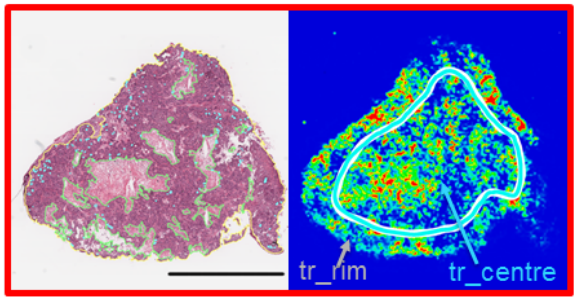


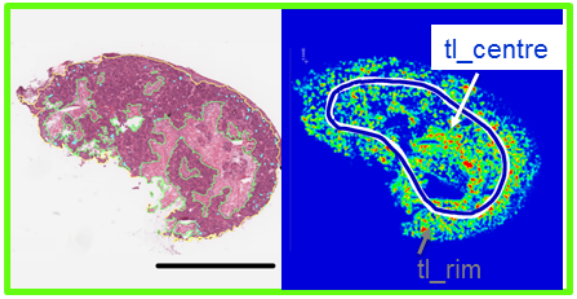
40 minutes



PE

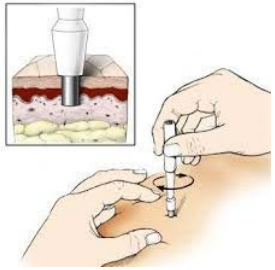




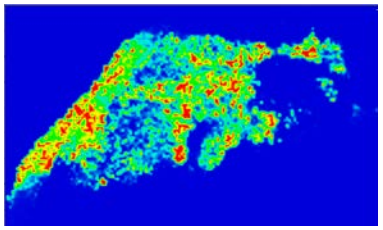


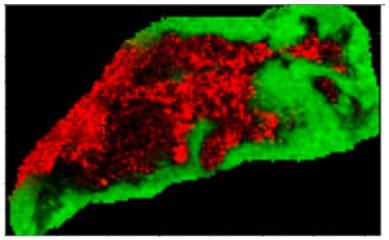
Early contrast agent distribution correlates with vascularity, mimics veliparib distribution at later timepoints

Clinical Applications

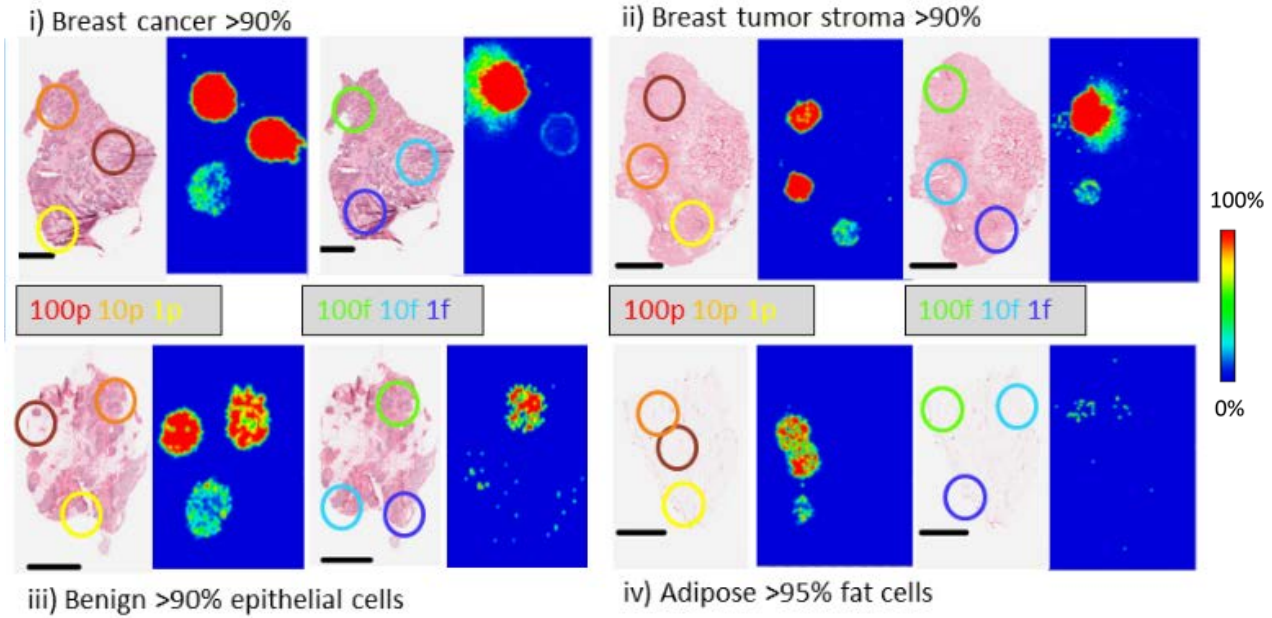


ABT888 *m/z* 245.140





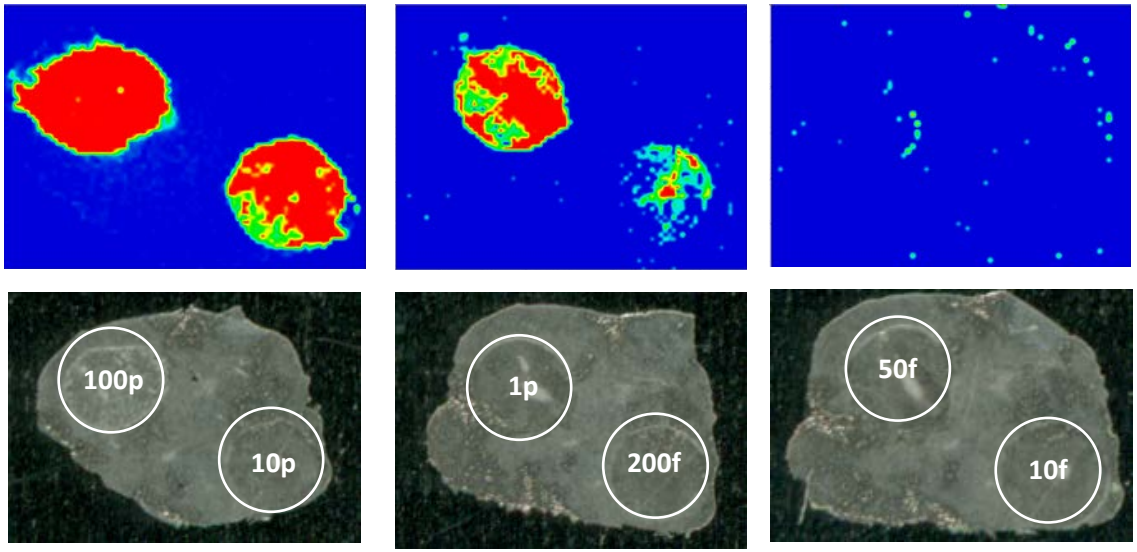
Veliparib
OCT



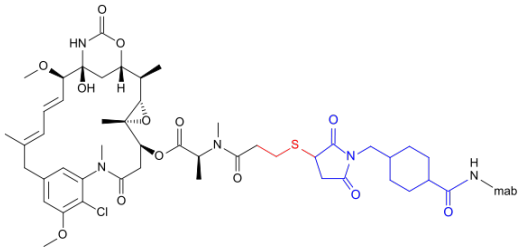
Veliparib can be imaged by MALDI-MSI when spiked on tumor tissue at clinically-relevant concentrations

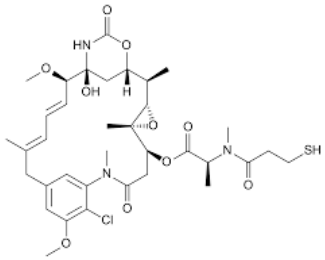
Visualizing DM1 in tumor tissue - feasibility

DM1 $[M+K]^+$

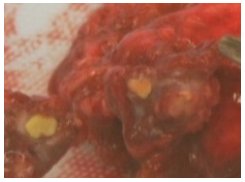


- DM1 acts as tublin inhibitor
- Dosed as antibody-drug conjugate
- T-DM1 when conjugated to trastuzumab

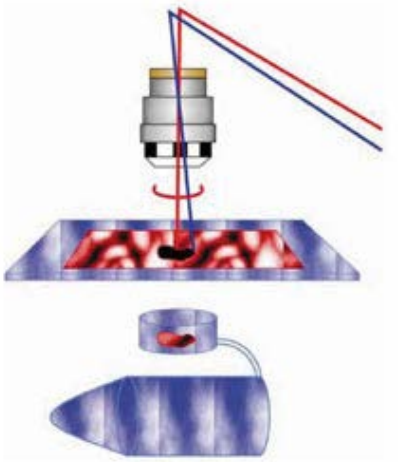




Future directions - spatial quantification (LCM)

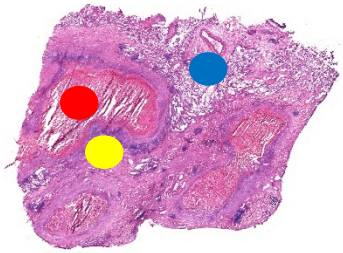


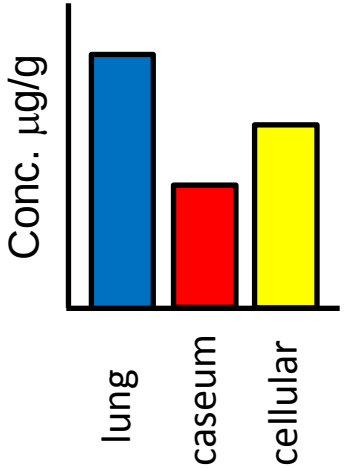
Sectioning



extraction

Quantification

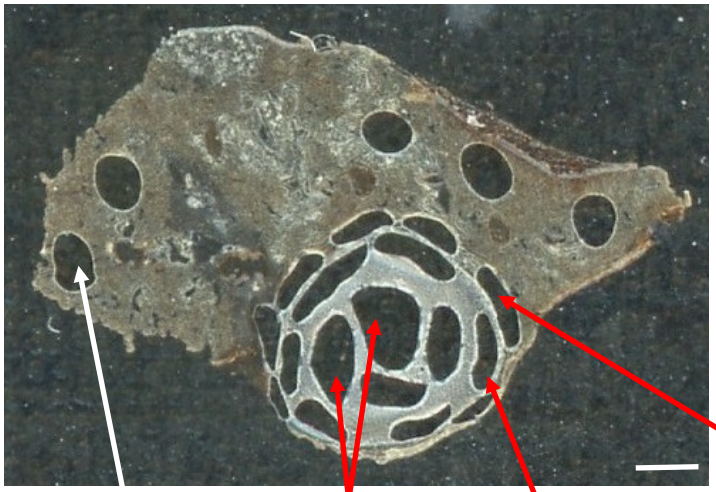




Increased sensitivity and full quantification. Complementary information to high spatial resolution MALDI MSI .

Laser capture microdissection (TB example)

Optical scan under LCM microscope



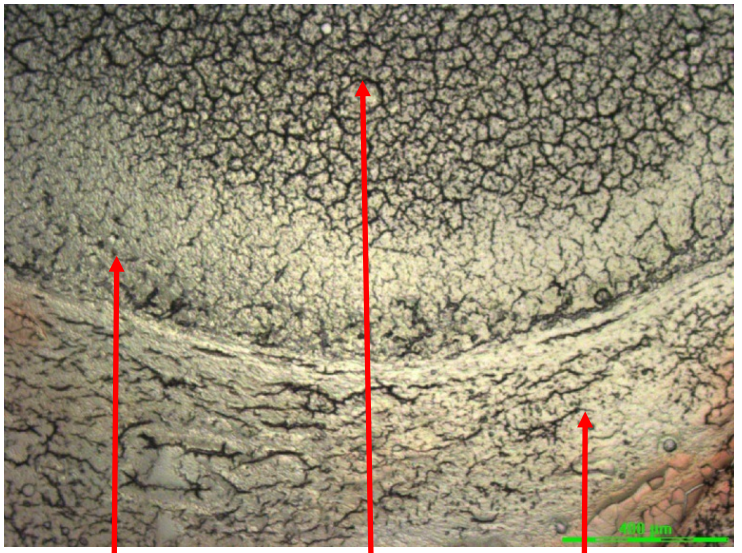
Uninvolved lung

Inner caseum

Outer caseum ring

Cellular

Optical scan under LCM microscope



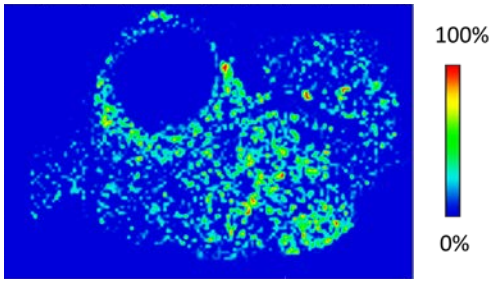
Inner caseum

Outer caseum/ring

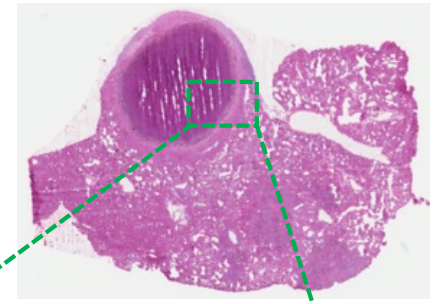
Cellular rim

Quantifying MALDI MS images by LCM-LC-MS/MS

BDQ



LCM serial section



Caseum core | Caseum edge | Cellular | Lung

BDQ ug/g	0.03	1.80	7.90	4.50

Summary

- MALDI-MSI is a powerful tool for visualizing drug distributions in tissue down to the cellular level.
- MALDI-MSI has been applied to visualize drug distribution into necrotic tissue areas in pulmonary TB disease and triple negative breast cancer.
- Necrotic tissue has low vascularity so little to no active drug delivery.
- Penetration of drug into necrotic tissue is crucial to target pathogens or cells residing within.
- Drug distribution into caseum correlated with several key compound physiochemical properties.
- A combined MRI and MALDI-MSI approach has value for correlating anti-cancer drug distribution with tumor vascularity.
- MALDI-MSI has significant potential for assessing anti cancer drug distribution within clinical tissues.
- Future steps – Quantification. Direct quantification by MALDI is possible, but challenging. LCM-LC-MS/MS is a viable complementary approach.

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