Imaging Investigations using AP-MALDI: drugs and metabolites distribution

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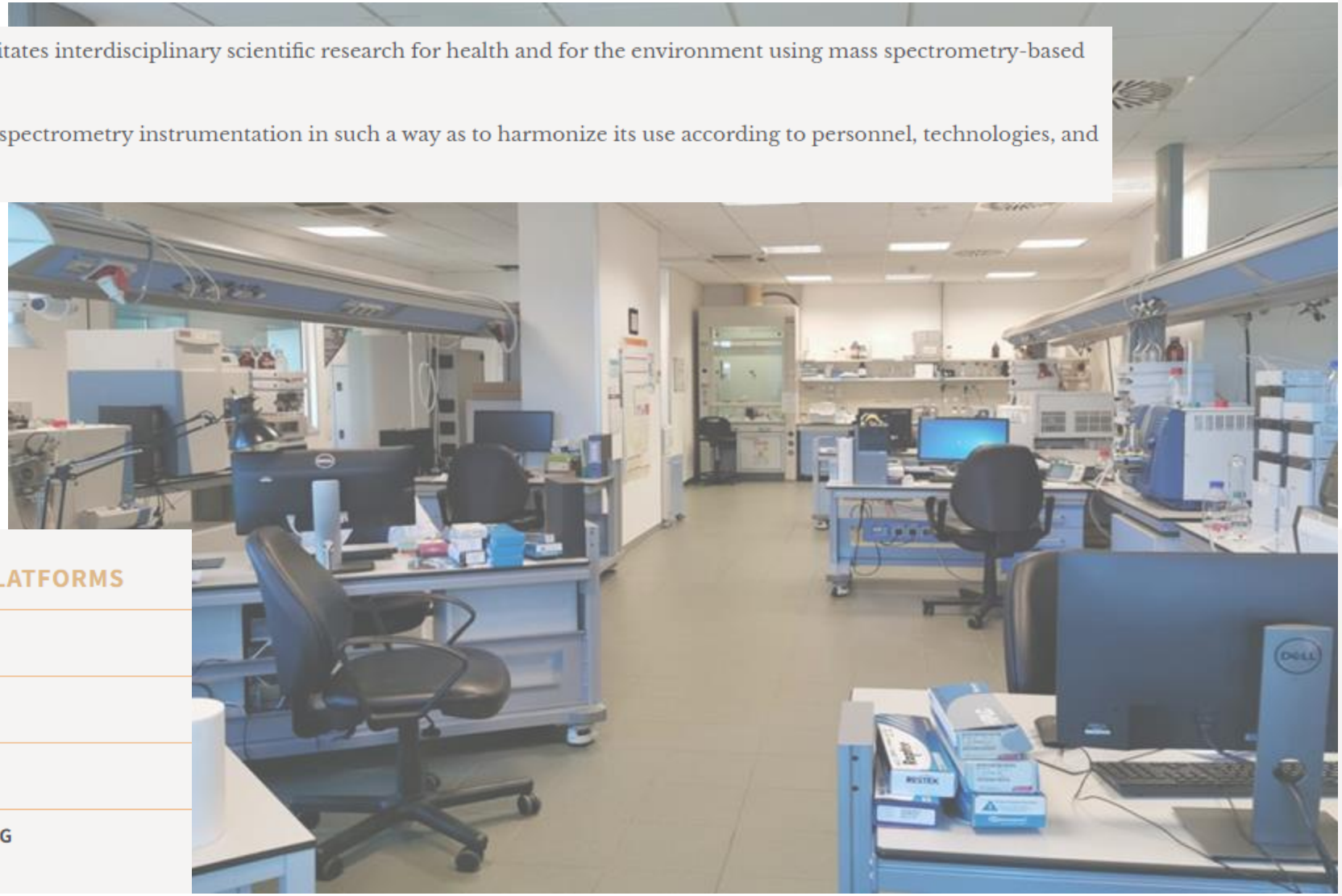
MASS SPECTROMETRY CENTRE FOR HEALTH AND ENVIRONMENT

https://www.marionegri.it/centro-di-ricerca-spettrometria-di-massa-per-la-salute-e-ambiente

The center promotes and facilitates interdisciplinary scientific research for health and for the environment using mass spectrometry-based technologies.

The center manages the mass-spectrometry instrumentation in such a way as to harmonize its use according to personnel, technologies, and available economic resources.



MASS SPECTROMETRY PLATFORMS

- GC/MS
- HPLC/MS
- MALDI TOF
- MASS SPECTROMETRY IMAGING

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MASS SPECTROMETRY CENTRE FOR HEALTH AND ENVIRONMENT

MASS SPECTROMETRY PLATFORMS

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MASS SPECTROMETRY CENTRE FOR HEALTH AND ENVIRONMENT: IMAGING APPROACHES

HUNTINGTON'S DISEASE

LEYOMIOSARCOMA

MASS SPECTROMETRY CENTRE FOR HEALTH AND ENVIRONMENT: IMAGING APPROACHES

STUDY OF THE SPATIAL DISTRIBUTION OF CHOLESTEROL IN BRAIN

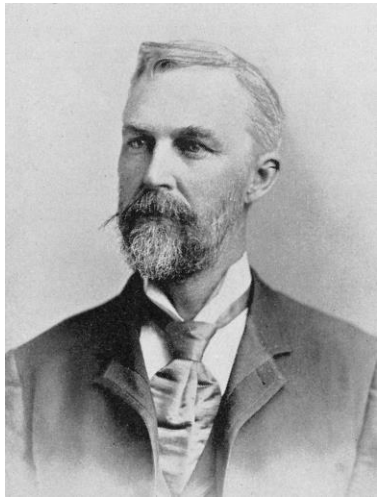
STUDY OF THE SPATIAL DISTRIBUTION OF DOCETAXEL IN TUMOR

HUNTINGTON'S DISEASE is an autosomal dominant neurodegenerative disease

First accurate description: 1872 by George Huntington

Epidemiology: 4-8 per 100000

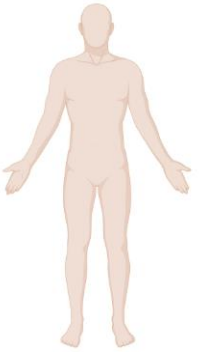
Causes:
mutation in Huntingtin gene (HTT) causes the translation for the mutant HTT protein
Normal individuals: less than 36 CAG repeats in HTT gene
Individuals with > 39 CAG repeats will develop HD



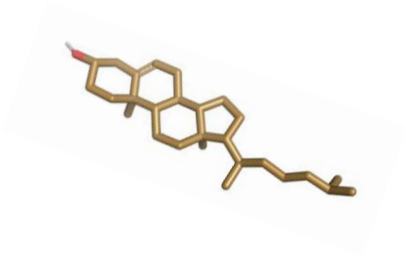
Neurological and peripheral symptoms

Classic neurological symptoms
Motor symptoms
Cognitive deterioration
Psychiatric and behavioural problems

Other symptoms:
Weight loss
Atrophy of skeletal muscle
Sleep disturbance



CHOLESTEROL IS ESSENTIAL FOR THE MAINTENANCE OF BRAIN HOMEOSTASIS



The brain is the richest organ in cholesterol:
70/80% is localized in the myelinated sheets
30/20% is a structural component of astrocytes and neuronal membranes

The new cholesterol produced in the brain every day is needed for the formation and remodeling of synaptic vesicles

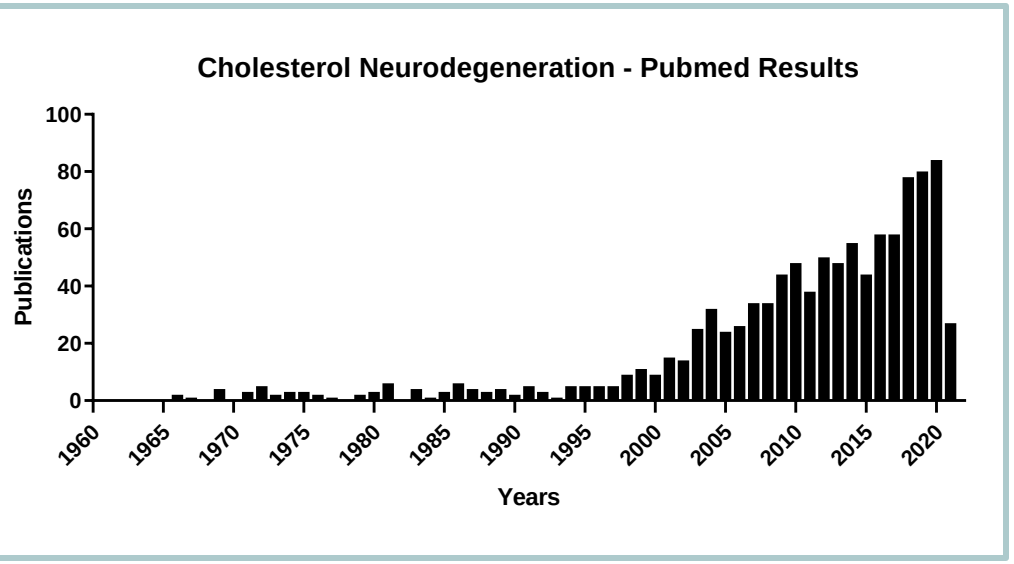
REVIEW ARTICLE
Front. Physiol., 04 January 2013 | https://doi.org/10.3389/fphys.2012.00486

Cholesterol homeostasis: a key to prevent or slow down neurodegeneration

Laura Anchisi[1,2], Sandra Dessi[4], Alessandra Pani[3] and Antonella Mandas[4]*

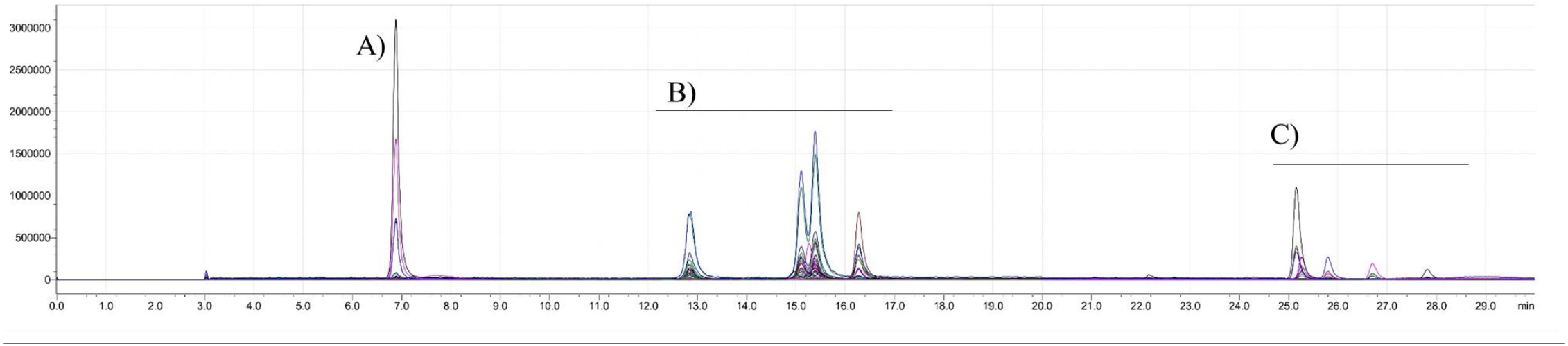
[1]Child Neuropsychiatry Unit, Azienda Sanitaria Locale (ASL) n°5, Oristano, Italy
[2]Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Messina, Italy
[3]Department of Biomedical Sciences, University of Cagliari, Monserrato, Cagliari, Italy
[4]Department of Medicine Sciences, University of Cagliari, Monserrato, Cagliari, Italy

Neurodegeneration, a common feature for many brain disorders, has severe consequences on the mental and physical health of an individual. Typically human neurodegenerative diseases are devastating illnesses that predominantly affect elderly people, progress slowly, and lead to disability and premature death; however they may occur at all ages. Despite extensive research and investments, current therapeutic interventions against these disorders treat solely the symptoms. Therefore, since the underlying mechanisms of damage to neurons are similar, in spite of etiology and background heterogeneous, it



Huntington's disease is characterized by an impairment of cerebral cholesterol homeostasis due to the alteration of both biosynthesis and catabolism

THE COMBINATION OF TEMPERATURE AND CHROMATOGRAPHIC GRADIENT GUARANTEES THE ANALYSIS OF BOTH OXYSTEROLS AND CHOL PRECURSORS

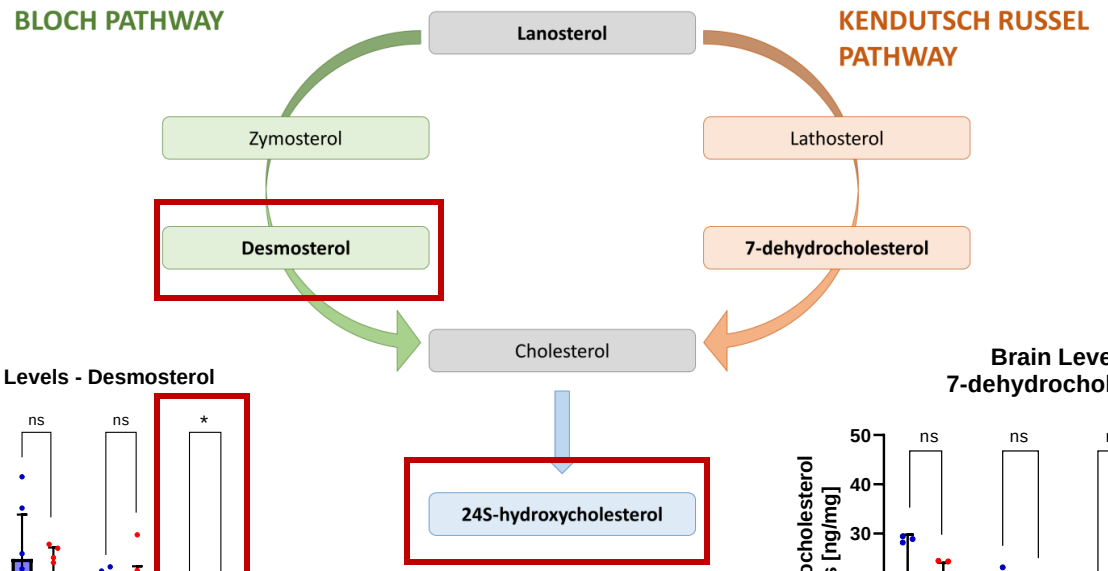


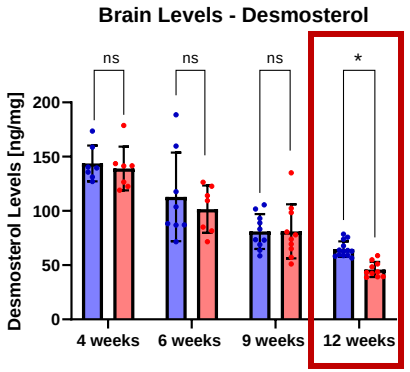
A) Bile Acids synthesis rate-limiting step: 7-alpha-hydroxycholesterol
B) Oxysterols: 22ROHC, 24SOHC, 25OHC, 27OHC
C) Cholesterol Precursors: Desmosterol, 7-dehydrocholesterol, Lanosterol

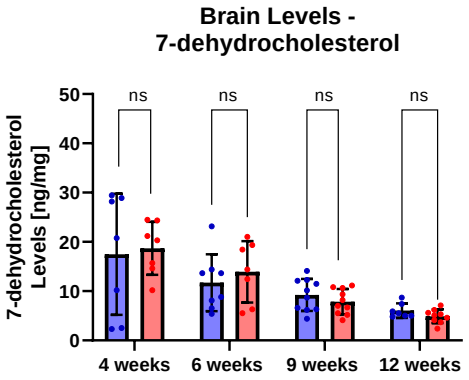
A combined solvent and temperature gradient to obtain the simultaneous analysis of different classes of compounds

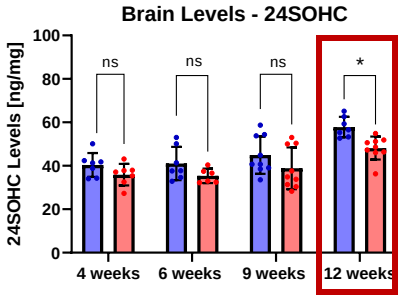
Passoni et al., 2024

THE METABOLIC STUDY IN WT AND R6/2 MICE HIGHLIGHTED SIGNIFICANT DIFFERENCES IN CHOL METABOLITES LEVELS







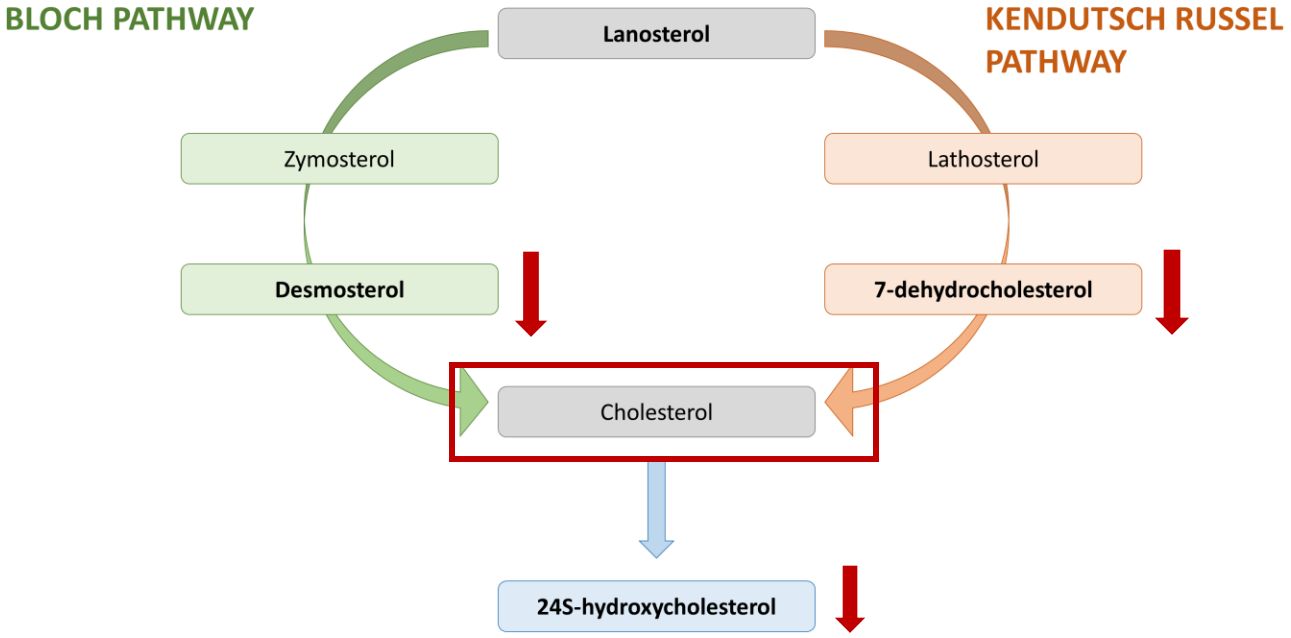


The results show in striatum a **significant difference** between WT and R6/2 animals at 12 weeks in desmosterol (Bloch pathway), but not in 7-dehydrocholesterol (Kandutsch-Russel pathway). **These results suggest that the Bloch pathway, used mostly in astrocyte cells, is the most affected.**

In the striatum, R6/2 mice show **decreased level of 24OH-cholesterol** in comparison with WT mice at 12 weeks of age, suggesting that the catabolism of cholesterol is affected too.

Passoni et al., 2024

THE METABOLIC STUDY IN WT AND R6/2 MICE HIGHLIGHTED SIGNIFICANT DIFFERENCES IN CHOL METABOLITES LEVELS



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Cholesterol homeostasis: a key to prevent or slow down neurodegeneration

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Efficacy of Cholesterol Nose-to-Brain Delivery for Brain Targeting in Huntington's Disease

Alice Passoni [1], Monica Favagrossa [1], Laura Colombo [1], Renzo Bagnati [1], Marco Gobbi [1], Luisa Diomede [1], Giulia Birolini [2 3], Eleonora Di Paolo [2 3], Marta Valenza [2 3], Elena Cattaneo [2 3], Mario Salmona [1]

Affiliations – collapse

Affiliations

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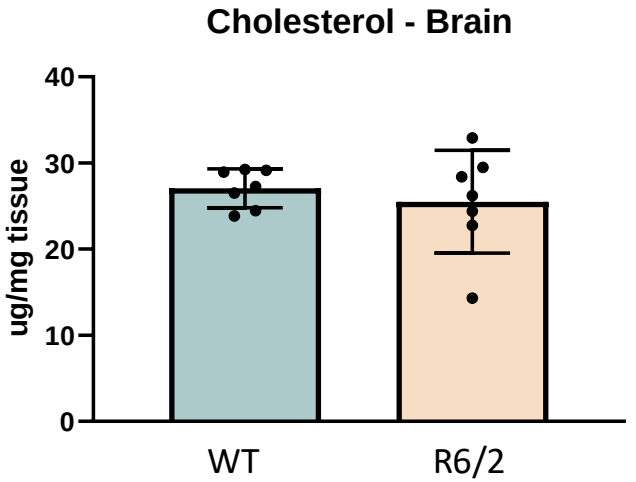
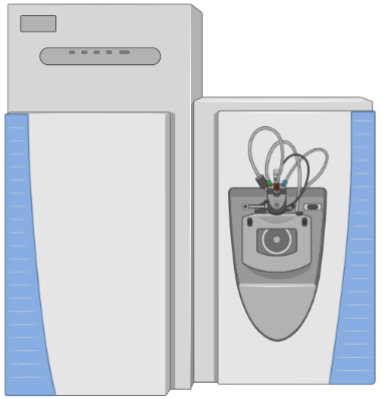


N=7/experimental group (12 weeks mice)

STUDY OF CHOL METABOLITES DISTRIBUTION IN BRAIN: MASS SPECTROMETRY IMAGING

Swansea University
Prifysgol Abertawe
Medical School
Ysgol Feddygaeth

Development of an imaging MS method for the study of the spatial distribution of the free fraction of cholesterol in brain slices from R6/2 and WT mice



Visualizing Cholesterol in the Brain by On-Tissue Derivatization and Quantitative Mass Spectrometry Imaging

Roberto Angelini, Eylan Yutuc, Mark F. Wyatt, Jillian Newton, Fowzi A. Yusuf, Lauren Griffiths, Benjamin J. Cooze, Dana El Assad, Gilles Frache, Wei Rao, Luke B. Allen, Zeljka Korade, Thu T. A. Nguyen, Rathnayake A. C. Rathnayake, Stephanie M. Cologna, Owain W. Howell, Malcolm R. Clench, Yuqin Wang, and William J. Griffiths*

> Anal Chem. 1997 Dec 1;69(23):4751-60. doi: 10.1021/ac970888i.

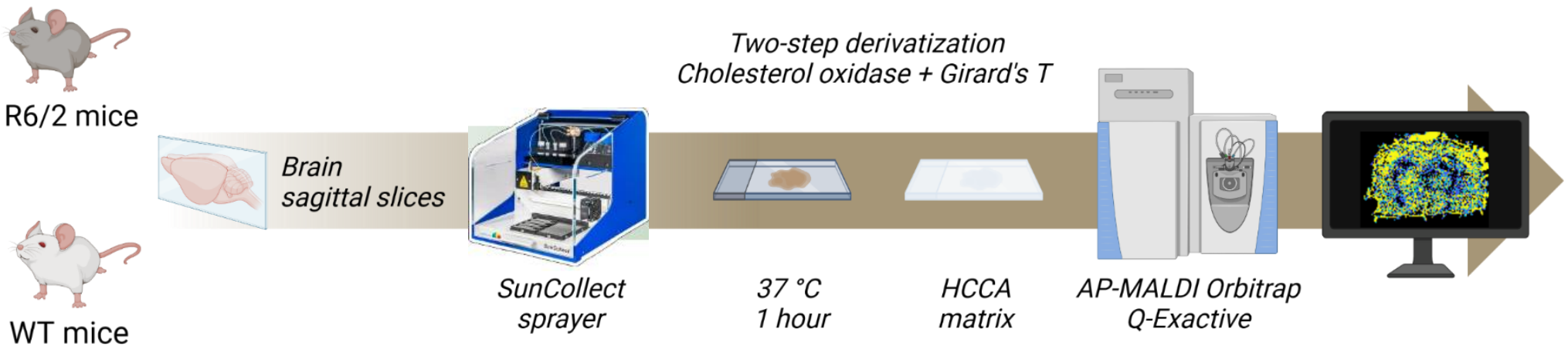
Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS

R M Caprioli [1], T B Farmer, J Gile

Mass spectrometry imaging (MSI):

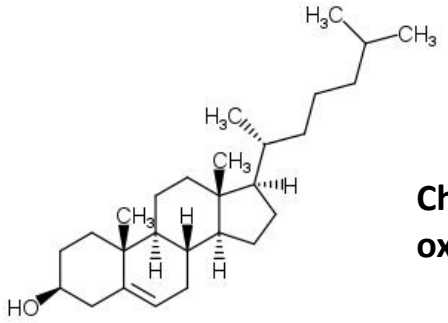
technique used in mass spectrometry to visualize the **spatial distribution of molecules in tissue slices by detecting their molecular masses**

IMAGING OF CHOLESTEROL IN BRAIN



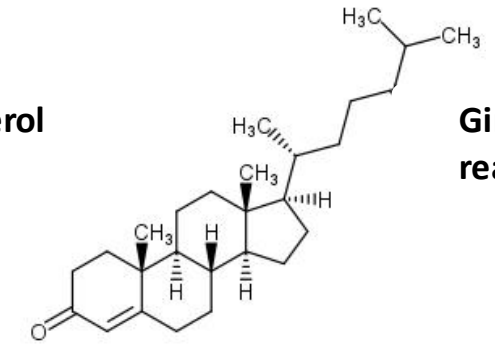
Spatial distribution of free fraction of chol metabolites using high resolution AP-MALDI imaging

GIRARD'S T DERIVATIZATION REACTION



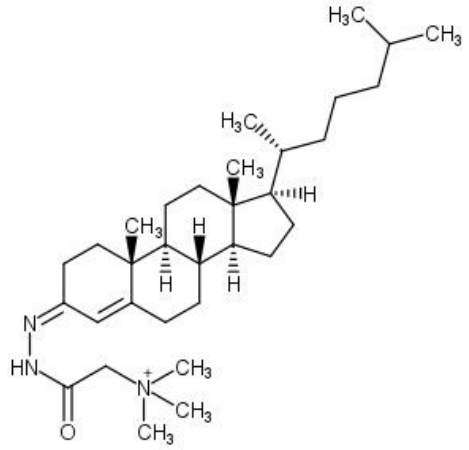
Exact Mass: 386.3548

Cholesterol oxidase



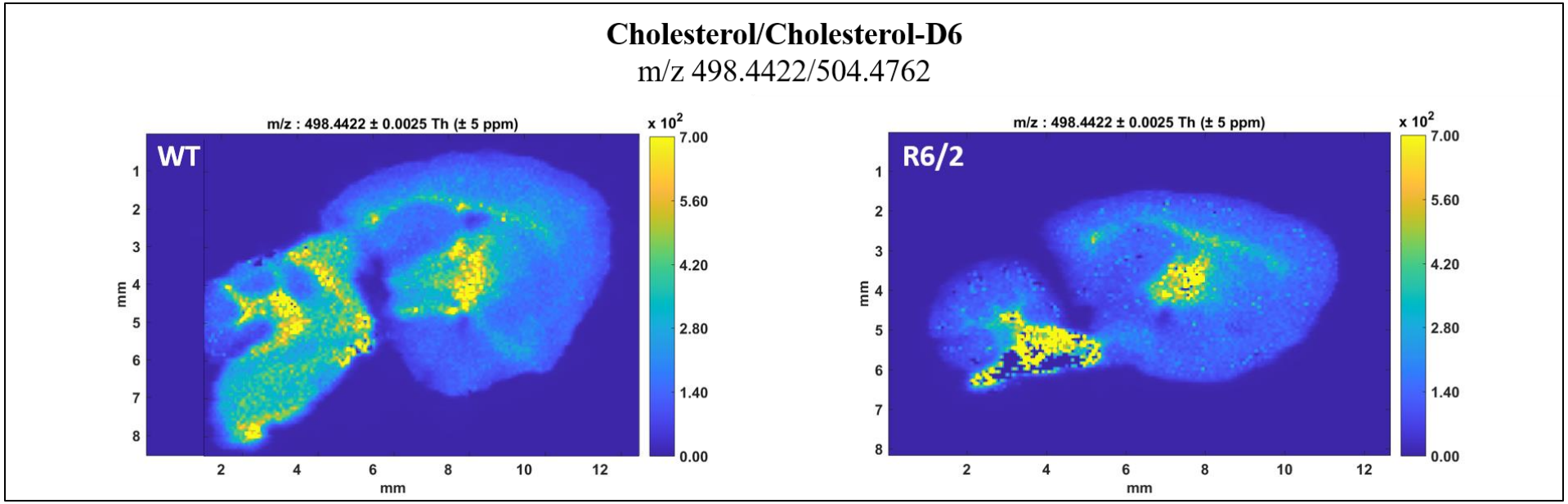
Exact Mass: 384.3392

Girard's reagent T



Exact Mass $[M]^+$: 498.4417

CHOLESTEROL DISTRIBUTION IN BRAIN SLICES FROM R6/2 AND WT MICE

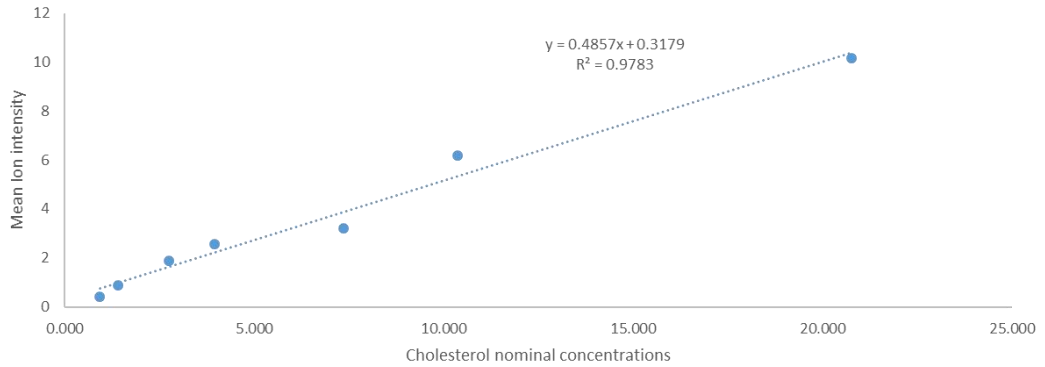


- **The images highlight the different levels of free Cholesterol R6/2 and WT brain slices thanks to the different color intensity in each pixel.**
- Integration of the method with a quantitative approach to better investigate the concentration and spatial distribution of cholesterol metabolites

QUANTITATIVE MASS SPECTROMETRY IMAGING
highlighted significant differences in cholesterol levels between WT and R6/2 mice

Swansea University
Prifysgol Abertawe
Medical School
Ysgol Feddygaeth

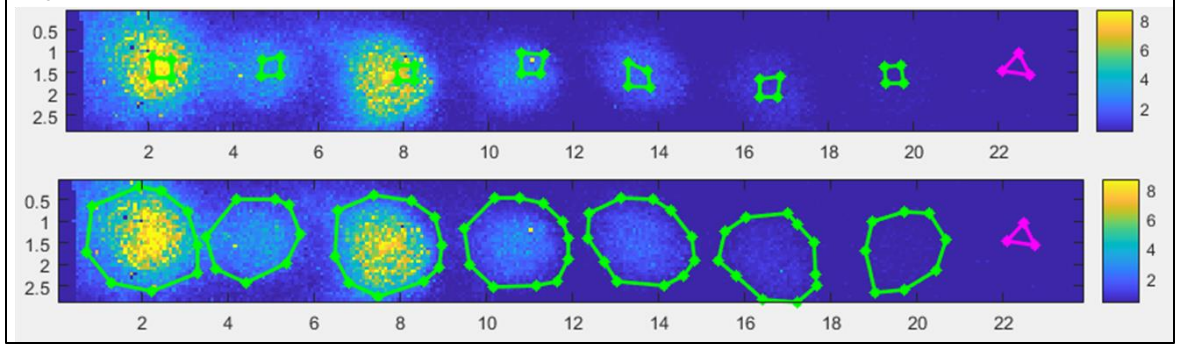
A)



B)

SAMPLE	Abundance	mm2	ng/mm2	Calculated ng/tot
WT	238.092	55.187	489.55	27016.76
R6/2	180.22	39.076	370.40	14473.66

C)



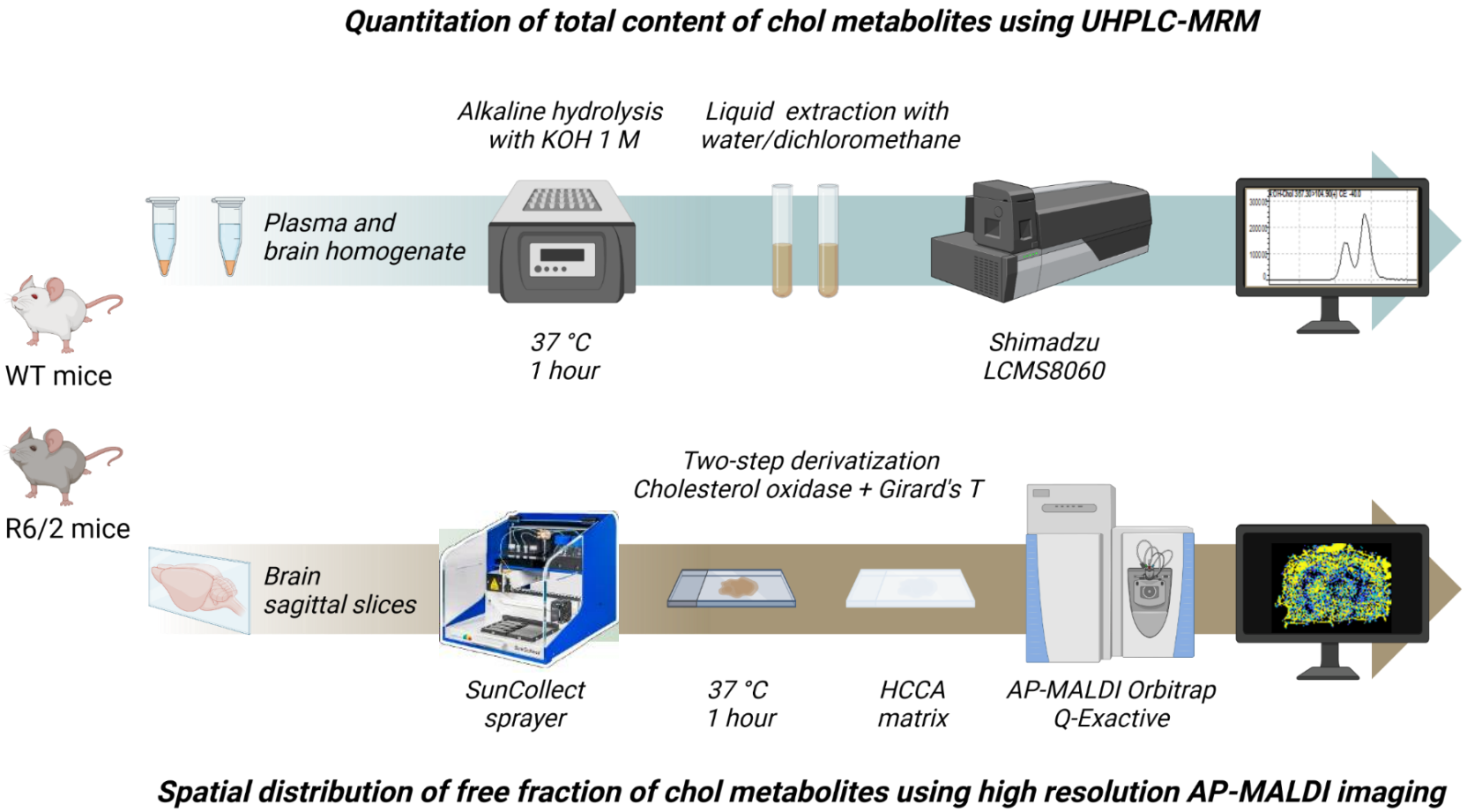
Past-in-the-Future. Peak detection improves targeted mass spectrometry imaging

Francesca Falcetta [a, 1], Lavinia Morosi [a, 1], Paolo Ubezio [a], Silvia Giordano [b], Alessandra Decio [a], Raffaella Giavazzi [a], Roberta Frapolli [a], Mridula Prasad [c, d, e], Pietro Franceschi [d], Maurizio D'Incalci [a], Enrico Davoli [b]

Quantitative imaging mass spectrometry highlighted differences in the free cholesterol levels between WT and R6/2 mice

The results suggested that although the total content of cholesterol is maintained at the steady-state, the available fraction of cholesterol is affected by the reduction of cholesterol synthesis

INTEGRATION OF MASS SPECTROMETRIC APPROACHES
is a powerful tool for the metabolic investigation of pathological conditions



The results obtained by the **integration of the two different mass spectrometric techniques** highlighted how mass spectrometry could be a powerful tool for a deep undestanding of the metabolic alterations related to pathological states

ACKNOWLEDGEMENTS

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UNIVERSITÀ DEGLI STUDI DI MILANO

FONDAZIONE I.R.C.C.S. ISTITUTO NEUROLOGICO CARLO BESTA

Swansea University Prifysgol Abertawe
Medical School
Ysgol Feddygaeth

Laboratory of Mass Spectrometry

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Dr. Renzo Bagnati, Dr.ssa Alessia Lanno

Laboratory of Biochemistry and Protein Chemistry

Dr. Mario Salmona, Dr.ssa Laura Colombo, Dr.ssa Monica Favagrossa

Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases

Prof.ssa Elena Cattaneo

Laboratory of neurogenetics

Dr.ssa Caterina Mariotti

Swansea University Medical School

Prof. William J. Griffiths, Prof. Yuqin Wang

MASS SPECTROMETRY CENTRE FOR HEALTH AND ENVIRONMENT: IMAGING APPROACHES

HUNTINGTON'S DISEASE

LEYOMIOSARCOMA

Leiomyosarcoma (LMS)

Leiomyosarcoma, or LMS, is a type of rare cancer that grows in the smooth muscles

LMS is an aggressive cancer and it is found most often in the abdomen or in the uterus

Leiomyosarcoma can occur at any age, but it is most commonly diagnosed in adults between the ages of 50 and 70

It accounts for approximately 5-10% of all soft tissue sarcomas

Symptoms

Common symptoms include abdominal pain, a noticeable mass, or unusual bleeding (especially in uterine LMS)

LMS TREATMENT

Surgery: Preferred treatment; aims for wide margins of tumor removal

Chemotherapy/Radiation: Used when surgical margins are narrow or tumor cells remain. **Provides survival benefits despite LMS resistance**.

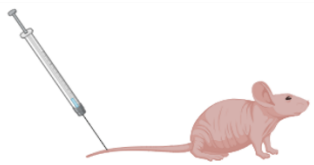
For Metastatic Disease:

Tratments include doxorubicin/ifosfamide, doxorubicin/gemcitabine, **docetaxel**, trabectedin.
Most of treatments include the combination of two anti-tumor drugs

AIM:

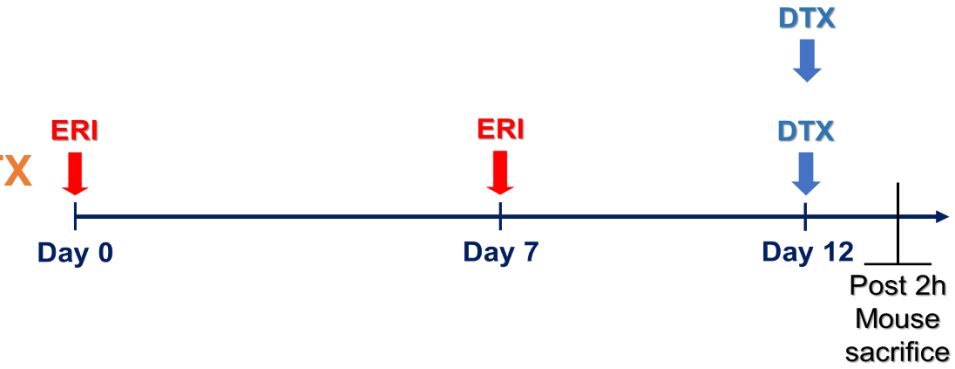
Evaluating the effect of a pre-treatment with eribulin on docetaxel distribution and penetration in tumor tissue

EXPERIMENTAL DESIGN



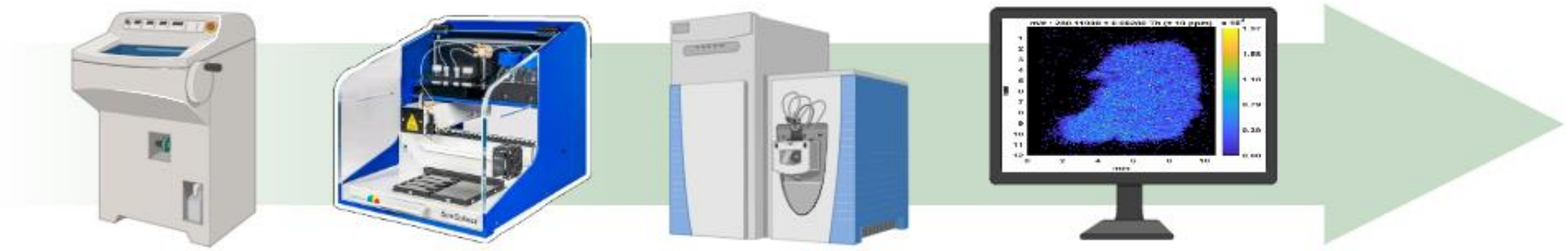
DTX

2ERI + DTX



DTX: 30 mg/kg, i.v.

ERI: 1 mg/kg, q7dx2, i.v.



Tissue sectioning

SunCollect
Sprayer
IS and Matrix

AP-MALDI Orbitrap
Q-Exactive

MSiReader
Quantification

QUANTITATIVE IMAGING MASS SPECTROMETRY APPROACH

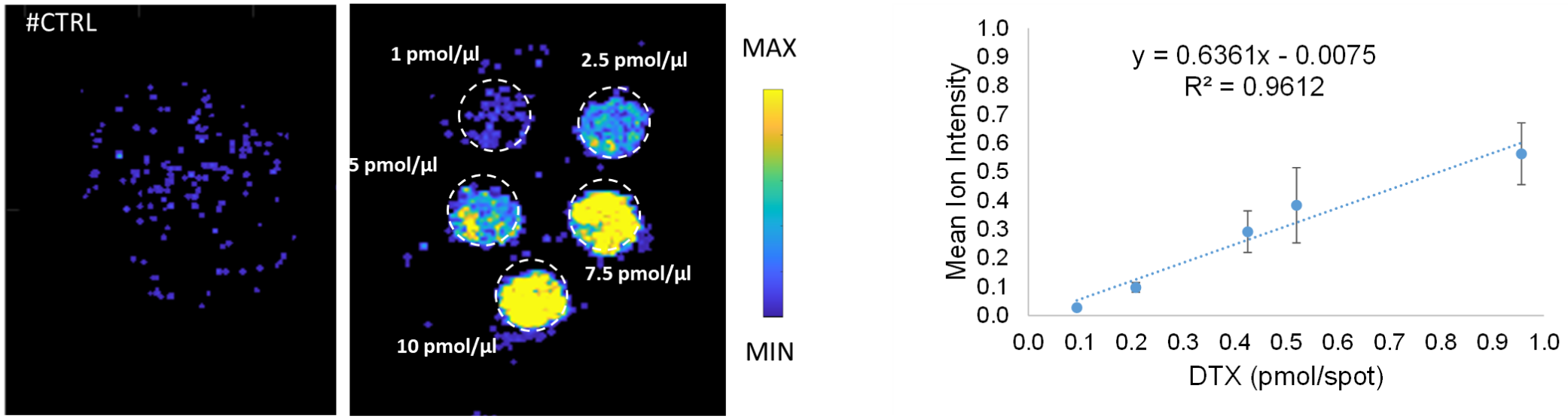
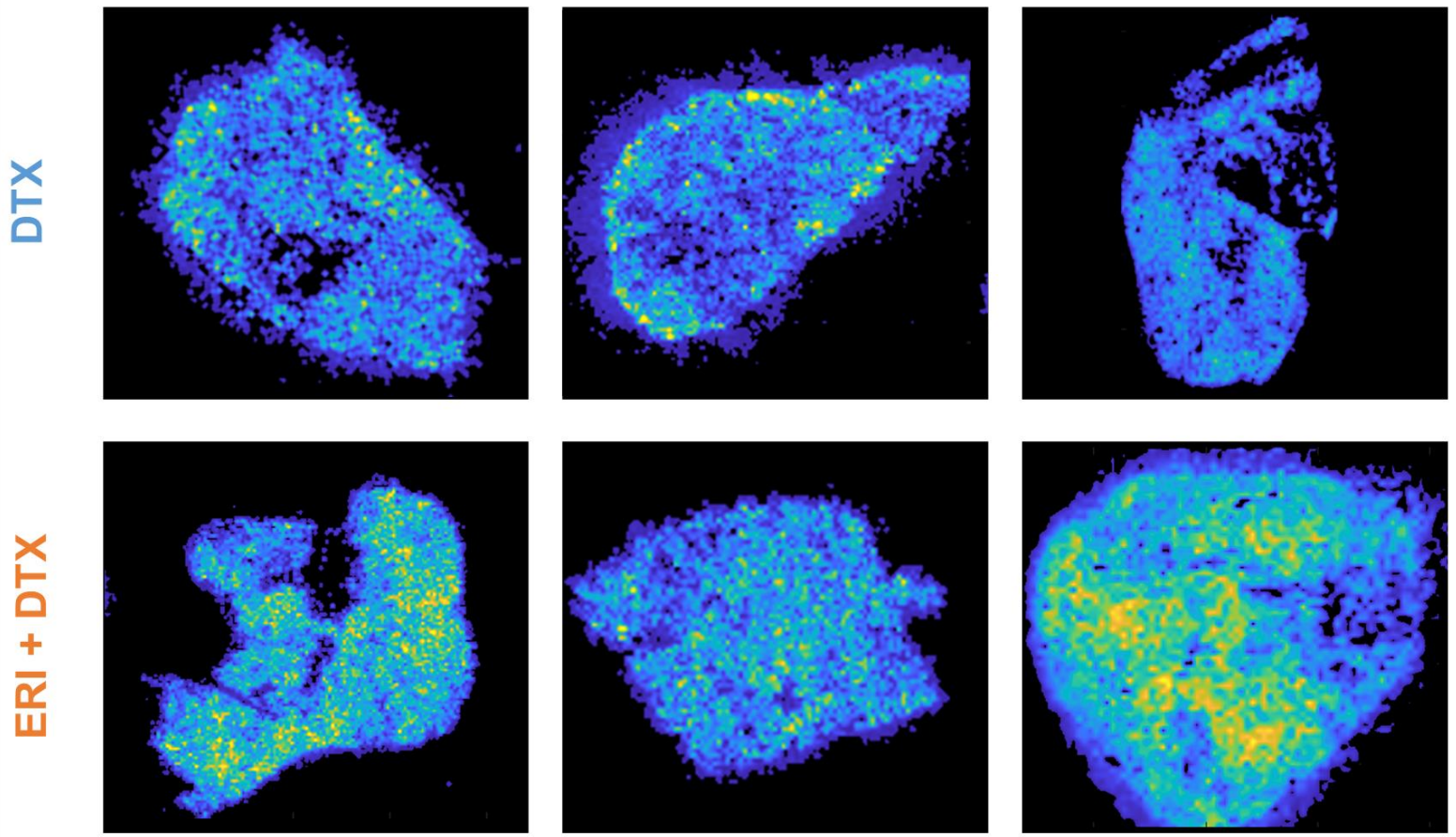
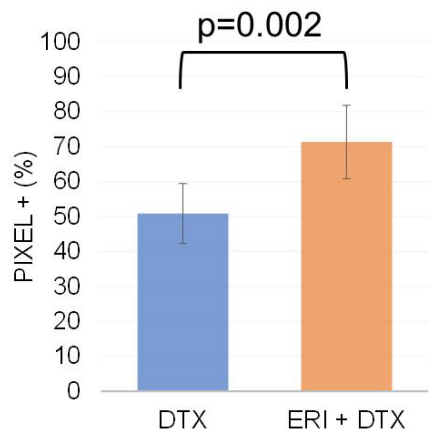
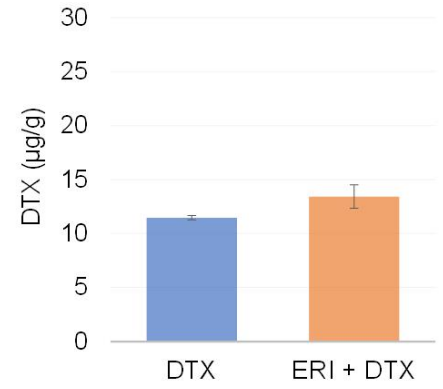


Figure 1: DTX standards spotted on CTRL tissue (DTX: m/z 280.1190; PTX: m/z 284.0931). IS normalized calibration curve (mean of three different curves).

ERIBULIN PRETREATMENT INCREASES DOCETAXEL PENETRATION IN TUMOR TISSUE







Statistical analysis: t-test

TUMOR TISSUE TREATED WITH ERIBULIN PRESENTS A REDUCTED NECROTIC AREA

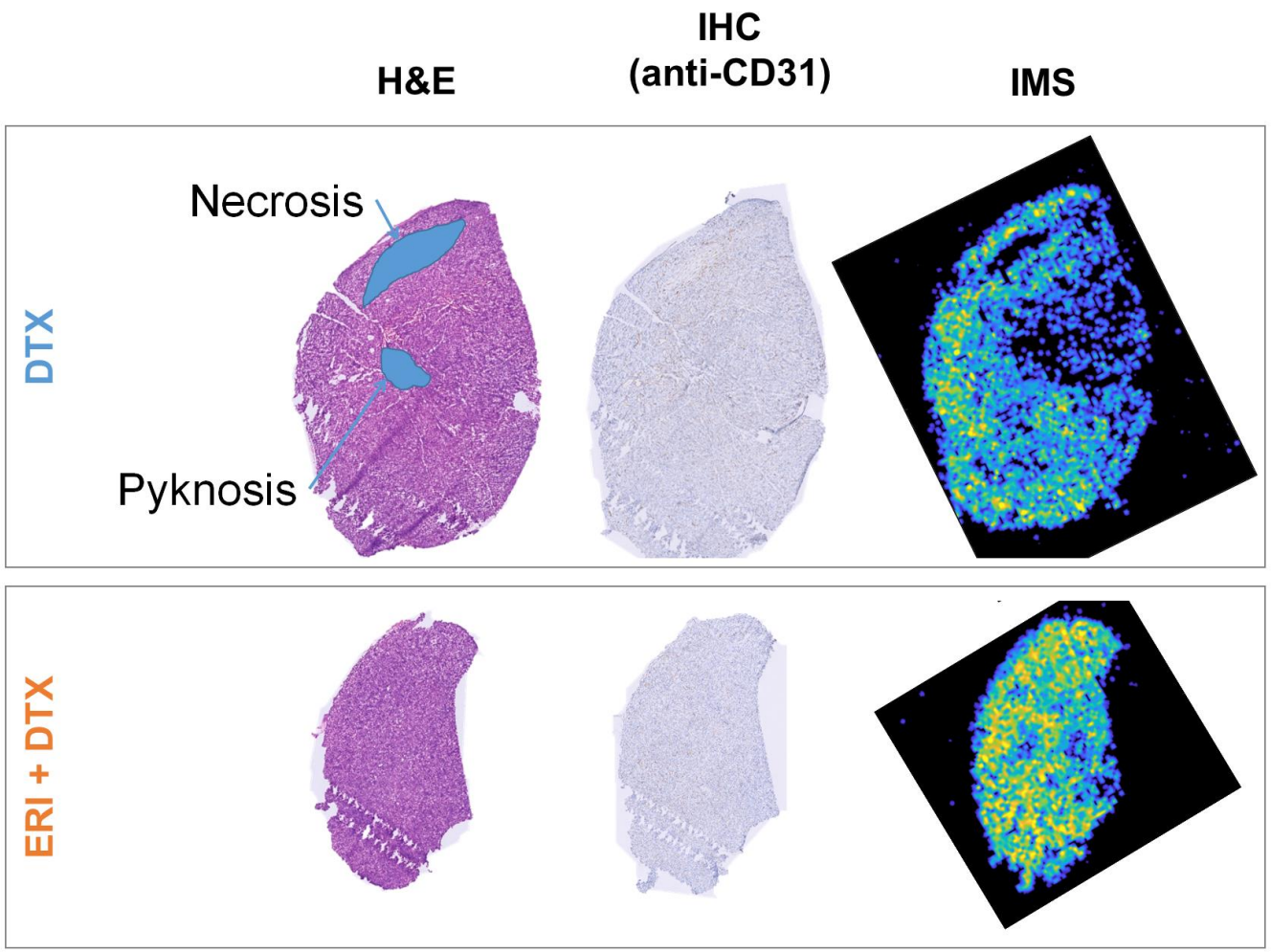


Figure 3: Comparation between the IMS distribution of DTX with the H&E and IHC staining of two adjacent slides.

ERI pretreatment improved the vascolarization

The histological analysis highlighted the reduction of necrotic areas in tumors, an essential factor in improving DTX penetration in tumors

CONCLUSIONS

- ERI pretreatment improved the penetration and distribution of DTX in the tumors of treated xenografts.
- IMS allowed us to appreciate the difference between treatments that were undetectable with classic quantitative approaches.

- **Future studies will be focused on:**

- Definition of treatment scheme, starting with the test of repeated doses of DTX
- IMS of metabolites in tumors to understand if there are altered metabolic pathways.

ACKNOWLEDGEMENTS

ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI · IRCCS

IEO Istituto Europeo di Oncologia

IRCCS HUMANITAS RESEARCH HOSPITAL

Sarcoma SFA Foundation of America
finding the cure in our time

Laboratory of Mass Spectrometry

Dr. Enrico Davoli
Dr. Stefano Mauro Carabellese

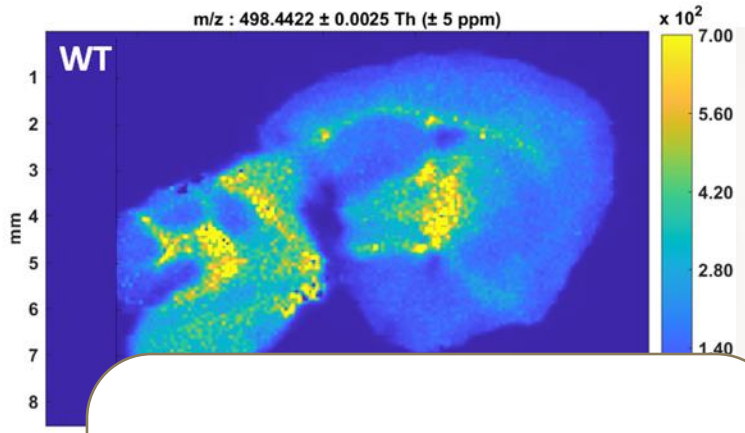
Laboratory of Cancer Pharmacology

Dr. Roberta Frapolli
Cristina Matteo, PhD
Dr Marta Cancelliere

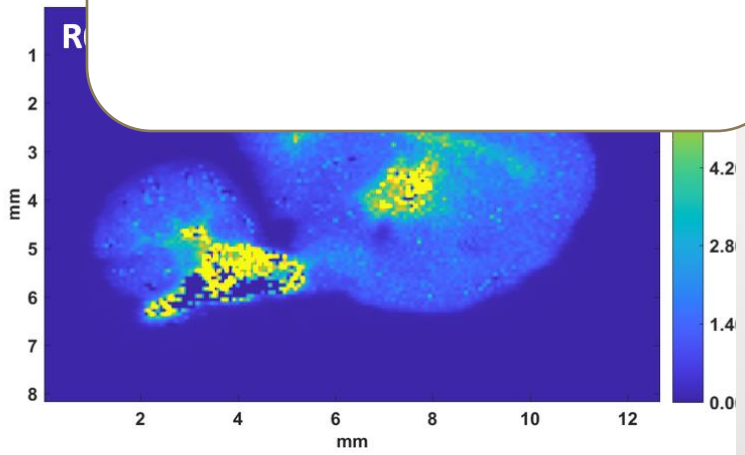
Lavinia Morosi, PhD

Supported by Sarcoma Foundation of America, R. and V. Aronsohn Memorial Research Award to R. Frapolli.

MASS SPECTROMETRY CENTRE FOR HEALTH AND ENVIRONMENT: IMAGING APPROACHES



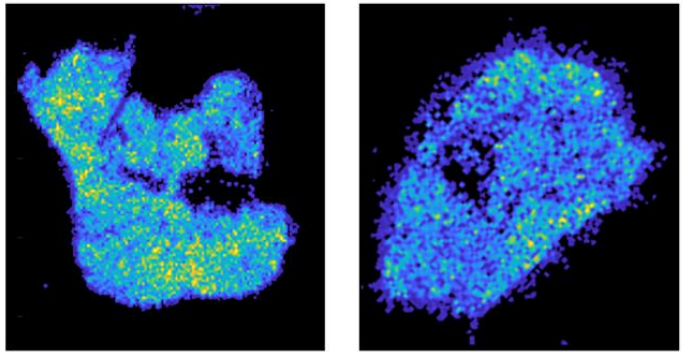
HUNTINGTON'S DISEASE



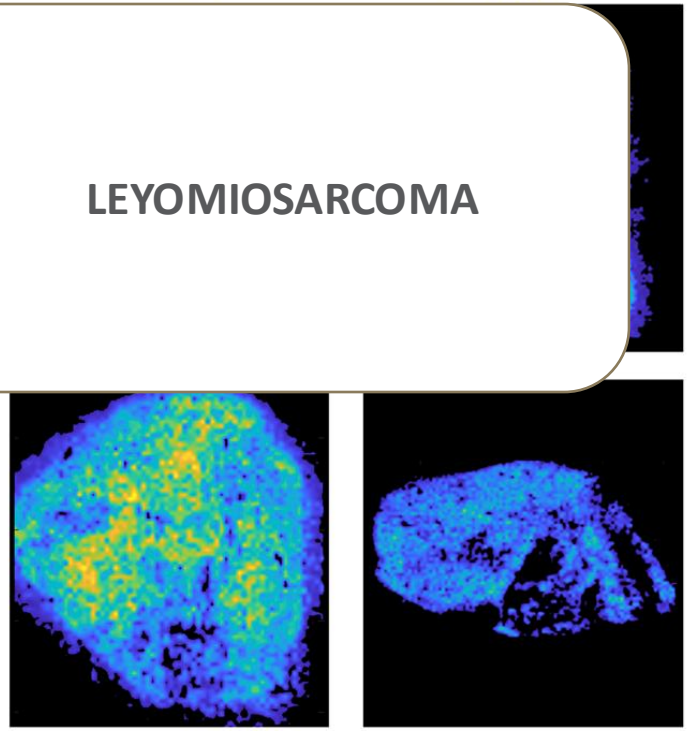
MassTech

AP/MALDI(ng) UHR Ion Source

..with ultra-high spatial resolution (better than 10 μm)



LEYOMIOSARCOMA



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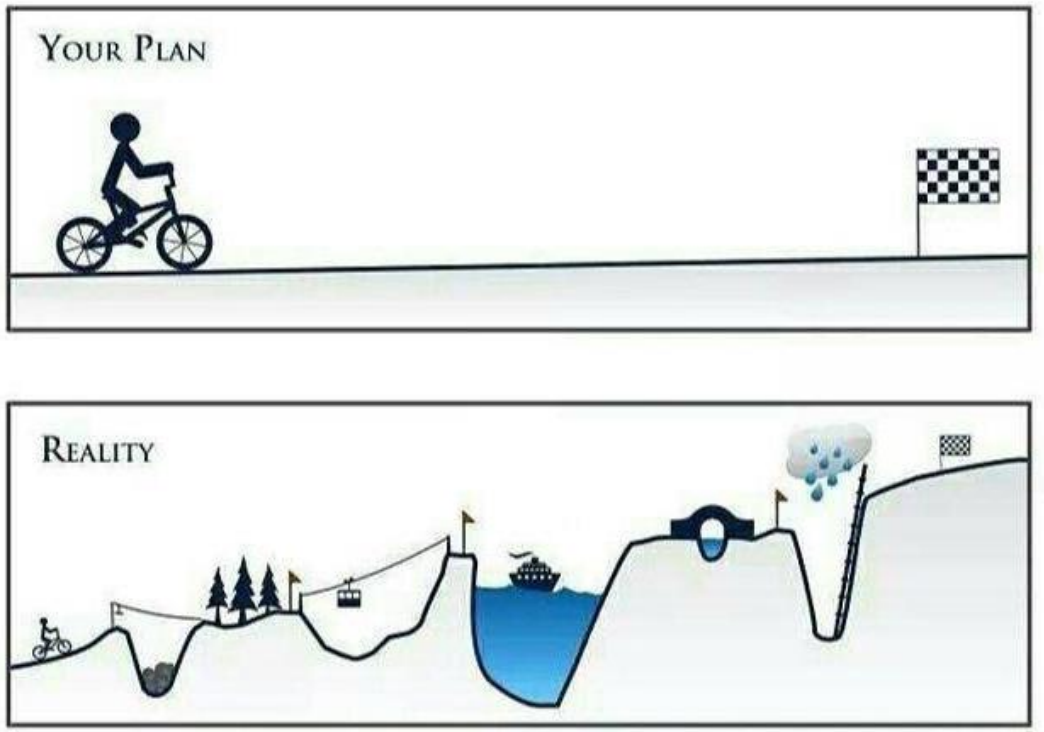

Imaging Investigations using AP-MALDI: drugs and metabolites distribution

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