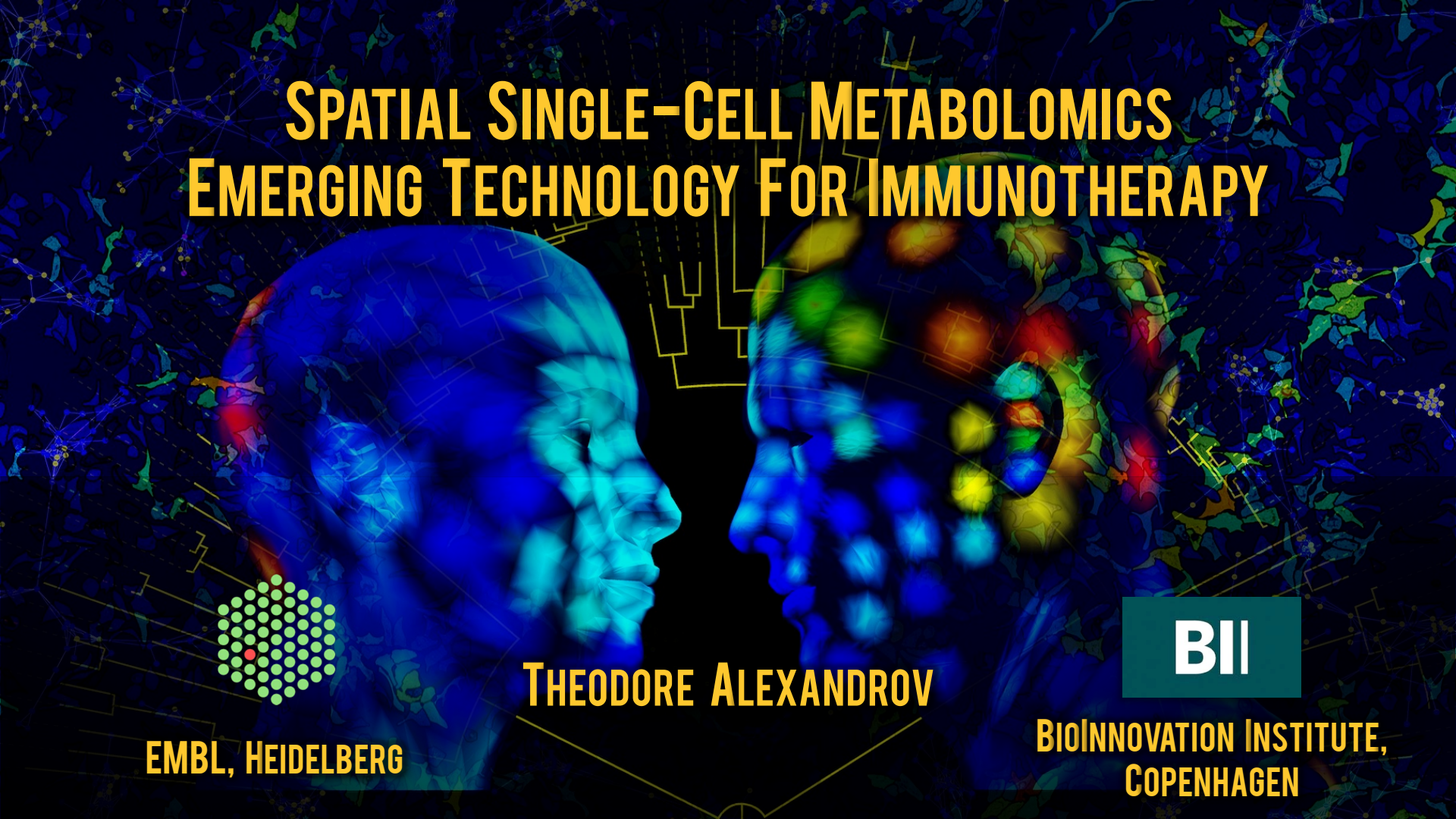
Spatial Single-Cell Metabolomics
Emerging Technology For Immunotherapy



Theodore Alexandrov

EMBL, Heidelberg

BII

BioInnovation Institute, Copenhagen

DISCLOSURES

Present

- SpaceM @ BioInnovation Institute – incubation phase (co-founder, equity holder)

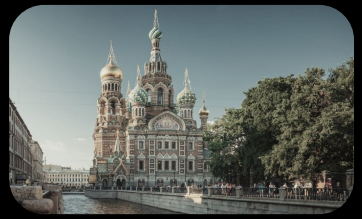
Past

- SCiLS GmbH (co-founder, equity holder, SAB)
- Grant from OpenTargets partnership funded by Sanofi, GSK, BMS

Patents inventor on spatial and single-cell metabolomics

CAREER

ST.PETERSBURG



Mathematics, computer science

BREMEN

Mass spectrometry

SAN DIEGO

Metabolomics

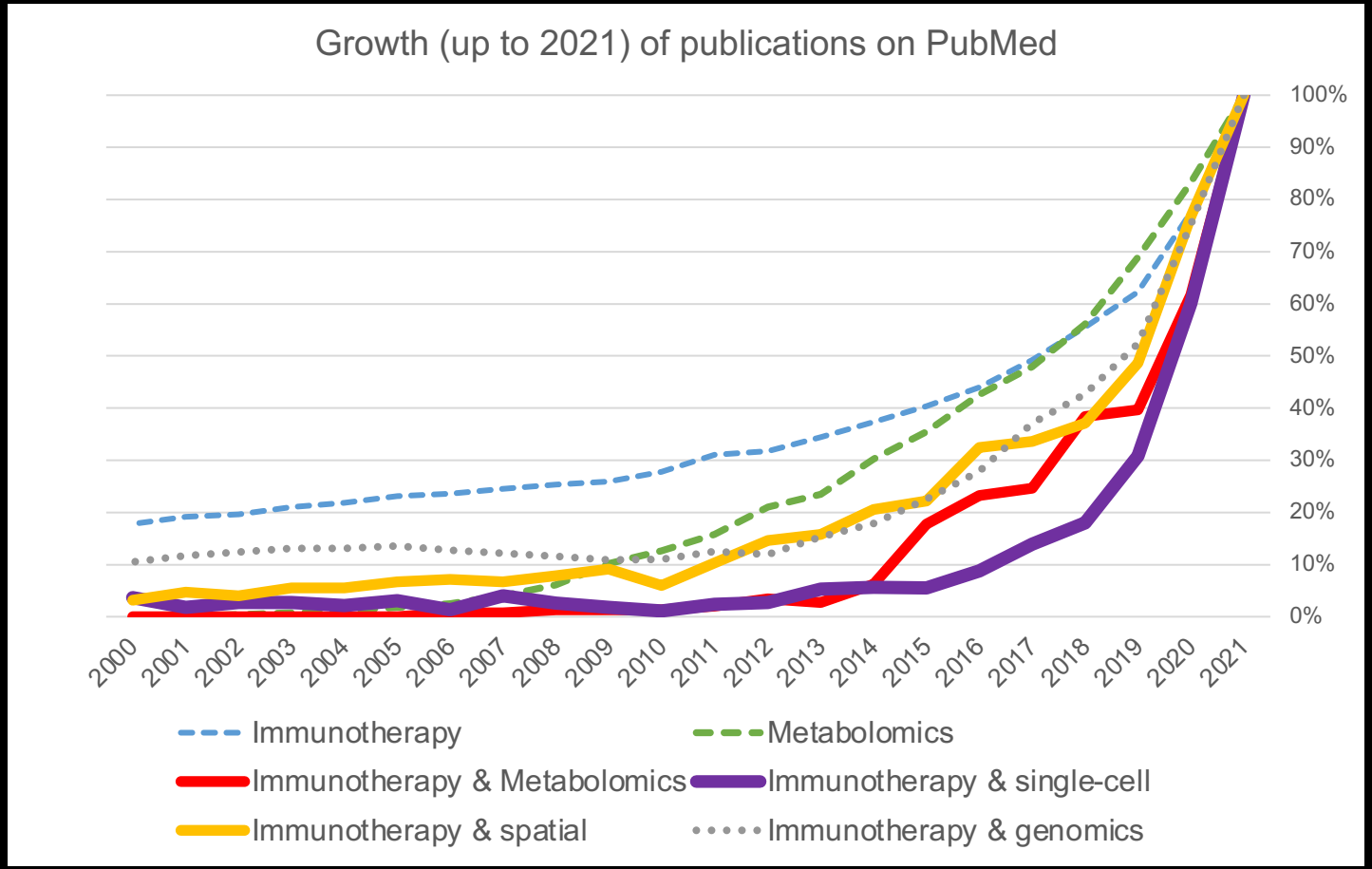
HEIDELBERG

Spatial & single-cell metabolomics

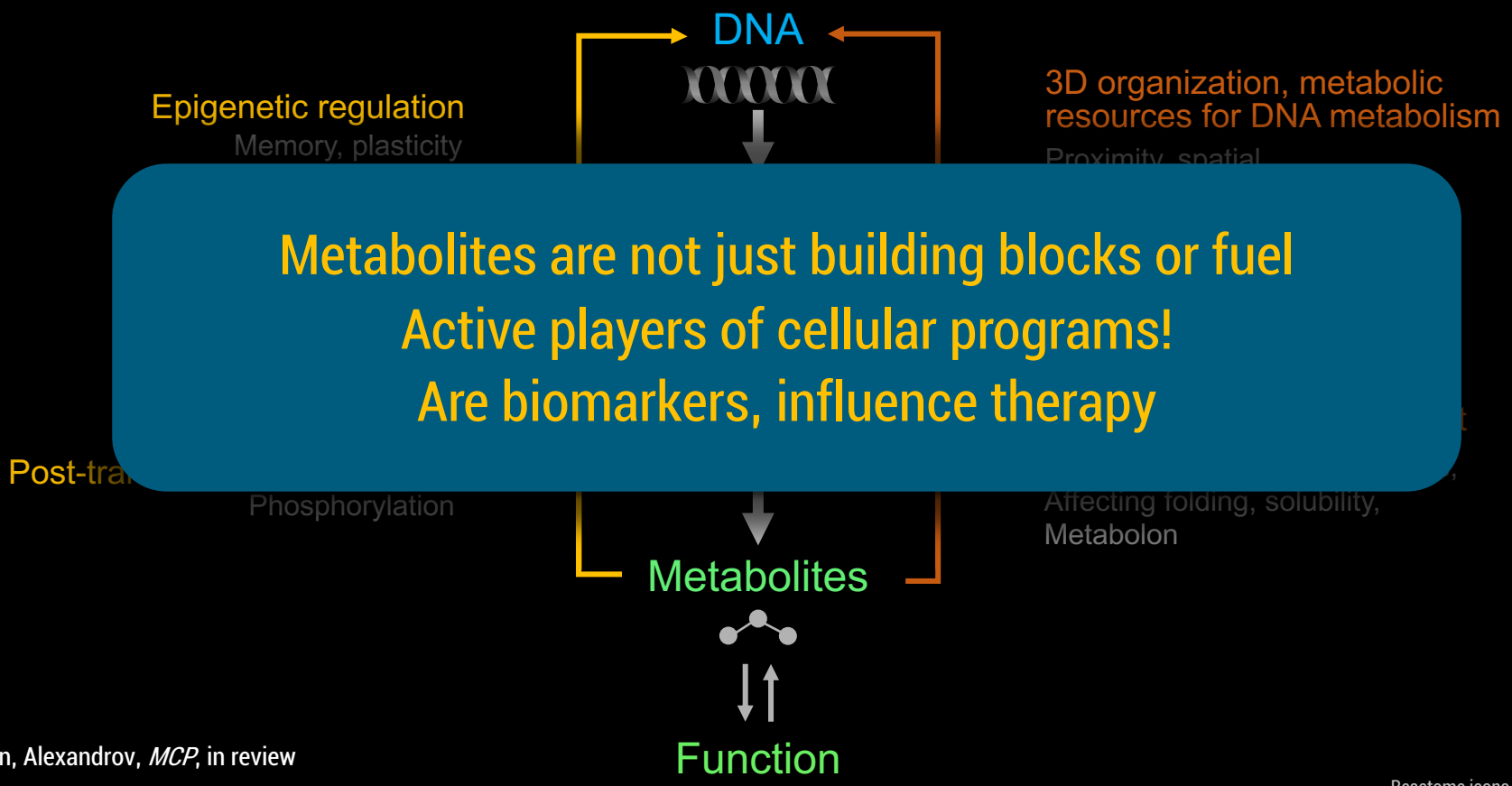
COPENHAGEN

Startup on single-cell metabolomics for drug discovery and therapy

IMMUNOTHERAPY + (SPATIAL & SINGLE-CELL) + METABOLOMICS?



WHY METABOLOMICS?

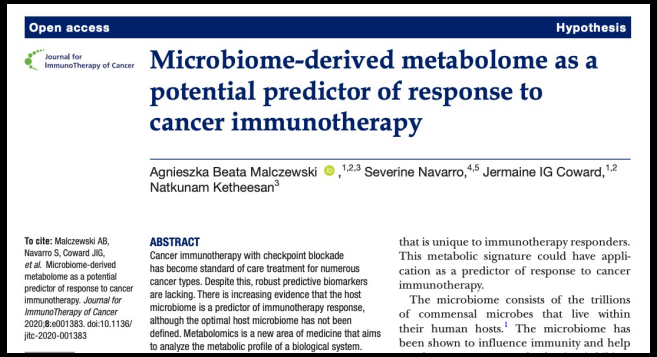


Gavin, Alexandrov, *MCP*, in review

Reactome icons

IMMUNOTHERAPY MEETS METABOLOMICS

Biomarkers



Open access Hypothesis

Journal for ImmunoTherapy of Cancer

Microbiome-derived metabolome as a potential predictor of response to cancer immunotherapy

Agnieszka Beata Malczewski ,[1,2,3] Severine Navarro,[4,5] Jermaine IG Coward,[1,2] Natkunam Ketheesan[3]

To cite: Malczewski AB, Navarro S, Coward JIG, *et al*. Microbiome-derived metabolome as a potential predictor of response to cancer immunotherapy. *Journal for ImmunoTherapy of Cancer* 2020;**8**:e001383. doi:10.1136/jitc-2020-001383

ABSTRACT

Cancer immunotherapy with checkpoint blockade has become standard of care treatment for numerous cancer types. Despite this, robust predictive biomarkers are lacking. There is increasing evidence that the host microbiome is a predictor of immunotherapy response, although the optimal host microbiome has not been defined. Metabolomics is a new area of medicine that aims to analyze the metabolic profile of a biological system. that is unique to immunotherapy responders. This metabolic signature could have application as a predictor of response to cancer immunotherapy.

The microbiome consists of the trillions of commensal microbes that live within their human hosts.[1] The microbiome has been shown to influence immunity and help

Metabolic barriers of tumor microenvironment

nature reviews immunology

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nature › nature reviews immunology › review articles › article

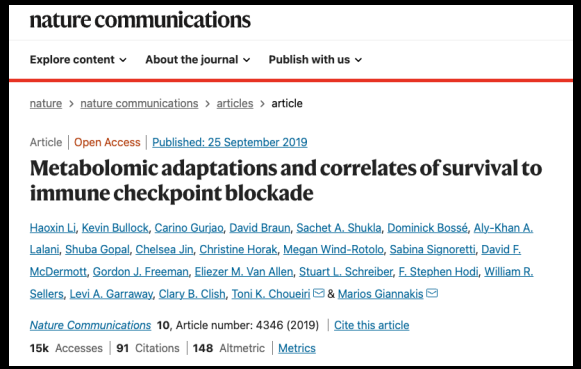
Review Article | Published: 29 April 2021

Metabolic barriers to cancer immunotherapy

Kristin DePeaux & Greg M. Delgoffe ✉

Nature Reviews Immunology **21**, 785–797 (2021) | Cite this article

Predicting non-responders and poor survival



nature communications

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nature › nature communications › articles › article

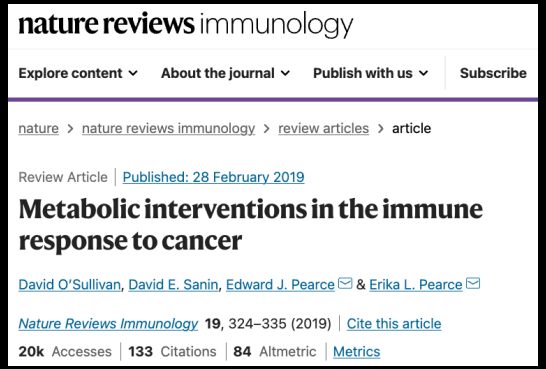
Article | Open Access | Published: 25 September 2019

Metabolomic adaptations and correlates of survival to immune checkpoint blockade

Haoxin Li, Kevin Bullock, Carino Gurjao, David Braun, Sachet A. Shukla, Dominick Bossé, Aly-Khan A. Lalani, Shuba Gopal, Chelsea Jin, Christine Horak, Megan Wind-Rotolo, Sabina Signoretti, David F. McDermott, Gordon J. Freeman, Eliezer M. Van Allen, Stuart L. Schreiber, F. Stephen Hodi, William R. Sellers, Levi A. Garraway, Clary B. Clish, Toni K. Choueiri ✉ & Marios Giannakis ✉

Nature Communications **10**, Article number: 4346 (2019) | Cite this article

Leveraging metabolism to enhance immunotherapy



nature reviews immunology

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nature › nature reviews immunology › review articles › article

Review Article | Published: 28 February 2019

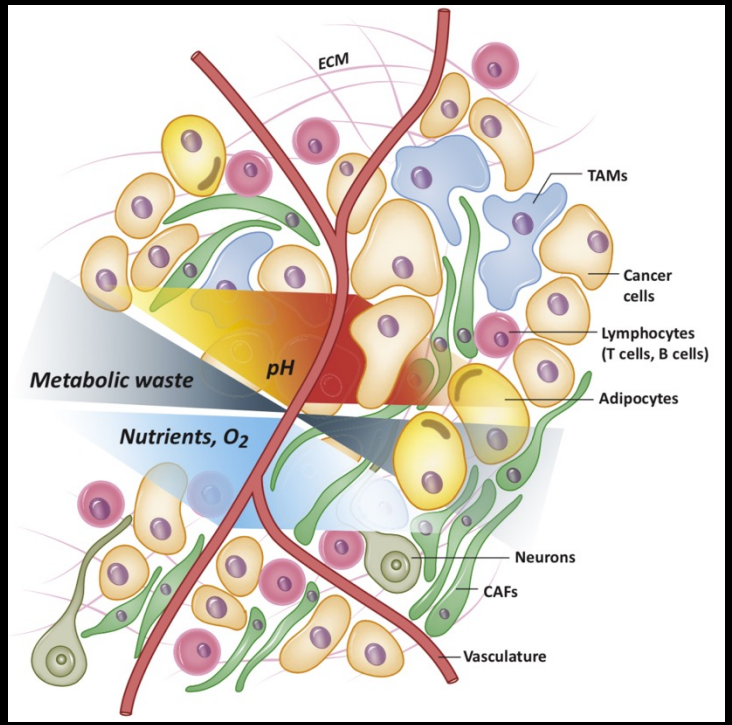
Metabolic interventions in the immune response to cancer

David O'Sullivan, David E. Sanin, Edward J. Pearce ✉ & Erika L. Pearce ✉

Nature Reviews Immunology **19**, 324–335 (2019) | Cite this article

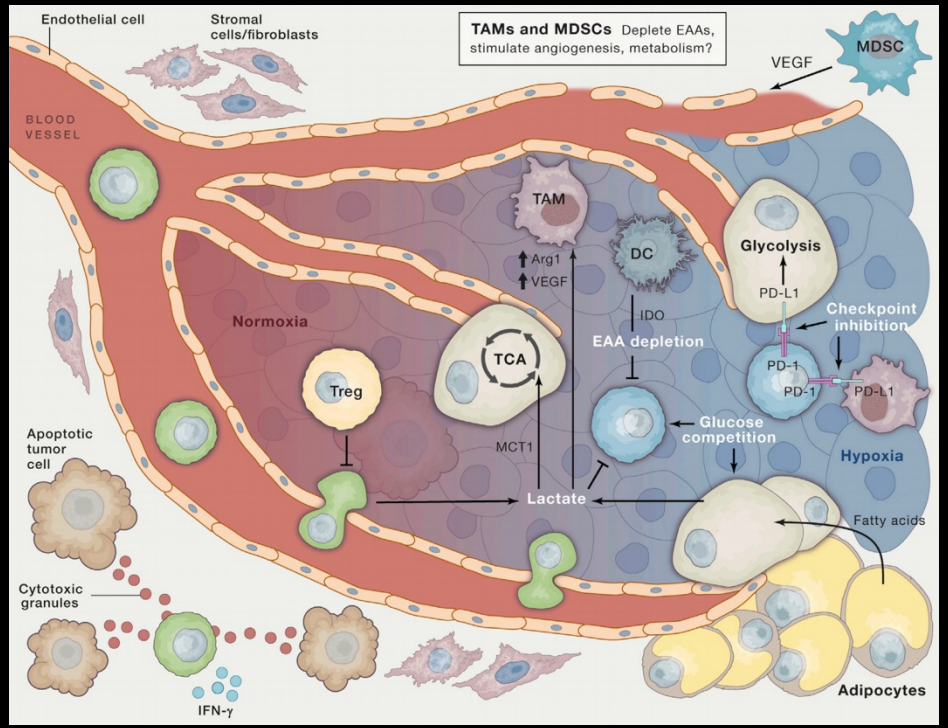
WHY SPATIAL SINGLE-CELL METABOLOMICS?

Tumor microenvironment



Lyssiotis, Kimmelman, *Trends Cell Biology* 2017

Spatial roles of immune system



Buck et al, *Cell* 2017

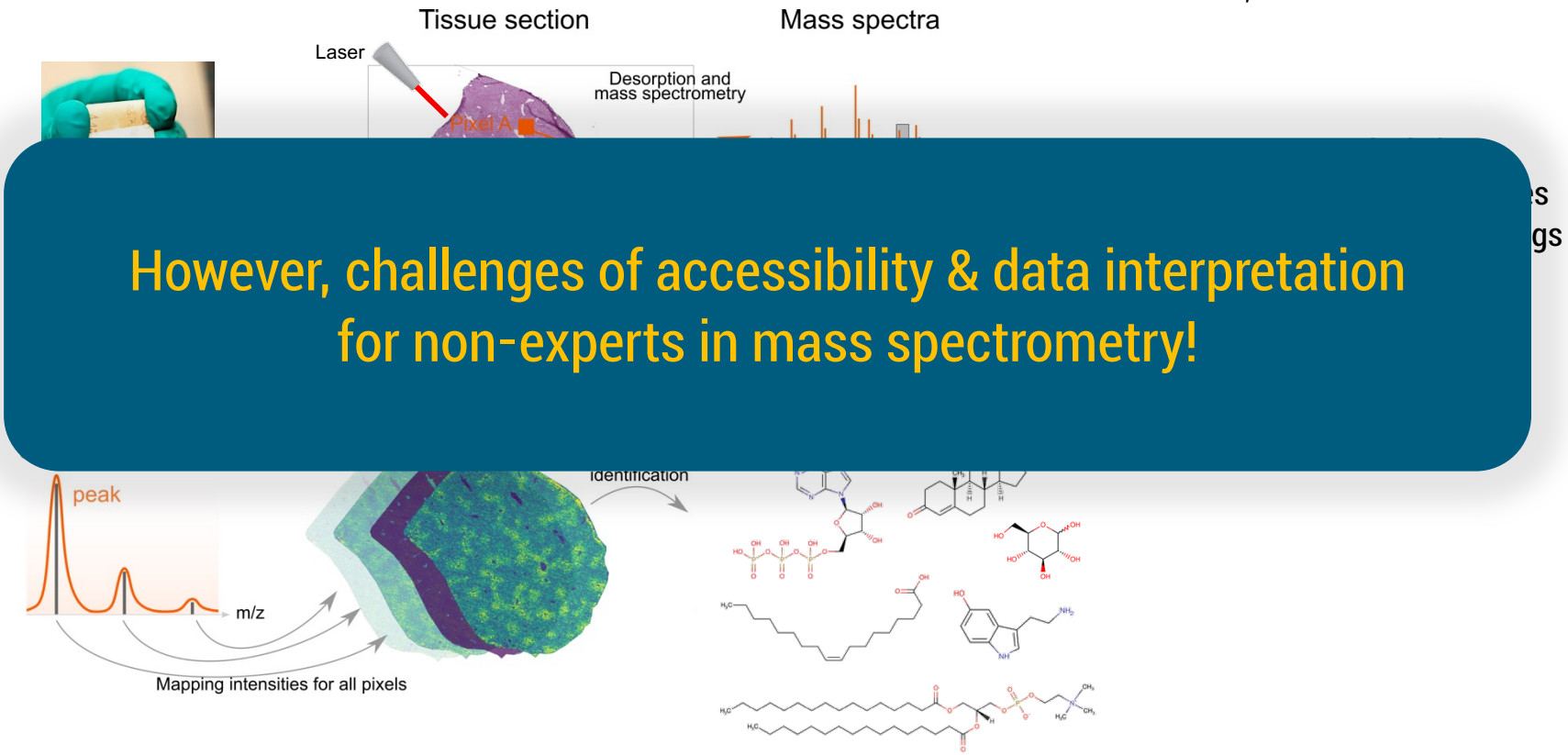
MALDI–IMAGING MASS SPECTROMETRY

Matrix Assisted Laser Desorption Ionization

MALDI–IMAGING MASS SPECTROMETRY

Matrix Assisted Laser Desorption Ionization

Alexandrov, *Annu Rev Biomed Data Sci* 2020



However, challenges of accessibility & data interpretation for non-experts in mass spectrometry!

METASPACE:
TRANSLATING SPECTRA INTO MOLECULAR DATA

HR imaging MS

Upload

Finds molecules

METASPACE

METASPACE2020.EU

Metabolite images

Metabolite imaging knowledge base

100+ labs / 1000+ users / 150+ publications

NIH

Palmer et al, *Nat Methods* 2017

https://metaspace2020.eu

METASPACE Upload Annotations Datasets Projects ♡EMBL♡ Help Theodore Alexandrov

Welcome to

METASPACE

Platform for metabolite annotation of imaging mass spectrometry data

Metabolite Annotation

Submit your high-resolution imaging mass spectrometry data to our high-throughput metabolite annotation engine

Explore the Knowledgebase

Browse annotations from all datasets using our interactive interface

You can search, filter and compare youre annotations alongside those from the whole imaging mass spectrometry community

Get Going Fast

Head to the upload page to submit a dataset.

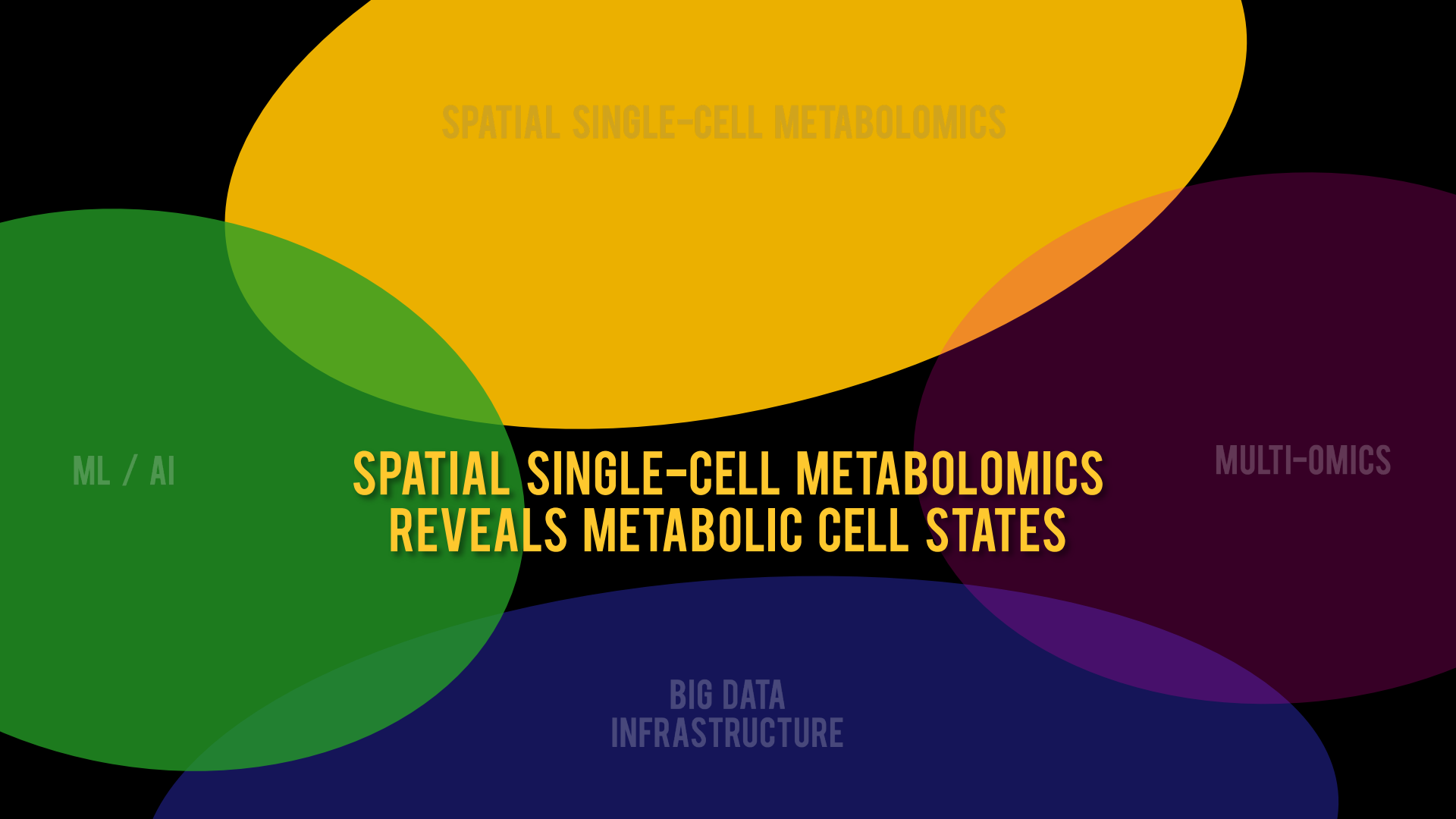
We also have interactive tutorials prepared to help you.

Open Access

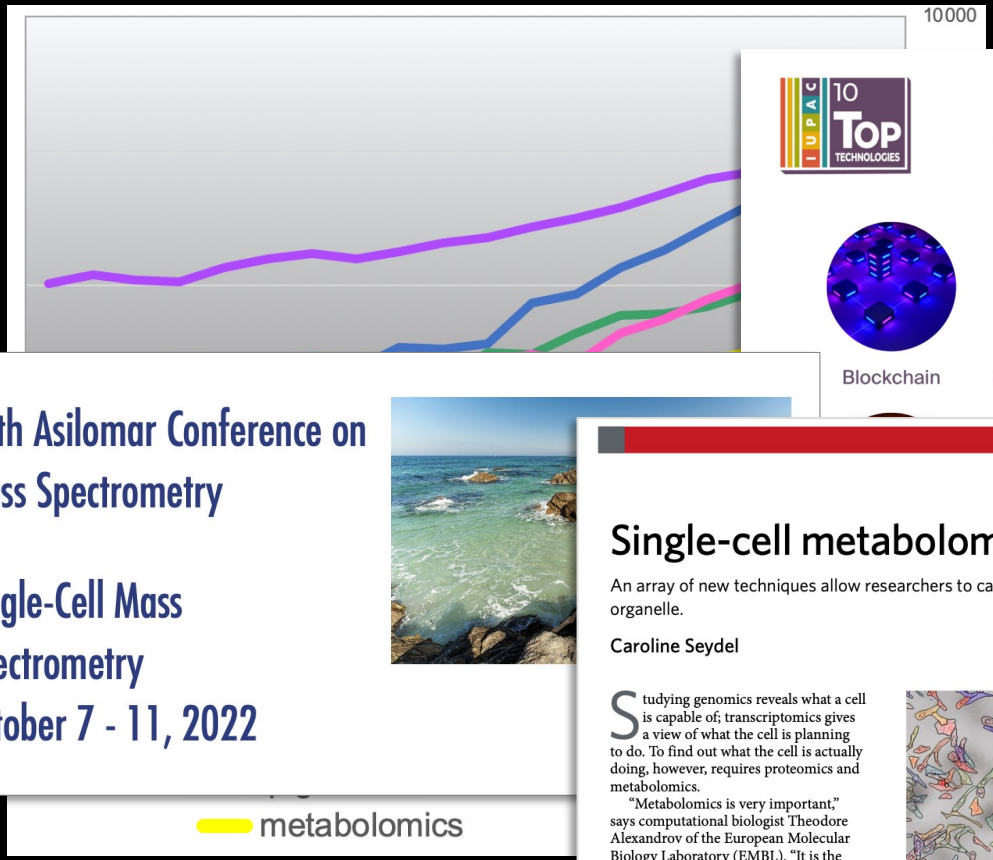
All code is open-source, the input format is the imzML supported by all mass spec major vendors, the metabolite annotations from the community datasets are public and can be browsed or exported.

SUMMARY #1

- Metabolism emerged as a key factor in biology & medicine
  - Metabolites are not only building blocks and fuel! "Two-ways street"
- Spatial metabolomics has maturated over the past decade
  - Used by top-10 pharma for DMPK, increasing interest in clinical applications
- Spatial metabolomics requires big data approaches
  - METASPACE: converting spectra into molecular data


SPATIAL SINGLE-CELL METABOLOMICS
ML / AI
SPATIAL SINGLE-CELL METABOLOMICS
REVEALS METABOLIC CELL STATES
MULTI-OMICS
BIG DATA
INFRASTRUCTURE

SINGLE-CELL METABOLOMICS: EMERGING FIELD



IUPAC Top Ten Emerging Technologies in Chemistry 2021

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

Single-cell metabolomics

37th Asilomar Conference on Mass Spectrometry

Single-Cell Mass Spectrometry

October 7 - 11, 2022

Check for updates

technology feature

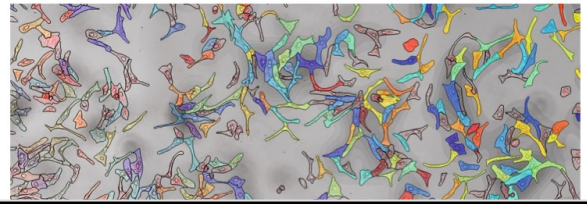
Single-cell metabolomics hits its stride

An array of new techniques allow researchers to catalog the chemical contents of a single cell, or even a single organelle.

Caroline Seydel

Studying genomics reveals what a cell is capable of; transcriptomics gives a view of what the cell is planning to do. To find out what the cell is actually doing, however, requires proteomics and metabolomics.

"Metabolomics is very important," says computational biologist Theodore Alexandrov of the European Molecular Biology Laboratory (EMBL). "It is the youngest of the omics, but it provides the



SpaceM: Spatial Single-Cell Metabolomics

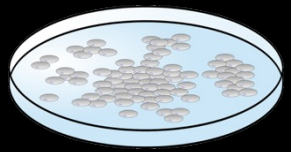
Rappez et al, *Nature Methods* 2021
Patent applications

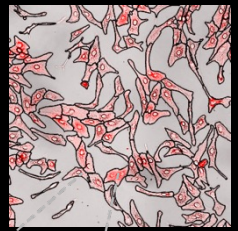
Cells → Microscopy → MALDI-imaging → Image analysis

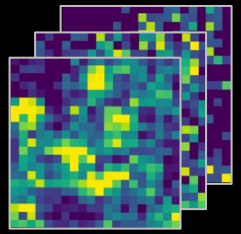
Microscopy → Cell image, morphology, spatial relationships

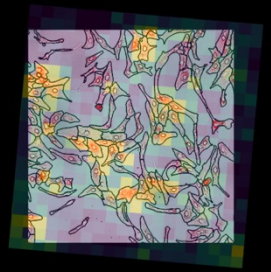
MALDI-imaging → METASPACE

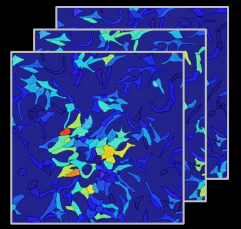
Image analysis → Single-cell metabolite images

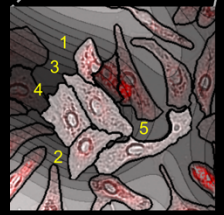


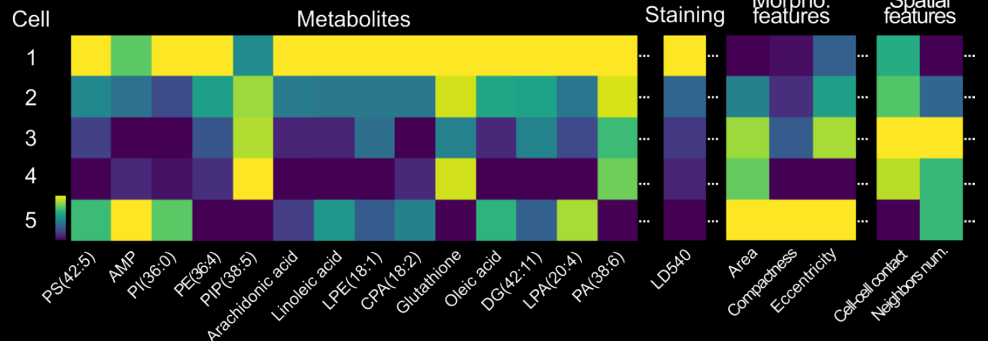






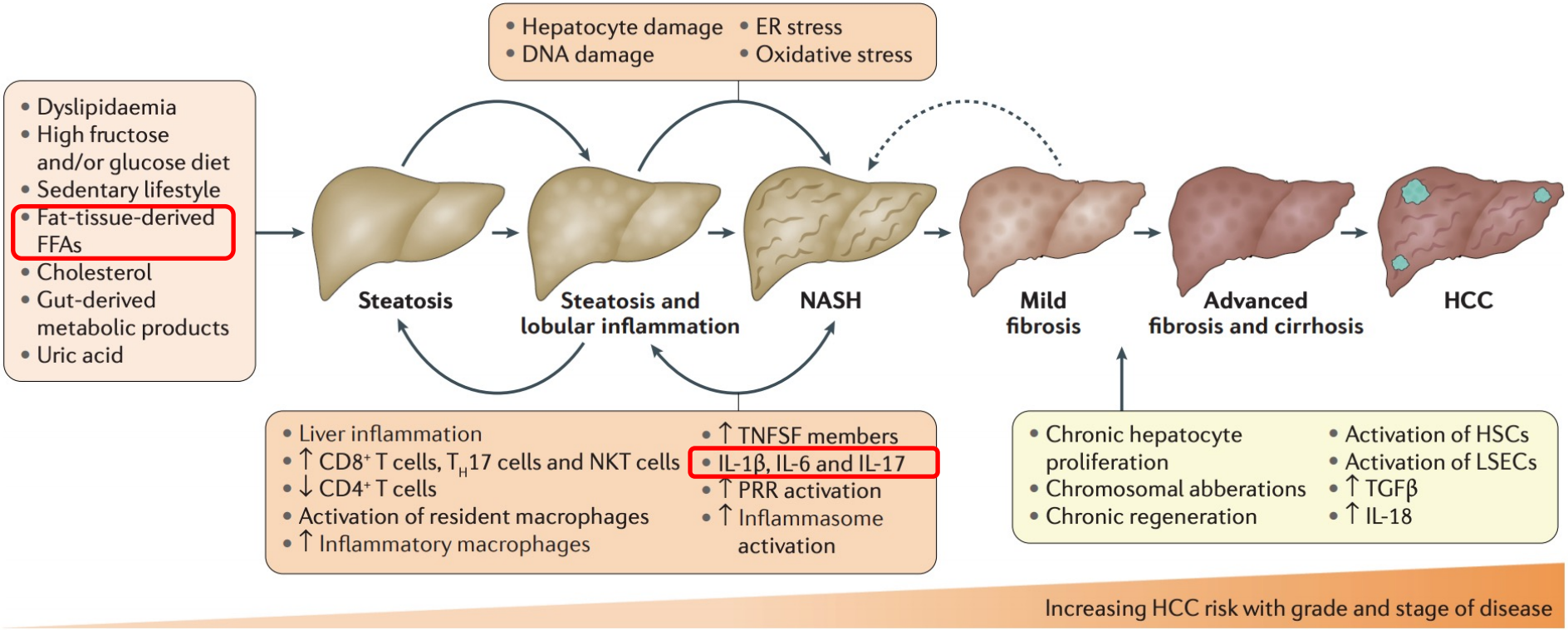






100+ molecules
1.000+ cells

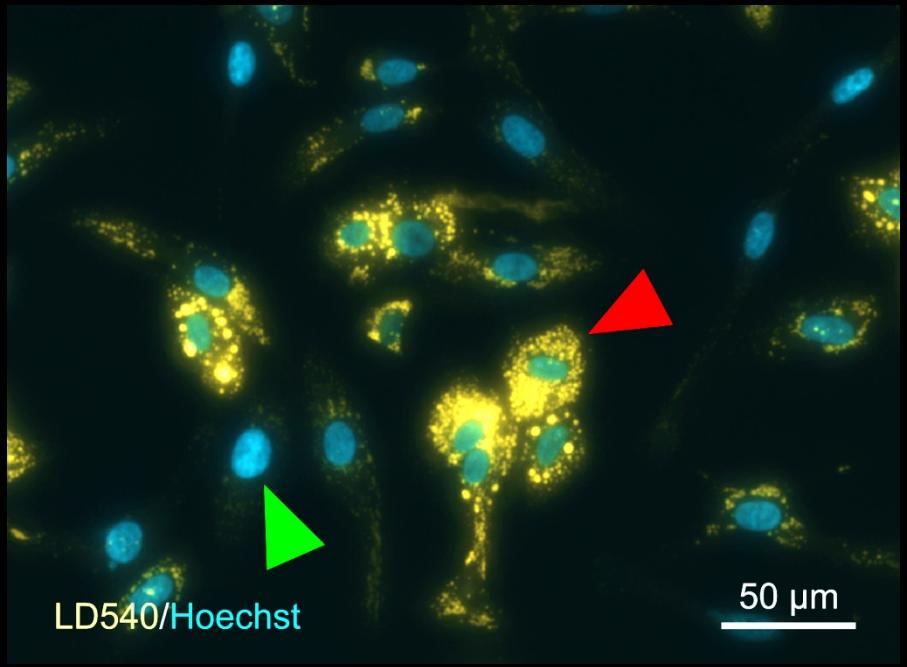
NON-ALCOHOLIC STEATOHEPATITIS
COMMON FACTOR FOR LIVER CANCER

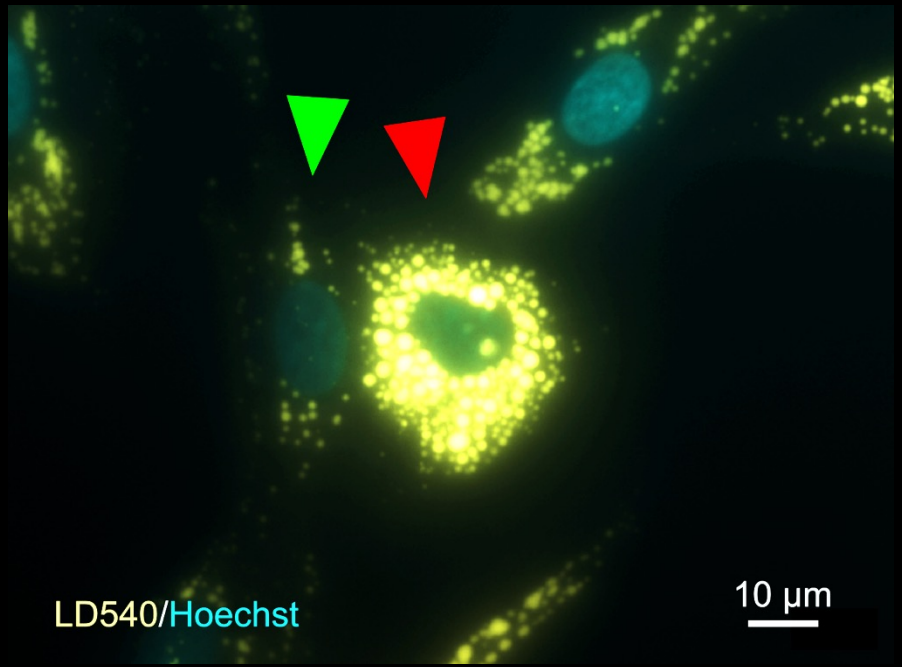


Anstee, ..., Heikenwalder, *Nat Rev Gastroenterol Hepatol*, 2019

SINGLE-CELL ANALYSIS OF NASH *IN VITRO*

Human hepatocytes dHepaRG +oleic acid +palmitic acid +TNFα





With Mira Stadler, Mathias Heikenwaelder, DKFZ

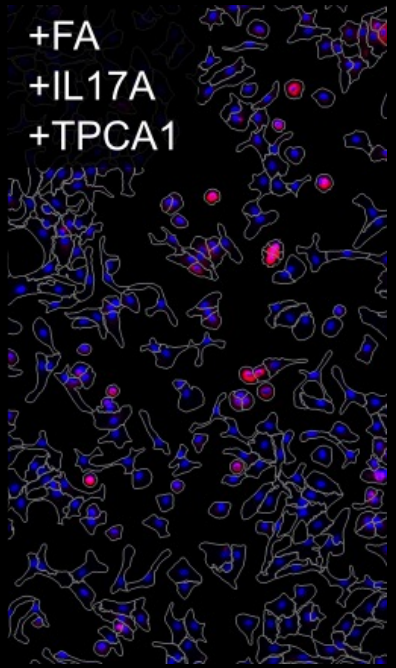
STIMULATED HEPATOCYTES

dHepaRG hepatocytes
control

+palmitic acid +oleic acid
"NAFLD"

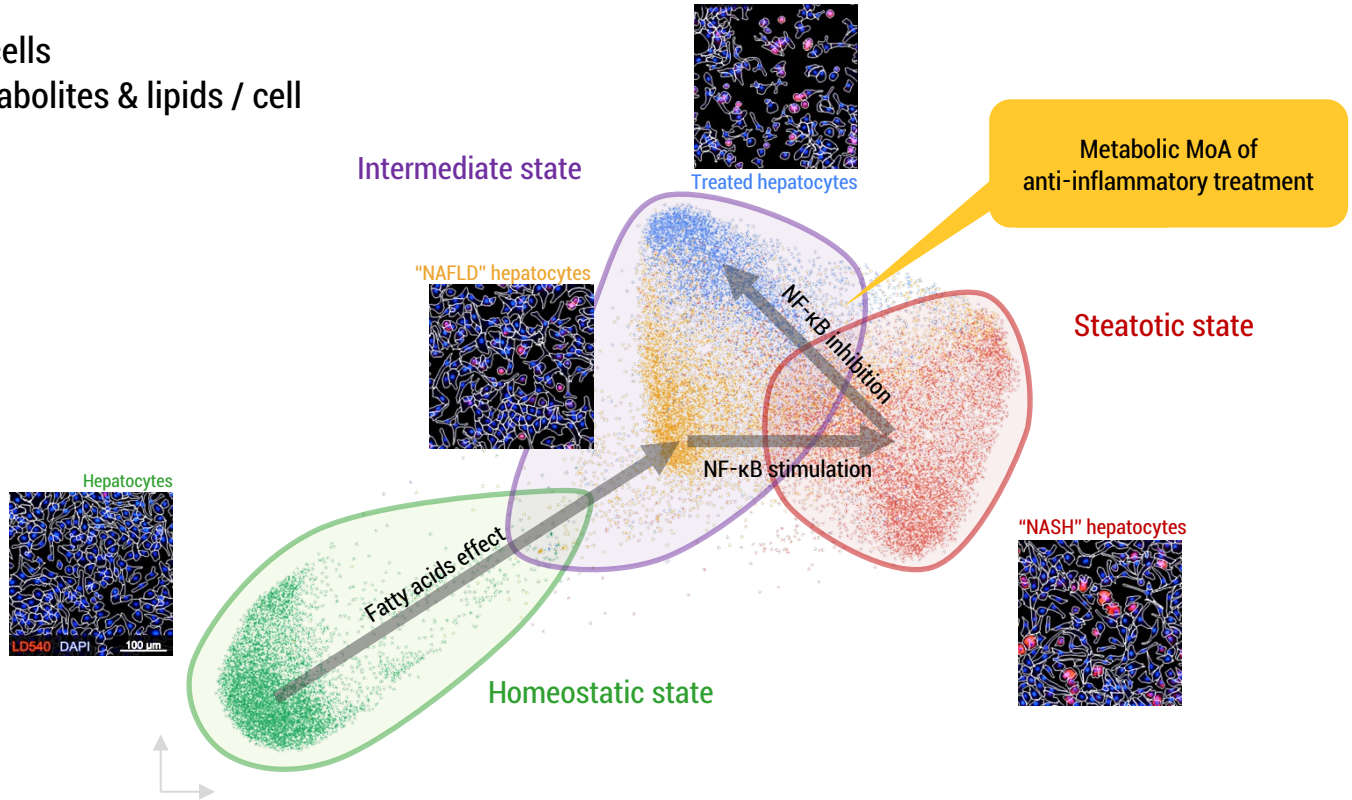
+IL17a
"NASH"

+TPCA-1, inhibitor of NFkB
anti-inflammatory treatment

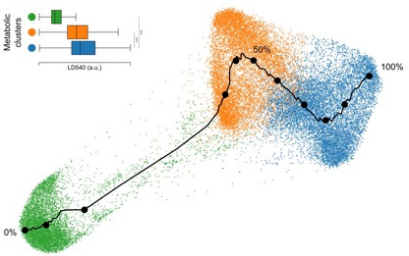


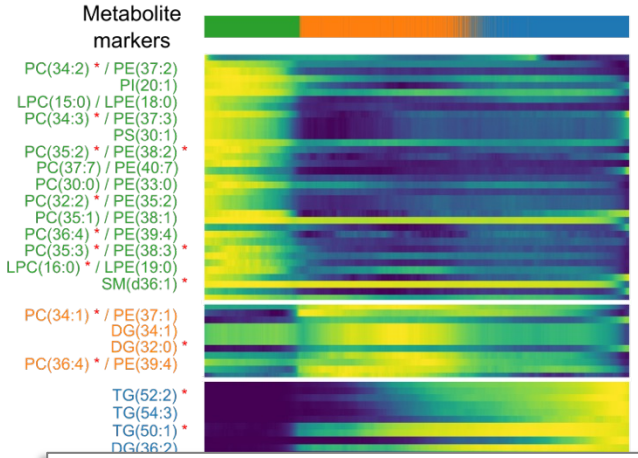
SINGLE-CELL METABOLOMICS OF NAFLD/NASH

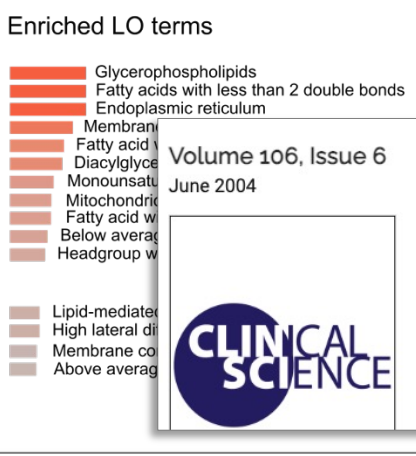
27.000+ cells
700+ metabolites & lipids / cell

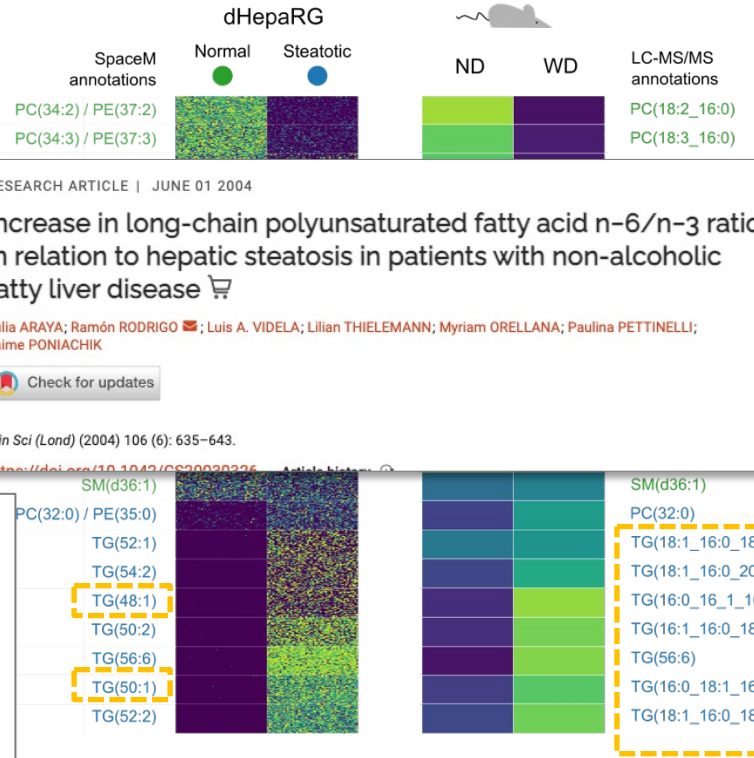


STEATOTIC LIPID MARKERS









Volume 106, Issue 6
June 2004

CLINICAL SCIENCE

RESEARCH ARTICLE | JUNE 01 2004

Increase in long-chain polyunsaturated fatty acid n–6/n–3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease

Julia ARAYA; Ramón RODRIGO ✉; Luis A. VIDELA; Lilian THIELEMANN; Myriam ORELLANA; Paulina PETTINELLI; Jaime PONIACHIK

Check for updates

Clin Sci (Lond) (2004) 106 (6): 635–643.

Research | Open Access | Published: 20 June 2018

Hepatic steatosis risk is partly driven by increased de novo lipogenesis following carbohydrate consumption

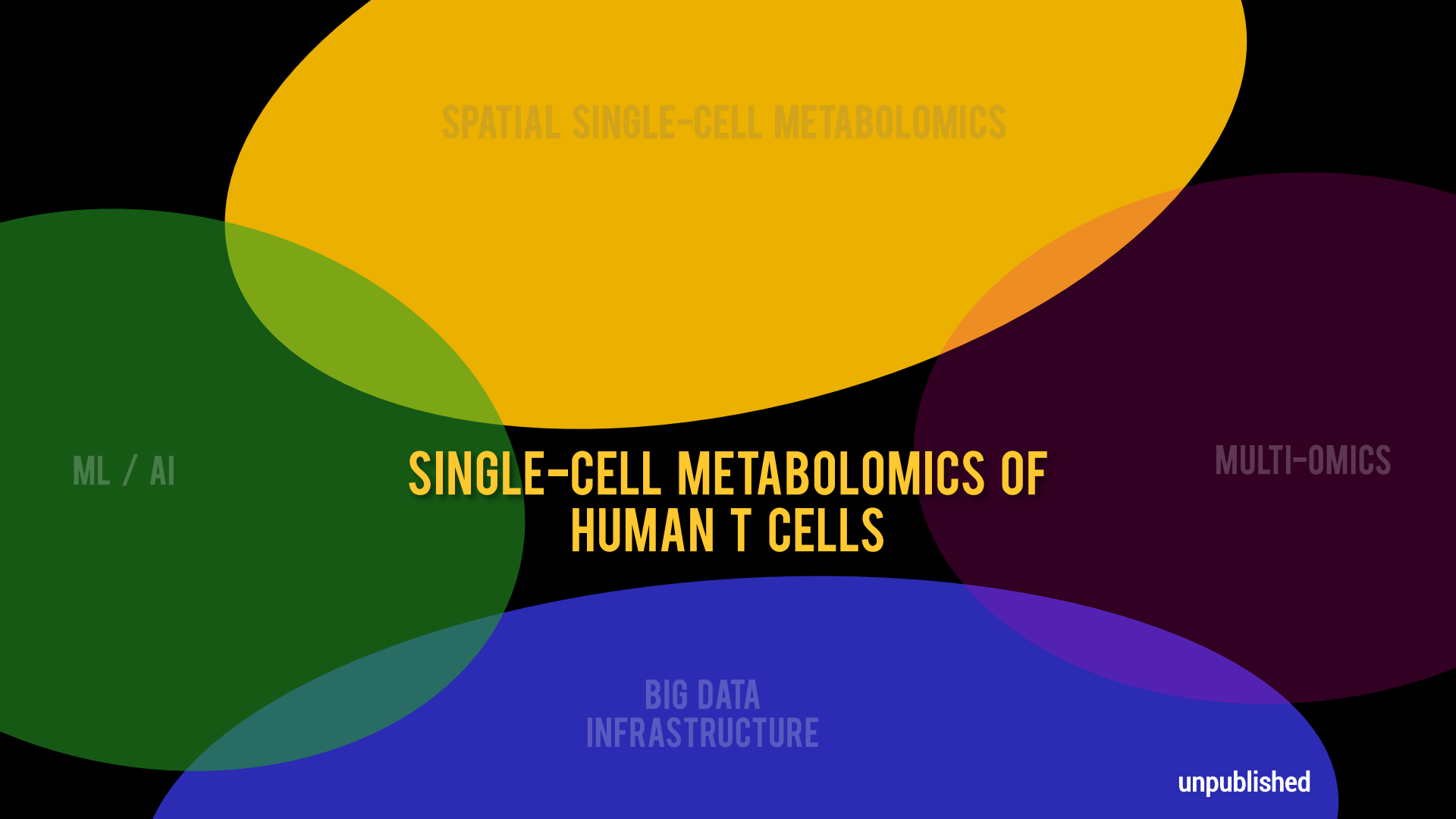
Francis W. B. Sanders, Animesh Acharjee, Celia Walker, Luke Marney, Lee D. Roberts, Fumiaki Imamura, Benjamin Jenkins, Jack Case, Sumantra Ray, Samuel Virtue, Antonio Vidal-Puig, Diana Kuh, Rebecca Hardy, Michael Allison, Nita Forouhi, Andrew J. Murray, Nick Wareham, Michele Vacca, Albert Koulman & Julian L. Griffin ✉

Sanders et al, *Genome Biol* 2018

Araya et al, *Clinical Science* 2004

SUMMARY #2

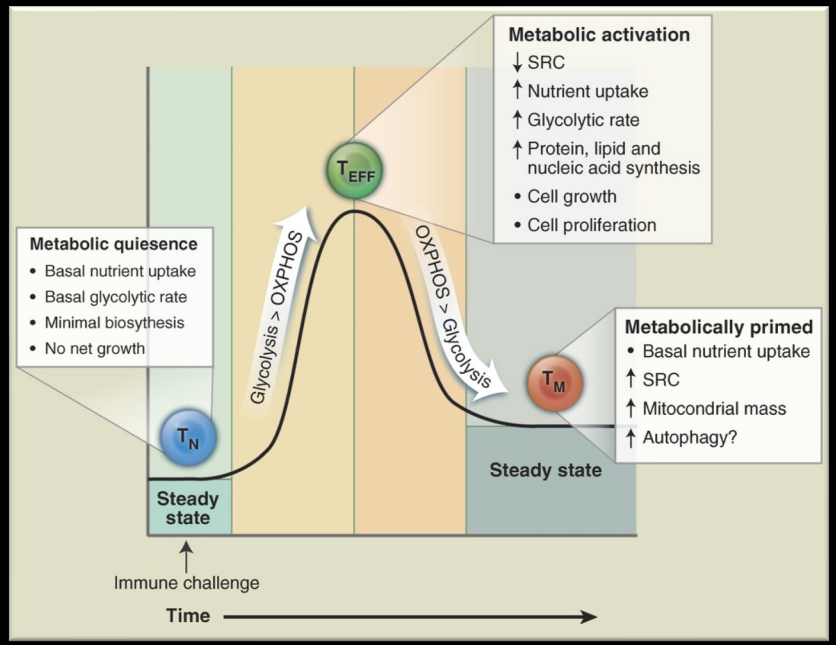
- Single-cell metabolomics is an emerging field
- SpaceM: method for spatial single-cell metabolomics
  - Reveals metabolic states in steatosis and NASH
  - Detects prognostic biomarkers
- Single-cell metabolomics: cheapest and fastest of all omics
  - x100 cheaper than scRNAseq
  - x10 faster than scRNAseq


SPATIAL SINGLE-CELL METABOLOMICS
ML / AI
SINGLE-CELL METABOLOMICS OF HUMAN T CELLS
MULTI-OMICS
BIG DATA INFRASTRUCTURE
unpublished

METABOLIC REPROGRAMMING OF T CELLS

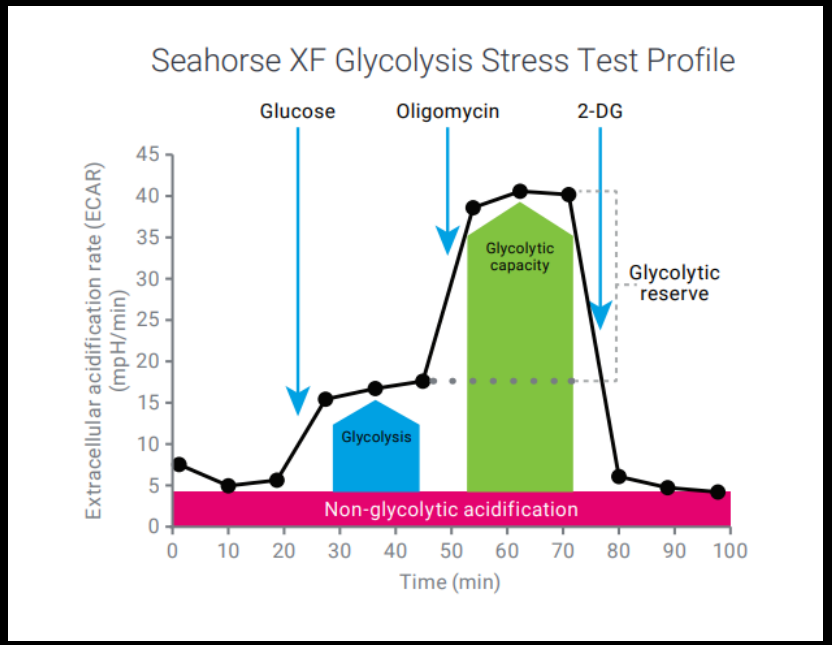
Luisa Abreu

Metabolic switch upon activation

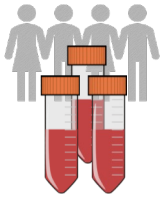


Pearce et al, *Science* 2013

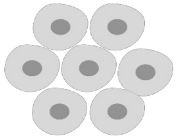
Seahorse: Bulk assay



EXPERIMENTAL DESIGN



Buffy coats from healthy donors peripheral blood

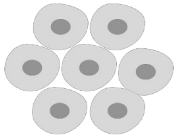


Naïve CD4^{+}T cells

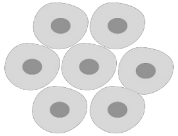
CTV labelling

FACS purity check
CD3, CD4, CD45RA, CD45RO

3-day culture

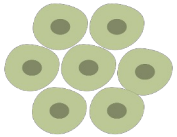


Non-stimulated

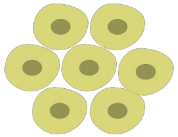


Stimulated

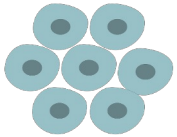
Beads with anti-CD3, -CD28



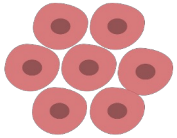
Non-stimulated



Stimulated



Stimulated + 2-DG

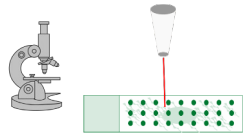


Stimulated + Oligomycin

30 min treatment

FACS activation, proliferation check
CD25, CD69, CTV

HT SpaceM



METABOLIC REPROGRAMMING OF HUMAN T CELLS

20K cells
80 metabolites

SUMMARY #3

- SpaceM profiles metabolism of single immune cells
  - Detects the metabolic reprogramming

- High-throughput SpaceM
  - Analyzes 64 samples, 1000s cells each (100.000+ cells) — overnight

LOOKING INTO THE FUTURE:
SPATIAL & SINGLE-CELL METABOLOMICS IN IMMUNOTHERAPY

nature immunology

Metabolic reprogramming of terminally exhausted CD8$^+$ T cells by IL-10 enhances anti-tumor immunity

Yugang Guo, Yu-Qing Xie, Min Gao, Yang Zhao, Fabien Franco, Mathias Wenes, Imran Siddiqui, Alessio Bevilacqua, Haiping Wang, Hanshuo Yang, Bing Feng, Xin Xie, Catherine M. Sabatel, Benjamin Tschumi, Amphun Chaiboonchoe, Yuxi Wang, Weimin Li, Weihua Xiao, Werner Held, Pedro Romero, Ping-Chih Ho & Li Tang

Nature Immunology **22**, 746–756 (2021) | Cite this article

28k Accesses | **58** Citations | **155** Altmetric | Metrics

Journal of Translational Medicine

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Review | Open Access | Published: 07 December 2021

Optimization of metabolism to improve efficacy during CAR-T cell manufacturing

Meng Zhang, Xin Jin, Rui Sun, Xia Xiong, Jiaxi Wang, Danni Xie & MingFeng Zhao

Journal of Translational Medicine **19**, Article number: 499 (2021) | Cite this article

4808 Accesses | **9** Citations | **2** Altmetric | Metrics

Metabolic barriers of tumor microenvironment

1. Spatial chemical profiling of microenvironment, ECM
2. Spatial profiling of metabolism of immune cells *in situ*

Metabolic revival of exhausted immune cells

Single-cell

Leveraging metabolism to enhance immunotherapy

Optimizing metabolic fitness of CAR T cells

TAKE HOME MESSAGES

- Metabolomics in immunotherapy is emerging
- Spatial metabolomics detects 100+ metabolites in tissues
- Spatial single-cell metabolomics (SpaceM) reveals metabolic reprogramming in NASH, T cells
- Prominent future applications include metabolic revival of exhausted immune cells and metabolic optimization of CAR T cells

Seeking collaborators on metabolic CAR T optimization

alexandrovteam

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Selected past members

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Uni Heidelberg

Jan Siemens
Julio Saez-Rodriguez

EMBL

ALMF (Sabine, Beate, Stefan)
FACS (Diana)

Wellcome Sanger

Gosia Trynka
Blagoje Soskic

Sanofi

Thomas Leeuw
Andreas Lindenschmidt

John Hopkins

Erika & Ed Pearce

Chica and Heinz Schaller Foundation
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Federal Ministry of Education and Research
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NIH
Open Targets