HOT TOPICS IN HIV

Vaccines, immune recovery and eradication

Block 2: Emerging Perspectives

New technologies to address HIV Research

Metabolomics: Searching for insights to understand Immunological Non-response to ART

iNIM Infection and Immunity

Anna Rull Aixa
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What's metabolomics?

The Need for Multi-Omics Biomarker Signatures in Precision Medicine

'c point of view, delineation of cessarily goal-oriented; that is, ...ssumed that information from different biochemical areas can provide added diagnostic value across substance boundaries – what can be referred to as "multi-omics" [2]. While the integration of genomics, metabolomics, proteomics, transcriptomics, etc. is attractive, it is hardly technically feasible with a single analytical platform. This is primarily due to diverse physiochemical properties and wide concentration ranges.

Applications of multi-omics analysis in human diseases

Integration of different types of omics data can elucidate underlying pathogenic changes of the disease, which can then be verified in further molecular researches. By integrating multi-omics, scientists can filter out novel associations between biomolecules and disease phenotypes, identify relevant signaling pathways, and establish detailed biomarkers of disease. Therefore, the integration of various omics data will facilitate the match of associations between molecular-disease and phenotype-environmental

The Need for Multi-Omics Biomarker Signatures in Precision Medicine

Overall, this ... that the concept of precision medicine is more than the use of genetic ... information to inform clinical practice and the fact that the use multi-omics approaches to achieve precision medicine in the clinic is extremely valuable but still in its infancy

PERSPECTIVE

Criteria for the use of omics-based predictors in clinical trials

Omics technologies are high throughput techniques that make it possible to gather, in a single experiment, large amounts of data about a specific type of molecules, such as the three billion base pairs of the human genome, the universe of proteins in a given tissue or a large collection of metabolites. Examples of these technologies are next generation sequencing, used for genomics and transcriptomics studies, and ... and metabolomics st...

OPINION ARTICLE

Human Genomics

Practicing precision medicine with intelligently integrative clinical and multi-omics data analysis

Box 1
Definition of 'omics'

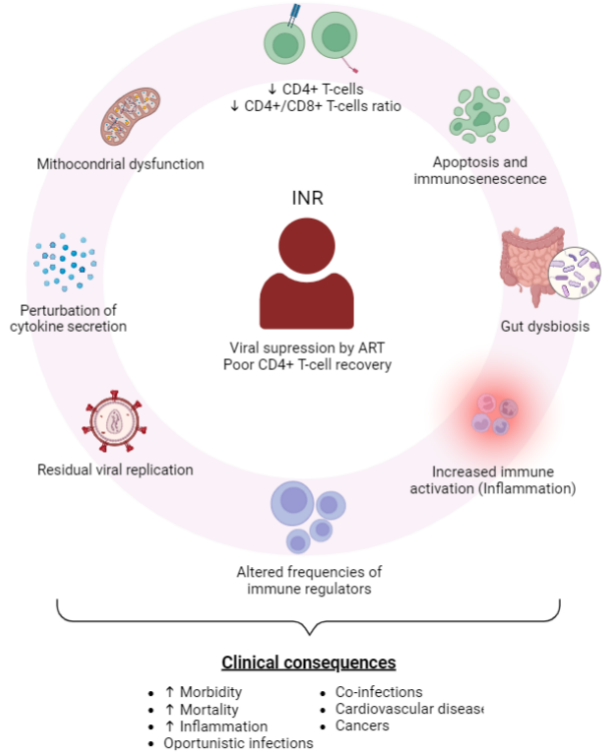
In its report, Evolution of Translational Omics: Lessons Learned and the Path Forward, the Institute of Medicine Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials defines 'omics' as the study of related sets of biological molecules in a comprehensive fashion. Examples of omics disciplines include genomics, transcriptomics, proteomics, metabolomics and epigenomics. An omics-based test is defined as "an assay composed of or derived from multiple molecular measurements and interpreted by a fully specified computational model to produce a clinically actionable result".

REVIEW ARTICLE

Omics-based biomarkers: current status and potential use in the clinic

Héctor Quezada, Ana Laura Guzmán-Ortiz, Hugo Díaz-Sánchez, Ricardo Valle-Rios, Jesús Aguirre-Hernández

potential indistinct results for patient care from highly expressed genes and disease-causing variants [9, 39]. Understanding how genetic variations contribute to health is one important aspect of precision medicine, where additional approaches involve measuring levels of proteins and metabolic products. By harnessing the power of metabolomics, we need to profile a patient's metabo-



Why is metabolomics important in the INR study?

Metabolomics involves the study of...?

- ❑ Lipids
- ❑ Alkaloids
- ❑ Amino acids
- ❑ Polymers

Sample preparation involves the following steps

- ❑ Extraction
- ❑ Processing
- ❑ Interpretation

To measure sets of metabolites, you can use

- ❑ Targeted metabolomics
- ❑ Untargeted metabolomics

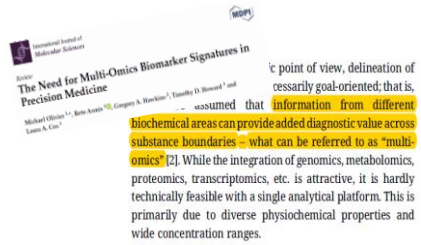
NMR is more sensitive than MS

- ❑ True
- ❑ False

Metabolomics can help to improve healthcare

- ❑ True
- ❑ False

What's metabolomics?



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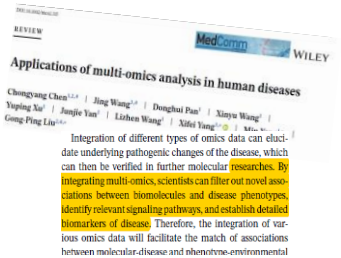
How is metabolomics used?



REVIEW

MedComm

WILEY

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BOX 1

Definition of 'omics'

PERSPECTIVE

OPEN

Criteria for the use of omics-based predictors in clinical trials

What's next for clinical applications of metabolomics?

OPINION ARTICLE

Human Genomics

Open Access

Practicing precision medicine with intelligently integrative clinical and multi-omics data analysis

potential indistinct results for patient care from highly expressed genes and disease-causing variants [9, 39]. Understanding how genetic variations contribute to health is one important aspect of precision medicine, where additional approaches involve measuring levels of proteins and metabolic products. By harnessing the power of metabolomics, we need to profile a patient's metabo-

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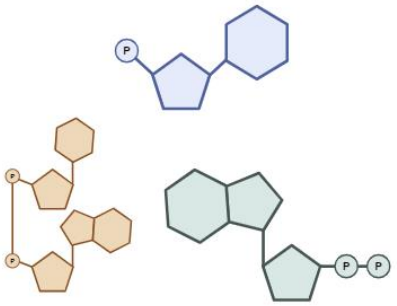
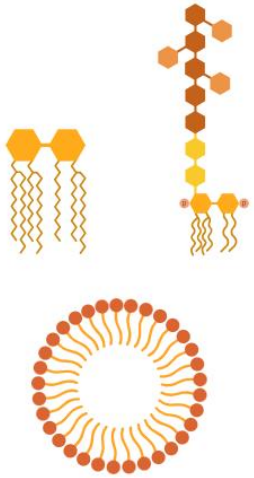
What's metabolomics?

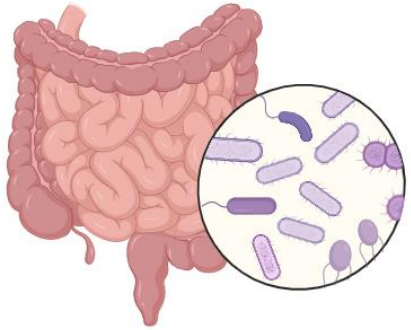
Metabolites



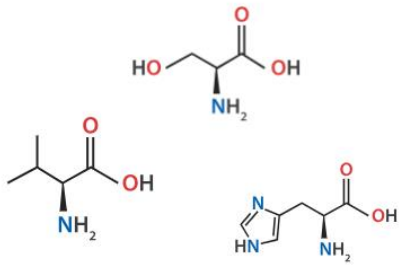
Lipids



Metabolites from microbiota



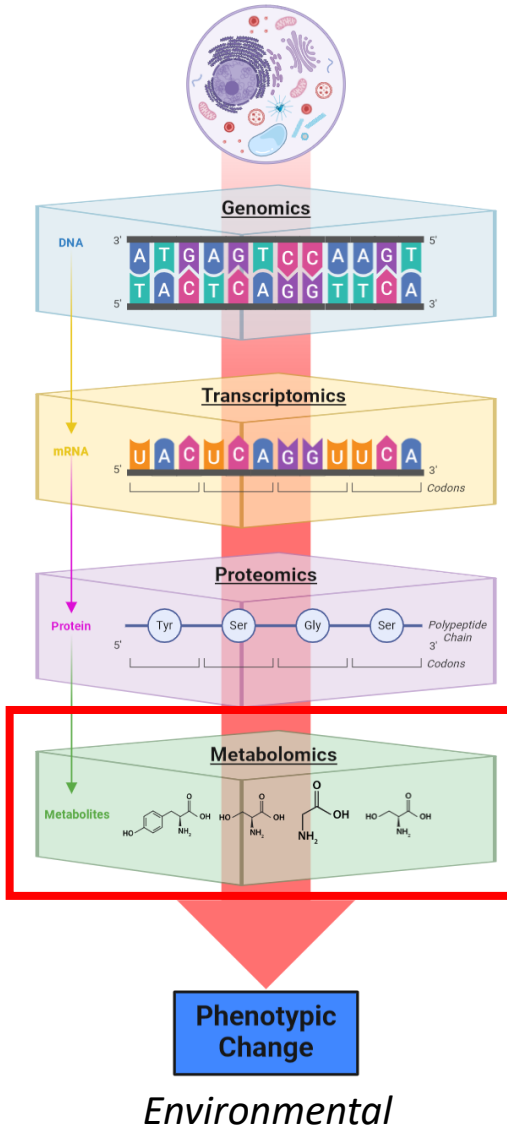
Amino acids



Comprehensive, qualitative, and quantitative study of low-molecular-weight molecules.

Examples of small molecules: sugars, lipids, amino acids, fatty acids, phenolic compounds, alkaloids, vitamins and many other types of molecules which are often the building blocks for larger compounds.

Why is metabolomics important?



What is possible?

What appears to happen?

What makes it happen?

What has happened and is happening?

Snapshot of the physiology of the cell most closely related to the phenotype.

How to design a metabolomics study ?

Polar compounds (GC semi-targeted)
(~100 molecules)
alanine, citric acid, d-fructose, ethanolamine, galacturonic acid, Glucose 6-phosphate, glutamine, linoleic acid, oxoproline, pyruvic acid, serine, succinic acid, urea….

Bile acids
Cholic acid, glycocholic acic, taurochenodeoxycholic acid …

Microbial phenolic metabolites

Lipid species
(~300 lipids)
Lyso-PCs, Lyso-PE, triglycerides, cholesteroyl esthers , sphingomyelins, ceramides

Prostaglandins

Steroid hormones

Short Chain Fatty Acids
Butyric acid, isovaleric acid, propionic acid, valeric acid…

Acylcarnitines

Oxylipins

Untargeted studies to identify a wide range of metabolites (profiling) to generate a hypothesis.

Targeted studies focus on a few specific metabolites when a hypothesis is already postulated.

How to design a metabolomics study?

Biological matrix


Blood

Urine



Salive

Tissue


Similar pipeline for Targeted and Untargeted metabolomics studies but different handling and preparation depending on the study design (approach selected and technique required).

Adapted from San-Martin Breno Sena De, et al . Arch. Endocrinol. Metab. 2020; 64: 654-663

How to design a metabolomics study?

Biological matrix

Blood

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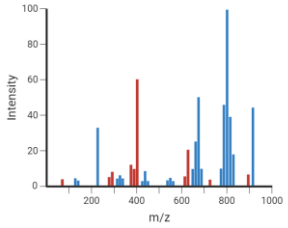
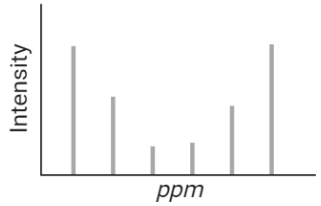
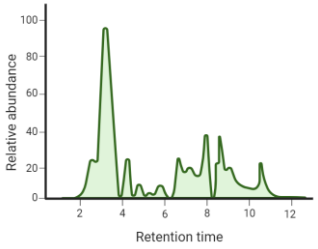
Data acquisition

MS SPECTRUM

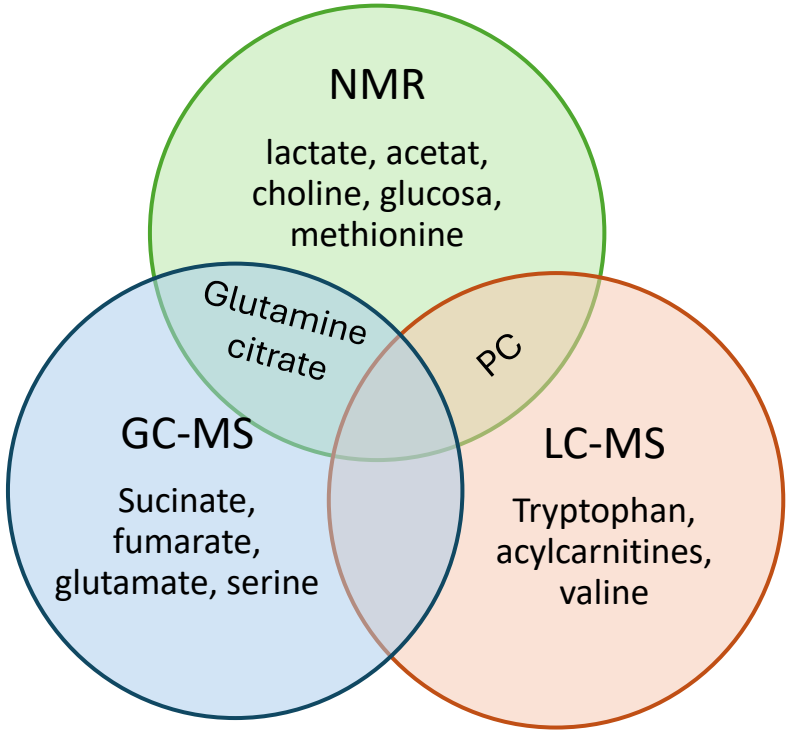


NMR SPECTRUM





MS more sensitive and accurate and offer a high mass resolution than NMR



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How to design a metabolomics study?

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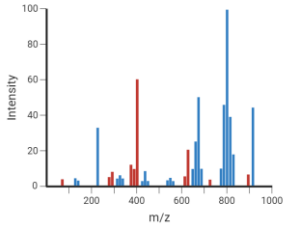
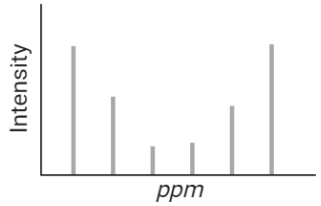
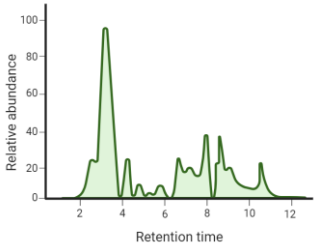


NMR SPECTRUM



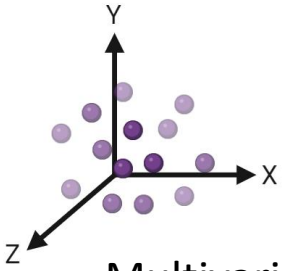


Data processing

Normalization data

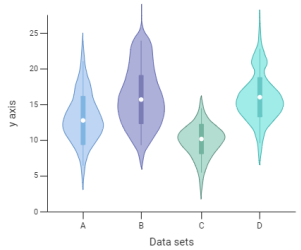
- ✓ Log transformation
- ✓ Alignment
- ✓ Scaling
- ✓ Peak detection

Statistical analysis



Multivariate

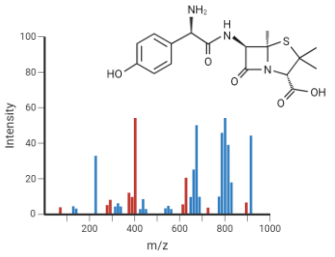
- ✓ PCA
- ✓ PLSA-DA
- ✓ OPLS-DA



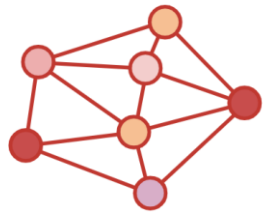
Univariate

- ✓ ANOVA
- ✓ T-Test
- ✓ Linear Model

Biomarker discovery



Feature identification



Pathways analysis

Adapted from San-Martin Breno Sena De, et al . Arch. Endocrinol. Metab. 2020; 64: 654-663

How is metabolomics used?

For diseases

- ✓ Risk assessment
- ✓ Screening
- ✓ Diagnosis
- ✓ Treatment
- ✓ Prognosis
- ✓ Monitoring

Technologies for identification

- ✓ NGS, Genome annotation
- ✓ Transcriptomics
- ✓ Proteomics: MS, Protein chips….
- ✓ Metabolomics: NMR, MS….

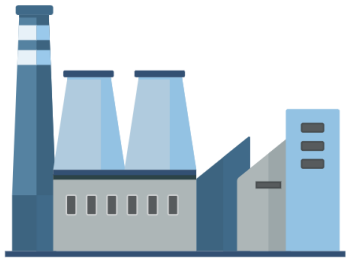
Biomarker contribution

- ✓ Identification of events
- ✓ Develop a dose-response
- ✓ Determine variability and effects
- ✓ Correlated events with disease

Biomarker evaluation

- ✓ Safe and easy to measure
- ✓ Low cost of follow-up test
- ✓ Proven to treatment to modify the biomarker

ACADEMIC



INDUSTRY

CLINICAL

Metabolomics involves the study of...?

- ❑ Lipids
- ❑ Alkaloids
- ❑ Amino acids
- ❑ Polymers

Metabolite is a low molecular weight organic compound, typically involved in a biological process as a substrate or product

Sample preparation involves the following steps

- ❑ Extraction
- ❑ Processing
- ❑ Interpretation

Sample preparation usually includes collection, storage, extraction and preparation

To measure sets of metabolites you can use

- ❑ Targeted metabolomics
- ❑ Untargeted metabolomics

Untargeted measures as many metabolites as possible from a range of biological samples.

NMR is more sensitive than MS

- ❑ True
- ❑ False

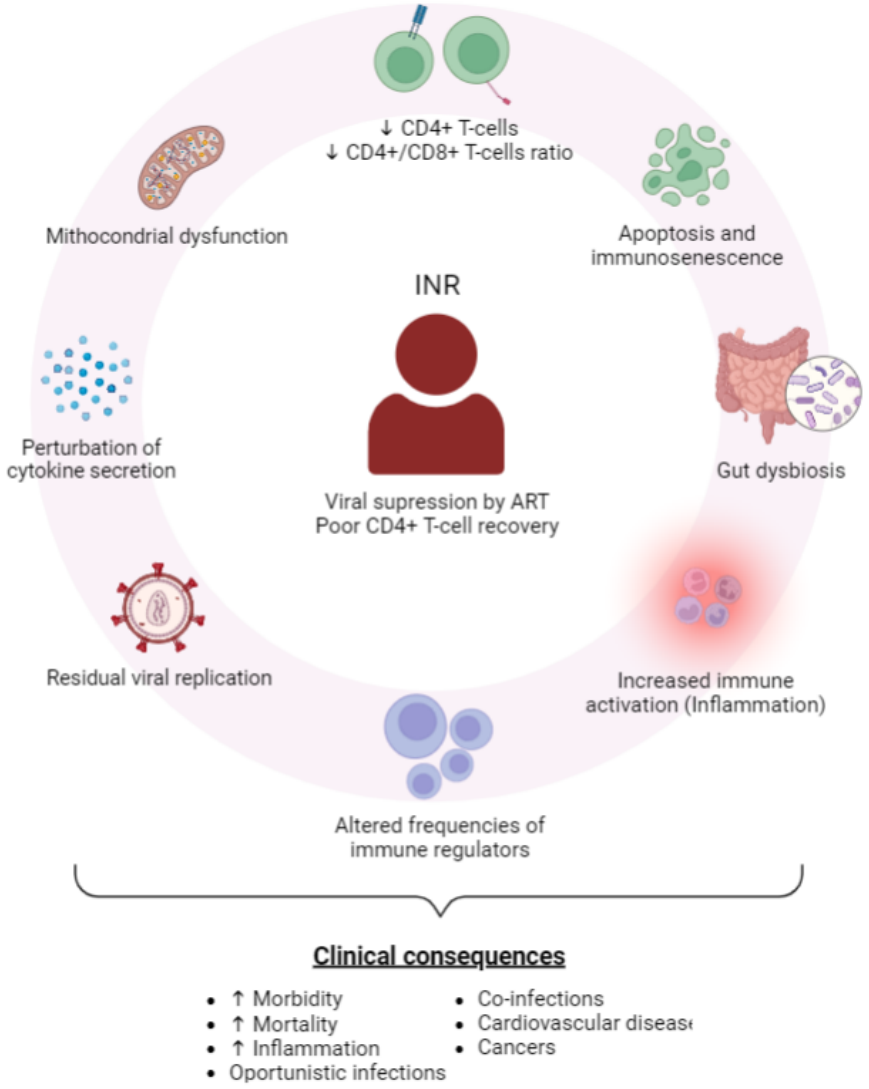
MS techniques are incredibly sensitive and accurate and offer a high mass resolution

Metabolomics can help to improve healthcare

- ❑ True
- ❑ False

To develop early-detection systems.

Metabolomics in PLHIV Immunological Non-Responders (INR)



Multi-omics in HIV: searching insights to understand immunological non-response in PLHIV.
doi: 10.3389/fimmu.2023.1228795

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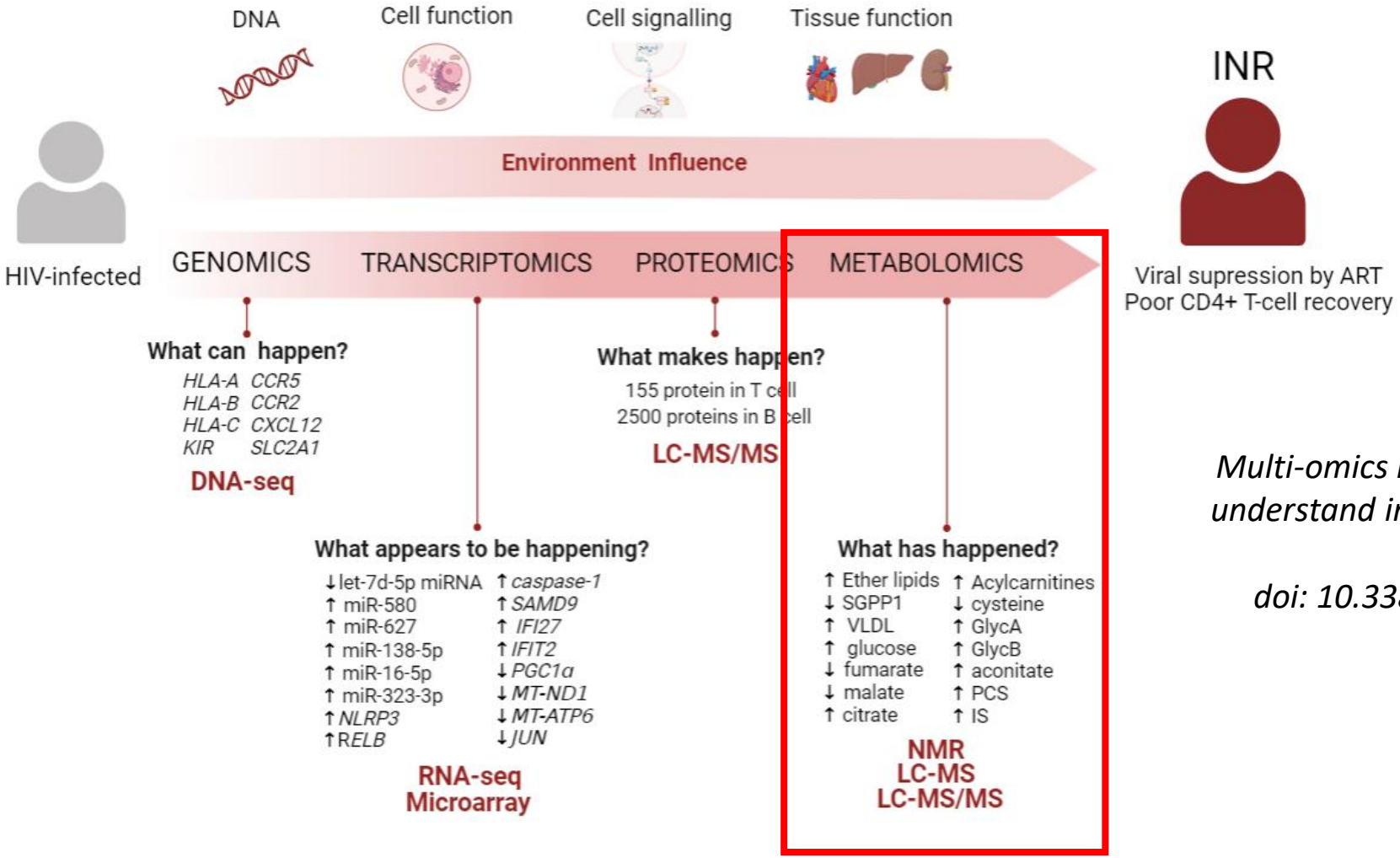

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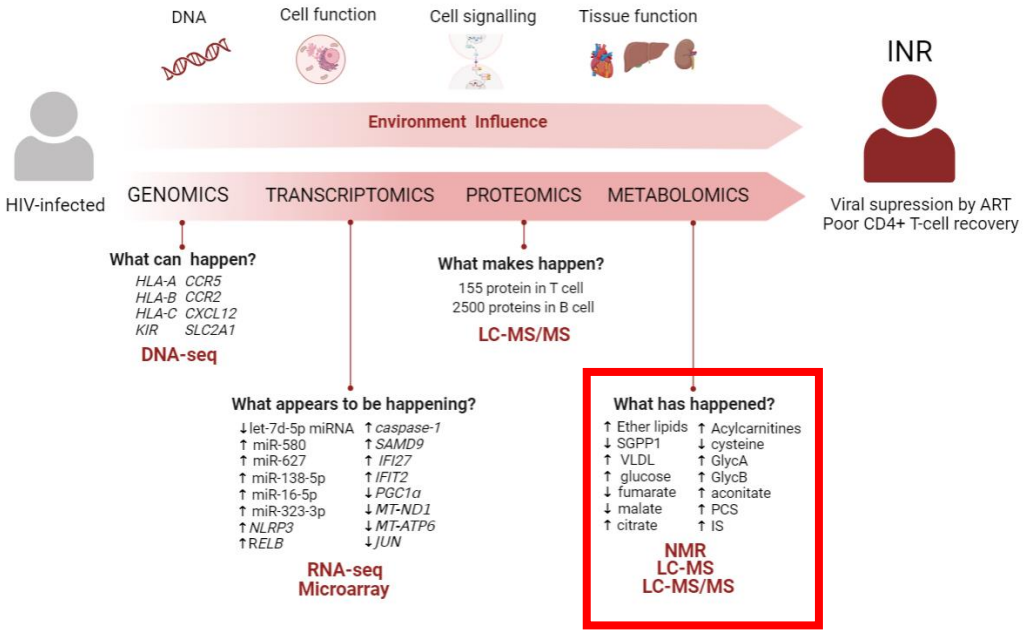
METABOLOMICS				
Scarpellini et al.	PLHIV with fail to increase CD4⁺ T-cell count by at least 30%	Targeted MS/MS	40 acylcarnitines 19 proteinogenic amino acids, ornithine and citrulline, 19 biogenic amines, the sum of hexoses, 76 phosphatidylcholines, 14 lyso-phosphatidylcholines 15 sphingomyelins	↑ Ether lipids ↓ SGPP1 β-oxidation
Rodríguez-Gallego et. al	pre-ART low nadir (<200 cells/µl) and CD4⁺ T-cell count lower than 250 cells/µl at 36 months on ART (baseline study).	NMR	HDL HDL-Cholesterol HDL- TGs VLDL VLDL-Cholesterol VLDL- TGs LDL/HDL	non-HDL lipoprotein particle ↑ VLDL particles ('medium' subclass) ↑ glucose
Masip et al.	pre-ART low nadir (<200 cells/µl) and CD4⁺ T-cell count lower than 250 cells/µl at 36 months on ART (longitudinal study).	NMR	HDL HDL-Cholesterol HDL- TGs VLDL VLDL-Cholesterol VLDL- TGs LDL/HDL	↑ large HDL-P ↑ small HDL-P (increased from baseline levels, Rodriguez-Gallego et. al)
Qian et al.	CD4⁺ T-cell count rise after 2 years of < 100 or > 300 cells/µl of ART.	PLC-MS/MS-ESI RP/UPLC-MS/MS-ESI+ RP/UPLC-MS/MS-ESI-	125 lipids 68 amino acids 7 peptides 14 carbohydrates 12 cofactors and vitamins 9 nucleotides 6 energy metabolites	↑ Acylcarnitines (MC, PC, OC, and SC) associated with INR
Ferrari et al.	CD4⁺ T-cells <350/µL receiving ART for 2 or more years	UPLC-MS/MS	125 metabolites	↑ citrate, aconitate,linolenate ↓ nicotinamide, fumarate, malate and phospholipids ↓ amino acids (isoleucina, alalnina glycine…)
Nyström et al.	pre-ART low nadir (<200 cells/µl) and rise in CD4⁺ T-cells <50 cells/year in the first 2 years following suppressive ART.	LC-MS technique	200 metabolites	↓ levels of cysteine could be associated with poor CD4+ T-cell recovery
Malo et al.	CD4⁺ T-cell count rises after 2 years of < 100 or > 300 cells/µl of ART.	NMR	Plasma glycoprotein profiles	↑ levels of GlycA and GlycB associated with a worse immunological state. ↑ levels of baseline glycoprotein concentrations tend to respond less to ART.

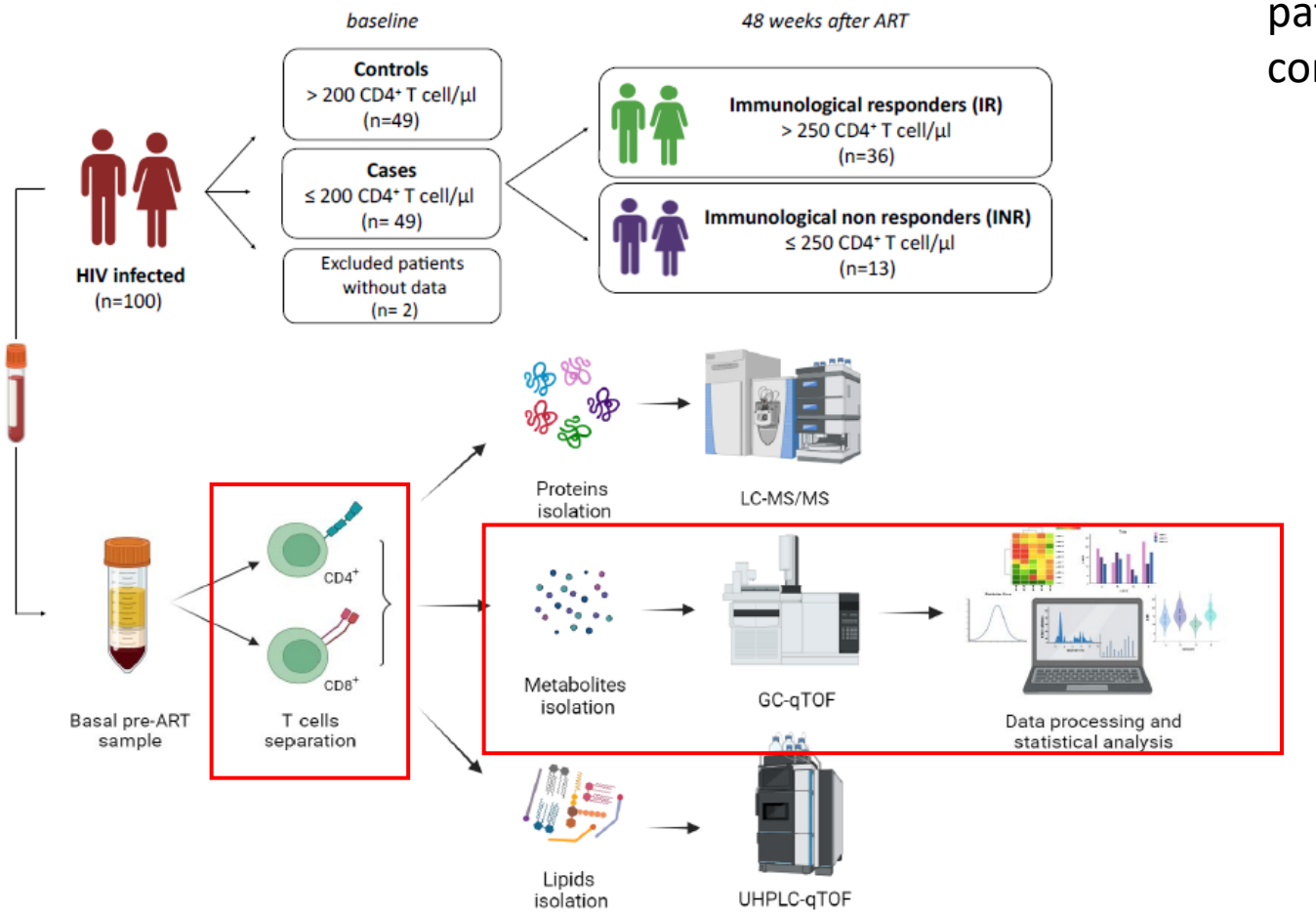


7 articles

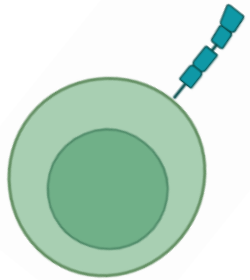
- 4MS
- 3 NMR

✓Plasma/Serum samples
✓Identification of possible biomarkers

Metabolomics in PLHIV Immunological Non-Responders (INR)



Identification of molecular pathways altered in INR condition (Preliminary results)

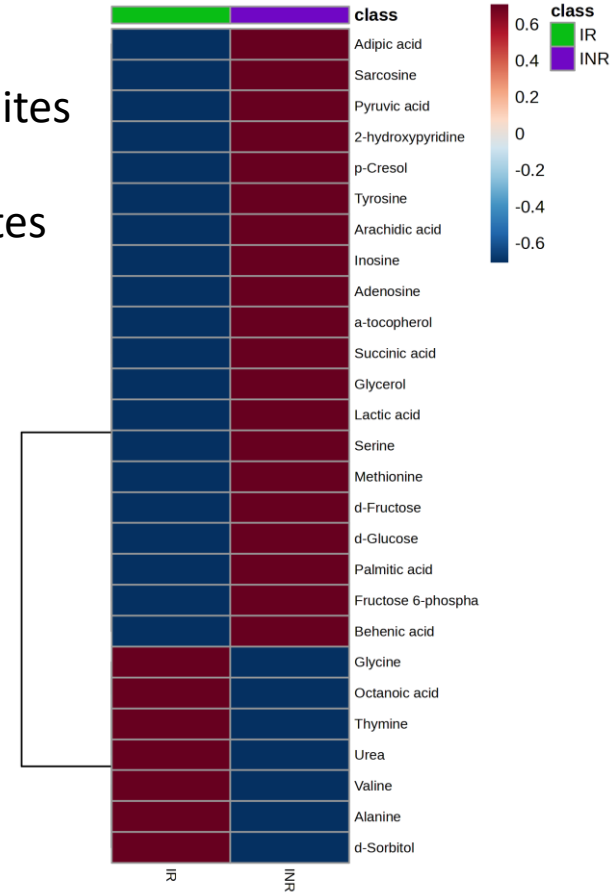


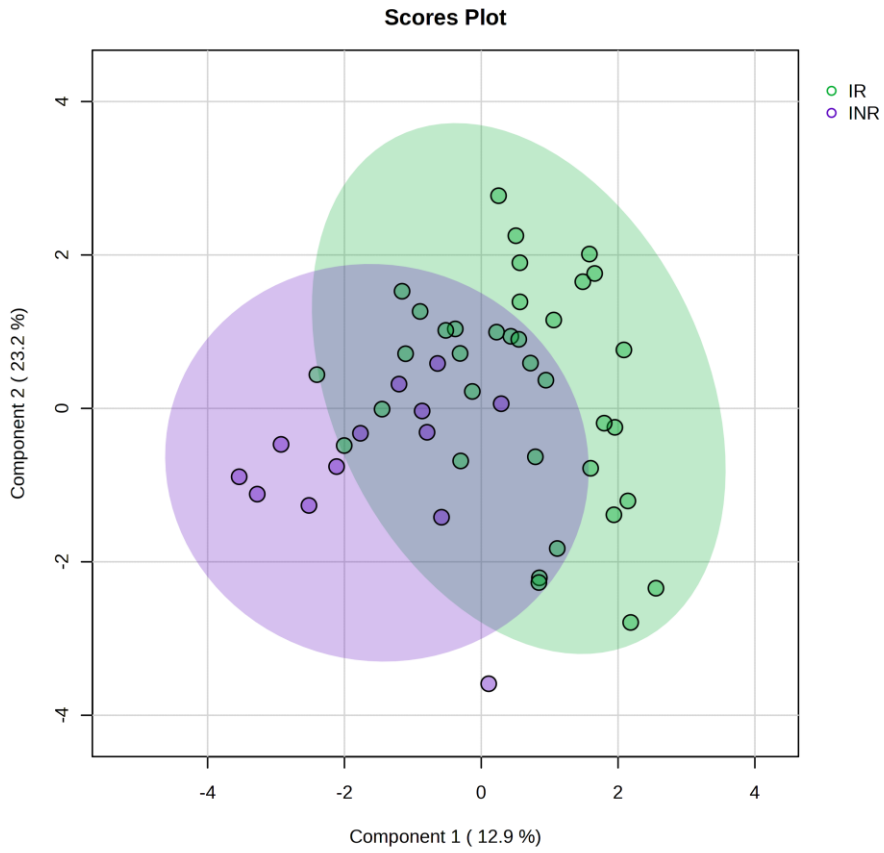
Metabolomics in CD4⁺ T Cells

55 metabolites detected
27 significant metabolites between IR vs INR (Mann-Whitney test)

↑20 metabolites

↓7 metabolites





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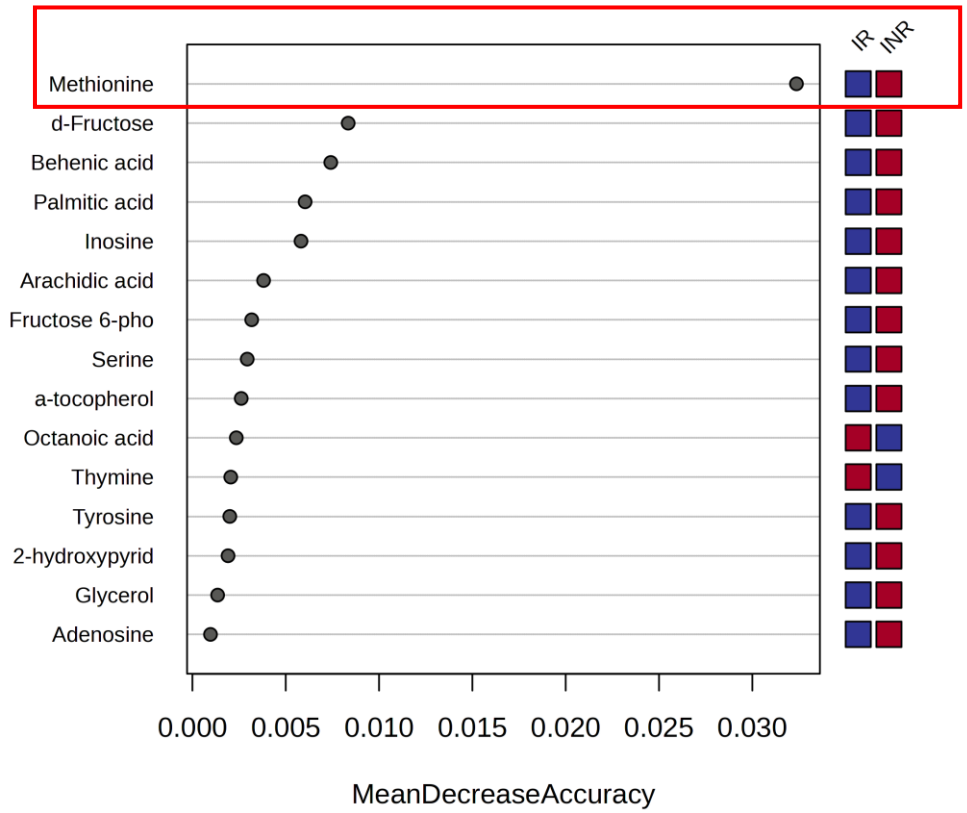

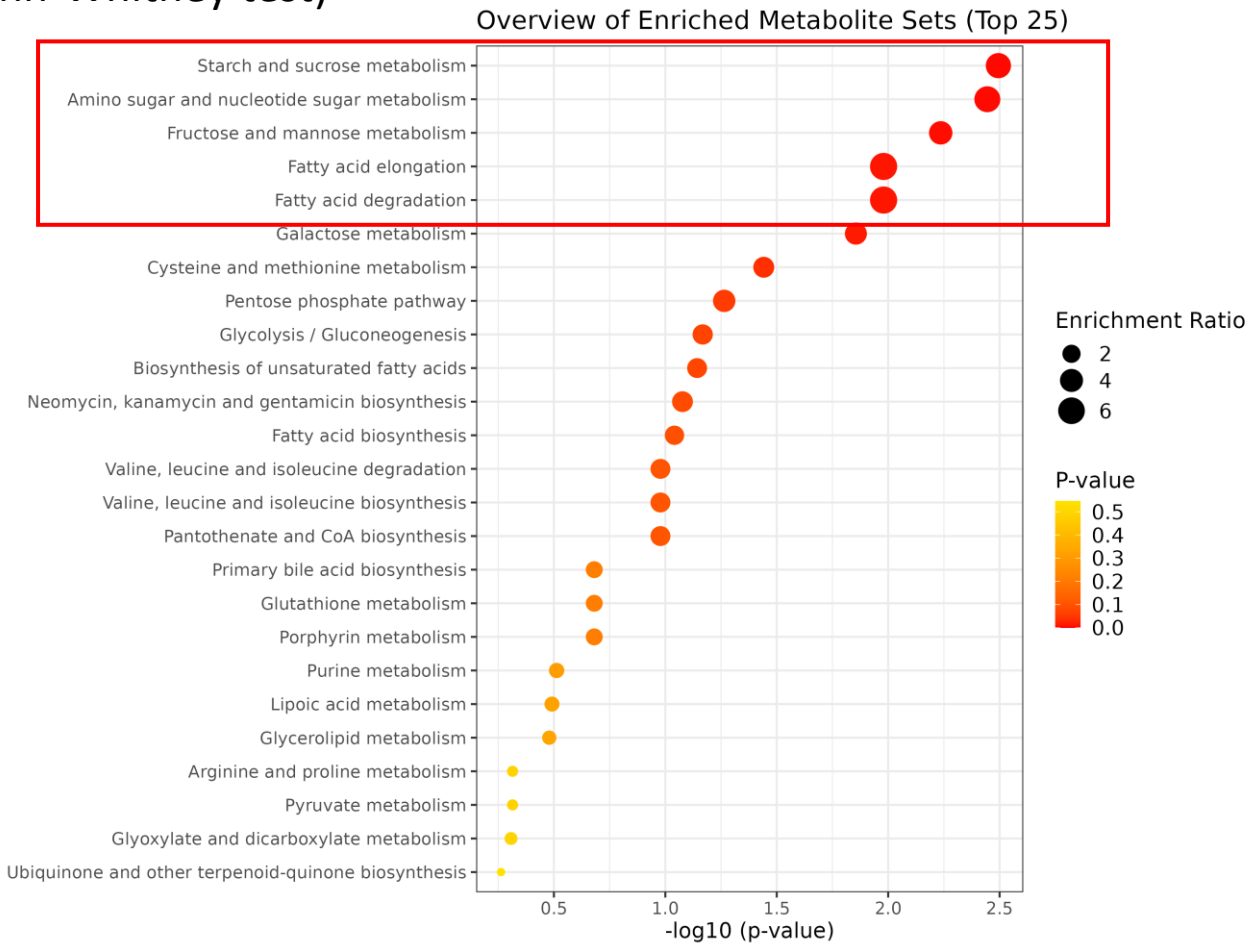

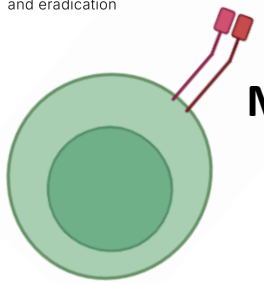

Metabolomics in CD8$^+$ T Cells

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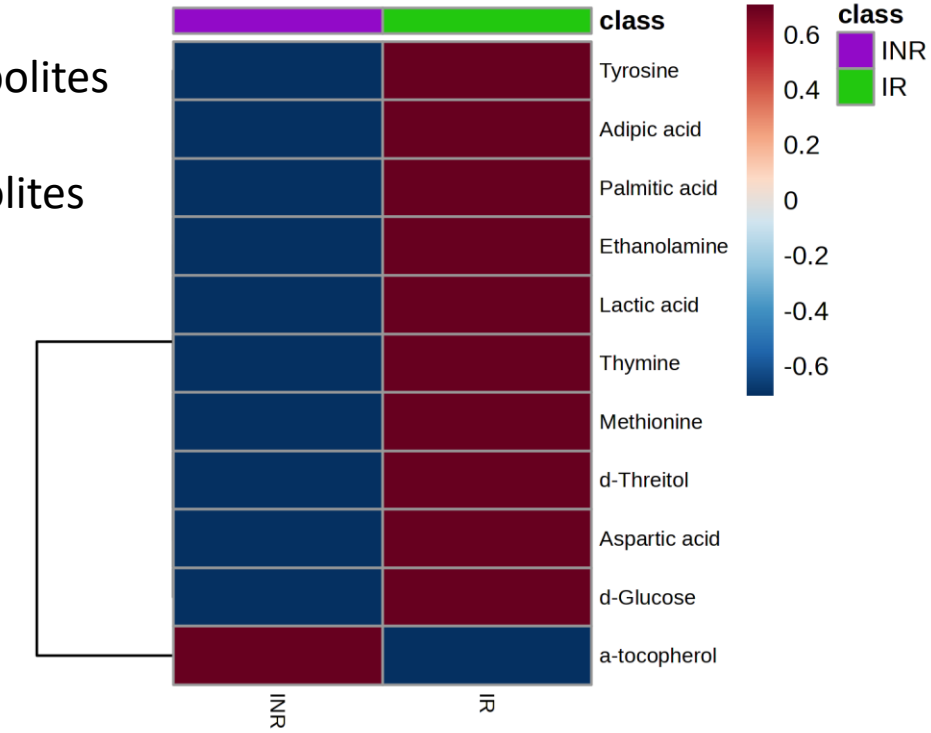
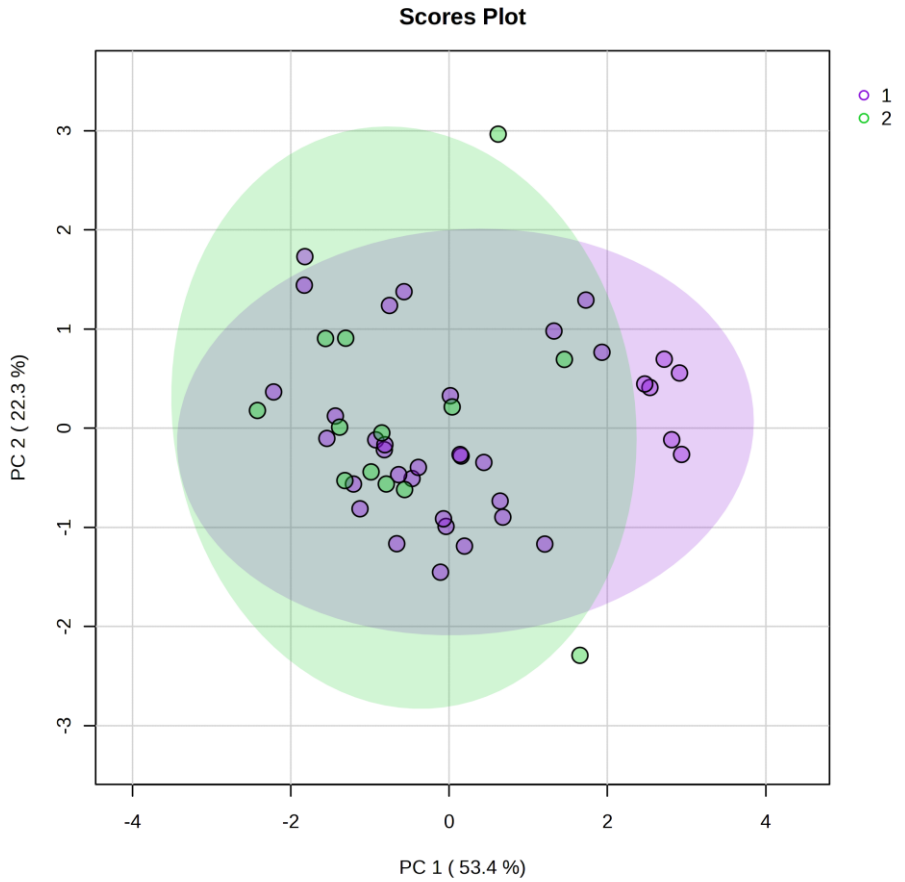
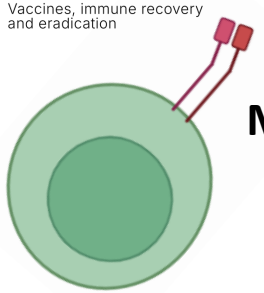
11 significant metabolites between IR vs INR (Mann-Whitney test)

↑10 metabolites

↓1 metabolites







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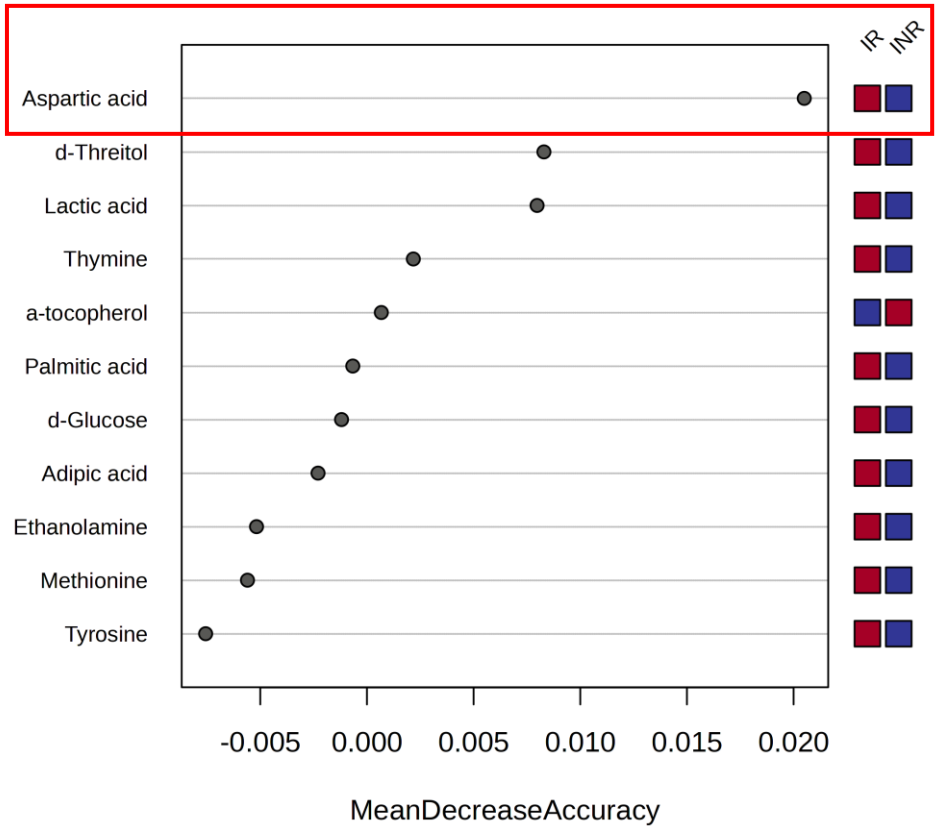
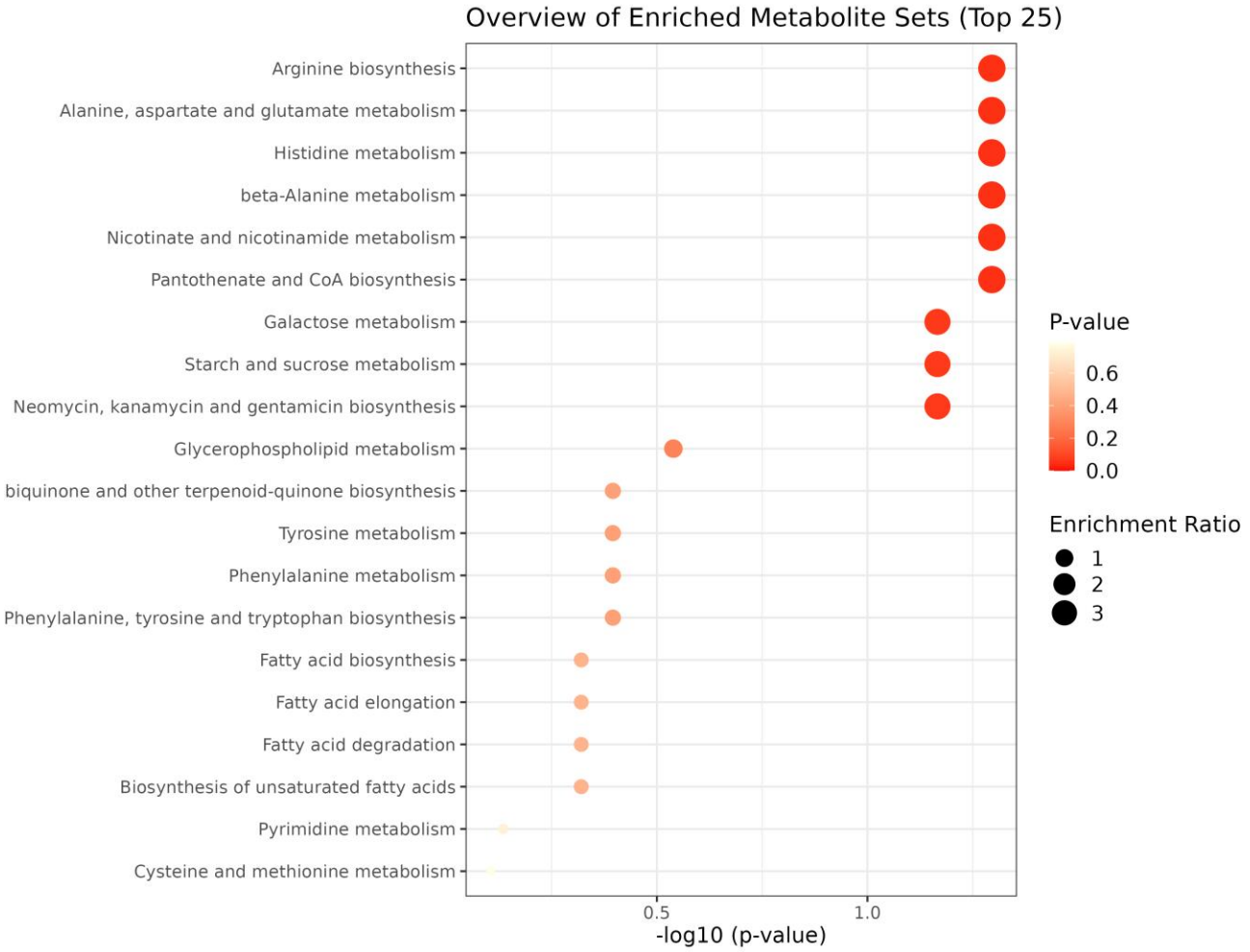
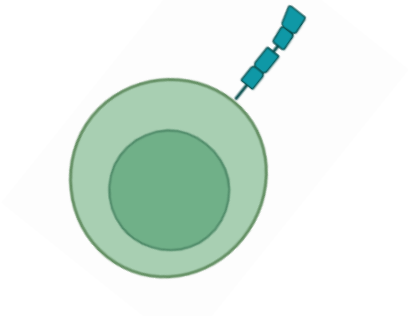


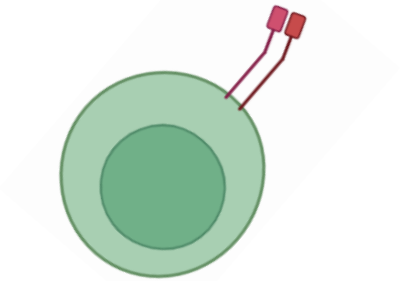


Conclusions



$CD4^+$ T Cells

- ✓ Variability in $CD4^+$ T cell response
- ✓ Sugar metabolism and fatty acid metabolism alteration



$CD8^+$ T Cells

- ✓ Less differences in $CD8^+$ T cell metabolic profile (11/55 metabolites)
- ✓ Amino acid metabolism alteration

Impaired mitochondrial activity

Take home message

- ✓ Metabolomics is the comprehensive, qualitative, and quantitative study of low-molecular-weight molecules.
- ✓ Metabolomics can be targeted or untargeted depending on the aim of the study.
- ✓ Applications: Clinical biomarkers, identification of molecular pathways….
- ✓ Metabolomics is a snapshot of the physiology of the cell most closely related to the phenotype.

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