The Regional Centre for Personalised Medicine: a Precision Medicine approach to Type 1 diabetes mellitus

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The Next Challenge in Medicine:

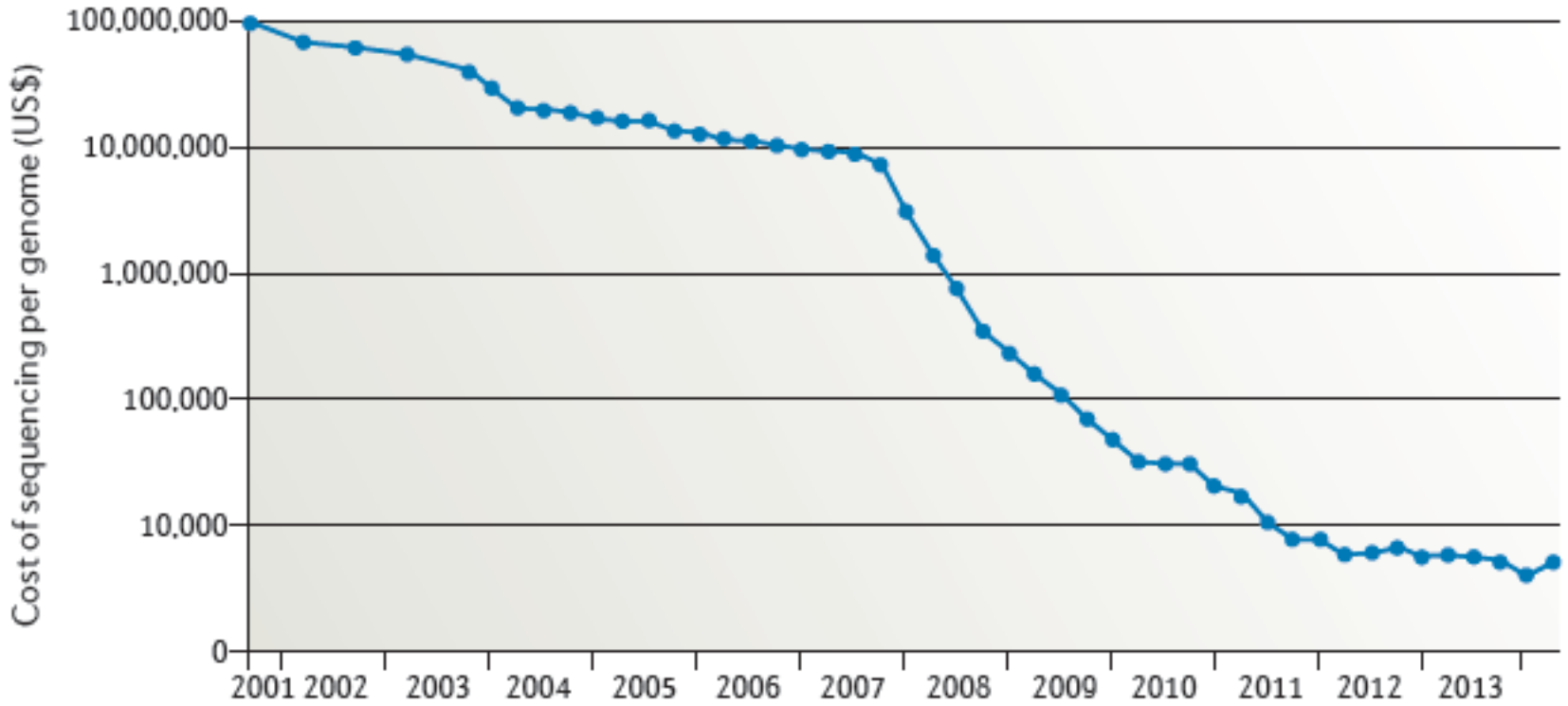
"Precision Medicine"

"Tonight, I'm launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes — and to give all of us access to the personalized information we need to keep ourselves and our families healthier."

— President Barack Obama, State of the Union Address, January 20, 2015

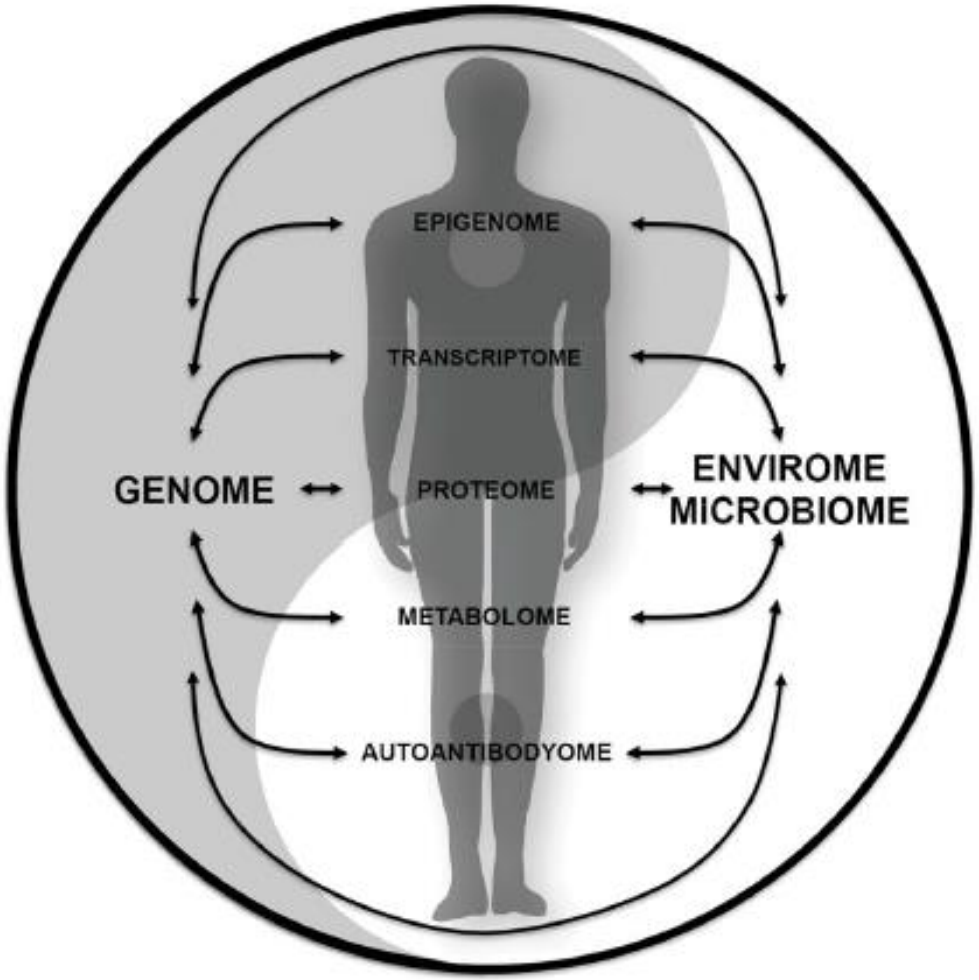
N ENGL J MED 372;9 NEJM.ORG FEBRUARY 26, 2015

Sequencing costs per genome



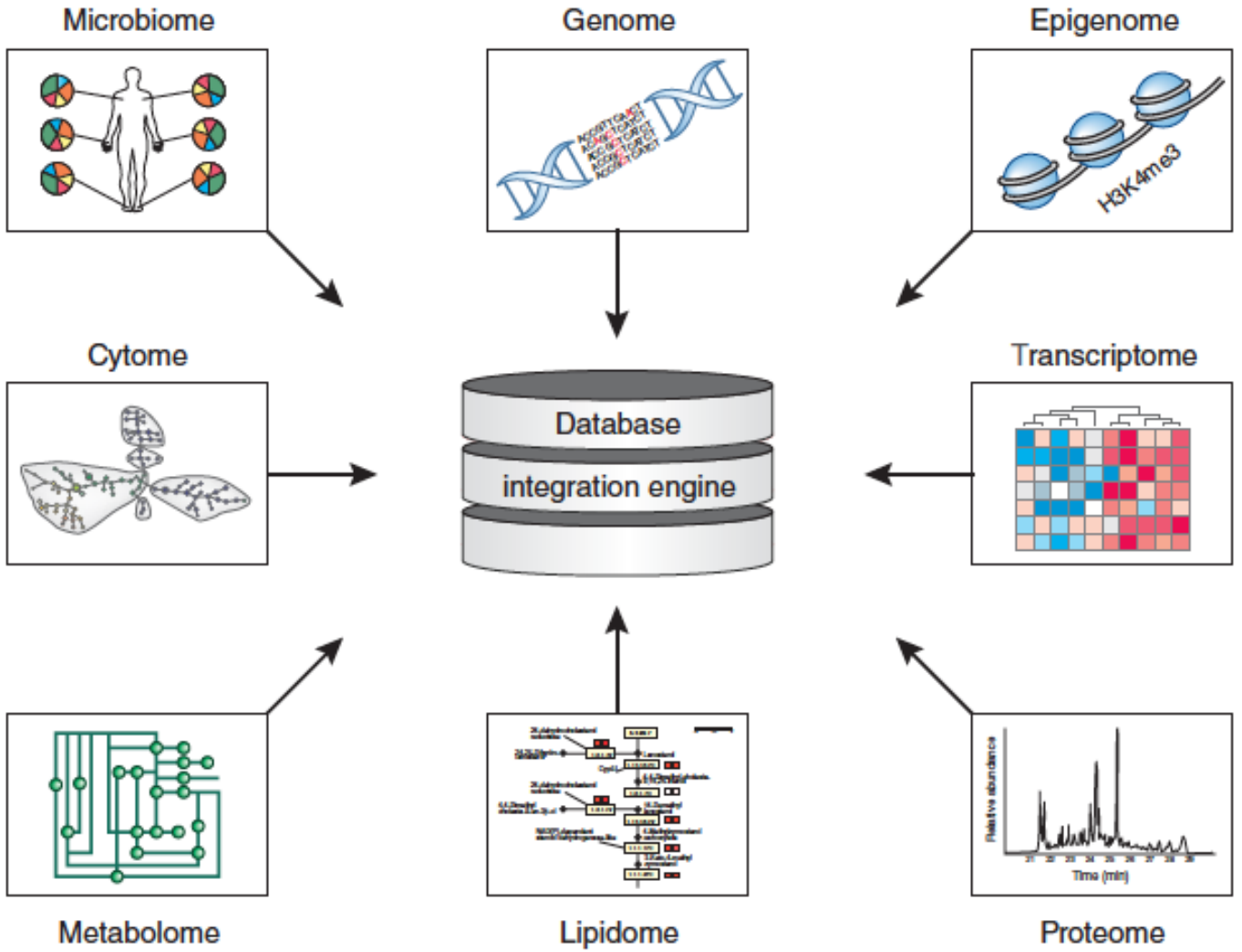
Nat.Rev.Immunol., doi:10.1038/nri3820 , March 2015

A new era for Precision Medicine



- ✓ **Large-scale biological databases;**
- ✓ **Powerful "omics" patient characterization tools;**
- ✓ **Tools for Big Data management**

Integrative omics for health and disease

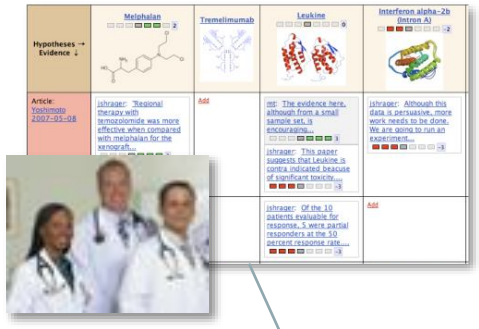


Nat.Rev.Immunol., doi:10.1038/nri3820

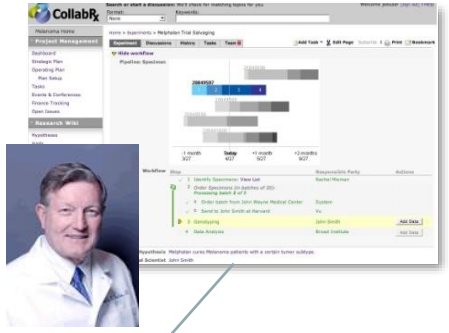
Aggregating Information

Scientists

Clinical Researchers



Physicians



Patients

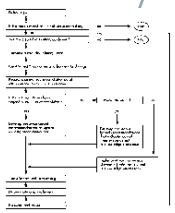
Data

Rapid Learning System

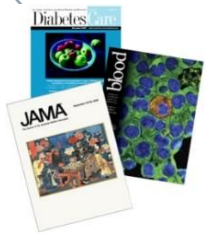
Molecular Disease Models

Trials

Treatments

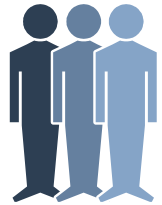


Guidelines



Literature

Tests



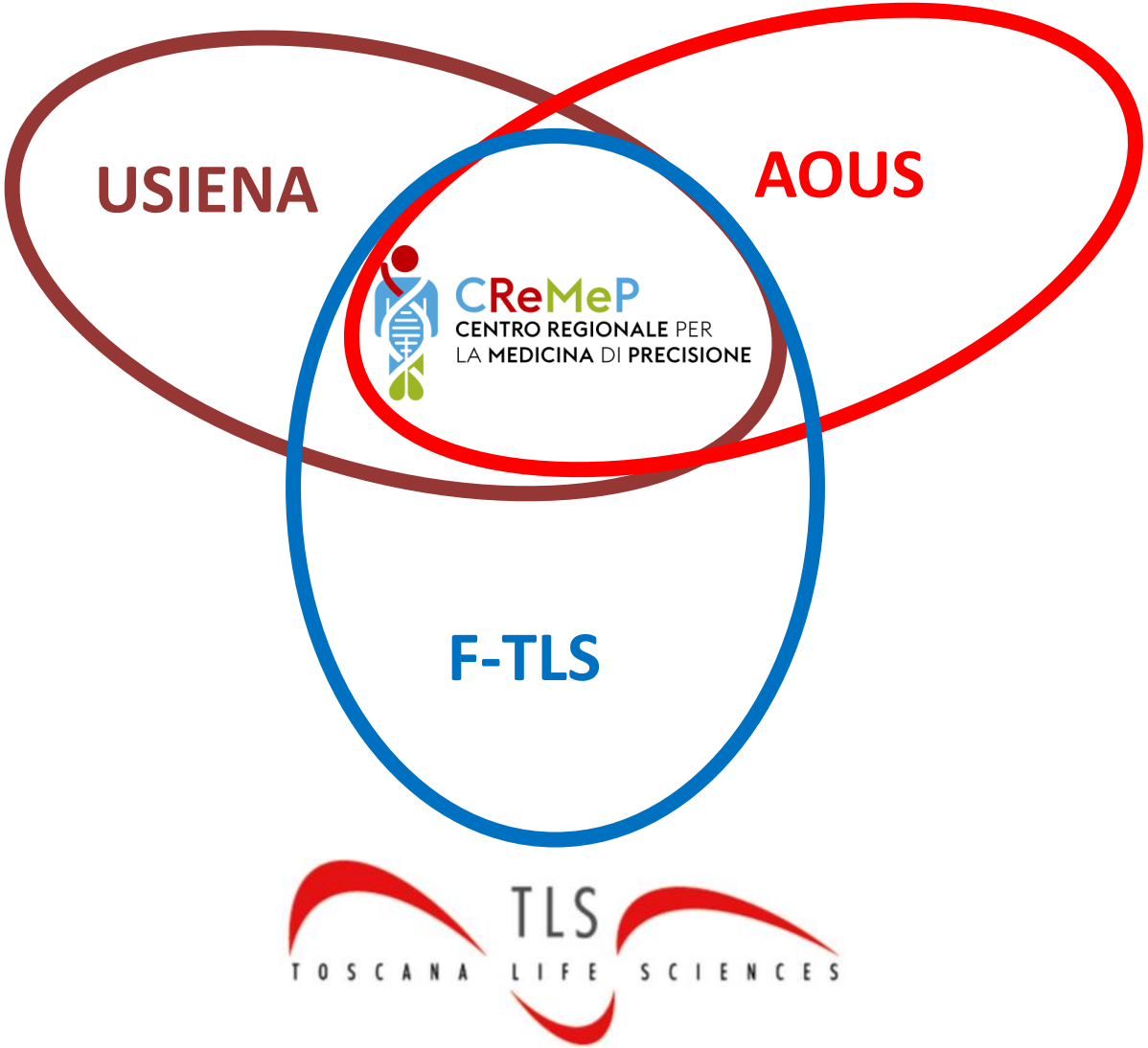
Cases

Changing how drugs are delivered

Identify non-responders and safety issues before prescribing or treating

Regional Center for Personalised Medicine

UNIVERSITÀ DI SIENA 1240



Azienda Ospedaliera Universitaria Senese

Center for Precision Medicine

- **Open access technical platforms (**e.g. NGS, imaging, Nanostring, etc**.)**
- **Bio-Bank (**shared SOPs for sample collection**)**
- **Bioinformatics and Big data management;**
- **Promote translational research and biomarker discovery for disease prediction, diagnosis and staging**
- **Clinical trials (targeted trials, virtual trials)**

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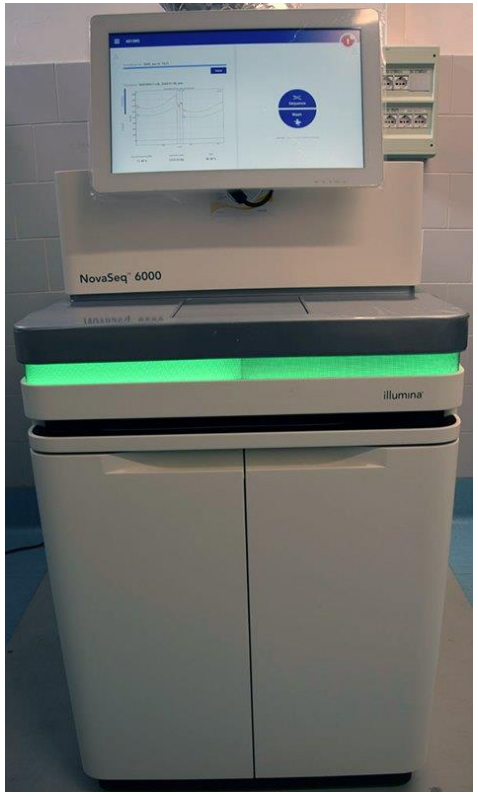
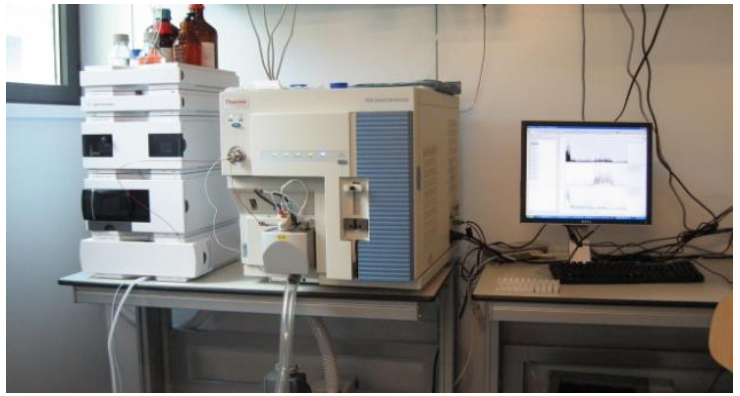
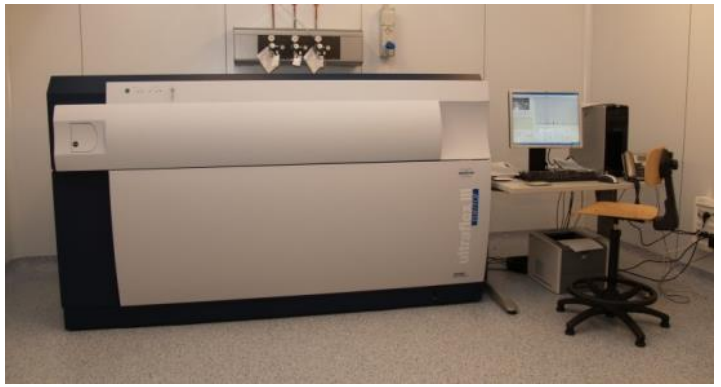
Open access technical platforms

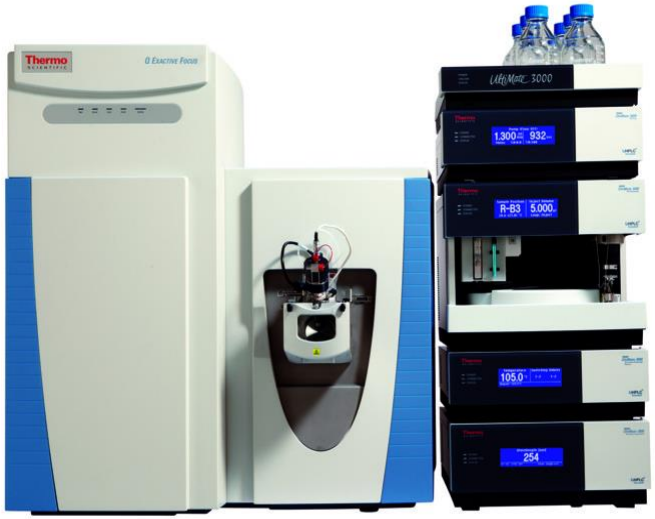
- **Nanostring nCounter FLEX Analysis System (Diatech Pharmacogenetics)**
- **Slide Scanner NanoZoomer mod.S60 (Nikon instruments)**
- **Quanterix SR-X Ultra-Sensitive Multiplexed Biomarker Detection Warranty & Service Programs (Quanterix)**
- **autoMACS Pro Separator – (Miltenyi Biotec).**
- **NovaSeq 6000**

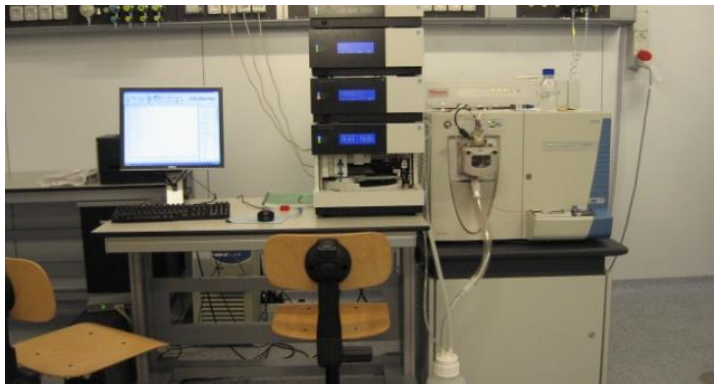


- Triple quadrupole Mass spectrometer
- MALDI-TOF/TOF Mass spectrometer
- 500Mhz dual channel NMR
- LTQ-Orbitrap mass spectrometer
- FT/MS Q Exactive Biopharma

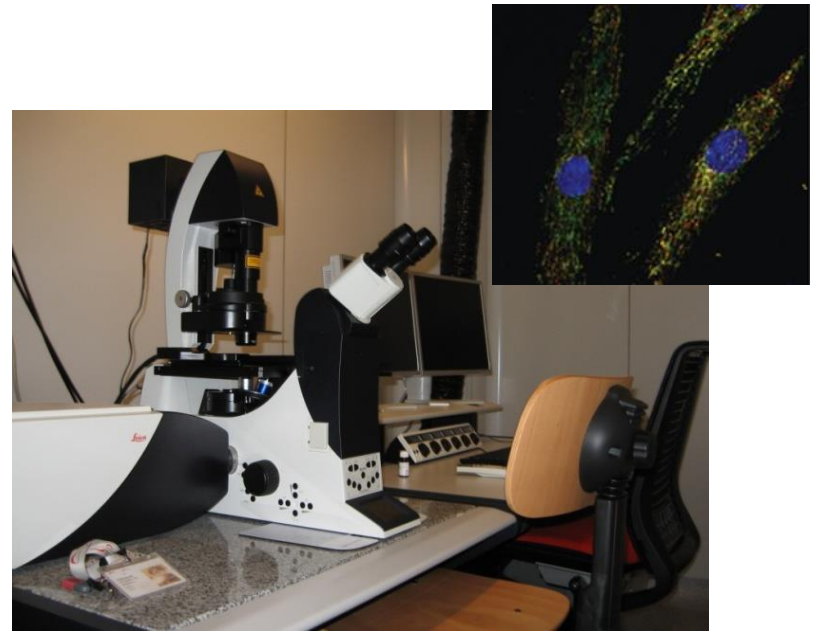


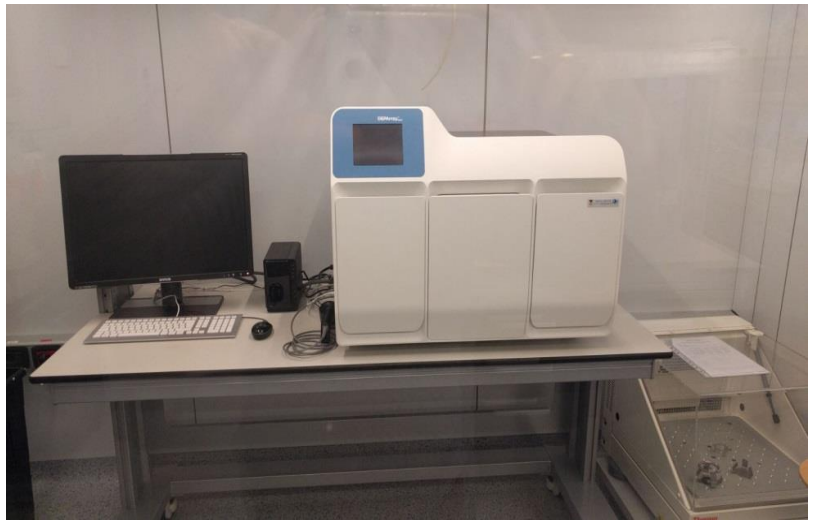


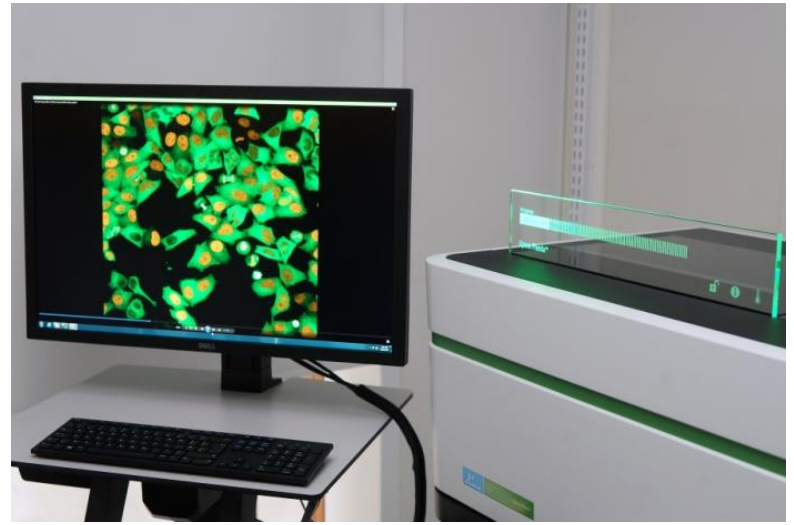




- DEPArray™ NxT Digital Cell-Sorting System
- Laser Capture Microdissector Arcturus XT
- BD FACS Canto TM II citofluorimetro (FACS)
- Confocal microscope LEICA TCS SP5
- Opera Phenix High-Content Screening System





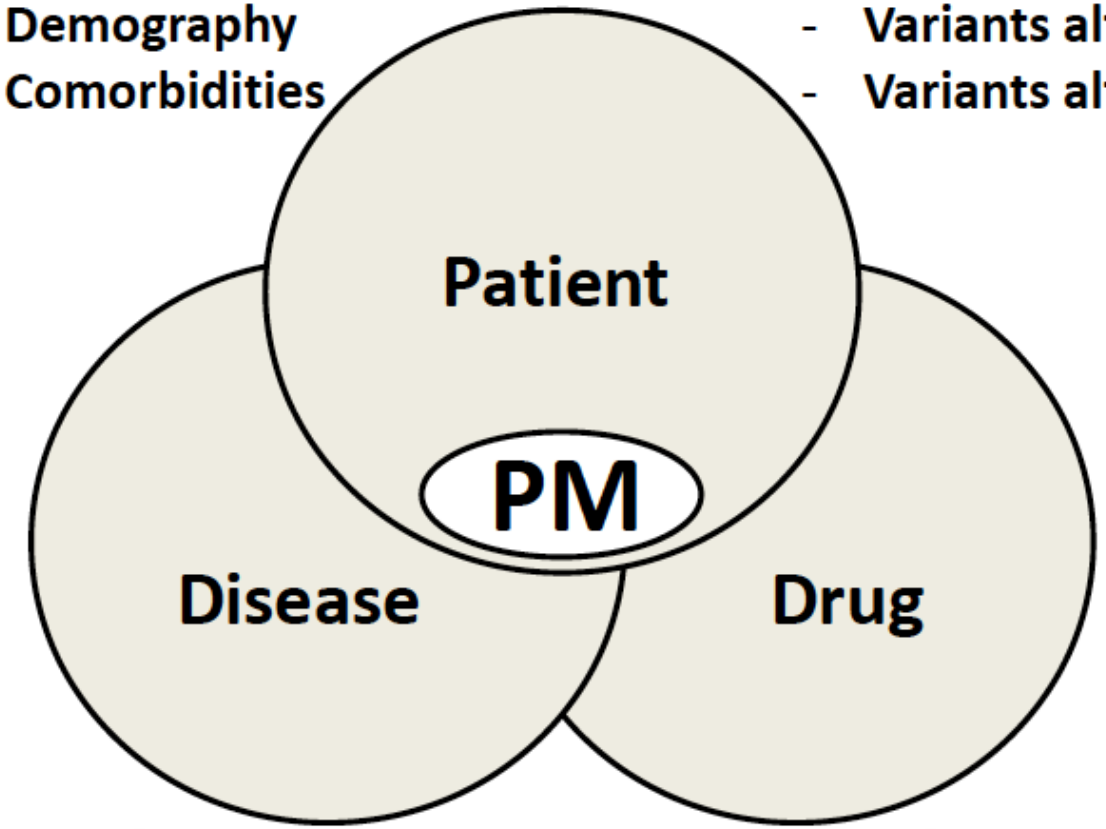


- Spectramax M2 Multi-modal microplate and cuvette readers
- Luminex Magpix Biorad - Multiplex immunoassay
- ViiA 7 Fast 96 Well Real Time PCR system
- FPLC - AKTA Explorer 100
- Droplet Digital PCR

Precision medicine: the future in diabetes care ?

Phenotype
- Demography
- Comorbidities

Genotype
- Variants altering PK
- Variants altering PD

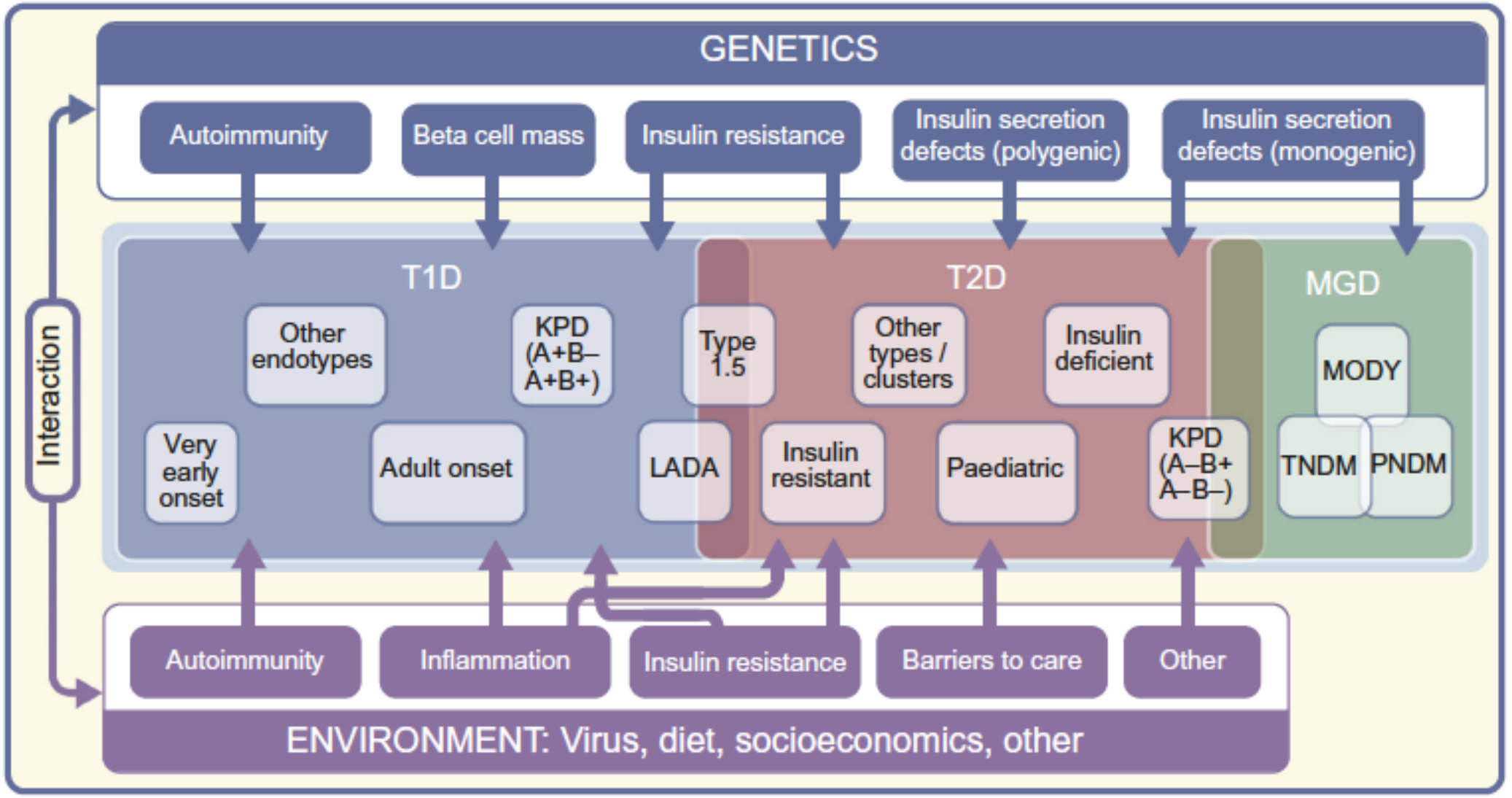


- Duration
- Severity (HbA1c)
- Fasting/postprandial
- Insulin secretion/resistance

- Pharmacokinetics (PK)
- Pharmacodynamics (PD)
- Cost

Diab Res Clin Pract. 2016, 117: 12–21

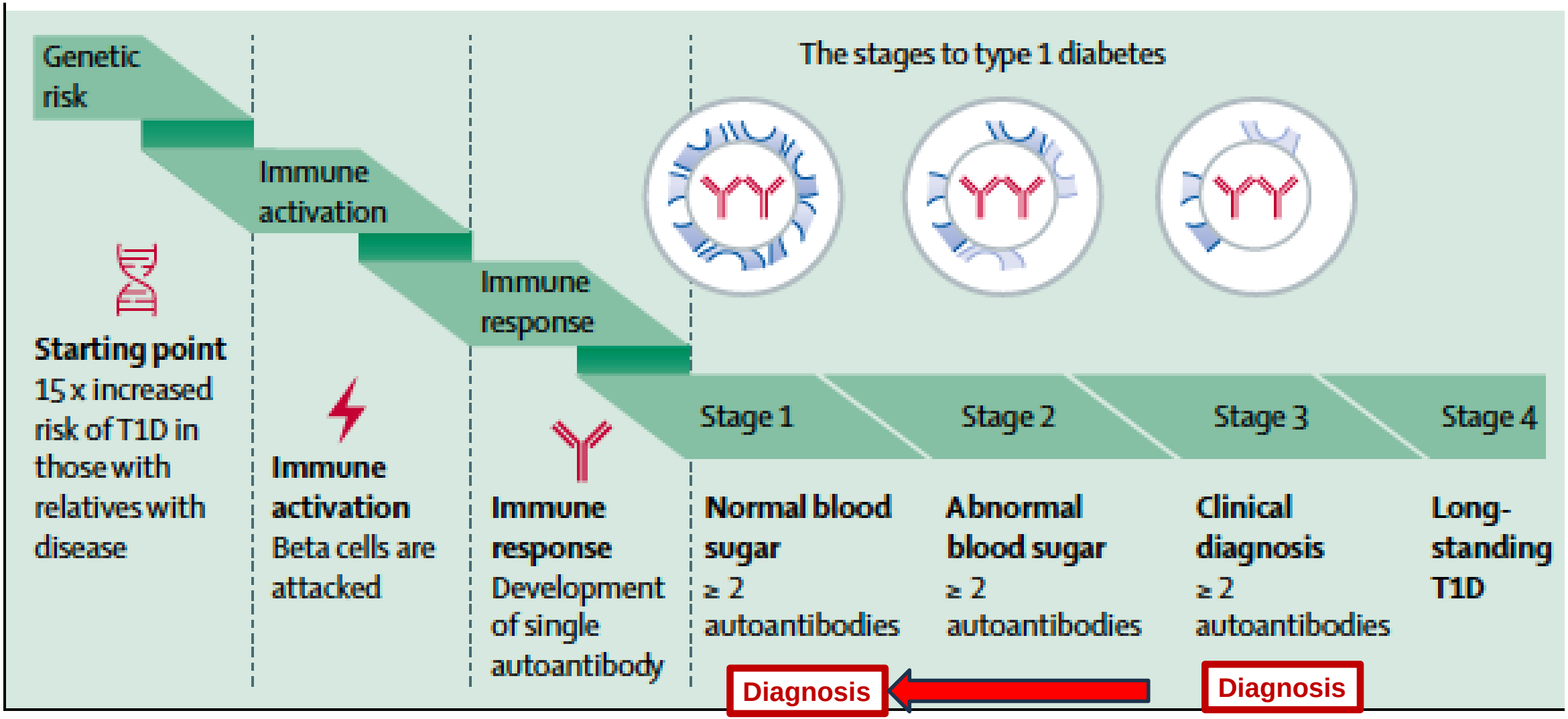
Heterogeneity in different forms of diabetes



Diabetologia (2020) 63:2040–2048

Type 1 diabetes natural hostory

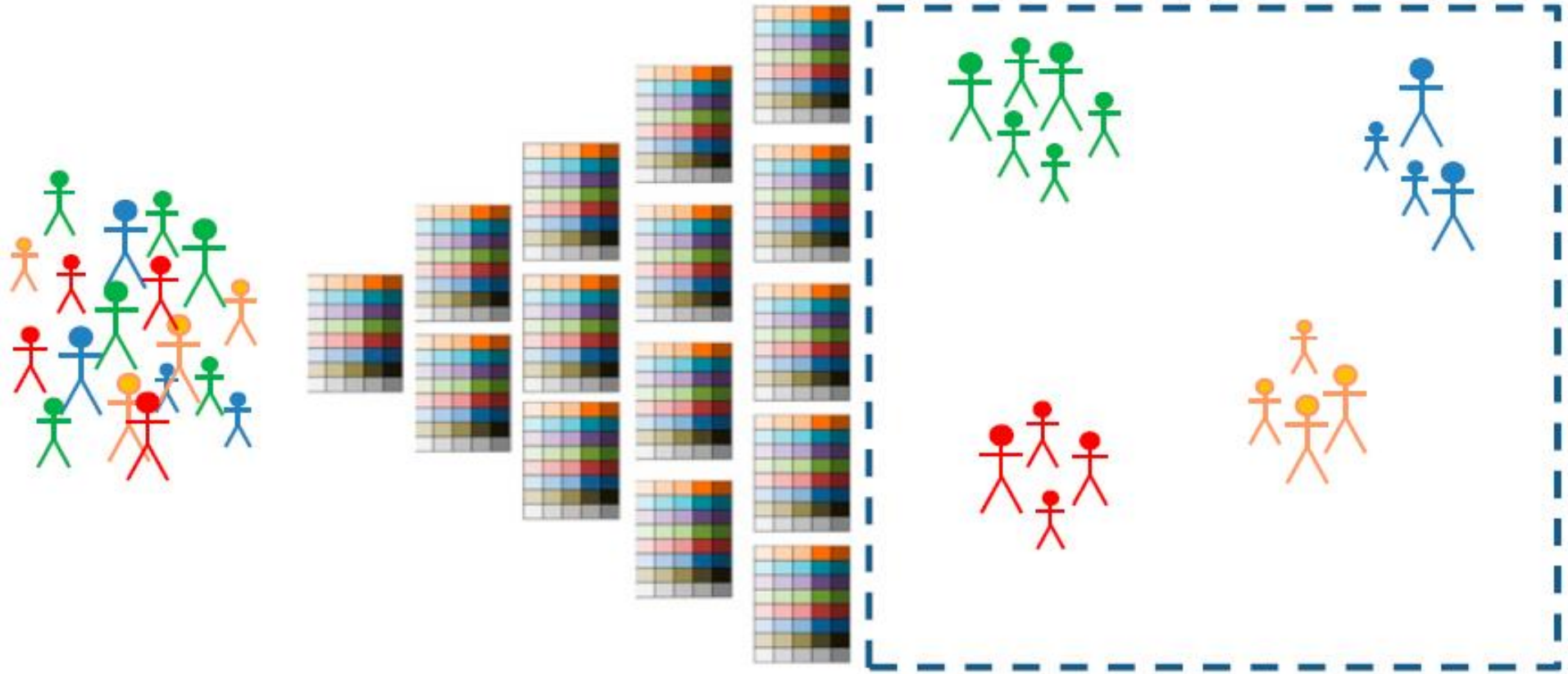
European Diabetes Forum Italia



Di Meglio L et. Al. Lancet 2018

Type 1 diabetes heterogeneity has implications for disease prediction, prevention, diagnosis and treatment

Diabetologia. 2020 October ; 63(10): 2040–2048. doi:10.1007/s00125-020-05211-7.



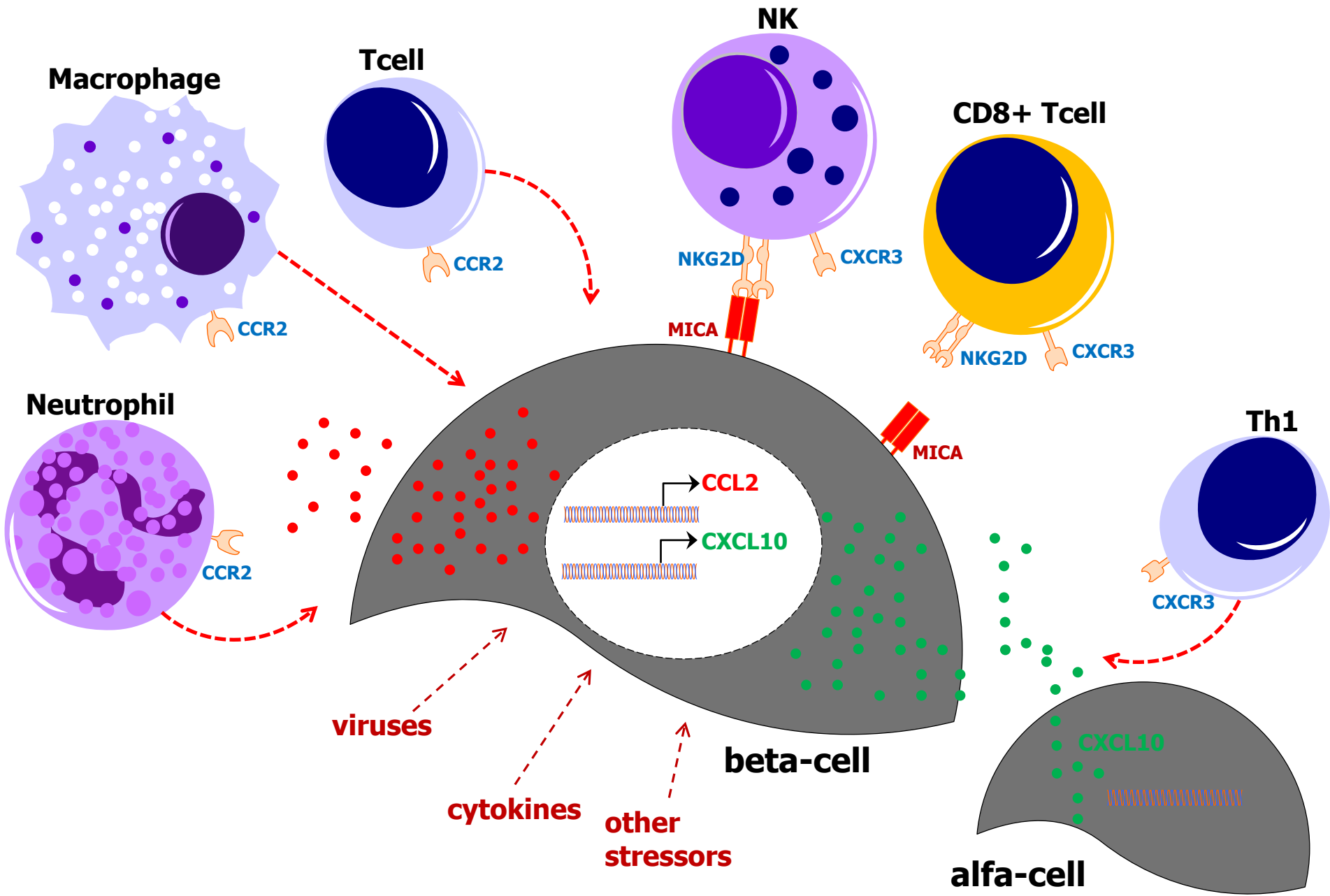
Diabetes Care 2020;43:5–12 | https://doi.org/10.2337/dc19-0880

T1D heterogeneity

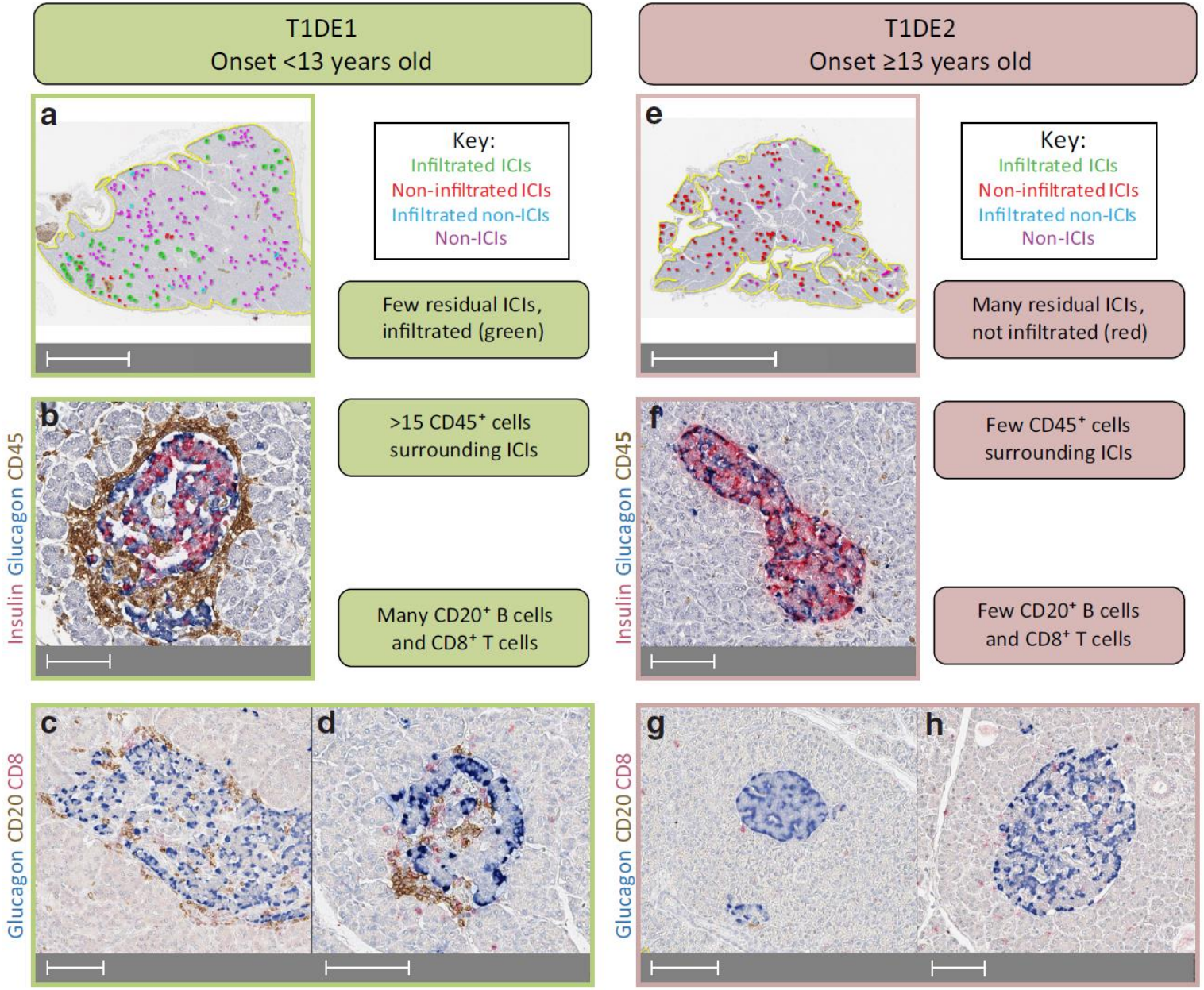
Clinical	• Age of onset of β cell–directed autoimmunity and hyperglycemia (from 6 months to adult); up to 40% onset in individuals over 30 years old • Other autoimmune endocrinopathies or diseases (thyroid, adrenal, celiac, etc.) • Degree and rate of development of diabetes-related complications (e.g., retinopathy, nephropathy, neuropathy)
Genetic	• Presence of HLA DR3/DR4 haplotypes • More than 50 genetic loci
Immunological	• Autoantibody frequency, profile, and target epitopes • Type 1 IFN, innate immunity, and T cell signatures
Metabolic	• Rate of decline in β cell function and mass in stages 2 and 3 • Degree and duration of residual C-peptide production • Response to immunomodulatory therapy
Pathological	• Insulitis (degree, location) differs between and within individuals • CD20+ B lymphocytes in younger-onset T1D • Degree of β cell loss • Differences between insulin-negative islets and islets with β cells

How do we find those who are at risk?
How do we identify who to treat?... And with what strategies?

Type 1 diabetes: Complex dialogue between islet and immune system

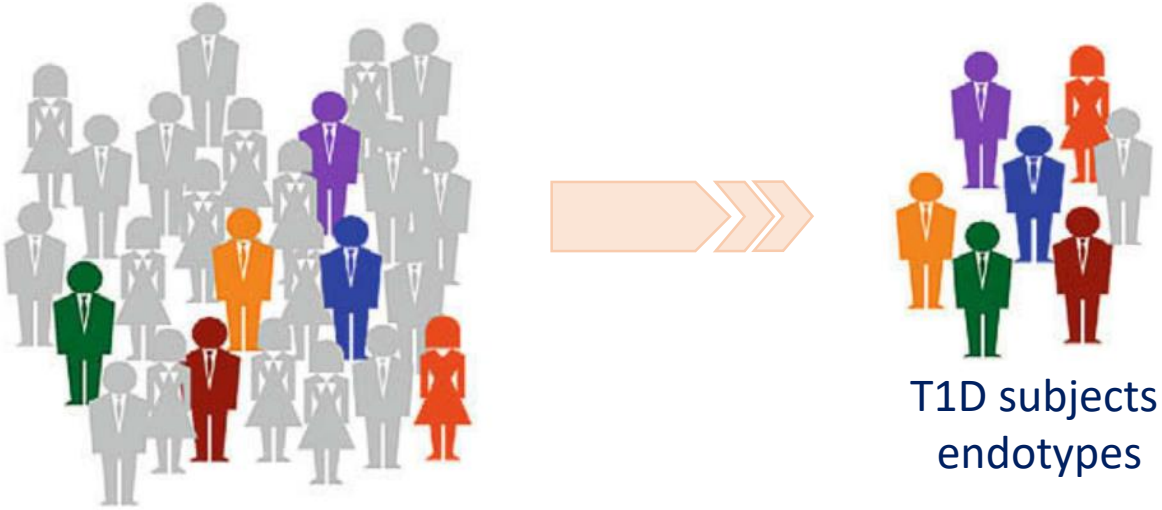


Age-related T1D Endotypes



Diabetologia (2021) 64:15–25

Is it possible to identify specific endotypes of T1D subjects on the basis of the expression profiles of circulating biomarkers and their correlation with clinical parameters and other molecular features?



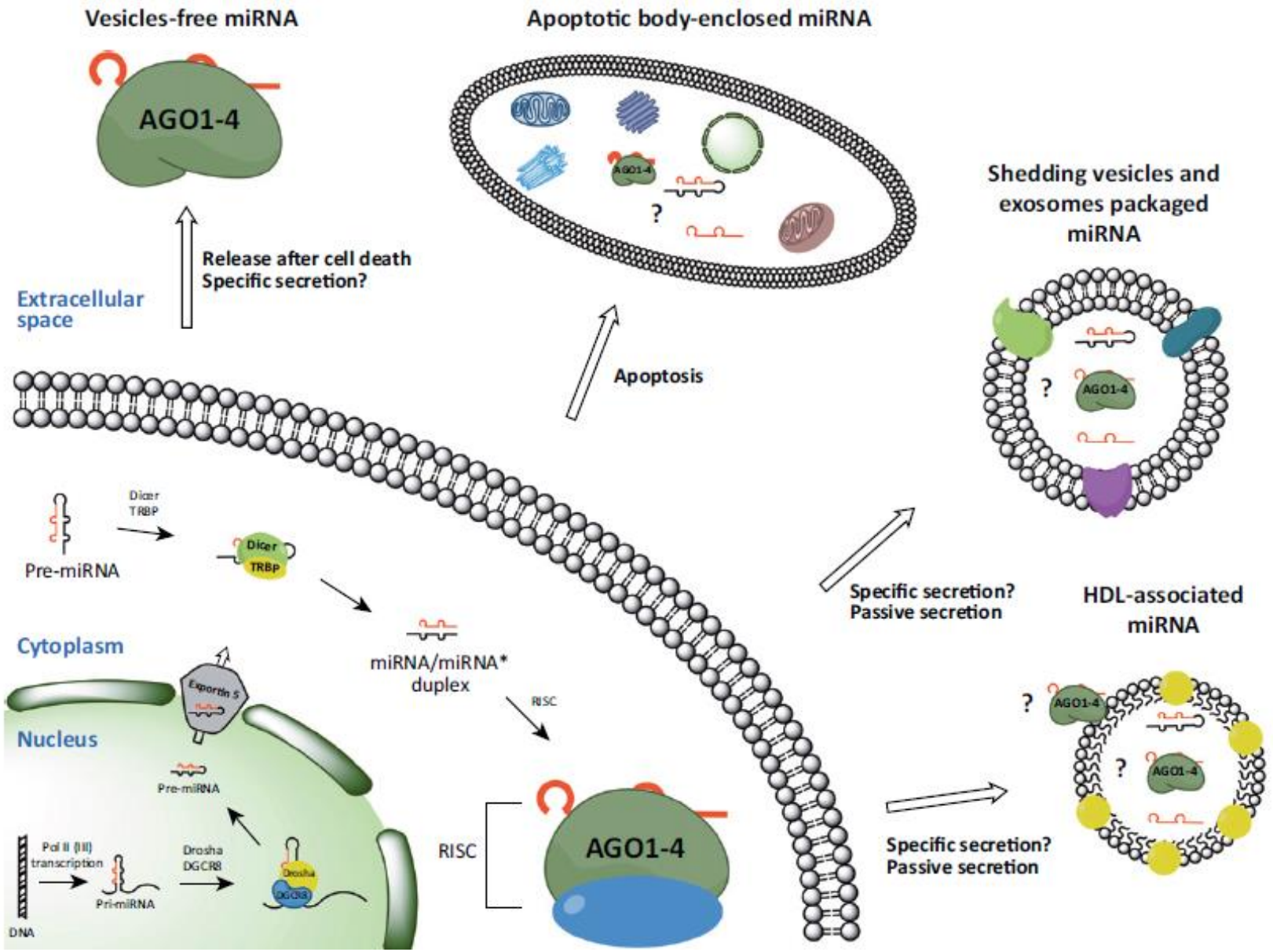
INNODIA Biomarkers Research study to identify novel T1D endotypes

MMTT		✓	✓	✓	✓
C-pep	✓	✓	✓	✓	✓
Serum	✓	✓	✓	✓	✓
Cells	✓	✓	✓	✓	✓
AA	✓			✓	✓
Omics*	✓			✓	✓
Stool /urine	✓	✓	✓	✓	✓

* Including micro-RNA and whole blood RNA

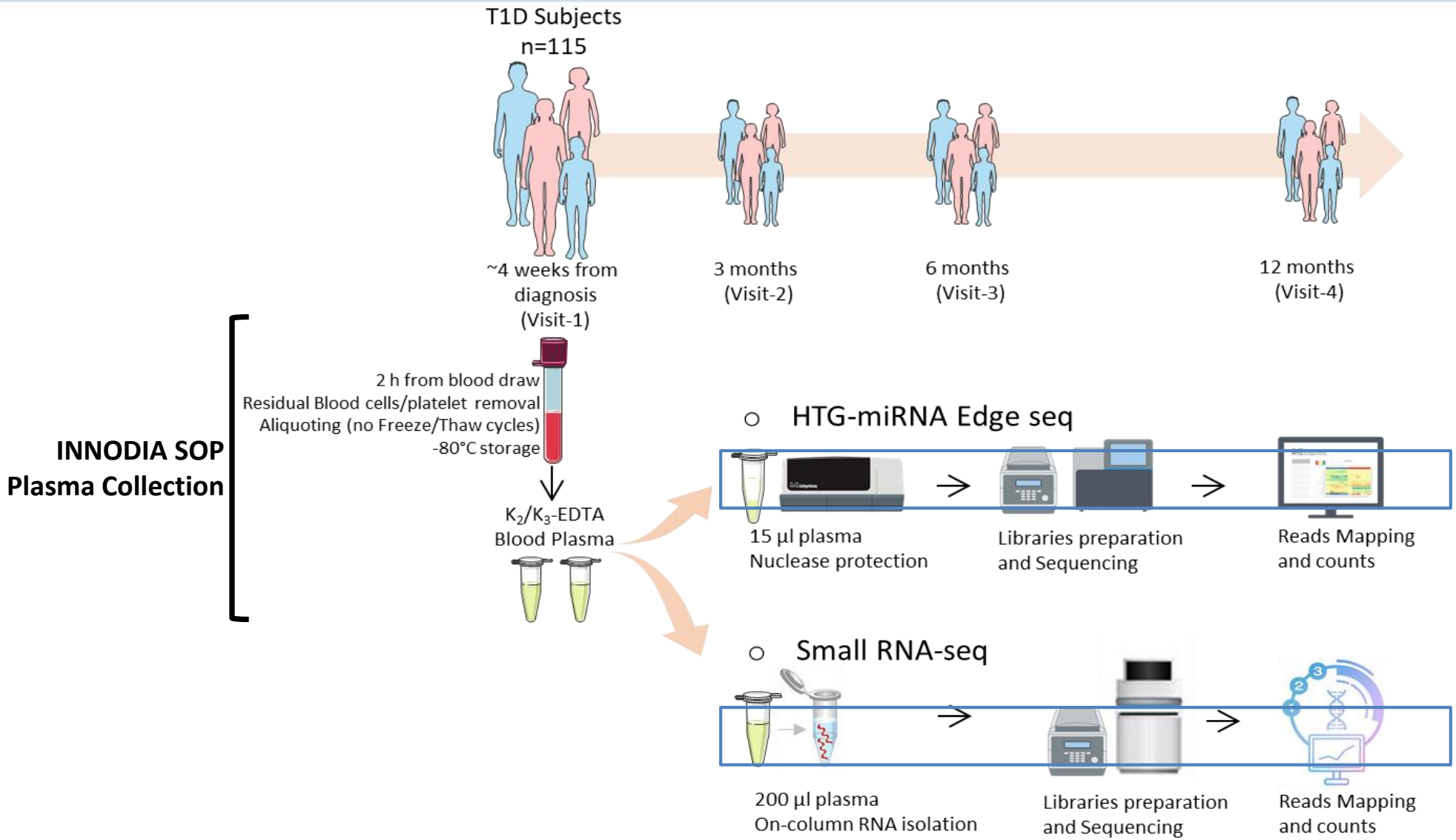
- Genotyping
- Immunomics
- Lipidomics
- Trancriptomics
- Proteomics
- Microbiome
- miRNA/Small RNAs

MicroRNA circolanti



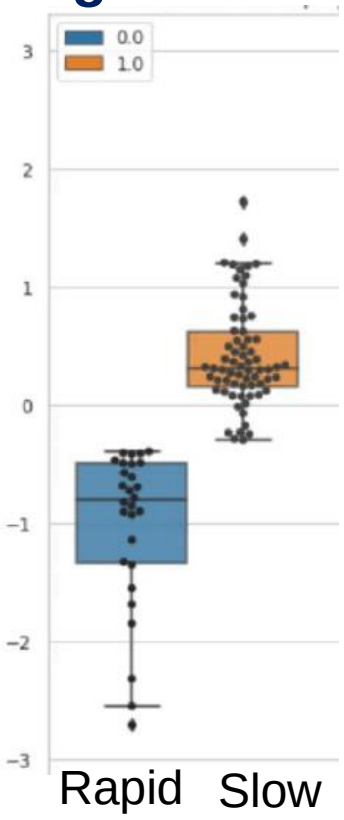
Turchinovich A. et al. 2012, Trends in Biochem Sci

Experimental Design for the analysis of miRNAs/small RNAs in T1D individuals

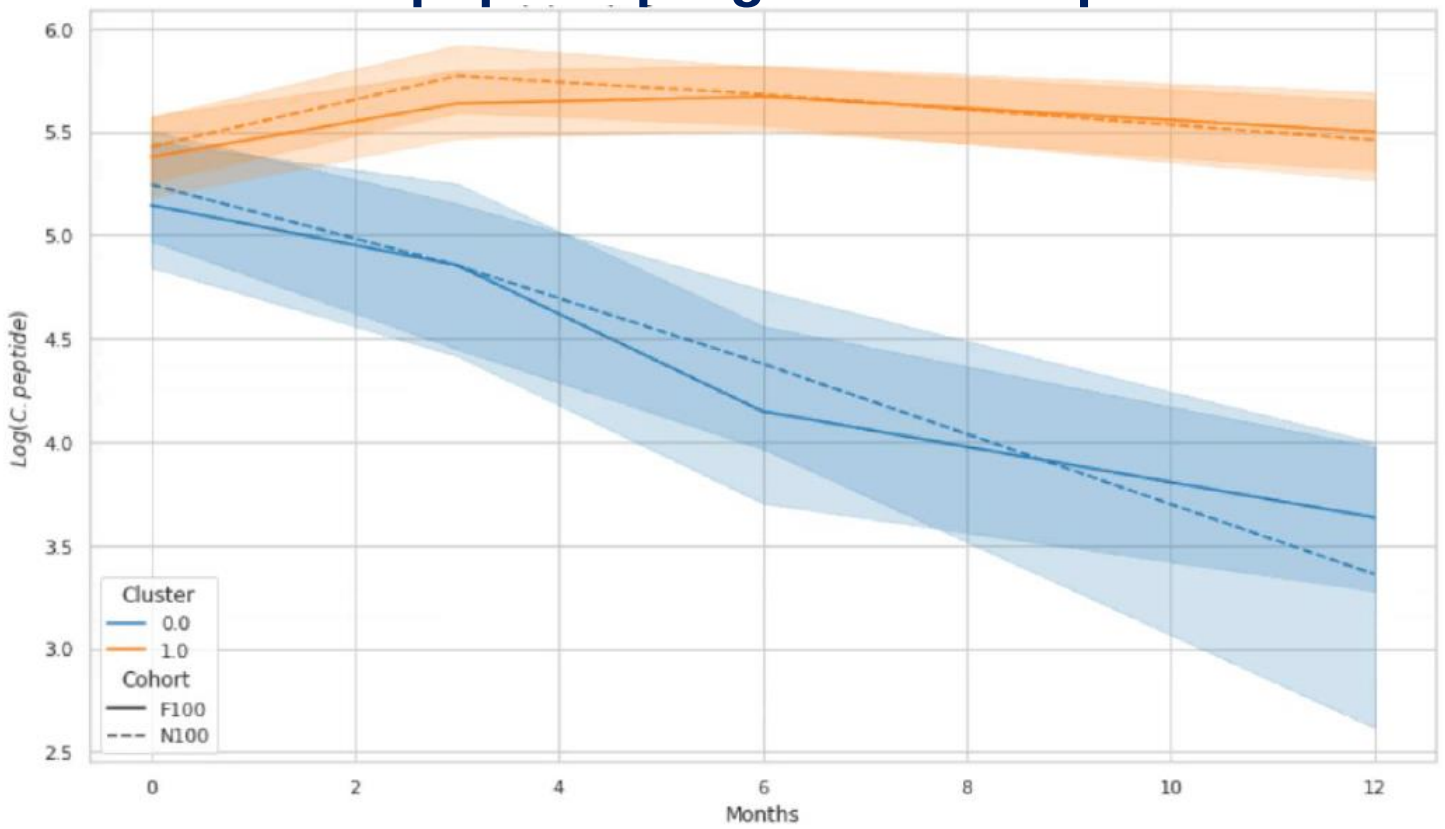


Identification of Rapid Progressors and Slow progressor among T1D individuals of INNODIA 100s cohort

Progression Clusters



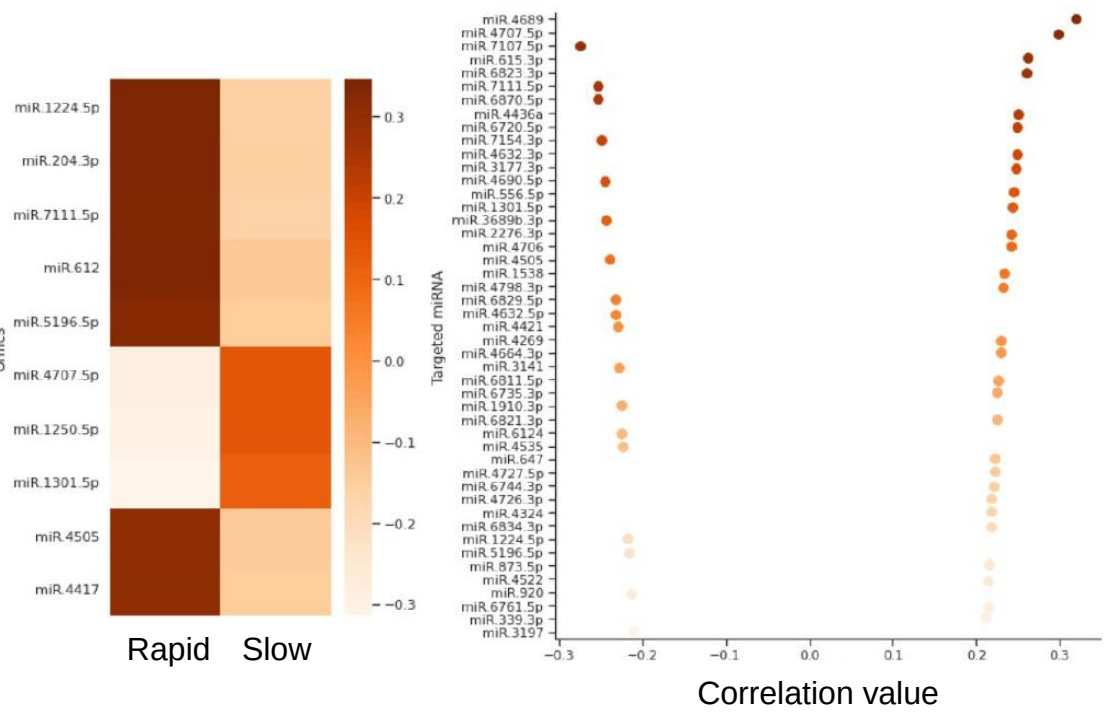
C-peptide progression slopes



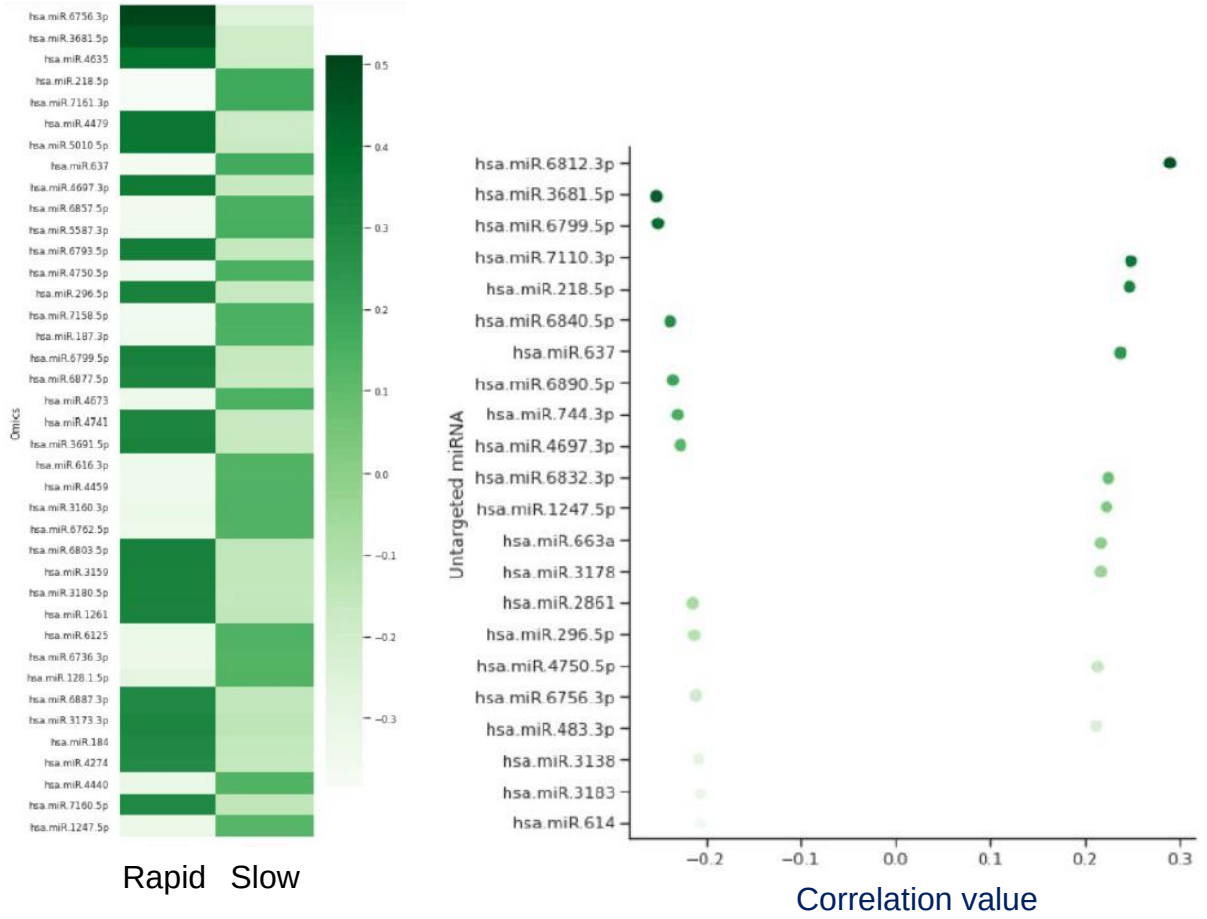
UCPH Team; Jose Juan Almagro Armenteros- Caroline Brorrson- Piotr Chmura- Soren Brunak

Most significant microRNAs correlated with progression clusters or slopes of T1D individuals

HTG-Edge Seq

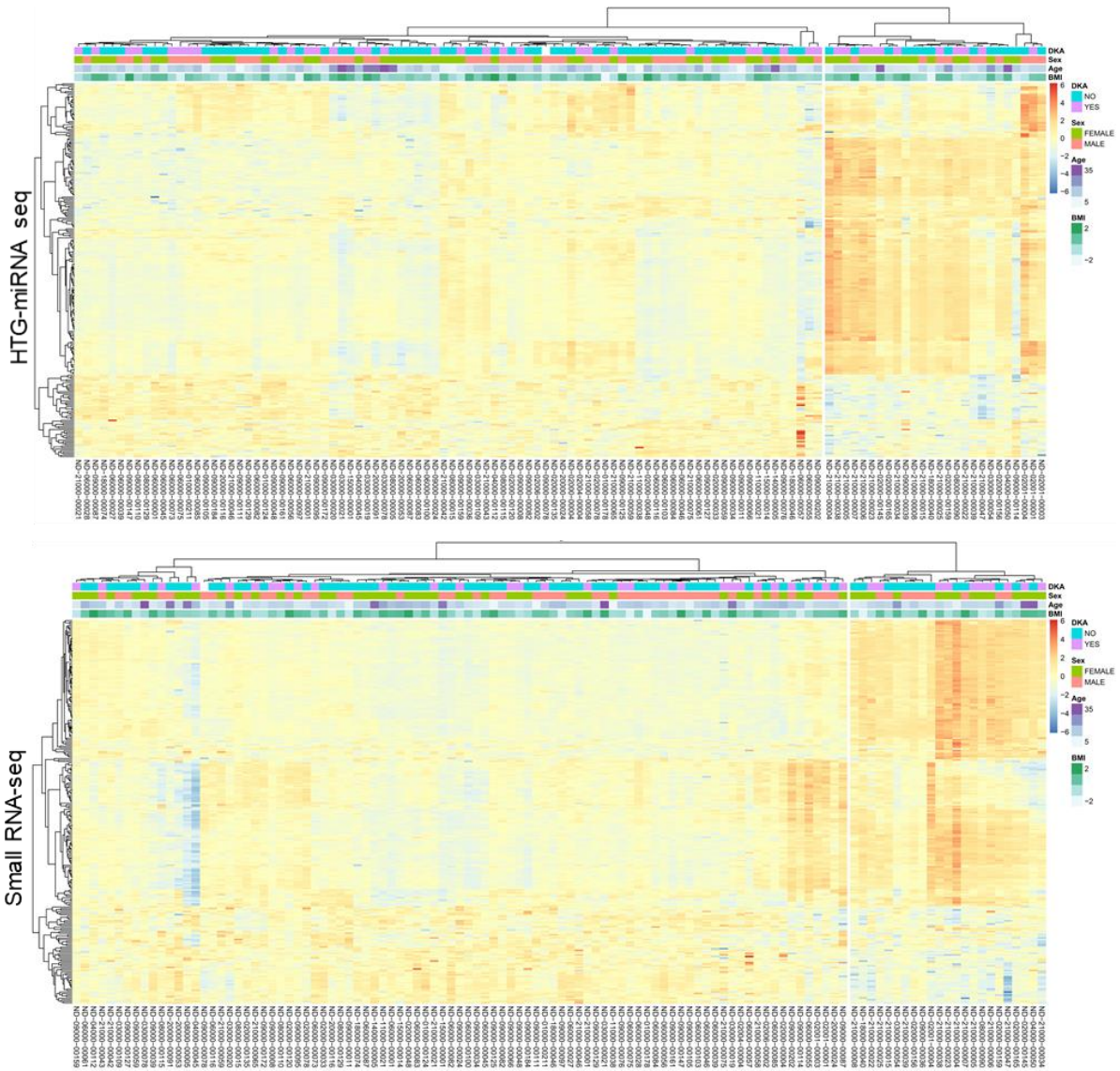


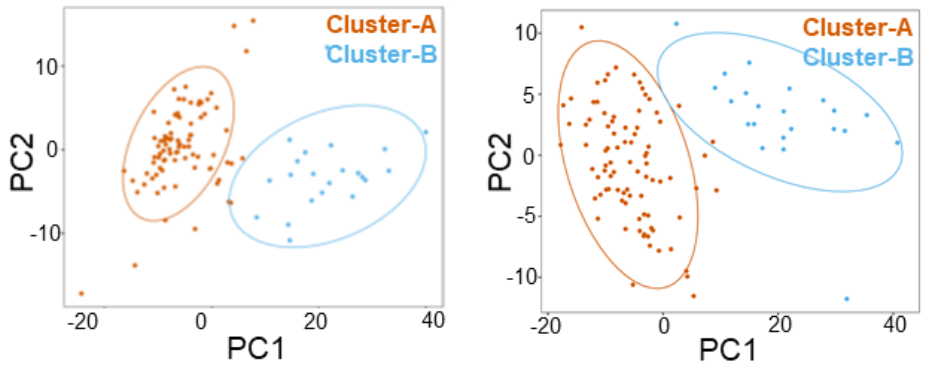
Small RNA-seq



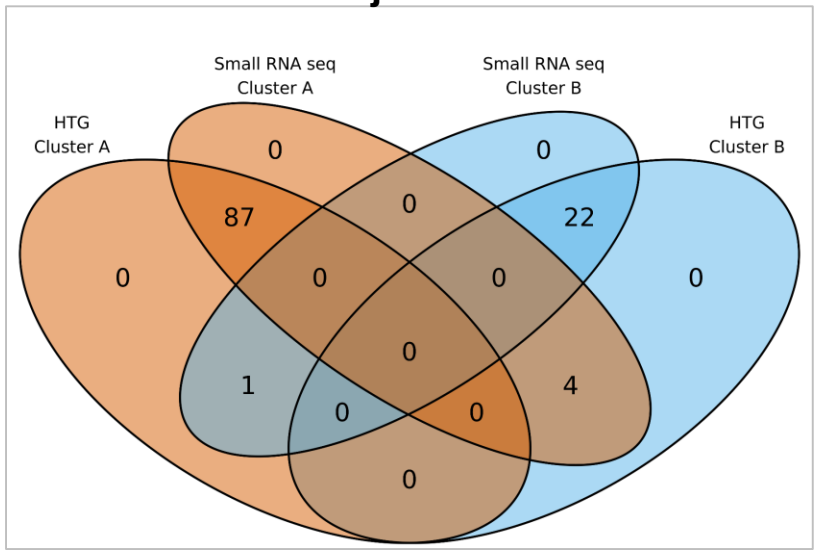
UCPH Team; Jose Juan Almagro Armenteros- Caroline Brorrson- Piotr Chmura- Soren Brunak

Two-seq platforms profiling reveals two distinct T1D subjects groups





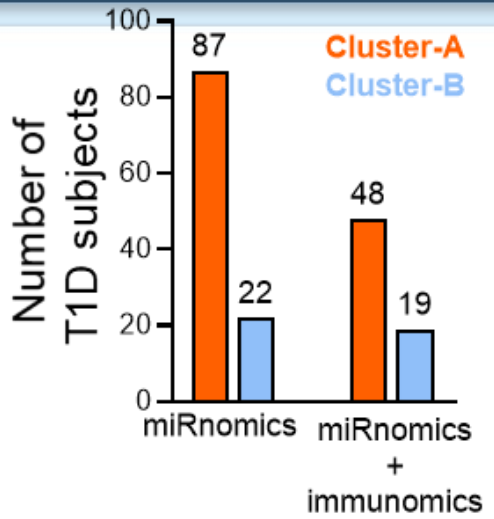
T1D subjects/Cluster

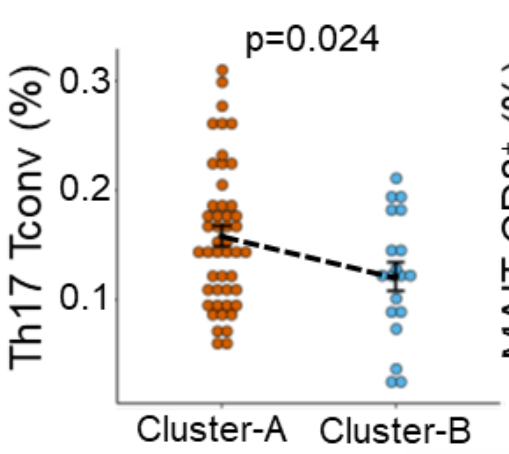


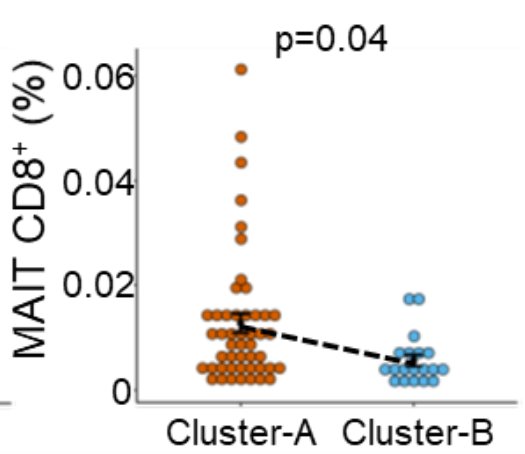
109/115 were consistently included in Cluster-A or Cluster-B in both platforms

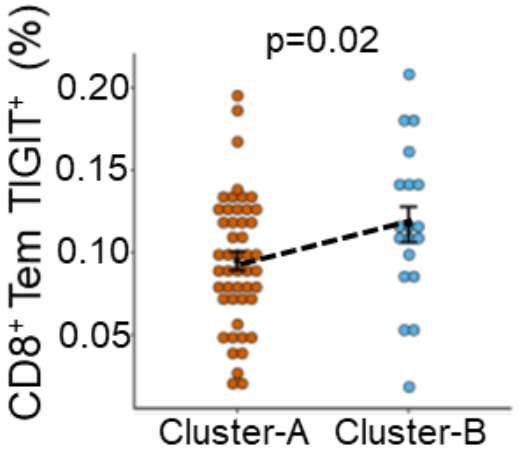
Can Immunomics tell us more about Cluster-A and Cluster-B T1D subjects?

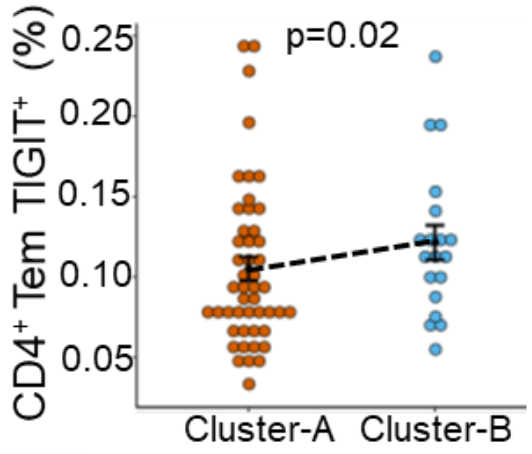
Tim Tree





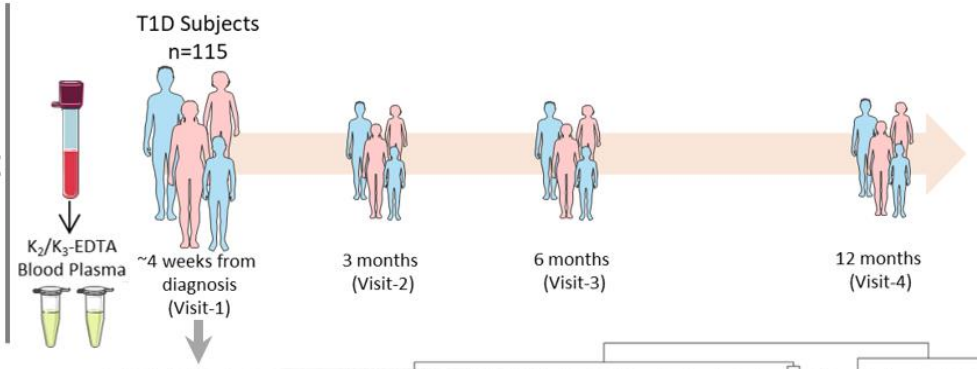




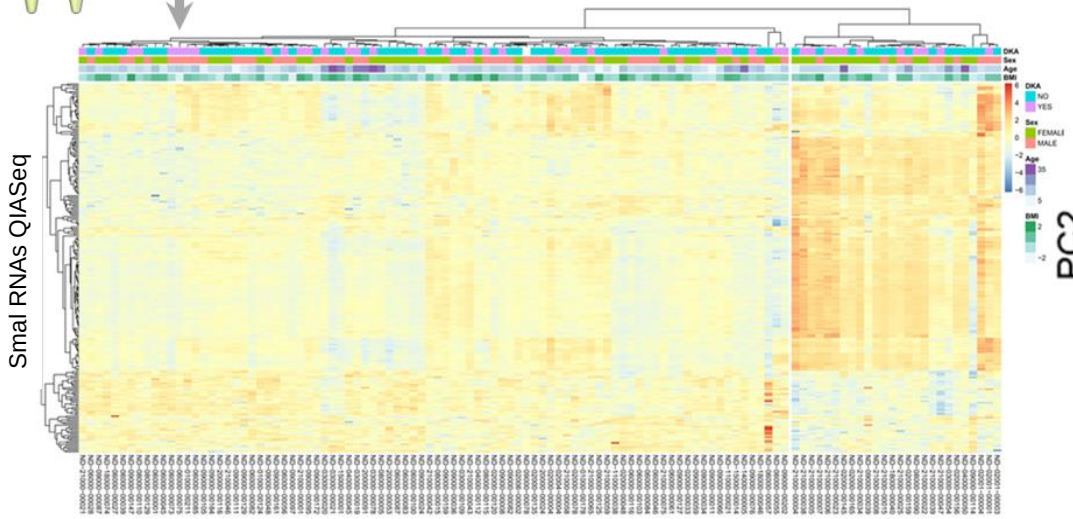


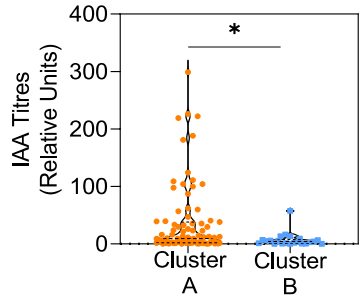
Circulating MicroRNAs signatures and T1D heterogeneity

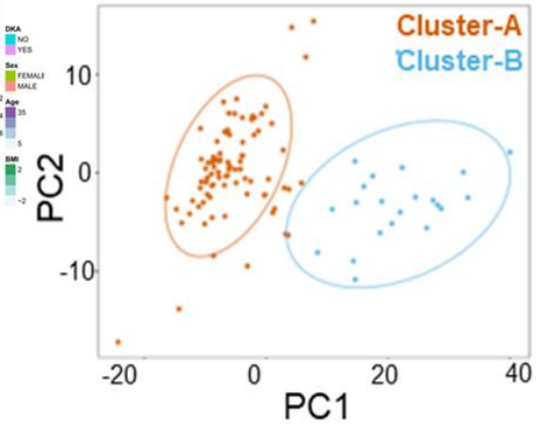
Patients recruitment and Follow-up



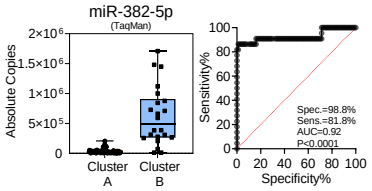
Circulating RNA analysis and patients stratification

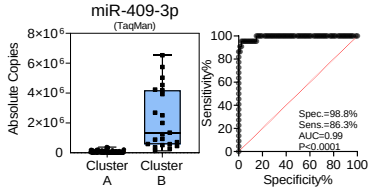


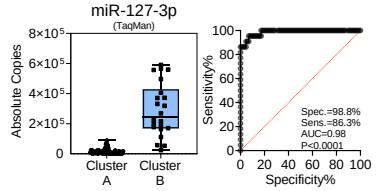




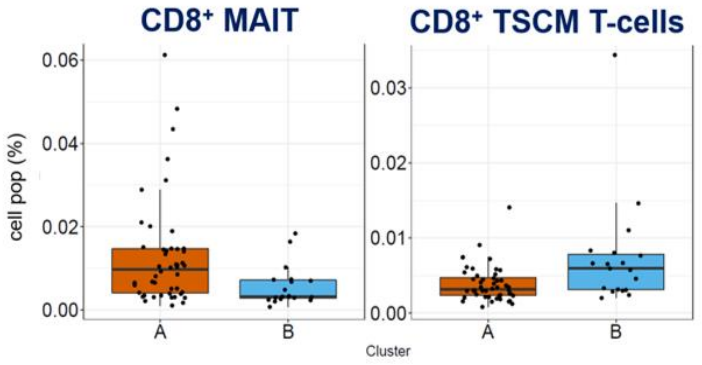
A set of microRNAs from chromosome 14q32 (miR-382, miR-409 and miR-127) distinguishes Cluster A and Cluster B: ddPCR validation



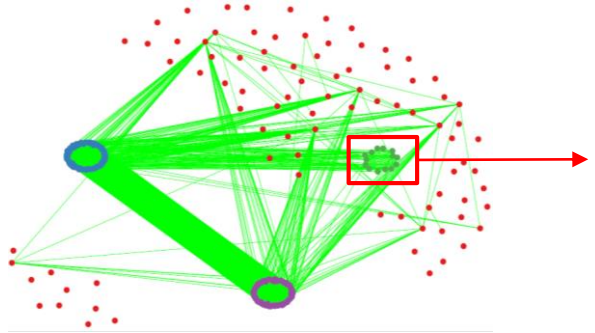




Clinical outcome prediction



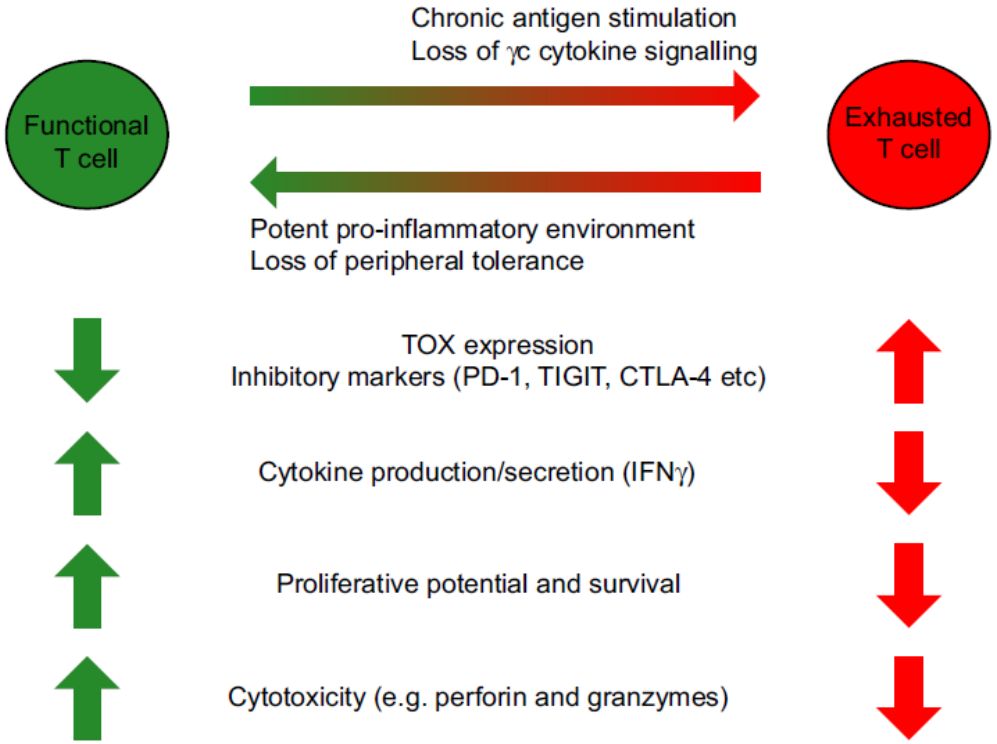
Cluster B is enriched of 14q32 miRNAs



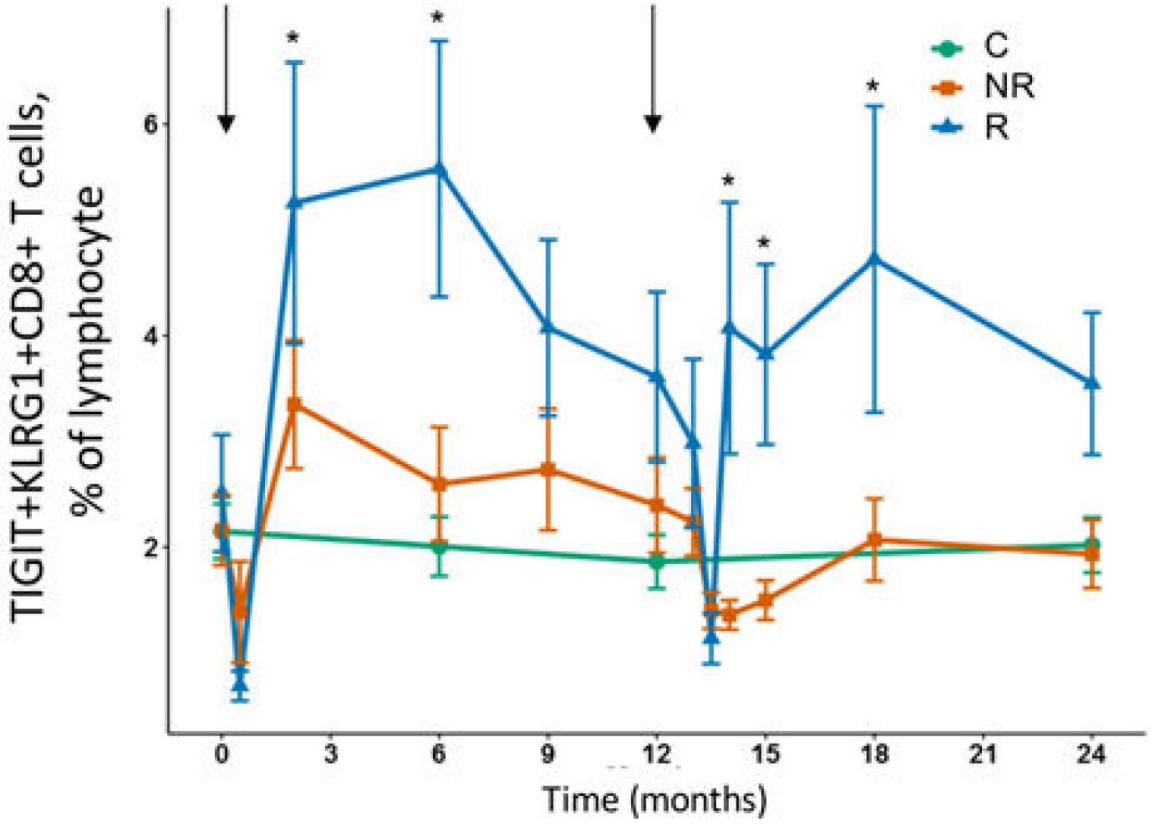
hsa-miR-127-3p
hsa-miR-134-5p
hsa-miR-323a-3p
hsa-miR-337-5p
hsa-miR-370-3p
hsa-miR-376c-3p
hsa-miR-381-3p
hsa-miR-382-5p
hsa-miR-409-3p
hsa-miR-431-5p
hsa-miR-485-3p
hsa-miR-494-3p
hsa-miR-589-5p
hsa-miR-654-3p

T-cell exhaustion in T1D

T-cell exhaustion is associated with reduced cytokines production

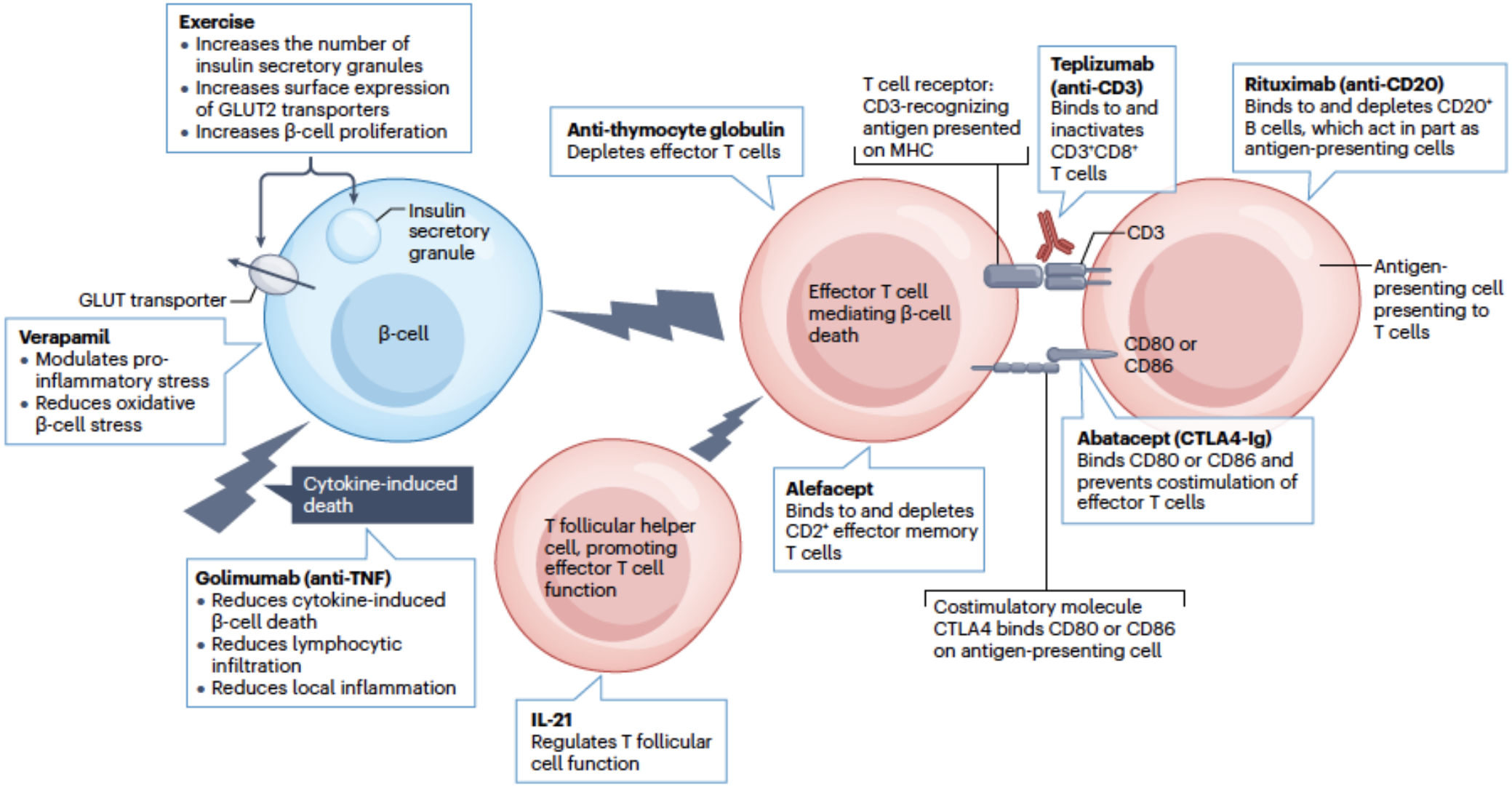


T1D subjects, **RESPONDERS** to aCD3 immunotherapy, showed higher levels of partially exhausted CD8 Tem KLRG1^{+} TIGIT^{+}



Long SA, et al .2016- *Sci. Immunol.*

Sites of action of therapeutic interventions for β-cell preservation.



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