

GISCoR

gruppo italiano screening colorettaile

Biomarcatori come test di screening primario e triage

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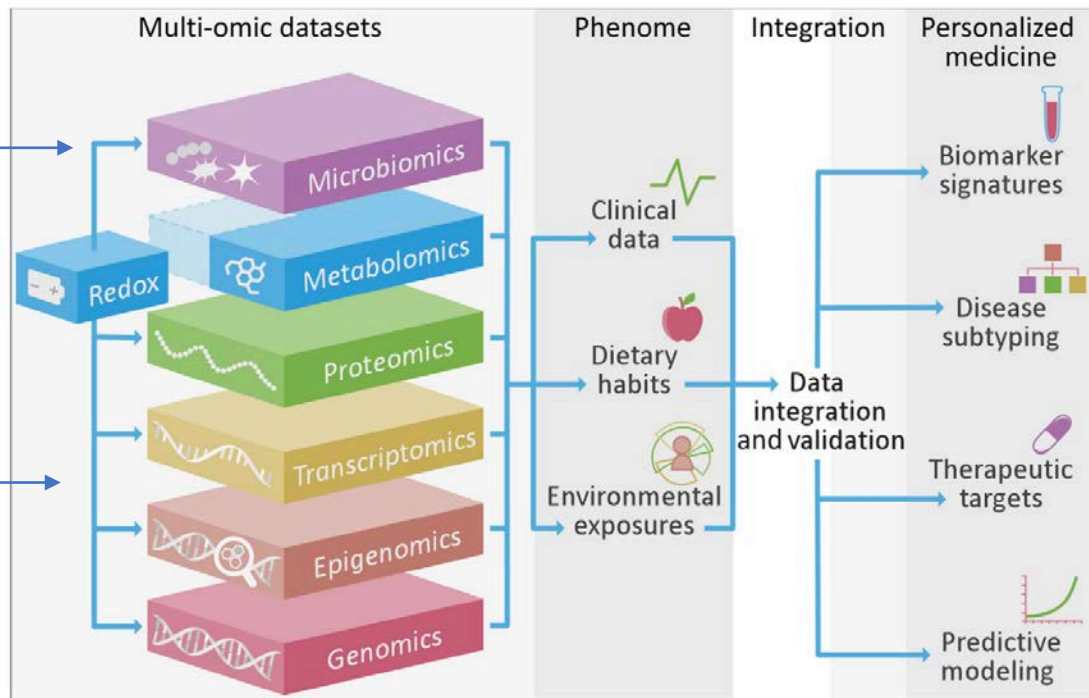


CONVEGNO
NAZIONALE
GISCoR 2023

Hotel Astoria Palace, Palermo



The spread of omic data – Applications



Some applications of integrative omics analysis:

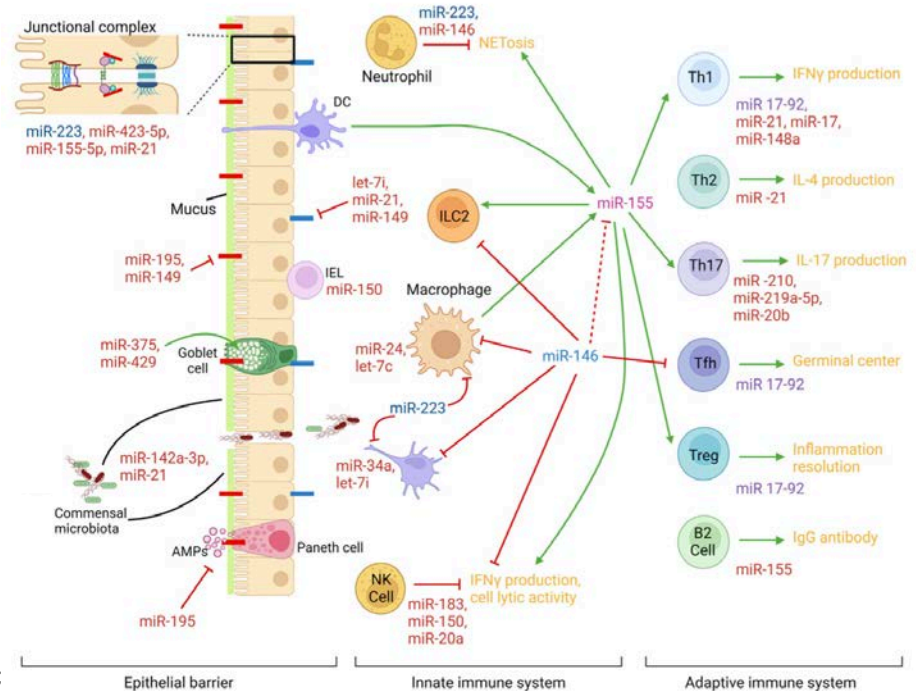
- Identification of **biomarker signatures** composed of heterogeneous entities
- **Stratification of patients** into specific subgroup or disease subtypes
- Identification of **novel therapeutic targets**
- Definition of **predictive models** for patient/disease classification
- Identification of **novel functional relationships**



microRNAs (miRNAs) - key regulators of the gut physiology

- miRNAs are key intra- and inter-cellular regulators of gene expression.
- miRNAs are released in the gut lumen and can be detected in fecal samples.
- Altered fecal miRNA levels may reflect:

miRNAs implicated in the regulation of gut homeostasis in mice and/or humans

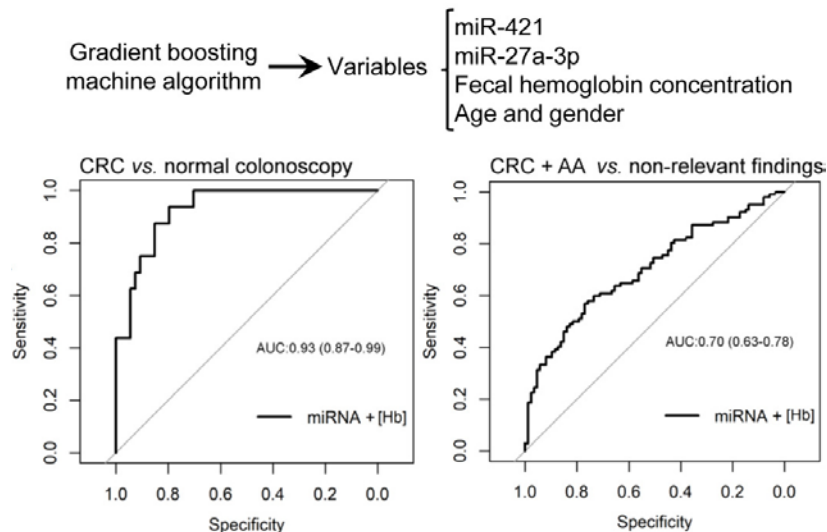




Fecal miRNA signatures for a non-invasive cancer detection

- Fecal tests (e.g., Fecal Immunichemical tests, FIT) currently used for colorectal cancer (CRC) screening show limited accuracy in detecting early tumors or precancerous lesions.
- Fecal miRNAs profiling may improve the CRC detection of current screening methods.
- A large-scale fecal miRNA analysis by small RNA-Seq is still needed for:
 - Unbiased identification of a miRNA signature
 - Signature evaluation on different populations
 - Comparison with different disease conditions

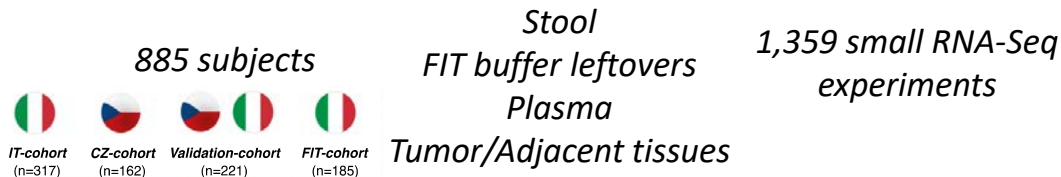
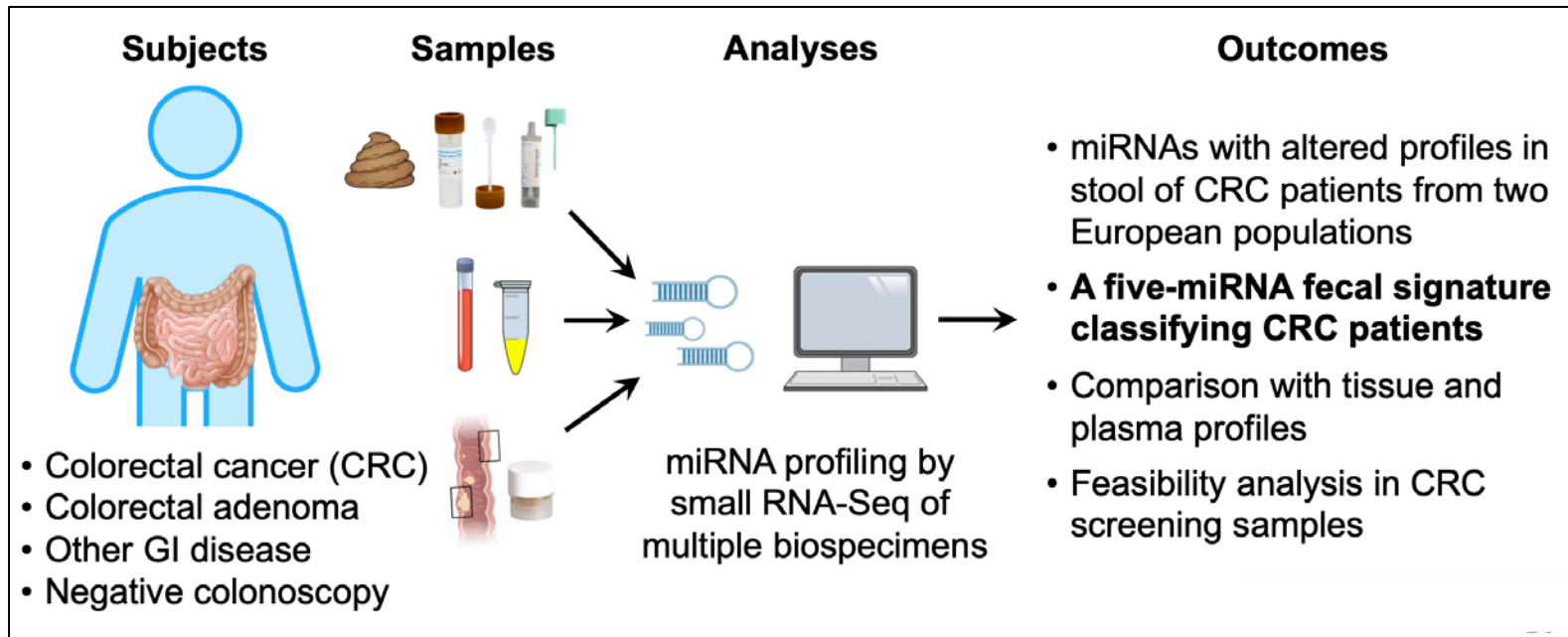
Fecal miRNA-based predictive model



(Duran-Sanchon et al., 2021, *Gastroenterology*)



Our experience – Summary of the study





Altered fecal miRNA levels overlapping in different populations

Fecal miRNome profiling and biomarker discovery

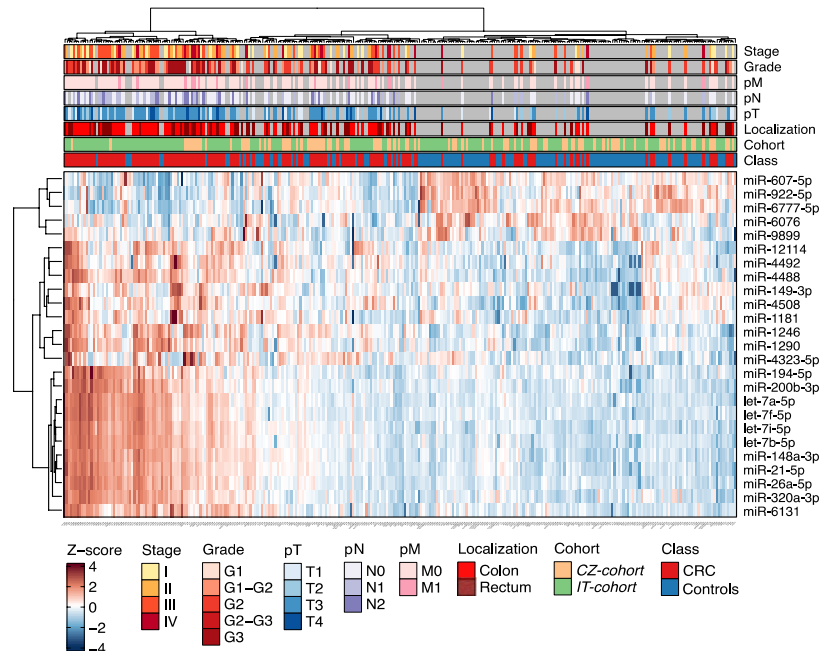
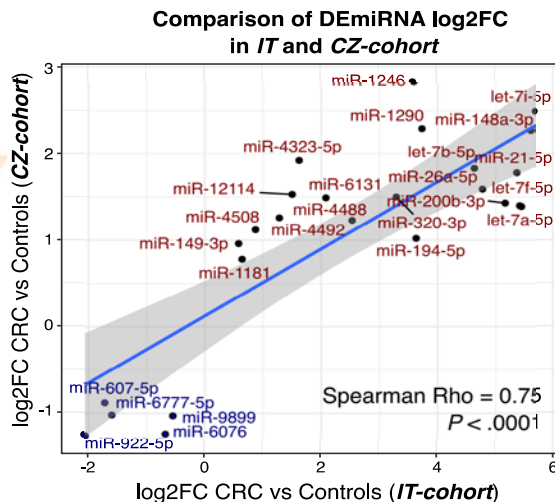
miRNome characterization

IT-cohort (89, CRC, 105 controls)
CZ-cohort (66 CRC, 36 controls)

Differential expression analysis



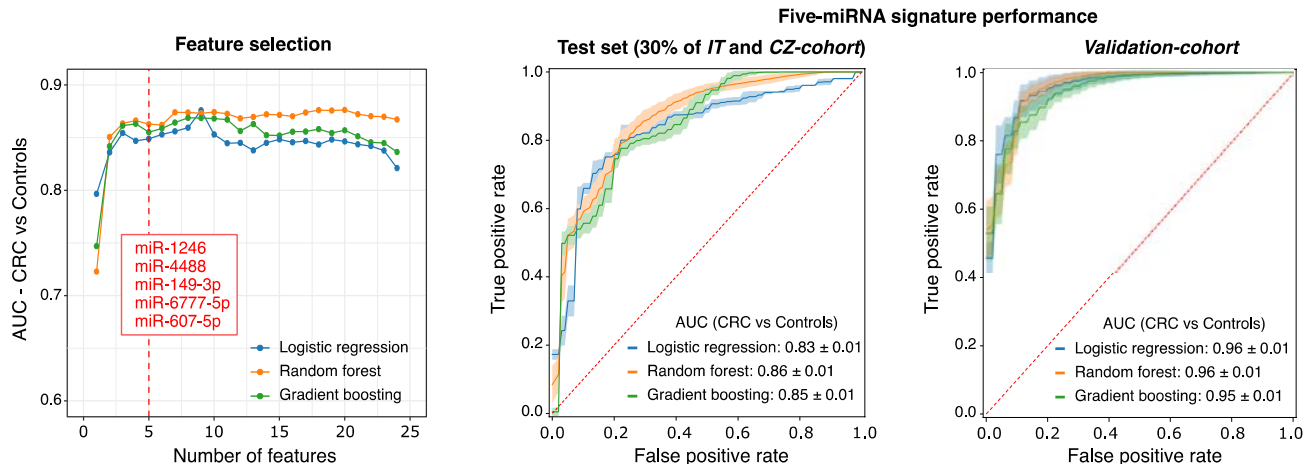
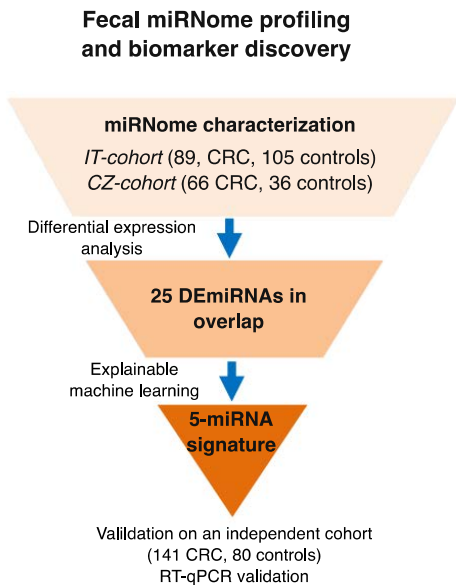
25 DE miRNAs in overlap



- **25** differentially expressed (DE) miRNA in CRC patients of both cohorts by sex- and age-adjusted differential expression analyses (adj. p < 0.05).
- Confirmed as coherently DE also after CRC patient stratification by tumor localization.
- Confirmed as CRC-associated by age, sex, BMI, smoking, and cohort-adjusted generalized linear model regression analysis.



A five miRNA signature accurately classifies CRC patient from controls



- A signature of **five** fecal miRNAs classifying CRC from controls was identified by stratified machine learning analysis tested on 30% of IT and CZ cohort set (AUC=0.86, 95% CI=0.79-0.94).
- The signature was validated in an independent cohort of CRC patients and controls (AUC=0.96, 95% CI=0.92-1.00)



miRNA signature validation with another technique

Fecal miRNome profiling and biomarker discovery

miRNome characterization

IT-cohort (89, CRC, 105 controls)
CZ-cohort (66 CRC, 36 controls)

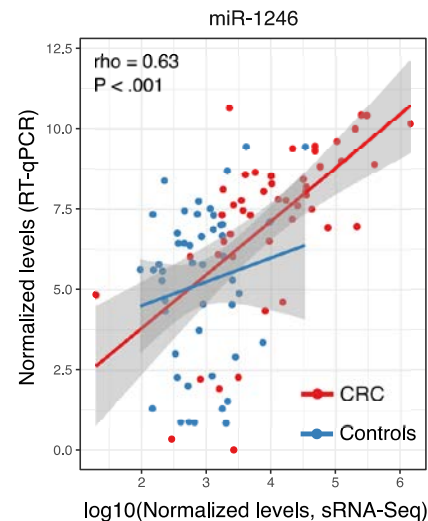
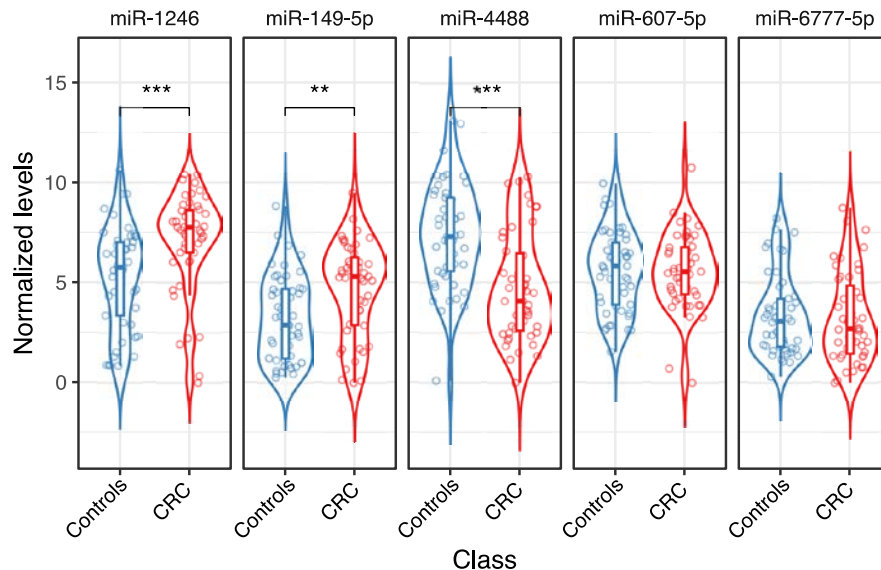
Differential expression analysis

25 DE miRNAs in overlap

Explainable machine learning

5-miRNA signature

Validation on an independent cohort
(141 CRC, 80 controls)
RT-qPCR validation

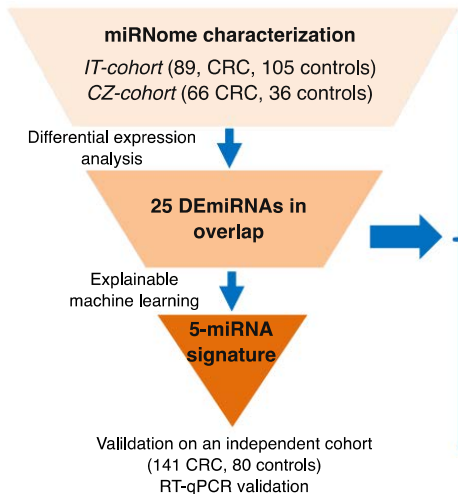


- The expression of the five miRNAs was validated by RT-qPCR on 96 samples from IT- and CZ-cohorts (** $p < 0.01$; *** $p < 0.001$).



Fecal miRNA dysregulation partially reflects tissue altered expression

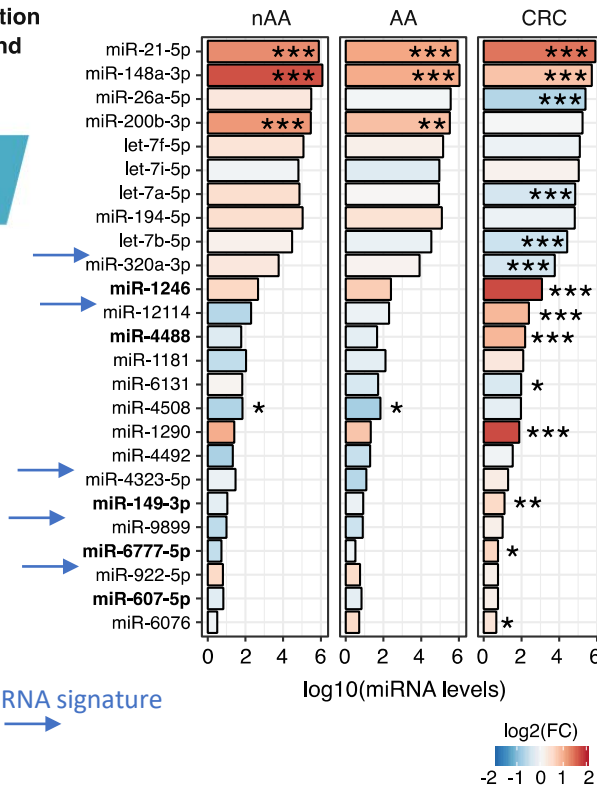
Fecal miRNome profiling and biomarker discovery



Fecal DE miRNA characterization in different sample types and diseases

Colonic tissue (n=264)
Tumor vs. adjacent mucosa
Adenoma vs. adjacent mucosa

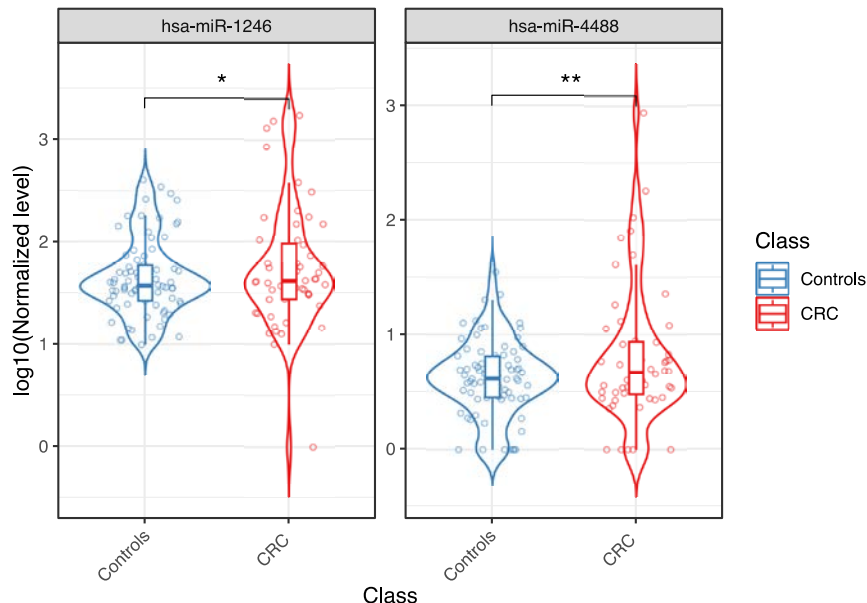
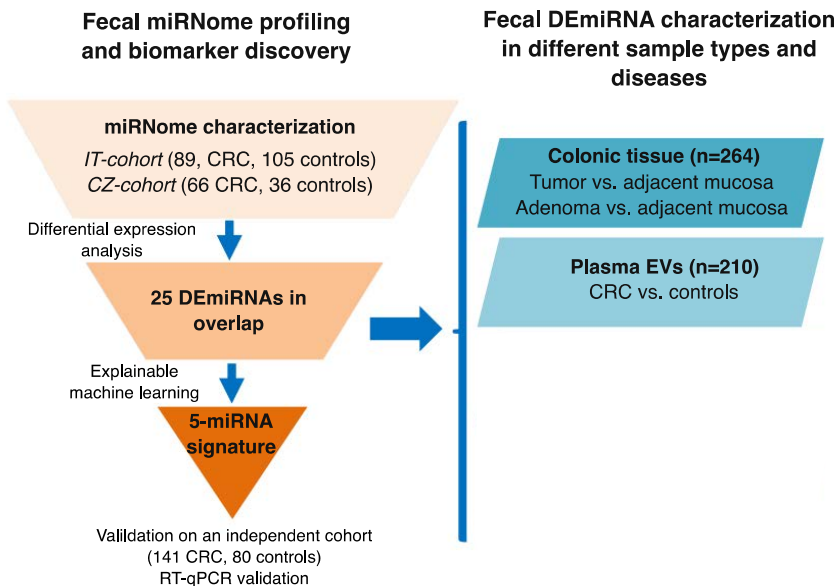
DE miRNA levels in tumor/adenoma and comparison with adjacent mucosa



- 14 out of the 25 fecal miRNAs were DE (adj. $p < 0.05$) in a paired DE analysis between CRC and adjacent mucosa tissues (**adj. $p < 0.001$; *adj. $p < 0.01$; *adj. $p < 0.05$).



miR-1246 and miR-4488 levels are altered in plasma EVs of CRC patients



- Only three miRNAs were DE in plasma EVs: two belong to our signature (**adj. $p < 0.01$; *adj. $p < 0.05$).

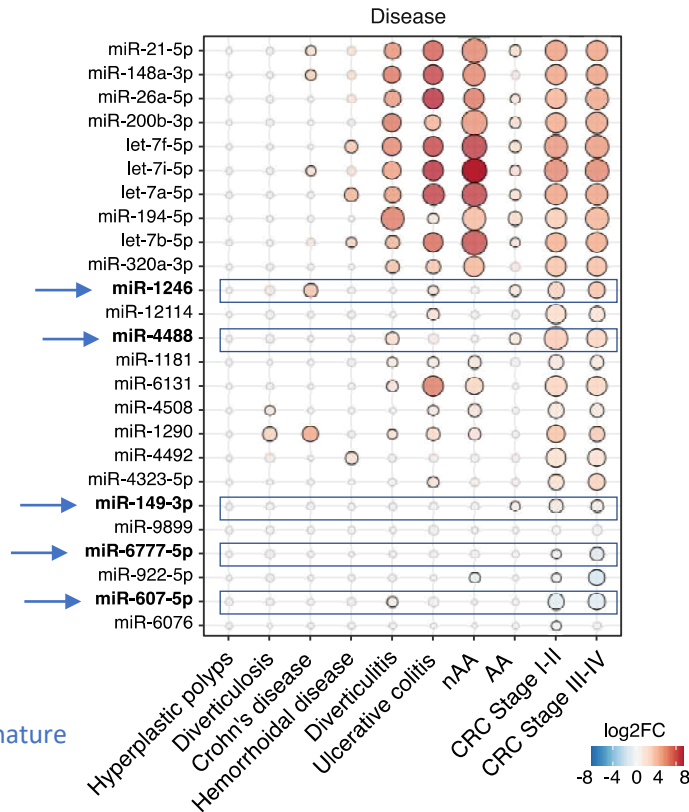
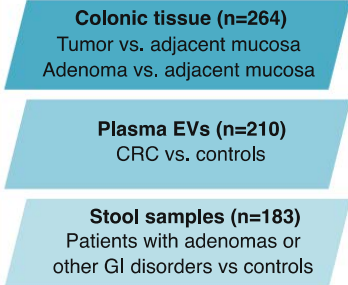
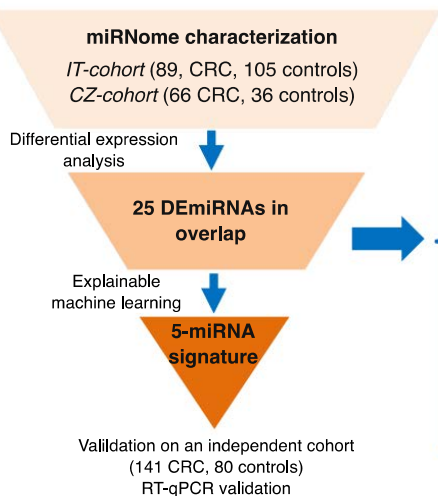


Some fecal miRNAs are also dysregulated in gastrointestinal diseases

DEmiRNA levels in stool of distinct GI disease
with respect to control subjects

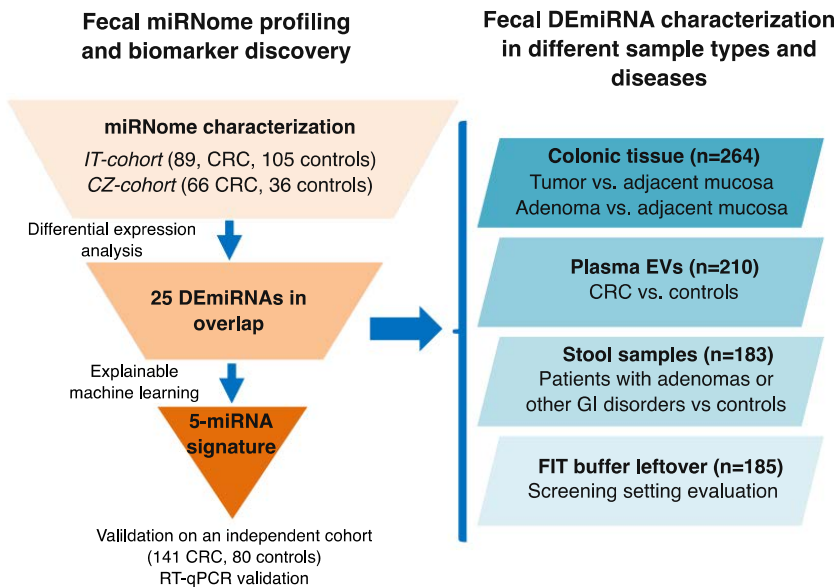
Fecal miRNome profiling and biomarker discovery

Fecal DEmiRNA characterization in different sample types and diseases





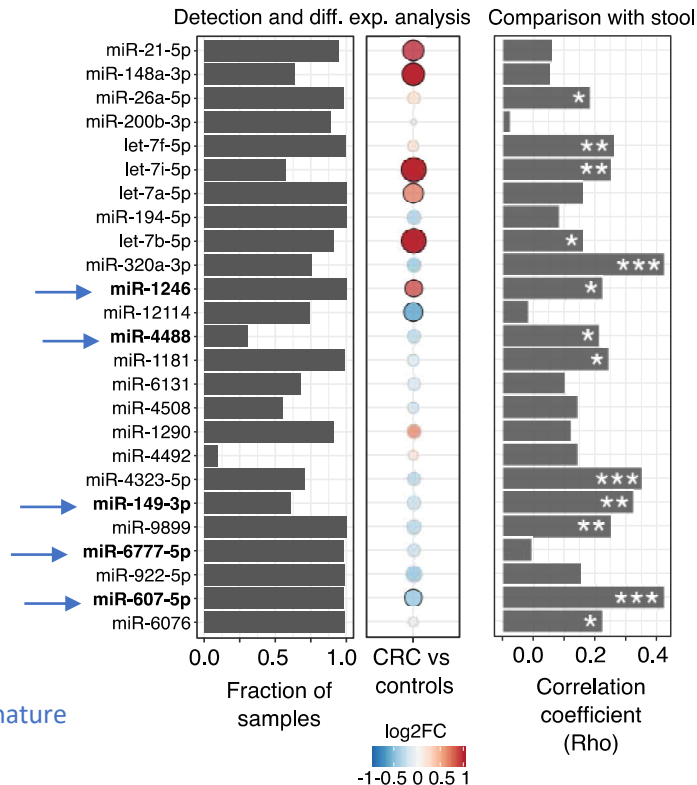
Fecal miRNA dysregulation can be observed in CRC screening samples



5-miRNA signature



DE miRNA levels in FIT positive buffer leftover samples

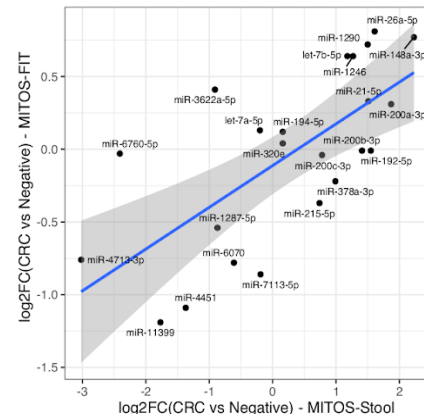
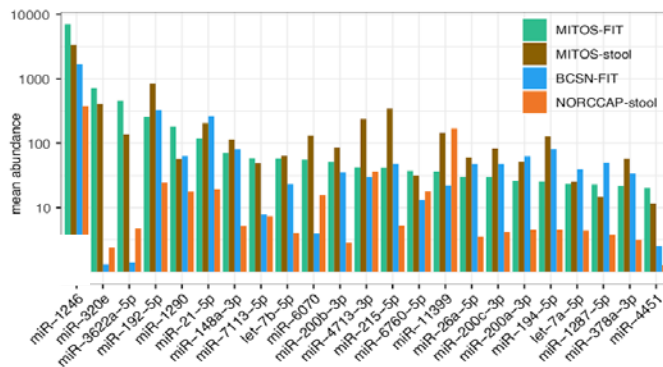




CRC miRNA biomarkers in FIT leftover samples: additional study

Implementation of miRNA profiling in FIT leftover samples, in collaboration with Prof Rounge (University of Oslo)

Feasibility assessment



**Comparison miRNA profiles in FIT
leftover/stool from the same subjects**



Improved meta-analysis of gut microbiome in CRC

nature medicine **ARTICLES**
<https://doi.org/10.1038/s41591-019-0405-7>

Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation

Andrew Maltez Thomas^{1,2,3,32}, Paolo Manghi^{1,32}, Francesco Asnicar¹, Edoardo Pasolli¹, Federica Armanini¹, Moreno Zolfo¹, Francesco Beghini¹, Serena Manara¹, Nicolai Karcher¹, João Carlos Setubal^{2,26}, Adrian Tett¹, Barbara Pardini^{5,27}, Maria Rescigno²⁸, Levi Waldron^{29,30,33}, Alessio Naccarati^{5,31,33} and Nicola Segata^{1,33*}



CRC gut microbiome samples			ONCOBIOME		US Cohort	
4 ONCOBIOME	16 studies	3,512 samples	4 Cohorts	494 CRC	Controls	448
1 US Cohort	11 countries		60 Adenomas	435	Adenomas	14
11 Public			95 Controls		CRC	

Public studies

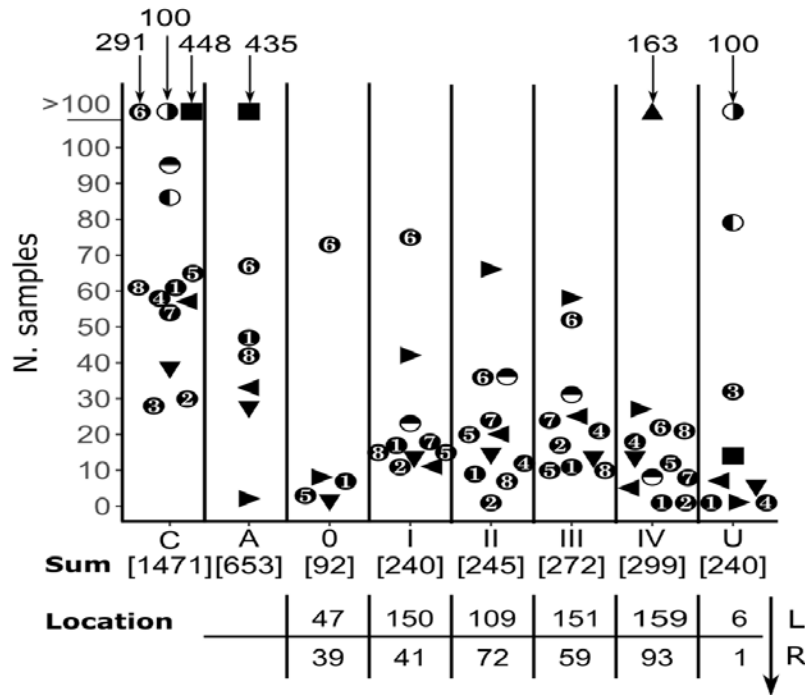
- ① Feng Q. (2015) ③ Thomas A.M. (2018) ⑤ Wirbel J. (2018) ⑦ Yu J. (2015)
 ② Gupta A. (2019) ④ Vogtmann E. (2016) ⑥ Yachida S. (2019) ⑧ Zeller G. (2014)

In previous meta-analyses

This study

- Cohort 1 ▼ Cohort 2
 ◄ Cohort 3 ▲ Cohort 4
 ■ Cohort 5

- ⑨ LiuNN_2022
 ⑩ YangJ_2020
 ⑪ YangY_2021

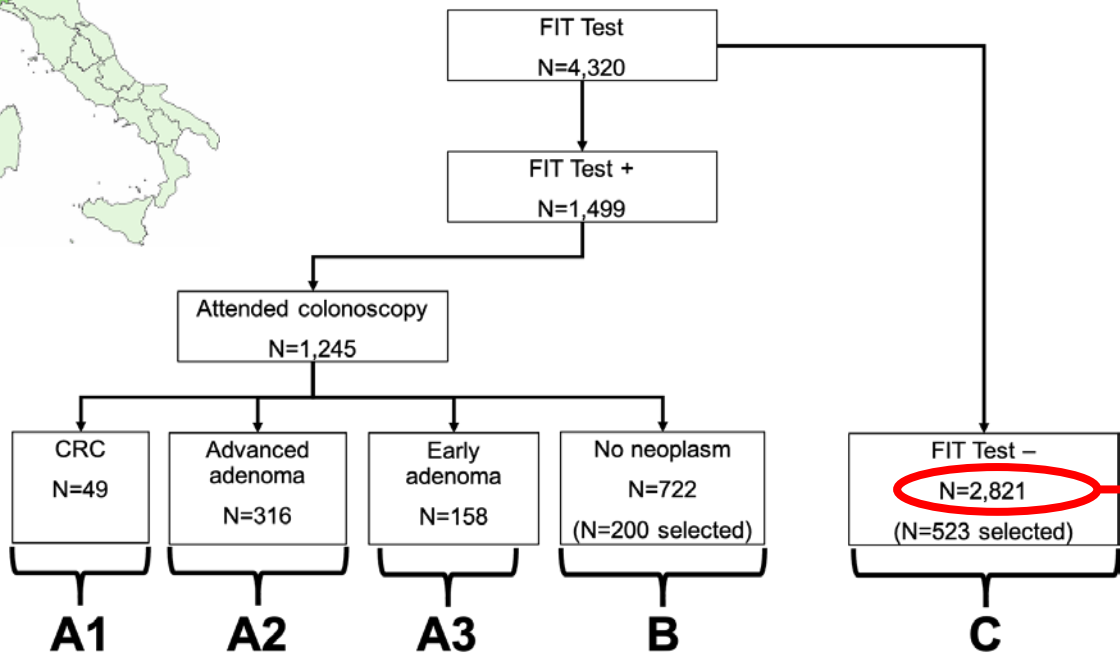


5 new cohorts
> 500 new CRC cases



CRC biomarkers in FIT leftover: gut microbiome analyses

Case-control study of FIT samples from the Turin-Piedmont CRC screening study



Total number of samples - 1246
Quality control samples - 66

We are collecting FIT Test negative
at second round (2-3 years later)
So far collected N>1000

40% of the FIT negative and 70%
of the FIT positive subjects have
provided dietary and lifestyle
questionnaires

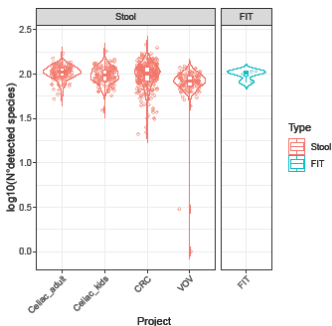
Selection based on matching by age, sex, season of collection in collab. with NCI (Dr Sinha's group)



Analyses in FIT leftover samples: from the biobank creation to omics analyses

Sample collection and DNA extraction

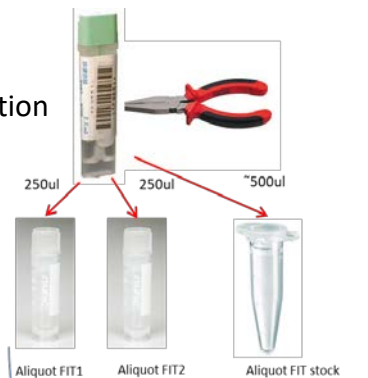
Analysis of FIT tubes in the screening



Shotgun metagenomics

FIT leftovers

Sample collection



biobanking

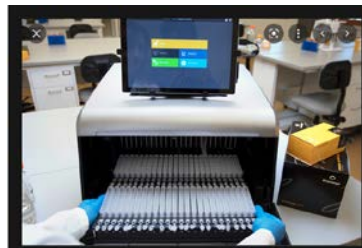
Aliquots stored at -80C until use

DNA concentration, according to MGI guidelines



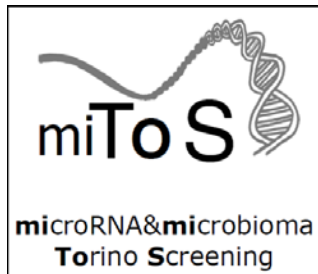
QC checking

Sample purity
 $OD_{260/280}=1.8-2.0$
 $OD_{260/230}>2.0$



DNA extraction

Fecal Microbiome DNA kit on Promega Maxwell RSC 48 Instrument (FIT samples, QCs and blanks)



Ongoing prospective study



AIRC IG 2019

“Combining faecal biomarkers to improve prediction of individual’s risk of pre-invasive and invasive colorectal lesions”

PI Dr Carlo Senore (Cancer Prevention Center of Piedmont Region)

IIGM Partner collaborator

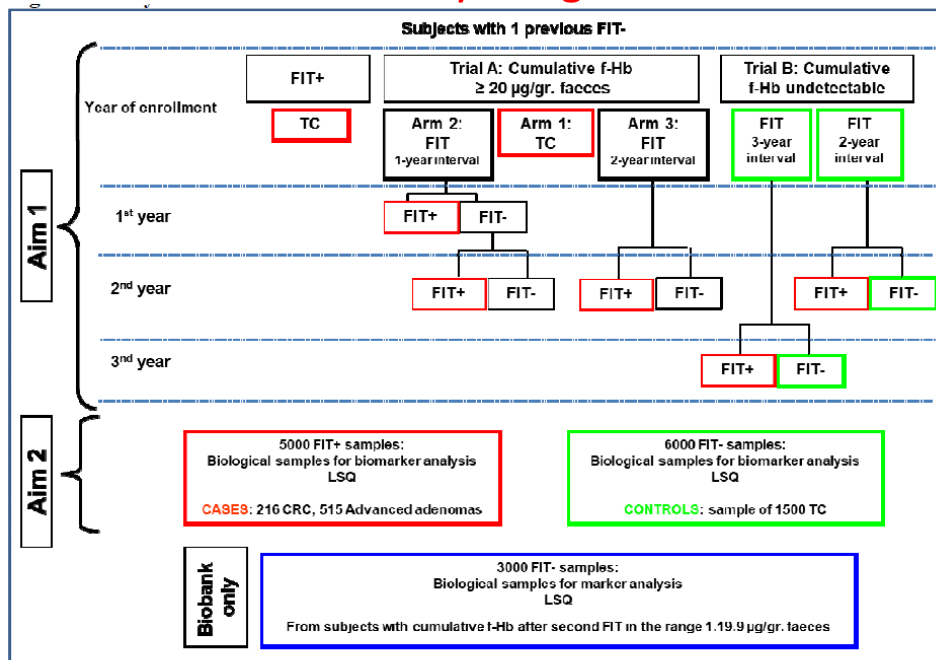
A new project supported by AIRC and within the miToS study has just started on January 2020

Study Hypothesis:

A tailored approach, using refined combinations of biomarkers, could allow a more effective use of current resources, offering more intensive screening to those subjects most likely to benefit, and less intensive screening to those at lower risk



Study design



TC: total colonoscopy; HB: haemoglobin; LSQ: Life-styles questionnaire

AIMS:

- To assess the potential impact of screening protocol tailored to the subsequent advanced neoplasia risk by class of cumulative f-HB level in a large cohort of screenees.
- To investigate whether altered expression of selected stool miRNA signature or gut microbiome profiles previously found associated with CRC risk are significantly more frequent in samples of patients with CRC or advanced adenoma, compared to matched healthy controls and if they satisfy pre-specified true- and false positive rates that are considered minimally acceptable in the screening setting.



Conclusions:

- Fecal miRNA profiles are extensively altered in CRC patients with respect to healthy controls
- A coherent fecal miRNA dysregulation characterises three independent cohorts of individuals
- A signature of five miRNAs is able to accurately discriminate CRC patients from healthy controls
- Fecal miRNAs are detectable **in CRC screening samples** and show levels coherent with those measured in stool samples from the same subjects

Ongoing analyses :

- Investigation of miRNA-mediated host-microbial interactions
- Studies on fecal miRNA profiles in longitudinal samples from CRC patients
- Analysis in other disease contexts (e.g., Familial Adenomatosis Polyposis, Lynch Syndrome, Obesity)
- Microbiome analyses in FIT
- Large scale investigations on miRNA profiles in cancer and precancer cases in FIT leftover samples
 - New more practical methodologies are needed (biosensors?)



Grazie per l'attenzione!



Centro di Riferimento per l'Epidemiologia
e la Prevenzione Oncologica in Piemonte



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Torino Screening**



Microbiota against cancer
International research program



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