

The promise of dPCR to measure circulating microRNAs as predictors of response to therapy in melanoma

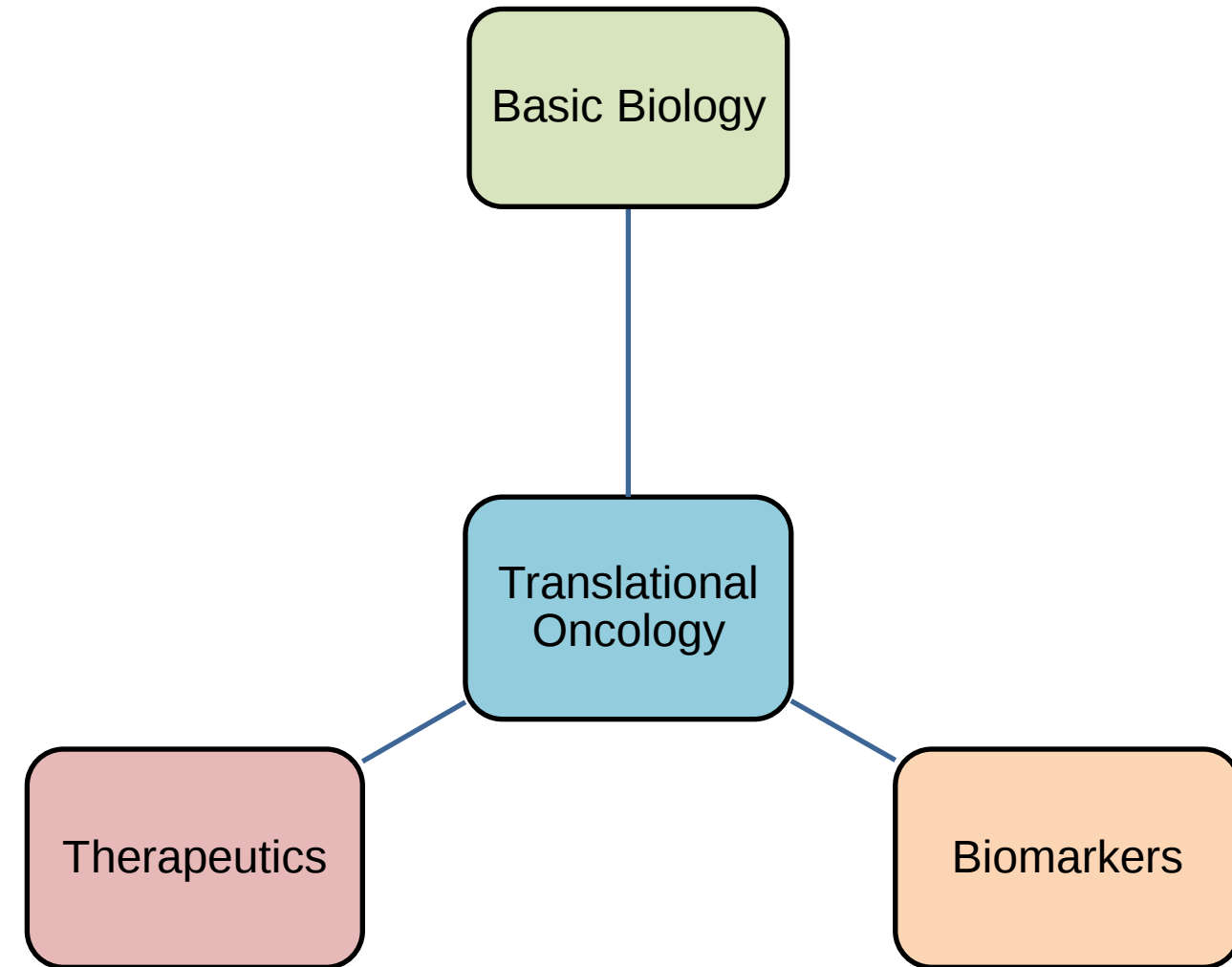
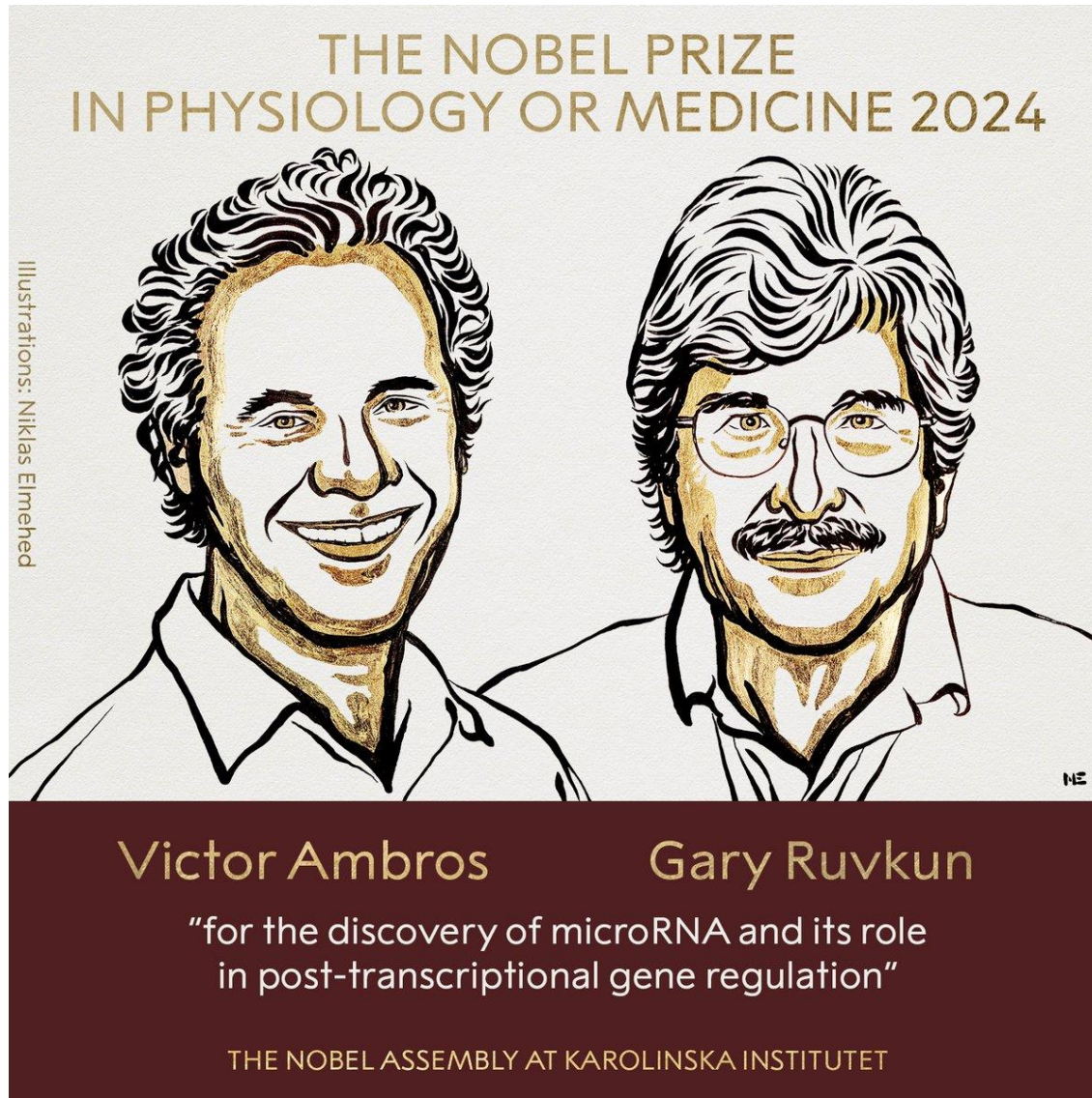
Luigi Fattore, PhD

26/11/2024

IRCCS Regina Elena National Cancer Institute

General assembly of the ELBS 26th – 27th of November 2024 Barcellona, Spain

microRNAs: back to the future



microRNAs could be ideal non invasive biomarkers in liquid biopsy

Available kits for miRNA diagnostics



- qPCR-based
- Liver function, bone quality, platelet function
- Blood

hepatomiR®
microRNA Biomarkers of Liver Function and Disease

osteomiR®
microRNA Biomarkers of Bone Quality

thrombo miR®
microRNA Biomarkers of Platelet Function

<https://www.tamirna.com/>

- qPCR-based
- Gastric cancer
- blood

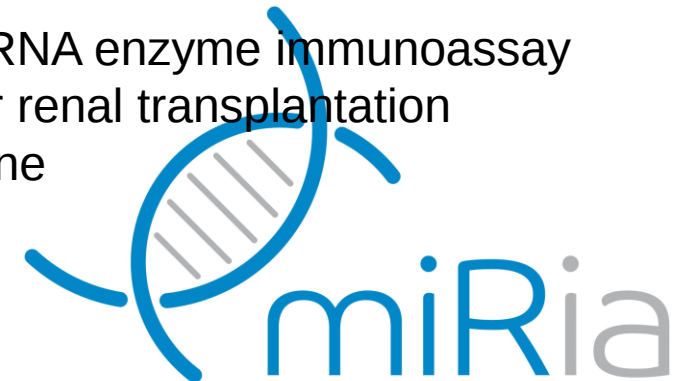


<https://gastroclear.mirxes.com/>



- qPCR-based
- Detection of neurodegenerative diseases
- Blood

- miRNA enzyme immunoassay
- For renal transplantation
- Urine



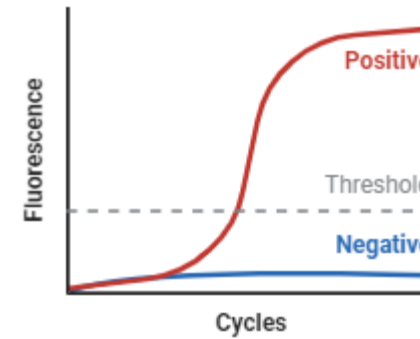
<https://www.biovendor.com/mirna>

Limitations in the use of circulating microRNAs

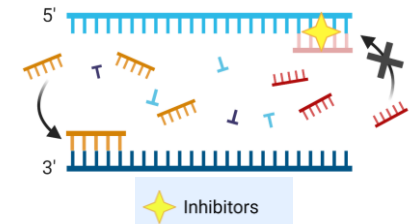
- low abundance in body fluids



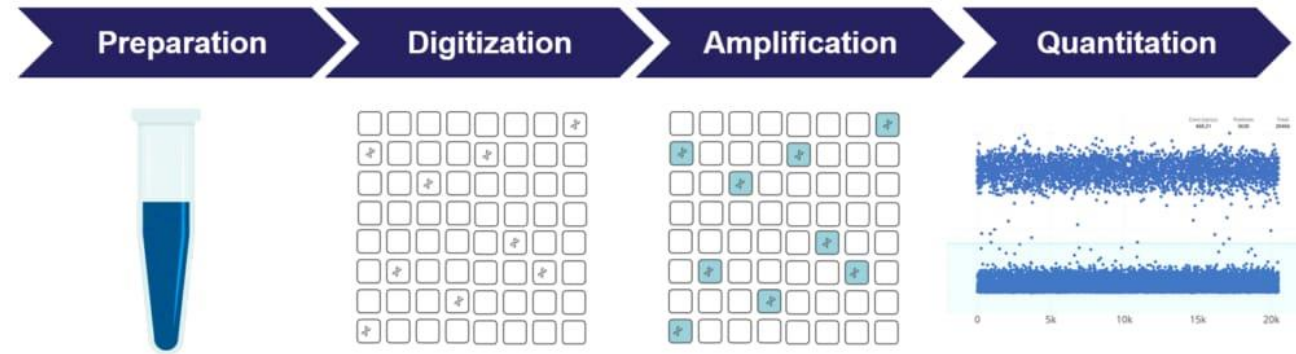
- difficulty in normalizing the results



- high levels of enzyme inhibitors



The promise of digital PCR (dPCR) to evaluate circulating miRNAs



Precision

High Levels of Accuracy and Reproducibility

Absolute Quantification

No standard curves required

Multiplexing

Simple strategies to maximize sample utilization

Sensitivity

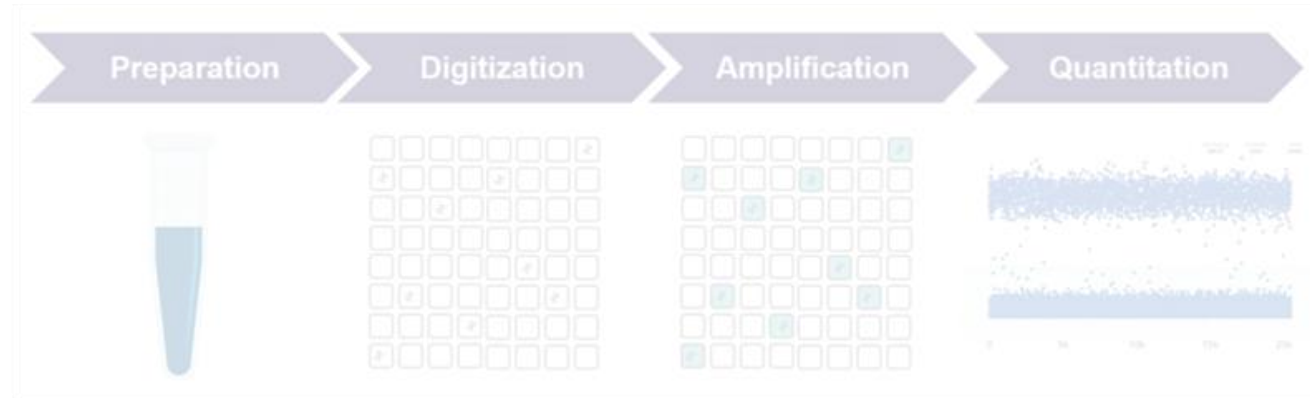
Detection of rare targets in complex backgrounds

Tolerant of PCR Inhibitors

Cycle to end point, partitioning

<https://www.thermofisher.com/it/en/home.html>

The promise of digital PCR (dPCR) to evaluate circulating miRNAs



Precision

High Levels of Accuracy and Reproducibility

However, there are no commercially available assays for multiplex dPCR

Absolute Quantification

No standard curves required

Multiplexing

Simple strategies to maximize sample utilization

Sensitivity

Detection of rare targets in complex backgrounds

Tolerant of PCR Inhibitors

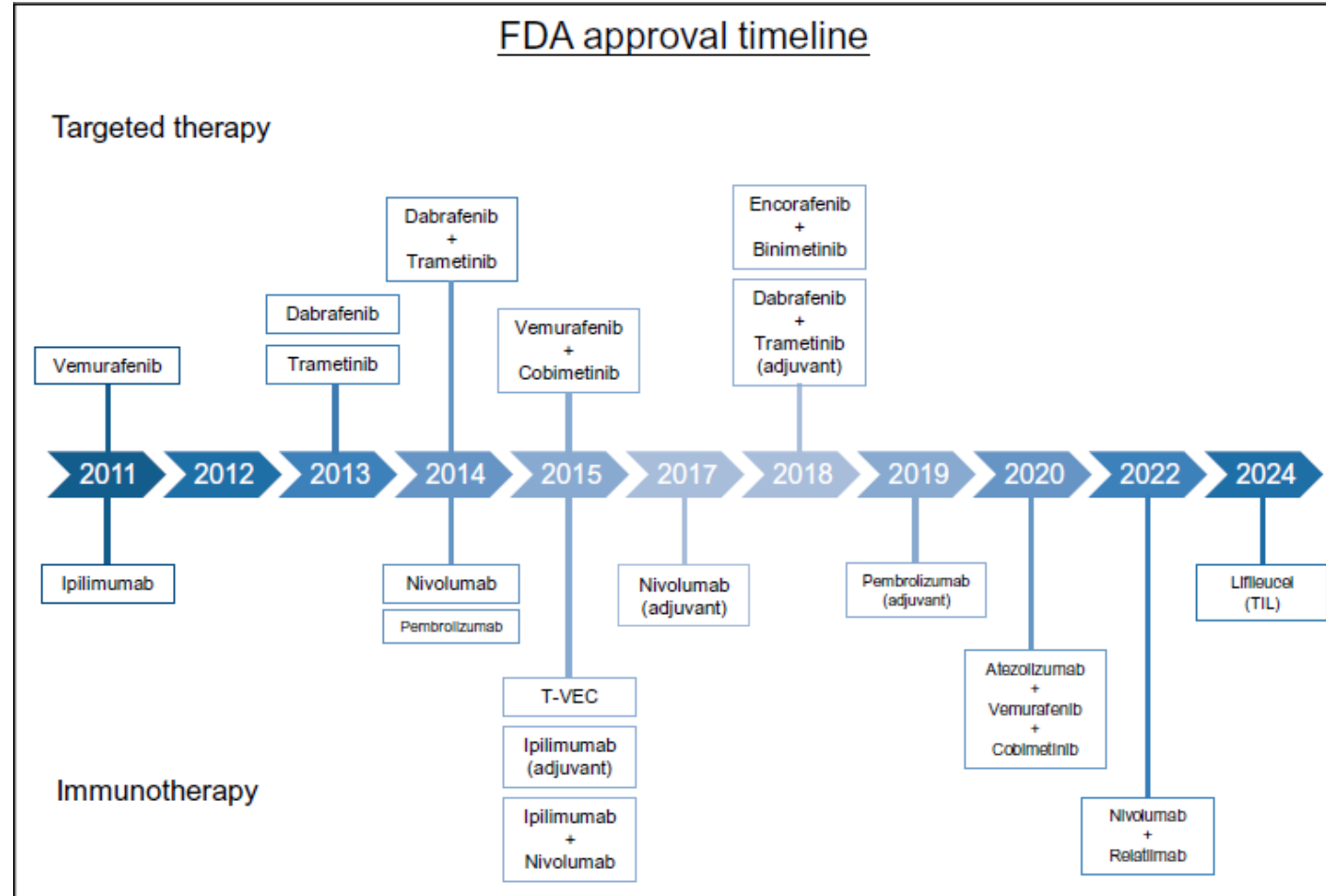
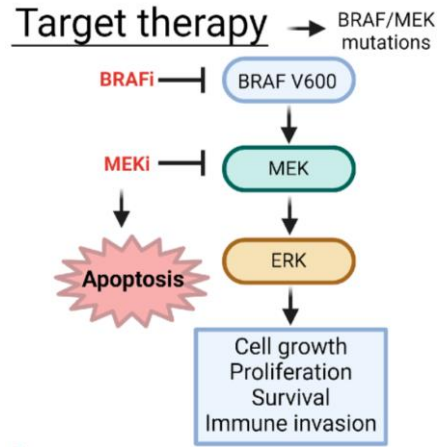
Cycle to end point, partitioning

<https://www.thermofisher.com/it/en/home.html>

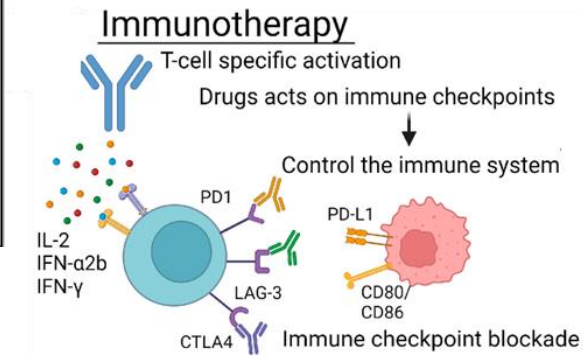
Proof of concept: metastatic melanoma

The therapeutic landscape for melanoma patients

Only for BRAF-mutant patients



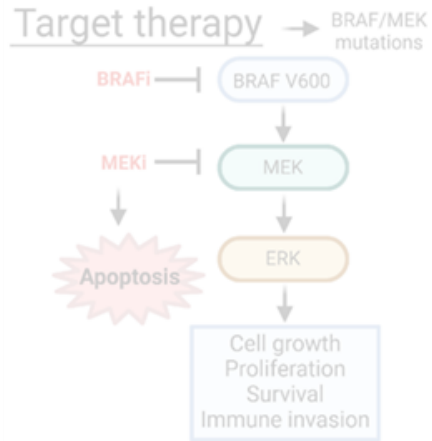
Independent from BRAF-mutational status



(Dougherty, et al, Journal of Nuclear Medicine 2024)

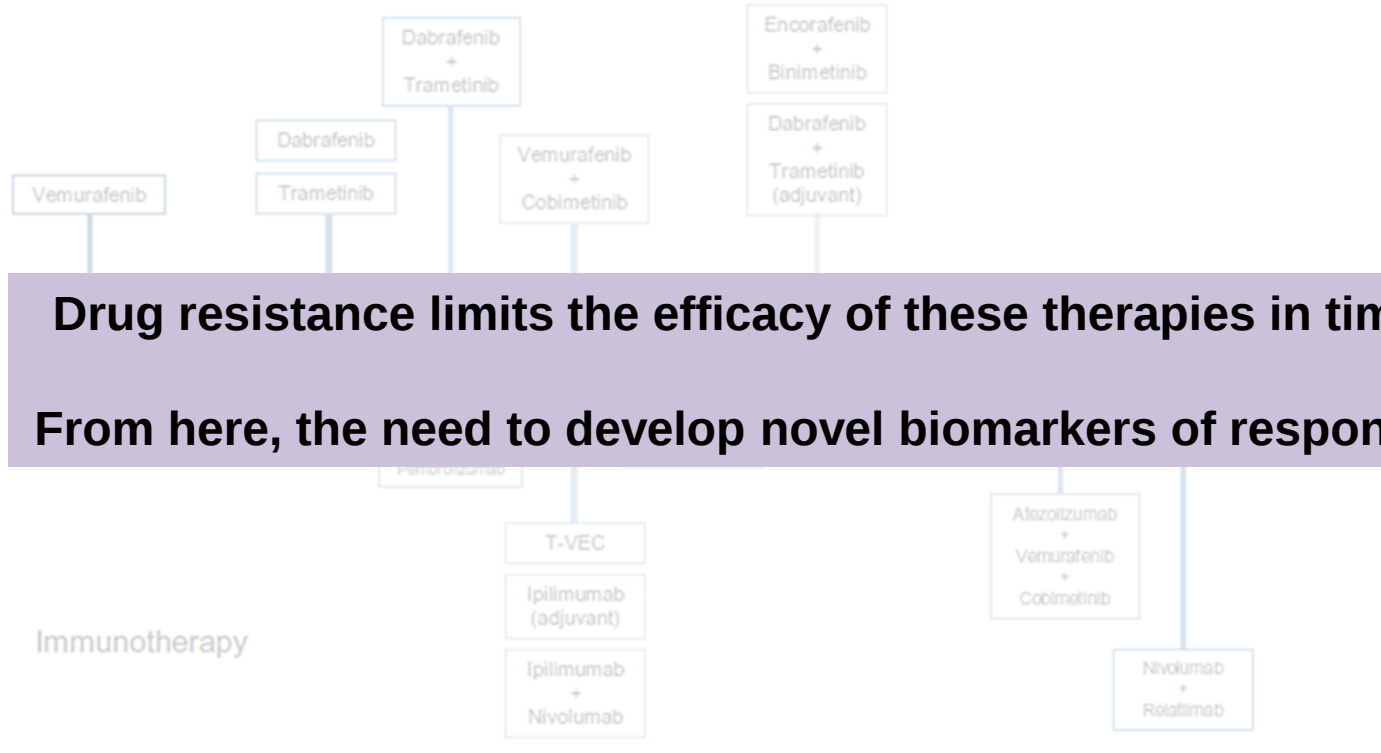
The therapeutic landscape for melanoma patients

Only for BRAF-mutant patients



FDA approval timeline

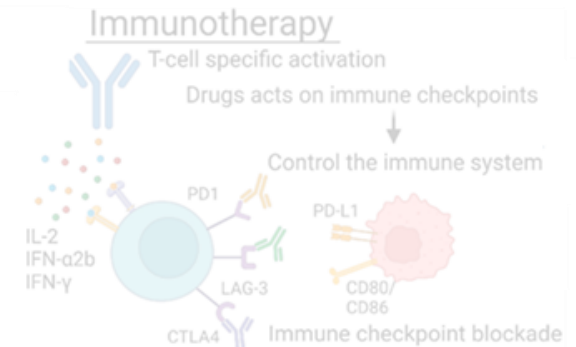
Targeted therapy



Drug resistance limits the efficacy of these therapies in time.

From here, the need to develop novel biomarkers of response.

Independent from BRAF-mutational status



(Dougherty, et al, Journal of Nuclear Medicine 2024)

Agenda of the presentation

- First study: focus on targeted therapies
- Second study: focus on immunotherapy



Agenda of the presentation

- First study: focus on targeted therapies
- Second study: focus on immunotherapy



miRatio predicts response to targeted therapy in BRAF-mutant melanoma patients

Theranostics 2022, Vol. 12, Issue 17

7420



Theranostics

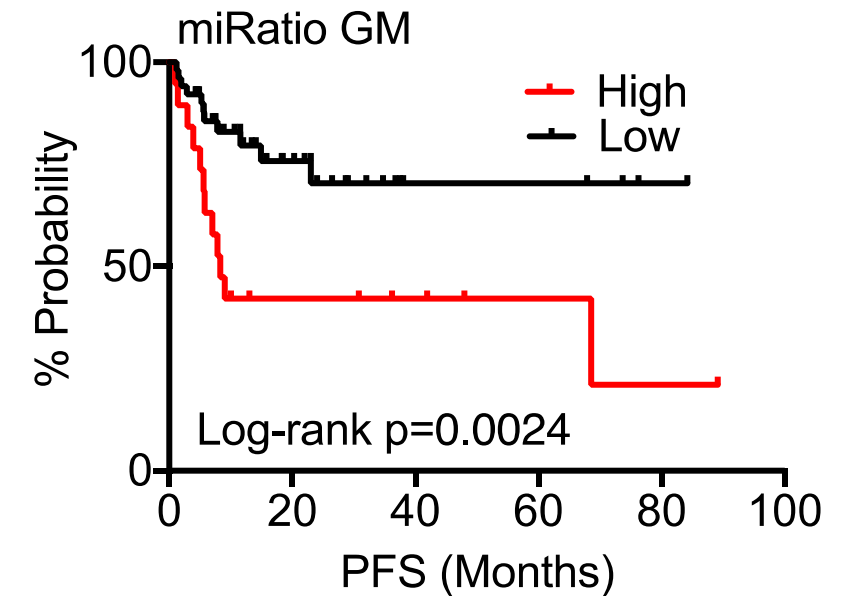
2022; 12(17): 7420-7430. doi: 10.7150/thno.77761

Research Paper

Identification of a miRNA-based non-invasive predictive biomarker of response to target therapy in BRAF-mutant melanoma

- ➡ Serum samples from 70 BRAF-mutant melanoma
- ➡ miRNAs evaluated through qRT-PCR
- ➡ miR-4488/miR-579-3p (miRatio): best predictive value

miRatio: miR-4488/miR-579-3p



(Ruggiero, Fattore et al, Theranostics, 2022)

miRatio predicts response to targeted therapy in BRAF-mutant melanoma patients

Theranostics 2022, Vol. 12, Issue 17

7420



Theranostics

2022; 12(17): 7420-7430. doi: 10.7150/thno.77761

Research Paper

Identification of a miRNA-based non-invasive predictive biomarker of response to target therapy in BRAF-mutant melanoma

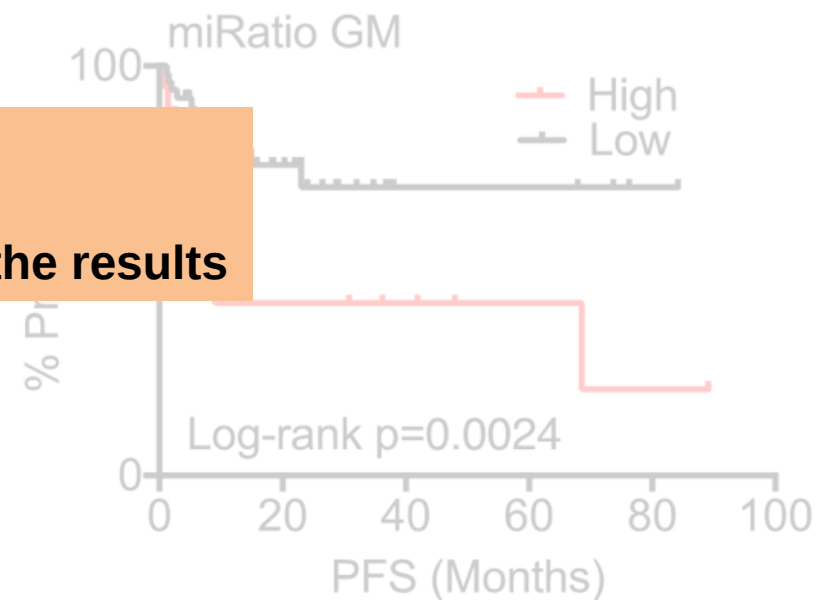
miRatio: miR-4488/miR-579-3p



Limits of this study

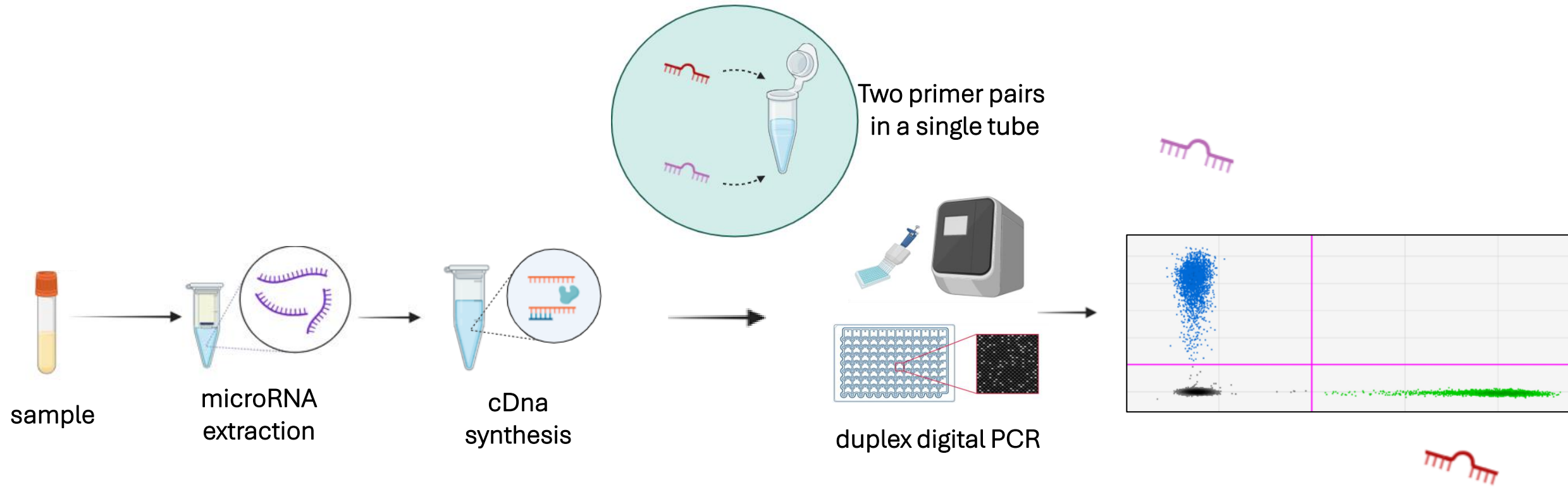
- ➡ Serum samples from 70 BRAF-mutant melanoma patients
- ➡ miRNAs evaluated through qRT-PCR
- ➡ miR-4488/miR-579-3p (miRatio): best predictive value

- Normalization and scalability of the results

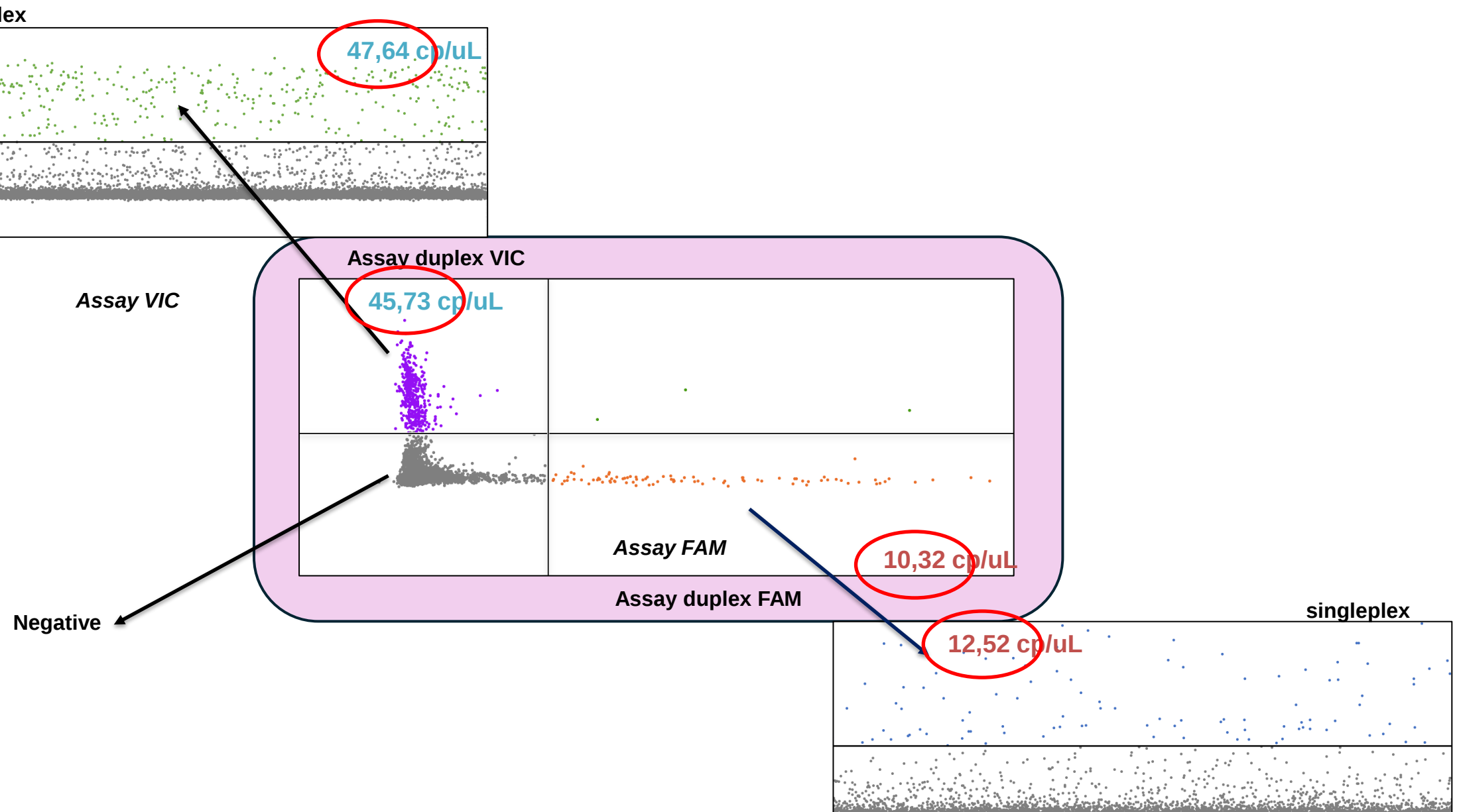


(Ruggiero, Fattore et al, Theranostics, 2022)

Workflow of duplex digital PCR



Development of a dPCR-based duplex assay to detect miRatio



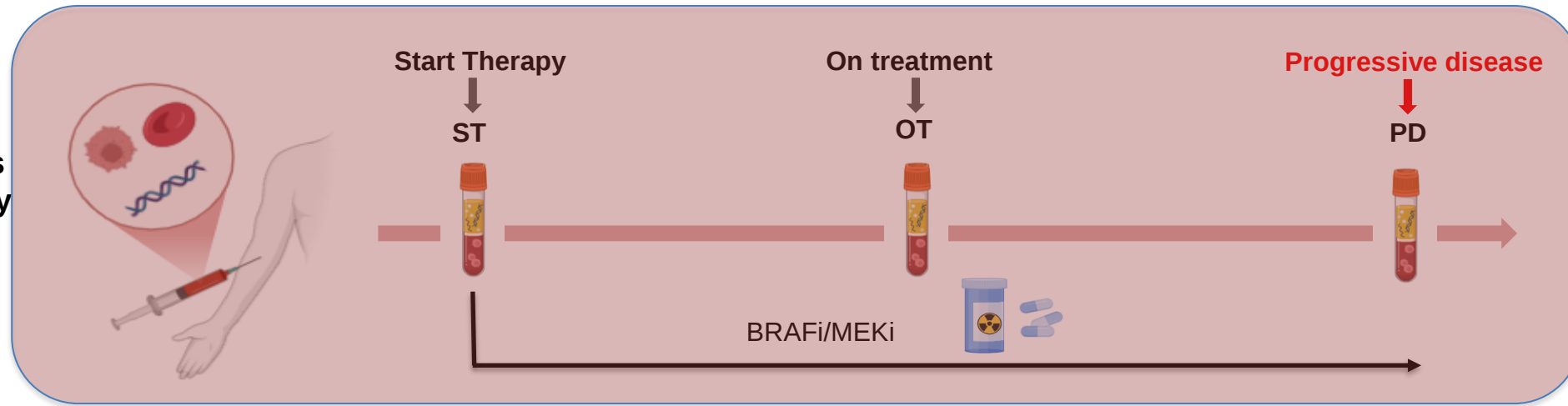
Testing miRatio duplex assay in predicting targeted therapy response

SinglePlex vs Duplex «miRatio» analysis in longitudinal samples



N= 7

Fast progressors
to MAPKi therapy



(Unpublished results)

First study: focus on target therapy

Duplex miRatio has been set up and demonstrated the ability to predict targeted therapy response



Next steps

- To validate the “miRatio” in a larger cohort of patients
- To demonstrate the predictive value of the duplex miRatio assay in a prospective trial



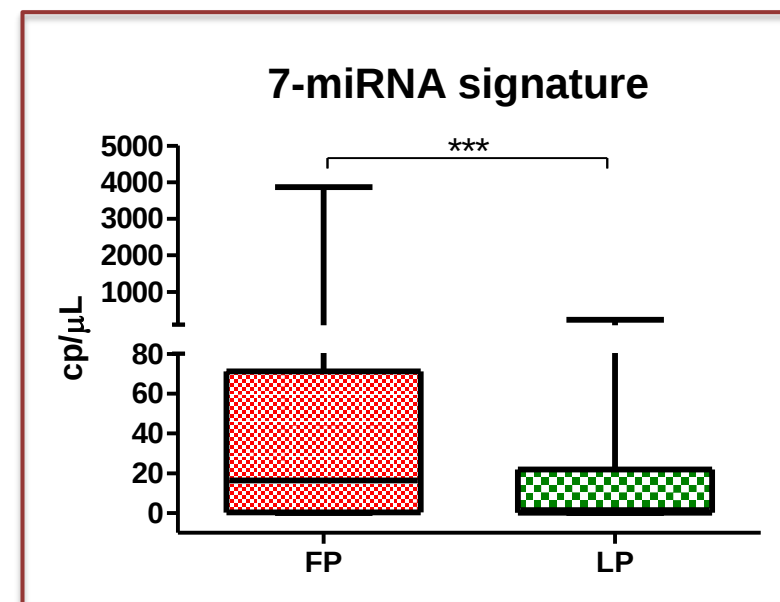
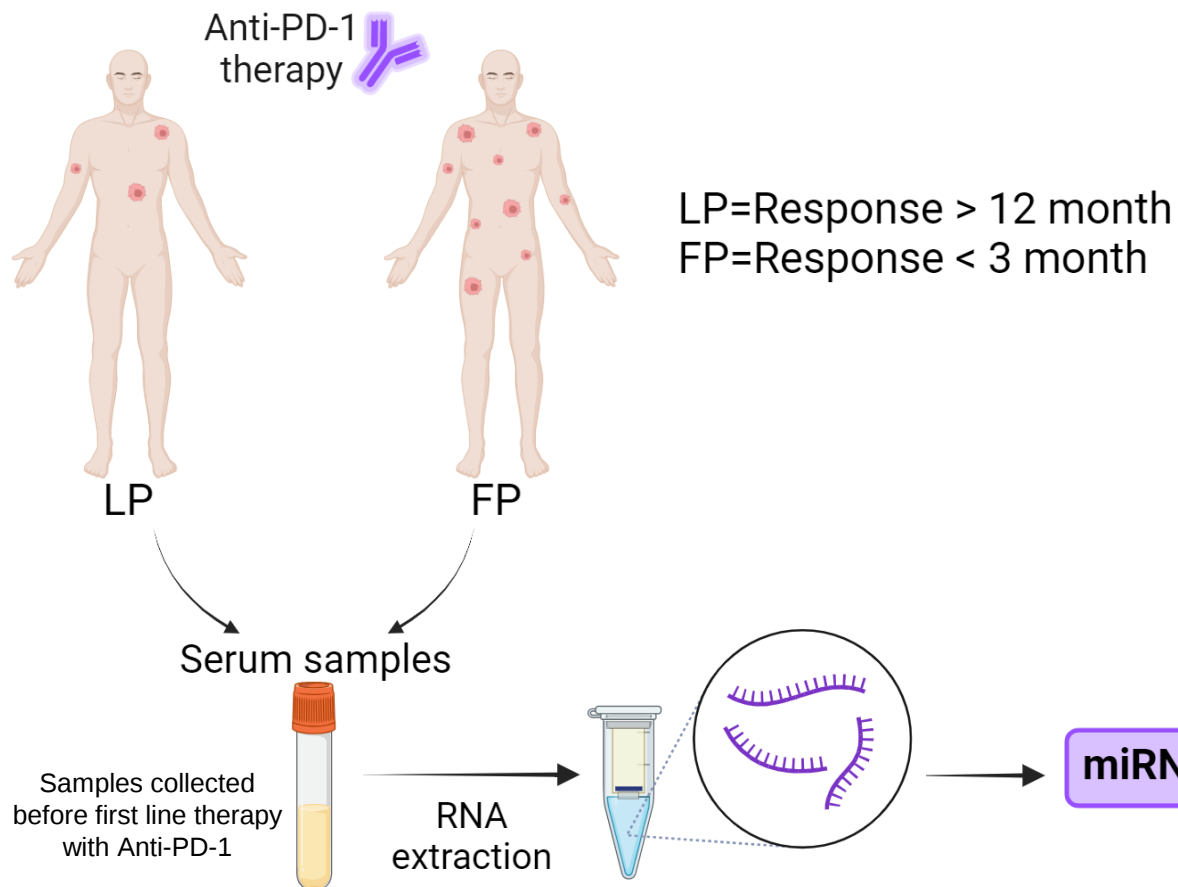
Agenda of the presentation

- First study: focus on targeted therapies
- Second study: focus on immunotherapy



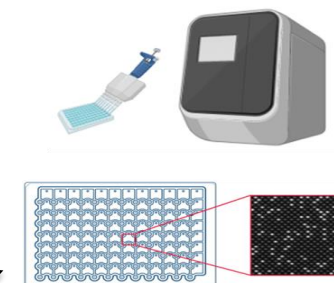
Identification of a miRNA signature able to predict immunotherapy response in melanoma

Melanoma patients = 48



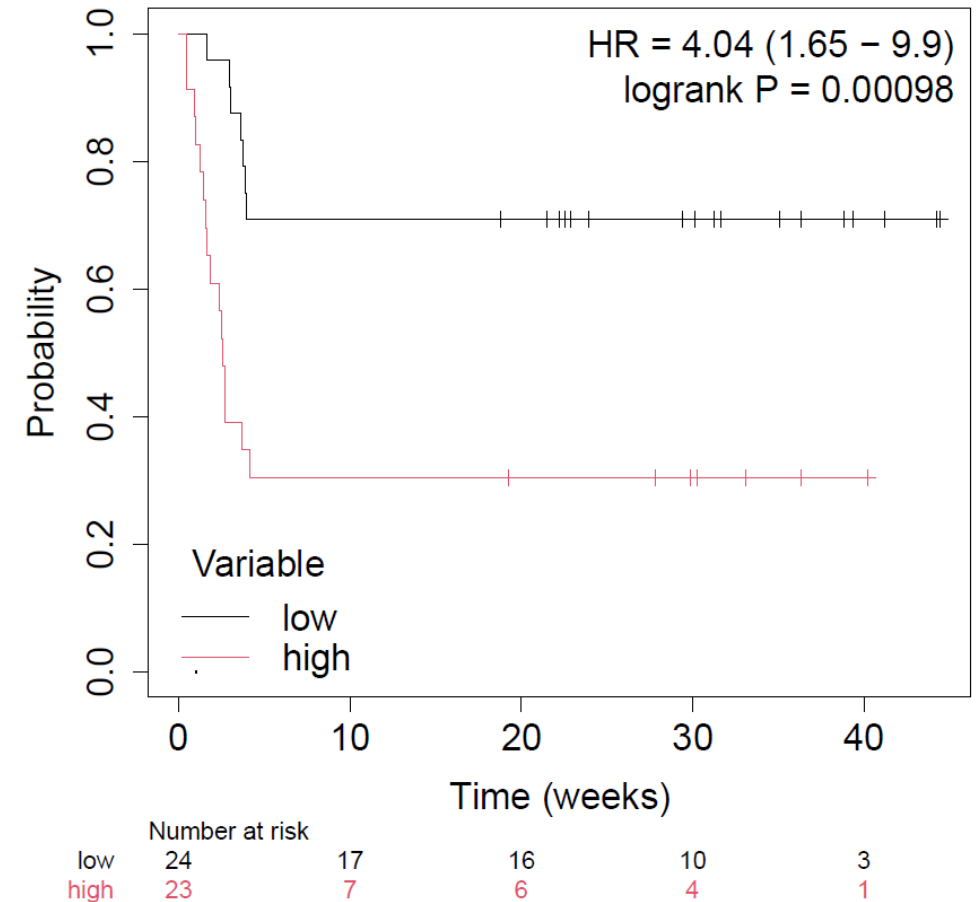
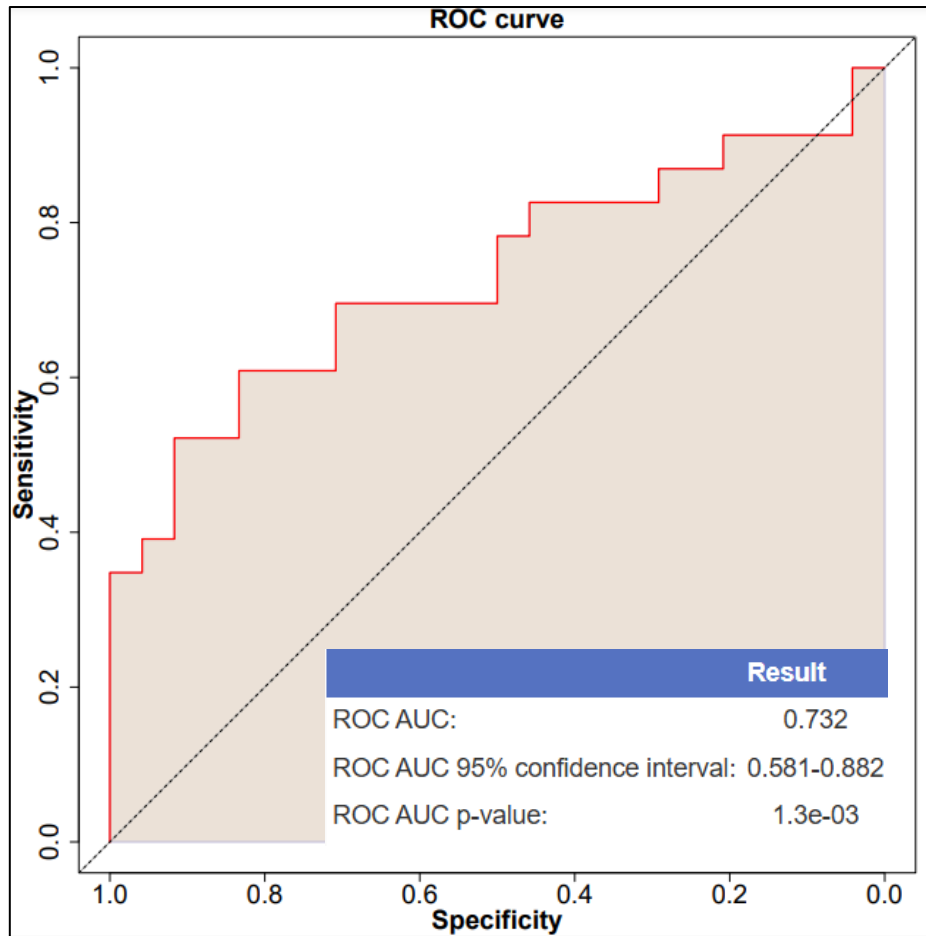
miRNA-seq

19 miRNAs
upregulated in
FP vs LP



(Data can't be disclosed because are pending for a patent application)

A 7-miRNA signature predicts response to immunotherapy in advanced melanoma patients



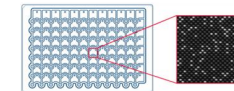
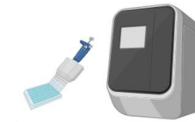
(Data can't be disclosed because are pending for a patent application)

Second study: focus on immunotherapy



Next steps

- To develop a multiplex dPCR on 7-miRNA signature
- To confirm miRNA deregulation in a validation cohort of patients



Thank you!



Alex De Gregorio

Domenico Liguoro
Andrea Sacconi
Valentina De Pascale
Matteo Allegretti
Irene Terrenato
Francesca Sperati
Simona Di Martino
Fabio Valenti
Maurizio Fanciulli

Patrizio Giacomini
Giovanni Blandino

Gennaro Ciliberto



Rachele Frigerio
(Antonella Esposito)



(Ciro Francesco Ruggiero)
Gabriele Madonna
Maria Elena Capone
Domenico Mallardo
Susan Costantini
Alfredo Budillon

Paolo A. Ascierto



SAPIENZA
UNIVERSITÀ DI ROMA

Arianna Ortolano
Sara Bruschini
Francesca Ascenzi
Elena Messina
(Vittorio Castaldo)
Claudia De Vitis
Valeria De Arcangelis
Giulia Rassu
Flavia Busu

Rita Mancini

Supporters



Finanziato
dall'Unione europea
NextGenerationEU



Special thanks to the patients and their familiars



ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO