IRE
ISTITUTO NAZIONALE TUMORI
REGINA ELENA
ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO

Clinical Genomics

Maurizio Fanciulli (Director SAFU Unit)

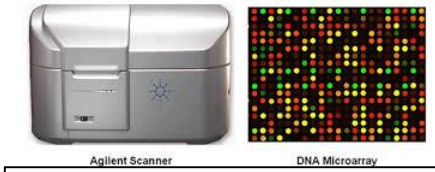
IRCSS Regina Elena National Cancer Institute

NGS Technology at IRE

Illumina Technology

Thermo Technology

NanoString Technology



Microarray Technology

DEPArray Technology

QuantStudio Digital PCR

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PATHOLOGY UNIT: Integrated Molecular Diagnostics

NGS activity

- To select patients who can benefit from targeted therapy
- To monitor the response and the onset of resistance to therapy
- To identify the molecular alterations necessary for the enrollment of patients in clinical trials
- To participate in studies promoted by ACC (Alliance Against Cancer)

NGS panels for analysis on tumor tissue (histological and cytological samples):

- Oncomine ™ Solid Tumor kit (CE-IVD): mutational analysis of 22 genes
- Oncomine™ Focus Assay: analysis of molecular alterations (mutations, amplifications and fusion genes) in 52 genes;
- IonAmpliSeq™ CancerHotspot Panel v2: mutational analysis of 50 genes
- Archer Fusion Plex Sarcoma for IonTorrent panel for simultaneous identification of fusions in 26 genes.
- Oncomine™ BRCA: analysis of BRCA1 and BRCA2 mutations (tumor analysis only).
- Oncochip ACC Lung genes panel (DNA panel: 250 genes, SNP, CNV, insertions and deletions; RNA panel: 93 fusion targets)

PATHOLOGY UNIT: Integrated Molecular Diagnostics

Research activity

- International multicenter study (sponsored by ThermoFisher) for the validation / introduction of the panel Oncomine Precision Assay in routine diagnostics

- Feasibility study for the joint genomic diagnosis of genetic risk and sensitivity to new drugs in breast, ovarian and colon neoplasms using the ACC GerSom Panel (somatic and germline variants ~ 500 genes)

- Multicenter study for the optimization of the detection of NTRK1,2,3 fusions in thyroid cancer (funded by Bayer)

- "VITA" multicenter study on the prevalence of rare molecular alterations susceptible to agnostic treatments

- "Testing service" mergers of RET as part of the ARROW study (phase 1/2 - Blueprint - Pralsetinib)

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Oncogenomics and Epigenetics UNIT: LIQUID BIOPSY in Oncology practice

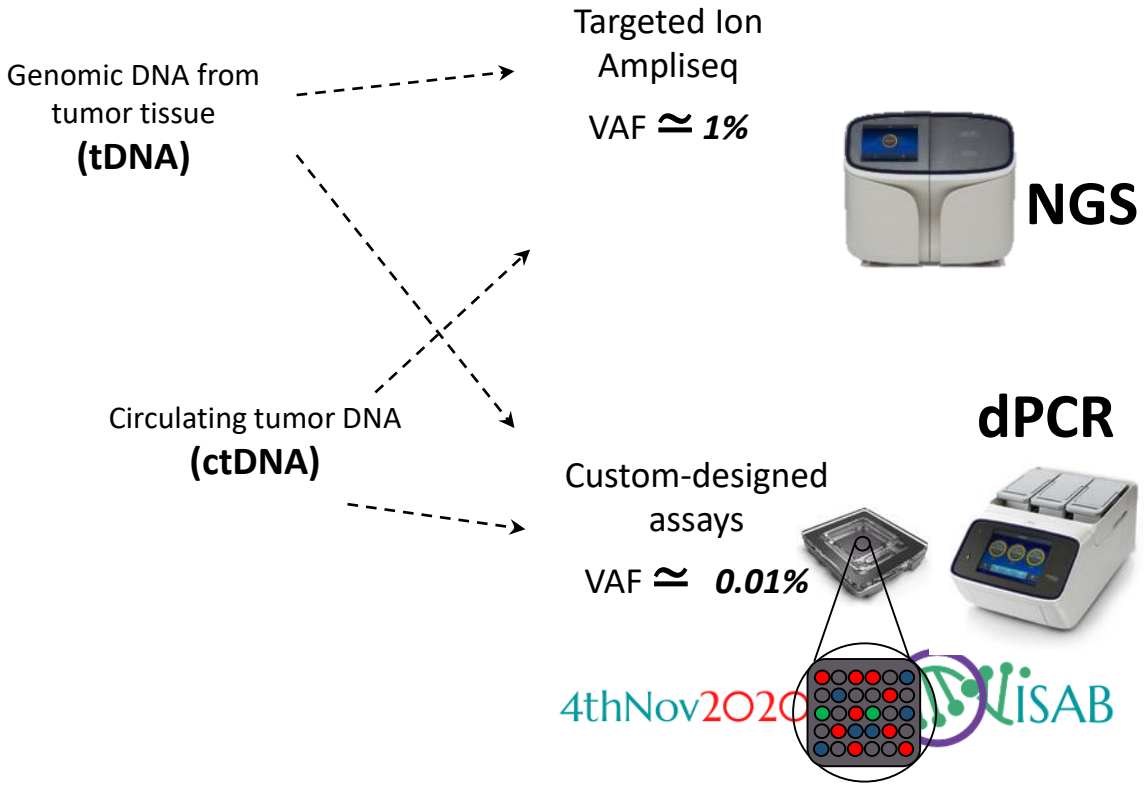
At IRE a classical two-step liquid biopsy protocol is performed:

1. **Genomic complexity of tumor DNA (tDNA) is captured with NGS *targeted* panels of appropriate size;**

2. **Captured mutations (index mutations) are investigated in blood. 'index' mutations are investigated by both ultra-sensitive *'capture'* NGS and dPCR. Particularly the latter lowers the Limit of Detection (LOD) down to few copies per ml of plasma.**

Activities:

- **Colorectal cancer**
- **Breast cancer**
- **Rare tumors: sarcomas and medullary thyroid carcinoma.**
- **Melanoma 4P (in collaboration between IRE and ISG).**
- **Molecular Tumor Board: assignment of therapy for vulnerabilities exclusively present in the blood.**



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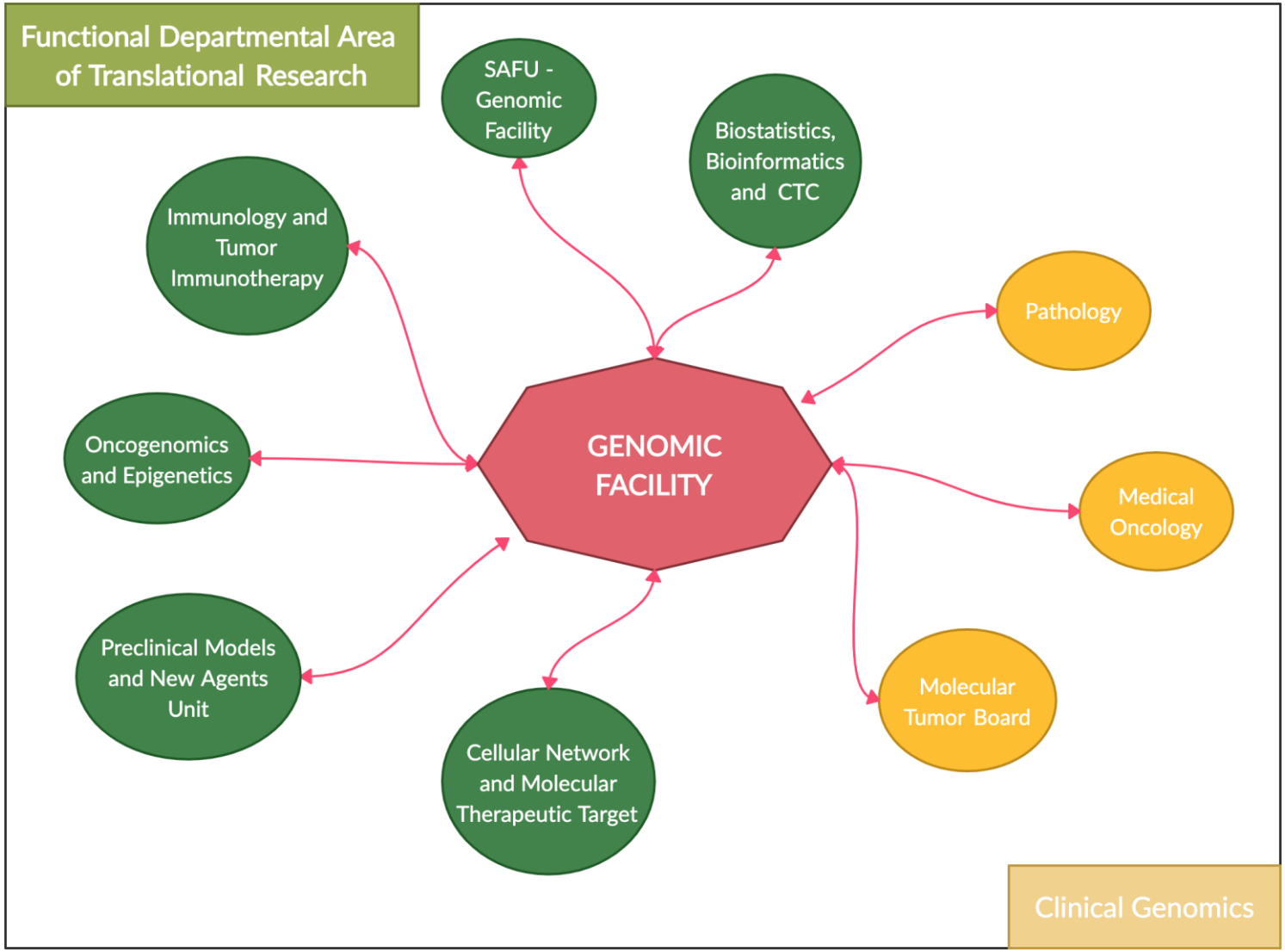
SAFU UNIT: Genomic Facility

The Genomic facility in strong collaboration with Bionformatic Unit carries out and implements several translational research projects to identify and validate new biomarkers that influence prognosis and therapeutic response:

- **Transcriptome analysis: in addition to the study of differential gene expression, gene ontology (GO) and gene pathway analysis, it is now possible to perform computational deconvolution**
- **Exome analysis: enhancement of the identification of pathogenetic variants together with CNV, TMB and MSI.**
- **Participation in the ACC network for projects within the WG Immunotherapy and WG Haematology.**
- **Epigenetic analyzes such as ChIP-Seq, ATAC-Seq, HiC-Seq to characterize the phenotype of tumors as well as gene mutations that influence prognosis and response to therapy.**

Genomic Facility



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Previous ISAB report

"They should consider integrating more discovery type assays with diagnostic workflows which will enable them to integrate research with patient care and provide opportunities for research with substantial efficiencies and potential external investment by pharma and other industry."

Clinical Genomic Projects at IRE

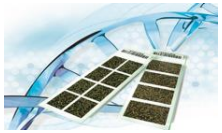
- colon cancer-liver metastases
- Immunotherapy
- Gliomas
- Rare Tumors
- Liquid biopsy
- Immuno response in covid patients with or without cancer

IRE colon cancer-liver metastases casuistry

Fresh-Frozen tissues

NUMBER OF PATIENTS	164
Histotype	
colon adenocarcinoma matched	18
liver metastases from colon adenocarcinoma matched	18
liver metastases from colon adenocarcinoma unmatched	121
secondary liver metastases from colon adenocarcinoma	4 matched 17 unmatched
liver metastases from other primitive tumors	25
SEX	
M	93
F	71
AGE	
<50	5
>50 <60	26
>60 <70	41
>70 <80	54
80+	38
colon adenocarcinoma	
Tumor Size (T)	
T1	1
T2	2
T3	11
T4	4
Nodal Status (N)	
N0	3
N1	11
N2	4
Metastasis (M)	
M>1	18
Grade (G)	
G1	0
G2	16
G3	2

DNA	RNA < 200nt	total RNA
17 matched patients	11 matched patients	6 matched patients + 11 liver metastases
mutational analysis *by Next generation Sequencing*	**microRNA profiling** *by Agilent platform*	**TruSeq RNA Exome** *by illumina platform*



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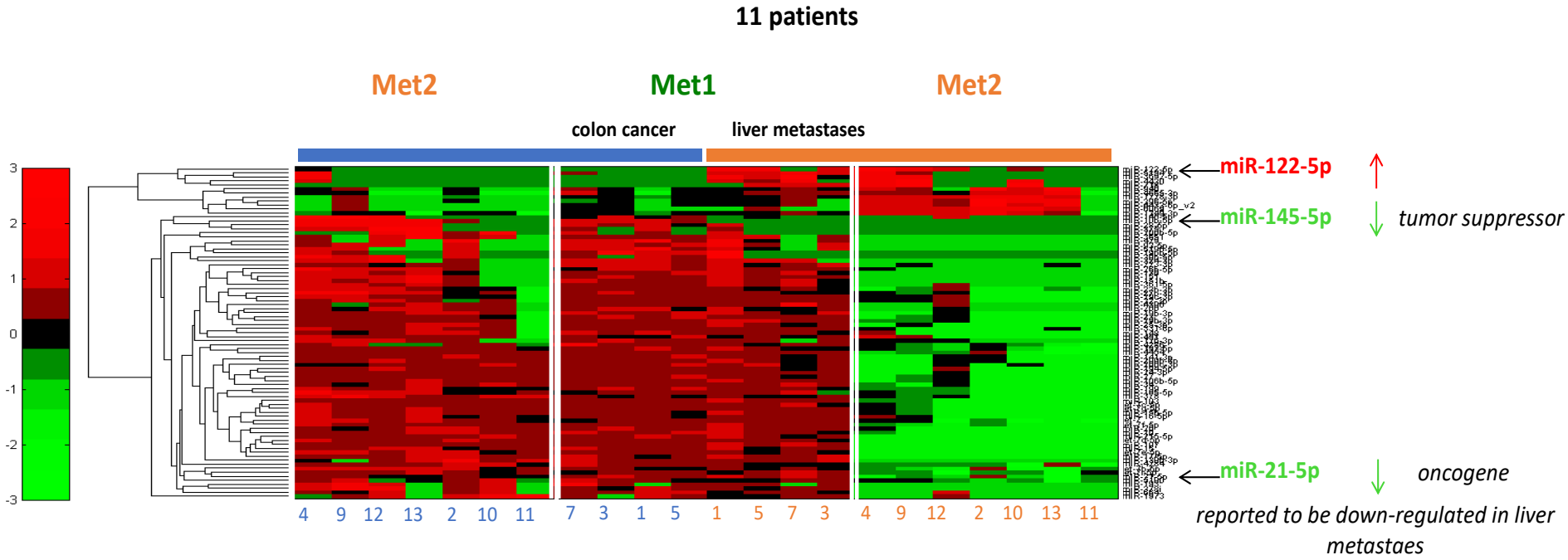
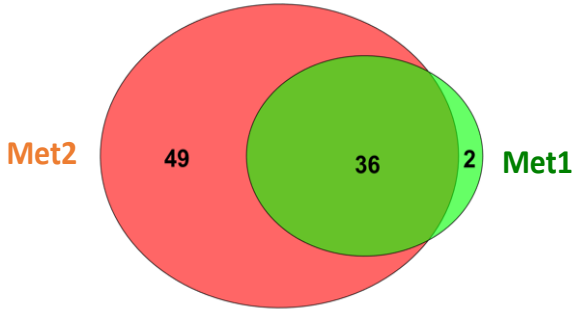
83 miRNAs deregulated in colon cancer-liver metastases



83 miRNAs signature divided patients in two population :

- **Met1**, in which liver metastases are quite similar to primary colon cancers
- **Met2**, in which liver metastases and primary colon cancers are very different

The two population share 36 miRNAs that are differentially expressed in primary colon cancers



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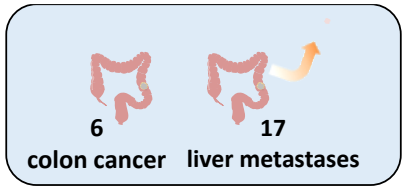
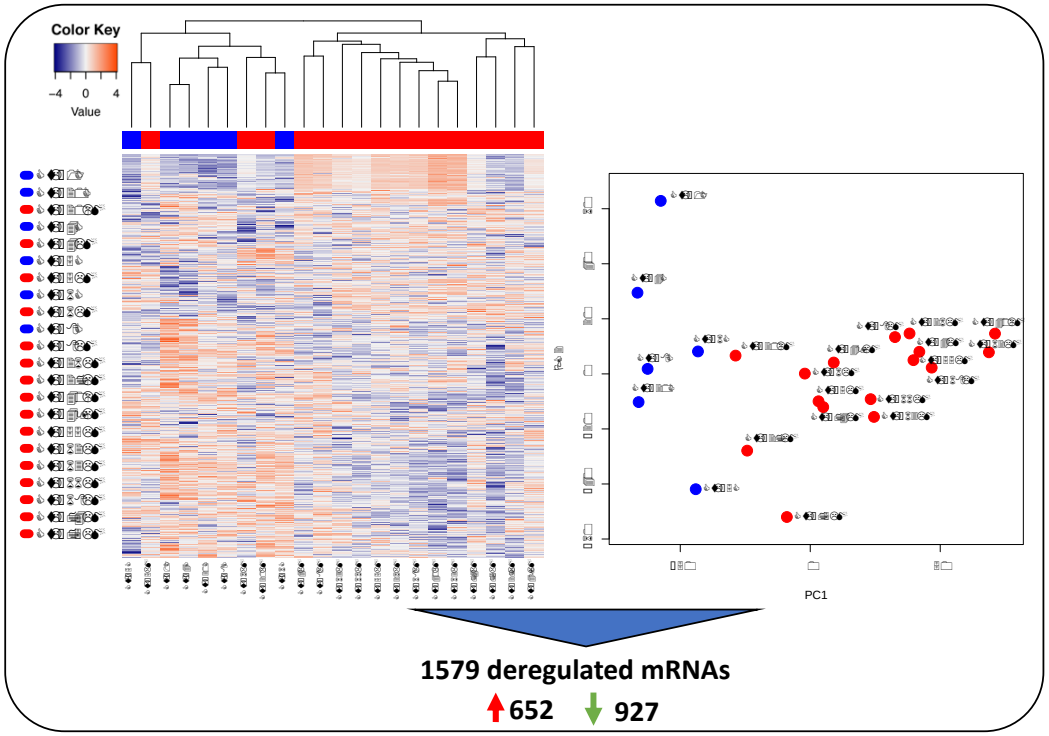
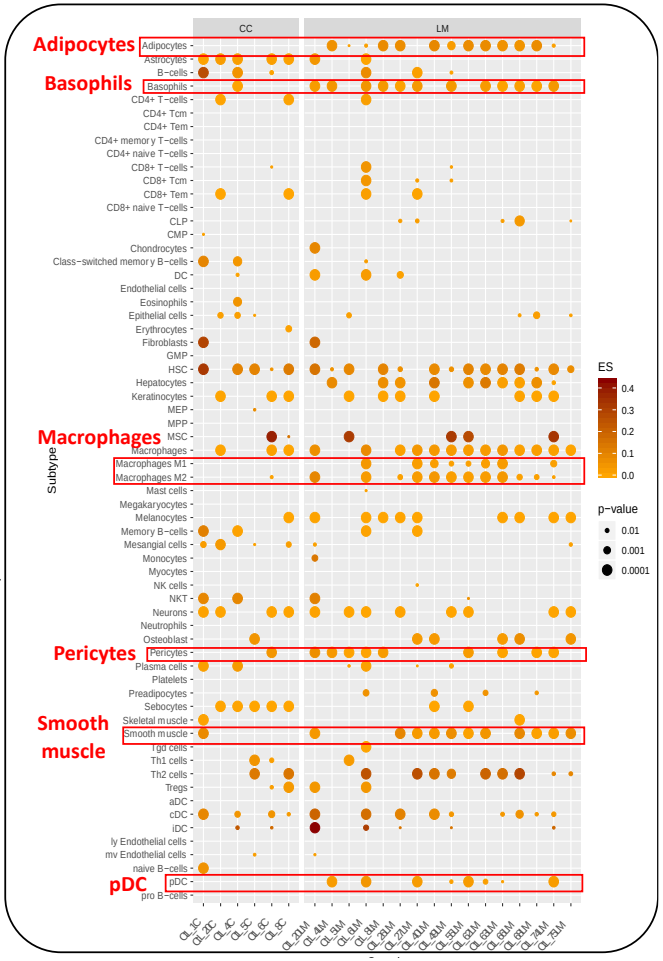
IRE colon cancer-liver metastases casuistry



RNAseq analysis



Deconvolution



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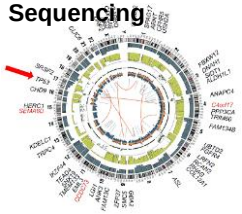
MAIN OBJECTIVES

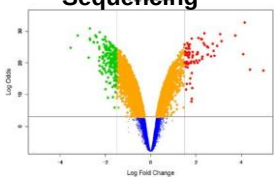
- **Shedding light on responsiveness to immunotherapy in NSCLC**
- **Identification of highly predictive biomarkers of response to new immunotherapeutic approaches in order to early identify those patients who can benefit from them**
- **Conversion of non-responsive patients into responsive ones with the development of new combination therapies.**

Multi-Omics Integration

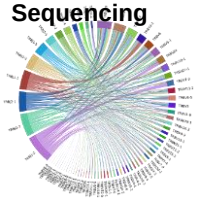
Whole Exome Sequencing



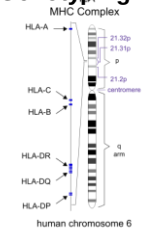
RNA Sequencing

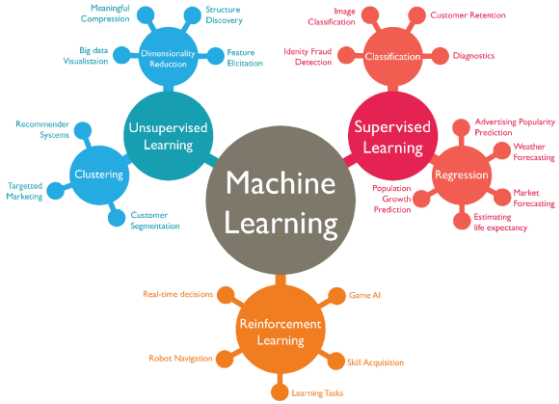


TCR Sequencing



HLA Genotyping





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Preliminary Results

24 samples collected:

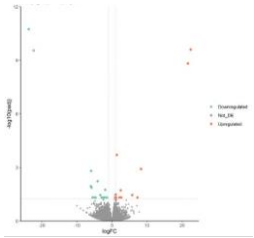
Good Responder at 10 months (13)

Fast Progressor at 3 months (11)

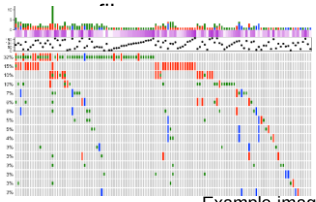
3 samples from Humanitas
12 samples from Regina Elena
9 samples from San Raffaele

Expression level of mutated genes

Differentially expressed genes



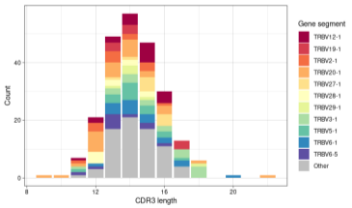
Mutational

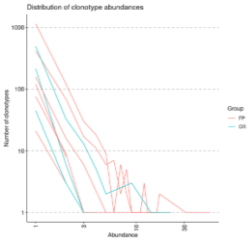


Example image

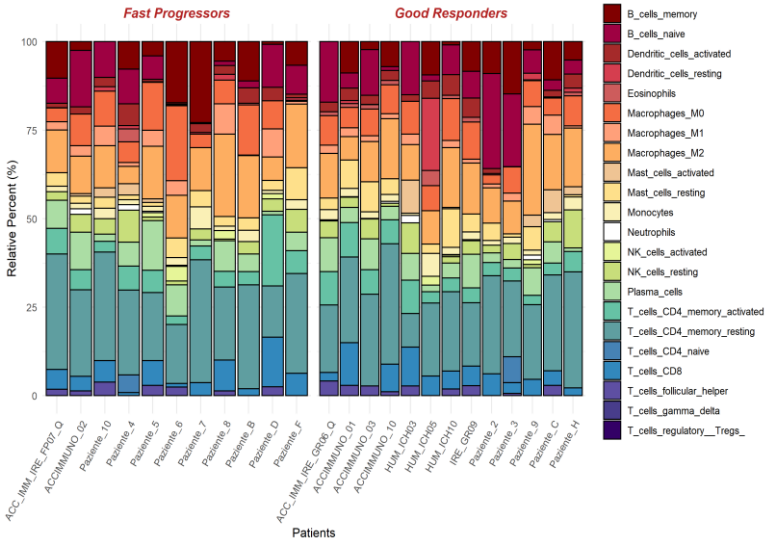
Hyper-expanded clones and oligoclonality

TCR analysis





Machine Learning on RNA-SEq



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ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

KEAP1-driven co-mutations in lung adenocarcinoma unresponsive to immunotherapy despite high tumor mutational burden

D. Marinelli[1†], M. Mazzotta[2†], S. Scalera[3†], I. Terrenato[4], F. Sperati[5], L. D'Ambrosio[3], M. Pallocca[3], G. Corleone[3], E. Krasniqi[2], L. Pizzuti[2], M. Barba[2], S. Carpano[2], P. Vici[2], M. Filetti[1], R. Giusti[6], A. Vecchione[7], M. Occhipinti[8], A. Gelibter[8], A. Botticelli[8], F. De Nicola[3], L. Ciuffreda[3], F. Goeman[9], E. Gallo[10], P. Visca[10], E. Pescarmona[10], M. Fanciulli[3], R. De Maria[11,12], P. Marchetti[1,8], G. Ciliberto[13] & M. Maugeri-Saccà[2*]

- Immune checkpoint inhibitors (ICIs) have demonstrated significant overall survival (OS) benefit in lung adenocarcinoma (LUAD).
- Nevertheless, a remarkable interpatient heterogeneity characterizes immunotherapy efficacy, regardless of programmed death-ligand 1 (PD-L1) expression and tumor mutational burden (TMB).
- KEAP1 mutations are associated with shorter survival in LUAD patients receiving chemotherapy.
- **We hypothesized that the pattern of KEAP1 co-mutations and mutual exclusivity may identify LUAD patients unresponsive to immunotherapy.**

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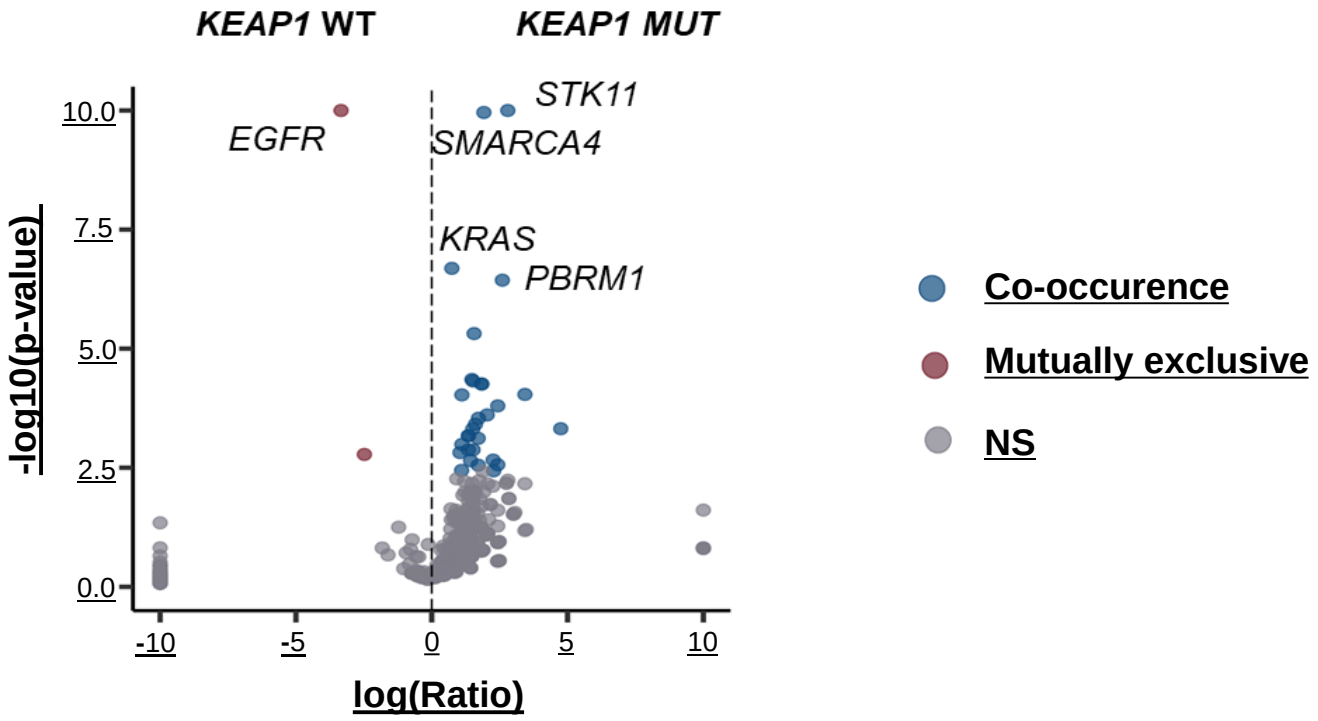
KEAP1 mutational co-occurrences and somatic interactions were studied in the whole MSKCC LUAD dataset



The impact of coexisting alterations on survival outcomes in ICI-treated LUAD patients was verified in the randomized phase II/III POPLAR/OAK trials (blood-based sequencing, bNGS cohort, N = 253).

Three tissue-based sequencing studies (Rome, MSKCC and DFCI) were used for independent validation (tNGS cohort, N = 289).

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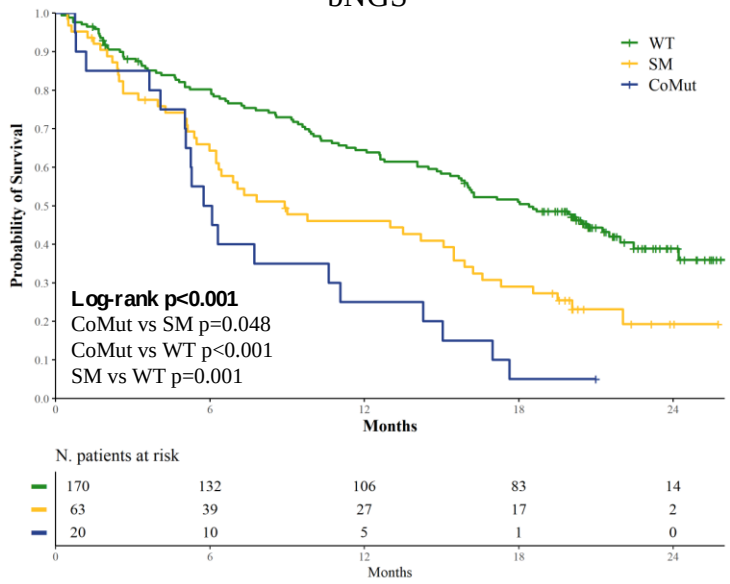
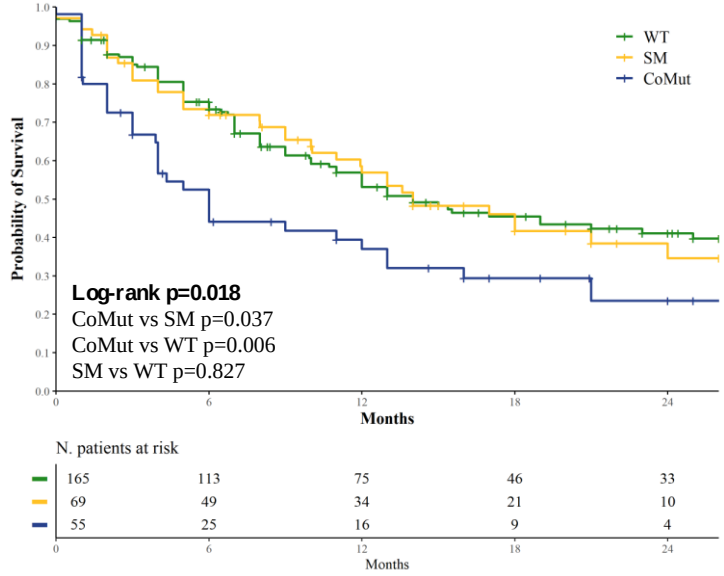
bNGS



tNGS



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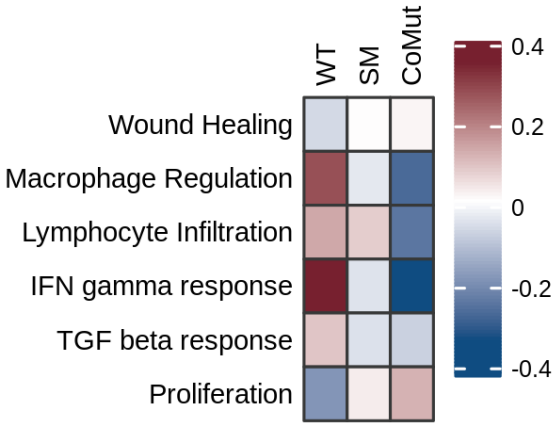
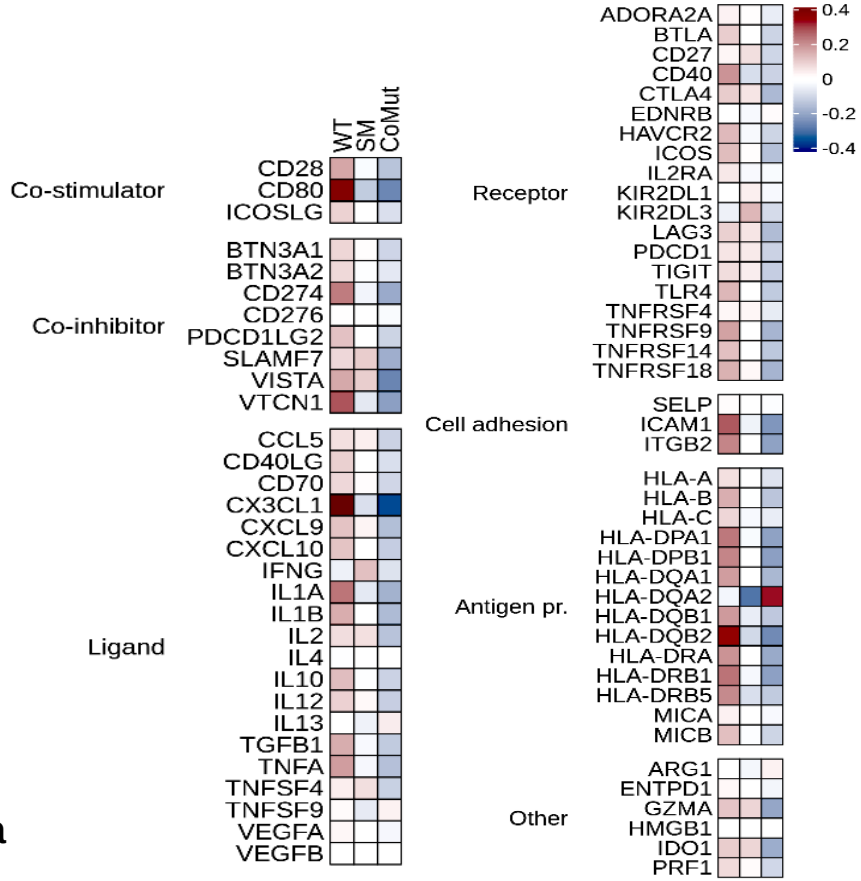
Immunogenomic features were analyzed using The Cancer Genome Atlas (TCGA) LUAD study

Immuno signatures core



Immunomodulatory genes



Conclusions:

This study indicates that coexisting alterations in a limited set of genes characterize a subset of LUAD unresponsive to immunotherapy and with high TMB.

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GLIOMA Project

Multicenter prospective observational study

CENTRO PROPONENTE:
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PRINCIPAL INVESTIGATOR/COORDINATORE:
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A. Fabi

Patologia Clinica
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Radiologia
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Radioterapia
A. Farneti, L. Marucci, G. Sanguineti

MAIN OBJECTIVE:

- **Radiomics: demonstrate if there is a correlation between non-morphological data on brain MRI obtained with diffusion and perfusion techniques with molecular data**
- **Implementation of a new model for molecular diagnostics**

RNA – seq Analysis:

- **Differential Expression Analysis**
- **Immune Deconvolution (+ differential deconvolution)**
- **Survival Analysis**
- **Others**

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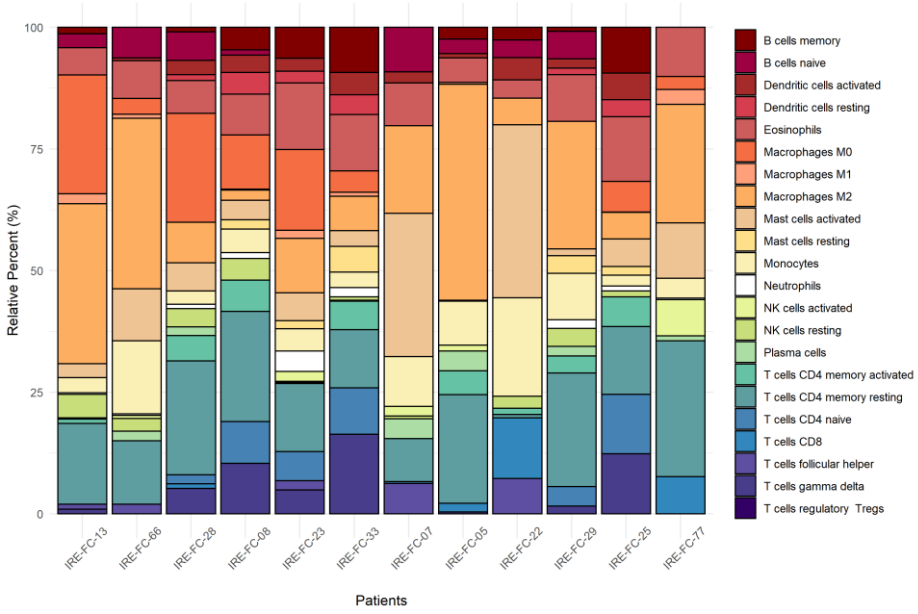
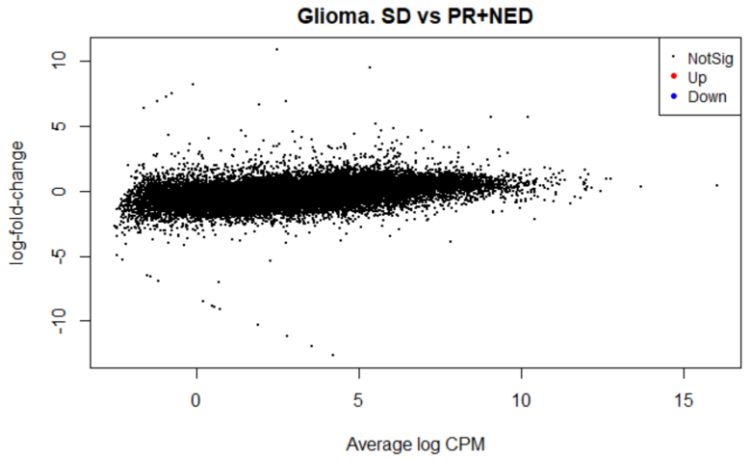
Preliminary results

12 sequenced samples

Immune cell deconvolution





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Dissecting the germline background of Pancreatic Carcinoma

- **Started collecting blood samples in 2017**
- **Both familiar and non-familiar PDACs**
- **First Italian targeted-germline screening for DDR defects in PDAC**

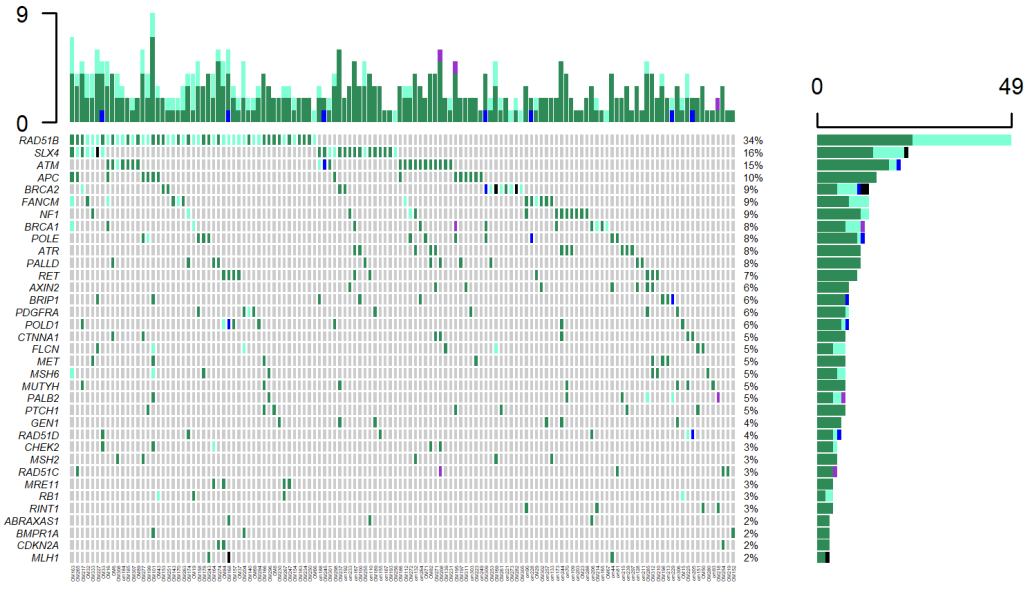
DDR targeted panel

- **65 DDR genes panel design**
- **200KB panel size**
- **10 Million Reads / Sample**
- **Amplicon-Based with UMIs (Unique Molecular Tags)**

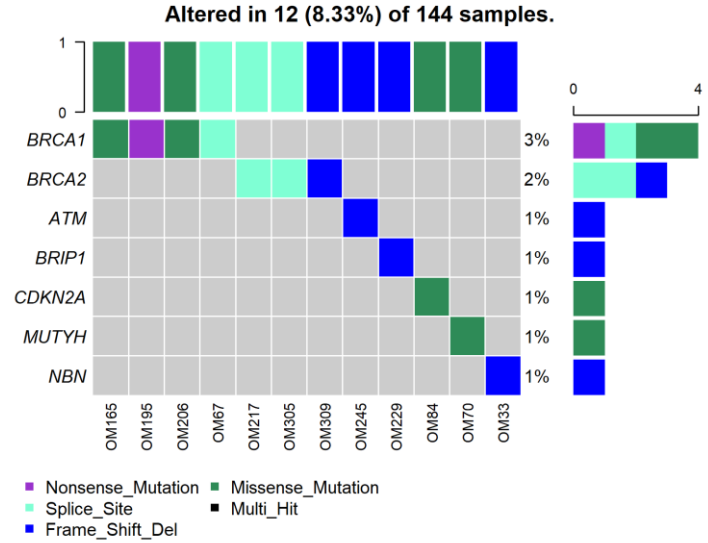
GENE PANEL								
AKT1	BRCA1	CTNNA1	HOXB13	MUTYH	PMS2	RAD51B	SDHB	XRCC2
APC	BRCA2	FAM175A	MEN1	NBN	POLD1	RAD51C	SDHC	
ATM	BRIP1	FANCM	MET	NF1	POLE	RAD51D	SDHD	
ATR	CDH1	FH	MITF	NTHL1	POT1	RB1	SLX4	
AXIN2	CDK4	FLCN	MLH1	PALB2	PRKAR1A	RECQL	SMAD4	
BAP1	CDKN2A	GALNT12	MRE11A	PALLD	PRSS1	RET	SMARCA4	
BARD1	CHEK1	GEN1	MSH2	PDGFRA	PTCH1	RINT1	TP53	
BMPR1A	CHEK2	GREM1	MSH6	PIK3CA	PTEN	RPS20	VHL	

Dissecting the germline background of Pancreatic Carcinoma

DDR Germline variants in 144 PDAC cases



Filtering in only ClinVar Pathogenic



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Cholangiocarcinoma Project

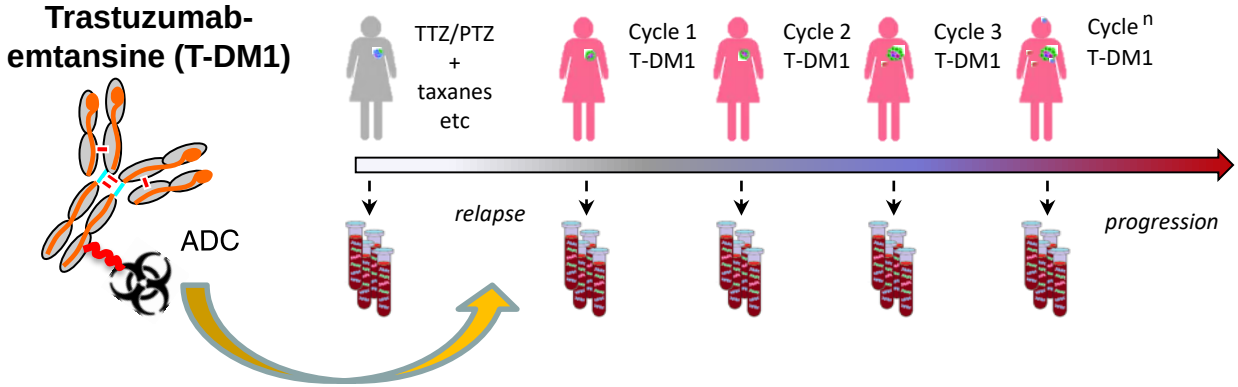
Identification of molecular markers by target sequencing, for the prediction of clinical outcomes, in patients with advanced cholangiocarcinoma (n = 130)

- Enrollment completed
- In progress: DNA extraction. Library preparation. Sequencing with ICGC Panel

LiqBreastTrack: monitoring T-DM1 treated patients by liquid biopsy

Primary Aims

- **Longitudinal cancer monitoring by LB (lead time, outcome)**
- **Discovery of novel mutation patterns associated with progression**



NGS

dPCR

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From LiqBreastTrack to LiqERBcept (GIM21)

Kick-off:
April 2019

PI	City	Center
Chairman: F. Cognetti Study coordinator: A. Fabi, P. Giacomini	RM	IRCSS Istituto Nazionale Tumori Regina Elena
C. Tondini	BG	A.O. Papa Giovanni XXIII
L. Moscetti	MO	A.O.U. Modena
L. Del Mastro	GE	IRCSS A.O.U. San Martino IST
P. Marchetti	RM	A.O.U. Sant'Andrea
G. De Placido	NA	Università degli Studi Federico II

AIRC id. 19503

Aiom Associazione Italiana di Oncologia Medica

Roche

https://www.oncotech.org/gim21

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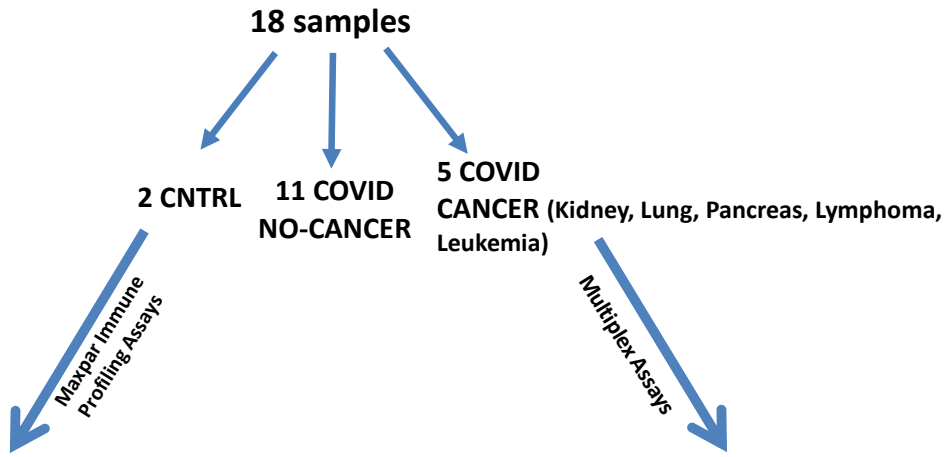
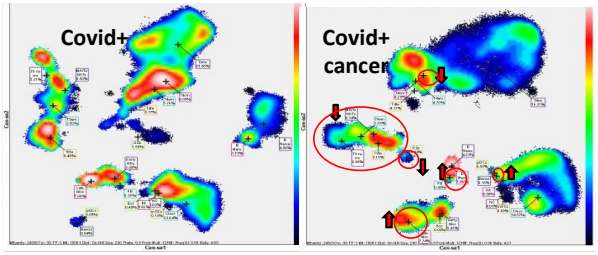
To assess immunological responses to Sars-CoV-2 in patients with cancers



PBMC subclass populations

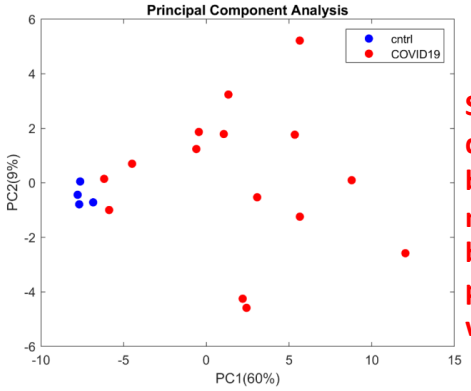
In COVID+cancer patients:



- CD8 T
- CD4 T centrl memory
- MAIT cells NK
- gdT cells

- B memory cells
- Plasmablasts
- Late NK
- Plasmacytoid Dendritic cells

Serum citochinome



Serum cytochines discriminate between covid and no-covid but not between covid patients with and without cancer

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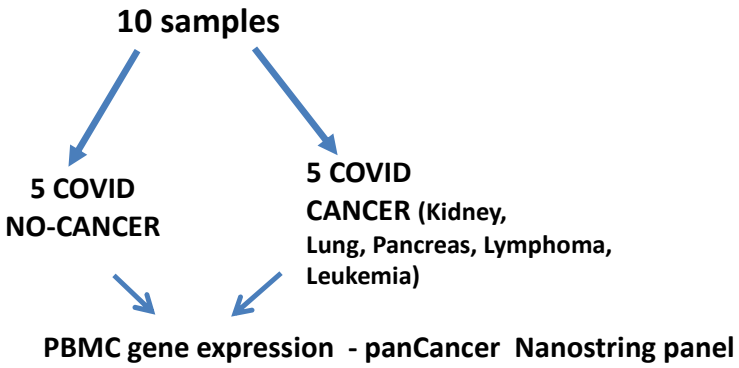
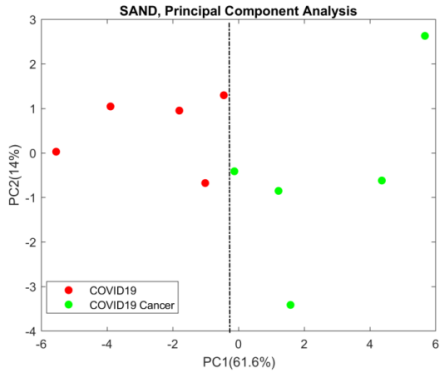
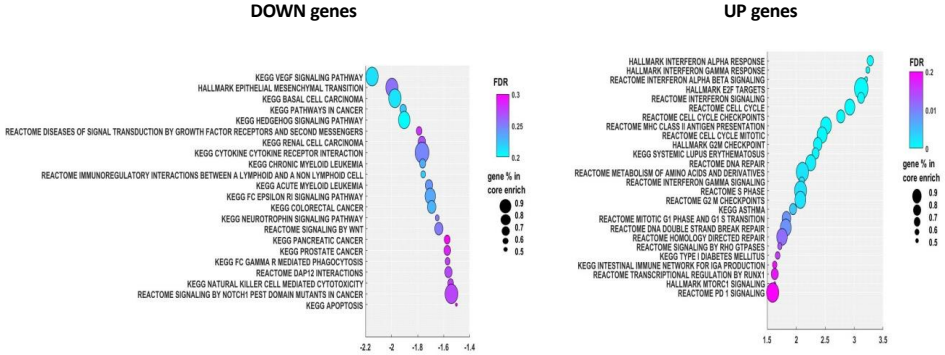
IRE

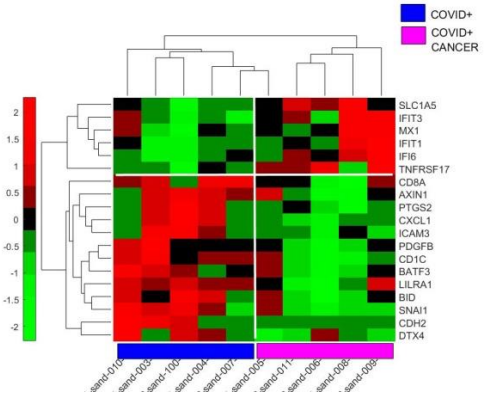
To assess immunological responses to Sars-CoV-2 in patients with cancers





Gene Set Enrichment Analysis, all ranked genes





- **VEGF signaling pathway is downregulated in Covid/cancer patients**
- **Interferon and cell cycle related pathways are upregulated in Covid/cancer patients**

- **A signature of 19 genes discriminates covid patients with and without cancer**

4thNov2020

The Future

NovaSeq 6000

Chromium Controller

GeoMx Digital Spatial Profiler

- **NovaSeq 6000: increases throughput and reduces costs.**
- **Single Cell Chromium: characterizes single cells and thus detects information from under-represented populations that would otherwise be lost (RNA-Seq, ATAC-Seq, CNV, Immunoprofiling).**
- **GeoMX Digital Spatial Profiler: spatial characterization of proteins and mRNAs in their morphological context of the tissue.**