XIX CONGRESSO NAZIONALE SIES 2026

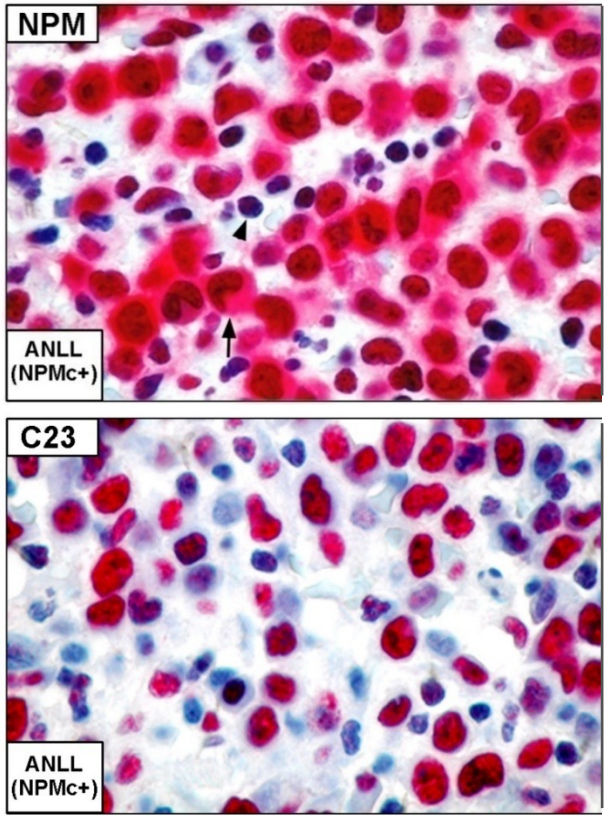
DEEP PROFILING DELLA LEUCEMIA ACUTA MIELOIDE NPM1 MUTATA FLT3-ITD NEGATIVA ATTRAVERSO L'INTEGRAZIONE DI MULTIPLI DATASET DI RNA-SEQ

M. Caridi*, G. Cimino*, W. Villiers*, M. Lim, F. Milano, S. De Santis, R. Ranieri, V. Cardinali, P. Aucken, R. Dillon, M. P. Martelli

Firenze | 4-6 marzo 2026
Palazzo degli Affari

No disclosures to declare

✓ 2005



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cytoplasmic Nucleophosmin in Acute Myelogenous Leukemia with a Normal Karyotype

Brunangelo Falini, M.D., Cristina Mecucci, M.D., Ph.D., Enrico Tiacci, M.D., Myriam Alcalay, M.D., Ph.D., Roberto Rosati, Ph.D., Laura Pasqualucci, M.D., Roberta La Starza, M.D., Ph.D., Daniela Diverio, M.D., Emanuela Colombo, Ph.D., Antonella Santucci, M.D., Barbara Bigerna, Roberta Pacini, Alessandra Pucciarini, Ph.D., Arcangelo Liso, M.D., Marco Vignetti, M.D., Paola Fazi, M.D., Natalia Meani, Ph.D., Valentina Pettirossi, Ph.D., Giuseppe Saglio, M.D., Franco Mandelli, M.D., Francesco Lo-Coco, M.D., Pier-Giuseppe Pelicci, M.D., Ph.D., and Massimo F. Martelli, M.D., for the GIMEMA Acute Leukemia Working Party*

N ENGL J MED 352;3 WWW.NEJM.ORG JANUARY 20, 2005

NPM1-mutated AML represents a distinct leukemia entity with a significant impact on prognosis

WHO 5ed

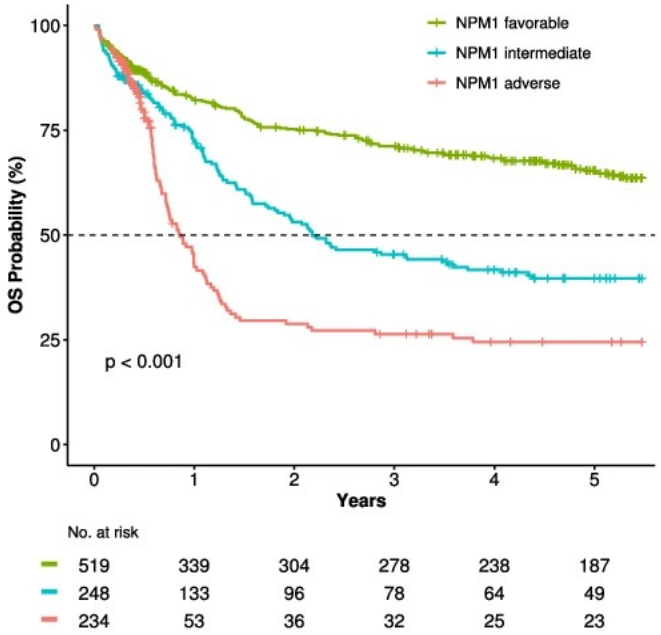
Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with *PML*::*RARA* fusion
Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB*::*MYH11* fusion
Acute myeloid leukaemia with *DEK*::*NUP214* fusion
Acute myeloid leukaemia with *RBM15*::*MRTFA* fusion
Acute myeloid leukaemia with *BCR*::*ABL1* fusion
Acute myeloid leukaemia with *KMT2A* rearrangement
Acute myeloid leukaemia with *MECOM* rearrangement
Acute myeloid leukaemia with *NUP98* rearrangement
Acute myeloid leukaemia with *NPM1* mutation
Acute myeloid leukaemia with *CEBPA* mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with other defined genetic alterations

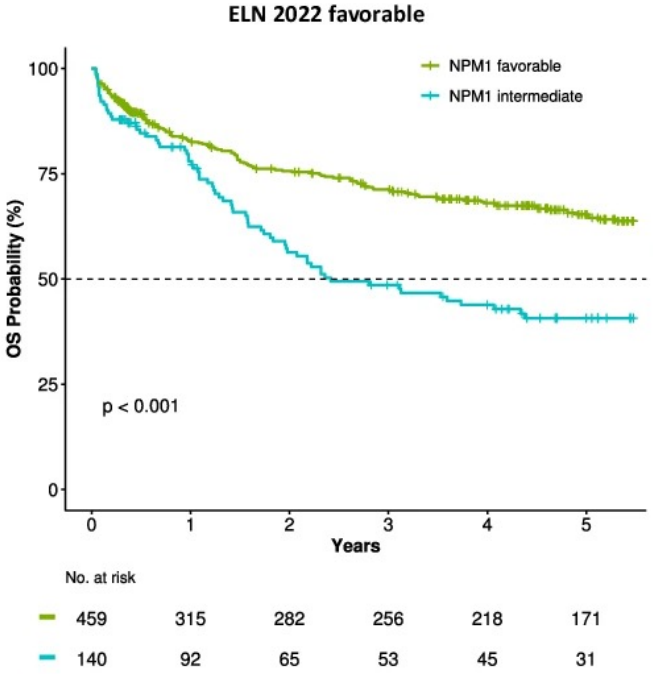
ELN 2022

Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖

Khoury J.D. et al. Leukemia 2022
Döhner H. et al. Blood 2022

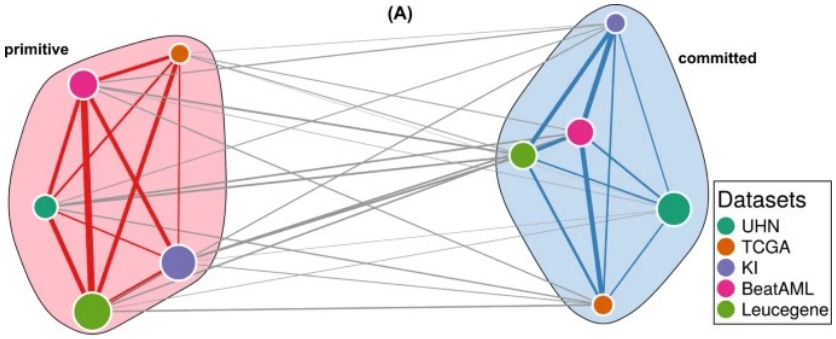
Unmet need: almost 40% of patients affected by ‘favourable’ *NPM1*-mut/*FLT3*wt AML experience Relapse/Refractory disease

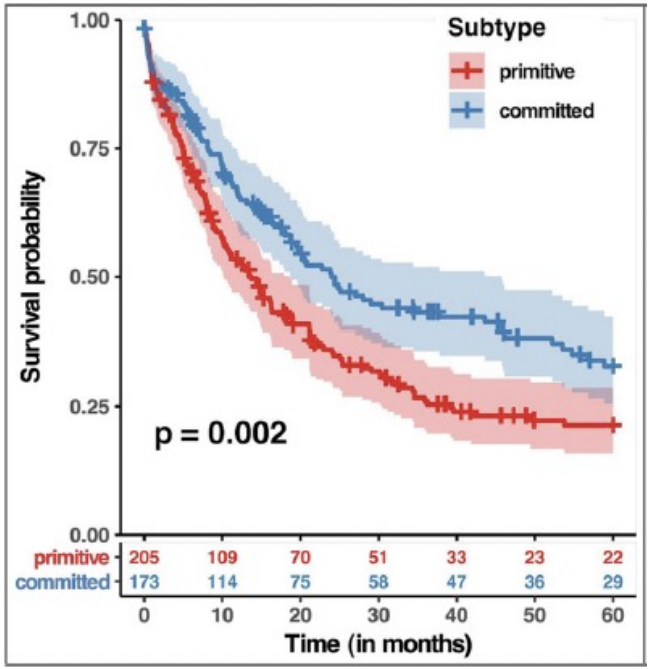




Hernández-Sánchez A et al. Leukemia. 2026

NPM1-mutated AML risk stratification: still an unmet need. Any role for gene expression profile?



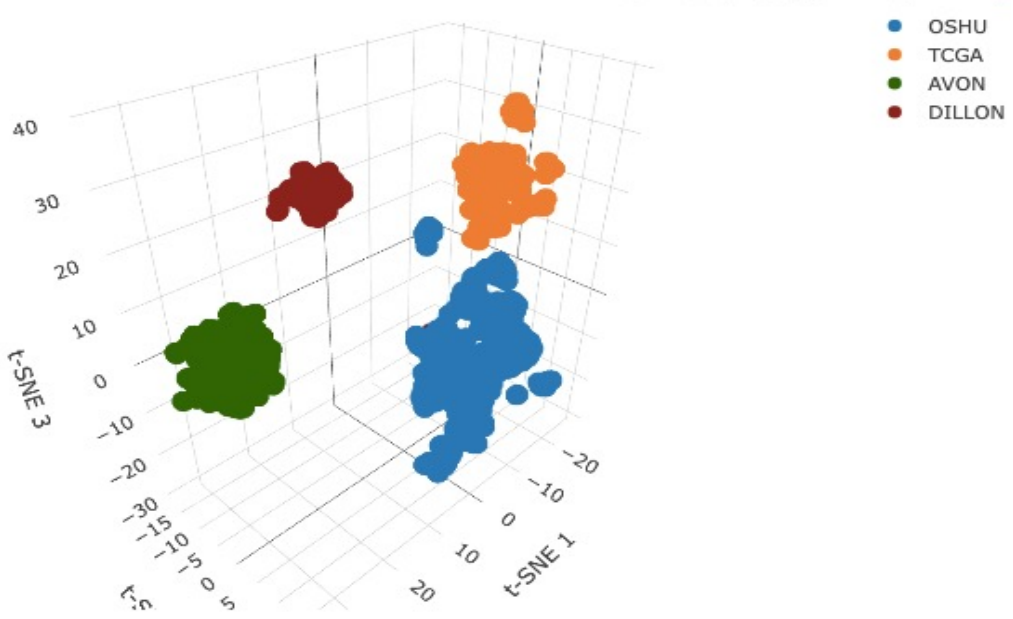


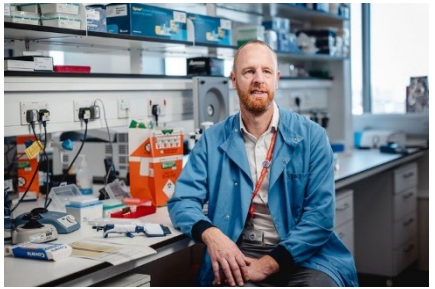
- Age
- HSCT
- *FLT3*-ITD

Mer et al. Nat Comm. 2021

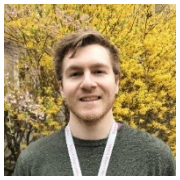
Standard normalization approaches do not ensure adequate unification of different RNA-seq datasets

t-SNE 3D Before ComBat – Log2 – transformed – Perplexity 30





Dr. Richard Dillon

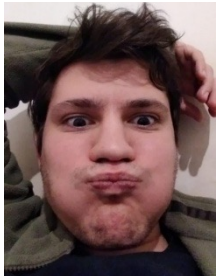


Dr.
W. Villiers

Dr.
M. Lim

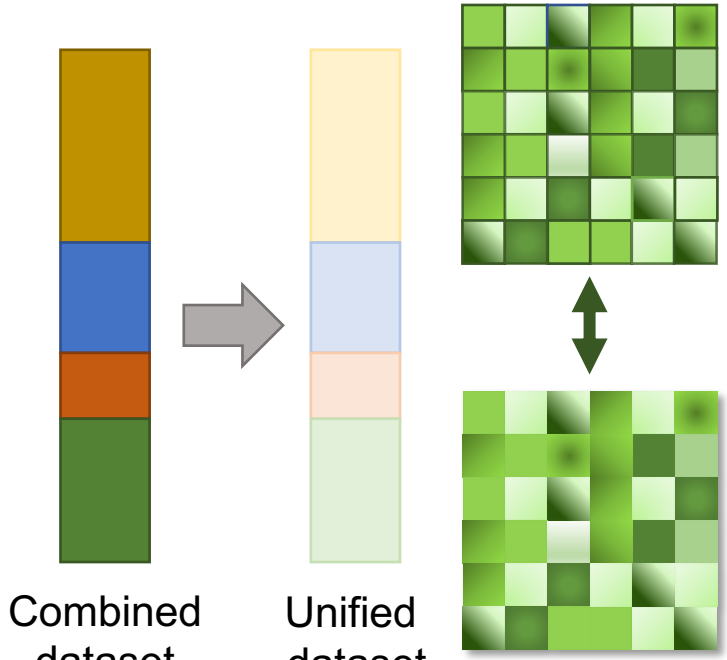
Dr.
P. Aucken

The 'Gu' algorithm generates a unified RNA-seq dataset

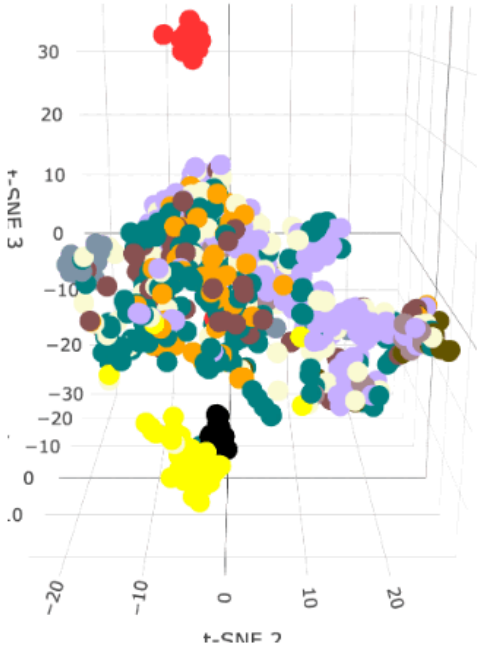


In loving memory of Guglielmo de Simone 'Gu'

1. Filtering common genes
2. Normalization of individual matrices with several technique
3. Dataset integration
4. Applying the batch adjustment
5. Assessment of different combinations



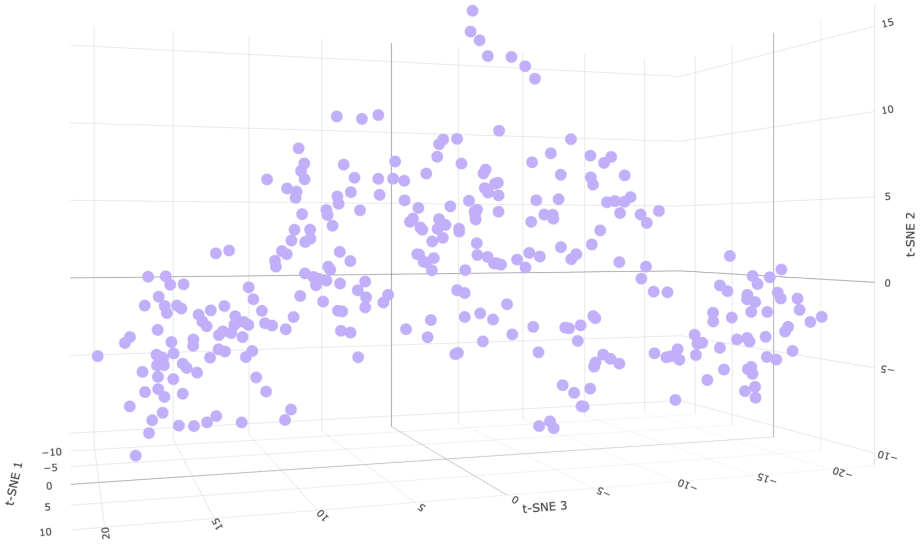
The unified dataset of n=894 AML patients maintains strong biological coherence



- AML with CEBPA mutations
- AML with inv(16) or t(16;16)
- AML with mutated NPM1
- AML with mutated TP53
- AML with myelodysplasia-related cytogenetic abnormalities
- AML with myelodysplasia-related gene mutations
- AML with other KMT2A rearrangements
- AML with t(8;21)
- AML with t(9;11)
- AML-NOS
- Acute promyelocytic leukemia (APL)

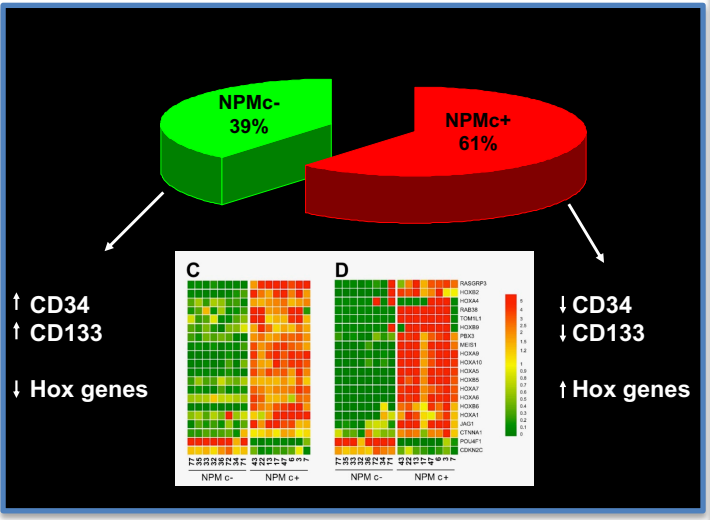
Dr. Gaetano Cimino

NPM1-mutated AML displays a heterogeneous transcriptomic profile

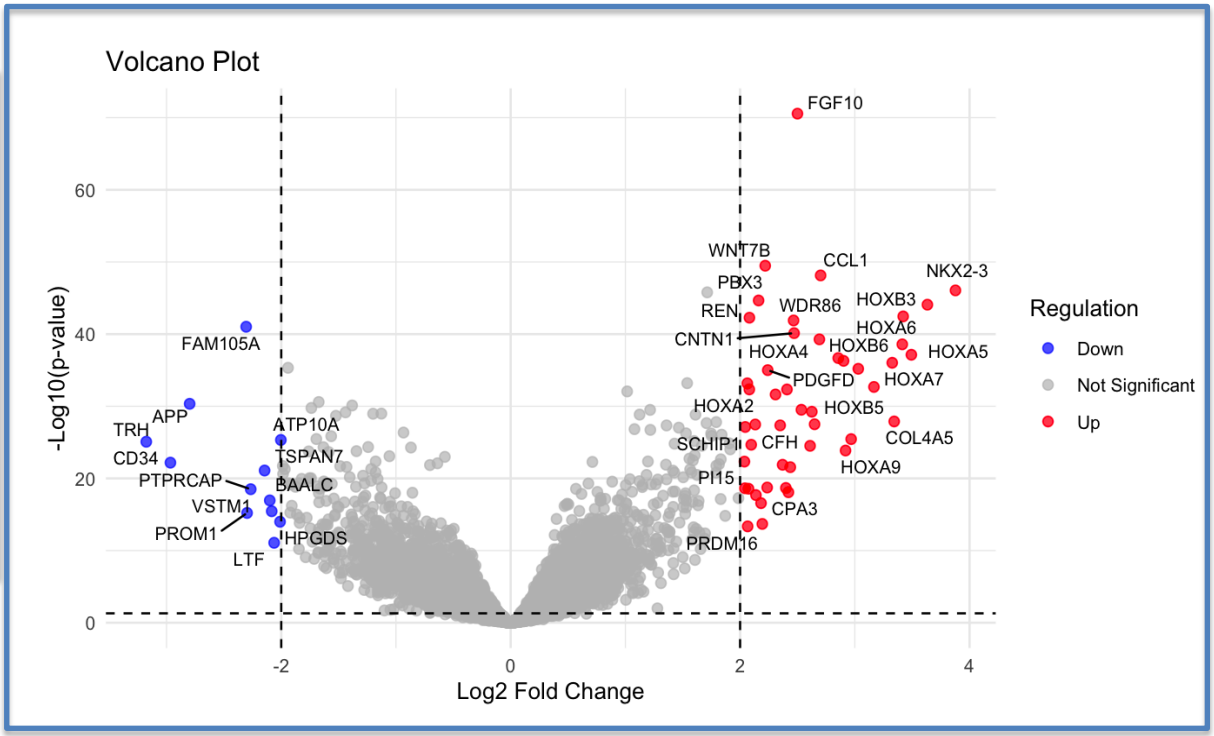


NPM1-mut AML (n=308, of which 187 *FLT3*-wt) is widely distributed

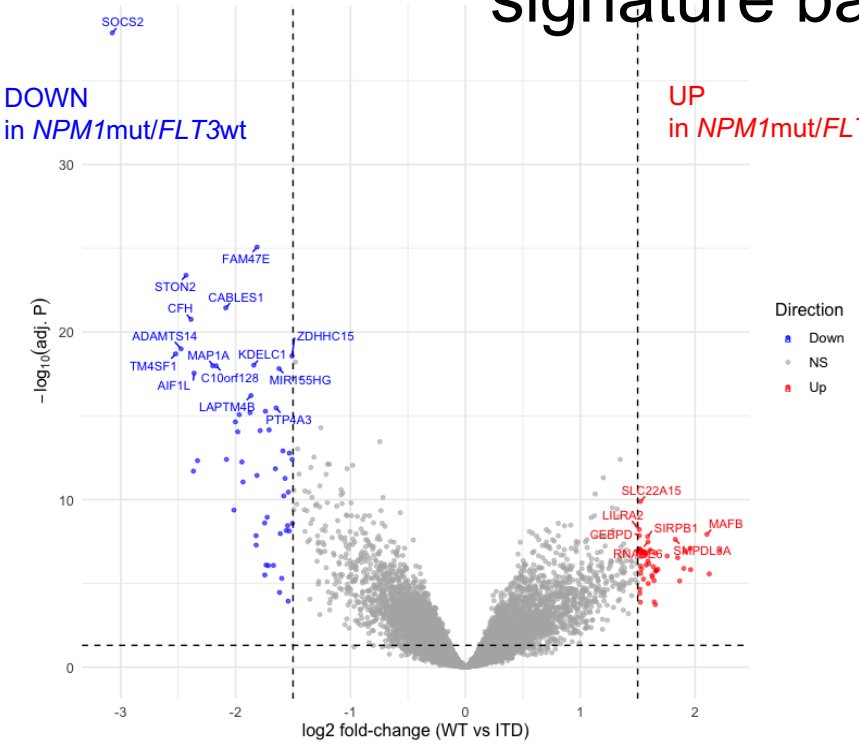
The *NPM1*-mutated AML (N=308) signature is confirmed in the unified dataset



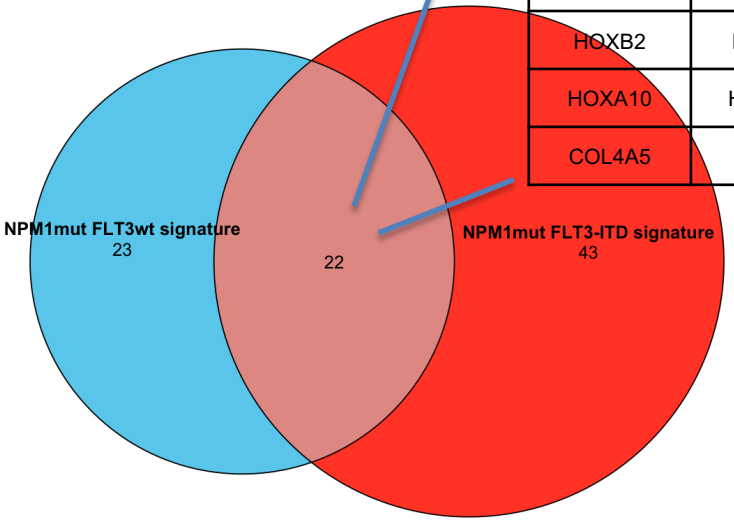
Alcalay M et al., Blood 2005



A '*NPM1*-mutated core' alongside a distinct transcriptomic signature based on *FLT3*-ITD



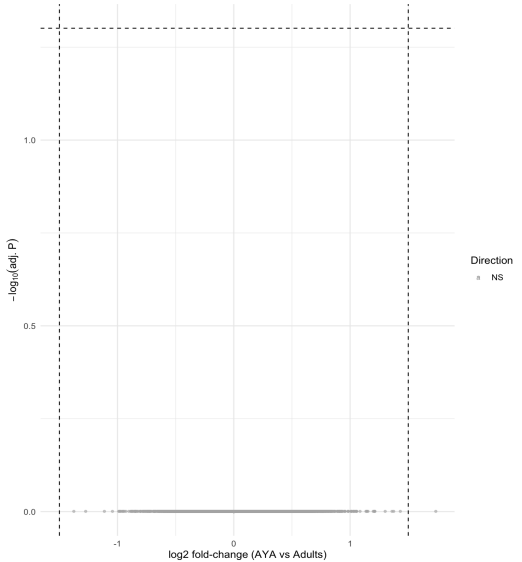
WNT7B	HOXB6	HOXA2
HOXB5	HOXB3	HOXA3
HOXA6	HOXA4	NKX2-3
HOXB4	HOXA5	HOXA11
IRX3	HOXA7	PI15
HOXB2	MEIS1	IRX5
HOXA10	HOXA9	FOXC1
COL4A5		



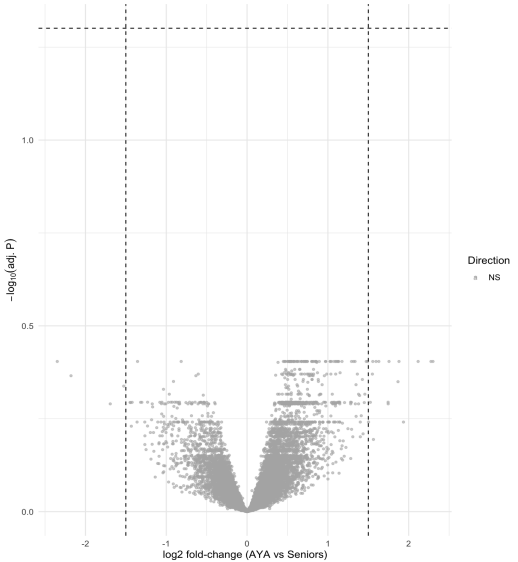
NPM1-mut/*FLT3*-wt *vs* *NPM1*-mut/*FLT3*-ITD

Transcriptomic profiling of *NPM1*-mut/*FLT3*-wt AML shows no/minimal variation across age groups

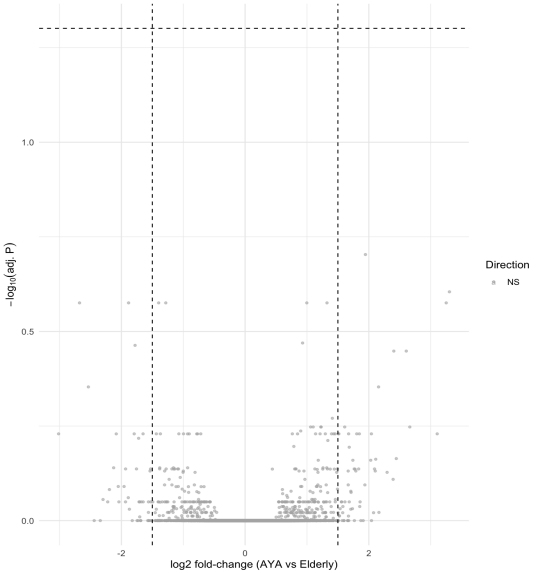
AYA (15–39 years)
vs
Adults (40–64 years)



AYA (15–39 years)
vs
Seniors (65–74 years)



AYA (15–39 years)
vs
Elderly (75+ years)



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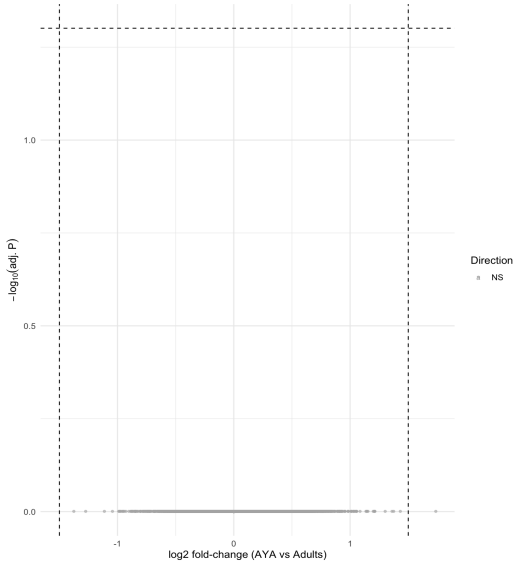

AYA (15–39 years)
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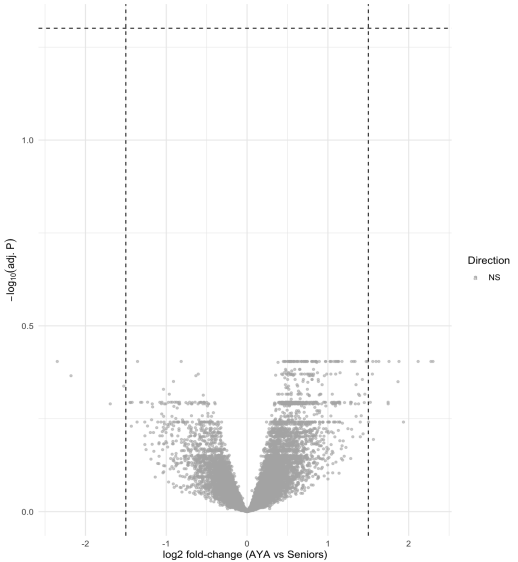

AYA (15–39 years)
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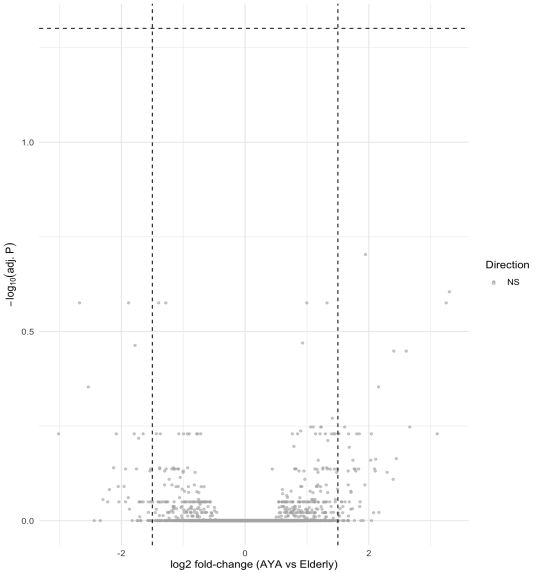
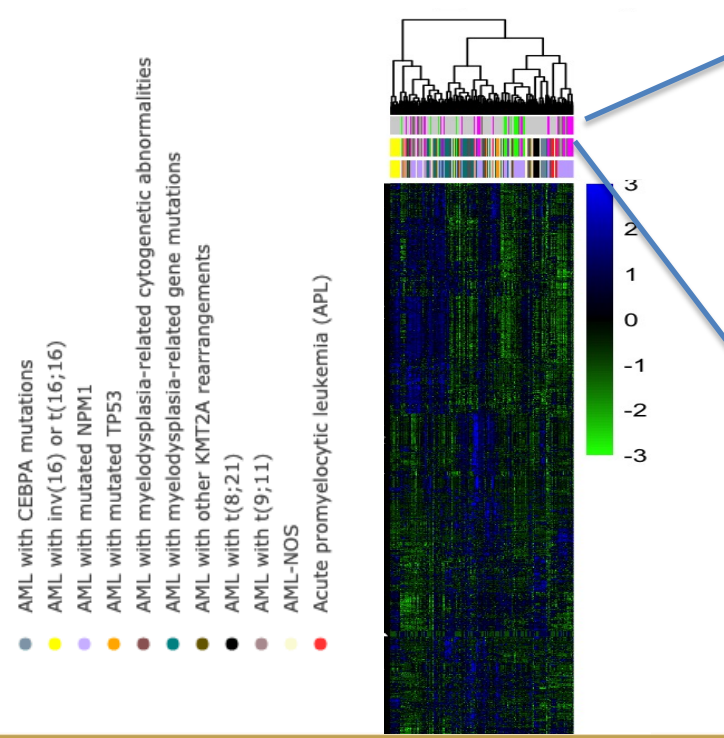
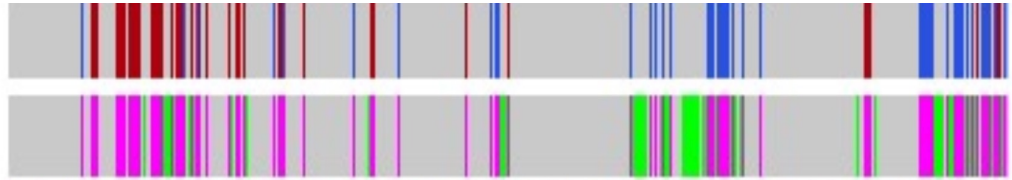
Elderly (75+ years)



Transcriptomic Dissection of NPM1mut/FLT3-ITDwt AML Reveals Two Biologically Distinct Subgroups





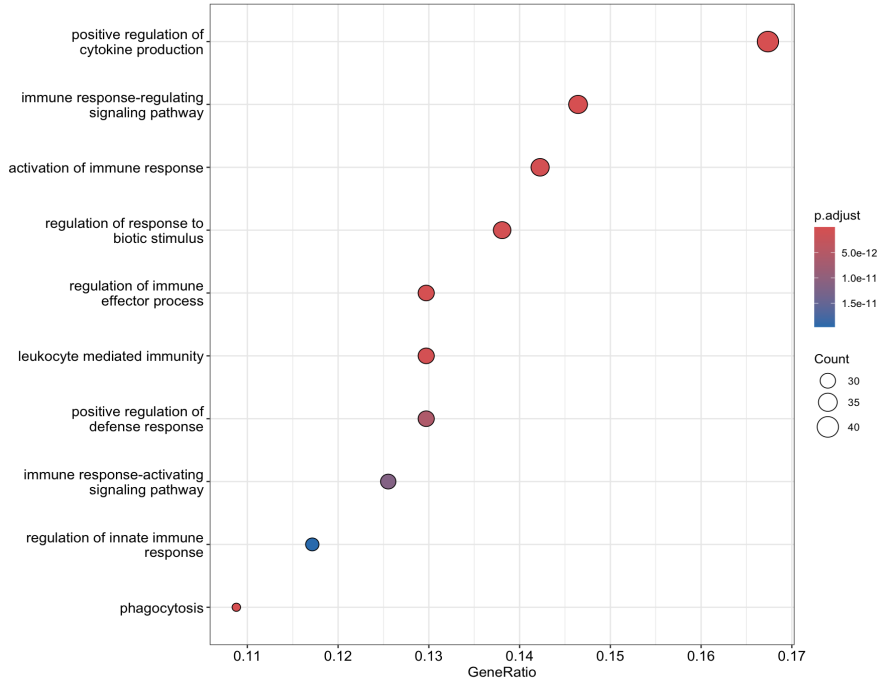
NPM1mut Flt3ITD-neg AML

NPM1mut Flt3ITD AML

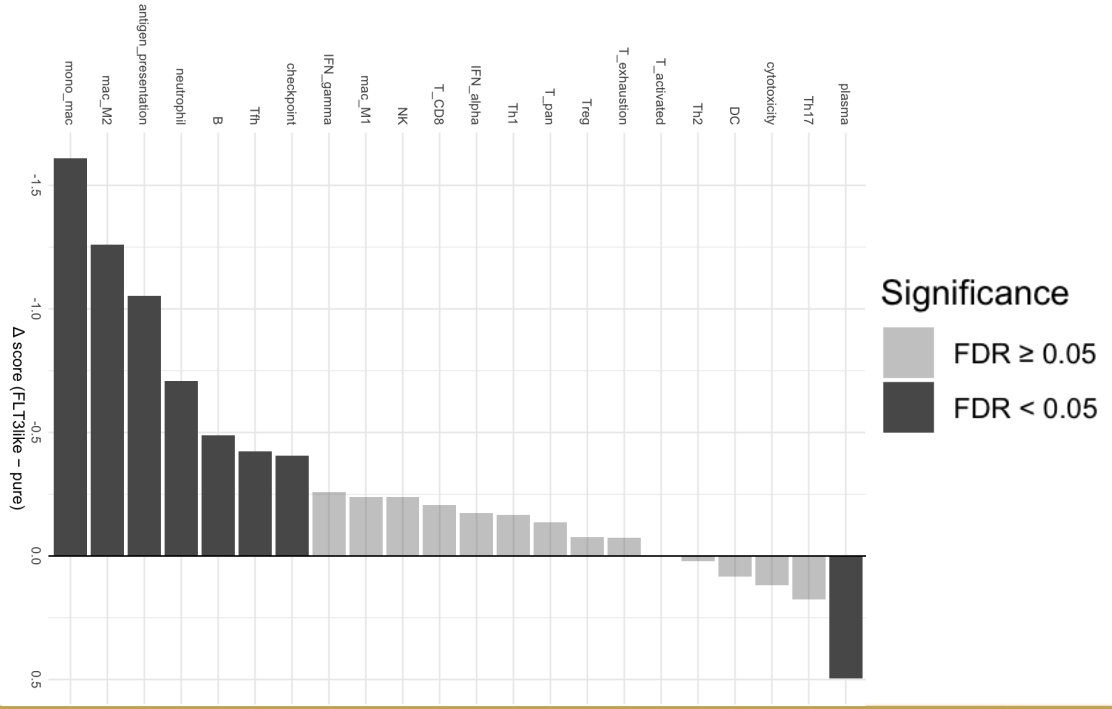
NPM1- “Pure” AML

“FLT3^{LIKE}” NPM1mut AML

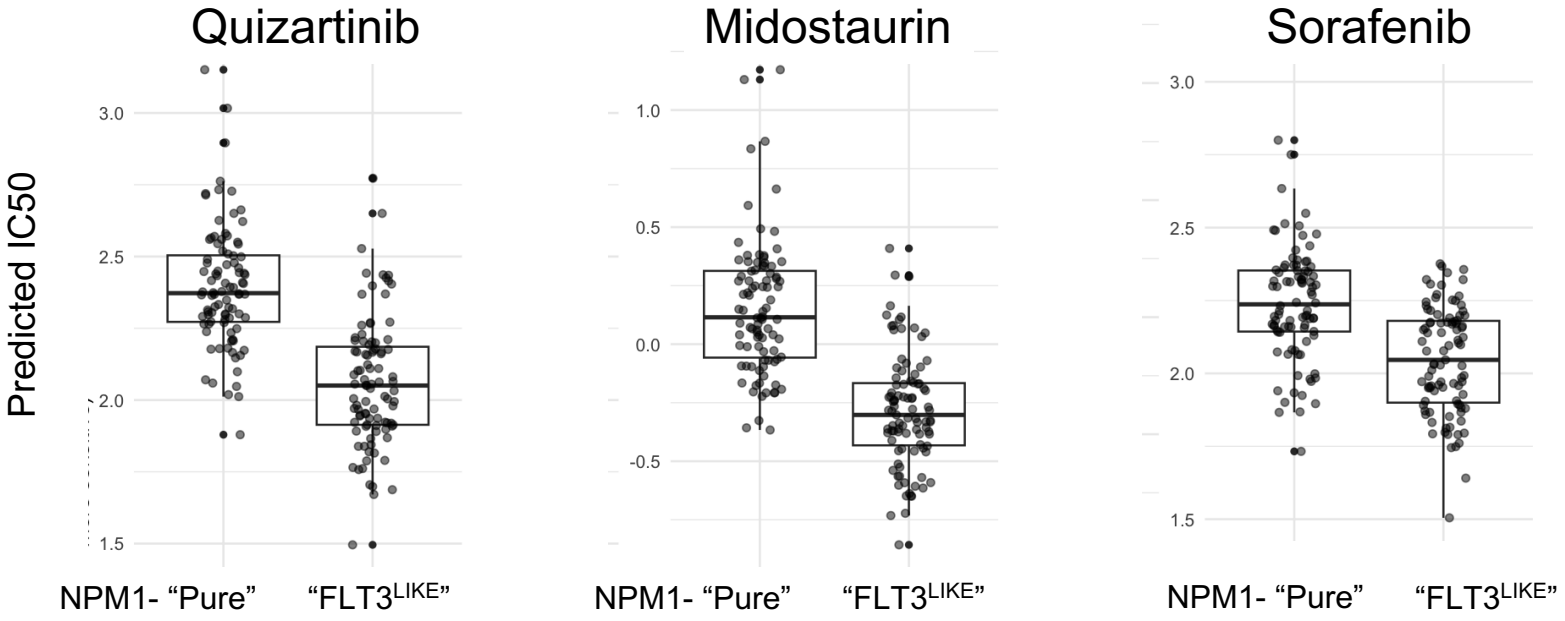
NPM1- “Pure” AML subgroup is highly enriched in genes related to inflammation and immune response



NPM1mut/FLT3-ITDneg subgroups shows different immune signatures

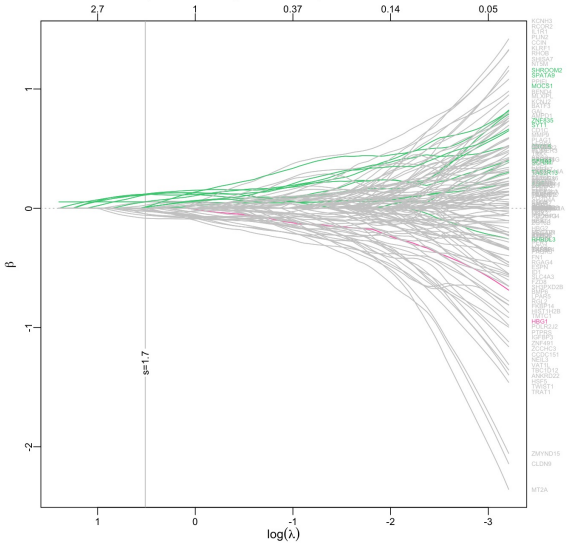


"FLT3^{LIKE}" NPM1mut AML showed a predicted drug sensitivity to FLT3-inhibitors

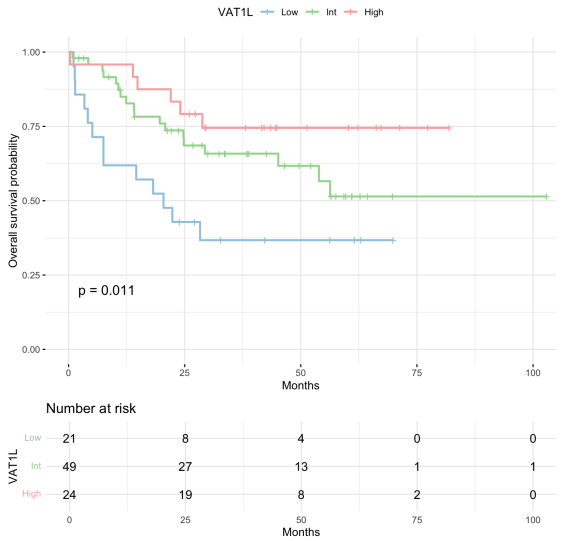


Genes expression may stratify prognosis within the NPM1-mut/FLT3-wt AML

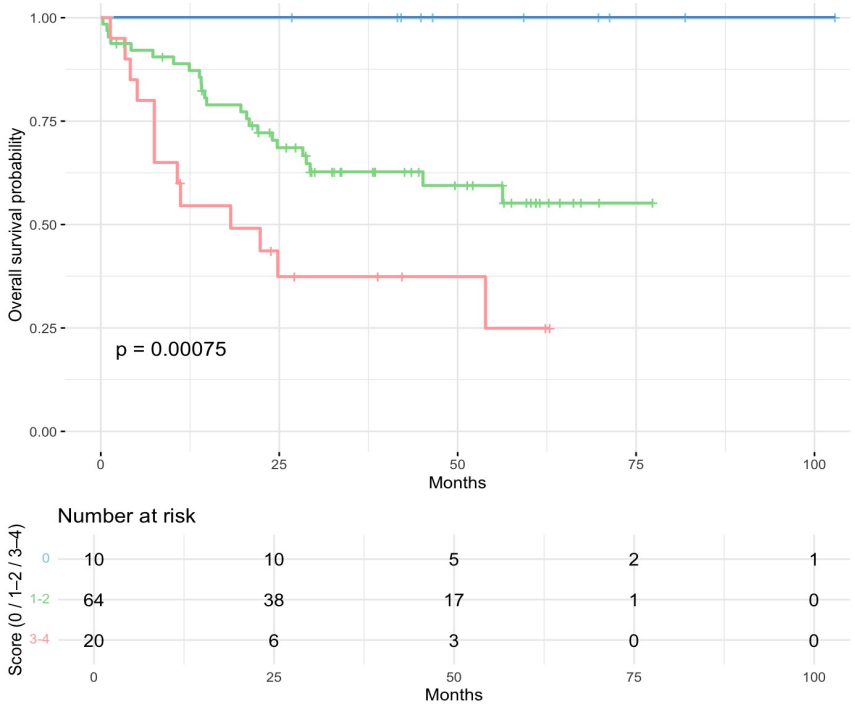
Adaptive Lasso Cox model
NPM1-mut/*FLT3*-wt AML, Age < 60 years



Kaplan-Meier
NPM1-mut/FLT3-wt, Age < 60 years



Genes expression may stratify prognosis within the NPM1-mut/FLT3-wt AML



4-gene-panel scoring system

- 0 point
- 1-2 points
- 3-4 points

Summary (1)

We designed the **“Gu” algorithm** to harmonize large-scale RNA-seq data integration across studies, assembling one of the most extensive AML dataset to date (n=894).

The integrated dataset maintains **clinical and biologic coherence** and allows deep molecular exploration within clinically significant AML subgroups (i.e. n=308 *NPM1*-mut AML (187 *FLT3*-wt – ‘favourable’, and 121 *FLT3*-ITD – ‘intermediate’).

Summary (2)

Our analysis confirms the peculiar characteristics of ***NPM1*-mut AML entity** (i.e. HOX signature). Transcriptomics is consistent across age, suggesting there are no biological differences within the same entity.

Our analyses identify two biologically distinct subgroups with different mutational profile, immune microenvironment and drug sensitivity, paving the way for new translational insights.

Our dataset represents a valuable resource for searching for **new prognostic/biological biomarkers**, even through machine-learning (ML) algorithms.

University and Hospital of Perugia

Director:

- **Maria Paola Martelli**

Lab Team:

- **Gaetano Cimino**
- Francesca Milano
- Roberta Ranieri
- Valeria Cardinali
- Barbara Bigerna

… and all the clinicians and lab collegues!

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- M. Lim
- P. Aucken

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- Marika Alborghetti

… and all the clinicians and lab collegues!

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- Daniele Remotti
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- Giuseppe Topini
- Loredana Bassi
- Gloria Pessina

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