miRNA and lncRNA as support for myeloma plasma cells

Antonino Neri
Scientific Directorate

Laboratory of Translational Research
Azienda USL-IRCCS di Reggio Emilia

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale di Reggio Emilia
IRCCS Istituto in tecnologie avanzate e modelli assistenziali in oncologia

No disclosures

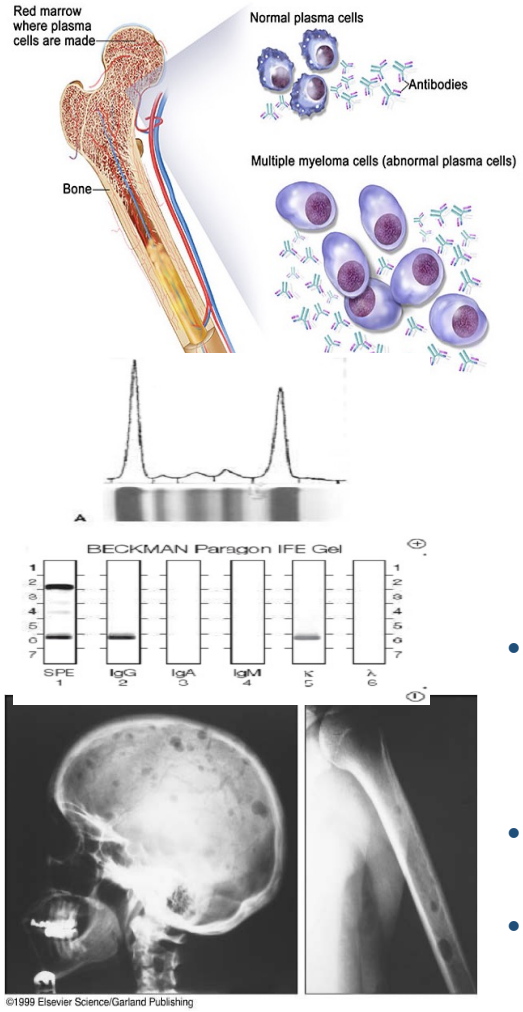
AUSL-IRCCS
TRANSLATIONAL RESEARCH
LABORATORY
REGGIO EMILIA - ITALY

OECI
COMPREHENSIVE
Cancer Centre

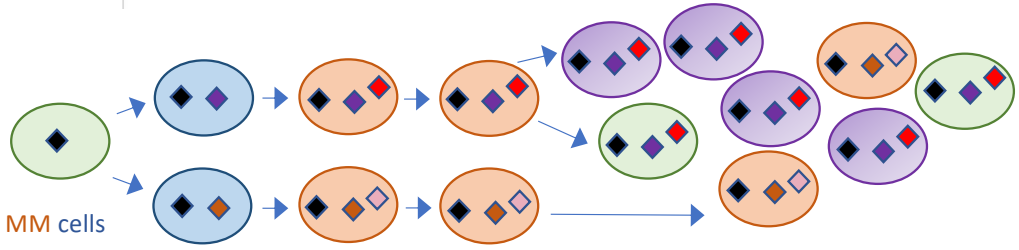
Multiple Myeloma

Plasma cell: post germinal switched terminally differentiated B-cell

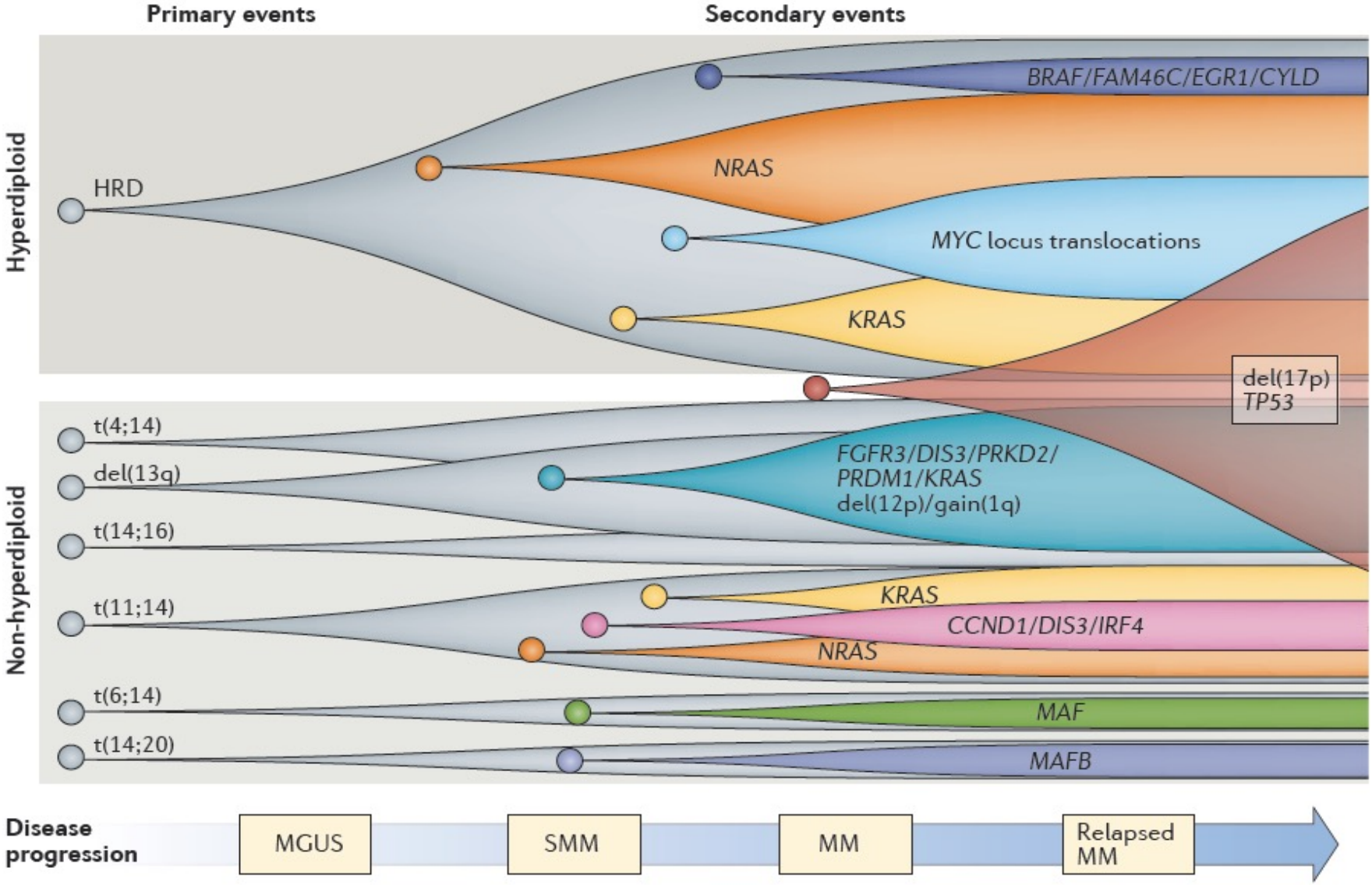
- 1% of cancer
- 10% of hematologic malignancies
- 20% of deaths from hematologic malignancies



- Monoclonal component in the serum
- End-organ damage
- High genomic instability



Molecular Pathogenesis and genetic architecture of MM: Two main models



HYPERDIPLOIDY

Mechanisms ???

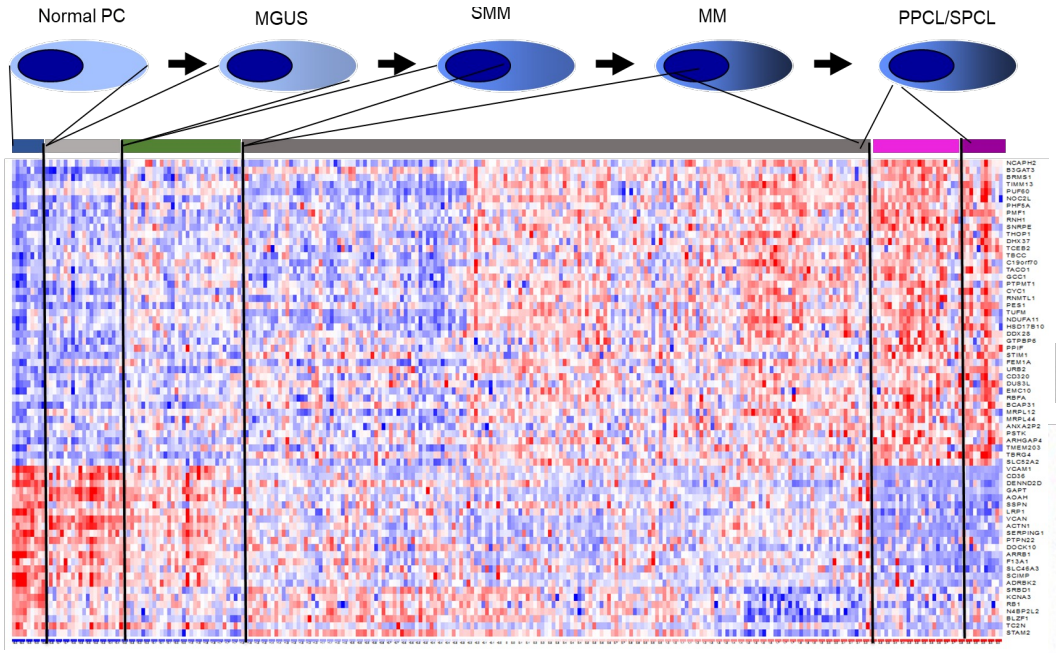
Trisomies
3,5,7,9,11,15,19,21

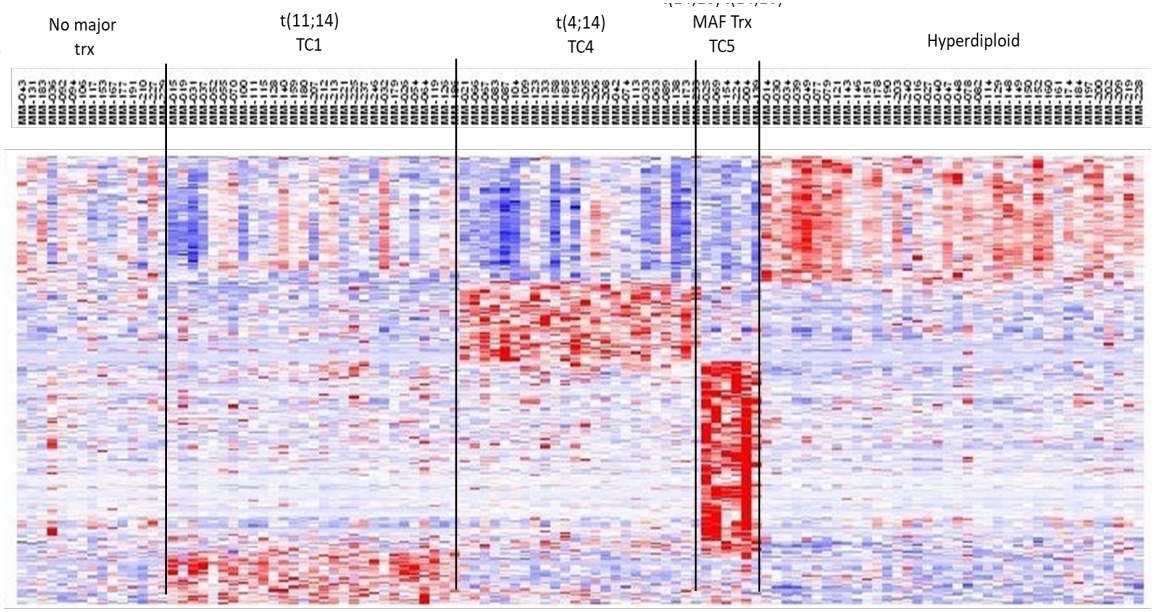
NON-HYPERDIPLOIDY

Class-switch recombination
In germinal center

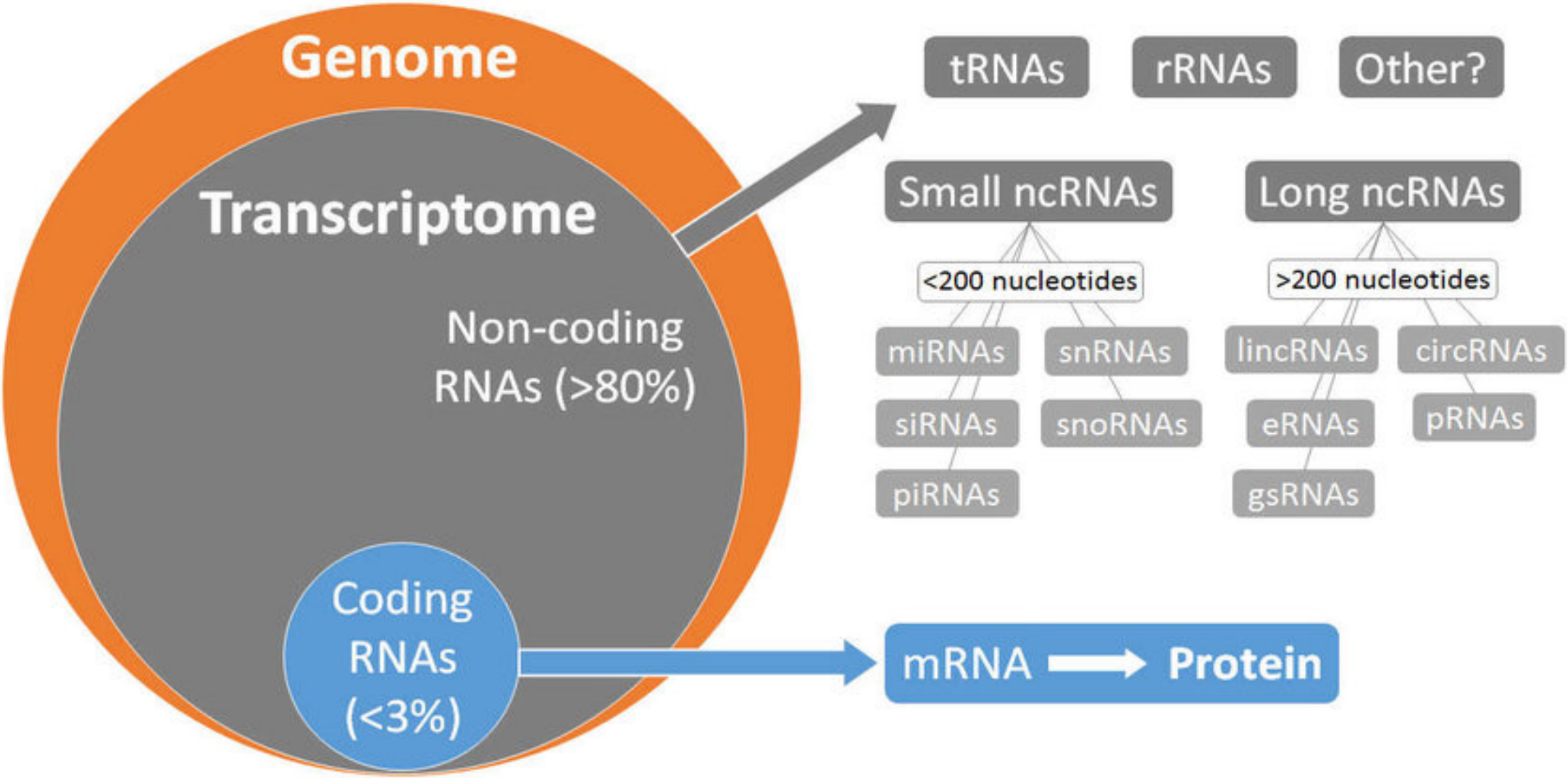
IGH TRX

Gene expression profiling reveals MM-stages specific transcriptomic signatures and molecular patterns associated with distinct genomic lesions





Mattioli et al. Oncogene, 2005; Agnelli et al., J Clin Oncol 2005; Todoerti K et al., Clin Cancer Res 2013


Genome
Transcriptome
Non-coding
RNAs (>80%)
Coding
RNAs
(<3%)
tRNAs
rRNAs
Other?
Small ncRNAs
Long ncRNAs
<200 nucleotides
>200 nucleotides
miRNAs
snRNAs
siRNAs
snoRNAs
piRNAs
lincRNAs
circRNAs
eRNAs
pRNAs
gsRNAs
mRNA
Protein

blood
2009 114: 20-26
Prepublished online Oct 21, 2009;
doi:10.1182/blood-2009-08-237495

Identification of microRNA expression patterns and definition of a microRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma

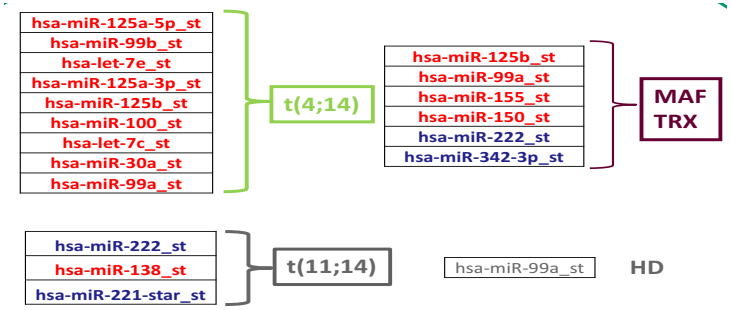
Marta Lionetti, Marta Biasiolo, Luca Agnelli, Katia Todoerti, Laura Mosca, Sonia Fabris, Gabriele Sales, Giorgio Lambertenghi Deliliers, Silvio Bicciato, Luigia Lombardi, Stefania Bortoluzzi and Antonino Neri

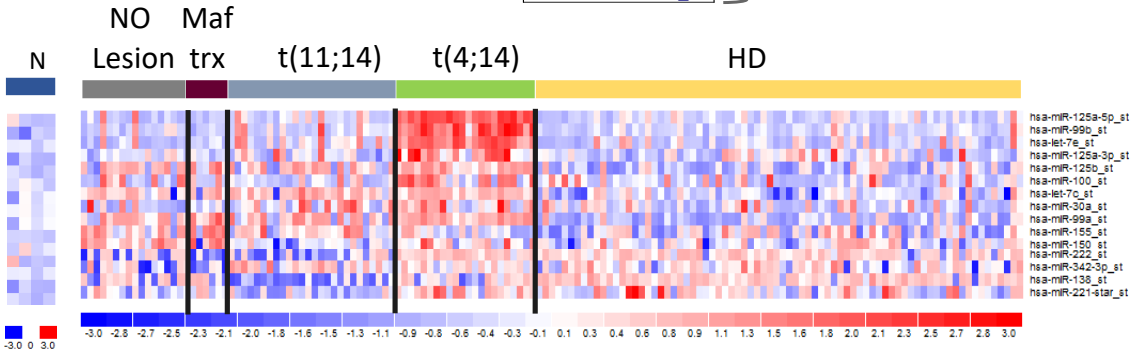
bjh research paper

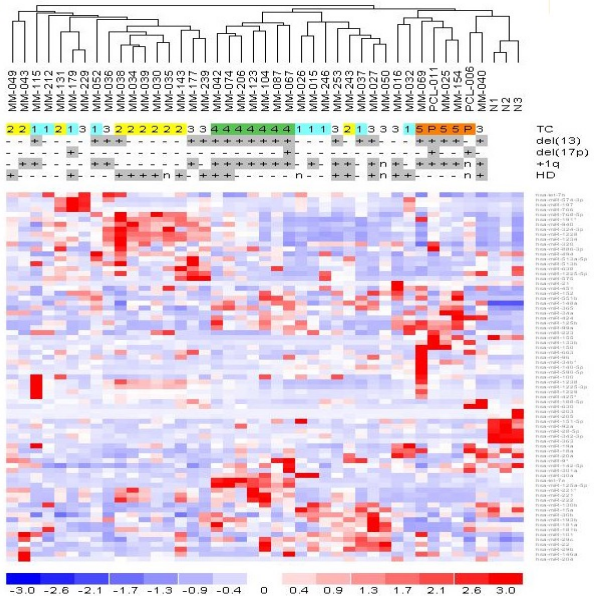
Improved risk stratification in myeloma using a microRNA-based classifier

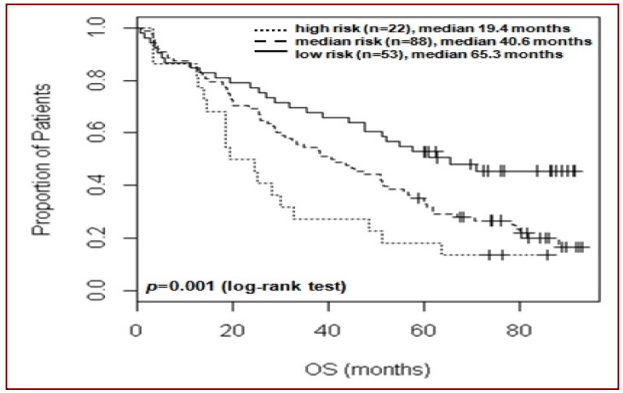
Ping Wu,[1,*] Luca Agnelli,[2,3,*] Brian A. Walker,[1] Katia Todoerti,[2,3] Marta Lionetti,[2,3] David C. Johnson,[1] Martin Kaiser,[1] Fabio Mirabella,[1] Christopher Wardell,[1] Walter M. Gregory,[4] Faith E. Davies,[1] Daniel Brewer,[5] Antonino Neri[2,3,†] and Gareth J. Morgan[1,†]

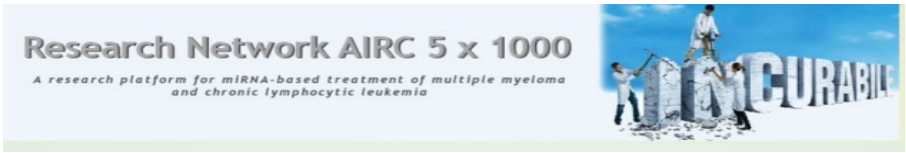
MRC Myeloma IX trial: 163 patients





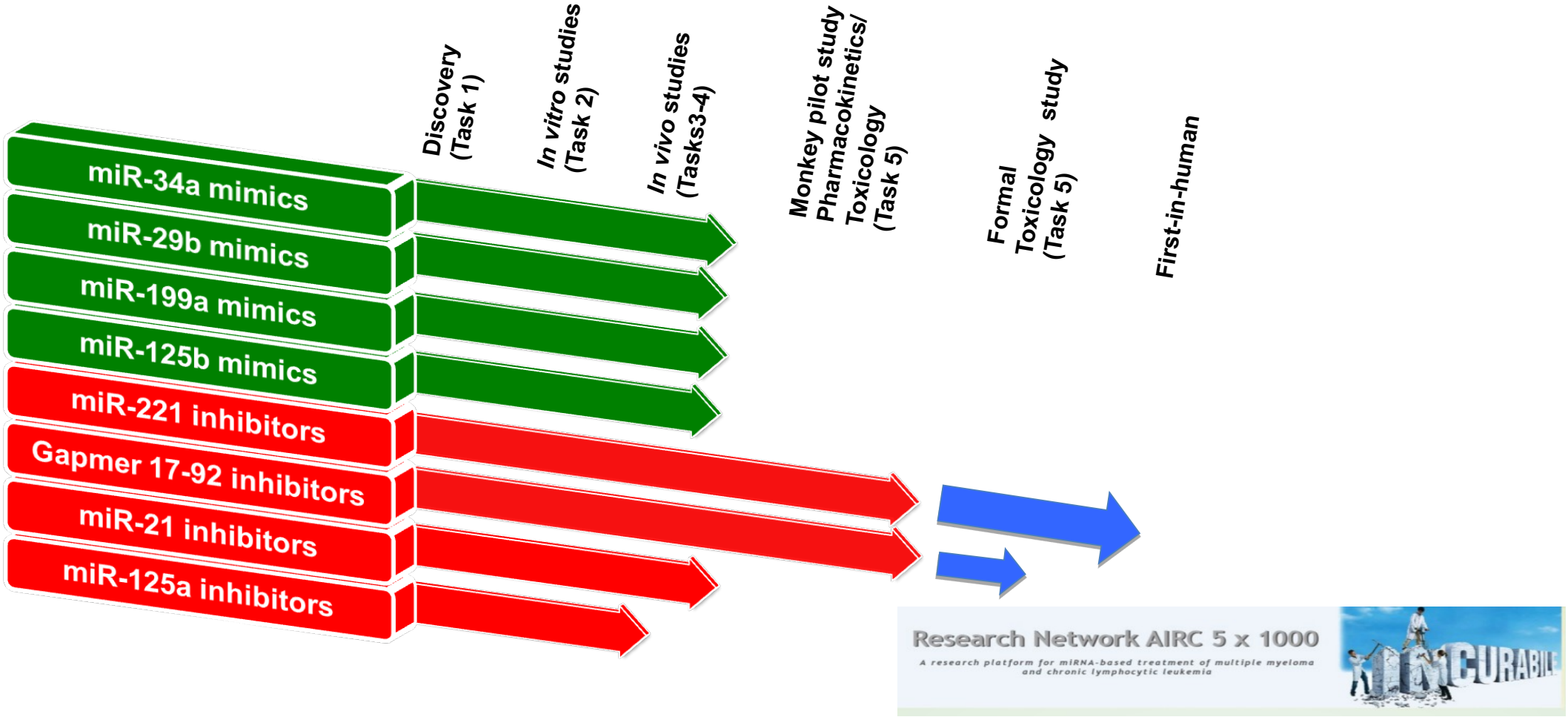






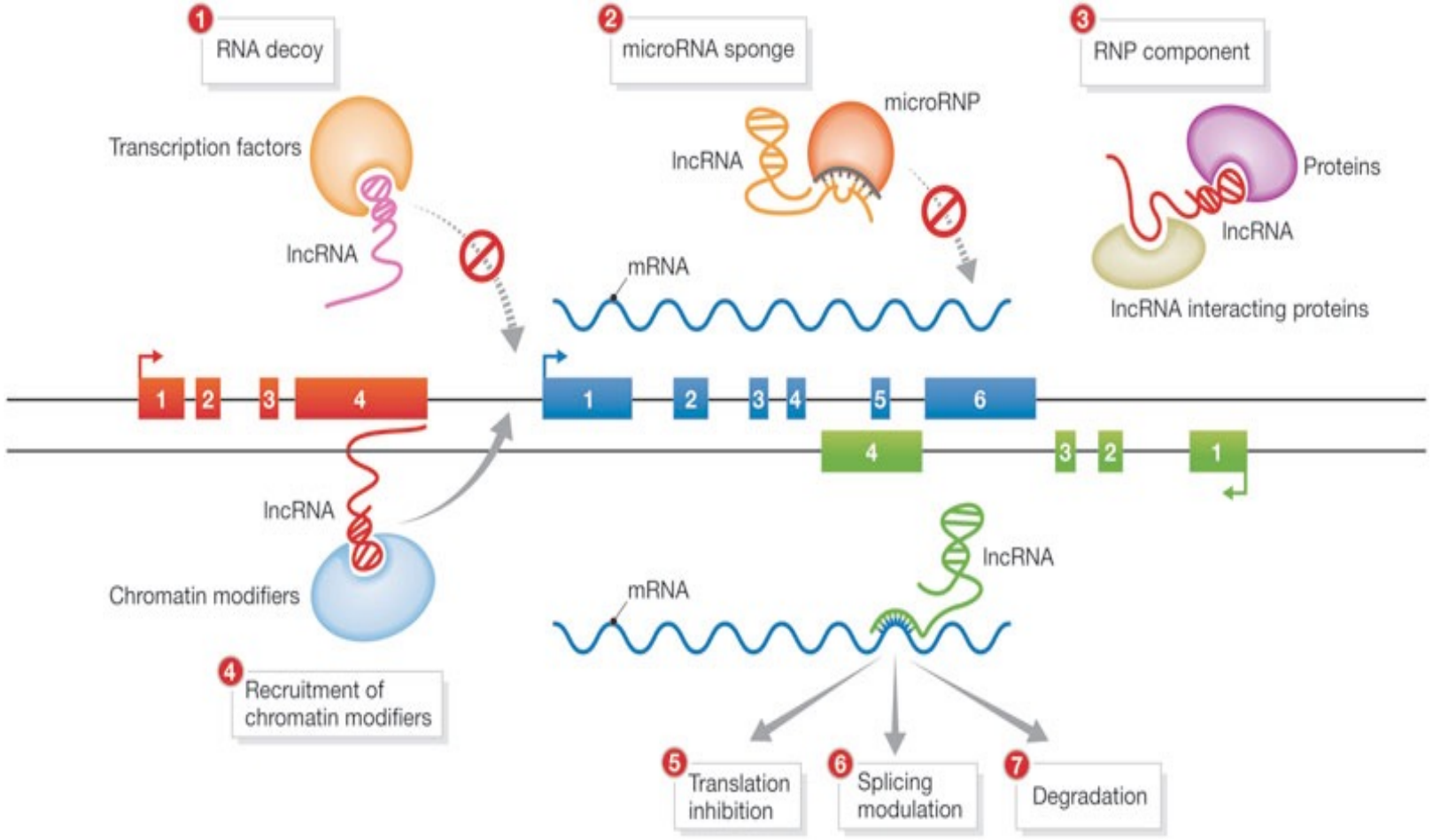
Results achieved within the AIRC 5x1000 Network

Project pipeline for Multiple Myeloma treatment

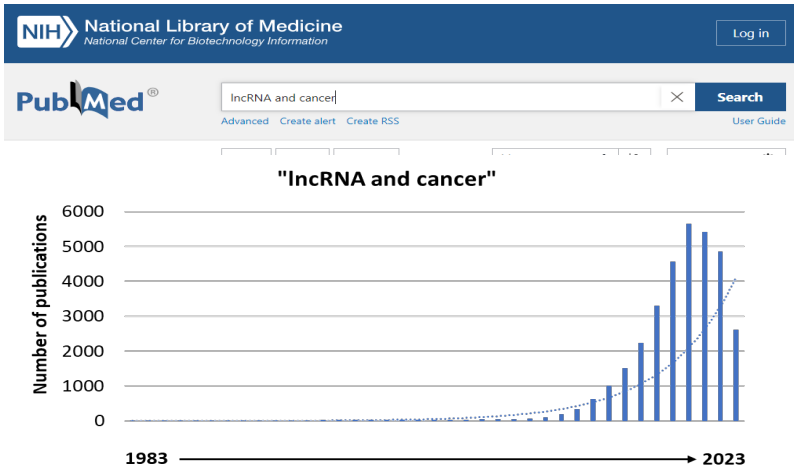


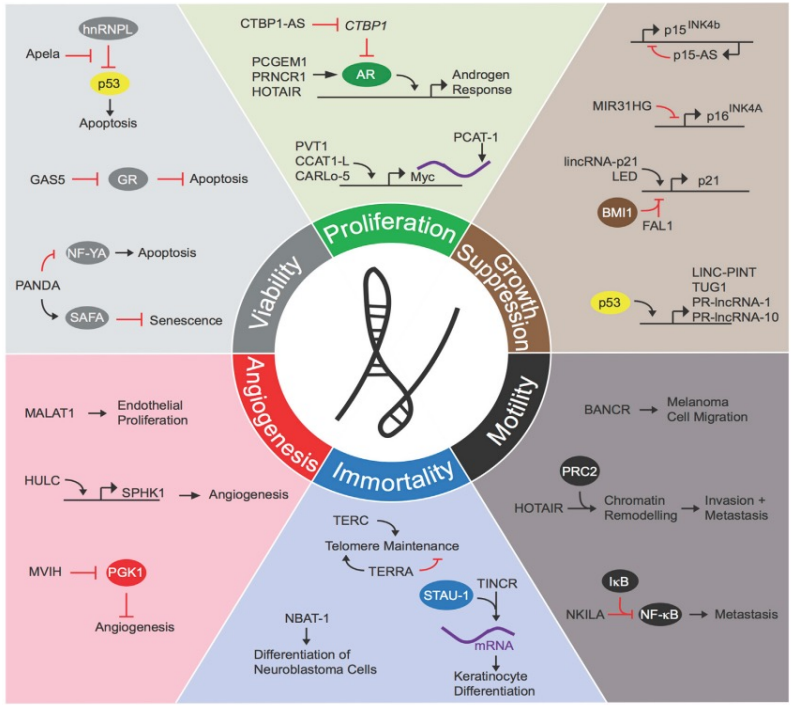
Long non-coding RNA: *non coding transcripts longer than 200 bp*

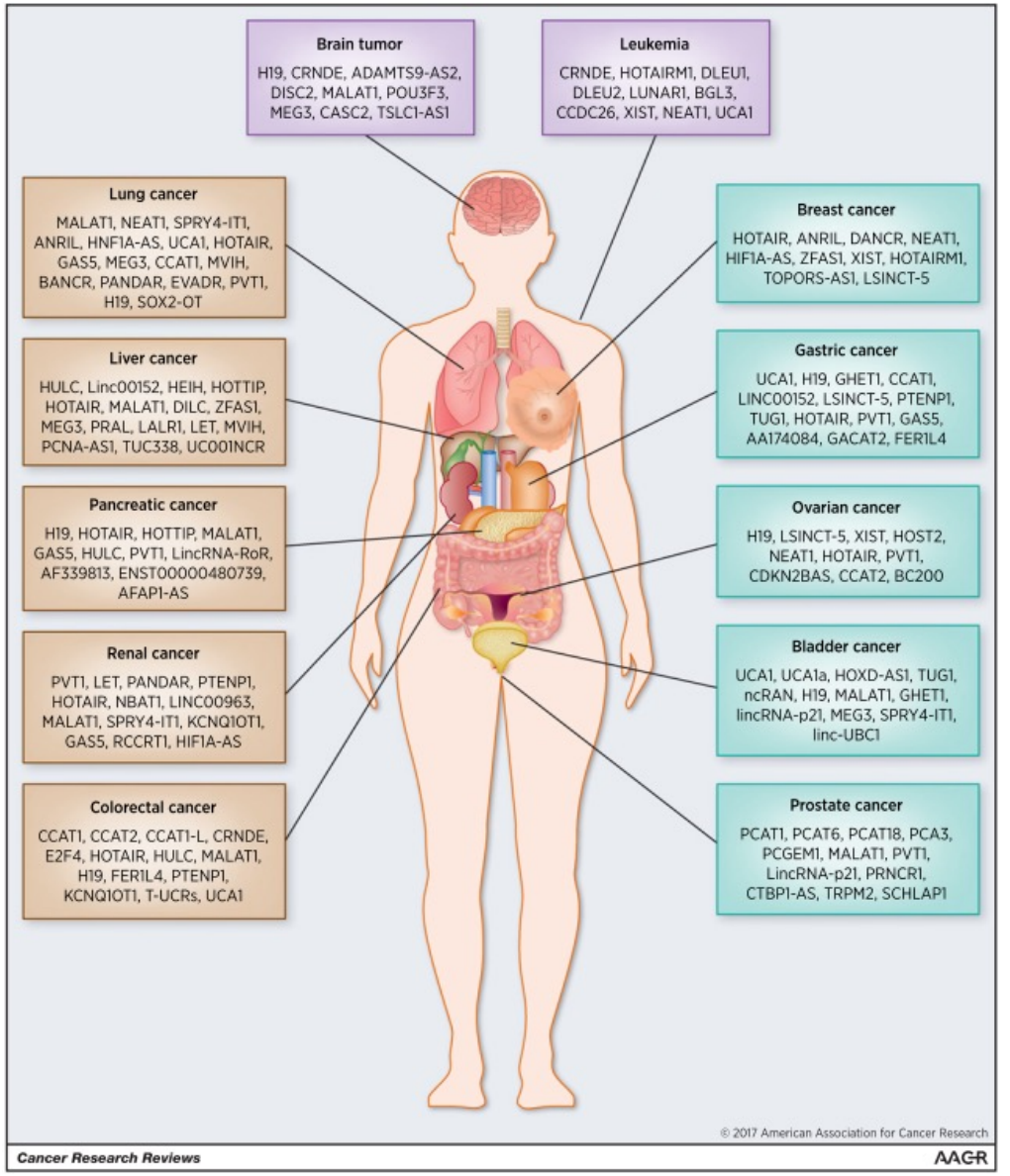
- **≈ 100.000 distinct sequences annotated**
- **Low expression compared to mRNA**
- **not evolutionary conserved**
- **specific expression in normal and pathological tissues**



lncRNA and cancer







Bhan, A. et al. (2017). Cancer research

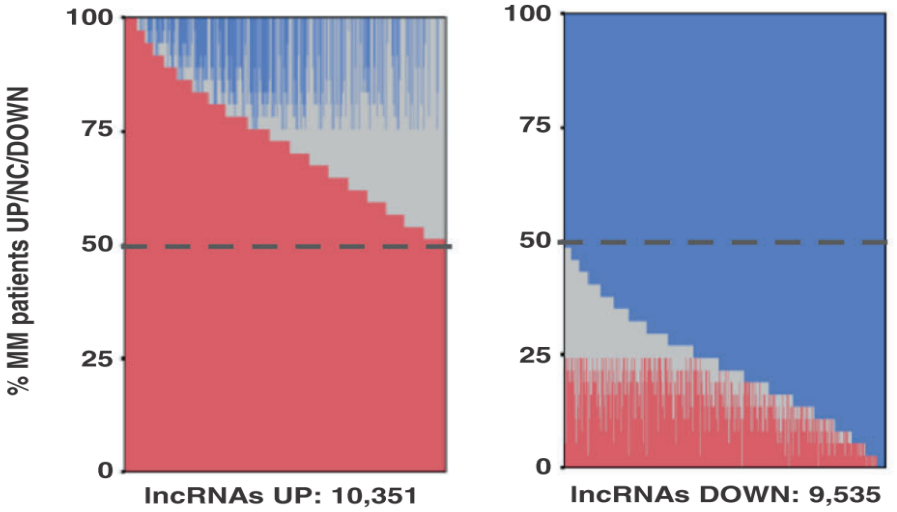
Leukemia (2021) 35:1438–1450
https://doi.org/10.1038/s41375-021-01147-y

ARTICLE

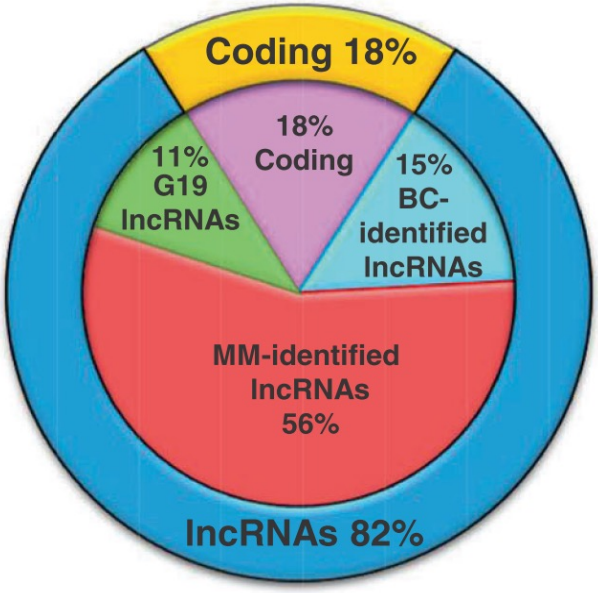
MULTIPLE MYELOMA, GAMMOPATHIES

Characterization of complete lncRNAs transcriptome reveals the functional and clinical impact of lncRNAs in multiple myeloma

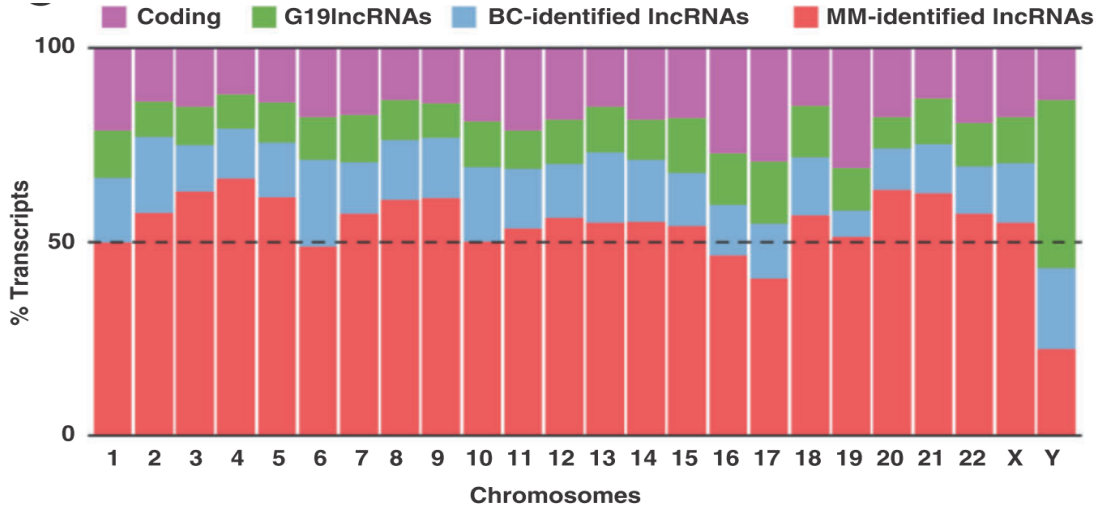
Arantxa Carrasco-Leon[1,2] · Teresa Ezponda[1,2] · Cem Meydan[3,4,5] · Luis V. Valcárcel[1,6] · Raquel Ordoñez[1,2] · Marta Kulis[7,8] · Leire Garate[1,2] · Estíbaliz Miranda[1,2] · Victor Segura[9] · Elisabeth Guruceaga[9] · Amaia Vilas-Zornoza[1,2] · Diego Alignani[2,10] · Marién Pascual[1] · Ane Amundarain[1,2] · Laura Castro-Labrador[1] · Patxi San Martín-Uriz[1] · Halima El-Omri[11] · Ruba Y. Taha[11] · Maria J. Calasanz[2,12] · Francisco J. Planes[6] · Bruno Paiva[2,10,12] · Christopher E. Mason[3,4,5] · Jesús F. San Miguel[2,13] · José I. Martin-Subero[2,8,14,15] · Ari Melnick[3] · Felipe Prosper[1,2,13] · Xabier Agirre[1,2]



LncRNAs represent the vast majority of MM transcriptome



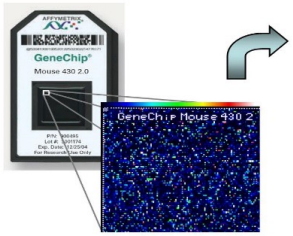
Coding and long non-coding genes are uniformly distributed among chromosomes



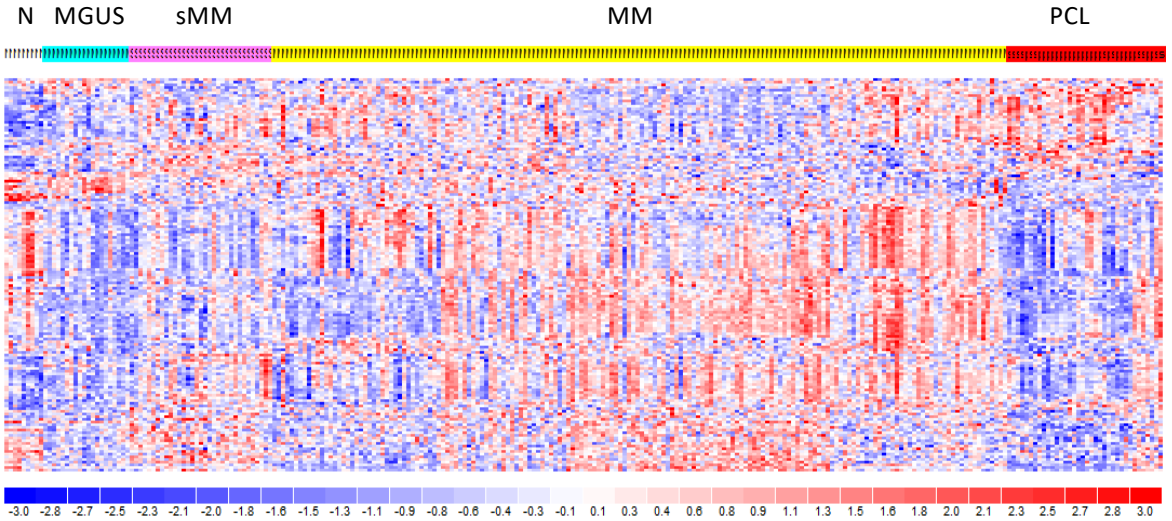
www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

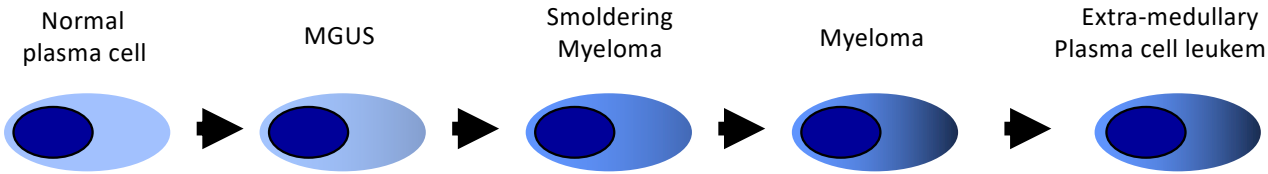
Distinct lncRNA transcriptional fingerprints characterize progressive stages of multiple myeloma

Domenica Ronchetti[1,2,*], Luca Agnelli[1,2,*], Elisa Taiana[1,2], Serena Galletti[1,2], Martina Manzoni[1,2], Katia Todoerti[3], Pellegrino Musto[3], Francesco Strozzi[4], Antonino Neri[1,2]



1852 LncRNAs investigated





lnc-LRRC47-1
lnc-SNX29P2-3
lnc-HSFY2-10
lnc-IRF2-3
lnc-VKORC1L1-3
lnc-STOM-7

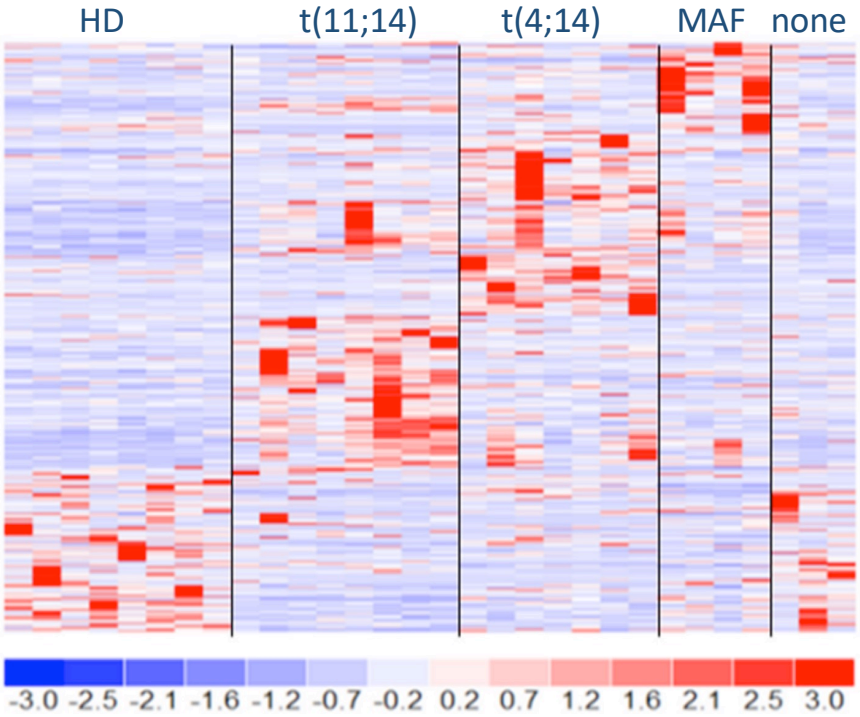
lnc-RLIM-6
lnc-ANGPTL1-3
lnc-SENP5-4
lnc-WHAMM-2
lnc-SERPINC1-1
lnc-CPSF2-2
lnc-RALGAPB-1
lnc-MC2R-2
lnc-SAFB2-3
lnc-KIF20B-7
lnc-DNAJC16-1
lnc-WDR11-7
lnc-PNRC1-1
lnc-DLX5-4
lnc-ZC3H12B-10

Ronchetti et al., Oncotarget 2016

lncRNA transcriptional signatures in MM

RNAseq 14202 lncRNAs

Expressed in MM 9540 lncRNAs



OPEN

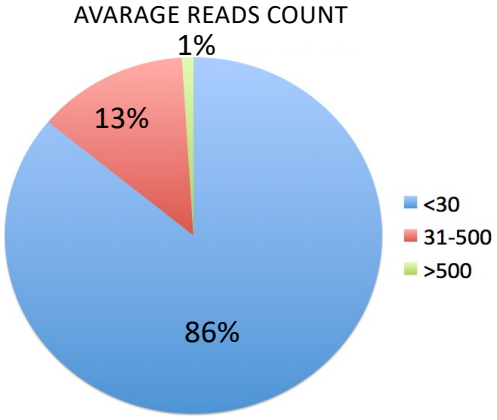
A compendium of long non-coding RNAs transcriptional fingerprint in multiple myeloma

Received: 19 January 2018
Accepted: 5 April 2018
Published online: 26 April 2018

Domenica Ronchetti[1,2], Luca Agnelli[1,2], Alessandro Pietrelli[3,4], Katia Todoerti[1], Martina Manzoni[1,2], Elisa Taiana[1,2] & Antonino Neri[1,2]

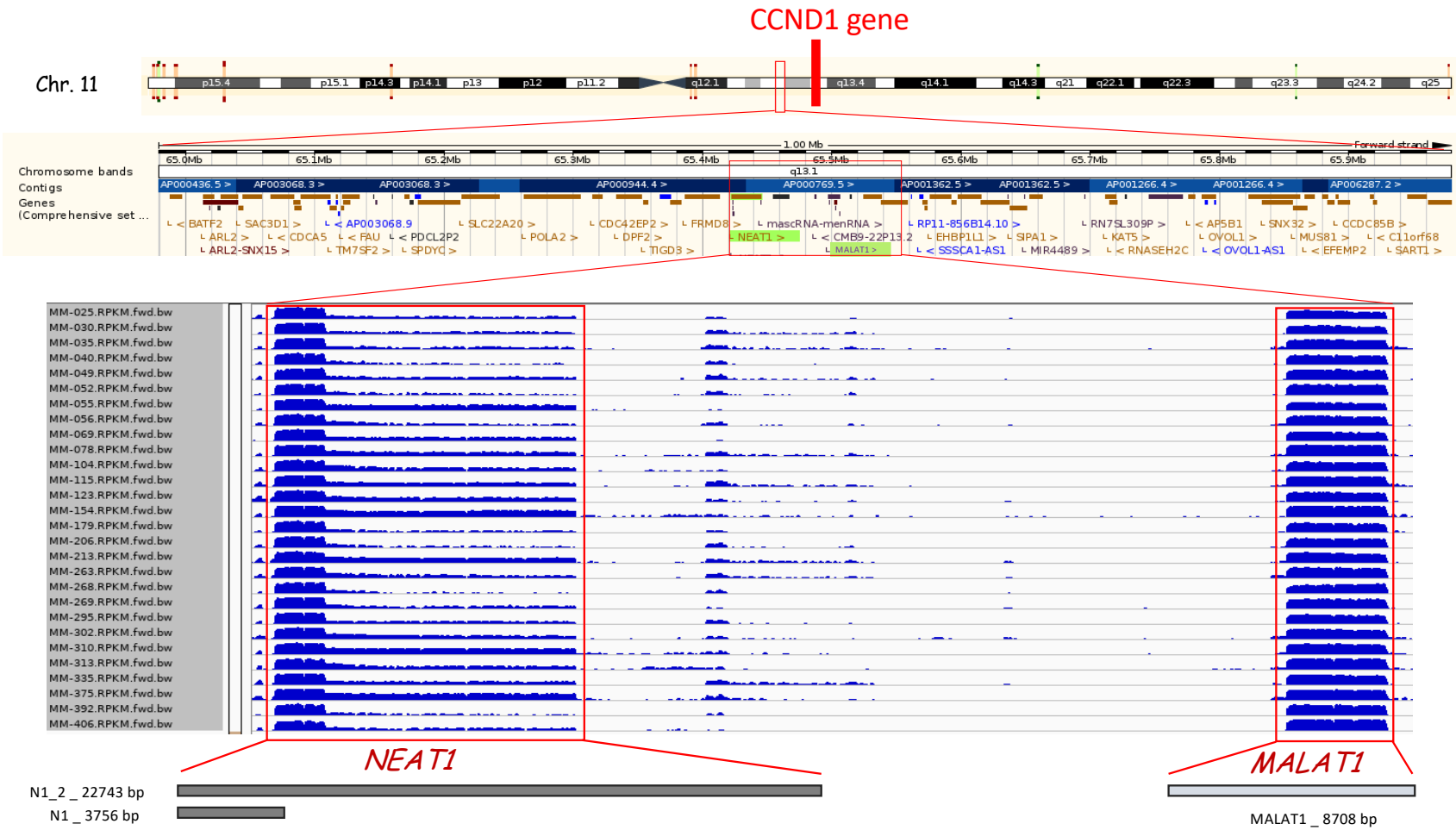
Ronchetti et al. Scientific Reports, 2018

12 lncRNAs are very highly expressed displaying an average read counts >5000, counting 64% of the reads assigned to lncRNAs



Log2 expression >10	chromosome
LINC01001	11p15
NEAT1	11q13
MALAT1	11q13
RP11-658F2.8	11q13
RP11-736K20.5	11q14
LINC01089	12q24
LRRC75A-AS1-014	17p11
LINC01480	19q13
RP11-161I10.1	1q31
TUG1	22q12
AC074289.1	2p14
FGD5-AS1	3p25
RP11-325F22.2	7q22
LINC-PINT	7q32
EBLN3	9p13
FTX	Xq13

Genomic localization of lncRNA NEAT1 and MALAT1 and RNAseq profile in MM patients stratified according the major genomic aberrations



Ronchetti et al, Scientific Report, 2018
Taiana et al , Haematologica, 2018

MALAT1 and NEAT1: two cancer-associated lncRNAs

The Long Noncoding RNA Malat1: Its Physiological and Pathophysiological Functions

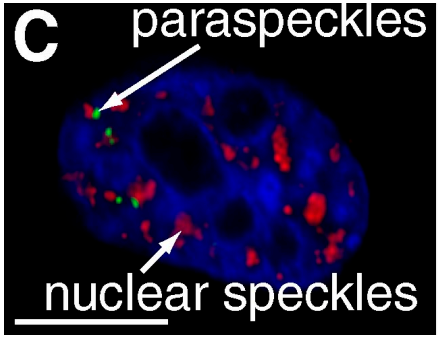
Xuejing Zhang, Milton H. Hamblin & Ke-Jie Yin

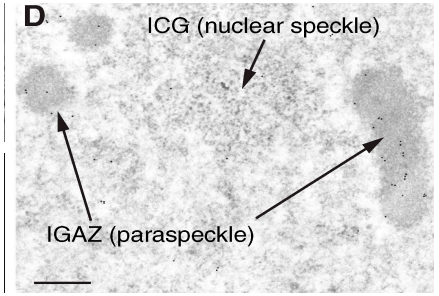
Biomed Pharmacother. 2017 Nov;95:822-828. doi: 10.1016/j.biopha.2017.09.005. Epub 2017 Sep 12.

Long non-coding RNA MALAT1 promotes proliferation and invasion via targeting miR-129-5p in triple-negative breast cancer.

Zuo Y[1], Li Y[2], Zhou Z[2], Ma M[2], Fu K[2].

MALAT1 **Localized to nuclear speckles**





Upregulated in many human malignancies including lung cancer, bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, melanoma, neuroblastoma, ovarian cancer, prostate cancer and renal cell carcinoma

MALAT1 knockdown significantly inhibits cell motility in vitro and significantly limits metastasis formation in mouse cancer models

Received: 3 September 2016 | Accepted: 10 December 2016
DOI: 10.1111/cpr.12329

REVIEW ARTICLE

WILEY Cell Proliferation

NEAT1: A novel cancer-related long non-coding RNA

Xin Yu[1] | Zheng Li[2] | Heyi Zheng[1] | Matthew T. V. Chan[3] | William Ka Kei Wu[3,4]

2017, VOL. 16, NO. 2, 137–138
http://dx.doi.org/10.1080/15384101.2016.1235847
Taylor & Francis Taylor & Francis Group

EDITORIALS: CELL CYCLE FEATURES

NEAT1-containing paraspeckles: Central hubs in stress response and tumor formation

Carmen Adriaens[a,b] and Jean-Christophe Marine[a,b]

[a]Laboratory for Molecular Cancer Biology, Center for the Biology of Disease, VIB, Leuven, Belgium; [b]Laboratory for Molecular Cancer Biology, Center for Human Genetics, KULeuven, Leuven, Belgium

NEAT1 **Scaffold for nuclear paraspeckles**

Upregulated in many human malignancies, including lung, esophageal and gastric cancers but downregulated in acute promyelocytic leukaemia

NEAT1 knockdown significantly suppressed cell proliferation and increased apoptosis in laryngeal squamous cell carcinoma, breast cancer and many other cell line

In colorectal cancer functionally, knockdown of NEAT1-1 decreased cell proliferation and invasion in vitro. Furthermore, high expression of NEAT1-1 was associated with poorer overall survival

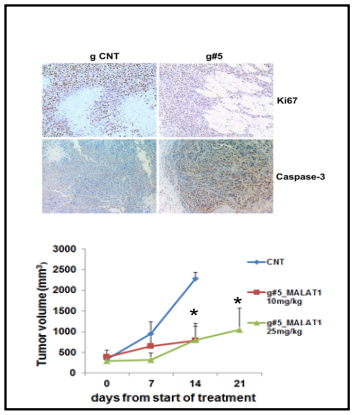
Leukemia

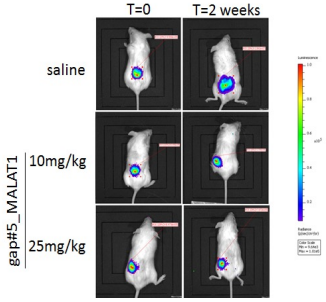
Leukemia. 2018 Feb 22. doi: 10.1038/s41375-018-0067-3. [Epub ahead of print]

Drugging the lncRNA MALAT1 via LNA gapmeR ASO inhibits gene expression of proteasome subunits and triggers anti-multiple myeloma activity.

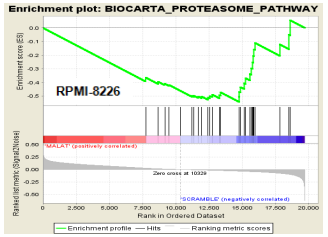
Amodio N[1], Stamato MA[1], Juli G[1], Morelli E[1], Fulciniti M[2], Manzoni M[3,4], Taiana E[3,4], Agnelli L[3,4], Cantafio MEG[1], Romeo E[1], Raimondi L[5], Caracciolo D[1], Zuccalà V[6], Rossi M[1], Neri A[3,4], Munshi NC[2,7], Tagliaferri P[1], Tassone P[8,9].

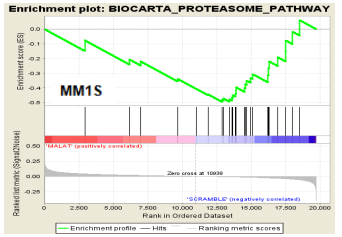
Strong antiproliferative effect of MALAT1 silencing by gymnotic Gapmer delivery in myeloma cells in vitro and in vivo



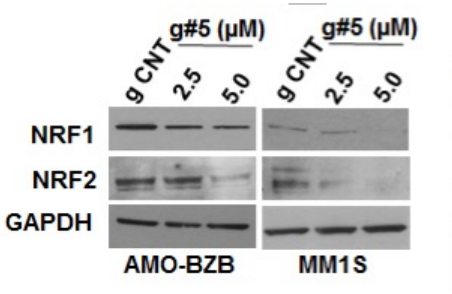


MALAT-1 depletion reduces proteasome gene expression in MM cells in vitro

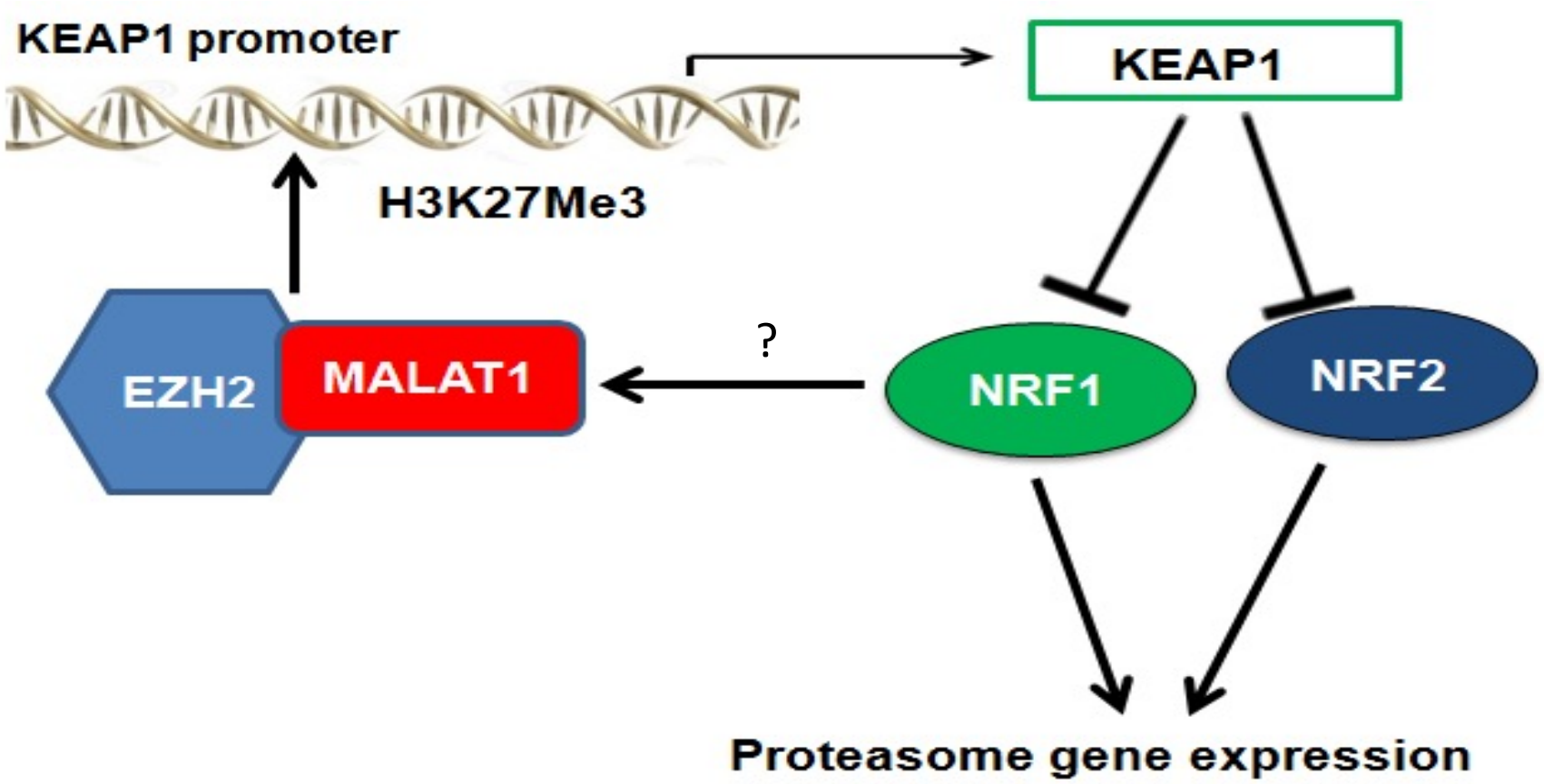




MALAT-1 silencing induces down regulation of transcription factors involved in proteasome gene activation

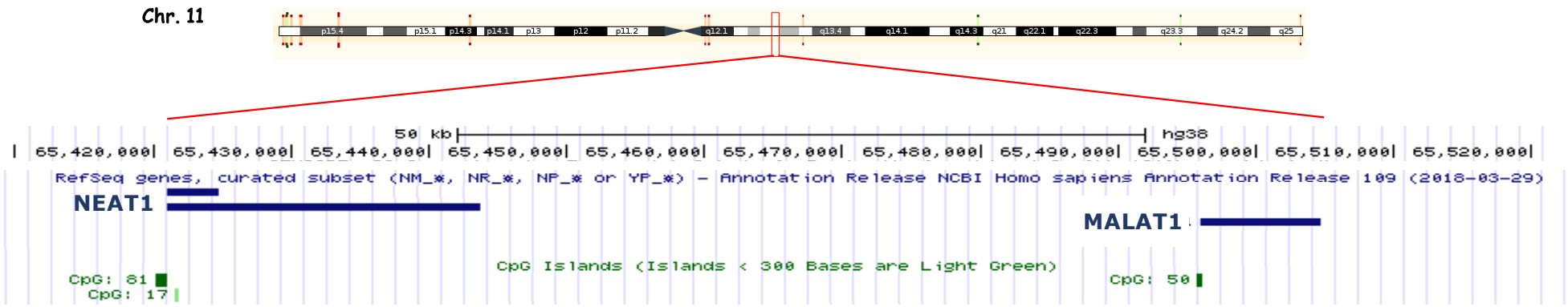


MALAT1 inhibition may target the proteasome in MM cells
by upregulating KEAP1 which in turn negatively regulates NFR2 and NFR2



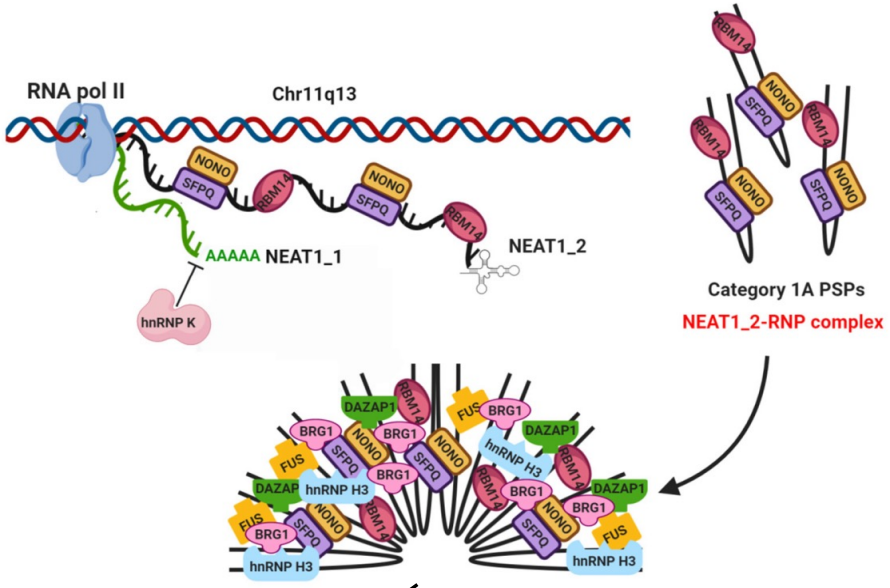
Amodio et al. Leukemia, 2018

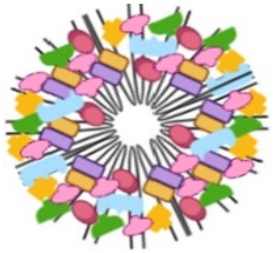
NEAT1



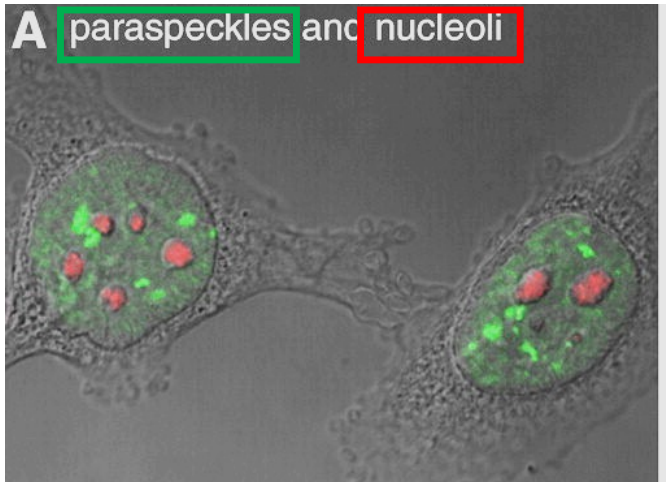
- architectural lncRNA
- located on chromosome 11
- abundantly express
- nuclear localization
- encodes for 2 different transcript variants (both single exon, 3.7 Kb and 23 Kb)
- **the long NEAT1 variant (NEAT1_2) represents the essential architectural component of paraspeckle nuclear bodies**

Paraspeckles

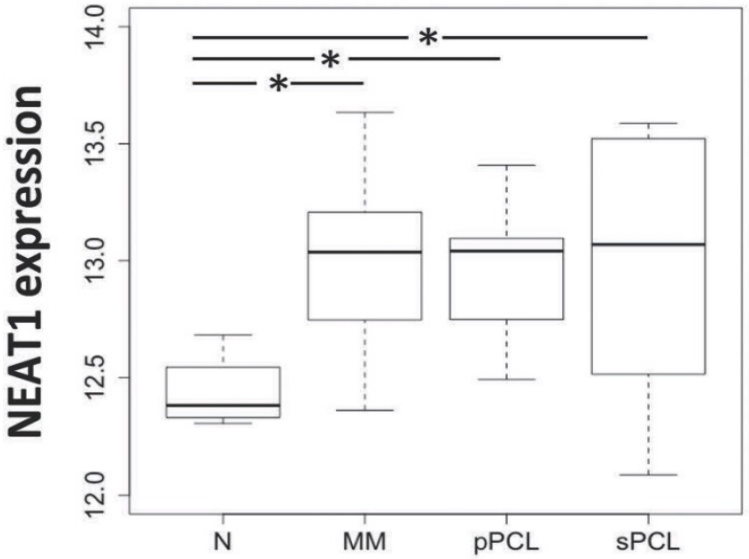




Paraspeckle



- **Paraspeckles (PSs)** are a class of dynamic subnuclear bodies found in the interchromatin space of mammalian cells.
- They are RNA-protein structures formed by the interaction between **NEAT1** and **essential proteins**: **NONO, SFPQ and FUS**. It is shown that more than 60 different RNA-binding proteins and TFs are in PSs.
- **PSs** control gene expression through the dynamic sequestration/release of proteins directly involved in transcriptional/translational activities
- Strongly involved in **cellular stress response**.



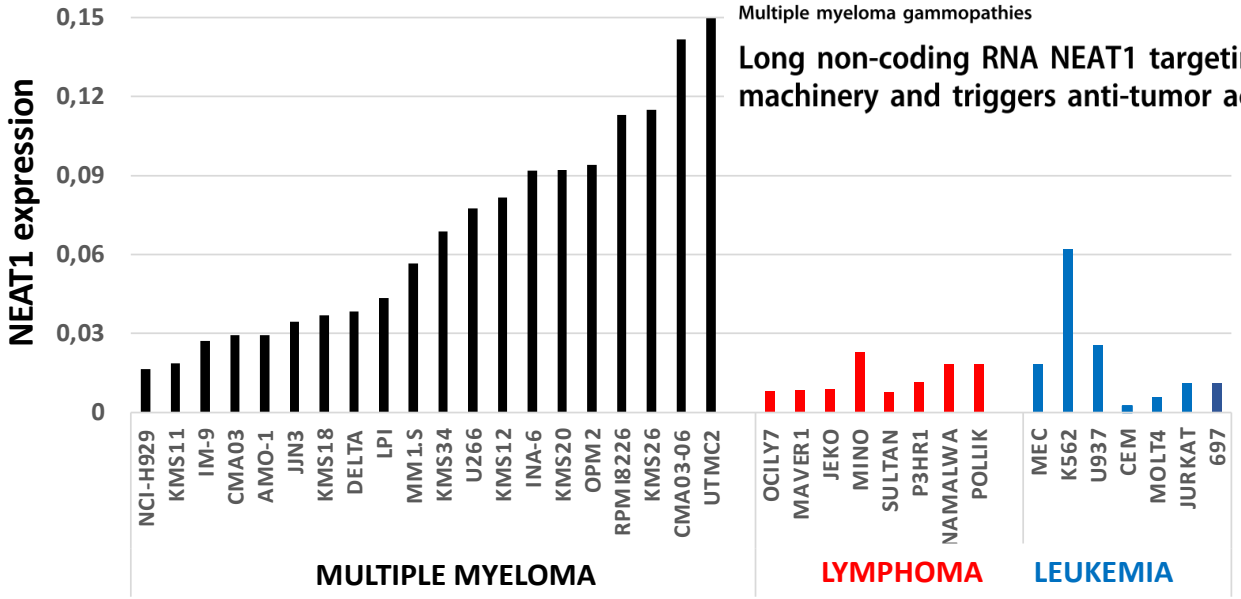
haematologica

Long non-coding RNA NEAT1 shows high expression unrelated to molecular features and clinical outcome in multiple myeloma

Taiana E. et al. (2019). Haematologica

Taiana E. et al. (2019). Haematologica
Taiana E. et al. (2020). Leukemia

NEAT1 is overexpressed in MM cells

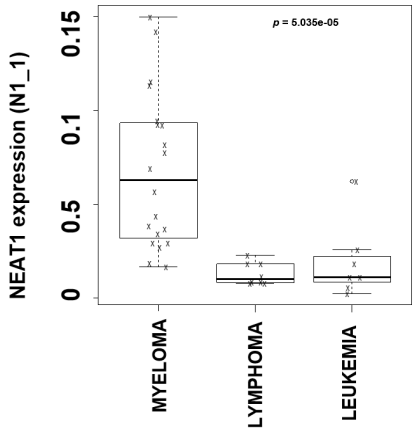


Leukemia (2020) 34:234–244
https://doi.org/10.1038/s41375-019-0542-5

ARTICLE

Multiple myeloma gammopathies

Long non-coding RNA NEAT1 targeting impairs the DNA repair machinery and triggers anti-tumor activity in multiple myeloma



Could **NEAT1** represent a candidate for a new **anticancer approach in MM**?

Functional investigation of lncRNA NEAT1 role in MM

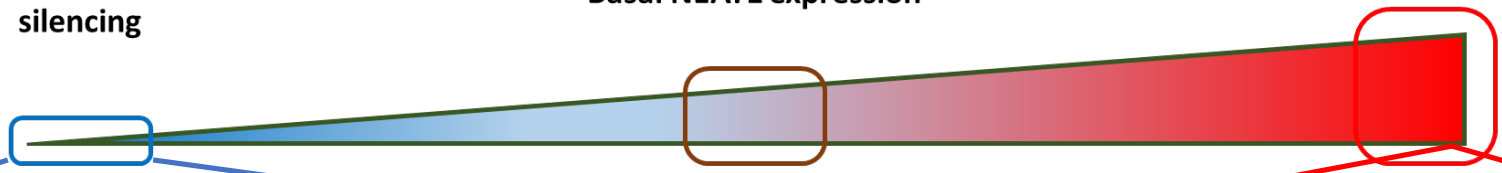
Integrated functional genomic approach reveals an emerging role of lncRNA NEAT1 in DNA damage response and drug resistance in multiple myeloma

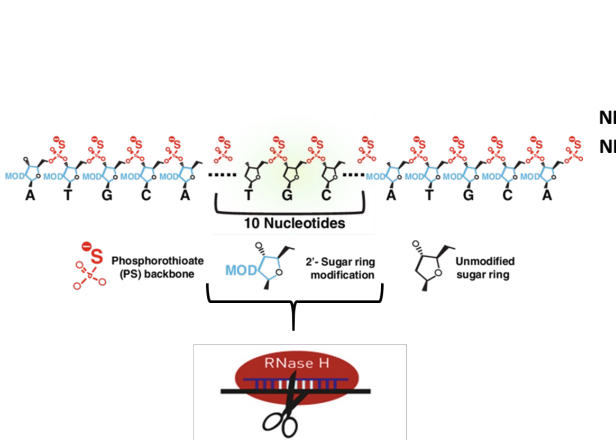
Experimental strategy

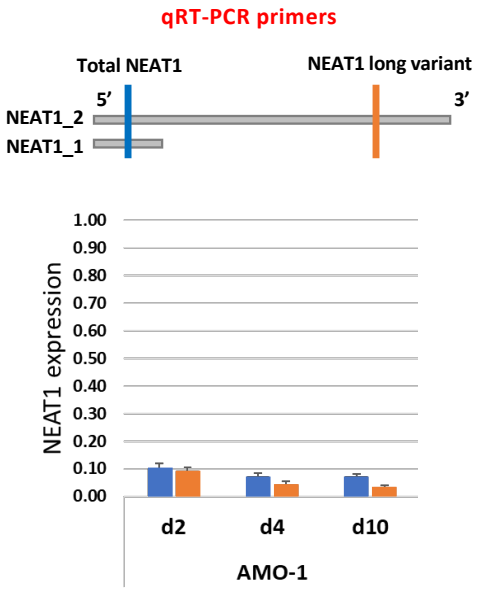
Antisense LNA GapmeRs-mediated NEAT1 silencing

Basal NEAT1 expression

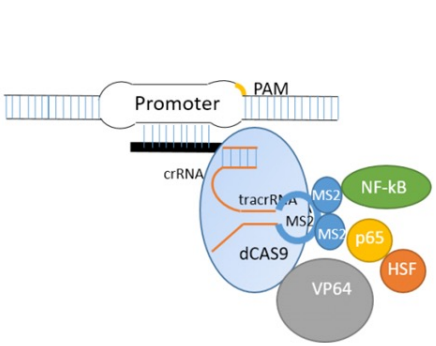
CRISPR/CAS9-mediated NEAT1 forced expression

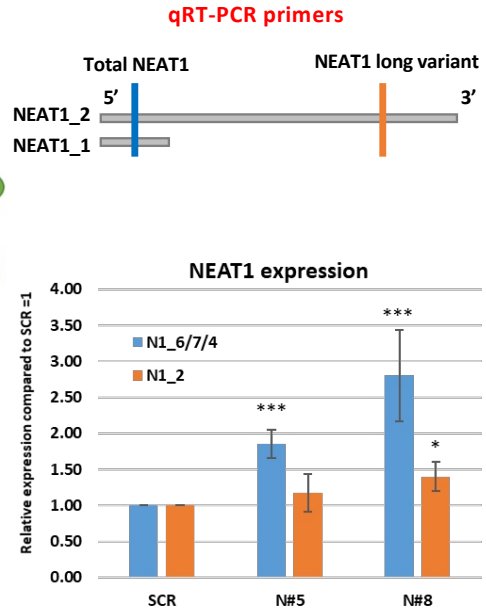






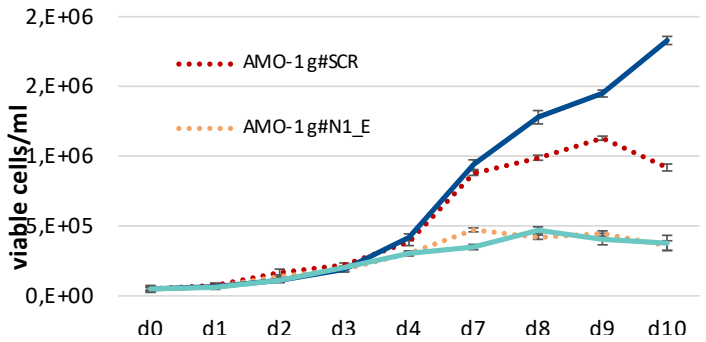
Taiana E. et al., Leukemia. 2020. PMID: 31427718.

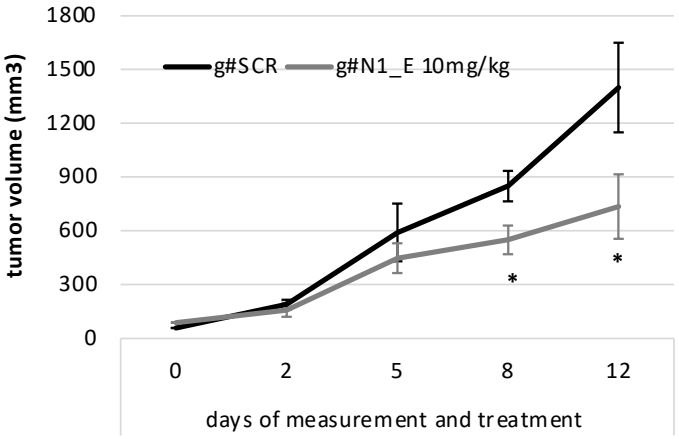




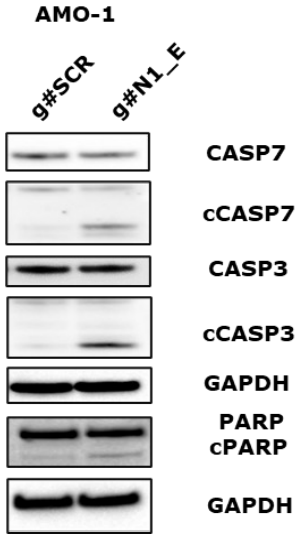
Taiana E. et al., Haematologica. 2023. PMID: 36073514

NEAT1 regulates proliferation and viability of MM cells

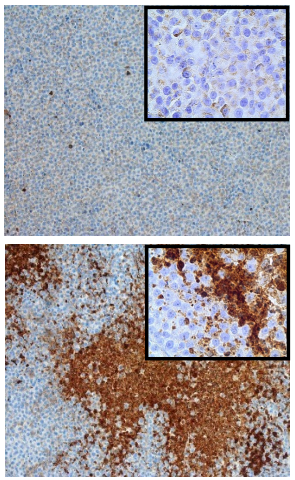




NEAT1 silencing induce apoptosis in MM cells



Caspase 3



NEAT1 silencing sensitizes MM cells to standards anti-MM treatments

PROTEASOME INHIBITORS

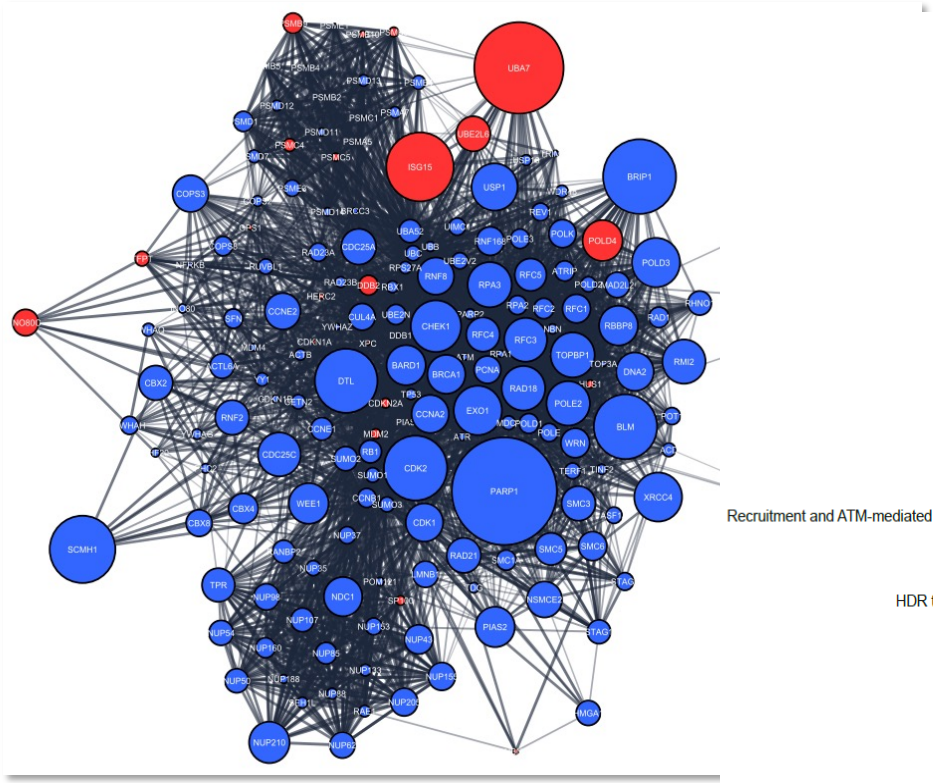
NCI-H929	g#N1_E	
	2.5µM	5µM
Carfilzomib (nM)	–	–
1.5	0.64	0.39
2	0.17	0.13
Bortezomib (nM)	–	–
0.75	0.69	0.5
1.5	0.8	0.54

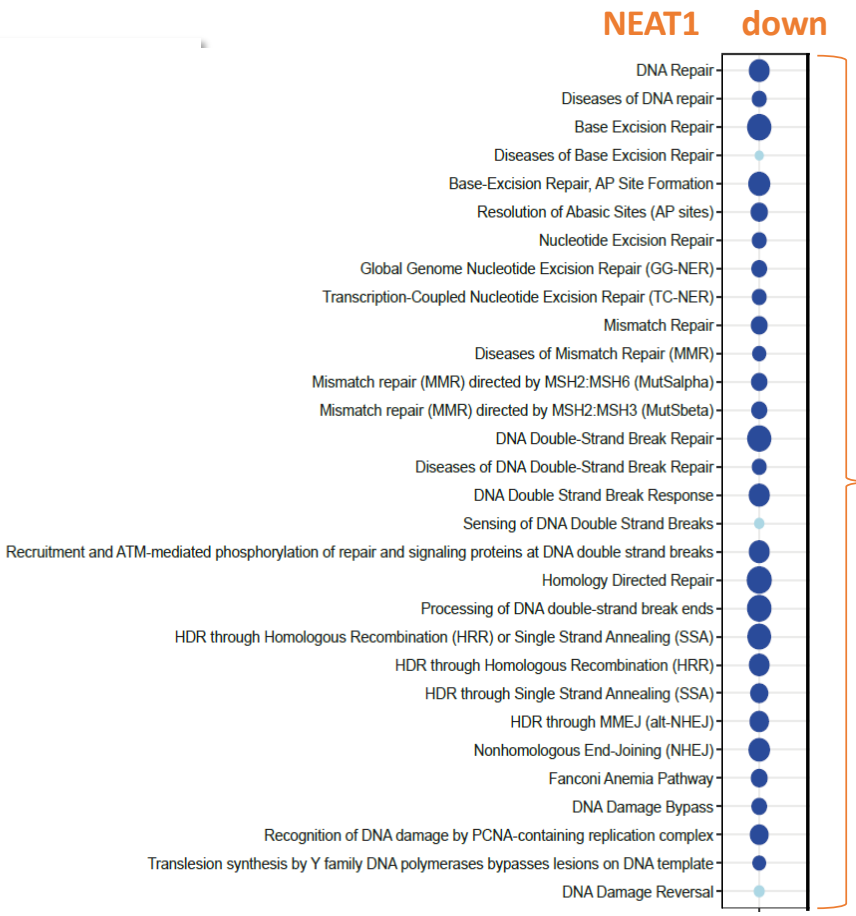
AMO-1	g#N1_E	
	2.5µM	5µM
Carfilzomib (nM)	–	–
1	0.74	0.62
1.5	0.75	0.73
Bortezomib (nM)	–	–
0.75	0.72	0.61
1.5	0.8	0.76

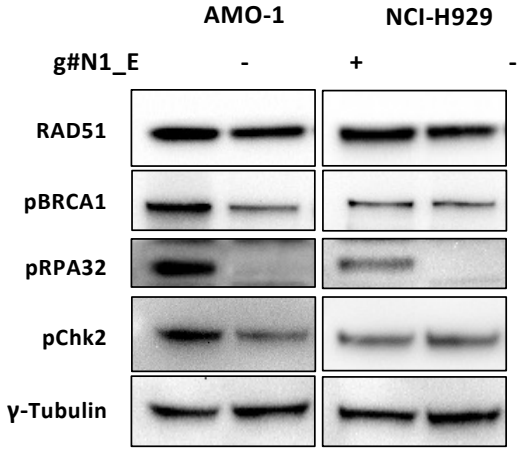
CI			Additive			
	1.45-1.2	1.20-1.10	1.10-0.90	0.90-0.85	0.85-0.7	0.7-0.3
	Moderate	Slight		Slight	Moderate	Regular
	Antagonism			Synergism		

Taiana, E., et al. (2020). Leukemia

NEAT1 KD transcriptomic signature reveals an impairment of DNA repair processes



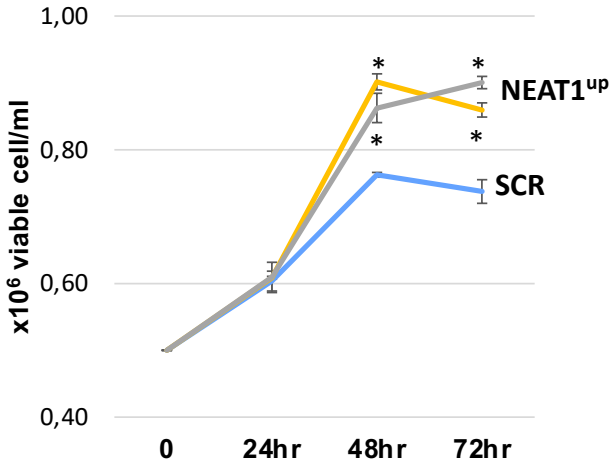


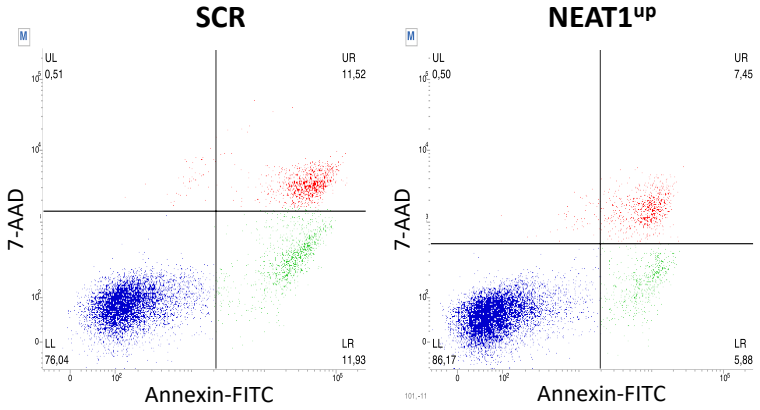


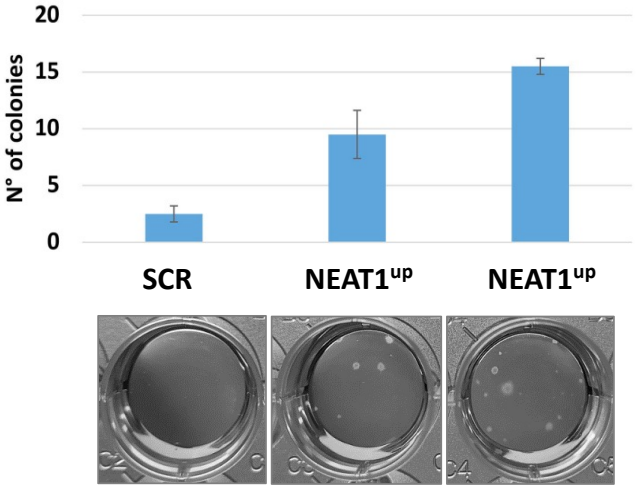
Taiana E. et al Leukemia, 2020

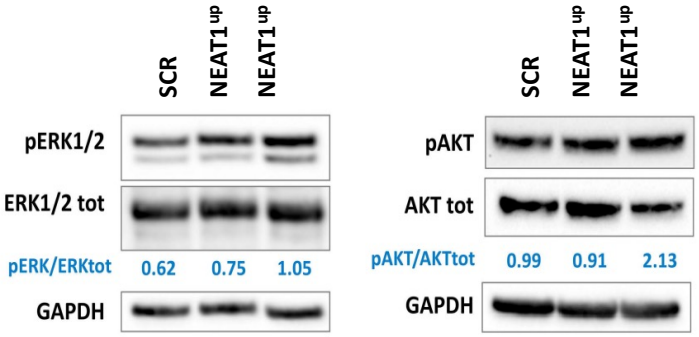
Taiana E, Neri A. et al., Manuscript in preparation

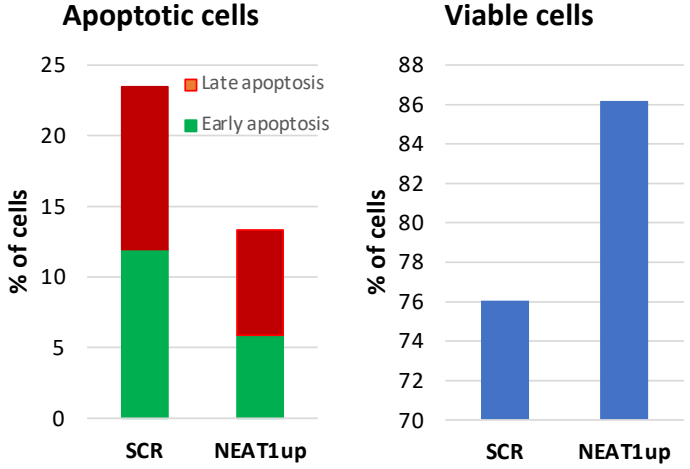
NEAT1 overexpression is crucial to **sustain the growth and the survival of MM cells** when maintained in serum starvation or hypoxia

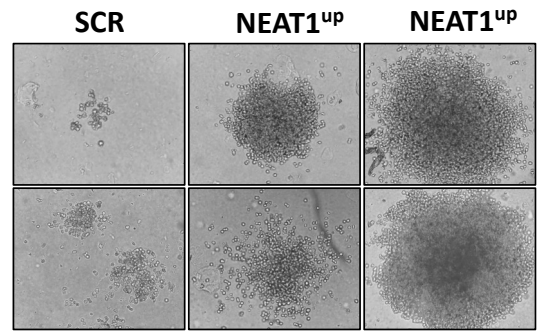










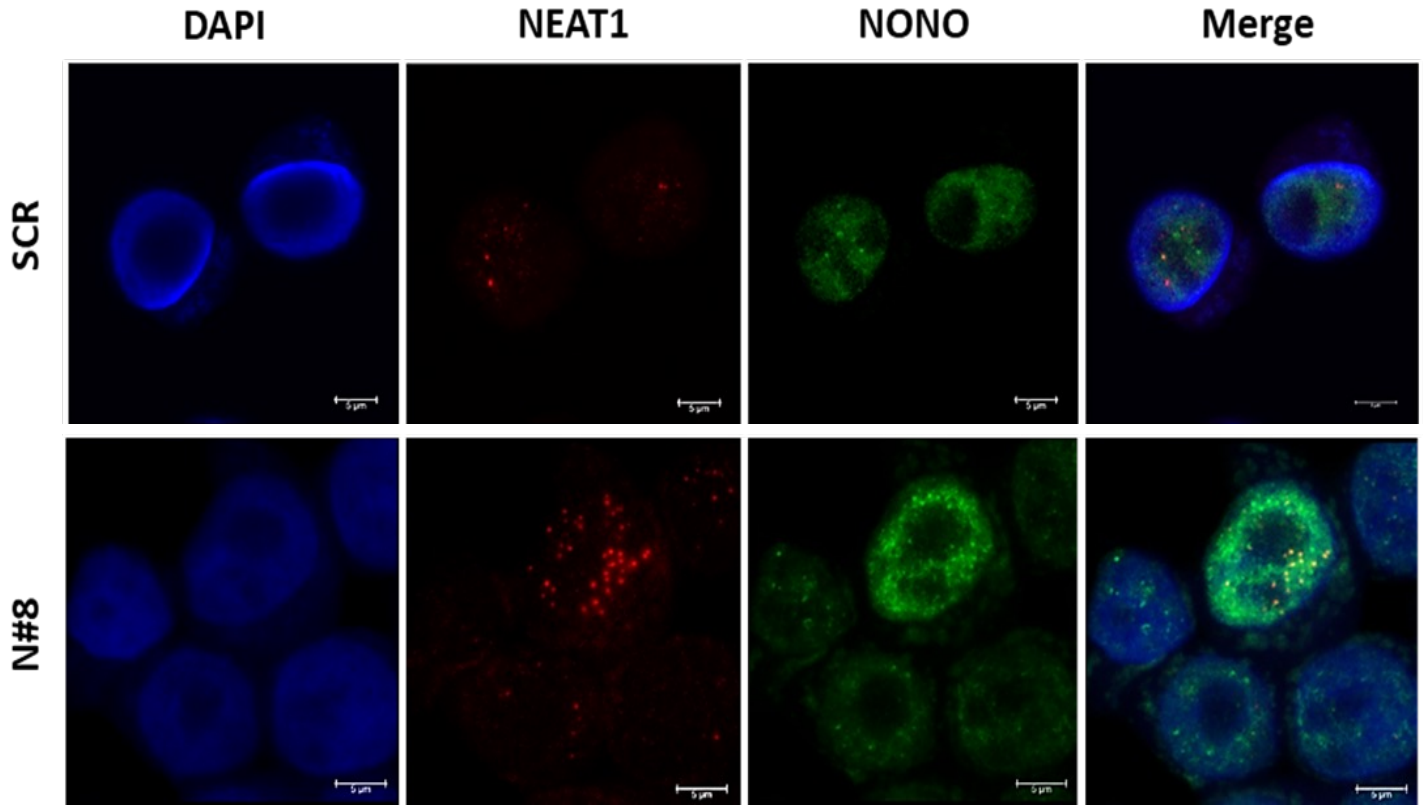


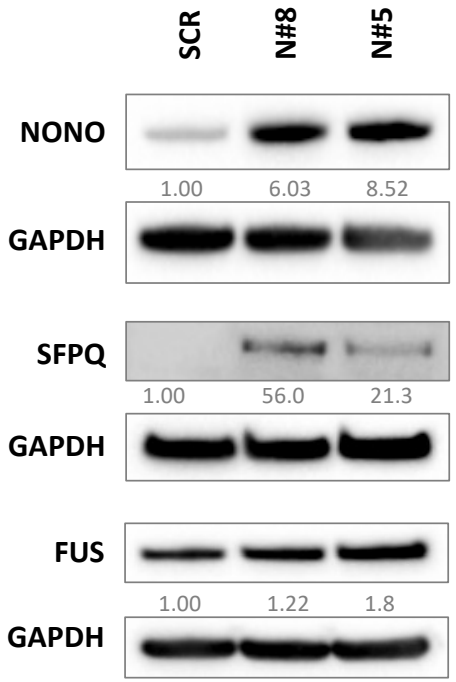
Taiana E. et al., Haematologica. 2023.

Taiana E. et al., Haematologica. 2023. PMID: 36073514

By means of a **CRISPR-Cas9 transactivating approach**, we demonstrated that NEAT1 is involved in the maintenance of the **genome integrity** supporting the **acquisition of a pro-survival** and **pro-oncogenic phenotype** by **MM cells** through:

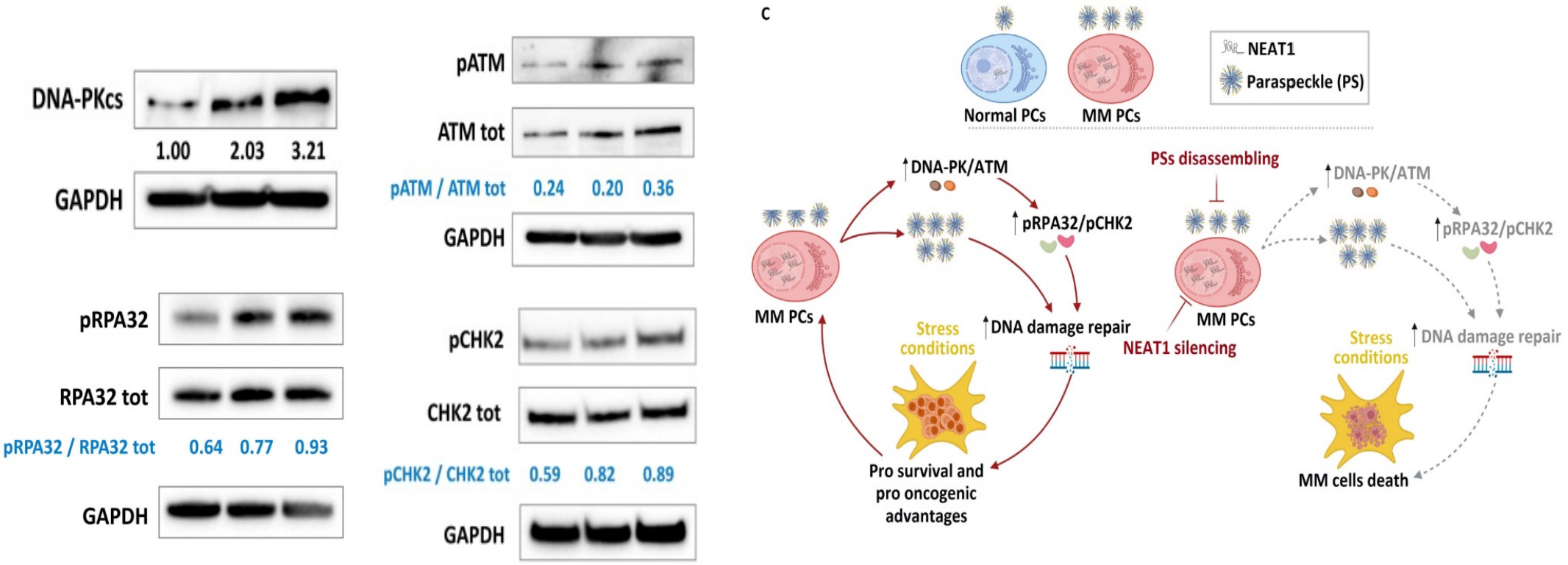
1. Increasing PSs and post-transcriptional stabilization of essential PS Proteins (NONO, SFPQ, FUS)





Taiana E. et al., Haematologica. 2023.

2. Positive regulation of the molecular axis involving **ATM** and **DNA-PKs** kinases and their direct targets **pRPA32 and pCHK2**



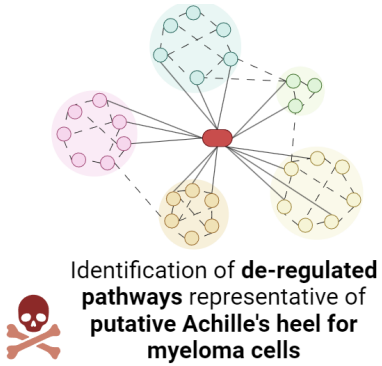
NEAT1 overexpression could be considered a generalized **rescue mechanism for MM plasma cells** strongly suggesting that **NEAT1 and PSs targeting** could be considered a novel promising strategy for **innovative anti-MM therapies**.

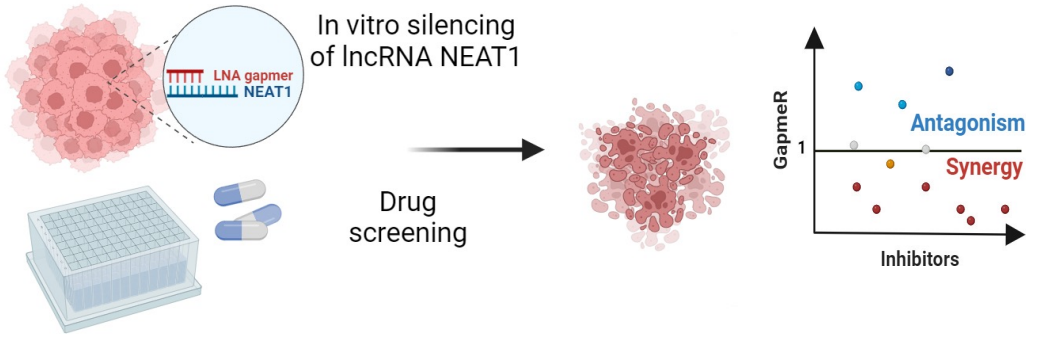
Taiana E. et al., Haematologica. 2023.

What's next?

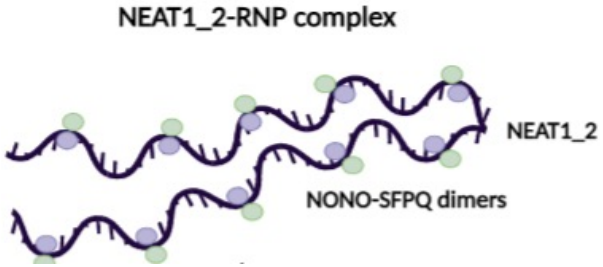
Given the **strong antiproliferative effect** observed upon **NEAT1 silencing** in MM cells we aim:

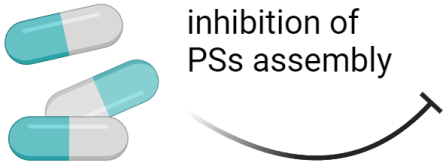
1. To provide novel insights concerning the potential effects that NEAT1 exerts on **gene expression** and its putative role in **chemo-resistant mechanisms,** priming the development of **novel combinatorial strategies in MM.**

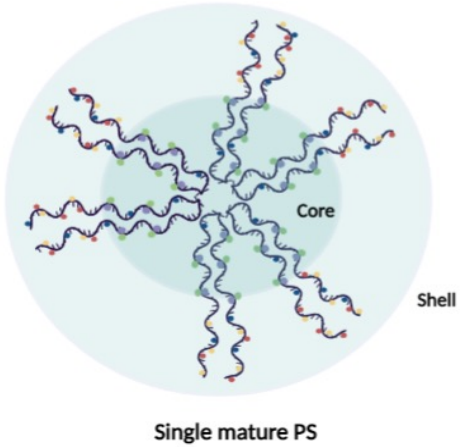




2. To identify possible chemical compounds able to **abrogate NEAT1-proteins and proteins-proteins interactions** considered to be essential for paraspeckle assembly and activity.







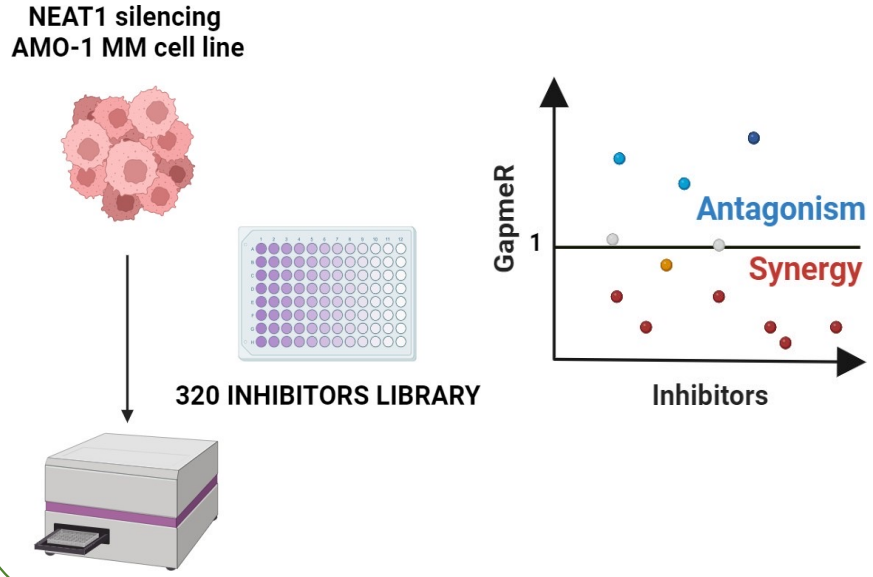
Schell, B., Legrand, P., & Fribourg, S. (2022). Crystal structure of SFPQ-NONO heterodimer. *Biochimie*, *198*, 1–7. Advance online publication.

Task 1

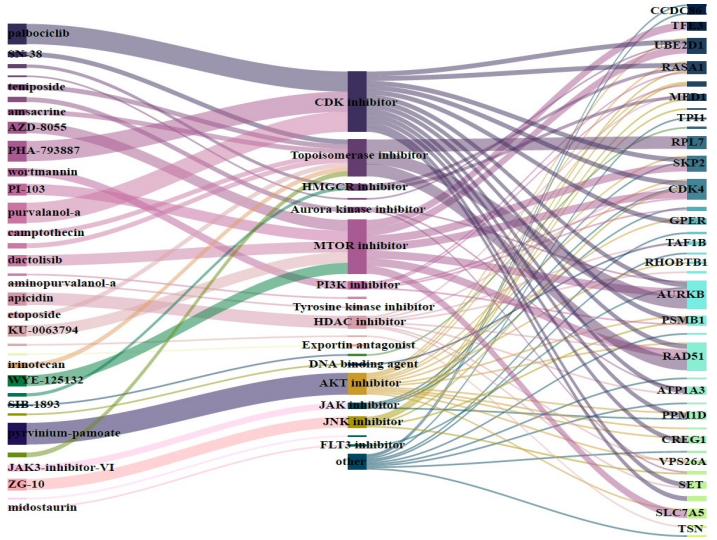
Identification of molecular targets having a synergistic effect in combination with NEAT1 silencing

NEAT1 silencing

In vitro high-throughput drug screening



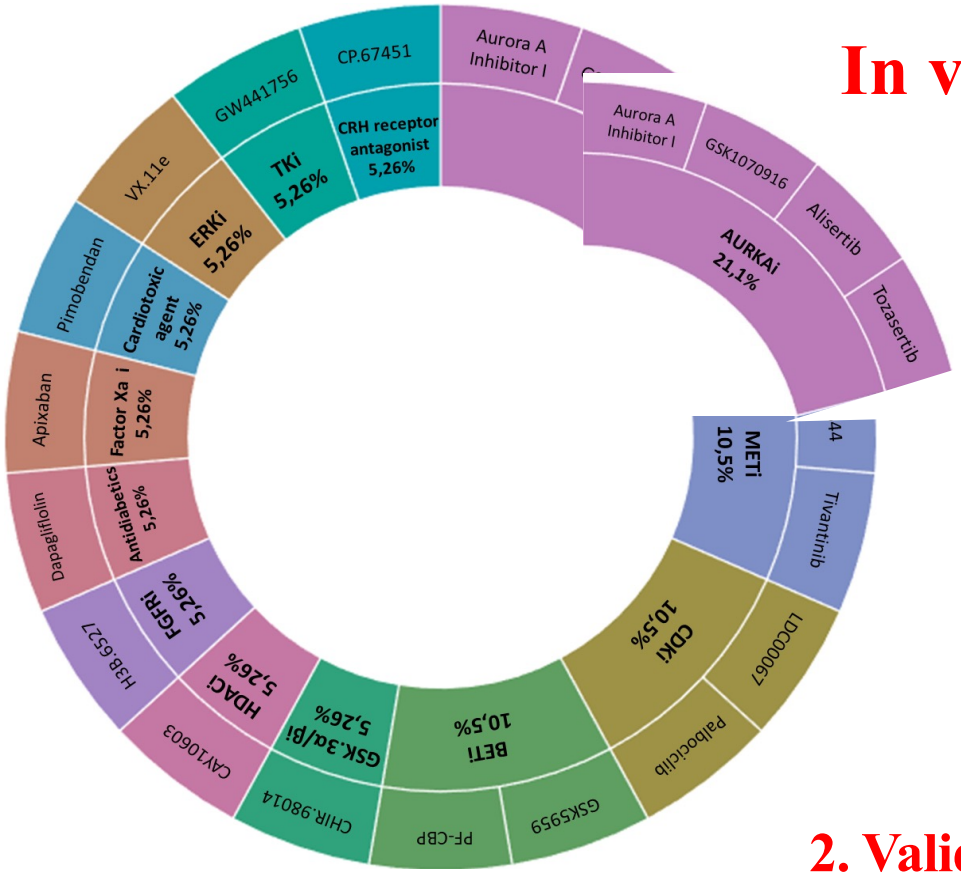
In silico drug identification



Unpublished. Please, do not post

Puccio N et al., Manuscript in preparation

In vitro highthroughput drug screening



Puccio N et al., Manuscript in preparation

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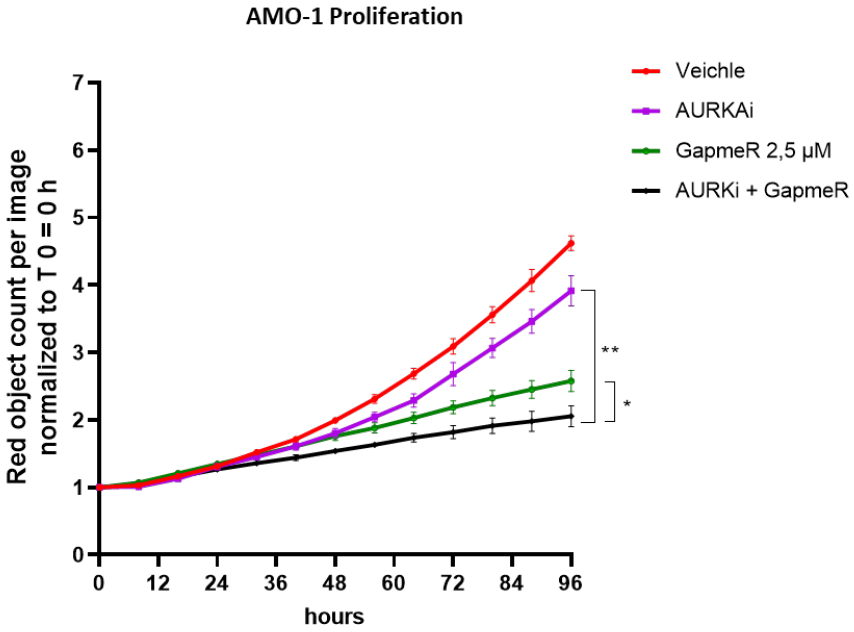
19 compounds have a **synergistic effect** in combination with **NEAT1 silencing,** leading to a **decreased multiple myeloma cells viability**.

Aurora kinase inhibitors are the most promising compounds able to **individually exert a synergistic activity with NEAT1 silencing** in vitro

2. Validation phase on a panel of MM cell lines with Incucyte S3™

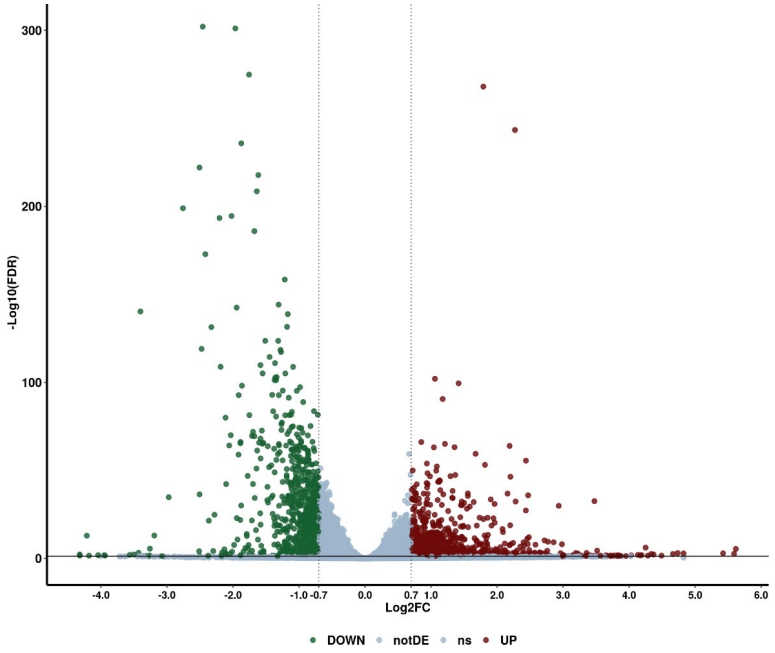


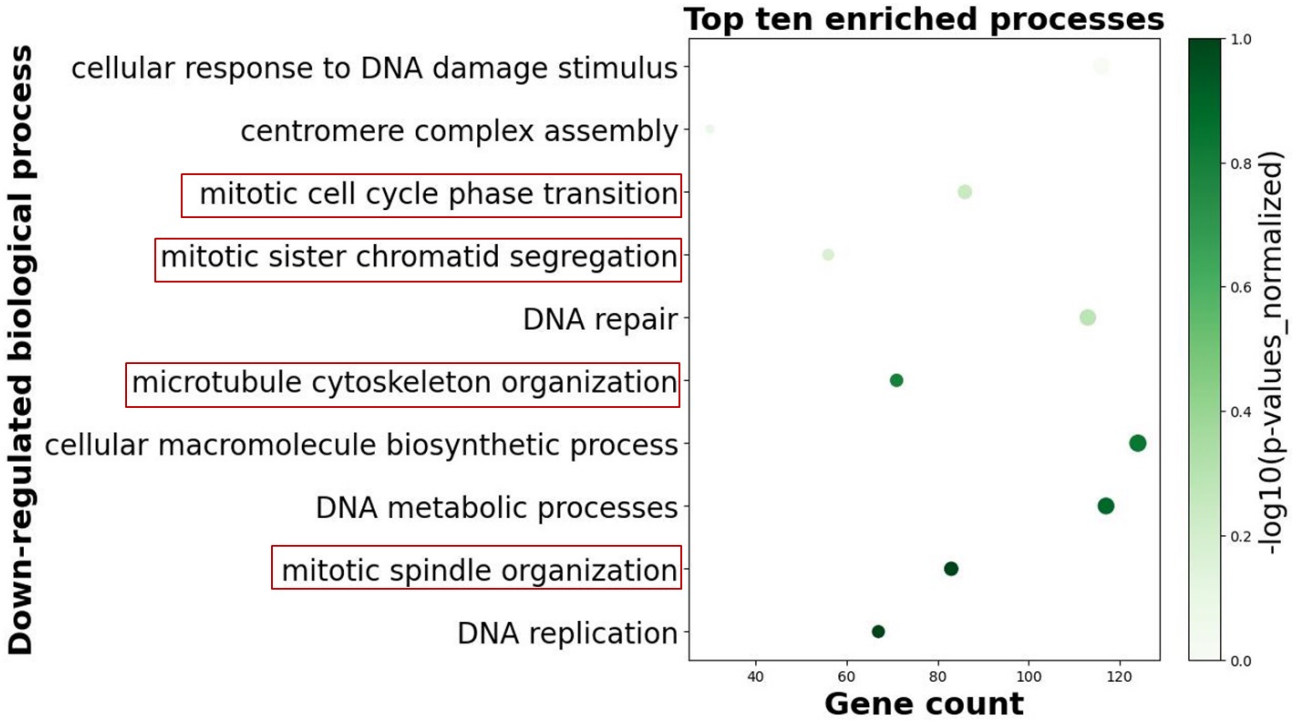
In silico drug identification approach

Transcriptome analysis by bulk RNA sequencing in **NEAT1 silenced AMO-1 MM cells and the relative control**

Identification of 752 down-regulated genes and 957 up-regulated genes **in NEAT1 silenced samples** compared to the control

Enrichment analysis





Unpublished. Please, do not post

Puccio N et al., Manuscript in preparation

Unpublished. Please, do not post

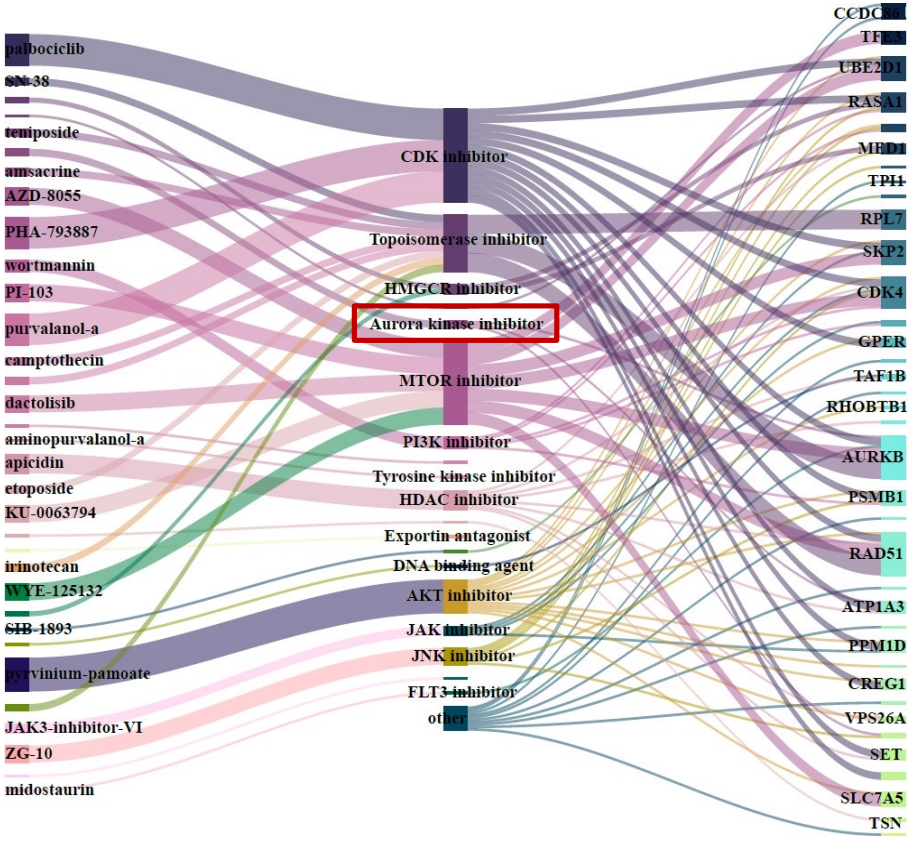
In silico drug identification approach

Transcriptome analysis by bulk RNA sequencing in **NEAT1 silenced AMO-1 MM cells and the relative control**

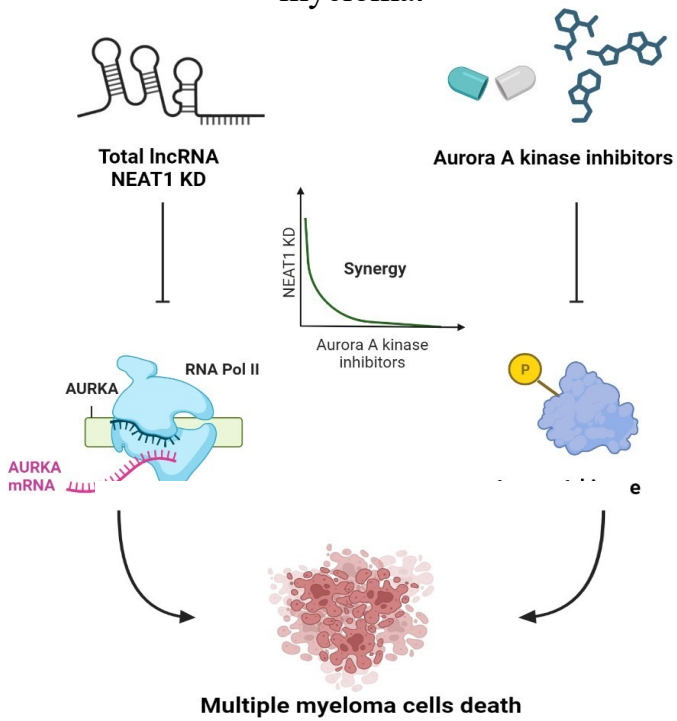
Identification of 752 down-regulated genes and 957 up-regulated genes **in NEAT1 silenced samples** compared to the control

ConnectivityMap

Compounds mimicking NEAT1 silencing signature were selected with the highest connectivity score



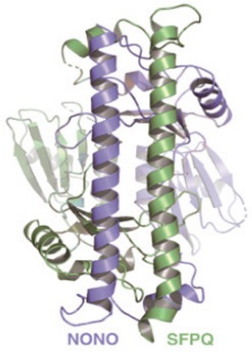
Both ***in silico*** and ***in vitro*** approaches identified **Aurora kinase inhibitors** as compounds able to **synergize with NEAT1 silencing**, suggesting that **this combination** could represent a **prominent therapeutic option** for multiple myeloma.

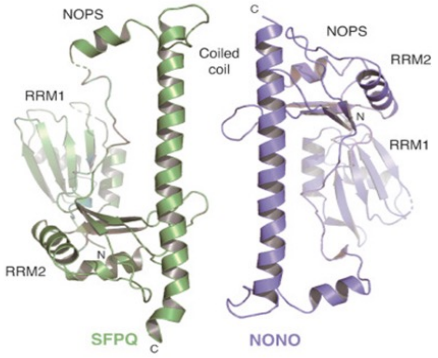


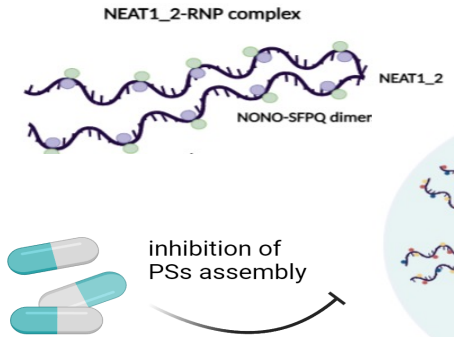
Identification of novel potential druggable vulnerabilities in MM

Task 2

NONO/SFPQ heterodimers and PSs structure







Discover Oncology

Ronchetti et al., 2022

Discover

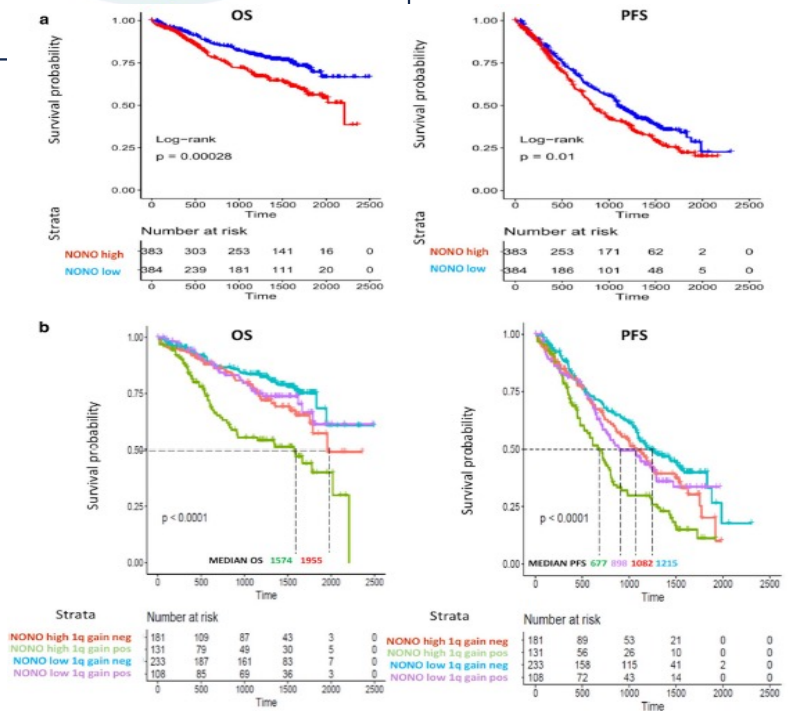
Brief Communication

Expression levels of NONO, a nuclear protein primarily involved in paraspeckles function, are associated with several deregulated molecular pathways and poor clinical outcome in multiple myeloma

Domenica Ronchetti[1,2] · Vanessa Katia Favasuli[1,2] · Ilaria Silvestris[1,2] · Katia Todoerti[1,5] · Federica Torricelli[3] · Niccolò Bolli[1,2] · Alessia Ciarrocchi[3] · Elisa Taiana[1] · Antonino Neri[4]

Higher NONO levels were correlated with **poorer OS and PFS of MM patients**.

The combination of **higher NONO expression level with the occurrence of 1q-gain was associated with the poorest survival rate in OS and PFS.**



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