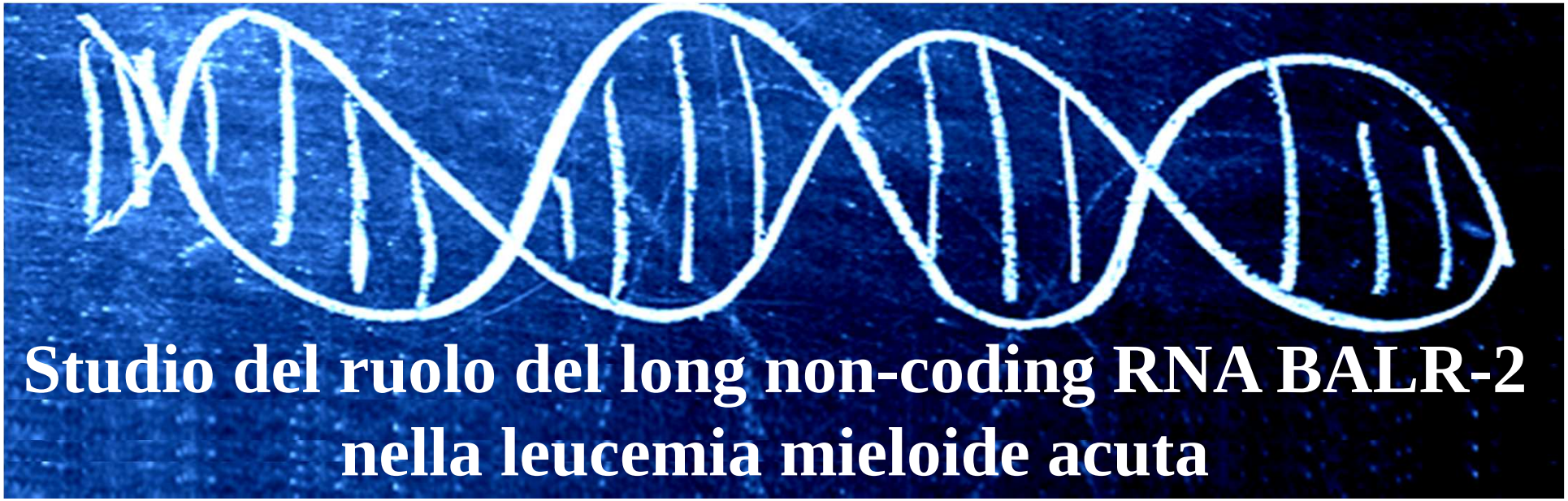
UNIVERSITÀ DEGLI STUDI DI PADOVA



Studio del ruolo del long non-coding RNA BALR-2 nella leucemia mieloide acuta

Bisio Valeria, PhD

Tregnago C, Borella G, Benetton M, Zanon C, Santoro N, Pierani P, Micalizzi C, Locatelli F, Basso G, Pigazzi M.

V Workshop AIEOP in LAB – 28-29 Maggio 2018 - Bologna

Long non coding RNA

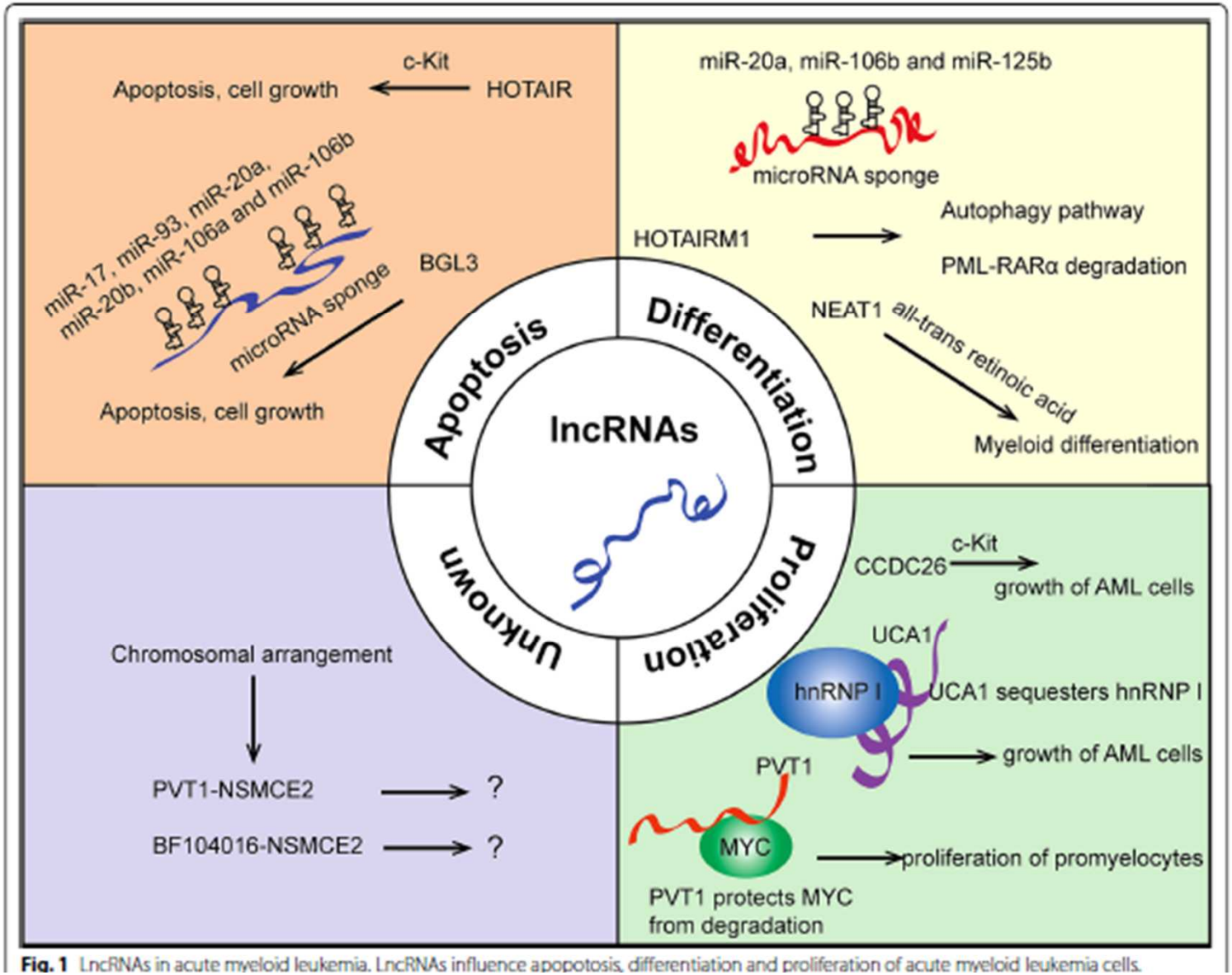


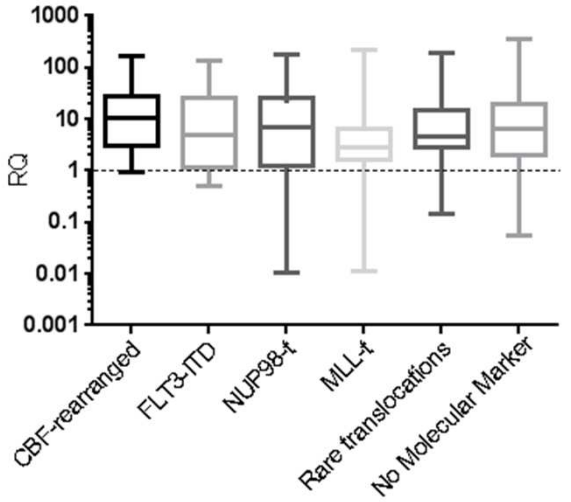
Fig. 1 LncRNAs in acute myeloid leukemia. LncRNAs influence apoptosis, differentiation and proliferation of acute myeloid leukemia cells.

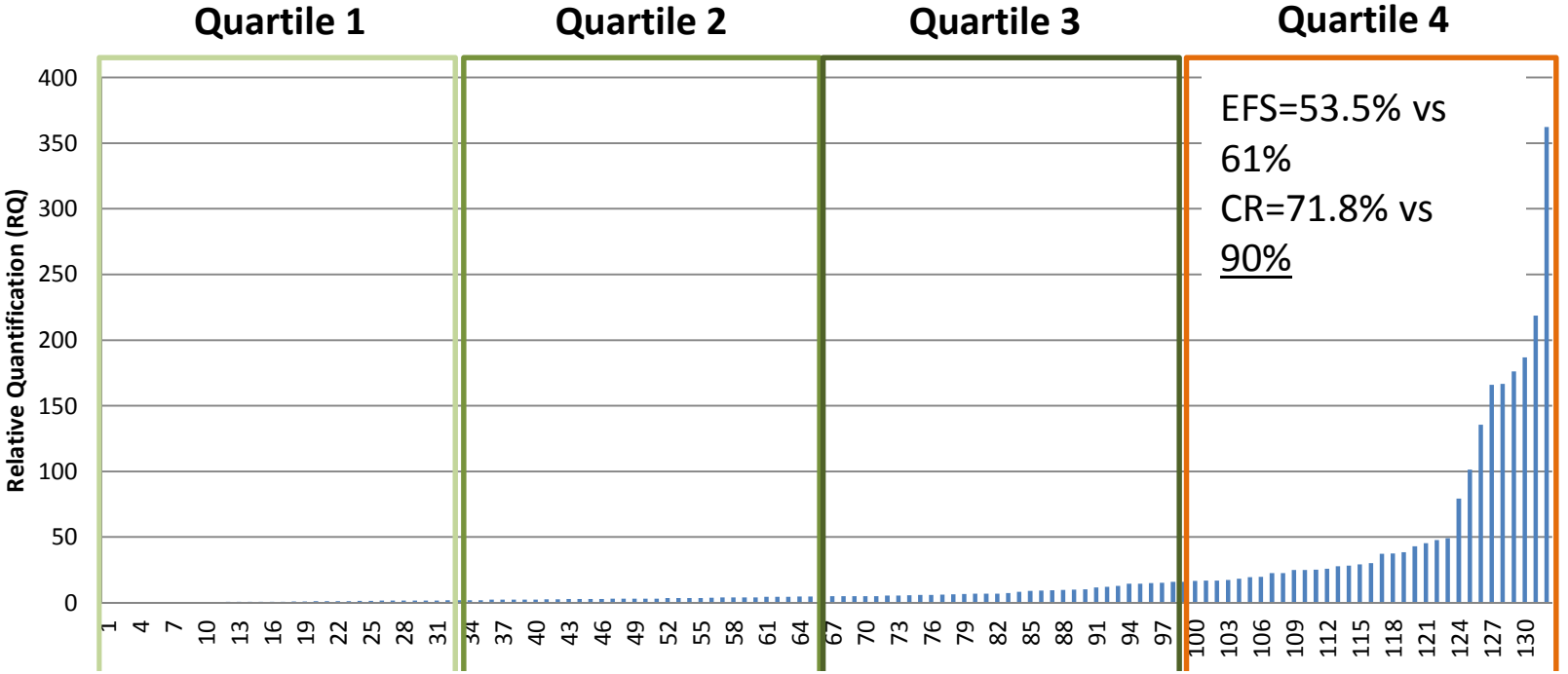
Shuyong et al. Experimental hematology and Oncology 2016

Long non coding RNA-BALR-2 nelle LAM

Rao et al. Molecular Cancer Cell 2015

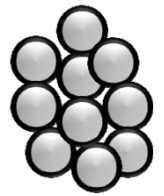
Pazienti: 132 LAM *de novo AIEOP 2002/01*





EFS=53.5% vs 61%

CR=71.8% vs 90%



BALR-2 Exp segue BULK leucemico

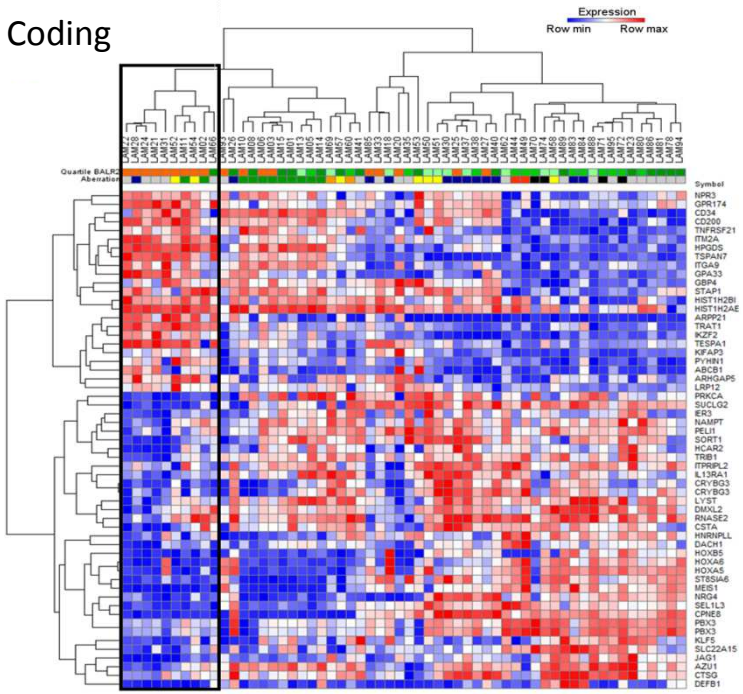
Espressione di BALR-2 nelle LAM

Analisi superisionata su 85 pazienti LAM *de novo* con diverse abberrazioni genetiche tramite Array HTA 2.0

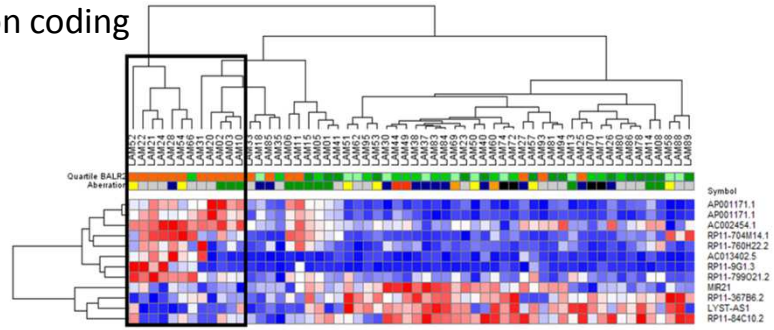
Quartile BALR-2

- Quartile 1
- Quartile 2
- Quartile 3
- Quartile 4

Coding



Non coding

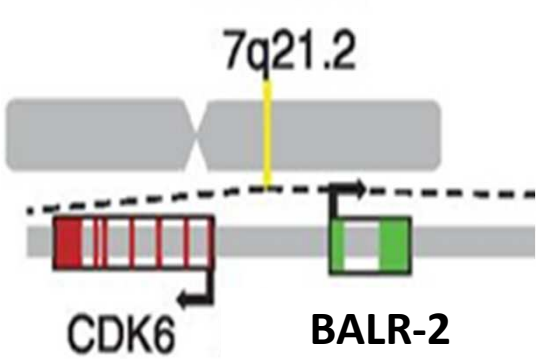


Aberration

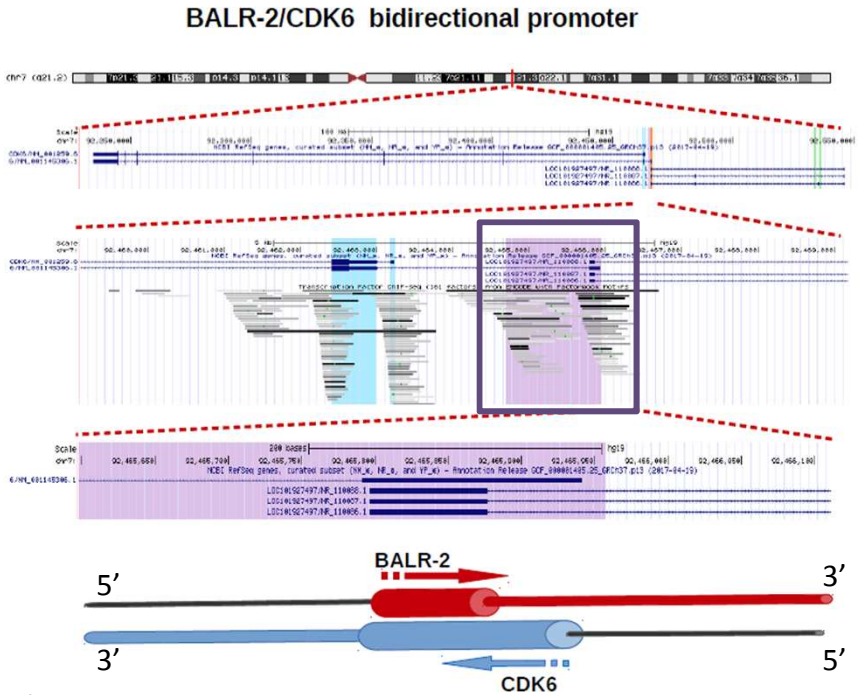
- t(8;21)
- DEK-NUP214
- FLT3-ITD
- inv(16)
- MLL-t
- Negative
- NUP98-t

57 geni codificanti e 12 geni non codificanti differenzialmente espresse tra 18 e 40 pazienti con LAM *de novo* con alta con bassa espressione di BALR-2, rispettivamente.
(p-value<0.01, FC>|2|).

Localizzazione cromosomica

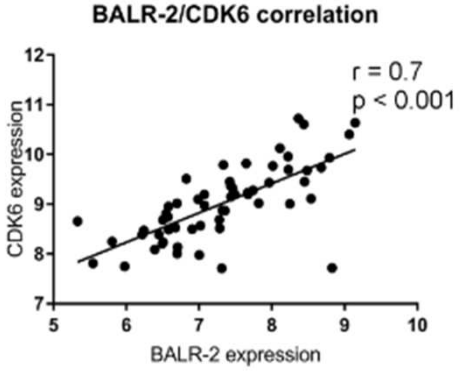


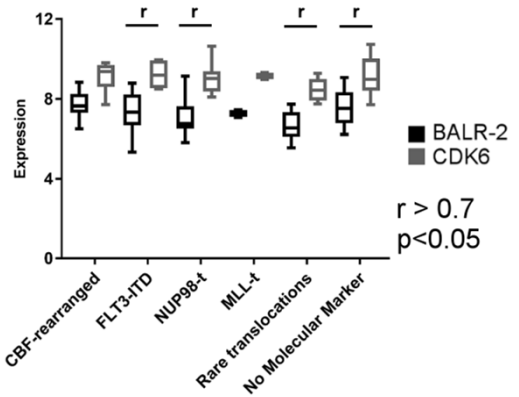
LncRNA = REGOLATORI DI ESP LOCALI



Asse BALR-2 /CDK6

Nei pazienti



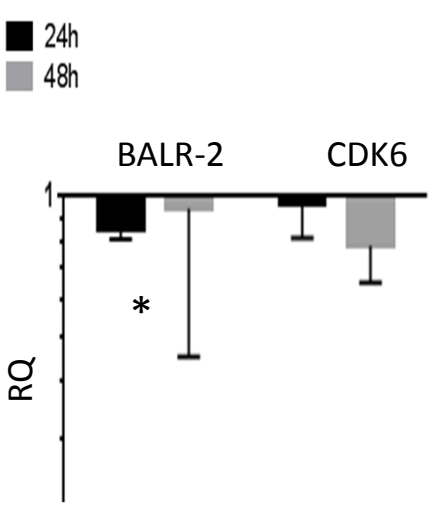


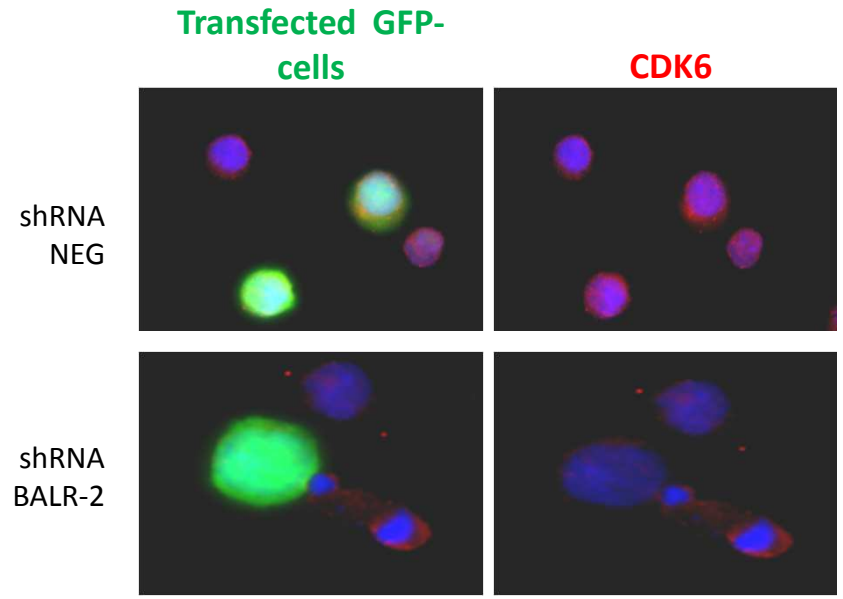
Carlo Zanon

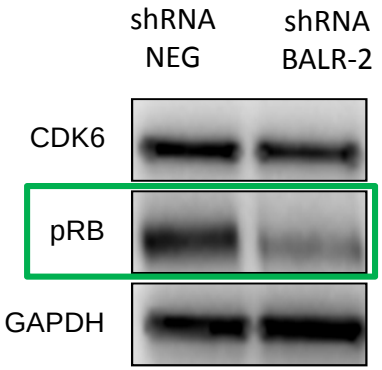
Ruolo di BALR-2 nelle LAM pediatriche

Asse BALR-2 /CDK6

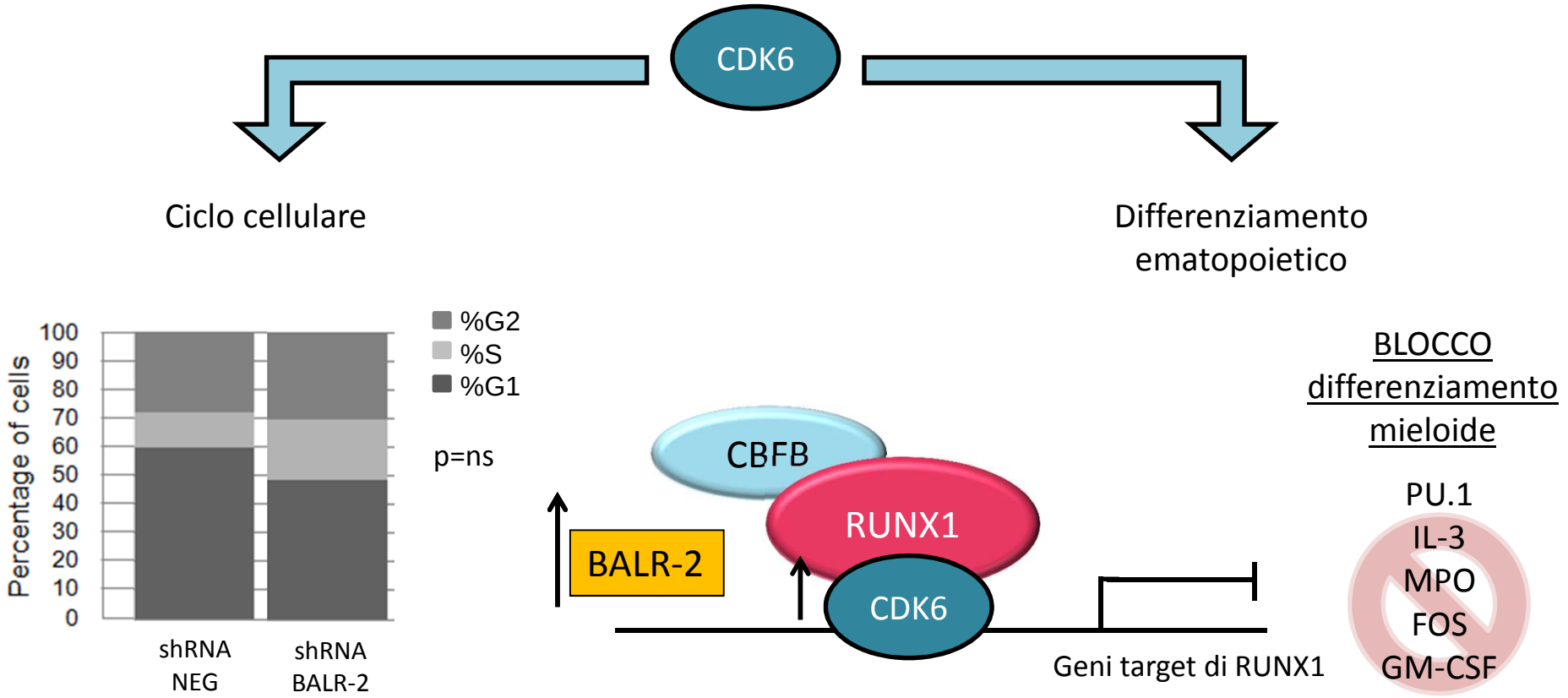
In vitro







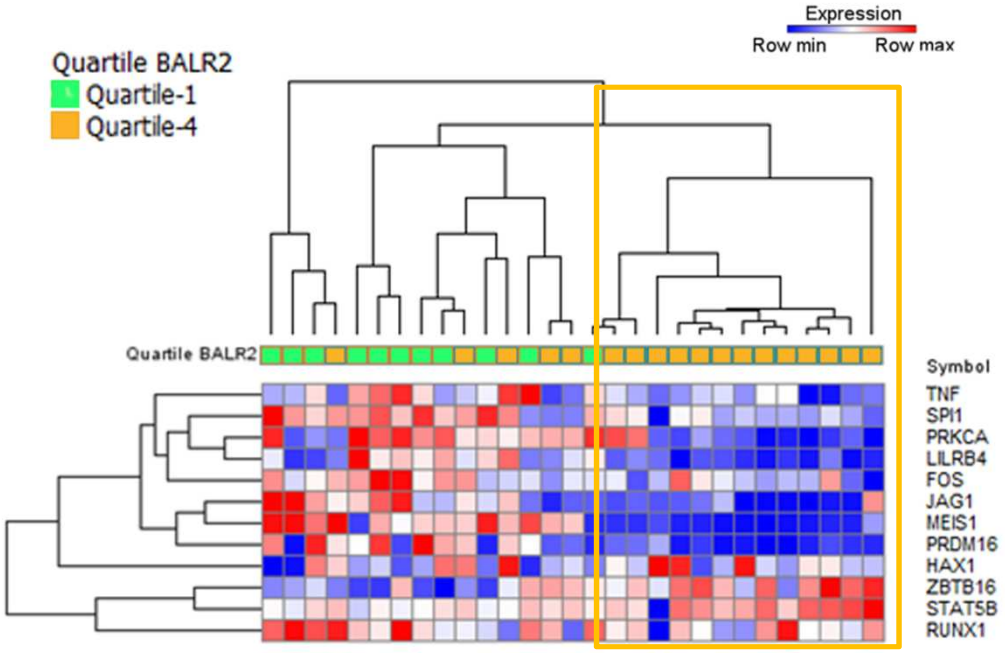
Ruolo di BALR-2 nelle LAM pediatriche

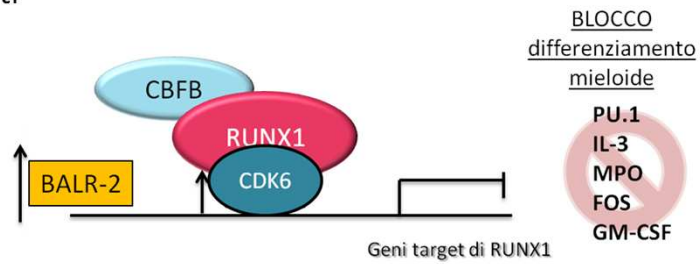


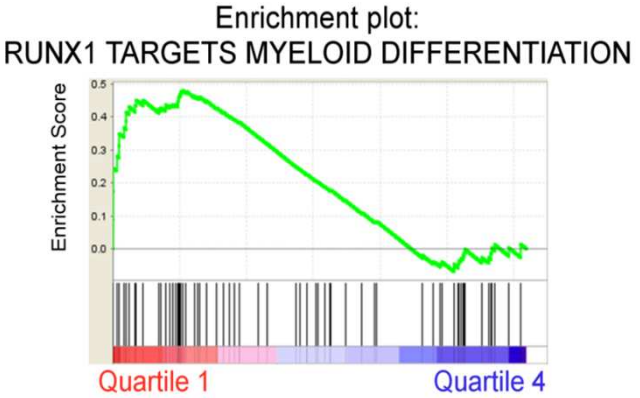
Modificato da T Fujimoto et al. The EMBO journal 2007

Ruolo di BALR-2 nelle LAM pediatriche

Geni target di RUNX1 e coinvolti nel differenziamento mieloide nei pazienti



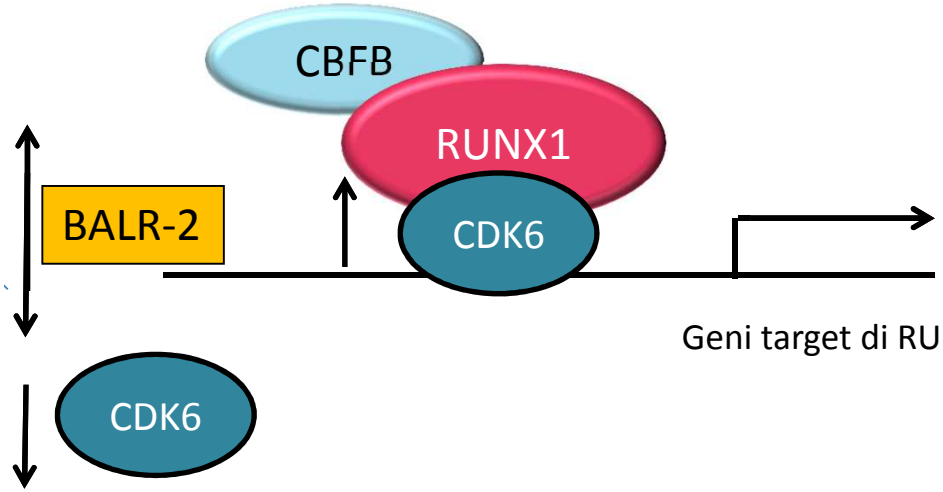




Ruolo di BALR-2 nelle LAM pediatriche

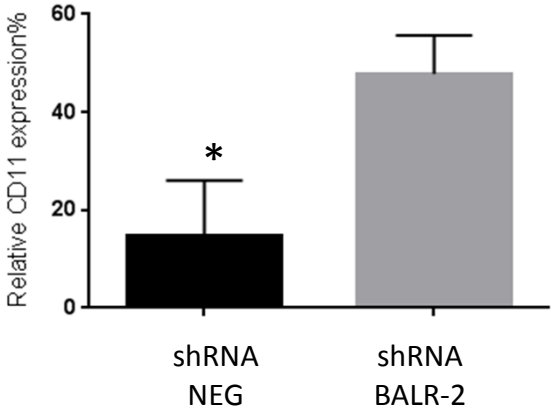
Silencing BALR2

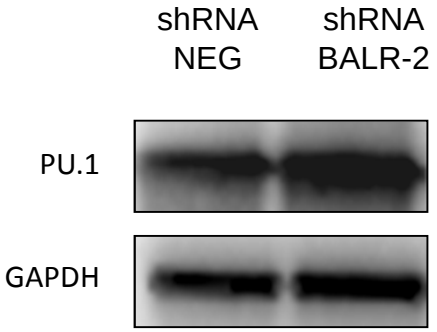
In vitro



SBLOCCO differenziamento mieloide

Geni target di RUNX1

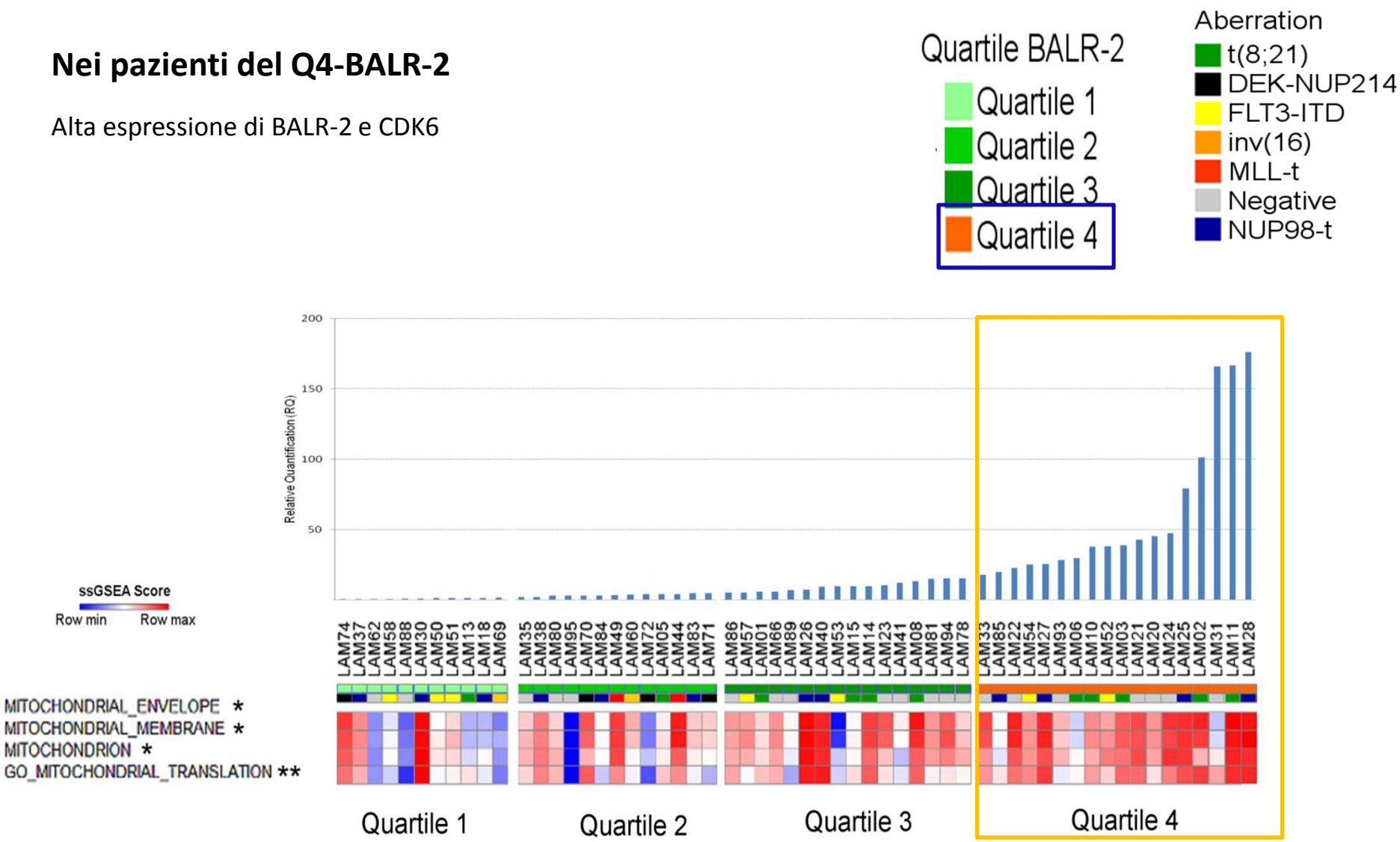




Ruolo di BALR-2 nelle LAM pediatriche

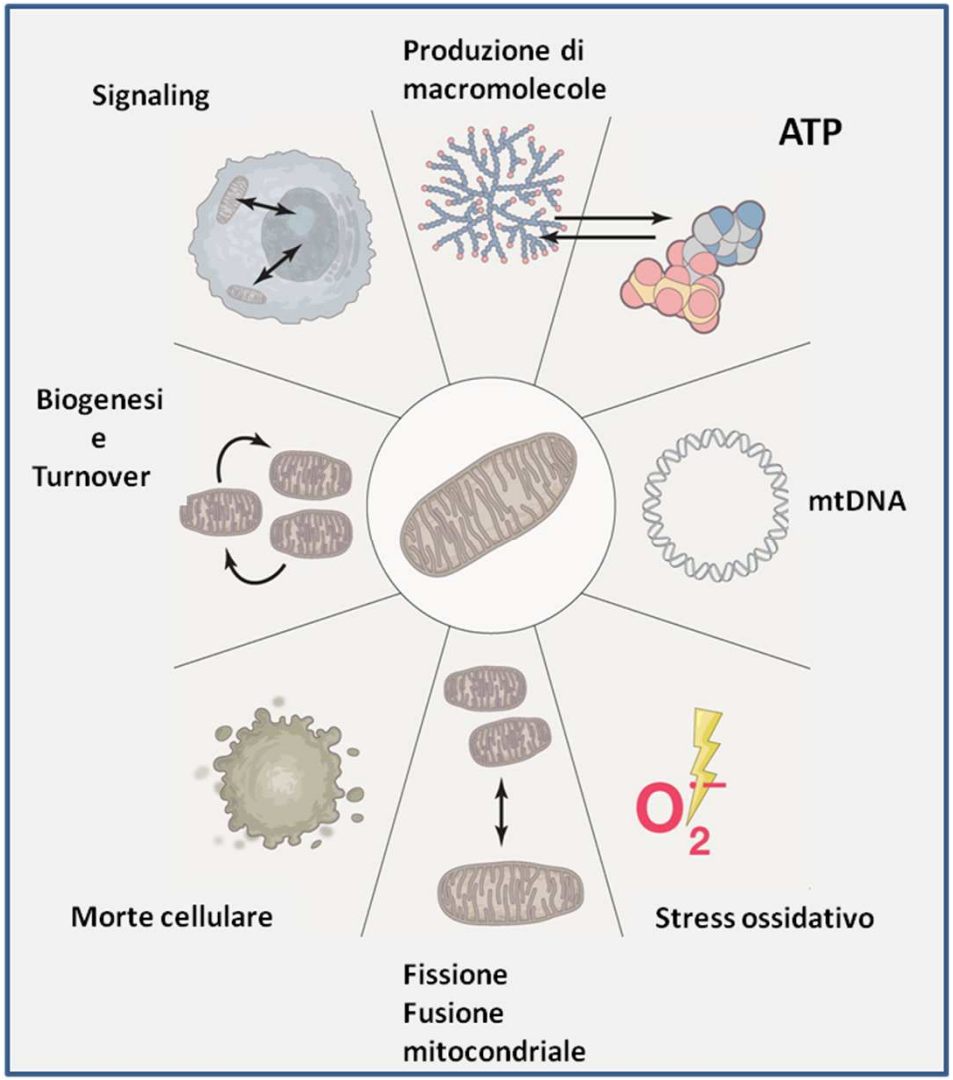
Nei pazienti del Q4-BALR-2

Alta espressione di BALR-2 e CDK6



single-sample GSEA

Ruolo di BALR-2 nelle LAM pediatriche



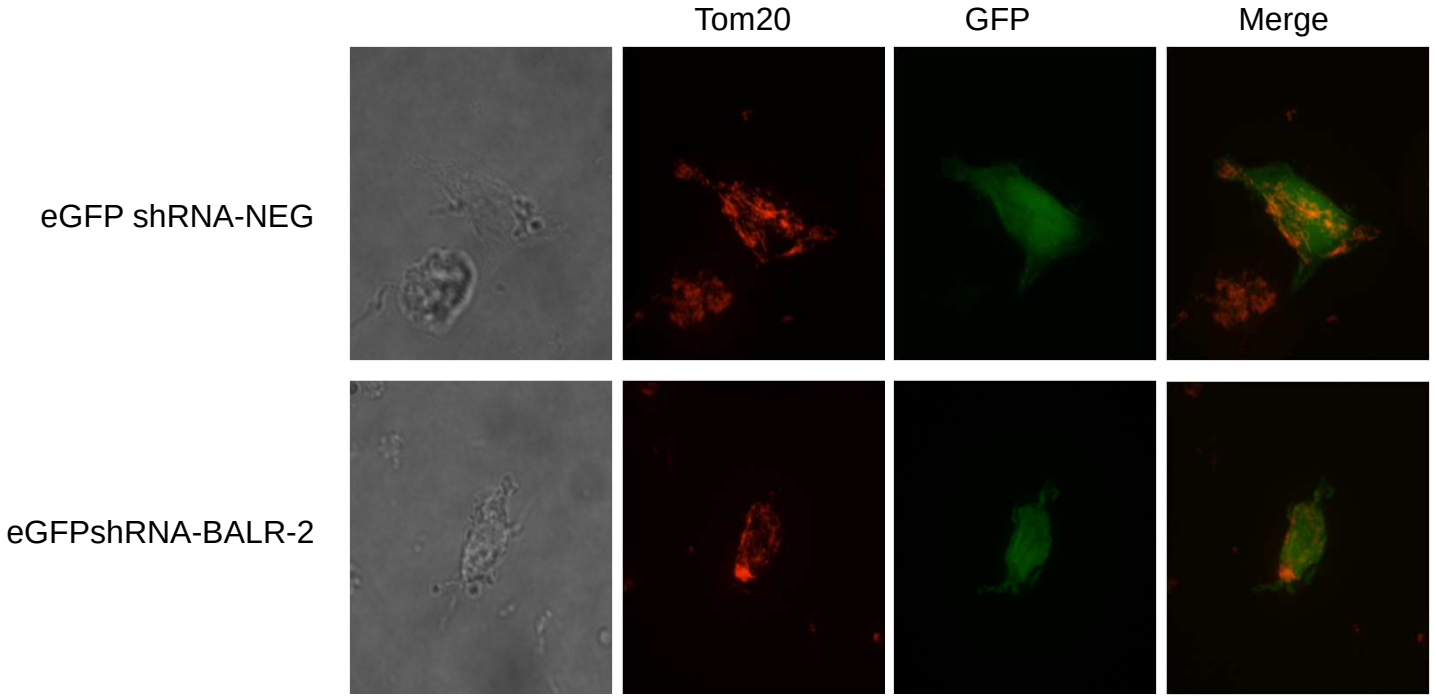
Modificato da Vyas et al. Cell 2016

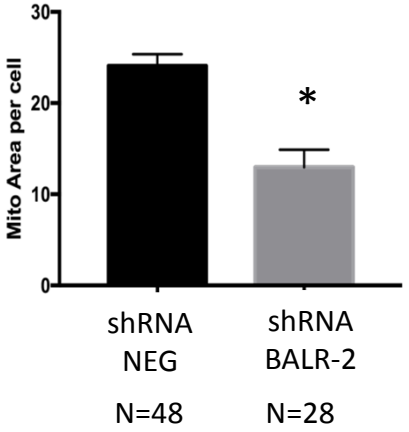
-Stadi differenziativi della cellula

-Resistenza alla terapia

Ruolo di BALR-2 nelle LAM pediatriche

Morfologia mitocondriale



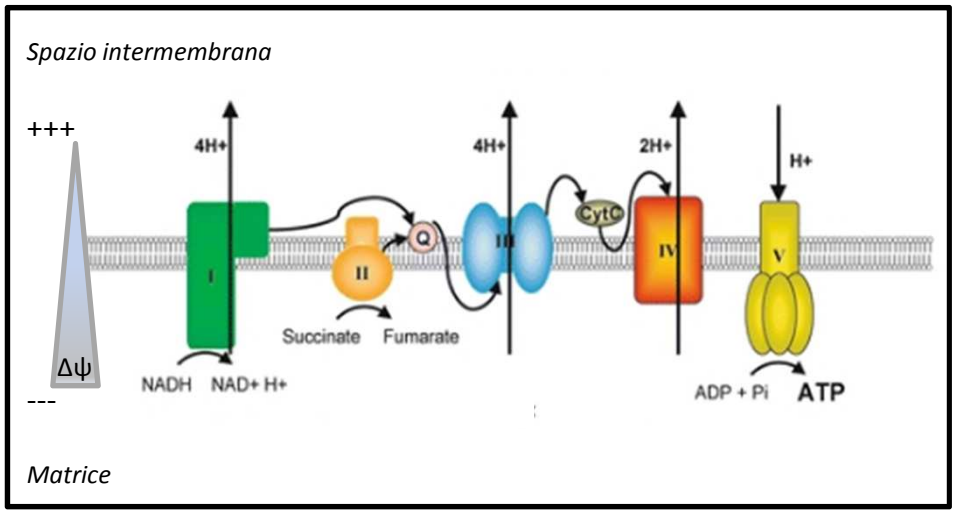


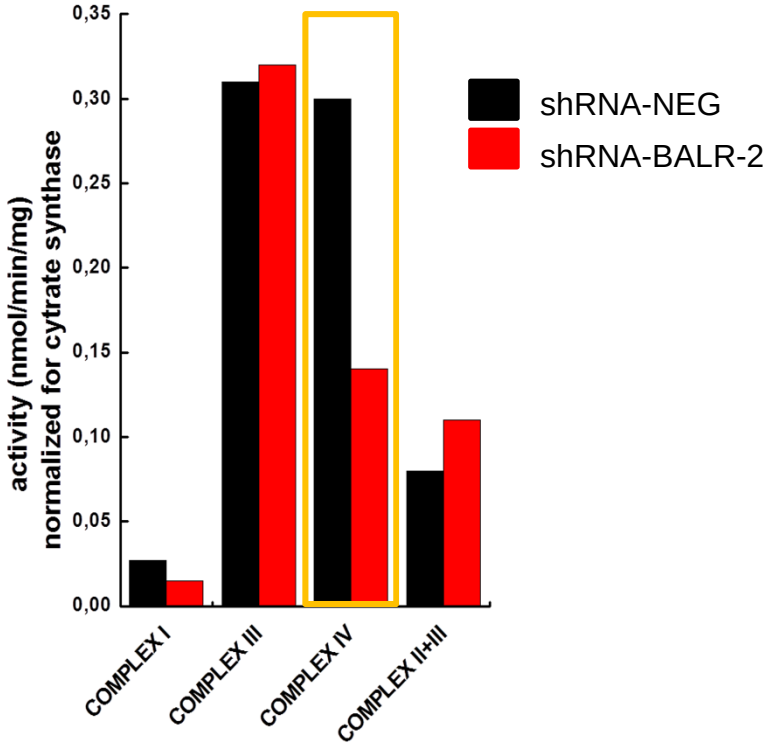
I livelli di BALR-2 modulano la massa mitocondriale

Ruolo di BALR-2 nelle LAM pediatriche

Funzionalità mitocondriale

ATTIVITA' DEI COMPLESSI RESPIRATORI

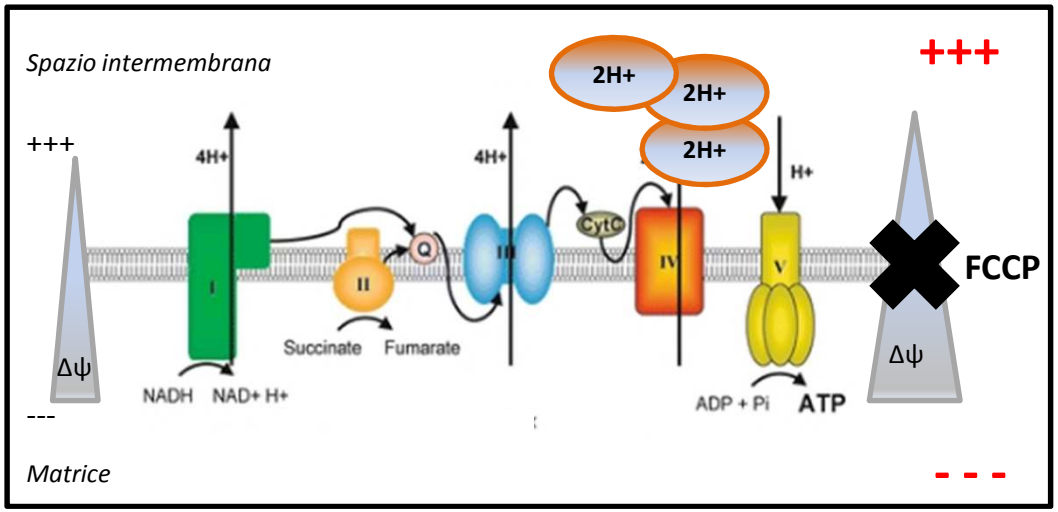


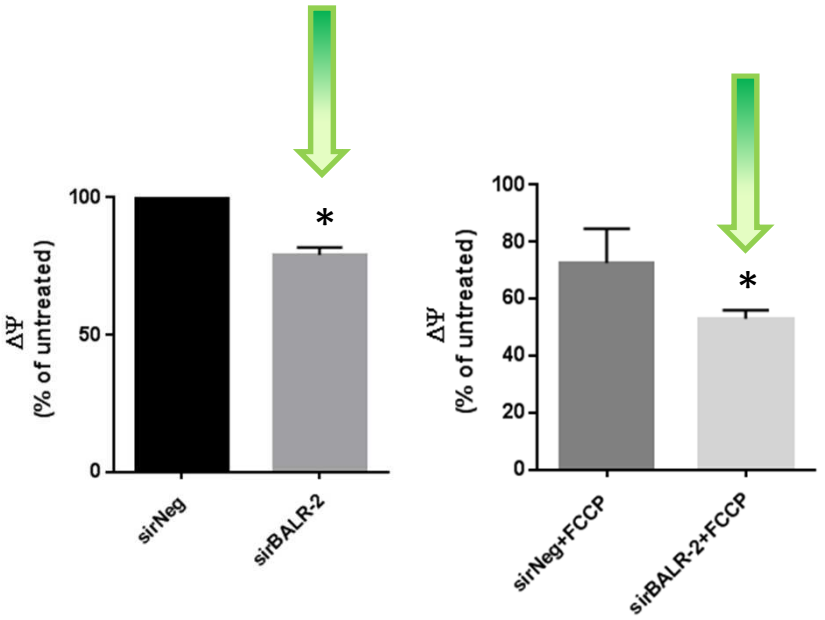


Alti livelli di BALR-2 inducono un aumentata attività del COMPLESSO IV (citocromo c ossidasi) della catena respiratoria

Ruolo di BALR-2 nelle LAM pediatriche

Funzionalità mitocondriale

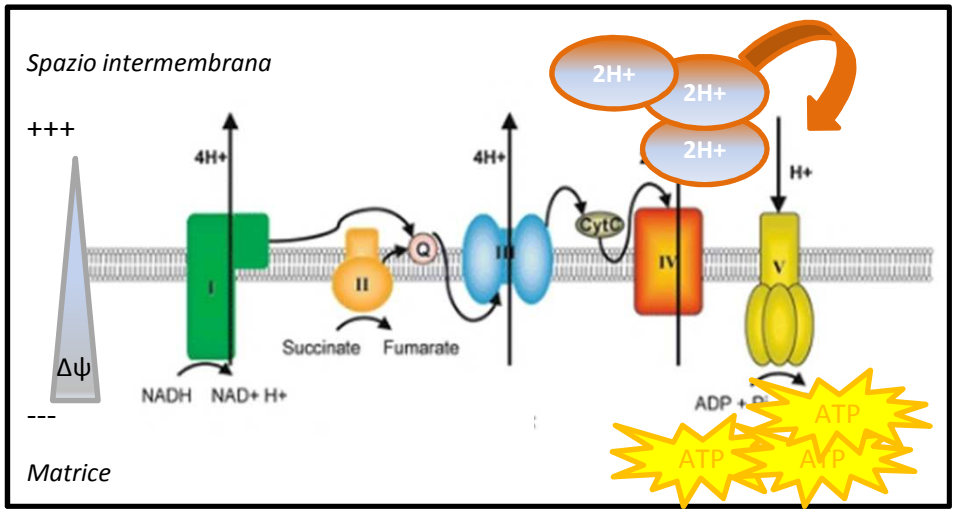


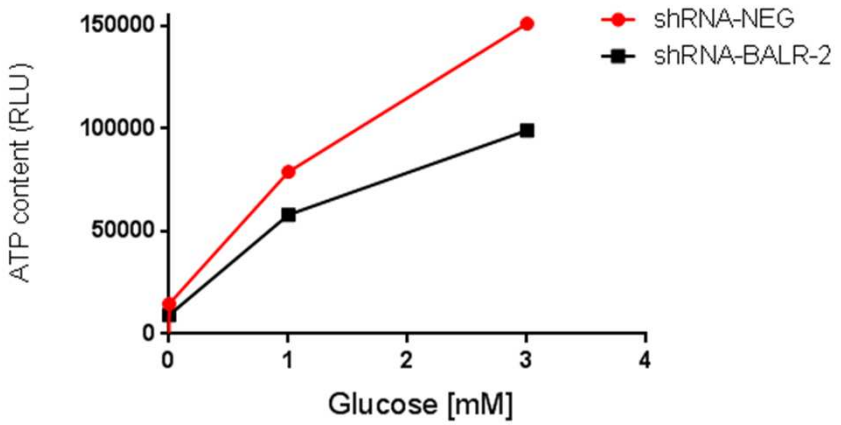


Differente risposta agli inibitori mitocondriali in base ai livelli di BALR-2

Ruolo di BALR-2 nelle LAM pediatriche

Funzionalità mitocondriale

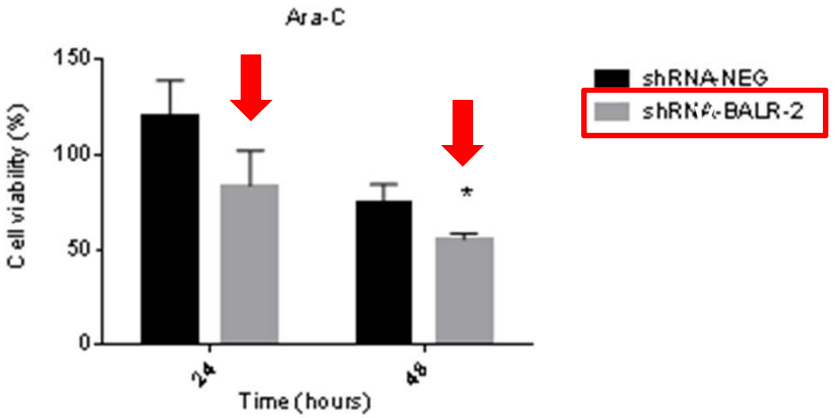


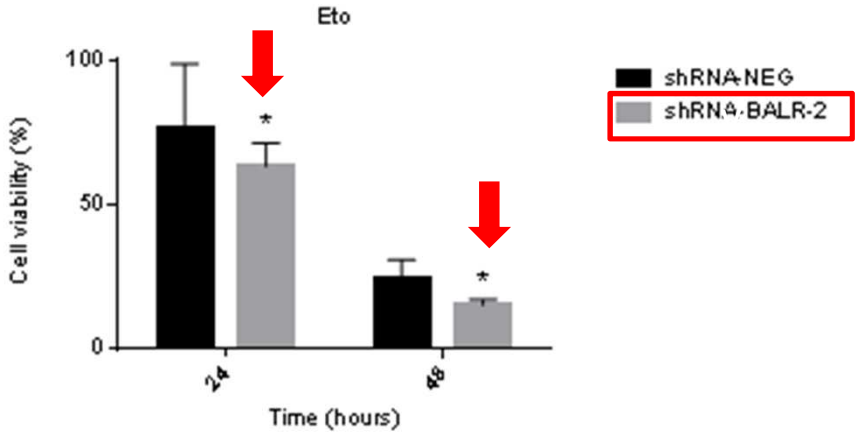


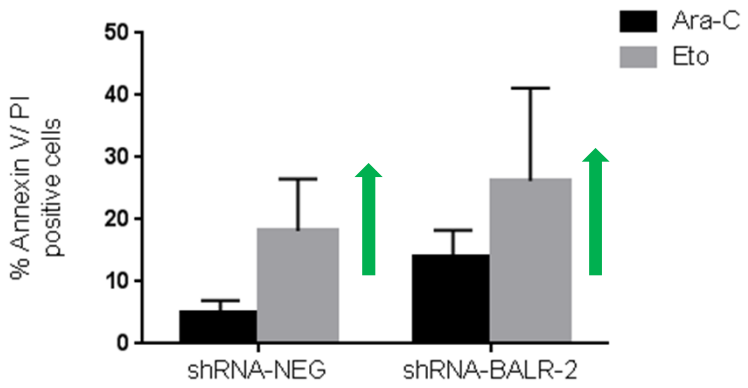
I livelli di BALR-2 modulano la produzione di ATP

Ruolo di BALR-2 nelle LAM pediatriche

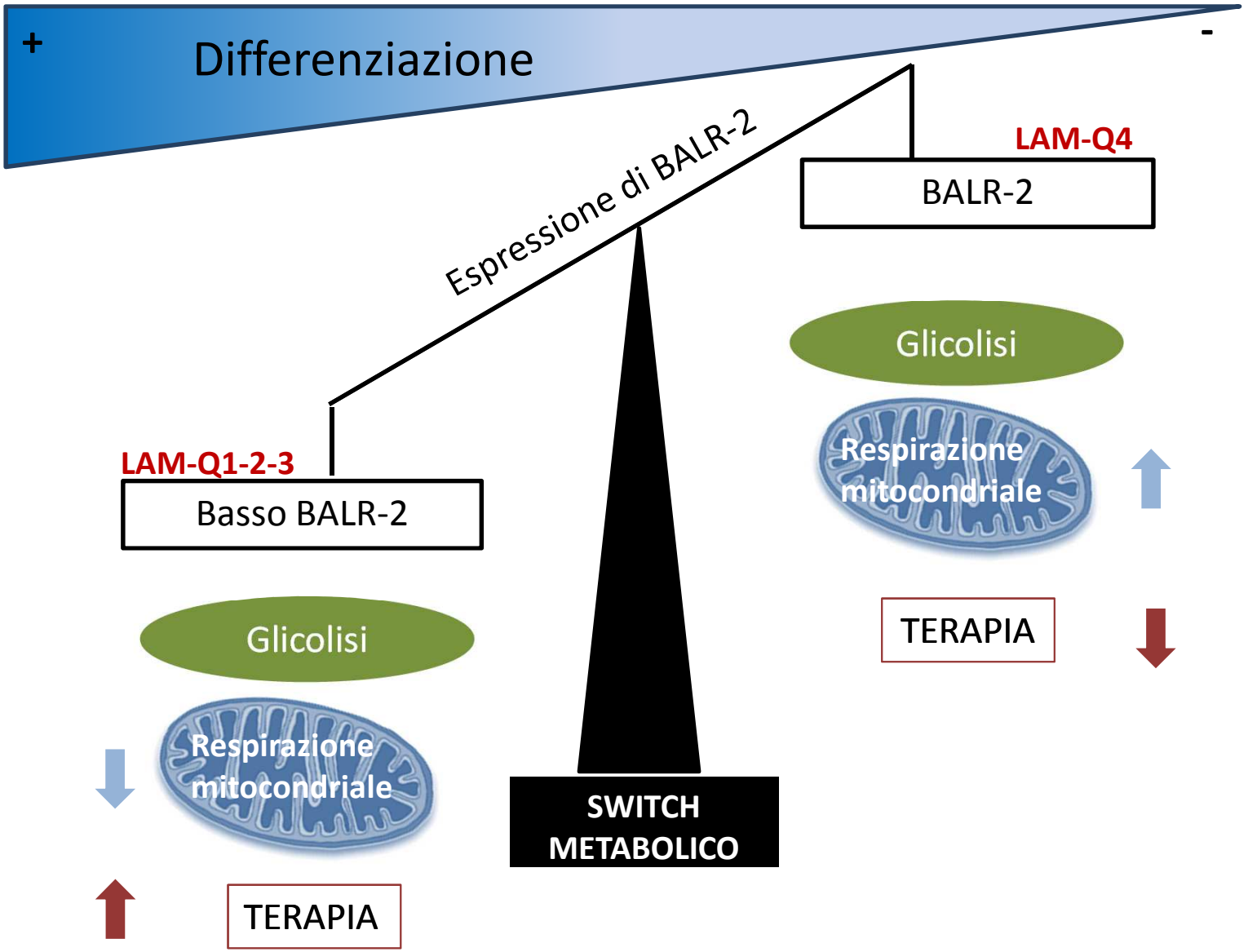
Risposta terapeutica







Conclusioni



PROSPETTIVE FUTURE

- Approfondire il ruolo dei mitocondri nella LAM resistenza alla terapia ⟺ inibitori mitocondriali come nuova strategia terapeutica
- Produrre virus sh-BALR2: silenziamento stabile in CD34+ per validare se BALR-2 è cruciale nel blocco del differenziamento tipico della leucemia

Ringraziamenti

PI: Dr. Martina Pigazzi
Dr. Claudia Tregnago
Dr. Matteo Zampini
Giulia Borella PhD student
Maddalena Benetton PhD student
Ambra dal Ros PhD student
Katia Polato tecnico

Laboratorio di Oncoematologia
Dipartimento di Salute della Donna e del Bambino-
Università degli Studi di Padova
Direttore Prof. G Basso

Genetica Medica
Dr. Eva Trevisson
Dr. Cristina Cerqua

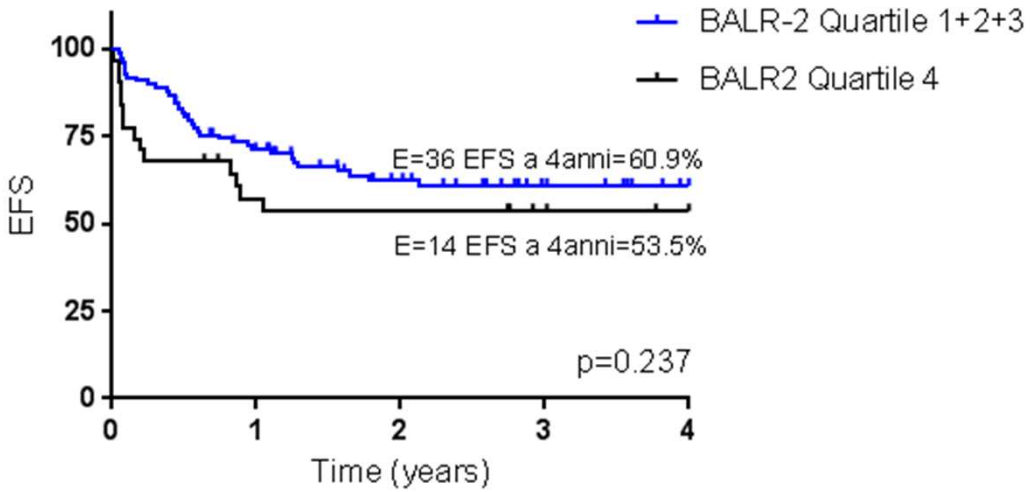
Facility di Bioinformatica IRP:
Carlo Zanon

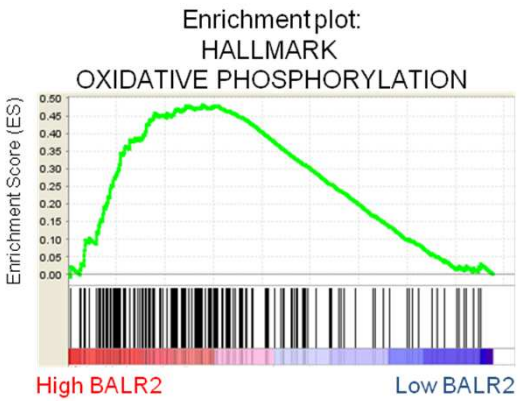
AML working group
Coordinator: Prof. Franco Locatelli

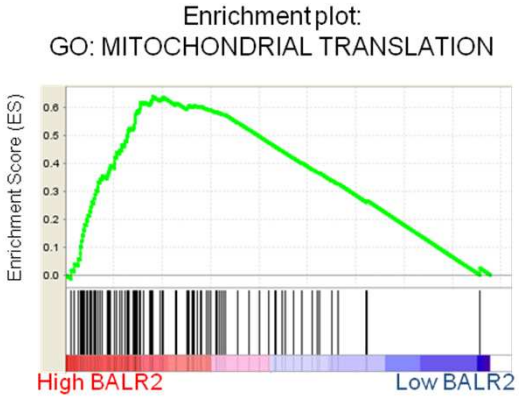
Prof. Andrea Pession
Riccardo Masetti
Pietro Merli
Carmelo Rizzari
Roberto Rondelli
Franca Fagioli
Maria Caterina Putti
Concetta Micalizzi
Marco Zecca
Angela Mastronuzzi
Annamaria Testi
Giuseppe Menna
Nicola Santoro
Paolo Pierani
Luca Lonigro

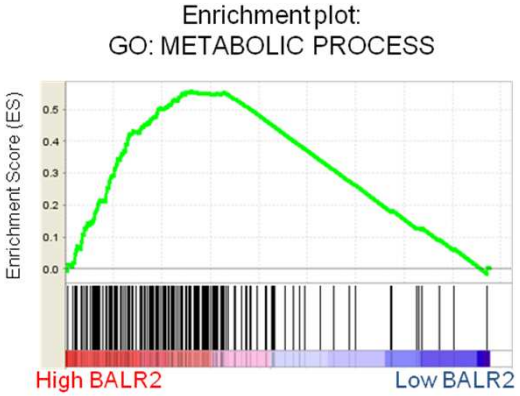
Prof. Silvia Campello
Dr. Luca Simula
Dr. Matteo Borghi

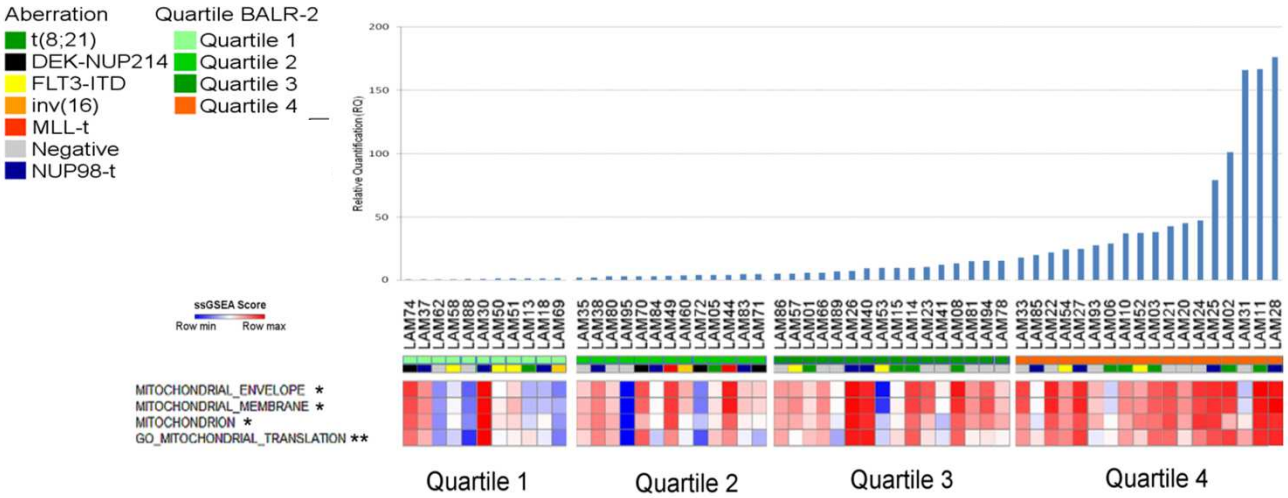
	BALR-2 exp		
	I+II+III quartile	IV quartile	*p-value*
TOTAL	100 (75%)	32 (24.2%)	
Sex			*ns*
F	38 (38%)	21 (65.5%)	
M	62 (62%)	11 (34.4%)	
age			*0,006*
<2 yrs	27 (27%)	1 (3%)	
2-10 yrs	36 (36%)	11 (34%)	
>10 yrs	37 (37%)	20 (62.5%)	
mean	7,4	10,1	
WBC count (x10^9/L)			*ns*
<10	29 (29%)	7 (21.8%)	
>10<99	47 (47%)	14 (43.7%)	
>100	16 (16%)	9 (28.1%)	
N/A	8 (8%)	2 (6.2%)	
mean	49447	101279	
FAB			*ns*
M0	10 (10%)	3 (9.3%)	
M1	11 (11%)	8 (25%)	
M2	17 (17%)	7 (21.8%)	
M4	13 (13%)	4 (12.5)	
M5	27 (27%)	5 (15.6)	
M6	1 (1%)	0 (0%)	
M7	4 (4%)	3 (9.3%)	
N/A	17 (17%)	2 (6.2%)	
Molecular Marker			*ns*
CBF-rearranged	13 (13 %)	7 (21.8%)	
FLT3-ITD	11 (11%)	4 (12.5)	
NUP98-t	9 (9%)	6 (18,7)	
MLL-t	33 (33%)	4 (12.5)	
Rare translocations	8 (8%)	1 (3,1%)	
No Molecular Marker	26 (26%)	10 (31,2)	
Karyotype			*ns*
Complex	8 (8%)	4 (12.5%)	
Monosomal	2 (2%)	1 (3.1%)	
Normal	65 (65%)	21 (65.6%)	
N/A	25 (25%)	6 (18.7)	
Primary Induction Failure (PIF)			
YES	8 (25%)	5 (15.6%)	*ns*
NO	92 (92%)	27 (13.5)	
Remission			
YES	88 (88%)	23 (71.8%)	*0,03*
NO	12 (12%)	9 (28.1%)	
Relapse			
YES	25 (25%)	9 (28.1%)	*ns*
NO	75 (75%)	23 (71.8%)	

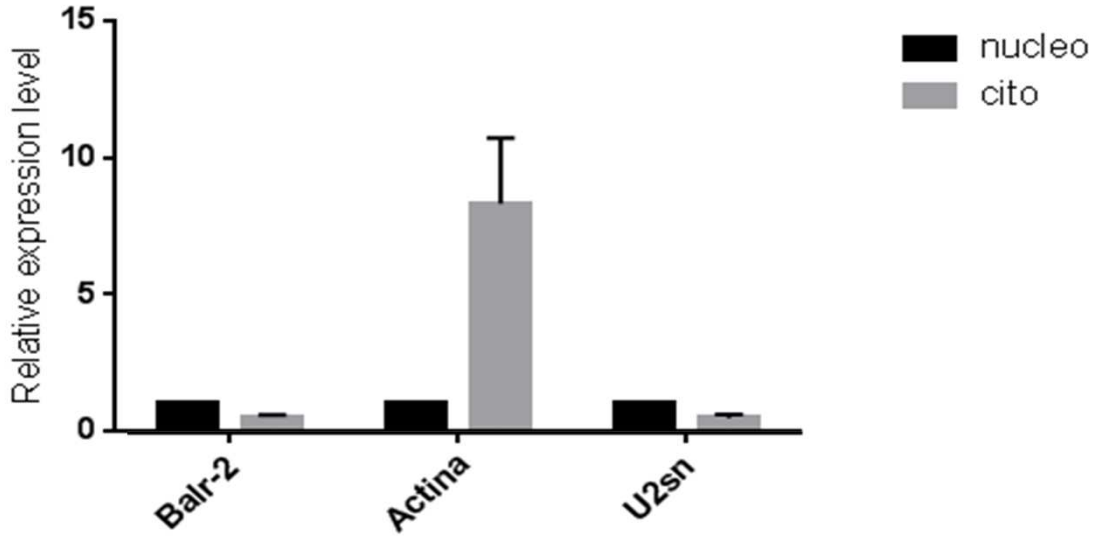

BALR-2 Quartile 1+2+3
BALR2 Quartile 4
100
75
50
25
0
EFS
E=36 EFS a 4anni=60.9%
E=14 EFS a 4anni=53.5%
p=0.237
0
1
2
3
4
Time (years)


Enrichment plot:
HALLMARK
OXIDATIVE PHOSPHORYLATION
Enrichment Score (ES)
High BALR2
Low BALR2


Enrichment plot:
GO: MITOCHONDRIAL TRANSLATION
Enrichment Score (ES)
High BALR2
Low BALR2


Enrichment plot:
GO: METABOLIC PROCESS
Enrichment Score (ES)
High BALR2
Low BALR2


Aberration
t(8;21)
DEK-NUP214
FLT3-ITD
inv(16)
MLL-t
Negative
NUP98-t
Quartile BALR-2
Quartile 1
Quartile 2
Quartile 3
Quartile 4
Relative Quantification (RQ)
ssGSEA Score
Row min
Row max
MITOCHONDRIAL_ENVELOPE *
MITOCHONDRIAL_MEMBRANE *
MITOCHONDRION *
GO_MITOCHONDRIAL_TRANSLATION **
Quartile 1
Quartile 2
Quartile 3
Quartile 4


15
10
5
0
Relative expression level
Balr-2
Actina
U2sn
nucleo
cito