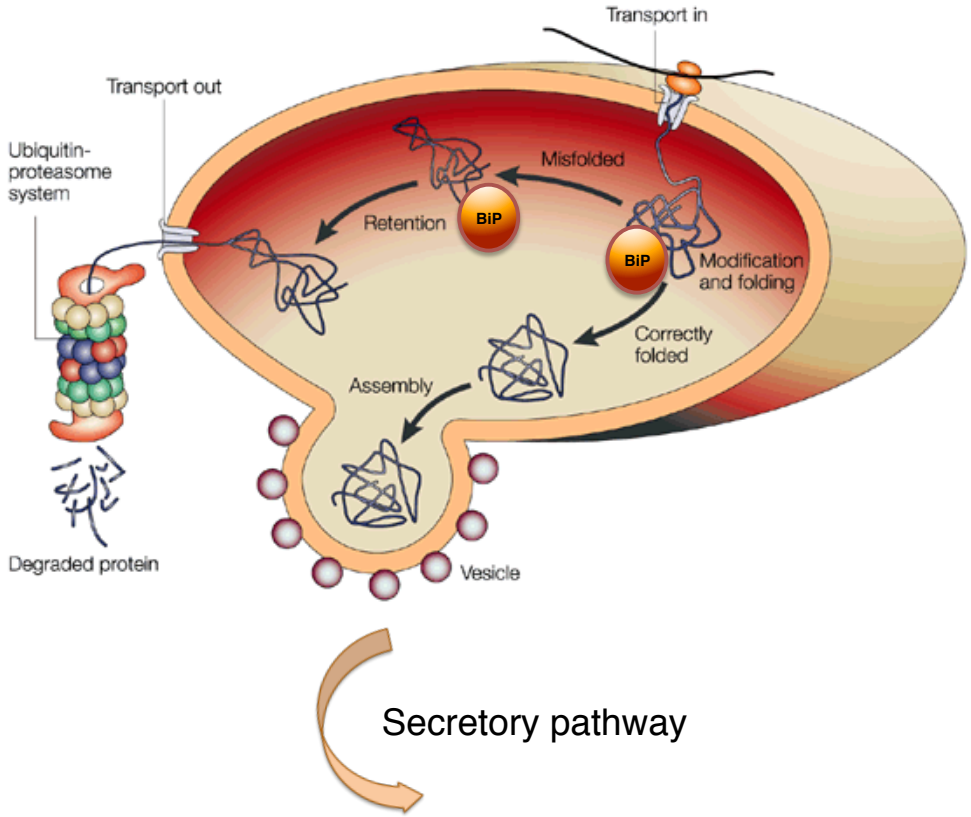
SAPIENZA
UNIVERSITÀ DI ROMA

Retinoic Acid and Arsenic Trioxide sensitize Acute Myeloid Leukemia cells to ER stress

7th international symposium on Acute Promyelocytic Leukemia
September 24-27, 2017

The Endoplasmic Reticulum

Secretory protein folding and processing

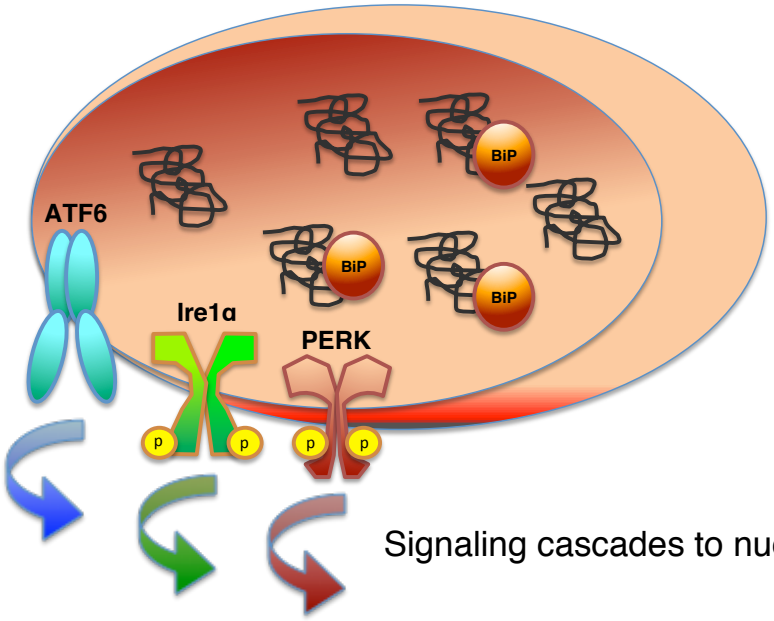


The endoplasmic reticulum (ER) is a multifunctional organelle

- ✓ lipid biosynthesis
- ✓ calcium storage
- ✓ protein folding and processing of membrane and secreted proteins

Dobson CM Nature Reviews Drug Discovery 2, 154-160 (February 2003)

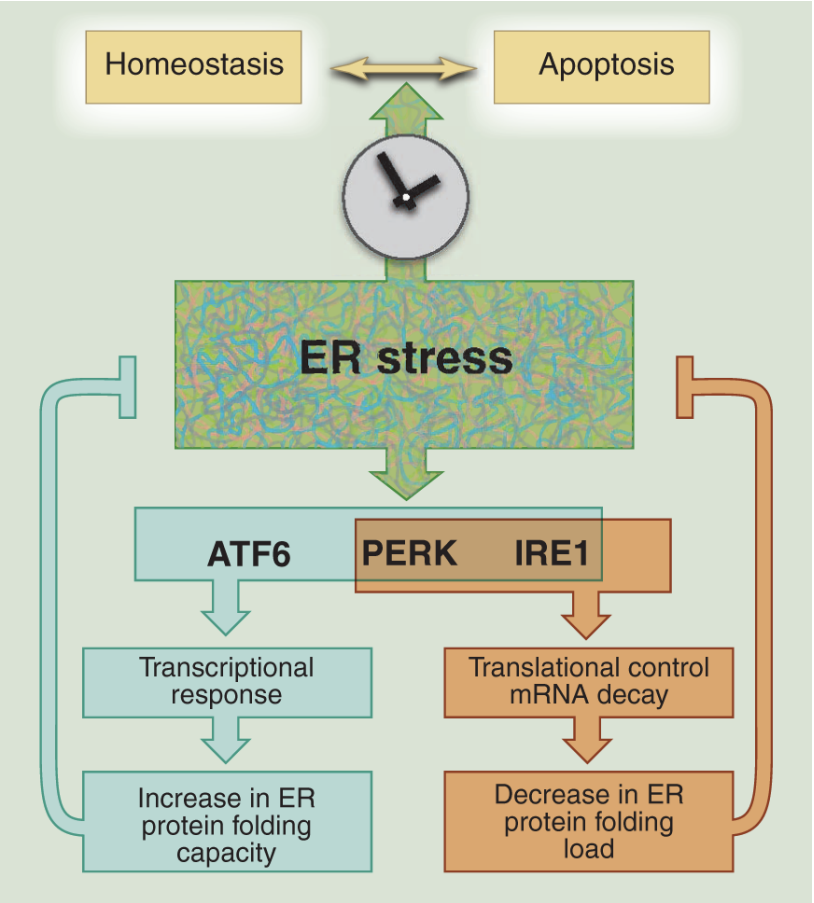
ER stress triggers the Unfolded Protein Response (UPR)



Signaling cascades to nucleus

ER stress is a condition of imbalance between the folding capacity and the amount of unfolded client proteins.

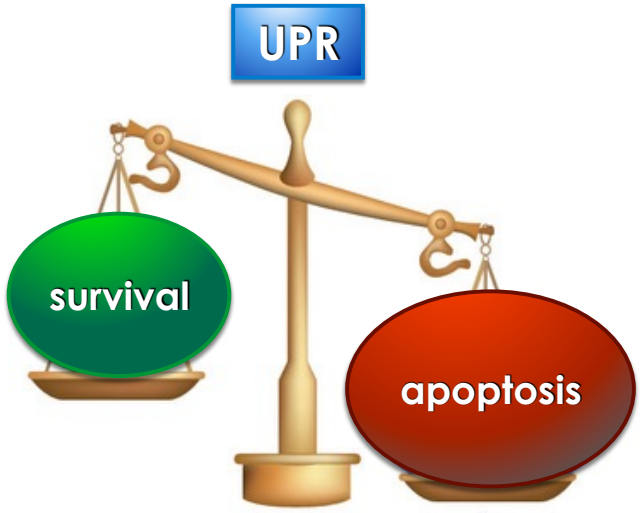
- ✓ physiological and pathological conditions
- ✓ pharmacological agents



from Walter and Ron 2011, SCIENCE VOL 334: 1081

Rationale

Aggravating ER stress in cancer to shift the UPR balance toward apoptosis



Rationale

Amounts of ER stress that can be coped with by most cells can be detrimental in cells with altered ER homeostasis

ER intrinsic causes

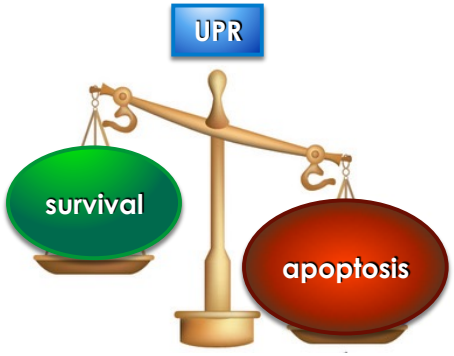
- ✓ adaptation to increased folding demand
- ✓ presence of mis-folded or aggregation prone proteins
- ✓ Ca++ unbalance
- ✓ …

ER extrinsic causes

- ✓ oxidative stress
- ✓ Ca++ unbalance
- ✓ presence of mis-folded or aggregation prone proteins
- ✓ impairment of protein degradation systems (proteasome, autophagy)
- ✓ …

Rationale

Aggravating ER stress in cancer to shift the UPR balance toward apoptosis



AML

Characterized by the presence of chimeric or mutant protein, possibly prone to mis-folding and aggregation.
(Data discussed by Ernestina Capuano in poster **PO002,** 01 APL biology topic)

APL

Expression of PML-RARα

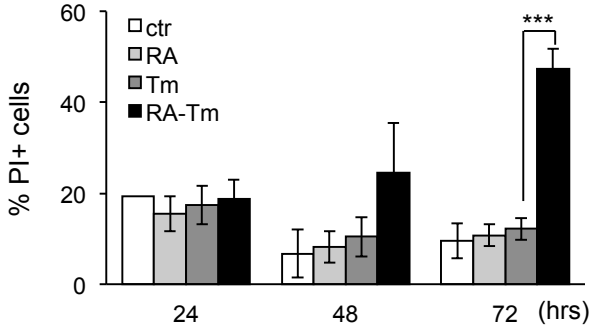
Granulocytic differentiation induced by *all-trans* retinoic acid requires increased folding capacity in the ER

Masciarelli et al., Leukemia 2017

RA-induced granulocytic differentiation sensitizes NB4 cells to ER stress

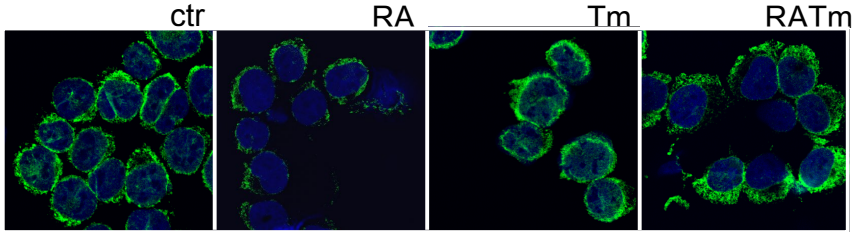
Treatment of the APL cell line NB4 with physiological doses of RA (10^{-8}) in combination with low doses of Tunicamycin (Tm, 50ng/ml) causes:

cell death

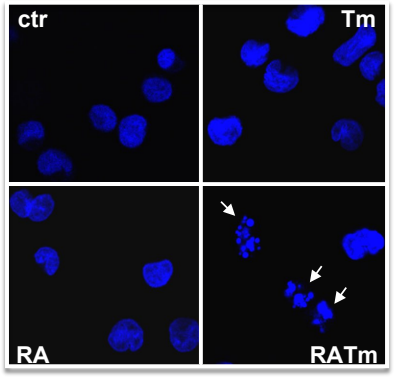


ER stress

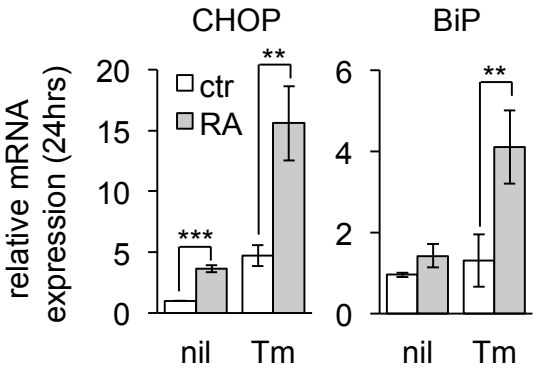
ER swelling



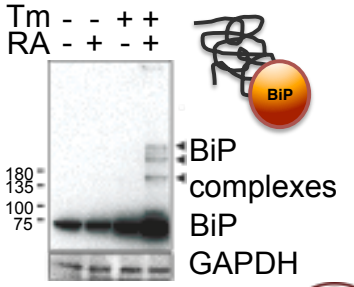
Staining for the ER chaperone calreticulin (72hrs)



Up-regulation of UPR target genes



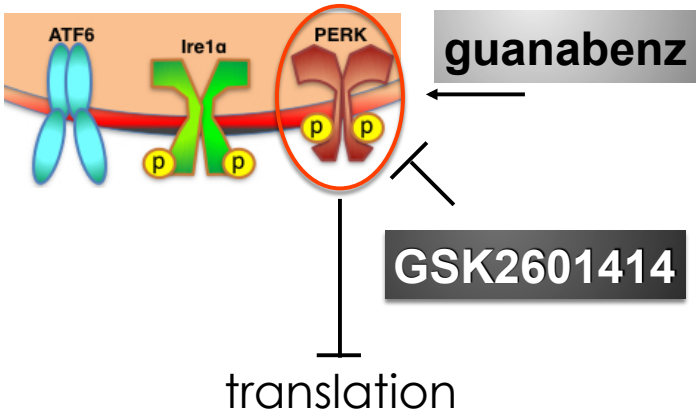
Accumulation of BiP complexes



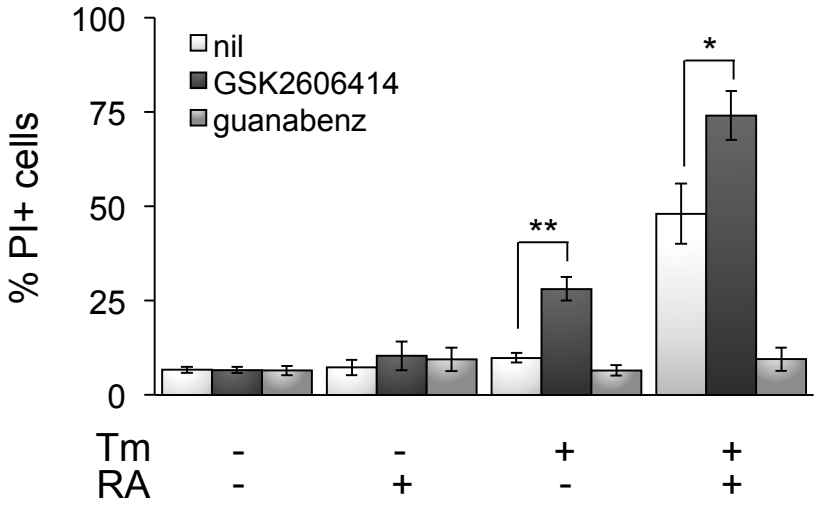
(72hrs)

n ≥ 3; * p value < 0.05, ** <0.02, ***< 0.005

Attenuation of general translation protects differentiating NB4 cells from ER stress-induced death



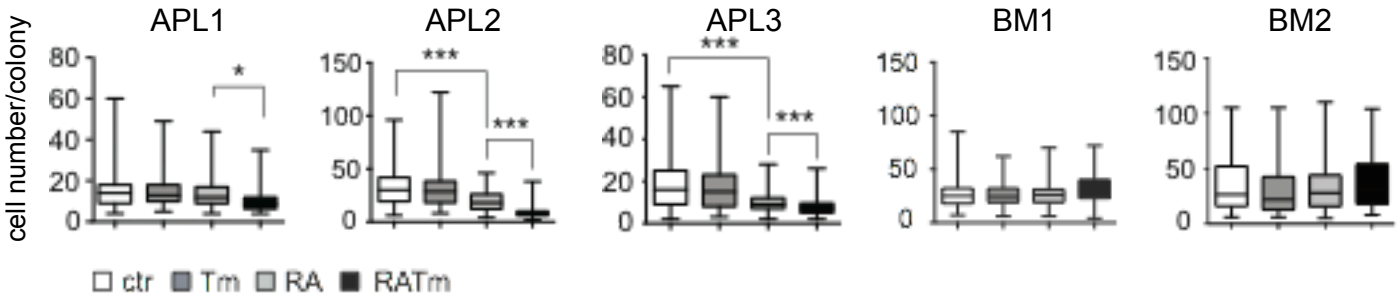
Activation of the PERK pathway results in attenuation of global translation with consequent reduction of the ER load



n ≥ 3; * p value < 0.05, ** <0.02, ***< 0.005

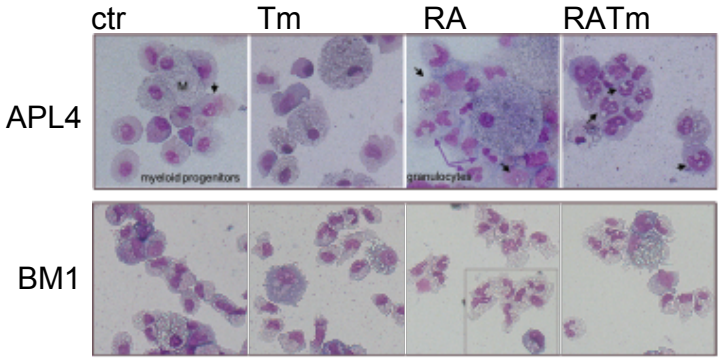
RA sensitizes APL primary blasts to ER stress

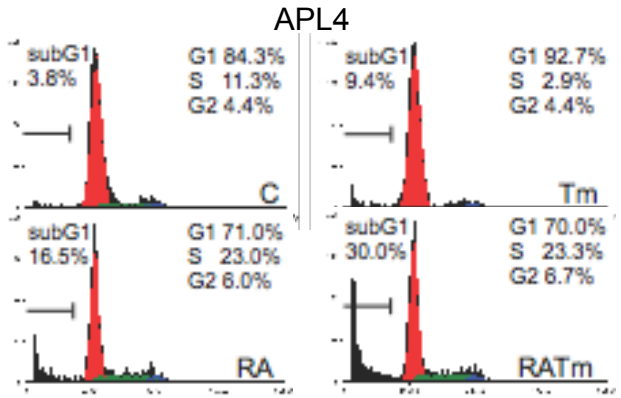
Treatment of APL primary blasts with 10^{-8} RA in combination with 50ng/ml Tm leads to:

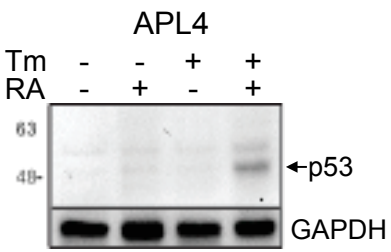


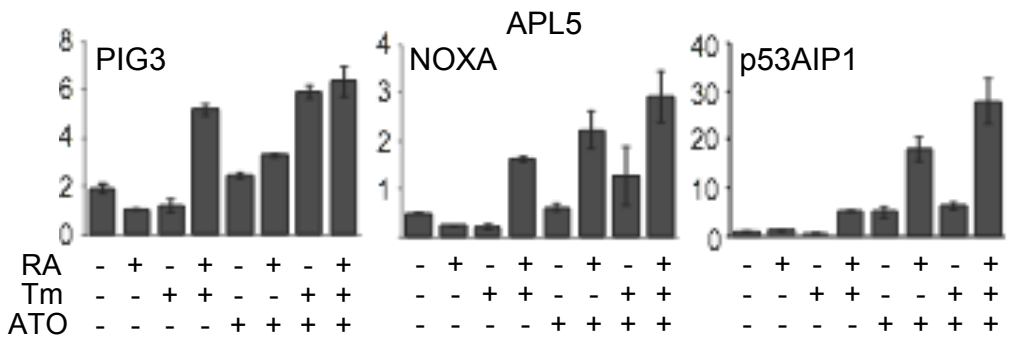
Formation of smaller colonies in CFU assay

Apoptotic cell death





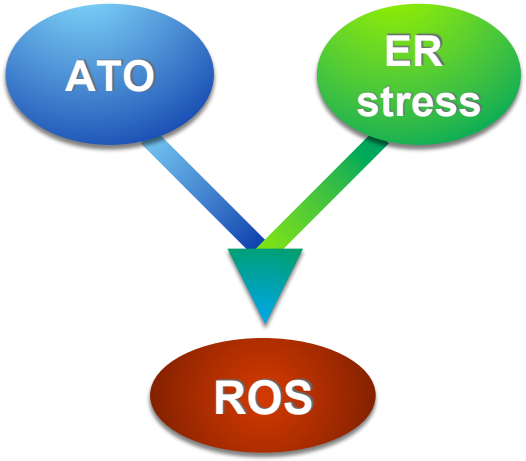




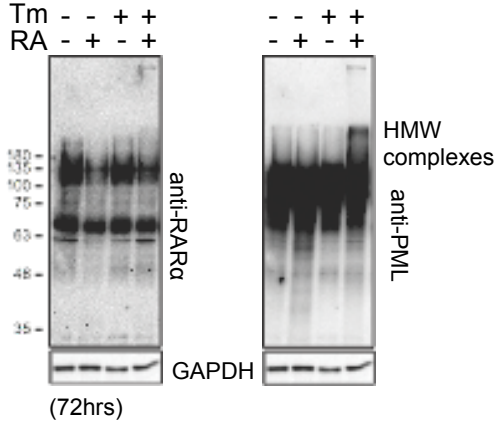
Activation of the TP53 pathway

ER stress and ATO exhibit synergistic toxicity in RA-sensitive NB4 and RA-resistant NB4-R4 cells

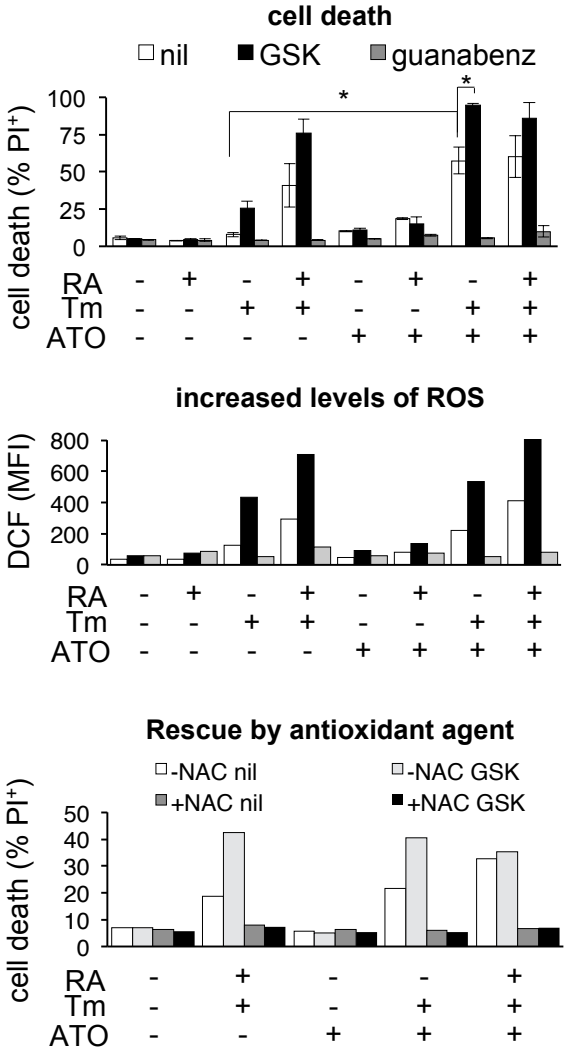
ER stress generates oxidative stress that results lethal in cells concomitantly treated with RA or ATO



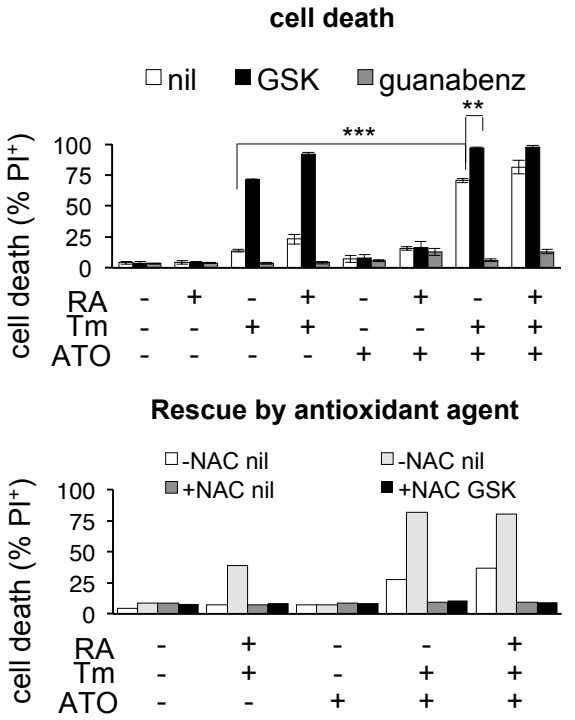
Disulphide bond-dependent aggregation of PML and PML-RARα



RA-responsive NB4



RA-resistant NB4-R4

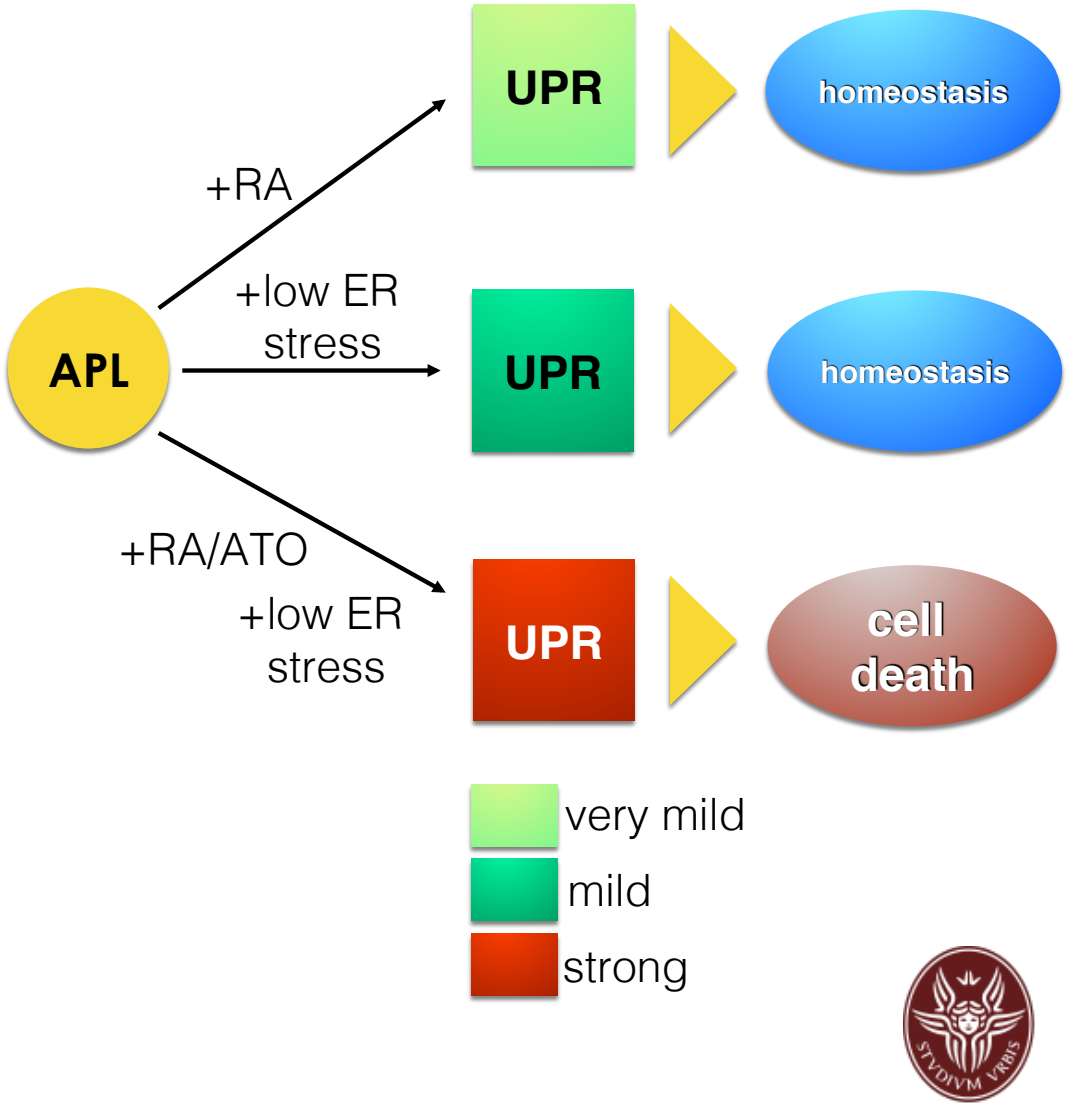


RA 10^{-8}
ATO 500nM
Tm 50ng/ml

Induction of ER stress in combination with RA and/or ATO shifts the balance of the UPR from recovery of homeostasis to apoptosis

CONCLUSION

- ✓ Low ER stress in combination with RA and/or ATO efficiently targets APL cells, *in vitro*, without affecting normal hematopoietic progenitors
- ✓ Doses of RA and ATO below the therapeutical reference range are sufficient to syngergize with Tm
- ✓ The toxic effect is mediated by activation of the UPR as a pro-apotic response and by generation of oxidative stress
- ✓ Pharmacological inhibition of the PERK pathway amplified the toxicity of the combined treatments making it an interesting molecular target



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