APOPTOSIS INDUCTION AND IMMUNOTHERAPY-HOW TO IMPROVE RESULTS OF IMMUNOTHERAPY

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IMMUNOTHERAPY DEPENDS ON INDUCTION OF APOPTOSIS!

- IF WE UNDERSTAND THE RESISTANCE MECHANISMS AGAINST APOPTOSIS WE CAN TARGET THESE AND IMPROVE THE RESULTS OF IMMUNOTHERAPY

CELL KILLING MECHANISMS USED BY LYMPHOCYTES DEPEND ON INDUCTION OF APOPTOSIS

1. Granzyme – Perforin Mediated Killing

CD8 CTL (CD4 CTL)

NK Cells and ADCC

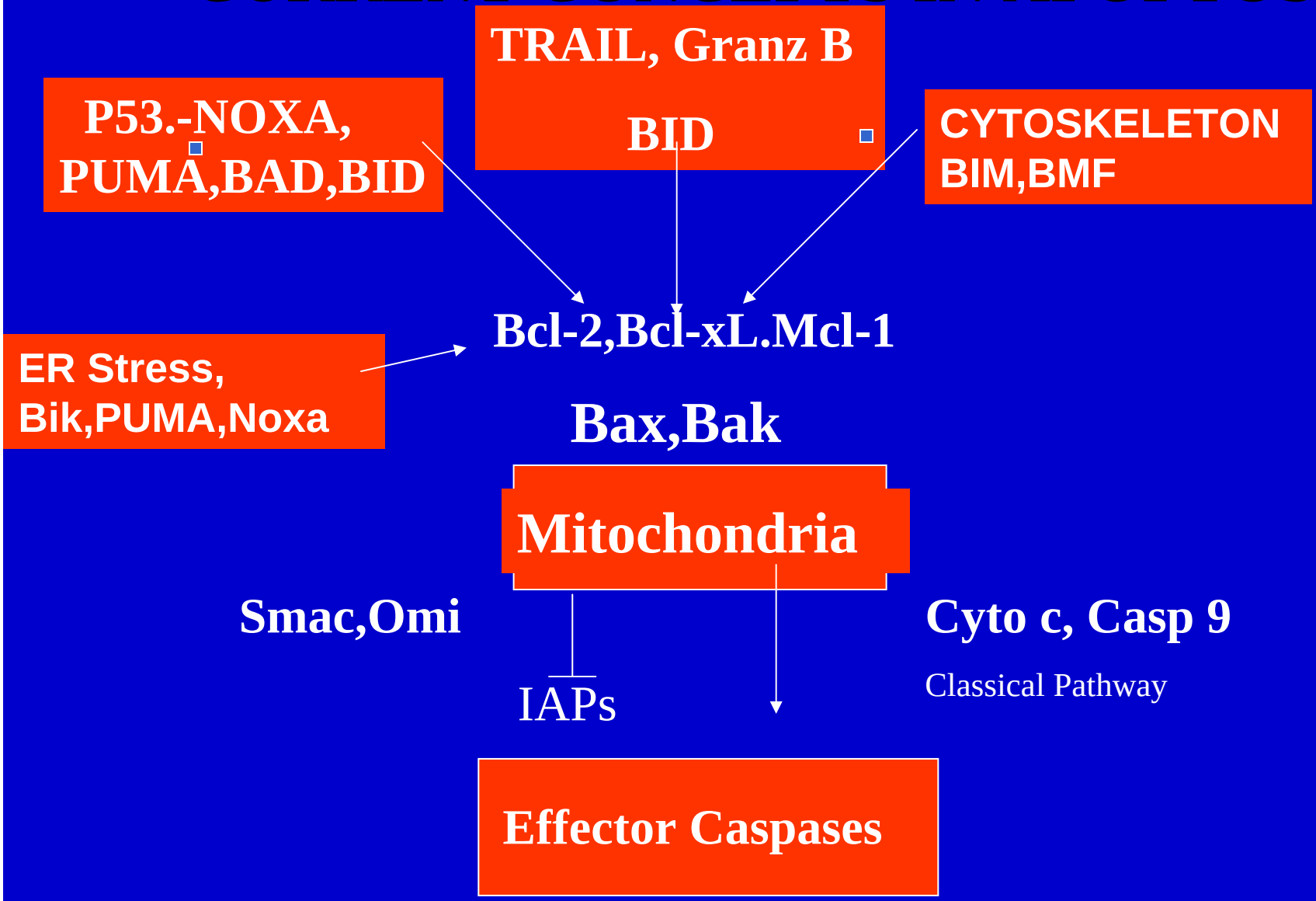
2.Death Ligand Mediated Killing

TRAIL, FasL, TNF

CD4 T Cells

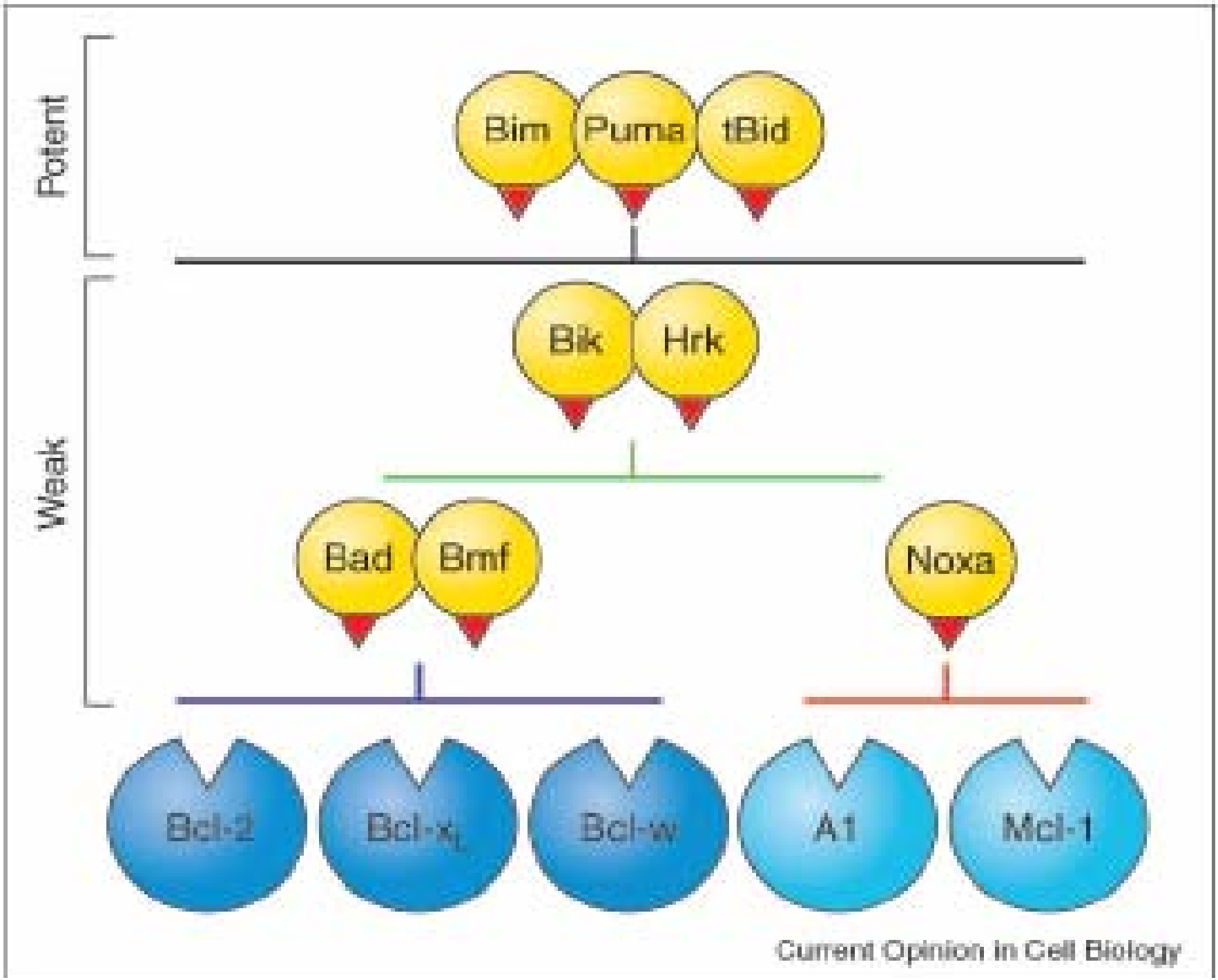
Monocytes, Dendritic Cells

CURRENT CONCEPTS IN APOPTOSIS



MITOCHONDRIAL PATHWAYS TO APOPTOSIS ARE REGULATED BY BCL-2 FAMILY PROTEINS

- Pro-apoptotic BH3 only damage sensor proteins (Bid, Bik,Bim, Bmf, Noxa,Puma,Bad,Hrk)
- Pro-apoptotic multidomain proteins: BAX, BAK
- Anti-apoptotic proteins: BCL-2, BCL-Xl, MCL-1, Bcl-W,A1


Potent
Weak
Bim
Puma
tBid
Bik
Hrk
Bad
Bmf
Noxa
Bcl-2
Bcl-xL
Bcl-w
A1
Mcl-1
Current Opinion in Cell Biology

WE ALREADY HAVE AGENTS THAT TARGET THE ANTI APOPTOTIC PROTEINS!

Targetting Anti Apoptotic Proteins

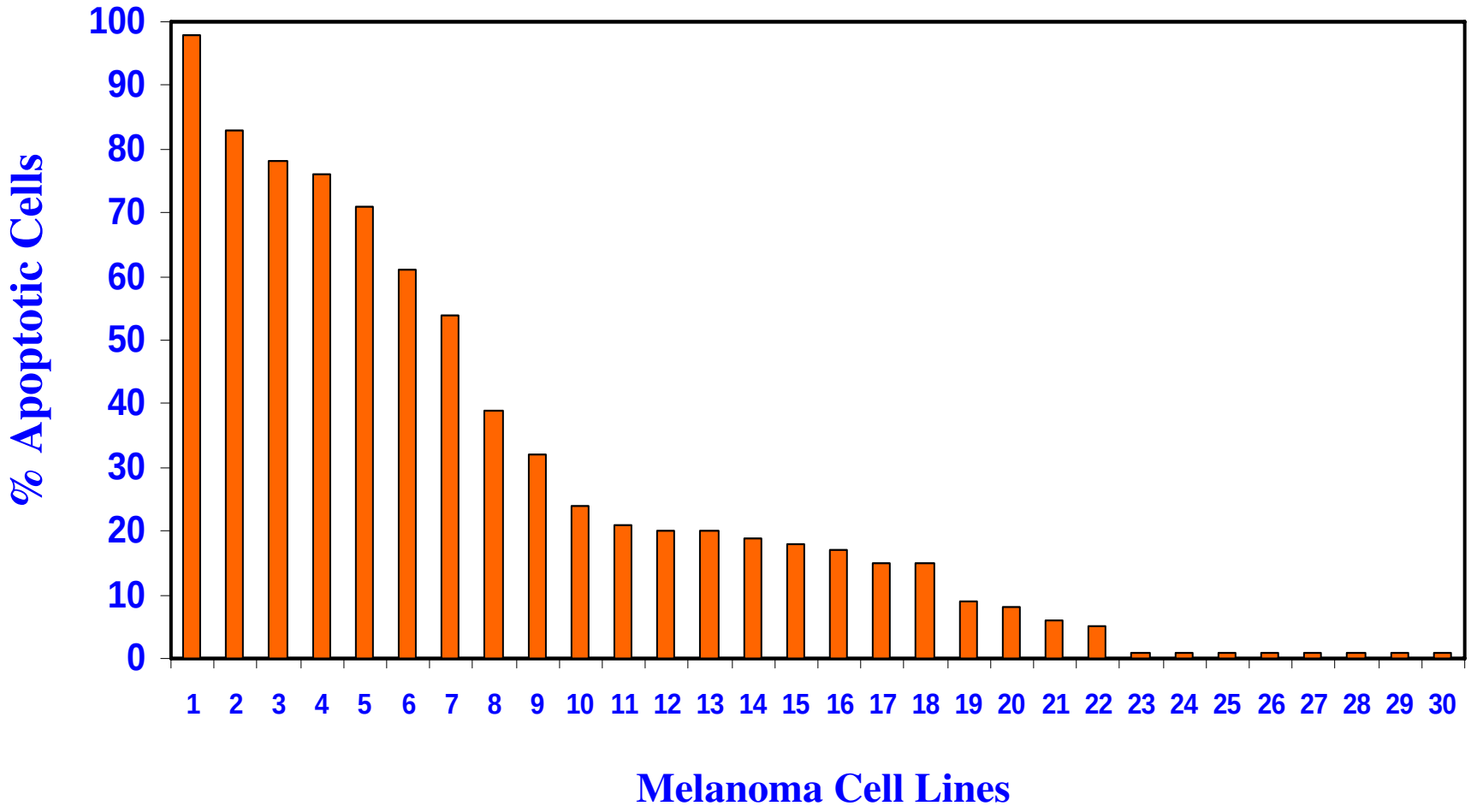
- Genasense against Bcl-2.
- Inhibition of production of the IAP protein Survivin YM155 (Astellas Pharm
- BH3 mimics that bind Bcl-2 proteins(Abbott ABT-737)

Targetting Anti-Apoptotic Proteins

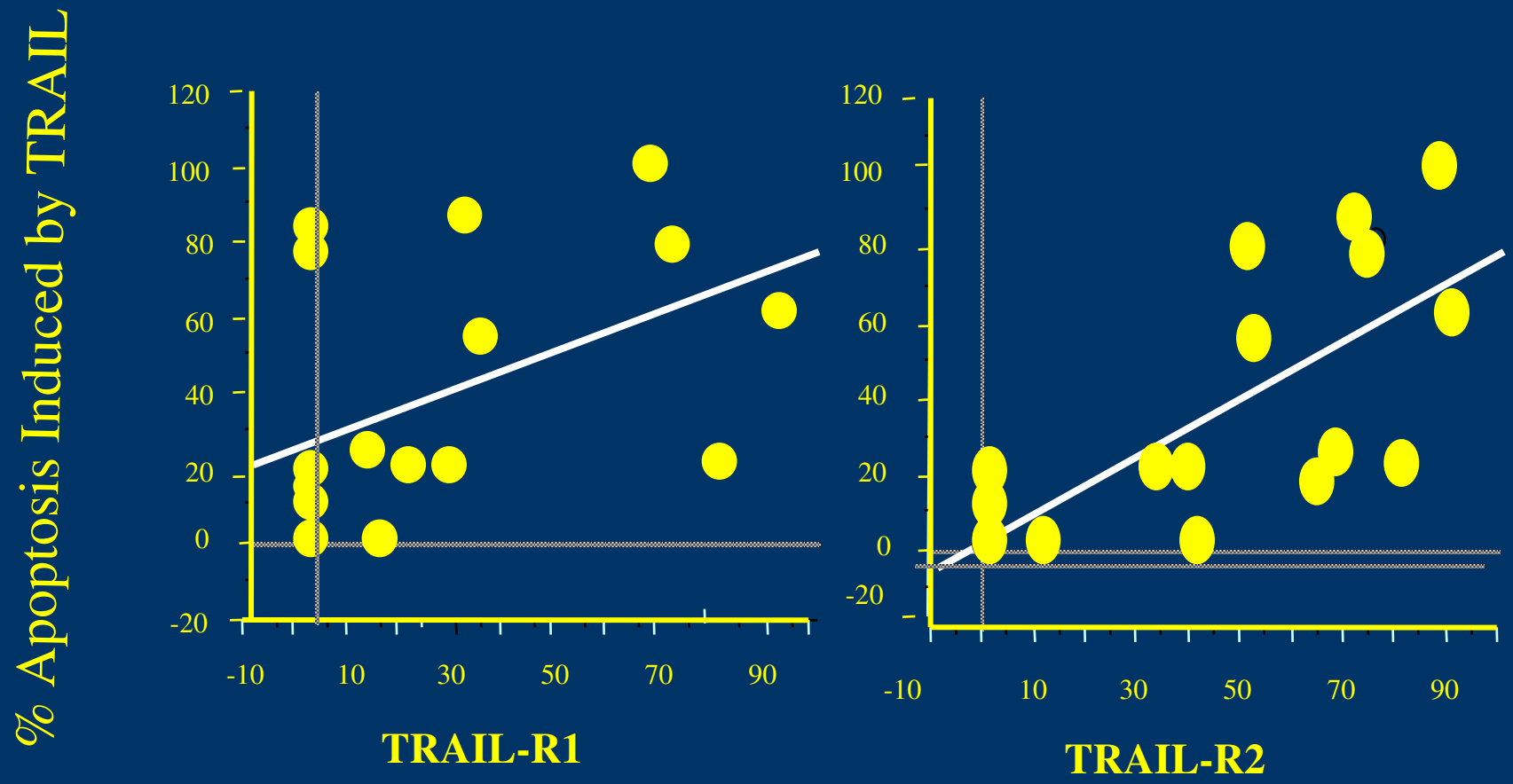
- **AT-101 (Gossypol) Oral inhibitor of Bcl-2 Bcl-XL,Mcl-1 . Ascenta Therapeutics**
- **TW37- Small mw mimic of Bim that inhibits Bcl-2, Bcl-XL,Mcl-1. Univ Michigan**
- **Obatoclax (GX015-070) Small mw BH3 mimic . Inhibits Bcl-2,Bcl-XL,Mcl-1. (Gemin X)**

TRAIL INDUCED KILLING REQUIRES DEATH RECEPTORS!

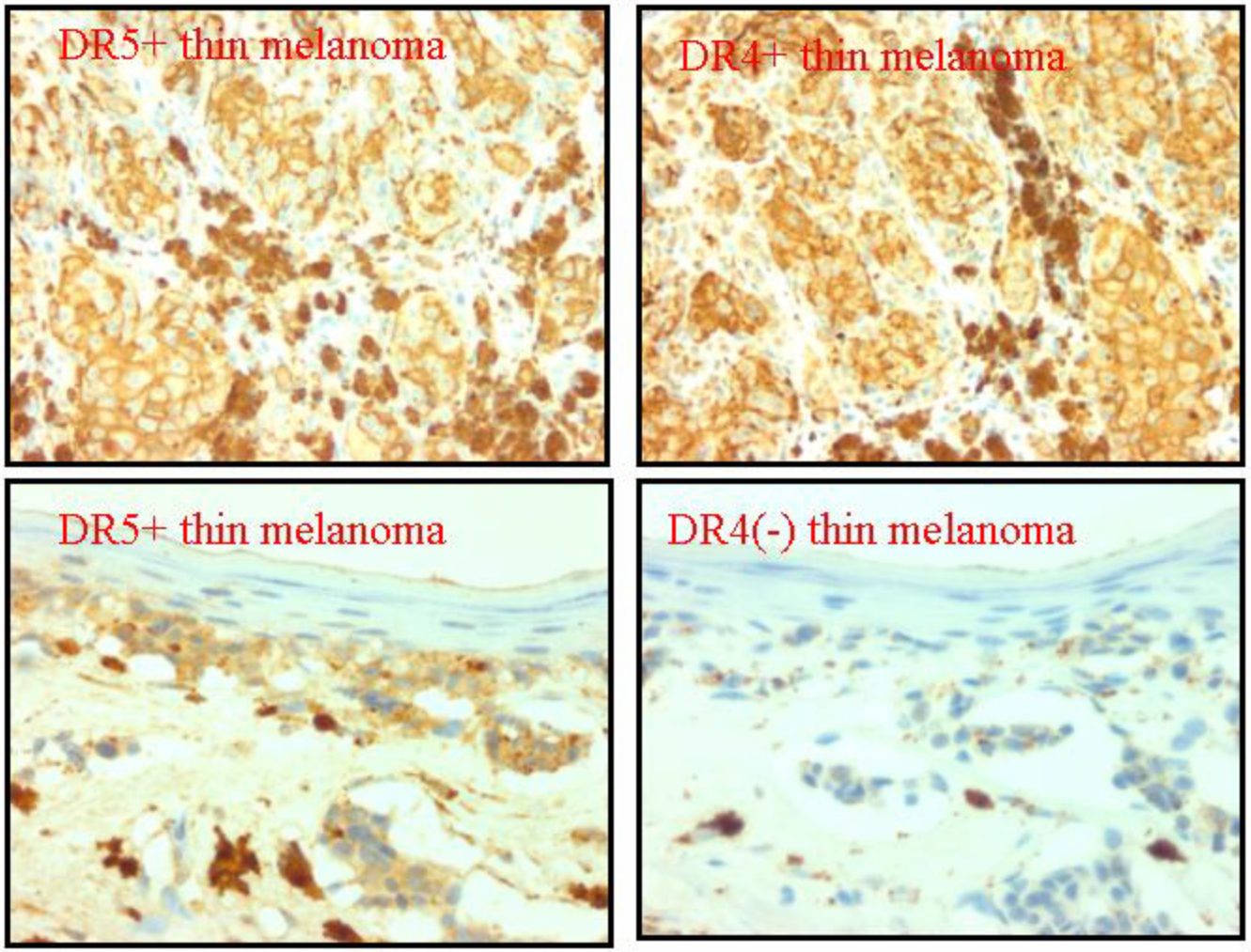
TRAIL Induces Apoptosis in the Majority of Melanoma Cell Lines



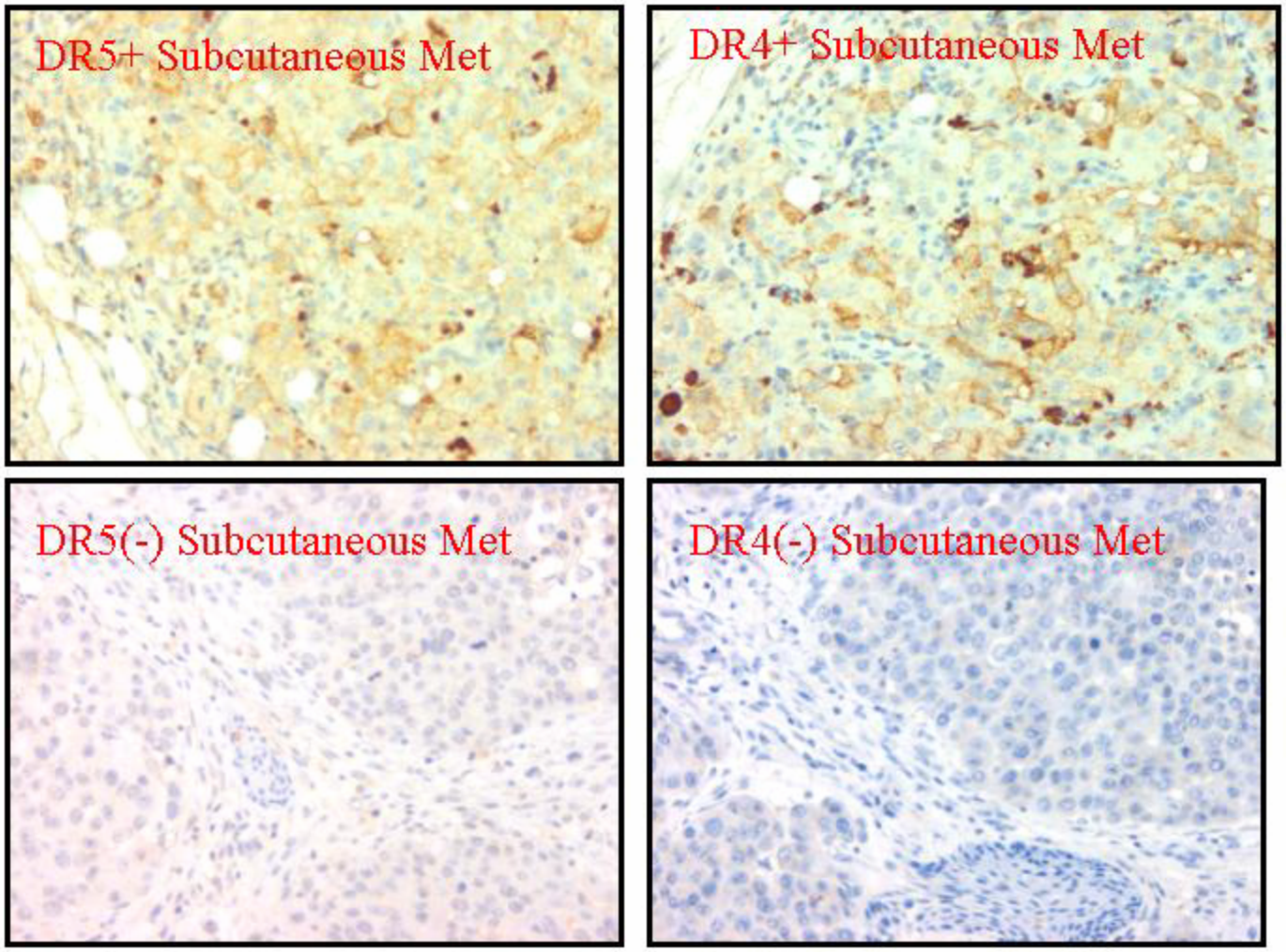
TRAIL-R1 & R2 Expression Correlates with Degree of Apoptosis



Zhang et al. Cancer research.59:2747 1999



Zhuang et al. Human Pathology 37,1286-94 .2006

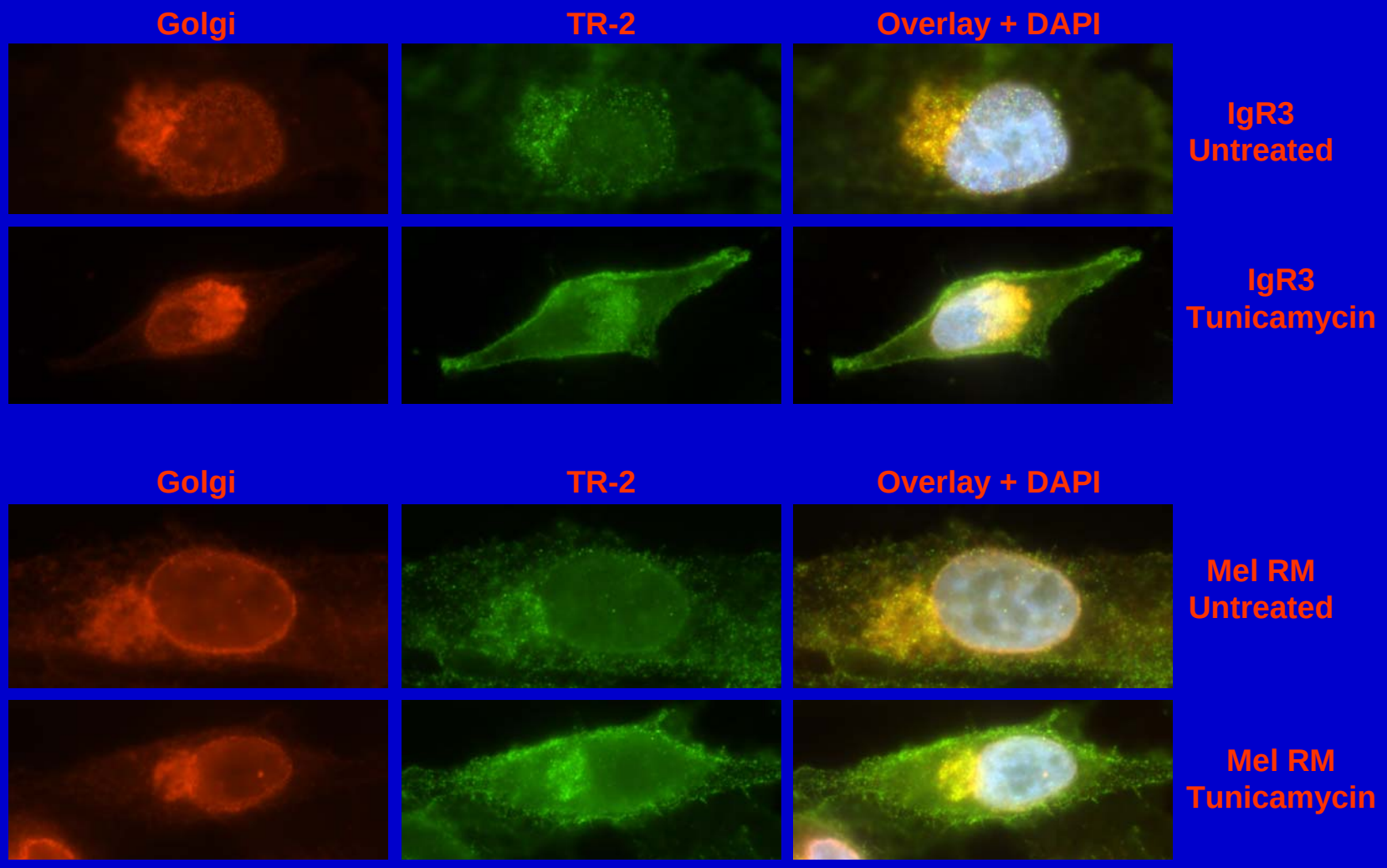

DR5+ Subcutaneous Met
DR4+ Subcutaneous Met
DR5(-) Subcutaneous Met
DR4(-) Subcutaneous Met

CAN TRAIL RECEPTORS BE UPREGULATED?

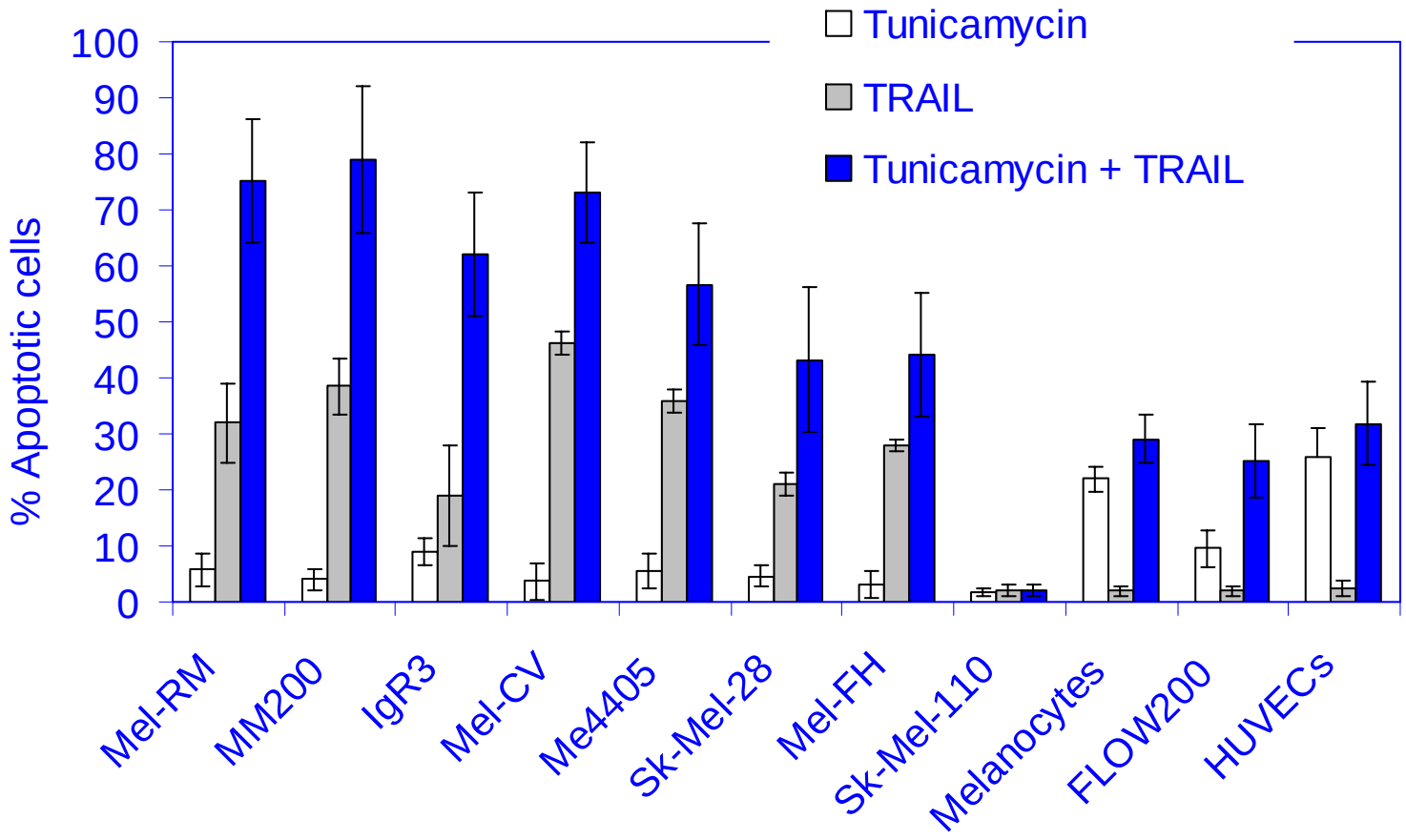
AGENTS THAT UPREGULATE TRAIL DEATH RECEPTORS

- Tunicamycin- Glycosylation inhibitor. Induces ER Stress
- Cox 2 Inhibitors. Upregulates Gadd 153(CHOP)
- Dipyrimadole-nucleoside transport inhibitor
  Upregulates Gadd153
- Curcumin-Upregulates Gadd153
- Perifosine (AKT inhibitor) in some cells

Tunicamycin selectively upregulates TRAIL-R2



Tunicamycin sensitizes melanoma cells to TRAIL-induced apoptosis



Jiang et al Canc Res 67:5880 2007

STATUS OF CURRENT TRIALS WITH TRAIL (ASCO 2006,2007)

Genentech/Amgen. 58 patients with various cancers. 7 with melanoma. 1 hr infusion, 5 days each 3 weeks. Well tolerated Abst3013

HGS. ETR2,Lexatumumab.Up to 10mg/kg each 2 weeks.31 patients Abst3012

HGS. ETR1,Mapatumumab. 1CR,2PR in follicular Lymphoma. Now in phase 2 studies in NHL

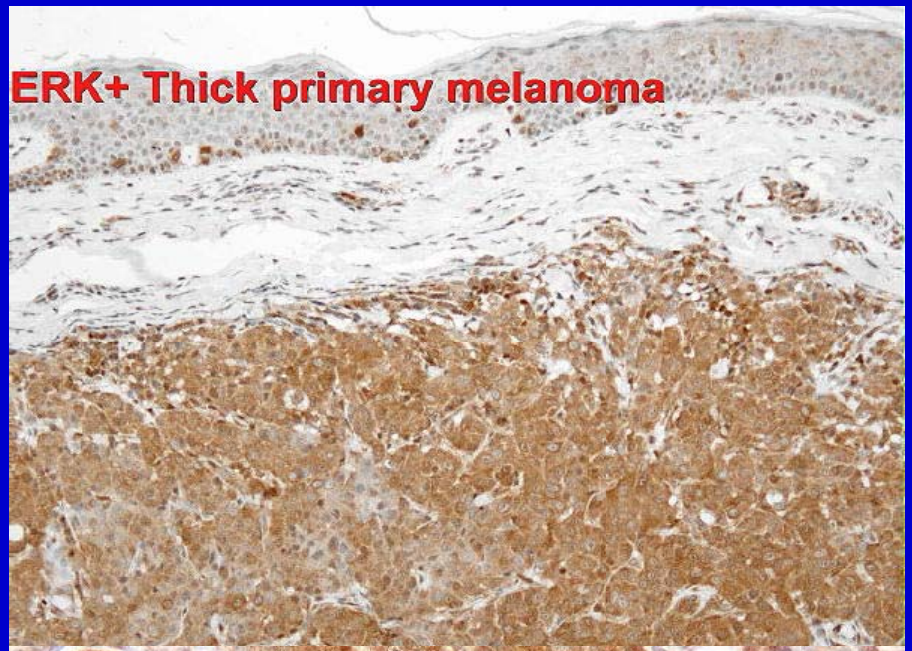
Amgen. AMG655 Ab.Dose finding in16 patients

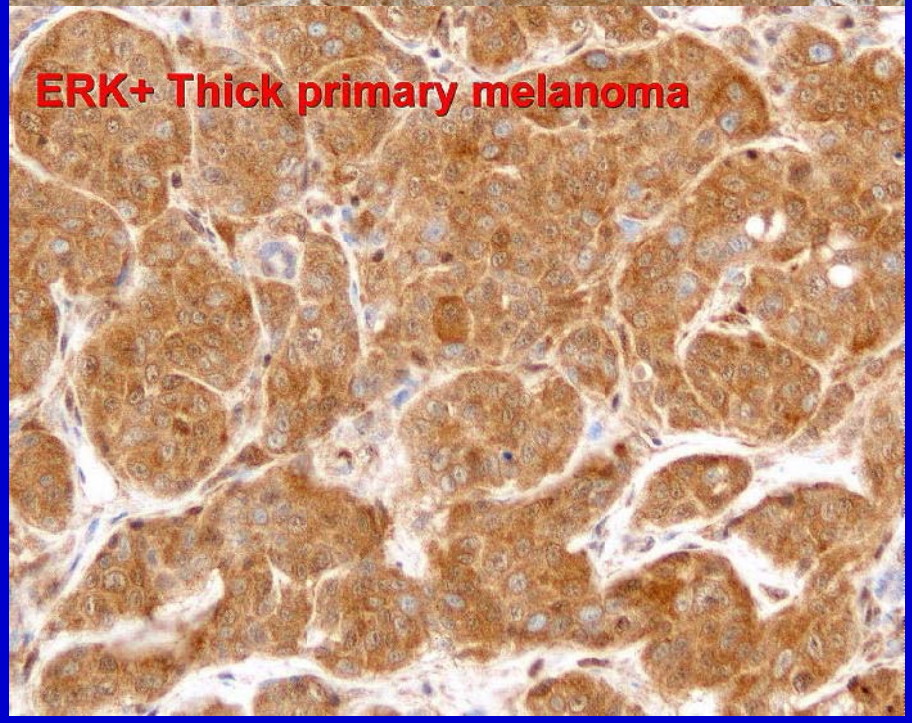
IS MANIPULATION OF THE BCL-2 FAMILY ENOUGH?

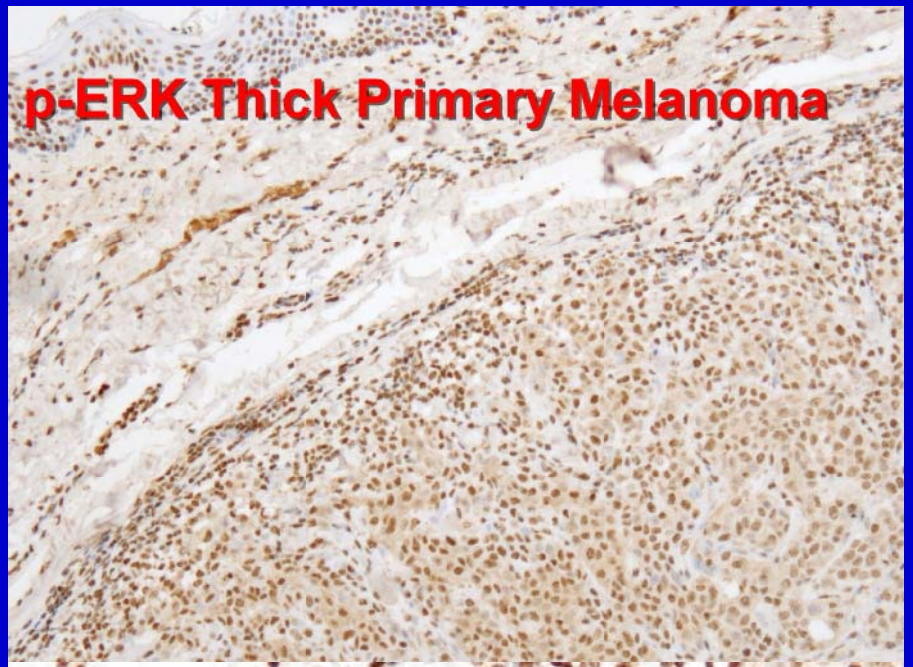
NO-THE MEK/ERK & Akt SIGNAL PATHWAYS SHUT DOWN APOPTOSIS

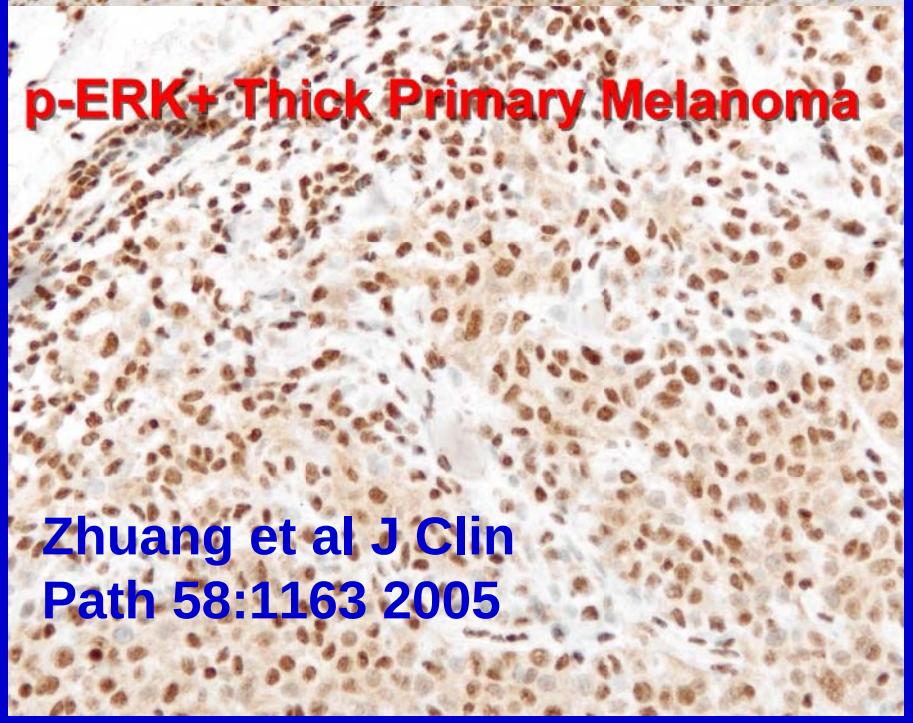
THE RAS/RAF/MEK/ ERK1/2 INHIBITORY PATHWAY IN MELANOMA

- May be activated by mutations in BRAF
- Cytokines such as TRAIL, chemotherapy such as the TAXOLS
- Adhesion molecule interactions
- ER Stress
- Activation of ERK related to the PKC epsilon phenotype in Melanoma cells (Mhaidat et.al. Mol. Can Res 5:1073 2007)

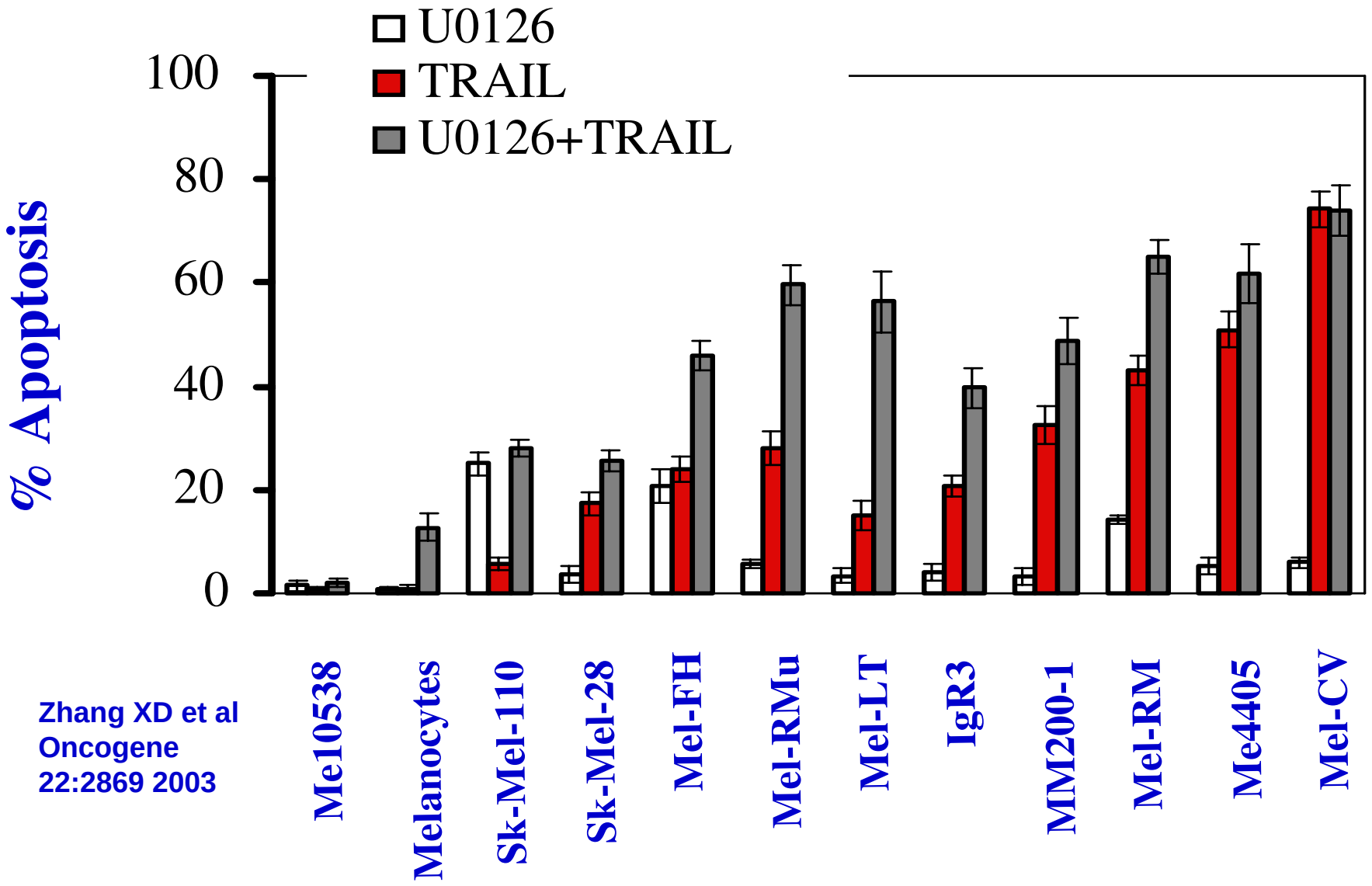

ERK+ Thick primary melanoma


ERK+ Thick primary melanoma


p-ERK Thick Primary Melanoma


p-ERK+ Thick Primary Melanoma
Zhuang et al J Clin
Path 58:1163 2005

U0126 Sensitises Melanoma to TRAIL-Induced Apoptosis



Zhang XD et al
Oncogene
22:2869 2003

THE ERK1/2 PATHWAY BLOCKS APOPTOSIS AT MULTIPLE SITES

- Inhibits Bim EL by phosphorylation Ser 69
- Phosphorylates Bad
- Induces Mcl-1 (Wang et al 2007)
- Induces GRP78-(GRP78 binds Bik, casp4)
- Induces IL-8 and upregulation of ICAM
- Increases HIF-1A expression

ADDITIONAL WAYS THE RAS /MEK PATHWAY INHIBITS CTL ACTIVITY

- **Downregulation of Mart-1, gp100 and Tyrosinase in Melanoma (Kono et al 2006)**
- **MEK inhibitors decreased production of IL-10,VEGF,IL-6 from melanoma cells (Sumimoto et al 2006)**
- **Knockdown of BRAF reduced IL-8 and ICAM-1 expression and reduced Melanoma cell extravasation (Liang et al 2007).**

WE ALREADY HAVE MANY AGENTS WHICH TARGET THE ANTI APOPTOTIC PATHWAYS

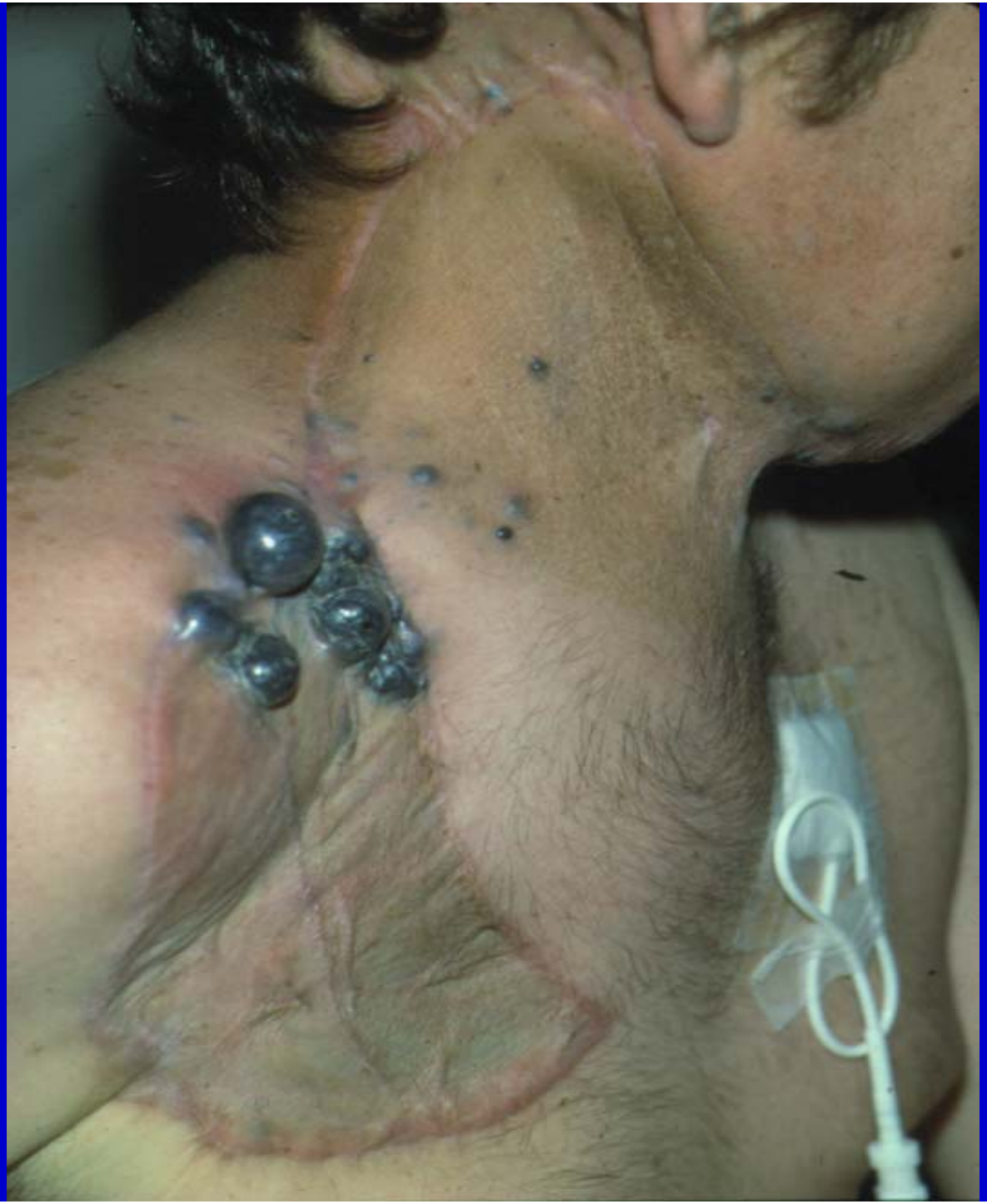
RAF Signal Pathway Inhibitors

- **Sorafenib/Nexavar RAF/VEGFR2,3 Onyx/Bayer**
- **KOS-953(17-AAG).Tanespimycin. Geldanomycin derivative. Hsp90 inhibitor. Raf,Akt,and others (Kosan/ Roche)**
- **Chir-265. Mutant BRAF/VEGFR2 (Novartis)**
- **PLX4032. Mutant BRAF ,Plexxicon/Roche**

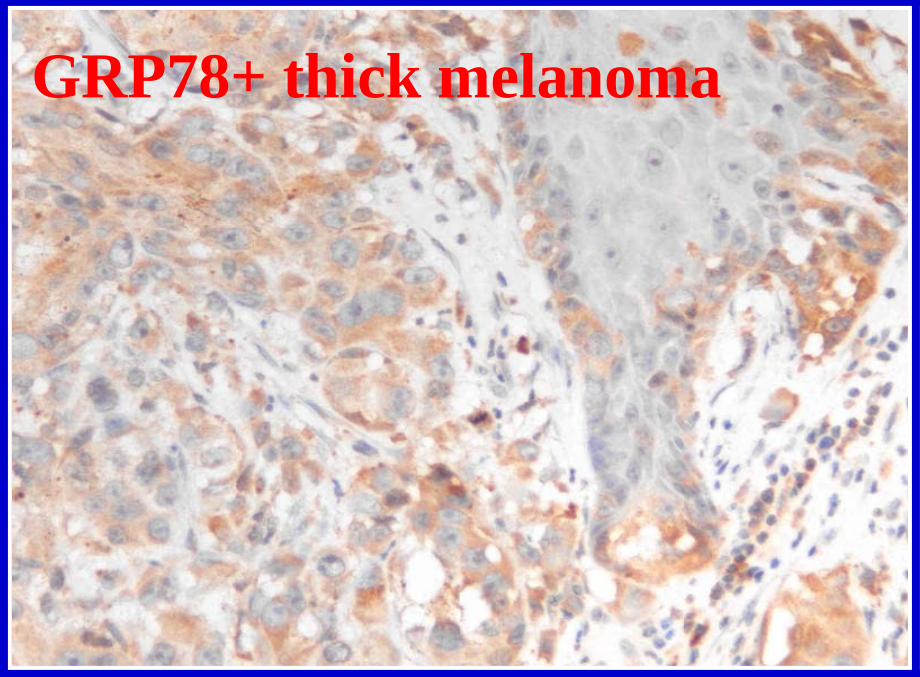
MEK & RTK Signal Pathway Inhibitors

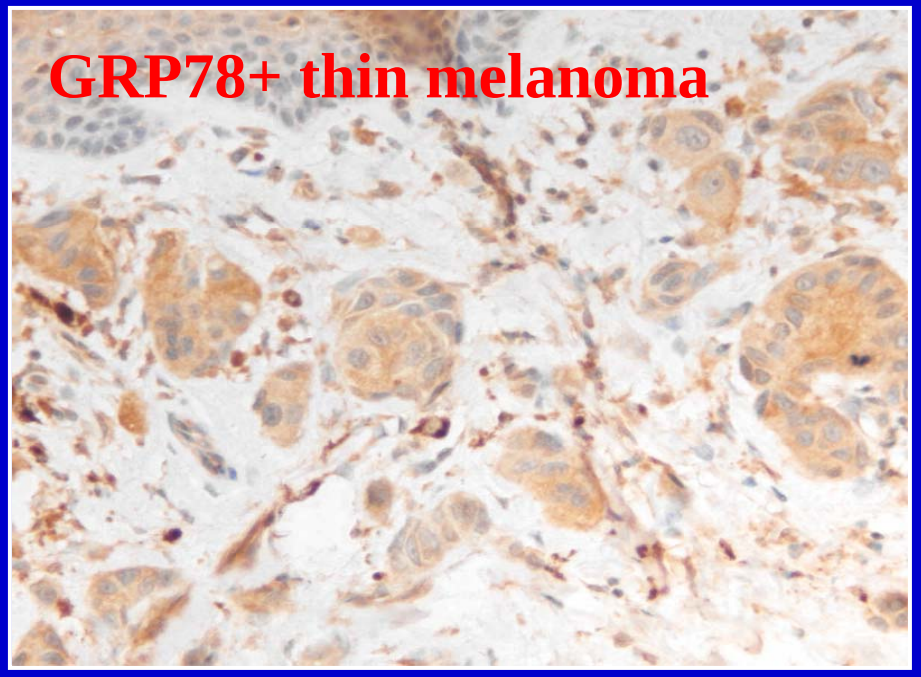
- AZD6244, Specific MEK inhibitor (Astra Zeneca)
- PD0325901,MEK inhibitor (Pfizer)
- RTKinases, Imatinib, Sutent,Erlotinib
- Farnesyl transferase Inhibitors R11577

THE MOST IMPORTANT CAUSE OF FAILURE OF IMMUNOTHERAPY ARE ADAPTIVE PROCESSES IN CANCER CELLS AGAINST ER STRESS

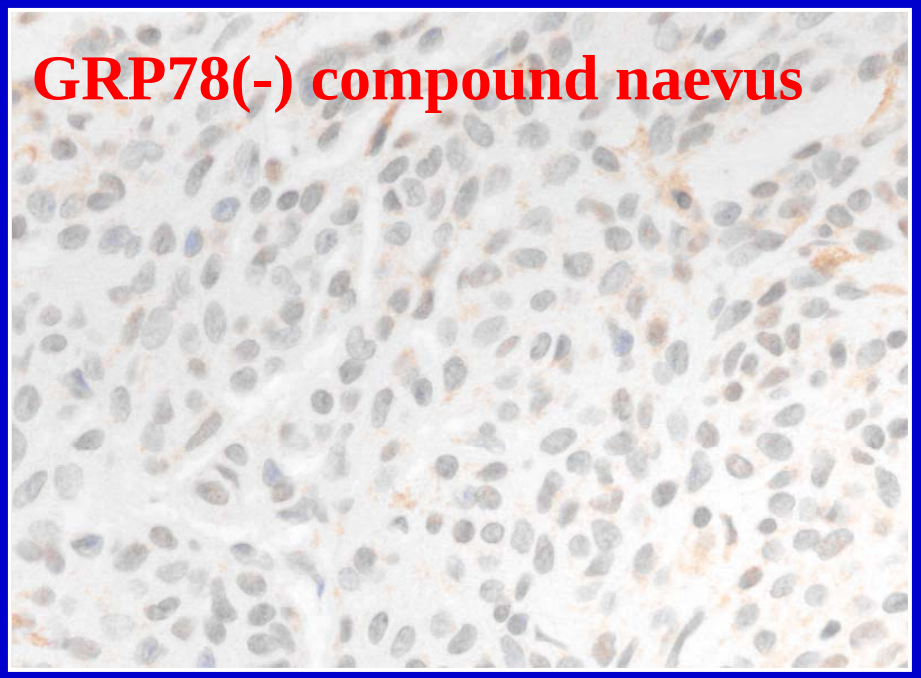


GRP78 CHAPERONE PROTEIN EXPRESSION IS AN INDICATOR OF ER STRESS

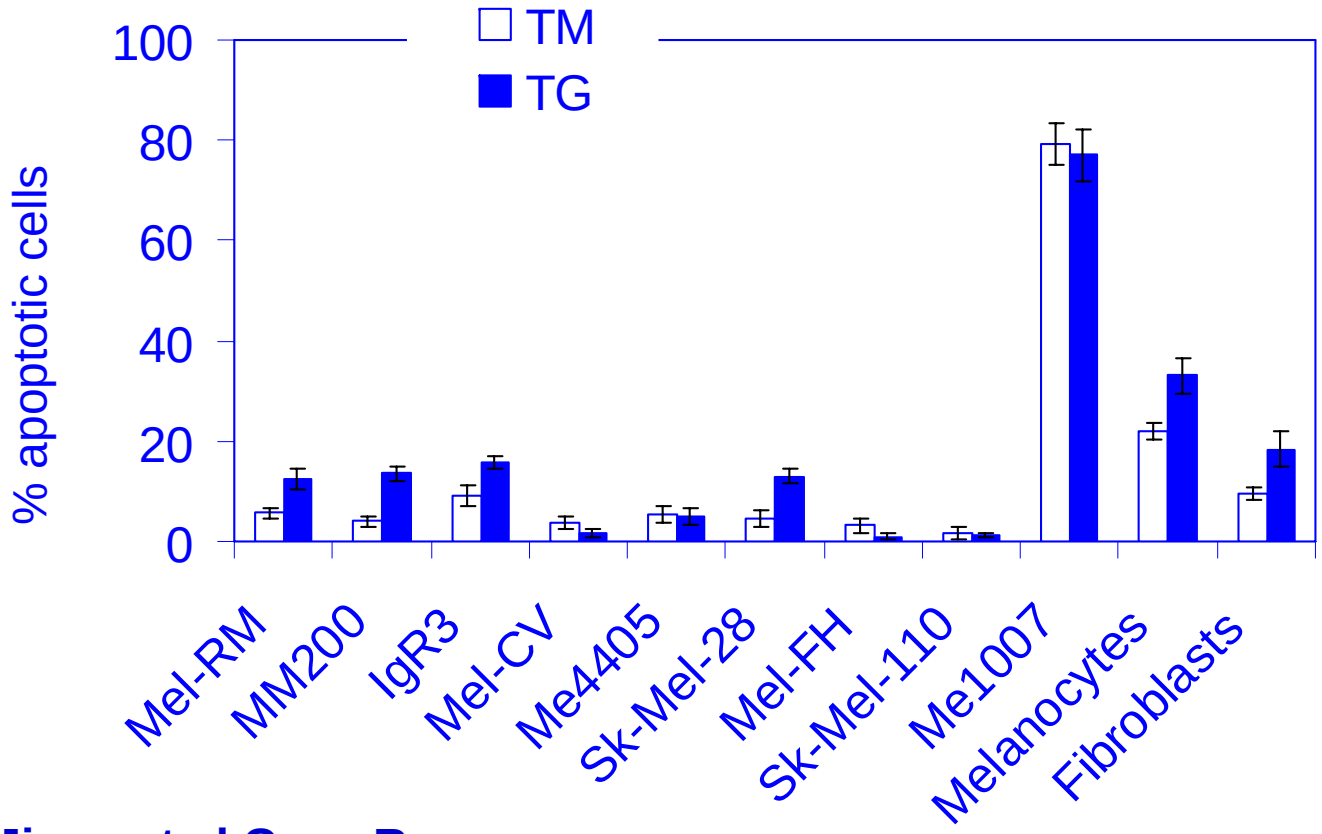

GRP78+ thick melanoma


GRP78+ thin melanoma


GRP78+ subcutaneous met


GRP78(-) compound naevus

Cultured melanoma cells are relatively resistant to ER stress-induced apoptosis

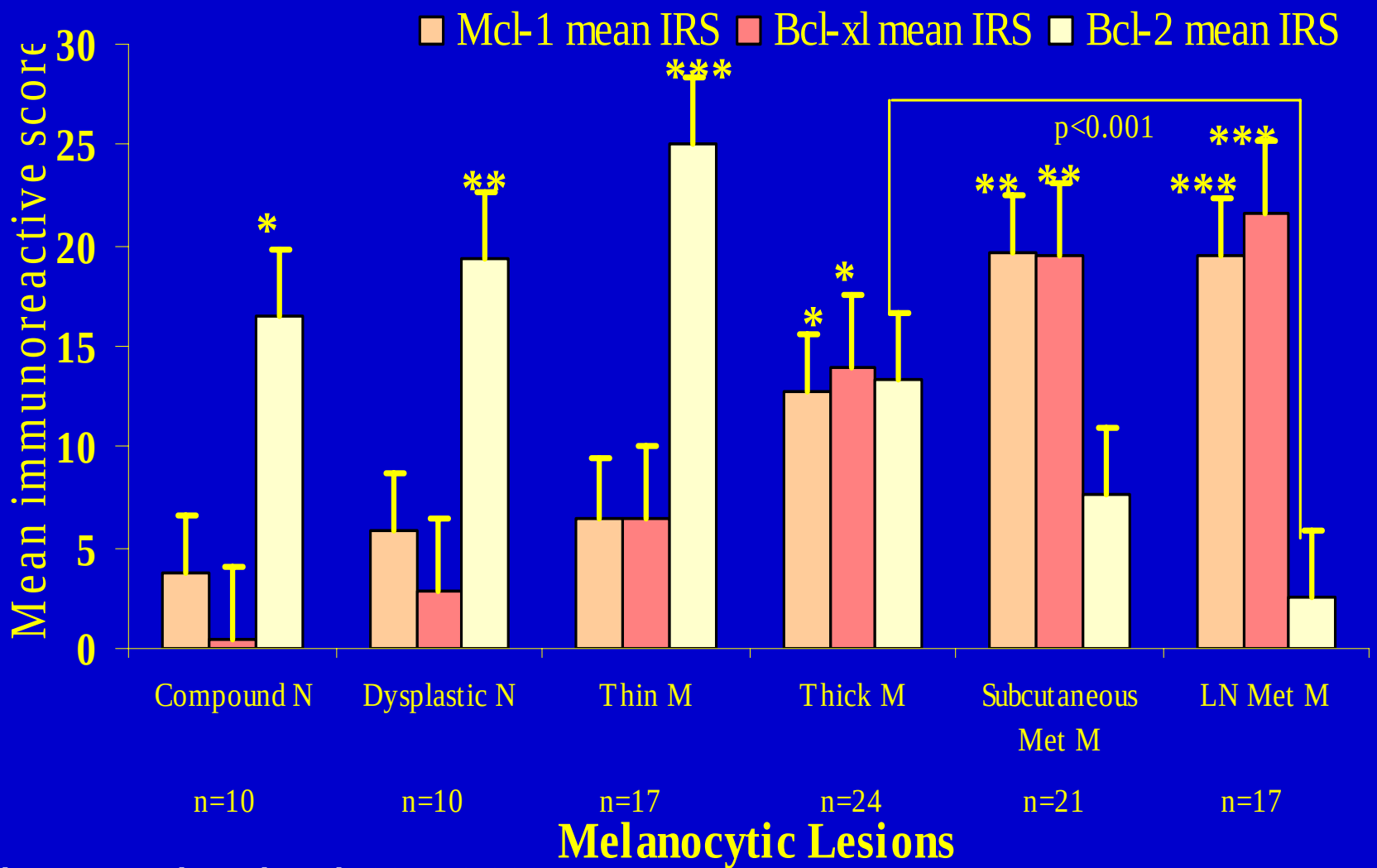


Jiang et al Canc Res 67:9750 2007

ER STRESS INDUCES ANTI APOPTOTIC EFFECTS

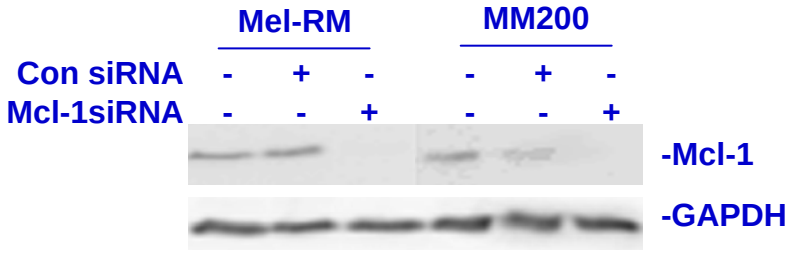
- Upregulation of BCL-XL,Mcl-1. Down of Bcl-2
- Activation of Akt., (MEK/ERK in normal cells)
- Upregulation of GRP78
- Downregulation of p53 (via?HDM2,viaGSK3b)
- Glycolysis and acidification of the microenvironment

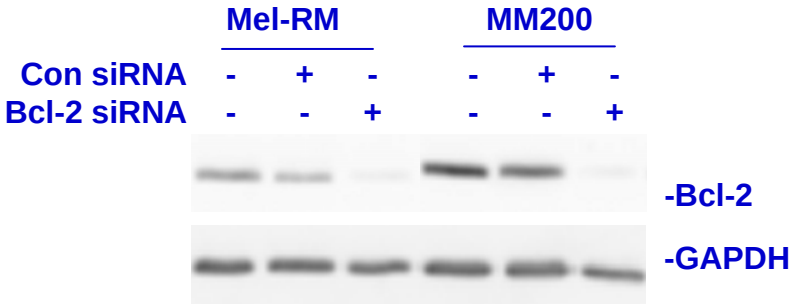
Down regulation of Bcl-2 during progression of melanoma

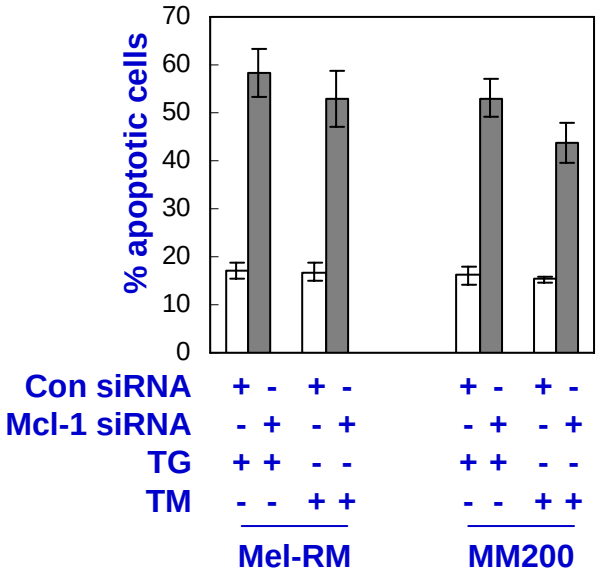


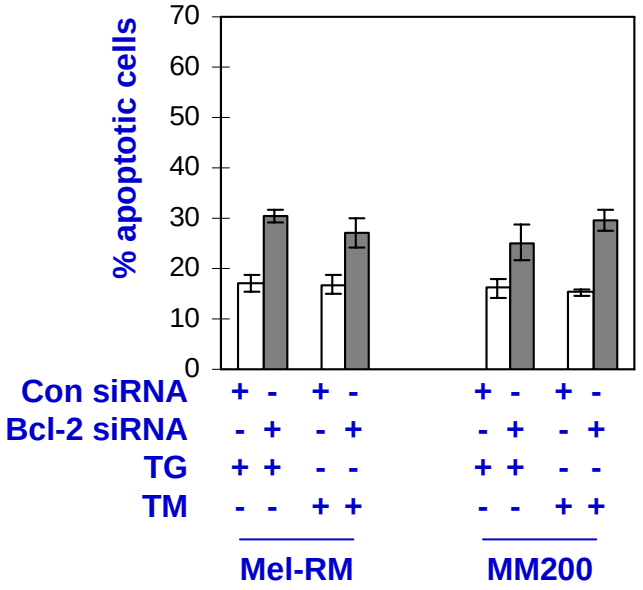
Zhuang et al Mod Path 20:416 2007

KNOCKDOWN OF MCL-1 RATHER THAN BCL-2 IS MORE EFFECTIVE IN SENSITISING MELANOMA CELLS TO ER STRESS

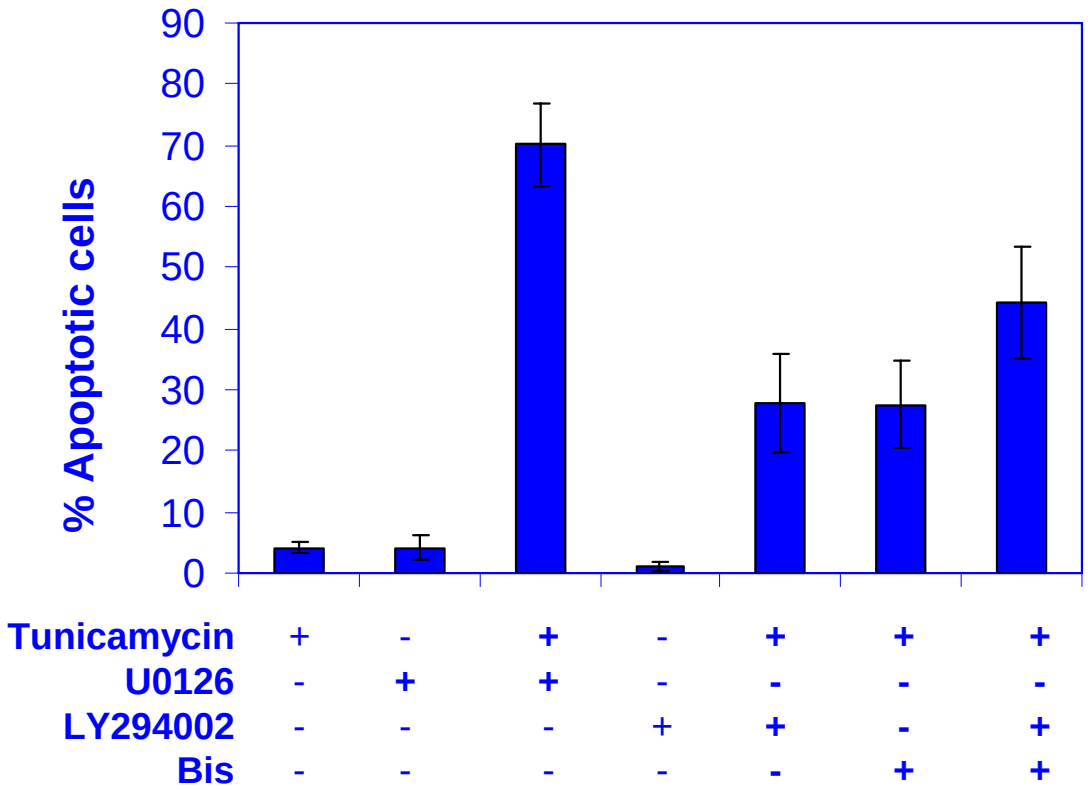








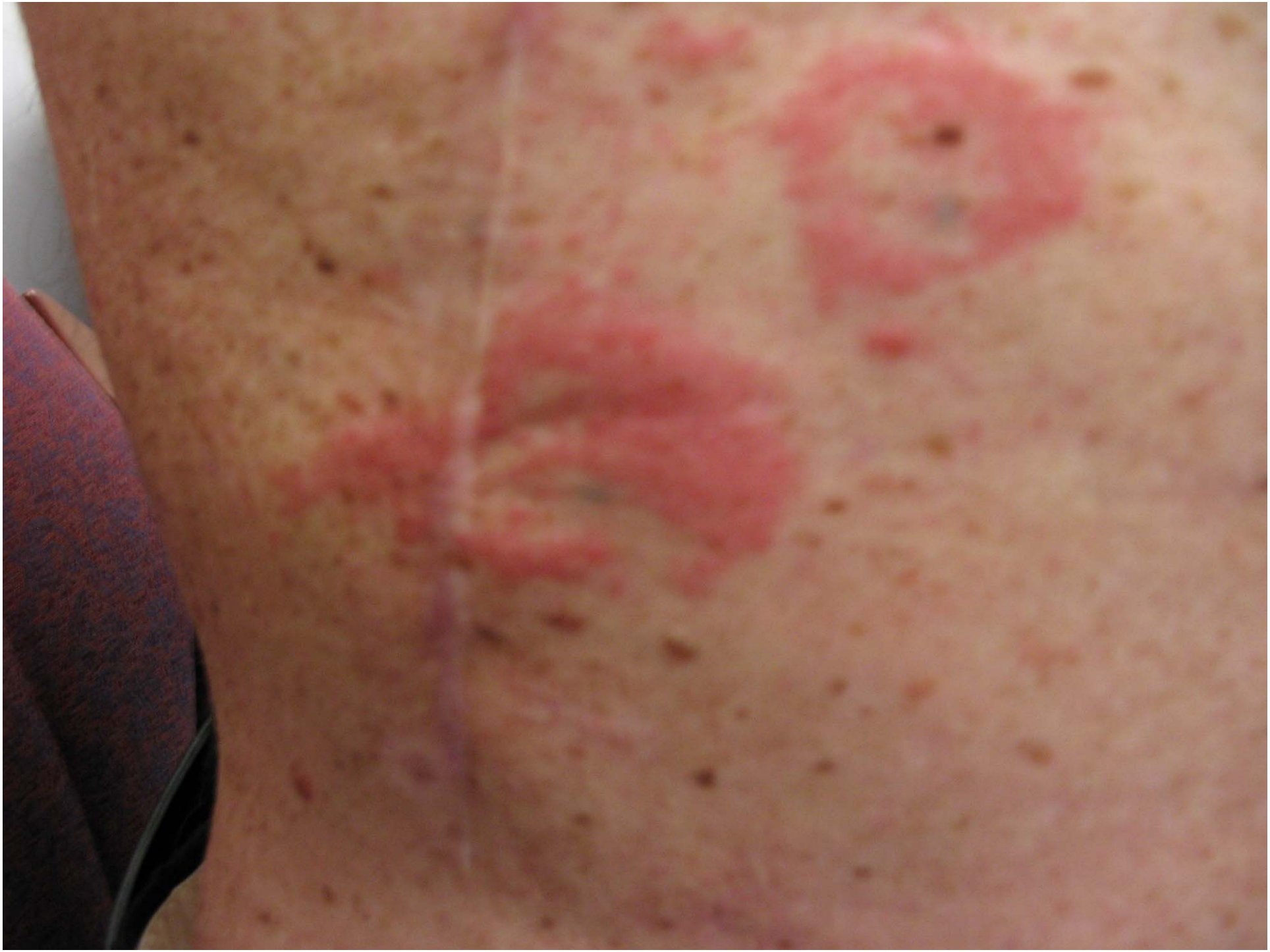
Inhibition of MEK, Akt, or PKC sensitizes melanoma cells to ER stress-induced apoptosis



SHOULD WE USE ADDITIONAL AGENTS TO TARGET ER STRESS INDUCED RESISTANCE TO APOPTOSIS?

- Agents that target HDM2 & increase p53 Eg Nutlin 3a
- Inhibitors of GSK3beta that targets S 315,376 on p53 eg DW1/2
- Inhibitors of GRP78,IRE1a eg Irestatin
- VEGF R inhibitors,eg AZD 2171,
- Proton pump inhibitors? Eg Omeprazole

CONCLUSION - THE TUMOR HAS EVOLVED INHERENT RESISTANCE MECHANISMS THAT NEED TO BE INHIBITED BEFORE IMMUNOTHERAPY WILL BE EFFECTIVE

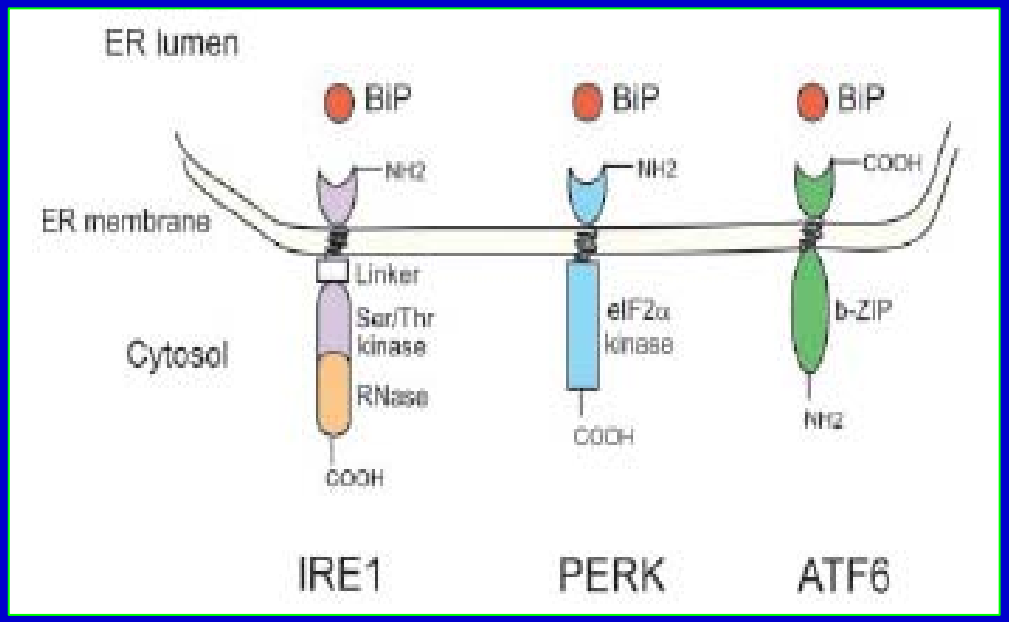


DOES THE WAY A CELL DIES INFLUENCE THE IMMUNE RESPONSE?

- **Necrosis said to release inflammatory mediators which stimulate NF-Kb,ERK and STAT 1,3 ,release of TNF,IL-1,IL-6, IL-8, IL-23 cytokines which can stimulate tumor growth and or induce Th17 helper T cells.**
- **HMGB1(High Mobility group B1) and Heat shock proteins may be key stimulators of DC maturation and immune responses ?**
- **Apoptosis said to be a more silent death which delivers antigens to APC without the marked release of Inflammatory mediators and no generation of CD4 Tcells.**
- **Resulting "Help-less" CD8 Tcells said to be tolerant and to prevent auto immune disease**

CONCLUSION
LETS HEAR WHAT THE PANEL SAYS!!

How is UPR initiated?



dimerization/oligomerization of IRE1, PERK, and ATF6 upon released from Bip initiates UPR signaling

IRE, inositol-requiring enzyme

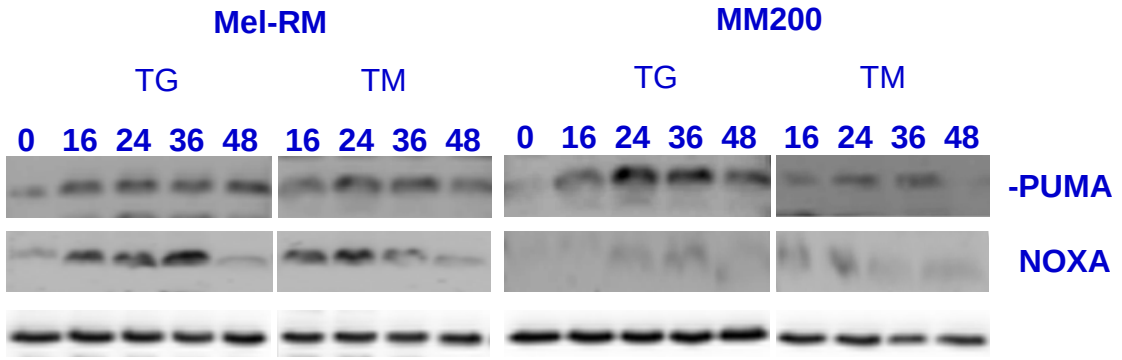
PERK, double-stranded RNA-activated protein kinase-like ER kinase

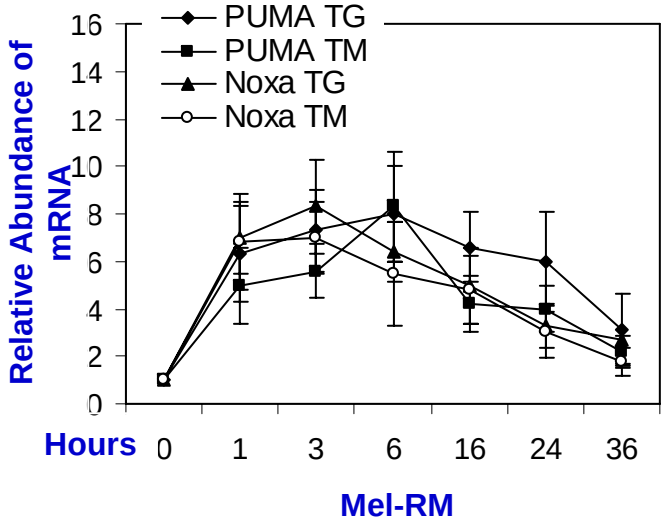
ATF, activating transcription factor

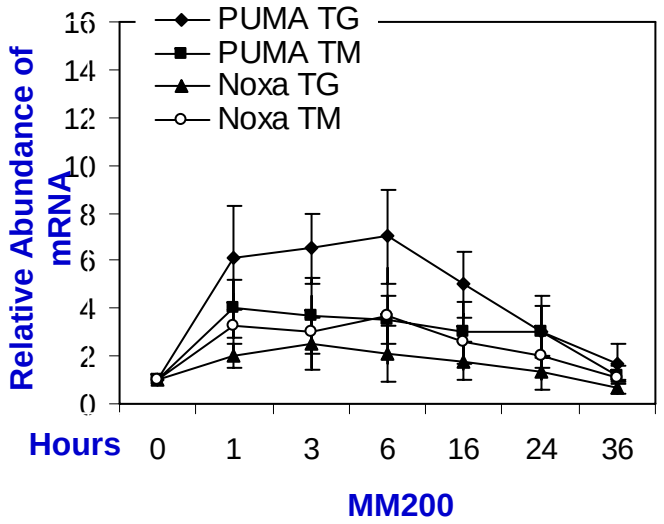
b-ZIP, basic leucine zipper

Bip, also called GRP78; GRP, glucose-regulated protein

ER stress up-regulates the expression of pro-apoptotic proteins







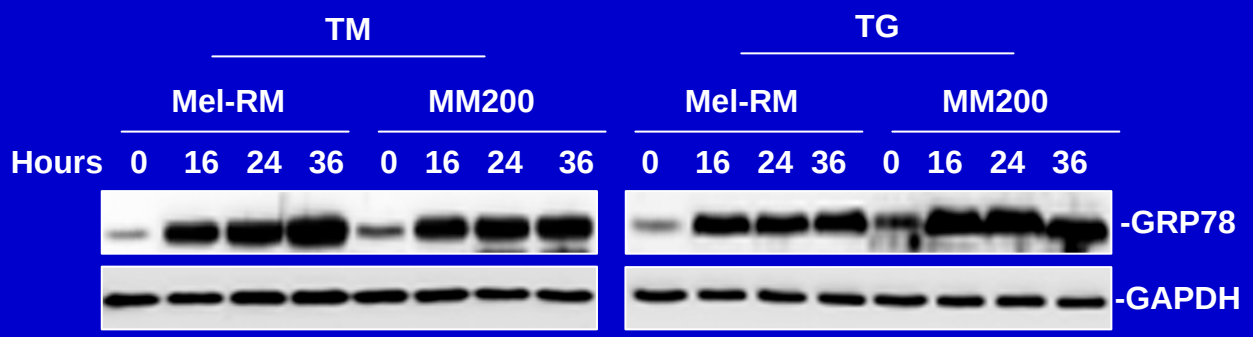
Akt –A KEY PLAYER IN ER STRESS

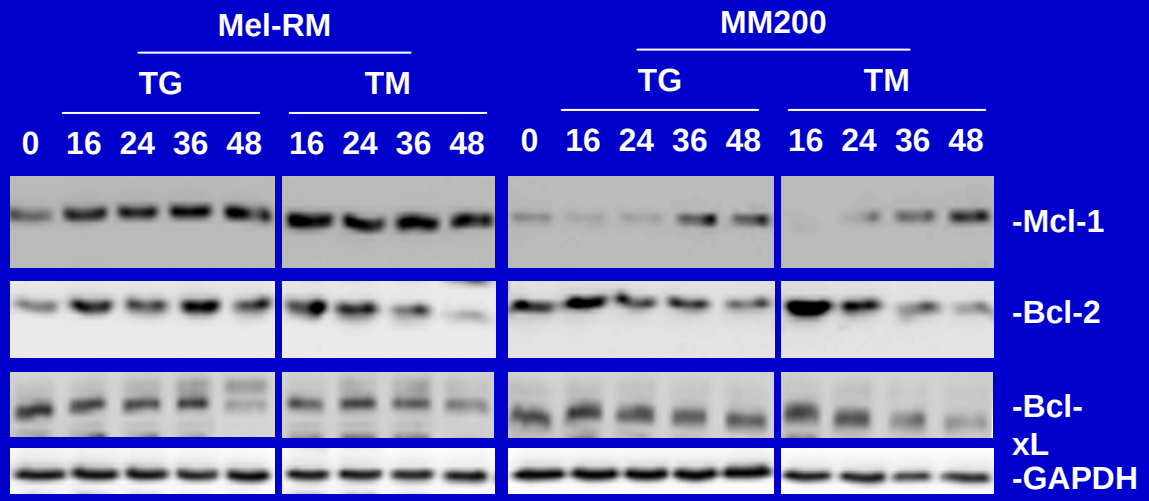
- Down regulates p53 by facilitating HDM2 entry into Nucleus
- Phosphorylates FOXO and decreases its entry into nucleus &BIM transcription
- Activates NF-kB
- Inhibits GSK3beta??

Akt related Signal Pathway inhibitors

- PI3K inhibitors-PI 103 (Workman ICR UK)
- Akt3 inhibitors- Perifosine , CMEP (Zhang Virginia)
- mTOR Inhibitor, RAD 001,CCI-779,Everolimus(Novartis)
- GSK3beta inhibitors SB216763?DW1/2
- Nutlin-3a HDM2 inhibitor

Regulation of the expression of anti-apoptotic proteins by the ER stress inducers, TG and TM





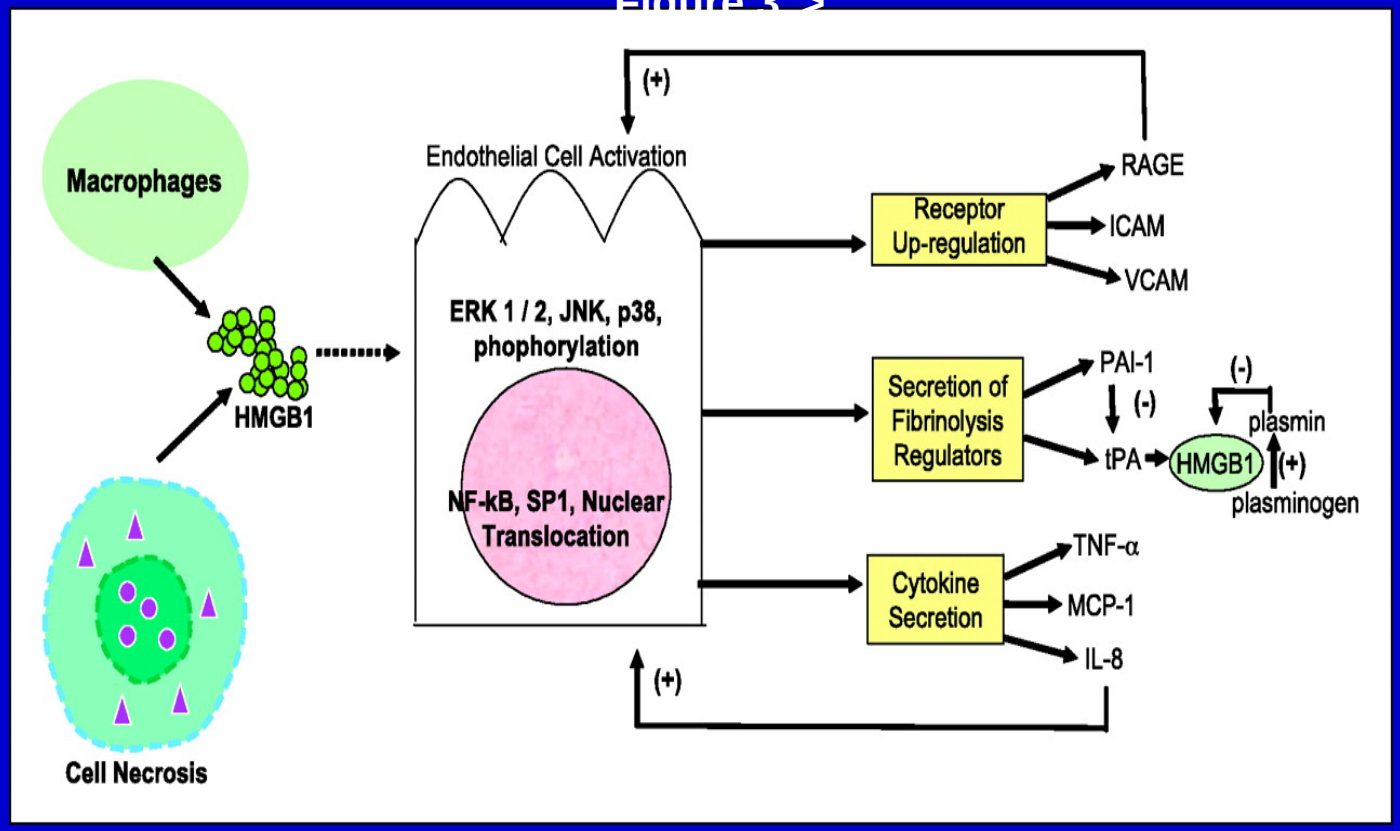
STRATEGY TO OVERCOME ER STRESS INDUCED RESISTANCE

- Use signal pathway inhibitors particularly those targetting ERK1/2 and Akt pathways
- Combine with Anti apoptotic strategies.In particular agents like TW37 ,Obatoclax or ?AT101 which can bind Mcl-1
- Target metabolic pathway?Anti LDH-A,, DCA (dichloroacetate),PTK/XK, Anti VEGFR eg AZD 2171

SUMMARY

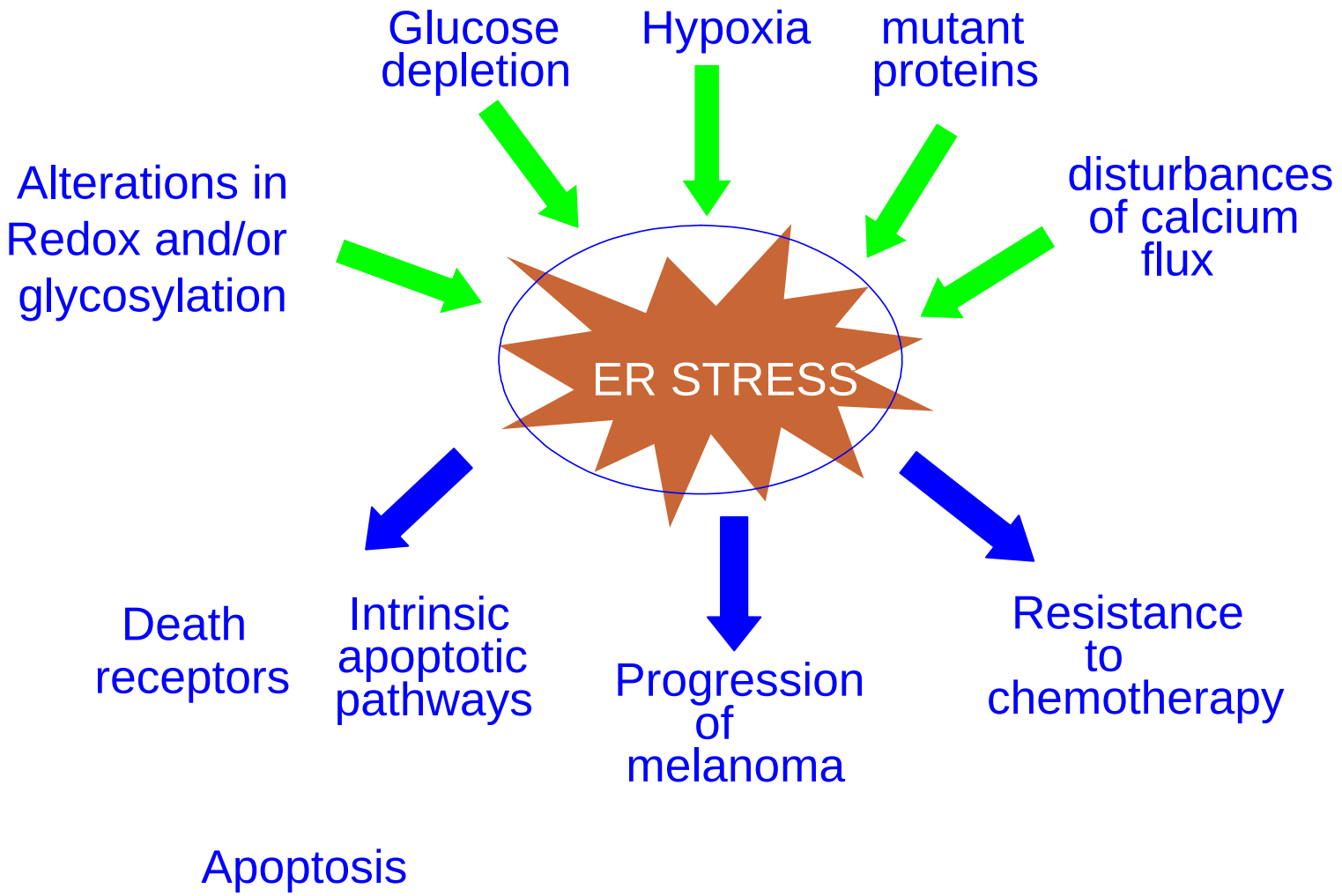
- **The immune system and ER stress are key drivers of progression and are responsible for the resistance of melanoma to treatment.**
- **Key roles for signal pathways ERK1/2,Akt and possibly GRP78 . Down regulation of p53 likely to be a consequence of ER stress**
- **Heterogeneity in the disease implies single agents unlikely to have much impact.**
- **We have a wealth of new agents waiting to be applied but we need better ways of selecting treatments for patient subgroups**

Figure 3">



Ellerman, J. E. et al. Clin Cancer Res 2007;13:2836-2848

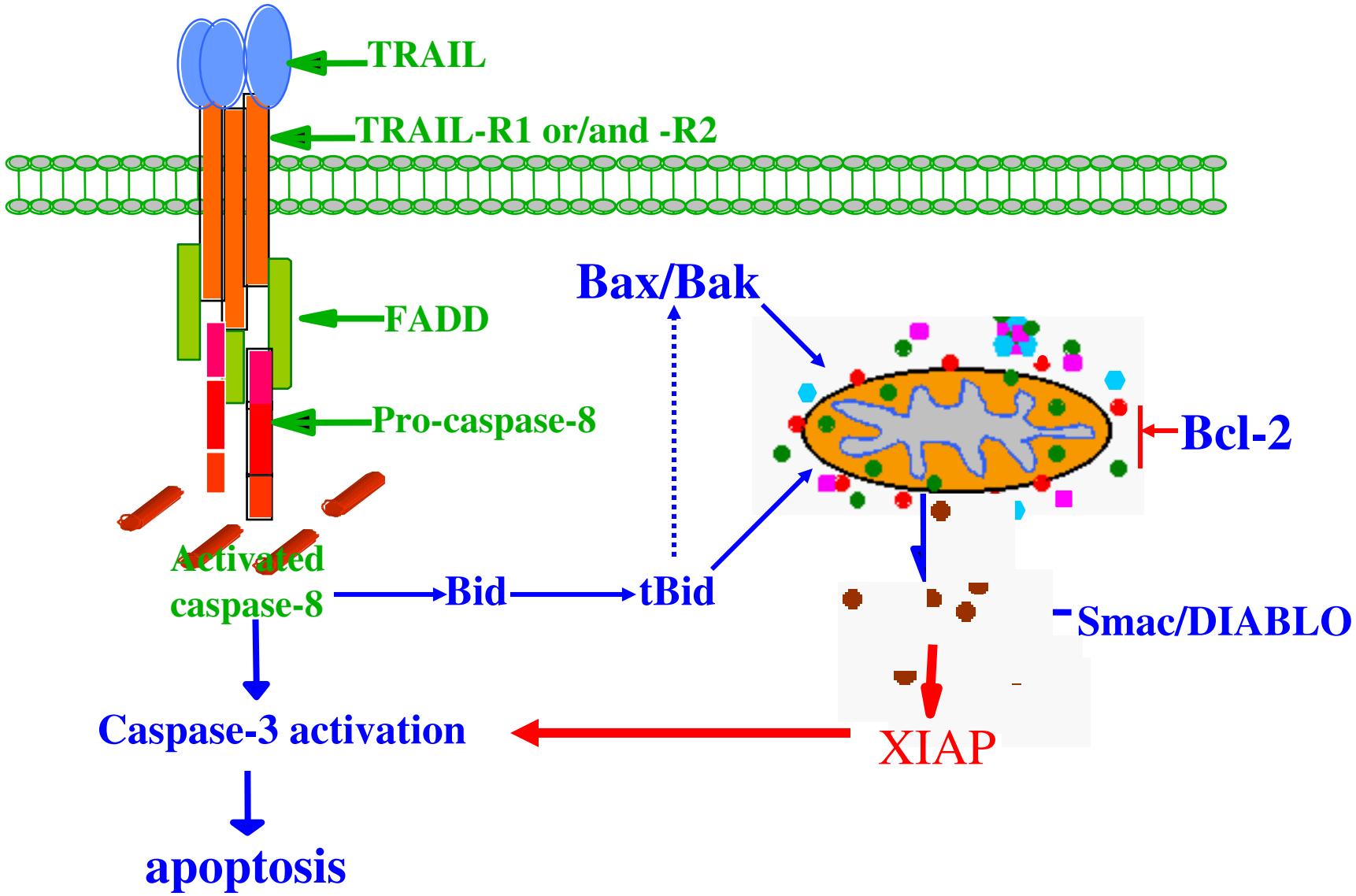
The ER stress response in melanoma: friend or foe?

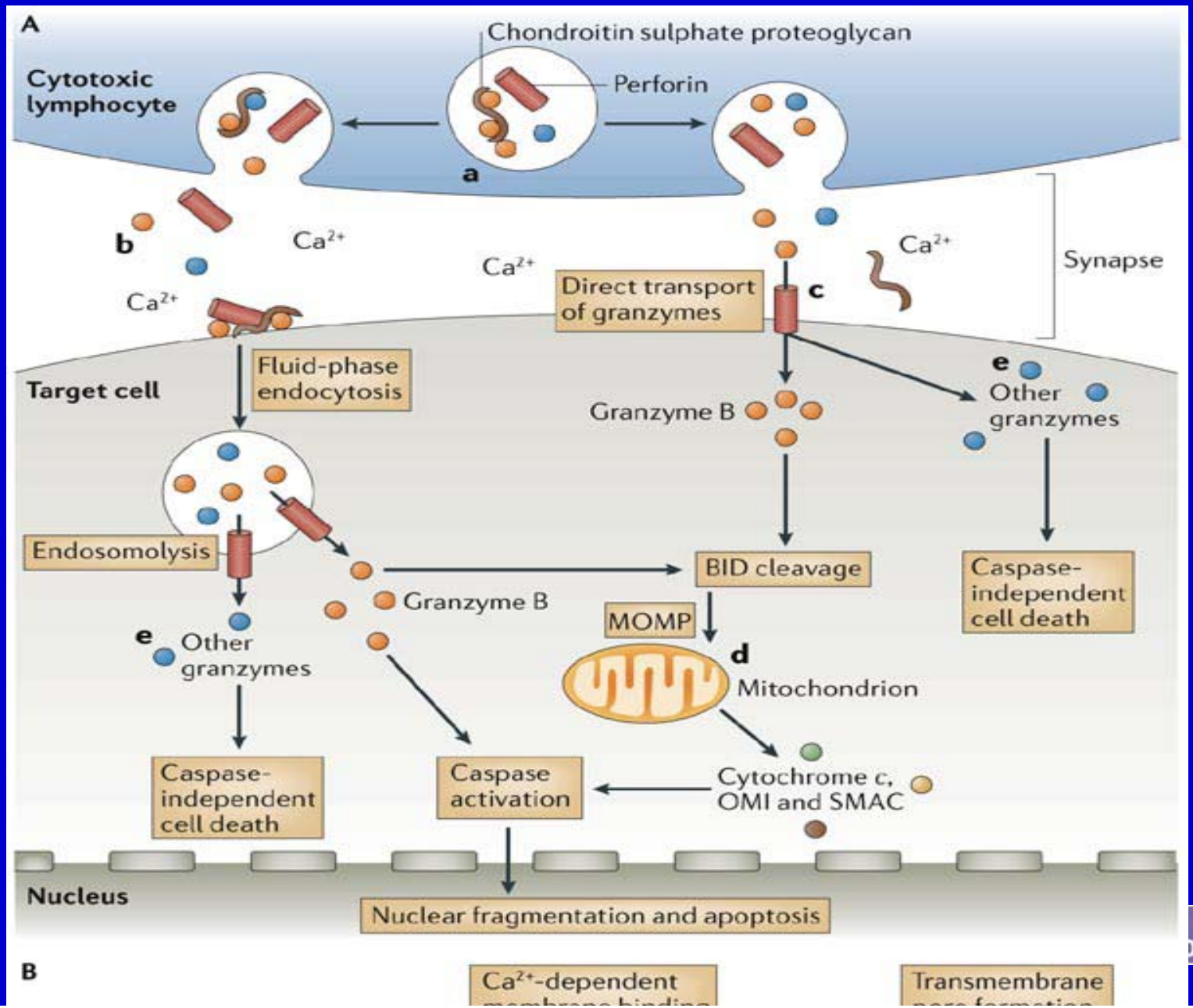


What about the serine protease inhibitor 9 against Granzyme B?

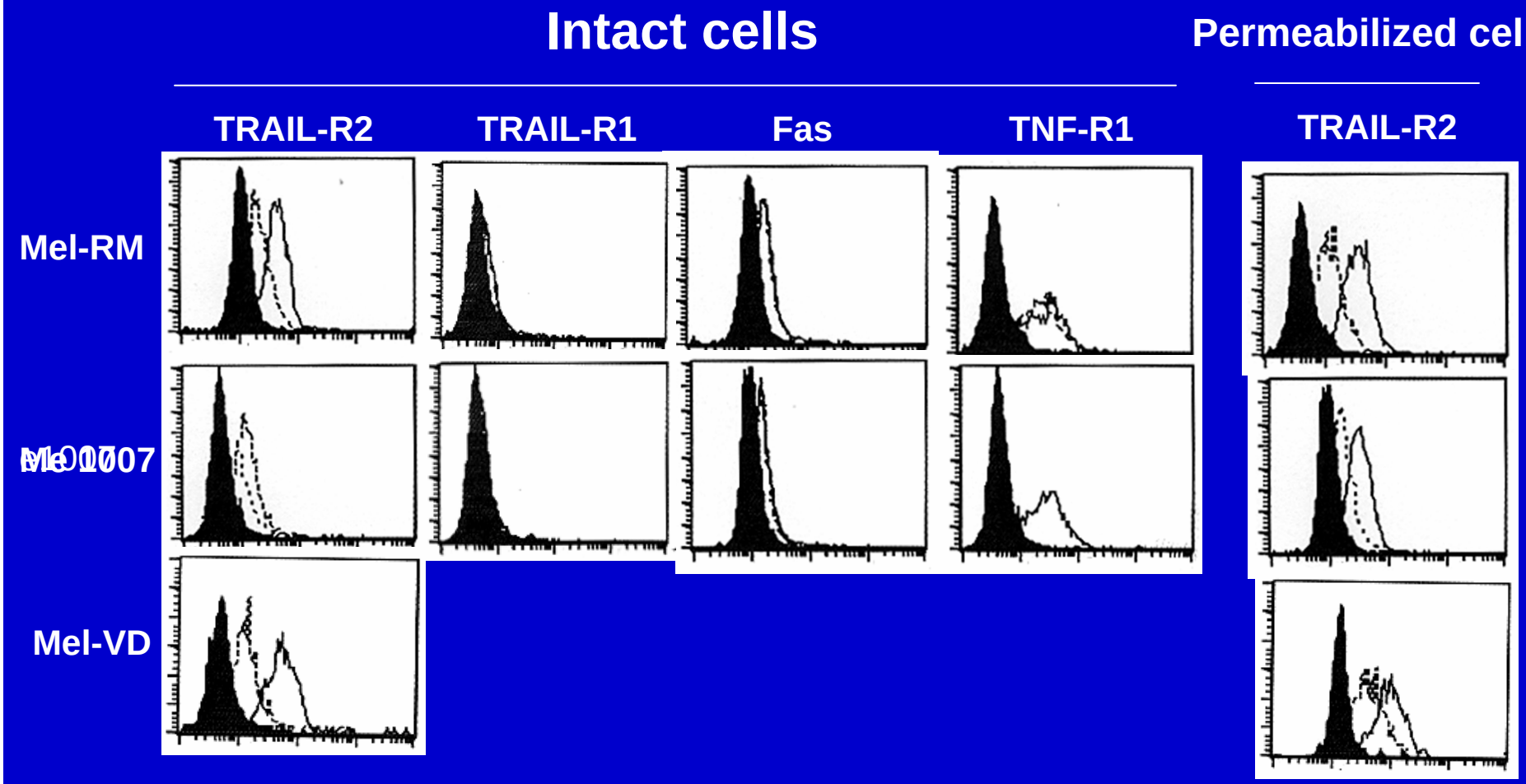
- Induced by Estrogens
- High levels in some melanoma and breast carcinoma cells

The TRAIL-Induced Apoptotic Pathway in Melanoma Cells




A
Chondroitin sulphate proteoglycan
Perforin
Cytotoxic lymphocyte
a
b
Ca2+
Ca2+
Ca2+
Ca2+
Synapse
Direct transport of granzymes
c
Fluid-phase endocytosis
Target cell
Granzyme B
e
Other granzymes
Endosomolysis
BID cleavage
Caspase-independent cell death
Granzyme B
MOMP
d
Mitochondrion
e
Other granzymes
Caspase-independent cell death
Caspase activation
Cytochrome c, OMI and SMAC
Nucleus
Nuclear fragmentation and apoptosis
B
Ca2+-dependent
Transmembrane

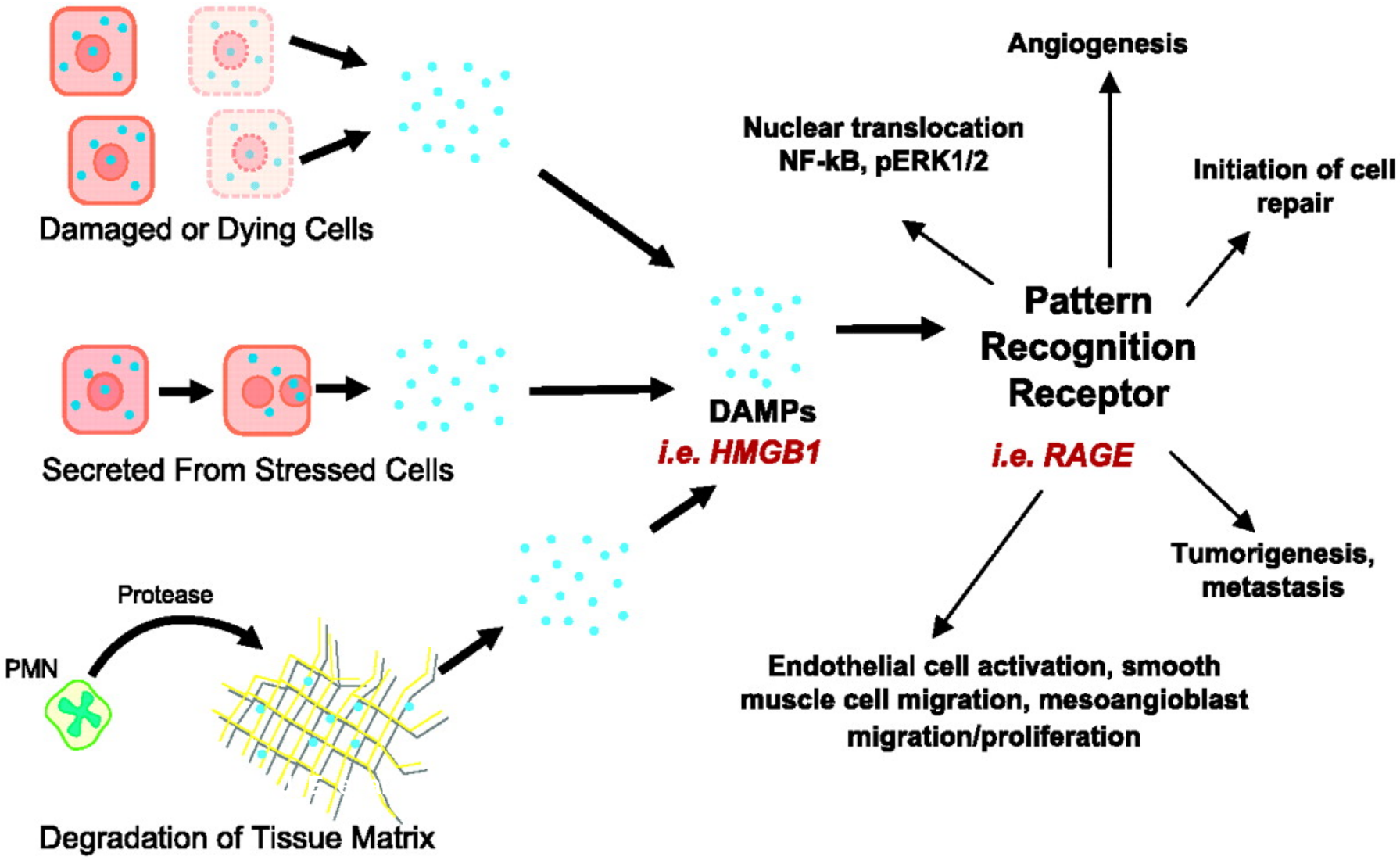
TUNICAMYCIN SELECTIVELY UPREGULATES TRAIL-R2



Jiang et al Cancer Research In press, 2007

Intact or permeabilized melanoma cells without (dotted lines) or with (solid lines) treatment with tunicamycin for 16 hours. were stained with mAB against TRAIL-R2, Fas, or TNF-R1 and analyzed by flow cytometry The filled hisgrams are isotype controls.

Mode of Cell Death is Important for Immune Cell Recruitment and Activation



INHIBITOR OF APOPTOSIS PROTEINS (IAPs)IN MELANOMA

- XIAP, IAP1,2, ML-IAP
- XIAP BINDS TO ACTIVATED CASPASE 3,6,7 & INHIBITS ACTIVATION OF CASPASE 9.
- IAP1&2 MAY HAVE AN INDIRECT EFFECT UPSTREAM OF CASPASE 8