« Apoptotic pathway as a promising targeted therapy in cancer»

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Programmed cell death or Apoptosis

APOPTOSIS: A BASIC BIOLOGICAL PHENOMENON WITH WIDE-RANGING IMPLICATIONS IN TISSUE KINETICS

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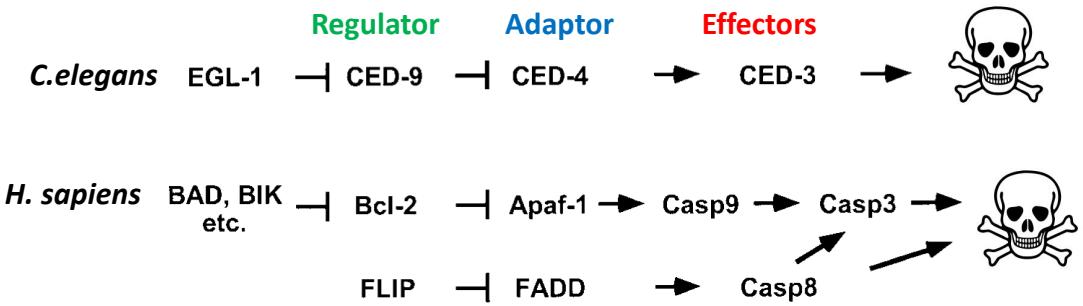
From the Department of Pathology, University of Aberdeen

Received for publication April 1972

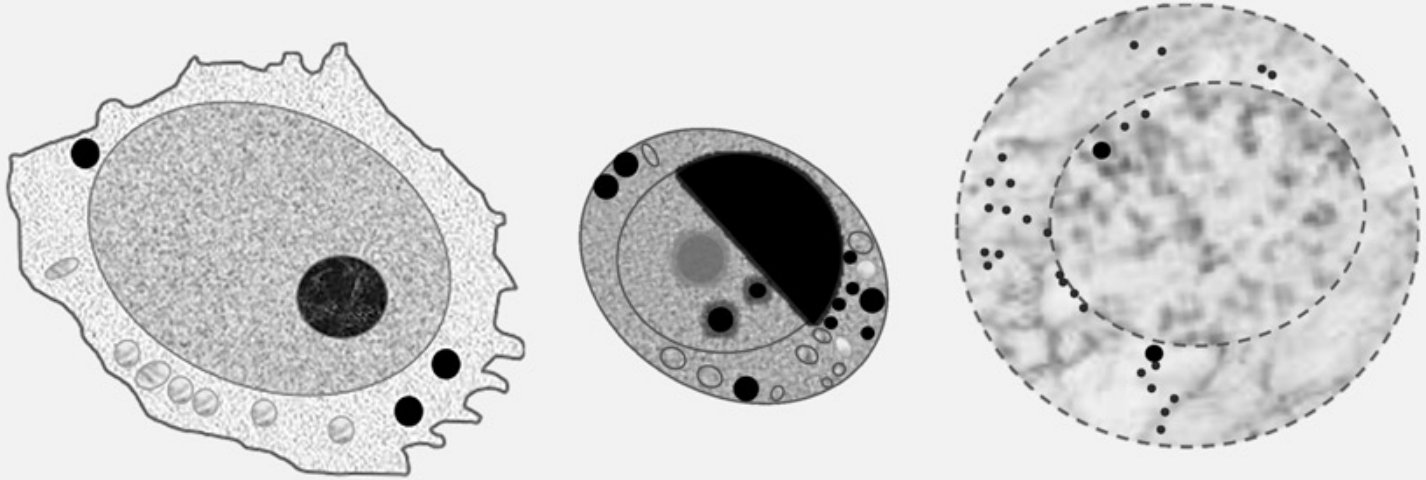
Br. J. Cancer (1972) **26**, 239

- Greek: apo, « from » and ptosi, « fall »: *falling of*
- *Active, programmed initiated by physiological or pathological stimuli*
- Role in development: Ex: formation of fingers and toes of the fetus
- To destroy cells: autoreactive lymphocytes, virus infected cells, cancer cells
- Highly conserved process



Vaux and Korsmeyer; Cell 1999

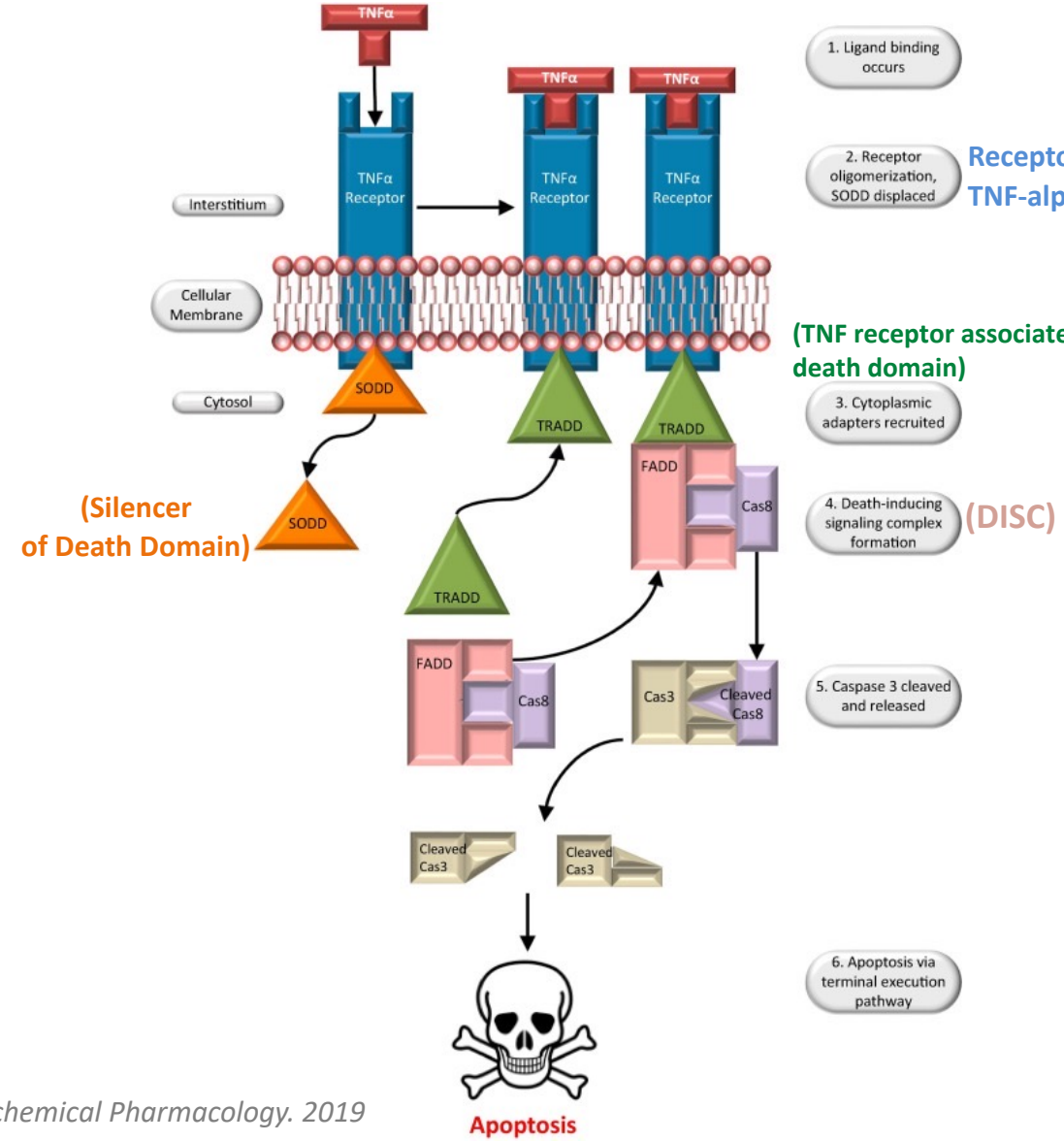
The morphological distinction of apoptosis and necrosis



Characteristics	Normal cell	Apoptotic cell	Necrotic cell
Cell shape	varies according to function	rounded and shrunk	rounded and swollen
Chromatin	well-defined domains of euchromatin and heterochromatin	Condensed, marginalized, later fragmented	lighter, disorganized
Cell membrane	intact	intact, later apoptotic bodies	ruptured
Nuclear envelope	intact	intact	disintegrating
Organelles (EM only)	well defined, numerous	shrunk	not discernable
Cytoplasm	contained	contained	spilling out

Voss AK and Strasser A. The essentials of developmental apoptosis [version 1]. F1000Research 2020, 9(F1000 Faculty Rev):148 (doi: 10.12688/f1000research.21571.1)

Extrinsic Apoptotic Pathway



Receptors from the TNF family: Fas/Apo-1, TNF-alpha, TRAIL receptors DR4 and DR5

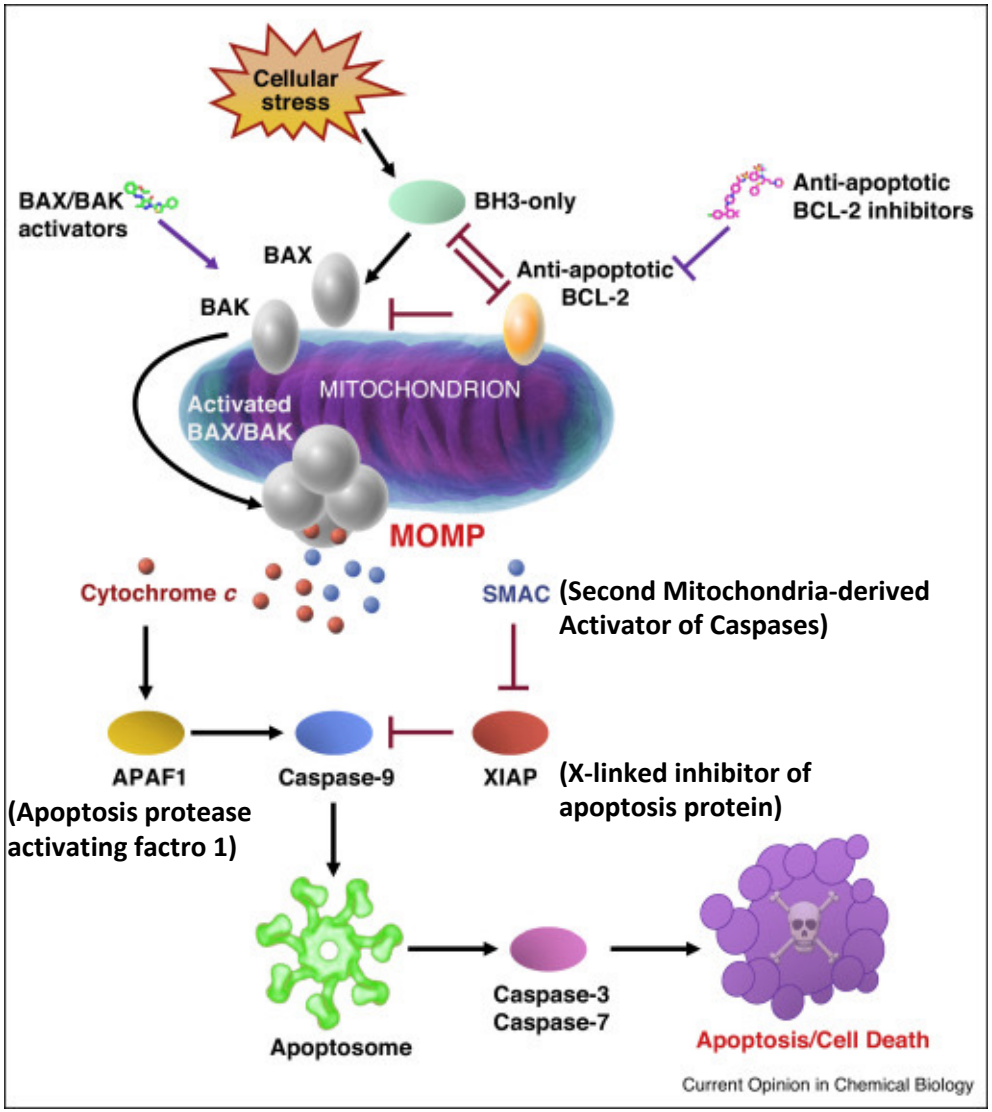
(TNF receptor associated death domain)

(Silencer of Death Domain)

(DISC)

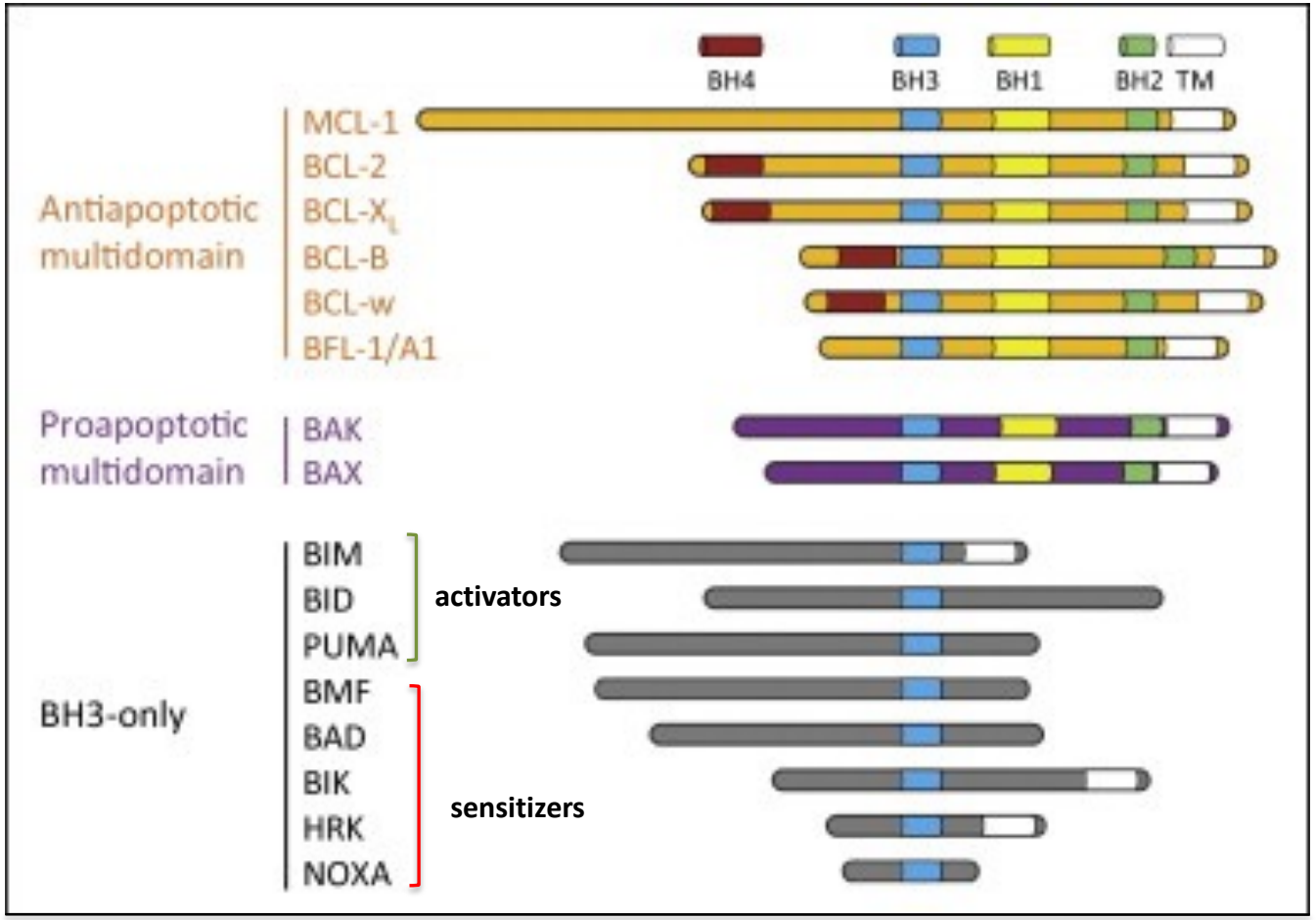
Knigth T, Biochemical Pharmacology. 2019

Intrinsic Apoptotic Pathway



Knigth T, Biochemical Pharmacology. 2019

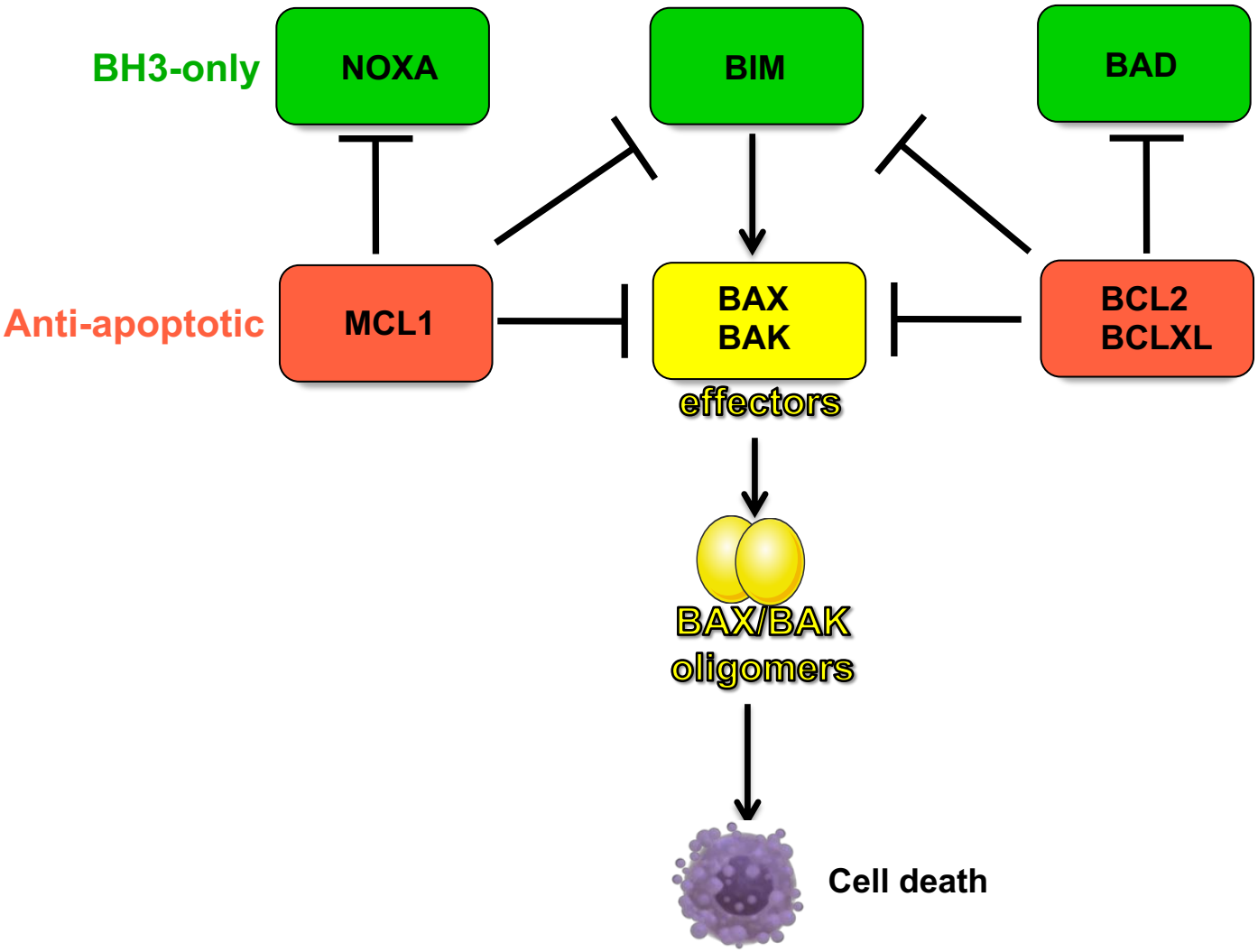
A BCL2 family Portrait



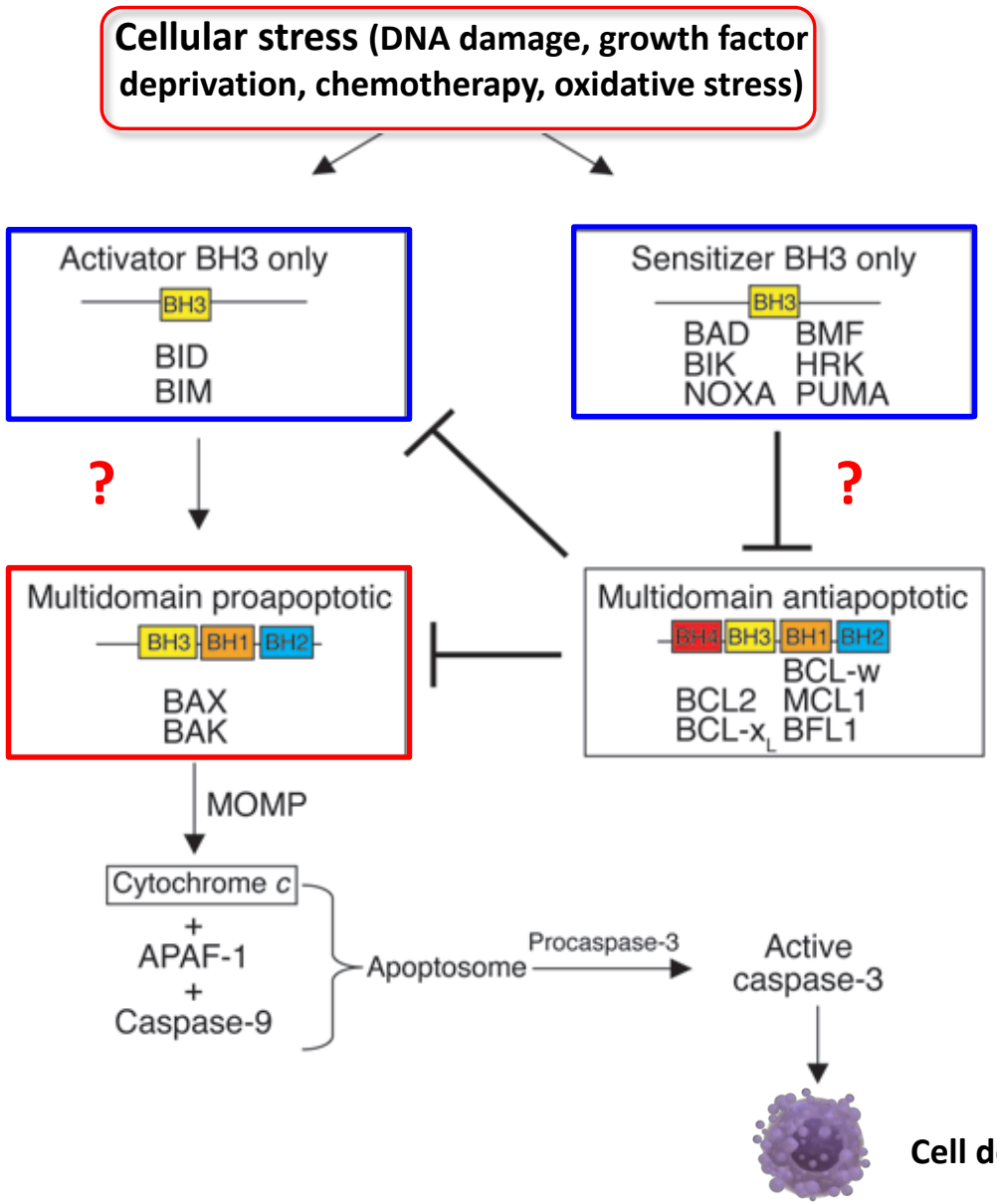
Gimenez-Cassina and Danial, Trends in Endrocrinol Metab 26, April, 2015

BH= BCL2 homology domain

Regulation of the function of BCL2 family proteins



The Bcl-2 family protein interactions mediate the apoptotic pathway under cellular stress

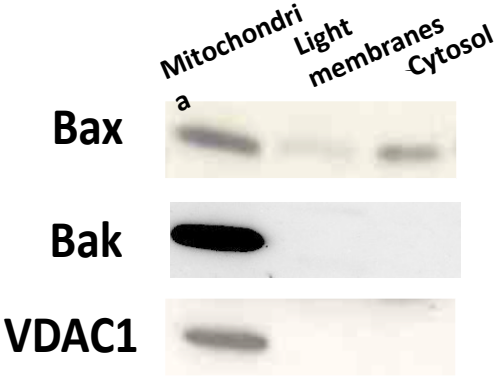


Initiators of the mitochondrial apoptotic pathway

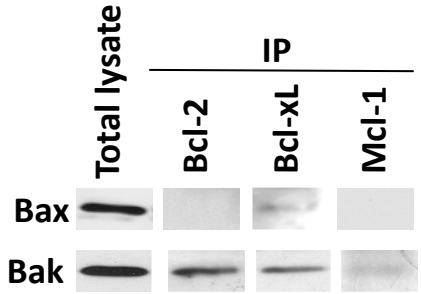
Adapted from Letai, Nature Rev Cancer, Oncogene 2008

Effectors regulation: a multistep process

- **Localisation**

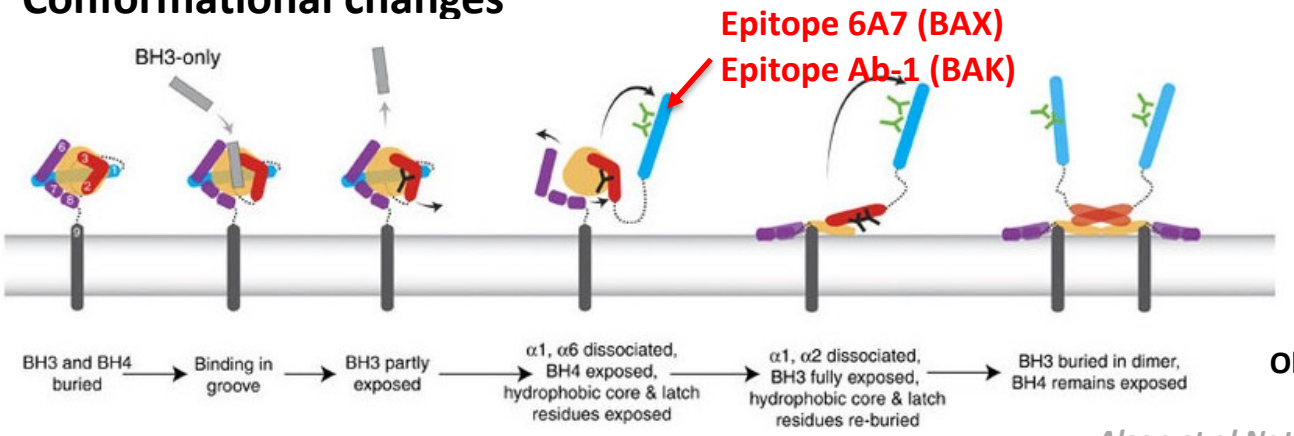


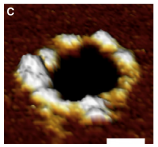
- **Inhibitory interactions**



Bodet, Gomez Bougie et al. Blood 2011

- **Conformational changes**

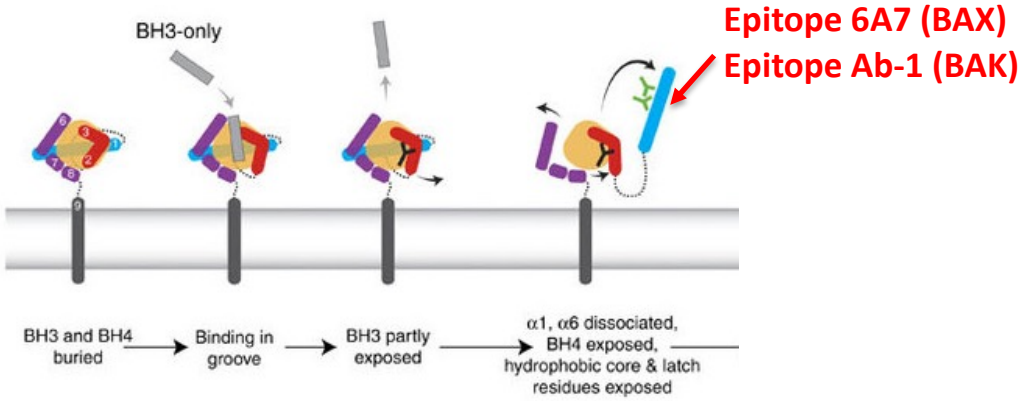


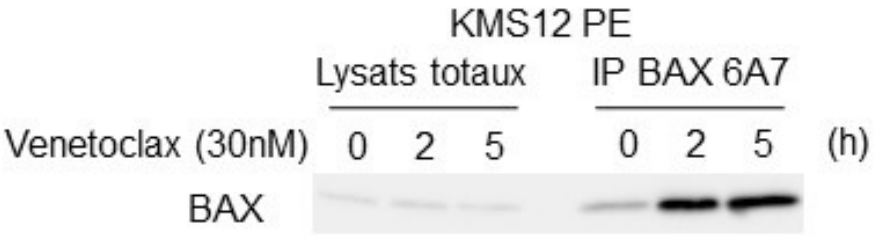


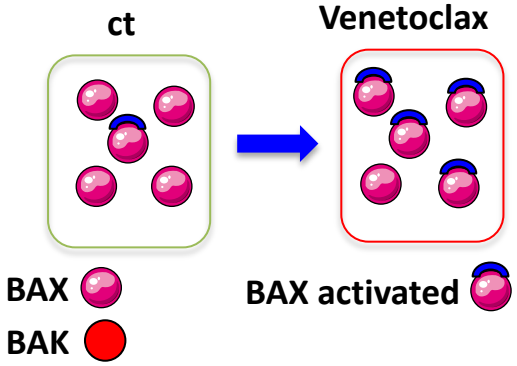
Oligomerization

Alsop et al Nat Comm 2014

Activation status of BAX and BAK effectors: exposure of epitopes (N' terminal) and heterodimers formation







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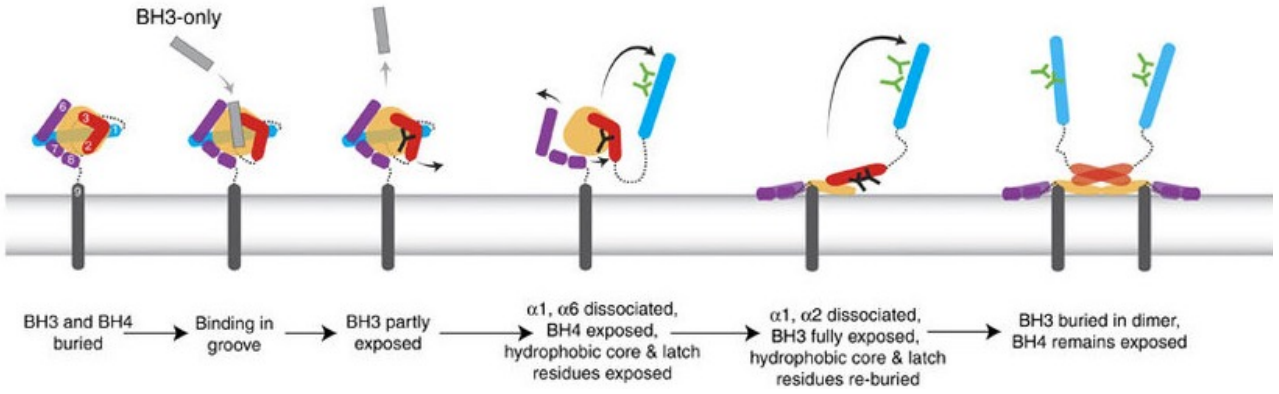
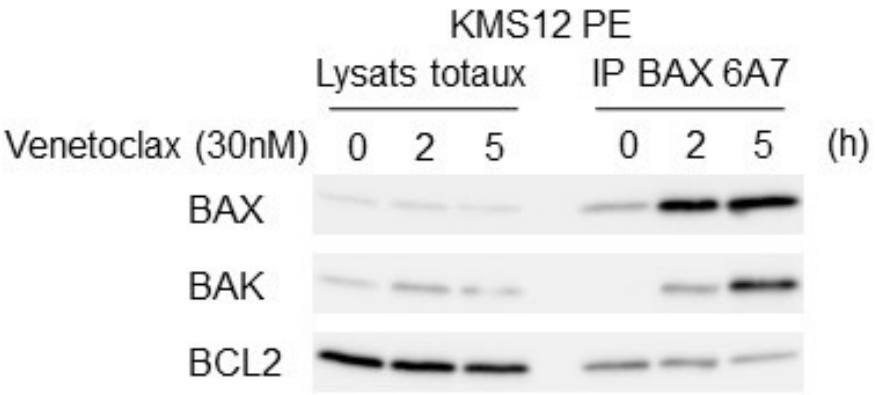
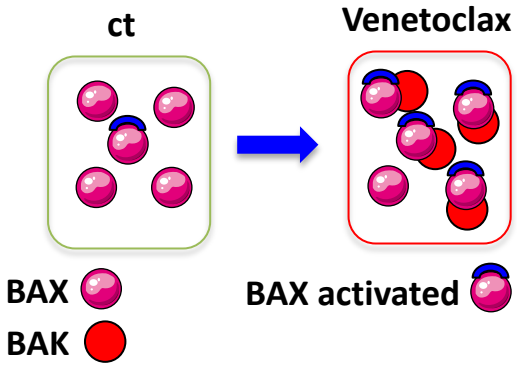
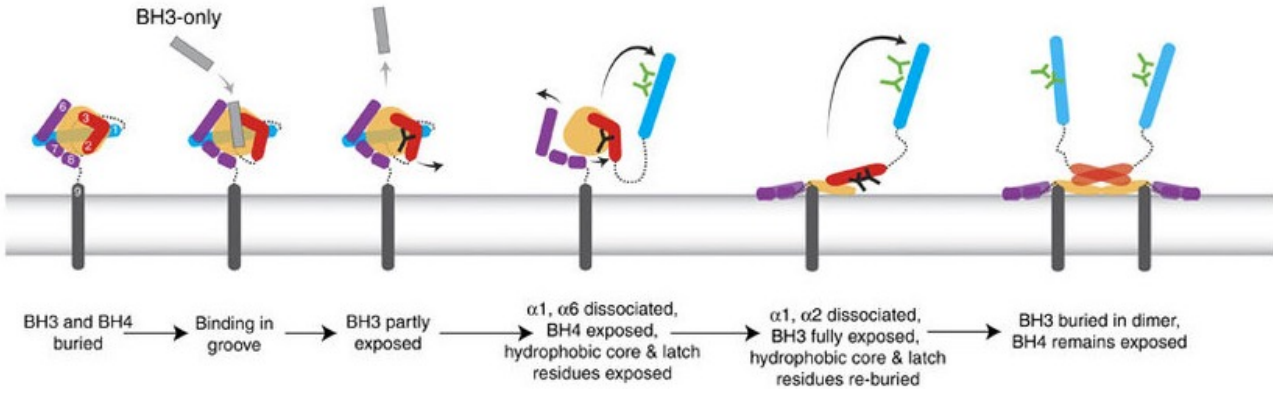


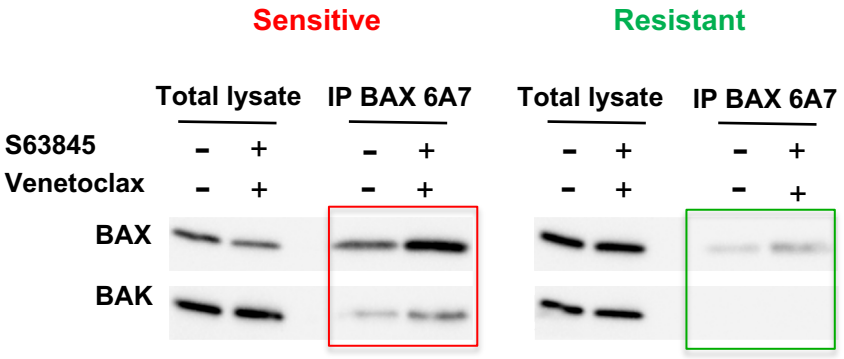


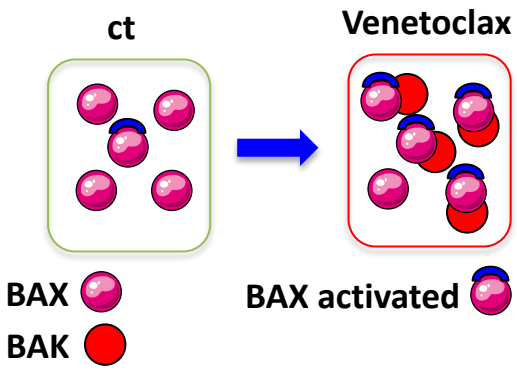


Unpublished data

Activation status of BAX and BAK effectors: exposure of epitopes (N' terminal) and heterodimers formation

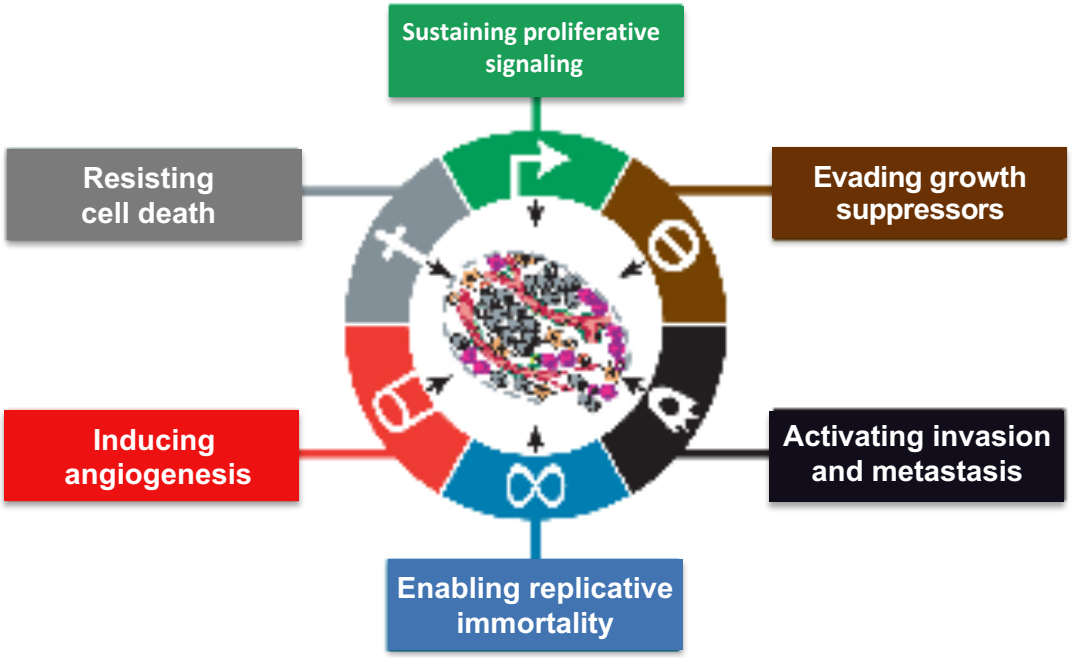






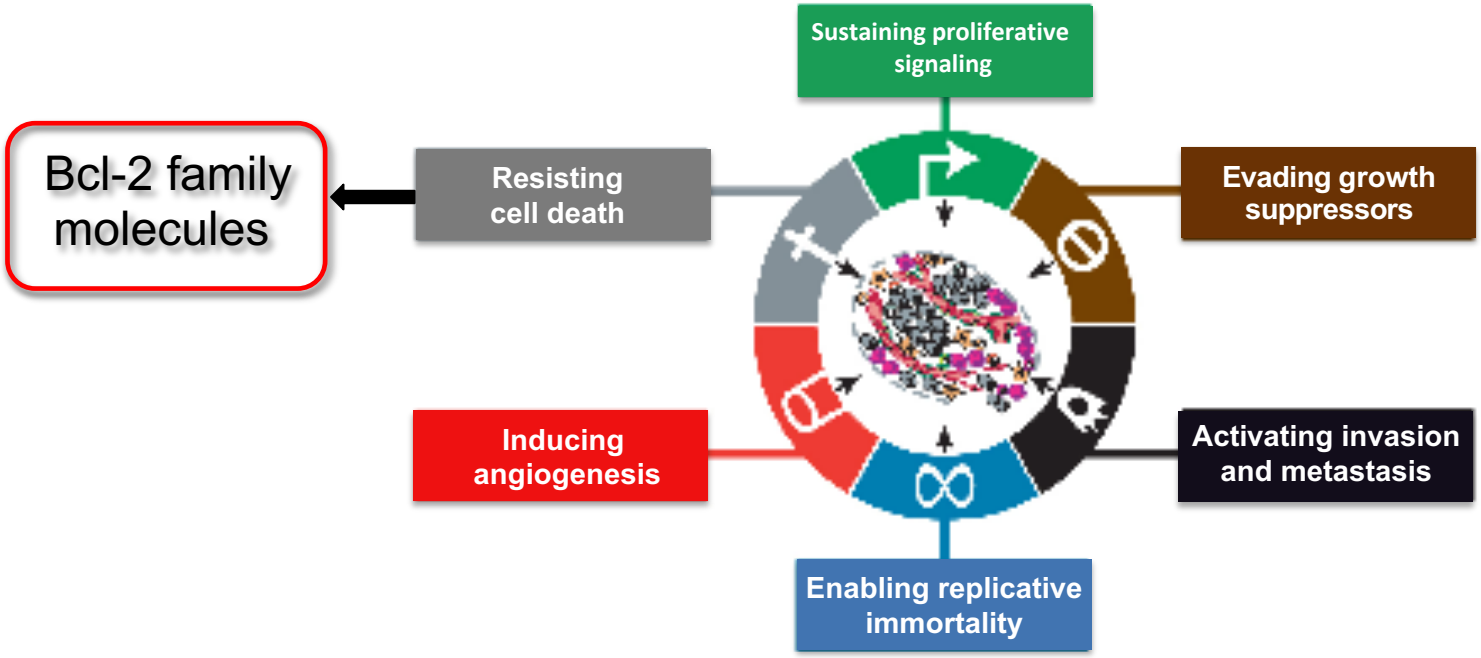
Sellier, Gomez Bougie, Amiot Cell Death & Dis 2011

Apoptosis evasion one of the hallmarks of cancer



Hanahan and Weinberg, Cell 144, March 4, 2011

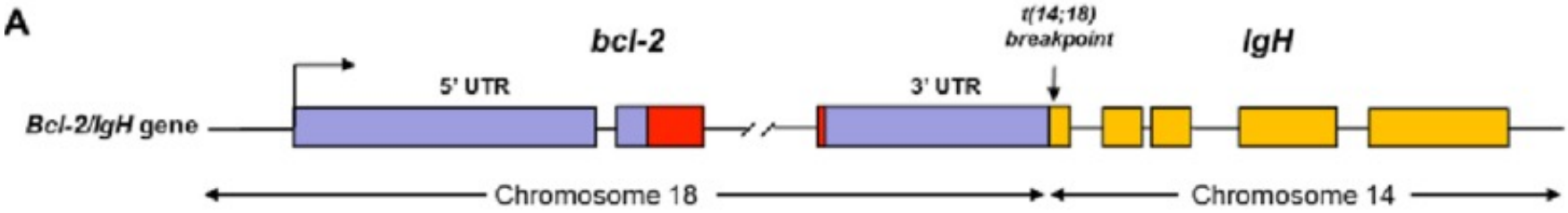
Apoptosis evasion one of the hallmarks of cancer

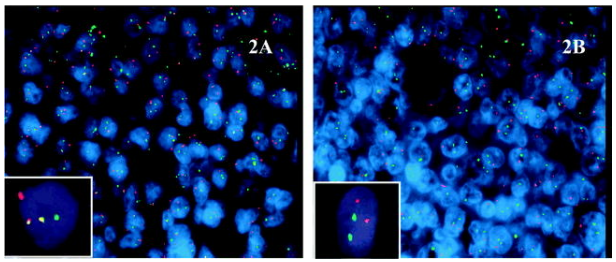


Hanahan and Weinberg, Cell 144, March 4, 2011

BCL2 family deregulation in Cancer cells: *BCL2 gene* translocation

- 1984 : *BCL2* (*B cell leukaemia or lymphoma gene number 2*)
- BCL2 gene (ch18): strong association translocation BCL2/IGH t(14;18) with follicular lymphoma





Fluoresence in situ hybridization (FISH)

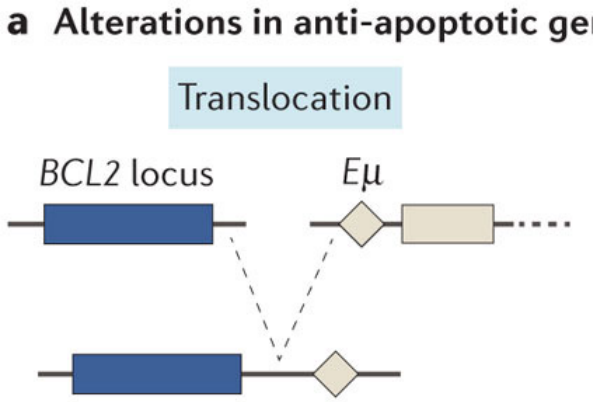
positive t(14;18) **negative t(14;18)**

KU G et al. Arch Pathol Lab Med. 2008

- BCL2 promoted cell survival but no effect in cell proliferation
- Overexpression of BCL2 block apoptosis triggered by diverse cellular stresses.

Delbridge A et al. Nat Rev Cancer 2016

BCL2 family deregulation in Cancer cells: overexpression of anti-apoptotic members



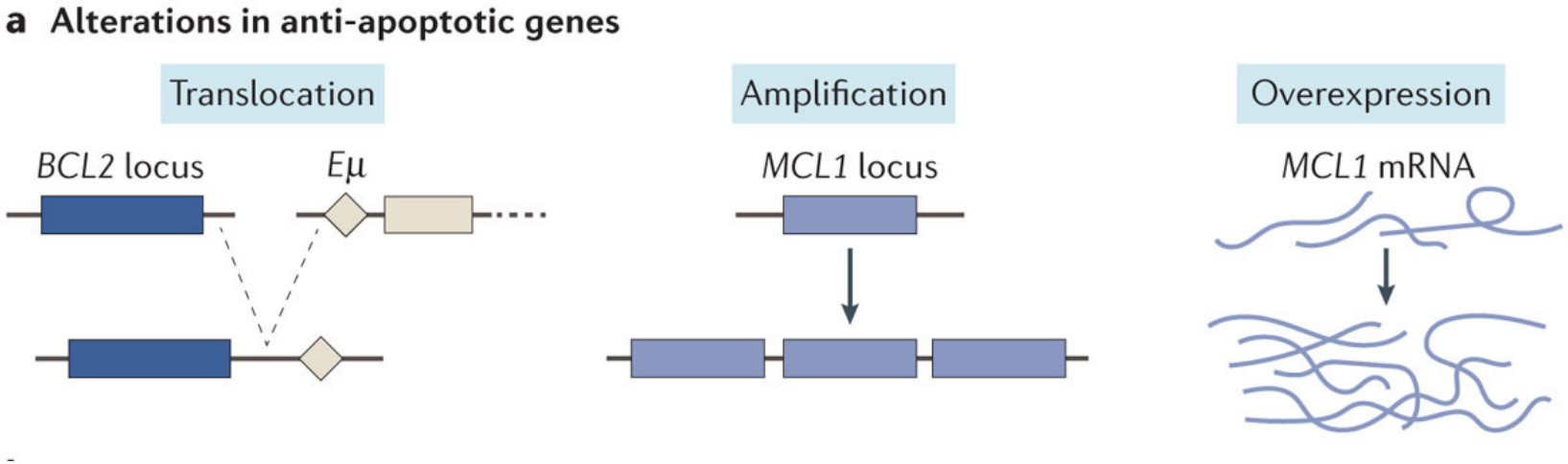
- BCL2 amplification in some cases of Diffuse large B cell lymphoma (DLBCL).
- Most CLL (chronic lymphocytic leukaemia) : BCL2 overexpression

miR-15a and/or
miR-16.1 deletion

BCL2 expression

Delbridge A et al. Nat Rev Cancer 2016

BCL2 family deregulation in Cancer cells: overexpression of anti-apoptotic members



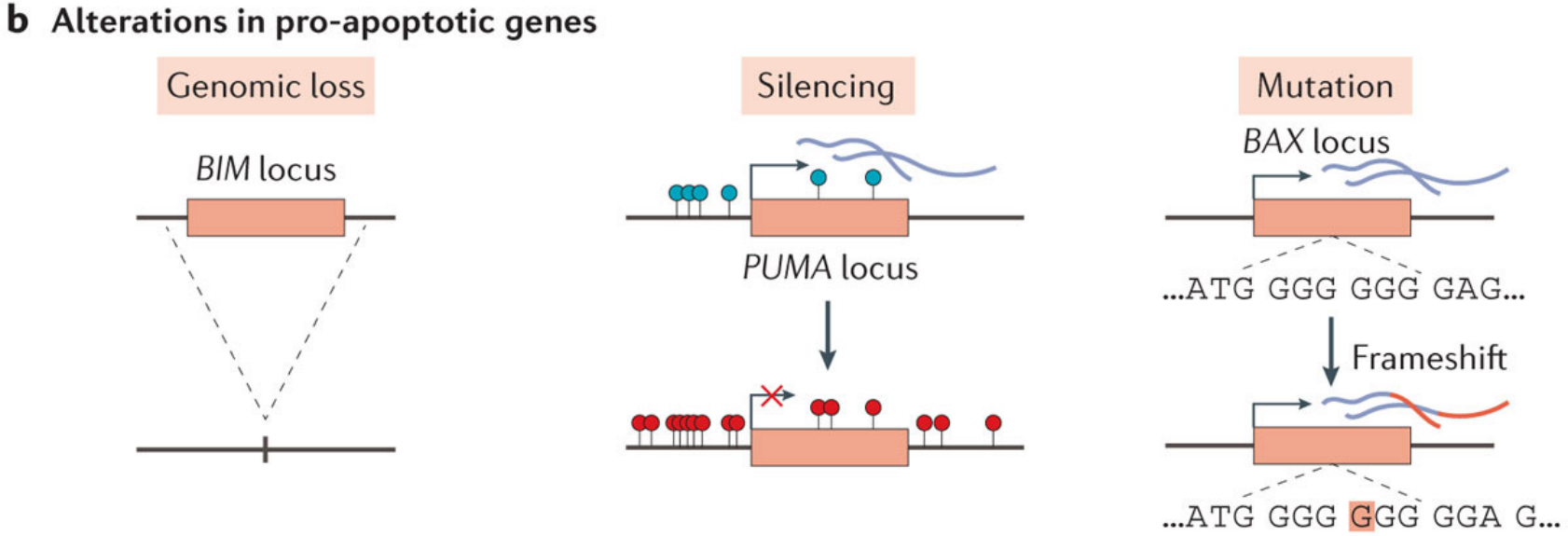
- 1q amplification: *MCL1* gene (Multiple Myeloma)
- Loss or silencing of miR

miR-29 and/or
miR-125, miR193 ⊣ *MCL1* expression

- Somatic copy number alterations (SCNA) of BCLXL => BCLXL increase

Delbridge A et al. Nat Rev Cancer 2016

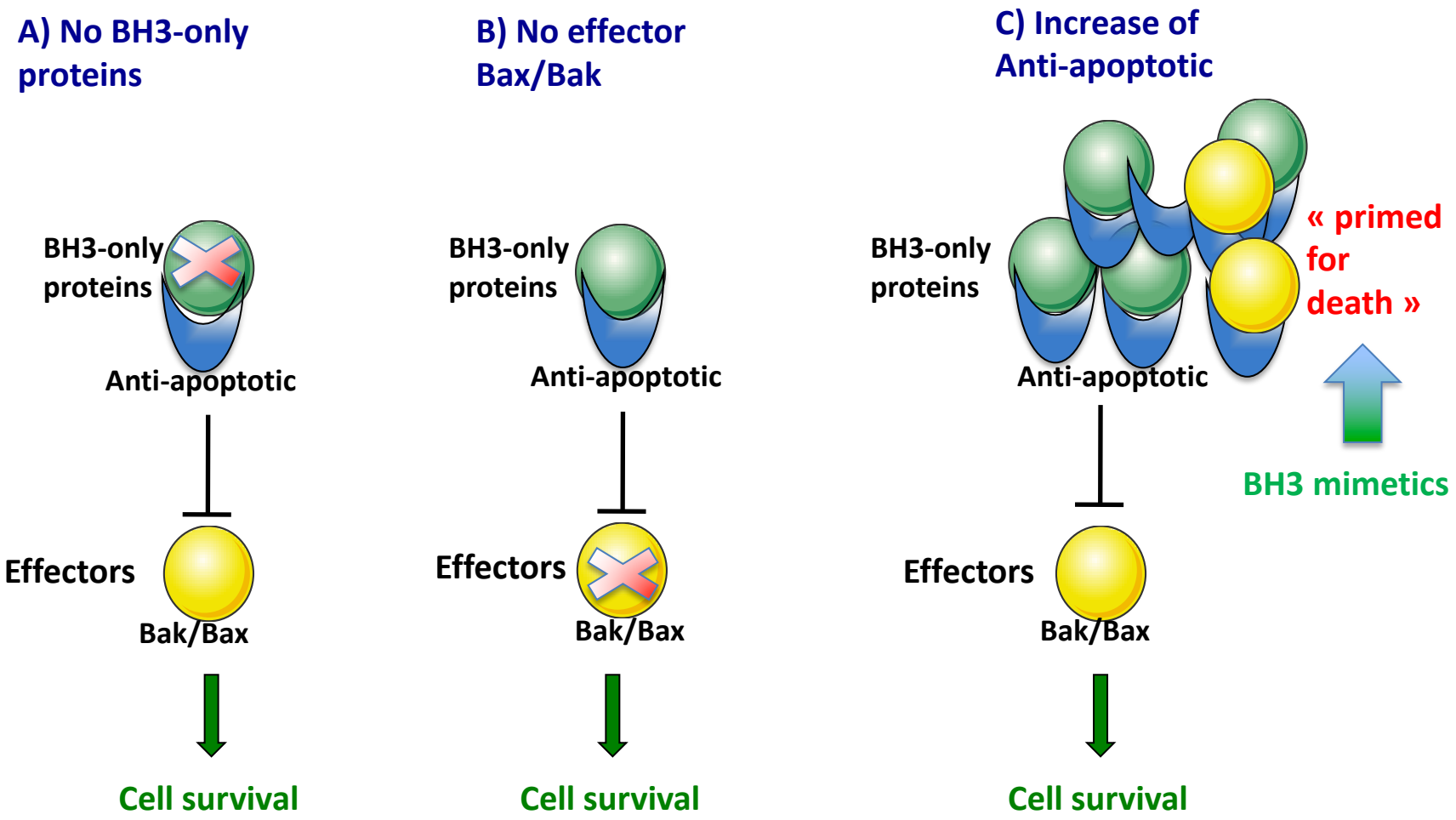
BCL2 family deregulation in Cancer cells: inhibition of pro-apoptotic members



- Homozygous deletion of *BIM* (20% Mantle cell lymphoma)
- Epigenetic silencing of *PUMA* or *BIM* : Renal carcinoma, Burkitt lymphoma
- Mutations (frame shift mutation *BAX*)

Delbridge A et al. Nat Rev Cancer 2016

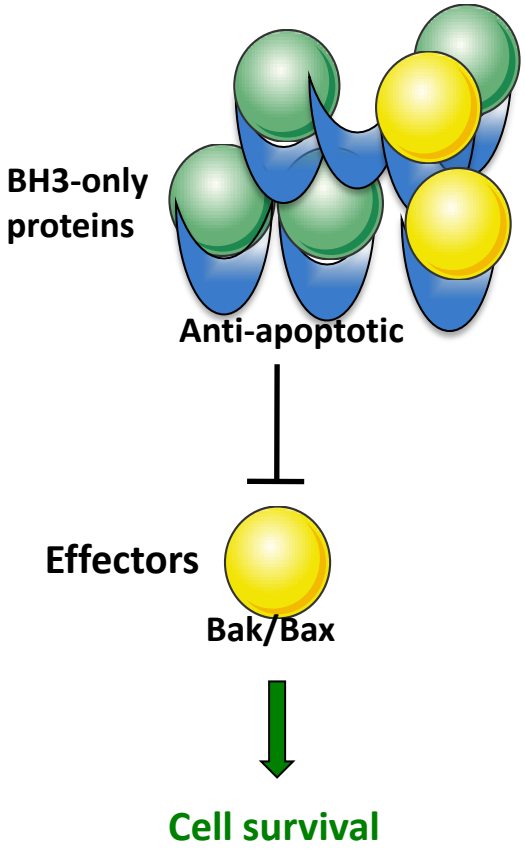
Mechanisms of apoptosis blockage in Cancer cells

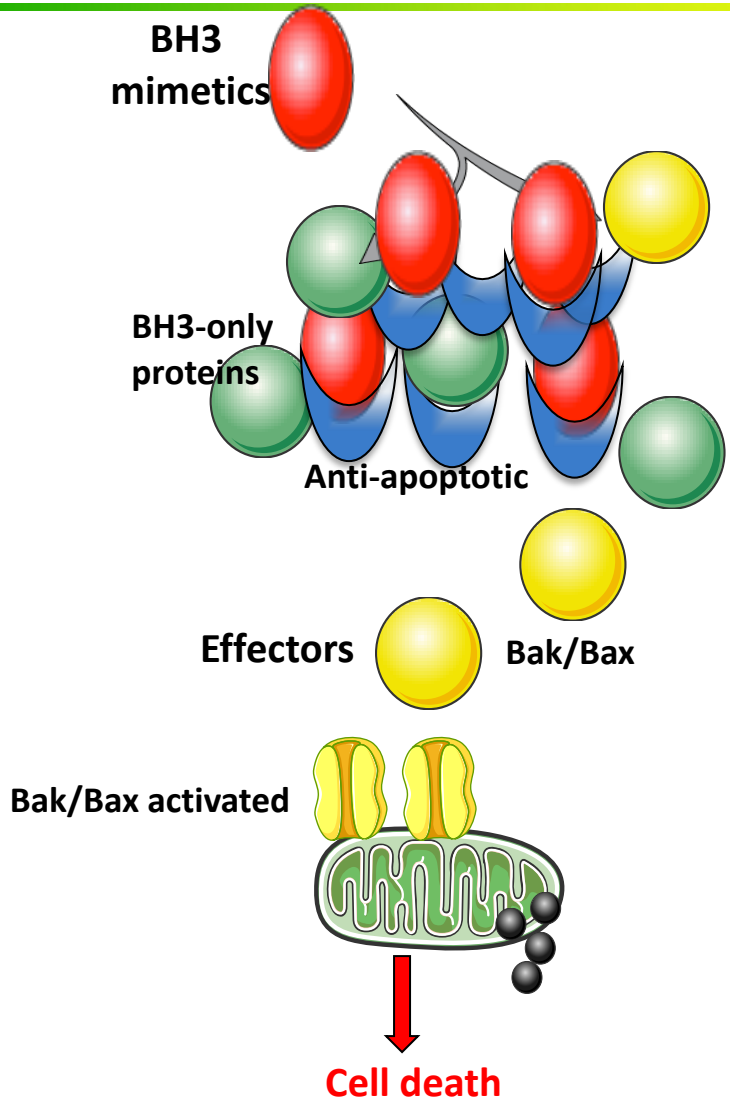


The lymphoid tumor cells are "primed for death"

Targeting of “primed for death” cells

« primed for death »

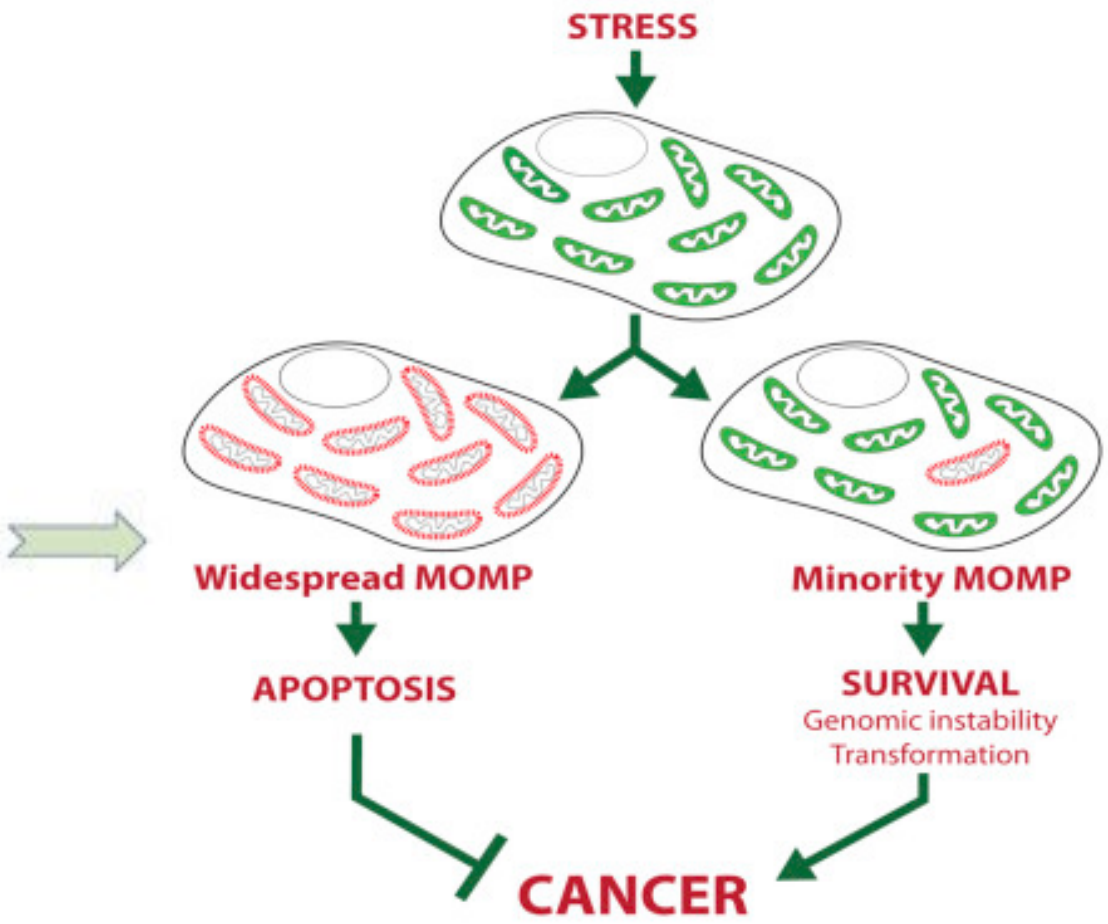




The lymphoid tumor cells are primed for death

Role of the permeabilization of the mitochondrial outer membrane in cell death

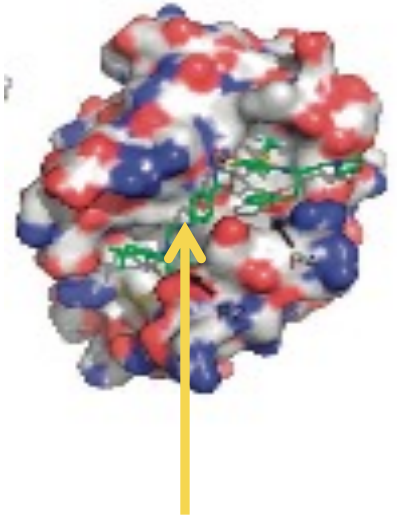
Anti-cancer targeted therapies



The History of the BCL2 family: from BCL2 discovery to Venetoclax FDA approval

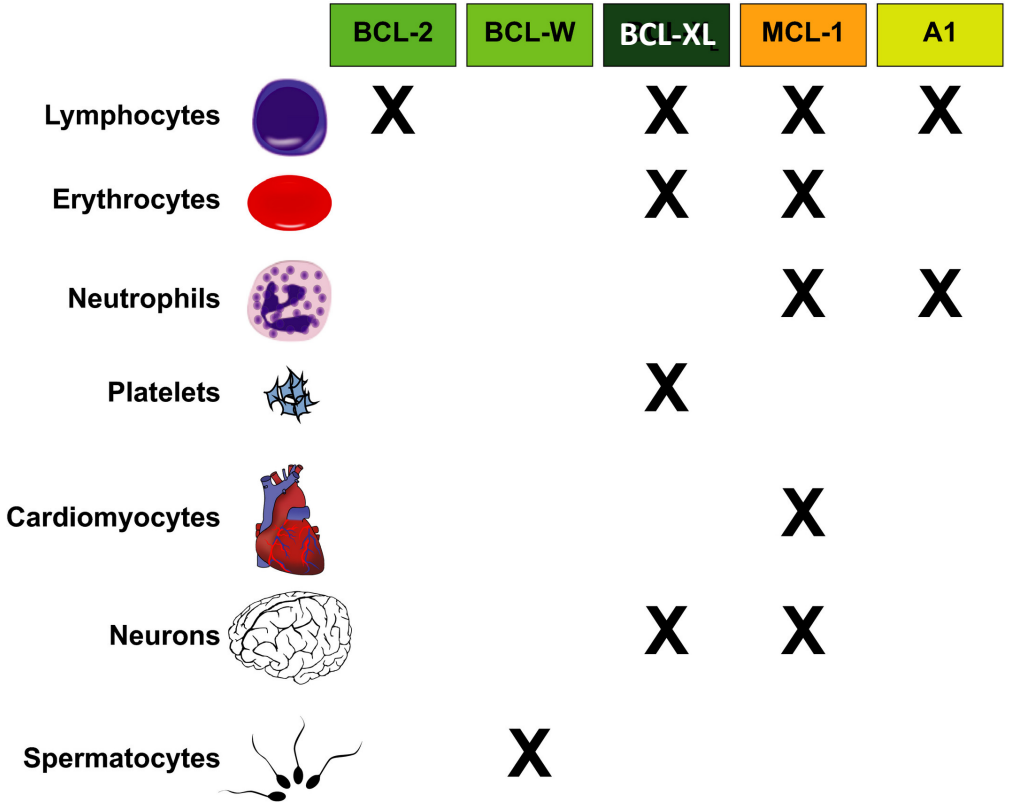
Milestones in the history of the Bcl-2 family Research	
1984	**Discovery of the gene coding Bcl-2**
1988	Role in apoptosis suppression
1990	Bcl-2 localized to mitochondria
1992	Chemoresistance
1993	Bax dimerizes with Bcl-2
1993	Bcl-2 overexpressed in CLL
1993	Bcl-2 ASO reverses chemoresistance
1996	BH3 domain mediates dimerization
1996	Bcl-x 3D structure
2001	Bcl-2 3D structure
2005	**Discovery of ABT-737 (1st BH3 mimetic)**
2007	Bcl-xL required for platelets
2008	Discovery of Navitoclax (ABT-263)
2011	Navitoclax Phase1
2013	Discovery of Venetoclax (ABT-199)
2016	**FDA approval Venetoclax (ABT-199) for CLL**

BCL2



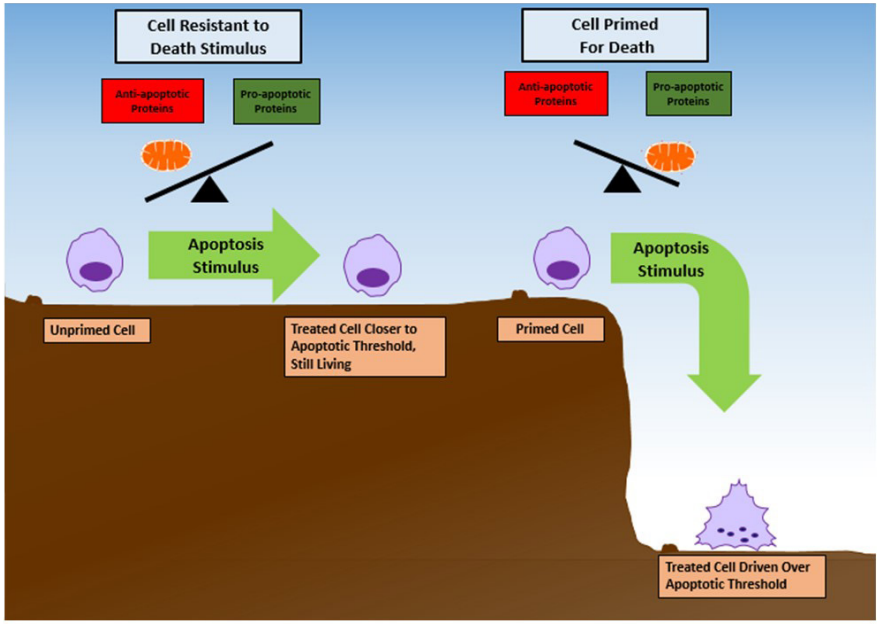
BH3 mimetic ABT-737

Anti-apoptotic members are implicated in normal cell biology



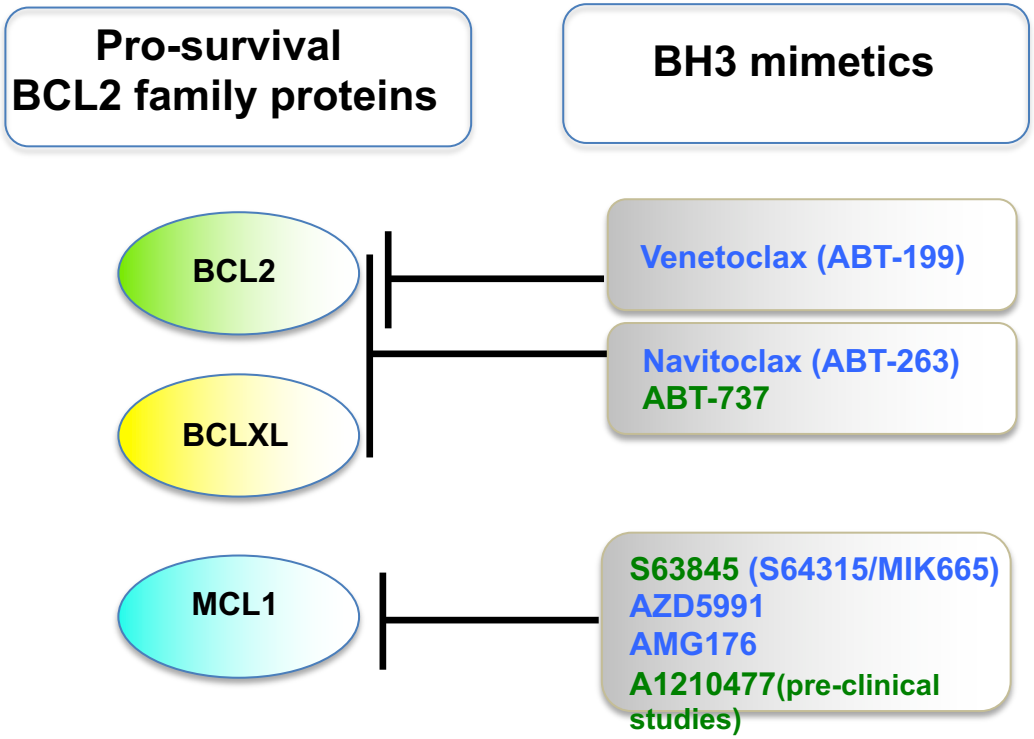
Opferman J, FEBS J. 2015

The “priming” condition as a therapeutic window

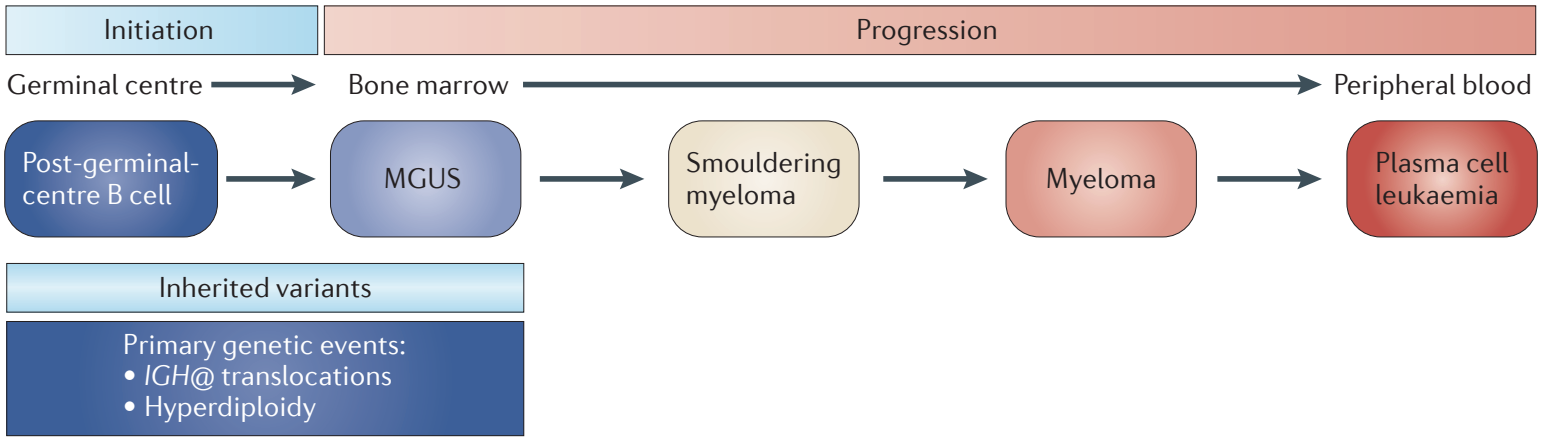


Tompkings and Thorburn, Yale J. 2019

Targeting the pro-survival BCL2 proteins



Initiation and progression of Myeloma



IGH translocations: **(% patients)**

- t(4;14): FGFR3 and MMSET (10-15%)
- t(14;16): C-MAF (3%)
- t(14;20): MAFB (1.5%)
- t(11;14): CCND1 (15-20%)
- t(6;14): CCND3 (1%)

Poor prognosis

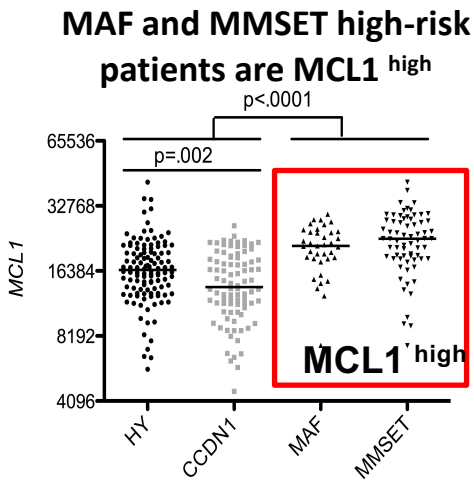
Hyperdiploidy HY

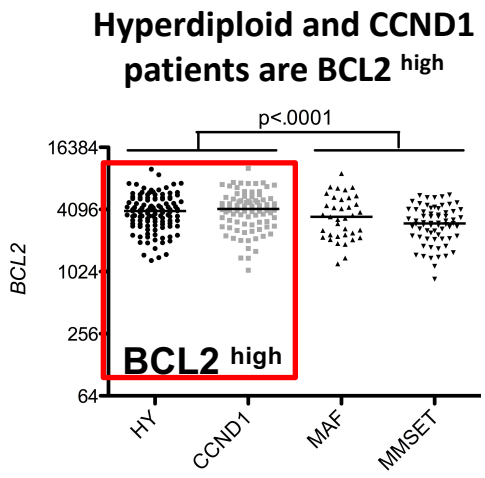
Trisomies of chromosomes 3,5,7,9,11,15,19 and 21 (57%)

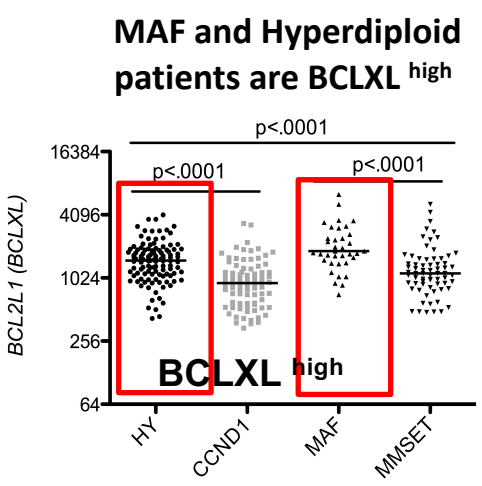
Myeloma is not a single disease entity: many multiple myeloma

Adapted from Morgan et al. , Nature Reviews Cancer 2013

Myeloma cells are “addicted” to pro-survival Bcl-2s



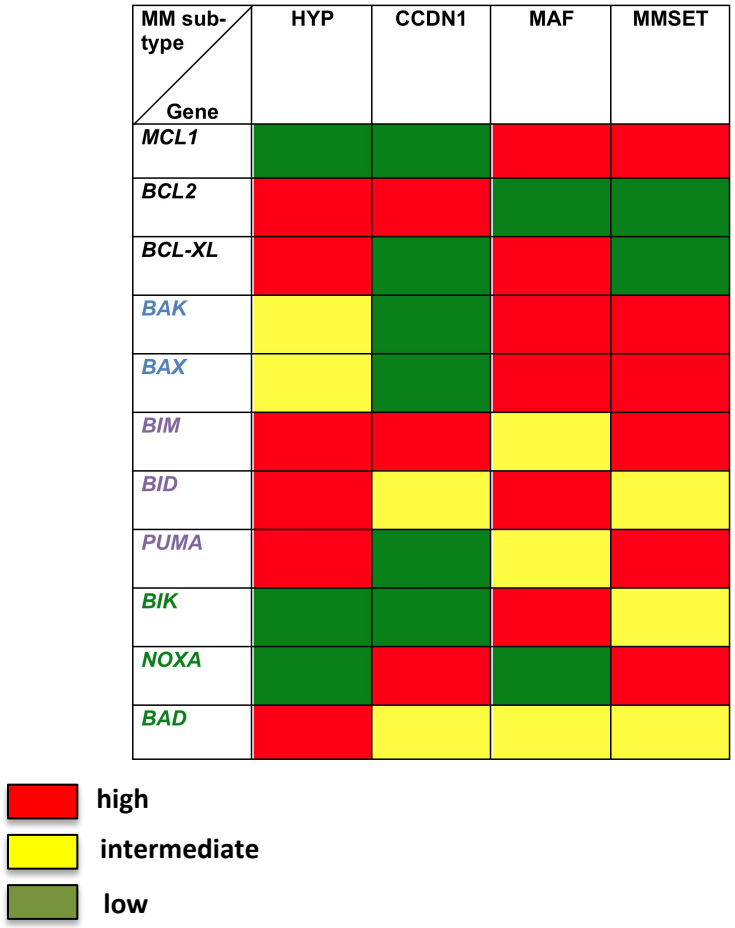




Multiple Myeloma heterogeneity is extended to the expression of Bcl-2 family members

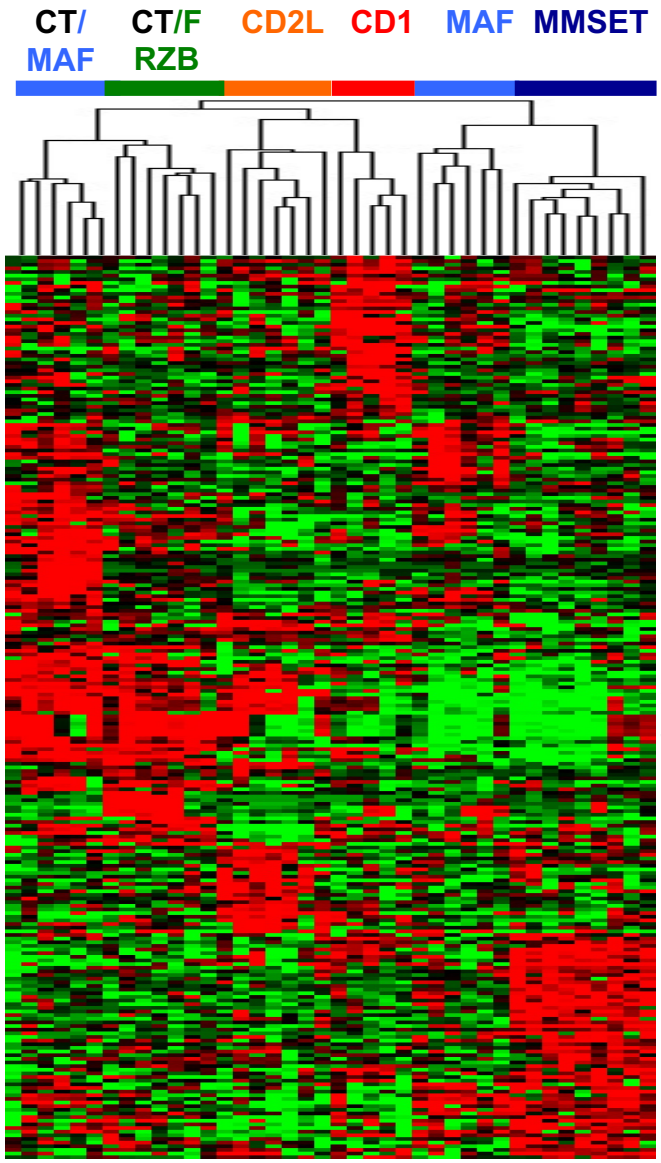
Gomez-Bougie P & Amiot M Front Immunol 2013

Multiple Myeloma cells are “primed for death”



Gomez-Bougie P & Amiot M Front Immunol 2013

Our MM tumor collection is mainly representative of the molecular diversity of patients

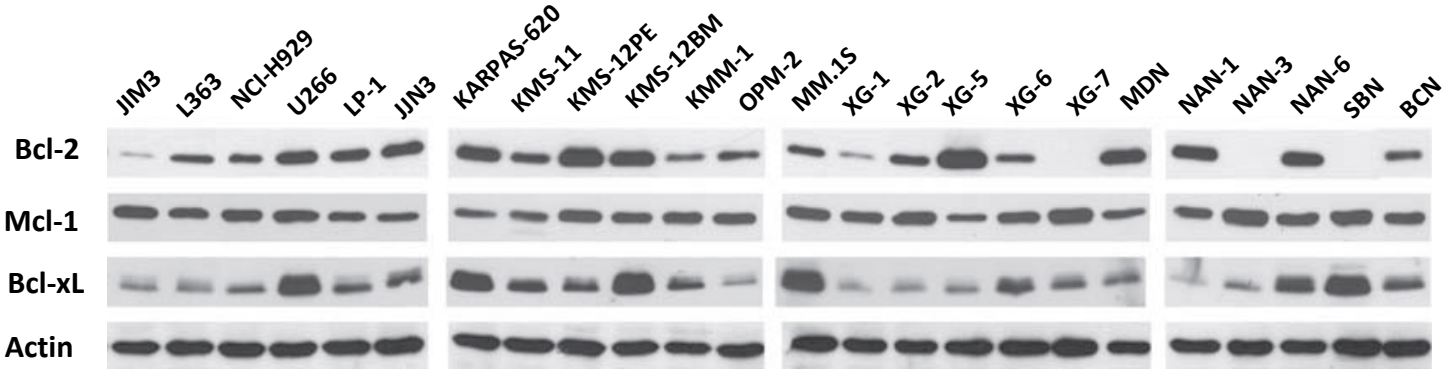


Moreaux J Pellat-Deceunynck C Haematologica 2011

- The genomic expression profile show that our cell line collection (n=40) is heterogeneous and mainly representative of the molecular diversity of patients.
- In particular, the group of patients harboring the recurrent translocations are well represented while the hyperdiploid patients are poorly represented.

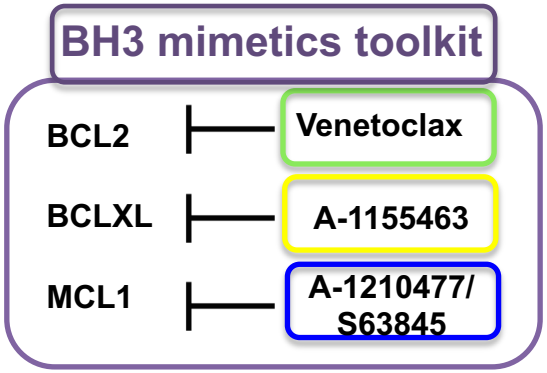
Myeloma cells dependency on anti-apoptotic Bcl-2's: the Achilles Heel?

- MM cells exhibit an elevated expression of anti-apoptotic proteins

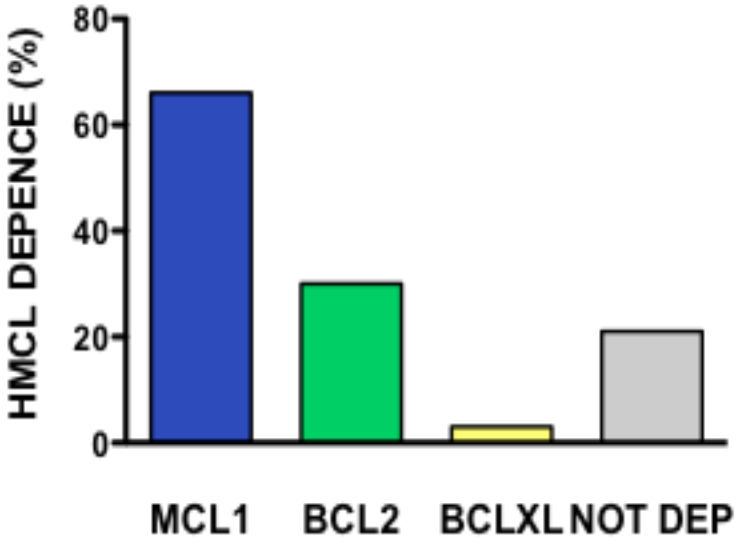


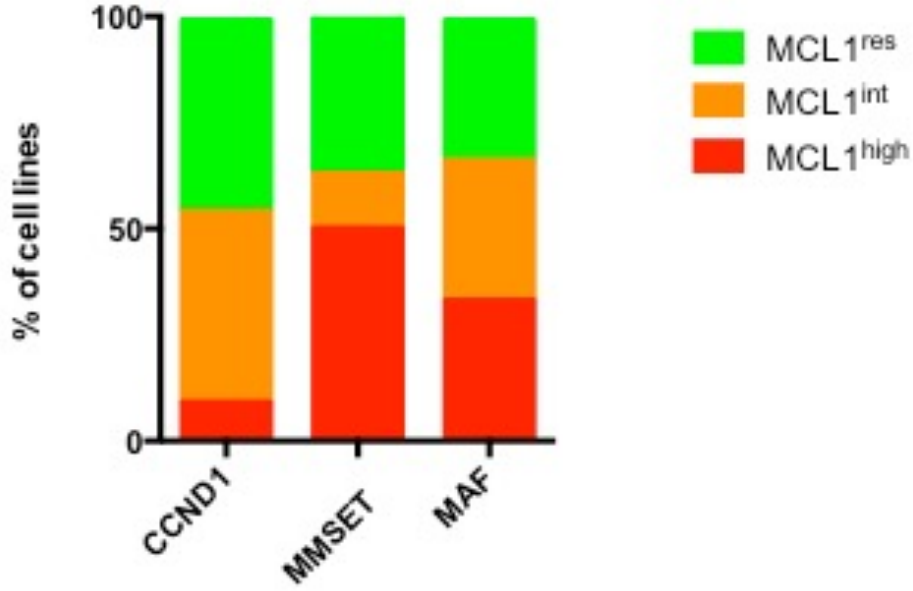
Bodet L, Gomez-Bougie P et al Br J Cancer 2010,

Are myeloma cells addicted to anti-apoptotic BCL2 members?



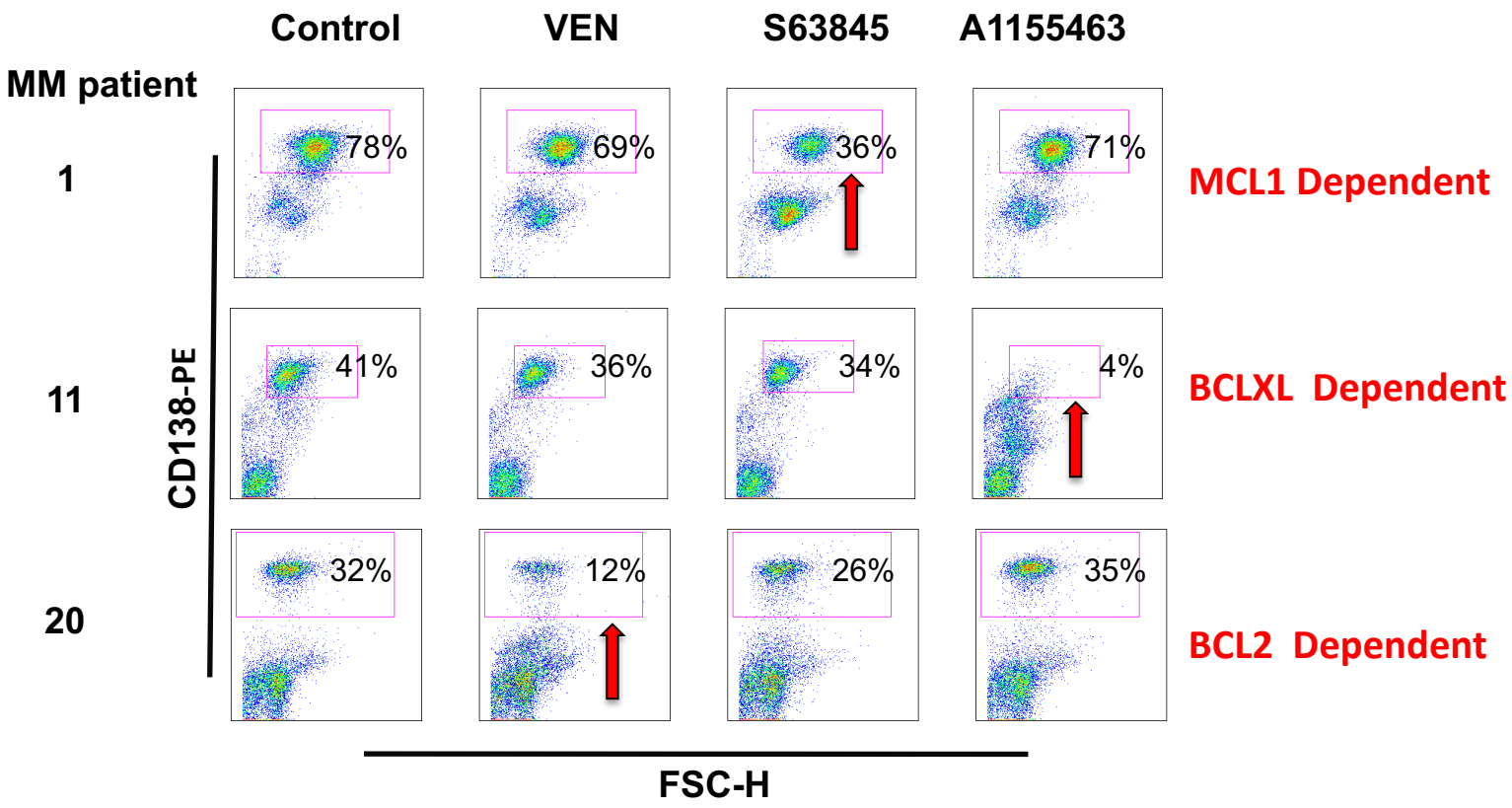
Multiple Myeloma Cell lines are MCL1 dependent





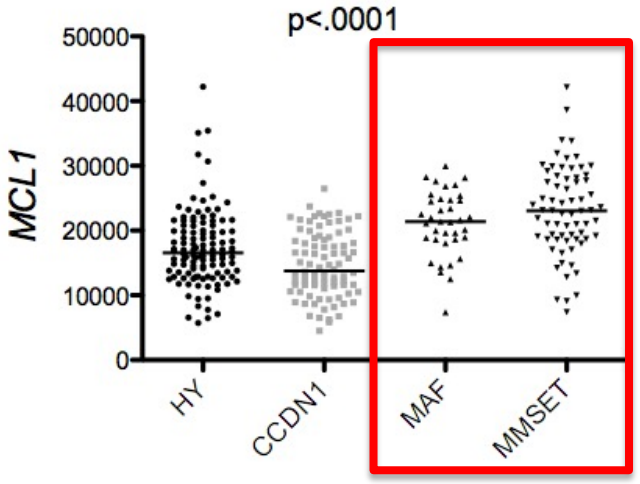
Gomez-Bougie P et al. BLOOD 2018 and unpublished data

Ex vivo analysis of cell dependencies in patients



MCL1 as target of therapeutic intervention in Multiple Myeloma?

Targeting Mcl-1 remains a priority in MM treatment

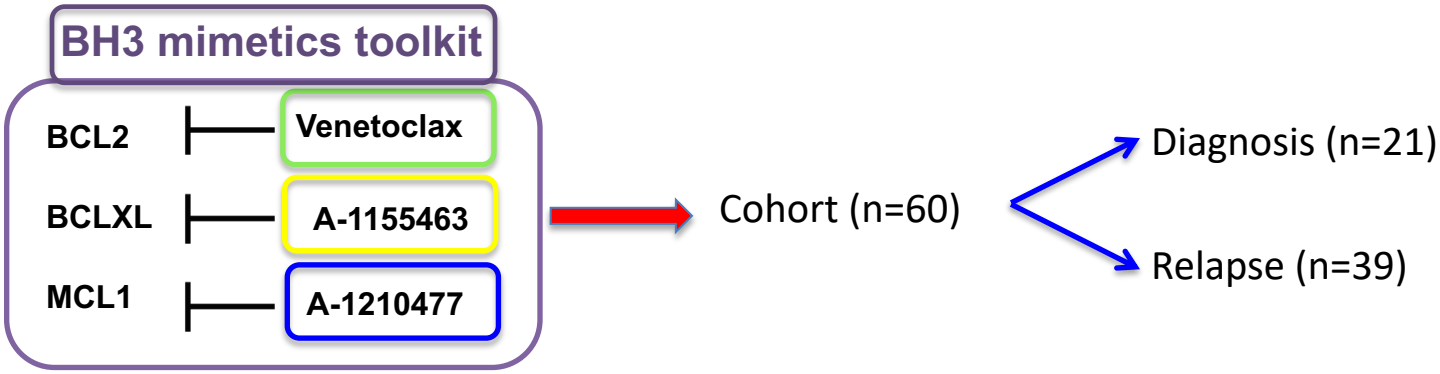


Gomez-Bougie P et Amiot M. Front Immunol 2013

-The amplification of 1q Chr during disease progression lead to MCL1 overexpression

Background

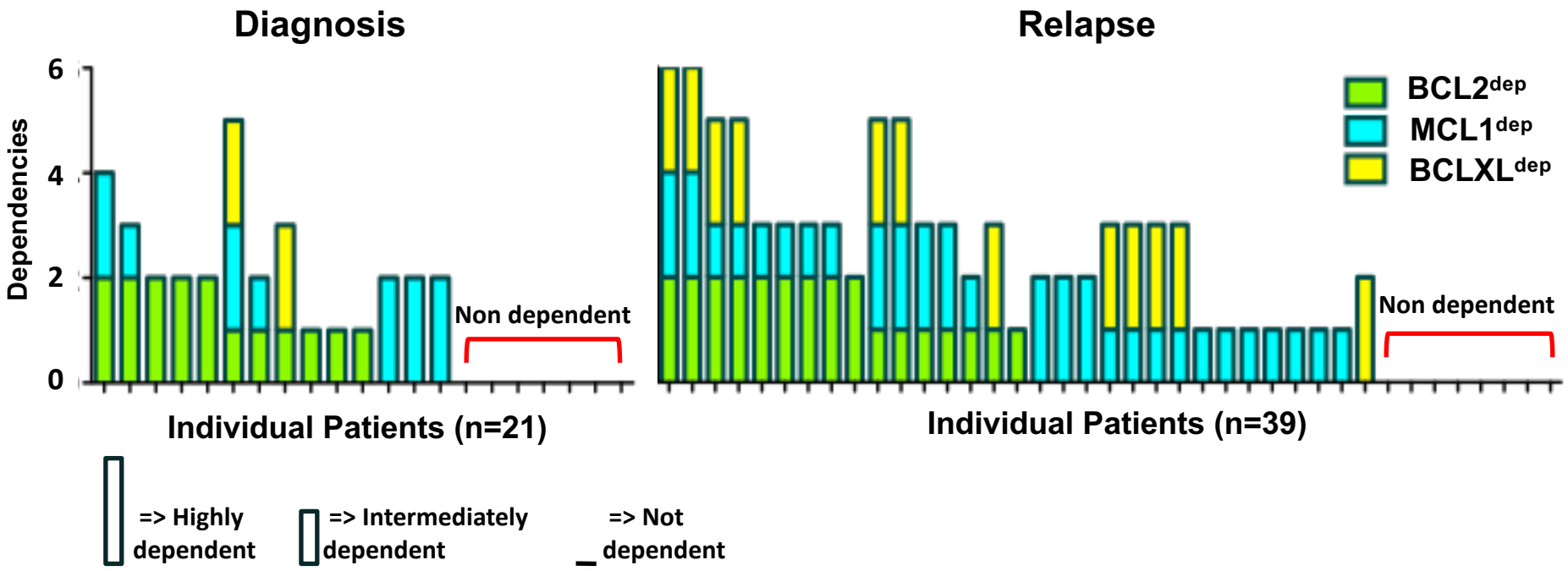
➢ **Study**: BH3 mimetics toolkit for ex vivo testing of primary myeloma cells:



➢**Dependencies** according to **cell death** were stratified using PCA analysis in 3 groups:

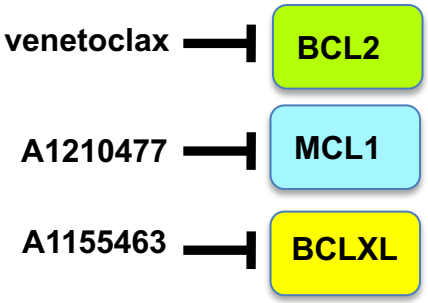
- Highly dependent
- Intermediate dependent
- Not dependent

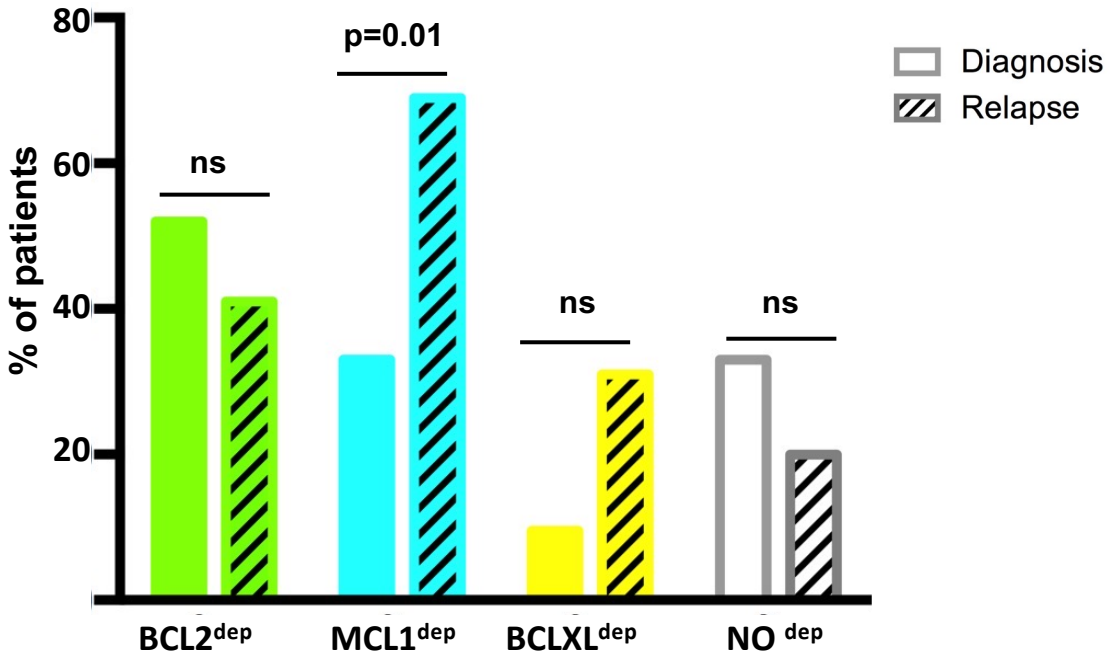
Individual dependencies on BCL2 pro-survival molecules



- Co-dependencies observed at Diagnosis (24%) and Relapse (46%)
- No dependence was observed in both groups

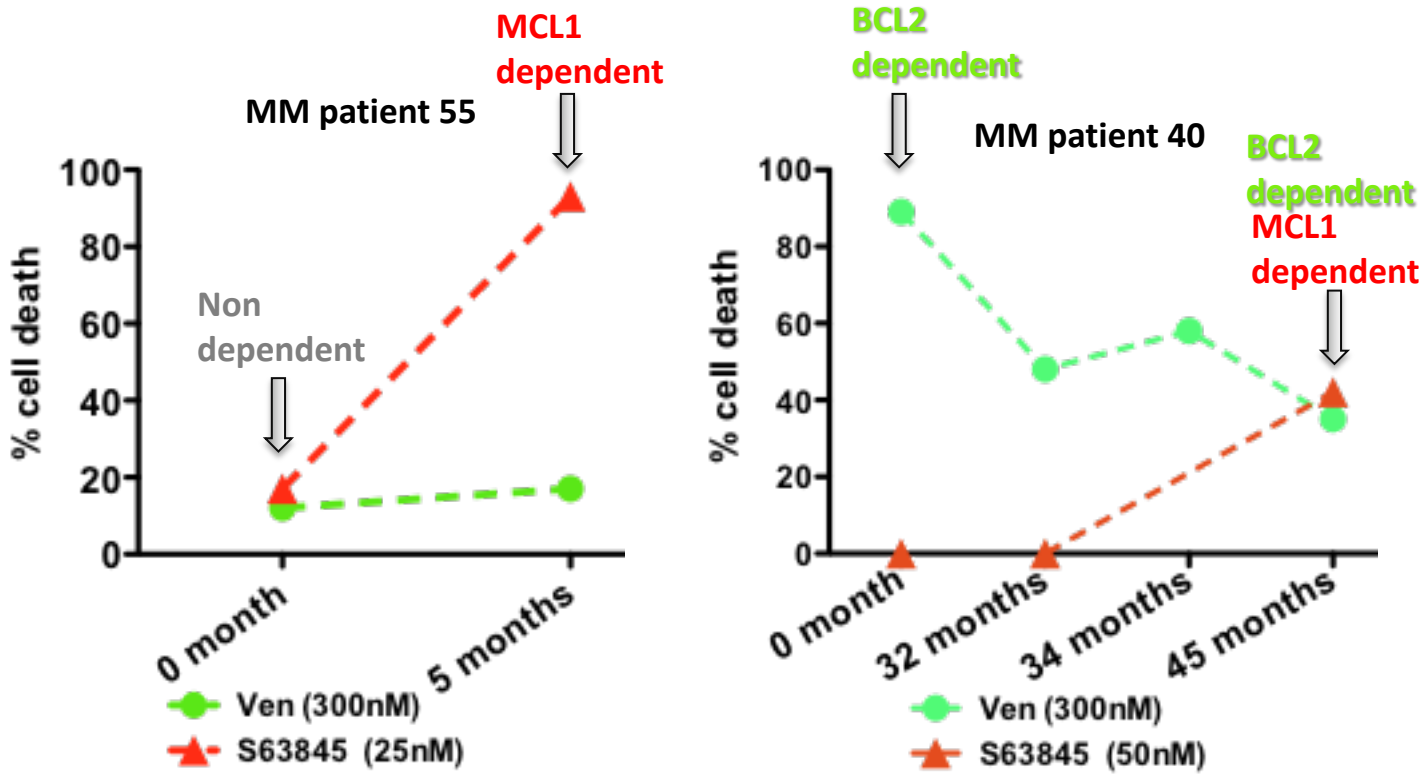
Dependencies of primary cells at Diagnosis and Relapse





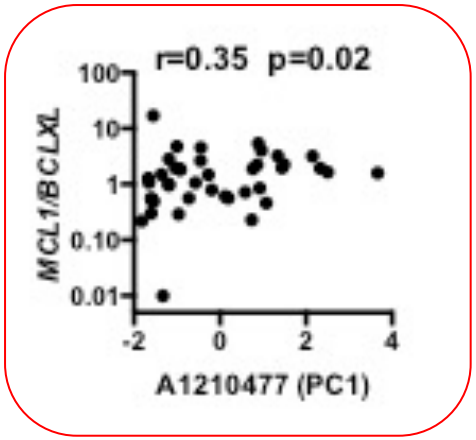
- BCL2 similar at Diagnosis and Relapse
- MCL1 dependency is increased at Relapse
- No dependent samples where identified in both stages

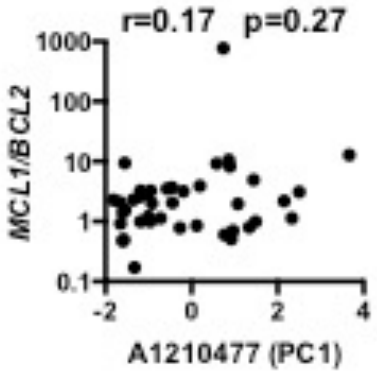
Plasticity of dependence in primary cells

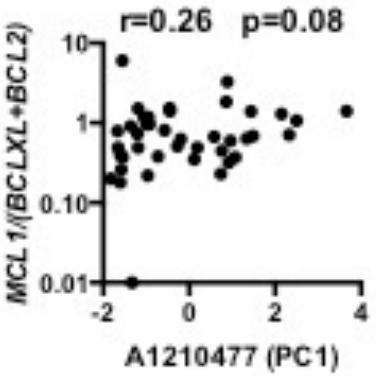


Unpublished data

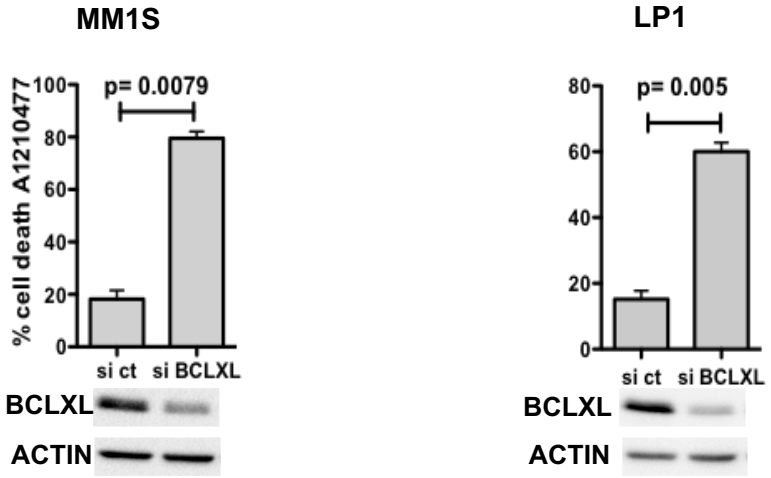
BCLXL has a role in resistance to MCL1 BH3 mimetics



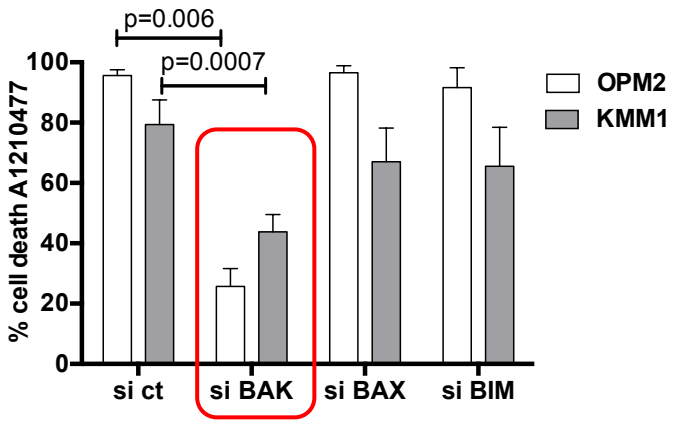


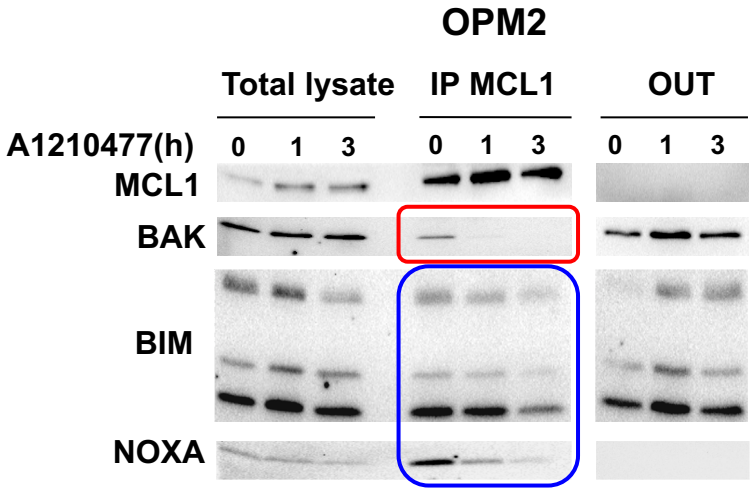


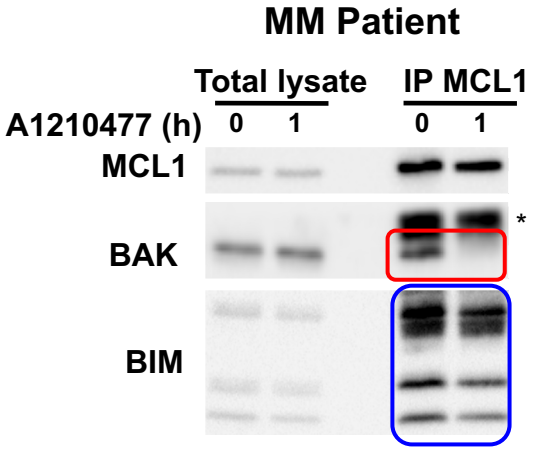
- BCLXL negatively influences MCL1i sensitivity
- MCL1/BCLXL mRNA ratio suggests a role of BCLXL in MCL1i A1210477 resistance



MCL1 mimetic induced apoptosis in a Bak dependent manner

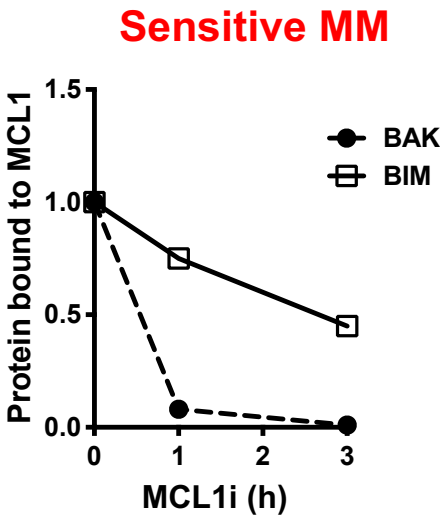


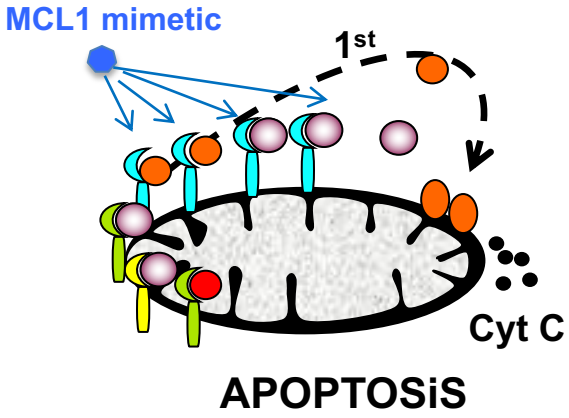


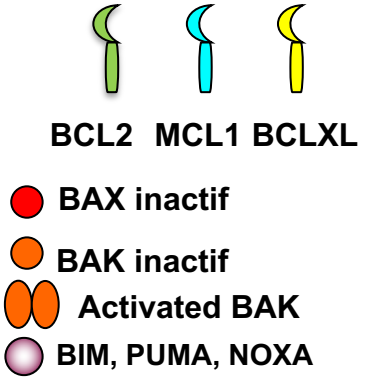


➢ BAK is essential for Apoptosis induced by MCL1 mimetics

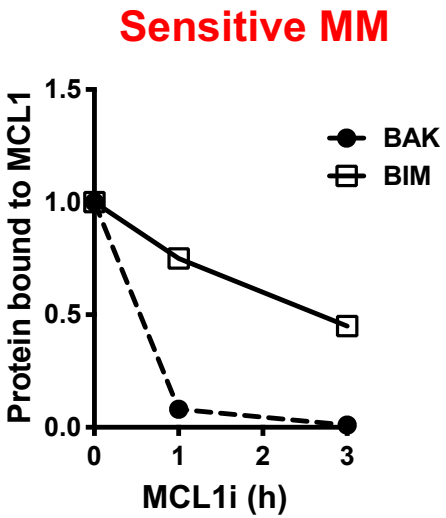
BCL2 protein interactions as target of BH3 mimetics

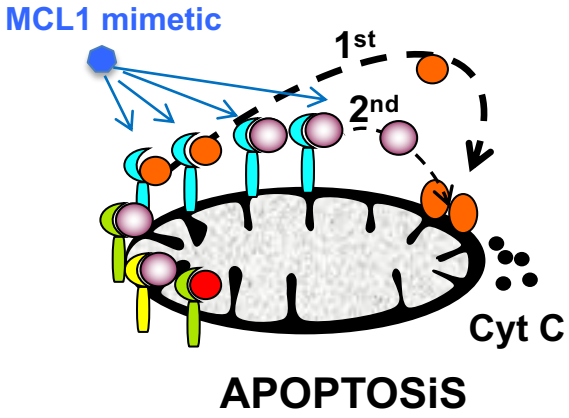


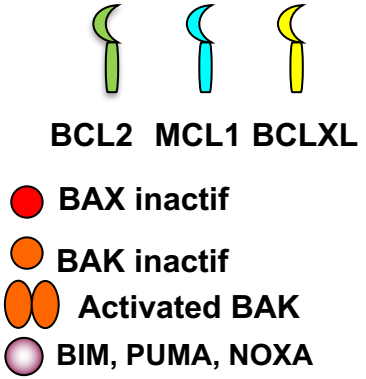




BCL2 protein interactions as target of BH3 mimetics

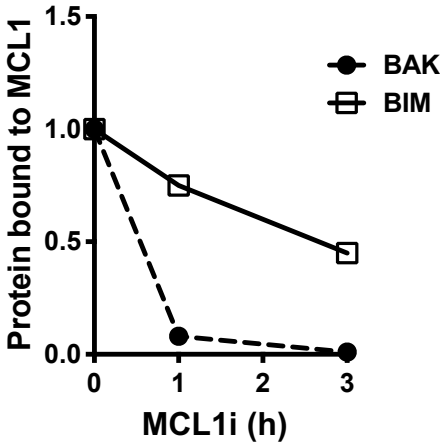


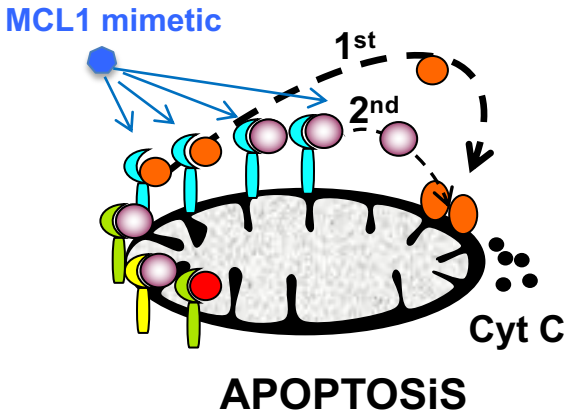


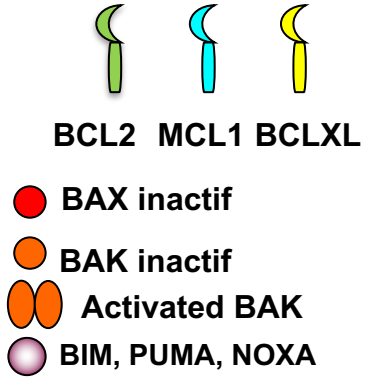


BCL2 protein interactions as target of BH3 mimetics

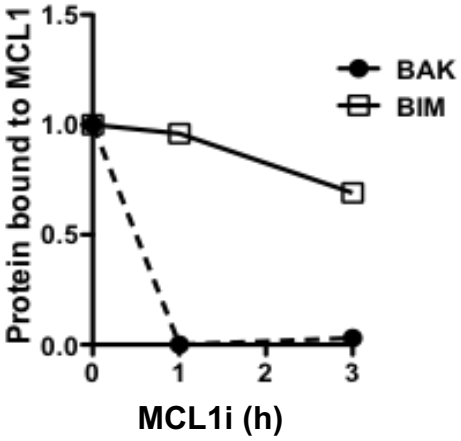
Sensitive MM





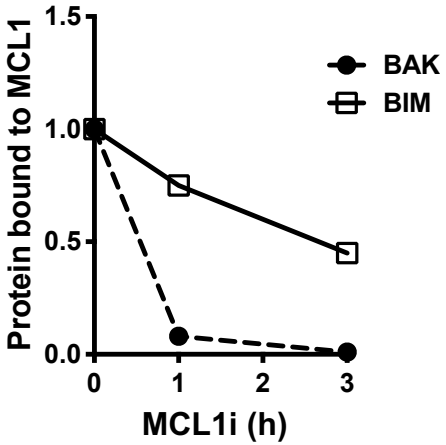


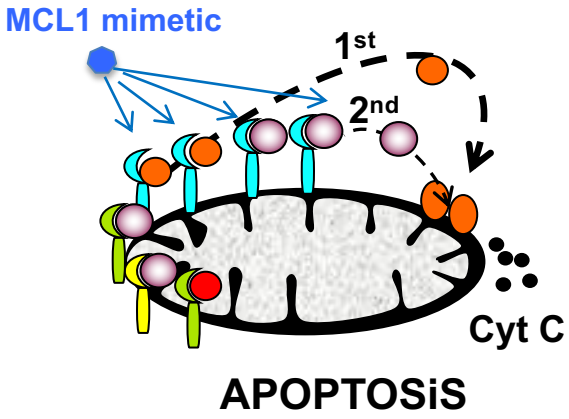
Resistant MM

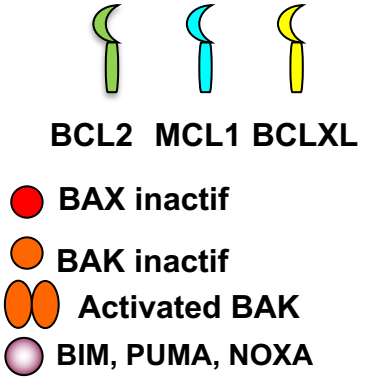


BCL2 protein interactions as target of BH3 mimetics

Sensitive MM







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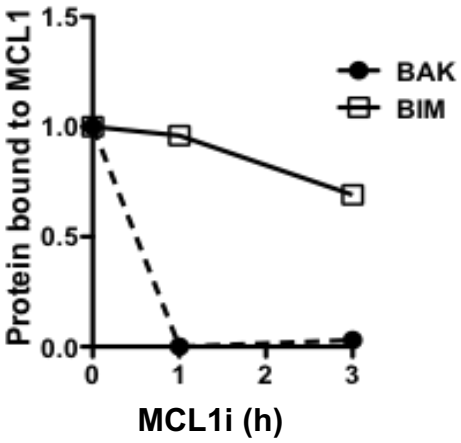
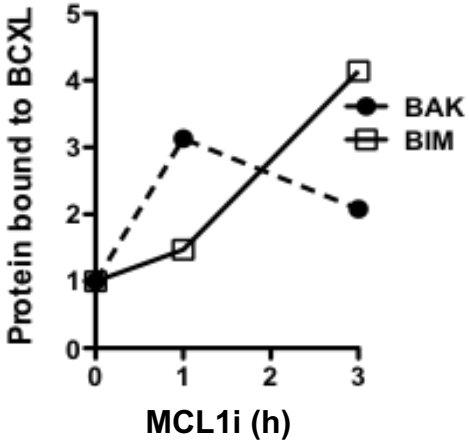
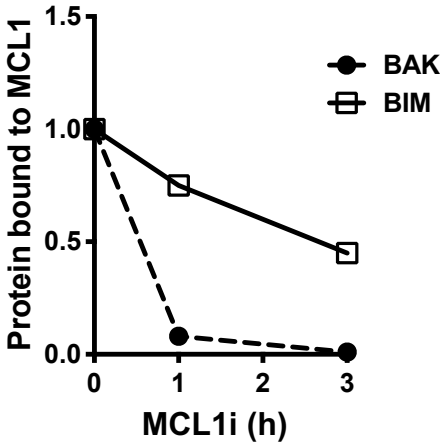
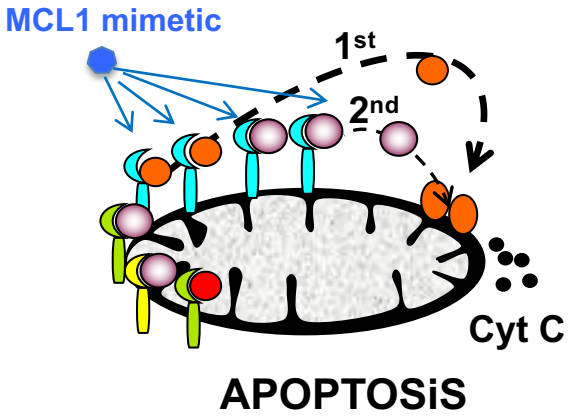


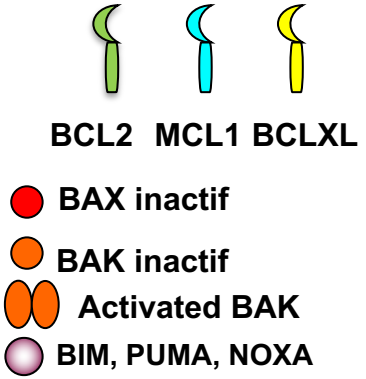


BCL2 protein interactions as target of BH3 mimetics

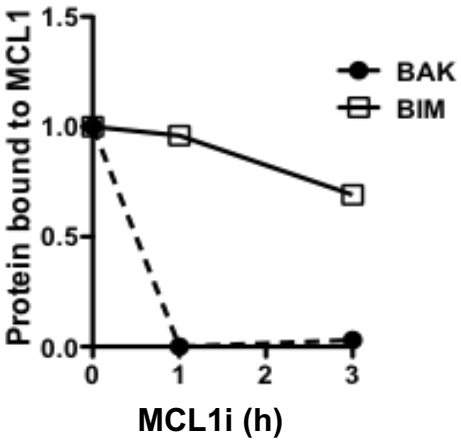
Sensitive MM

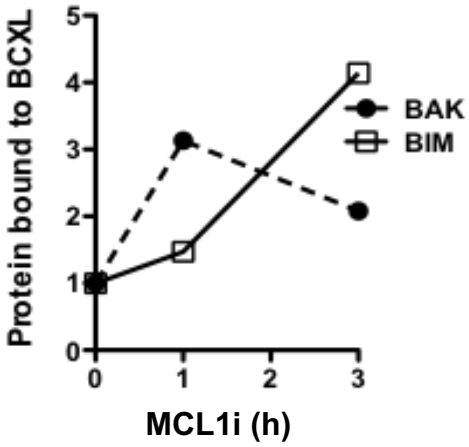


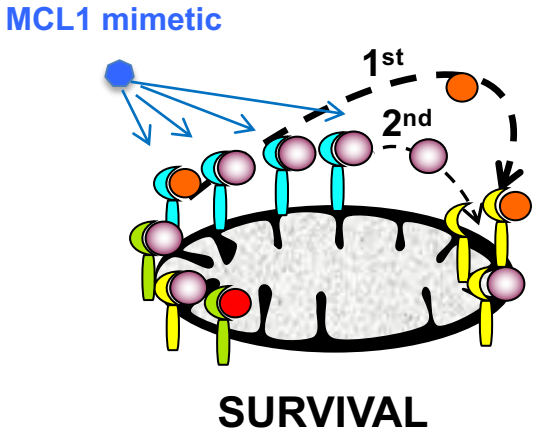




Resistant MM

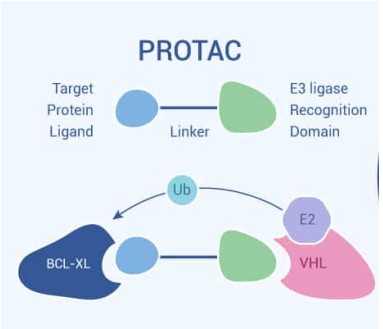






Targeting BCL2 family: what's next?

- Venetoclax: CLL, AML in combination with Azatidine, MM
- MCL1 BH3 mimetics: clinical trials halted => Vectorisation of MCL1i
- BCLXL protact: E3 ubiquitin ligases : degradation of BCLXL



- Inducers of Effectors activation:
  - BTSA1: induces BAX activation
  - 7D10 monoclonal antibody: BAK activation

CRCI²NA Team-11

Project re-MOVE-B

Molecular Vulnerabilities of tumor Escape in mature B-cell malignancies

D. BOULET
POST-DOC

R. DURAND
POST-DOC

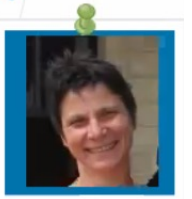
S. DECOMBIS
PHD STUDENT

C. SEILLER
PHD STUDENT

A. TATOUG
PHD STUDENT

M. Amiot

Researcher



A. Aubry

Researcher

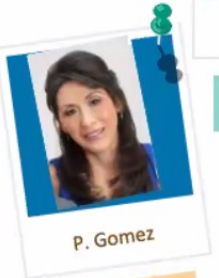
Catherine Pellat & David Chiron

C. Pellat

Team Leader

D. Chiron

Co Leader



P. Gomez

Researcher