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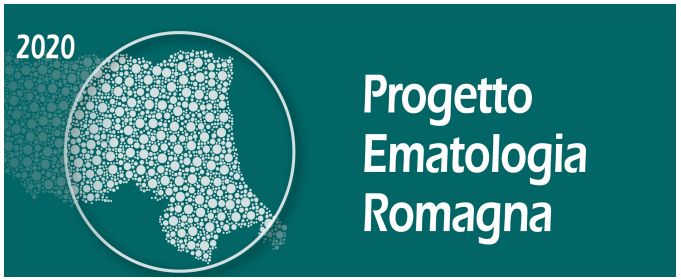
Ospedale Niguarda Cancer Center

Sistema Socio Sanitario
Regione Lombardia



7 Novembre, 2020

La terapia con anti-BCL2: nuovi target
INTRODUZIONE

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Ematologia
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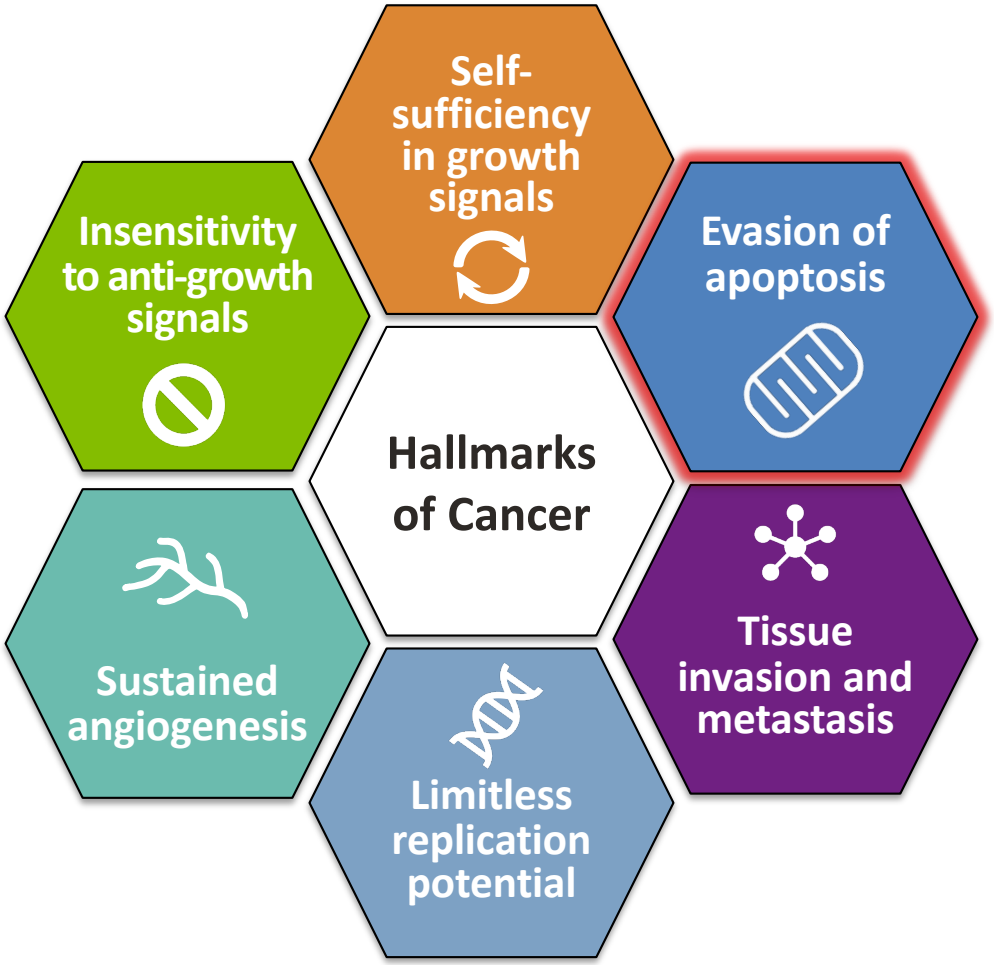
Milano

Marco Montillo
Conflict of Interest

- Abbvie
- Acerta/Astra Zeneca
- Gilead
- Janssen
- Roche
- Verastem

Cancer Pathogenesis Is Dependent on Numerous Mechanisms

Oncogenesis is driven by a complex interplay of genetic alterations and mechanisms that ensure cell survival and proliferation



Evasion of apoptosis is a hallmark of malignant cell survival

Hanahan D & Weinberg RA. *Cell* 2011; **144**:646–674.

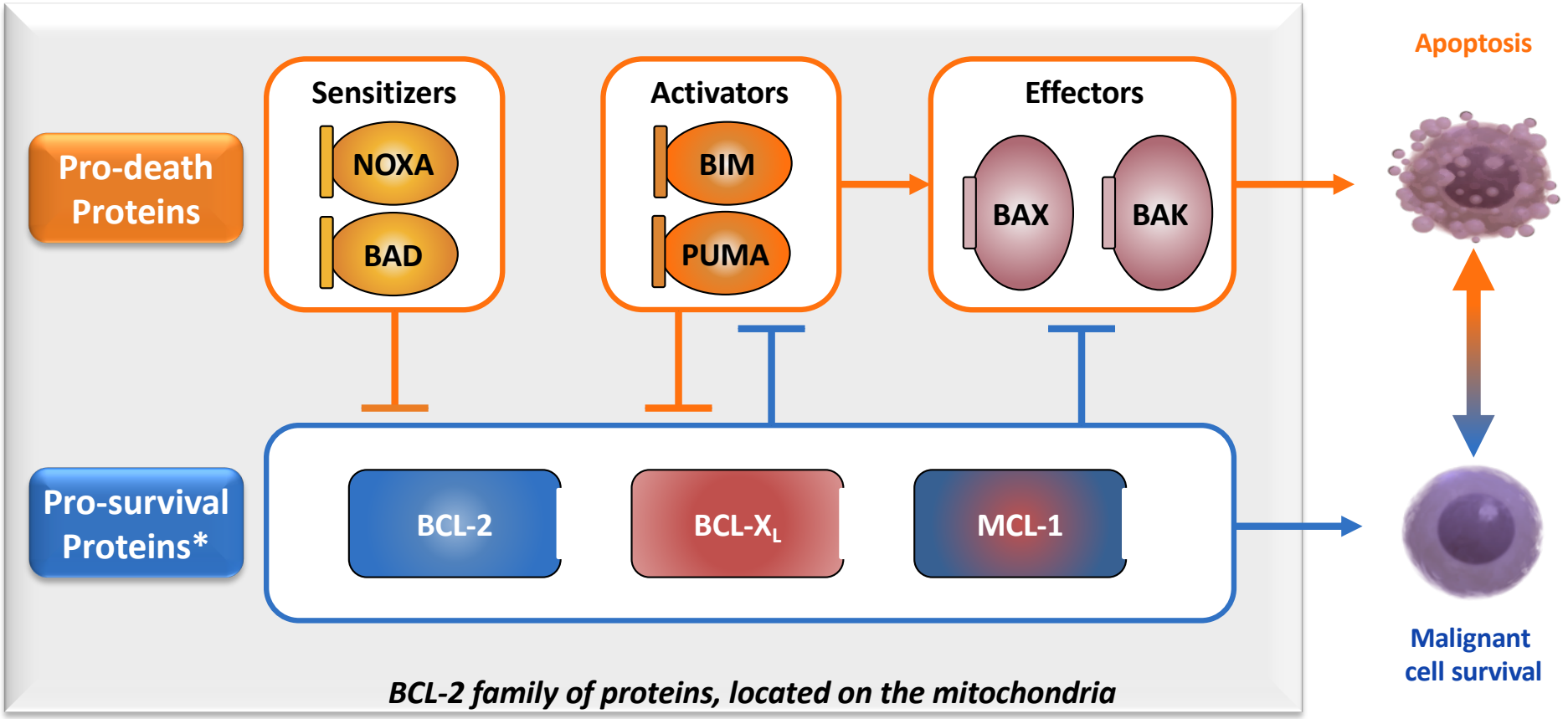
BCL2 family proteins

- Bcl2 was the first discovered regulator of apoptosis when it was found as translocated in patients suffering from follicular B cell lymphoma, 30 years ago.
- Nineteen proteins of the human 'Bcl-2 family' have been described.
- Seven members of this family (Bcl-2, Bcl-XL, Mcl-1, Bcl-B, Bcl-w and A1/Bfl1) are anti-apoptotic proteins while the rest (Bax, Bak, Bok, Bim, Bmf, Puma, Noxa, Bad, Bid, Bcl-XS, BiNP3 and Hrk) have a pro-apoptotic function.
- The balance between protein anti-apoptotic and pro-apoptotic proteins defines the fate of cell

Vela Current opinion in Pharmacology 2015, 23:74-81
Hanahan D & Weinberg RA. Cell 2011; 144:646–674;
Sochalska FEBS J. 2015 March ; 282(5): 834–849.

Apoptosis Is Regulated by the BCL-2 Family of Proteins

BCL-2 family proteins include both pro-survival (anti-apoptotic) and pro-death (pro-apoptotic) proteins with opposing functions[1–4]

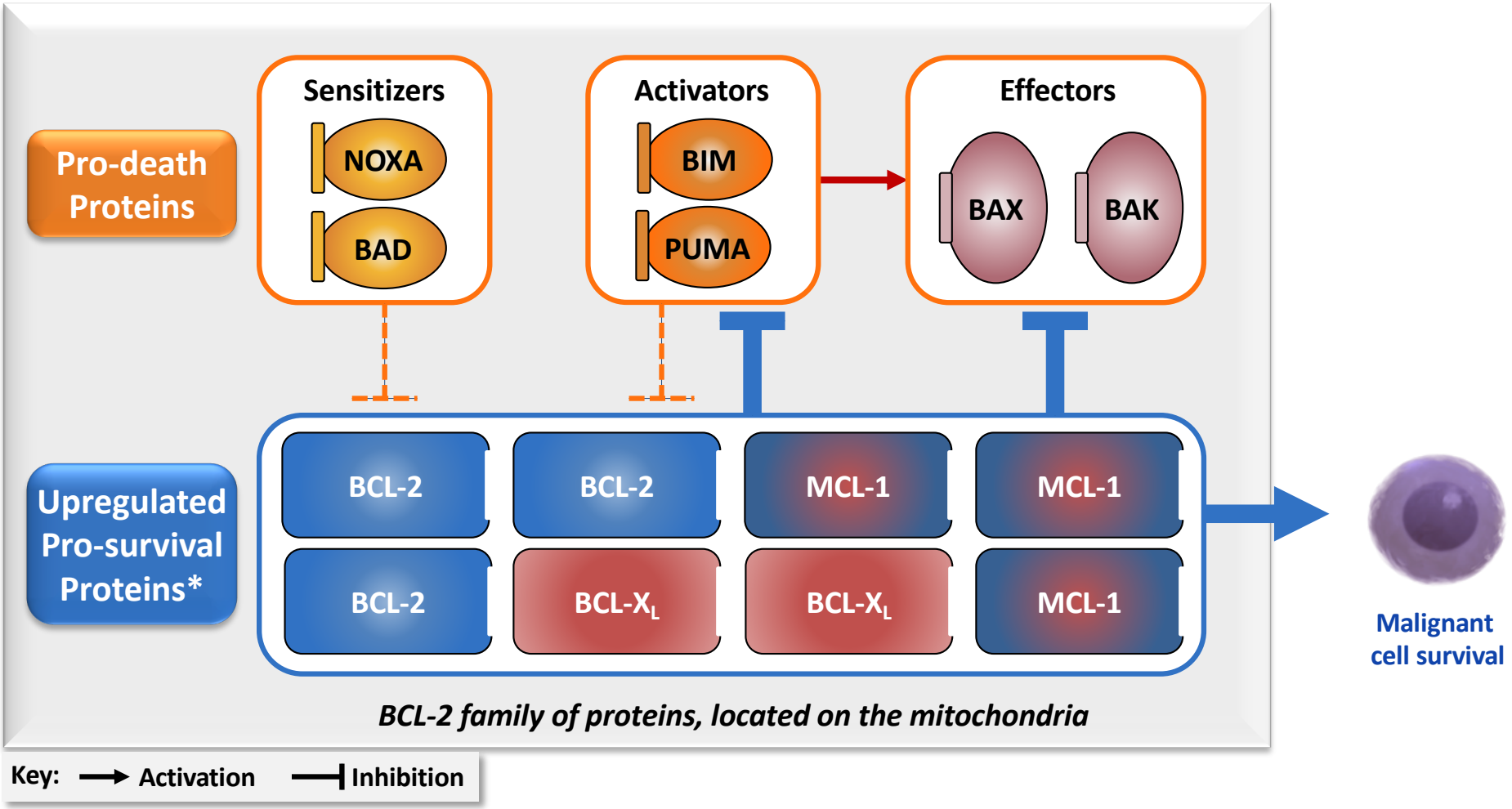


* Also includes: BCL-w and BFL-1/BCL2-A1.
BCL-2, B-cell lymphoma 2.

1. Plati J, *et al. Integr Biol (Camb)* 2011; **3:**279–296; 2. Czabotar PE, *et al. Nat Rev Mol Cell Biol* 2014; **15:**49–63; 3. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393; 4. Valentin R, *et al. Blood* 2018; **132:**1248–1264.

Malignant Cells Can Evade Apoptosis by Upregulating BCL-2 and Other Pro-survival Proteins

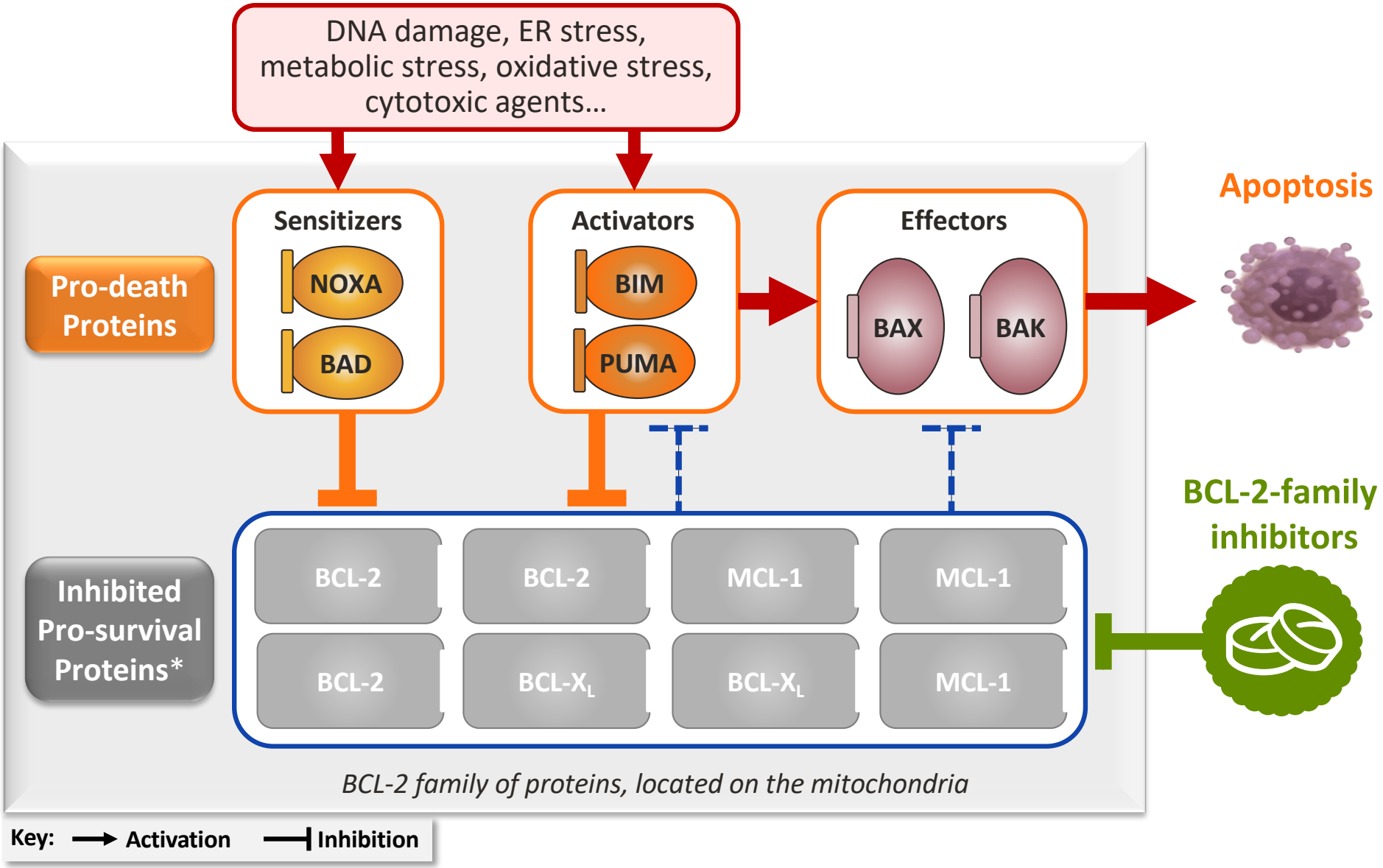
Malignant cells often evade apoptosis by upregulation of pro-survival proteins, such as BCL-2, MCL-1, and BCL-X_L



* Also includes: BCL-w and BFL-1/BCL2-A1.

1. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393;
2. Czabotar PE, *et al. Nat Rev Mol Cell Biol* 2014; **15:**49–63; 3. Adams JM & Cory S. *Oncogene* 2007; **26:**1324–1337;
4. Letai A, *et al. Cancer Cell* 2002; **2:**183–192; 5. Certo M, *et al. Cancer Cell* 2006; **9:**351–365.

Agents that Inhibit Pro-survival Proteins Can Promote Apoptosis in Malignant Cells



* Also includes: BCL-w and BFL-1/BCL2-A1.

1. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393;
2. Czabotar PE, *et al. Nat Rev Mol Cell Biol* 2014; **15:**49–63; 3. Adams JM & Cory S. *Oncogene* 2007; **26:**1324–1337;
4. Letai A, *et al. Cancer Cell* 2002; **2:**183–192; 5. Certo M, *et al. Cancer Cell* 2006; **9:**351–365.

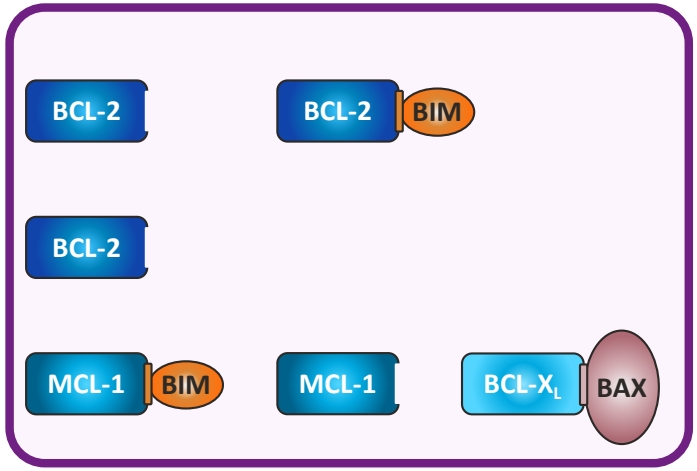
Malignant Cells with High Dependence on the BCL-2 Protein for Survival Are "Primed for Death"

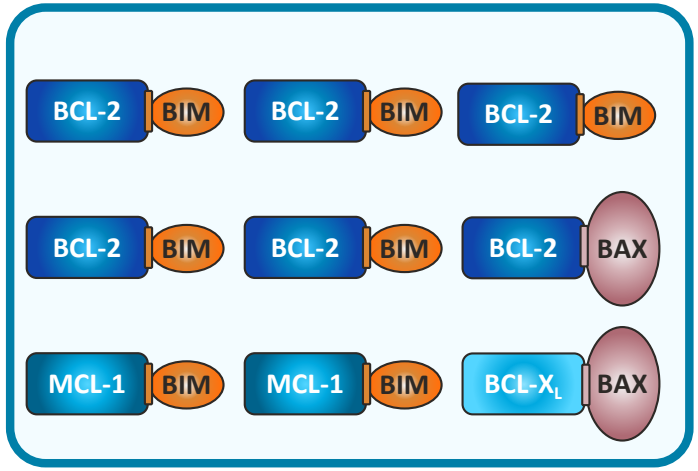
Unprimed healthy cell: High apoptotic threshold

- **Low level of sequestered pro-death proteins**
- **Released pro-death proteins may be re-captured by available pro-survival proteins and may not be sufficient to trigger apoptosis**

Primed malignant cell: Low apoptotic threshold

- **High levels of pro-survival proteins and high levels of sequestered pro-death proteins**
- **Sufficient release of pro-death proteins is likely to initiate apoptosis**
- **"BCL-2 primed": primed due to high levels of BCL-2 relative to other pro-survival proteins**





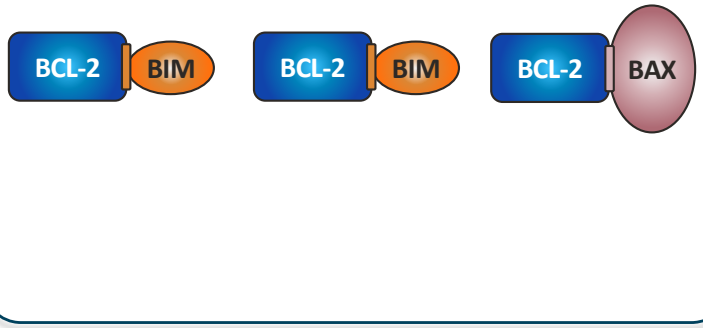
Key:

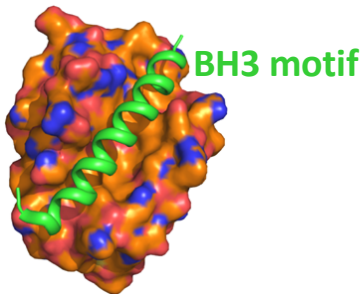
BCL-2 BIM Bound BIM–BCL-2

Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393;
Potter DS & Letai A. *Cold Spring Harb Symp Quant Biol* 2016; **81:**131–140.

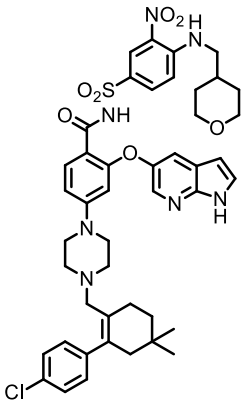
Venetoclax Is a Highly Selective, Potent, Oral BCL-2 Inhibitor Designed to Induce Apoptosis in Malignant Cells

Pro-survival proteins (e.g. BCL-2) sequester pro-death proteins by binding to their BH3 motifs[1,2]

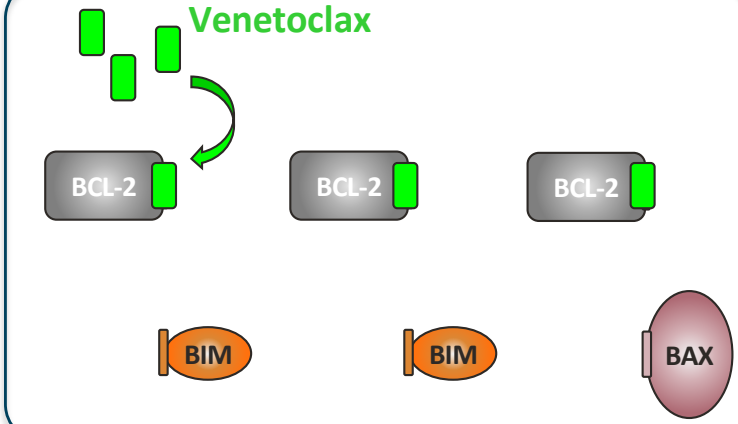


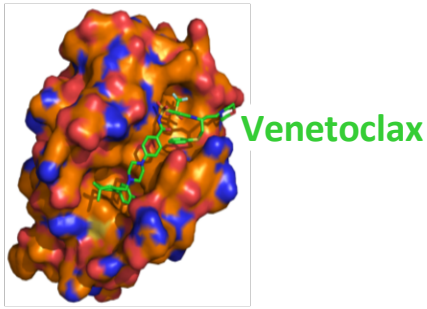


Venetoclax[3]



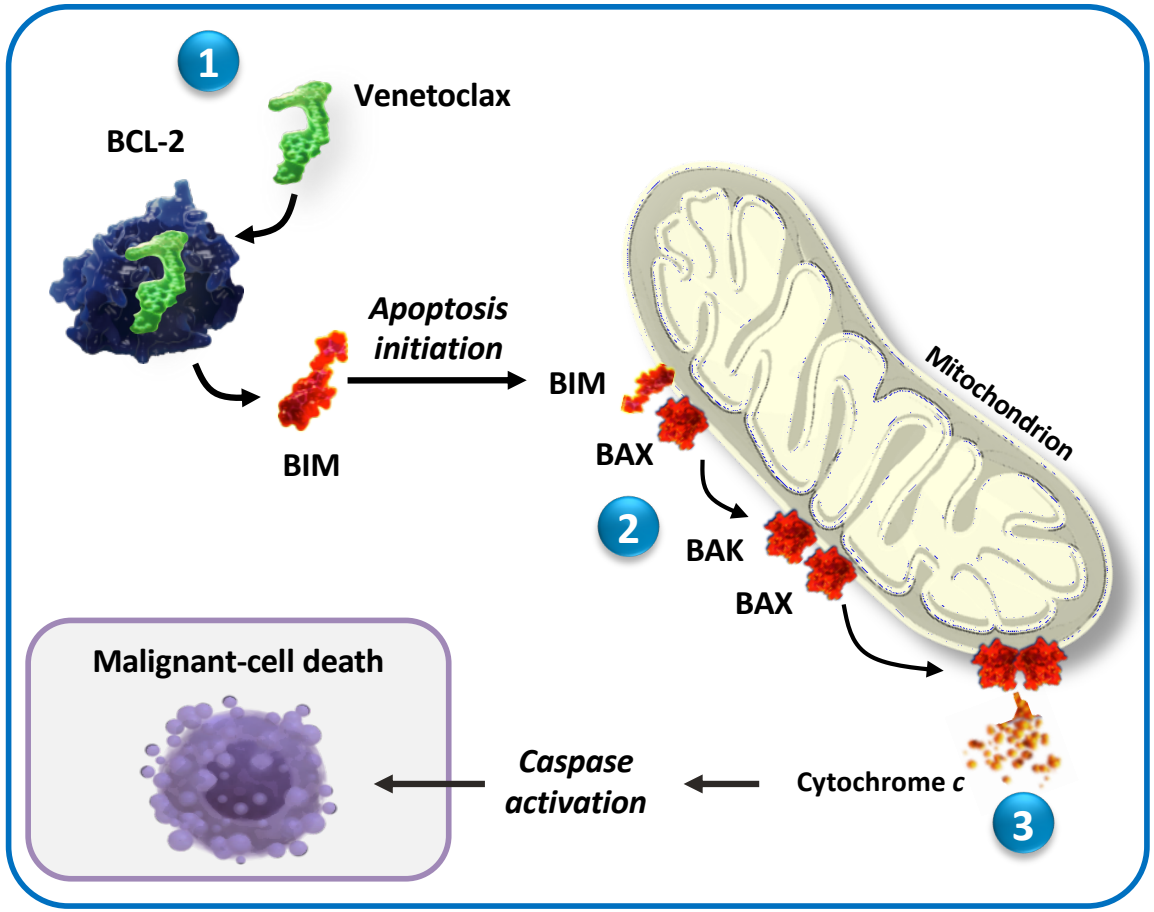
Venetoclax competes for binding to pro-survival proteins, freeing pro-death proteins[3–5]





1. Plati J, *et al. Integr Biol (Camb)* 2011; **3:**279–296; 2. Czabotar PE, *et al. Nat Rev Mol Cell Biol* 2014; **15:**49–63; 3. Souers AJ, *et al. Nat Med* 2013; **19:**202–208 (incl. suppl.); 4. Oltersdorf T, *et al. Nature* 2005; **435:**677–681; 5. Tse C, *et al. Cancer Res* 2008; **68:**3421–3428.

Venetoclax Induces Apoptosis in Malignant Cells

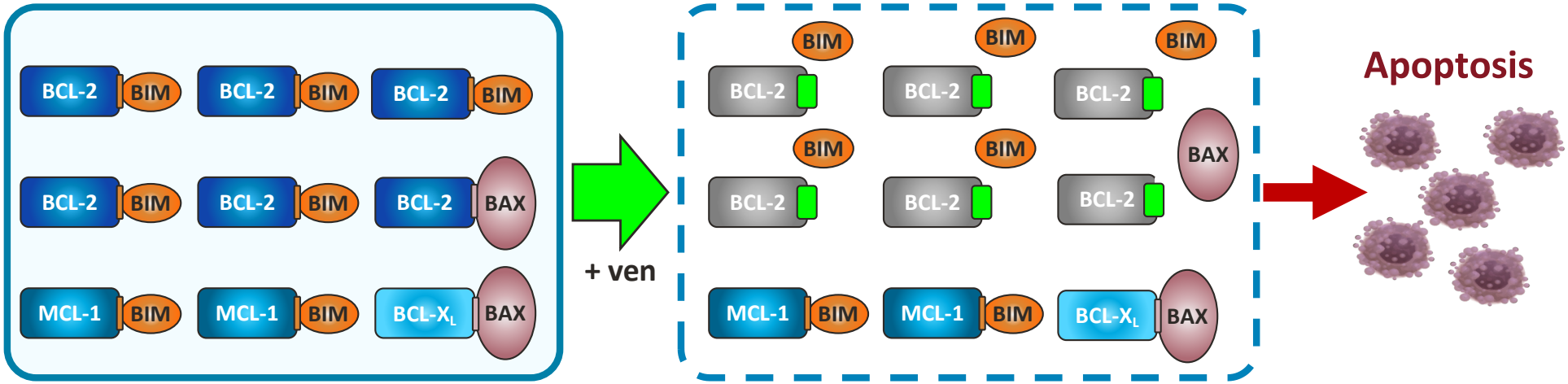


1. **Venetoclax binds to BCL-2 and frees pro-death proteins (BIM, BAX)**
2. **The freed pro-death proteins interact and initiate an apoptotic cascade, which results in formation of a pore in the outer mitochondrial membrane**
3. **Cytochrome *c* is released from mitochondria and activates caspases, which dismantle the cell, resulting in malignant-cell death (apoptosis)**

1. Letai A, *et al. Cancer Cell* 2002; **2:**183–192; 2. Adams JM & Cory S. *Oncogene* 2007; **26:**1324–1337;
3. Souers AJ, *et al. Nat Med* 2013; **19:**202–208; 4. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393.

Malignant Cells with High Dependence on the BCL-2 Protein for Survival Are Inherently Sensitive to Venetoclax

Highly dependent on BCL-2 (increased levels of BCL-2 relative to other pro-survival proteins, including MCL-1 and BCL-X_L)



Inhibition of BCL-2 can free enough BIM and BAX to trigger apoptosis

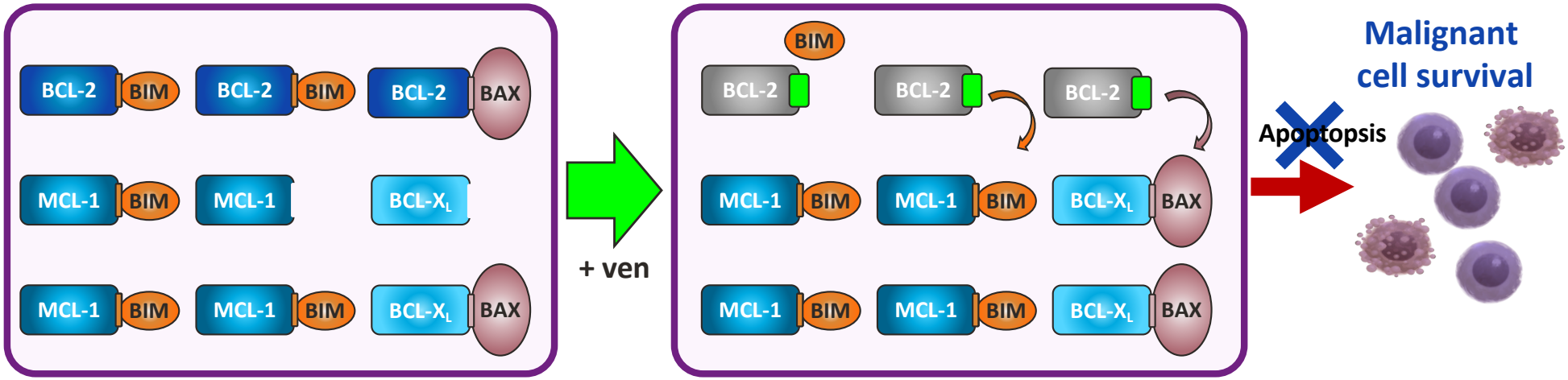
Key:

- Bound BIM–BCL-2
- Free BIM & inactivated BCL-2
- Venetoclax

1. Certo M, *et al. Cancer Cell* 2006; **9:**351–365; 2. Souers AJ, *et al. Nat Med* 2013; **19:**202–208 (incl. suppl.); 3. Punnoose EA, *et al. Mol Cancer Ther* 2016; **15:**1132–1144; 4. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393.

Malignant Cells That Are Co-dependent on Other Pro-survival Proteins May Be Less Sensitive to BCL-2 Inhibition Alone

Co-dependent on pro-survival proteins (increased levels of other pro-survival proteins, MCL-1 and BCL-X_L)



Inhibition of BCL-2 <u>may not</u> free enough BIM and BAX to trigger apoptosis in <u>all malignant cells</u>

Key:

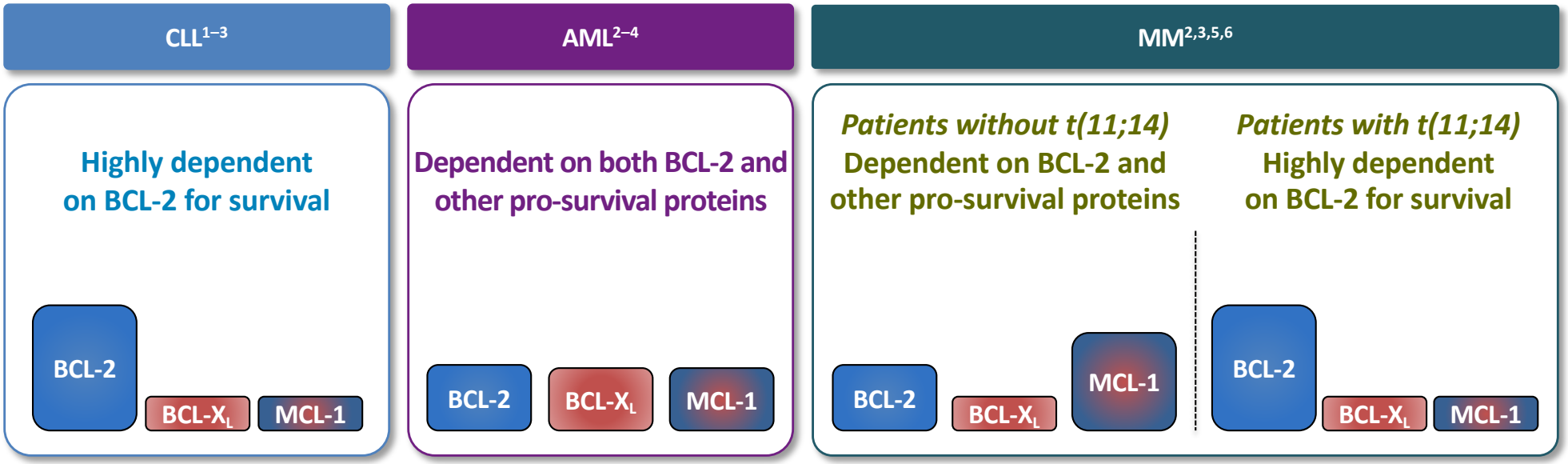
- Bound BIM–BCL-2
- Free BIM & inactivated BCL-2
- Venetoclax

Leverson JD, *et al. Cancer Discov* 2017; **7**:1376–1393.

Patterns of expression of BCL2 family in selected lymphoid malignancies

	Major	Level of bcl2 expression	Variability	Comment on mechanism
CLL	BCL2> MCL>> BCLxL	High	Some variability but always high	BCL2: loss of repression by miRNA15/16 MCL1 and BCLxL induced by CD40 ligation and microenviromental stimuli
Follicular Lymphoma	BCL2, MCL1, BCLxL	High	Rare to not be expressed	T(14;18) leads to constitutive expression. CD40L stimulates BCLxL expression MCL1 in centroblasts
DLBCL	BCL2> MCL1	High GC type low in many ABC type	High where MYC driven MCL1>BCL2	Varies including gene apmplification t(14;18) in double hit lymphoma, conseuqences of MYC dysregualtion
MCL	BCL2> MCL1	High	Minor	Consequence of cyclin D1 dysregualtion MCL1 high in blastoid
Myeloma	BCL2, MCL1, BCLxL	High Especially t(11;14)	Moderate	BCL2 consequence of cyclin dysregulation MCL1 constitutively expressed in plasma cells BCLXL increased through microenviroment stimulation
ALL	BCL2, MCL1, BCLxL	variable	Significant	Appears to mimic expression pattern of precursor cell Patterned by oncogenic driver

Malignant Cell Dependence on BCL-2 for Survival Has Been Shown across Several Hematologic Malignancies



Size of rectangles indicates relative dependency on specific protein for survival

Malignant cells' dependence on pro-survival proteins makes the BCL-2 family members rational targets for anticancer therapies

1. Del Gaizo Moore V, *et al. J Clin Invest* 2007; **117:**112–121; 2. Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393;
3. Valentin R, *et al. Blood* 2018; **132:**1248–1264; 4. Pan R, *et al. Cancer Discov* 2014; **4:**362–375;
5. Touzeau C, *et al. Leukemia* 2018; **32:**1899–1907; 6. Gomez-Bougie P, *et al. Blood* 2018; **132:**2656–2669.

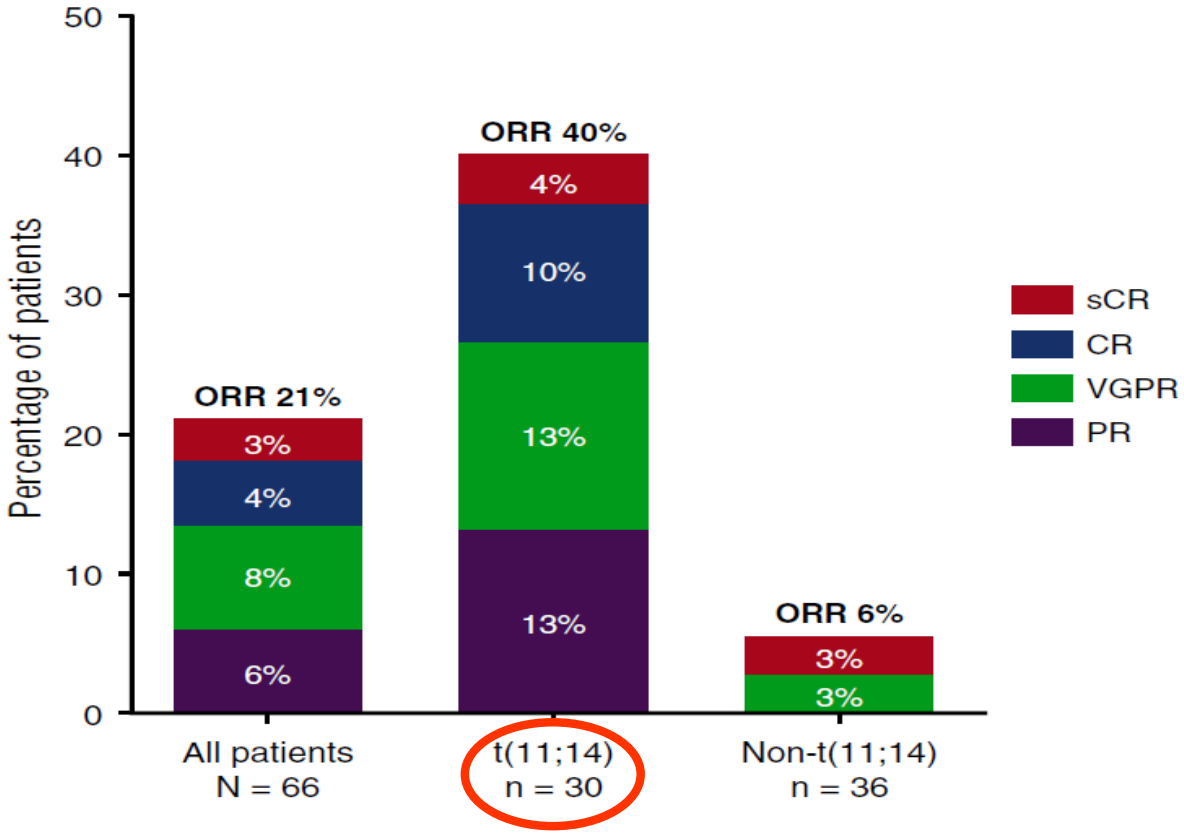
Rationale for anti-BCL2 agents use in multiple myeloma

- Gene-expression profiling (GEP) has shown that MM harboring t(11;14) has a specific pattern, characterized by CCND1 overexpression.
- GEP profile of t(11;14) plasma cells share similarities with GEP profile of lymphoma cells.
- Interestingly, t(11;14) plasma cells frequently express CD20, unlike all other myeloma subgroups.
- Plasma cells harboring t(11;14) are associated with increased dependency upon BCL2 for myeloma cell survival.

Bersagel et al. JCO 2005
Zhan et al, Blood 2006
Cleyen et al, Blood 2018

Venetoclax for t(11;14) MM

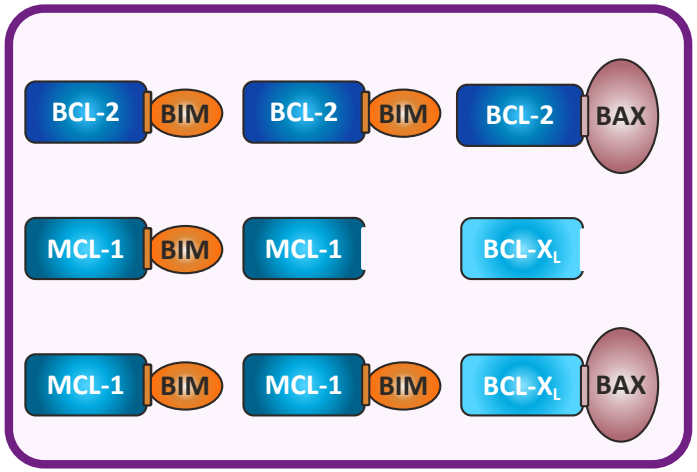
ORR rate by t(11;14) status



Kumar et al. Blood 2017

Venetoclax Can Combine with Agents That Increase BCL-2 Dependency or Target Other Pro-survival Proteins

Co-dependent on pro-survival proteins (increased levels of other pro-survival proteins, MCL-1 and BCL-X_L)



+ ven

+ other agents

Sensitivity to BCL-2 inhibition can be promoted by increasing BCL-2 dependency (upregulation of BIM and BCL-2)*

and/or

Sensitivity to BCL-2 inhibition can be promoted by directly or indirectly downregulating or neutralizing other pro-survival proteins†

Key:

Bound BIM–BCL-2

Free BIM & inactivated BCL-2

Venetoclax

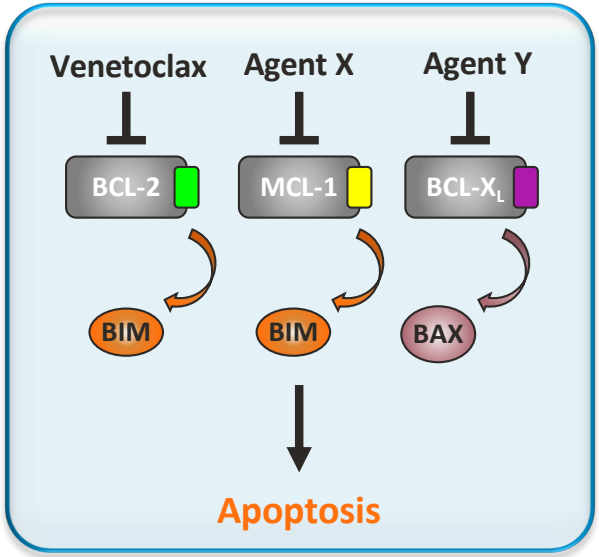
* e.g. dexamethasone; † e.g. proteasome inhibitors, HMAs, or LDAC.
HMA, hypomethylating agent; LDAC, low-dose cytarabine.

Leverson JD, *et al. Cancer Discov* 2017; **7**:1376–1393.

Venetoclax Can Also Combine with Agents Targeting Apoptotic and/or Non-apoptotic Complementary MoAs

Overlapping MoAs

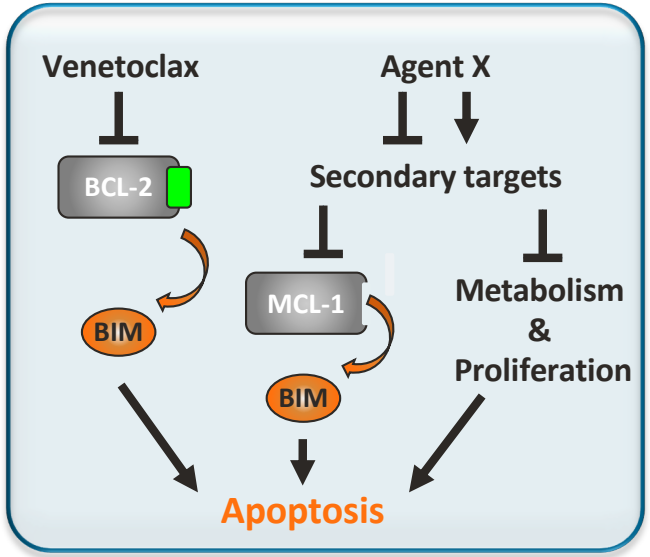
Direct intrinsic apoptosis



- MCL-1 inhibitors (S63845)
- BCL-X$_L$ inhibitors (navitoclax)

Partially Overlapping MoAs

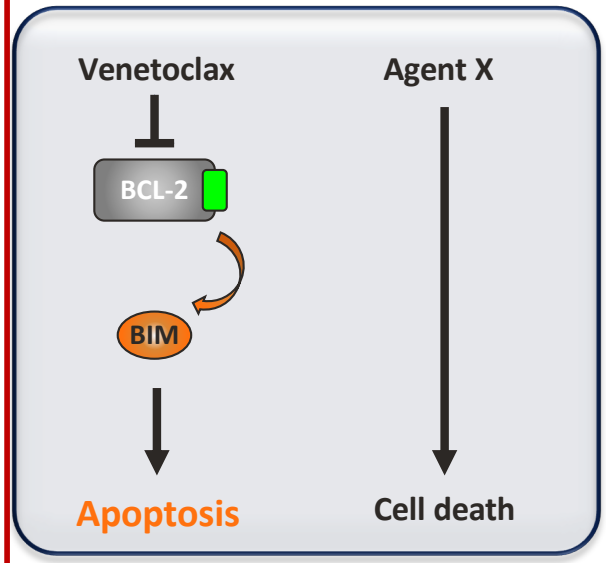
Indirect apoptosis + pleiotropic effect



- HMA, LDAC
- CDK9 inhibitors (alvocidib, dinaciclib)
- PIs (bortezomib, carfilzomib)
- FLT3 inhibitors (midostaurin, quizartinib, gilteritinib)
- MDM2 inhibitor (idasanutlin)

Distinct MoAs

Cell death other than apoptosis



- Anti-CD20 antibodies (rituximab, obinutuzumab)
- Anti-CD38 antibodies (daratumumab)
- IMiDs (lenalidomide)
- Checkpoint inhibitors (pembrolizumab, nivolumab)

Key: → Activation ⊣ Inhibition

CDK9, cyclin-dependent kinase 9; FLT3, FMS-like tyrosine kinase 3; IMiD, immunomodulatory imide drug; PI, proteasome inhibitor.

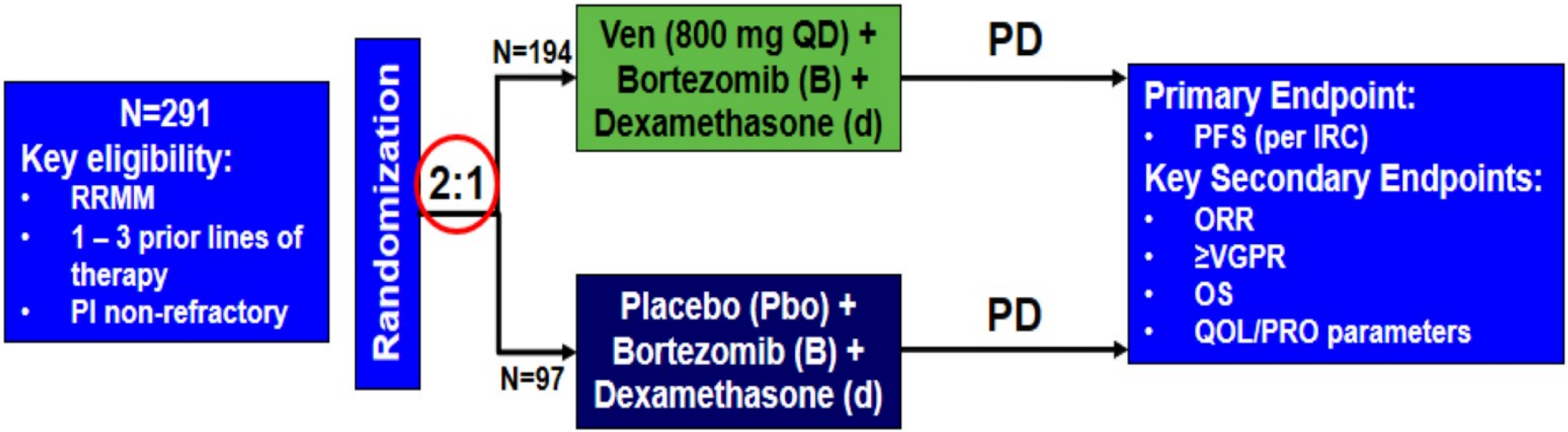
Leverson JD, *et al. Cancer Discov* 2017; **7**:1376–1393.

Rational combinations with the BCL2-selective inhibitor venetoclax

	Mechanism	Agent(s)	Key target
BCL2	Direct inhibitor, BH3 mimetic	Venetoclax S55746 (BCL201)	BCL2
BCL2 BIM	Elevated BCL2 priming–BIM stabilization/upregulation	UO126, selumetinib Ibrutinib Dexamethasone Tamoxifen	MEK BTK HR ER
$BCLX_L$	Direct inhibitor, BH3 mimetic	Navitoclax A-1331852 A-1155463 WEHI-539	$BCLX_L$
MCL1	Direct inhibitor, BH3 mimetic	S63845 A-1210477	MCL1
MCL1 NOXA	Reduced proteolysis of NOXA, a BH3-only protein which can bind/neutralize MCL1	Bortezomib	Proteasome
MCL1	Reduced RNA POLII-mediated transcriptional elongation of *MCL1* mRNA	Dinaciclib Alvocidib	CDK9
MCL1	Inhibition of cap-dependent translation of *MCL1*	AZD8055	mTORC1/2
	Unknown	Anthracyclines Hypomethylators	DNA/RNA DNA/RNA

Leverson JD, *et al. Cancer Discov* 2017; **7:**1376–1393.

BELLINI Study Design



Cycles 1 – 8: 21-day, Bortezomib 1.3 mg/m^2 Days 1, 4, 8, 11 and dexamethasone 20 mg Days 1, 2, 4, 5, 8, 9, 11, 12

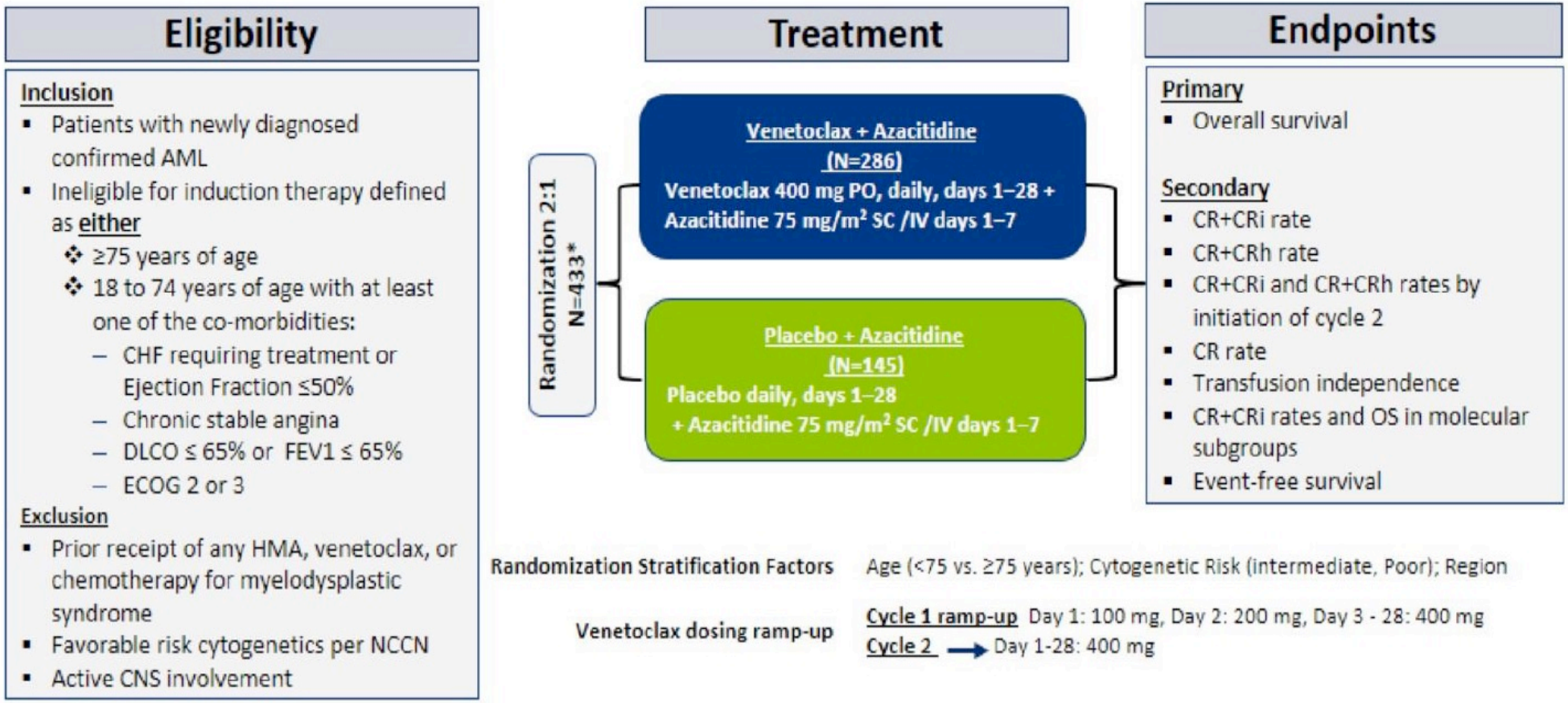
Cycles 9+: 35-day, Bortezomib 1.3 mg/m^2 Days 1, 8, 15, 22 and dexamethasone 20 mg Days 1, 2, 8, 9, 15, 16, 22, 23

Stratification factors	• Bortezomib sensitive vs naïve • Prior lines of therapy: 1 vs 2–3
Non-ranked secondary endpoints	PFS in BCL-2^{high} (IHC), DOR, TTP, MRD negativity rate, other PROs (GHS, fatigue)
Key subgroup analyses	t(11;14), high/standard-risk cytogenetics, and *BCL2* expression (gene expression)

DOR, duration of response; GHS, global health status; IHC, immunohistochemistry; MRD, minimal residual disease; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PI, proteasome inhibitor; PRO, patient reported outcome; QD, daily; QOL, quality of life; RRMM, relapsed/refractory multiple myeloma; TTP, time to progression; VGPR, very good partial response.

Kumar et al. ASH 2019

VIALE-A Study Design (NCT02993523)



Dinardo C et al, EHA 2020

Take Home Messages

- Malignant cell dependence on pro-survival proteins makes the BCL-2 family members rational targets for anticancer therapies
- Malignant cells with high dependence on the BCL-2 protein for survival (like CLL cells) are Inherently sensitive to BCL-2 inhibition with Venetoclax
- However, malignant cells that are co-dependent on other pro-survival proteins (like AML and MM cells) may be less sensitive to BCL-2 inhibition alone.
- For this reason, Venetoclax can combine with agents targeting apoptotic and/or non-apoptotic complementary mechanism of action in diseases like AML, MM and potentially also NHL