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EMILIA-ROMAGNA
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

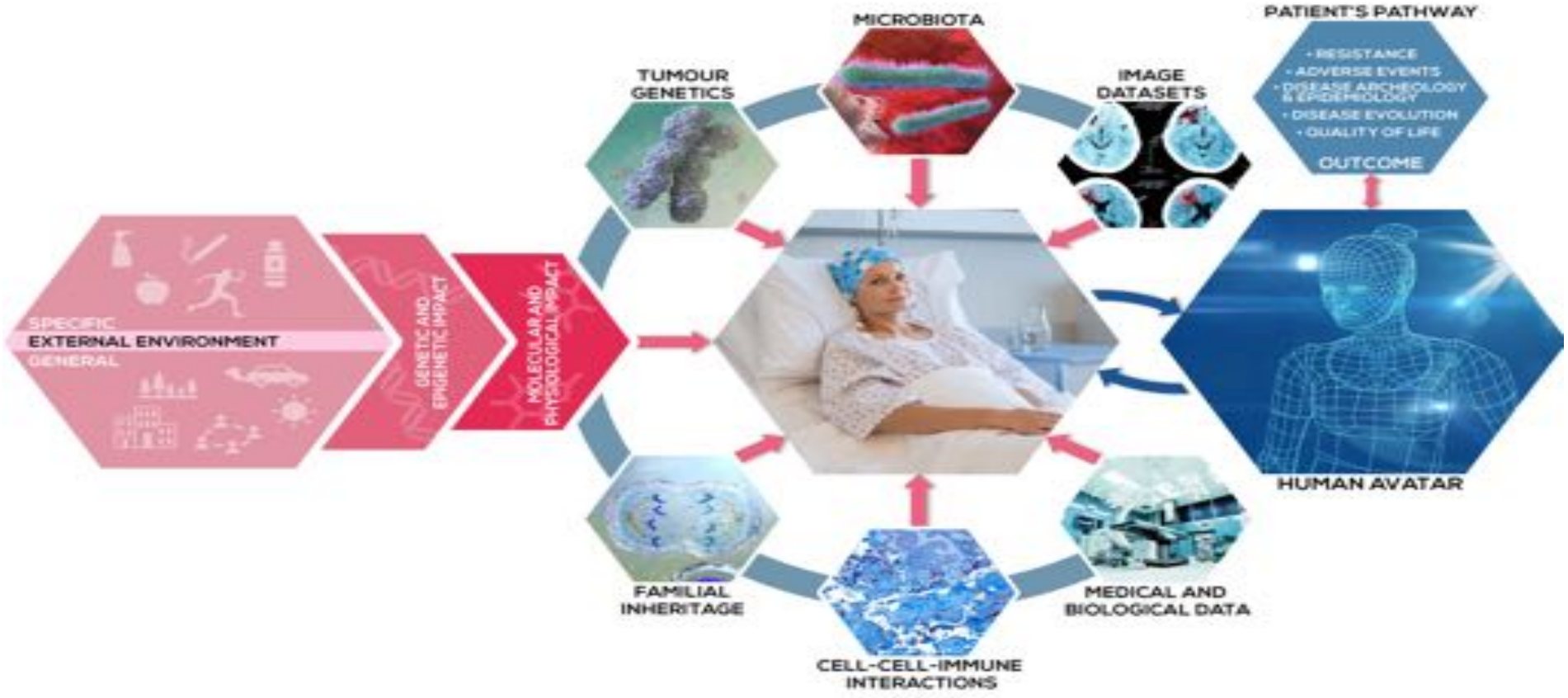
ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI

Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori IRST-IRCCS

Inibitori dell' apoptosi o molecular driven therapy

Prof. Giovanni Martinelli, MD, PhD
Scientific Director IRCCS della Romagna

Cancer Complexity in the 21st Century needs large databases and the support of artificial intelligence to produce and take advantage of the Human Avatar



Italian IRCCS networks

Institute of Medical Technologies and Sciences

Preventing the diseases, personalizing the treatment, improving the quality of life

IRCCS Cancer Network

ALLEANZA CONTRO IL CANCRO

IRCCS
Cardiology
Network

MEF via MoH: €55 Ml (2019-2024)

ARTICLE

DOI: 10.1038/s41467-018-06992-7 OPEN

Network integration of multi-tumour omics data suggests novel targeting strategies

Ítalo Faria do Valle[1,2], Giulia Menichetti[3], Giorgia Simonetti[4], Samantha Bruno[4], Isabella Zironi[1], Danielle Fernandes Durso[4,5], José C.M. Mombach[6], Giovanni Martinelli[4,7], Gastone Castellani[1] & Daniel Remondini[1]

Canonical translocation
with
TK or TF activation
ex. t (8;21) or inv(16)

TK activation
ex. FLT3 ITD +

Aneuploidy
or
p53 mut

Table 2 Common biological categories in gene signatures

	NF-κB signaling	Chromosomal instability	Ubiquitin-proteasome system
Cluster 1	*BTRC, CUL1, SRC, NFKBIA, NFKB1, NFKB2, REL, RELB, CHUK*	*CDC20, BUB1, MLFPIP, CENPC1, MIS12, PMF1, NDC80, RAD21, STAG1*	*BTRC, CUL1, PSMB9, PSMC2, PSMF1*
Cluster 2	*BTRC, SRC, NFKBIA, TNFRS10B, IL6R*	*MIS12, DSN1, MLFPIP, CENPC1, PLK1, NEDD1, TUBGCP5, TUBGCP6*	*BTRC, PSMB3, PSMC3*
Cluster 3	*FBXW11, AKT2, TNFR1A*	*CDC16, CDC27, NEDD1, TUBGCP5, KIF2B, KIF2C*	*FBXW11, PSMC3, PSMD9*

All signatures have genes that can be grouped into the following categories: NF-κB signaling, chromosomal instability and ubiquitin-proteasome system

Curable

Less Curable

MCL1 not degradable
uncurable

AML stratification based on molecular test

❖ **Prognosi molto favorevole:**

- *PML-RARα*, *CEBPA* double-mutated.

❖ **Prognosi favorevole:**

- *RUNX1-RUNX1T1*, *CBFB-MYH11*, *NPM1mut/FLT3-ITD-*

❖ **Prognosi intermedia:**

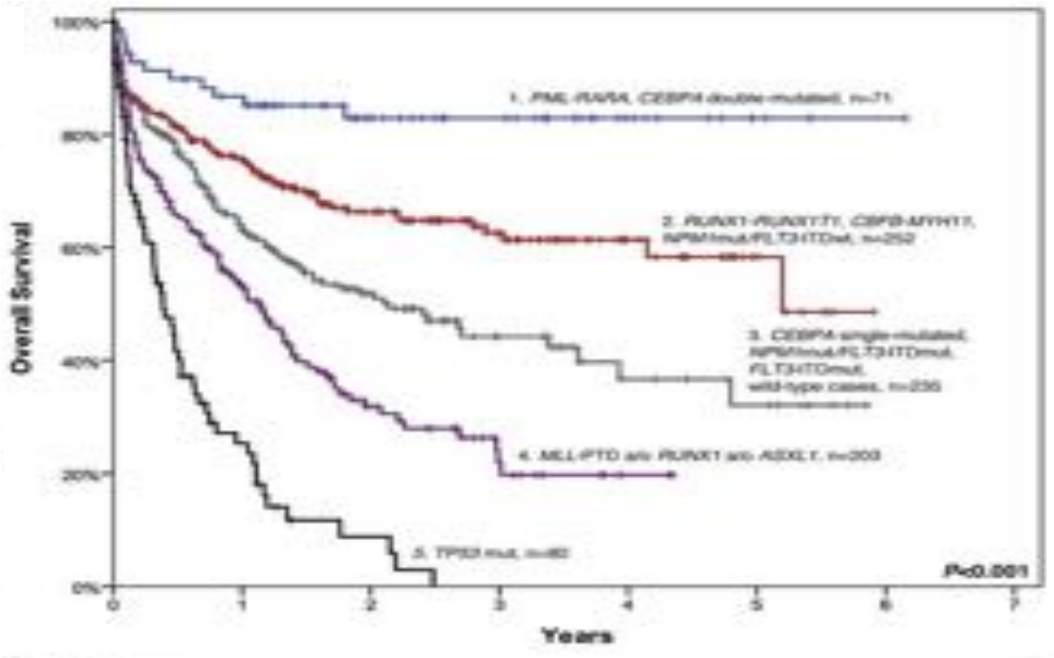
- *CEBPA* single-mutated, *FLT3-ITD+*, casi wild-type.

❖ **Prognosi sfavorevole:**

- Mutazioni in *MLL-PTD* e/o *RUNX1* e/o *ASXL1* .

❖ **Prognosi molto sfavorevole:**

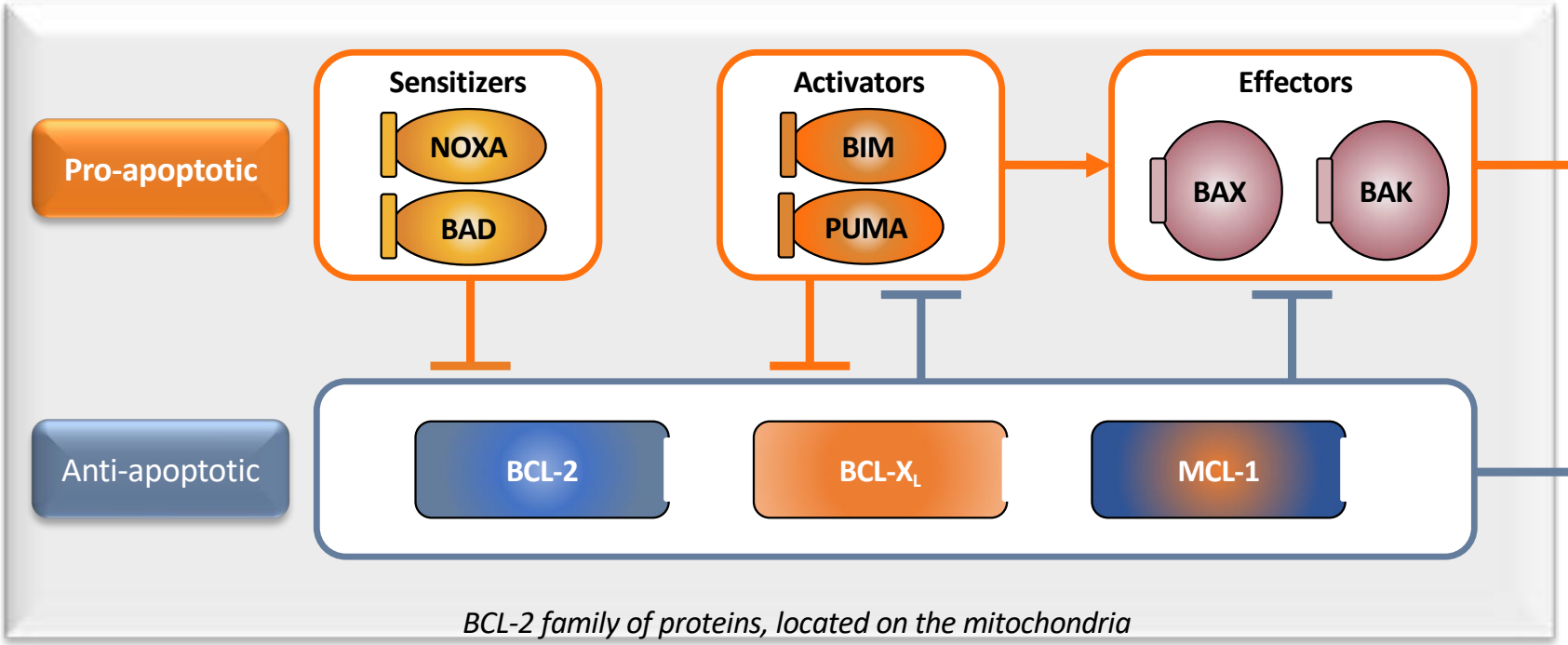
- Mutazioni in *TP53*.



Grossmann et al., Blood, 2012

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]

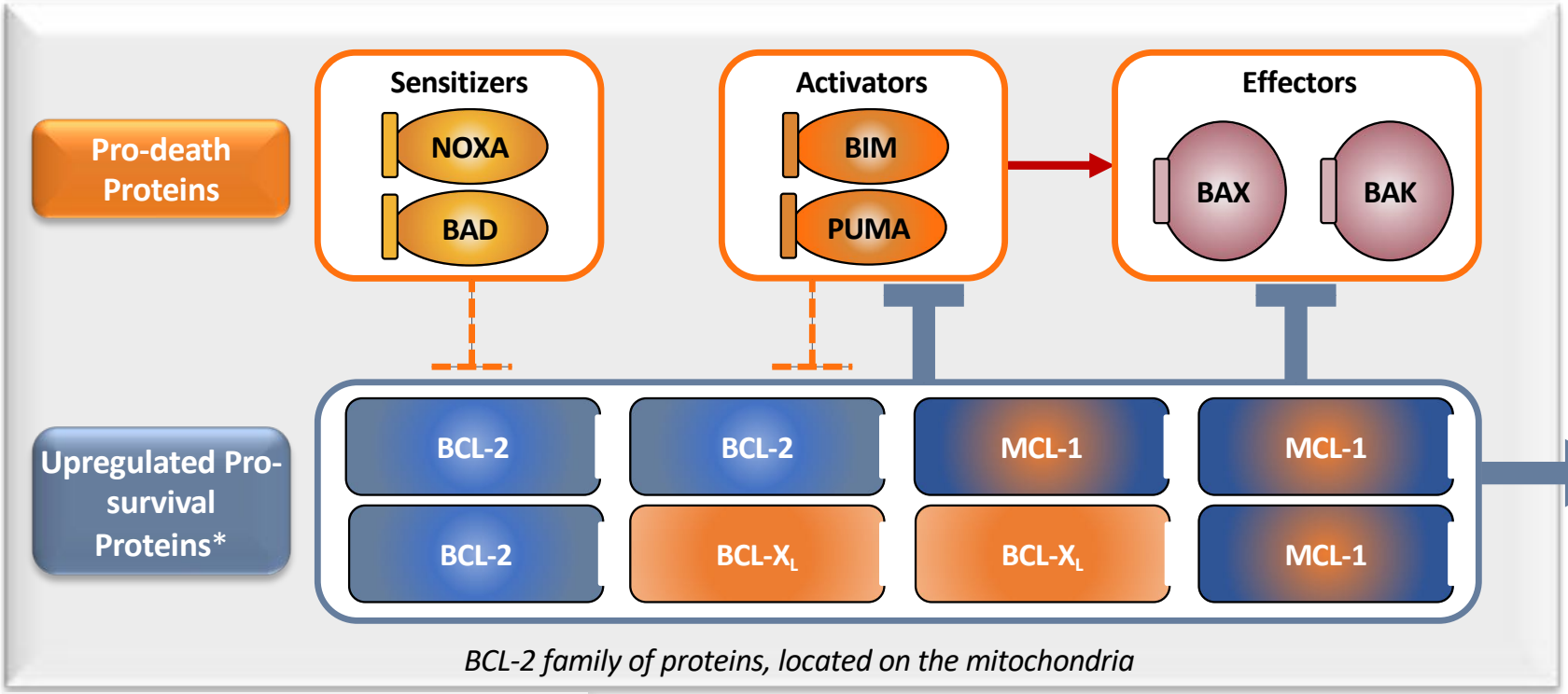


* Also includes: BCL-w and BFL-1/BCL2-A1.
BCL-2, B-cell lymphoma 2.

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



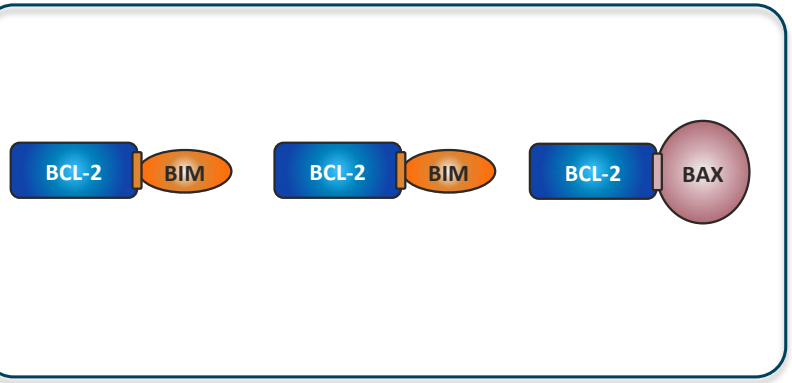
Key: → Activation ⊣ Inhibition

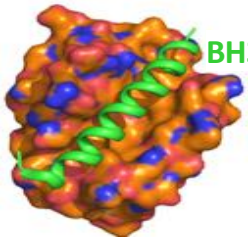
* Also includes: BCL-w and BFL-1/BCL2-A1.

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.

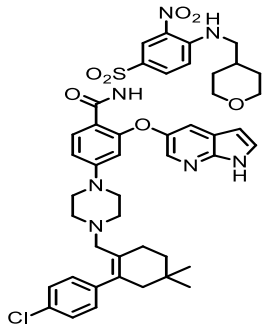
Venetoclax Is a Highly Selective, Potent, Oral BCL-2 Inhibitor Designed to Induce Apoptosis in Malignant Cells

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

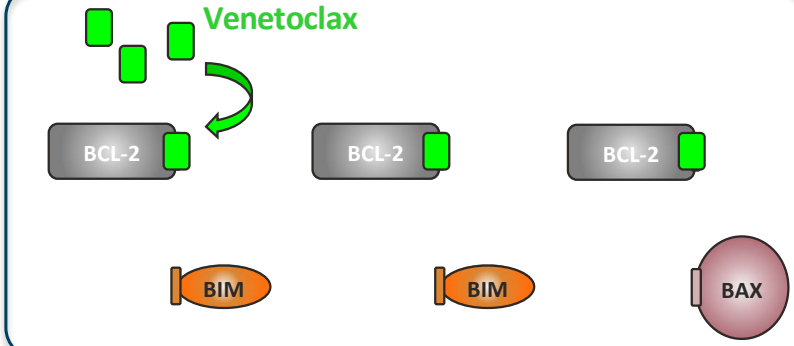


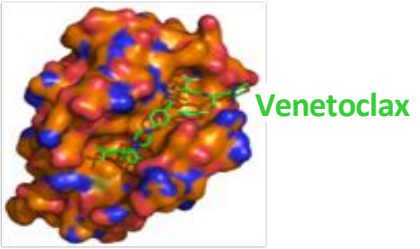


Venetoclax[3]



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 Has a High Selectivity for BCL-2, Which Correlates with Its Anti-tumor Activity

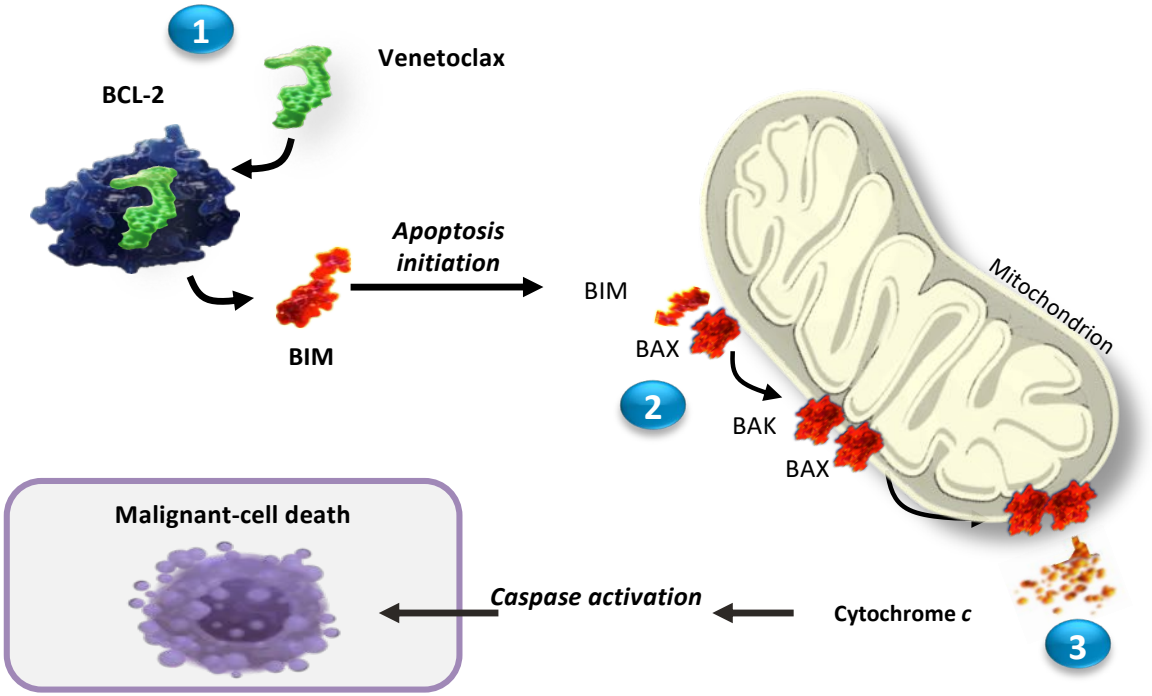
Venetoclax binding affinity by TR-FRET

Protein	K_i (nM)
BCL-2	**<0.0100**
BCL-X_L	48
MCL-1	>444
BCL-W	245

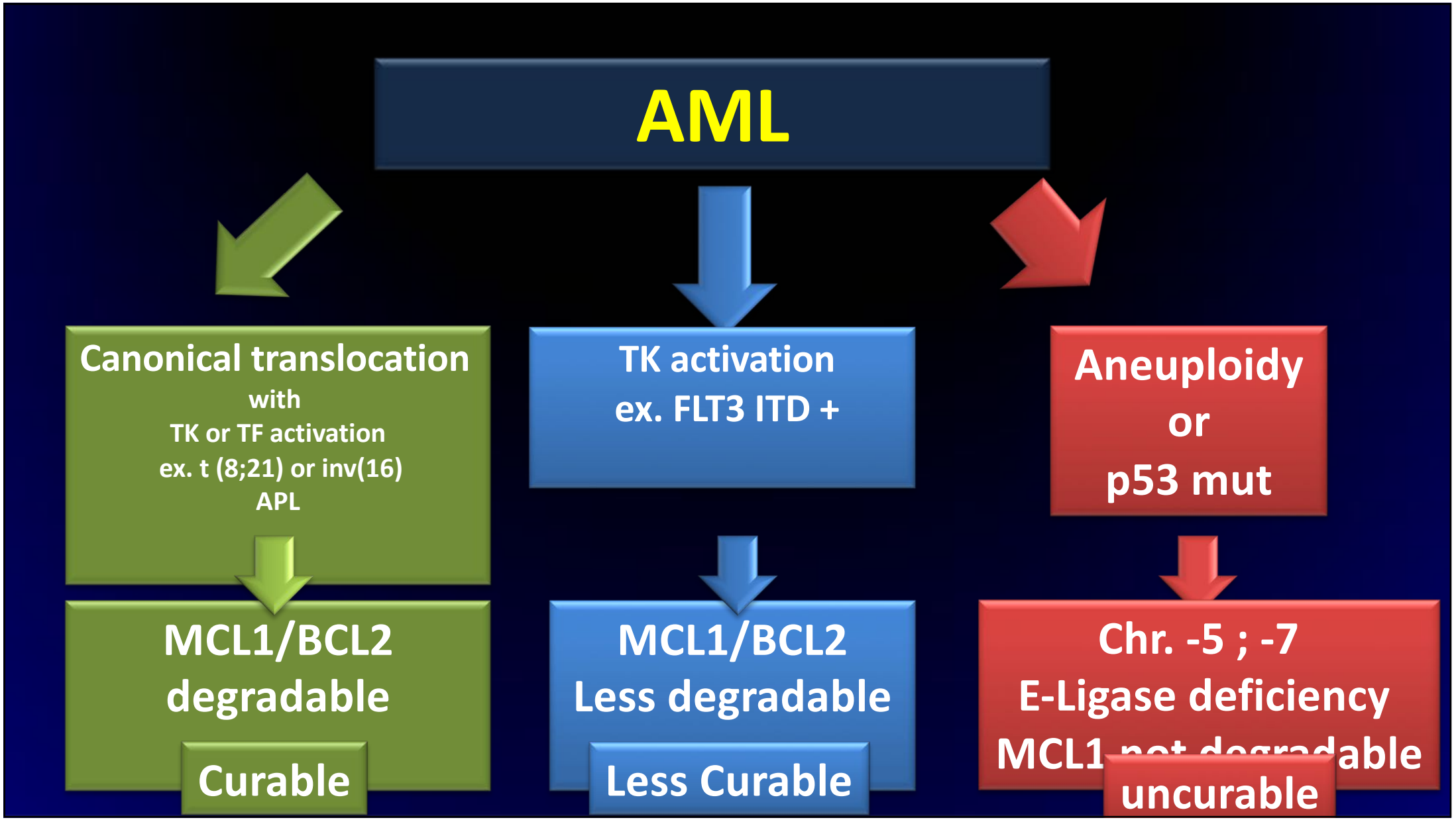
EC_{50}, half-maximal effective concentration; K_i, inhibition constant; TR-FRET, time-resolved fluorescence energy transfer.

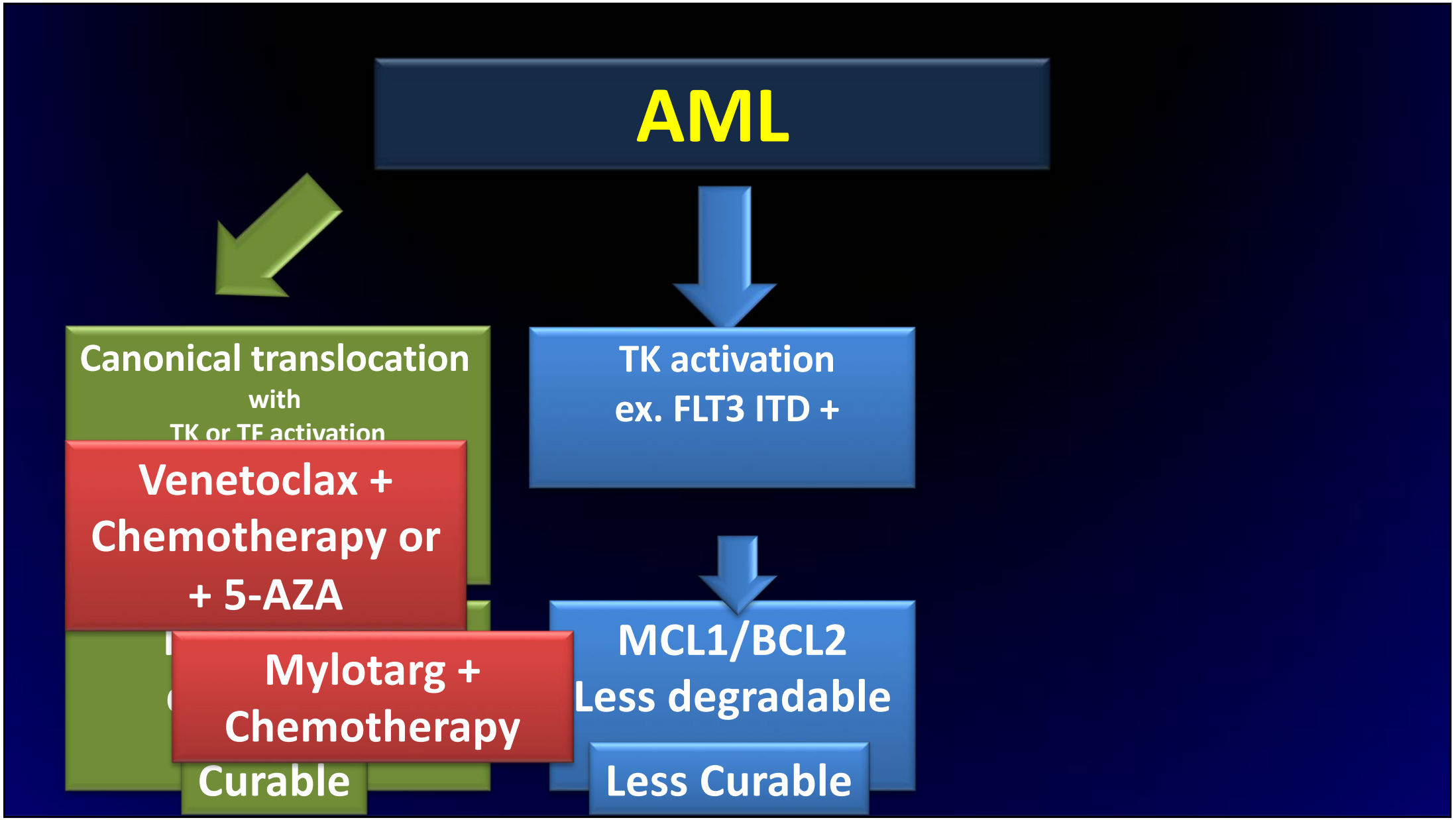
Souers AJ, *et al. Nat Med* 2013; **19:**202–208 (incl. suppl.).

Venetoclax Induces Apoptosis in Malignant Cells



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.


AML
Canonical translocation
with
TK or TF activation
ex. t (8;21) or inv(16)
APL
TK activation
ex. FLT3 ITD +
Aneuploidy
or
p53 mut
MCL1/BCL2
degradable
Curable
MCL1/BCL2
Less degradable
Less Curable
Chr. -5 ; -7
E-Ligase deficiency
MCL1 not degradable
uncurable


AML
Canonical translocation
with
TK or TF activation
Venetoclax +
Chemotherapy or
+ 5-AZA
Mylotarg +
Chemotherapy
Curable
TK activation
ex. FLT3 ITD +
MCL1/BCL2
Less degradable
Less Curable

Frontline Venetoclax + HMAs in Elderly AML Pts

- Open-label, nonrandomized, 2-arm, 2-stage, phase Ib study of BCL-2 inhibitor venetoclax

Pats with untreated AML, 65 yrs of age or older, adverse or intermediate-risk cytogenetics, ineligible for standard induction therapy (N = 34)

Safety, PK, dose finding

Venetoclax* + Decitabine
20 mg/m^2 Days 1-5, IV 28-day cyc
(n = 18)

Venetoclax* + Azacitidine
75 mg/m^2 Days 1-7, IV/SC 28-day cycle
(n = 16)

Expansion stage: safety and efficacy confirmation

Venetoclax + HMA
(n = 40)

84% CR

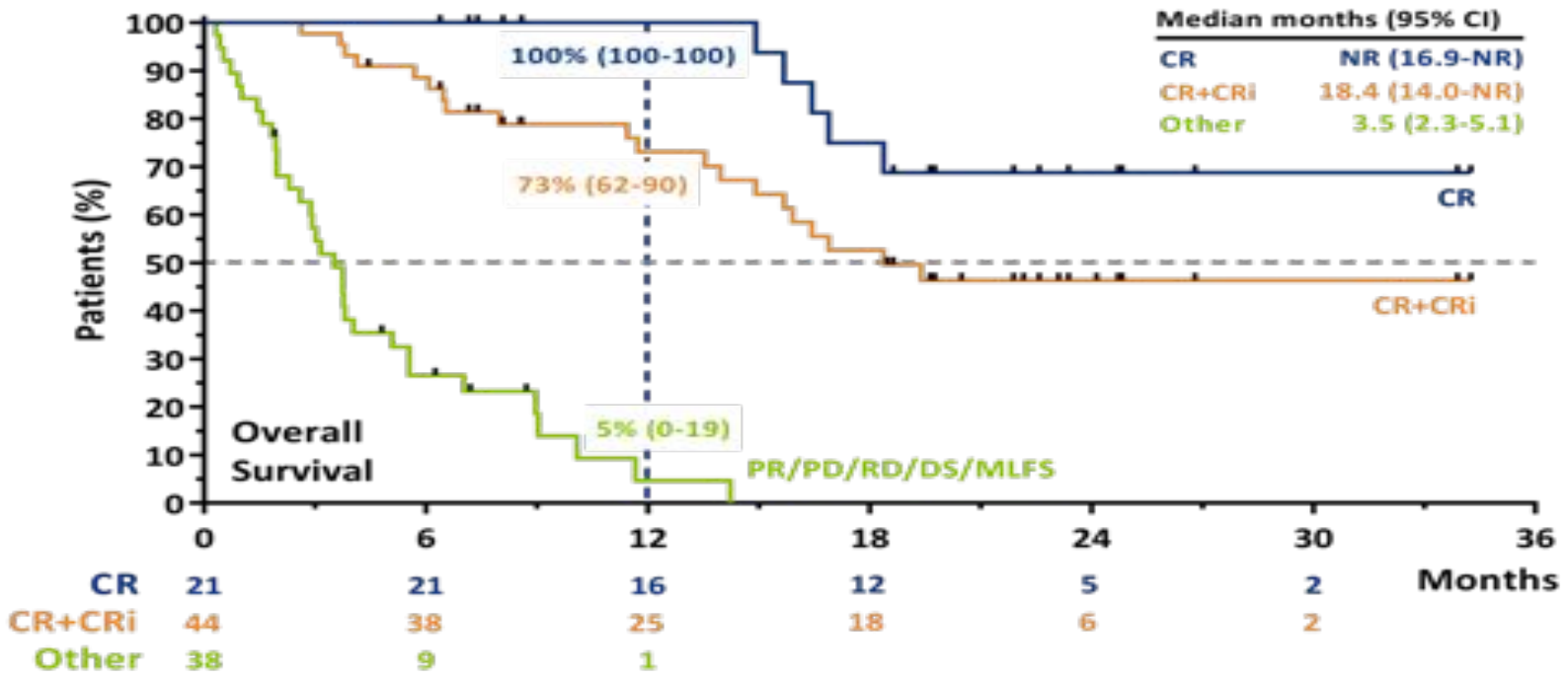
82% CR

*In each arm, 1 cohort received veneto
PO and 2 cohorts received 800 mg PO d 2-28 (cycle 1) and d 1-28 (following cycles) of 28-day cycle.

DiNardo C, et al. ASH 2015. Abstract 327.

ASH 2015

Overall Survival by Response



Venetoclax with LDAC induces durable responses in older patients with AML | ASH 2018

AVALON IRST204.04

ITALIAN OBSERVATIONAL STUDY OF PATIENTS WITH ACUTE MYELOID LEUKEMIA TREATED WITH SMALL MOLECULE INHIBITING BCL-2

PI – G. Martinelli/ E. Todisco/N. Fracchiolla

Disegno: Studio multicentrico osservazionale retrospettivo

Obiettivo: valutazione tossicità/efficacia trattamento con **Venetoclax** pazienti con AML

Sample size: 70 Pts, ~ 35 centri
Study duration: 2 yrs

Inclusion criteria:

- ☑ LAM Patients who received any anti-BCL2 immunoconjugate from 1 Jan 2015 to 1 Jan 2019 outside clinical
- ☑ Informed consent

Milestones	
Approvazione CEROM	10/04/2019
Attivazione primo centro	19/08/2019
Attivazione ultimo centro	Dicembre 2020

Take home massages

- **Venetoclax in combination with HMA results in a significant rate of CR even in high risk patients and TP53 mutated**

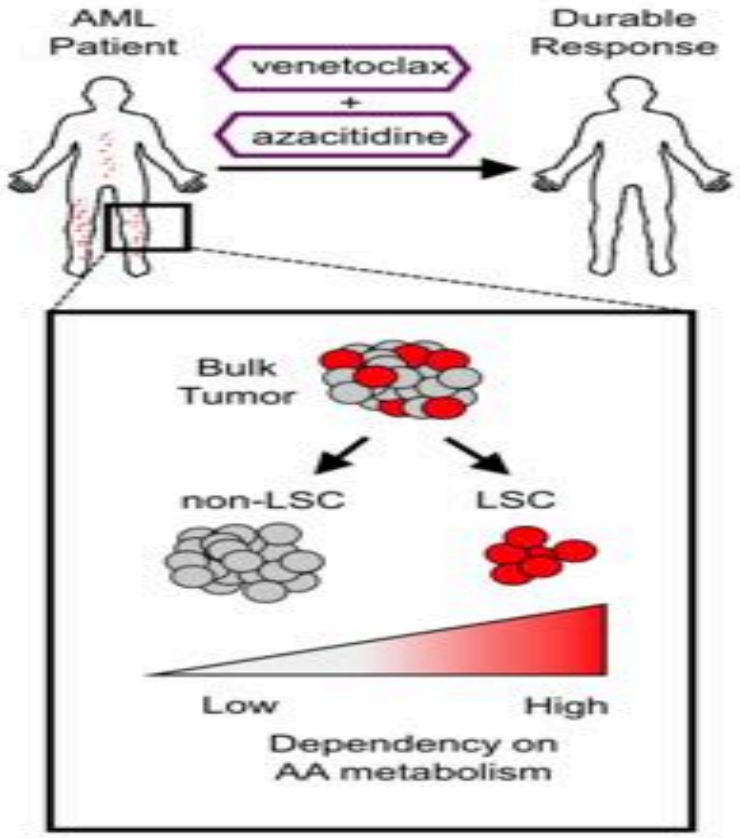
- Ven+ Comimetinib phase 1 completed and successful
- Ven + Idasanutlin completed and successful
- Ven + Gilteritinib completed and successful
- Ven + IMG CD123 + AZA ongoing

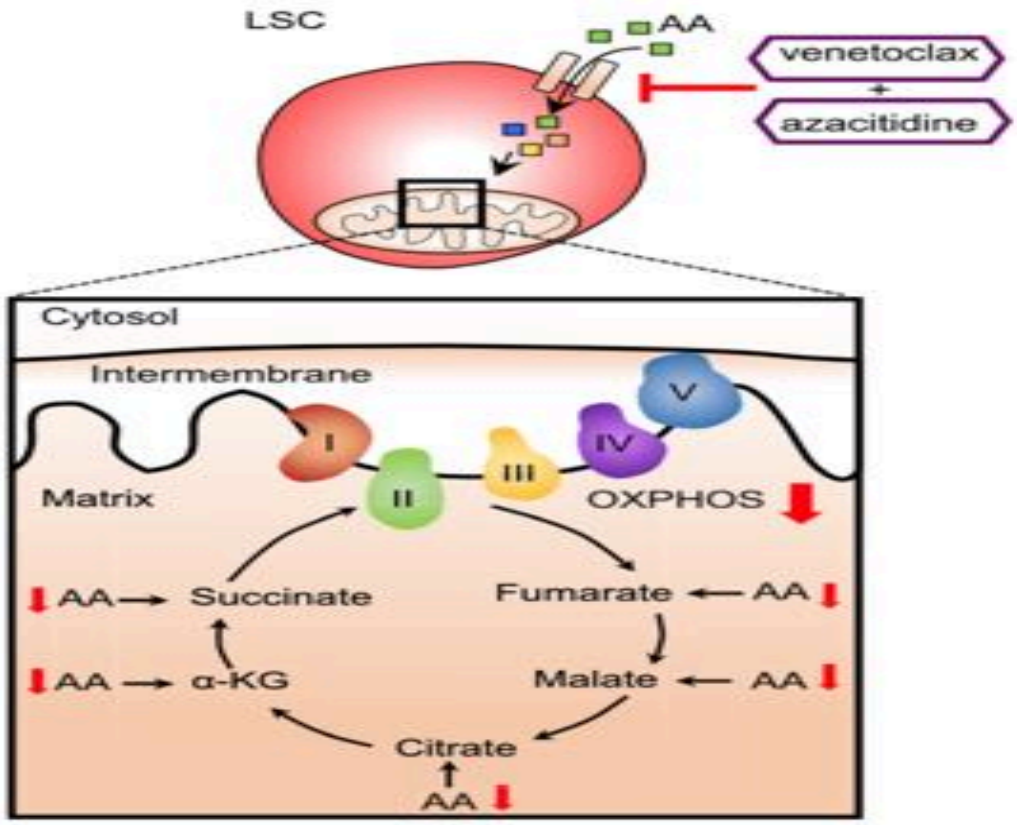
- Venetoclax demonstrated activity in R/R AML

- For patients relapsed after HMA the results are still poor

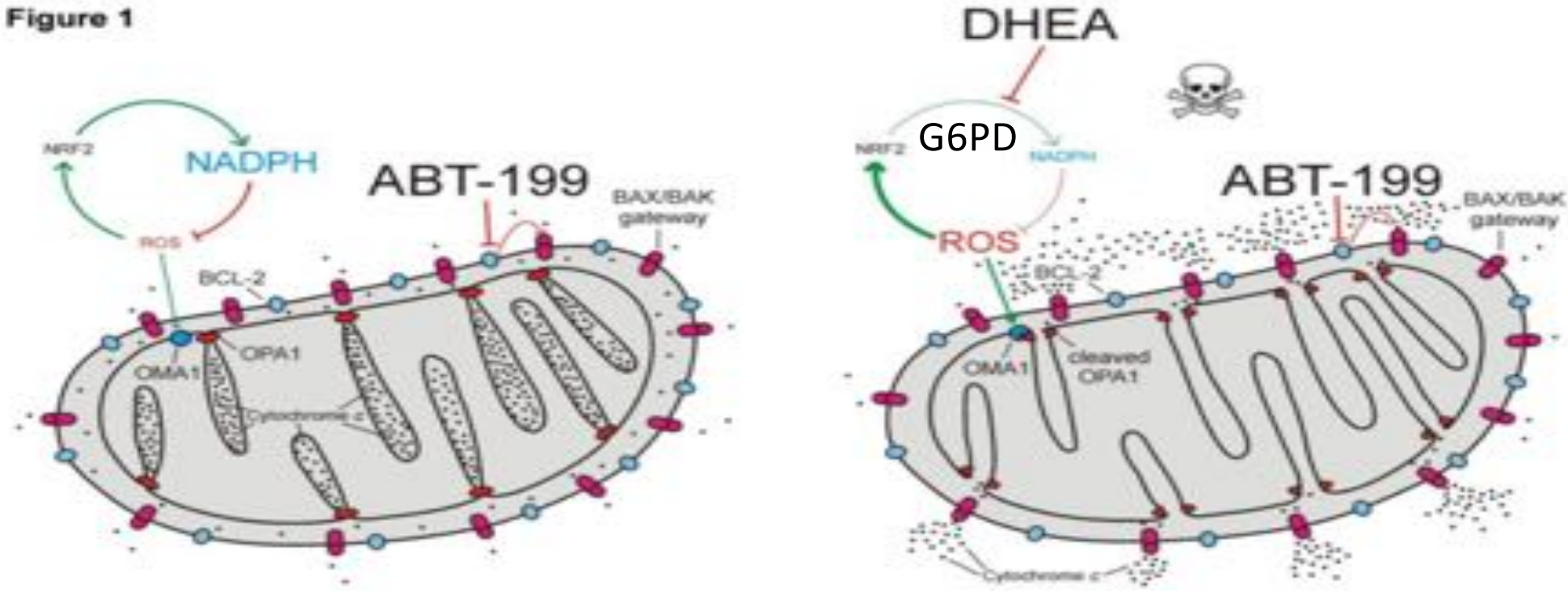
- There are new combinations on the horizon (venetoclax and FLT3 inhibitors)

Why venetoclax works ?
LSC dependency from AA metabolism and G6PD activity





Potentiating apoptosis in ALL and AML



Gimema Clinical Trial Open
V-FLAI

AML de novo patients with intermediate or complex karyotype

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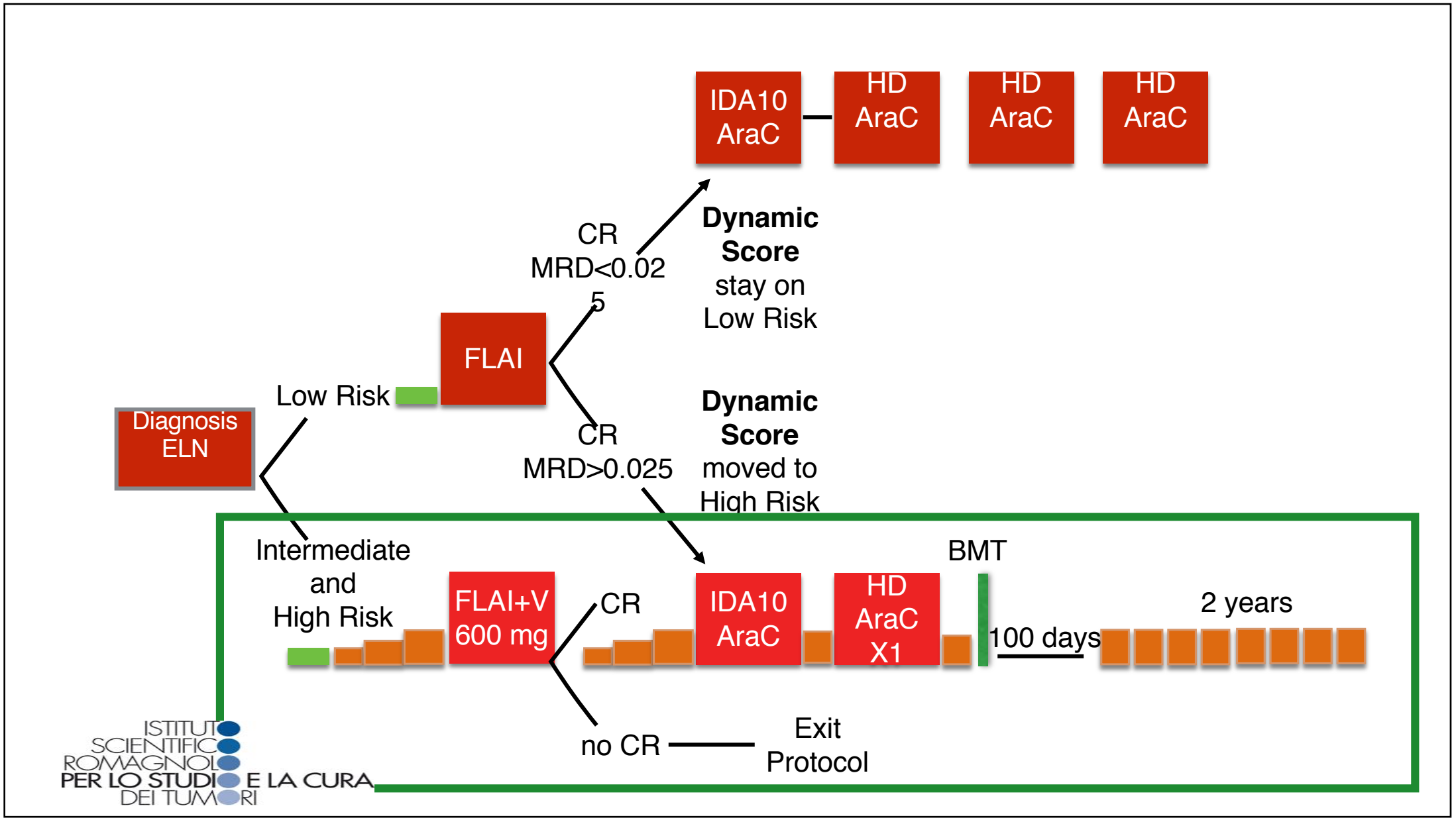
IDA10 AraC
HD AraC
HD AraC
HD AraC
CR MRD<0.025
Dynamic Score stay on Low Risk
FLAI
Low Risk
Diagnosis ELN
CR MRD>0.025
Dynamic Score moved to High Risk
Intermediate and High Risk
FLAI+V 600 mg
CR
IDA10 AraC
HD AraC X1
BMT
100 days
2 years
no CR
Exit Protocol
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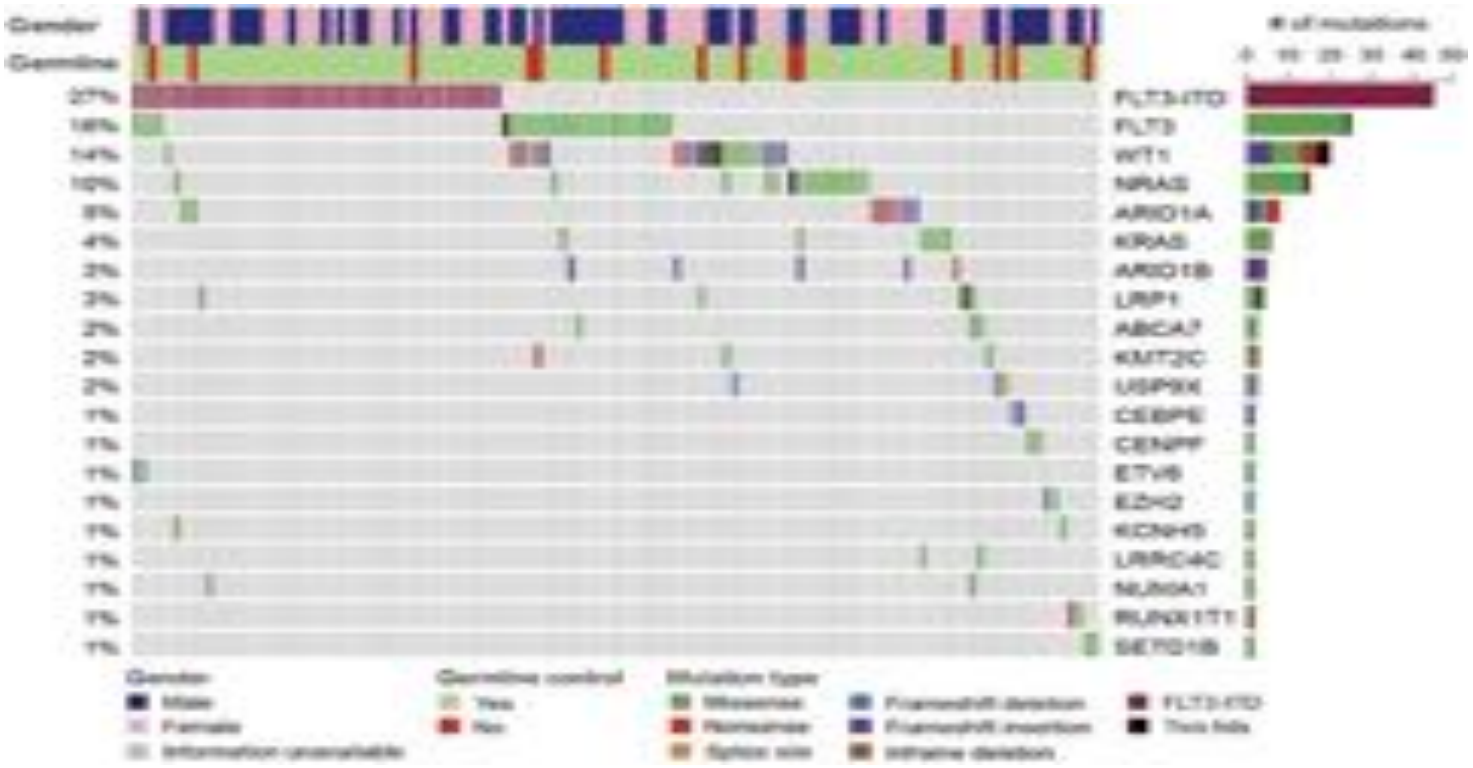
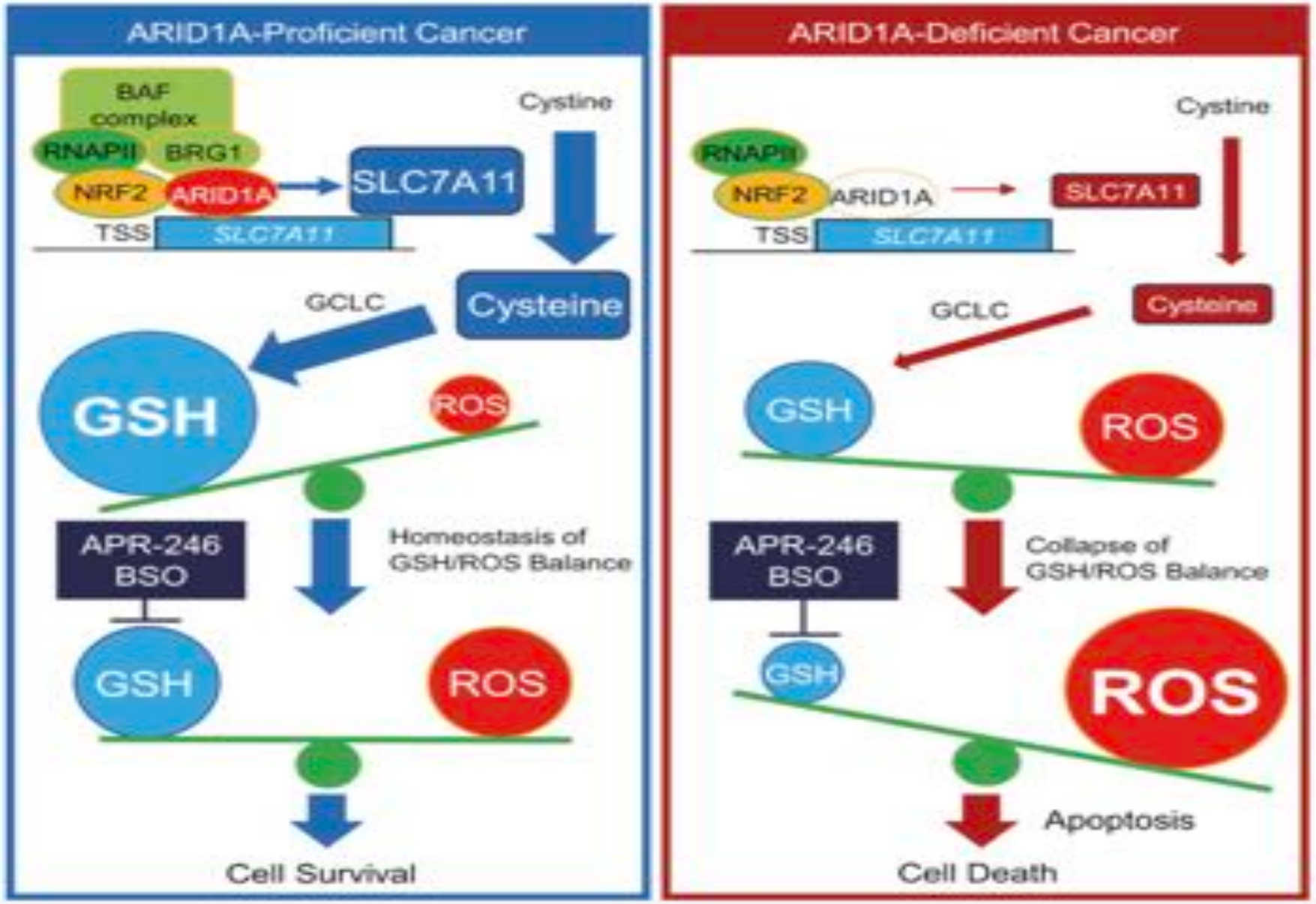
Might Venetoclax works more?

ARID1a and ARID1b



Synthetic Lethality in Leukemias

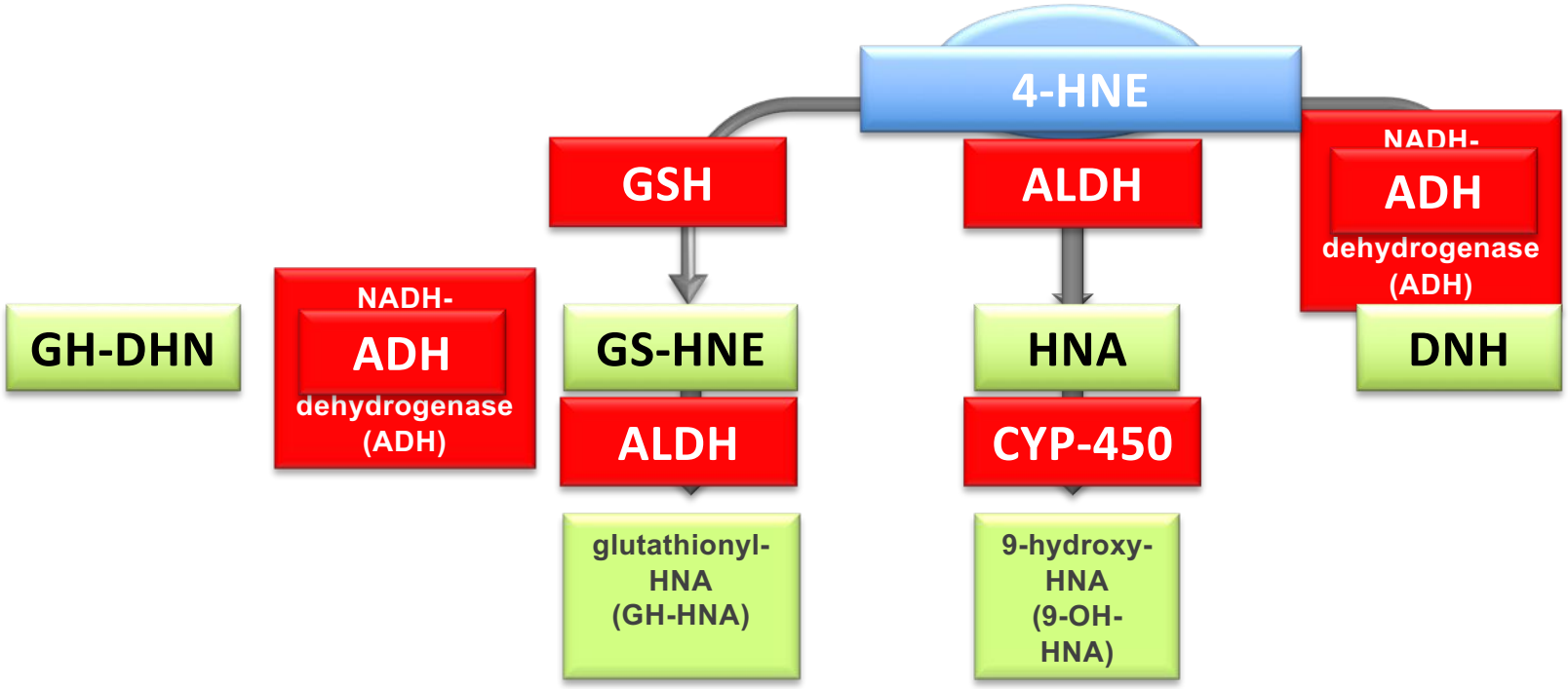

ARID1A-Proficient Cancer
BAF complex
RNAPII
BRG1
NRF2
ARID1A
SLC7A11
TSS
SLC7A11
Cystine
Cysteine
GCLC
GSH
ROS
APR-246
BSO
Homeostasis of GSH/ROS Balance
GSH
ROS
Cell Survival
ARID1A-Deficient Cancer
RNAPII
NRF2
ARID1A
SLC7A11
TSS
SLC7A11
Cystine
Cysteine
GCLC
GSH
ROS
APR-246
BSO
Collapse of GSH/ROS Balance
GSH
ROS
Apoptosis
Cell Death

Potentiating apoptosis in AML associated with translocations

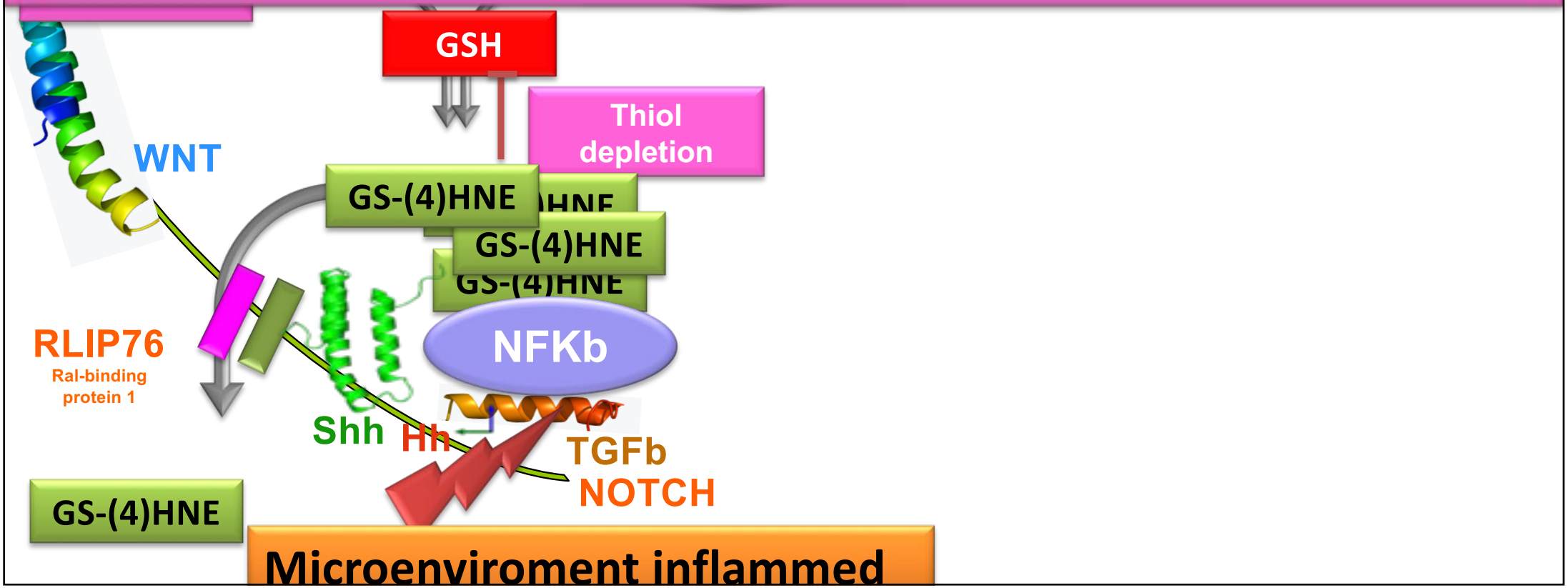
G6PD low > high GSSH/GSH

Why 4-HNE accumulate in CD34+ AML ?
To remove 4-HNE glutathione is depleted

Enzymatic production of 4-HNE and metabolism



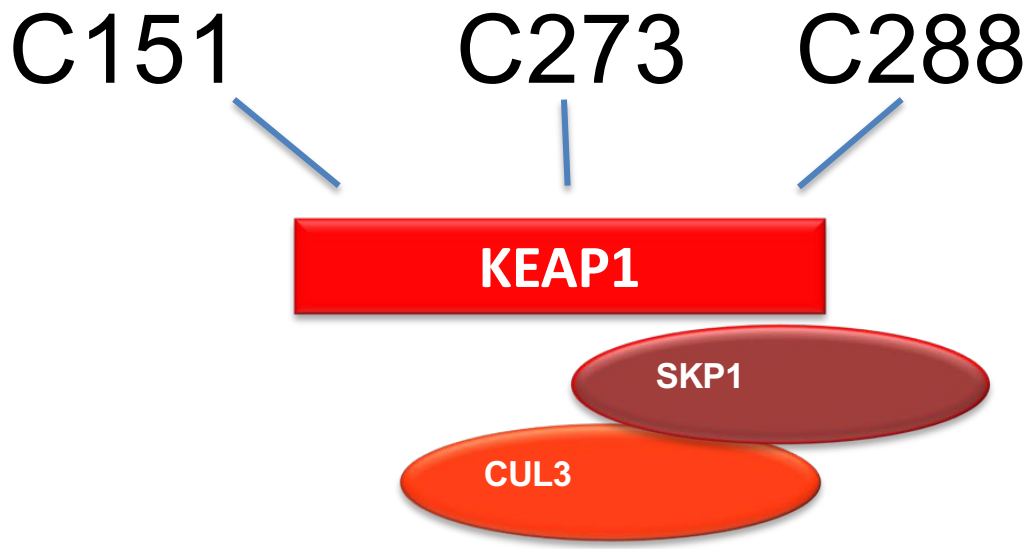
GS-4HNE is a potent inhibitor of the activity of glutathione S-transferase and produces Thiol Depletion



Thiol depletion produces a structural modification of cysteine C151, C273, e C288 of

Keap1

a Ubiquitin E-Ligasi-protein



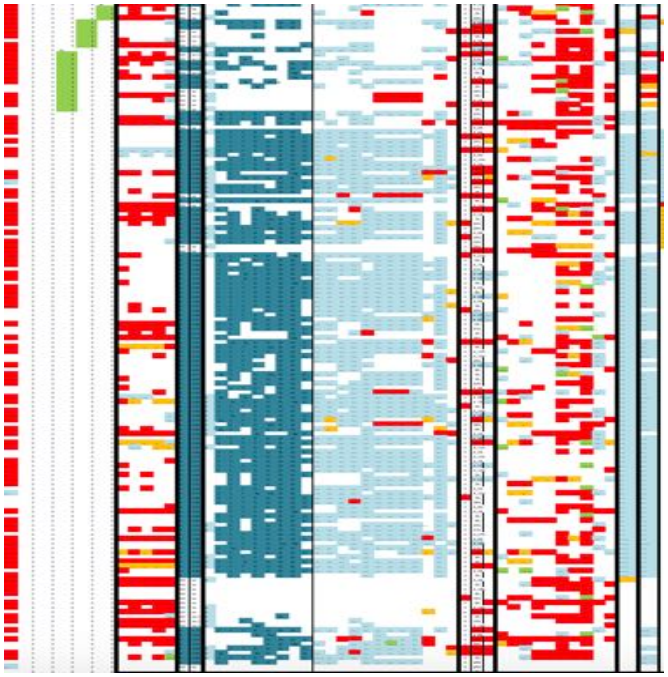
What's Keap1?

In MM
if Keap1 is amplified
= NO traslocation

Keap1

Translocation
t(4;14)

No
Translocations



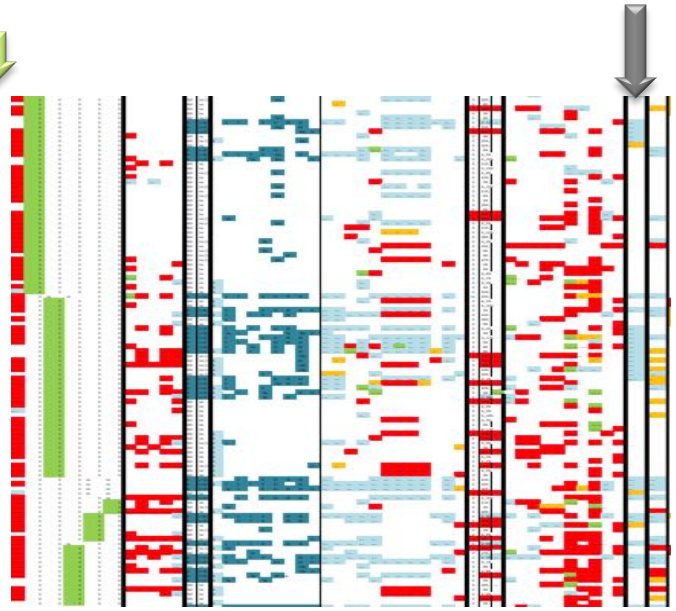
In Mm
If Keap1 is not amplified
= translocation!

Translocation
t(4;14)

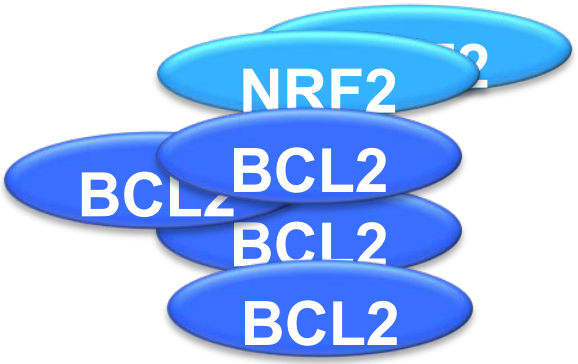
Translocation
t(14;16)

Translocation
t(4;14)

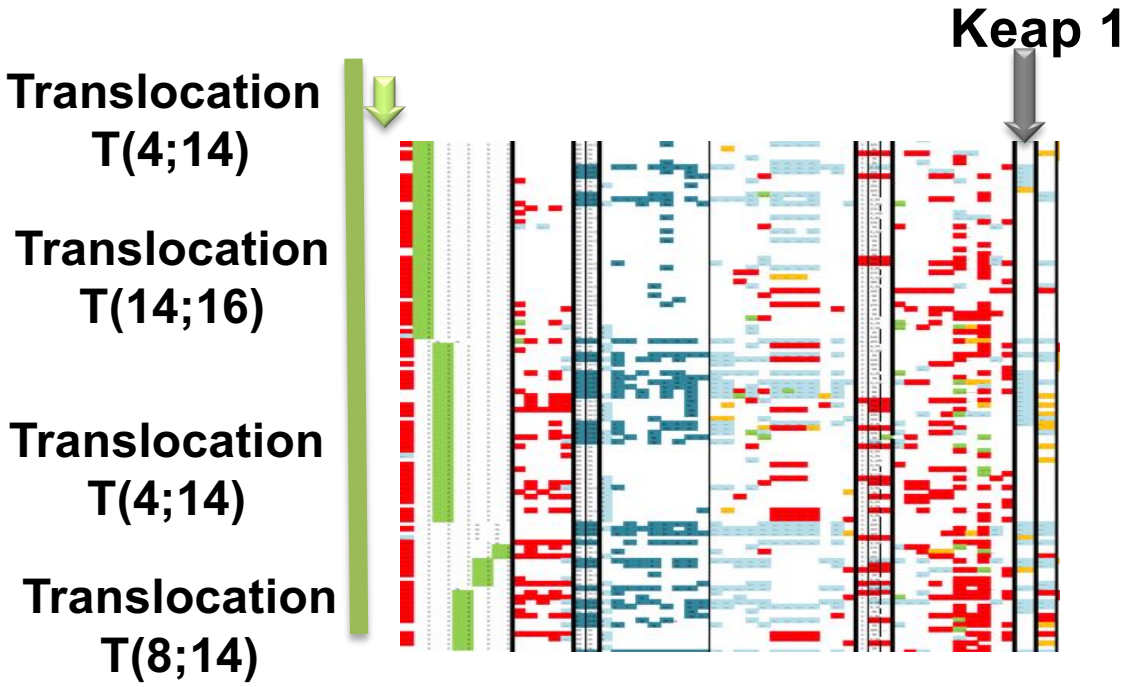
Translocation
t(8;14)

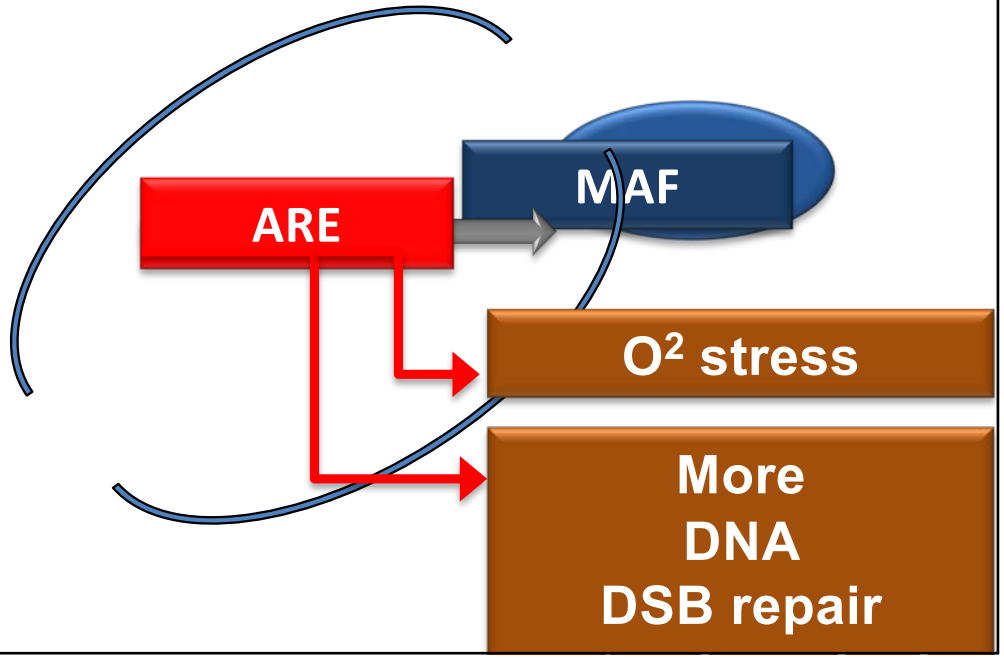


Less APOPTOSIS
Less Chemio-sensibility?



If Keap1 is not amplified
= translocation!





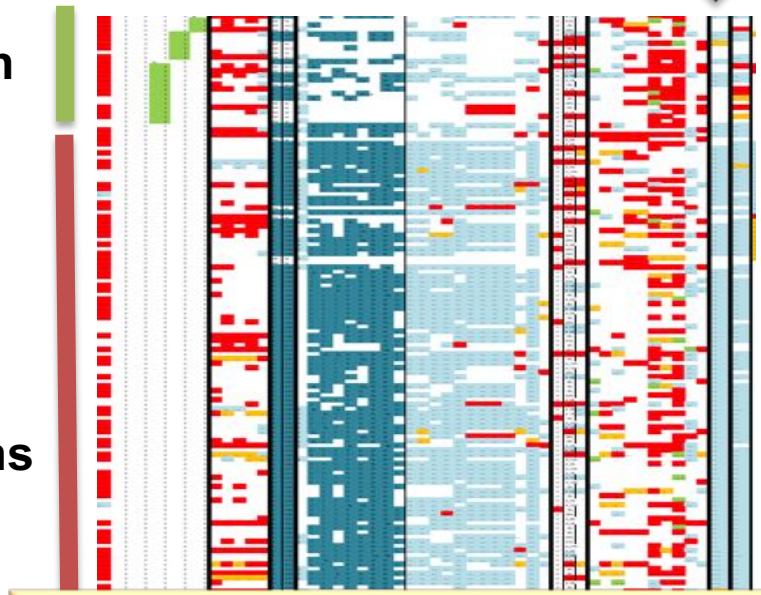
More APOPTOSIS
More Chemio-sensibility?

In MM
if Keap1 is amplified
= NO traslocation

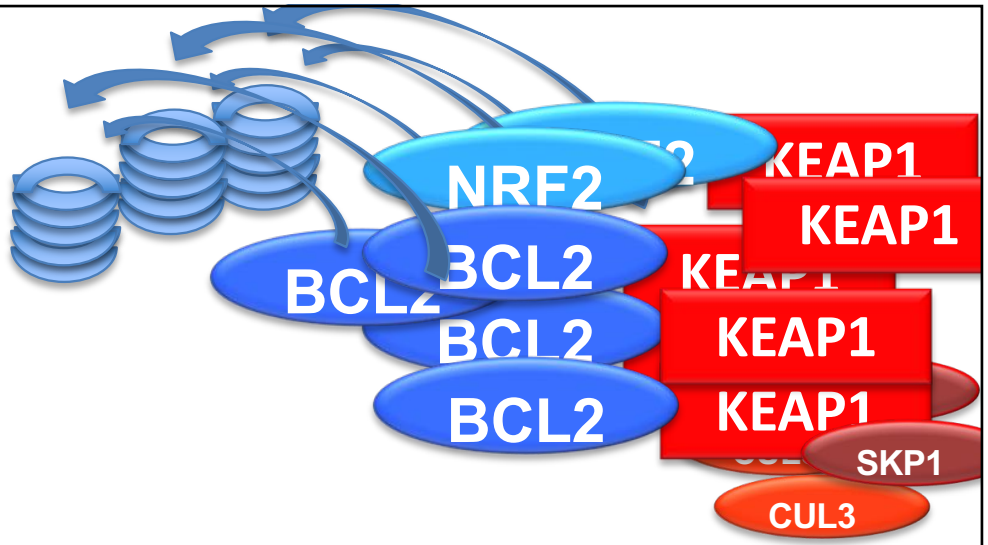
Keap1

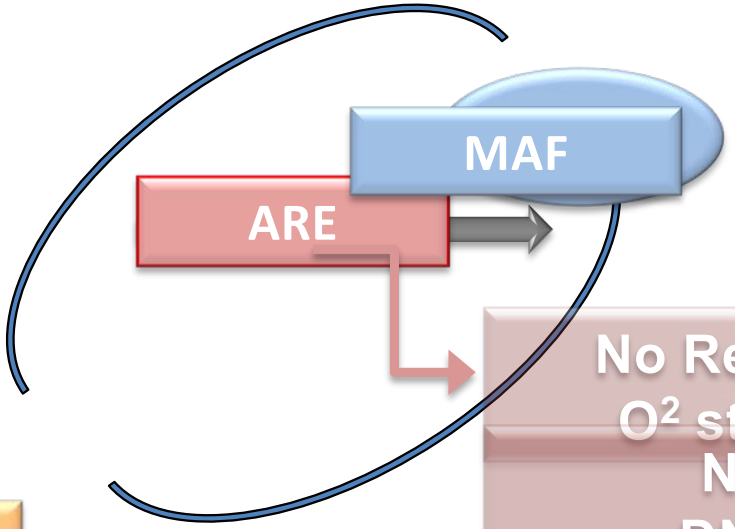
Translocation
t(4;14)

No
Translocations

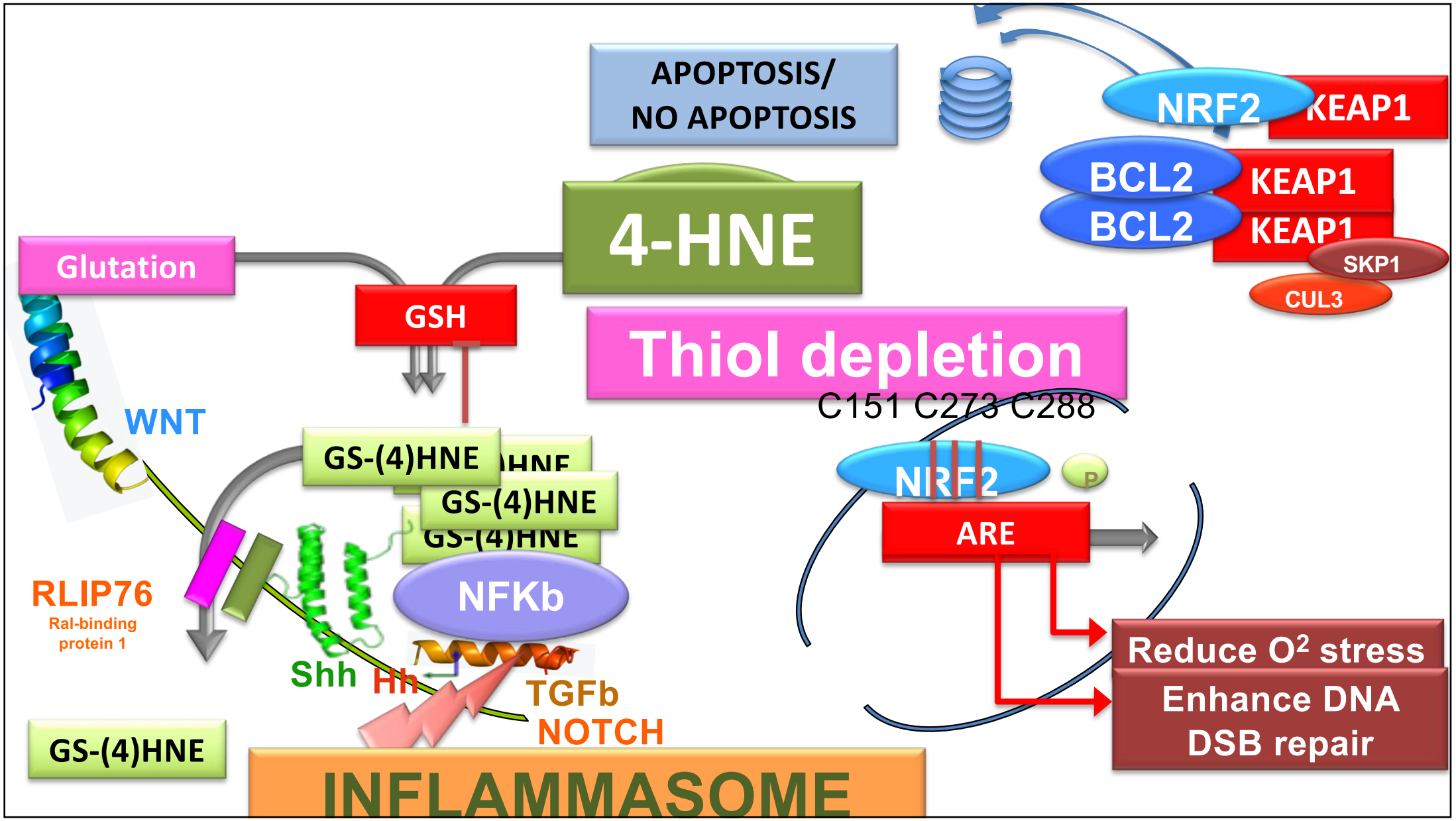


More Aneuploidy ?





No Reduce
O^2 stress
No
DNA
DSB repair


APOPTOSIS/
NO APOPTOSIS
NRF2
KEAP1
BCL2
KEAP1
BCL2
KEAP1
SKP1
CUL3
4-HNE
Glutation
GSH
Thiol depletion
C151 C273 C288
WNT
GS-(4)HNE
GS-(4)HNE
GS-(4)HNE
NRF2
P
ARE
RLIP76
Ral-binding
protein 1
NFKb
Shh
Hh
TGFb
NOTCH
Reduce O2 stress
Enhance DNA
DSB repair
GS-(4)HNE
INFLAMMASOME

AML CK

Translocations
Aneuploidy or
p53 mut

Chr. -5 ; -7
E-Ligase deficiency
MCL1 not degradable
uncurable

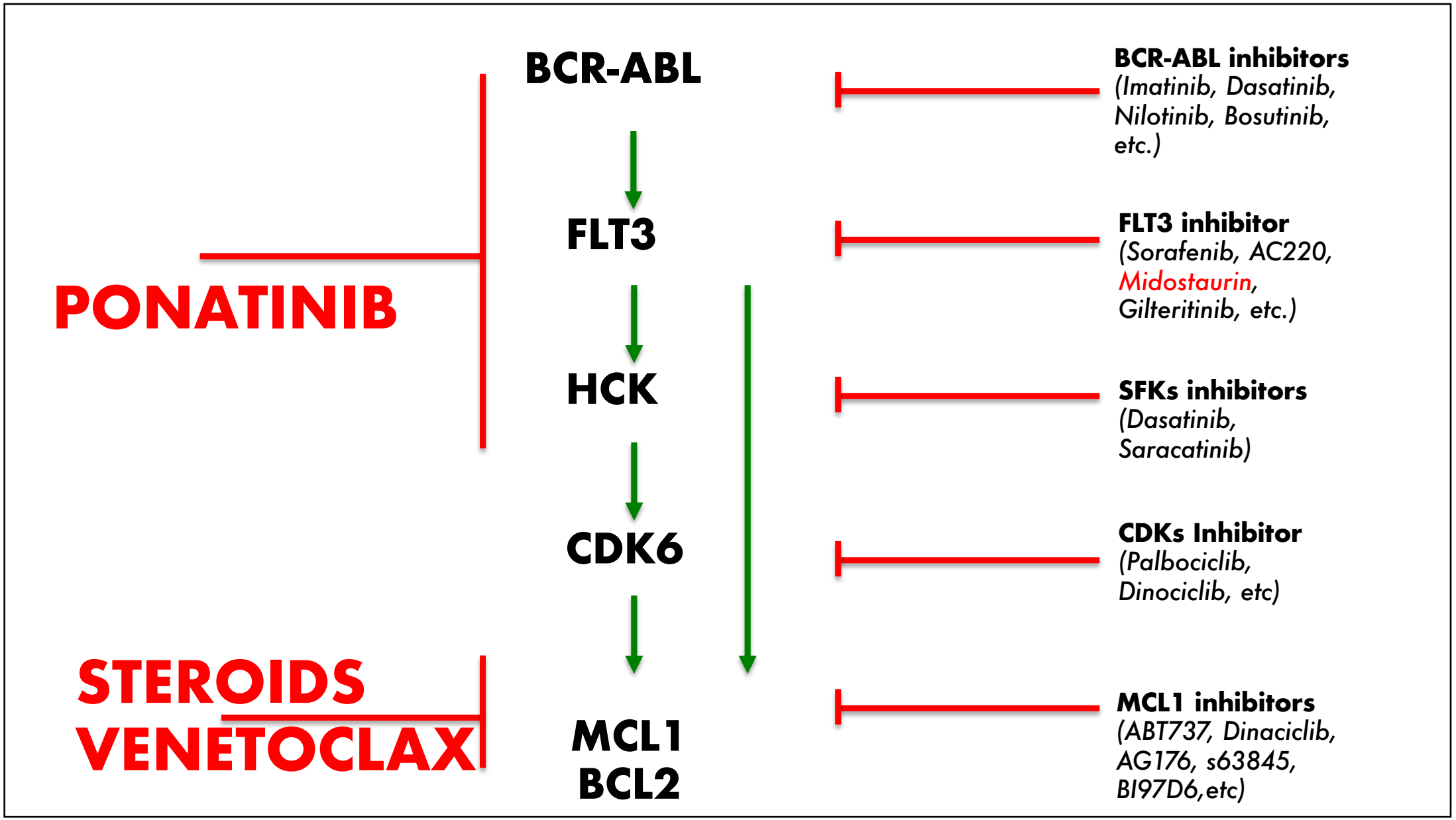
Translocations

1. Helicases are frequently affected in CK AML

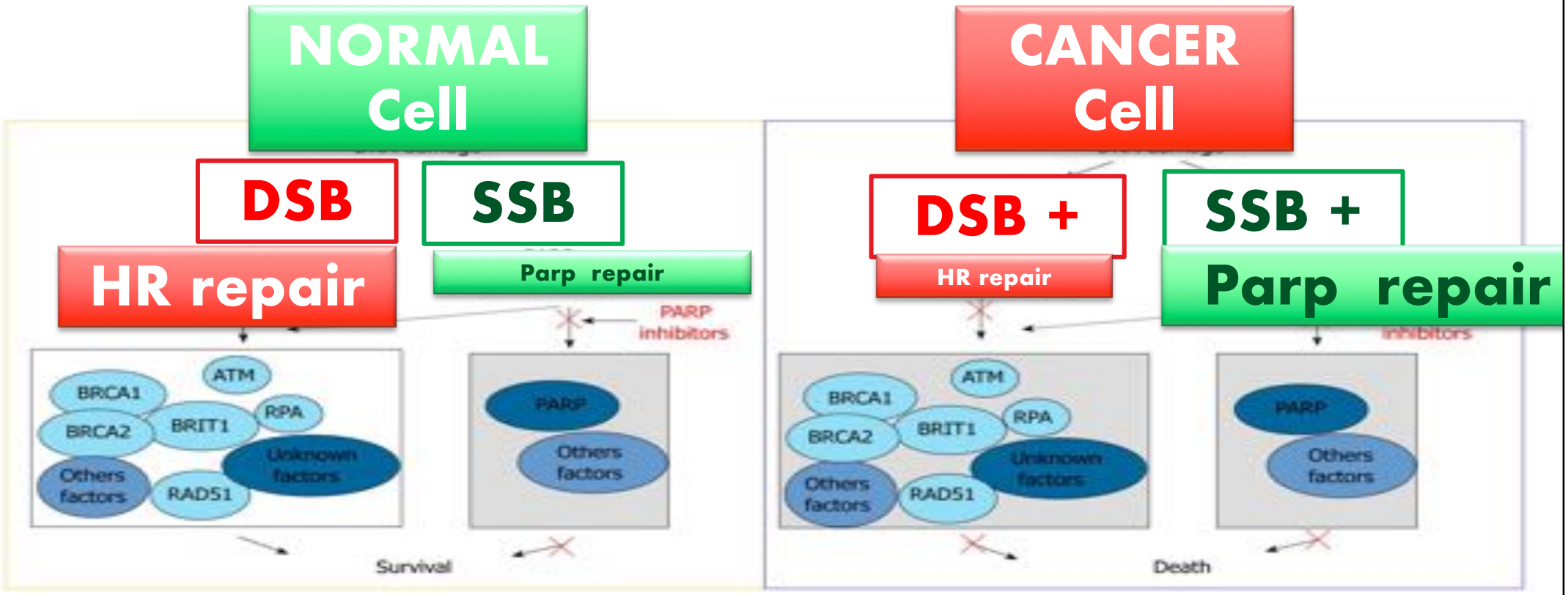
CTD1 +/+ and Helicases (RecQ +/+) studies suggest that by **homologous recombination (HR)** their catalyze dissolution of **double Holliday junctions**.

Like other RecQ helicases, **RECQ5b** promotes branch migration of **Holliday junctions** and exhibits strand-annealing and strand-exchange activities in vitro.

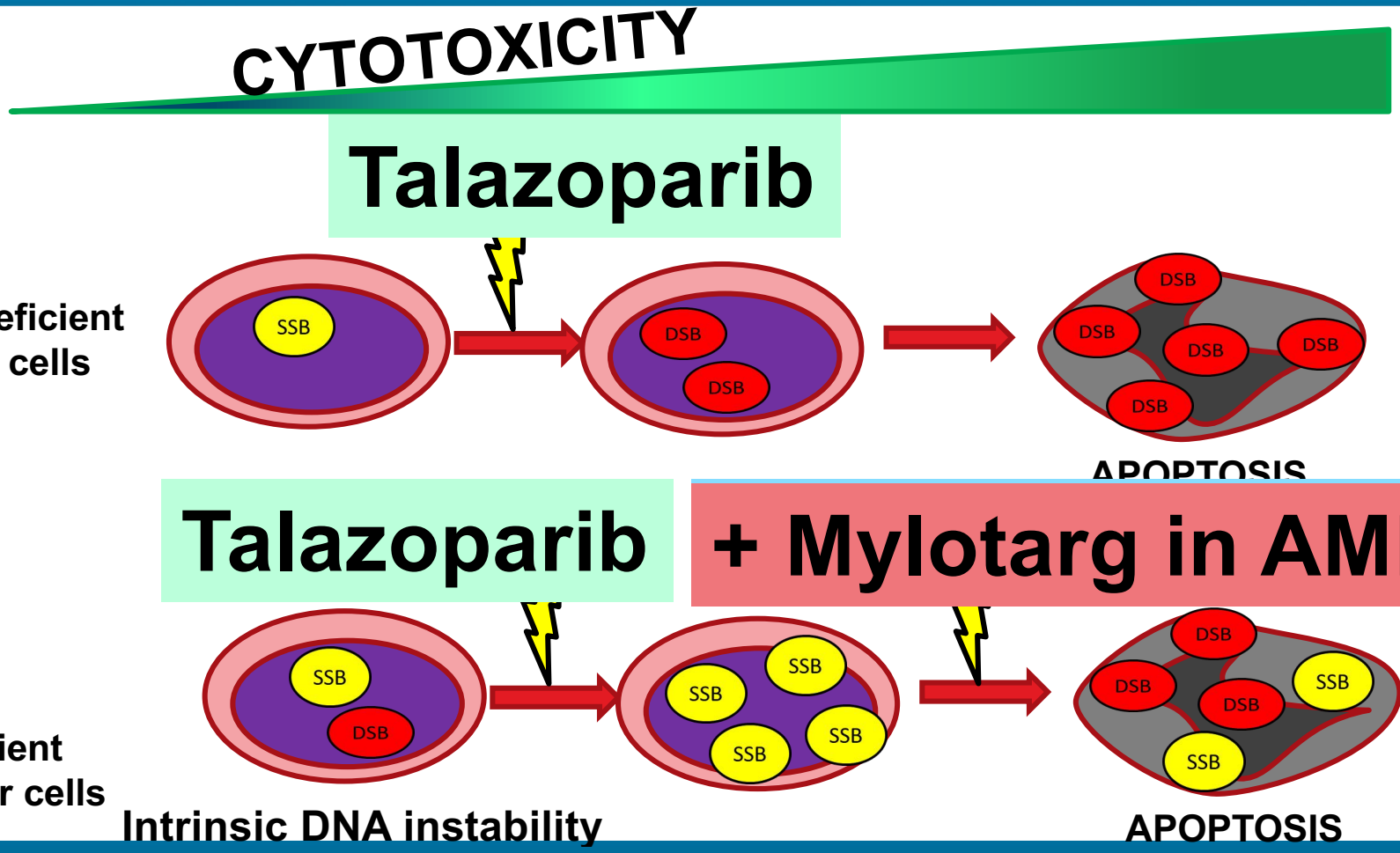
Synthetic Lethality in Leukemias


BCR-ABL
FLT3
HCK
CDK6
MCL1
BCL2
PONATINIB
STEROIDS
VENETOCLAX
BCR-ABL inhibitors
(Imatinib, Dasatinib, Nilotinib, Bosutinib, etc.)
FLT3 inhibitor
(Sorafenib, AC220, Midostaurin, Gilteritinib, etc.)
SFKs inhibitors
(Dasatinib, Saracatinib)
CDKs Inhibitor
(Palbociclib, Dinociclib, etc)
MCL1 inhibitors
(ABT737, Dinaciclib, AG176, s63845, BI97D6,etc)

RATIONALE FOR PARP1 INHIBITION IN SINGLE AGENT



ENHANCE PARP1 INHIBITOR EFFICACY



Thank you!

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