"The anti apoptosis way in cancer: **BCL2 inhibition**"

Istituto "Seragnoli"
University of Bologna

4° International Conference

Translational Research in Oncology Meldola, IRCS

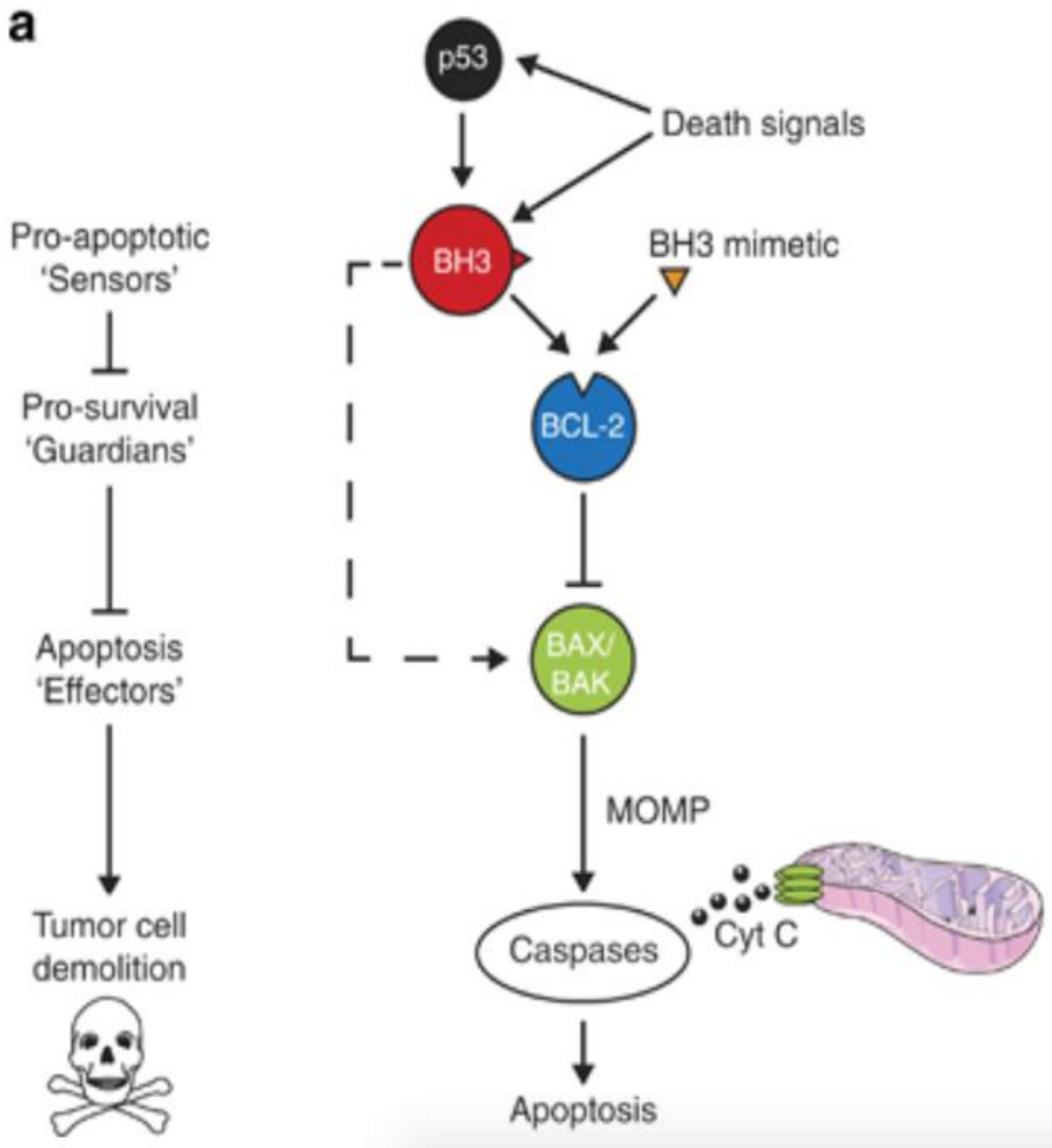
Novembre 9, 2016

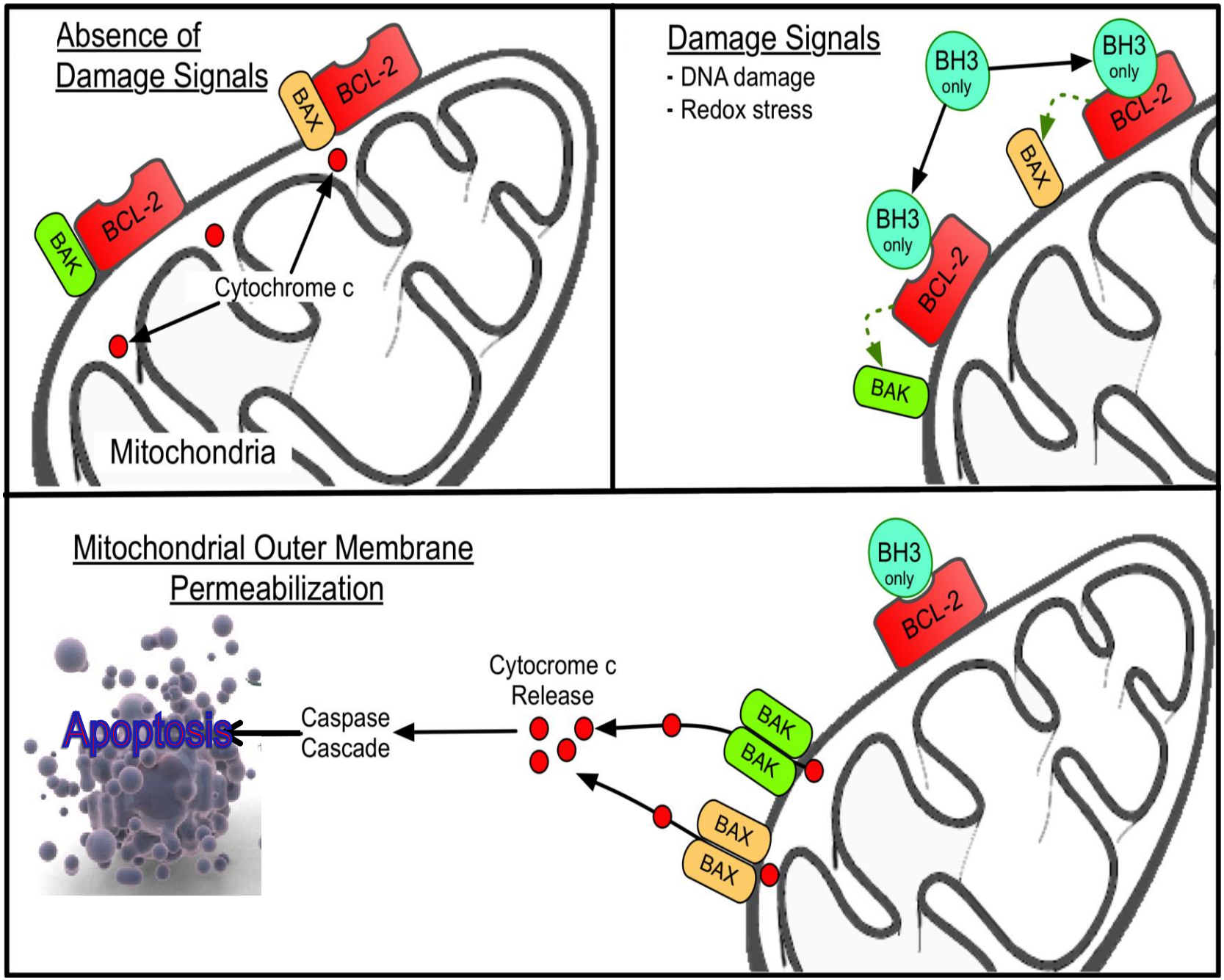
Forli Hotel Globus Cyty

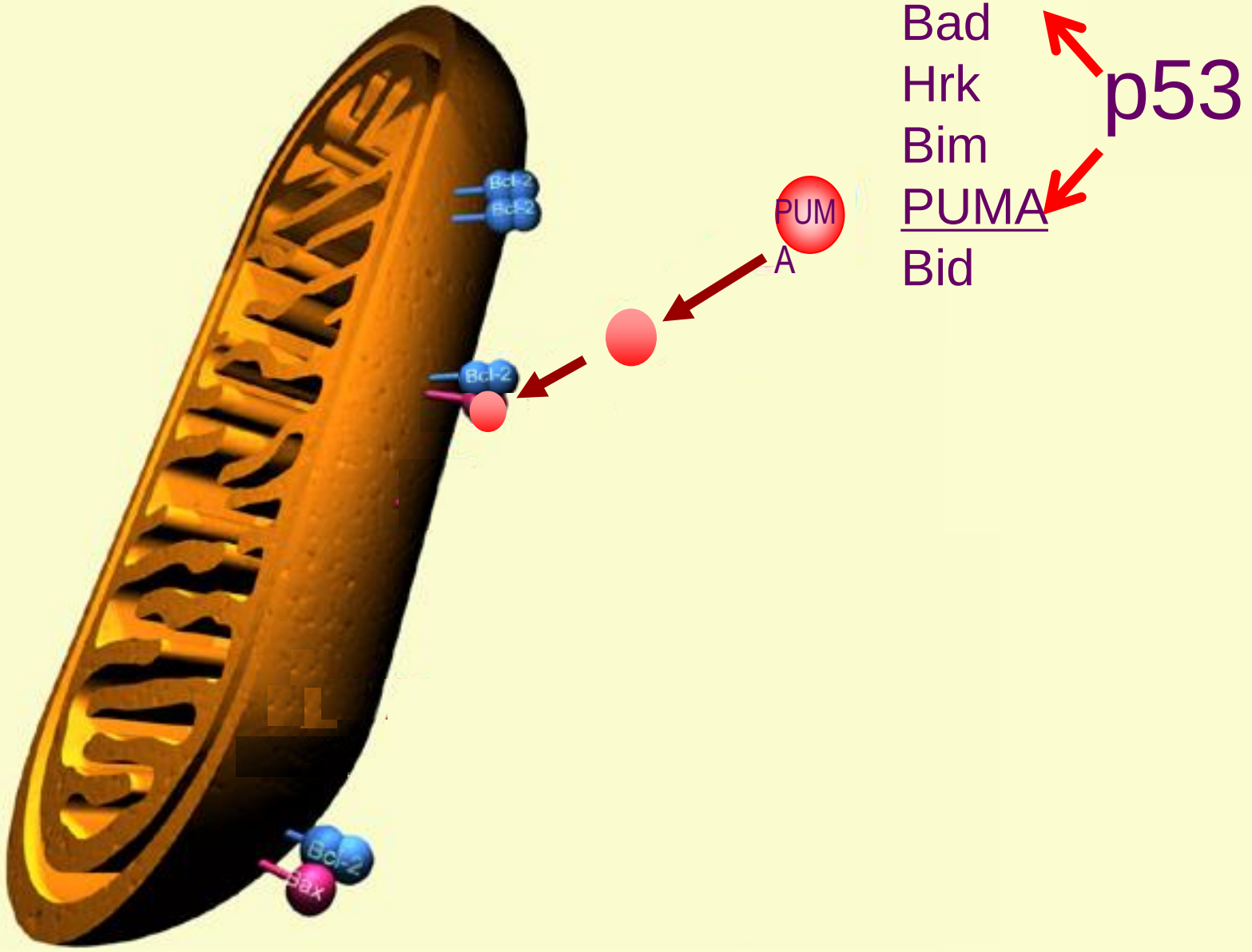
SEVENTH FRAMEWORK PROGRAMME

NEXT GENERATION SEQUENCING for Targeted Personalized Therapy of Leukemia

BCL2 is a well known oncogene in Leukemia



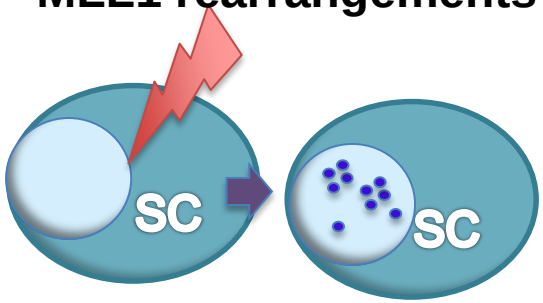

Absence of
Damage Signals
BAX
BCL-2
BCL-2
BAK
Cytochrome c
Mitochondria
Damage Signals
- DNA damage
- Redox stress
BH3
only
BH3
only
BCL-2
BAX
BH3
only
BCL-2
BAK
Mitochondrial Outer Membrane
Permeabilization
BH3
only
BCL-2
Apoptosis
Caspase
Cascade
Cytocrome c
Release
BAK
BAK
BAX
BAX


NOXA
Bad
Hrk
Bim
PUMA
Bid
p53
PUM
A
Bcl-2
Bcl-2
Bcl-2
Bcl-2
Bax

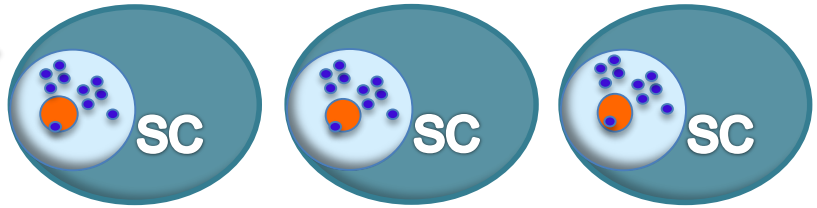
P53 (and BCL2) as a target !

Transient DNA Damage or
Oncogene expression
AML-ETO
BCR-ABL
MLL1 rearrangements

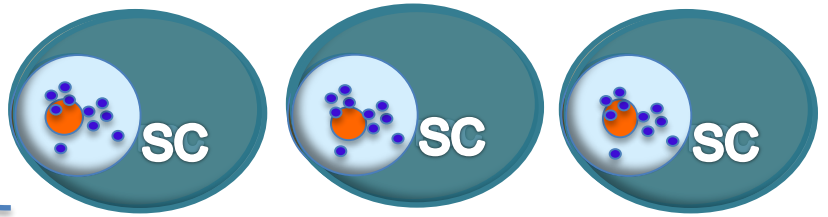
(Numb1, Prospero, BRAT, etc.)



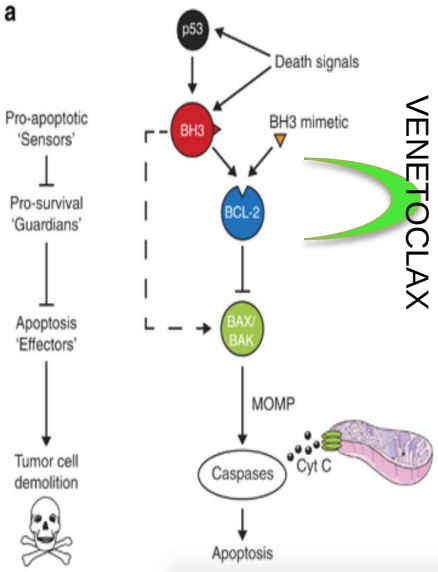
p21 ⊣ p53



asymmetric division



impeding apoptosis
leading to cell cycle entry
and changing asymetric division to
symmetric self-renewing divisions



VENETOCLAX

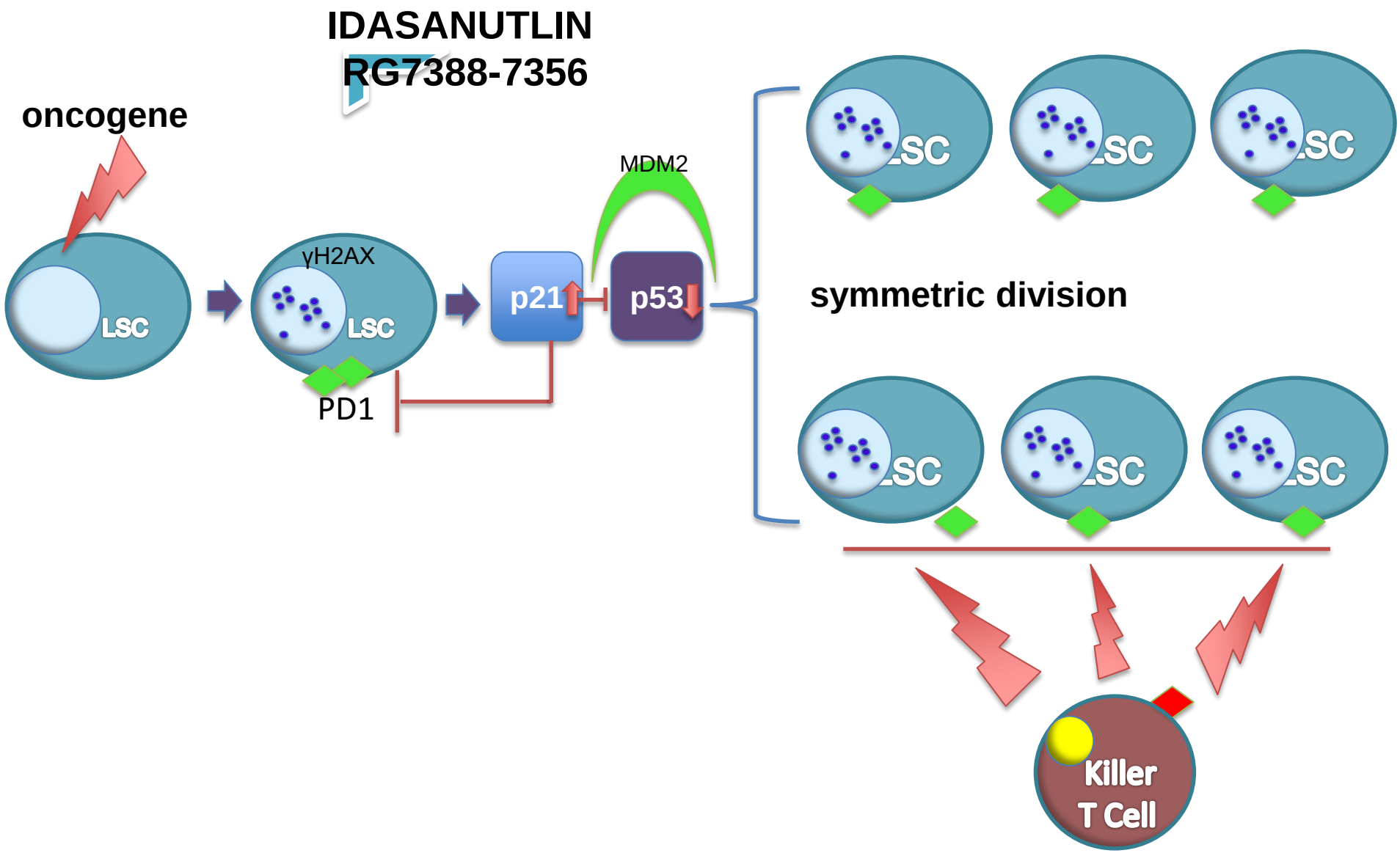
Removing transcriptional inhibition on Myc promoter
Enhancing Myc transcription
Switching to symmetric self-renewing divisions

symmetric division

p53 ⊣ MYC

asymmetric division

The mdm2-mdm4 inhibition restore P53 dependent activation of apoptosis and of immunological surveillance

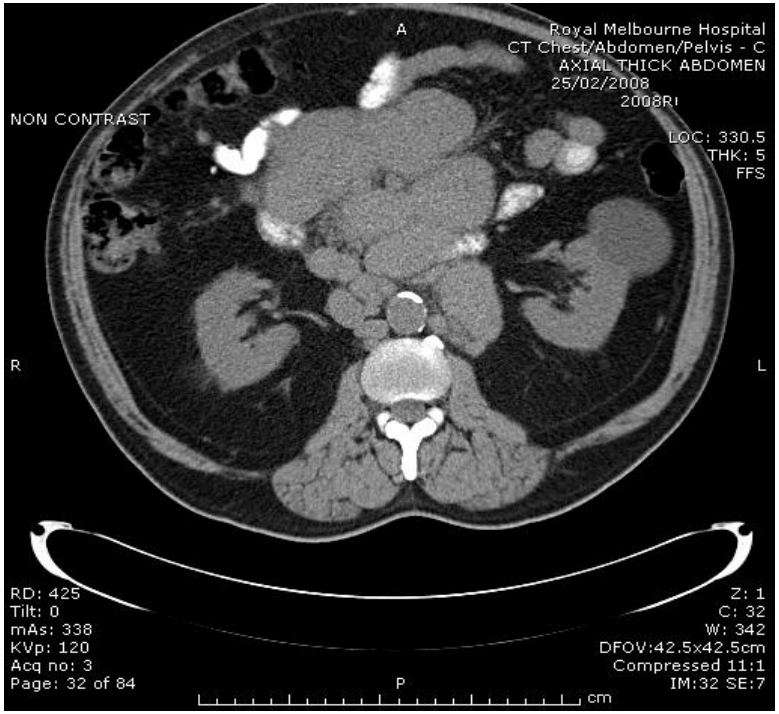


Small Molecule Inhibitors Of Bcl-2 Proteins And IC_{50} Of BID Peptide Displacement

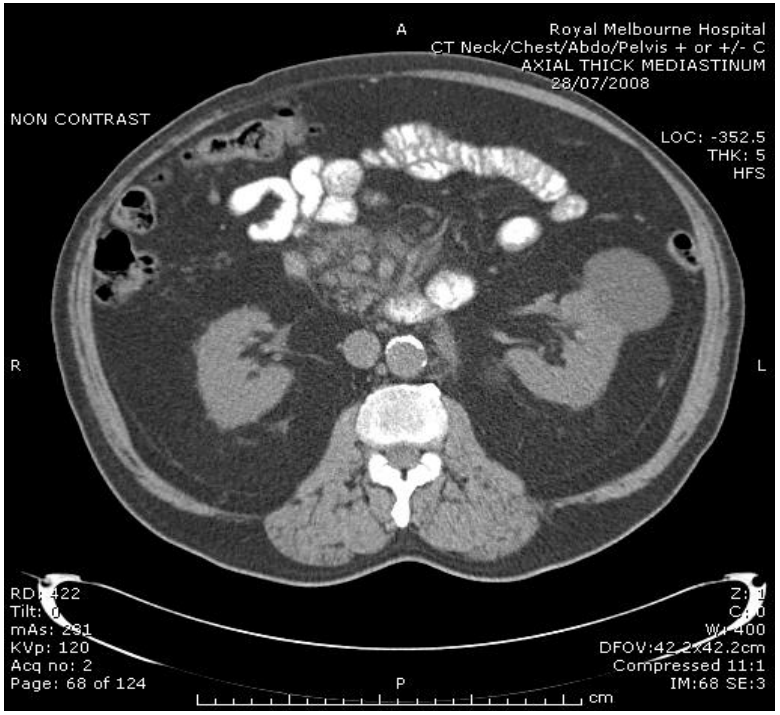
Compound IC_{50} (μM)					
Protein	Gossypol	Apogossypol	Obatoclax	ABT-737	ABT-199
Bcl-xL	3.0	2.8	4.69	0.064	0.048
Bcl-2	0.28	0.64	1.11	0.10	<0.10nM
Bcl-w	1.4	2.1	7.01	0.024	N/A
Bcl-B	0.16	0.37	2.15	>10	N/A
Mcl-1	1.75	3.35	2.90	>20	N/A

Partial Response in a Heavily Pre-treated CLL Patient

72-Year-Old Male, 7 prior regimens, Fludarabine refractory, 17p del



Pre-Treatment

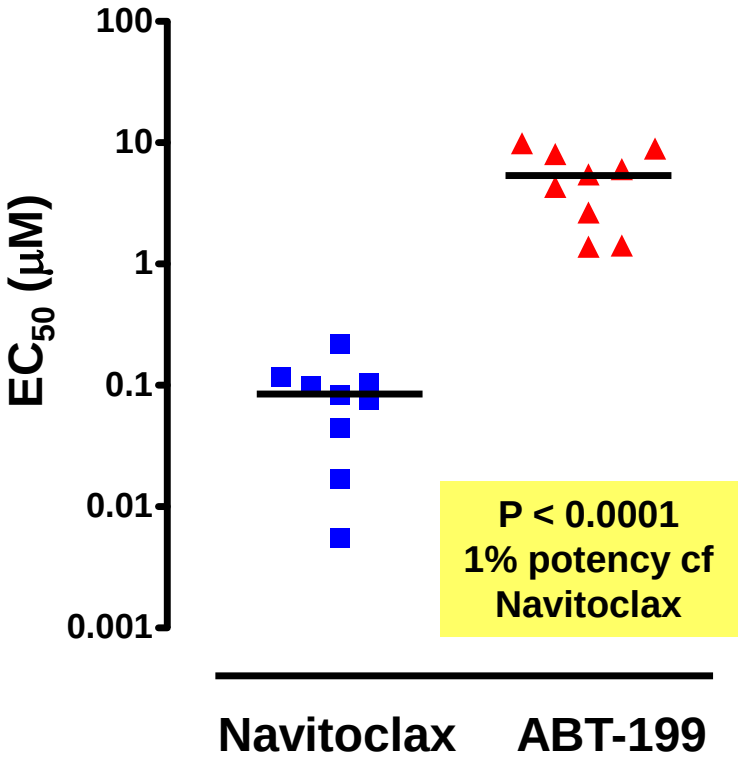


7 Cycles ABT-263

Roberts et al, JCO 30: 488, (2012)

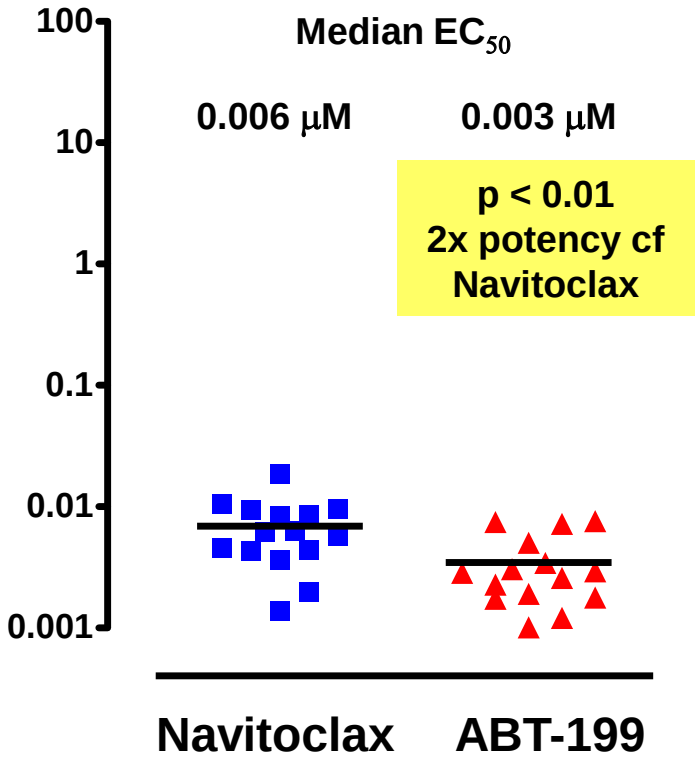
ABT-199 (Venetoclax): Preclinical Evidence of Selective Killing

Human platelets *ex vivo*



Normal human donor platelet rich plasma samples exposed to drug for 24 hours (n=9)

CLL samples *ex vivo*

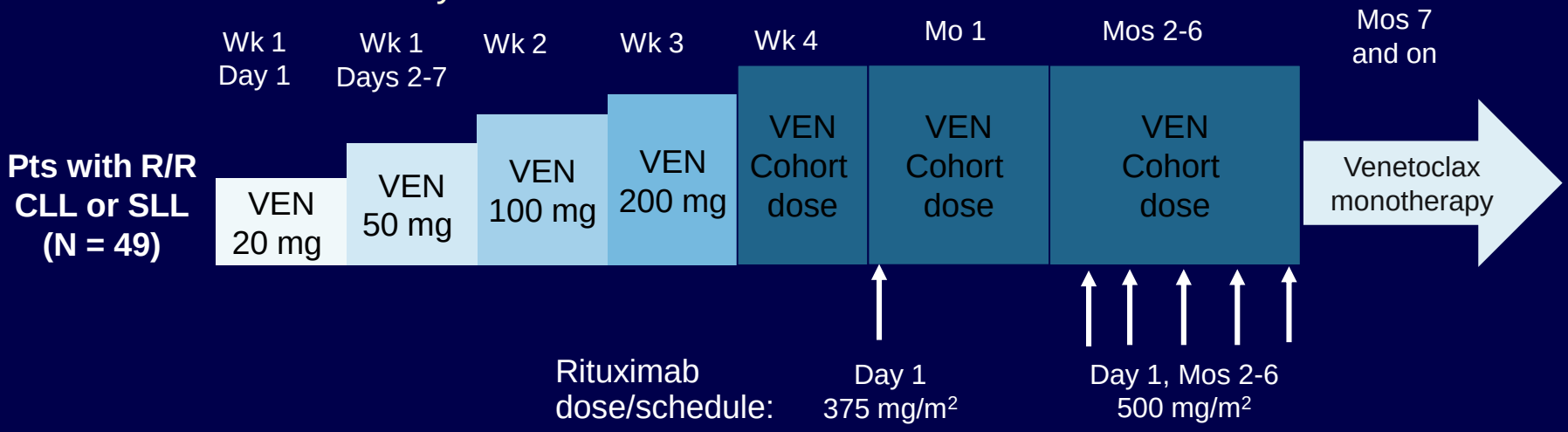


Primary CLL patient samples exposed to drug in 10% BCS for 24 hours (n=15)

S Khaw, K Mason, A Roberts, D Huang unpublished data

1. Venetoclax and Rituximab in R/R CLL: Phase Ib Study Design

- Dose-escalation study



Pts had protocol-defined option to stop venetoclax if CR or MRD-negative PR achieved

- Dose-escalation phase: 5 cohorts—200, 300, 400, 500, and 600 mg/day (n = 41)
- Safety expansion phase included 400-mg/day cohort (n = 8)
- Data pooled for safety, efficacy analyses
- Study aims: assess MRD status, evaluate PFS, OS, response durability in pts who stop venetoclax, retreatment of pts with PD off therapy

Ma S, et al. ASH 2015. Abstract 830.

Venetoclax and Rituximab in R/R CLL: Baseline Characteristics

Characteristic	Pts (N = 49)
Median age, yrs (range)	68 (50-88)
Male, %	61
Diagnosis: CLL/SLL, n	48/1
Lymphocyte count x 10^9/L, median (range) ▪> 5 x 10^9/L, %	18.6 (0.3-207.1) 65
Bulky nodes > 5 cm, %	45
Prior therapies, median n (range) ▪≥ 3, % ▪Rituximab containing, % ▪Rituximab refractory, % ▪Fludarabine containing, % ▪Fludarabine refractory, %	2 (1-5) 43 90 29 57 18
del(17p) and/or *TP53* mutation (n = 45)	33
Unmutated *IGHV* (n = 27)	70

Ma S, et al. ASH 2015. Abstract 830.

Venetoclax and Rituximab in R/R CLL: Response and Survival

- Median time on study: 21 mos (range: < 1-37)
  - 37 pts remain on study; median time 23 mos (range: 15-37)
  - 12 discontinuations: PD, 6 (including 5 Richter's transformation); AE, 3; consent withdrawn, 3

Best Objective Response, %	All (N = 49)	del(17p) or Mut *TP53* (n = 15)	Fludarabine Refractory (n = 9)	Rituximab Refractory (n = 14)	*IGHV* Unmut (n = 19)	Age ≥ 70 Yrs (n = 22)
Overall Response	86	87	56	64	84	77
CR (incl 7 CRi)	47	47	44	29	47	41
PR (incl 1 nPR)	39	40	11	36	37	36
SD	8	7	22	21	5	18
PD	4	0	11	7	5	5
Death due to TLS*	2					

*1 fatal TLS event reported in a pt with del(17p), *IGHV* unmutated, and who was fludarabine and rituximab refractory; no others after protocol amendment

- 2-yr PFS: 83%
- 2-yr OS: 94%

Ma S, et al. ASH 2015. Abstract 830.

Venetoclax and Rituximab in R/R CLL: MRD, DoR, Response Durability

Response Classification, %	MRD Negative	MRD Positive	Not Evaluable
CR (n = 23)	35	10	2
PR (n = 19)	20	16	2
Other (n = 7)	0	2	12
Total (n = 49)	**55**	**29**	**16**

- Response maintained in 100% of MRD-negative and ~ 75% of MRD-positive pts
- 11 pts stopped venetoclax after achieving response, including 9 MRD-negative pts
  - 9 in follow-up; median time off venetoclax of 16 mos
  - 2 pts with MRD-positive CR/CRi had asymptomatic progression

Ma S, et al. ASH 2015. Abstract 830.

Venetoclax and Rituximab in R/R CLL: Conclusions

- Venetoclax + rituximab highly active in R/R CLL
  - ORR: 86% with 47% CR/CRi
  - MRD negativity in bone marrow in 55% of pts, and all MRD-negative pts maintained response at time of reporting
- 11 pts stopped venetoclax after achieving MRD negativity or CR
  - MRD-positive pts (n = 2) who stopped treatment progressed; PR at 3 mos with retreatment in 1 pt
  - No MRD-negative pts progressed after stopping therapy
- Most frequent grade 3/4 toxicity: neutropenia
- Ongoing phase III trial comparing venetoclax/rituximab vs bendamustine/rituximab in previously treated pts with CLL

Ma S, et al. ASH 2015. Abstract 830. ClinicalTrials.gov NCT02005471.

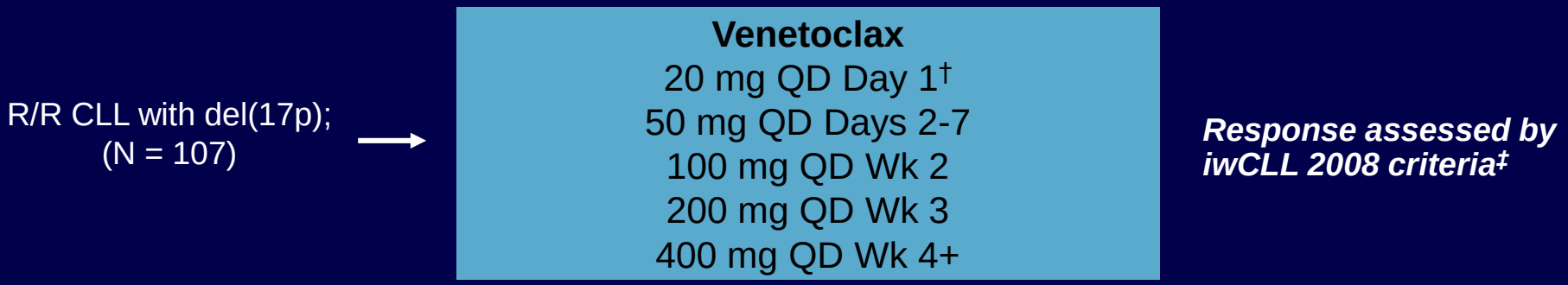
2. Venetoclax in R/R CLL With del(17p): Background

- Pts with R/R CLL with del(17p) have limited options and face poor prognoses
  - Median PFS < 12 mos with frontline chemotherapy
- Venetoclax: oral selective BCL2 inhibitor[1]
  - Induces p53-independent apoptosis of CLL cells
- Phase I study showed 79% response rate to venetoclax in pts with R/R CLL[2]
  - ORR for R/R CLL with del(17p): 71% (95% CI: 52% to 86%)
- Current study evaluated the efficacy and safety of single-agent venetoclax for the treatment of R/R CLL with del(17p)[3]

1. Souers AJ, et al. Nat Med. 2013;19:202-208.
2. Roberts AW, et al. N Engl J Med. 2015;[Epub ahead of print].
3. Stilgenbauer S, et al. ASH 2015. Abstract LBA-6.

Venetoclax in R/R CLL With del(17p): Study Design

- Single-arm, multicenter phase II study



- Primary endpoint: ORR (IRC assessment)
- Exploratory endpoint: MRD

Stilgenbauer S, et al. ASH 2015. Abstract LBA-6.

Venetoclax in R/R CLL With del(17p): Best Responses

Response, %	Investigator	IRC
Overall response	73.8	79.4
▪ CR or CRi	15.9	7.5
▪ nPR	3.7	2.8
▪ PR	54.2	69.2
No response	26.2	20.6
▪ Stable disease	22.4	NA

- 25/48 pts (52%) had no evidence of CLL in bone marrow by IHC
- 18/45 pts assessed (40%) were MRD negative in peripheral blood samples
- Among 87 pts with baseline lymphocytosis, only 4 failed to normalize ALC count to < 4 x 10^9/L
  - Median time to normalization: 22 days (range: 2-122)
- Among 96 pts with baseline lymphadenopathy, 89 had ≥ 50% reduction in nodal size of the largest target lesion (by SPD)
  - Median time to ≥ 50% reduction: 2.7 mos (range: 0.7-8.4)

Stilgenbauer S, et al. ASH 2015. Abstract LBA-6.

Venetoclax in R/R CLL With del(17p): Response Duration and Survival

Parameter	
iwCLL response, median mos (range)	
▪ Time to first response	0.8 (0.1-8.1)
▪ Time to CR/CRi	8.2 (3.0-16.3)
Duration of response: 12-mo estimates, % (n = 85)	
▪ All responders	84.7
▪ CR/CRi/nPR	100
▪ MRD negative	94.4
Survival rates: 12-mo estimates, % (95% CI) (n = 107)	
▪ PFS	72 (61.8-79.8)
▪ OS	86.7 (78.6-91.9)

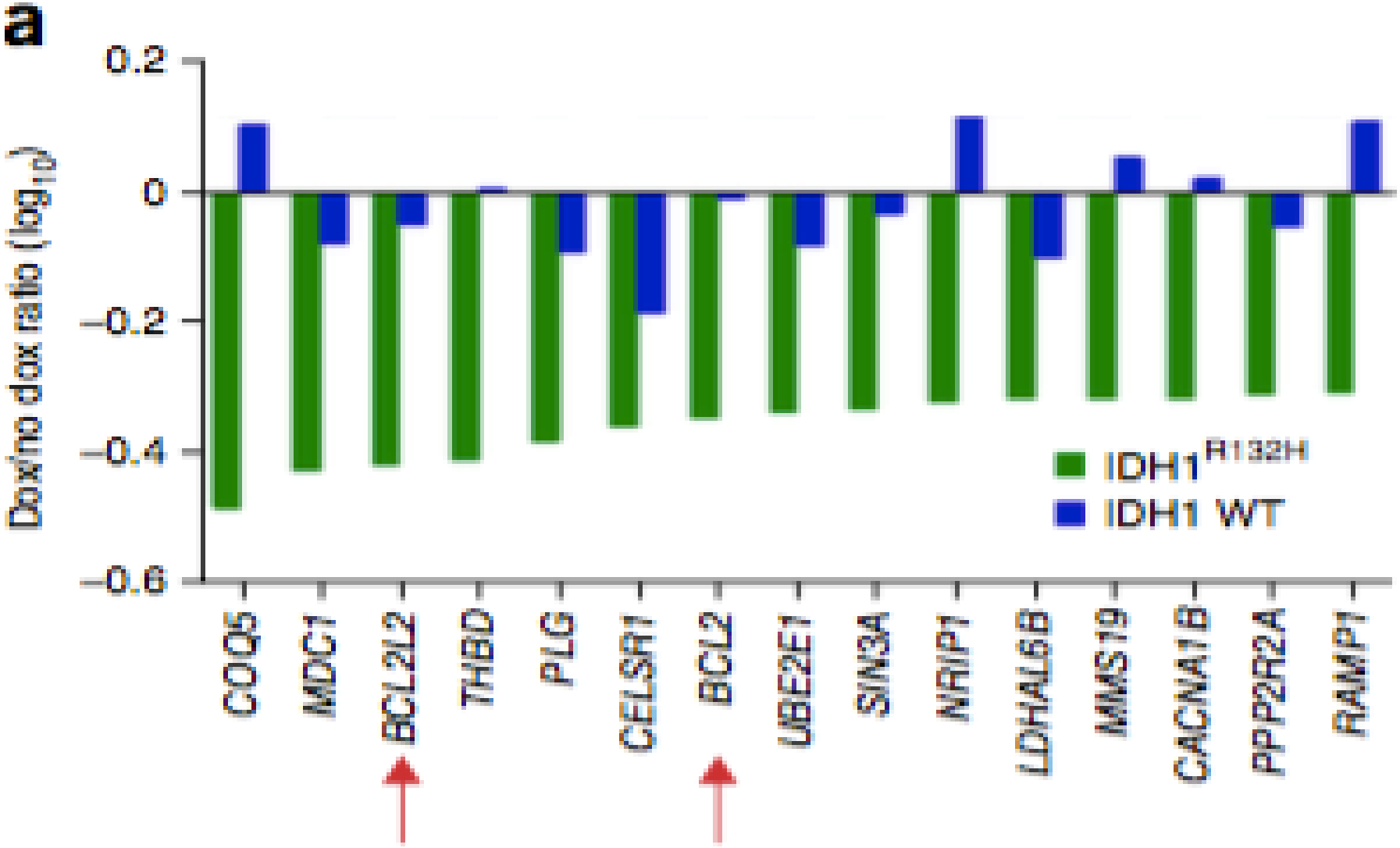
Stilgenbauer S, et al. ASH 2015. Abstract LBA-6.

Venetoclax in R/R CLL With del(17p): Conclusions

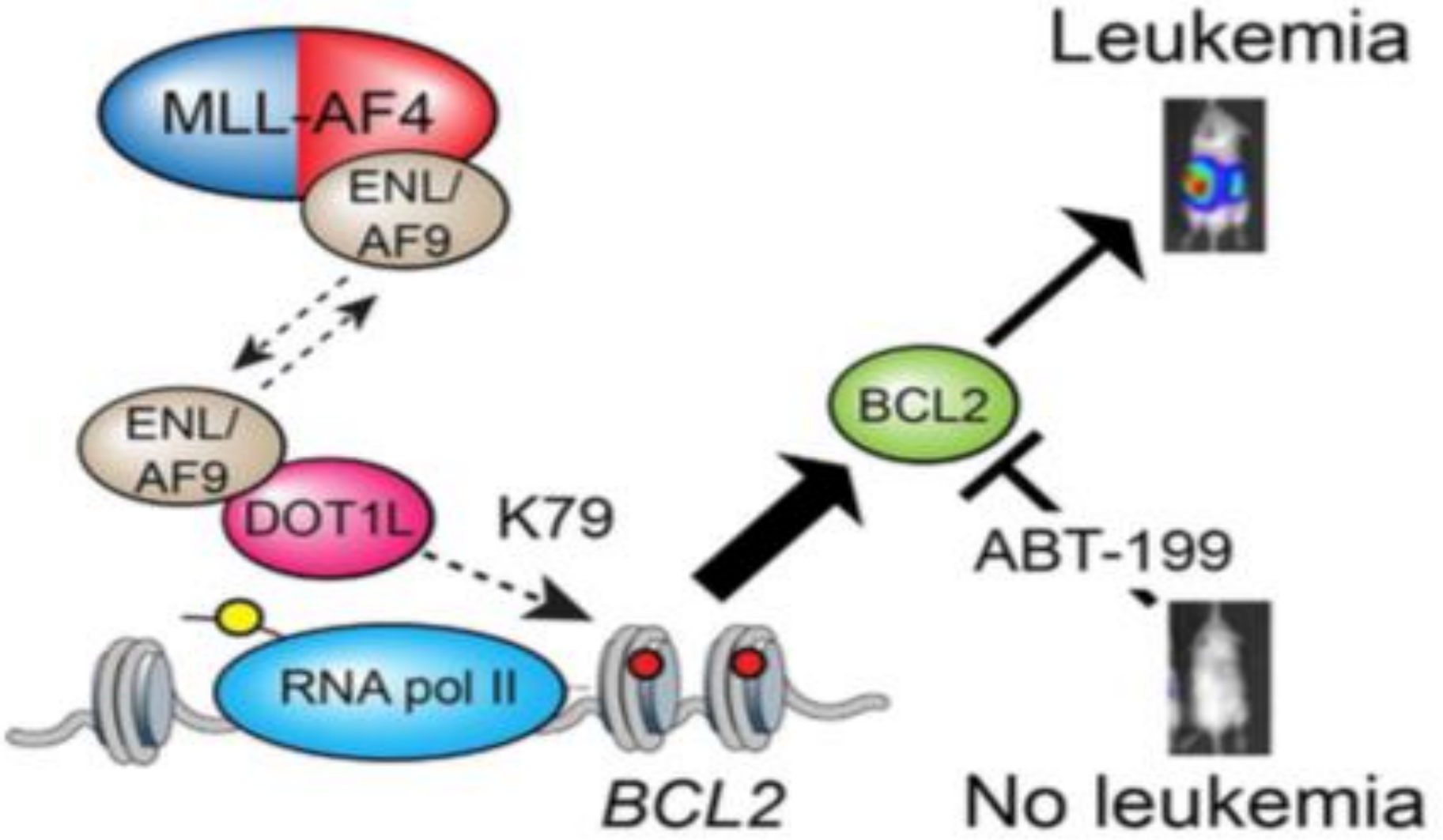
- Venetoclax active with deep responses in R/R CLL with del(17p)
  - > 10% had IRC-confirmed CR, CRi, or nPR
  - > 20% of responders MRD negative
- Authors conclude that venetoclax toxicity and risk-benefit profiles are acceptable
  - Risk of TLS effectively mitigated; no clinical TLS
  - Similar incidence of neutropenia, infection as with frontline chemoimmunotherapy

Stilgenbauer S, et al. ASH 2015. Abstract LBA-6.

.. but less is known regarding which gene are synthetic lethality with BCL2: ex. IDH1



BCL2 in AML is critical



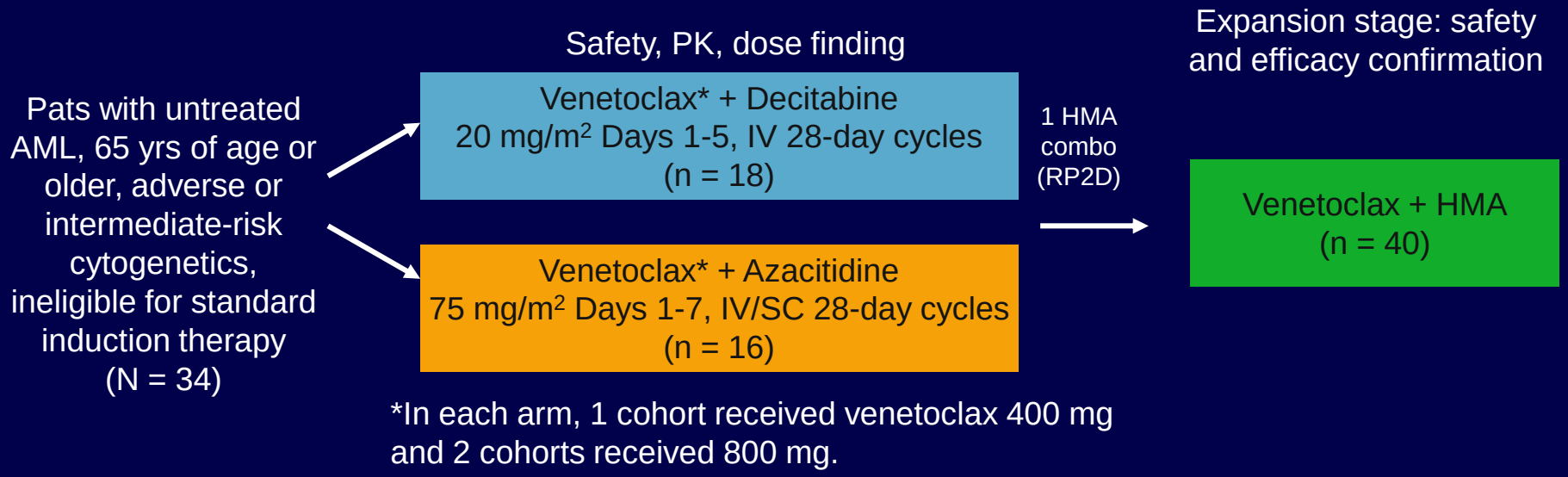
Venetoclax + HMAs in AML: Background

- Outcomes for elderly pts (65 yrs of age or older) with AML remain poor, with low rates of response, remission, and survival.
- BCL-2 protein a promising therapeutic target for AML
  - Overexpression enhances survival of AML cells; associated with poor survival, chemotherapy resistance
  - synthetic letal with IDH1 and MLL1
- Venetoclax: potent, orally available, selective BCL-2 inhibitor

1. Eleni L, et al. J Oncol. 2010; 2010:943823. 2. Hiddemann W, et al. J Clin Oncol. 1999;17:3569-3576. 3. Klepin HD, et al. Oncologist. 2009;14:222-232. 4. Tzifi F, et al. Adv Hematol. 2012;2012:524308. 5. Tsao T, et al. Ann Hematol. 2012;91:1861-1870. 6. Mehta SV, et al. Neoplasma. 2013;60:666-675. 7. Souers A, et al. Nat Med. 2013;19:202-208. 8. Pan R, et al. Cancer Discov. 2014;4:362-375. 9. DiNardo C, et al. ASH 2015. Abstract 327.

Phase Ib Study: Frontline Venetoclax + HMAs in Elderly AML Pts

- Open-label, nonrandomized, 2-arm, 2-stage study



*In each arm, 1 cohort received venetoclax 400 mg and 2 cohorts received 800 mg.

- Endpoints
  - Safety: MTD, DLTs, RP2D, AEs, early deaths, PK
  - Efficacy: ORR per IWG AML criteria, response duration, TTP, PFS, OS, MRD (assessed after cycles 1 and 4, then every 12 weeks)
  - Exploratory: mutational profiling and BCL-2 characterization, molecular markers, ex vivo testing of pt samples

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

Frontline Venetoclax + HMAs in Elderly AML Pts: Best Response

- 30/34 pts had bone marrow assessment at end of cycle 1
  - > 50% reduction in bone marrow blasts: 28 (93%)
  - CR/CRi: 24 pts; median time to CR/CRi: 29.5 days (range: 24-112)

Best Response, %	Venetoclax/Decitabine		Venetoclax/Azacitidine		ITT Response (N = 34)
	400 mg (n = 6)	800 mg (n = 12)	400 mg (n = 4)	800 mg (n = 12)	
ORR (CR/CRi/PR)	50	83	100	75	76
CR	33	17	75	42	35
CRi	17	50	25	33	35
PR	0	17	0	0	6
MLFS	0	8	0	0	3
RD	17	8	0	17	12
Not evaluable	33	0	0	8	9

- Median days on study: 106.5 (range: 6-305)

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

Frontline Venetoclax + HMAs in Elderly AML Pts: Conclusions

- Combination venetoclax with decitabine or azacitidine tolerable, similar safety profile in both treatment arms
  - No TLS or DLTs
- No effect of decitabine or azacitidine on venetoclax exposure in early pharmacokinetic data
- Early CR/CRi observed across all treatment cohorts and arms as compared with HMA alone
- MTD not reached in either arm; dose escalation ongoing
- Study will progress to expansion stage
- Alternative venetoclax schedule to address dose delays due to neutropenia

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

Investigational therapy ABT199 (Venetoclax)

A Phase 1/2 Study of ABT-199 in Combination with Low-Dose Cytarabine in Treatment-Naïve Subjects with Acute Myelogenous Leukemia Who Are ≥ 65 Years of Age and Who Are Not Eligible for Standard Anthracycline-Based Induction Therapy

Oral Comunication
To be presented at ASH San Diego 2016

abbvie

ABT-199
M14-387 Protocol
EudraCT 2014-002610-23

Phase 1/1b Study of RG7388, a Potent MDM2 Antagonist, in Acute Myelogenous Leukemia (AML) Patients (Pts)

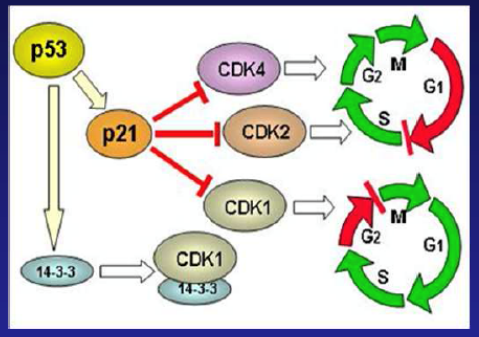
Karen Yee[1], Giovanni Martinelli[2], Norbert Vey[3], Michael J. Dickinson[4], Karen Seiter[5], Sarit Assouline[6], Mark Drummond[7], Sung-Soo Yoon[8], Margaret Kasner[9], Je-Hwan Lee[10], Kevin R. Kelly[11], Steven Blotner[12], Brian Higgins[12], Steven Middleton[12], Gwen Nichols[12], Gong Chen[12], Hua Zhong[12], William E. Pierceall[12], Jianguo Zhi[12] and Lin-Chi Chen[12]

[1]Princess Margaret Hospital, Toronto, Canada; [2]Seràgnoli Institute of Hematology, Bologna University School of Medicine, Bologna, Italy; [3]Hematology Department, Institut Paoli Calmettes, Marseille, France; [4]Department of Haematology, Peter MacCallum Cancer Centre, Melbourne, Australia; [5]New York Medical College, Valhalla, NY; [6]Division of Hematology, Jewish General Hospital, McGill University, Montreal, QC, Canada; [7]Beatson West of Scotland Cancer Centre, Gartnavel General Hospital, Glasgow, Scotland; [8]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea; [9]Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; [10]Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [11]Cancer Therapy and Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX; [12]Roche Innovation Center New York, Roche Pharma Research & Early Development, New York, NY

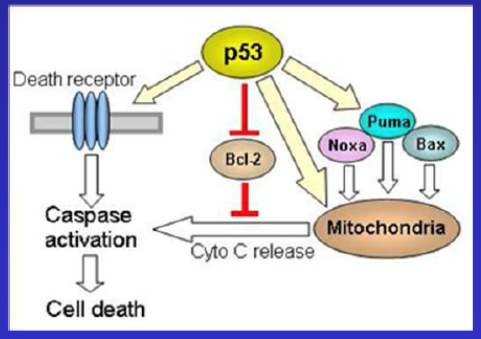
Rationale for Using MDM2 Antagonist RG7388

Activate p53, block proliferation and induce apoptosis

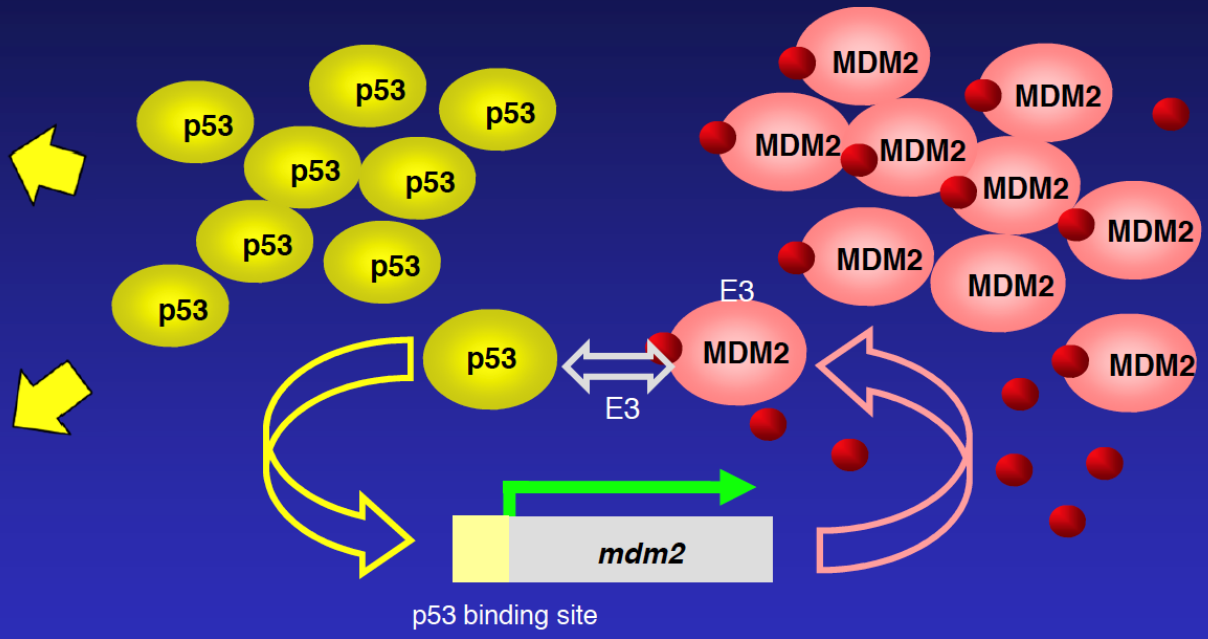
Cell cycle arrest



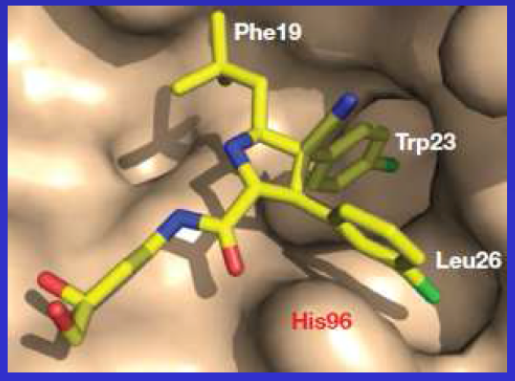
Apoptosis



The p53-MDM2 loop



RG7388

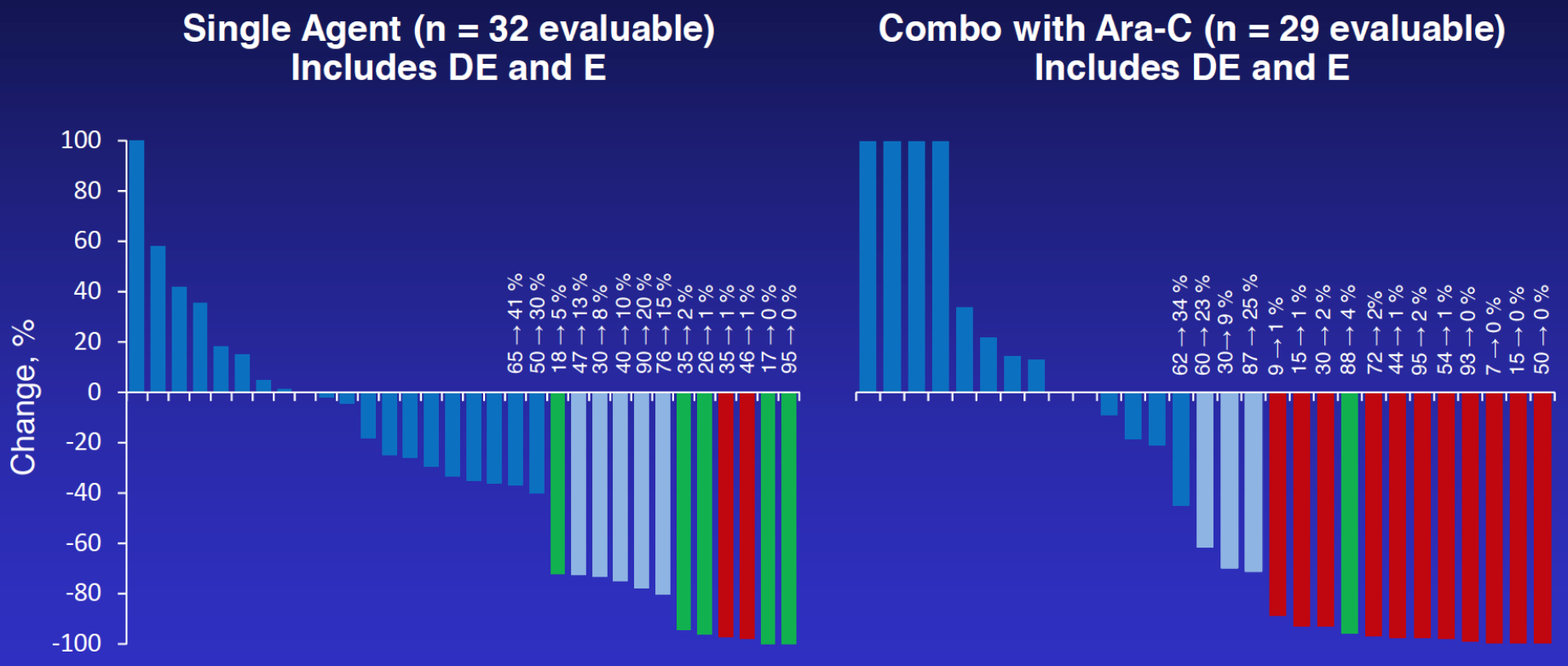


Vassilev LT, et al. *Trends Mol Med.* 2007; Ding Q, et al. *J Med Chem.* 2013.

RG7388 AML Phase 1/1b Responses

Change in bone marrow blasts from baseline

Response assessment*: CR CRi / MLFS PR

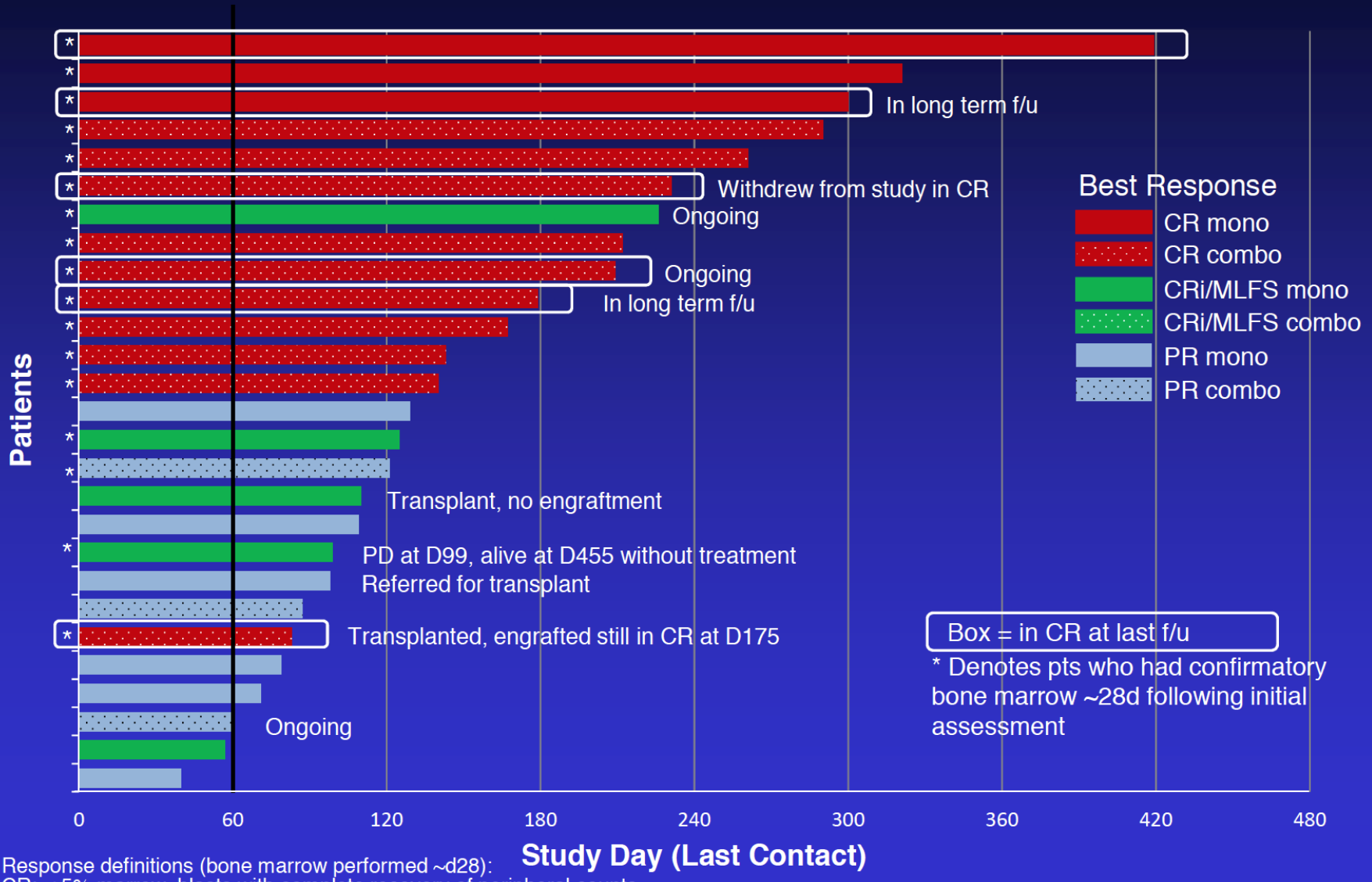


Response definitions:
CR: < 5% marrow blasts with complete recovery of peripheral counts
CRi/MLFS: < 5% marrow blasts with incomplete/no recovery of peripheral counts
PR: > 50% decrease in marrow blasts

*All bone marrow assessments performed at d28 or later except for one patient each in the single agent and combination therapy arms.

RG7388 AML Phase 1/1b Responses on Study

All CRs confirmed ~28d following initial CR assessment



Response definitions (bone marrow performed ~d28):
CR: < 5% marrow blasts with complete recovery of peripheral counts.
CRi/MLFS: < 5% marrow blasts with incomplete/no recovery of peripheral counts.
PR: > 50% decrease in marrow blasts.

Summary of RG7388 +/- Ara-C in AML

- RG7388 was tolerated as both monotherapy and in combination with Ara-C
- ORR (CR + CRp + CRi/MLFS)
  - Monotherapy: 7/46 (15%)
  - Combination with Ara-C: 12/42 (29%)
- Rapid and durable responses to treatment with RG7388 were seen
  - Most responses occurred after one cycle of therapy
  - Patients who achieved a CR had a confirmatory assessment ~28d following initial CR
- A gene signature may potentially identify patients more likely to respond to RG7388 containing therapy

+ Idasanutlin (MDM2)
+ Venetoclax (BCL2)
+ Cobimetinib (MAPK1 Inhibitor)

phase 1a/1b, multiple-arms, multicenter, open-label, dose findings

Patient population
- AML
- R/R multiple lines
- Age >=18
- ECOG =<2

→

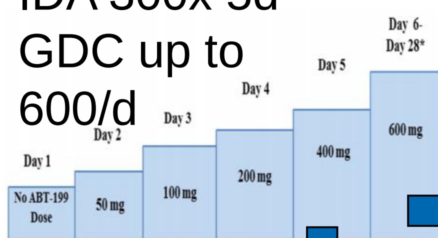
Venetoclax +Idasanutlin
or
Venetoclax+Cobimetinib

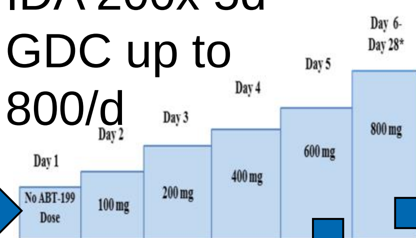
Relapsed refractory
First-line >65 y or unfit (all ages)

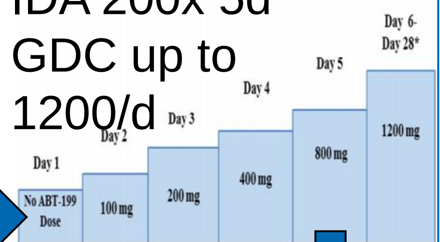
- ***Phase I – safety, feasibility+ secondary endpoint: efficacy, biomarker of response, MRD evaluation.***
- ***Recruitment Ongoing***

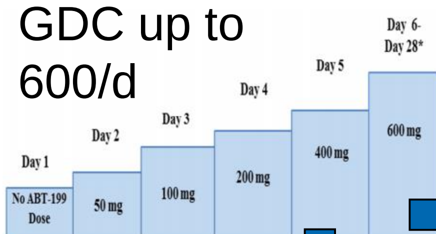
Dose Escalation Cohorts

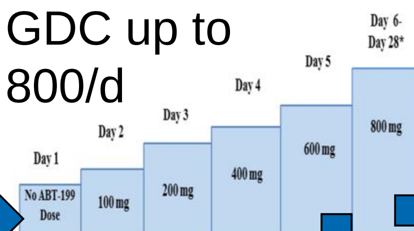
Dose escalation	GDC-0199 600 mg	GDC-0199 800 mg	GDC-0199 1200 mg
Idasanutlin 200 mg	IDA 300x 5d GDC up to 600/d	IDA 200x 5d GDC up to 800/d	IDA 200x 5d GDC up to 1200/d
Idasanutlin 300 mg	IDA 300x 5d GDC up to 600/d	IDA 300x 5d GDC up to 800/d	IDA 300x 5d GDC up to 1200/d
Idasanutlin 400 mg	IDA 400x 5d GDC up to 600/d	IDA 400x 5d GDC up to 800/d	IDA 400x 5d GDC up to 1200/d

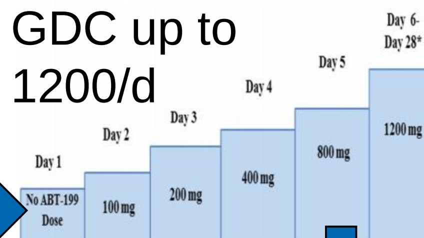


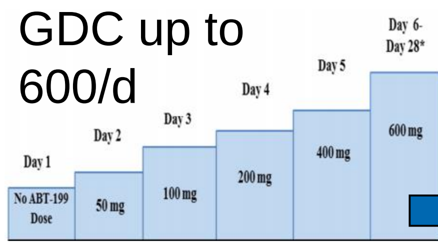


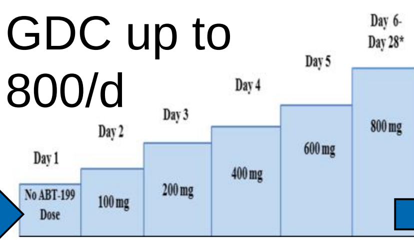


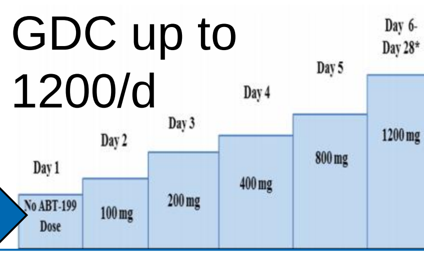












Conclusion.

- **Venetoclax alone or in combination is very active in CLL, AML, and MM.**

- **Venetoclax is active on synthetic lethal mechanism that has to be better explored to improve efficacy and specificity on leukemia stem cells.**

- **Clinical trial with combination of Venetoclax with Idasanutlin are extremely promising as new frontier of leukemia therapy**

Acknowledgments

Supported by: FP7, European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna

Acknowledgments

Hematology/Oncology University of Bologna:

Manuela Mancini
Caterina De Benedittis
Luca Zazzeroni
Cristina Papayannidis
Giovanni Martinelli

Personal Genomics & University of Verona:

Massimo Delledonne
Alberto Ferrarini
Elisa Zago
Marianna Garonzi
Anna Scandola

Department of Hematology and Hemostaseology, Medical University of Vienna:

Peter Valent
Sabine Cerny-Reiterer

U.O.Corelab- Hematology Cesena:

Michela Rondoni

SEVENTH FRAMEWORK PROGRAMME

NEXT GENERATION SEQUENCING for Targeted Personalized Therapy of Leukemia

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