BCL2 inhibition in CLL:

From Undruggable Target to Standard of Care

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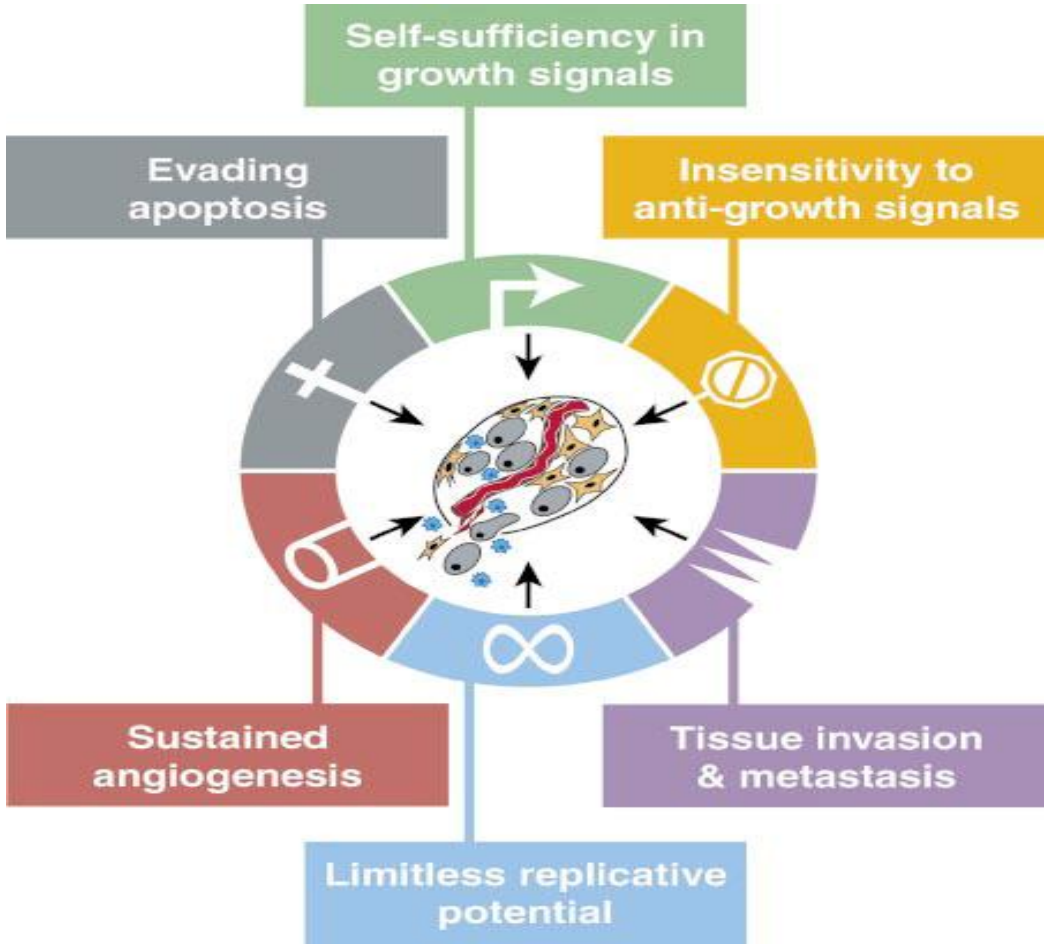
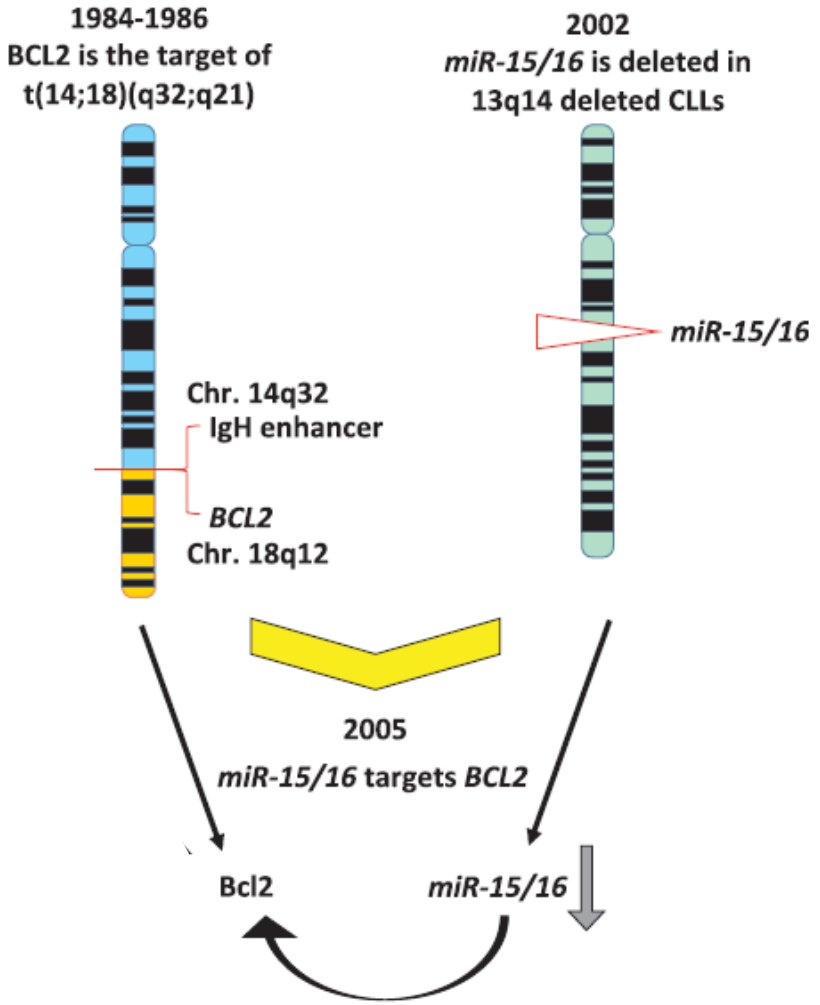
Apoptosis

- A hallmark of cancer is the development of ways to avoid apoptotic cell death
  (Hanahan and Weinberg, Cell, 2000, 2011)



Mechanisms of BCL-2 dysregulation in CLL vs FL

Follicular Lymphoma



CLL

Peransky, Balatti & Croce. Cell Death Diff 2017

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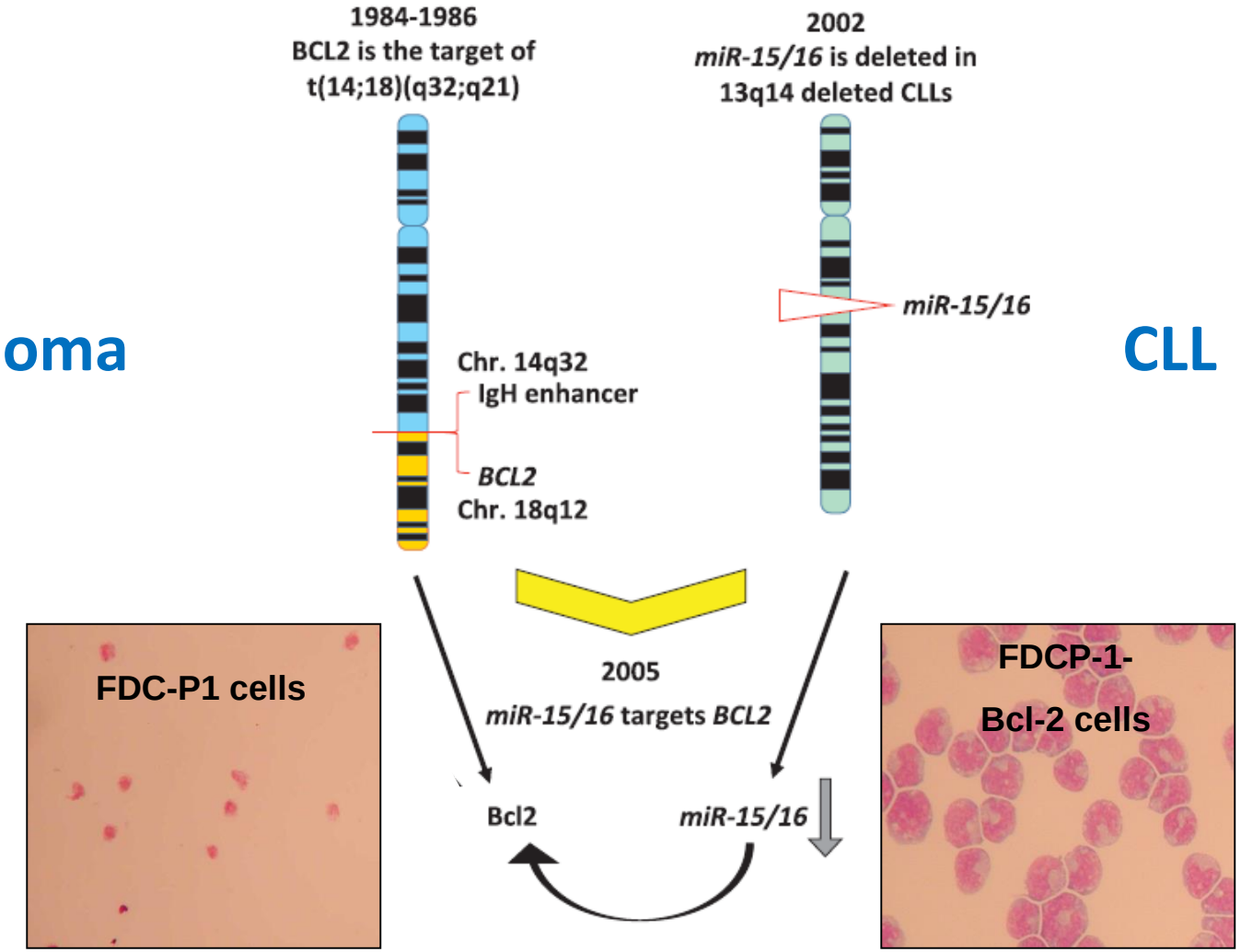
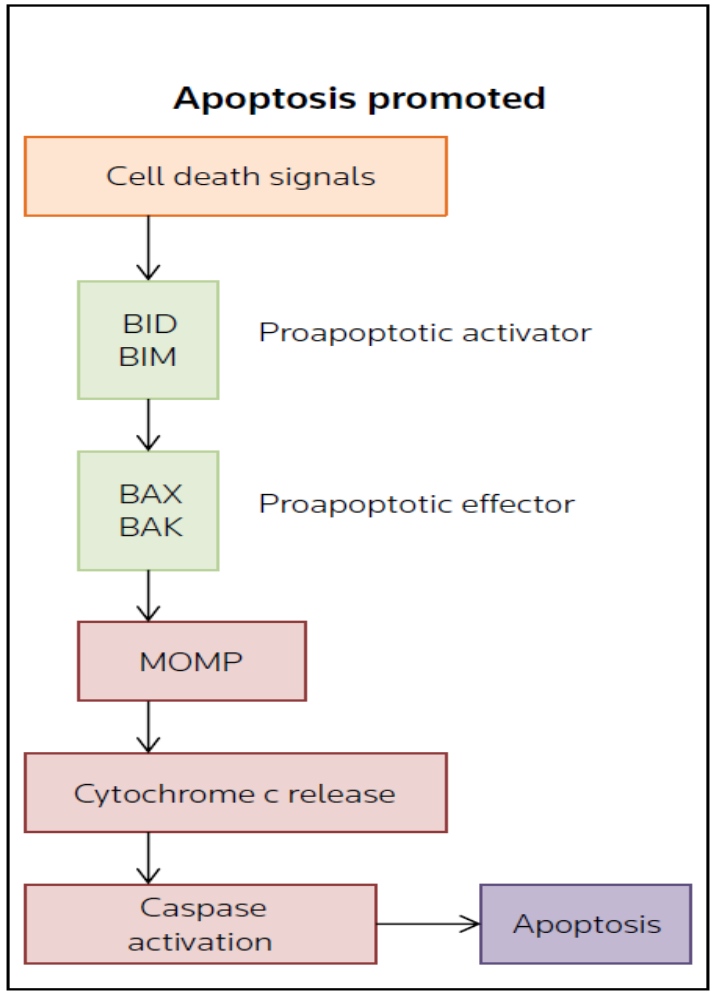


CLL

Vaux *et al*, Nature 1988: BCL2 enhances cell survival.

Peransky, Balatti & Croce. Cell Death Diff 2017

Pro- and anti-apoptotic rheostat



Del Poeta et al. Drugs of Today. 2016;52(4):253.

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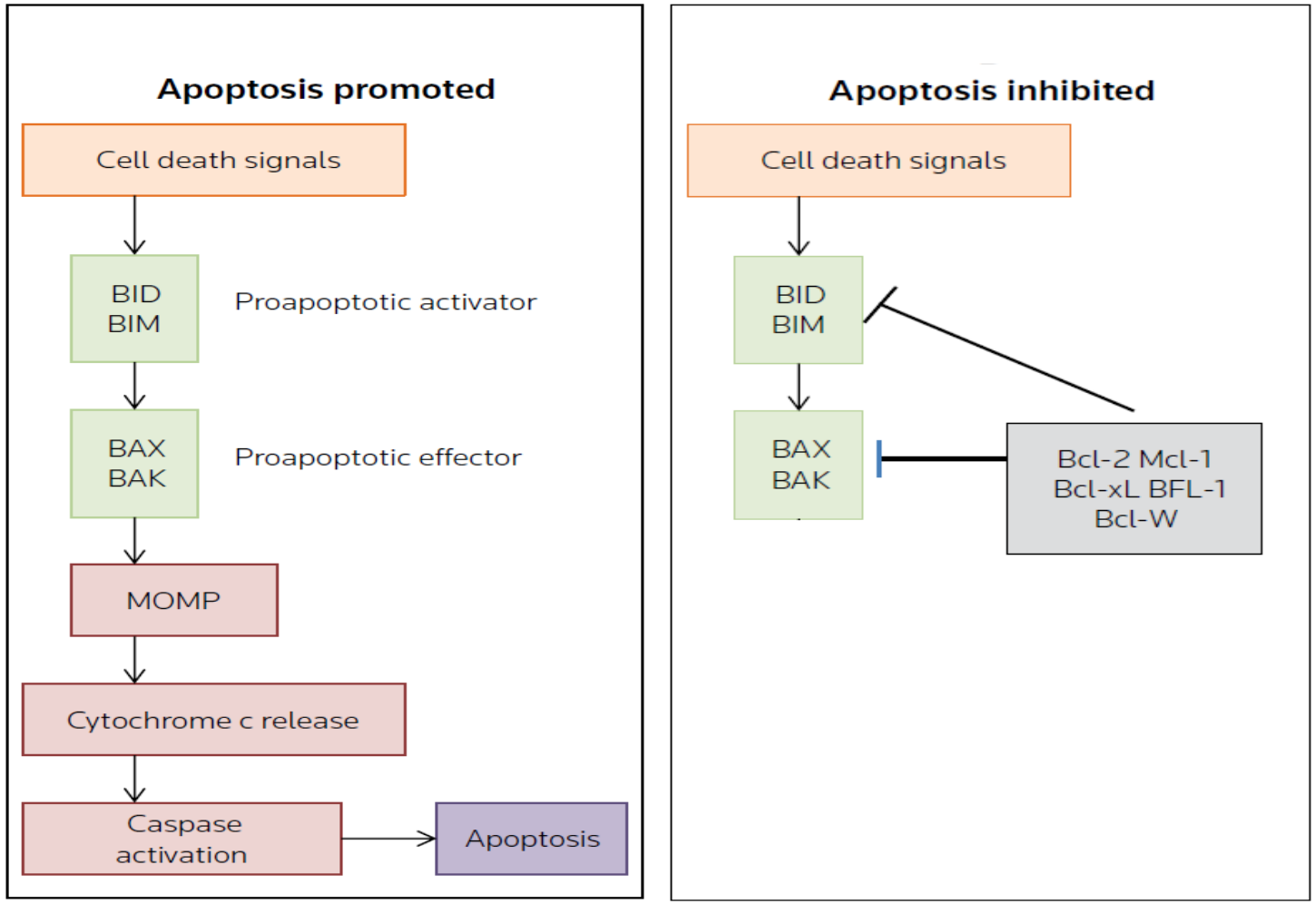

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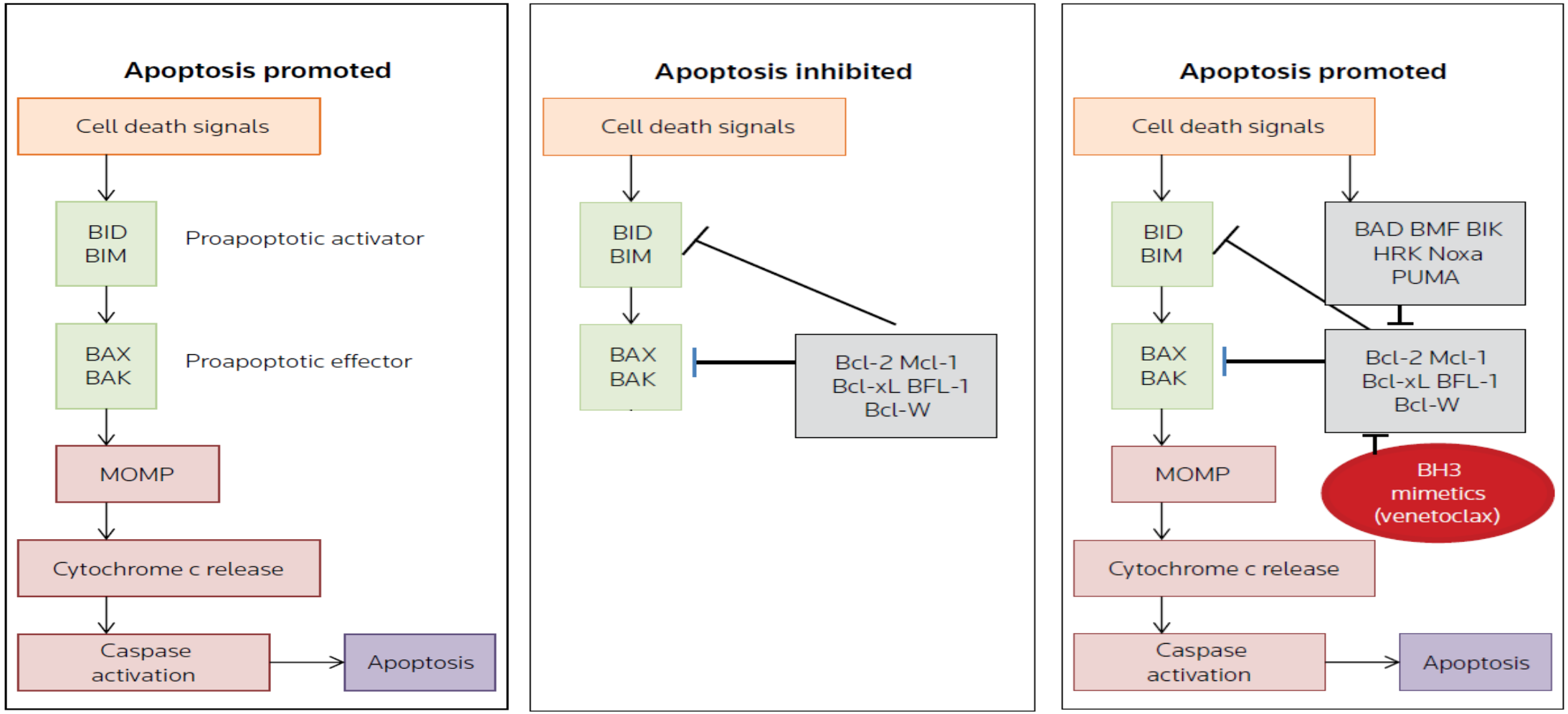
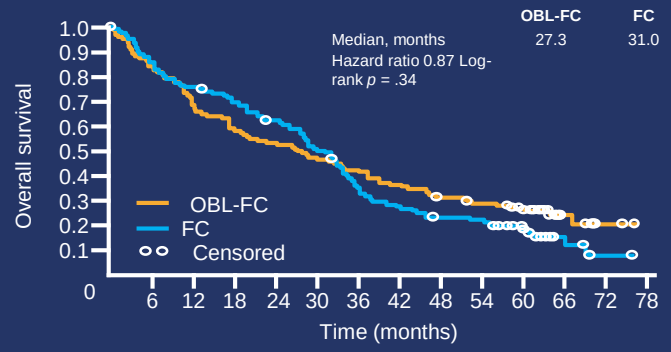


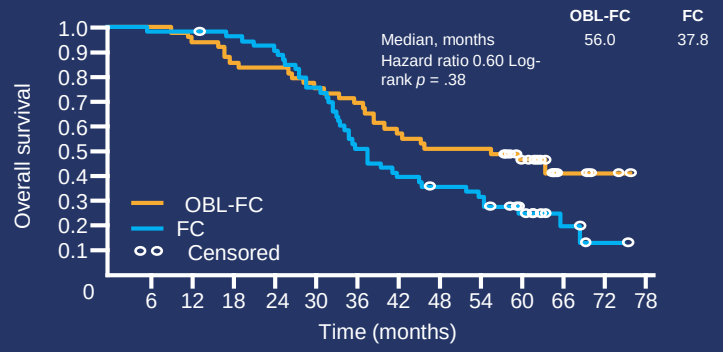
Del Poeta et al. Drugs of Today. 2016;52(4):253.

Targeting bcl-2 in CLL: Oblimersen

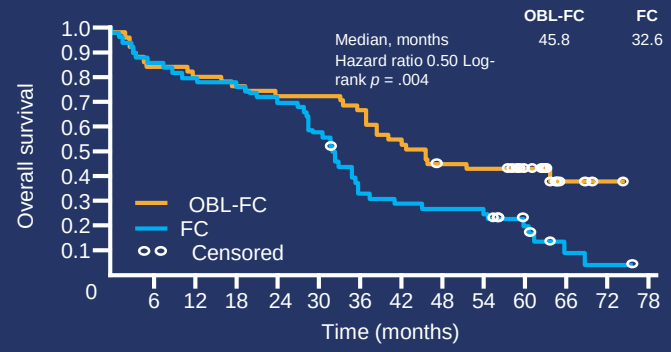
- Bcl-2 antisense 23mer oligonucleotide
- Toxic to CLL cells *in vitro*[1]
- Modest single-agent activity in CLL (8% PR, ↓ lymphocytes in 50% pts)[2] and FL[3]
- When added to FC attains statistically significant, though clinically modest, improvement in survival for some patients with R/R CLL (fludarabine sensitive, patients with CR/PR)[4]



All



Fludarabine sensitive



CR/PR

PR, partial response.

1. Pepper C, *et al*. *Br J Haematol* 1999; 107:611–615.
2. O'Brien S, *et al*. *J Clin Oncol* 2005; 23:7697–7702.
3. Waters JS, *et al*. *J Clin Oncol* 2000; 18:1812-1823.
4. O'Brien S, *et al*. *J Clin Oncol* 2009; 27:5208–5212.

A Plethora of Misleading BH3-mimetics Mol Cancer Ther 2016

The good

BH3 mimetic	Proposed targets	Induces NOXA in cells	Activates the ISR	Kills CLL cells *ex vivo*	Kills platelets *ex vivo*
ABT-737/ABT263	BCL2, BCLXL	No	No	Yes	Yes
ABT-199	BCL2	No	No	Yes	No
WEHI-539	BCLXL	No	No	No	Yes
A-1155463	BCLXL	No	No	No	Yes

The bad

Putative BH3 mimetics	**Proposed targets**	**Induces NOXA in cells**	**Activates the ISR**	**Kills CLL cells *ex vivo***	**Kills platelets *ex vivo***
Gossypol and AT-101	pan-BCL2	Yes	Yes	No	No
Apogossypol	pan-BCL2	Yes	Yes	No	nd
S1	pan-BCL2	Yes	Yes	No	No
HA14-1	BCL2	Yes	Yes	No	nd
2 methoxy antimycin A_3	BCL2, BCLXL	Yes	Yes	No	nd
Obatoclax (GX15-070)	pan-BCL2	Yes	Yes	No	nd
BXI-61	BCLXL	Yes	Yes	No	No
BXI-72	BCLXL	Yes	Yes	No	No
TW37	BCL2, BCLXL, MCL1	Yes	nd	nd	nd
MIM1	MCL1	Yes	Yes	No	nd
UMI-77	MCL1	Yes	Yes	No	nd

Abbreviations: ISR, integrated stress response; nd, not determined; N/A, R.S. Soderquist and A. Eastman unpublished observations.

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Req Bax / Bak

The bad

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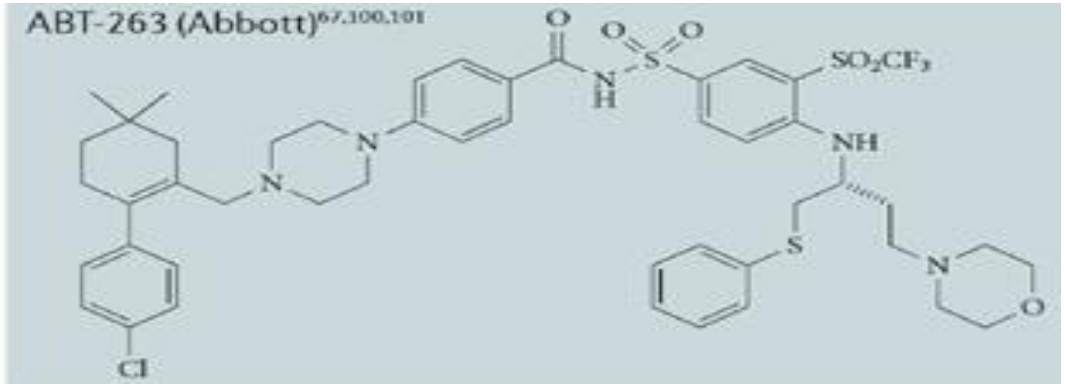
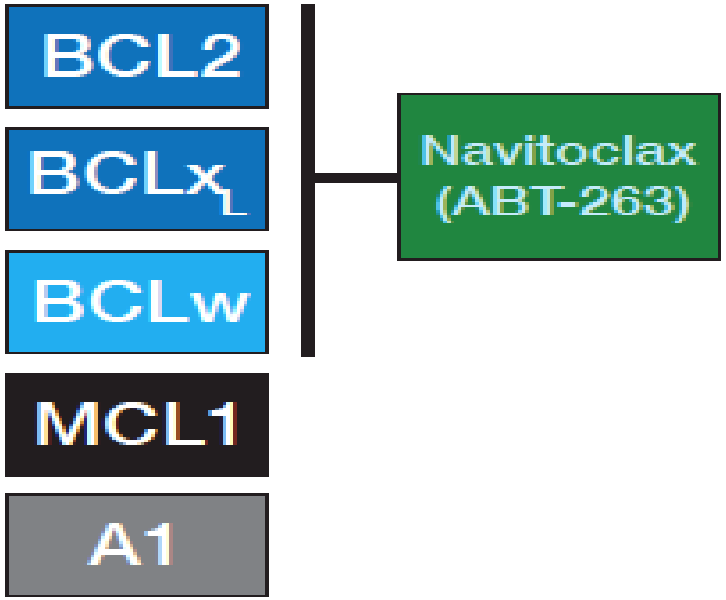
Navitoclax

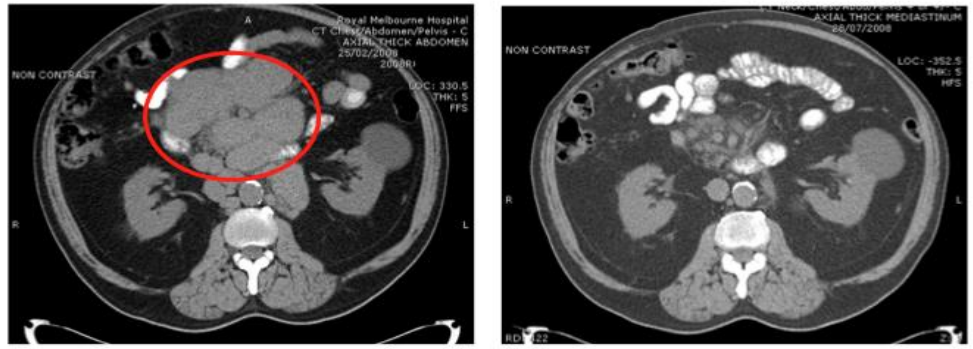


(Park, J Med Chem, 2008)



35% ORR as single agent in R/R CLL.

Dose limiting on-target thrombocytopenia (Roberts, JCO, 2012)

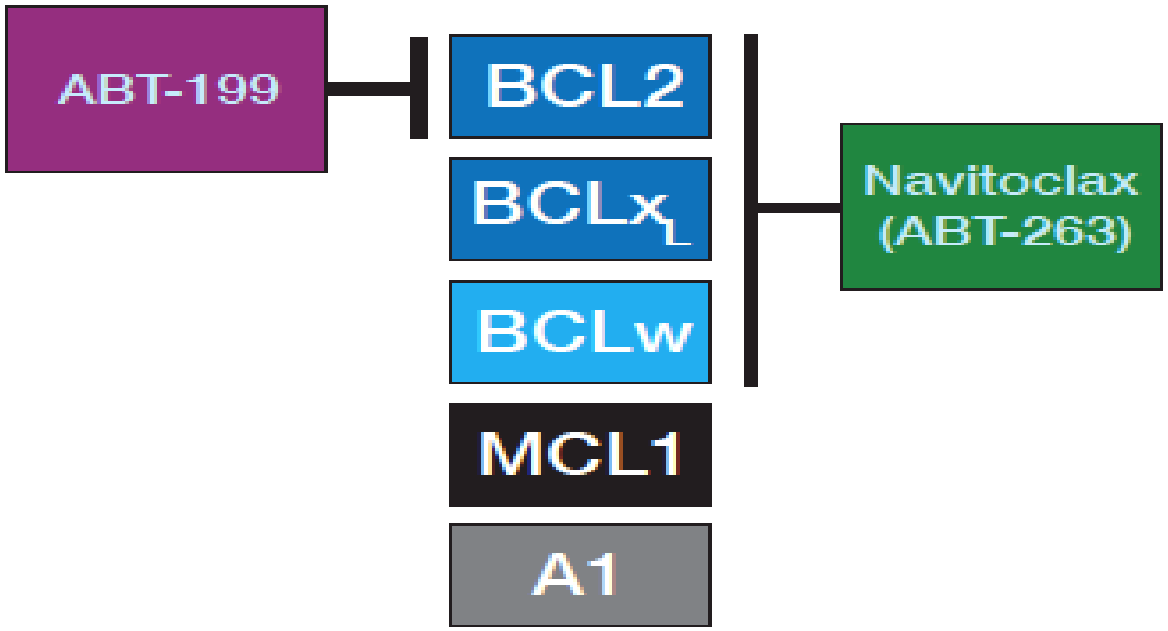


Pre-Treatment

7 Cycles ABT-263

(Images courtesy of Andrew Roberts)

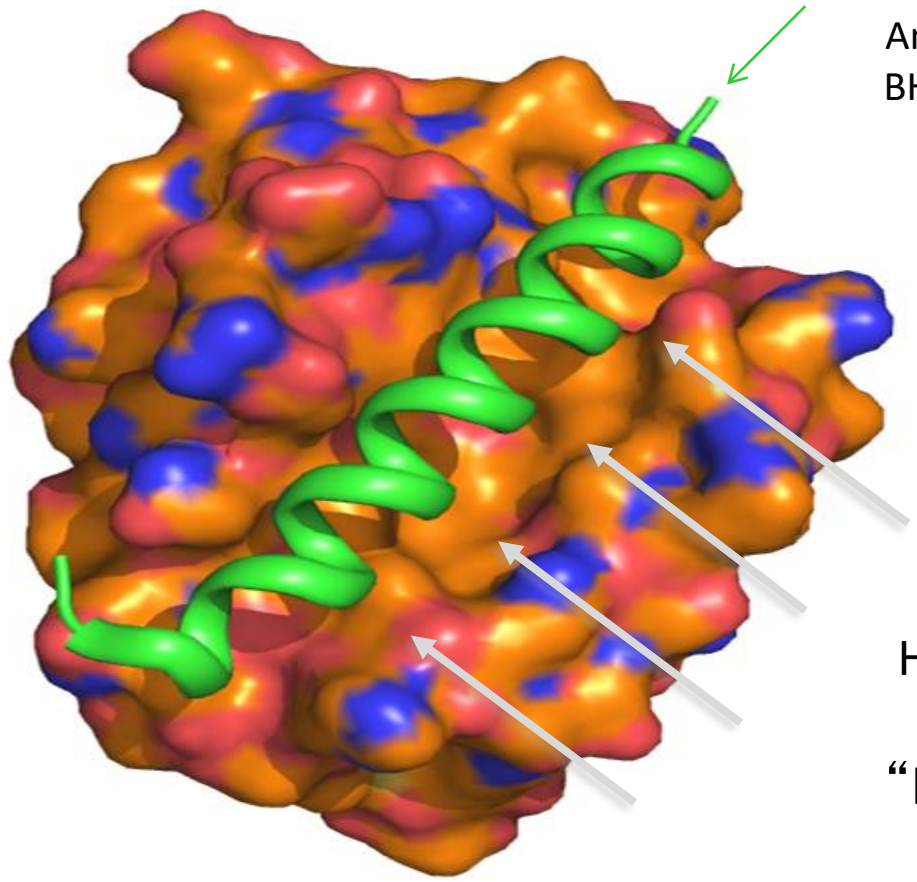
ABT-199 is a selective Bcl-2 inhibitor



(Anderson, et al; Semin Hematol; 2014)

The Challenges of Drugging Specific Protein-Protein Interactions

- Large, hydrophobic surface area – may necessitate large, greasy molecules (not drug-like)
- Protein structure is dynamic
- High-affinity interactions of natural binding ligands - requires pM affinity binders to compete adequately in cells
- BH3-binding grooves of BCL-2 and BCL-X_L are highly related
  - Only 4 amino acid residues differ



BAD BH3 motif bound to BCL-X_L

Amphipathic α-helix BH3 Domain "ligand"

Hydrophobic groove "binding site"

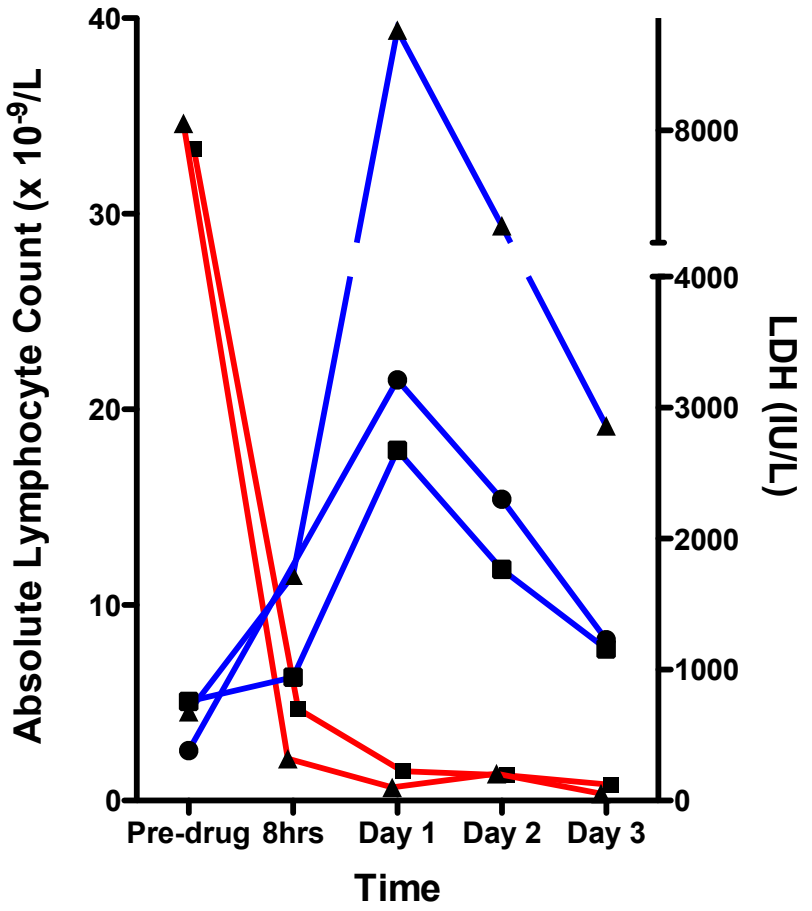
SA ~620 Å^2

NPSA ~440 Å^2

Souers, et al, Nat Med. 2013

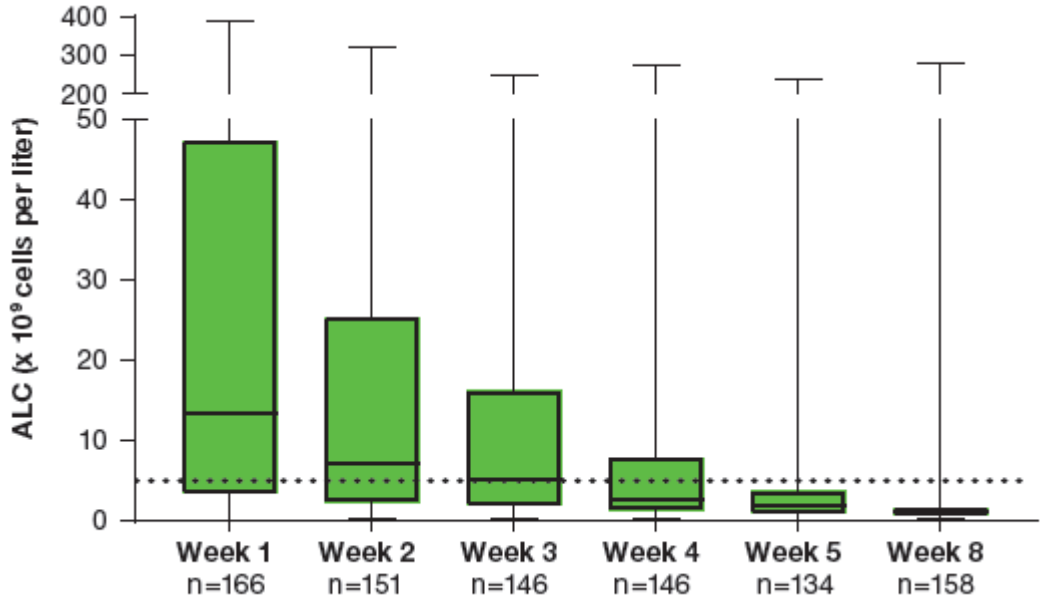
June 2011: Single Dose of ABT-199 Achieved Rapid Reduction of CLL Burden in Patients Enrolled in Cohort 1

- **ABT-199 200 mg, 200 mg, 100 mg given as single oral dose**
  - >95% reduction in lymphocytosis within 24h
  - Rapid reduction in palpable lymphadenopathy
  - Dose-limiting laboratory TLS:
    - ↑ LDH, phosphate
    - ↑ K^+ (max 5.9 mmol/L)
  - Daily dosing (50 – 100mg) resumed within 7 days



Lymphocyte Counts (Red; n = 2) and LDH (Blue; n = 3) post first dose

TLS Manifestations with Venetoclax in CLL



Ven mg/d: 20 50 100 200 400 →

Event, n (%)	N=168
TLS Adverse Event	4 (2)
Clinical TLS	0
Laboratory TLS*	1 (1)
Other reported TLS†	3 (2)
Fatal AE	0
Single laboratory abnormality AEs leading to dose interruptions:	
Potassium elevation	5 (3)
Phosphate elevation	3 (2)
Calcium decrease	0
Uric acid elevation	0

Analyte Event, n (%)		N=168
Potassium	>ULN	116 (69)
	>6 mmol/L	1 (1)
	Treated*	22 (13)
Phosphate	>ULN	121 (72)
	>1.45 mmol/L	78 (46)
	Treated*	25 (15)
Calcium	<LLN	148 (88)
	<1.75 mmol/L	8 (5)
	Treated*	11 (7)
Uric acid	>ULN	13 (8)
	>476 µmol/L	9 (5)
	Treated*	5 (3)

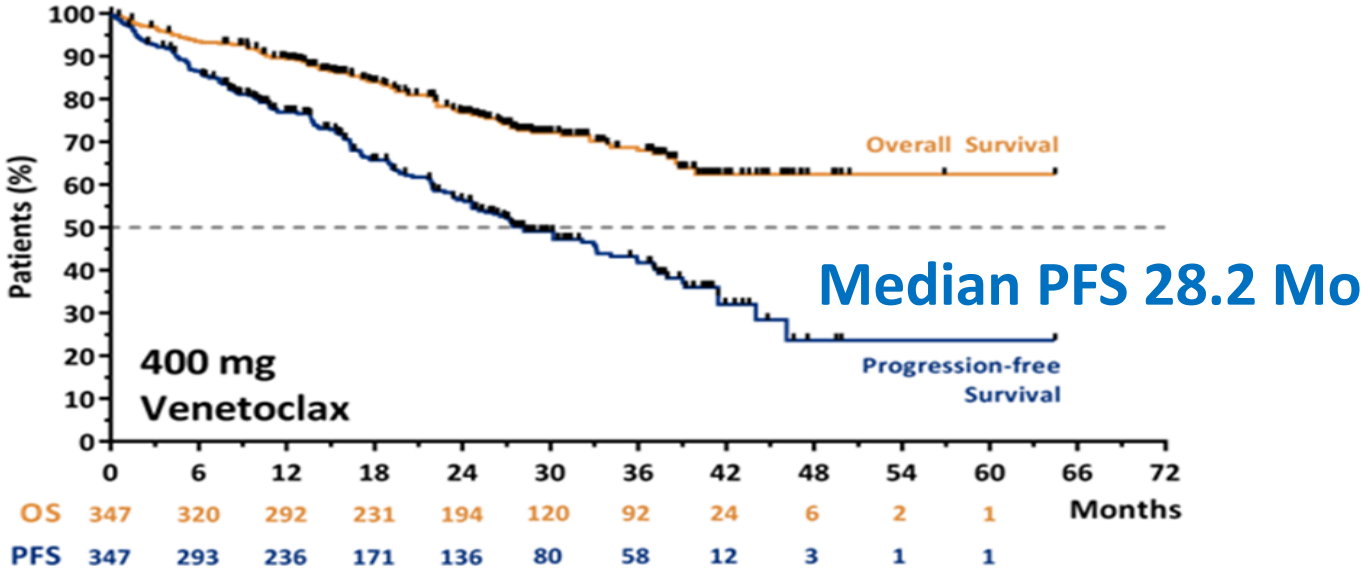
Davids M, et al. ASCO 2018

Continuous single agent venetoclax in R/R CLL

Aggregate 347 patients from 4 phase 1/2 studies; 42% prior BCRi

Median age 66 (28 – 85), median 3 prior therapies, 76% del(17p)/TP53mut

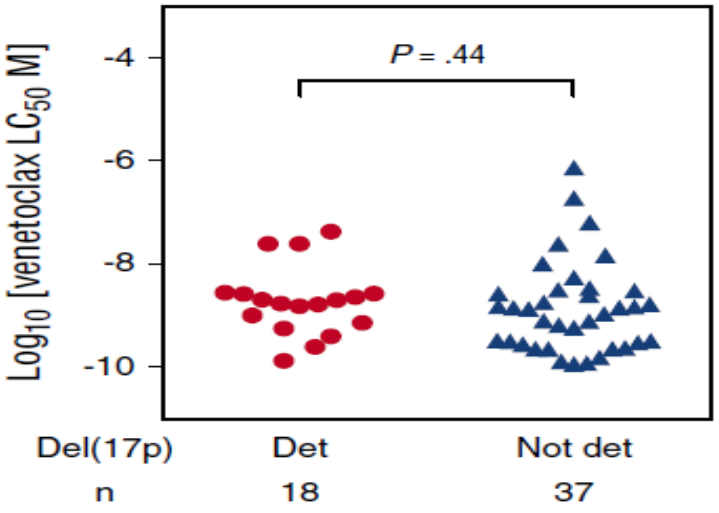
Median follow-up 28.8 months

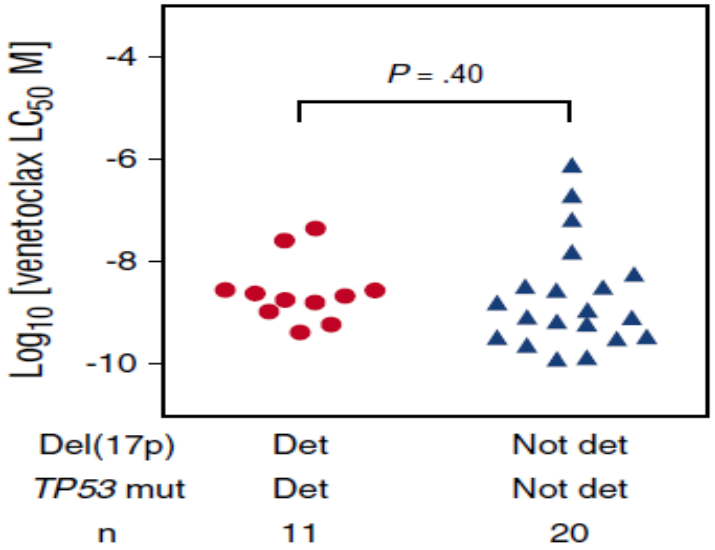


Roberts AW, *et al*. Blood 134:111-22, 2019

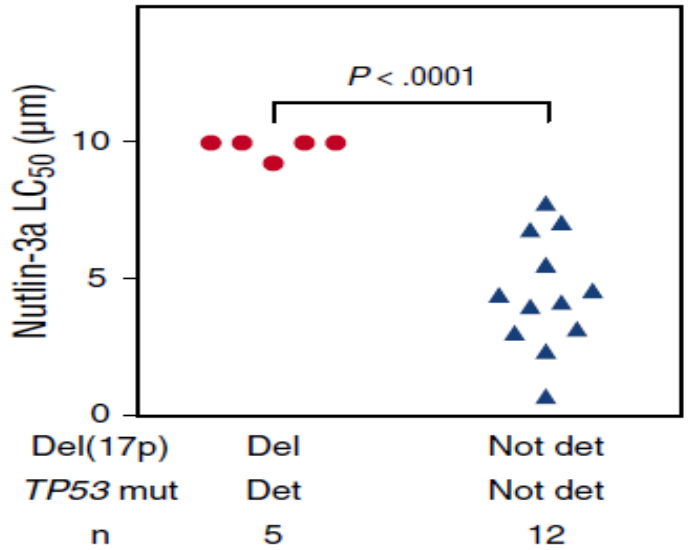
Venetoclax kills primary CLL cells independent of del(17p) or *TP53* mutational status

Venetoclax Sensitivity *in vitro*





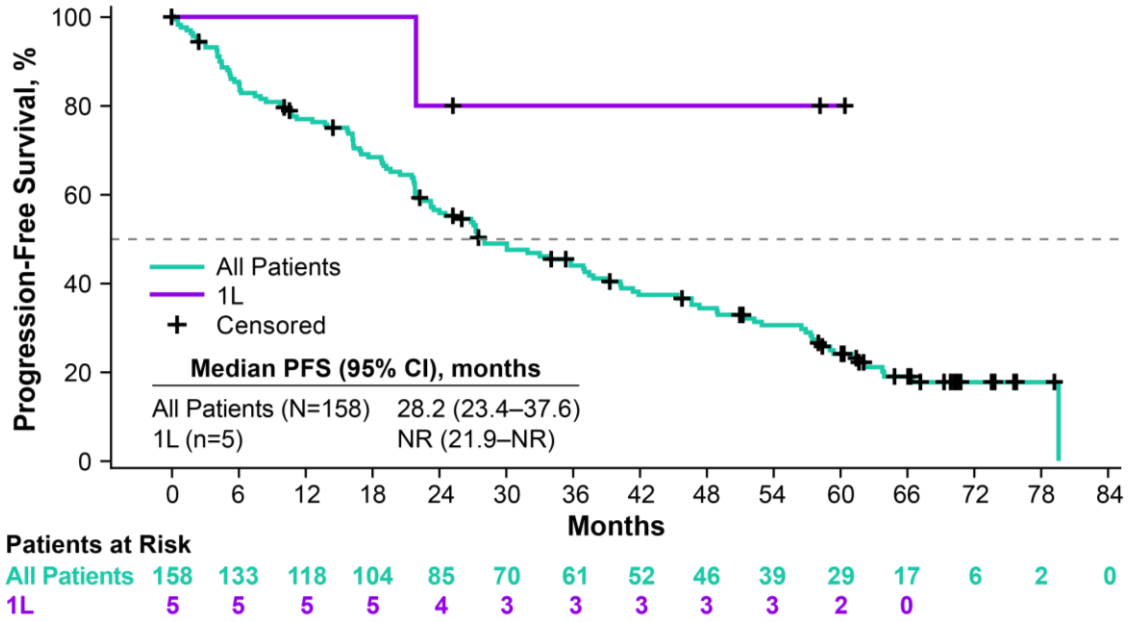
Nutlin Sensitivity



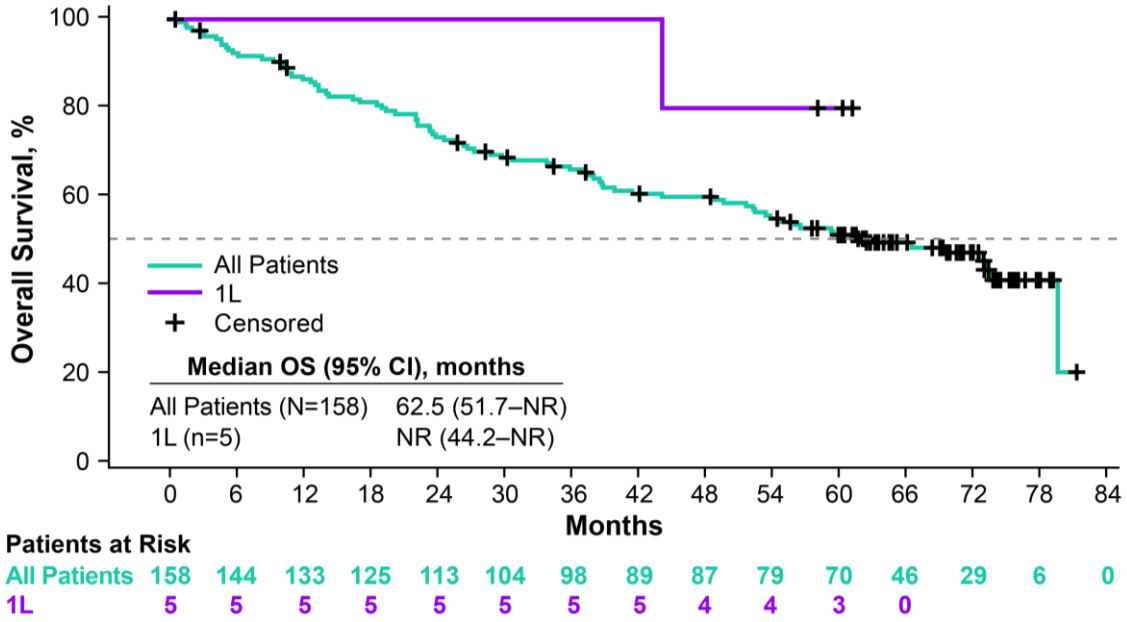
Anderson MA *et al* Blood 127:3215-24, 2016

Del(17p) Phase 2 continuous monotherapy study (median F/U 70 months)

Progression-Free Survival



Overall Survival



- In previously treated cohort, 5-year rate for PFS was 24% and OS was 52%

1L, first-line.

Stilgenbauer S, *et al*. Blood Adv 2024

Development timeline for Venclexta

Date	Article
May 15, 2019	Approval AbbVie Announces US FDA Approval of Venclexta (venetoclax) as a Chemotherapy-Free Combination Regimen for Previously Untreated Chronic lymphocytic Leukemia Patients
Jun 8, 2018	Approval Genentech Announces FDA Approval for Venclexta Plus Rituxan for People With Previously Treated Chronic Lymphocytic Leukemia
Apr 11, 2016	Approval FDA Approves Venclexta (venetoclax) for Chronic Lymphocytic Leukemia with 17p Deletion
Jan 12, 2016	FDA Grants Priority Review for Venetoclax New Drug Application
Dec 6, 2015	Pivotal Phase II Study Showed Nearly 80 Percent of People with Hard-to-treat Type of Chronic Lymphocytic Leukemia Responded to Investigational Medicine Venetoclax

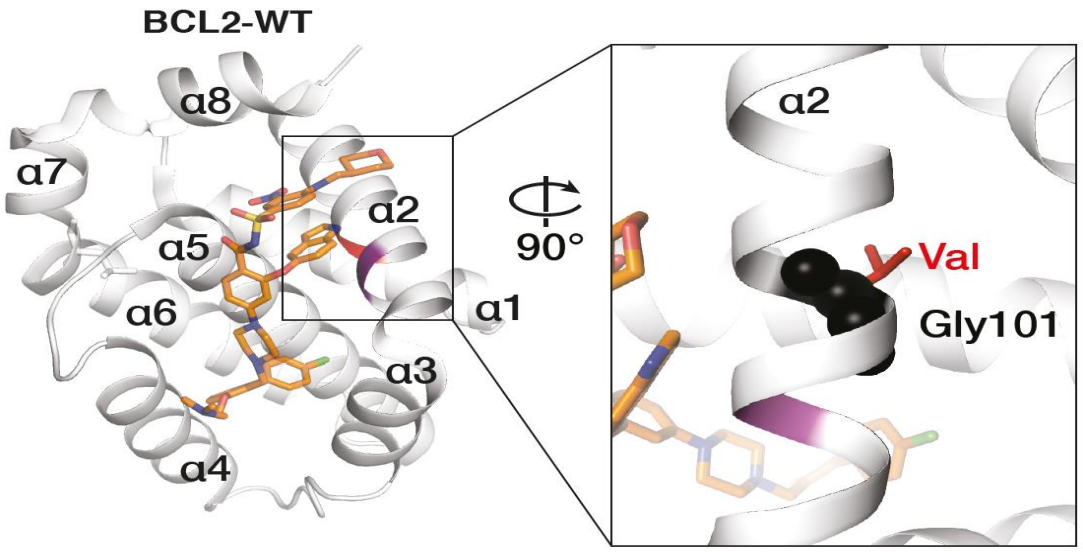
Mechanism of secondary Venetoclax resistance in CLL

Multiple *BCL2* mutations in 11 of 26 (42%) CLL progressions after median 36 (range 13 – 70) months

Detection of mutations preceded clinical PD by up to 25 months

Mutations lead to variably reduced drug sensitivity *via* abrogation of displacement of Bax from BCL2

Also examples of up-regulation of alternative BCL2 family members; BCL-X_L, MCL1



Blombery P, *et al*. Cancer Discov 2019

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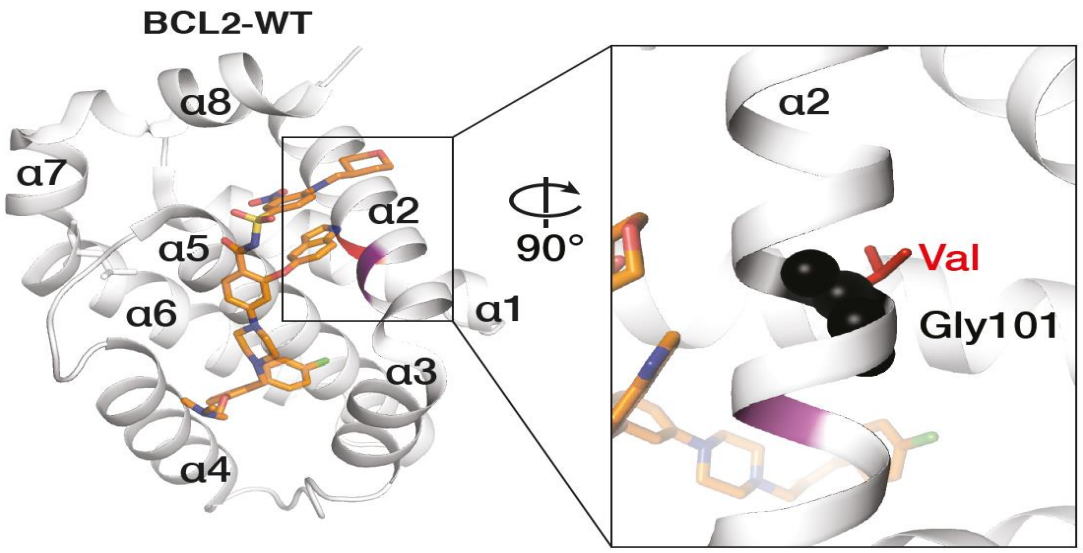
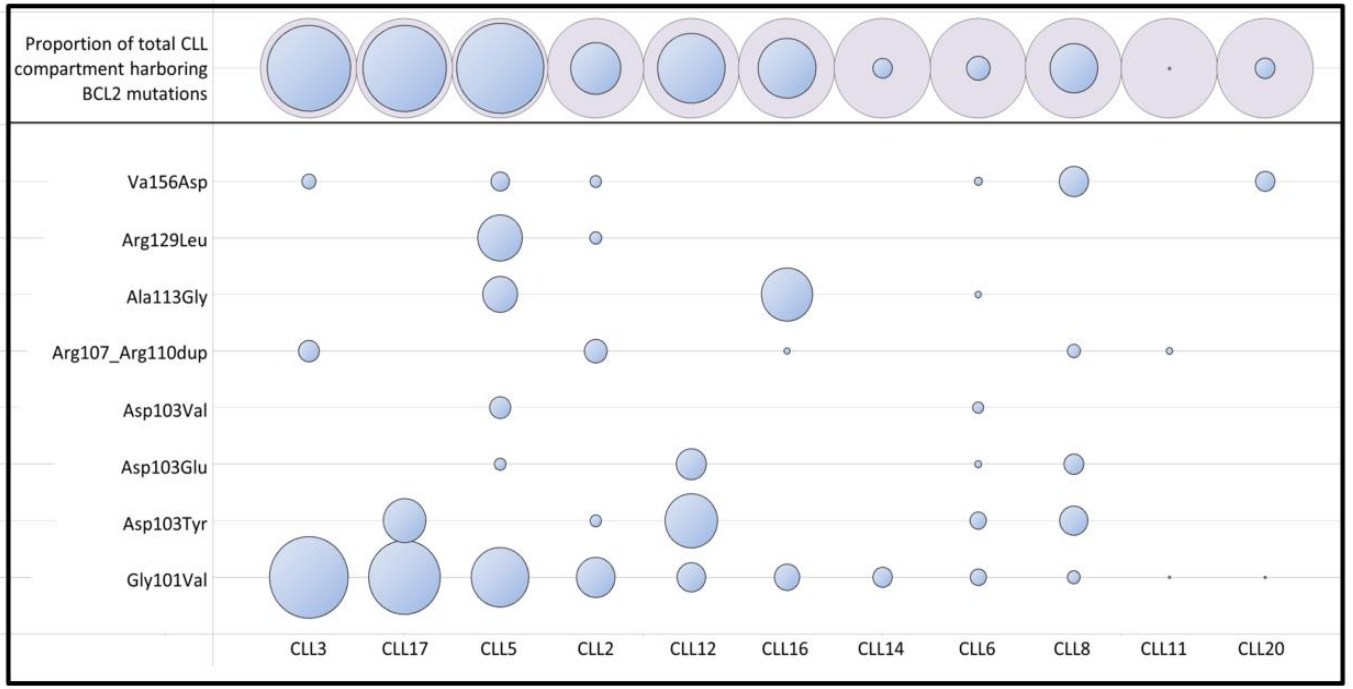




Blombery P, *et al*. Cancer Discov 2019

Blombery P, *et al*. Blood 2020

Potentially reversible up-regulation of MCL-1 via NFkB activation

Serial single-cell analysis of 13 pts with secondary resistance at least 24 months Ven, incl 4 on subsequent BTKi

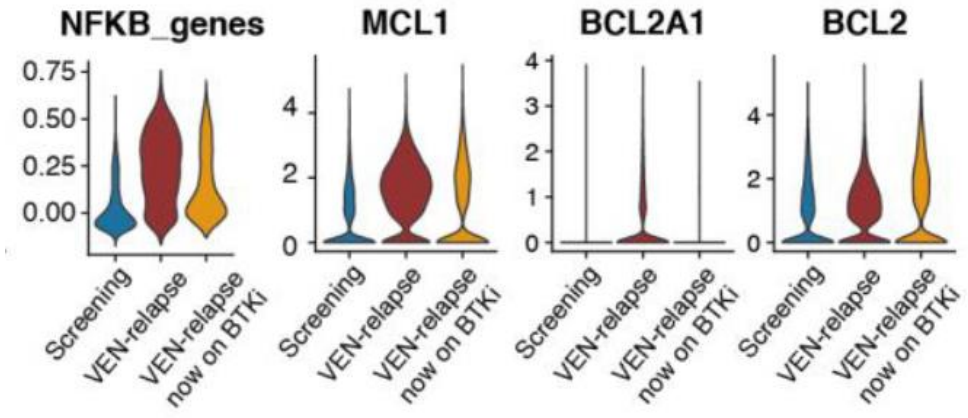
All had *MCL-1* over-expression, but gene amplification only present in 3

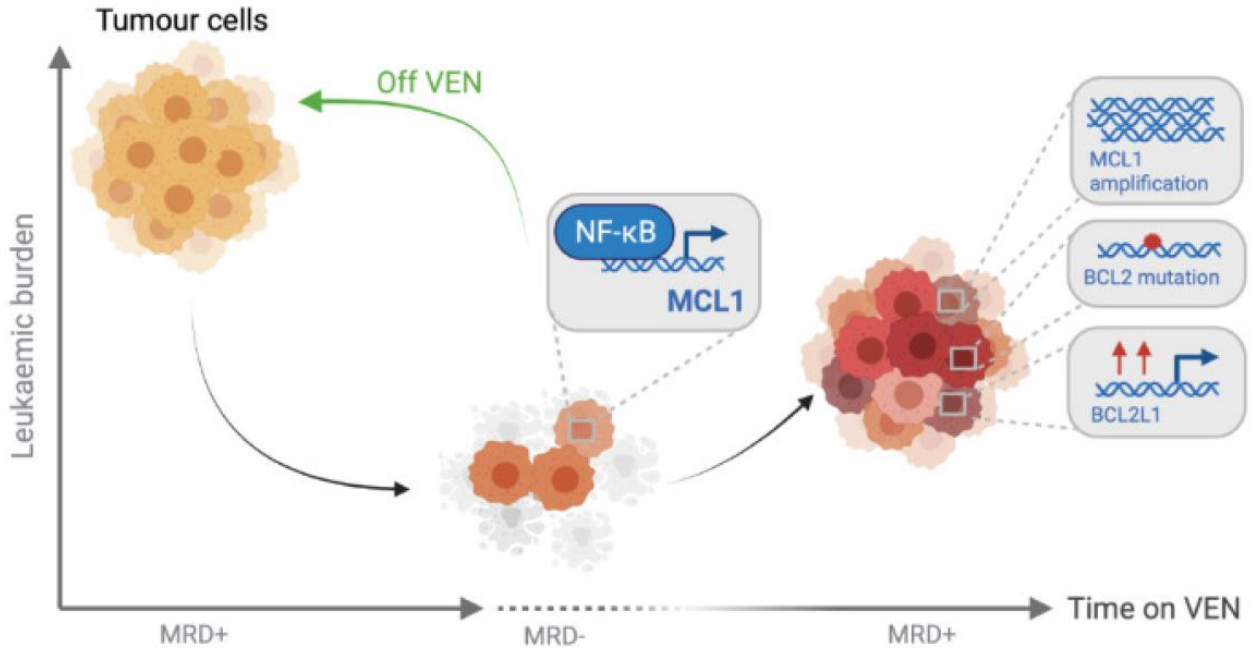
Transcriptional activation of *MCL-1* via NFkB as early as 6 Mo on Ven Rx

Reversion of transcriptional profile to "pre-Ven" in all 4 pts studied after withdrawal / on subsequent BTKi

Reversion of NFkB / MCL1 expression

CLL23



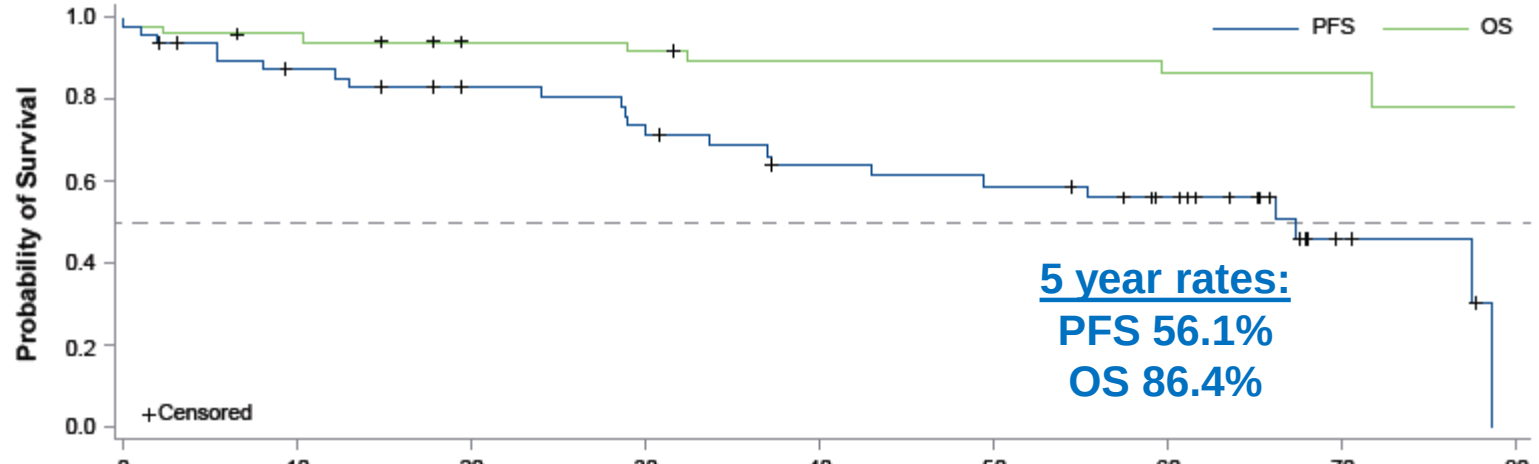


Thijssen R, *et al*. Blood 2022

Venetoclax & Rituximab in R/R CLL

n = 49, median age 68, median 2 prior therapies, 19% del(17p)

61% of patients attained BM uMRD
PFS: median F/U 5.3 years
19 pts ceased Ven after median 17 Mo; median 38.4 Mo off drug

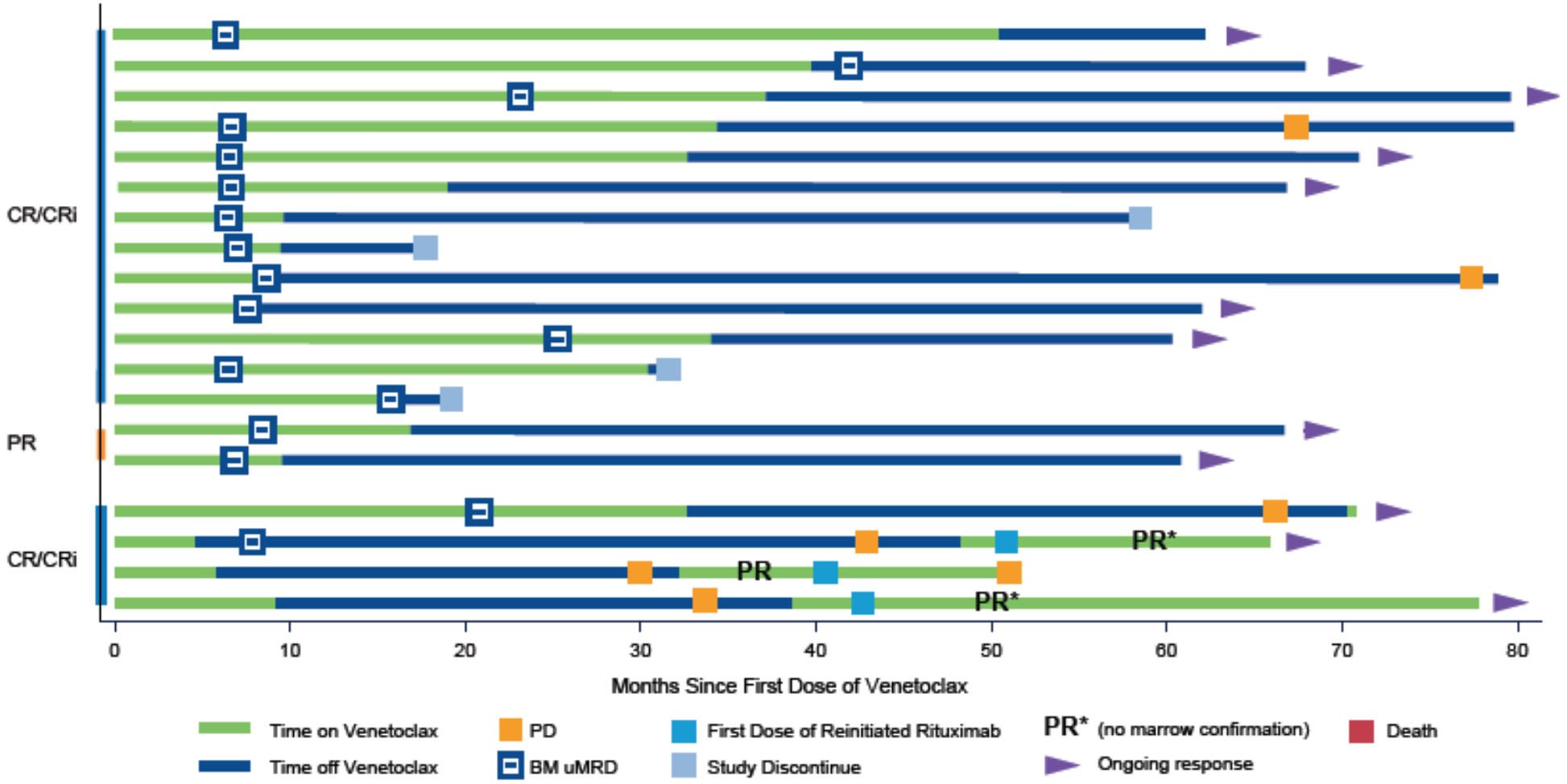


Patients at Risk									
PFS	49	40	35	30	25	23	8	4	0
OS	49	45	41	40	38	37	31	13	0

		Survival Estimate (95% CI)				
	Events	Month 24	Month 36	Month 60	Month 72	Median months (95% CI)
PFS (N=49)	23	80.7% (66.2, 89.5)	68.8% (52.9, 80.3)	56.1% (39.9, 69.5)	45.9% (27.8, 62.2)	67.3 (37.1, 78.6)
OS (N=49)	7	93.8% (82.0, 98.0)	89.2% (75.8, 95.3)	86.4% (71.9, 93.7)	78.5% (54.7, 90.8)	- (-, -)

Ma S, *et al.* Blood 138:836-46, 2021

Patient Disposition: Discontinued with Deep Response



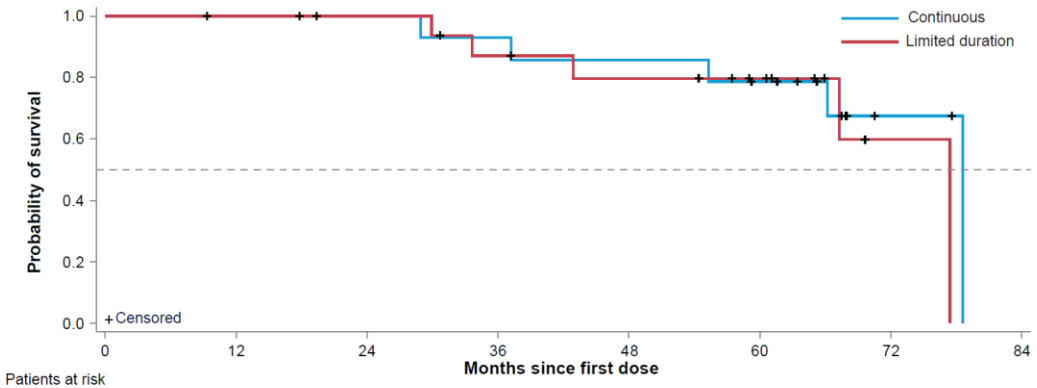
Ma S, *et al.* Blood 138:836-46, 2021

Venetoclax & Rituximab in R/R CLL

Continuous versus limited-duration treatment

33 pts achieved uMRD/CR and were eligible to cease drug;
14 continuous (2 *TP53abn*), 19 limited-duration (5 *TP53abn*)
Median total time on drug 5.6 vs 2.1 years

Progression-free survival

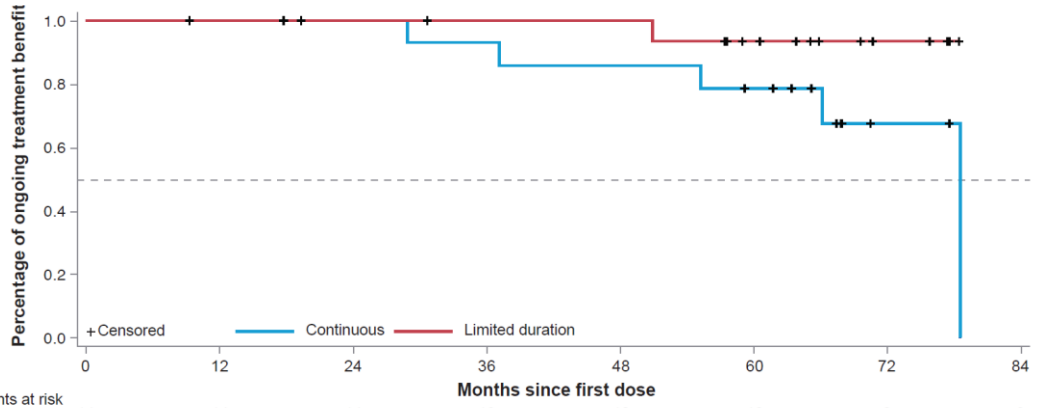


Patients at risk								
Continuous	14	14	14	13	12	10	2	0
Limited duration	19	18	16	13	11	8	1	0

Survival estimate (95% CI)

	Events	Month 24	Month 36	Month 60	Month 72	Median months (95% CI)
Continuous (N = 14)	5	100% (100–100)	92.9% (59.1–99.0)	78.6% (47.2–92.5)	67.3% (33.0–86.9)	78.6 (55.3–78.6)
Limited duration (N = 19)	5	100% (100–100)	87.1% (57.3–96.6)	79.8% (49.4–93.0)	59.8% (17.9–85.8)	77.4 (42.9–77.4)

Time-to-venetoclax failure



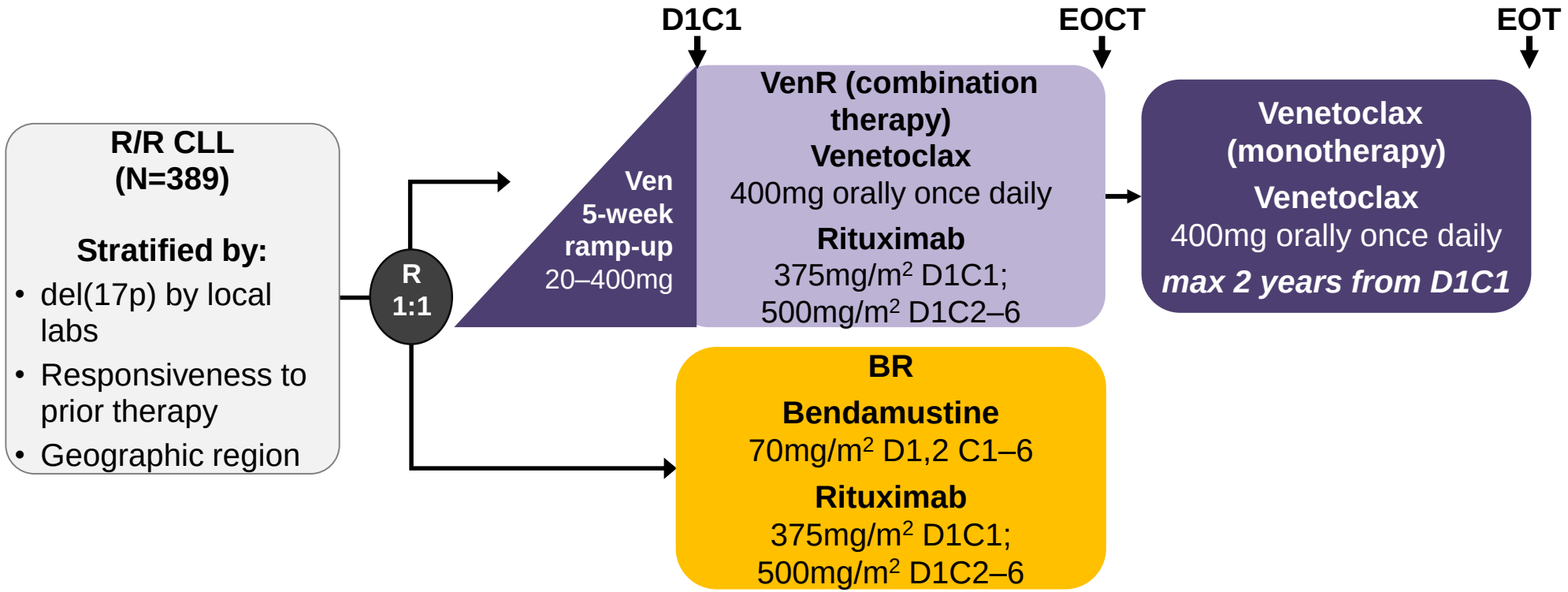
Patients at risk								
Continuous	14	14	14	13	12	10	2	0
Limited duration	19	18	16	15	15	11	4	0

Survival estimate (95% CI)

	Event	Month 24	Month 36	Month 60	Month 72	Median months (95% CI)
Continuous (N = 14)	5	100% (100–100)	92.9% (59.1–99.0)	78.6% (47.2–92.5)	67.3% (33.0–86.9)	78.6 (55.3–78.6)
Limited duration (N = 19)	1	100% (100–100)	100% (100–100)	93.3% (61.3–99.0)	93.3% (61.3–99.0)	NR (NR–NR)

Ma S, *et al.* Blood 138:836-46, 2021

MURANO study design

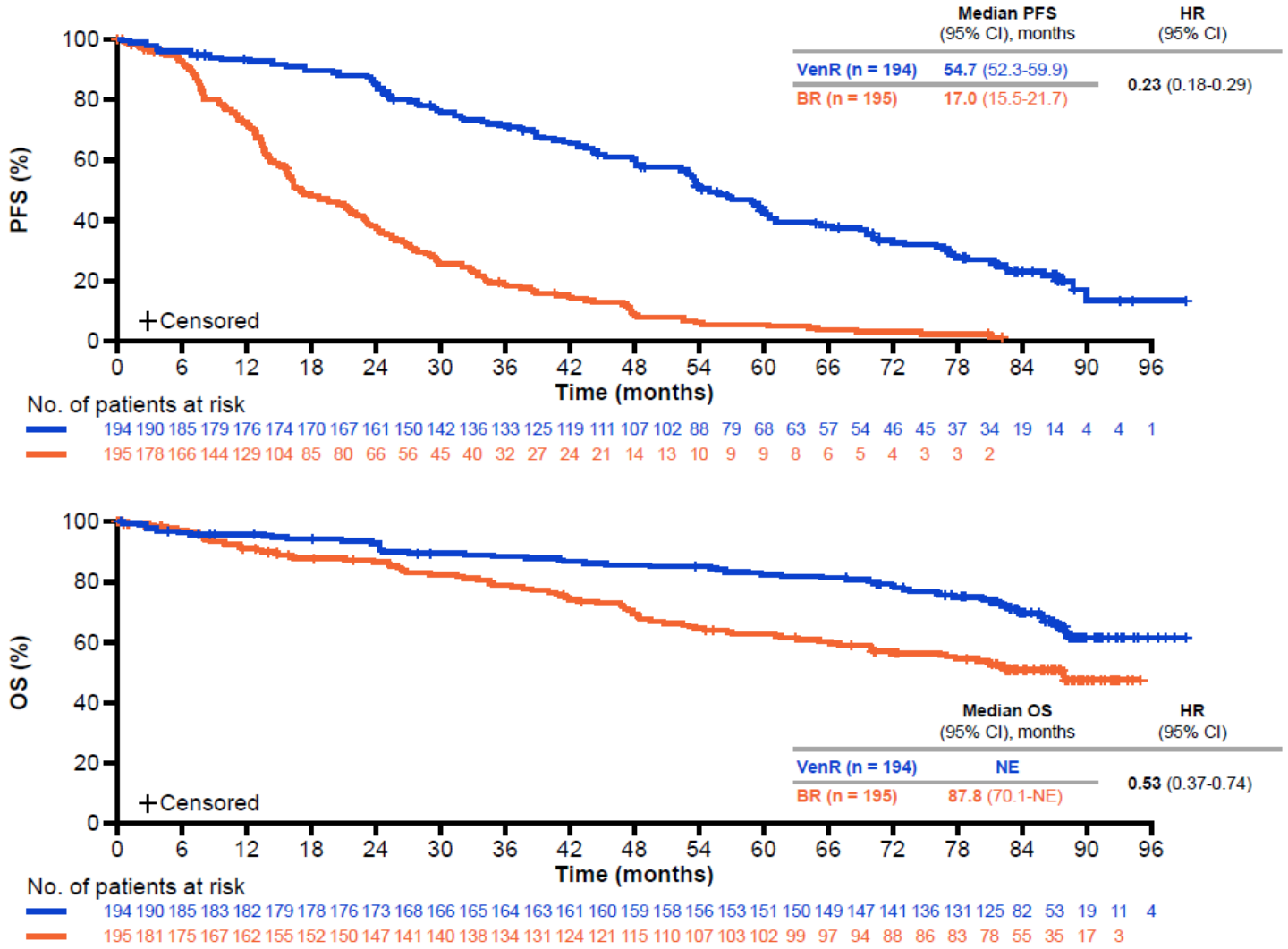


- **Primary endpoint:** investigator-assessed PFS
- **Secondary endpoint:** rates of clearance of MRD
- Clinical response and MRD* in PB during Ven monotherapy and follow-up visits were assessed every 3 months for 3 years, then every 6 months thereafter, or until PD

*Undetectable MRD defined as <1 CLL cell/10,000 leukocytes, determined by ASO-PCR or flow cytometry per iwCLL recommendations for reporting of MRD.
BR, bendamustine–rituximab; D1C1, day 1, cycle 1; D1C2-6, day 1, cycles 2-6; EOCT, end of combination treatment; EOT, end of treatment; MRD, minimal residual disease; PB, peripheral blood; PD, progressive disease/disease progression; R, randomization; R/R, relapsed/refractory VenR, venetoclax–rituximab

Seymour JF, et al. N Engl J Med. 2018 Mar 22;378(12):1107-1120

MURANO: Final Analysis (7 year median F/U)



P < 0.0001

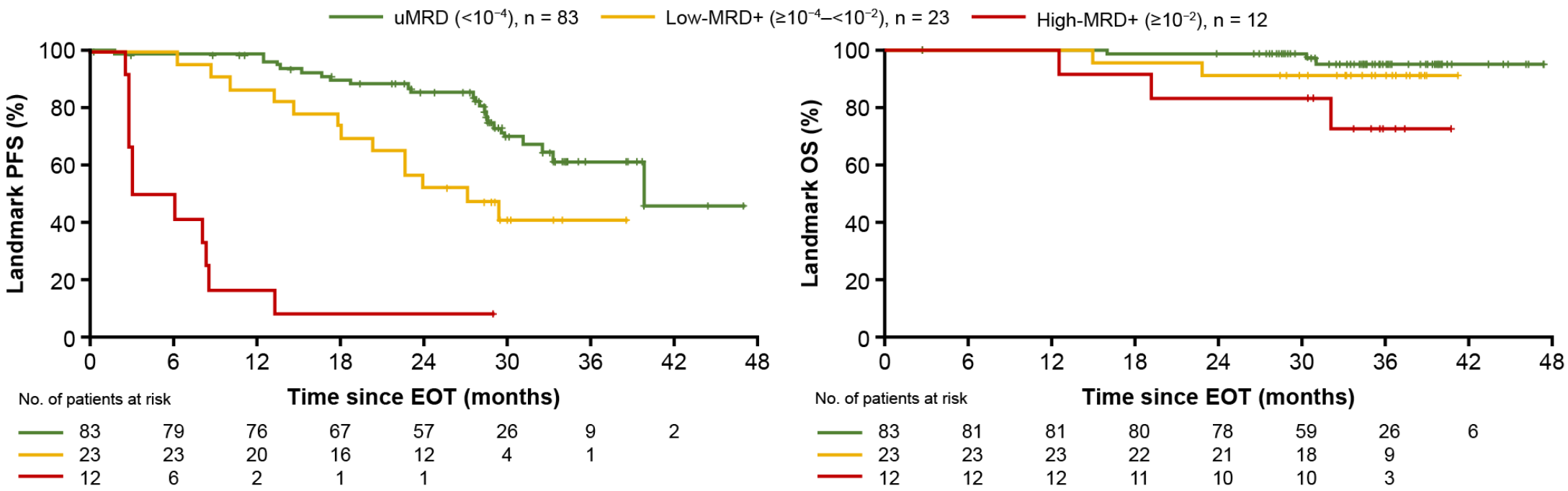
At 7 years
23 vs 0%

At 7 years
70 vs 51%

P = 0.0002

Kater AP, *et al.* Blood in press

MURANO: uMRD at EOT is associated with improved outcomes post-EOT in the VenR arm

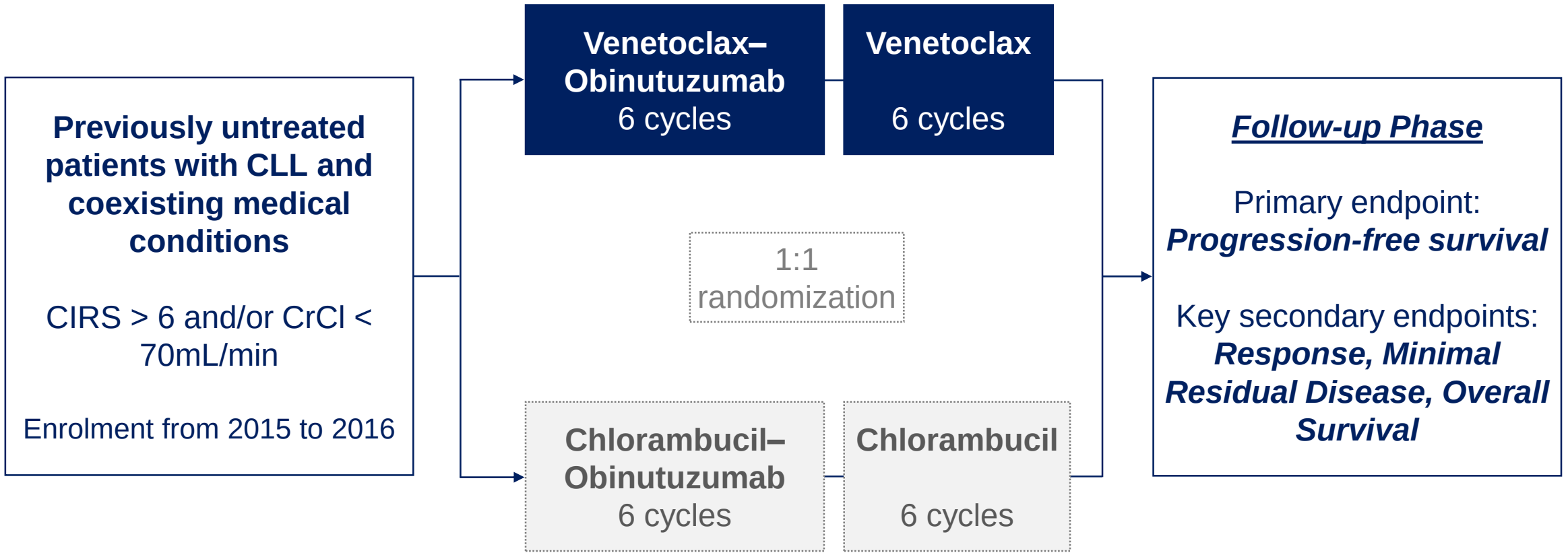


Category	PFS (95% CI) since EOT 24-month	36-month
uMRD (<10⁻⁴)	85.4 (77.4, 93.4)	61.3 (47.3, 75.2)
Low-MRD+ (≥10⁻⁴–<10⁻²)	52.2 (31.8, 72.6)	40.7 (19.2, 62.2)
High-MRD+ (≥10⁻²)	8.3 (0.0, 24.0)	NE
	HR (95% CI)	***P* value***
uMRD vs low-MRD+	0.40 (0.18, 0.91)	.0246
uMRD vs high-MRD+	0.02 (<0.01, 0.18)	< .0001
Low-MRD+ vs high-MRD+	0.32 (0.10, 0.99)	.0410

Category	OS (95% CI) since EOT 24-month	36-month
uMRD (<10⁻⁴)	98.8 (96.4, 100.0)	95.3 (90.0, 100.0)
Low-MRD+ (≥10⁻⁴–<10⁻²)	91.3 (79.8, 100.0)	91.3 (79.8, 100.0)
High-MRD+ (≥10⁻²)	83.3 (62.3, 100.0)	72.9 (46.4, 99.5)
	HR (95% CI)	***P* value***
uMRD vs low-MRD+	0.72 (0.11, 4.84)	.7334
uMRD vs high-MRD+	0.12 (0.01, 1.24)	.0385
Low-MRD+ vs high-MRD+	0.34 (0.02, 5.33)	.4414

Seymour JF, *et al.* Blood 2022

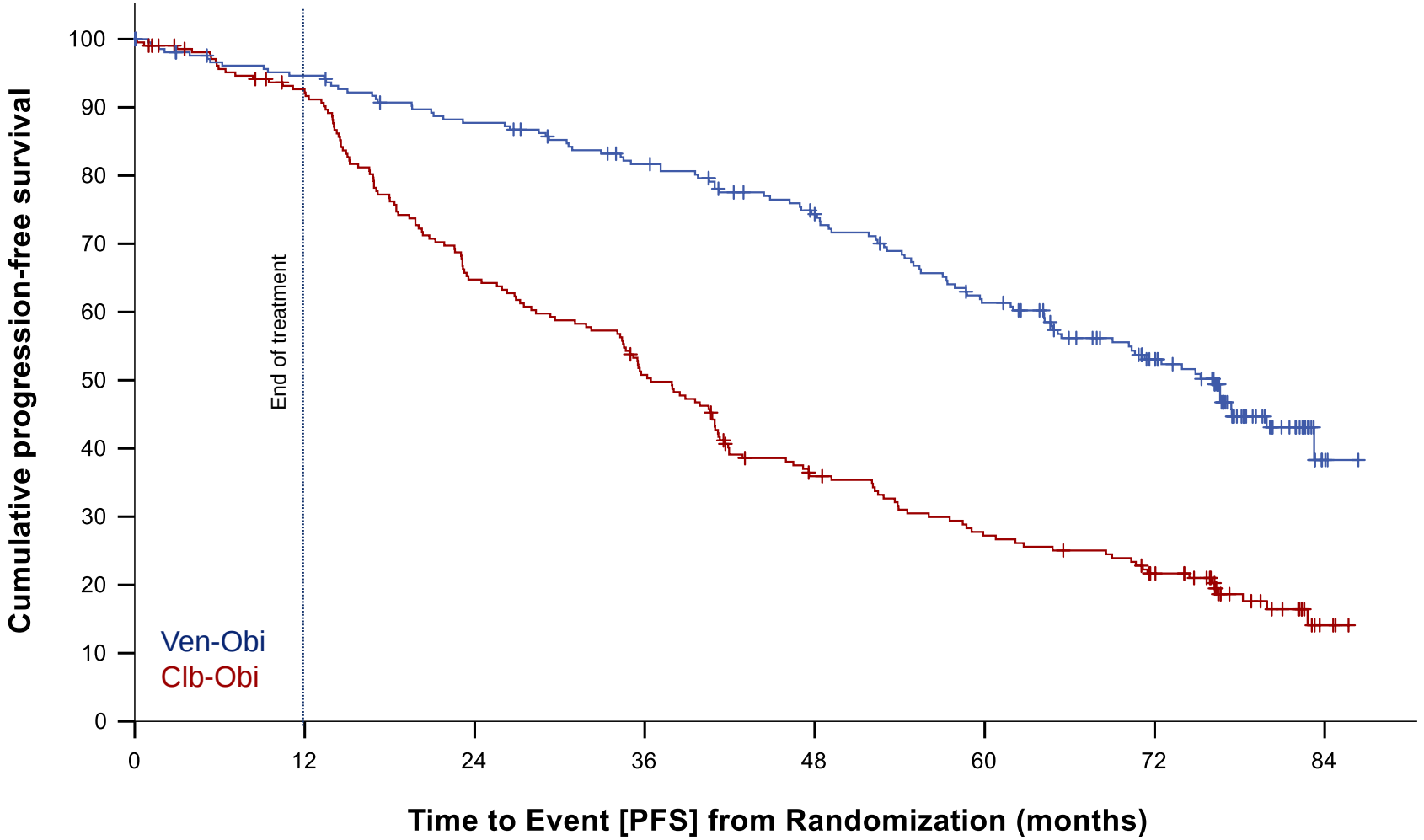
CLL14: TRIAL DESIGN



Current median observation time: 76.4 months

PROGRESSION-FREE SURVIVAL

Investigator-assessed PFS



Median PFS

Ven-Obi: 76.2 months

Clb-Obi: 36.4 months

6-year PFS rate

Ven-Obi: 53.1%

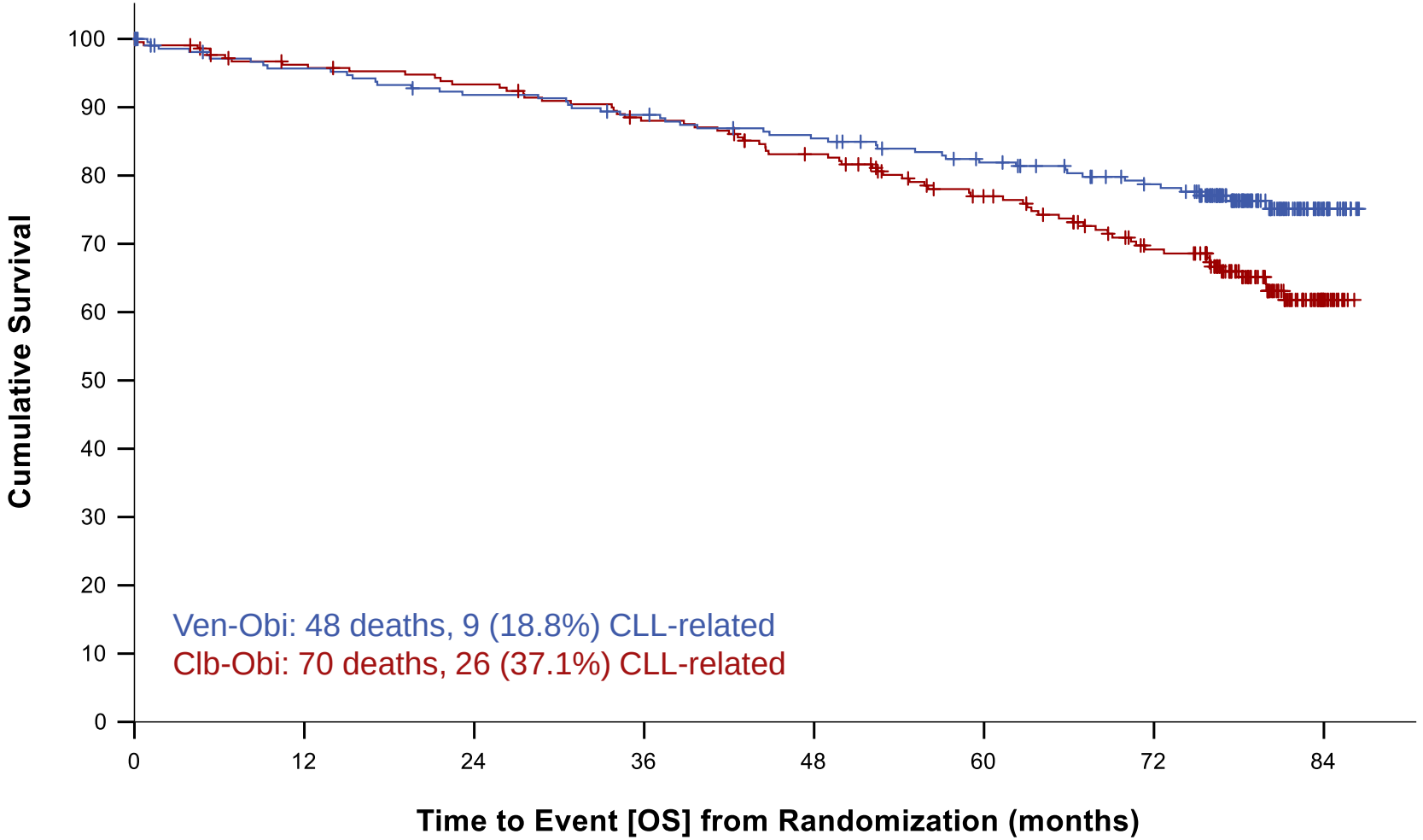
Clb-Obi: 21.7%

HR 0.40, 95% CI [0.31-0.52]
P<0.0001

Al-Sawaf O, *et al.* Blood 2024

OVERALL SURVIVAL

Median observation time 76.4 months



Ven-Obi	216	198	189	182	173	160	144	23
Clb-Obi	216	201	194	181	167	144	118	16

Median OS

Ven-Obi: not reached

Clb-Obi: not reached

6-year OS rate

Ven-Obi: 78.7%

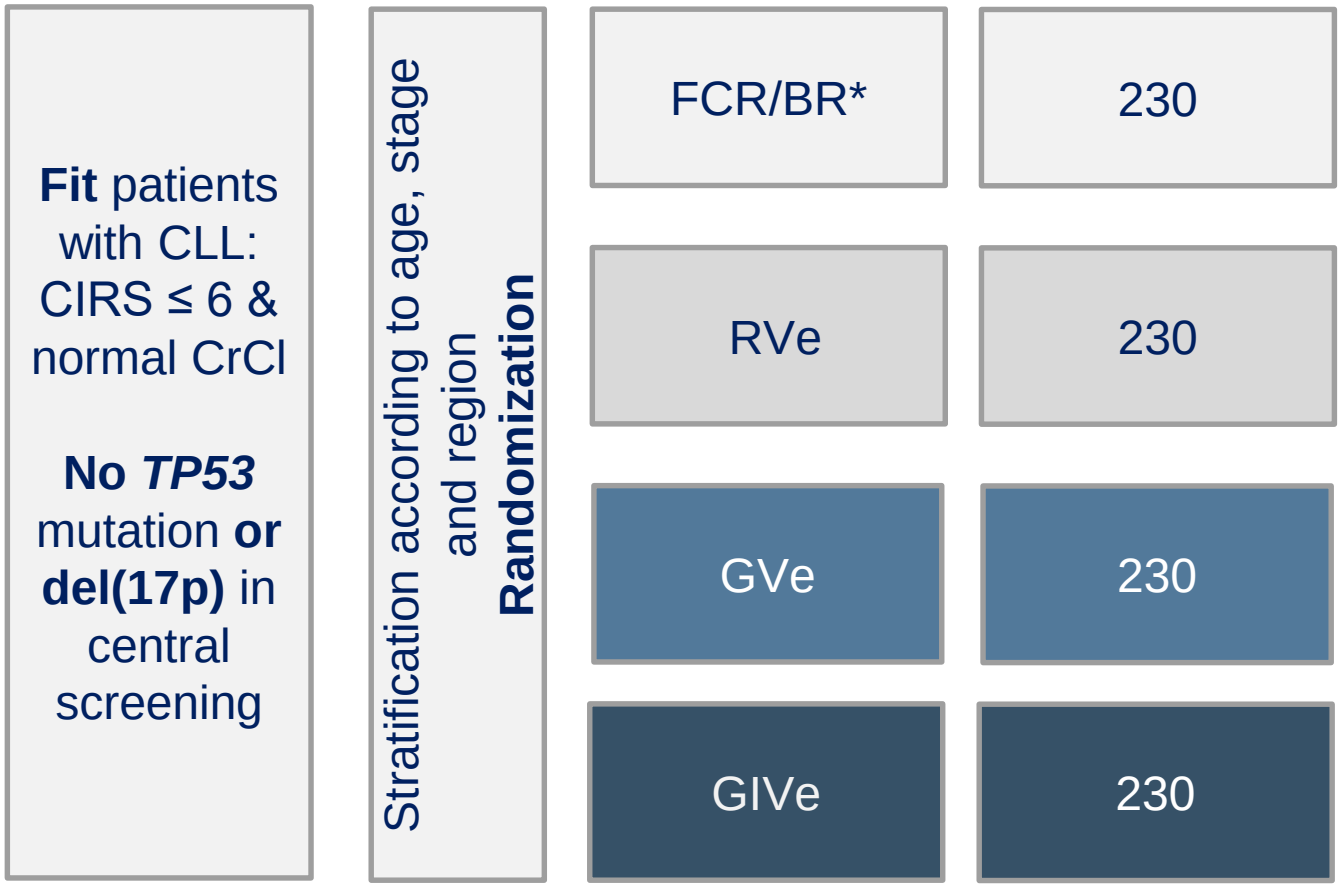
Clb-Obi: 69.2%

HR 0.69, 95% CI [0.48-1.01], p=0.052

Al-Sawaf O, *et al.* Blood 2024

GAIA/CLL13 Study : Design

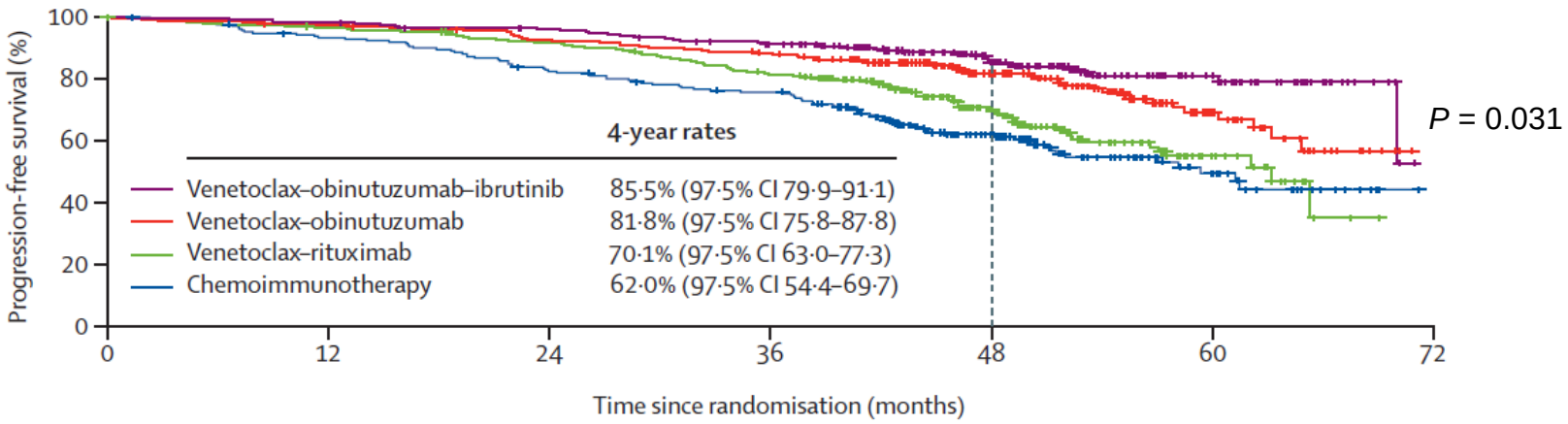
Chemoimmunotherapy (**FCR/BR**) versus **R**ituximab + **Ve**netoclax versus Obinutuzumab (**G**) + **Ve** versus **G** + **I**brutinib + **Ve**
Recruitment in 10 countries (DE, AU, CH, NL, BE, DK, SE, FL, IR, IL)



Eichhorst B, *et al.* NEJM 2023

GAIA/CLL13 Study : PFS at 4 year median follow-up

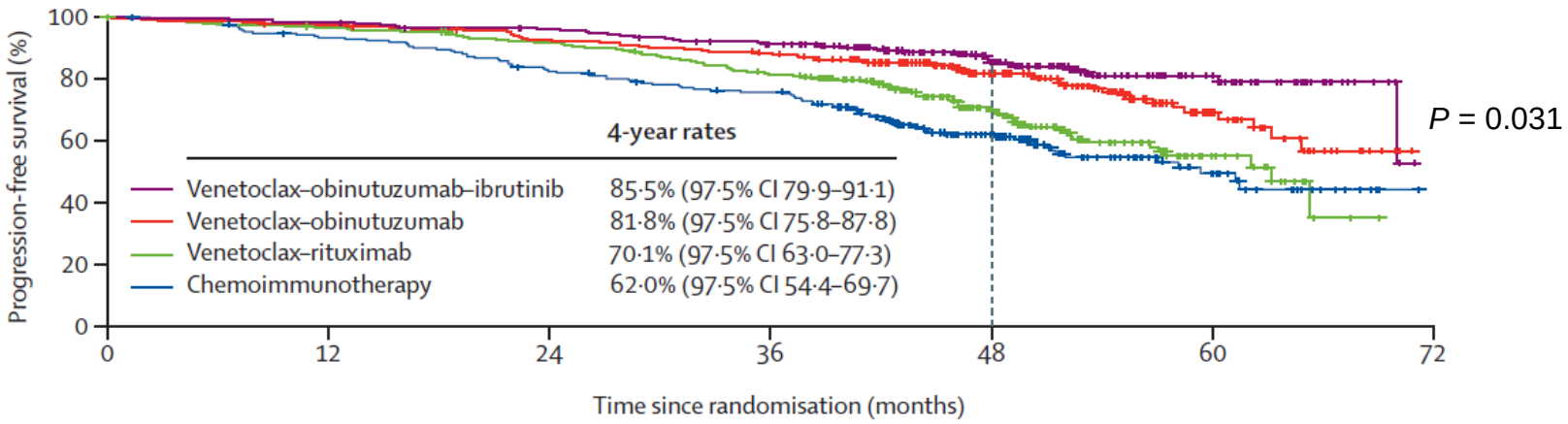
Whole cohort



Fürstenau M, *et al.* Lancet Oncol 2024

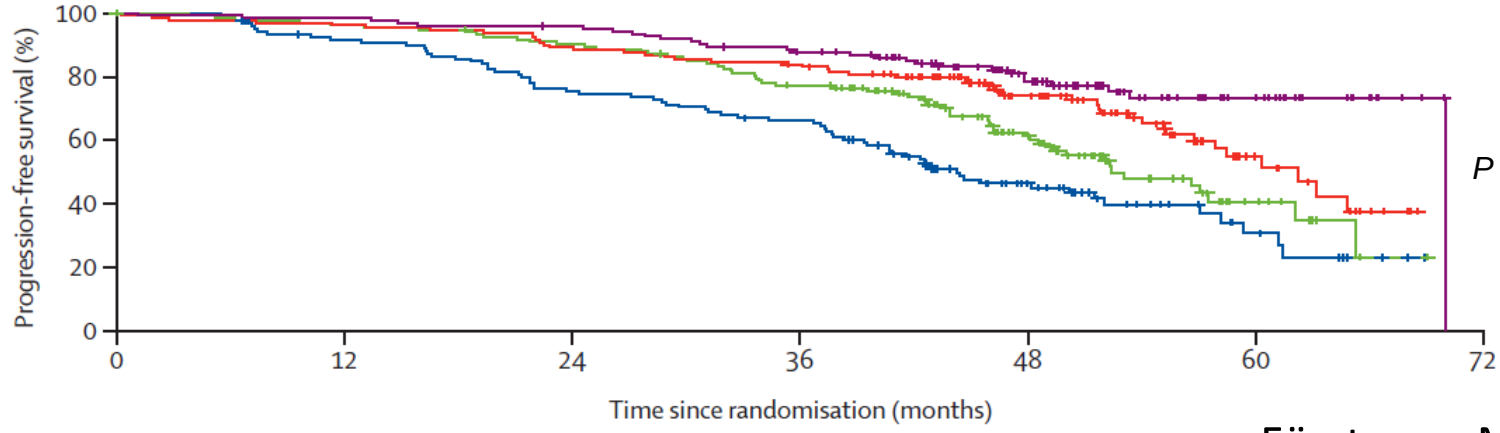
GAIA/CLL13 Study : PFS at 4 year median follow-up

Whole cohort



P = 0.031

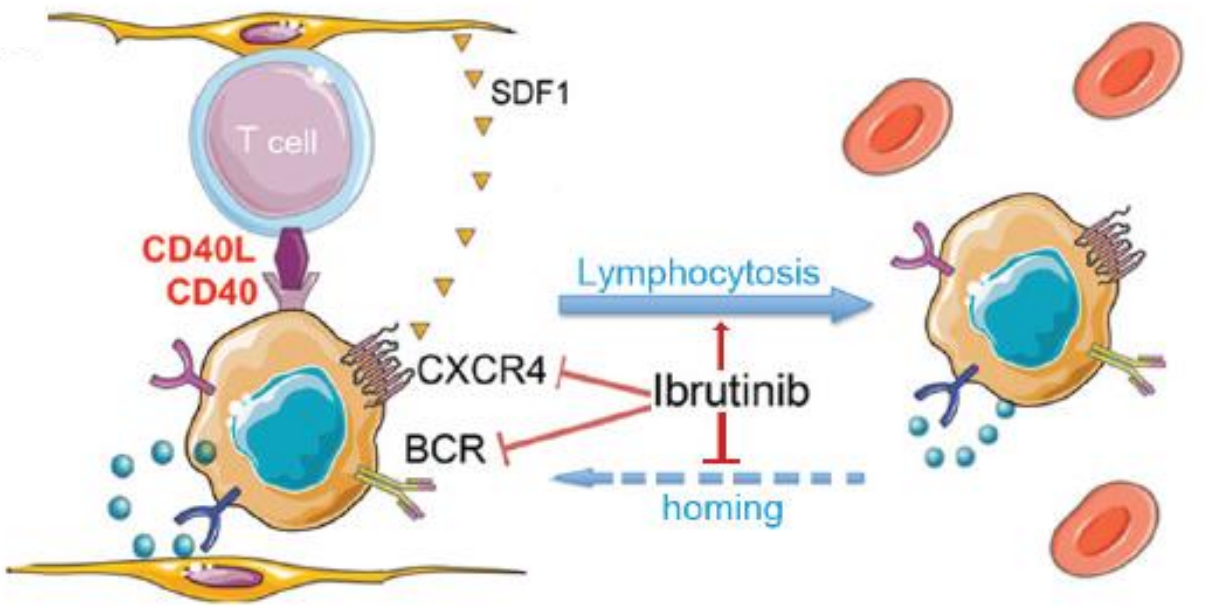
IGVH unmutated



P = 0.025

Fürstenau M, *et al.* Lancet Oncol 2024

BTKi-mediated stromal disruption leads to Venetoclax sensitisation

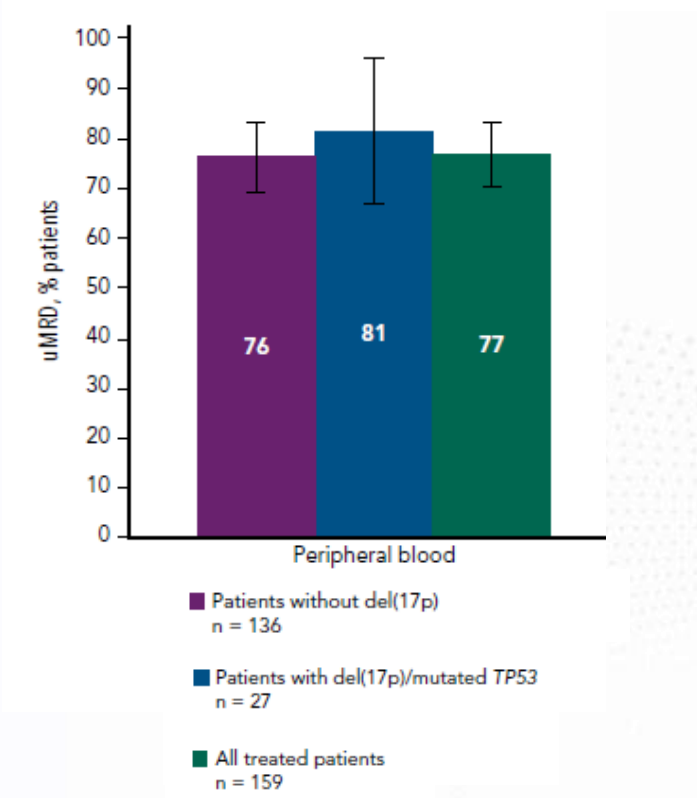


Chiron et al. Oncotarget. 2015;6(11):8755.

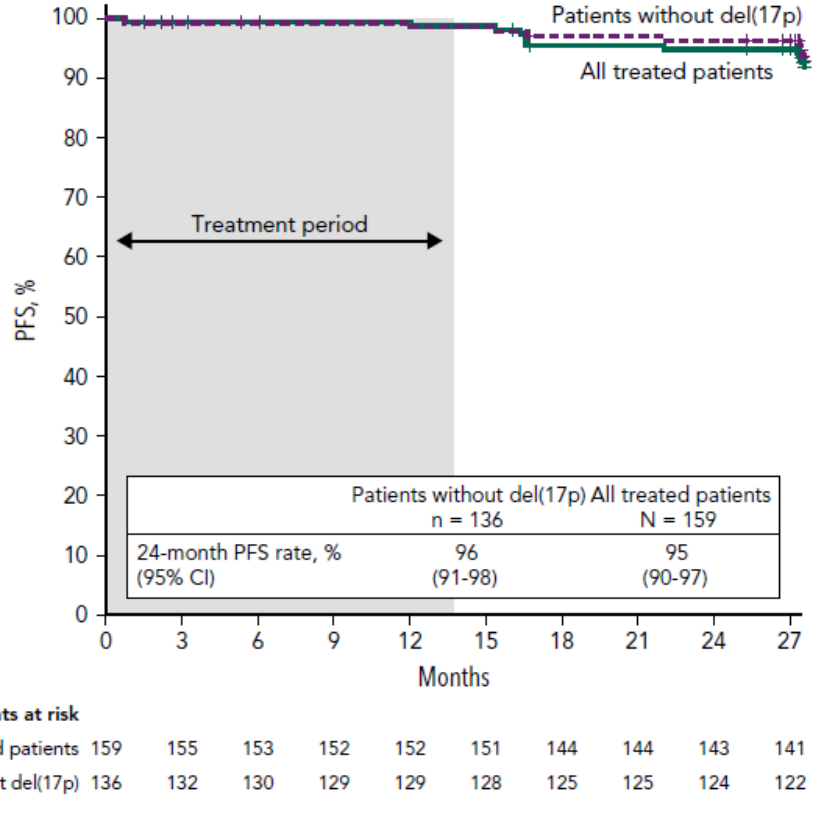
Time-limited Ven+I in 1L CLL

CAPTIVATE fixed duration cohort: Phase 2, venetoclax + ibrutinib in untreated CLL patients aged <70 years (N=159)

Best PB MRD response



PFS (median follow-up: 27.9 months)



	Patients without del(17p) n = 136	All treated patients N = 159
24-month PFS rate, % (95% CI)	96 (91-98)	95 (90-97)

Patients at risk

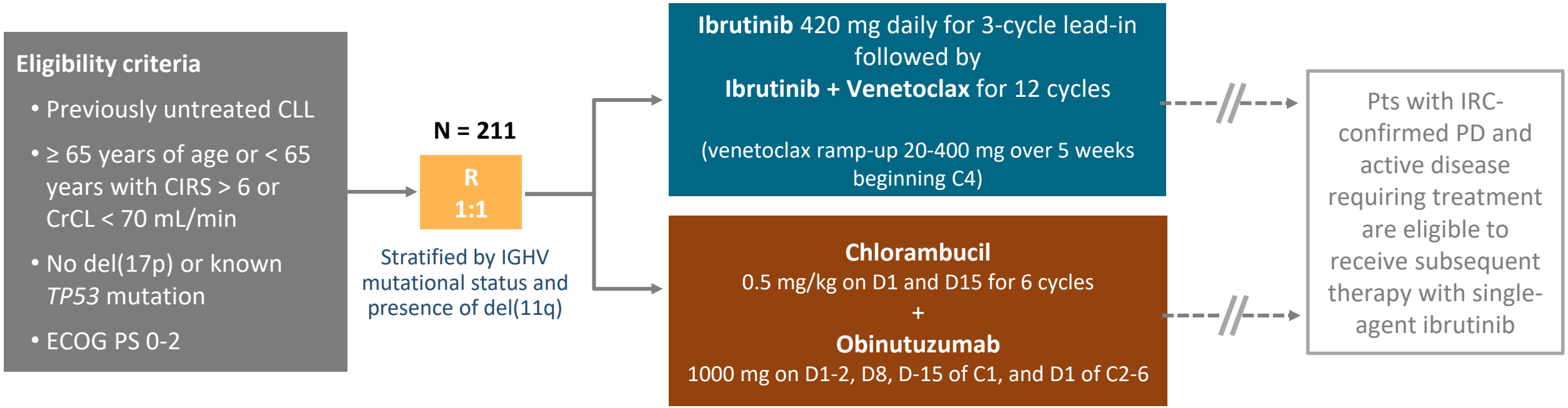
All treated patients	159	155	153	152	152	151	144	144	143	141
Patients without del(17p)	136	132	130	129	129	128	125	125	124	122

Safety

- No clinical TLS
- AEs generally decreased after the first 6 months of Ven+I
- Grade ≥3 AEs: infrequent
  - Most common Grade ≥3 AEs: neutropenia, hypertension, and infections

0/13 pts analysed at PD had detectable *BCL2* or *BTK* mutations

Phase 3 GLOW Study Design (NCT03462719)



Primary end point: Progression-free survival by independent review committee (IRC)

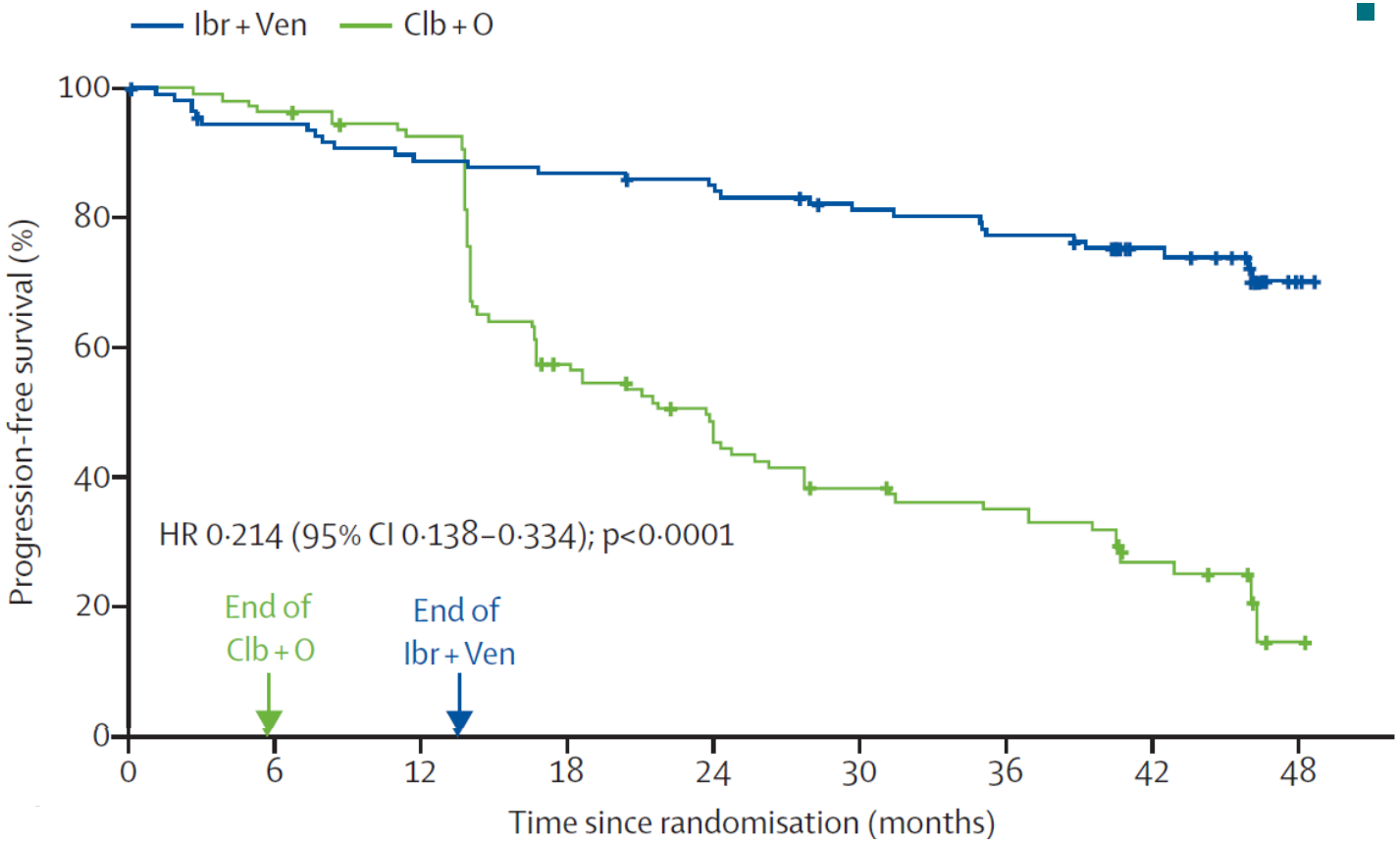
- 71 PFS events to detect an effect size with an HR = 0.5 (80% power at a 2-sided significance level of 0.05)

Key secondary end points: Undetectable MRD in BM, CR rate (IRC), ORR (IRC), OS; safety was also evaluated.

CLL, chronic lymphocytic leukemia; CIRS, Cumulative Illness Rating Scale score; CIRS, Cumulative Illness Rating Scale; CrCl, creatinine clearance; ECOG, Eastern Cooperative Oncology Group performance status; C, cycle (28 days); D, day; PD, progressive disease; PFS, progression-free survival; HR, hazard ratio; MRD, minimal residual disease; BM, bone marrow; CR, complete response; ORR, overall response rate; OS, overall survival

Kater AP, et al. NEJM Evid. 2022

GLOW: PFS at median 46 months of Follow-up



- **With median follow-up of 46 months:**
  - IRC-assessed PFS superior for Ibr+Ven (HR 0.214, 95% CI, 0.138-0.334; $p < 0.0001$)
  - 42-month PFS: 74.6% for Ibr+Ven vs 24.8% for Clb+O
  - Overall survival HR 0.487 (95% CI, 0.262-0.907), with 15 deaths for Ibr+Ven vs 30 for Clb+O
  - 4 sudden cardiac deaths in Ibr+Ven

Niemann CU, et al. Lancet Oncol. 2023

Conclusions

- Effective therapeutic targeting of apoptosis requires:
  - Understanding the cellular biology and BH3-mimetic mechanism of action
  - Detailed profiling of cellular Bcl2-family proteins & their binding partners
- Substantial single-agent clinical activity in CLL
- Resistance is multifactorial but usually involves acquired *BCL2* mutations
- Deep remissions with CD20 mAb combinations allow time-limited treatment
- Apparent lower rate of resistance mutations enables effective re-treatment
- Synergy with BTKi
- Multiple frontline time-limited combinations approved globally as standard of care

Melbourne pioneers in apoptosis research

David Vaux

Andreas Strasser

David Huang