Targeting Bruton's Tyrosine Kinase (BTK)

Lapo Alinari, MD, PhD

4th International Conference,
Translational Research in Oncology
Forli', November 9-11/2016

The James

THE OHIO STATE UNIVERSITY
COMPREHENSIVE CANCER CENTER

Conflict of Interest for Lapo Alinari

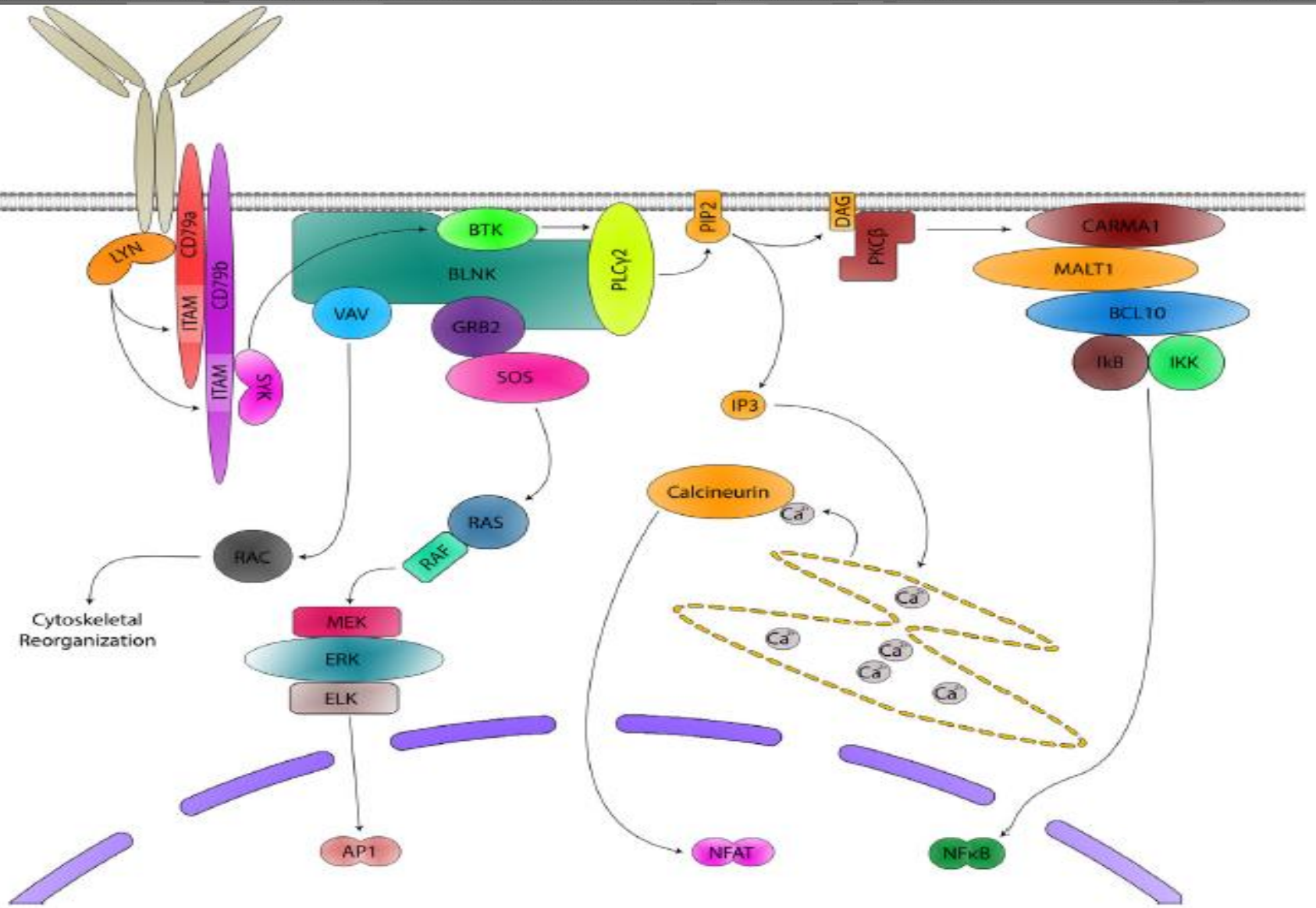
Investigator role in clinical trials	Pharmacyclics (an AbbVie Company), Merck, Bristol-Myers Squibb
Research Support	BetaCat Pharmaceuticals
Employee	None
Consultant	None
Major Stockholder	None
Scientific Advisory Board	None

Objectives

- Explore the B-cell receptor signaling pathway
- Discuss BTK as a therapeutic target
- Discuss activity of and resistance to BTK inhibitors in the treatment of lymphoid malignancies
- Identify mechanisms to improve upon BTK inhibition

Objectives

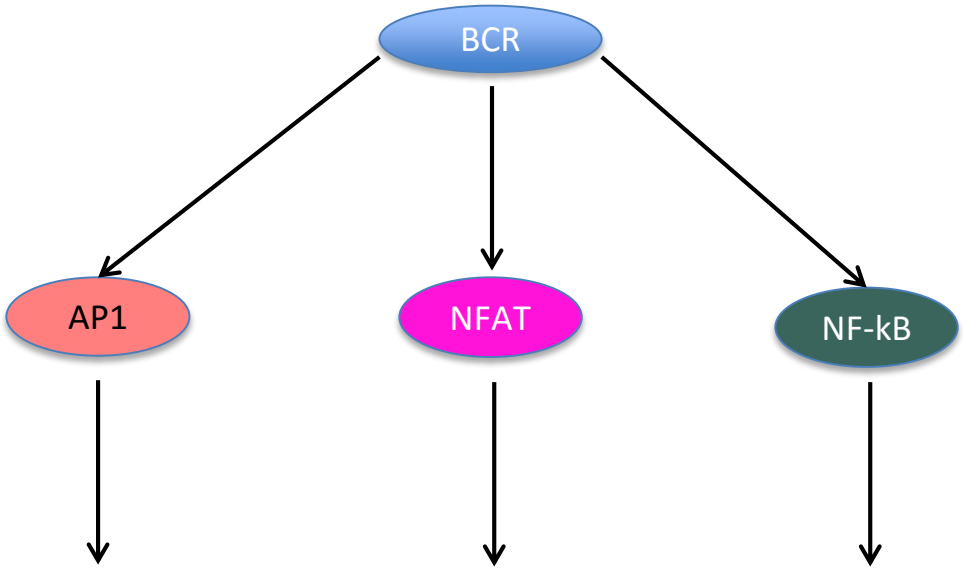
- Explore the B-cell receptor signaling pathway
- Discuss BTK as a therapeutic target
- Discuss activity of and resistance to BTK inhibitors in the treatment of lymphoid malignancies
- Identify mechanisms to improve upon BTK inhibition



ITAM: immunoreceptor tyrosine-based activation motif

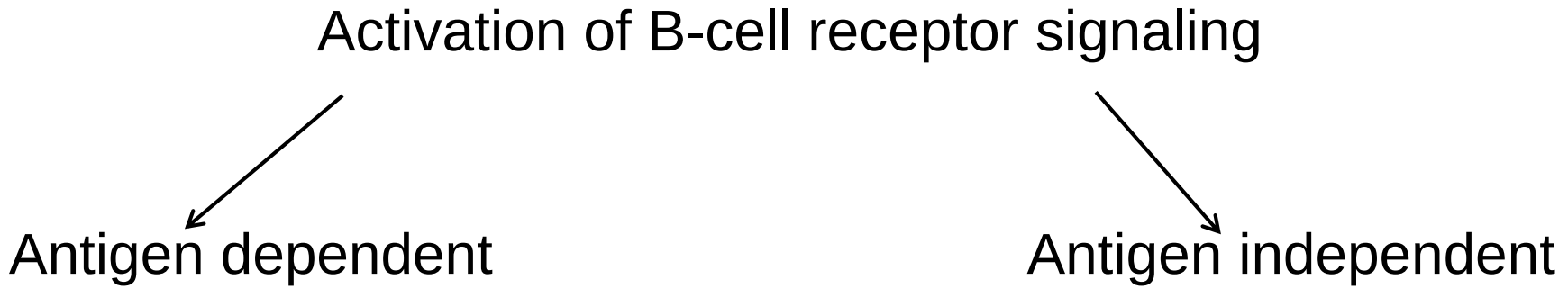
Zhong Y et al. Seminars in Hematology 2014

B-cell receptor signaling pathway



Cyclin D1	TNFα	c-MYC
Cyclin A	CD154	Bcl2
Cyclin E	IL2	Bax
p53	IL3	Bcl-xl
p21	IL4	IRF4
p16	IL5	Blimp1
P19	INFϒ	ILs
PTPRO		

B-cell receptor signaling



Normal B cells

Chronic lymphocytic leukemia (CLL)

Mantle cell lymphoma (MCL)

ABC-diffuse large B-cell lymphoma (ABC-DLBCL)

Objectives

- Explore the B-cell receptor signaling pathway
- Discuss BTK as a therapeutic target
- Discuss activity of and resistance to BTK inhibitors in the treatment of lymphoid malignancies
- Identify mechanisms to improve upon BTK inhibition

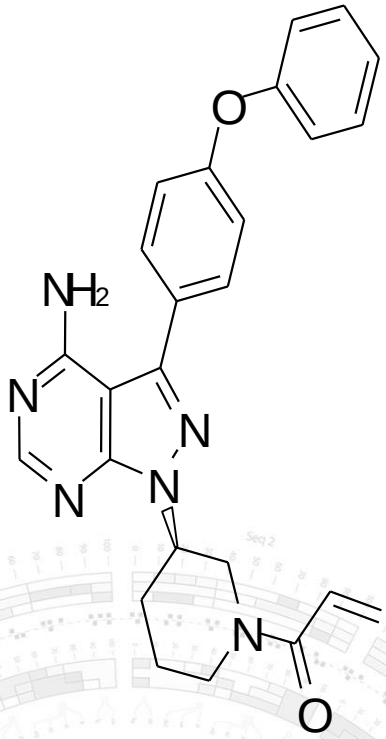
BTK as a therapeutic target

- BTK is a member of the Tec family kinases and plays a central role in B-cell receptor signaling pathway.
- Although BTK is expressed in multiple hematopoietic cells the primary defect in BTK-/- mice is B-cell specific.
- Loss of BTK function in humans give rise to X-linked agammaglobulinemia, an inherited disorder characterized by complete lack of mature B cells.
- BTK was identified in preclinical models as an essential signaling kinase for survival of chronic lymphocytic leukemia (CLL) and certain B-cell lymphomas.

Pros and cons of targeting BTK

- Pros:
  - BCR signaling is vital to malignant B cell survival, proliferation
  - BTK activation leads to activation of PI3K, PLCγ2, MAPK, and NF-kB pro-survival pathways
  - Mouse models of BTK deficiency suggest predominantly a B-cell defect, without impairment of T-cells
- Cons:
  - Targeting BTK may enhance immune suppression due to influence on normal B-cells function, neutrophils maturation, and NK cells mediated ADCC

Ibrutinib: a potent Btk Inhibitor



- Binds irreversibly to cysteine-481 in Btk
- Inhibits BCR signaling
- Active in preclinical models of CLL and lymphoma
- Orally available
- Once daily dosing results in 24-hr sustained target inhibition

Honigberg LA et al. PNAS 2010
Herman S et al. Blood 2011
Ponader L et al. Blood 2012

Table S1. IC_{50} values and fold selectivity for inhibition of enzymatic activity by PCI-32765

Kinase	IC_{50}, nM	Btk selectivity, fold
BTK	0.5	—
BLK*	0.5	1
BMX*	0.8	1.6
CSK	2.3	4.6
FGR	2.3	4.6
BRK	3.3	6.6
HCK	3.7	7.4
EGFR*	5.6	11.2
YES	6.5	13
ErbB2*	9.4	18.8
ITK*	10.7	21.4
JAK3*	16.1	32.2
FRK	29.2	58.4
LCK	33.2	66.4
RET	36.5	73
FLT3	73	146
TEC*	78	156
ABL	86	172
FYN	96	192
RIPK2	152	304
c-SRC	171	342
LYN	200	400
PDGFRα	718	1436
FMS	5545	>10,000
FER	8070	>10,000
JAK1	>10,000	>10,000
JAK2	>10,000	>10,000
NEK2	>10,000	>10,000
p38	>10,000	>10,000
PI3K	>10,000	>10,000
PLK1	>10,000	>10,000
RSK1	>10,000	>10,000
SYK	>10,000	>10,000

*Kinases that contain a cysteine residue aligning with Cys-481 in Btk.

Interleukin-induced tyrosine kinase

Honigberg LA et al. PNAS 2010

Objectives

- Explore the B-cell receptor signaling pathway
- Discuss BTK as a therapeutic target
- Discuss activity of and resistance to BTK inhibitors in the treatment of lymphoid malignancies
- Identify mechanisms to improve upon BTK inhibition

Ibrutinib clinical development

Phase I open-label study of ibrutinib in relapsed refractory B-cell malignancies

Patient characteristics	N = 56	Overall response rate
Histologic subtype:		
Follicular lymphoma	16	37.5%
CLL	16	67.5%
Mantle cell lymphoma	9	78%
DLBCL	7	28%
Other	8	50%

Advani RH et al. JCO 2013

Disease focus

- Chronic lymphocytic leukemia
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma

Chronic lymphocytic leukemia (CLL)

- CLL is the most prevalent adult leukemia and is characterized by a progressive accumulation of functionally incompetent B cells
- Presentation usually indolent
- Standard treatment regimen for symptomatic CLL patients: Options include purine analogs, alkylating agents, monoclonal antibodies, ibrutinib.
- Options at relapse include ibrutinib, monoclonal antibodies, bcl2 inhibitor, PI3K inhibitors, CDK inhibitors, steroids, chemo-immunotherapy, enrollment in a clinical trial, transplant

A Phase Ib/II Study of Ibrutinib in Relapsed CLL

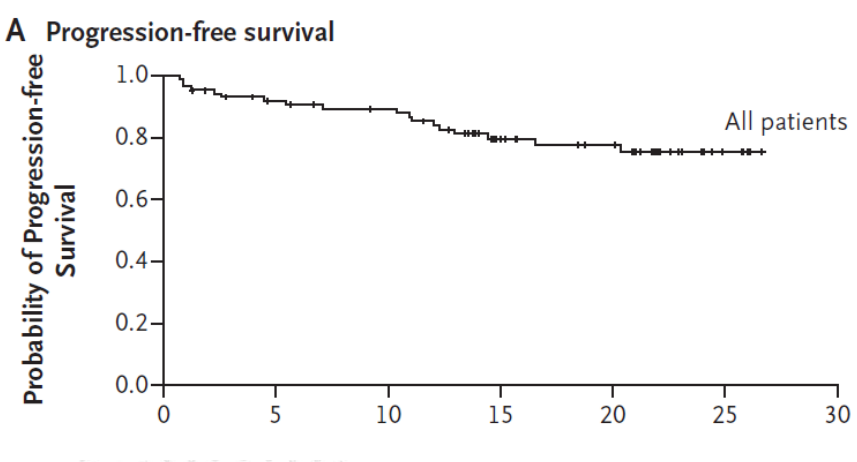
PCYC-1102-CA	N = 86
Age, years	
Median (Range)	66 (37 – 82)
≥ 70 years, (%)	35%
ECOG Status	
0	41%
1	56%
2	2%
Median Prior Therapies	4 (1-12)
β_2 Microglobulin > 3mg/L, %	49%
Rai Stage III/IV at Baseline	65%
Prognostic Markers, %	
IgV_H unmutated	85%
del(17p13.1)	35%
del(11q22.3)	39%

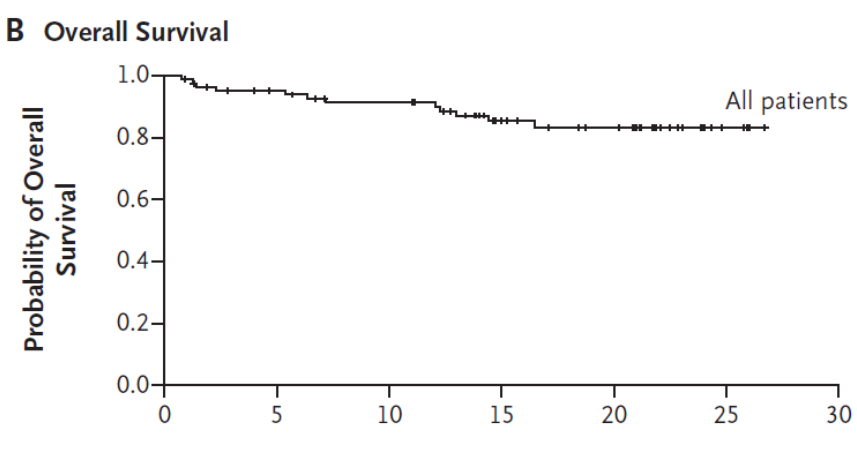
Byrd et al NEJM. 2013

The James

The Ohio State University
Comprehensive Cancer Center

A Phase Ib/II Study of Ibrutinib in Relapsed CLL



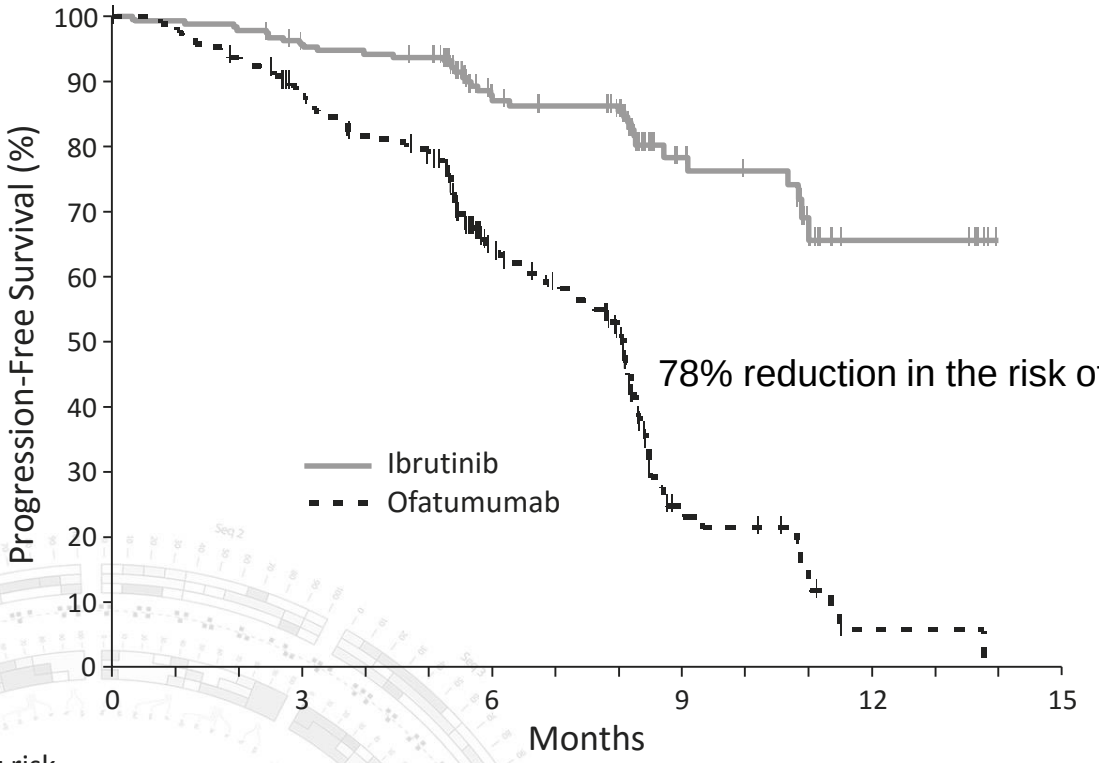


PFS at 26 months 75%

OS at 26 months 75%

Byrd JC et al. NEJM. 2013

Phase III study of ibrutinib versus ofatumomab in patients with relapsed/refractory CLL (RESONATE)

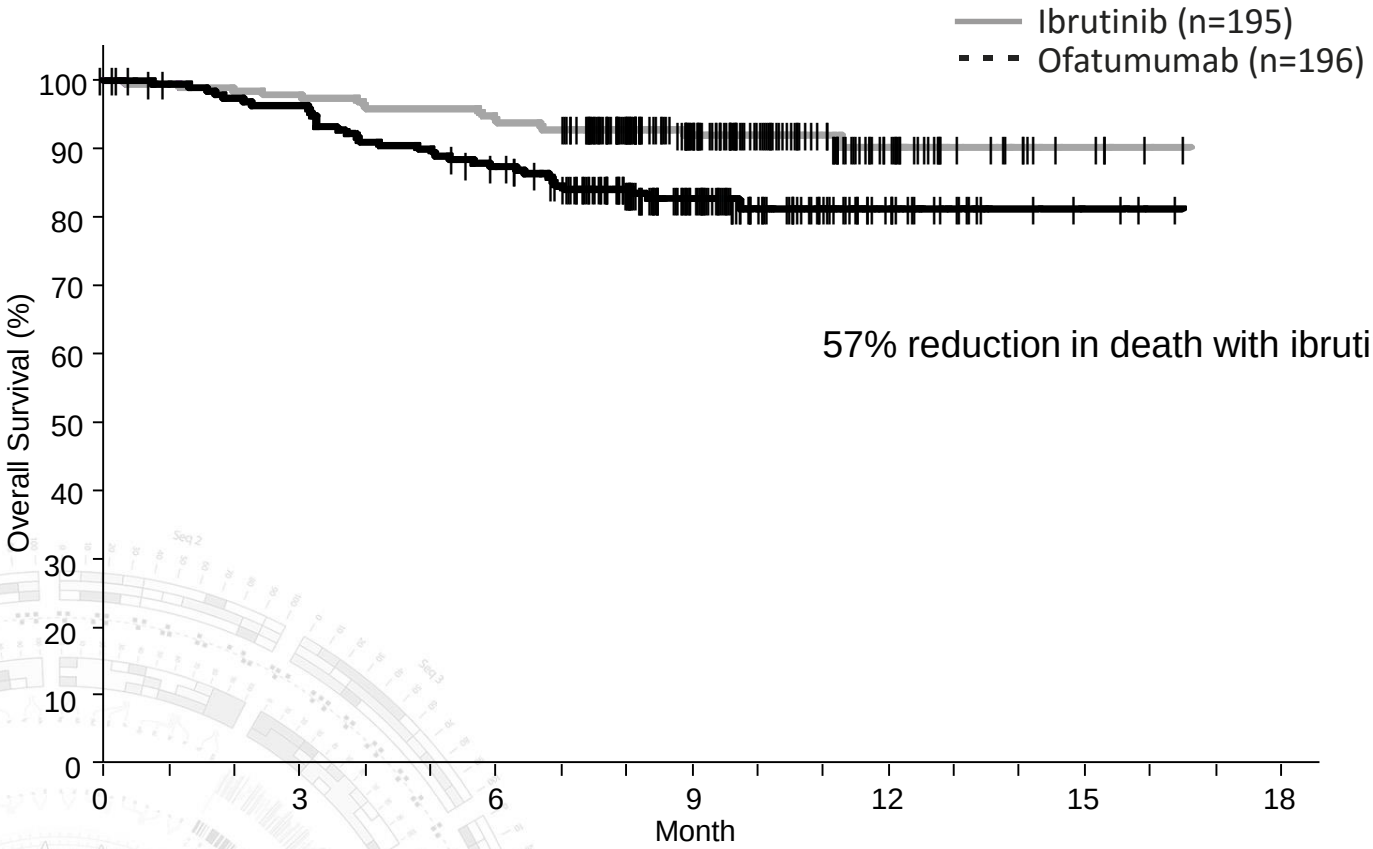


78% reduction in the risk of progression with ibrutinib (p<0.001)

No. at risk						
Ibrutinib:	195	183	116	38	7	
Ofatumumab:	196	161	83	15	1	0

Byrd JC et al. NEJM 2014

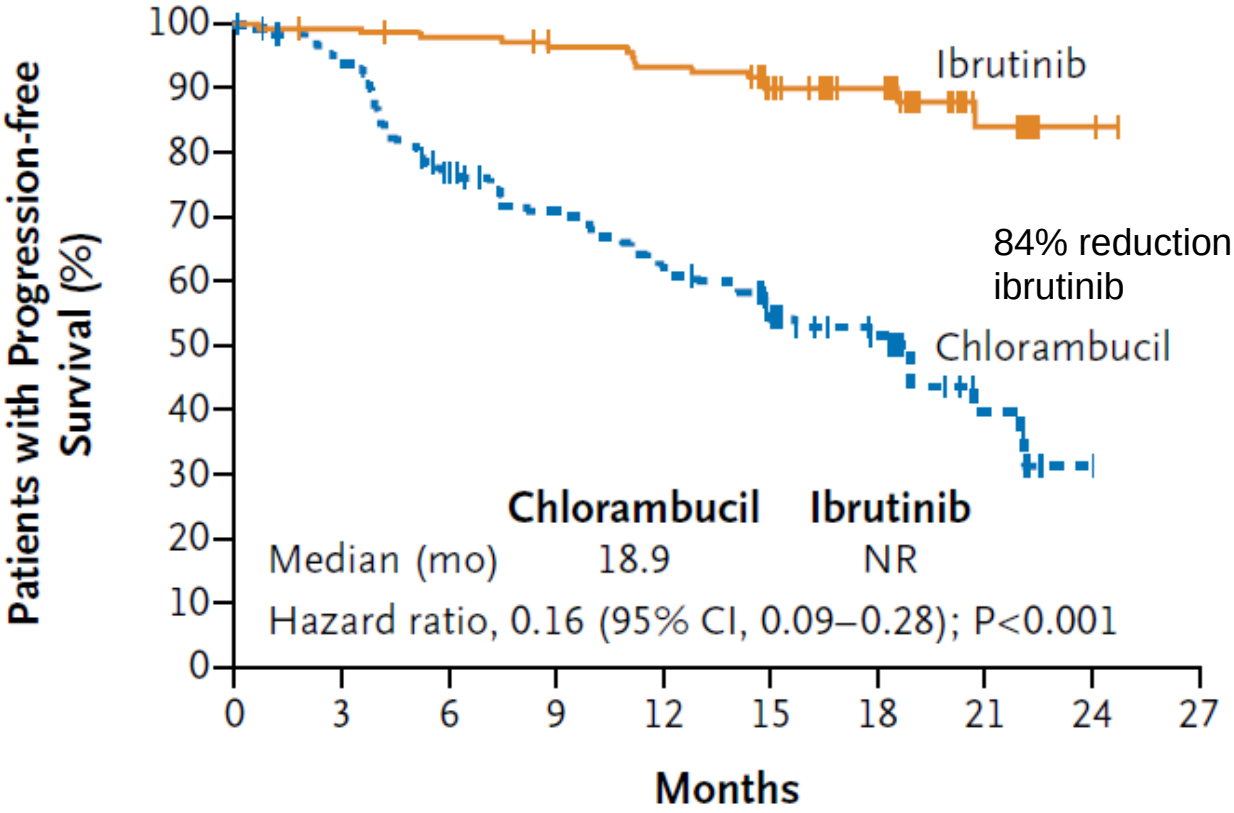
Phase III study of ibrutinib versus ofatumomab in patients with relapsed/refractory CLL (RESONATE)



57% reduction in death with ibrutinib (p=0.0049)

Byrd JC et al. NEJM 2014

Phase III study of ibrutinib versus chlorambucil in patients with treatment naive CLL (RESONATE-II)



84% reduction in the relative risk of progression with ibrutinib

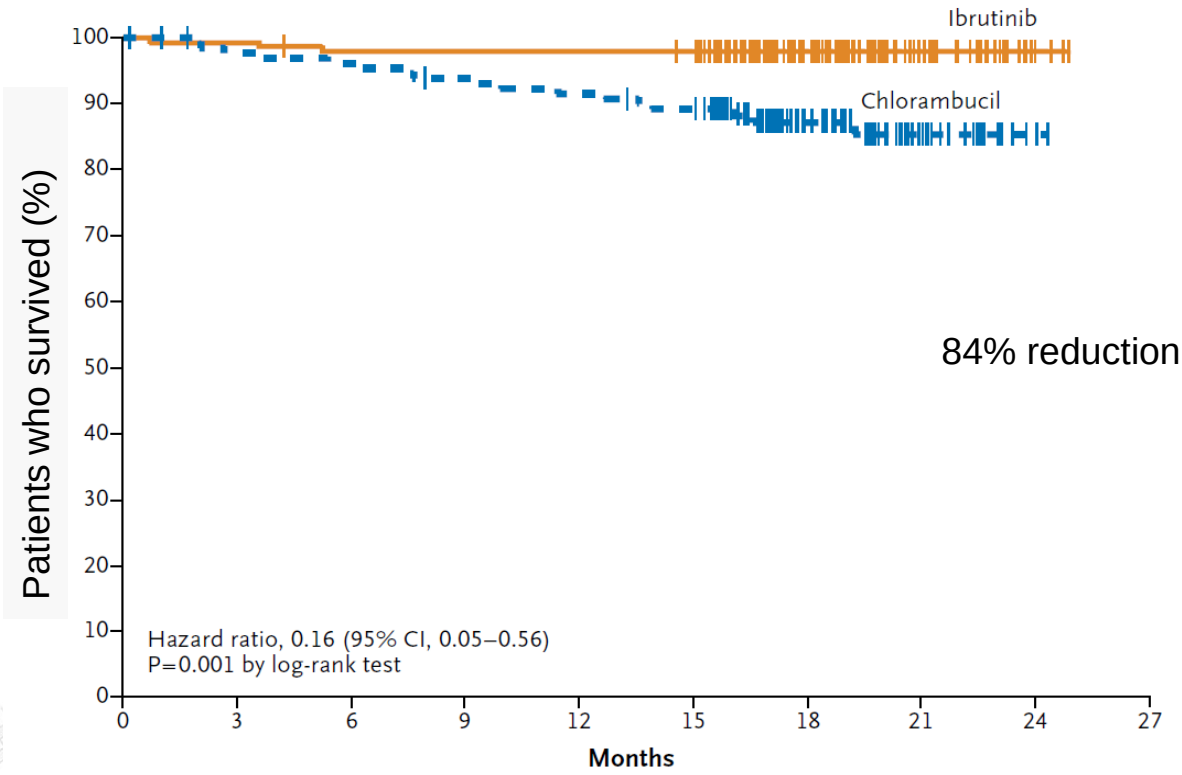
18 month PFS 90% vs 52%

Burger JA et al. NEJM 2015

The James

The Ohio State University
Comprehensive Cancer Center

Phase III study of ibrutinib versus chlorambucil in patients with treatment naive CLL (RESONATE-II)

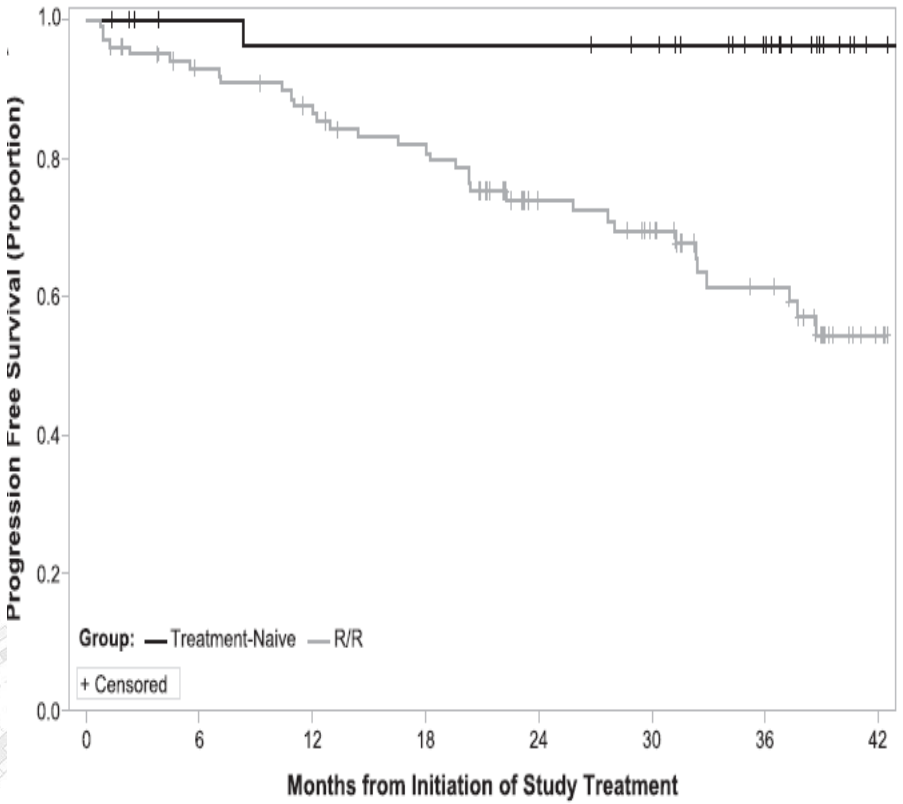


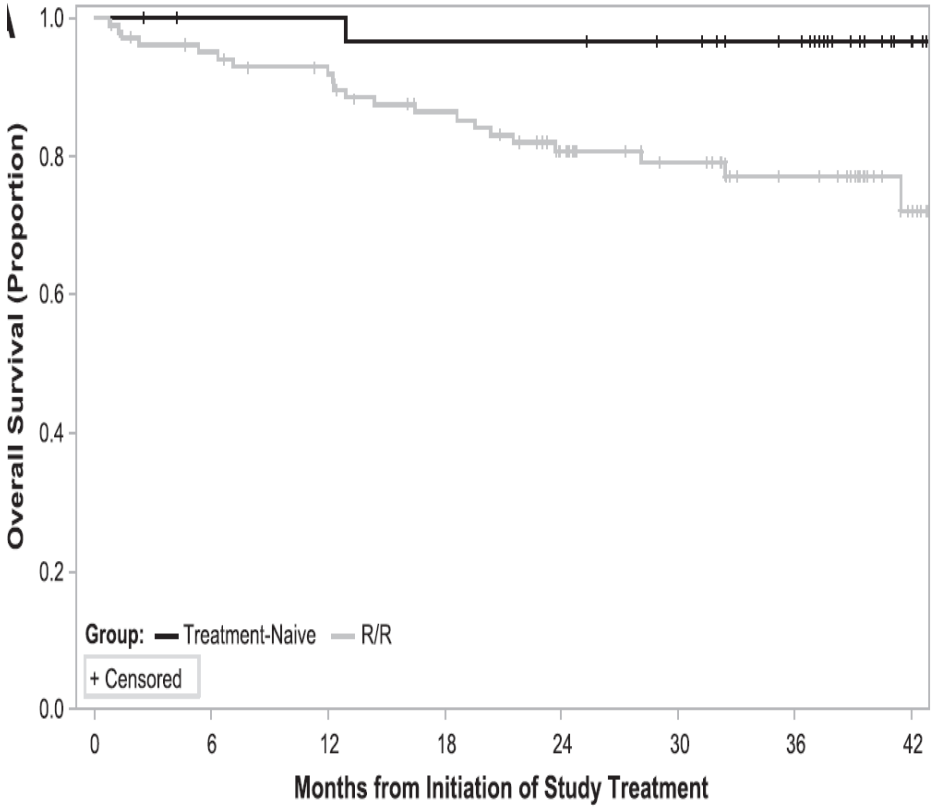
84% reduction in relative risk of death with ibrutinib

24 month OS 98% vs 85%

Burger JA et al. NEJM 2015

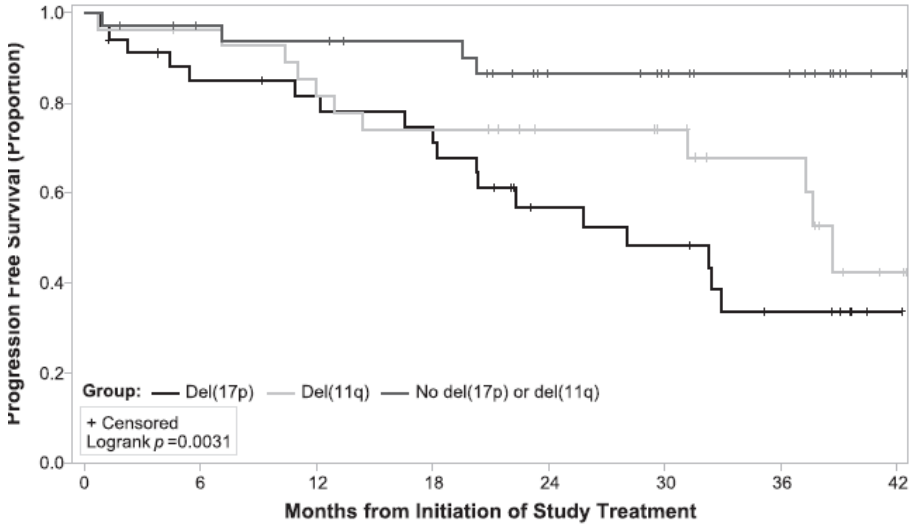
3-year follow-up of treatment-naïve and relapsed CLL patients receiving ibrutinib

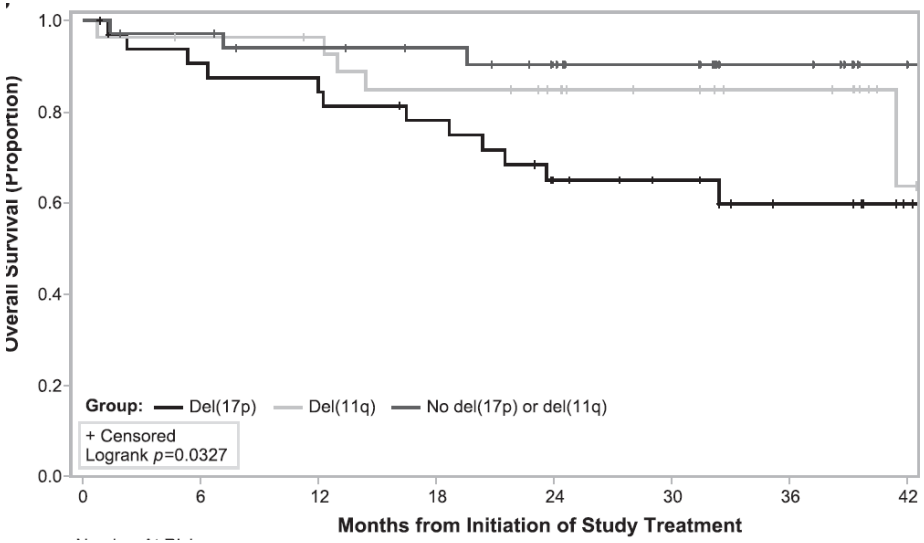




Byrd JC et al, Blood 2015

Limited duration of response to ibrutinib: del17p

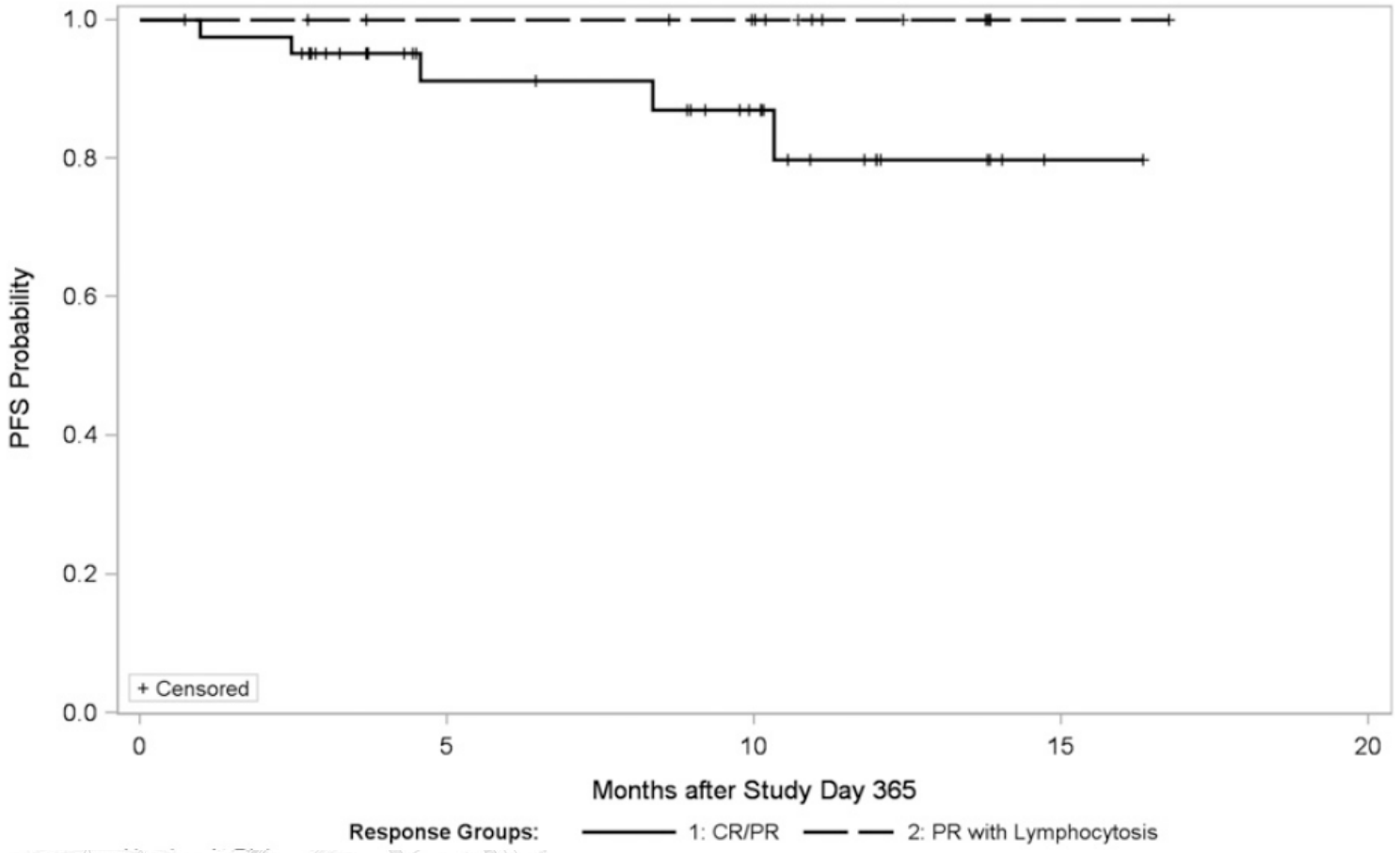




	30-months PFS
No 17p-/11q-	89.0%
17p-	45.9%

Byrd JC et al, Blood 2015

Prolonged lymphocytosis during ibrutinib treatment does not indicate suboptimal response



Woyach JA et al, Blood 2014

Mantle Cell Lymphoma (MCL)

- MCL is a rare and incurable B-cell non-Hodgkin's lymphoma
- Presentation can be indolent or aggressive
- No standard front-line regimen:
  - Rituximab(R)-chemo, R-chemo followed by ASCT
- Consolidation with autologous transplant in first remission may prolong progression-free survival
- Options at relapse include ibrutinib, bortezomib, R-bendamustine, lenalidomide, mTOR inhibitor, enrollment in a clinical trial, transplant in selected patients

The NEW ENGLAND JOURNAL *of* MEDICINE

established in 1812 august 8, 2013 vol. 369 no. 6

Targeting BTK with Ibrutinib in Relapsed or Refractory Mantle-Cell Lymphoma

Michael L. Wang, M.D., Simon Rule, M.D., Peter Martin, M.D., Andre Goy, M.D., Rebecca Auer, M.D., Ph.D., Brad S. Kahl, M.D., Wojciech Jurczak, M.D., Ph.D., Ranjana H. Advani, M.D., Jorge E. Romaguera, M.D., Michael E. Williams, M.D., Jacqueline C. Barrientos, M.D., Ewa Chmielowska, M.D., John Radford, M.D., Stephan Stilgenbauer, M.D., Martin Dreyling, M.D., Wieslaw Wiktor Jedrzejczak, M.D., Peter Johnson, M.D., Stephen E. Spurgeon, M.D., Lei Li, Ph.D., Liang Zhang, M.D., Ph.D., Kate Newberry, Ph.D., Zhishuo Ou, M.D., Nancy Cheng, M.S., Bingliang Fang, Ph.D., Jesse McGreivy, M.D., Fong Clow, Sc.D., Joseph J. Buggy, Ph.D., Betty Y. Chang, Ph.D., Darrin M. Beaupre, M.D., Ph.D., Lori A. Kunkel, M.D., and Kristie A. Blum, M.D.

Wang ML et al, NEJM 2013

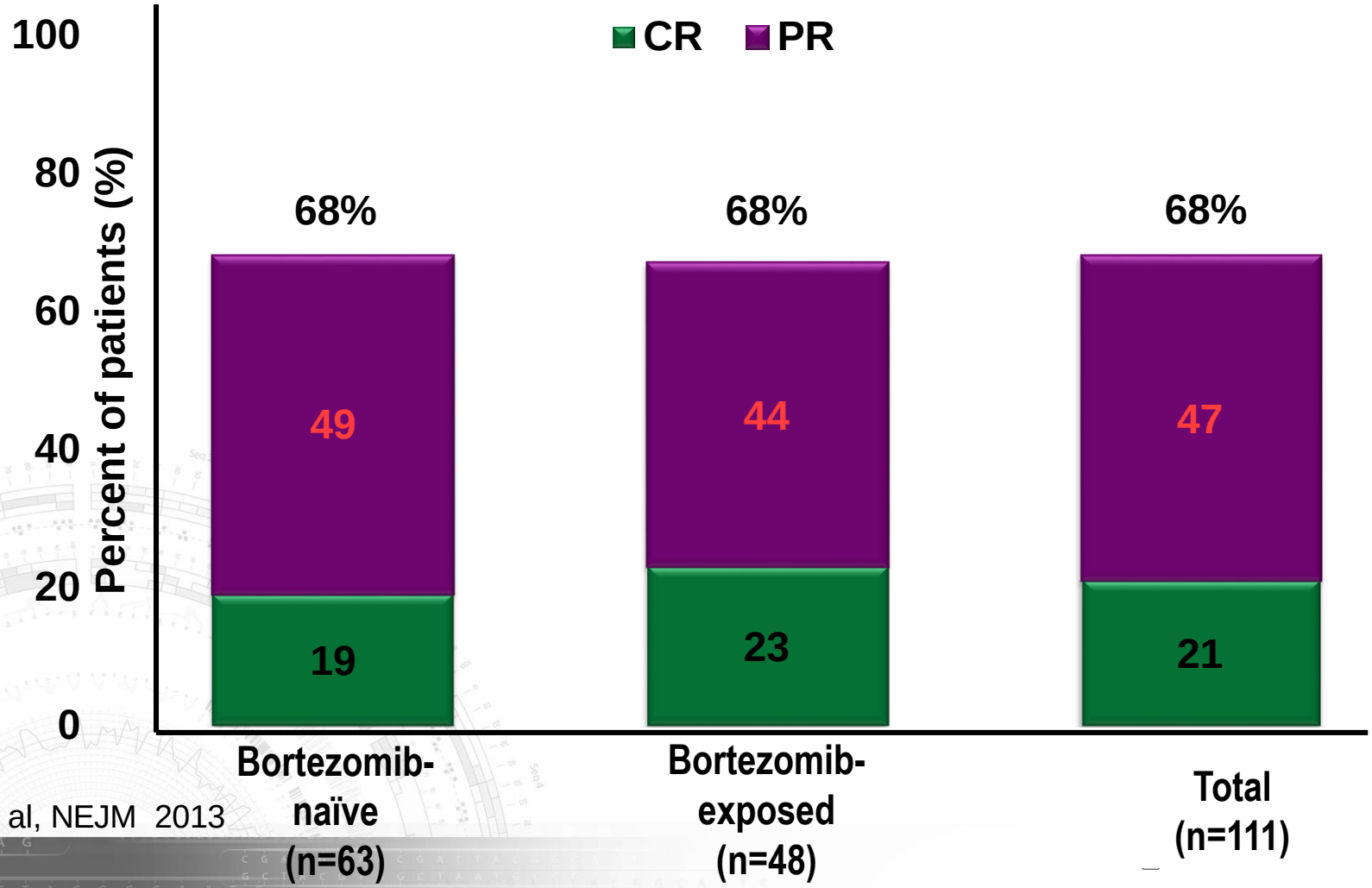
Patient Characteristics

	Bortezomib-Naïve (N=63)	Bortezomib-Exposed (N=48)	Total (N=111)
Median Age	66 (46-83)	69 (40–84)	68 (40–84)
Median number prior regimens: (Range) ≥ 3 regimens	2 (1-5) 31 (49%)	3 (1-5) 30 (62%)	3 (1-5) 61 (55%)
Simplified MIPI Low risk (0-3) Intermediate (4-5) High risk (6-11)	 9 (14) 24 (38) 30 (48)	 6 (12) 18 (38) 24 (50)	 15 (14) 42 (38) 54 (49)
Refractory (less than PR to last tx)	27 (43)	23 (48)	50 (45)

Wang ML et al, NEJM 2013

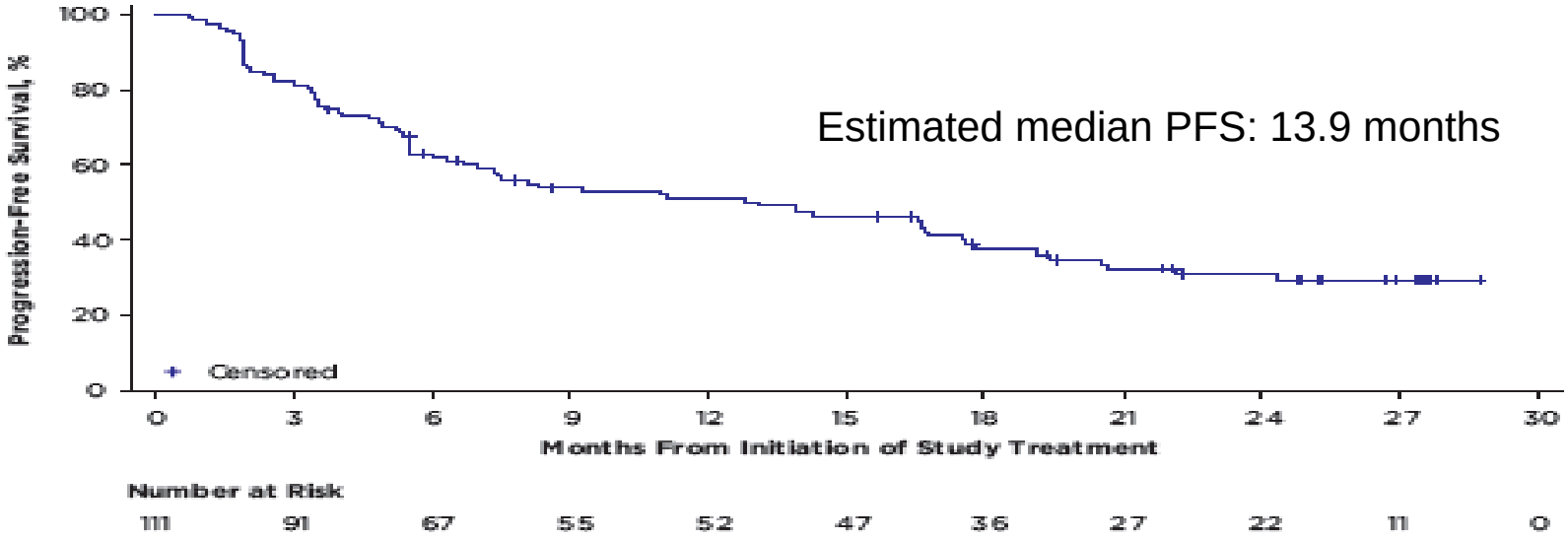
Overall response

Efficacy Population n=111, Median Follow Up ~15.3 months (range, 1.9-22.3)

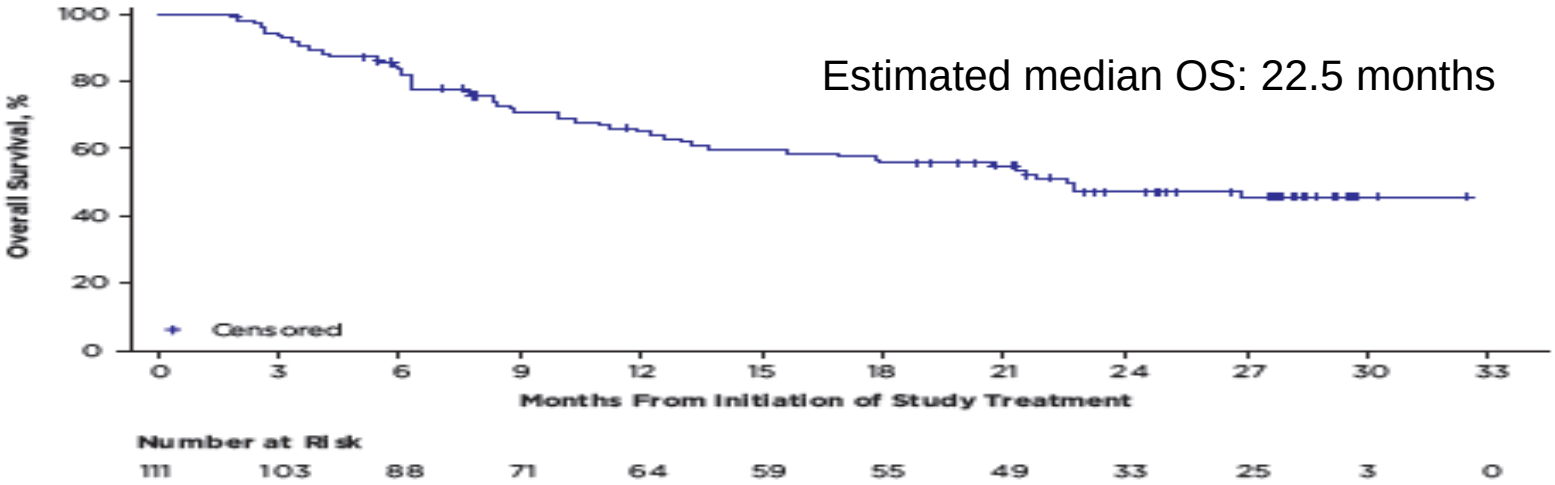


Wang ML et al, NEJM 2013

Ibrutinib survival curves



Estimated median PFS: 13.9 months



Estimated median OS: 22.5 months

Wang ML et al, NEJM 2013

Outcomes for patients relapsing/progressing on ibrutinib

Cheah CY, Ann Onc, 2015

- ***42 discontinued ibrutinib (or R-ibrutinib)***
  - 19% primary progression
  - 47% relapsed
  - 14% AE
  - 10% transplant
  - 10% patient choice
- ***Median 6.5 cycles (1-43)***
- ***31 patients salvage with ORR 32%***
  - regardless of regimen
- MEDIAN OS 8.4 months

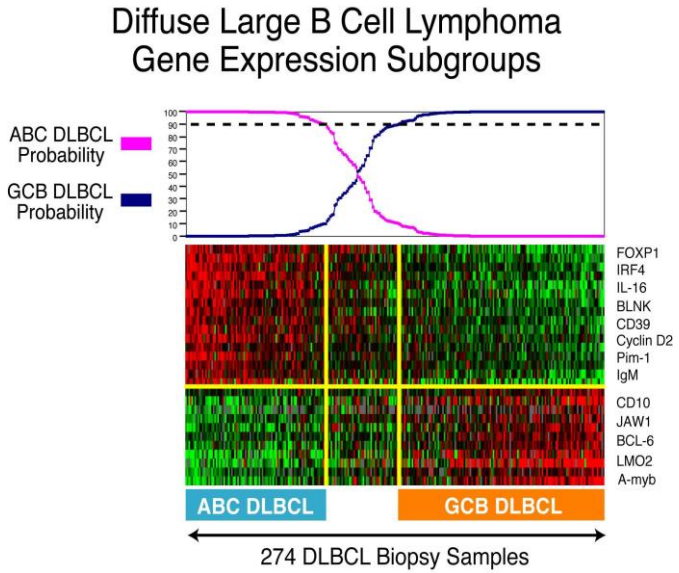
Martin P, Blood, 2016

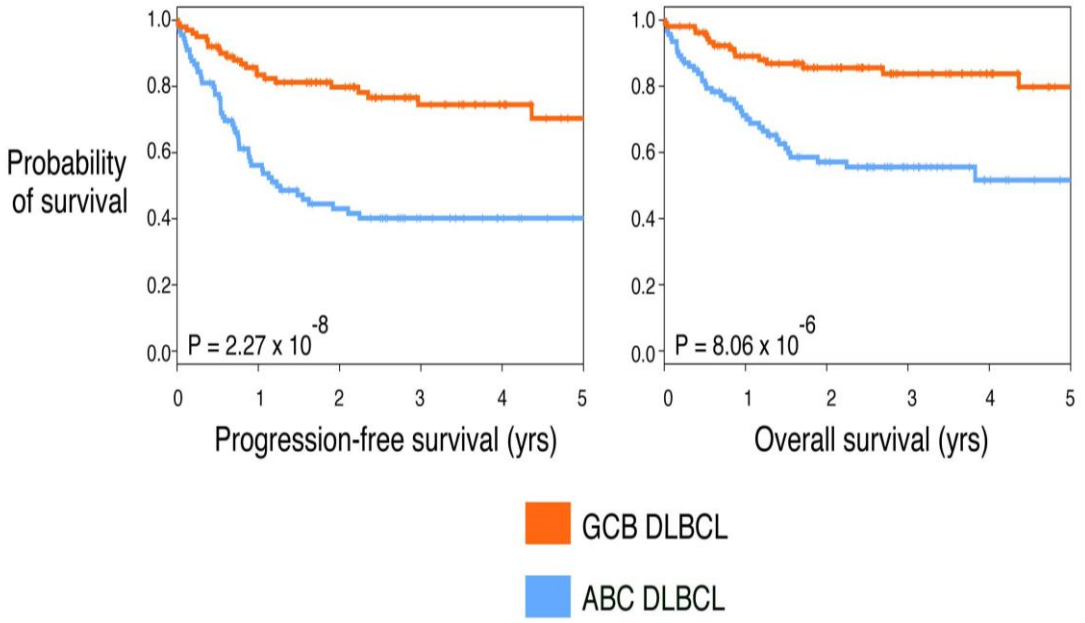
- ***114 discontinued ibrutinib***
  - 32% primary progression
  - 54% relapsed
  - 2% AE
  - 1% patient choice
- ***Median 4.7 cycles (0.7-43.6)***
- ***73 patients salvage with ORR 36%***
  - no differences in median OS with any specific regimen
- MEDIAN OS 2.9 months

Diffuse large B-cell lymphoma (DLBCL)

- DLBCL is the most common NHL
- remarkable heterogeneity with diverse histologic and molecular variants: germinal center B-cell (GCB) vs activated B-cell (ABC)-DLBCL.
- ABC-DLBCL, but not GC-type, relies on constitutive activation of NF-kB for proliferation and survival and has been associated with worse outcome
- Standard front-line regimen: R-CHOP
- High dose chemotherapy followed by autologous stem cell transplant (ASCT) is standard for DLBCL patients with chemosensitive relapse
- DLBCL patients that relapse after ASCT have a very poor prognosis with reported median OS of 5-10 months
- Options at relapse after ASCT include enrollment in a clinical trial, chemotherapy, ibrutinib, lenalidomide

Molecular Subtypes of DLBCL





Lenz et al, N Engl J Med. 2008

Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma

Wyndham H Wilson[1], Ryan M Young[1], Roland Schmitz[1], Yandan Yang[1], Stefania Pittaluga[2], George Wright[3], Chih-Jian Lih[4], P Mickey Williams[4], Arthur L Shaffer[1], John Gerecitano[5,6], Sven de Vos[7], Andre Goy[8], Vaishalee P Kenkre[9], Paul M Barr[10], Kristie A Blum[11], Andrei Shustov[12], Ranjana Advani[13], Nathan H Fowler[14], Julie M Vose[15], Rebecca L Elstrom[16], Thomas M Habermann[17], Jacqueline C Barrientos[18], Jesse McGreivy[19], Maria Fardis[19], Betty Y Chang[19], Fong Clow[19], Brian Munneke[19], Davina Moussa[19], Darrin M Beaupre[19] & Louis M Staudt[1]

Nature Medicine 2015

Wilson WH et al, Nat Med 2015

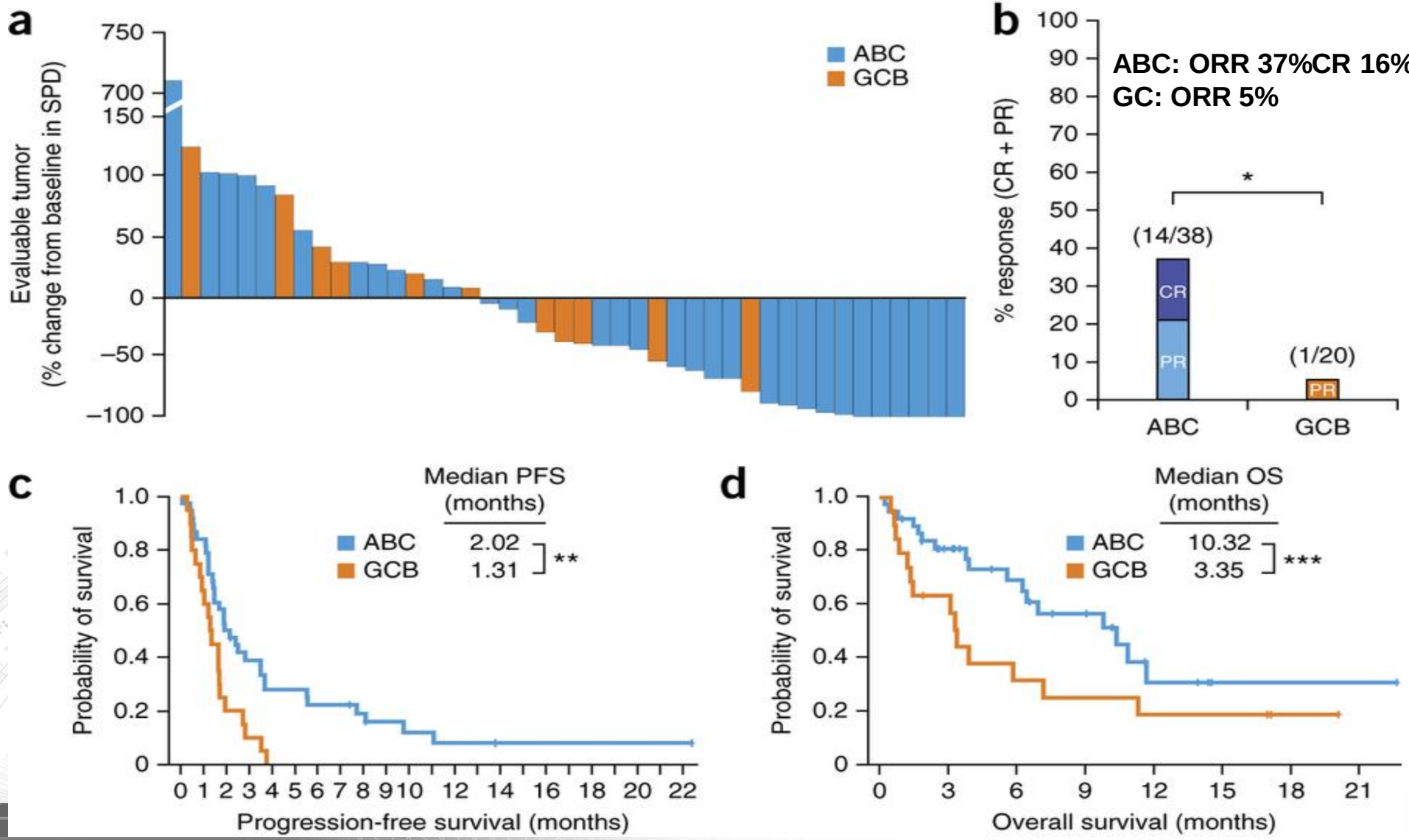
Patient characteristics

(Total 70 pts, 29 ABC, 20 GCB, 16 Unclassifiable, 5 Unknown)

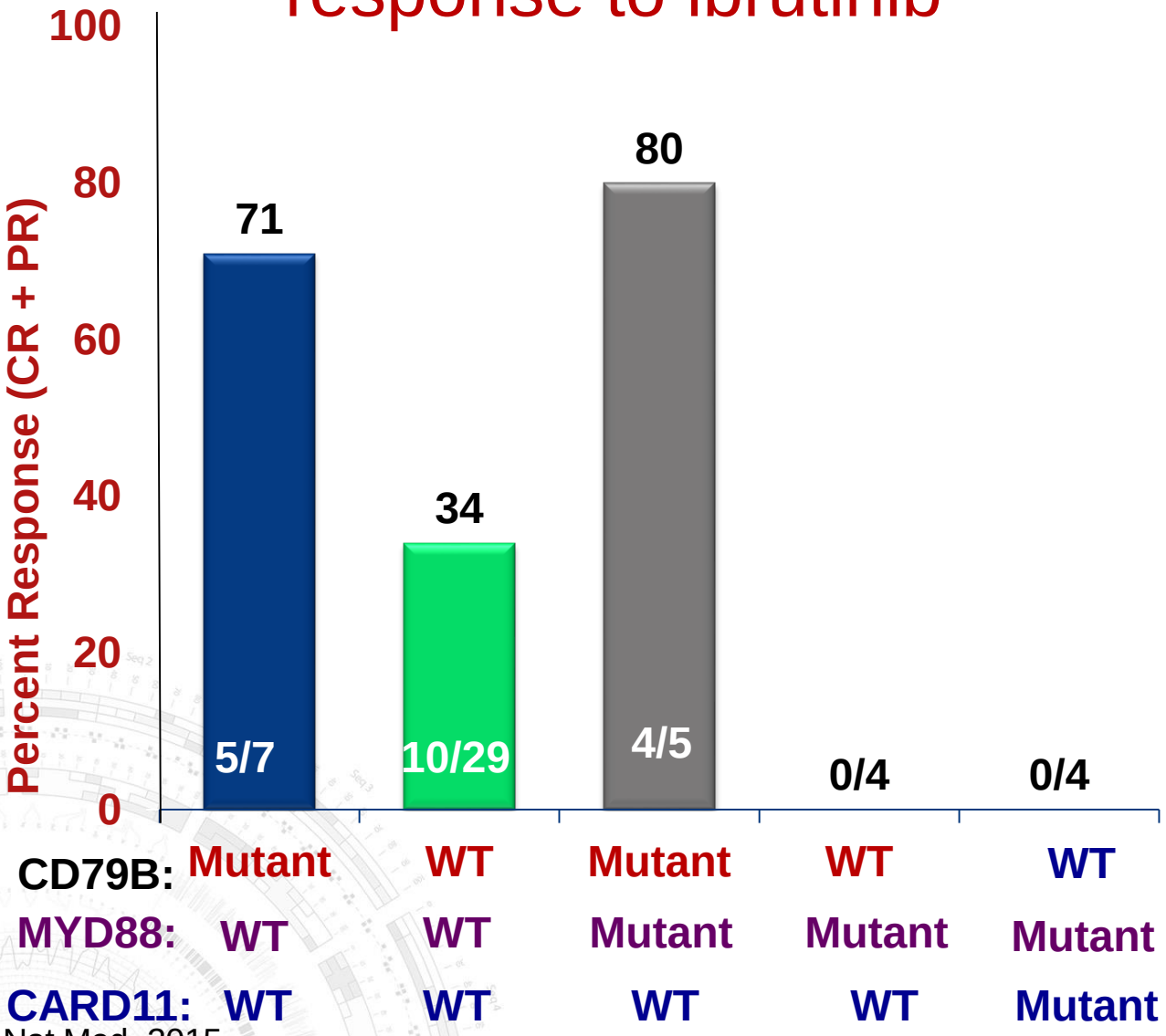
Characteristics	Total (N=70)	ABC (N=29)	GCB (N=20)
Median Age (range)	64 (28-92)	62 (34-89)	65 (28-92)
IPI (HI/High)	37 (59%)	61%	58%
Prior regimens median (range)	3 (1-7)	3 (1-7)	3.5 (1-7)
Prior ASCT	16 (23%)	17%	30%
Refractory disease	38 (54%)	41%	70%

Wilson WH et al, Nat Med 2015

Ibrutinib improves PFS/OS in ABC DLBCL compared to GC-subtype



CD79B mutant ABC DLBCL predicts higher rate of response to ibrutinib



Wilson WH et al, Nat Med 2015

The James

The Ohio State University
Comprehensive Cancer Center

Primary and acquired resistance to ibrutinib

The James

The Ohio State University

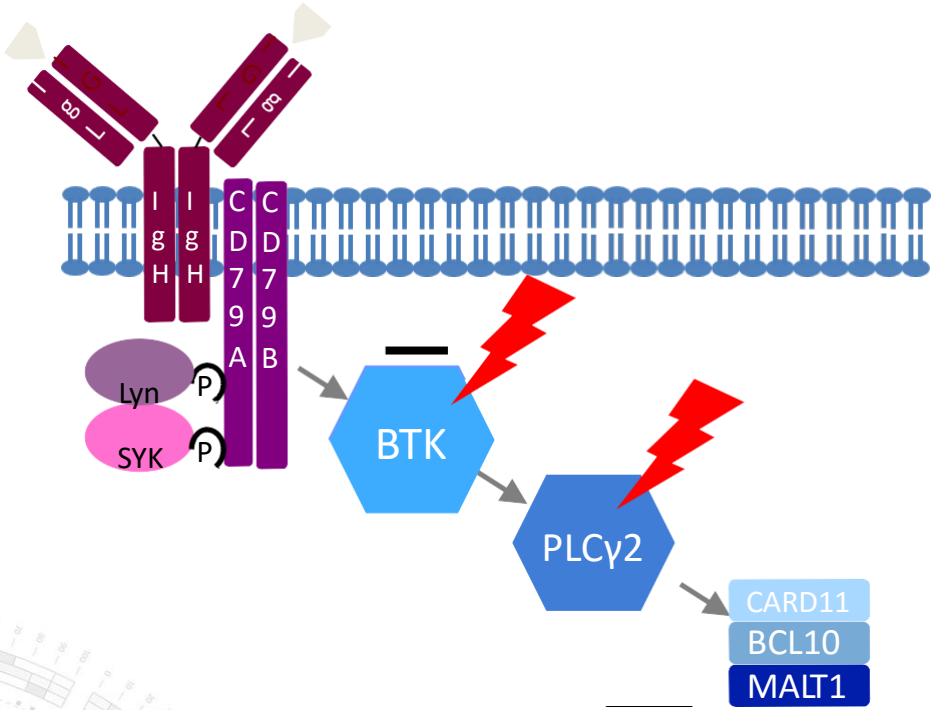
Comprehensive Cancer Center

Characteristics of 6 CLL patients with acquired resistance to ibrutinib

Patient	Age	No. Prior Therapies	Cytogenetics	Study Treatment	Duration on Ibrutinib	Best Response	Identified Mutation
1	59	5	del(17p13.1), +12	560 mg qd	621 days	PR	**C481S, BTK**
2	75	2	del(17p13.1), complex karyotype	420 mg qd	673 days	PR	**R665W, PLCγ2**
3	59	3	del(11q22.3)	BR x 6 cycles, 420 mg qd	388 days	CR	**C481S, BTK**
4	51	2	complex karyotype	Ofatumumab x 24 weeks, 420 mg qd	674 days	CR	**C481S, BTK**
5	69	9	del(17p13.1), complex karyotype	840 mg qd	868 days	PR	**C481S, BTK**
6	61	4	del(17p13.1), complex karyotype	Ofatumumab x 24 weeks, 420 mg qd	505 days	PR	**L845F, PLCγ2; C481S, BTK**

Woyach J et al. NEJM 2014

BTK and PLCγ2 mutations

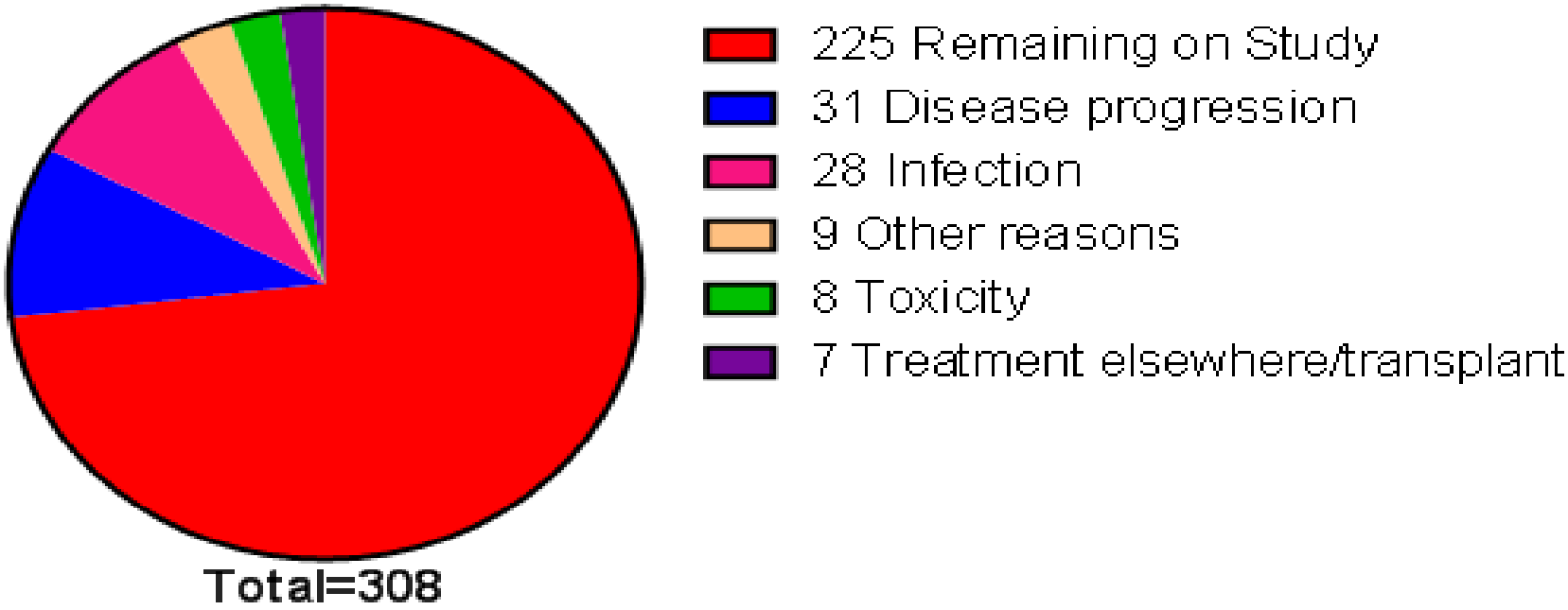


WES discovery of BTK and PLCG2 mutations: Woyach J et al, NEJM 2014

Characterization of ibrutinib resistant disease: Maddocks et al, JAMA Oncol 2015

Modeling PLC γ2 mutation: Liu et al, Blood 2015

What is the pattern of ibrutinib failure?



Patients	BTK	PLCγ2	Both
11/13	7	2	2

Maddocks K et al. Jama Oncol 2015

Mechanisms of ibrutinib resistance in MCL

- Balasubramian D, et al. (ASH 2014, abstract 78)
  - 25 patients refractory to ibrutinib in multi-center, phase 2 trial
    - 23 patients had **pre-treatment** tumor or CD19-selected peripheral blood samples sequenced
    - No BTK C481S mutations and 1 PLCγ2 mutation identified

- Chiron D, et al. (Cancer Discovery, 2014)
  - 8 patients with ibrutinib failure with BTK and PLCγ2 mutational analysis **at recurrence**
    - 2 patients with C481S mutations, treated for 14 and 30 months
    - 6 patients without mutations, all treated < 5 months

- Martin P, et al. (Blood, 2016)
  - 114 patient with ibrutinib failure
  - 10 patients had BTK and PLCγ2 mutational analysis **at recurrence**
    - 2 patients with C481S mutations, treated for 12.1 and 12.6 months
    - 8 patients without mutations, treated 0.4-43 months

Mechanisms of ibrutinib resistance in MCL

- CARD11 mutation
  - Identified in 1 patient at relapse (Wu et al, ICML 2015)
- Genetic lesions in the alternative NF-kB pathway occur in patients with MCL (Chiron D et al, Cancer Discovery 2014)
  - TRAF2 (6%), TRAF3 (10%), BIRC2, BIRC3, MAP3K14
- BTK WT
  - Despite ongoing inhibition of BTK, high level of PI3K-AKT expression and activation has been found (Chiron D et al, Cancer Discovery 2014)

What we know about single agent ibrutinib

- Most CLL patients have durable remissions to ibrutinib
- Acquired mutations in BTK and PLCϒ2 appear to be the main driver to ibrutinib resistance in CLL
- 30-40% of MCL patients do not respond to ibrutinib and 10-20% have very short remissions.
- Among the MCL patients that achieve a durable remission, relapse appears universal.
- Responses in DLBCL is limited to a subset of patients.
- Duration of response in DLBCL is also short

Objectives

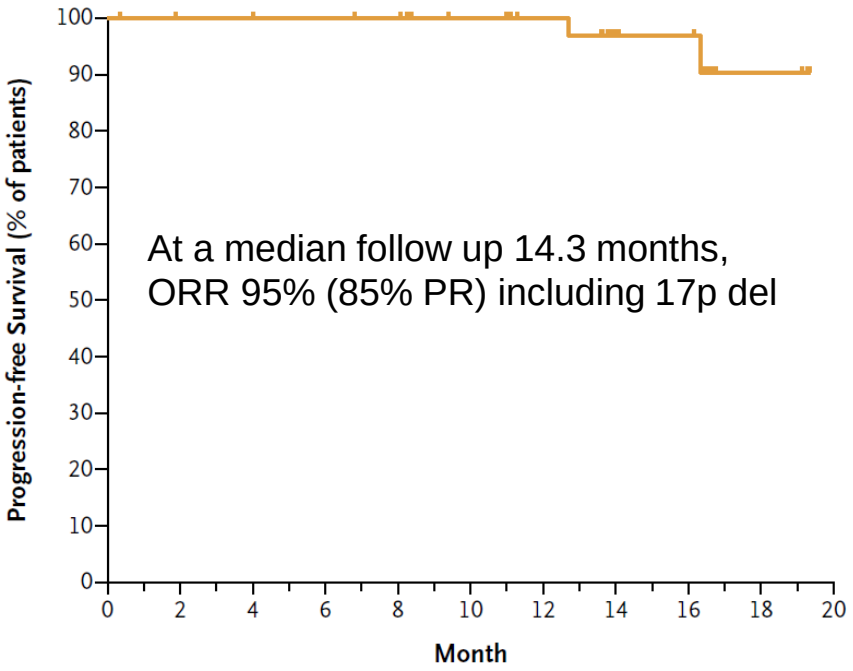
- Explore the B-cell receptor signaling pathway
- Discuss BTK as a therapeutic target
- Discuss activity of and resistance to BTK inhibitors in the treatment of lymphoid malignancies
- Identify mechanisms to improve upon B-cell receptor inhibition

Novel BTK inhibitors: ACP-196 in CLL

- Acalabrutinib (ACP-196) is a second generation, selective, irreversible inhibitor of BTK characterized by the absence of irreversible inhibition of other kinases (TEC, EGFR, ITK)

Table 1. Baseline Demographic and Clinical Characteristics of the Patients.

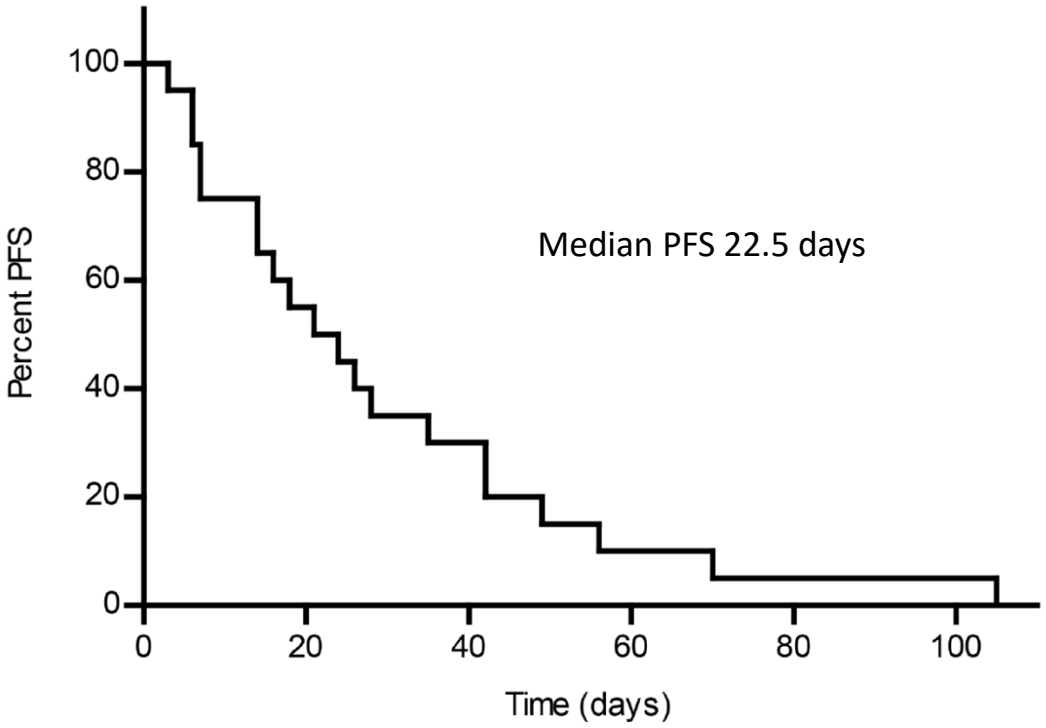
Characteristic	Value (N = 61)
Age	
Median — yr	62
Rai risk classification — no. (%)†	
Low	1 (2)
Intermediate	19 (31)
High	41 (67)
No. of previous therapies	
Median	3
Range	1–13
Cytopenia at baseline — no. (%)	
Absolute neutrophil count ≤1500 μl	15 (25)
Hemoglobin ≤11.0 g/dl	21 (34)
Platelet count ≤100,000/μl	32 (52)
Prognostic factors — no./total no. (%)	
Unmutated immunoglobulin variable-region heavy-chain gene	38/51 (75)
Chromosome 17p13.1 deletion‡	18/59 (31)
Chromosome 11q22.3 deletion‡	17/59 (29)



At a median follow up 14.3 months, ORR 95% (85% PR) including 17p del

Byrd JC et al. NEJM 2016

ACP-196 in lymphoma preclinical models



24 dogs with spontaneous DLBCL received escalating dose of ACP-196 (range 2.5-20mg/kg BID)

Harrington B, et al. PlosOne 2016

Rationale for combination therapy

- Improved ORR and convert PR to CR
  - Deeper remissions = longer PFS/OS?
- Prevent relapse

Combination therapy

- With conventional chemotherapy
- With biological agents

What does chemotherapy add to ibrutinib in CLL?

	Ibrutinib+BR
Phase	3
Number	289
Age	64
17p permitted ?	no
# prior regimens	1 – 11 (2)
ORR	**83%**
CR	**10%**
Median follow up	17 mo
PFS	**72% at 24 mo**
OS	**88% at 24 mo**

Chanan-Khan A et al, Lancet Oncol 2016

What does chemotherapy add to ibrutinib in CLL?

	Ibrutinib+BR	Ibrutinib alone
Phase	3	3
Number	289	101
Age	64	64
17p permitted ?	no	yes
# prior regimens	1 – 11 (2)	1-- 12 (4)
ORR	**83%**	**90%**
CR	**10%**	**7%**
Median follow up	17 mo	36 mo
PFS	**72% at 24 mo**	**69% at 30 mo**
OS	**88% at 24 mo**	**79% at 30 mo**

Chanan-Khan A et al, Lancet Oncol 2016
Byrd JC et al, NEJM 2014

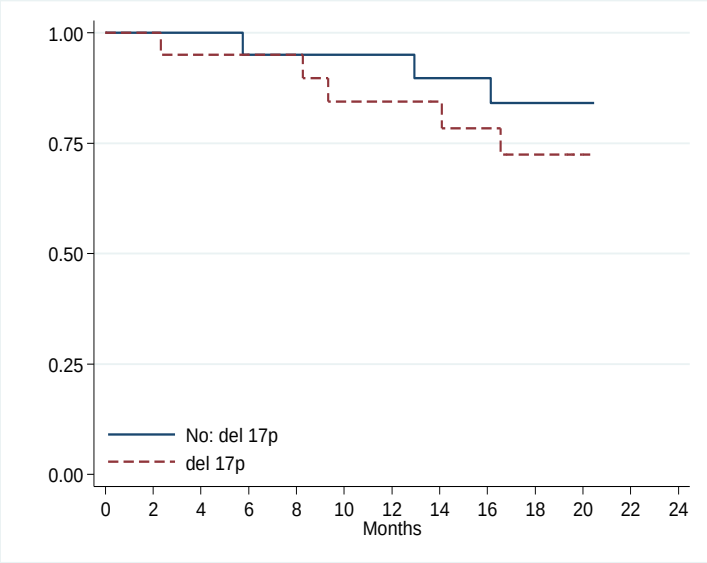
What does chemotherapy add to ibrutinib in lymphoma?

	Ibrutinib+BR	Ibrutinib alone
ABC-DLBCL		
ORR	**37%**	**37%**
CR	**27%**	**16%**
Median PFS	**2.5 months**	**2 months**
Median OS	Not provided	**10 months**
MCL		
ORR	**94%**	**68%**
CR	**76%**	**21%**
Median PFS	Not reached	**13.9 months**
Median OS	Not reached	**22.5 months**

Wang ML et al, NEJM 2013
Wilson WH et al, Nat Medicine 2015
Maddocks K et al, Blood 2015

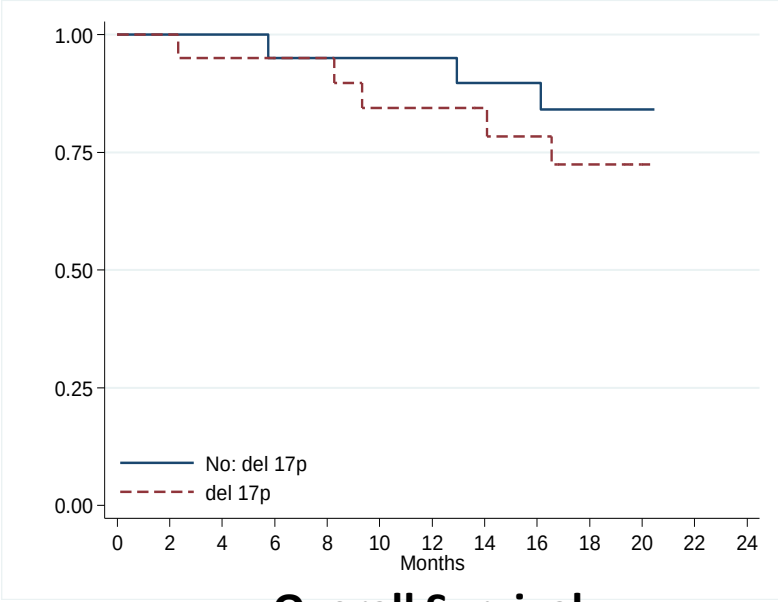
Ibrutinib + Rituximab for patients with high risk CLL

- 40 patients (some previously untreated)
- ORR 95% (8% CR)



Progression Free Survival

18 month PFS 78%



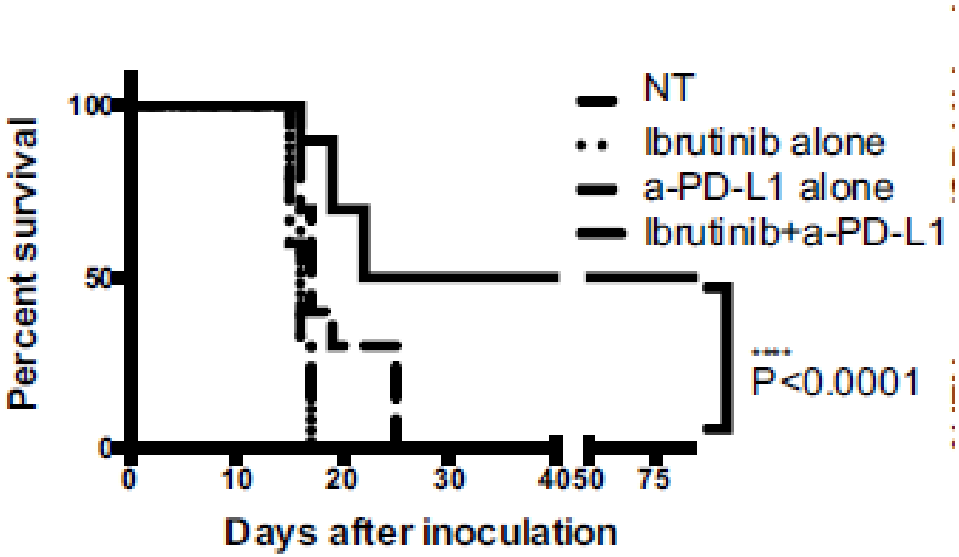
Overall Survival

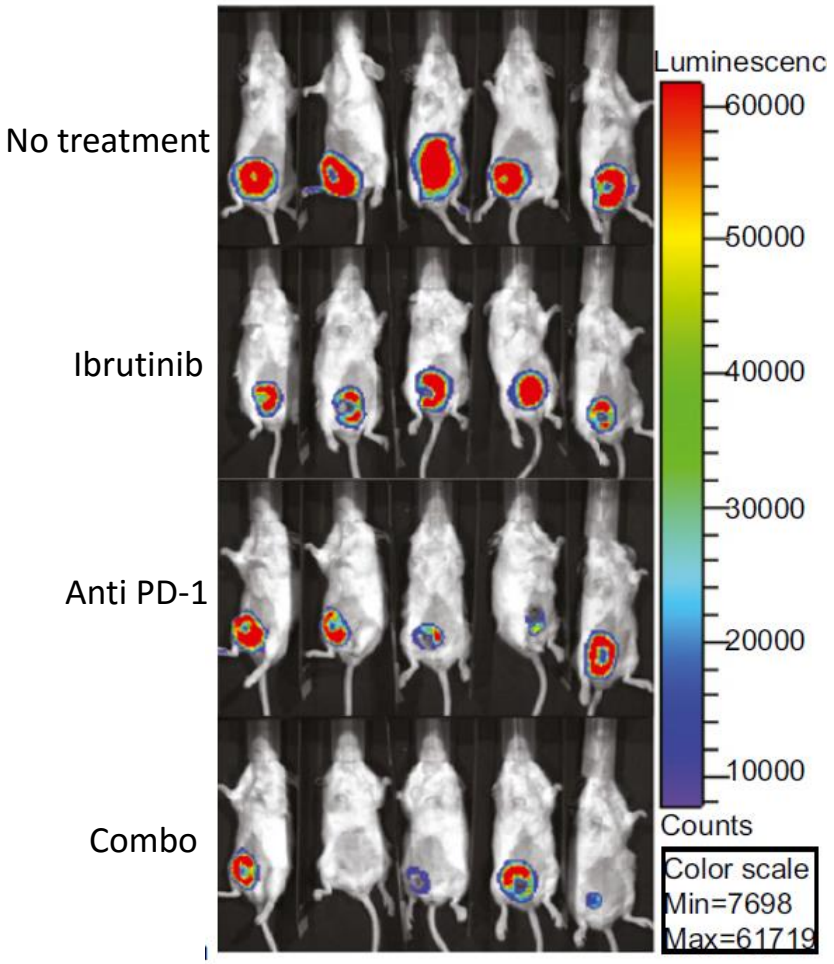
18 month OS 84%

PFS at 30 months 69%; OS 79%; **Byrd JC Blood 2015**

Burger JA, et al. Lancet Oncol 2014

In vivo ibrutinib and an anti-PD-1 blocking agent controls BTK resistant B cell lymphoma





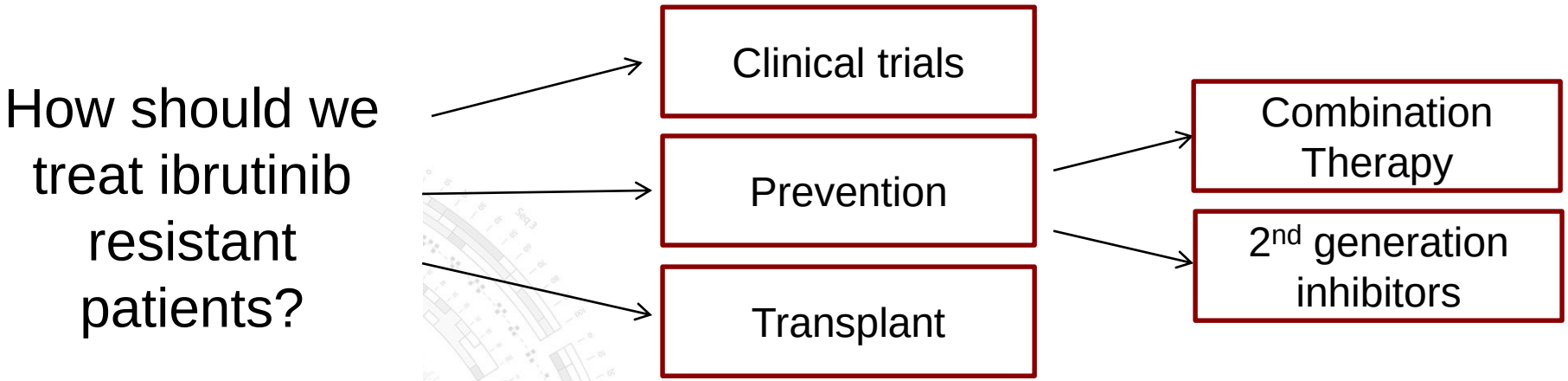
Sagive-Barfi et al. PNAS 2015

The James

The Ohio State University
Comprehensive Cancer Center

Conclusions

- BTK inhibitors have dramatically changed the treatment paradigm for CLL
- Ibrutinib has high single agent activity in several subtypes of NHL but most patients ultimately relapse and survival after relapse is short
- Multiple mechanisms of resistance likely exist, and these are better defined in CLL than B-cell NHL



The new James Cancer Hospital

The James

Thank You

Interested in a collaboration?
Interested in a research experience?
Please contact me: Lapo.Alinari@osumc.edu

To learn more about Ohio State's cancer program, please visit **cancer.osu.edu** or follow us in social media:

The James

The Ohio State University
Comprehensive Cancer Center