Detection of Burkitt Lymphoma immunoglobulin rearrangements in blood may have prognostic value

Katharine Lombardo

Outline

- B cell receptor in normal cells and in B cell malignancies

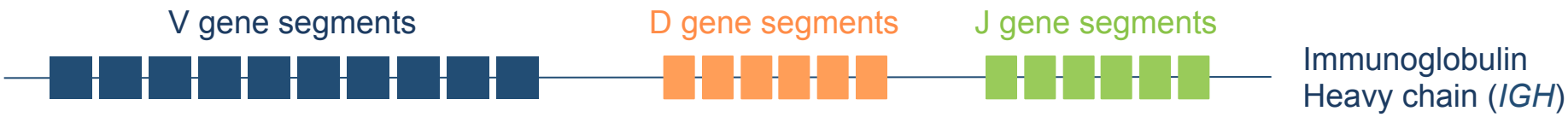
- B cell receptor in Burkitt Lymphoma

Outline

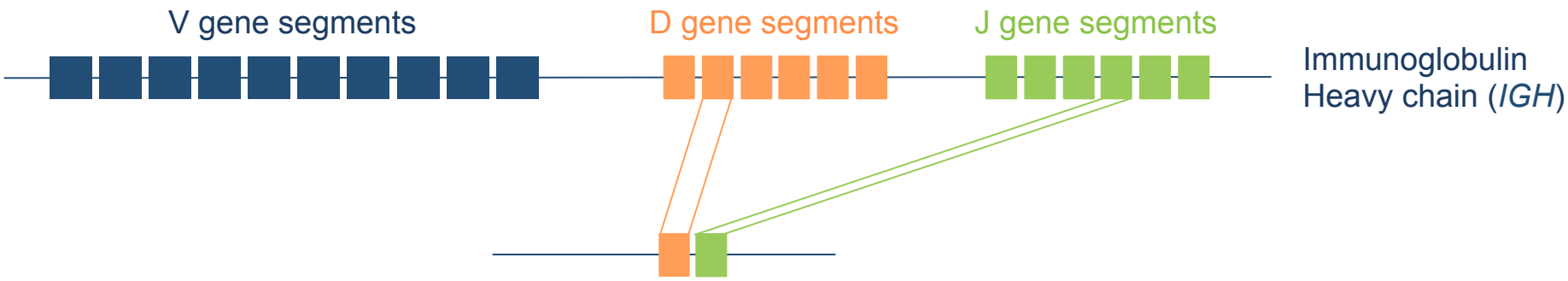
- B cell receptor in normal cells and in B cell malignancies

- B cell receptor in Burkitt Lymphoma

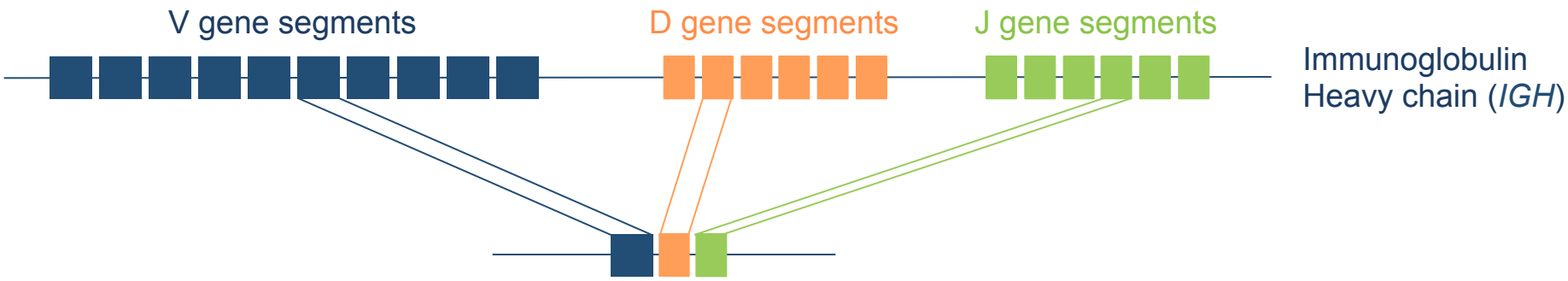
All B cells express a unique B cell receptor (BCR)



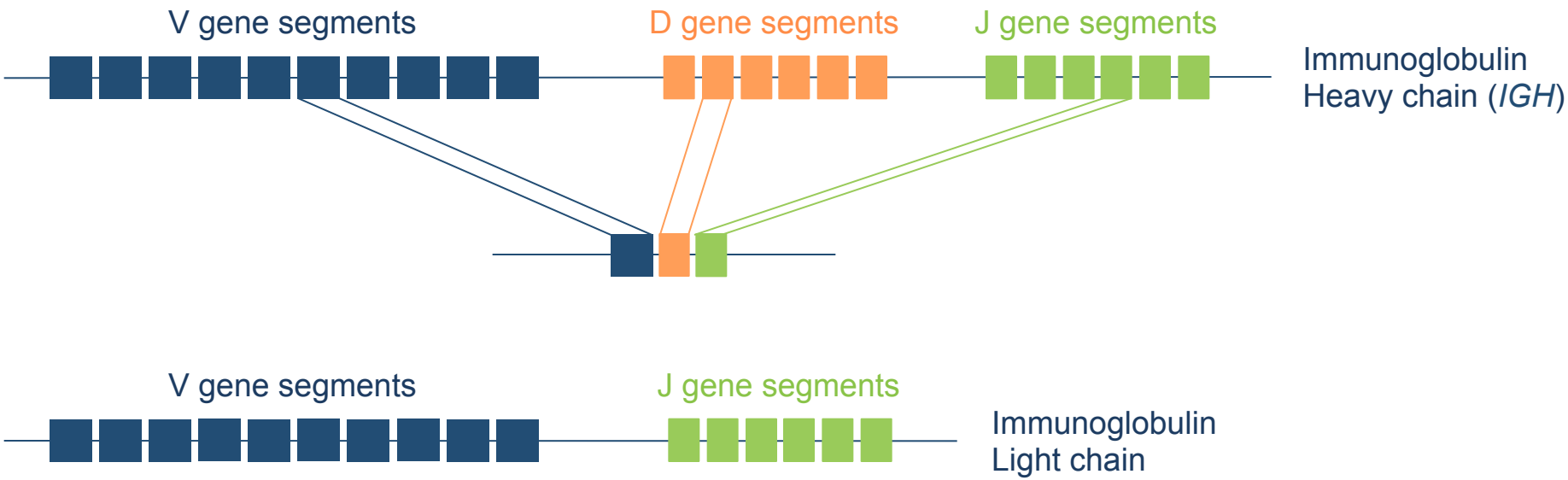
All B cells express a unique B cell receptor (BCR)



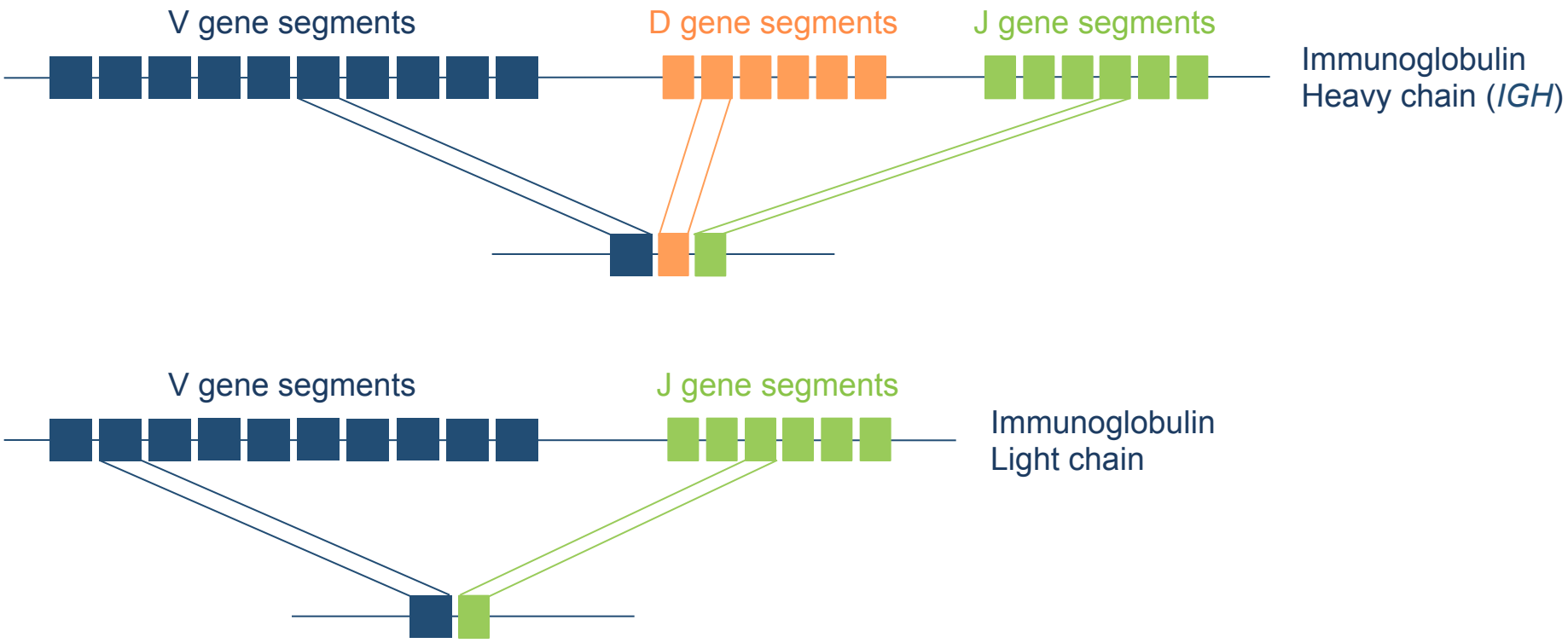
All B cells express a unique B cell receptor (BCR)



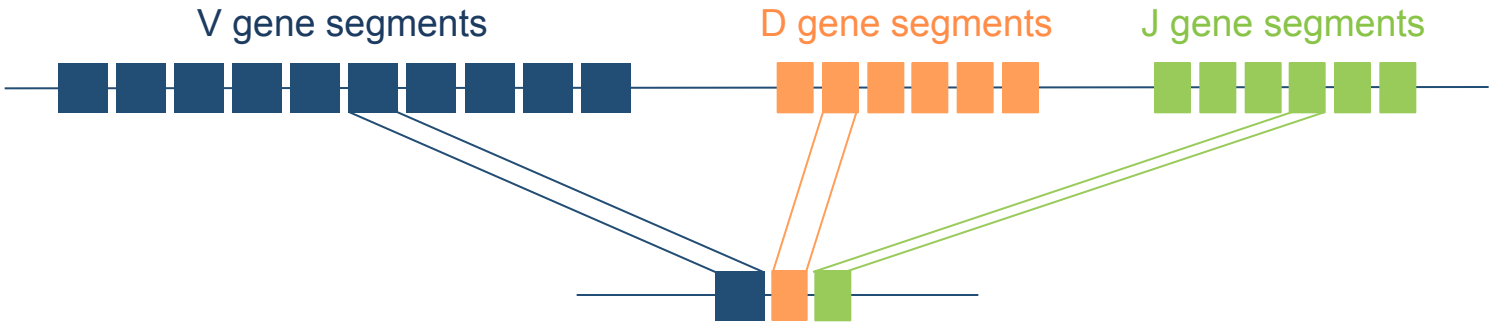
All B cells express a unique B cell receptor (BCR)



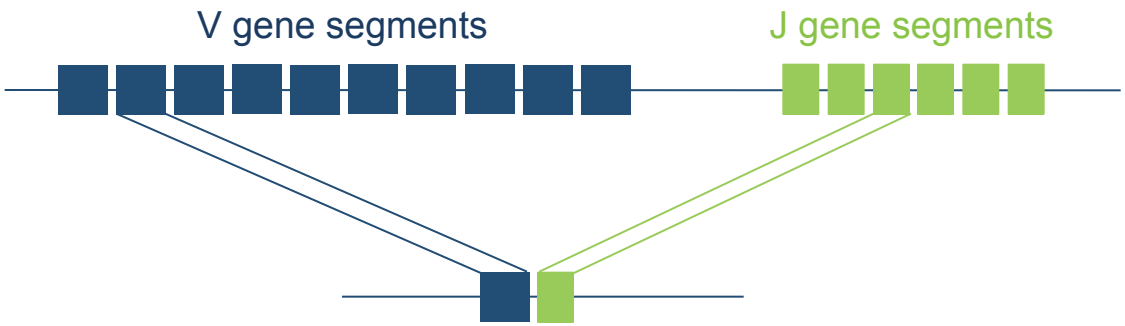
All B cells express a unique B cell receptor (BCR)



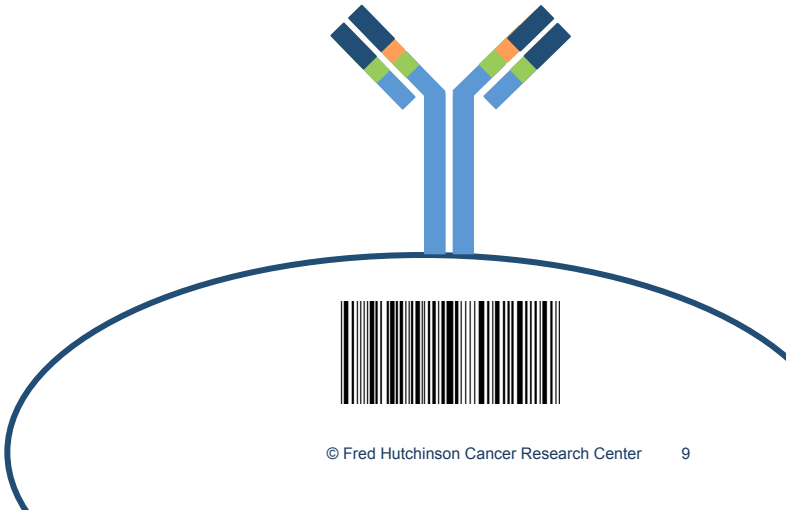
All B cells express a unique B cell receptor (BCR)



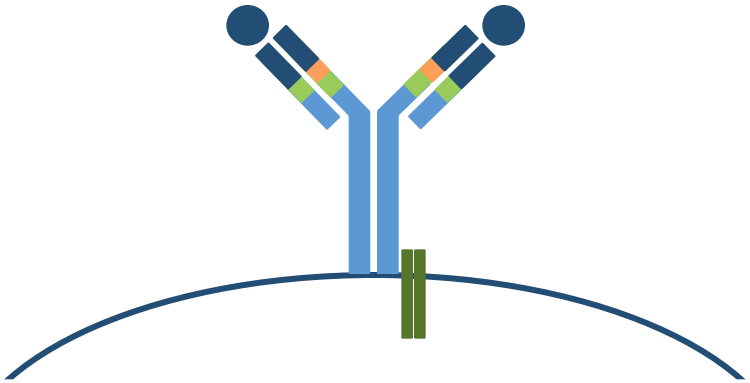
Immunoglobulin
Heavy chain (*IGH*)



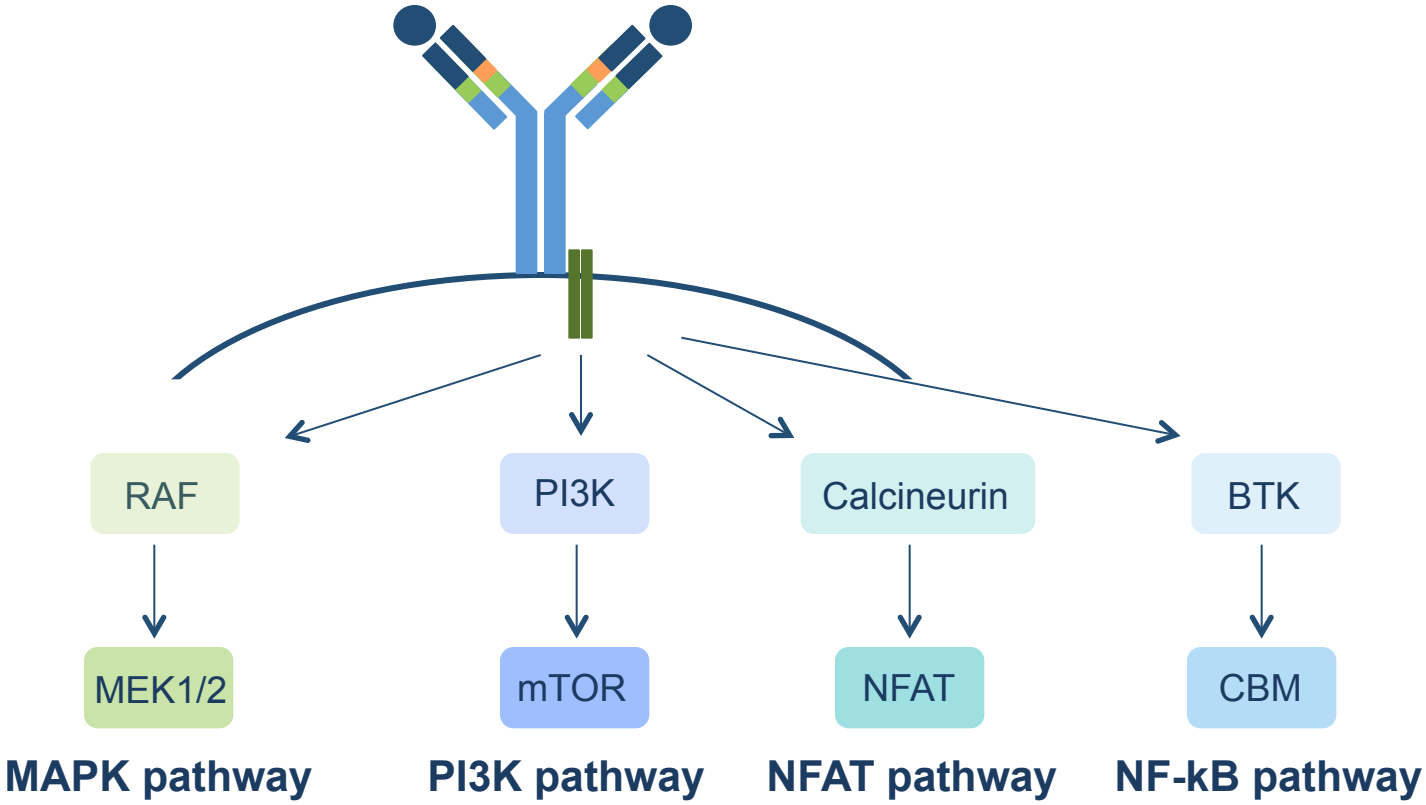
Immunoglobulin
Light chain



The BCR sends survival, differentiation and proliferation signals



The BCR sends survival, differentiation and proliferation signals



Adapted from Young & Staudt. *Nature Reviews*. 2013

BCR signaling in B cell lymphomas

Role of BCR Signaling	Malignancy
BCR signaling required for cell viability	ABC DLBCL, BL
Activating mutations within BCR signaling pathways	ABC DLBCL, BL
BCR stereotypy	CLL, SMZL, MALT lymphoma
BCRs activated by specific antigens	CLL, MALT lymphoma, FL, BL

BCR signaling in B cell lymphomas

Role of BCR Signaling	Malignancy
BCR signaling required for cell viability	ABC DLBCL, BL
Activating mutations within BCR signaling pathways	ABC DLBCL, BL
BCR stereotypy	CLL, SMZL, MALT lymphoma
BCRs activated by specific antigens	CLL, MALT lymphoma, FL, BL

Outline

- B cell receptor in normal cells and in B cell malignancies

- B cell receptor in Burkitt Lymphoma

Outline

- B cell receptor in normal cells and in B cell malignancies

- B cell receptor in Burkitt Lymphoma

BL patient cohort

- 19 BL tumor samples from Uganda Cancer Institute with 14 diagnostic patient-matched blood samples (13 post-treatment)

BL patient cohort

- 19 BL tumor samples from Uganda Cancer Institute with 14 diagnostic patient-matched blood samples (13 post-treatment)

Ugandan BL Patient Cohort	
Characteristic	**BL Patients (n=19)**
Gender	
Female	37%
Male	63%
Age at enrollment	
Median	7 yrs
Range	4-12 yrs
HIV Status	
Negative	95%
Positive	5%
Ziegler Disease Stage	
A	37%
B	16%
C	16%
D	31%

BL patient cohort

- 19 BL tumor samples from Uganda Cancer Institute with 14 diagnostic patient-matched blood samples (13 post-treatment)

Ugandan BL Patient Cohort	
Characteristic	**BL Patients (n=19)**
Gender	
Female	37%
Male	63%
Age at enrollment	
Median	7 yrs
Range	4-12 yrs
HIV Status	
Negative	95%
Positive	5%
Ziegler Disease Stage	
A	37%
B	16%
C	16%
D	31%

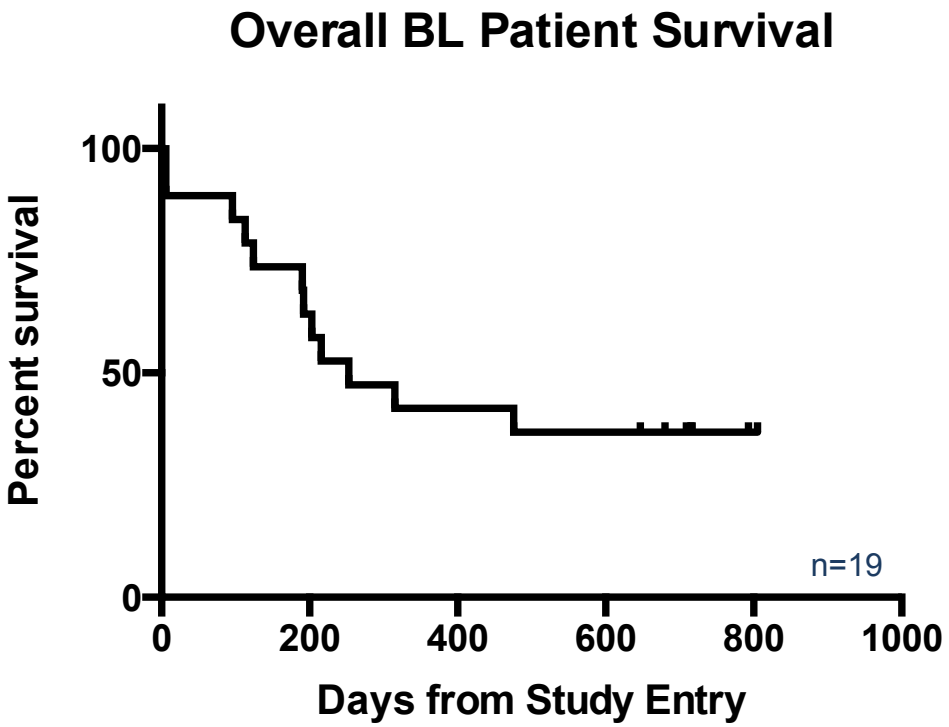
- 9 BL tumor samples from NCI Ghana BL Study with 6 patient-matched serum samples and 9 patient-matched CSF samples

Long-term BL patient survival is 37%

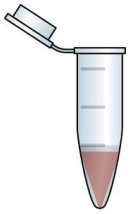
Standard treatment regimen: 6 cycles of cyclophosphamide, vincristine and methotrexate chemotherapy

Long-term BL patient survival is 37%

Standard treatment regimen: 6 cycles of cyclophosphamide, vincristine and methotrexate chemotherapy

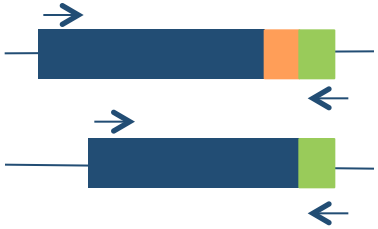


Deep sequencing of BL immunoglobulin genes

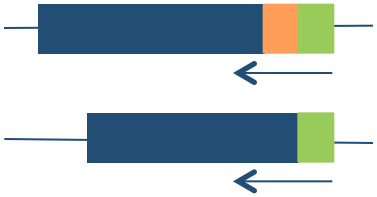


Cryopreserved BL tumor, blood, serum or CSF sample

Digested and lysed to extract gDNA



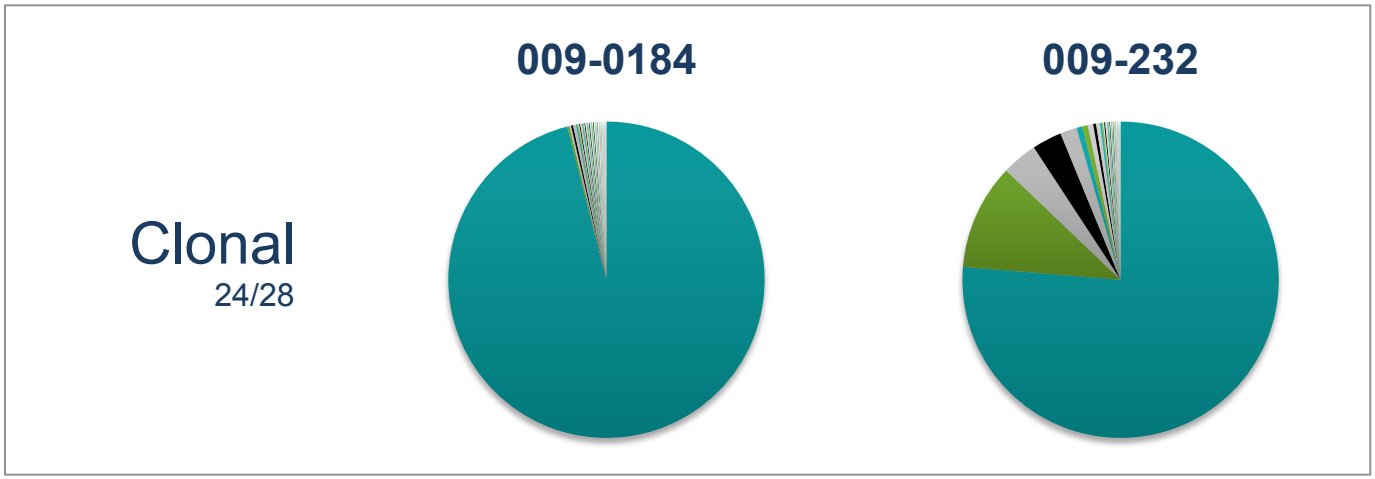
Amplification of Ig genes



High-throughput sequencing of the Ig repertoire in the tumor or blood sample

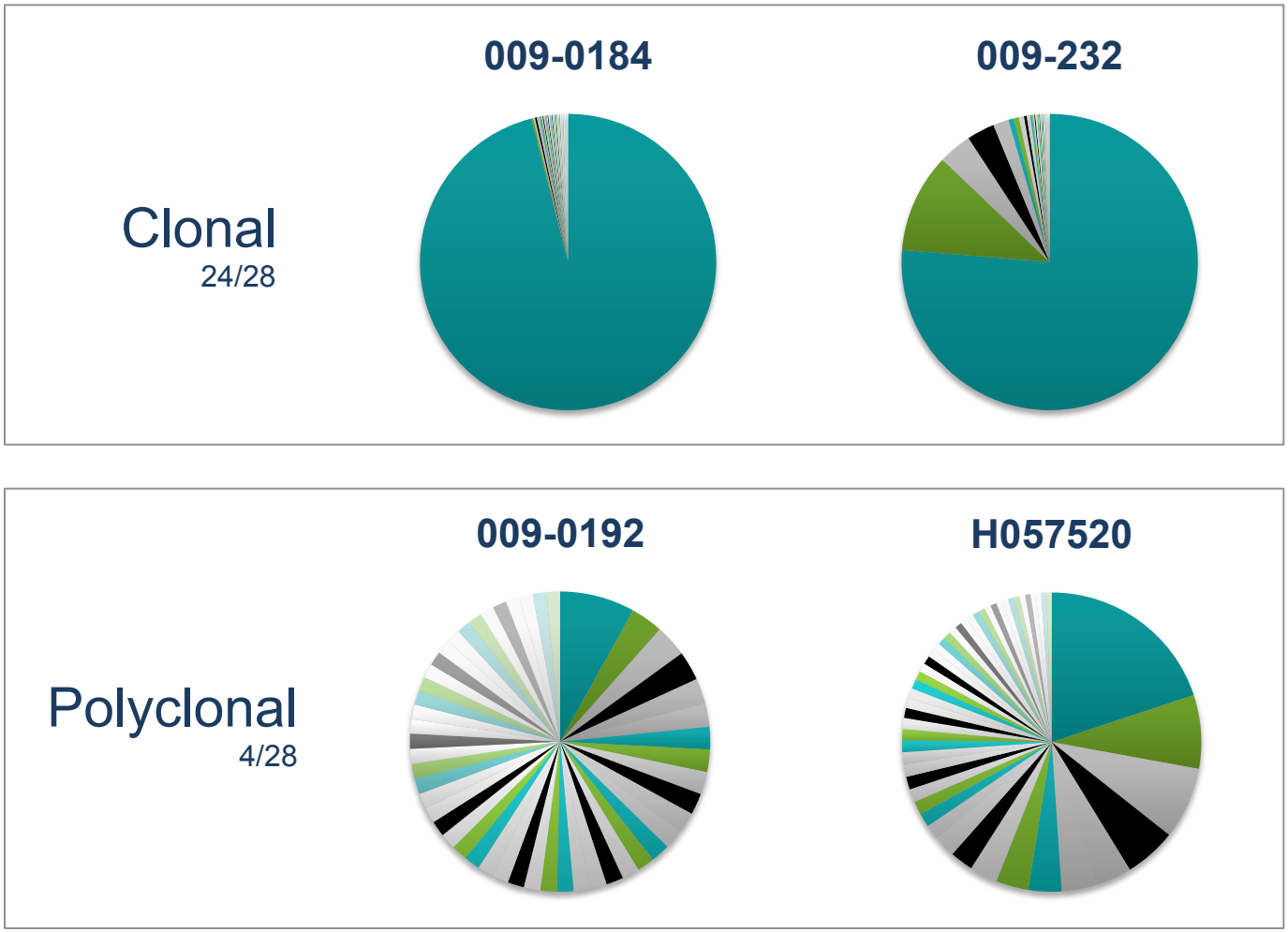
Clonal *IGH* rearrangements identified in 24/28 BL tumors

Top 50 unique sequences from representative BL patient tumors

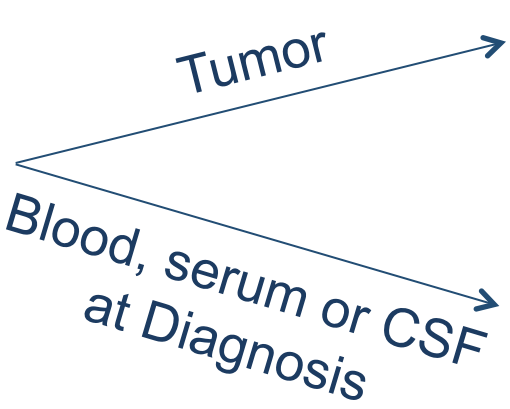


Clonal *IGH* rearrangements identified in 24/28 BL tumors

Top 50 unique sequences from representative BL patient tumors



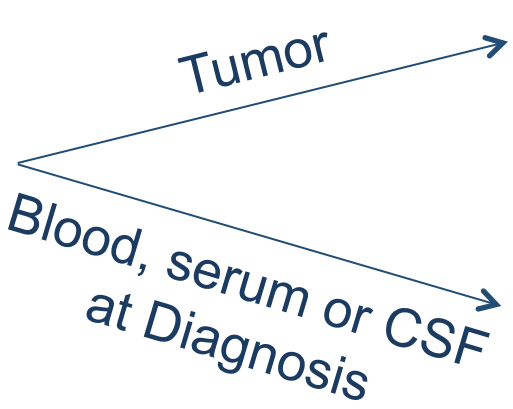
BL BCR as a patient-specific biomarker



1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

BL BCR as a patient-specific biomarker

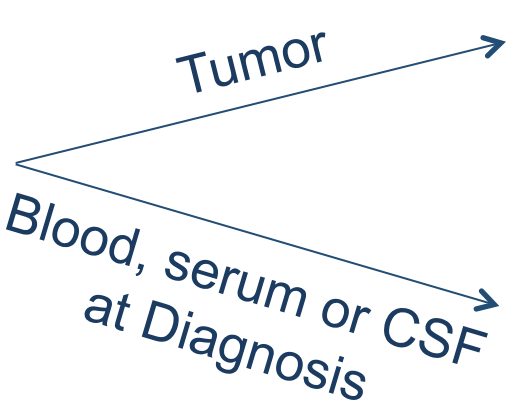


1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

Source	Negative for ct-DNA	Positive for ct-DNA
Blood	4/14 patients	10/14 patients

BL BCR as a patient-specific biomarker



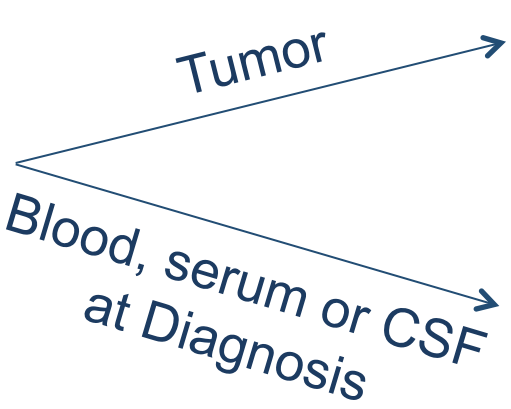
1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

Source	Negative for ct-DNA	Positive for ct-DNA
Blood	4/14 patients	10/14 patients
Serum	2/5 patients*	3/5 patients*

*Serum data from 1 patient still pending

BL BCR as a patient-specific biomarker



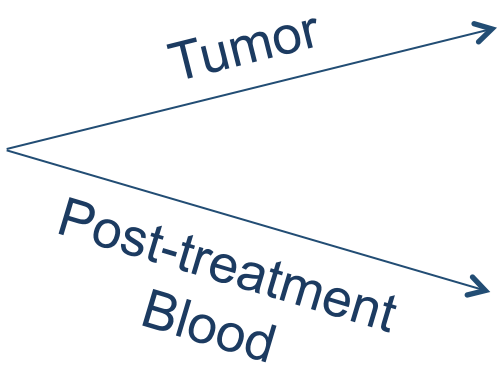
1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

Source	Negative for ct-DNA	Positive for ct-DNA
Blood	4/14 patients	10/14 patients
Serum	2/5 patients*	3/5 patients*
CSF	7/9 patients	2/9 patients**

*Serum data from 1 patient still pending
**1 positive CSF sample did not match patient

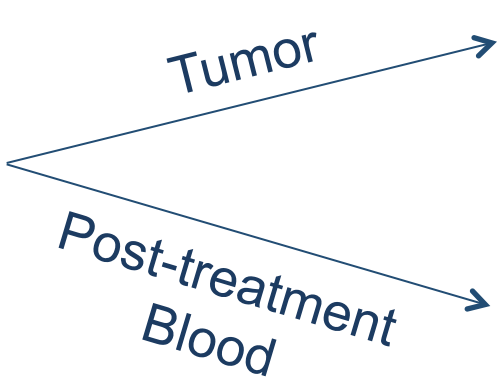
BL BCR as a patient-specific biomarker



1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

BL BCR as a patient-specific biomarker

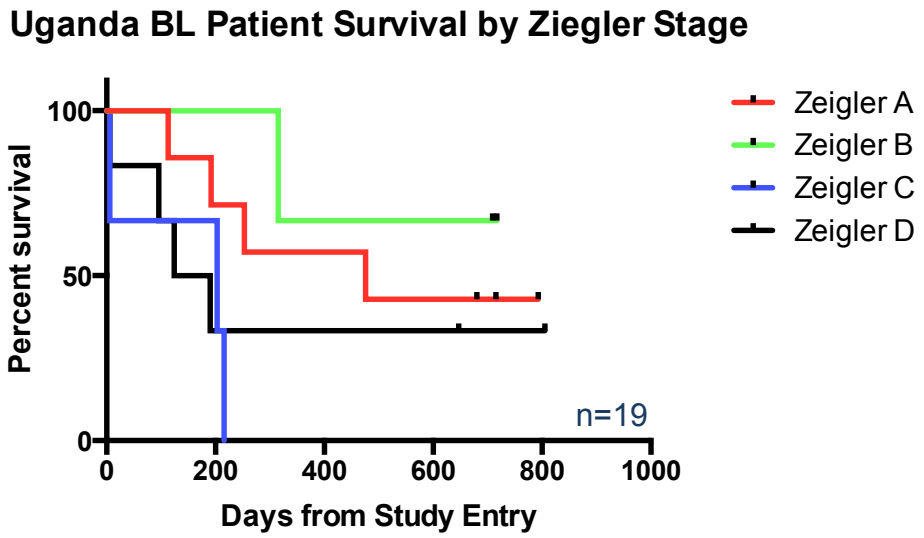


1. Identify unique *IGH* rearrangement

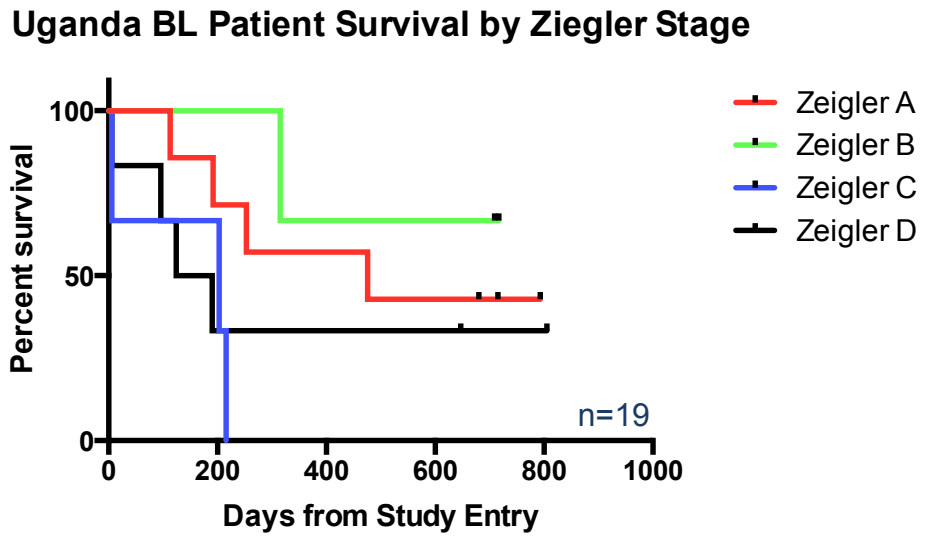
2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

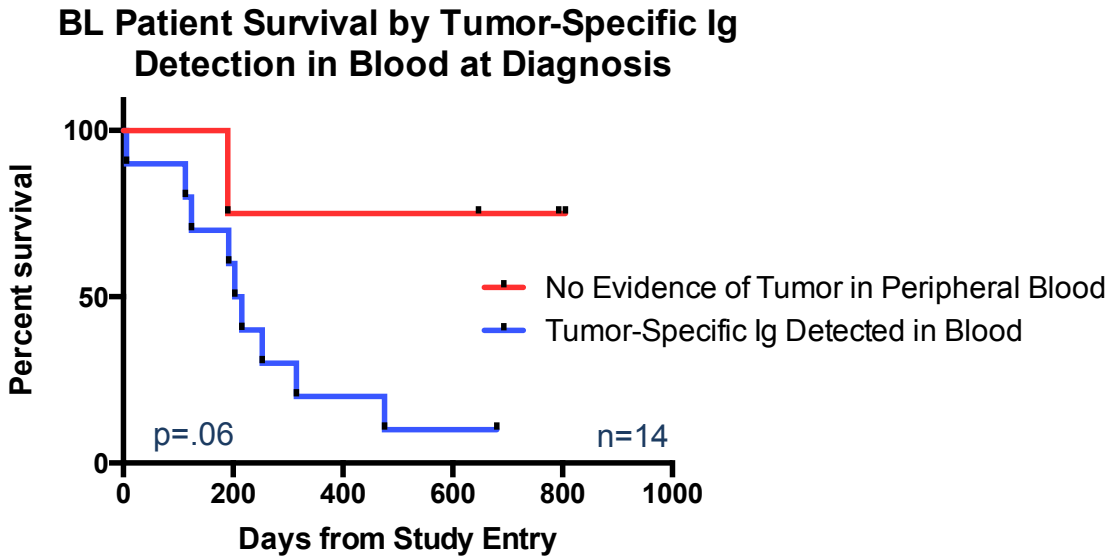
Source	Negative for ct-DNA	Positive for ct-DNA
Blood	11/13 patients	2/13 patients

BL BCR as a prognostic indicator at diagnosis

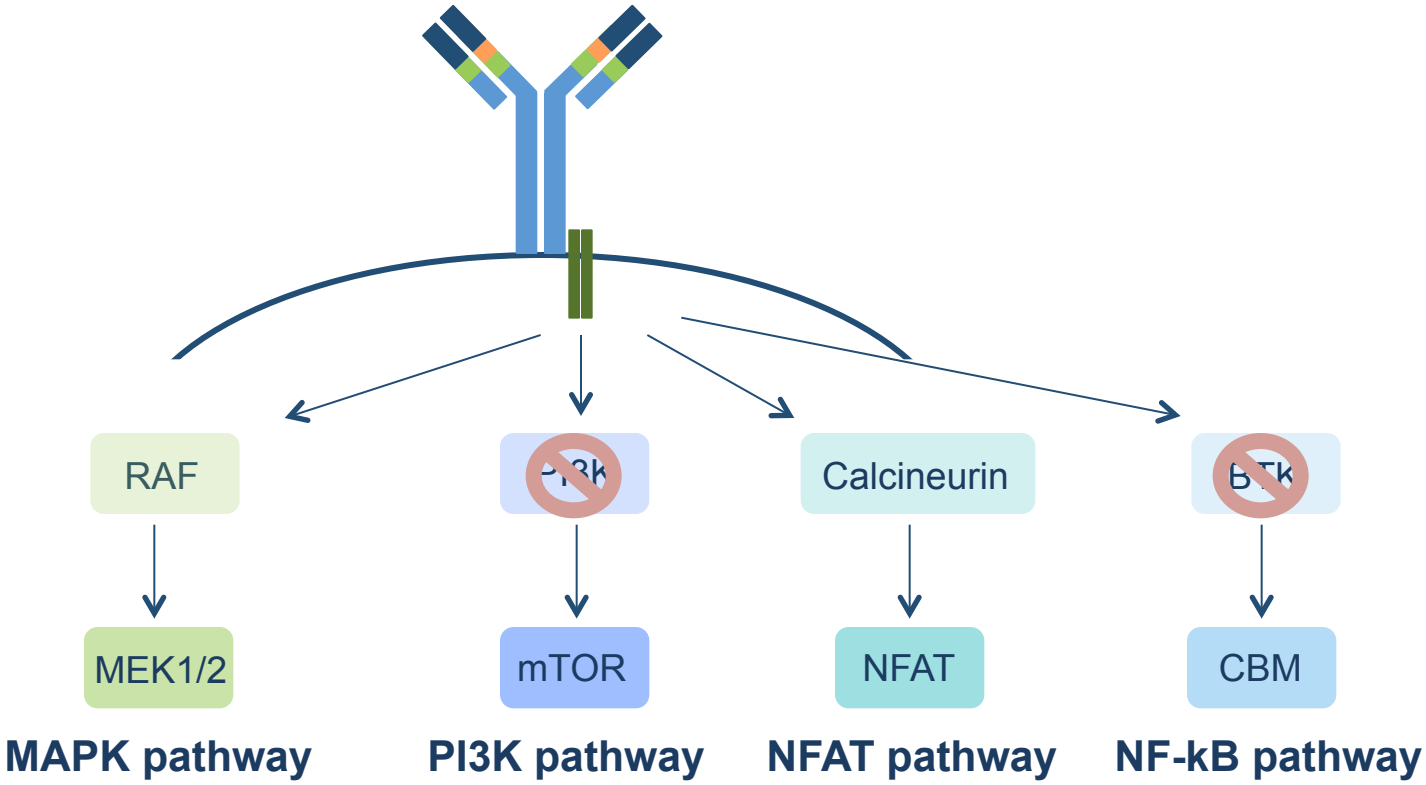


BL BCR as a prognostic indicator at diagnosis

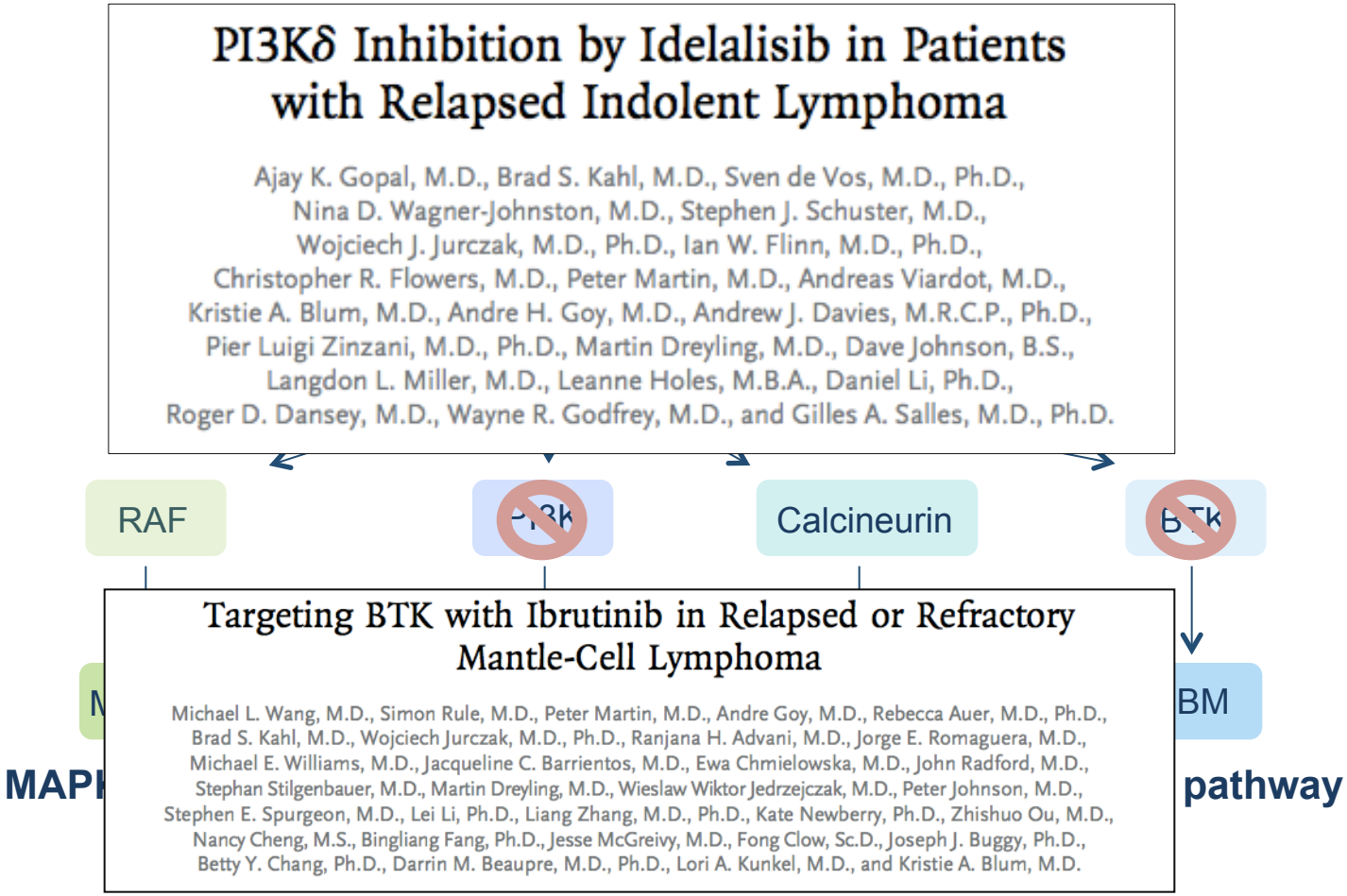




BCR as a therapeutic target



BCR as a therapeutic target



Conclusions & future directions

- The BCR in BL:
  - Patient-specific prognostic biomarker
  - Oncogene
  - Therapeutic target

- Measurement of residual disease to identify patients for salvage therapy

Acknowledgements

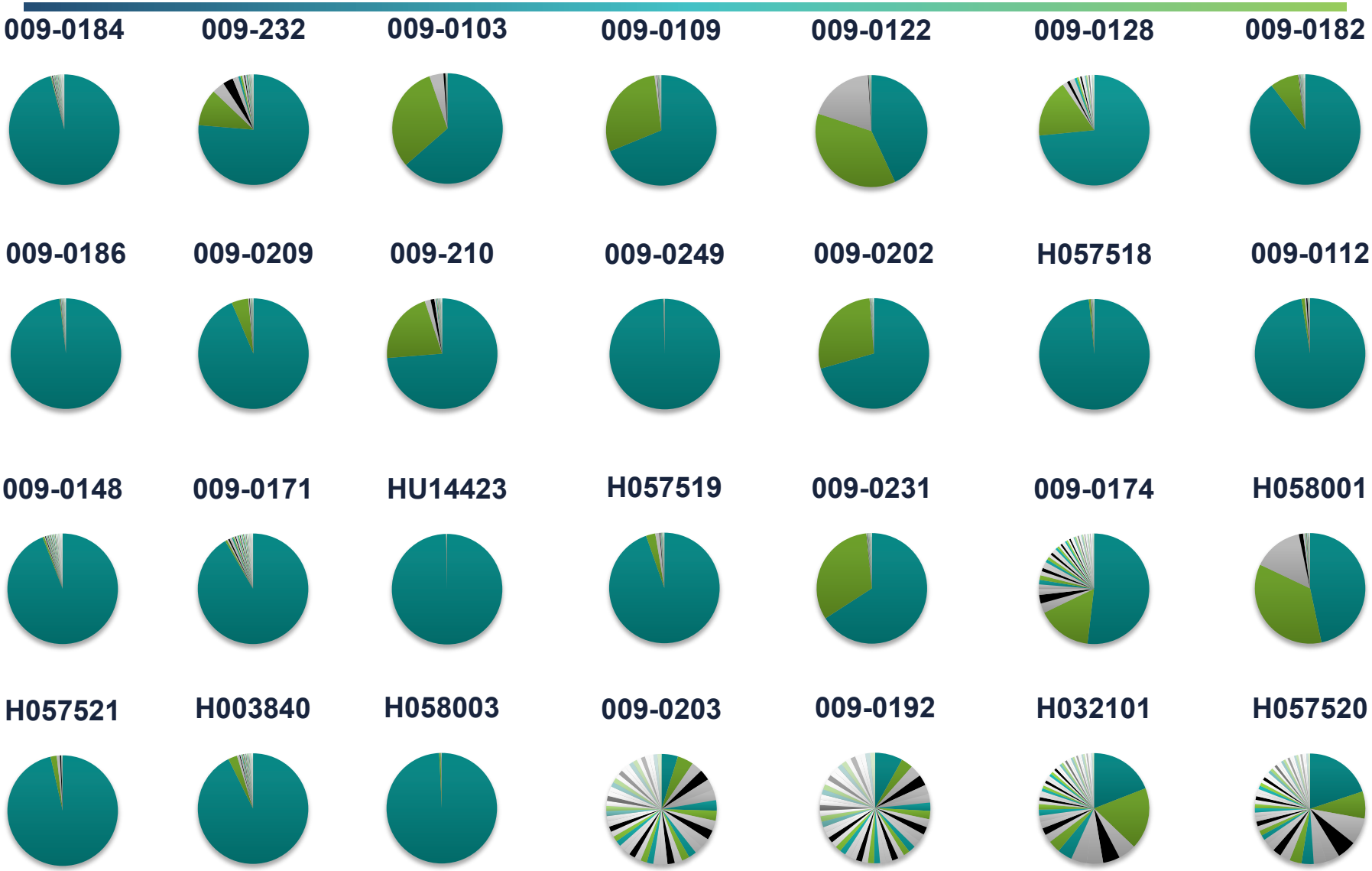
- **BL patients and their families**
- Warren Lab
  - Edus H. Warren
  - Andrea Towlerton
  - David Coffey
  - Yuexin Xu
  - Alicia Morales
  - Scott Tykodi
  - Sharon Ornales
- Fred Hutch
  - Chris Carlson
- NCI Ghana BL Repository
  - Robert Biggars
  - Sam Mbulaiteye
- Fred Hutch Global Oncology
  - Sarah Gerdts
  - Corey Casper

THANK YOU

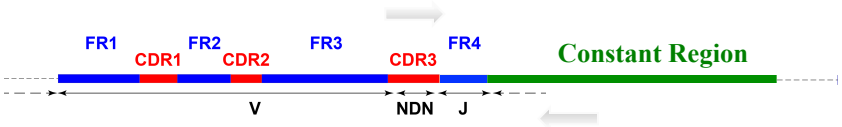
FRED HUTCH
CURES START HERE™

fredhutch.org

Top 50 unique *IGH* sequences from all BL tumors



A.



B.

IGH Expression	
Isotype	**BL Sample Number (N=16)**
IgM^+ IgD^+	13
IgG^+	2
No Expression	1

IG κ / λ Expression	
Expression	**BL Sample Number (N=16)**
IG κ^+	12
IG λ^+	3
No Expression	1