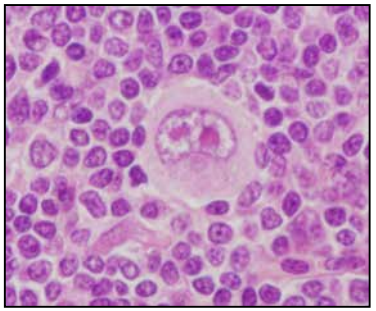
Mutational Landscape of Hodgkin Lymphoma

Juan F García

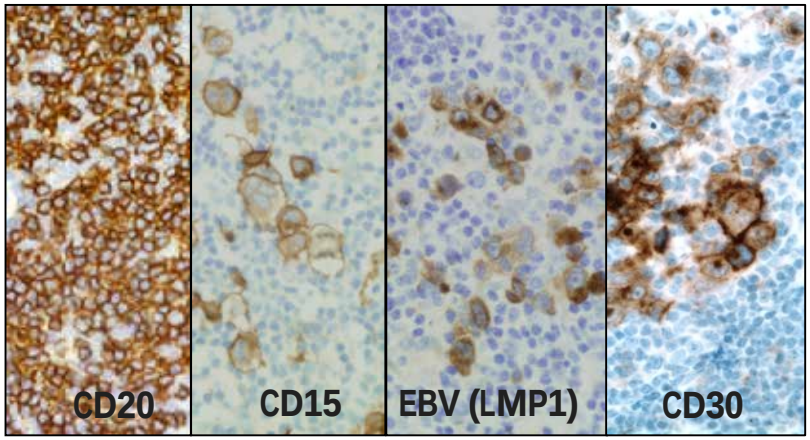
MD Anderson Cancer Center
Madrid • España

Classical Hodgkin Lymphoma

Lymphoid malignancy in which tumor (HRS) cells usually represent a minor population (< 1-2 %) within the affected tissue, whereas the majority of the malignancy is composed of benign lymphocytes, eosinophils, macrophages, fibroblasts, … (inflammatory microenvironment)



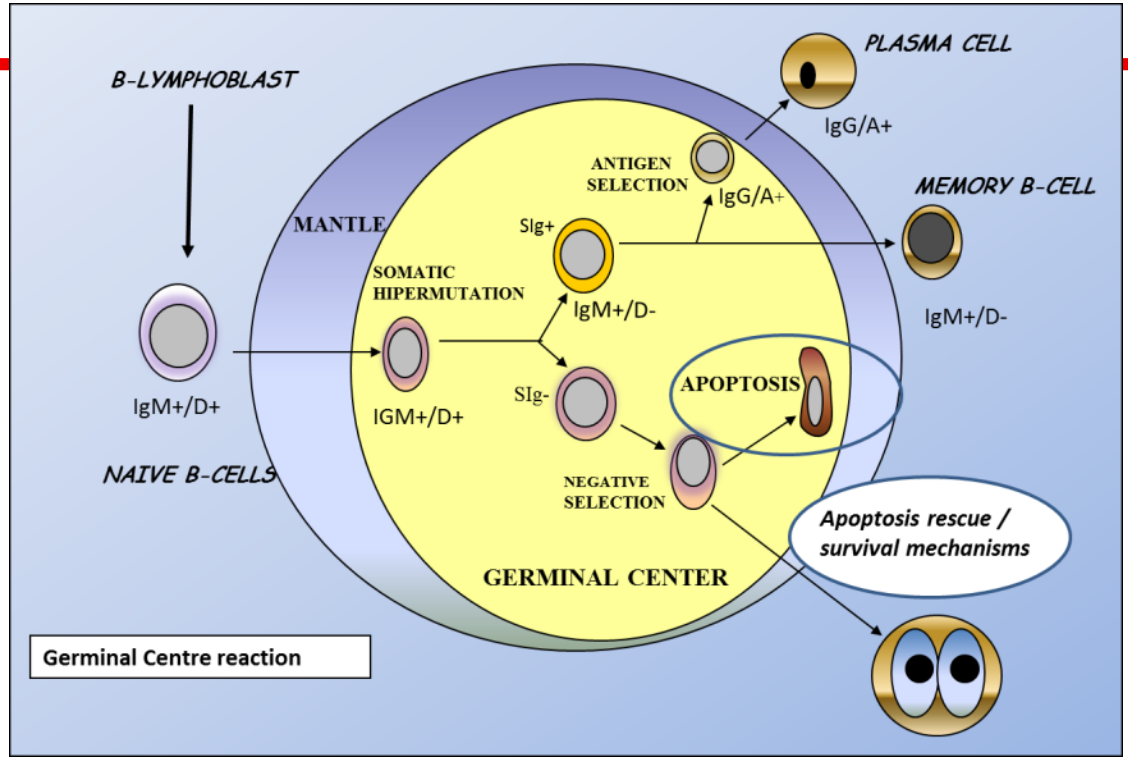
- Origin: GC B-lymphocytes
- Phenotype:
  - PAX5+, MUM1+, CD20-/+, CD79a-, Bcl6-,
  - **Igs(BCR)-**, OCT2-/+
  - CD30+, CD15+ (70-80%), EBV(LMP1)+ (40-70%)
- Genetics:
  - Rearranged and somatically mutated Ig genes
  - Gains of 9p and 2p



MD Anderson Cancer Center
Madrid · España

Classical Hodgkin Lymphoma: absence of BCR expression

- Defective transcription of Ig genes (crippling mutations, deficit of transcription factors, epigenetic deregulation,…)



- Immune to the physiological CD95/FAS-mediated apoptosis that occur in normal GC B-lymphocytes that do not express functional Igs

MD Anderson Cancer Center
Madrid · España

Gene Mutations Previously Described

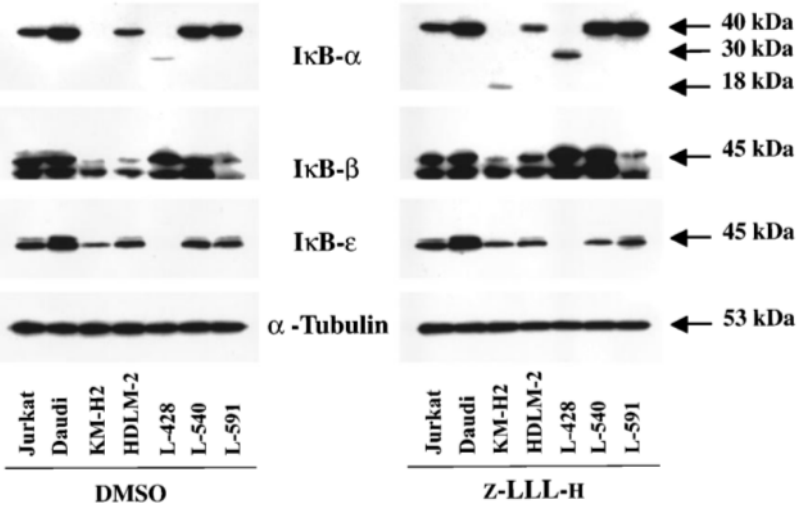
Mutations	References
Associated with constitutive NF-κB activity:	
NFKBIA (IκBα), is inactivated by somatic mutations in about 15-20% of cHL, NFKBIE (IκBε) is also mutated in a few cases	Cabannes et al. Oncogene 1999 Emmerich et al. Blood 1999 Emmerich et al. J Pathol 2003 Jungnickel et al. J Exp Med 2000
TNFAIP3 (A20) mutations in 16/36 cHLs (microdisected HRS cells) A20 mutations in 5/15 cHLs	Schmitz et al, J Exp Med. 2009 Kato etal. Nature. 2009
Associated with JAK/STAT activation:	
SOCS-1 mutations, 8/19 cHL samples (microdisected HRS cells), and in 3/5 HL-derived cell lines	Weniger et al, Oncogene. 2006
Suppressor genes:	
TP53 mutations very rare	Montesinos-Rongen et al. Blood 1999 Maggio et al. Int J Cancer 2001
FAS/CD95 mutations very rare	Müschen et al. Cancer Res 2000
Latest results (NGS):	
PTPN1 mutations in PMBCL, 6/30 cHL samples	Gunawardana et al. Nature Genetics 2014
B2M mutations, 7/10 cHL samples (flow-sorted HRS cells)	Reichel et al. Blood 2015

Oncogene (1999) 18, 3063–3070

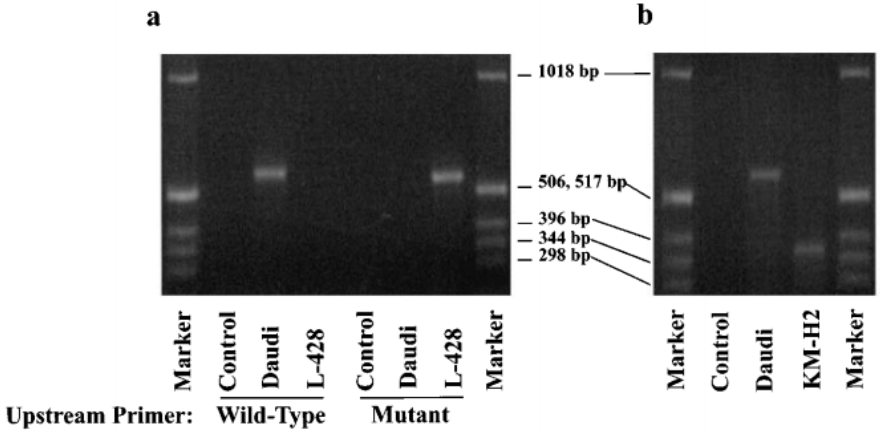
http://www.stockton-press.co.uk/onc

Mutations in the *IkBa* gene in Hodgkin's disease suggest a tumour suppressor role for IκBα

Eric Cabannes[1], Gulfaraz Khan[2], Fabienne Aillet[1], Ruth F Jarrett[2] and Ronald T Hay*,[1]

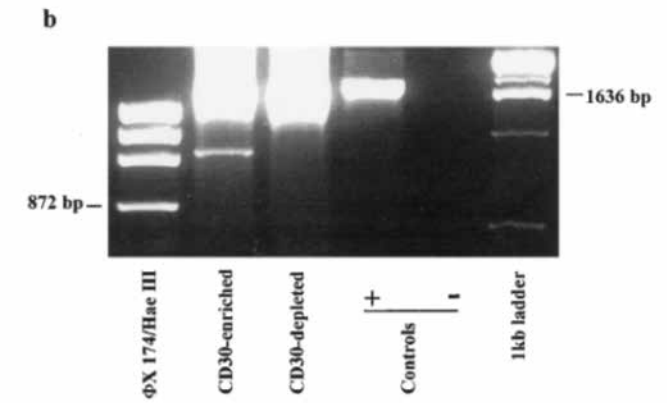


Aberrant IkBa protein in Hodgkin's disease-derived cell lines



The IkBa gene is mutated in L-428 and KM-H2 cell lines, demonstrated by PCR

Detection of an IkBa allele mutation in a biopsy sample from 1 out of 4 HD patient samples (Cd30+ enriched fraction by flow)



Brief Definitive Report *J. Exp. Med. 2000. 191; 2(17):395–401*

Clonal Deleterious Mutations in the IκBα Gene in the Malignant Cells in Hodgkin's Lymphoma

By Berit Jungnickel,* Andrea Staratschek-Jox,‡ Andreas Bräuninger,§ Tilmann Spieker,§ Jürgen Wolf,‡ Volker Diehl,‡ Martin-Leo Hansmann,§ Klaus Rajewsky,* and Ralf Küppers*‡

*From the *Institute for Genetics, and the ‡Department of Internal Medicine I, University of Cologne, 50931 Cologne, Germany; and the §Department of Pathology, University of Frankfurt, 60596 Frankfurt, Germany*

Abstract

Members of the nuclear factor (NF)-κB family of transcription factors play a crucial role in cellular activation, immune responses, and oncogenesis. In most cells, they are kept inactive in the cytosol by complex formation with members of the inhibitor of NF-κB (IκB) family, whose degradation activates NF-κB in response to diverse stimuli. In Hodgkin's lymphoma (HL), high constitutive nuclear activity of NF-κB is characteristic of the malignant Hodgkin and Reed-Sternberg (H/RS) cells, which occur at low number in a background of nonneoplastic inflammatory cells. In single H/RS cells micromanipulated from histological sections of HL, we detect clonal deleterious somatic mutations in the IκBα gene in two of three Epstein-Barr virus (EBV)-negative cases but not in two EBV-positive cases (in which a viral oncogene may account for NF-κB activation). There was no evidence for IκBα mutations in two non-HL entities or in normal germinal center B cells. This study establishes deleterious IκBα mutations as the first recurrent genetic defect found in H/RS cells, indicating a role of IκBα defects in the pathogenesis of HL and implying that IκBα is a tumor suppressor gene.

Key words: Hodgkin's lymphoma • IκBα • nuclear factor κB • tumor suppressor gene • Reed-Sternberg cell

In single H/RS cells micromanipulated from histological sections of HL, we detect clonal deleterious somatic mutations in the IkBa gene in two of three EBV- negative cases but not in two EBV-positive cases

J. Exp. Med. 2000. 191; 2(17):395–401

Table I. *Analysis of the IκBα Protein, Transcripts, and Gene in HL-derived Cell Lines*

Cell line	Origin/EBV status*	Full-length IκBα protein	IκBα transcripts		Mutations in IκBα gene‡
			Polymorphisms‡	Aberrations‡	
L428	B/−	−	175C, 399C, 1049C	893C→U§	2278C→T§
KMH-2	B/−	−	175C, 399C, 1049C	Del. 509–641 Ins. UCCAG‖	Del. 1497–1710
L1236	B/−	+	175Y, 399C, 1049U	–	
DEV	B/−	+	175Y, 399C, 1049C	–	
L591	B/+	+	175U, 399C, 1049U	–	
L540	T/−	+	175U, 399C, 1049Y	–	
HDLM-2	T/−	+	175C, 399C, 1049U	–	
HD-MyZ	Myeloid/−	+	175C, 399U, 1049C	–	

These sequence data are available from GenBank/EMBL/DDBJ under accession nos. AJ249290 and AJ249291.
*The presumptive origin of the cell lines, as indicated by the presence of rearrangements of the Ig or TCR gene loci, as well as the presence/absence of EBV in the cells, is given (reference 15).
‡Positions in the cDNA and gene refer to references 18 and 30. Y = C/U, R = A/G. Del., deletion; Ins., insertion.
§The mutation generates a premature stop codon.
‖The transcript in the line KMH-2 is generated by aberrant splicing that leads to insertion of intronic sequence.

J. Exp. Med. 2000. 191; 2(17):395–401

Table II. *Sequence Analysis of the IκBα Exons Amplified from Tissue Sections and Single H/RS Cells*

Case	Exon	Polymorphisms*	PCR-positive cells‡	Products sequenced	Mutations in H/RS cells	Alleles amplified§
EBV(-)						
1	1	212C	2/5	2	Deletion (254–255)	2WT/M
	2	1059C	2/5	2	–	
	3	1678G	5/8	5	–	
	4	2025C	6/8	6	Deletion (1994)	2WT, 1M, 3WT/M
	5	–	6/8	6	–	
	6	2787T	4/8	1	–	
		2921A				
2	1	212C	7/37	6	–	
	2	1059T	8/37	8	2 unique mutations‖	
	3	1678G	5/14	5	–	
	4	2025C	5/14	5	–	
	5	–	5/14	5	Deletion (2355–2356)	5WT/M
	6	2787C	4/14	4	–	
		2921G				
3	1	212Y	8/26	7	51C→T in 1 seq.	2C, 3T, 2Y
	2	1059Y	5/26	5	–	4C, 1T
	3	1678R	6/8	6	–	2A, 4G
	4	2025Y	5/8	5	–	4C, 1T
	5	–	5/8	5	–	–¶
	6	2787Y	6/8	6	–	4T, 1C, 1Y
		2921R				4A, 1G, 1R
EBV(+)						
4	1	212T	5/12	4	52C→T in 1 seq.	
	2	1059C	6/12	4	–	
	3	1678G	5/10	5	–	
	4	2025C	7/10	6	–	
	5	–	7/10	6	–	
	6	2787T	7/10	6	–	
		2921A				
5	1	212Y	8/17	4	–	1T, 3Y
	2	1059Y	7/17	7	6 unique mutations‖	4T, 3Y
	3	1678R	7/8	5	–	1A, 1G, 3R
	4	2025Y	5/8	5	–	3C, 1T, 1Y
	5	–	7/8	5	–	–¶
	6	2787Y	5/8	4	3035G→A in 1 seq.	1C, 3Y
		2921R				1G, 3R

These sequence data are available from GenBank/EMBL/DDBJ under accession nos. AJ249283–AJ249289 and AJ249294–AJ249295. seq., sequence.

*Nucleotides at polymorphic sites in the respective exons amplified from the whole tissue DNA. Y = C/T, R = A/G. Positions in the IκBα gene refer to reference 18.

‡Two experiments yielding PCR products from negative controls were disregarded.

§Indicates (in cases 1 and 2) amplification of wild-type (WT), mutant (M), or both (WT/M) copies of the respective exon from individual cells, and (in cases 3 and 5) the number of sequences containing either of the two polymorphic nucleotides, or both.

‖The following mutations were each detected in only one of the products of the respective exons. Case 2, exon 2: 942A→G, 1059T→C; case 5, exon 2: 760C→T, 766G→A, 777C→T, 825T→C, 1004A→C, 1053C→T.

¶Products spanning exons 4 and 5 were amplified. Unmutated exon 5 on both alleles was concluded from the detection of both polymorphic nucleotides at position 2025 in unmutated products.

J. Exp. Med. 2000. 191; 2(17):395–401

- In several PCR products amplified from the H/RS cells of cases 2–5 unique nucleotide exchanges were detected. …, and are thus apparently not due to mutations present in all malignant cells.
- These mutations might indicate enhanced mutability of the IkBa gene, reflect the genomic instability of H/RS cells, or be derived from Taq DNA polymerase errors.
- … the frequent occurrence of this type of mutation emphasizes an important issue: one can only invoke clonal genetic defects as an early event in tumorigenesis.

NEOPLASIA

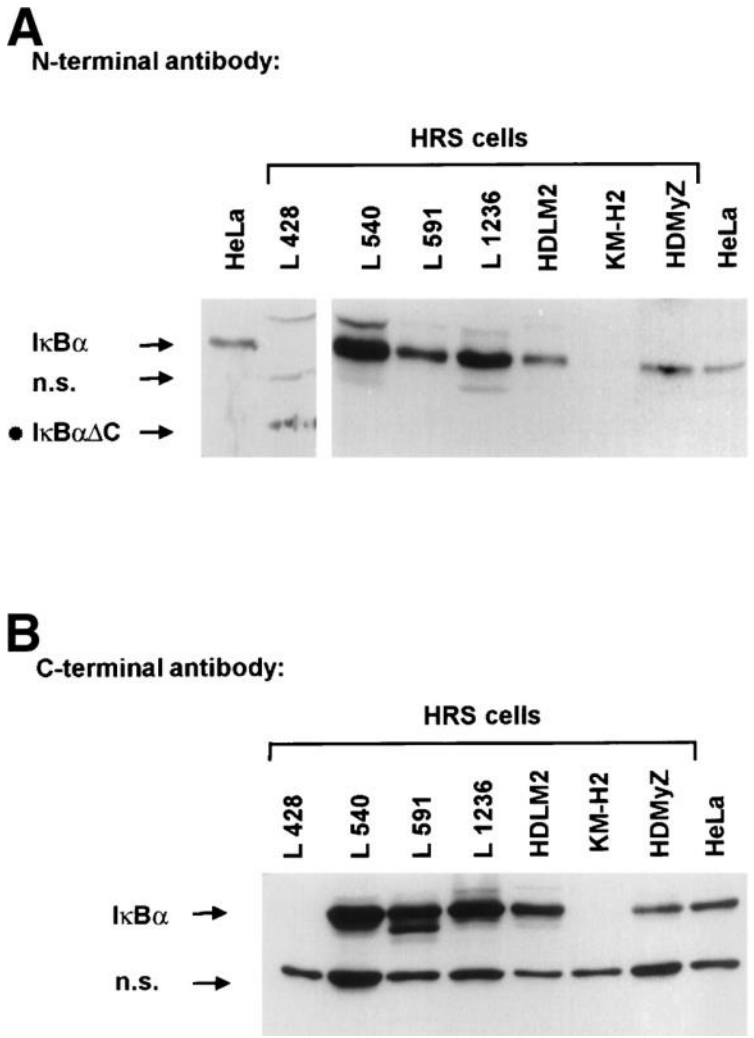
Overexpression of I Kappa B Alpha Without Inhibition of NF-κB Activity and Mutations in the I Kappa B Alpha Gene in Reed-Sternberg Cells

By Florian Emmerich, Martina Meiser, Michael Hummel, Gudrun Demel, Hans-Dieter Foss, Franziska Jundt, Stephan Mathas, Daniel Krappmann, Claus Scheidereit, Harald Stein, and Bernd Dörken

The transcription factor NF kappa B (NF-κB) mediates the expression of numerous genes involved in diverse functions such as inflammation, immune response, apoptosis, and cell proliferation. We recently identified constitutive activation of NF-κB (p50/p65) as a common feature of Hodgkin/Reed-Sternberg (HRS) cells preventing these cells from undergoing apoptosis and triggering proliferation. To examine possible alterations in the NF-κB/IκB system, which might be responsible for constitutive NF-κB activity, we have analyzed the inhibitor I kappa B alpha (IκBα) in primary and cultured HRS cells on protein, mRNA, and genomic levels. In lymph node biopsy samples from Hodgkin's disease patients, IκBα mRNA proved to be strongly overexpressed in the HRS cells. In 2 cell lines (L428 and KM-H2), we detected mutations in the IκBα gene, resulting in C-terminally truncated proteins, which are presumably not able to inhibit NF-κB–DNA binding activity. Furthermore, an analysis of the IκBα gene in single HRS cells micromanipulated from frozen tissue sections showed a monoallelic mutation in 1 of 10 patients coding for a comparable C-terminally truncated IκBα protein. We suggest that the observed IκBα mutations contribute to constitutive NF-κB activity in cultured and primary HRS cells and are therefore involved in the pathogenesis of these Hodgkin's disease (HD) patients. The demonstrated constitutive overexpression of IκBα in HRS cells evidences a deregulation of the NF-κB/IκB system also in the remaining cases, probably due to defects in other members of the IκB family.

Blood 1999;94:3129-3134

Western blot analysis of IκBα proteins in HD-derived cell lines.



Florian Emmerich et al. Blood 1999;94:3129-3134

Detection of Mutations in the Intron and Exon Regions of the IkBa Gene in Single HRS Cells

Table 4. Detection of Mutations in the Intron and Exon Regions of the IκBα Gene in Single HRS Cells

Case	No. of Isolated Cells	Type	No. of Amplificates	No. of Mutations	
				Intron	Exon
1	72	NS	22	3; del 19bp *	1 (⇒ Stop)
1 (control cells)	27	NS	21	3; del 19bp *	—
2	38	NS	22	3	—
3	24	NS	20	3; del 2bp	1†
4	43	NS	23	6	—
5	53	NS	24	6	—
6	38	NS	24	3	—
7	45	NS	13	3	1†
8	42	NS	17	1	—
9	24	MC	21	4; del 19bp *	1†
10	21	MC	19	6	1†

*This 19 bp deletion was identical.

†C to T transition at 2333 nt; as compared with the sequence published by Ito et al.[20]

Florian Emmerich et al. Blood 1999;94:3129-3134

Journal of Pathology
J Pathol 2003; **201**: 413–420.
Published online 18 August 2003 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1454

Original Paper

Inactivating I kappa B epsilon mutations in Hodgkin/Reed–Sternberg cells

Florian Emmerich,[1*†] Sebastian Theurich,[1†] Michael Hummel,[2] Antje Haeffker,[1] Magnus S Vry,[1] Konstanze Döhner,[3] Kurt Bommert,[4] Harald Stein[2] and Bernd Dörken[1,4]

[1]*Humboldt University of Berlin, Universitätsklinikum Charité, Robert-Rössle-Klinik, Lindenberger Weg 80, 13125 Berlin, Germany*
[2]*Institute for Pathology, Universitätsklinikum Benjamin Franklin, Free University of Berlin, Hindenburgdamm 30, 12200 Berlin, Germany*
[3]*Department of Internal Medicine III, University of Ulm, Robert-Koch-Str 8, 89081 Ulm, Germany*
[4]*Max Delbrück Center for Molecular Medicine, Robert-Rössle-Str 10, 13092 Berlin, Germany*

**Correspondence to:*
Dr Florian Emmerich, Humboldt University of Berlin, Universitätsklinikum Charité, Robert-Rössle-Klinik, Lindenberger Weg 80, 13125 Berlin, Germany.
E-mail: emmerich@mdc-berlin.de

†Florian Emmerich and Sebastian Theurich contributed equally to this work.

Received: 8 December 2002
Revised: 27 April 2003

Abstract

The pathogenesis of Hodgkin lymphoma (HL) is still unclear. Previous investigations have demonstrated constitutive nuclear activity of the transcription factor NF kappa B (NF-κB) in Hodgkin/Reed–Sternberg (HRS) cells as an important prerequisite in protecting these cells from apoptosis. As a molecular mechanism leading to constitutive NF-κB activity in HRS cells, mutations of the NF-κB inhibitor I kappa B alpha (IκBα) have recently been identified in classical (c) HL-derived cell lines in a patient with cHL. In the present study, the NF-κB inhibitor I kappa B epsilon (IκBε) has been analysed for somatic mutations in the same group of six patients already studied for IκBα mutations, as well as in cHL-derived cell lines. In one cHL-derived cell line (L428), a hemizygous frame-shift mutation generating a pre-terminal stop codon resulting in a severely truncated protein was found. Moreover, in the HRS cells of one patient, a hemizygous mutation affecting the 5′-splicing site of intron 1 of the IκBε gene was found. These results, in combination with recently described IκBα mutations, indicate that defective NF-κB inhibitors appear more frequent than previously thought and might explain the constitutive nuclear activity of NF-κB in a significant proportion of cHL cases.

J Exp Med. 2009
206(5):981-9.

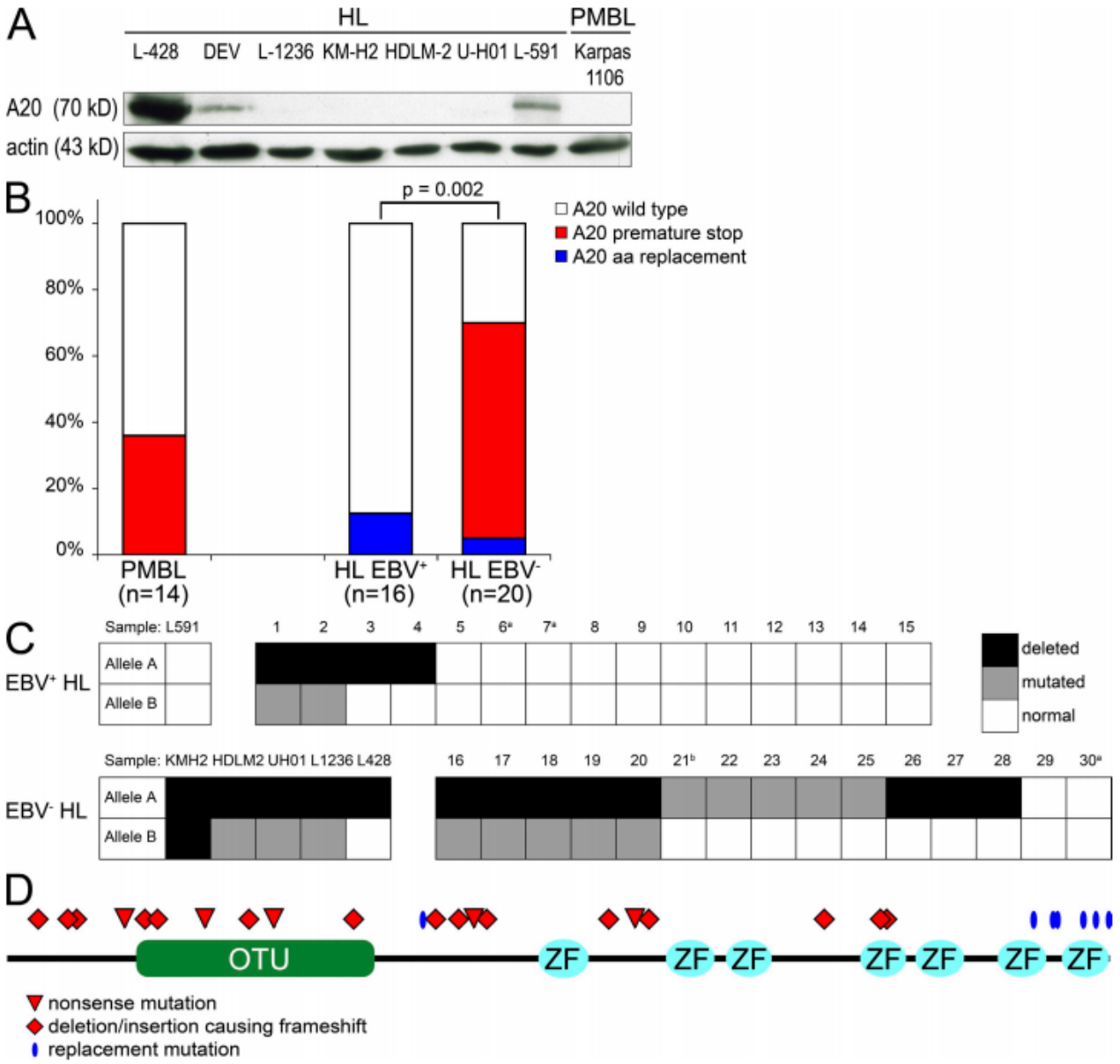
TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma

Roland Schmitz,[1] Martin-Leo Hansmann,[2] Verena Bohle,[1]
Jose Ignacio Martin-Subero,[3] Sylvia Hartmann,[2] Gunhild Mechtersheimer,[5]
Wolfram Klapper,[4] Inga Vater,[3] Maciej Giefing,[3,6] Stefan Gesk,[3]
Jens Stanelle,[1] Reiner Siebert,[3] and Ralf Küppers[1]

[1]Institute of Cell Biology (Cancer Research), Medical School, University of Duisburg-Essen, 45122 Essen, Germany
[2]Senkenberg Institute of Pathology, University of Frankfurt/Main, 60590 Frankfurt, Germany
[3]Institute of Human Genetics and [4]Department of Pathology, Hematopathology Section and Lymph Node Registry, Christian-Albrechts University Kiel, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany
[5]Institute of Pathology, University of Heidelberg, 69120 Heidelberg, Germany
[6]Institute of Human Genetics, Polish Academy of Sciences, 60-479 Poznan, Poland

Proliferation and survival of Hodgkin and Reed/Sternberg (HRS) cells, the malignant cells of classical Hodgkin lymphoma (cHL), are dependent on constitutive activation of nuclear factor κB (NF-κB). NF-κB activation through various stimuli is negatively regulated by the zinc finger protein A20. To determine whether A20 contributes to the pathogenesis of cHL, we sequenced *TNFAIP3*, encoding A20, in HL cell lines and laser-microdissected HRS cells from cHL biopsies. We detected somatic mutations in 16 out of 36 cHLs (44%), including missense mutations in 2 out of 16 Epstein-Barr virus–positive (EBV$^+$) cHLs and a missense mutation, nonsense mutations, and frameshift-causing insertions or deletions in 14 out of 20 EBV$^-$ cHLs. In most mutated cases, both *TNFAIP3* alleles were inactivated, including frequent chromosomal deletions of *TNFAIP3*. Reconstitution of wild-type *TNFAIP3* in A20-deficient cHL cell lines revealed a significant decrease in transcripts of selected NF-κB target genes and caused cytotoxicity. Extending the mutation analysis to primary mediastinal B cell lymphoma (PMBL), another lymphoma with constitutive NF-κB activity, revealed destructive mutations in 5 out of 14 PMBLs (36%). This report identifies *TNFAIP3* (A20), a key regulator of NF-κB activity, as a novel tumor suppressor gene in cHL and PMBL. The significantly higher frequency of *TNFAIP3* mutations in EBV$^-$ than EBV$^+$ cHL suggests complementing functions of *TNFAIP3* inactivation and EBV infection in cHL pathogenesis.

J Exp Med. 2009
206(5):981-9.



Nature. 2009 Jun 4;459(7247):712-6. doi: 10.1038/nature07969. Epub 2009 May 3.

Frequent inactivation of A20 in B-cell lymphomas.

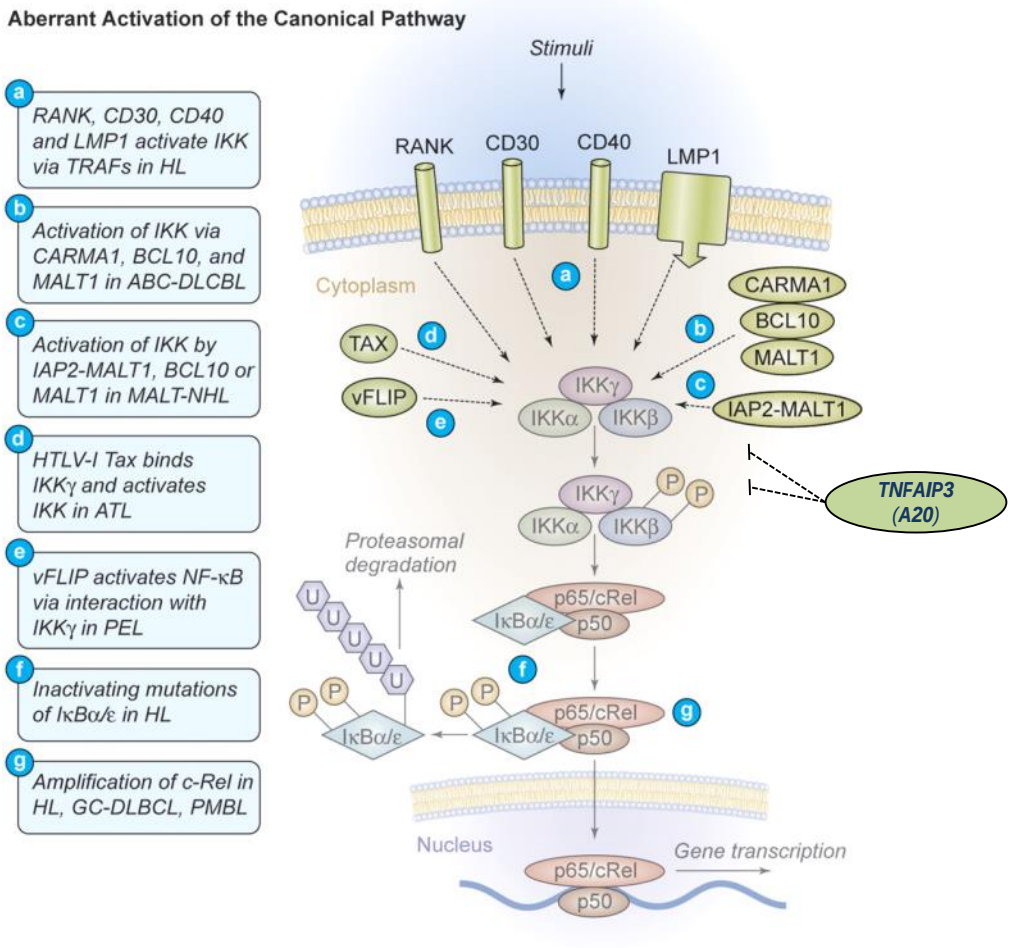
Kato M[1], Sanada M, Kato I, Sato Y, Takita J, Takeuchi K, Niwa A, Chen Y, Nakazaki K, Nomoto J, Asakura Y, Muto S, Tamura A, Iio M, Akatsuka Y, Hayashi Y, Mori H, Igarashi T, Kurokawa M, Chiba S, Mori S, Ishikawa Y, Okamoto K, Tobinai K, Nakagama H, Nakahata T, Yoshino T, Kobayashi Y, Ogawa S.

Author information

Abstract

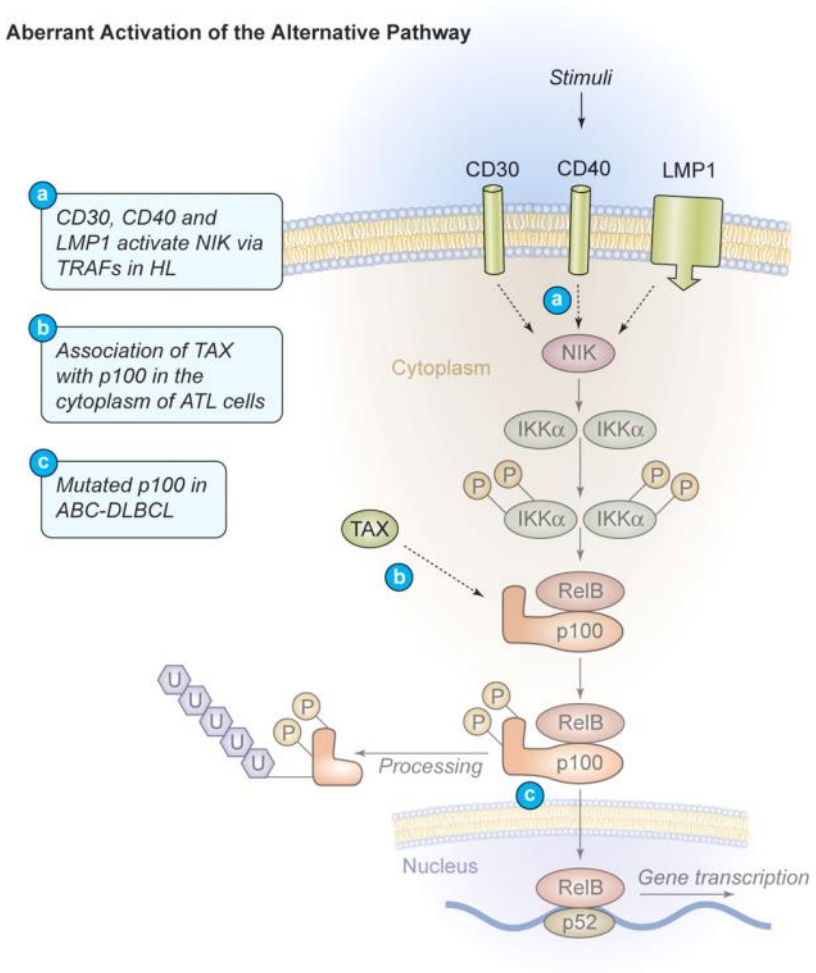
A20 is a negative regulator of the NF-kappaB pathway and was initially identified as being rapidly induced after tumour-necrosis factor-alpha stimulation. It has a pivotal role in regulation of the immune response and prevents excessive activation of NF-kappaB in response to a variety of external stimuli; recent genetic studies have disclosed putative associations of polymorphic A20 (also called TNFAIP3) alleles with autoimmune disease risk. However, the involvement of A20 in the development of human cancers is unknown. Here we show, using a genome-wide analysis of genetic lesions in 238 B-cell lymphomas, that A20 is a common genetic target in B-lineage lymphomas. A20 is frequently inactivated by somatic mutations and/or deletions in mucosa-associated tissue lymphoma (18 out of 87; 21.8%) and Hodgkin's lymphoma of nodular sclerosis histology (5 out of 15; 33.3%), and, to a lesser extent, in other B-lineage lymphomas. When re-expressed in a lymphoma-derived cell line with no functional A20 alleles, wild-type A20, but not mutant A20, resulted in suppression of cell growth and induction of apoptosis, accompanied by downregulation of NF-kappaB activation. The A20-deficient cells stably generated tumours in immunodeficient mice, whereas the tumorigenicity was effectively suppressed by re-expression of A20. In A20-deficient cells, suppression of both cell growth and NF-kappaB activity due to re-expression of A20 depended, at least partly, on cell-surface-receptor signalling, including the tumour-necrosis factor receptor. Considering the physiological function of A20 in the negative modulation of NF-kappaB activation induced by multiple upstream stimuli, our findings indicate that uncontrolled signalling of NF-kappaB caused by loss of A20 function is involved in the pathogenesis of subsets of B-lineage lymphomas.

Mechanism of aberrant NF-κB activation through the canonical signaling pathway in human lymphomas.



Philipp J. Jost, and Jürgen Ruland Blood 2007;109:2700-2707

Mechanism of aberrant NF-κB activation through the alternative signaling pathway in human lymphomas.



Philipp J. Jost, and Jürgen Ruland Blood 2007;109:2700-2707

Oncogene (2006) 25, 2679–2684

www.nature.com/onc

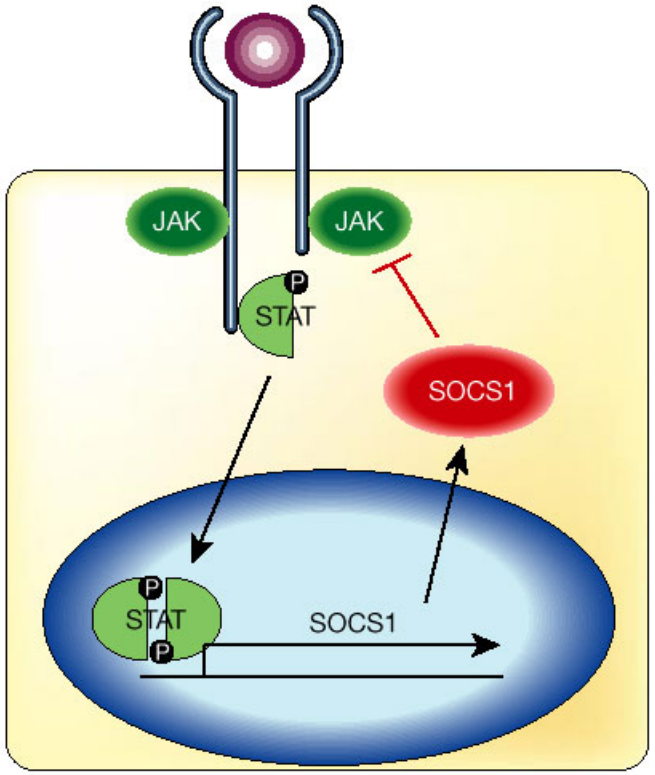
ONCOGENOMICS

Mutations of the tumor suppressor gene *SOCS-1* in classical Hodgkin lymphoma are frequent and associated with nuclear phospho-STAT5 accumulation

MA Weniger[1], I Melzner[1], CK Menz, S Wegener, AJ Bucur, K Dorsch, T Mattfeldt, TFE Barth and P Möller

Department of Pathology, University of Ulm, Ulm, Germany

- SOCS-1 mutations, 8/19 cHL samples (microdisected HRS cells), and in 3/5 HL-derived cell lines



Blood. 1999 Sep 1;94(5):1755-60.

Mutation of the p53 gene is not a typical feature of Hodgkin and Reed-Sternberg cells in Hodgkin's disease.

Montesinos-Rongen M[1], Roers A, Küppers R, Rajewsky K, Hansmann ML.

Author information

Abstract

Point mutations of the p53 tumor suppressor gene are a frequent finding in human carcinomas and are thought to be an important oncogenic event. In non-Hodgkin lymphomas, p53 mutations occur in a minor fraction of cases. However, conclusive data are still lacking for Hodgkin's disease (HD) where the analysis meets technical problems. The neoplastic tumor cell clone in HD is represented by the large Hodgkin and Reed-Sternberg (HRS) cells, which account for only a minority of all cells in the tumor tissue (often <1%). To identify putative HRS cell-specific mutations, single HRS cells were micromanipulated from frozen tissue sections of HD biopsy specimens. Exons 4 to 8 of the p53 gene (in which more than 90% of p53 mutations associated with human neoplasms occur) were amplified from these single cells and sequenced. Mutations of p53 were not found in HRS cells of any of 8 cases of HD analyzed. We conclude that mutation of the p53 gene is only rarely, if at all, involved in the pathogenesis of HD.

Int J Cancer. 2001 Oct 1;94(1):60-6.

TP53 gene mutations in Hodgkin lymphoma are infrequent and not associated with absence of Epstein-Barr virus.

Maggio EM[1], Stekelenburg E, Van den Berg A, Poppema S.

Author information

Abstract

Reed-Sternberg (RS) cells, the neoplastic cells of Hodgkin lymphoma (HL) have clonal immunoglobulin gene rearrangements. The presence of somatic mutations suggests a germinal center origin, whereas the presence of crippling mutations suggests rescue of RS precursors from apoptosis by a transforming event. Epstein-Barr virus (EBV), which can be detected in 30-50% of HL cases, probably plays a role in this transforming event. The frequent presence of p53 protein expression in RS cells also suggests a role of the TP53 gene in this escape from apoptosis. Although mutations of the TP53 gene occur infrequently in RS cells, it has been suggested that in EBV-negative cases this gene mutation may be fundamental for the inhibition of apoptosis. In this study, we tested the hypothesis that there is an inverse correlation between the presence of TP53 gene mutations and the presence of EBV. In 21 of 67 cases EBV encoded small RNA (EBER)1-2 mRNAs were detected. Immunostaining for p53 protein revealed positivity in all 67 cases with variable percentages of positive cells and staining intensity. Screening for mutations in exons 5, 6, 7 and 8 of the TP53 gene in single RS cells obtained by laser microdissection from 26 HL specimens and 4 HL-derived cell lines revealed mutations in 2 of 15 EBV-positive cases and in 1 of 11 EBV-negative cases. Our results confirm the presence of infrequent (11.5%) TP53 gene mutations in HL and suggest that mutations of the TP53 gene are not correlated to the absence of EBV.

Leuk Lymphoma. 2006 Sep;47(9):1932-40.

Dysfunctional p53 deletion mutants in cell lines derived from Hodgkin's lymphoma.

Feuerborn A[1], Möritz C, Von Bonin F, Dobbelstein M, Trümper L, Stürzenhofecker B, Kube D.

Author information

Abstract

Classical Hodgkin's lymphoma (cHL) is a distinct malignancy of the immune system. Despite the progress made in the understanding of the pathology of cHL, the transforming events remain to be elucidated. It has been proposed that mutations in the TP53 gene in biopsy material as well as cell lines derived from cHL are rare and therefore not notably involved in the pathogenesis of the malignant H&RS cells. Re-evaluating the expression in cHL-derived cell lines, we found that in 3/6 of these cell lines, TP53 transcripts are characterized by deletions within exon 4 (L428 cells) and nearly a complete loss of exons 10 - 11 (L1236) or exons 8 - 11 (HDLM-2), respectively. These changes were found in otherwise rarely mutated regions of TP53. Cell lines L1236 and HDLM-2 harbour fusions with alu-repeats in their TP53 mRNA 3'-ends, resulting in the carboxyterminal truncation and loss of the transcriptional activity of p53. Transcriptional inactivity was also found for p53 in L428 cells. This study characterizes mutations in TP53 transcripts within cHL cell lines with associated functional defects in the resulting p53 proteins and therefore reintroduces the concept that mutations of TP53 might be involved in the pathogenesis of Hodgkin's lymphoma.

Comment in

P53 gene alterations identified in classical Hodgkin's lymphoma cell lines. [Leuk Lymphoma. 2006]

nature genetics

Nature Genetics 46, 329–335 (2014)

Recurrent somatic mutations of *PTPN1* in primary mediastinal B cell lymphoma and Hodgkin lymphoma

Jay Gunawardana[1,2], Fong Chun Chan[1,3], Adèle Telenius[1], Bruce Woolcock[1], Robert Kridel[1,2], King L Tan[1], Susana Ben-Neriah[1], Anja Mottok[1], Raymond S Lim[1], Merrill Boyle[1], Sanja Rogic[4], Lisa M Rimsza[5], Chrystelle Guiter[6], Karen Leroy[6–8], Philippe Gaulard[6–8], Corinne Haioun[7,8], Marco A Marra[9,10], Kerry J Savage[1], Joseph M Connors[1], Sohrab P Shah[2], Randy D Gascoyne[1,2] & Christian Steidl[1,2]

Classical Hodgkin lymphoma and primary mediastinal B cell lymphoma (PMBCL) are related lymphomas sharing pathological, molecular and clinical characteristics. Here we discovered by whole-genome and whole-transcriptome sequencing recurrent somatic coding-sequence mutations in the *PTPN1* gene. Mutations were found in 6 of 30 (20%) Hodgkin lymphoma cases, in 6 of 9 (67%) Hodgkin lymphoma–derived cell lines, in 17 of 77 (22%) PMBCL cases and in 1 of 3 (33%) PMBCL-derived cell lines, consisting of nonsense, missense and frameshift mutations. We demonstrate that *PTPN1* mutations lead to reduced phosphatase activity and increased phosphorylation of JAK-STAT pathway members. Moreover, silencing of *PTPN1* by RNA interference in Hodgkin lymphoma cell line KM-H2 resulted in hyperphosphorylation and overexpression of downstream oncogenic targets. Our data establish *PTPN1* mutations as new drivers in lymphomagenesis.

Nature Genetics 46, 329–335 (2014)

Somatic mutations discovered by next-generation sequencing

- To discover somatic mutations in PMBCL, we used whole-genome sequencing, comparing tumor genomes for two index cases to matched constitutional genomes.
- …we found mutations in two negative regulators of the JAK-STAT signaling pathway, SOCS1 and PTPN1
- …Analysis of the transcriptome by RNA-seq of five additional cases and three PMBCL cell lines identified PTPN1 mutations in two more cases

PTPN1 is mutated in PMBCL and Hodgkin lymphoma

- We screened the complete coding sequence of *PTPN1*, comprising 10 exons, for genomic mutations in an additional 70 PMBCL samples by Sanger sequencing and deep amplicon sequencing.
- …we found 20 variants in our PMBCL cohort (18 mutations in 17 of 77 clinical samples and 2 mutations in 1 of 3 cell lines screened), with some cases harboring multiple mutations.
- Because classical Hodgkin lymphoma is a closely related disease entity, we also screened 9 Hodgkin lymphoma–derived cell lines and 30 samples of Hodgkin Reed-Sternberg (HRS) cells, microdissected from classical Hodgkin lymphoma. A total of 12 mutations were discovered (6 in 30 microdissected HRS cells and 6 in 9 cell lines screened

Plenary Paper

LYMPHOID NEOPLASIA

Flow sorting and exome sequencing reveal the oncogenome of primary Hodgkin and Reed-Sternberg cells

Jonathan Reichel,[1,2] Amy Chadburn,[3] Paul G. Rubinstein,[4] Lisa Giulino-Roth,[1,5] Wayne Tam,[1] Yifang Liu,[1] Rafael Gaiolla,[1,6] Kenneth Eng,[1] Joshua Brody,[7] Giorgio Inghirami,[1] Carmelo Carlo-Stella,[8,9] Armando Santoro,[8] Daoud Rahal,[8] Jennifer Totonchy,[1] Olivier Elemento,[1,10] Ethel Cesarman,[1] and Mikhail Roshal[1]

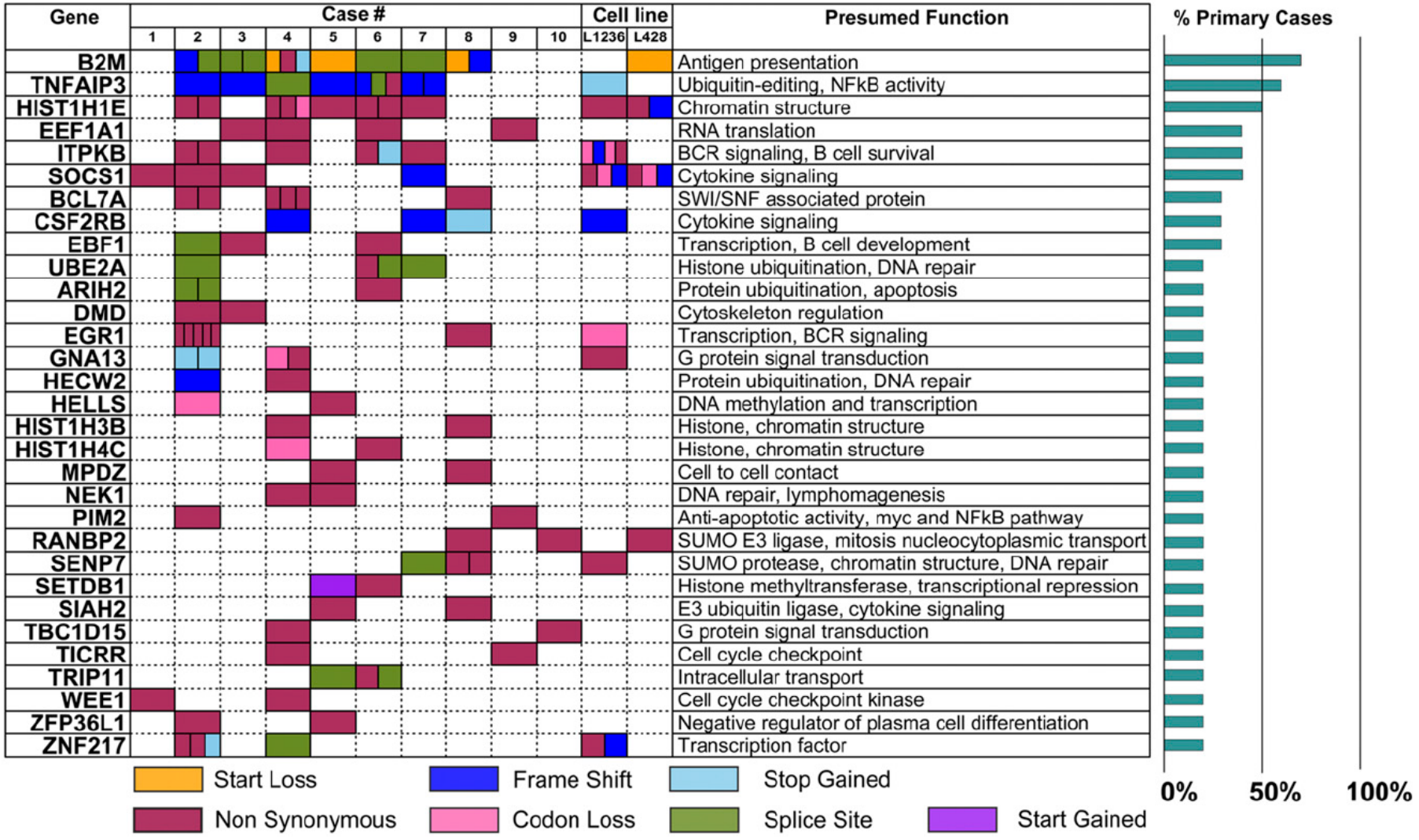
[1]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY; [2]Tri-Institutional Training Program in Computational Biology and Medicine, New York, NY; [3]Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [4]John H. Stroger Jr Hospital of Cook County, Rush University Medical Center, Ruth M. Rothstein CORE Center, Chicago, IL; [5]Department of Pediatrics, Weill Cornell Medical College, New York, NY; [6]Botucatu School of Medicine, Sao Paulo State University, Botucatu, Brazil; [7]Icahn School of Medicine at Mount Sinai, New York, NY; [8]Humanitas Cancer Center, Humanitas Clinical and Research Center, Rozzano (Milan), Italy; [9]School of Medicine, University of Milan, Milan, Italy; and [10]Institute for Computational Biomedicine, Weill Cornell Medical College, New York, NY

Key Points

- We show feasibility of whole-exome sequencing on purified primary HRS cells and report recurrent genetic alterations characterizing cHL.
- *B2M* is the most frequently mutated gene in cHL, strongly associated with nodular sclerosis subtype, younger age, and better overall survival.

Classical Hodgkin lymphoma (cHL) is characterized by sparsely distributed Hodgkin and Reed-Sternberg (HRS) cells amid reactive host background, complicating the acquisition of neoplastic DNA without extensive background contamination. We overcame this limitation by using flow-sorted HRS and intratumor T cells and optimized low-input exome sequencing of 10 patient samples to reveal alterations in genes involved in antigen presentation, chromosome integrity, transcriptional regulation, and ubiquitination. β-2-microglobulin (*B2M*) is the most commonly altered gene in HRS cells, with 7 of 10 cases having inactivating mutations that lead to loss of major histocompatibility complex class I (MHC-I) expression. Enforced wild-type B2M expression in a cHL cell line restored MHC-I expression. In an extended cohort of 145 patients, the absence of B2M protein in the HRS cells was associated with lower stage of disease, younger age at diagnosis, and better overall and progression-free survival. B2M-deficient cases encompassed most of the nodular sclerosis subtype cases and only a minority of mixed cellularity cases, suggesting that B2M deficiency determines the tumor microenvironment and may define a major subset of cHL that has more uniform clinical and morphologic features. In addition, we report previously unknown genetic alterations that may render selected patients sensitive to specific targeted therapies. (*Blood*. 2015;125(7):1061-1072)

Table 1. Recurrently mutated genes in cHL with potential pathogenic functions



B2M mutations = loss of cell-surface HLA-I, mechanisms of tumor evasion from the Immune Response?

Reichel et al. Blood 2015

NGS analyses of HL primary tumors

- We analyzed **57 cHL** tumor samples (FFPE) and 7 cHL-derived cell lines using massive parallel sequencing (Ion Torrent™).
- Previous tumor cell enrichment process by punch tissue cores from selected tumor-rich areas was implemented.
- Bioinformatics analysis, including alignment with germline sequences and SNPs filtering, were done using the Torrent Suite Software and Variant Caller. Pathogenic prediction of single nucleotide variants (SNVs) and mutation interpretation were performed using the Alamut and Provean softwares.
- Experiments were done in duplicate to minimize false positive rates.

Mata et al. Summited (2016)

MD Anderson Cancer Center
Madrid · España

cHL tumor samples series (FFPE)

FEATURE	N	%
Age		
< 45	37	68,52
≥ 45	17	31,48
Gender		
Male	26	48,15
Female	28	51,85
IPS		
0-2	35	64,81
≥ 3	19	35,19
Outcome		
Refractory	18	33,96
Complete response	35	66,04
Ann Arbor Stage		
< IV	36	67,92
≥ IV	17	32,08

FEATURE	N	%
Histology		
Nodular sclerosis	34	61,82
Mix celularity	13	23,64
Lymphocyte rich	6	10,91
NA	2	3,64
EBV		
Positive	15	28,30
Negative	38	71,70

Mata et al. Summited (2016)

MD Anderson Cancer Center
Madrid · España

Target gene selection

- Target genes were selected by previous analyses of the mutational profile of cHL with Illumina Hiseq and Sure Select technology (Agilent Technologies) in an initial discovery cohort of 7 tumor tissue samples (freshly frozen), with a targeted analysis of 522 genes involved in HL biology and B cell-related pathways.
- The final panel included 36 selected genes recurrently mutated in this initial discovery cohort (variants detected in at least 2 samples).

Mata et al. Summited (2016)

MD Anderson Cancer Center
Madrid · España

Target gene selection (I)

BCR pathway			DLBCL Pathway	NFKB pathway				STAT pathway		TCR pathway		HL pathway	
AKT1	JUN	PIK3CB	ADAM8	AHR	GADD45B	NFKB1	TNFAIP3	BCL2L1	IL6	CBL	MAPK3	TNFRSF5	CENPF
AKT2	KRAS	PIK3CD	AFMID	BAG4	ICAM1	NFKB2	TNFAIP6	IL10	IL6R	CBLB	NCK1	TNFSF5	MAPRE1
AKT3	LPL	PIK3CG	ARID3A	BANK1	ID2	NFKBIA	TNFRSF10A	IL10RA	IL6ST	CBLC	NCK2	CD80	BUB3
B2M	LYN	PIK3R1	BCL6	BATF	IGF1	NFKBIB	TNFRSF10B	IL10RB	IL7	CD247	PAK1	CD86	NBS1
BCL10	MAP2K3	PIK3R2	BCOR	BCL2	IKBKAP	NFKBIE	TNFRSF11A	IL11	IL7R	CD28	PAK2	FUT4	CCNH
BLNK	MAP2K4	PIK3R3	CD1A	BCL2A1	IKBKB	NFKBIL1	TNFRSF11B	IL11RA	IL9	CD3D	PAK3	FUT9	CSE1L
BTK	MAP2K7	PIK3R5	CKAP4	BCL3	IKBKE	NFKBIL2	TNFRSF12A	IL12A	IL9R	CD3E	PAK6	FUT7	NUMA1
CALM1	MAP3K13	PIM2	DGKG	BIRC2	IKBKG	NGFR	TNFRSF13B	IL12B	IRF9	CD3G	PAK7	FUT6	CDC2
CALM2	MAP3K3	PIM3	DUSP5	BIRC3	IL1A	PASK	TNFRSF13C	IL12RB1	JAK1	CD4	PDCD1	CASP8	HMMR
CALM3	MAP3K6	PLCG2	ENPP3	BIRC4	IL1B	PBEF1	TNFRSF14	IL12RB2	JAK2	CD8A	PDK1	CASP3	RSN
CARD11	MAP4K4	PRDM1	ERCC6L	BIRC5	IL1R1	PECAM1	TNFRSF17	IL13	JAK3	CD8B	PLCG1	CASP9	RAMP
CD19	MAPK8	PRKCB	EZH2	BIRC6	IL32	PLEK	TNFRSF18	IL13RA1	LIF	CDC42	PPP3CA	BAX	CCNH
CD22	MCL1	PRKD1	GSG2	BIRC7	IL8	PRKCA	TNFRSF19	IL13RA2	LIFR	CDK4	PPP3CB	BAK1	CDK7
CD27	MS4A1	PRKD3	IGLL1	BLK	IRAK1	PRKCD	TNFRSF1A	IL15	MPL	CSF2	PPP3CC	BID	CCNA2
CD38	MyD88	PTPN1	irak3	CCL2	IRF1	PRKCH	TNFRSF1B	IL15RA	MYC	CSK	PPP3R1	BIK	CDC6
CD5	NAIP	PTPRC	LFNG	CCL22	IRF3	PRKCI	TNFRSF21	IL19	PIAS1	CTLA4	PPP3R2	BCL2L11	CCNE2
CD70	NFAM1	RELT	LPP	CCL3	ITGAL	PRKCZ	TNFRSF25	IL2	PIAS2	DAPP1	PRKCQ	MCL1	CDC2
CD79A	NFAT5	RHOA	MME	CCL4	ITGAM	PTPN3	TNFRSF4	IL20	PIAS3	DOK1	PTEN	BCL2L10	BCCIP
CD79B	NFATC1	SMARCA4	NCF4	CCR4	JUNB	REL	TNFRSF6B	IL20RA	PIAS4	DOK2	PTPN7	BAD	CCNH
CD81	NFATC2	STK40	PECR	CCR7	KLF10	RELA	TNFRSF8	IL21	PIM1	ELK1	RAC1	CSF2RA	CDKN2C
CHP	NFATC2IP	SYK	PFTK1	CD36	LITAF	RELB	TNFRSF9	IL21R	PTPN6	FOS	RAF1	CSF3	H1F0
CHUK	NFATC3	TCL1A	PLTP	CD40	LSP1	RET	TNFSF10	IL22	SOCS1	GRAP2	RASA1	CSF2RB	H2AFX
CR2	NFATC4	TCL1B	PSTPIP2	CD40LG	LTA	RGS1	TNFSF11	IL22RA1	SOCS2	GRB2	RASGRP1	CSF3R	HIST1H3D
CYLD	NFKBIZ	TLR2	PTK2	CD44	LTB	RIPK1	TNFSF12	IL22RA2	SOCS3	HRAS	SHC1	CSF1R	DCK
DAPP1	NRAS	TP53	PTPRO	CD82	LTBR	RIPK2	TNFSF13	IL23R	SOCS4	ICOS	SOS1	CSF2	RRM2
GSK3B	OASL	TTRAP	RAB7L1	CD83	MALT1	RIPK3	TNFSF13B	IL26	SOCS5	IFNG	SOS2	CSF1	TYMS
IL17D	PAG1	VAV1	RASL11A	CEP110	MAP3K1	RRAS2	TNFSF14	IL28A	SOCS6	ITK	TCRA	CD274	MLH1
IL1RAP	PAK4	VAV2	RC3H1	CFLAR	MAP3K14	SDC4	TNFSF15	IL28B	STAM	LAT	TCRB	PDCD1LG2	TOP2A
IL27RA	PELI1	VAV3	RRAGB	CRADD	MAP3K4	SELL	TNFSF18	IL28RA	STAM2	LCK	TEC	DNAJA2	GRB2
IL4RA	PIK3C2B	VPREB1	RRAS2	CX3CL1	MAP3K5	SMAD7	TNFSF4	IL29	STAT1	LCP2	UBASH3B	HSPA4	MAPK9
INPP5D	PIK3C3	VPREB3	S100A8	CXCL1	MAP3K7	SMARCA2	TNFSF8	IL2RA	STAT2	MAP2K1	ZAP70	HSP90AA1	MAPK6
IRF4	PIK3CA	WSB2	SERPINA9	CXCL10	MAP3K7IP1	SOD2	TNFSF9	IL2RB	STAT3	MAP4K1	NOTCH1	HSPA9B	SH2D1A
MCL Pathway			SH3BP5	CXCL13	MAP3K7IP2	SPI1	TRADD	IL2RG	STAT4	Others		AURKA	ALDH1A1
ATM	CDKN2A	MDM2	TOX	CXCL2	MAP3K7IP3	SPIB	TRAF1	IL3	STAT5A	RYBP	MEL18	CENPE	ITGA4
BMI1	CEBPB	RB1	TPD52	CXCL9	MAP3K8	TANK	TRAF2	IL3RA	STAT5B	RNF2	GLI3	MAD2L1	LCP1
CCND1	CHEK1	SUZ12	ZBTB32	CXCR4	MAPK11	TBK1	TRAF3	IL4	STAT6	RING1		BUB1B	LGALS1
CDK6	CHEK2		ZNF185	CXCR7	MAPK12	TLR4	TRAF4	IL5	TSLP				
			ZNF230	FADD	MAPK13	TNF	TRAF5	IL5RA	TYK2				
			KLHL6	FAS	MAPK14	TNFAIP1	TRAF6						
				FYN	MYB	TNFAIP2							

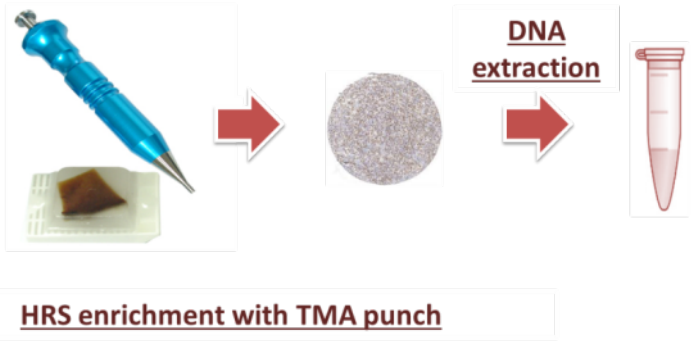
Target gene selection (II) (Ion Ampliseq DNA custom panel)

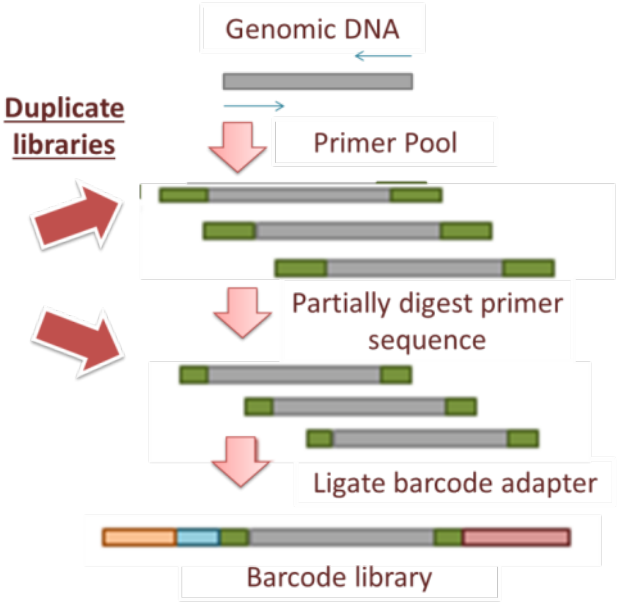
			COVERAGE STATUS			DESIGN CRITERIA			
GENE	SEQUENCING	AMPLICONS	TOTAL BASES	COVERED BASES	OVERALL COVERAGE (%)	cHL	PREVIOUS SEQUENCING	DLBCL	BIOLOGICAL RELEVANCE
B2M	COMPLETE GENE	6	393	393	100	X	X	X	
CARD11		49	3729	3674	98,52		X		
CASP8		23	2263	2232	98,63		X		
CSF1R		42	3150	3150	100		X		
BCL10		11	735	704	95,78		X		
NFKBIA		14	1020	970	95,09	X	X		
STAT6		37	2795	2795	100		X		
BTK		28	2178	2153	98,85		X		
IL32		10	850	760	89,41		X		
MYB		34	2787	2721	97,63				X
SH3BP5		16	1502	1296	86,28		X		
ABL1	REGION	8	3393	431	12,7		X		
ADAM8		4	2229	178	8		X		
CD19		3	1674	144	8,6		X		
CD38		2	903	88	9,7		X		
NFKB2		5	2700	238	8,8		X		
PIK3CD		5	3135	205	6,5		X		
RET		8	3219	391	12,14		X		
TNFRSF14		1	852	58	6,8		X		
CSF2RB		4	2694	164	6	X	X		
FAS		1	7536	41	0,5	X			X
LCP1		1	1866	41	2,2				X
LTB		2	234	110	47		X		
MAPK3		2	1074	82	7,6		X		
MDM2		2	966	82	8,4				X
NUMA1		5	6306	205	3,2		X		
PLCG2		3	3798	131	3,4		X		
SMARCA4		7	4842	322	6,6		X		
NOTCH1		4	7668	269	3,5		X	X	
CREBBP		3	7215	252	3,4			X	
EP300		8	7245	460	6,3			X	
STAT3		1	2169	103	4,7			X	
MYC		2	1365	82	6			X	
EZH2		1	2214	25	1,1			X	
MYD88		1	480	44	9,1			X	

The final panel included 36 selected genes (recurrently mutated in cHL samples) consisting in **353 amplicons:** complete coding regions from 11 genes and additional 81 selected regions from 25 genes.

MD Anderson Cancer Center
Madrid · España

Technical approach





MD Anderson Cancer Center
Madrid · España

Filtering criteria

- Duplicate concordance
- Coverage over 100 reads on both duplicates
- Allele frequency > 1%
- SNVs in the middle of the amplicon
- Exonic regions
- Damaging or deleterious prediction by Provean or Alamut software's.

MD Anderson Cancer Center
Madrid · España

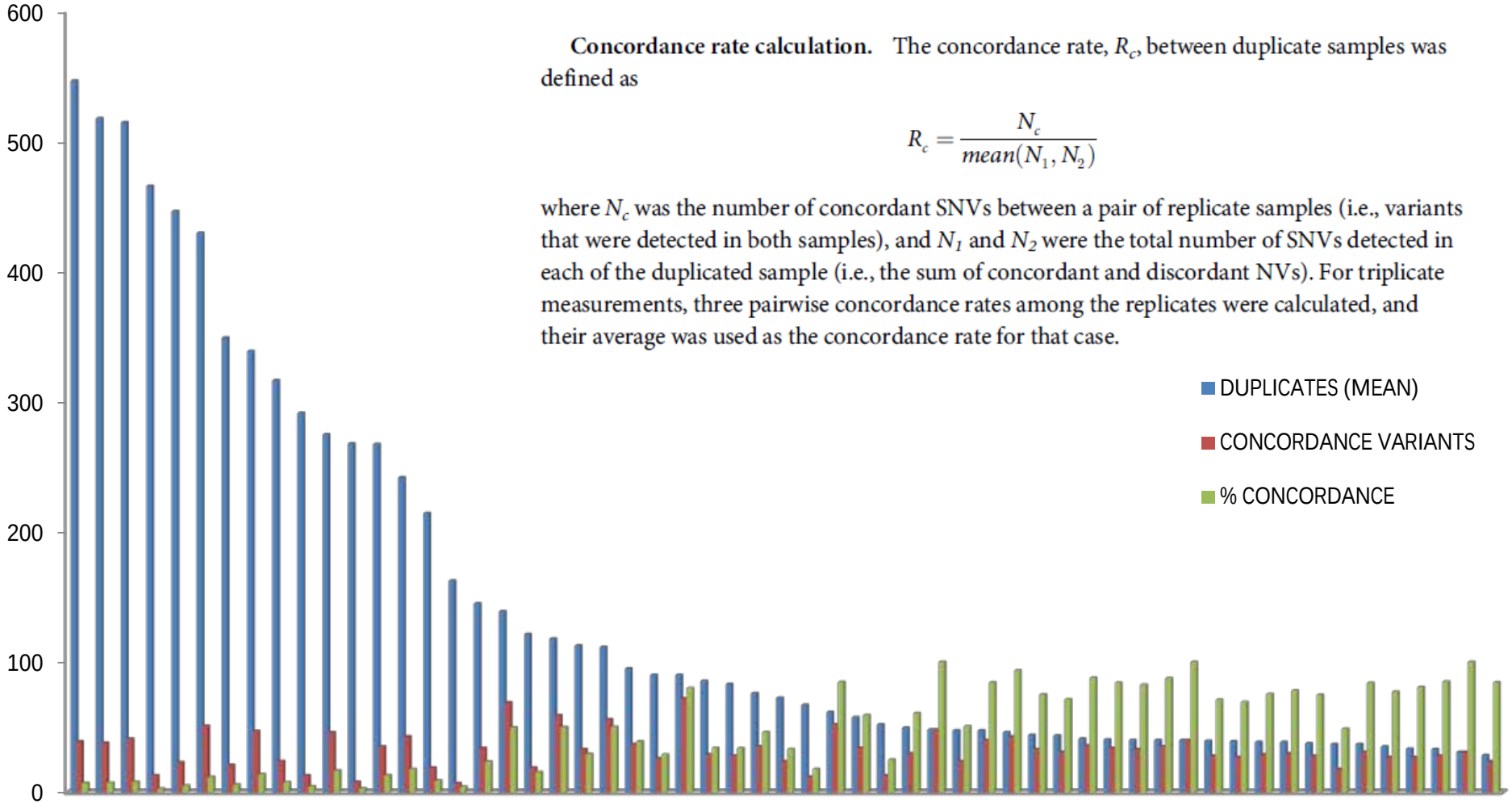
Results (I)

- Overall, the results show very high genomic heterogeneity with a range of 10-400 SNVs per sample, most of them (~60%) missense type.
- We found a relatively large number of genes recurrently mutated at low frequency and only a few genes mutated in up to 15-20% of the patients, reflecting a high level of genomic instability in the neoplasm.

Mata et al. Summited (2016)

MD Anderson Cancer Center
Madrid · España

Concordance ratio



Concordance rate calculation. The concordance rate, R_c, between duplicate samples was defined as

$$R_c = \frac{N_c}{mean(N_1, N_2)}$$

where N_c was the number of concordant SNVs between a pair of replicate samples (i.e., variants that were detected in both samples), and N_1 and N_2 were the total number of SNVs detected in each of the duplicated sample (i.e., the sum of concordant and discordant NVs). For triplicate measurements, three pairwise concordance rates among the replicates were calculated, and their average was used as the concordance rate for that case.

Mata et al. Summited (2016)

MD Anderson Cancer Center
Madrid · España

Results (II)

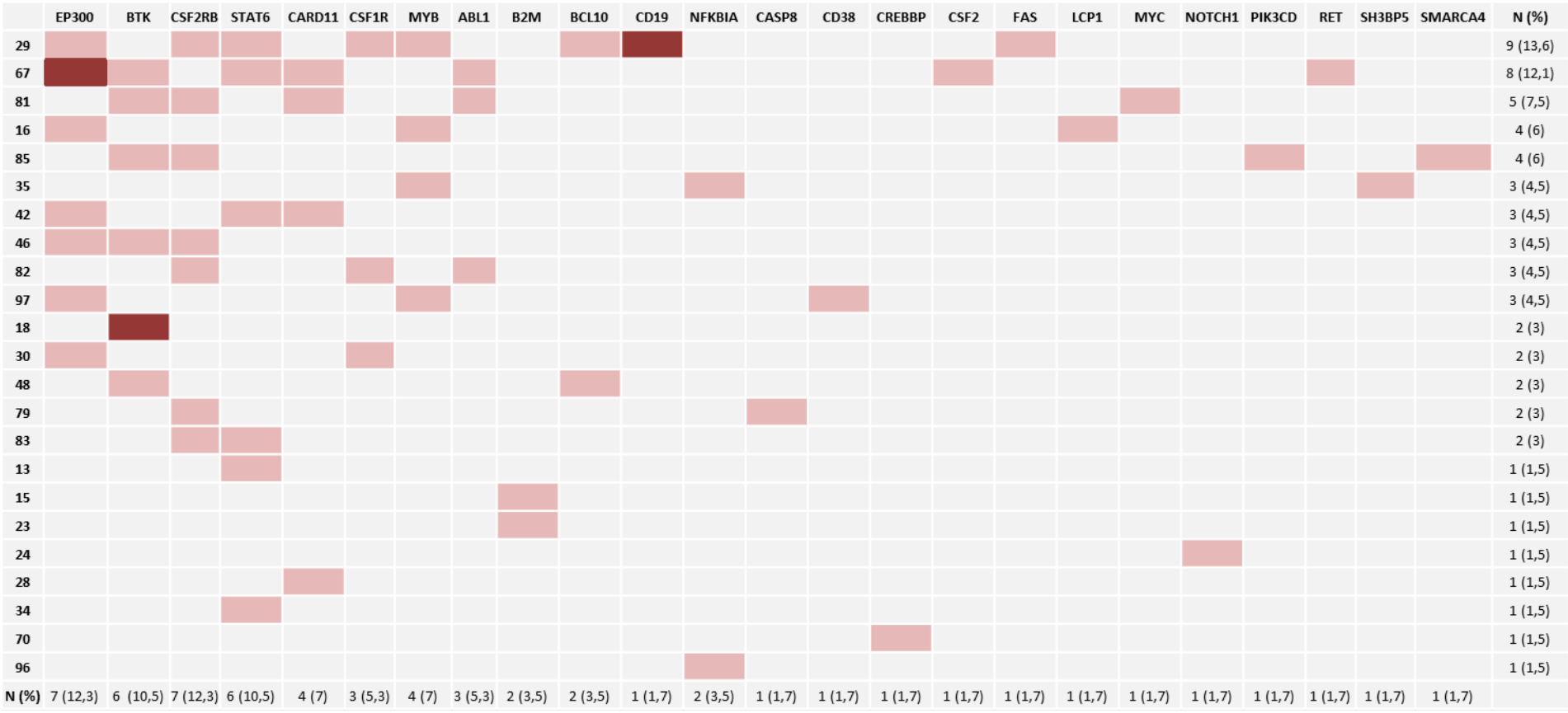
- Specific mutations in genes previously described in cHL (*NFKBIA*, *TNFRSF14, B2M)* and in diffuse large B-cell lymphomas (*CARD11*, *STAT6*, *CREBBP*, *CMYB*) were consistently found, as well as new SNVs in genes not previously described (*BTK*, *NFKB2*).

- Mutations affecting selected genes (*B2M*, *CARD11*, *NFKBIA*, *CSF2RB*, and *STAT3*) in cell lines were additionally validated by Sanger sequencing.

MD Anderson Cancer Center
Madrid · España

COVERAGE MEAN = 927,5
FREQUENCY MEAN = 16,34

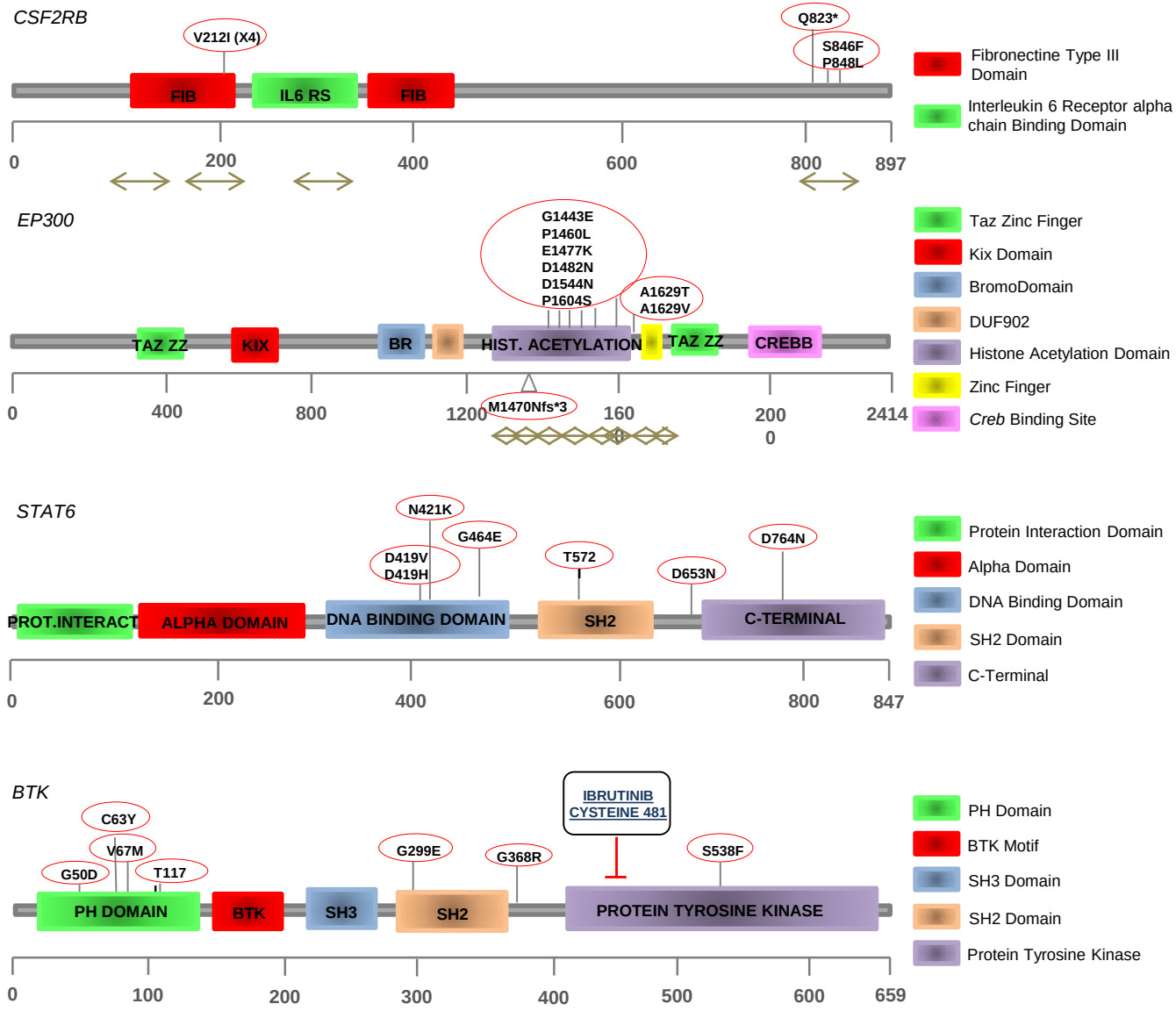
cHL tumor samples series results



	EP300	BTK	CSF2RB	STAT6	CARD11	CSF1R	MYB	ABL1	B2M	BCL10	CD19	NFKBIA	CASP8	CD38	CREBBP	CSF2	FAS	LCP1	MYC	NOTCH1	PIK3CD	RET	SH3BP5	SMARCA4	N (%)
29																									9 (13,6)
67																									8 (12,1)
81																									5 (7,5)
16																									4 (6)
85																									4 (6)
35																									3 (4,5)
42																									3 (4,5)
46																									3 (4,5)
82																									3 (4,5)
97																									3 (4,5)
18																									2 (3)
30																									2 (3)
48																									2 (3)
79																									2 (3)
83																									2 (3)
13																									1 (1,5)
15																									1 (1,5)
23																									1 (1,5)
24																									1 (1,5)
28																									1 (1,5)
34																									1 (1,5)
70																									1 (1,5)
96																									1 (1,5)
N (%)	7 (12,3)	6 (10,5)	7 (12,3)	6 (10,5)	4 (7)	3 (5,3)	4 (7)	3 (5,3)	2 (3,5)	2 (3,5)	1 (1,7)	2 (3,5)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	1 (1,7)	

MD Anderson Cancer Center
Madrid · España

Recurrent mutations in cHL

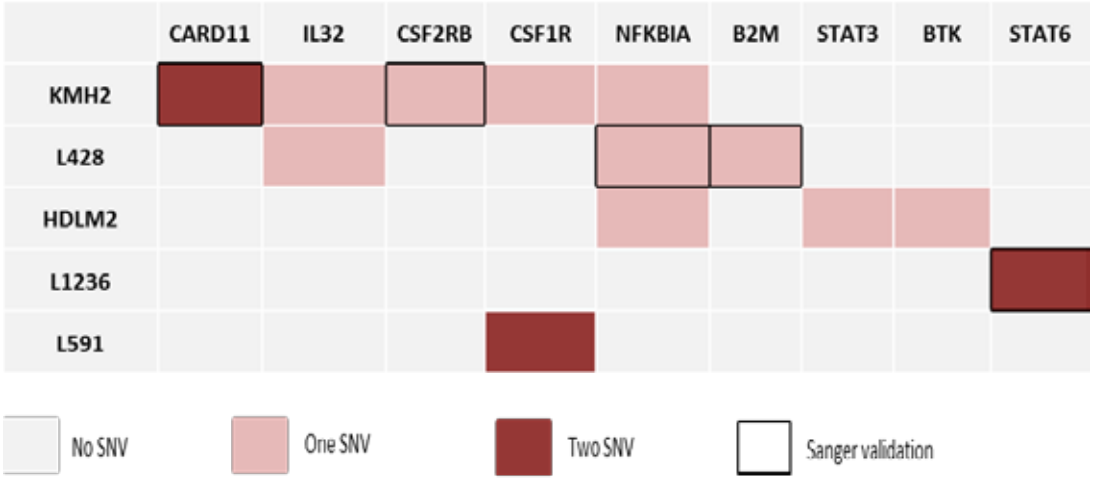


- **SNVs in > 10% of samples.**
- Double brown arrow represent sequenced regions per gene. In *STAT6* and *BTK* the design coverage is at least 98%.

MD Anderson Cancer Center
Madrid · España

Mutation analyses in cHL-derived cell lines

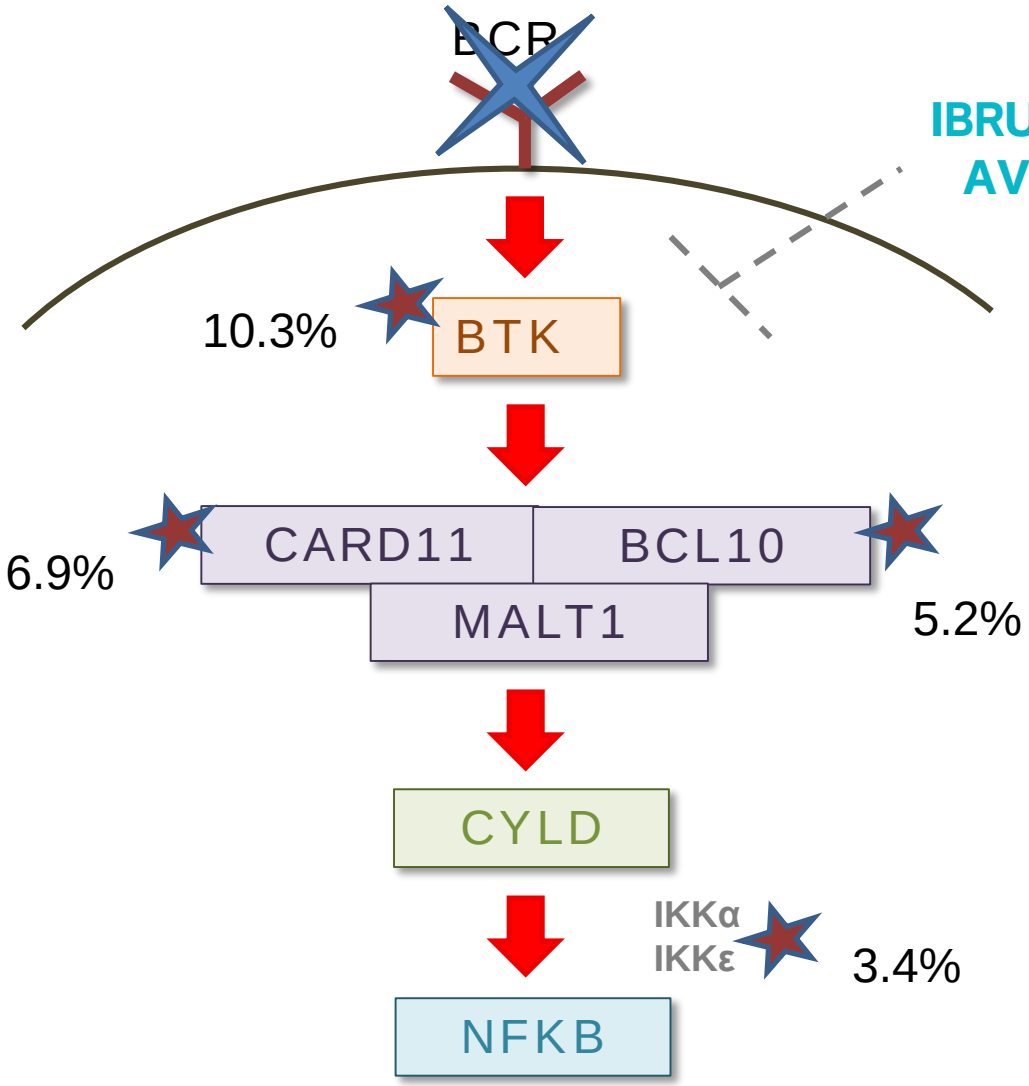
cHL-derived cell line	Chromosome	Chromosomal position	Base change	Frequency	Coverage	Gene ID	Type	Amino acid change
HDLM2	chr14	35871799	A>G	4.0	247	NFKBIA	SNV	L236P
HDLM2	chr17	40474420	C>A	58.0	100	STAT3	SNV	D661Y
HDLM2	chrX	100625061	A>G	3.2	158	BTK	SNV	Y106H
KMH2	chr7	2956982	T>A	45.7	608	CARD11	SNV	D882V
KMH2	chr7	2978436	GTCTGA>-	51.0	4935	CARD11	Deletion	DS296*
KMH2	chr16	3117390	G>A	24.1	2235	IL32	SNV	D10N
KMH2	chr22	37325765	G>A	48.4	3123	CSF2RB	SNV	V212I
KMH2	chr5	149450132	T>C	46.5	5884	CSF1R	SNV	H362R
KMH2	chr14	35871136	TTC>-	92.8	318	NFKBIA	Deletion	-
L1236	chr12	57496662	C>T	88.7	8647	STAT6	SNV	D419N
L1236	chr12	57496668	T>A	88.9	8580	STAT6	SNV	N417Y
L428	chr16	3117426	C>T	23.8	3483	IL32	SNV	L22F
L428	chr14	35871707	G>A	61.4	933	NFKBIA	SNV	Q267*
L428_N	chr15	45003746	T>A	7.9	865	B2M	SNV	M1K
HDMYZ	chr22	41568567	G>T	52.2	2251	EP300	SNV	G1506V
HDMYZ	chr11	71718315	C>T	33.6	373	NUMA1	SNV	D1795N
L591	chr5	149450132	T>C	46.2	492	CSF1R	SNV	H362R



Mutations affecting selected genes (**high frequency SNVs: B2M, CARD11, NFKBIA, CSF2RB, and STAT3**) in cell lines were additionally validated by Sanger sequencing.

MD Anderson Cancer Center
Madrid · España

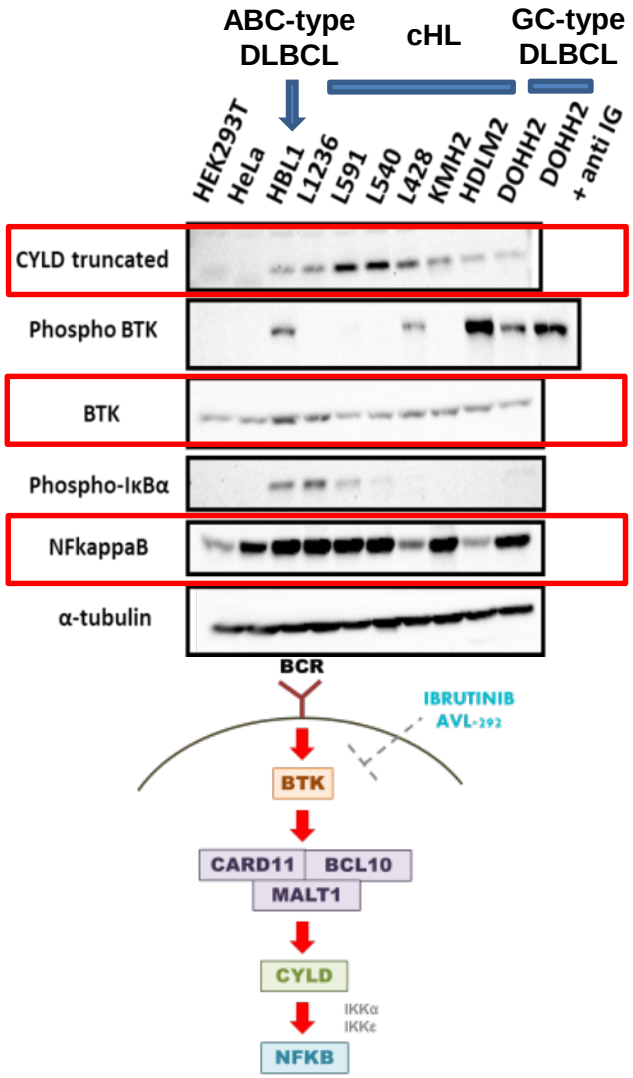
high prevalence of mutations affecting *BTK* and the BCR pathway

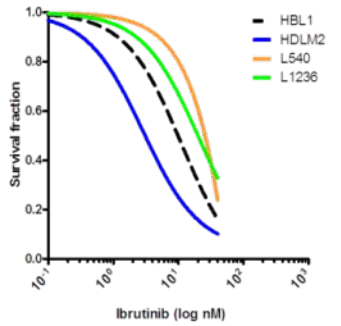


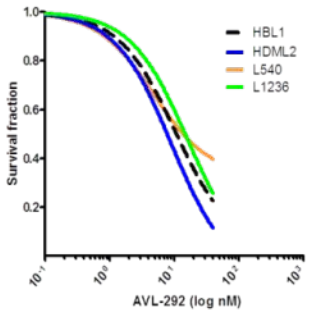
> 20% of cases accumulate mutations affecting members of the BCR signaling pathway.

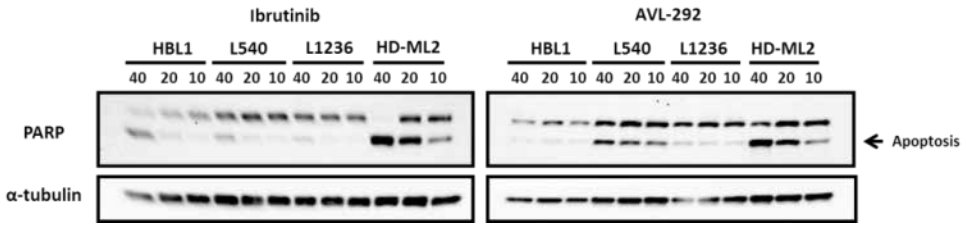
MD Anderson Cancer Center
Madrid · España

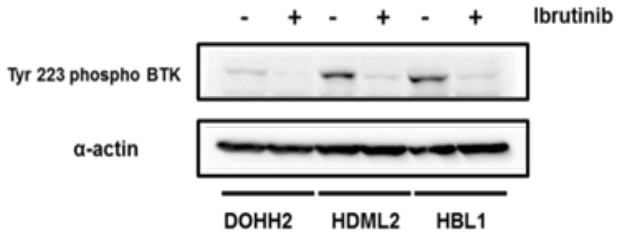
Functional studies: Ibrutinib and AVL-292 treatment (BTK inhibitors) in CLH cell lines









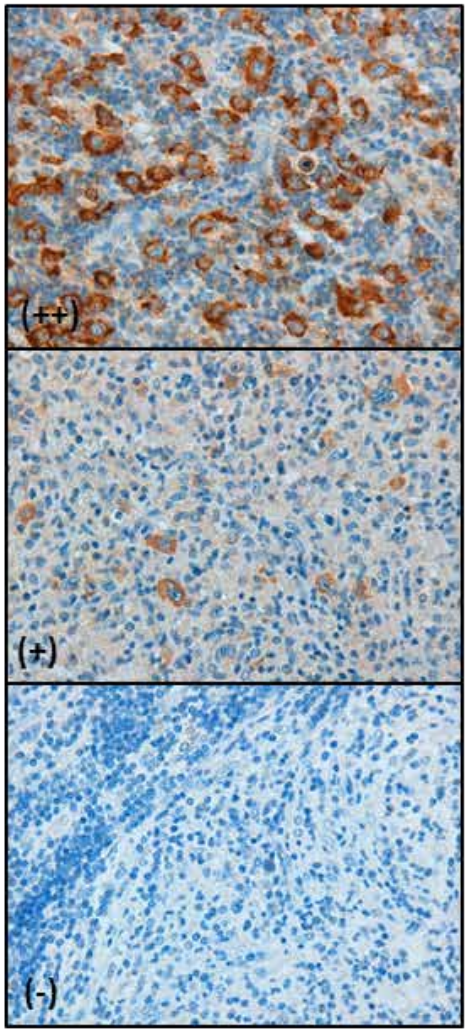


GC-type DLBCL | cHL | ABC-type DLBCL

MD Anderson Cancer Center
Madrid · España

BTK Expression in primary HRS Cells

A

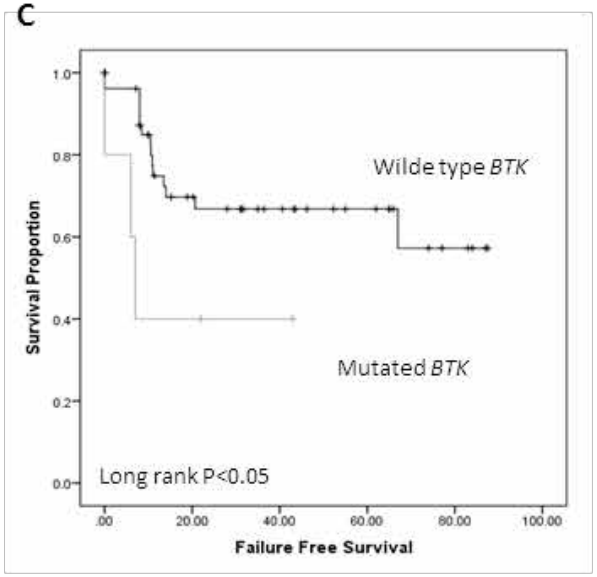


B

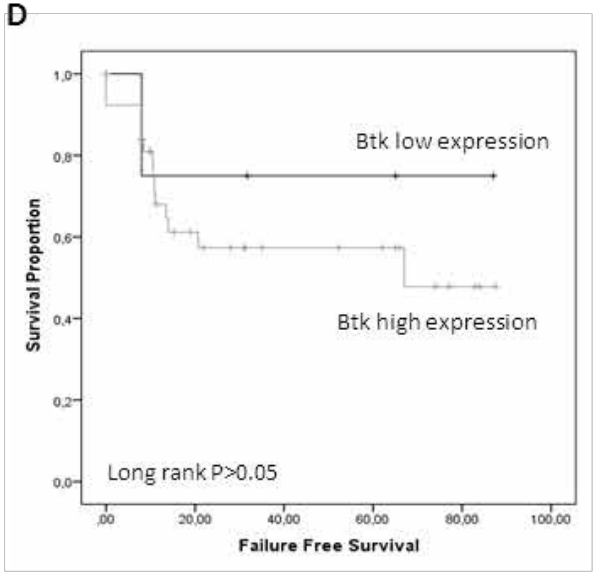
Expression level of Btk	# Patient N (%)
Strong positive (++)	4/53 (8)
Normal positive (+)	34/53 (64)
Negative (-)	9/53 (17)
Not assessable (NA)	6/53 (11)

72% of the tumors showed Btk protein expression in our series: Btk positivity (+) was concluded for cases with a level of expression comparable to that seen in normal germinal center B lymphocytes, as reference. (C) Kaplan–Meier survival curves demonstrate longer FFS in wt-BTK cases (P<0.05). (D) Survival curves demonstrate a longer FFS in cases with a low level of expression of Btk protein (P=n.s.).

C



D



The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE

Ibrutinib in Refractory Classic Hodgkin's Lymphoma

N Engl J Med 2015; 373:1381-1382 | October 1, 2015 | DOI: 10.1056/NEJMc1505857

Article

To the Editor:

Ibrutinib is an oral, small-molecule Bruton's tyrosine kinase (BTK) inhibitor that has activity in chronic lymphocytic leukemia, mantle-cell lymphoma, and Waldenström's macroglobulinemia.[1-3] We administered single-agent ibrutinib at a dose of 560 mg daily to two heavily pretreated patients who had primary refractory classic Hodgkin's lymphoma.

Ibrutinib as a single agent to two heavily pretreated patients with primary refractory cHL. One of the patients showed almost complete regression of the disease two months after initiation of a therapy that lasted for 4 months. The second patient showed a stable complete response four months after the initiation of treatment

Conclusions

- NGS technologies are suitable for gene mutation identification in cHL.
- Overall, the results show high genomic instability, including numerous mutations in genes related with B-cell function and specific signaling pathways: **similar mutational profile between cHL and DLBCL / PMLBCL**.
- High prevalent mutations (drivers?):
  - Affecting **NFkappaB** pathway:
    - NFKBIA (IκBα) and NFKBIE (IκBε)
    - TNFAIP3 (A20)
  - Associated with **JAK/STAT** activation:
    - SOCS-1 mutations, PTPN1 mutations (also in PMBCL), STAT6
  - **B2M** mutations, (loss of cell-surface HLA-I, immune scape?)
  - Mutations affecting ***BTK* and the BCR pathway**
  - **Epigenetic regulation,** EP300, CREBBP

MD Anderson Cancer Center
Madrid · España

Acknowledgements

Tumor Bank, CNIO
M.M. Morente

Bioinformatics IBBTEC, UNICAN
Ignacio Varela

Lymphoma Research Group, Instituto Investigación Sanitaria Puerta de Hierro (IDIPHIM), Madrid
Margarita Sánchez-Beato

H.U. Marqués de Valdecilla, IFIMAV
Miguel A. Piris

Spanish Hodgkin's Lymphoma Study Group
Hospital U. de Móstoles,
Hospital U. Infanta Sofía, San Sebastián de los Reyes,
Hospital Severo Ochoa, Leganés,
Hospital G.U. Gregorio Marañón

Elena Mata, BSc.

Ana M. Martín, PhD.

Antonio Díaz, PhD.

Mónica Estévez, MD.
Carlos Montalbán, MD.

This work was cofunded by ISCIII-FEDER (PI12/01832 and RTICC RD06/0020/0107 and the Spanish Association for Cancer Research (AECC)