The NOTCH pathway is recurrently mutated in diffuse large B-cell lymphoma associated with hepatitis C virus infection

Luca Arcaini,[1,2] Davide Rossi,[3] Marco Lucioni,[4] Marta Nicola,[1] Alessio Bruscaggin,[3] Valeria Fiaccadori,[1] Roberta Riboni,[4] Antonio Ramponi,[5] Virginia V. Ferretti,[2] Stefania Cresta,[3] Gloria Margiotta Casaluci,[3] Maurizio Bonfichi,[2] Manuel Gotti,[2] Michele Merli,[6] Aldo Maffi,[1] Mariarosa Arra,[1] Marzia Varettoni,[2] Sara Rattotti,[2] Lucia Morello,[1] Maria Luisa Guerrera,[1] Roberta Sciarra,[1] Gianluca Gaidano,[3*] Mario Cazzola,[1,2*] and Marco Paulli[1,4*]

[1]Department of Molecular Medicine, University of Pavia; [2]Department of Hematology Oncology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia; [3]Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara; [4]Department of Pathology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia; [5]Division of Pathology, Department of Health Science, Amedeo Avogadro University of Eastern Piedmont, Novara; and [6]Division of Hematology, Ospedale di Circolo e Fondazione Macchi, University of Insubria, Varese, Italy

**GG, MC and MP contributed equally to this work.*

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Correspondence: luca.arcaini@unipv.it

Immunohistochemistry

For morphological examination, sections were stained with haematoxylin and eosin and Giemsa. Automated paraffin section immunostainings (DakoCytomation Autostainer, Denmark) were performed using the streptavidin-biotin peroxidase conjugated method after antigen retrieval procedures, when required. The panel of antibodies and antisera included L26/CD20cy, CD79a, Bcl-6/PG-B6, LCA/CD45, CD30, MUM1/IRF4, Ki67/Mib1, Kappa, Lambda, IgA, IgD, IgM, IgE, IgG, LMP1, ALK, TdT (Dako), CD3, CD5, CD10/CALLA, CD23 (Novocastra), PAX5, BOB1, (Santa Cruz), CD15, Cytokeratin CAM5.2 (Beckton Dickinson), Cyclin D1 (Neomarkers), CD138 (IQ Products), CD56 (Zymed).

Mutation analysis

Sequences for all annotated exons and flanking splice sites were retrieved from the UCSC Human Genome database using the corresponding mRNA accession number as a reference. PCR primers, located ~50 bp upstream or downstream to target exon boundaries, were either derived from previously published studies or designed in the Primer 3 program (http://frodo.wi.mit.edu/primer3/) and filtered using UCSC in silico PCR to exclude pairs yielding more than a single product. All PCR primers and conditions are available upon request. Purified amplicons were subjected to conventional DNA Sanger sequencing using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), and compared to the corresponding germline sequences using the Mutation Surveyor Version 4.0.5 software package (SoftGenetics) after automated and/or manual curation. Candidate variants were confirmed from both strands on independent PCR products. The following databases were used to exclude known germline variants: Human dbSNP Database at NCBI (Build 138) (http://www.ncbi.nlm.nih.gov/snp); The 1000 Genomes Project (http://www.1000genomes.org/).

Supplemental table 1 - Comparison of clinical features of discovery panel and extension panel

	Discovery (n=19)	**Extension** (n=27)	**P**
Age, yrs: median (range)	72 (55-88)	66 (44-88)	0.184
Gender: n (%)			>0.900
M	7 (37%)	11 (41%)	
F	12 (63%)	16 (59%)	
Ann Arbor III-IV: n (%)	13 (68%)	17 (63%)	0.762
B symptoms: n (%)	8 (42%)	4 (15%)	0.049
ECOG PS 2-3*: n (%)	2 (11%)	4 (27%)	0.375
Low grade component: n (%)	6 (32%)	6 (22%)	0.513
Histogenesis: n (%)			0.749
GC	5 (26%)	9 (33%)	
Non GC	14 (74%)	18 (67%)	
Chang*: n (%)			0.261
A	2 (11%)	5 (28%)	
B	13 (68%)	8 (44%)	
C	4 (21%)	5 (28%)	
Ki67, %: median (range)	65 (30-95)	70 (30-90)	0.393
Genotype*: n (%)			0.242
1b	4 (67%)	1 (20%)	
2a/2c	2 (33%)	4 (80%)	
Cryoglobulins*: n (%)	4 (31%)	4 (22%)	0.689
Bone marrow involved: n (%)	1 (7%)	3 (14%)	>0.900

*Missing values
M, male; F, female; CG, germinal center

Supplemental table 2 - Clinical characteristics of the HCV-negative DLBCL cohort at diagnosis (n=64)

Clinical characteristics	**n**	**%**
Age >60 y	40	62.5
Male:female	31:33	
ECOG PS >1	14	21.9
Ann Arbor stage		
I	4	6.3
II	9	14.1
III	16	25.0
IV	34	53.1
B symptoms	22	34.4
Extranodal sites >1	13	20.3
LDH >ULN	38	59.4
IPI		
Low	10	15.6
Low-Intermediate	17	26.6
High-Intermediate	29	45.3
High	7	10.9
Non-GC phenotype	42	65.6

LDH, lactate dehydrogenase; ULN, upper limit of normal;
IPI, International Prognostic Index; CG, germinal center

Supplemental table 3 - Detailed description of mutations

			Discovery Panel	Extension Panel	HCV negative
NOTCH pathway	NOTCH 1	c.7544_7545DelCT p.P2515fs*4	1	1	0
	NOTCH 2	c. 6394 A>T p.K2132*	0	1	0
		c.6721_6724del4bp p.S2142fs*4	1	0	0
		c.6772 G>T p.E2258*;	0	1	0
		c.6791_6794 Del 4bp p.Y2264fs*18	0	1	0
		c.6902 T>A p.L2301*	0	1	0
		c.6909_6910insC p.I2304 fs*9	0	0	1
		c.7198 C>T p.R2400*	4	0	0
	SPEN	c.3308 C>A p.S1103*; c.4288 A>G p.K1430E	1	0	0
NF-kB pathway	MYD88	c.794 T>C p.L265P	1	n.e.	n.e.
	TNFAIP3	c. 1162 G>T p.E388*	1	n.e.	n.e.
		c.1269_1270insC p.K424fs*5	1	n.e.	n.e.
		c.130_137del8bp p.H44fs*54	1	n.e.	n.e.
		c.2267_2268InsC p.K759fs*10	1	n.e.	n.e.
		c.506delA; c.505_507insTT p.D169fs*85	1	n.e.	n.e.

Supplemental table 4 – Comparison of *NOTCH2* mutational status between our series of HCV-positive DLBCL and unselected series of DLBCL

Reference	**N of DLBCL cases**	***NOTCH2*+ cases**
Present study	46	9
Lee et al. Cancer Science 2009	63	5
Lohr et al. Lohr et al. PNAS 2012	49	2

Present study vs Lee et al. (Chi-square test p=0.07, Fisher exact test 0.08); present study vs Lohor et al. (Chi-square p=0.01, Fisher exact test 0.025 Fisher)