

Supplementary Material

IDENTIFICATION OF PROGNOSTIC FACTORS IN CLASSIC HODGKIN LYMPHOMA BY INTEGRATING WHOLE SLIDE IMAGING AND NEXT GENERATION SEQUENCING

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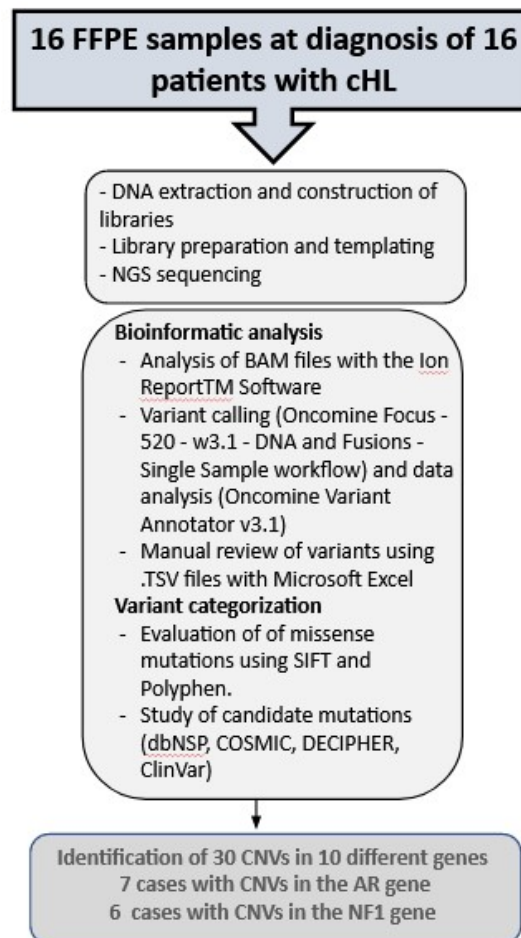
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Supplementary Figure 1. Workflow analysis of the sequencing process, bioinformatic analysis, and overall results.



Supplementary Table 1. Parameters used to define color deconvolution in the algorithms MembraneQuant and NuclearQuant in the QuantCenter application (version 2.3) (3DHitech Ltd., Budapest, Hungary).

Measurement of CD30 expression (MembraneQuant algorithm)	Measurement of CD20 and CD3 expression (MembraneQuant algorithm)	Measurement of MUM1 expression (NuclearQuant algorithm)
Membrane detection	Membrane detection	Nucleus detection

Blur (3) Limit (200)	Blur (3) Limit (200)	Blur (15) Limit (200)
Sensitivity (255)	Sensitivity (230)	Radius (8-20)
Filter	Filter	Area (≥ 100)
Intensity (100-255)	Intensity (0-255)	Filters
Area (40-300)	Area (40-300)	Intensity (≥ 50)
Nucleus	Nucleus	Contrast (≥ 60)
Blur (15)	Blur (15)	
Radius (3-8)	Radius (3-8)	
Area (≥ 10)	Area (≥ 10)	
Filters	Filters	
Intensity (≥ 60)	Intensity (≥ 60)	
Contrast (≥ 60)	Contrast (≥ 60)	

Supplementary Table 2. Filtered copy number variants (CNVs) in cHL.

Case number	Gene (mean copy number)	Cytoband	Confidence interval	CNV p-value
1	NF1 (4.08)	17q11.2(29422233-29685527)	5%:2.7, 95%:5.86	0.007553
	AR (3.94) */**	Xq12(66776186-66945230)	5%:3.23, 95%:4.77	0
	MED12 (3.06)	Xq13.1(70339117-70349268)	5%:1.82, 95%:4.84	0.001343
2	FGFR2 (7.7) **	10q26.13(123247505-123354466)	5%:4.47, 95%:11.77	0.001292

	NF1 (8.95)	17q11.2(29422233- 29685527)	5%:3.77, 95%:16.41	0.01721
	CDK4 (5.7)	12q14.1(58142052- 58146026)	5%:2.88, 95%:9.25	0.02549
3	KRAS (0)	12p12.1(25364761- 25400274)	5%:0, 95%:1.21	0.02638
	CDK4 (5.15)	12q14.1(58142052- 58146026)	5%:2.55, 95%:8.4	0.04025
	FGFR2 (6.95)	10q26.13(123247505- 123354466)	5%:3.99, 95%:10.64	0.002621
	NF1 (8.3)	17q11.2(29422233- 29685527)	5%:3.5, 95%:15.11	0.02101
4	CCND1 (0)	11q13.3(69456942- 69467039)	5%:0, 95%:1.4	0.02819
	NF1 (7.8)	17q11.2(29422233- 29685527)	5%:3.89, 95%:13	0.008046
5	AR (5.9) **	Xq12(66776186- 66945230)	5%:3.09, 95%:9.36	0.001647
	BIRC2 (11.1)	11q22.2(102234187- 102248277)	5%:3.36, 95%:21.65	0.04601
6	AR (4.8)	Xq12(66776186- 66945230)	5%:3.05, 95%:6.94	0.004492
7	None detected			
8	PIK3CA (0)	3q26.32(178916683- 178916683)	5%:0, 95%:0.22	0.003195

		178952097)		
	KIT (0)	4q12(55529117- 55599362)	5%:0, 95%:1.1	0.02359
	KRAS (0)	12p12.1(25364761- 25400274)	5%:0, 95%:0.73	0.01281
	CDK4 (5.8)	12q14.1(58142052- 58146026)	5%:3, 95%:9.14	0.02148
	NF1 (7.33)	17q11.2(29422233- 29685527)	5%:2.75, 95%:13.41	0.04989
9	AR (6.8)	Xq12(66776186- 66945230)	5%:3.04, 95%:11.23	0.03161
	NF1 (9.9)	17q11.2(29422233- 29685527)	5%:3.24, 95%:18.64	0.045
10	Read_count < 100,000			
11	None detected			
12	KRAS (0)	12p12.1(25364761- 25400274)	5%:0, 95%:0	0.003727
	PIK3CA (0)	3q26.32(178916683- 178952097)	5%:0, 95%:0	0.001038
	AR (7.9)	Xq12(66776186- 66945230)	5%:2.95, 95%:14.03	0.04624
13	AR (19.0)	Xq12(66776186- 66945230)	5%:4.21, 95%:37.89	0.04033
13	CDK4 (39.5)	12q14.1(58142052-	5%:8.32,	0.04062

		58146026)	95%:79.82	
14	NF1 (4.38)	17q11.2(29422233-	5%:2.66,	0.01473
		29685527)	95%:6.73	
15	FGFR2 (2.74)	10q26.13(123247505-	5%:2.24,	0.01093
		123354466)	95%:3.36	
16	CDK4 (10.9)	12q14.1(58142052-	5%:4.75,	0.01174
		58146026)	95%:18.71	

¹ *Filtered out since gene gain was less than 2 over normal for CNV calling. ** Marked as “Amplification. Gain-of-function” by Ion Reporter.

Supplementary Table 3. Results of the correlation analysis between CD30+,CD20+, CD3+ and MUM1+ cell percentages quantified by whole-slide imaging in classic Hodgkin lymphoma.

	%CD30 positivity	%CD20 positivity	%CD3 positivity	%MUM1 positivity
%CD30 positivity	1	$\rho=0.391$ ($p=0.134$)	$\rho=0.238$ ($p=0.374$)	$\rho= (-0.059)$ ($p=0.829$)
%CD20 positivity	$\rho=0.391$ ($p=0.134$)	1	$\rho=0.094$ ($p=0.729$)	$\rho= (-0.141)$ ($p=0.602$)
%CD3 positivity	$\rho=0.238$ ($p=0.374$)	$\rho=0.094$ ($p=0.729$)	1	$\rho= (-0.109)$ ($p=0.688$)
%MUM1 positivity	$\rho= (-0.059)$ ($p=0.829$)	$\rho= (-0.141)$ ($p=0.602$)	$\rho= (-0.109)$ ($p=0.688$)	1