THE OHIO STATE UNIVERSITY

Gene expression signatures predict time-to-progression after front-line chemoimmunotherapy for CLL

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Acknowledgements and Disclosures

Lynne V. Abruzzo

Carmen D. Herling

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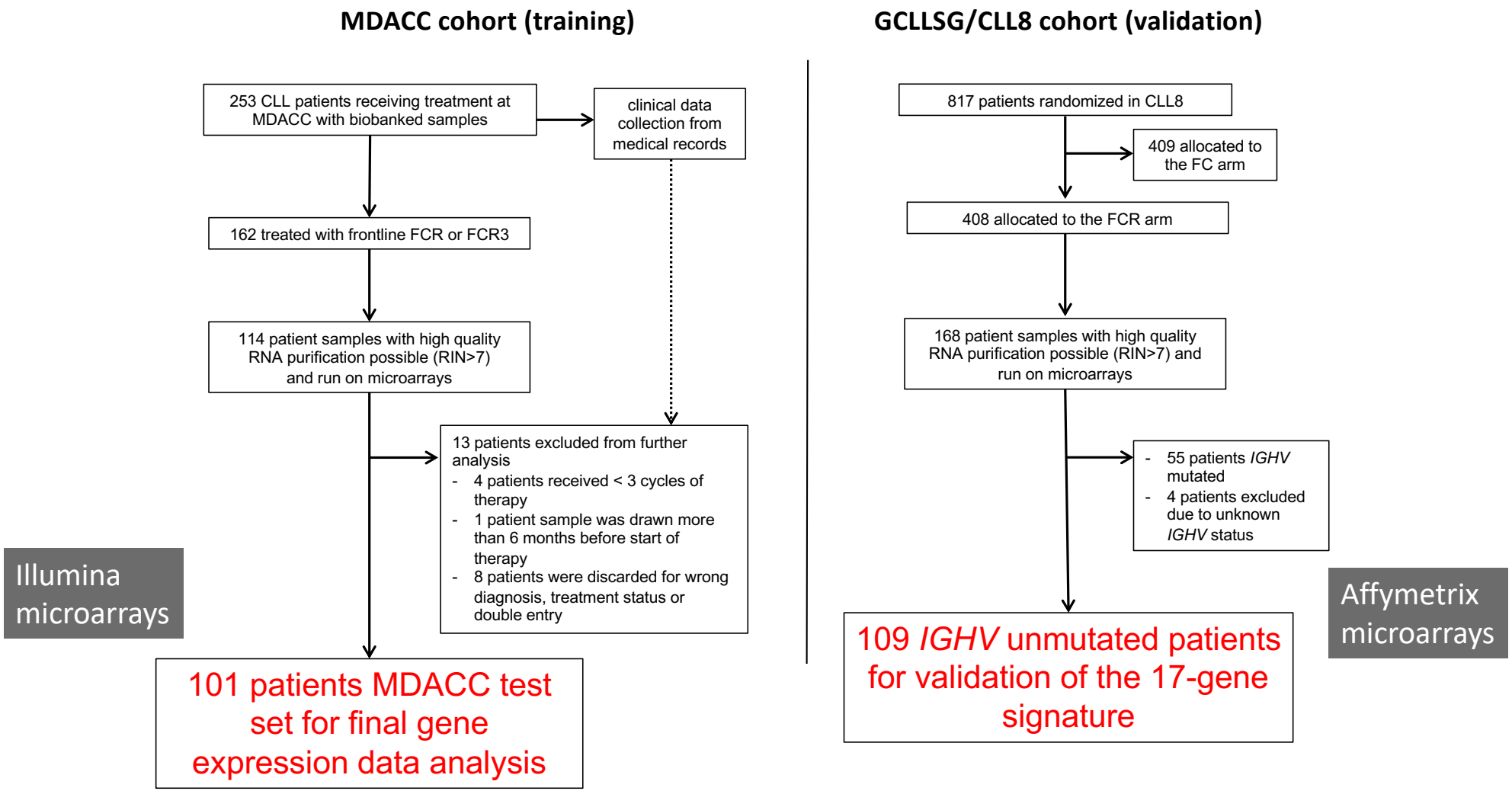
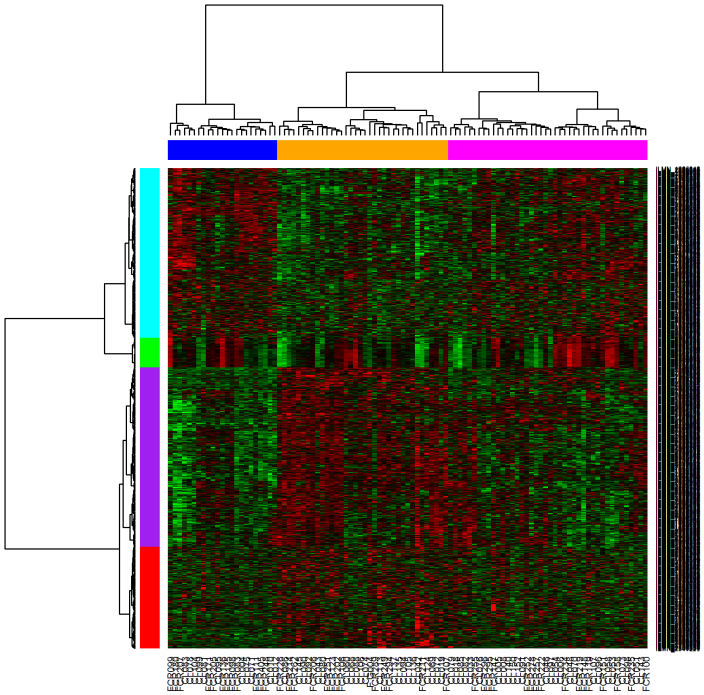

MDACC cohort (training)
253 CLL patients receiving treatment at MDACC with biobanked samples
clinical data collection from medical records
162 treated with frontline FCR or FCR3
114 patient samples with high quality RNA purification possible (RIN>7) and run on microarrays
13 patients excluded from further analysis
- 4 patients received < 3 cycles of therapy
- 1 patient sample was drawn more than 6 months before start of therapy
- 8 patients were discarded for wrong diagnosis, treatment status or double entry
Illumina microarrays
101 patients MDACC test set for final gene expression data analysis
GCLLSG/CLL8 cohort (validation)
817 patients randomized in CLL8
409 allocated to the FC arm
408 allocated to the FCR arm
168 patient samples with high quality RNA purification possible (RIN>7) and run on microarrays
- 55 patients IGHV mutated
- 4 patients excluded due to unknown IGHV status
Affymetrix microarrays
109 IGHV unmutated patients for validation of the 17-gene signature

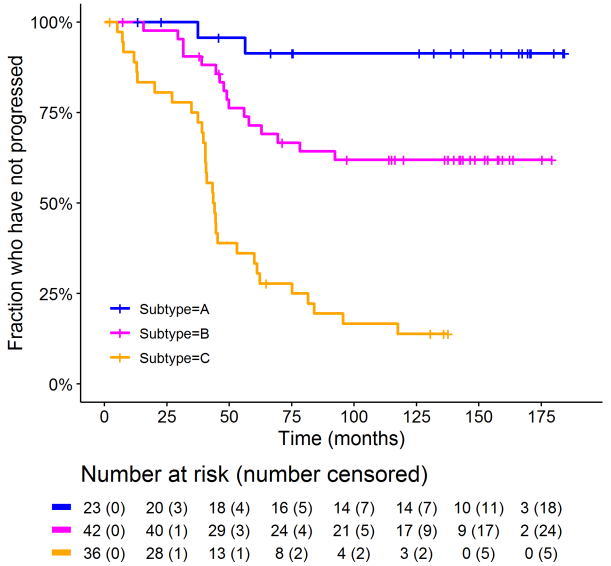
Clinical characteristics

		MDACC		GCLLSG/CLL8
		All (n = 101)	Unmutated (n = 66)	Unmutated (n = 109)
Median age at Dx		56 (49-62)	56 (49-63)	60 (53-65)
Gender	Male	76 (75.2%)	52 (78.8%)	86 (78.9%)
	Female	25 (24.8%)	14 (21.2%)	23 (21.1%)
Rai stage	0-2	77 (76.2%)	**52 (78.8%)**	63 (69.2%)
	≥3	24 (23.8%)	14 (21.2%)	**28 (30.8%)**
	Not available	0	0	18
***IGHV* status**	Mutated	35 (34.7%)	0 (0%)	0 (0%)
	Unmutated	66 (65.3%)	66 (100%)	109 (100%)
β2M	≤4 mg/L	68 (67.3%)	42 (63.6%)	**85 (78.7%)**
	>4 mg/L	33 (32.7%)	**24 (36.4%)**	23 (21.3%)
	Not available	0	0	1
WBC	≤150x10^9/L	83 (82.2%)	50 (75.8%)	84 (77.1%)
	>150x10^9/L	18 (17.8%)	16 (24.2%)	25 (22.9%)
	Not available	0	0	1

		MDACC		GCLLSG/CLL8
		All (n = 101)	Unmutated (n = 66)	Unmutated (n = 109)
ZAP70	Positive	49 (55.7%)	**43 (74.1%)**	31 (47.7%)
	Negative	39 (44.3%)	15 (25.9%)	**34 (52.3%)**
	Not available	13	8	44
CD38 expr.	<30%	75 (74.3%)	**45 (68.2%)**	59 (58%)
	≥30%	26 (25.7%)	21 (31.8%)	**42 (42%)**
	Not available	0	0	8
Cytogenetics				
	del17p	1 (1.0%)	1 (1.5%)	**13 (11.9%)**
	del11q	17 (16.8%)	16 (24.2%)	**42 (48.5%)**
	+12	17 (16.8%)	**10 (15.2%)**	3 (2.8%)
	FISH normal	31 (30.7%)	**24 (36.4%)**	22 (20.2%)
	del13q	35 (34.7%)	15 (22.7%)	29 (26.6%)

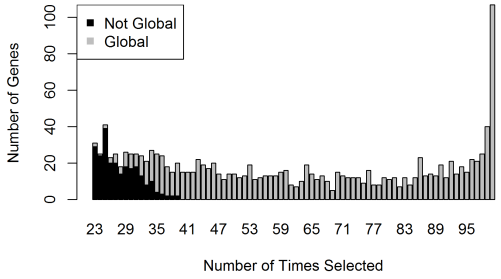
Hierarchical clustering using genes having univariate association with time to progression finds three subtypes with different outcomes

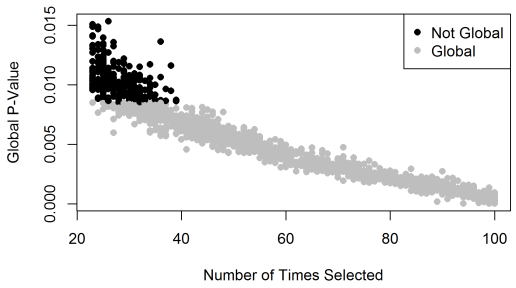


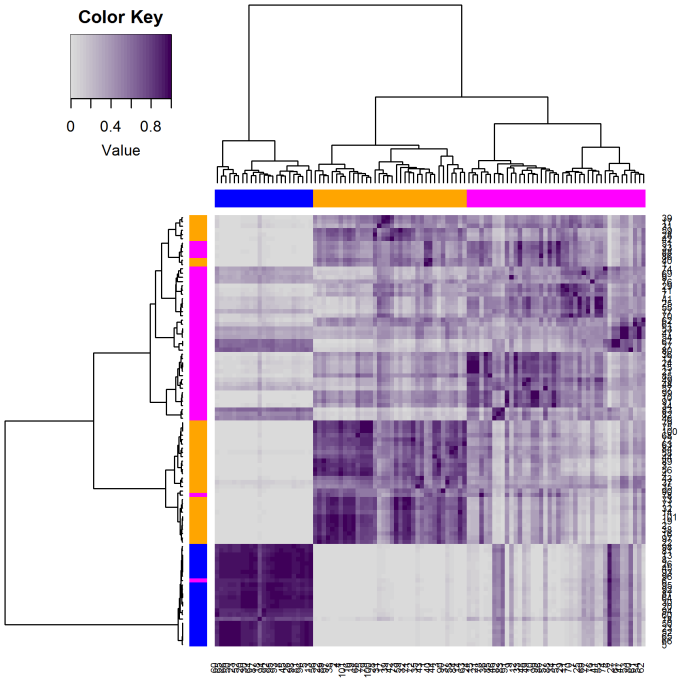


IGHV status	Blue	Magenta	Orange
Mutated	22	11	2
Unmutated	1	31	34

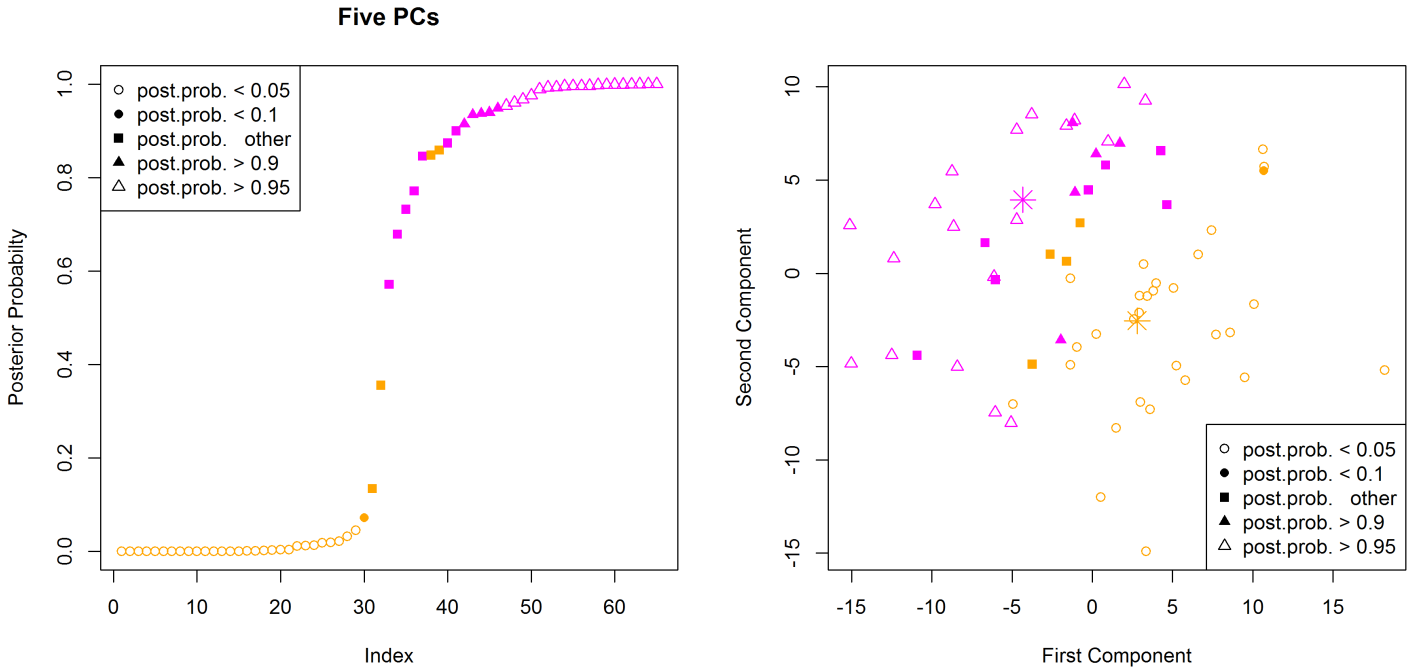
Bootstrap validated the consistency of gene selection and clusters in the MDACC cohort







Used LDA with 5 PCA predictors to identify unmutated samples that could be reliably separated into two groups

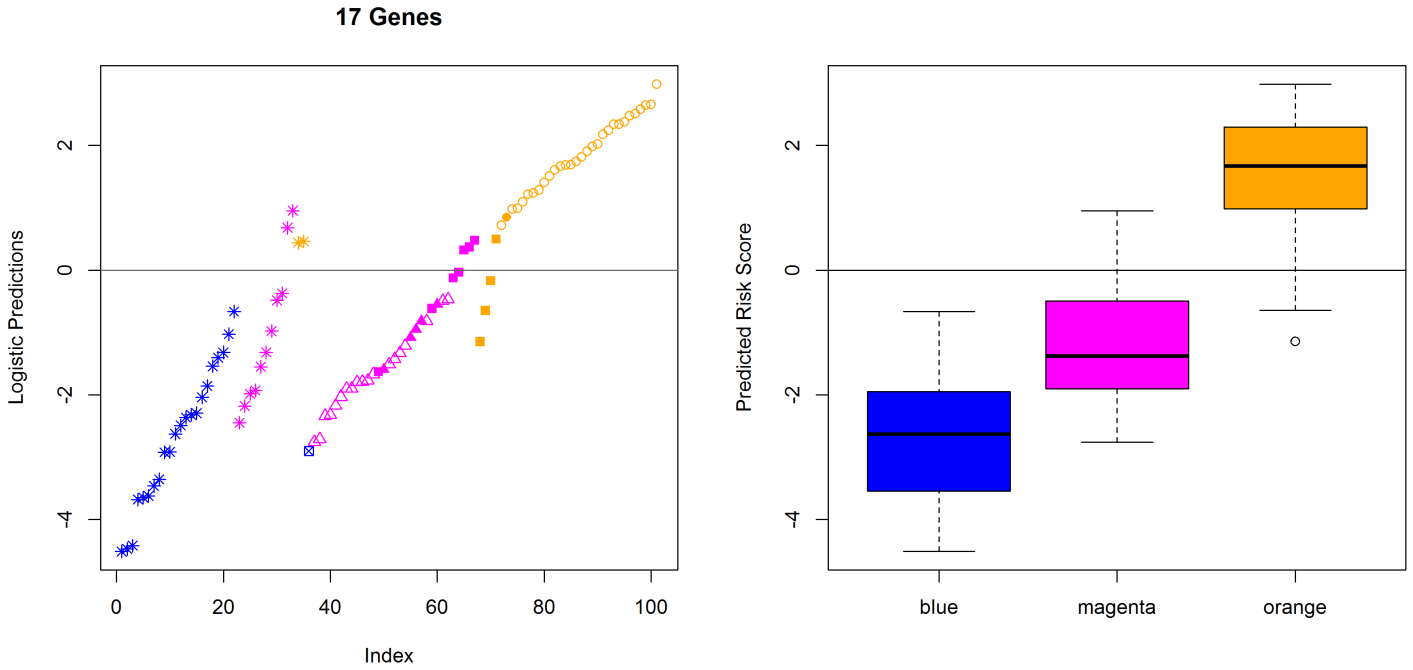


The 17-gene model (with coefficients) contrasts “purple” vs. “cyan” gene groups

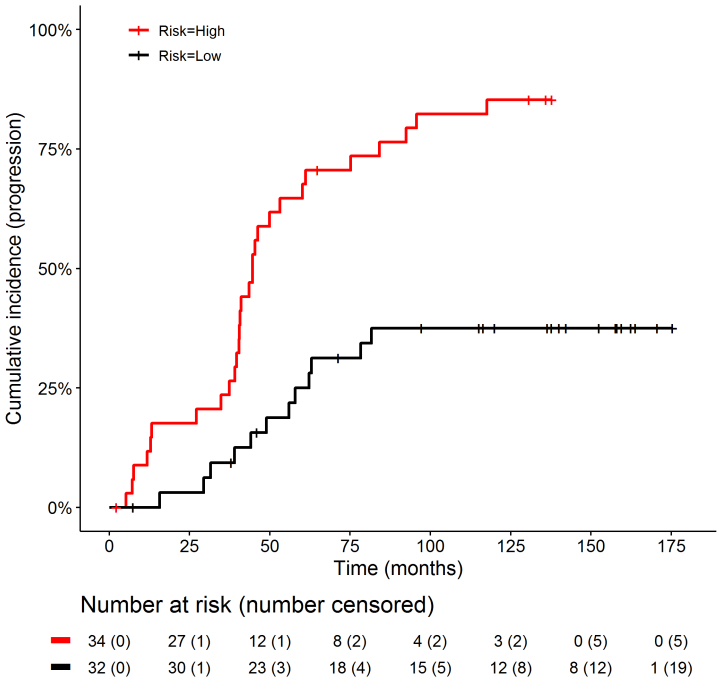
Rank	Symbol	Std. Coef.	Gene Name	Entrez Gene ID	Gene Cluster
1	OSBPL5	+0.633	Oxysterol binding protein like 5	114879	purple
2	MSI2	+0.234	Musashi RNA binding protein 2	124540	purple
3	KSR2	+0.219	Kinase suppressor of RAS2	283455	purple
4	NME1	+0.206	NME/NM23 nucleoside diphosphate kinase 1	4830	purple
5	SLC35A4	+0.199	Solute carrier family 35 member A4	113829	purple
6	TXN	+0.188	Thioredoxin	7295	purple
7	LAG3	+0.187	Lymphocyte activating 3	3902	red
8	ZNHIT1	+0.162	Zinc finger HIT-type containing 1	10467	purple

Rank	Symbol	Std. Coef.	Gene Name	Entrez Gene ID	Gene Cluster
9	PDE8A	-0.159	Phosphodiesterase 8A	5151	cyan
10	RGS10	+0.150	Regulator of G-protein signaling 10	6001	purple
11	TSPO	+0.145	Translocator protein	706	purple
12	CRLF3	-0.129	Cytokine receptor like factor 3	51379	cyan
13	DCAF12	+0.058	DDB1 and CUL4 associated factor 12	25853	purple
14	ADSL	+0.040	Adenylosuccinate lyase	158	purple
15	AQP1	-0.037	Aquaporin 1 (Colton blood group)	358	cyan
16	GRN	+0.025	Granulin	2896	purple
17	TTC38	+0.018	Tetratricopeptide repeat domain 38	55020	purple

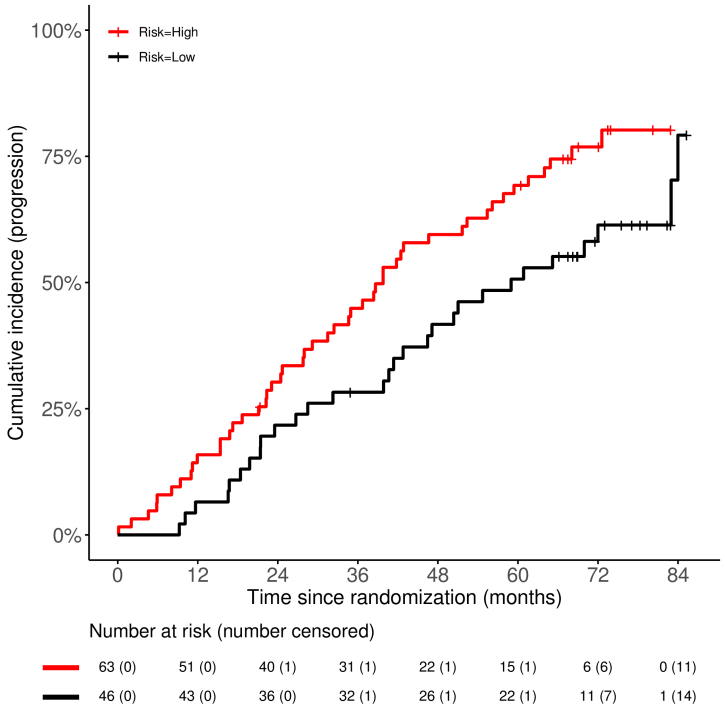
Performance of the 17-gene model on the MDACC cohort, including training samples



Validation of cumulative incidence of progression in the GCLLSGCLL8 cohort



MDACC



GCLLSG/CLL8

Gene Enrichment Analysis suggests that differences in metabolic activity distinguish patients with different outcomes

Purple gene cluster

- oxidoreductase activity (GO:0016491; FDR $q=1{\cdot}01 \times 10^{-8}$),
- glycosyl compound, nucleoside, and ribonucleoside metabolic processes (GO:1901657, GO:0009116, GO:0009119; all with FDR $q=1{\cdot}22 \times 10^{-11}$);
- mitochondrion (GO:0005739; FDR $q=9{\cdot}79 \times 10^{-16}$).
- metabolic pathways (KEGG:132956; FDR $q=3{\cdot}04 \times 10^{-13}$)
- oxidative phosphorylation (KEGG:82942; FDR $q=4{\cdot}31 \times 10^{-7}$).

Cyan gene cluster

- ATP binding (GO:0005524; FDR $q=2{\cdot}38 \times 10^{-3}$) and
- purine ribonucleoside triphosphate binding (GO:0035639; FDR $q=2{\cdot}38 \times 10^{-3}$).
- nucleic acid binding (GO:0003676; $p=6{\cdot}20 \times 10^{-4}$),
- DNA-templated transcription (GO:0006351; $p=5{\cdot}28 \times 10^{-6}$),
- the nucleus (GO:0005634; $p=2{\cdot}17 \times 10^{-8}$)
- zinc-finger transcription factors (UniProt keywords, $p=4{\cdot}78 \times 10^{-7}$; sequence features $p=1{\cdot}47 \times 10^{-5}$)

Lancet Oncology
Publication planned for 24 September 2019

- Time-to-progression after front-line fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy for chronic lymphocytic leukaemia: a retrospective, multicohort study
- Carmen D. Herling*, Kevin R. Coombes*, Axel Benner, Johannes Bloehdorn, Lynn L. Barron, Zachary B. Abrams, Tadeusz Majewski, Jolanta E. Bondaruk, Jasmin Bahlo, Kirsten Fischer, Michael Hallek, Stephan Stilgenbauer, Bogdan A. Czerniak, Christopher Oakes, Alessandra Ferrajoli, Michael J. Keating, Lynne V. Abruzzo

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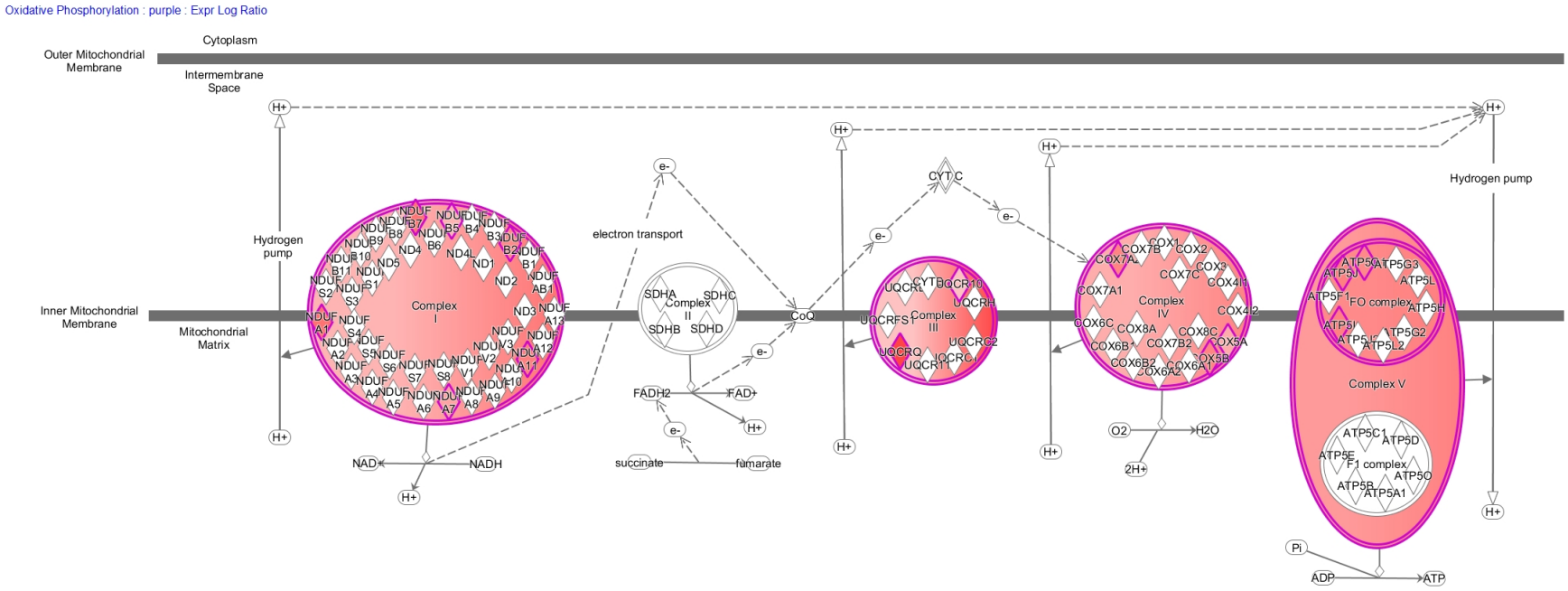

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