GC B−cell Positioning Pathway Mutations in DLBCL

Analysis of Chapuy + Duke/GAMBL Cohorts

Chapuy n=135 | Duke/GAMBL n=969

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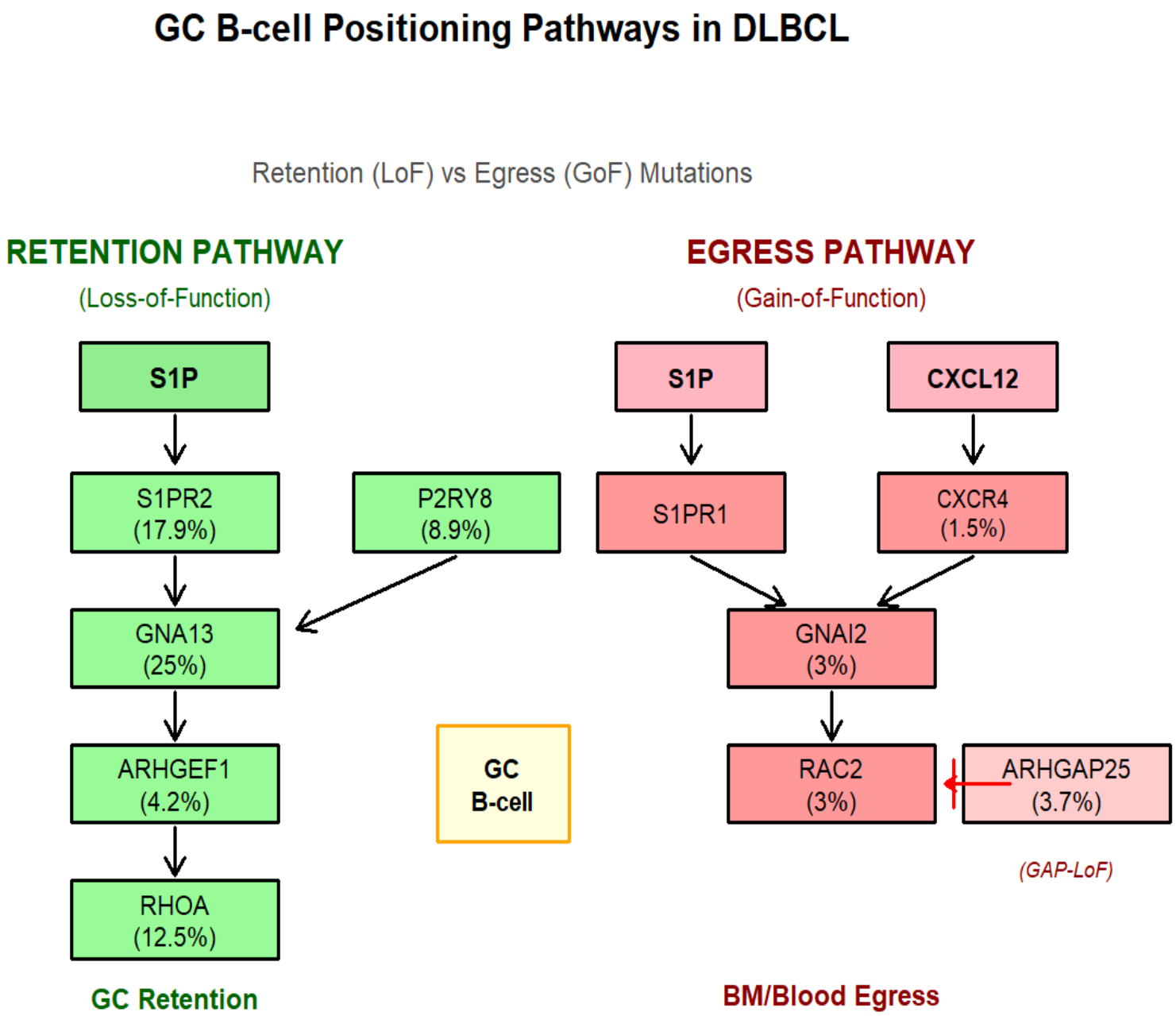
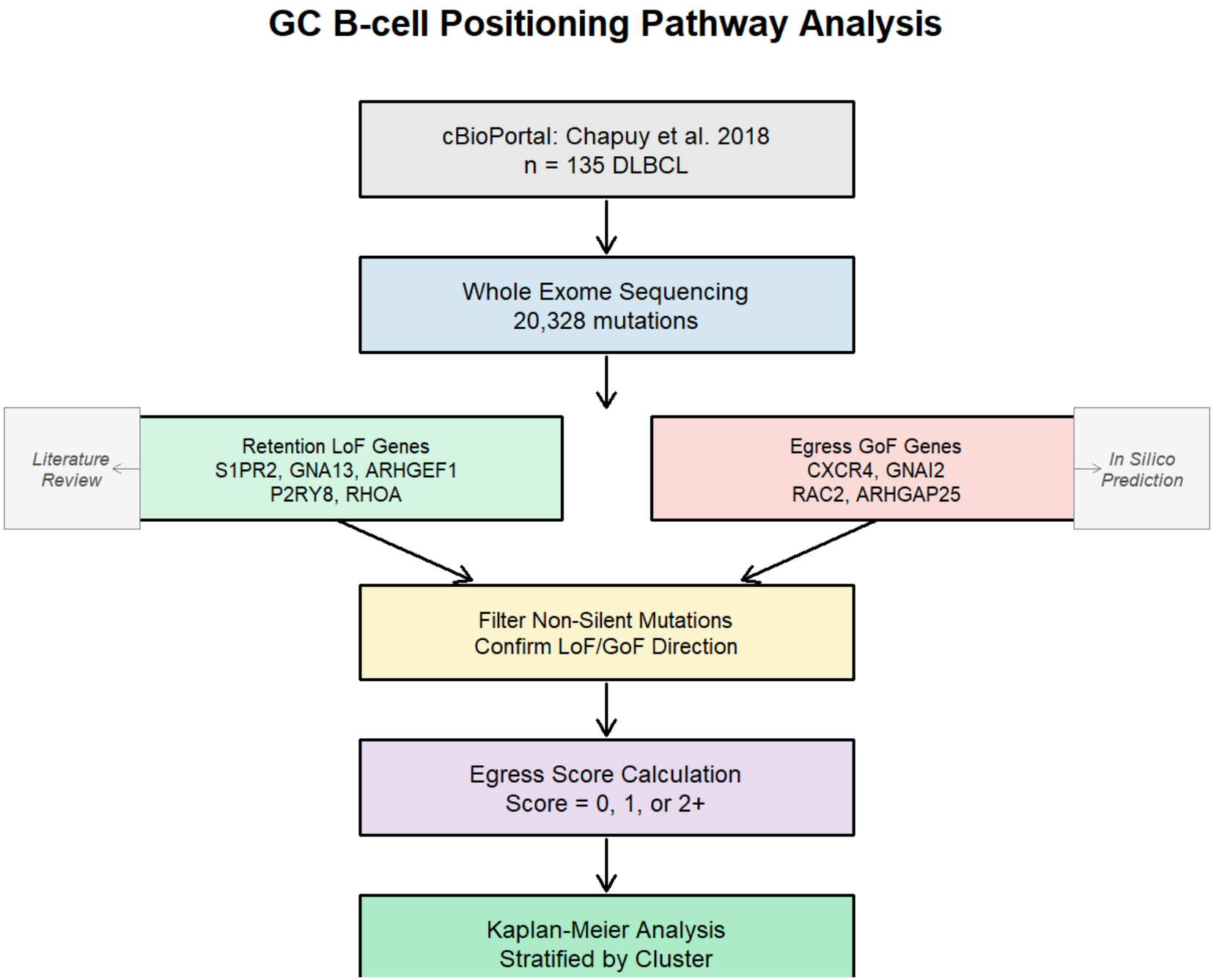
Analysis Date: 2026−01−17

Data Source: cBioPortal, GAMBL/bioRxiv

Background: S1P Signaling Pathways



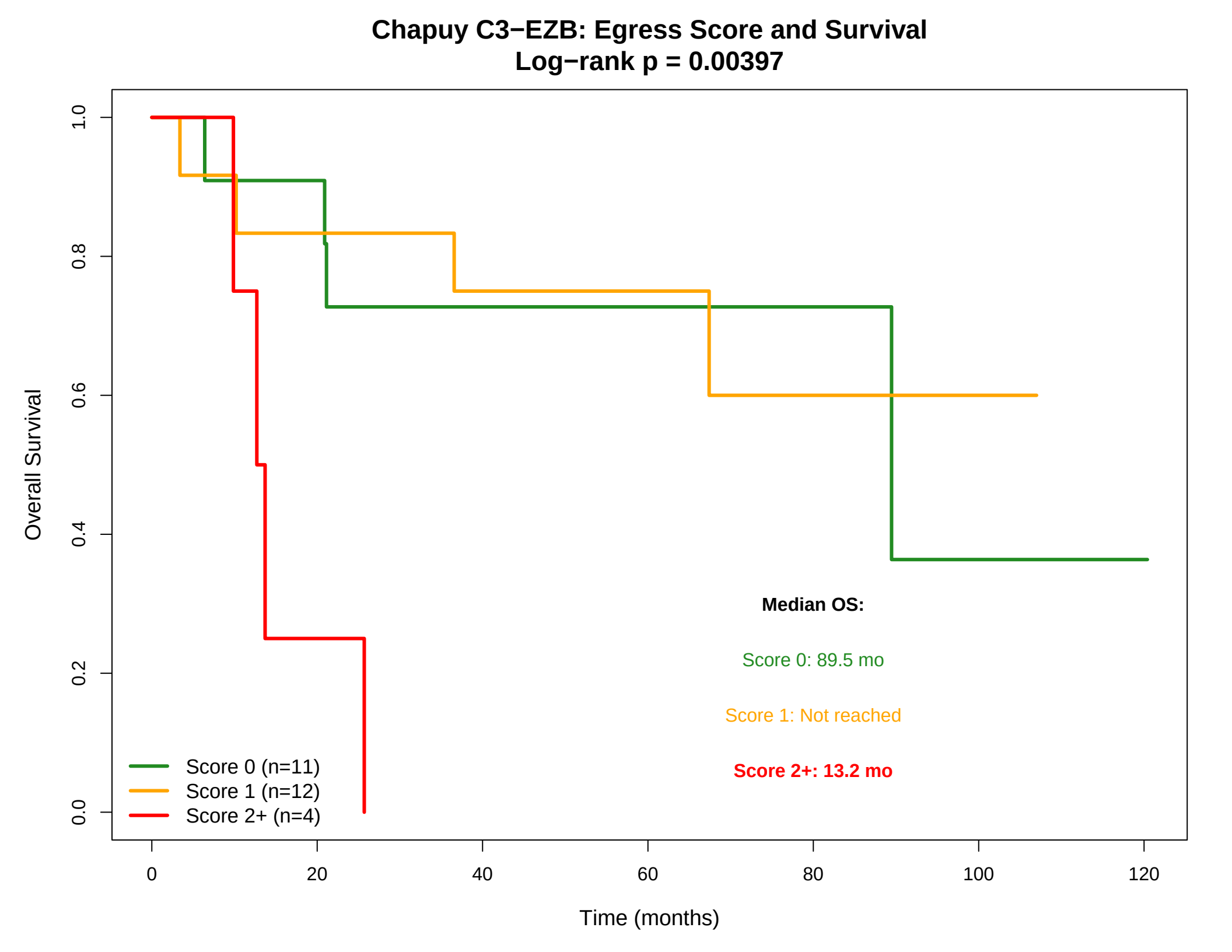
Methods: Analysis Pipeline



Chapuy Cohort: Mutations by Genetic Cluster

Cluster	N	Ret LoF	Egr GoF	Both	Any	Key Genes
C0−Unclass	4	0%	0%	0%	0%	−
C1−BN2	16	18.8%	18.8%	6.2%	31.2%	RAC2
C2−TP53	32	15.6%	0%	0%	15.6%	P2RY8
C3−EZB (GCB)	28	**50%**	**14.3%**	**3.6%**	**60.7%**	GNA13, S1PR2
C4−SGK1	24	29.2%	16.7%	4.2%	41.7%	RHOA, P2RY8
C5−MCD (ABC)	31	9.7%	6.5%	0%	16.1%	ARHGAP25
OVERALL	**135**	**23.7%**	**10.4%**	**3.7%**	**30.4%**	

C3−EZB (GCB) has highest pathway involvement: 60.7%


Chapuy C3−EZB: Egress Score and Survival
Log−rank p = 0.00397
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Time (months)
Score 0 (n=11)
Score 1 (n=12)
Score 2+ (n=4)
Median OS:
Score 0: 89.5 mo
Score 1: Not reached
Score 2+: 13.2 mo

Chapuy C3−EZB: Key Finding

Egress Score >= 2 in C3−EZB:

100% mortality (4/4 deaths)

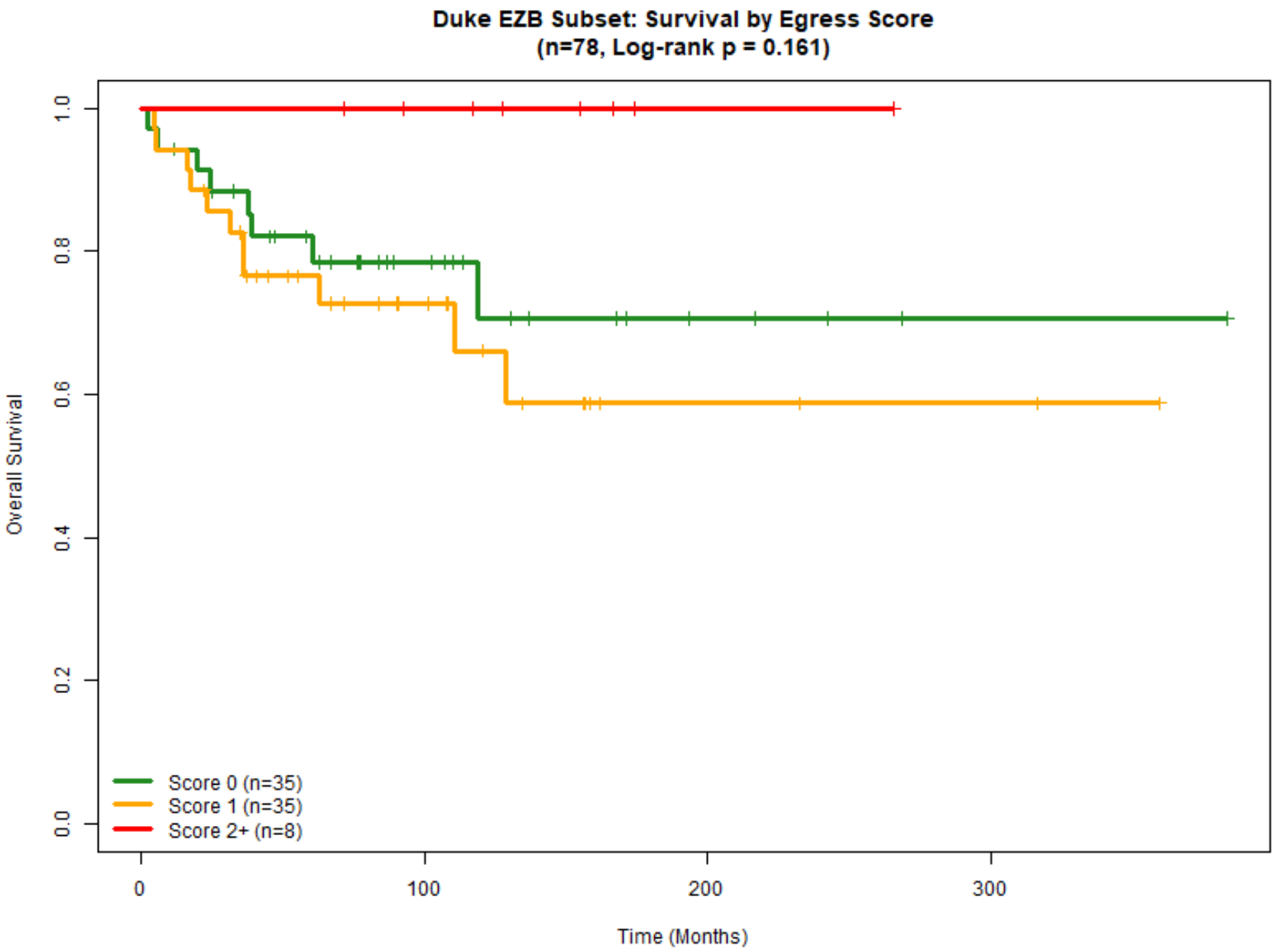
Median OS: 13.2 months

Egress Score 0−1 in C3−EZB:

33−36% mortality

Median OS: 89.5 mo (Score 0) / Not reached (Score 1)

Duke EZB−like (n=83): Opposite Direction (p = 0.16)



Duke EZB: Surprising Finding

Duke vs Chapuy EZB Comparison

Metric	Chapuy EZB	Duke EZB
Score 2+ Deaths	**4/4 (100%)**	**0/8 (0%)**
Score 0−1 Deaths	8/24 (33%)	19/70 (27%)

OPPOSITE DIRECTION in Duke EZB:

Score 2+ patients have BETTER survival (0% mortality)

Possible reasons:

1. Mutation−based classification may not match true EZB
2. Duke missing 4/9 pathway genes (P2RY8, ARHGEF1, RAC2, ARHGAP25)

GAMBL Reanalysis of Duke Data

Dreval et al. 'Revisiting Reddy: A DLBCL Do−over' (bioRxiv 2023)

GAMBL: Improved variant calling pipeline

Reanalyzed Reddy/Duke DLBCL data (n=969 samples)

Found thousands of missed mutations in original analysis

Pathway Gene Frequencies: GAMBL vs Original Duke

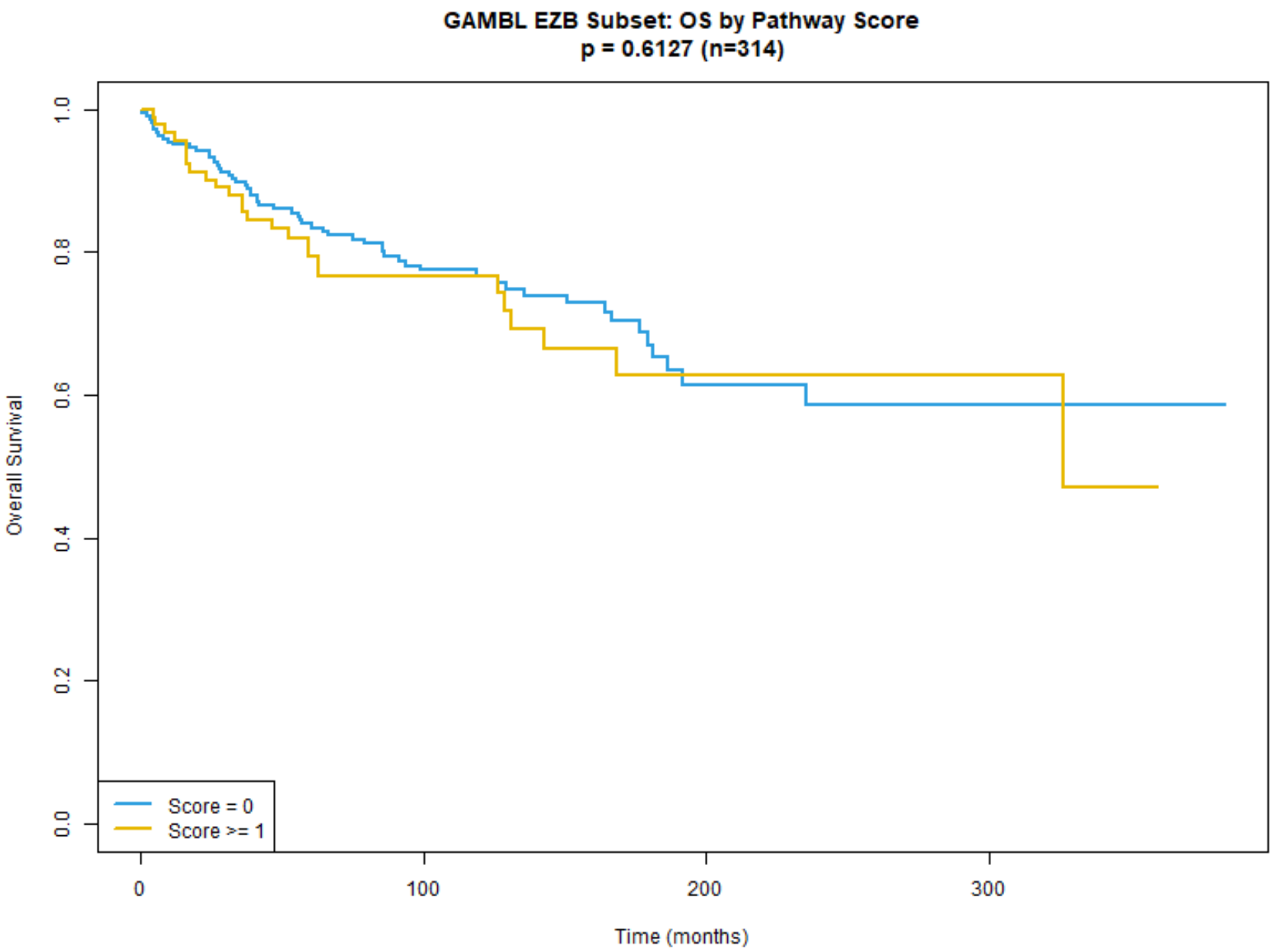
Gene	GAMBL	Duke	Difference
GNA13	8.6%	8.3%	+0.3%
RHOA	3.1%	3.0%	+0.1%
GNAI2	2.3%	2.2%	+0.1%
S1PR2	2.0%	1.9%	+0.1%
CXCR4	1.1%	1.4%	−0.3%

KEY FINDING: GAMBL didn't substantially change pathway frequencies

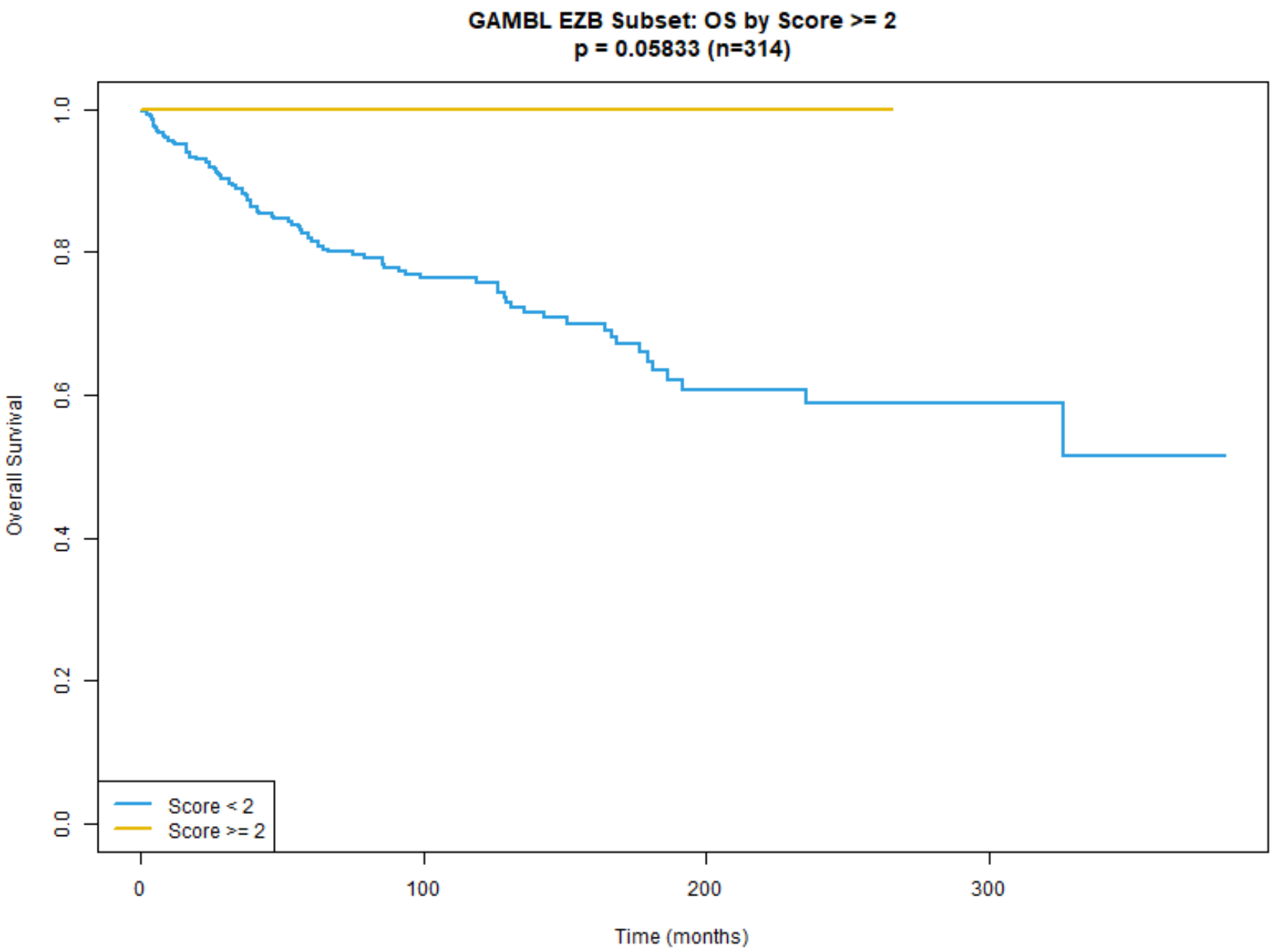
Missing genes (P2RY8, ARHGEF1, RAC2, ARHGAP25) still missing

These genes weren't in the original Reddy sequencing panel

GAMBL EZB Subset (n=314): Score >= 1 vs 0 (p = 0.61)



GAMBL EZB: Score >= 2 vs < 2 (p = 0.058) − APPROACHING SIGNIFICANCE



GAMBL EZB: Surprising Result

Score >= 2 in GAMBL EZB (n=11):

0 deaths (0%) − BETTER survival!

p = 0.058 (approaching significance)

Score < 2: 84/303 deaths (28%)

OPPOSITE DIRECTION vs Chapuy EZB!

Chapuy EZB Score >= 2: 100% mortality, 13.2 mo median OS

GAMBL EZB Score >= 2: 0% mortality, median OS not reached

Gene coverage issue explains discrepancy (4 genes missing)

INTERPRETATION:

GAMBL Score mainly reflects GNA13/RHOA (retention genes)

Missing egress genes (P2RY8, RAC2, ARHGAP25) may explain reversed effect

Cross−Cohort Comparison: EZB Subsets

Cohort	N	% Mut	Direction	P−value	Genes
Chapuy C3−EZB	28	60.7%	**WORSE**	**0.004**	9/9
Duke EZB	83	55.1%	**BETTER**	0.16	5/9
GAMBL EZB	314	29.6%	**BETTER**	0.058	5/9

GENE COVERAGE IS CRITICAL

Cohorts with 5/9 genes show OPPOSITE DIRECTION from Chapuy (9/9)

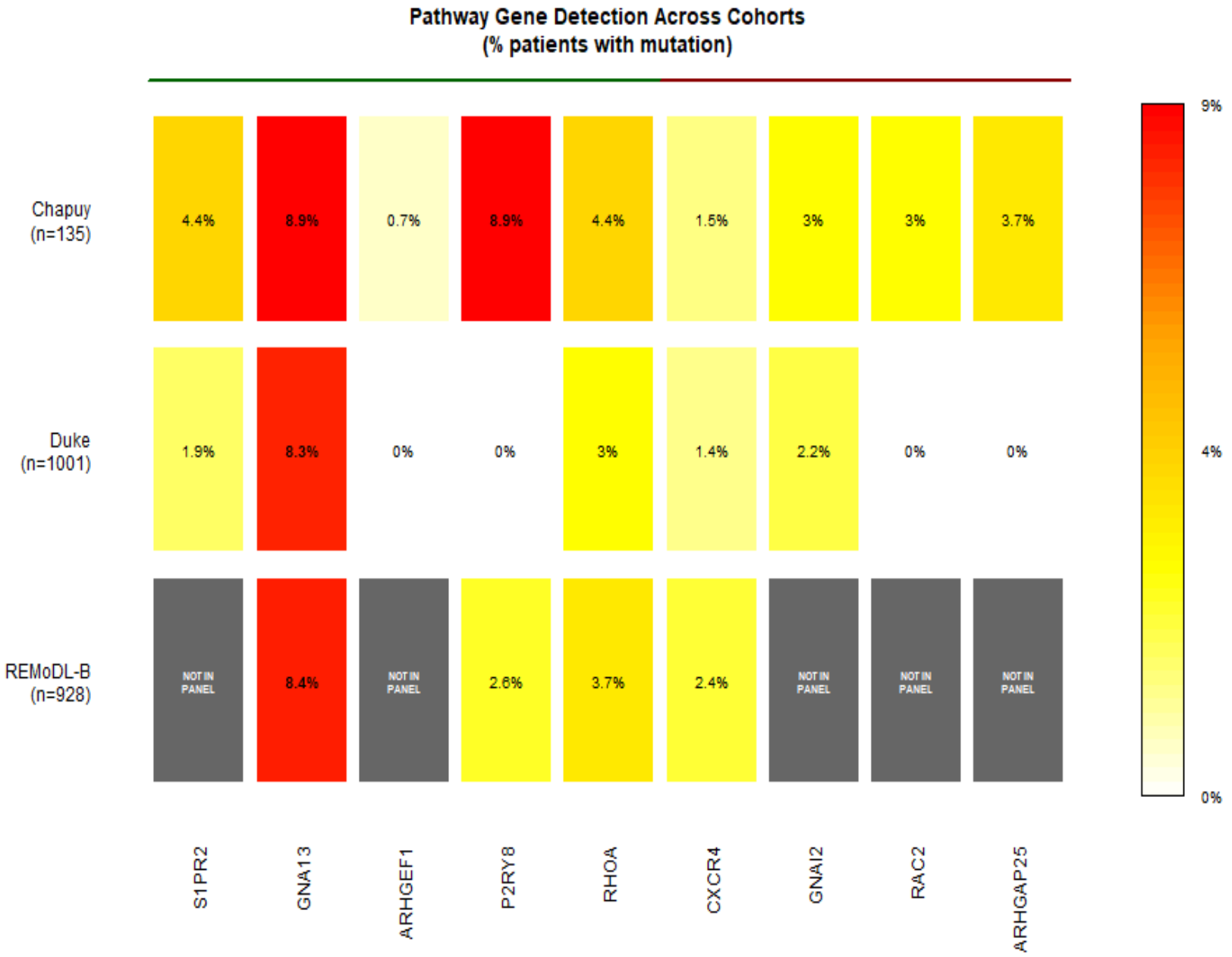
KEY INSIGHT:

Full pathway (9 genes) = WORSE survival (Chapuy)

Partial pathway (5 genes) = BETTER survival (Duke/GAMBL)

Missing egress genes (P2RY8, RAC2, ARHGAP25) likely drive poor prognosis

Pathway Gene Detection: Critical Gaps Across Cohorts



Gene Coverage: Key Differences Explain Discrepancies

Gene	Chapuy	Duke/GAMBL	Notes
RETENTION LoF			
S1PR2	4.4%	2.0%	Similar
GNA13	8.9%	8.6%	Similar
ARHGEF1	0.7%	–	GAMBL 0!
P2RY8	8.9%	–	GAMBL 0!
RHOA	4.4%	3.1%	Similar
EGRESS GoF			
CXCR4	1.5%	1.1%	Similar
GNAI2	3.0%	2.3%	Similar
RAC2	3.0%	–	GAMBL 0!
ARHGAP25	3.7%	–	GAMBL 0!

DUKE/GAMBL: 4 genes missing from Reddy sequencing panel

P2RY8, ARHGEF1, RAC2, ARHGAP25 − can't be reanalyzed

Conclusions & Next Steps

Key Findings:

1. Chapuy C3−EZB: Score >= 2 predicts poor survival (p = 0.004)

   All 9 pathway genes available in WES data

2. Duke/GAMBL EZB: Score >= 2 shows BETTER survival (p = 0.058)

   Only 5/9 genes in Reddy panel − cannot detect full pathway

CRITICAL: Full gene panel (9/9) required to see effect

Missing egress genes (P2RY8, RAC2, ARHGAP25) appear essential

Partial pathway (5/9 genes) shows OPPOSITE direction

Next Steps:

− Schmitz/NCI cohort (dbGaP pending) − has all 9 genes + large sample

− Need cohorts with complete pathway coverage

− Focus on finding Score 2+ patients with full gene panel

GAMBL confirms: Duke variant calling was reasonable − issue is gene panel coverage

Analysis updated: 2026−01−17