Biomarkers for JC Virus Infection in Multiple Sclerosis

PI: DR. MICHAEL GUREVICH
NEUROGENOMICS LABORATORY
MULTIPLE SCLEROSIS CENTER

Jaron Tepper
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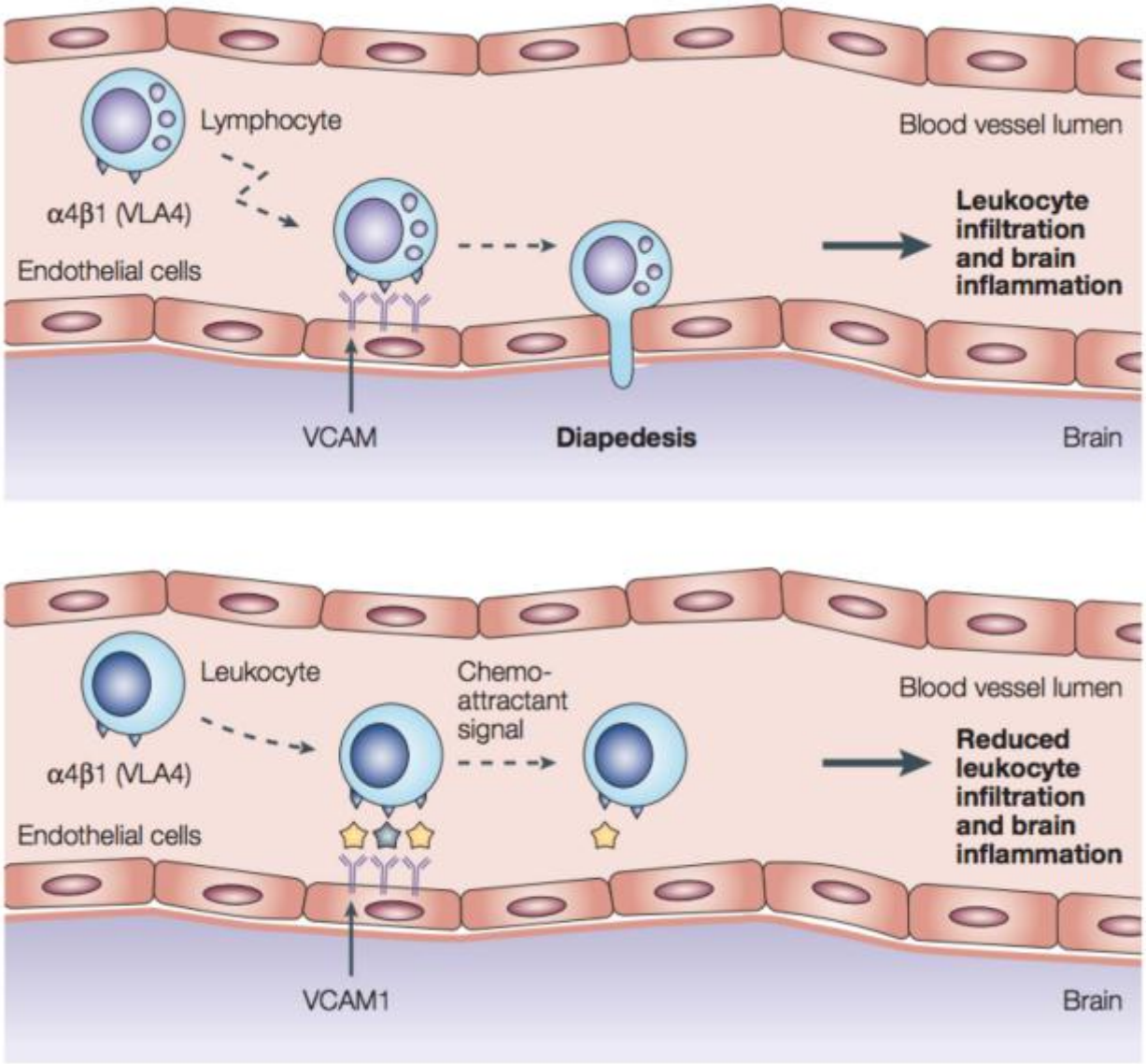
Common Treatment of Relapsing and Remitting MS

Treatment of Acute Relapses	Disease Modifying Therapies
• Corticosteroids	• Interferon Beta • Glatiramer acetate (Copaxone) • **Natalizumab (Tysabri)**

Natalizumab (Tysabri)

Humanized monoclonal antibody against α4β1 integrin on the surface of lymphocytes.

Interferes with lymphocyte migration across the blood brain barrier.

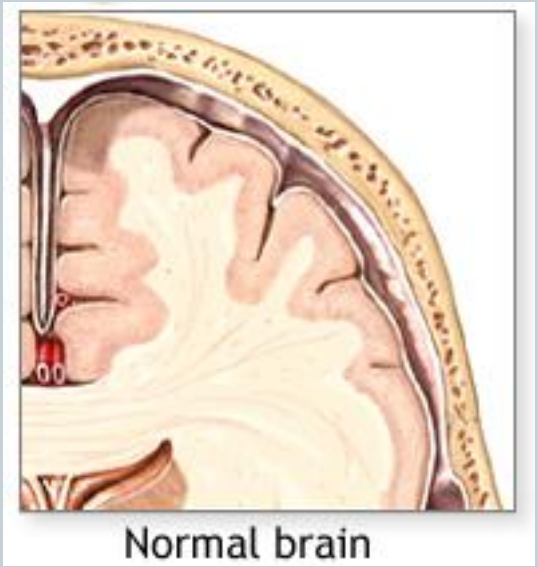


Tysabri (natalizumab)

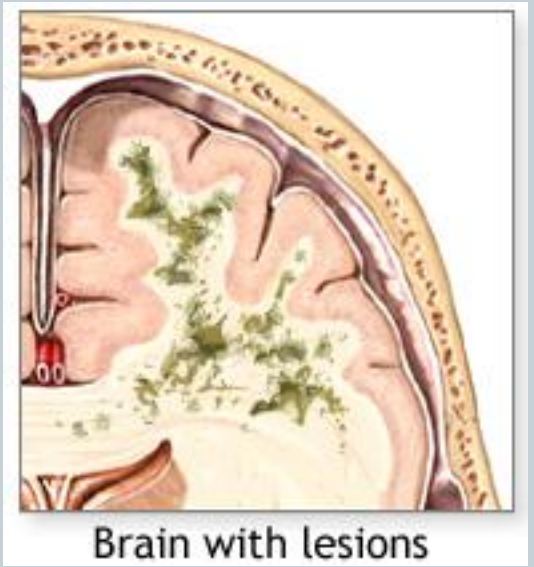
- 2003 clinical trials: two thirds decrease in risk of relapse rate over two year period vs. placebo
- 2005 – Three cases of Progressive Multifocal Leukoencephalopathy (PML) are associated with Tysabri, drug is withdrawn from market
- 2006 – Reintroduced to market controlling for risk of developing PML

Progressive Multifocal Leukoencephalopathy (PML)

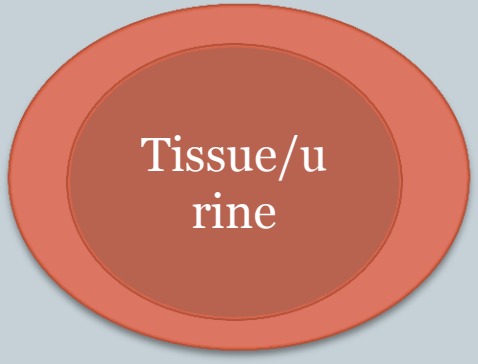
1. JC Virus
2. Immunocompromised state


Normal brain

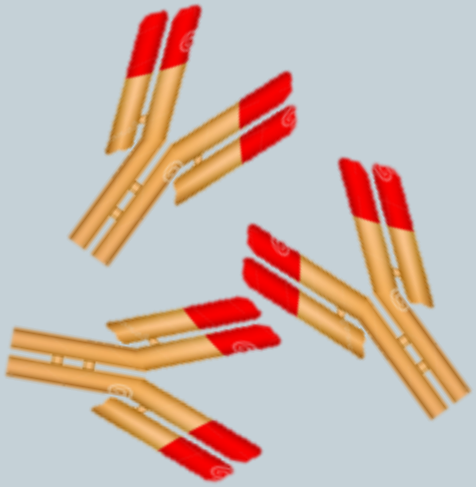
Pathogenesis of PML


Brain with lesions

JC Virus Testing Methods



PCR



ELISA

Project Proposal

- The discovery of novel biomarkers of JCV may provide alternate means of testing for infection:

JCV infection → altered gene expression →→→ anti-JCV Ab detectable

We propose the investigation of mRNA based biomarkers to detect early stages of JC virus proliferation

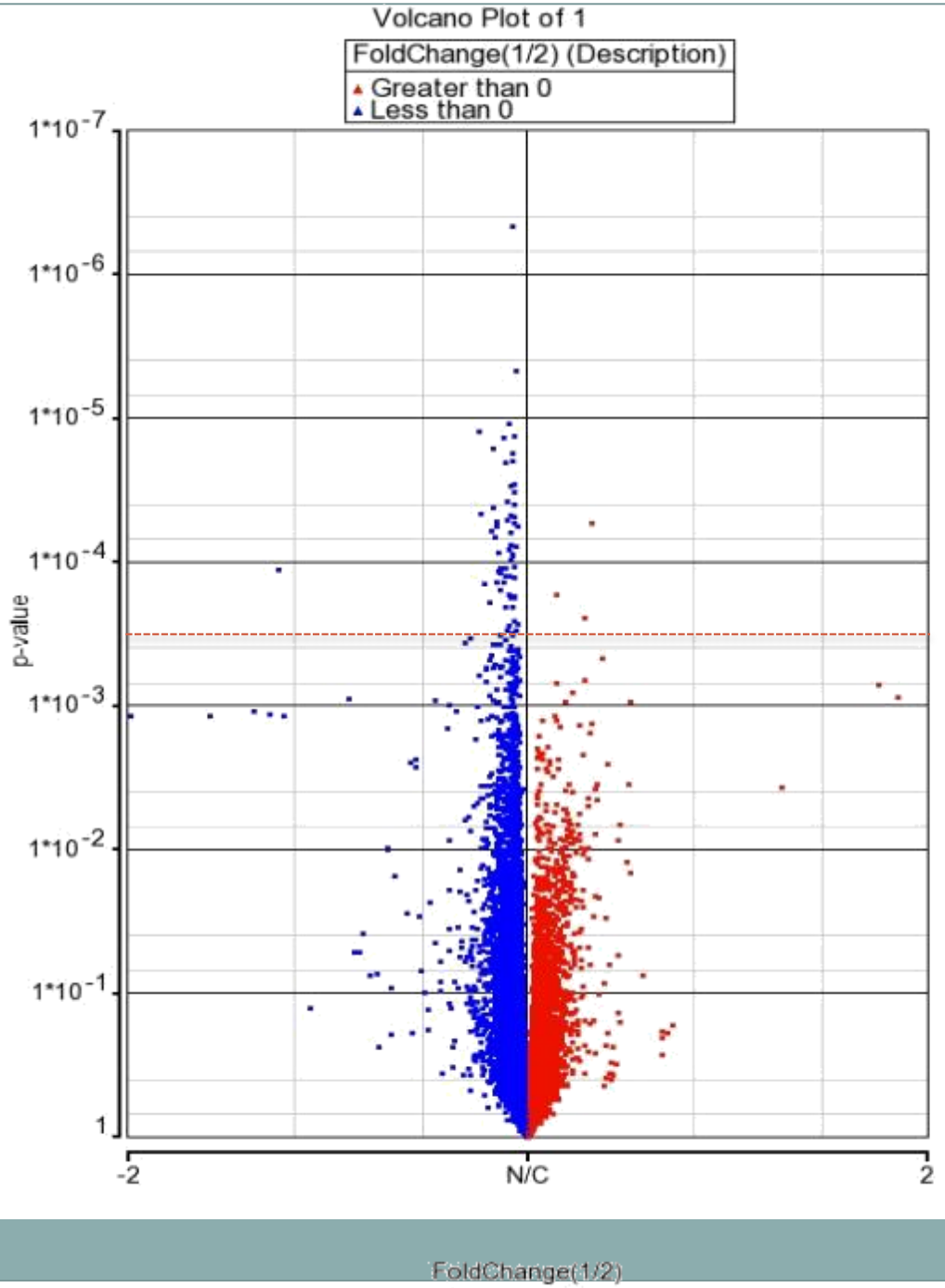
Methods

- Inclusion criteria:
  - RRMS (McDonald criteria) patients
  - Only confirmed JC virus positive or negative patients by ELISA
  - No previous treatment with natalizumab
- Gene expression comparison between JC virus positive and negative groups was performed

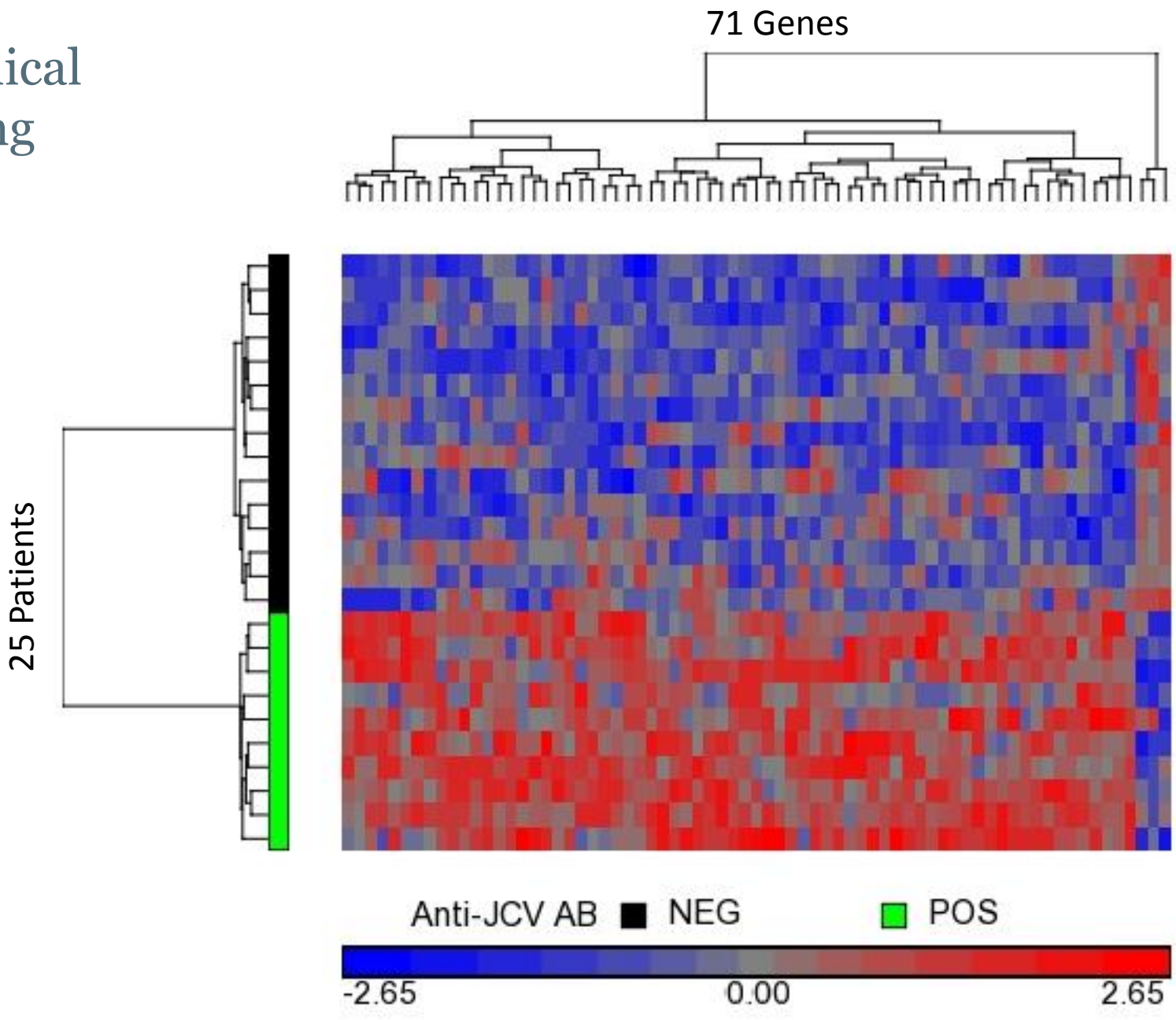
Table 1: clinical and demographic parameters of patients

Patient characteristics	n=25
Age (years)	47.4 ± 9.7
Women, n	21 (84%)
JCV positive	10 (40%)
EDSS	4.4 ± 2.1
Disease duration (years)	12.3 ± 8.1

- 71 genes were found to be statistically significant between the two groups after correction for multiple comparison using a FDR cutoff of 0.1

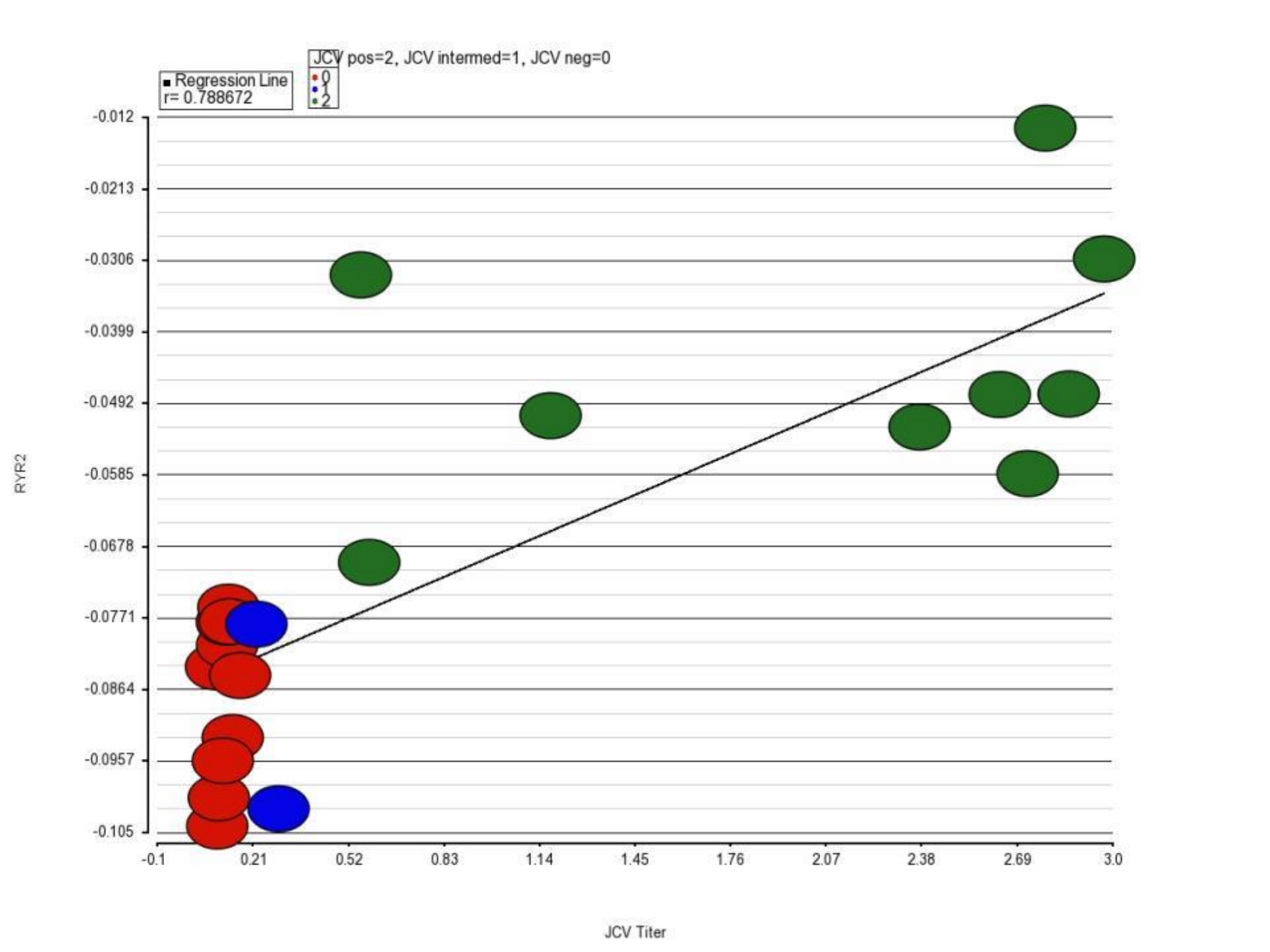


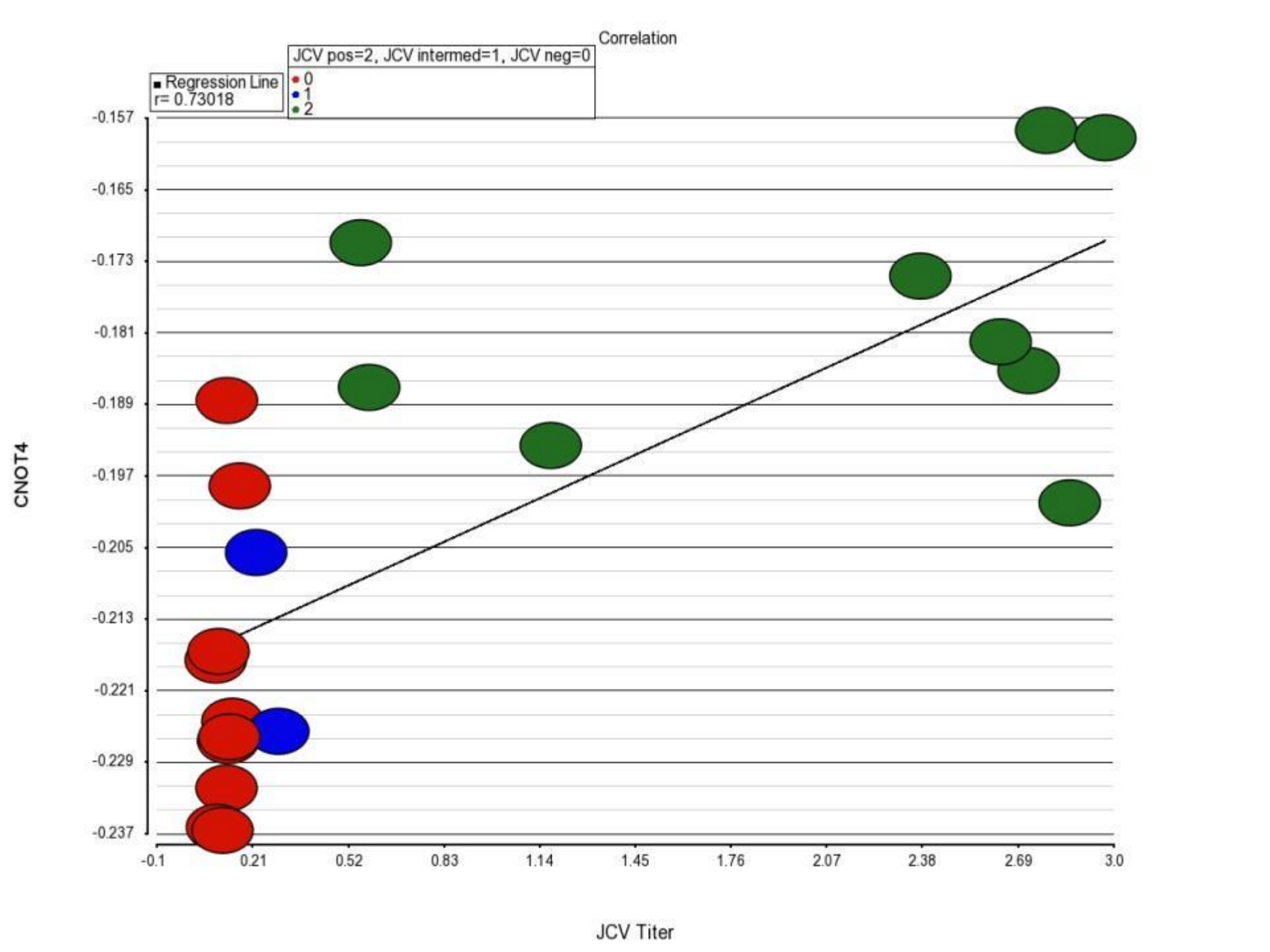
Hierarchical Clustering



Correlation analysis

- The correlation analysis adds a second layer of proof
- Additionally, without a correlation any test can only be binary i.e. positive or negative. Correlation allows prediction of titer level.
- Using the 71 genes, a correlation was performed (using 21 positive and negative patients with titer available)
- Result: 64/71 genes significantly (FDR<0.05) correlated with titer


JCV pos=2, JCV intermed=1, JCV neg=0
0
1
2
Regression Line
r= 0.788672
-0.012
-0.0213
-0.0306
-0.0399
-0.0492
-0.0585
-0.0678
-0.0771
-0.0864
-0.0957
-0.105
RYR2
-0.1
0.21
0.52
0.83
1.14
1.45
1.76
2.07
2.38
2.69
3.0
JCV Titer


Correlation
JCV pos=2, JCV intermed=1, JCV neg=0
0
1
2
Regression Line
r= 0.73018
CNOT4
-0.157
-0.165
-0.173
-0.181
-0.189
-0.197
-0.205
-0.213
-0.221
-0.229
-0.237
-0.1
0.21
0.52
0.83
1.14
1.45
1.76
2.07
2.38
2.69
3.0
JCV Titer

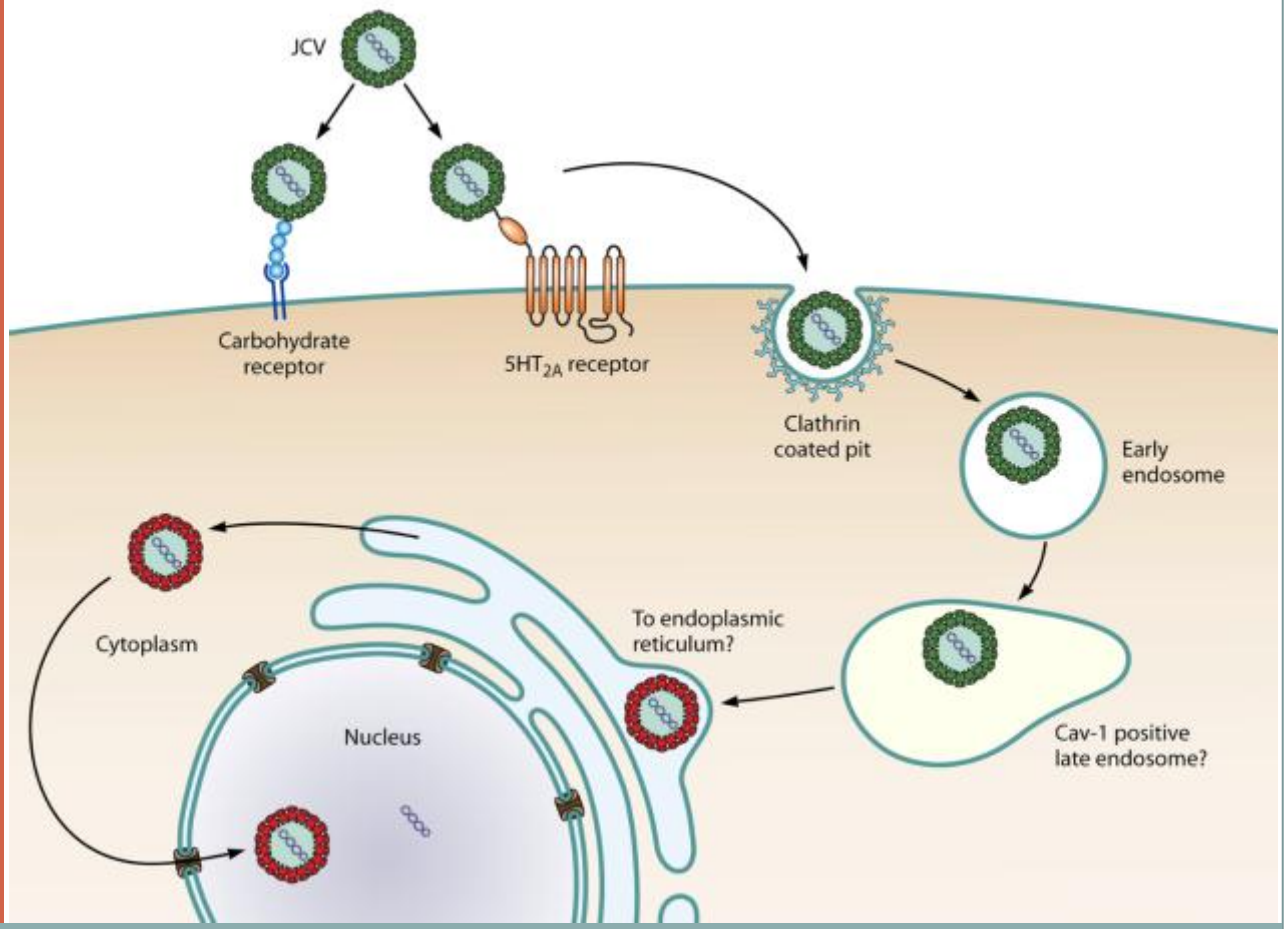
Choosing Promising Biomarkers

- Rationale
- Chosen using a scoring system of 5 categories:
  - Biological relevance via literature search
  - Genes with lowest *p*-value
  - Genes with the strongest correlation to JCV titer
  - Genes with greatest fold change
  - Genes affected by natalizumab

Gene Symbol	JCV pos vs. neg: p-value	JCV pos vs. neg: bonferroni(p-value)	JCV pos vs. neg: stepup(p-value)	JCV pos vs. neg: Bootstrap	JCV neg vs. pos FoldChange(1/2)	JCV Titer Correlation: r	JCV Titer Correlation: p-value(correlation)	JCV Titer Correlation: bonferroni(p-value(correlation))	JCV Titer Correlation: stepup(p-value(correlation))	Biological relevance (from literature)	5 genes with lowest p value	5 genes with strongest correlation	5 genes with better F/C	Changed by Nat	Score
MAP4K5	1.24E-05	2.62E-01	4.37E-02	1.90E-01	-1.09E+00	6.62E-01	1.07E-03	1.00E+00	2.48E-01		1		1	1	3
ARHGEF26	4.72E-06	9.99E-02	4.37E-02	8.50E-02	-1.02E+00	7.71E-01	4.23E-05	9.39E-01	1.31E-01	1	1	1			3
DERL1	4.70E-05	9.94E-01	4.59E-02	4.00E-01	-1.08E+00	5.70E-01	6.97E-03	1.00E+00	4.18E-01	1				1	2
KIAA0226	1.63E-05	3.45E-01	4.37E-02	2.15E-01	-1.06E+00	6.62E-01	1.07E-03	1.00E+00	2.48E-01	1				1	2
LGALS8	5.27E-05	1.00E+00	4.59E-02	4.10E-01	-1.05E+00	5.42E-01	1.12E-02	1.00E+00	4.84E-01	1				1	2
EPHB1	1.10E-05	2.33E-01	4.37E-02	1.65E-01	-1.03E+00	7.45E-01	1.05E-04	1.00E+00	1.31E-01		1	1			2
RYR2	7.69E-05	1.00E+00	5.29E-02	4.80E-01	-1.03E+00	8.05E-01	1.07E-05	2.37E-01	1.19E-01	1		1			2
CNOT4	4.68E-07	9.90E-03	9.90E-03	1.00E-02	-1.03E+00	7.66E-01	5.12E-05	1.00E+00	1.31E-01		1	1			2
EIF1AX	2.49E-04	1.00E+00	8.78E-02	7.25E-01	1.10E+00	-6.26E-01	2.40E-03	1.00E+00	3.22E-01				1	1	2
HLA-DRB4	1.13E-04	1.00E+00	5.98E-02	5.50E-01	-1.54E+00	5.64E-01	7.79E-03	1.00E+00	4.40E-01				1		1
UTY	3.40E-04	1.00E+00	1.01E-01	7.85E-01	-1.10E+00	5.56E-01	8.89E-03	1.00E+00	4.58E-01				1		1
CCZ1 /// CCZ1B	6.05E-05	1.00E+00	4.74E-02	4.45E-01	-1.06E+00	6.44E-01	1.62E-03	1.00E+00	2.67E-01	1					1
YIF1A	1.40E-04	1.00E+00	6.17E-02	6.20E-01	-1.05E+00	5.48E-01	1.01E-02	1.00E+00	4.77E-01	1					1

Identified Genes and JCV Replication

- Derlin-1
- KIAA0226
- CCZ1
- LGALS8
- YIF1A



Identified Genes related to cell adhesion

- LGALS8
- EPHB1
- ARHGEF26
- ITGB3

Natalizumab and JCV Seroconversion

- Other work in the lab found a list of >700 genes affected by natalizumab
- Some of these genes (derlin-1, LGAL8, were also identified in this analysis)
- This may point to a mechanism by which natalizumab stimulates the virus

Future plan

- Verification of biomarkers:
  - PCR
  - Another data set

THANK YOU!

QUESTIONS?