

Proteome analysis of cerebrospinal fluid: Cross sectional and longitudinal analysis of HIV infected patients

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58th ASMS Conference

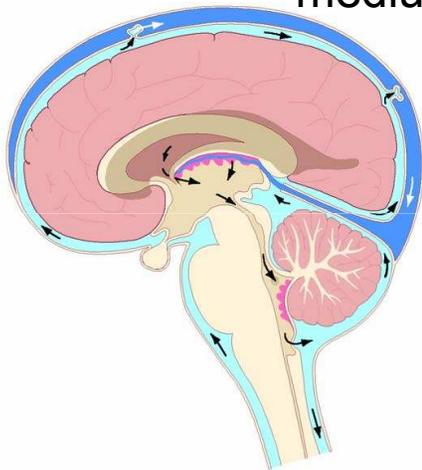
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Cerebrospinal Fluid

A window into the central nervous system

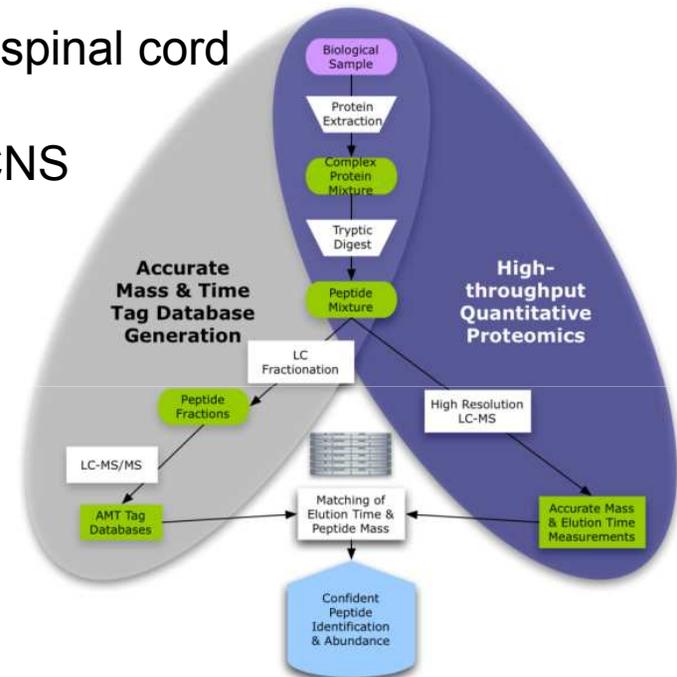
- **Goal: Measure changes in the cerebrospinal fluid proteome resulting from highly active antiretroviral therapy (HAART) in patient with HIV-associated dementia (HAD)**

- Cerebrospinal fluid baths the brain and spinal cord
 - providing mechanical protection
 - medium for communication within the CNS



- produced at rate of 500 mL/day
- Average volume ~150 mL
- Turns over ~ 3 times a day
- CSF protein 0.2-0.5 mg/mL

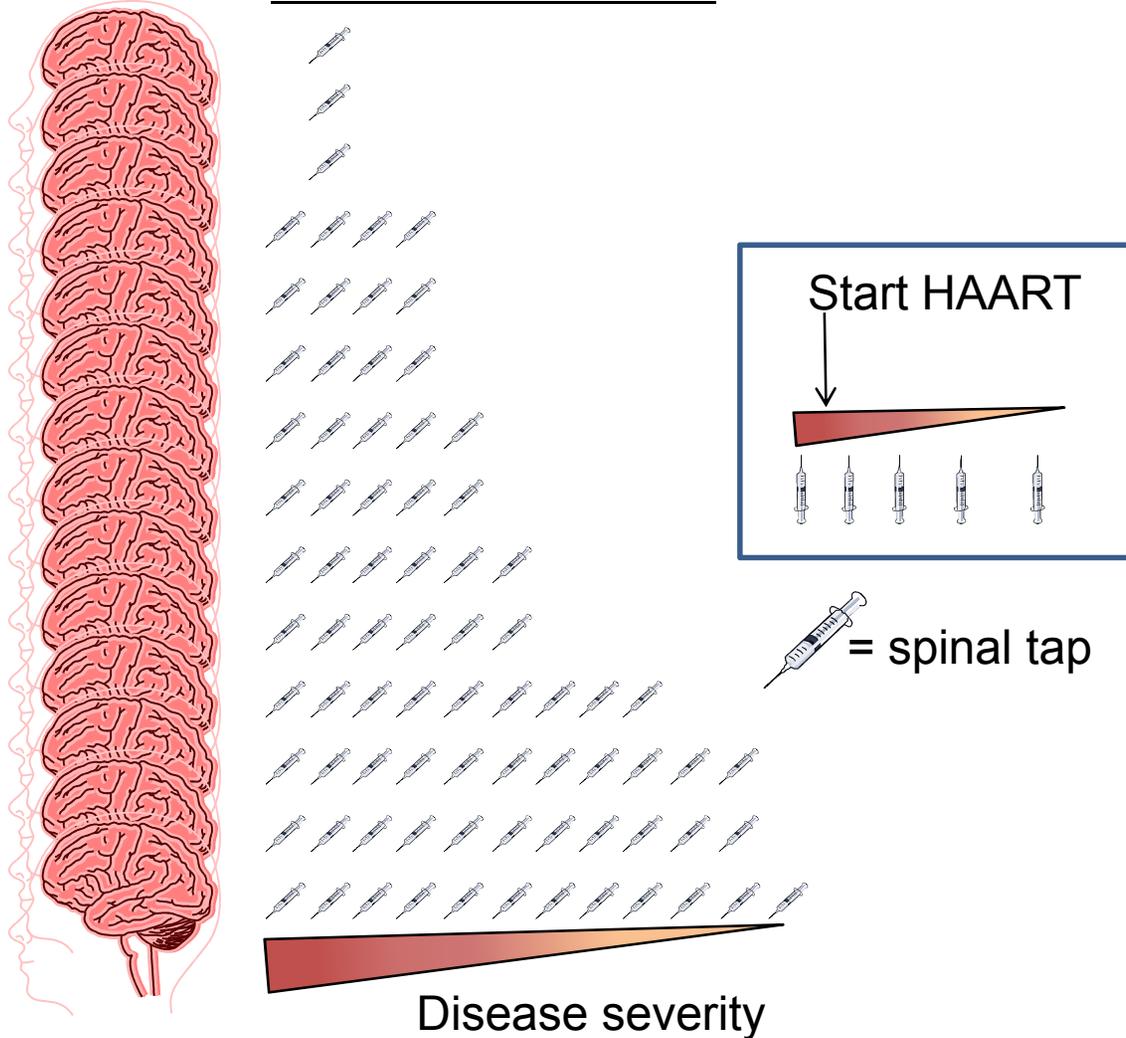
http://www.control.tfe.umu.se/lan/CSF/CSF_diagram.jpg



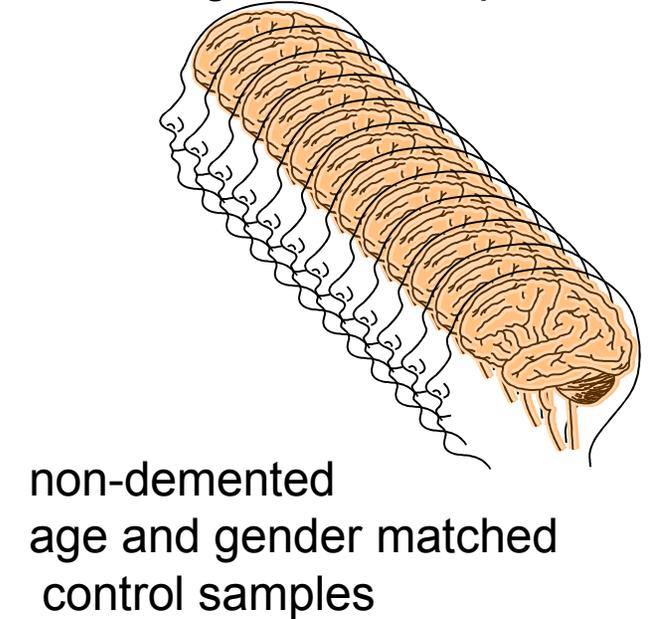
We are characterizing the CSF proteome in HIV-infected patients that present clinical manifestations of HAD employing established label free mass spectrometry based analytical techniques.

Characterization of CSF proteome in serial samples to detect changes resulting from HAART

CSF samples from 14 HIV infected individuals



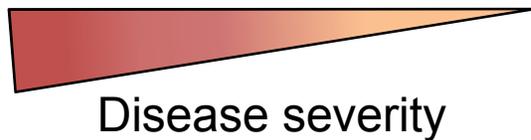
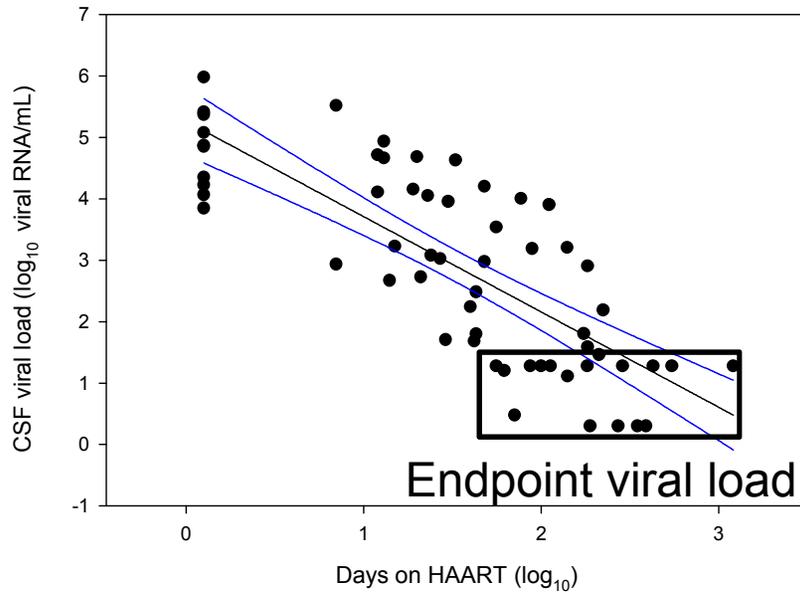
12 HIV negative control samples Single CSF samples



Serial CSF sample sets coupled with age and gender matched HIV negative controls allow for interrogation of CSF to determine HAART treatment effects.

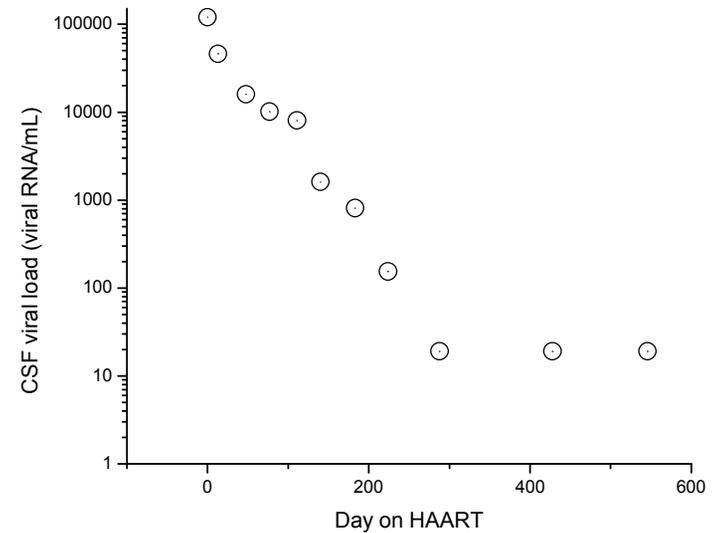
CSF HIV viral load reflecting patient response to HAART

CSF viral load decreases over course of HAART

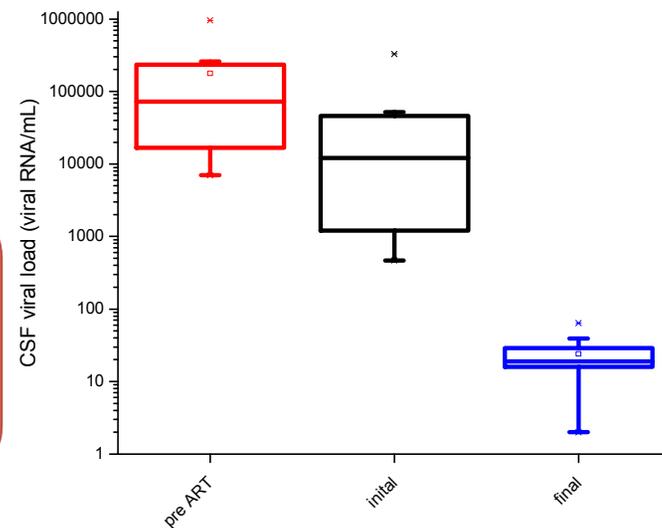


Patients show positive response to HAART

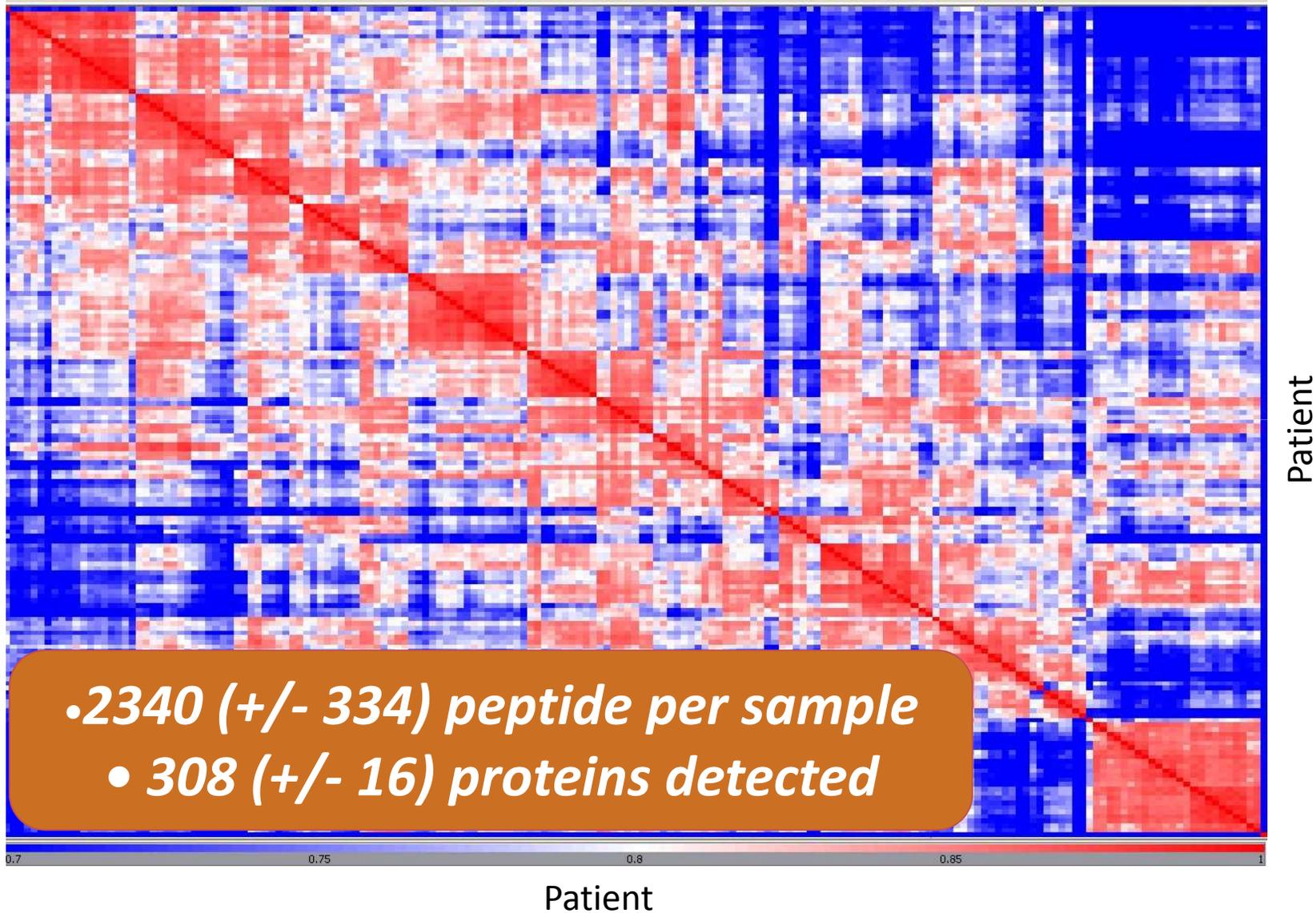
Example of patient response to HAART



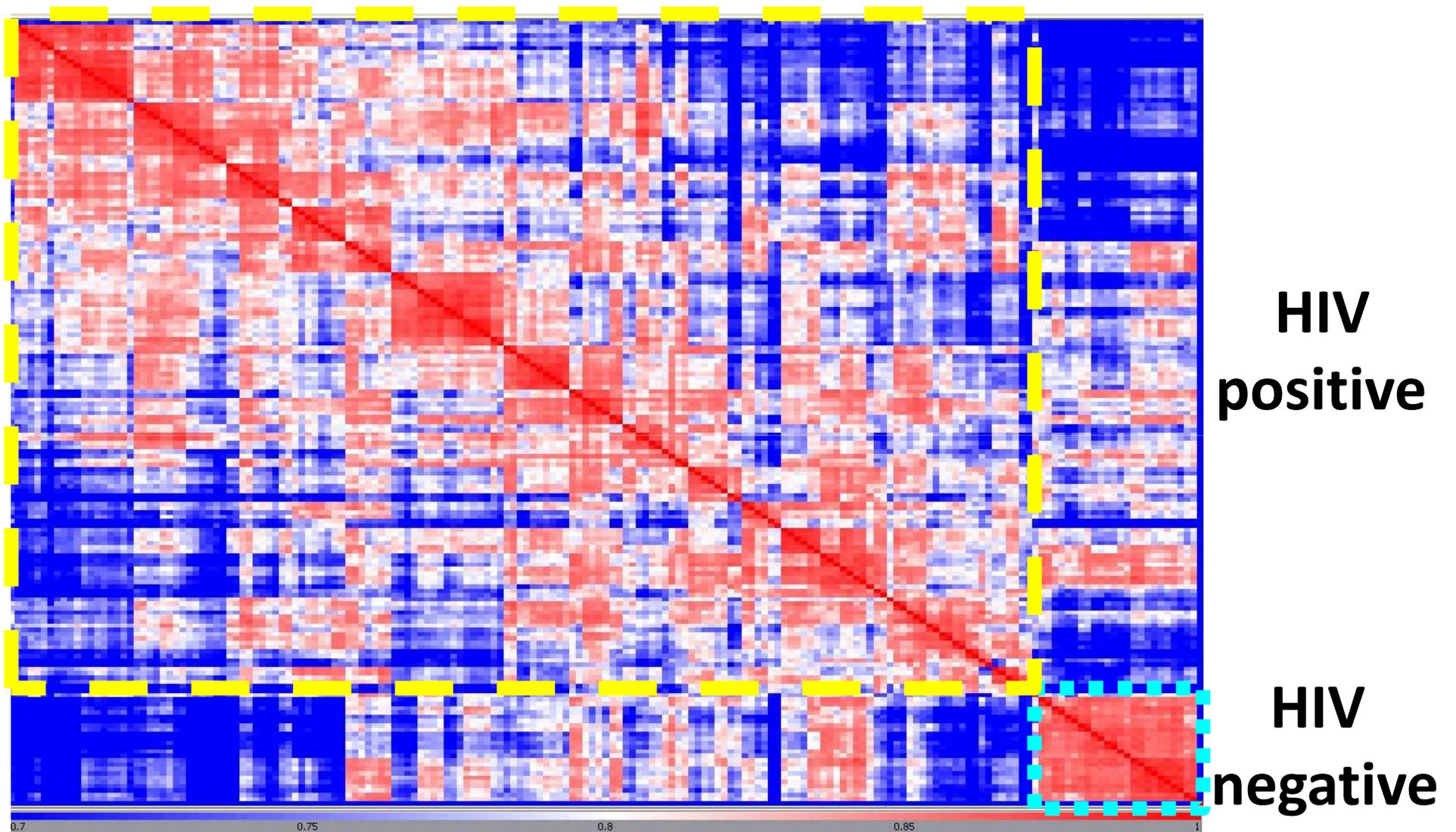
CSF viral load-response to HAART



Hierarchical clustering and cross correlation analysis of patient samples

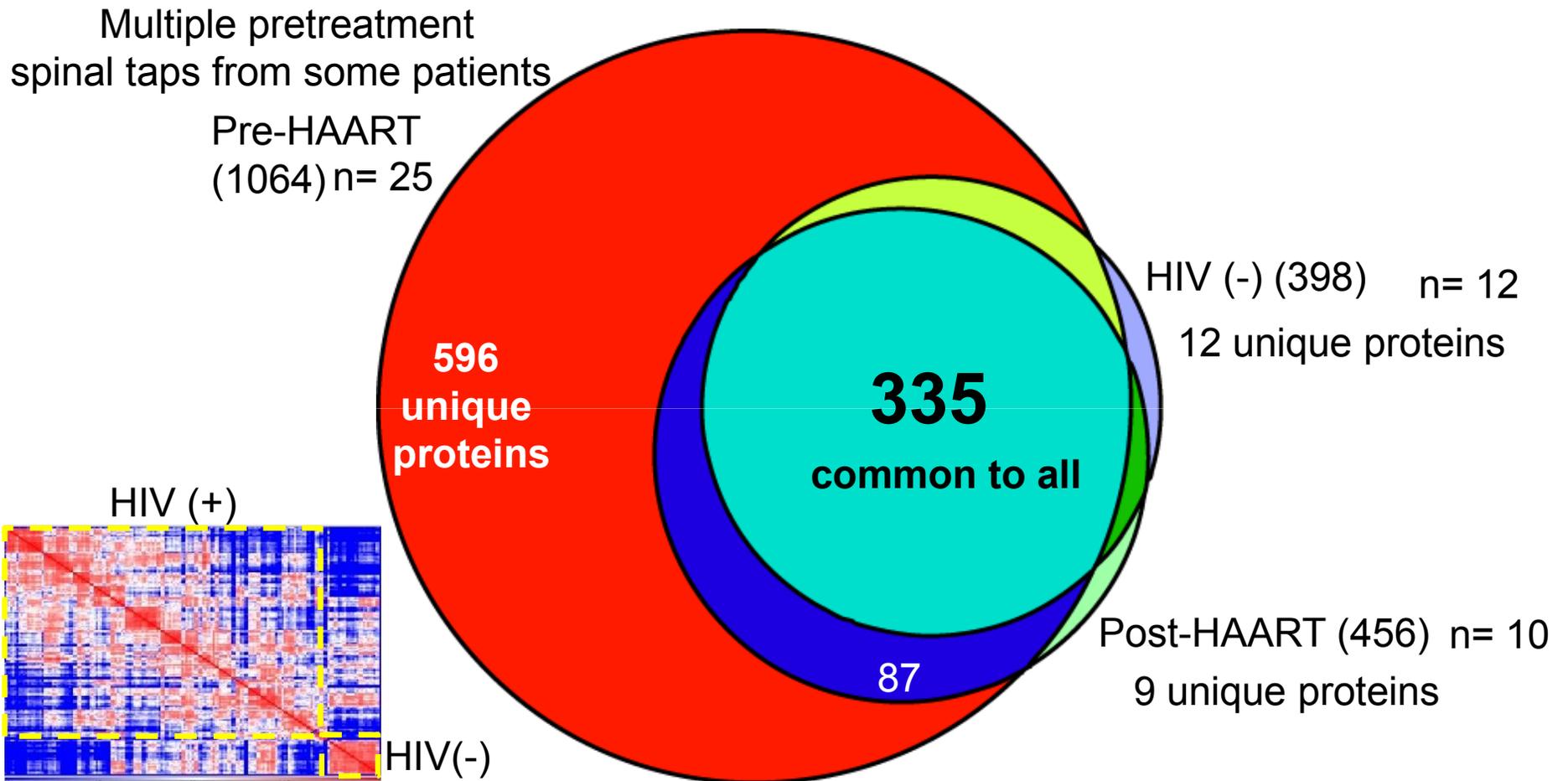


CSF proteome distinct for HIV(+) and HIV (-)



Defines two main populations

Assessment of proteins in HIV(+) untreated, treated end point measurement, and HIV(-) samples



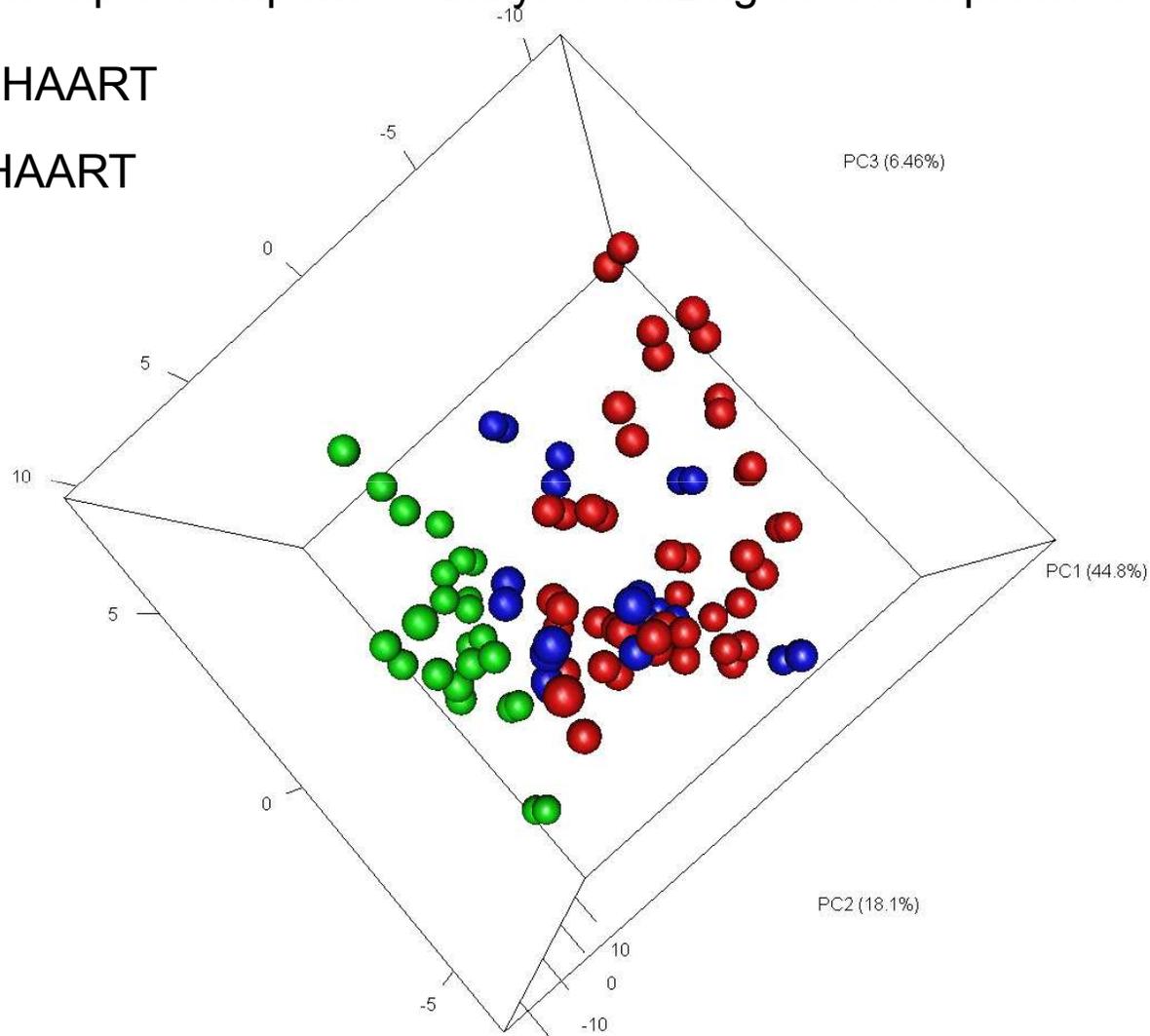
Common proteins between patient states and control samples form an analytical foundation

CSF proteome from HIV(+) samples becomes more like HIV (-) following HAART

● HIV(-) Principle Component Analysis utilizing common proteins

● HIV(+) Post-HAART

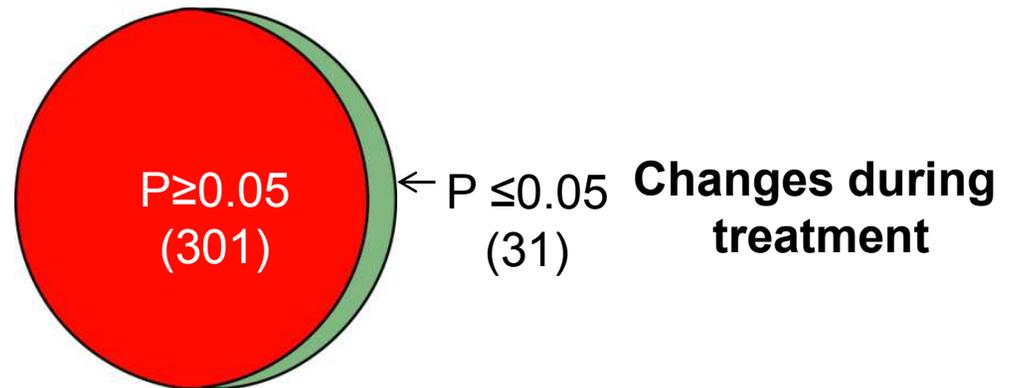
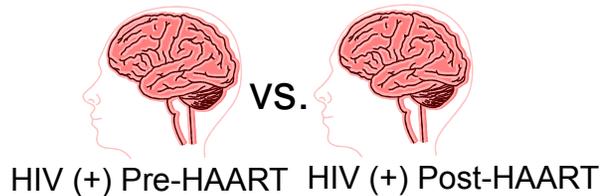
● HIV(+) Pre-HAART



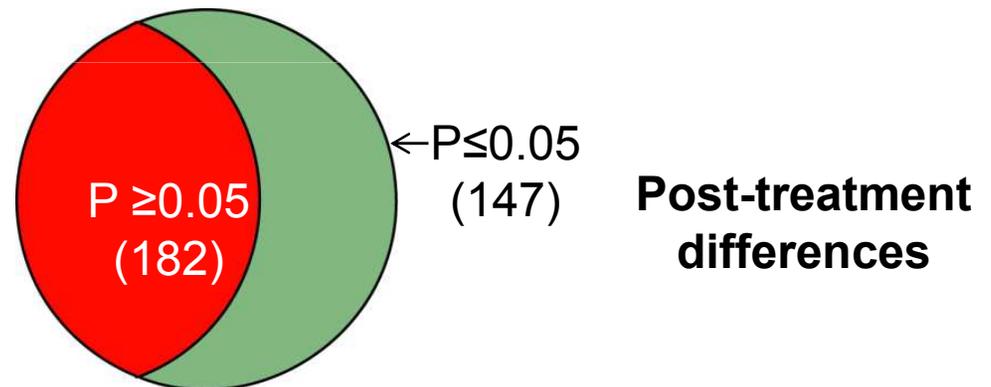
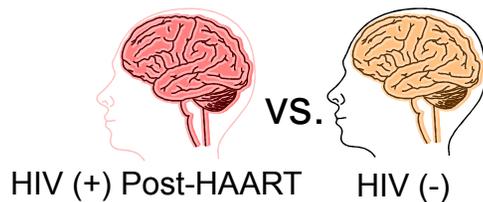
What proteins are “normalizing”?

Measured treatment ANOVA for protein abundance

ANOVA
Pre-HAART vs. Post-HAART

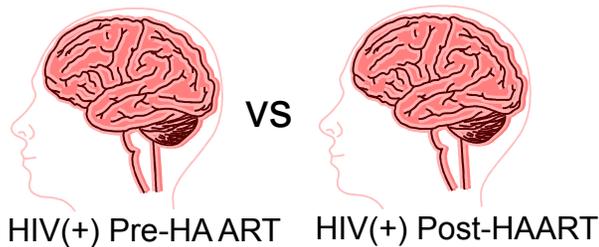


ANOVA
Post HAART vs. HIV (-)

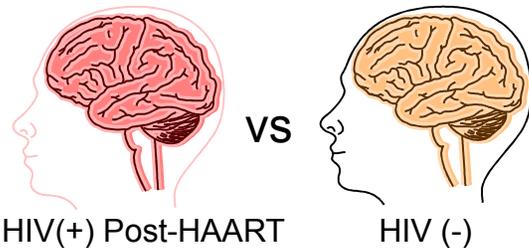
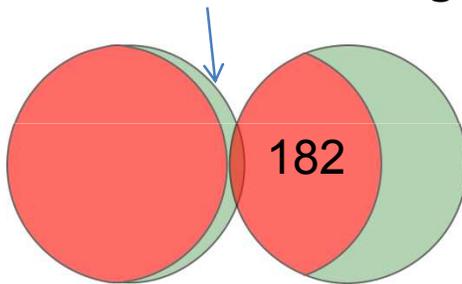


Determining the proteins that change in abundance over the course of HAART and attain baseline levels

What proteins in the CSF change over the course of HAART?

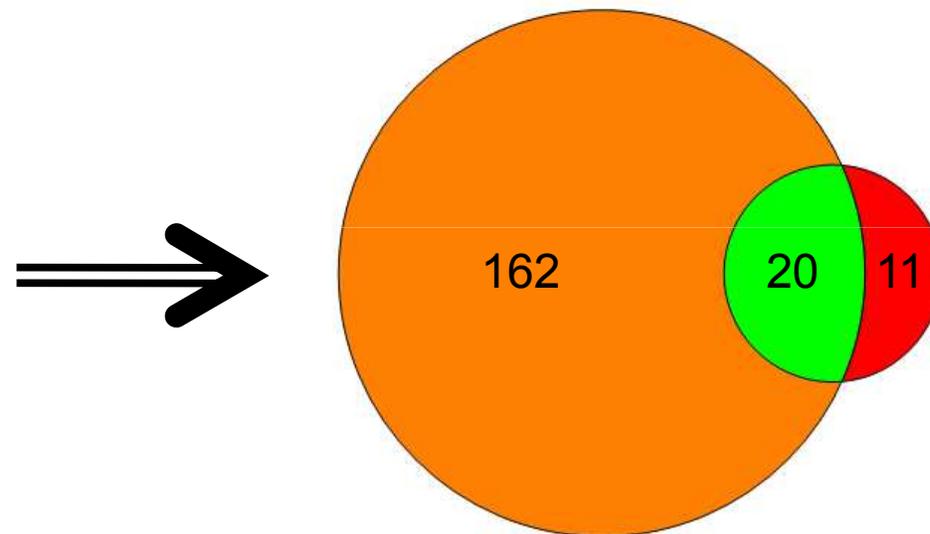


(31 proteins) $p < 0.05$
Proteins that change



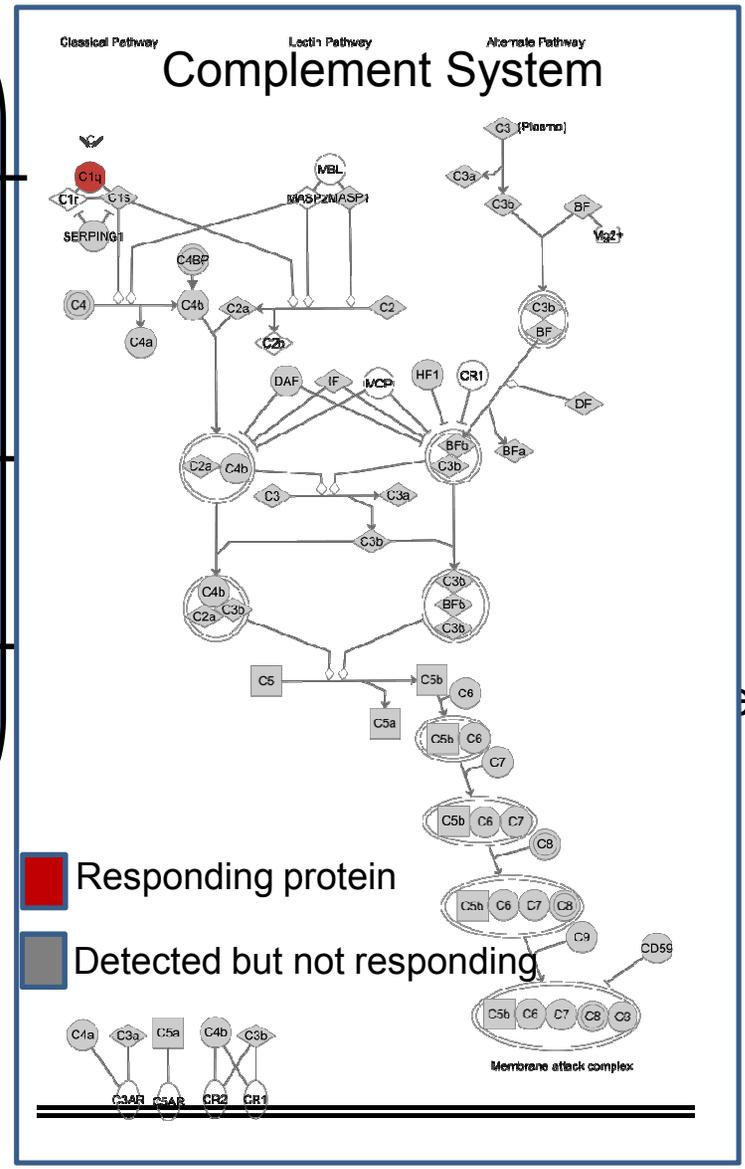
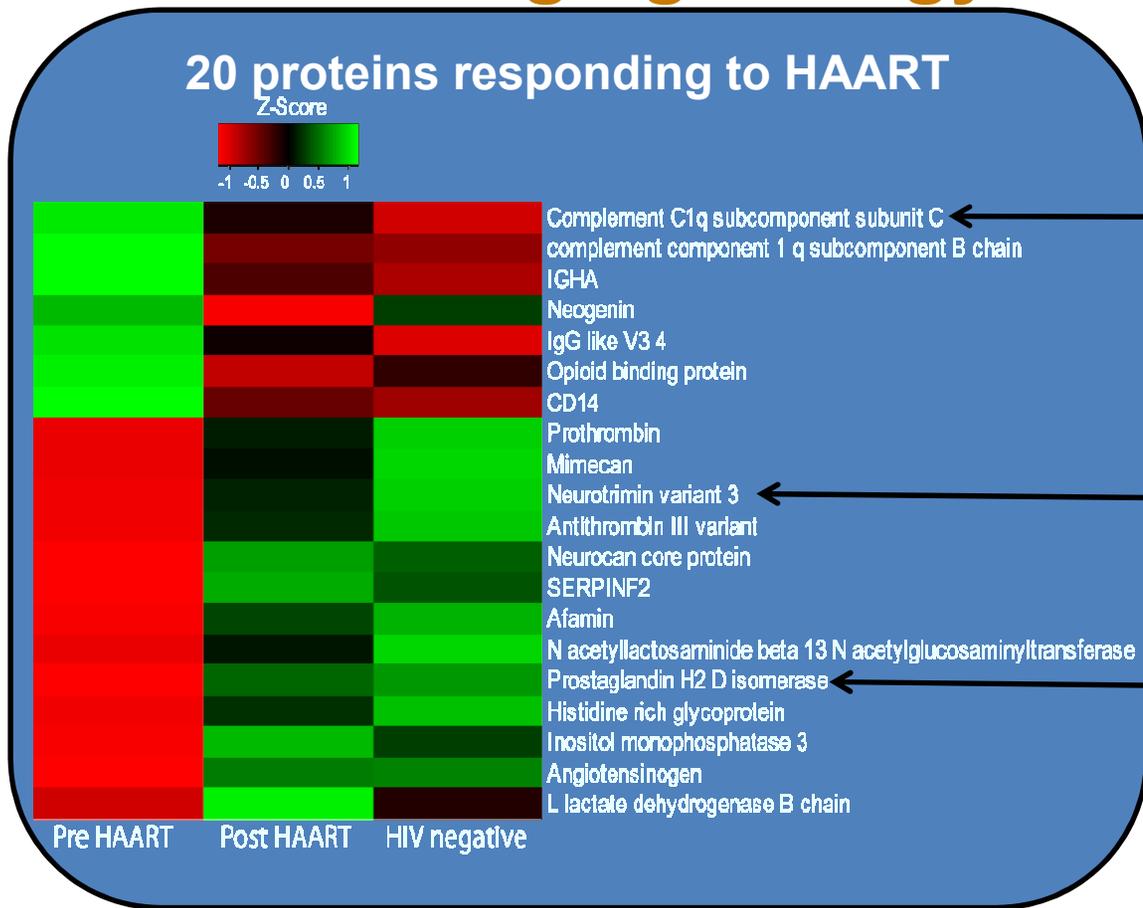
(182 proteins) $p > 0.05$
Proteins that are no longer differ between HIV (+) and (-)

Finding proteins that change over course of treatment and no longer differ between HIV(+) and HIV(-) samples

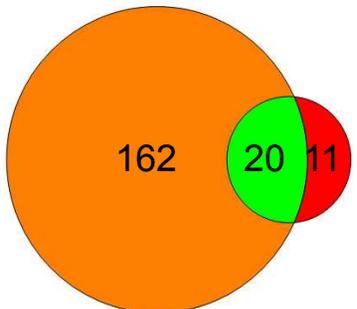


Proteins that change reflect biological response to treatment and decreased disease severity

Changes in protein abundance in CSF reflect changing biology and pathology

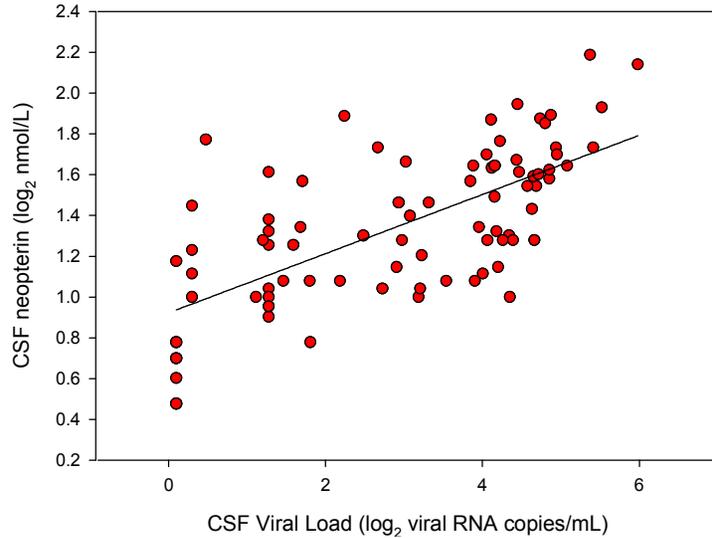


Protein level "normalization" detected over the course of HAART reflecting immune modulation, reduction in neuronal damage.

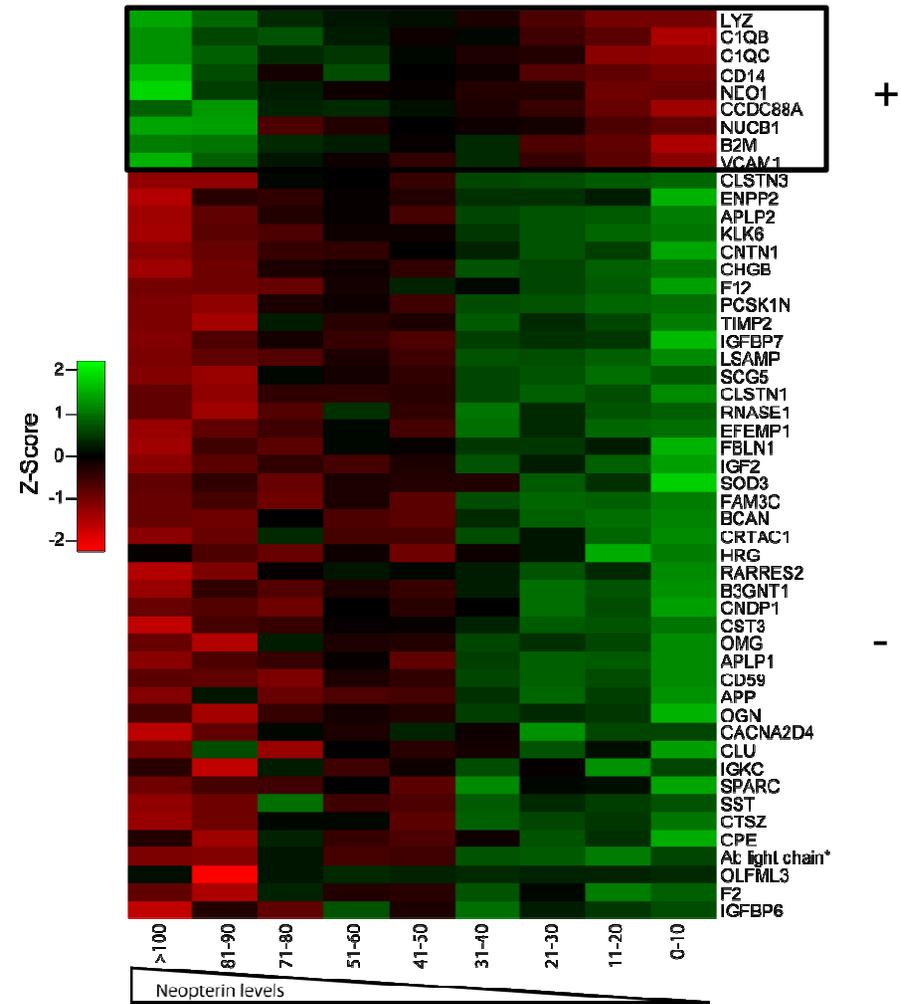
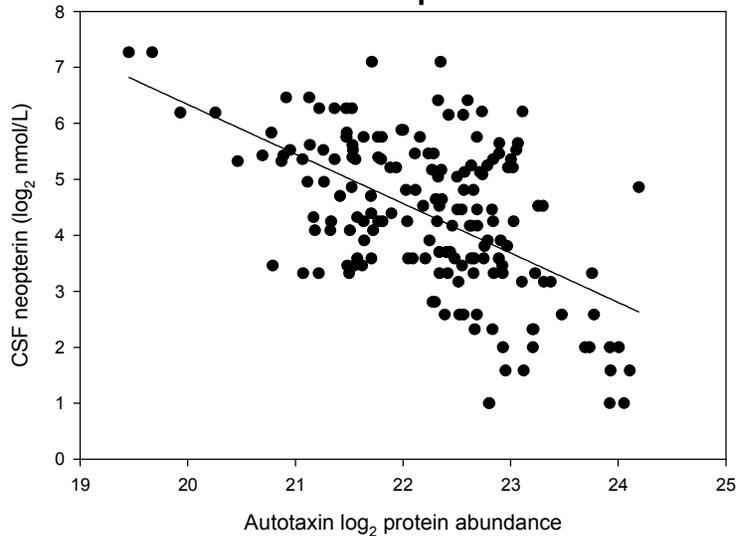


Immune activation and correlative changes in protein abundance

Marker of innate immune activation



Protein with significant correlation with neopterin



Demonstrates the successful use of analytical "guideposts" for analysis of patient innate immune response to HAART

Summary and Conclusion

- ▶ Proteome analysis of cerebrospinal fluid discriminates HIV negative and HIV positive samples.
- ▶ Detected proteins that “normalize” following HAART representing indicators for positive drug response.
- ▶ Integrating orthogonal measurements of analytical “guideposts” detected correlative changes in the CSF proteome that are coincident with the reduction of HAD and positive therapeutic outcome.

Collaborators

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Funding

**NIDA-HIV PROTEOMIC CENTER
FOR HOST-VIRAL
RESPONSE CHARACTERIZATION
(P01DA026134)**

**NIH National Center for
Research Resources
(RR018522)**