



Microarray comparisons show significant
prechallenge differences in a vaccine trial of
replicating Ad5-HIV/SIV recombinants
alone or with Env boosts

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Gene Expression Profiling in AIDS Vaccine Strategies – Goal: New and Better Correlates of Immunity

- Need:

While cell-mediated immunity appears to play the primary role in controlling disease progression, robust correlates of immunity based on such measures remain elusive.

- Hypothesis:

Gene expression changes observed in blood will correlate with the disease progression and/or extent of protection observed during trials of AIDS vaccine strategies in macaque models.

- Relevance of whole blood as sampling approach:

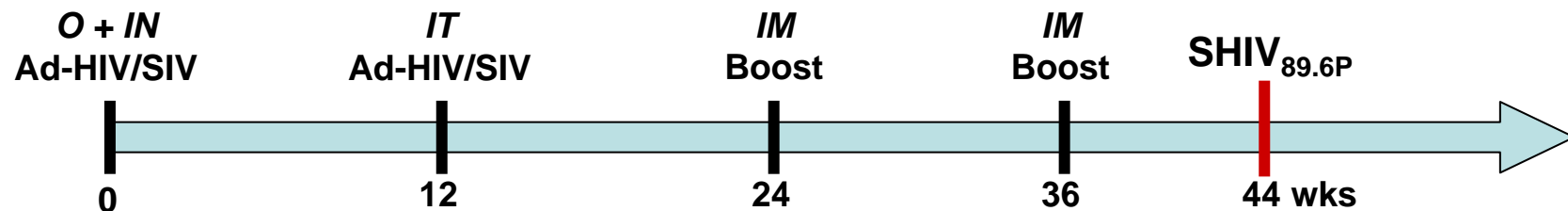
Readily obtained with *immediate* preservation of host transcriptome, without perturbations from lymphocyte isolation and/or expansion *ex vivo*.

- Potential long-term benefits:

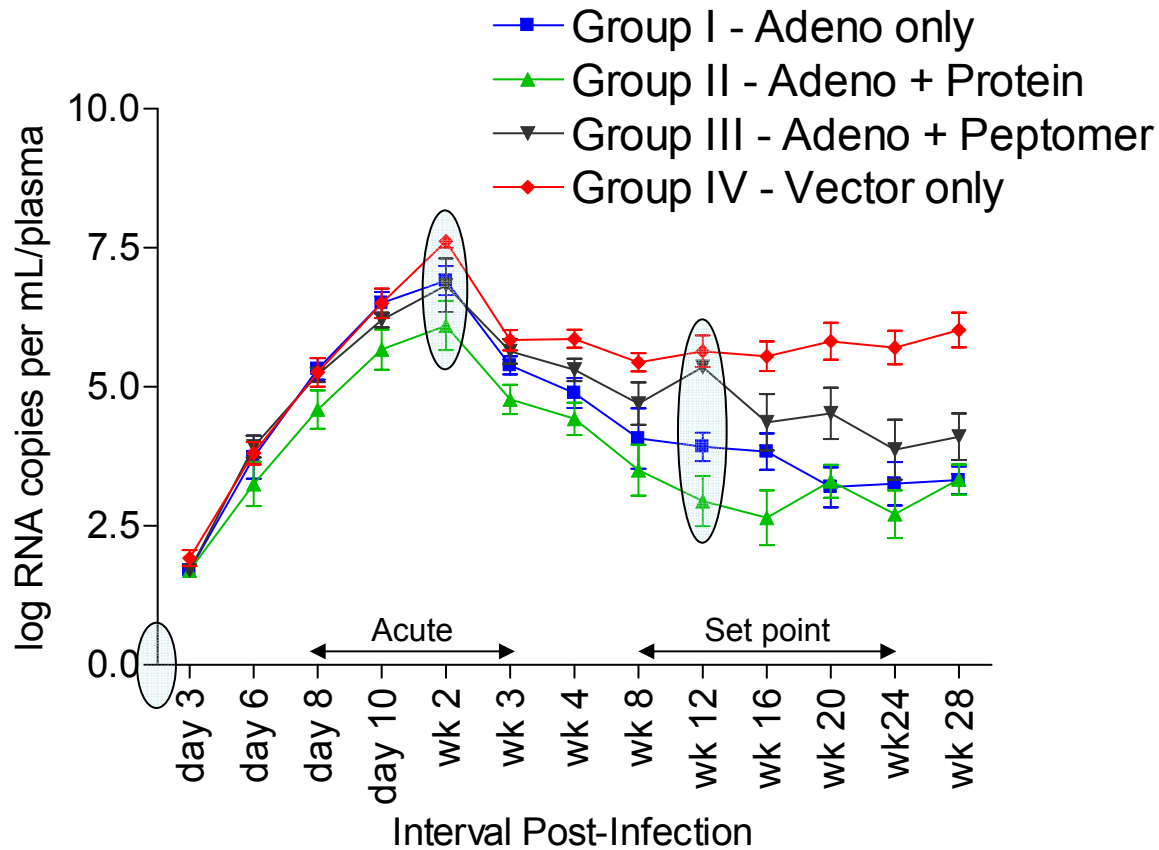
- Diagnostic and prognostic tools to apply to human AIDS vaccine trials, for identifying responders to treatment and their possible extent of protection.
- Mechanistic insights into the efficacy of particular vaccine regimens, thus suggesting strategies for improvements

Evaluating Envelope Subunit Boosts with a Replicating Ad-HIV/SIV Recombinant Vaccine - Protective Efficacy against SHIV_{89.6P}

- Ad vectors target mucosal sites.
- Ad serotypes available with little pre-existing immunity in human population
- Ad5 host-range mutant can replicate in macaque
- Previously shown to be highly effective in an SIV_{mac251} model



Gp140 Boosting Significantly Reduced Acute and Set Point Viremia Non-boosted Group Also Showed Reduced Set Point Viremia



- PAXgene blood samples from Day 0, Wk 2 and Wk 12 time points chosen for microarray analysis.
- Peptomer boosted group appeared intermediate between non-boosted and unimmunized animals and consequently was not advanced to microarrays.

Methodology of Macaque Functional Genomics



- V2 Macaque Oligonucleotide Microarrays
 - based on the rhesus macaque genome
 - 60-mer probe designs from collaboration of Katze lab with Agilent technologies
 - 17,641 distinct macaque sequences, derived from human orthology and macaque EST sequencescf. eArray 4.5, design number 013791
<http://earray.chem.agilent.com/earray/>
- Two color ratiometric array analyses
 - all measurements performed against a common reference.
 - common reference: PAXgene blood from 7 unassigned, untreated male rhesus macaques.



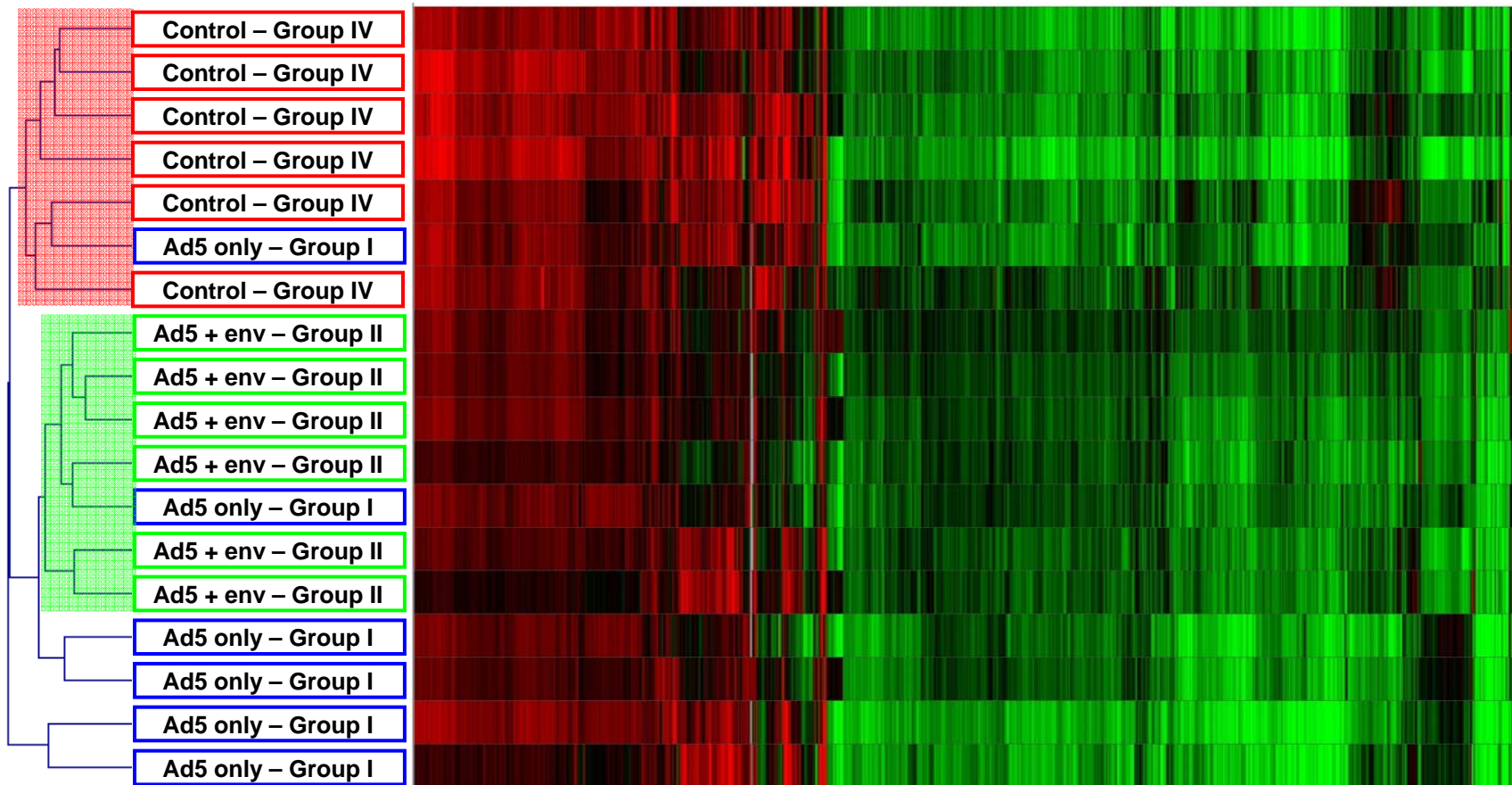
Up vs. Common Reference



Down vs. Common Reference

Day 0 Expression Profiles of Treatment Groups – Demonstration of Show Distinctions by Unsupervised 2D Hierarchical Clustering

- Relatively clean separation of Control Group IV
- Group II (Ad5-HIV/SIV + Env) also partitions into a sub-tree
- Fine structure within groups is also evident



Genes of Interest for Env-Boosted Group on Day 0

Why Day 0?

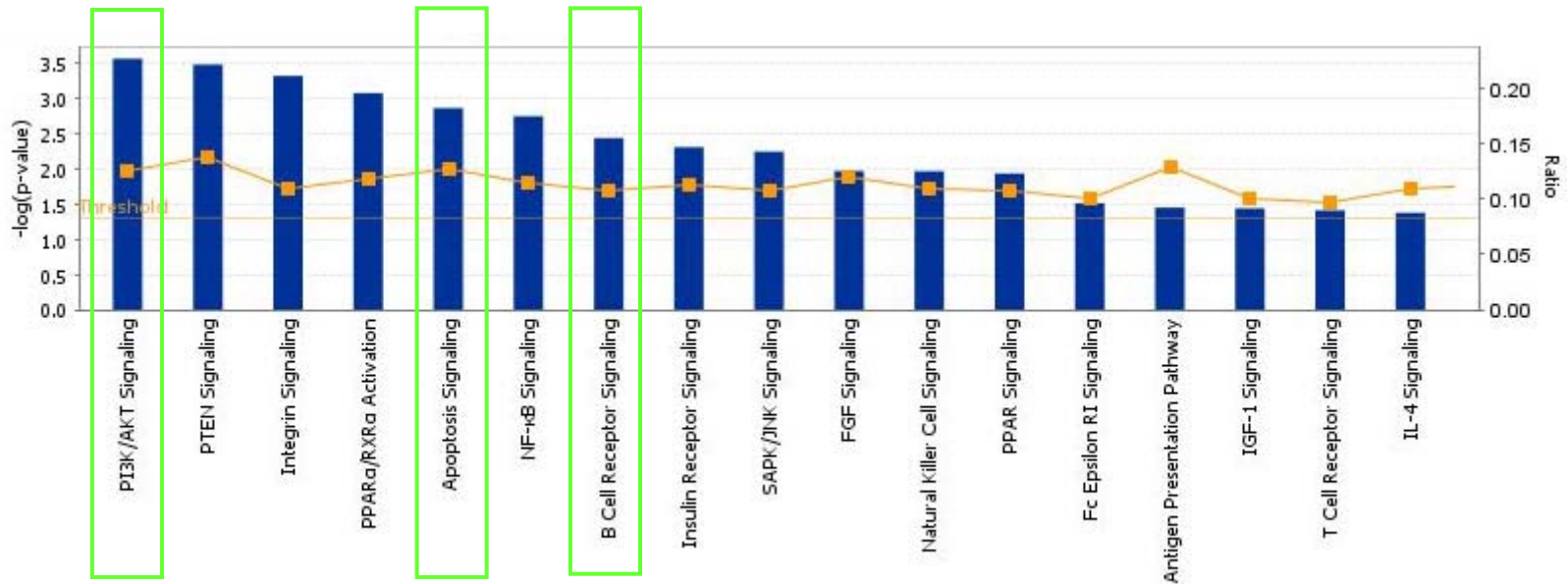
- Overall goal to determine prechallenge expression features that correlate with vaccine efficacy.

Why Group II (Ad-HIV/SIV + env boost) vs. Control Group IV?

- Group II (with the protein boost) had highest Env-binding antibody titers, but also highest increased ELISpot, proliferative and IFN- γ ⁺ memory T cell responses.
 - Comparison that should show the strongest immunological signature
 - Will features in the Env-boosted group help to identify expression features that may only be weakly evident in animals immunized with only the Ad-HIV/SIV recombinants (Group I)?
- 1367 genes distinguish Group II (Ad-HIV/SIV + env boost) from Control Group IV
 - Selected by ANOVA of Day 0 expression profiles followed by post hoc analysis
 - Input into Ingenuity Pathway Analysis
 - Refine on the basis of biological relevance

Functional Analysis Highlights Akt, Apoptosis and B Cell Receptor Pathways

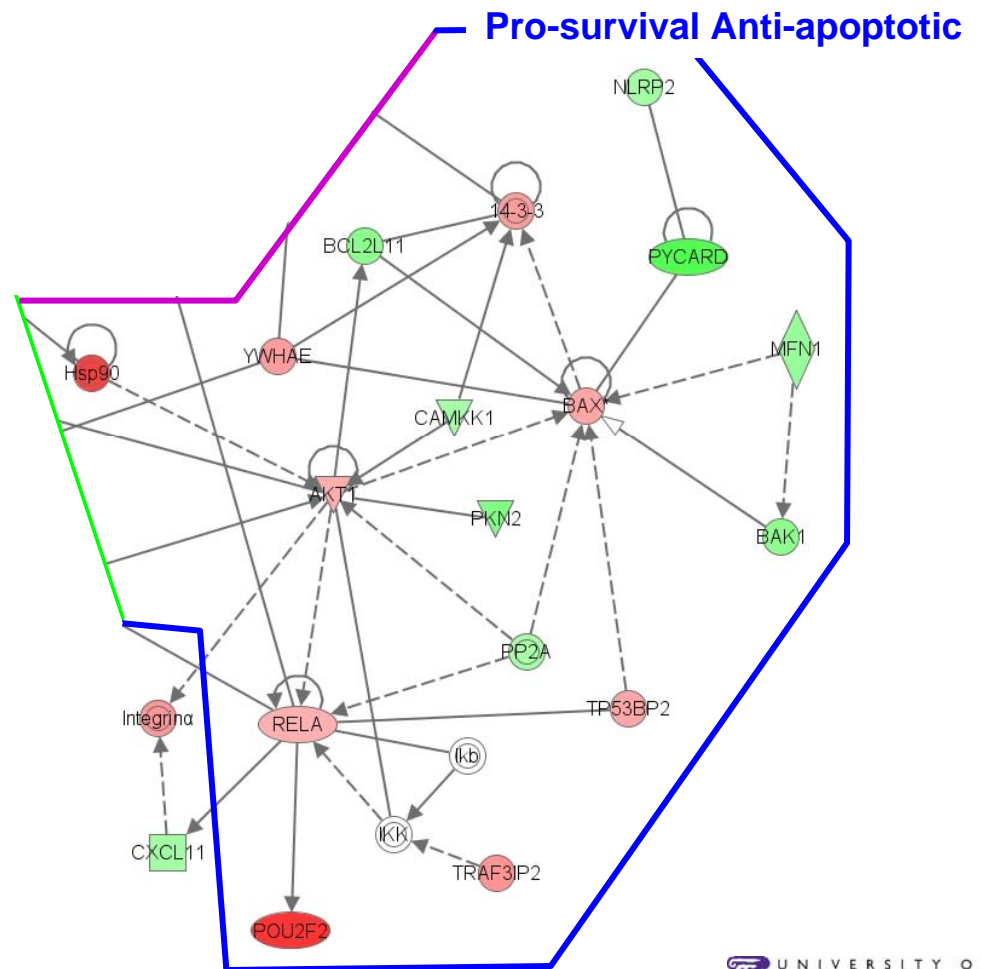
- List analysis by Ingenuity for membership in the indicated canonical pathways.



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- Other functional analysis: up regulation of many genes in protein synthesis and N-linked glycan synthesis
 - Consequence of antibody production?

Network Diagram of Immune-Associated Genes Reveals a Cluster of Anti-apoptotic Expression Changes



Conclusions and Future Directions

- We have demonstrated that gene expression profiling of whole blood can distinguish vaccine treatment groups as long as 8 weeks after the last immunization/boost.
- Detailed comparison of Group II (Ad-HIV/SIV + env boost) vs. Control Group IV revealed expression changes indicative of:
 - pro-survival signals accomplished by down-regulating pro-apoptotic genes (BAK1, BCL2L11, PYCARD, PKN) while up-regulating genes of anti-apoptotic activity (AKT1, RELA, YWHAE, HSP90)
 - up-regulation of many genes associated with T cell and B cell signaling pathways
 - up-regulation of transcriptional co-repressors (NCOR2, BCOR, BTBD14B) that also impact B cell fate

FUNCTIONAL GENOMICS DOES WORK!

Immediate directions

- Validation
- What are the biologically relevant gene expression changes for Group I (Ad-HIV/SIV only)?
- Are there day 0 expression features that correlate with the extent of protection?
- Gene expression changes at peak viremia and virological set point:
 - What are the gene expression changes that seem indicative of a protective immune response?
 - Do these help refine our examination of the day 0 expression data?

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