Insights into HIV/HCV-host Interactions: a High-throughput Genomics Approach

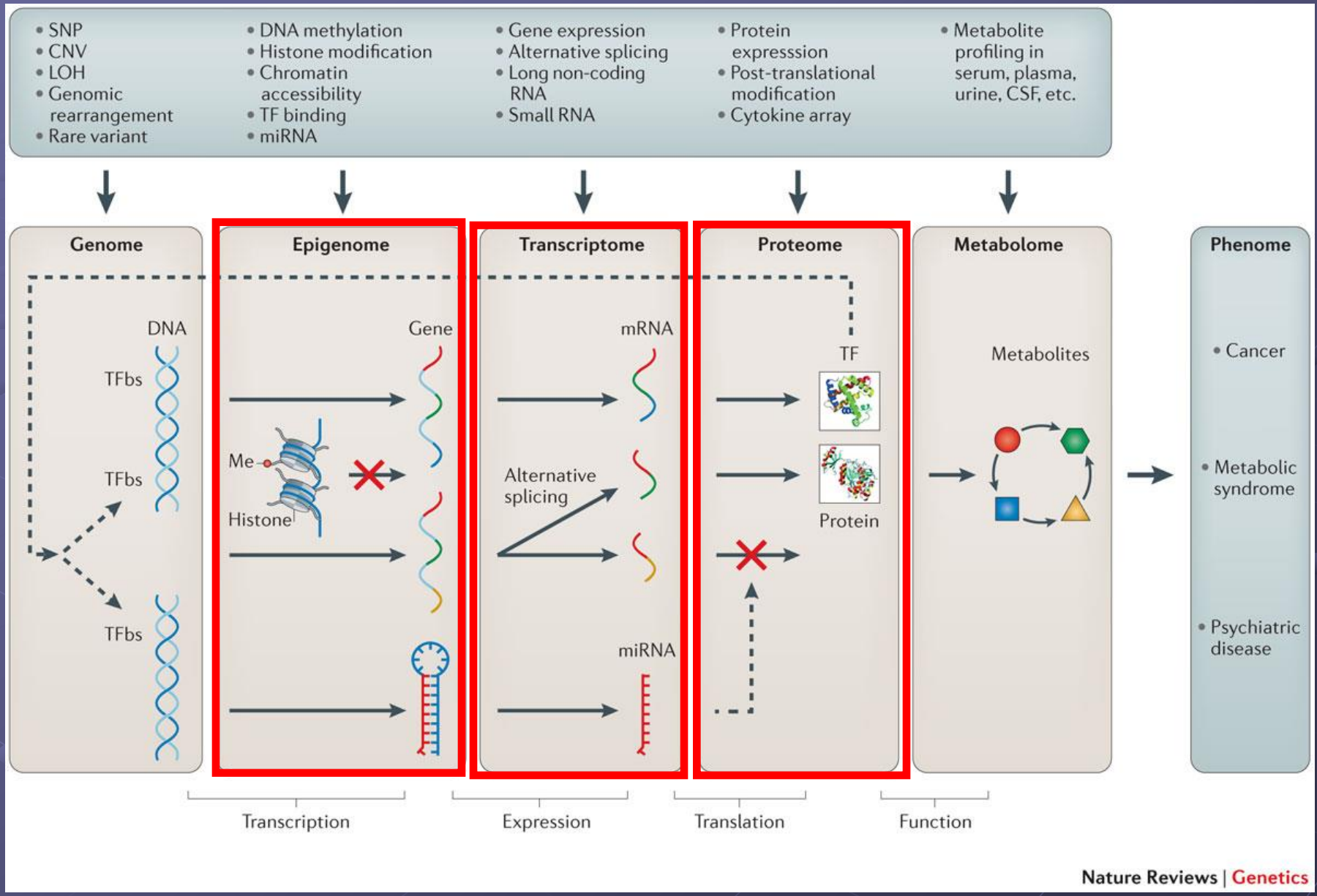
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Outline of my talk

- HIV/HCV co-infection
  - Transcriptome analysis of HIV/HCV co-infection
  - Genome-wide microRNA analysis of HIV/HCV co-infection
- HIV disease status and therapy effects
  - Transcriptome analysis: immune cell subsets
  - Antibody microarray assays for T cells and PBMCs

Levels of Biological Systems



HIV/HCV co-infection

- 30% of HIV+ individuals are co-infected with HCV in the United States and Europe
- HAART has substantially reduced AIDS-associated death
- HCV-related liver disease has become the leading cause of death in HIV+ individuals

Effect of co-infection on disease progression

- HIV accelerates HCV disease progression – doubles the risk for cirrhosis and increases the chance for liver cancer
- Higher HCV viral loads and more frequent HCV relapses
- Impaired CD4+ T cell count recovery during HAART
- Side effects of HAART such as rash and hepatoxicity in HIV patients are exacerbated

Microarray studies on coinfection

- Coinfection vs. HCV; paired liver biopsy and PBMC: increased inflammation & immune activation (Rasmussen AL et al, 2012,Virology)
- CD4+ T cells; HIV vs. co-infec: cell cycle up-regulated in HIV; HCV vs co-infec or HIV; GPCR signaling pathway up-regulated in HCV+ individuals (Yi L et al, 2014, Virol J)
- CD8+ T cells; HIV vs HCV: cell cycle up-regulated in HIV; coinfec vs. HCV: immune suppression (IL2, IFNγ $\downarrow$); coinfec vs. HIV: lack of difference (Zhao Z et al, 2012 Plos One)

Wu et al. Virology Journal (2015) 12:4
DOI 10.1186/s12985-014-0236-6

VIROLOGY JOURNAL

RESEARCH Open Access

Differential regulation of cytotoxicity pathway discriminating between HIV, HCV mono- and co-infection identified by transcriptome profiling of PBMCs

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Abstract

Background: Despite the easy accessibility and diagnostic utility of PBMCs and their potential to show distinct expression patterns associated with the accelerated disease progression in HIV/HCV co-infection, there has not been a systematic study focusing on the global dysregulations of the biological pathways in PBMCs from HIV, HCV mono- and co-infected individuals. This study aimed at identifying the transcriptome distinctions of PBMCs between these patient groups.

Methods: Genome-wide transcriptomes of PBMCs from 10 HIV/HCV co-infected patients, 7 HIV+ patients, 5 HCV+ patients, and 5 HIV/HCV sero-negative healthy controls were analyzed using Illumina microarray. Pairwise comparisons were performed to identify differentially expressed genes (DEGs), followed by gene set enrichment analysis (GSEA) to detect the global dysregulations of the biological pathways between HIV, HCV mono- and co-infection.

Results: Forty-one, 262, and 44 DEGs with fold change > 1.5 and FDR (false discovery rate) <0.05 for the comparisons of HCV versus co-infection, HIV versus co-infection, and HIV versus HCV were identified, respectively. Significantly altered pathways (FDR < 0.05), featured by those involved in immune system, signaling transduction, and cell cycle, were detected. Notably, the differential regulation of cytotoxicity pathway discriminated between HIV, HCV mono- and co-infection (up-regulated in the former versus the latter group: co-infection versus HIV or HCV, HIV versus HCV; FDR <0.001 ~ 0.019). Conversely, the cytokine-cytokine receptor interaction pathway was down-regulated in co-infection versus either HCV (FDR = 0.003) or HIV (FDR = 0.028). For the comparison of HIV versus HCV, the cell cycle (FDR = 0.016) and WNT signaling (FDR = 0.006) pathways were up- and down-regulated in HIV, respectively.

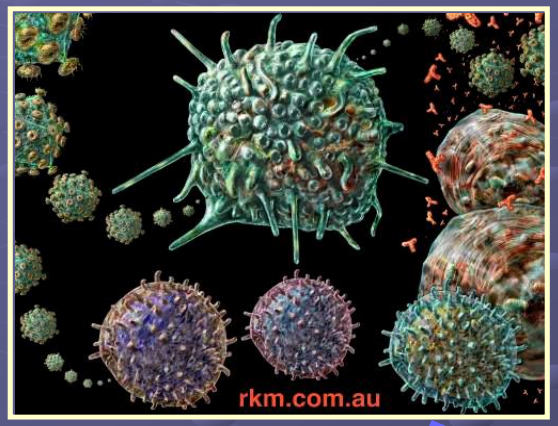
Conclusions: Our study is the first to identify the differential regulation of cytotoxicity pathway discriminating between

Patient groups and controls

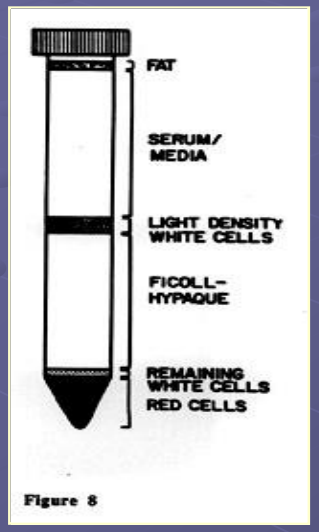
- HIV/HCV co-infected patients (HH; n=10)
  - No ART; liver fibrosis F0-1
  - CD4 > 400 cells/ul, HIV RNA <50 ~ 5000
- HIV+ patients (HIV; n=7)
  - no ART, CD4 > 500, HIV RNA <50 ~ 2000
- HCV+ patients (HCV; n=5)
- HIV HCV negative controls (CTR; n=5)

Experiment outline

PBMC



Ficoll Seperation



Total RNA isolation



RNA amplification



Gene Expression Chip



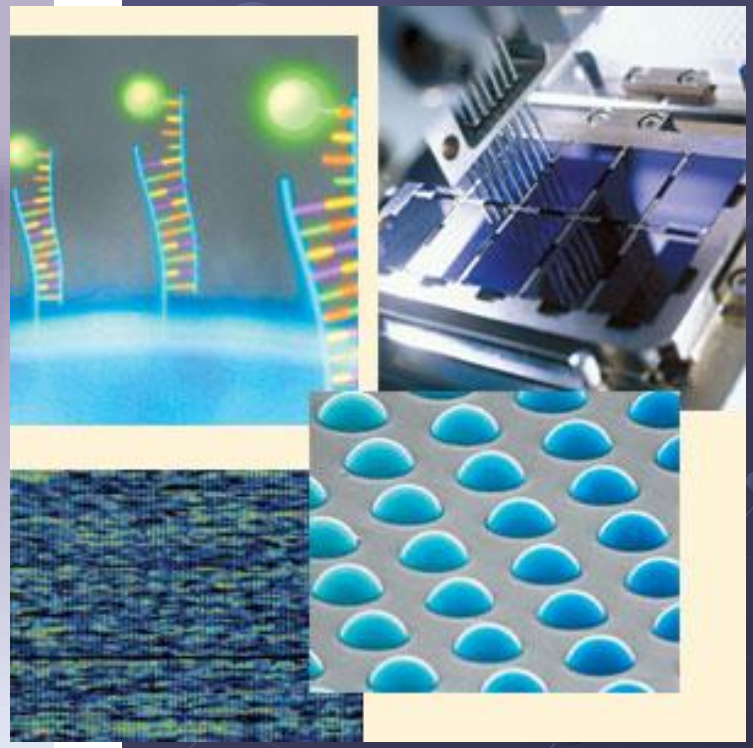
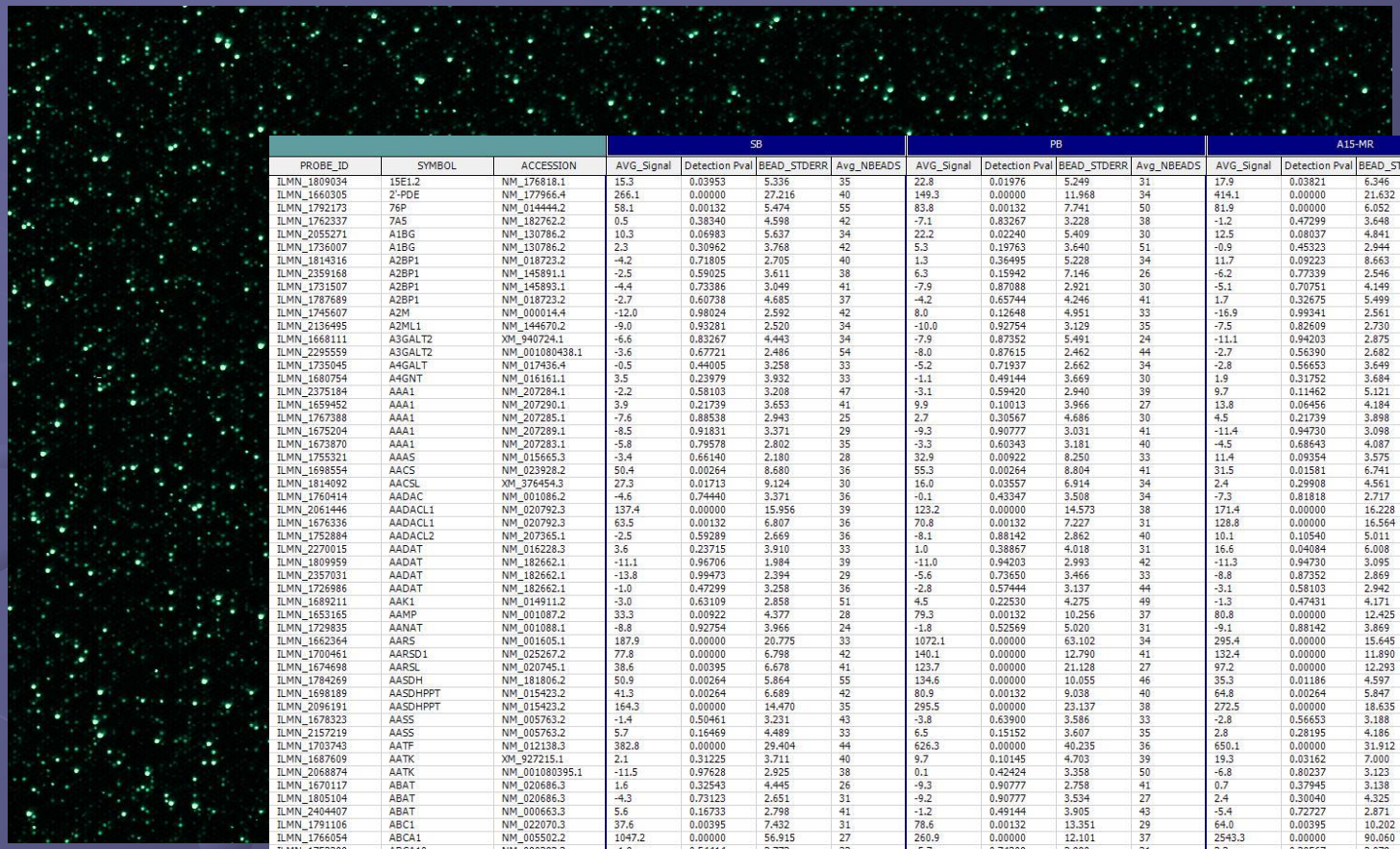


Image scanning and raw data



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ILMN_1809034	15E1.2	NM_176818.1	15.3	0.03953	5.336	35	22.8	0.01976	5.249	31	17.9	0.03821	6.346	29	28.3	0.01318	5.528	45
ILMN_1660305	2'-PDE	NM_177966.4	266.1	0.00000	27.216	40	149.3	0.00000	11.968	34	414.1	0.00000	21.632	40	219.5	0.00000	12.777	42
ILMN_1792173	76P	NM_014444.2	58.1	0.00132	5.474	55	83.8	0.00132	7.741	50	81.9	0.00000	6.052	48	152.1	0.00000	13.910	35
ILMN_1762337	7A5	NM_182762.2	0.5	0.38340	4.598	42	-7.1	0.83267	3.228	38	-1.2	0.47299	3.648	51	0.6	0.40448	5.002	40
ILMN_2055271	A1BG	NM_130786.2	10.3	0.06983	5.637	34	22.2	0.02240	5.409	30	12.5	0.08037	4.841	33	21.4	0.02240	5.006	40
ILMN_1736007	A1BG	NM_130786.2	2.3	0.30962	3.768	42	5.3	0.19763	3.640	51	-0.9	0.45323	2.944	53	2.1	0.33860	4.809	34
ILMN_1814316	A2BP1	NM_018723.2	-4.2	0.71805	2.705	40	1.3	0.36495	5.228	34	11.7	0.09223	8.663	25	-4.9	0.73123	3.216	44
ILMN_2359168	A2BP1	NM_145891.1	-2.5	0.59025	3.611	38	6.3	0.15942	7.146	26	-6.2	0.77339	2.546	37	8.5	0.10277	4.326	50
ILMN_1731507	A2BP1	NM_145893.1	-4.4	0.73386	3.049	41	-7.9	0.87088	2.921	30	-5.1	0.70751	4.149	41	-8.7	0.88669	2.183	45
ILMN_1787689	A2BP1	NM_018723.2	-2.7	0.60738	4.685	37	-4.2	0.65744	4.246	41	1.7	0.32675	5.499	28	-4.5	0.70751	3.914	33
ILMN_1745607	A2M	NM_000014.4	-12.0	0.98024	2.592	42	8.0	0.12648	4.951	33	-16.9	0.99341	2.561	34	-6.5	0.82213	3.596	41
ILMN_2136495	A2ML1	NM_144670.2	-9.0	0.93281	2.520	34	-10.0	0.92754	3.129	35	-7.5	0.82609	2.730	43	-4.4	0.70487	4.028	37
ILMN_1668111	A3GALT2	XM_940724.1	-6.6	0.83267	4.443	34	-7.9	0.87352	5.491	24	-11.1	0.94203	2.875	49	4.3	0.23320	4.236	54
ILMN_2295559	A3GALT2	NM_001080438.1	-3.6	0.67721	2.486	54	-8.0	0.87615	2.462	44	-2.7	0.56390	2.682	41	11.3	0.05797	4.925	40
ILMN_1735045	A4GALT	NM_017436.4	-0.5	0.44005	3.258	33	-5.2	0.71937	2.662	34	-2.8	0.56653	3.649	34	-5.3	0.74572	4.006	40
ILMN_1680754	A4GNT	NM_016161.1	3.5	0.23979	3.932	33	-1.1	0.49144	3.669	30	1.9	0.31752	3.684	30	13.7	0.04480	7.841	25
ILMN_2375184	AAA1	NM_207284.1	-2.2	0.58103	3.208	47	-3.1	0.59420	2.940	39	9.7	0.11462	5.121	36	7.9	0.11462	3.702	39
ILMN_1659452	AAA1	NM_207290.1	3.9	0.21739	3.653	41	9.9	0.10013	3.966	27	13.8	0.06456	4.184	45	22.0	0.01976	5.718	42
ILMN_1767388	AAA1	NM_207285.1	-7.6	0.88538	2.943	25	2.7	0.30567	4.686	30	4.5	0.21739	3.898	43	0.4	0.41502	4.922	30
ILMN_1675204	AAA1	NM_207289.1	-8.5	0.91831	3.371	29	-9.3	0.90777	3.031	41	-11.4	0.94730	3.098	44	-14.6	0.98946	2.281	43
ILMN_1673870	AAA1	NM_207283.1	-5.8	0.79578	2.802	35	-3.3	0.60343	3.181	40	-4.5	0.68643	4.087	38	-3.4	0.64032	2.872	39
ILMN_1755321	AAAS	NM_015665.3	-3.4	0.66140	2.180	28	32.9	0.00922	8.250	33	11.4	0.09354	3.575	45	67.0	0.00132	9.301	40
ILMN_1698554	AACS	NM_023928.2	50.4	0.00264	8.680	36	55.3	0.00264	8.804	41	31.5	0.01581	6.741	35	69.3	0.00000	7.650	44
ILMN_1814092	AACSL	XM_376454.3	27.3	0.01713	9.124	30	16.0	0.03557	6.914	34	2.4	0.29908	4.561	28	8.7	0.10145	4.204	32
ILMN_1760414	AADAC	NM_001086.2	-4.6	0.74440	3.371	36	-0.1	0.43347	3.508	34	-7.3	0.81818	2.717	42	-3.7	0.66008	3.558	37
ILMN_2061446	AADACL1	NM_020792.3	137.4	0.00000	15.956	39	123.2	0.00000	14.573	38	171.4	0.00000	16.228	36	179.0	0.00000	16.395	36
ILMN_1676336	AADACL1	NM_020792.3	63.5	0.00132	6.807	36	70.8	0.00132	7.227	31	128.8	0.00000	16.564	26	150.9	0.00000	11.795	36
ILMN_1752884	AADACL2	NM_207365.1	-2.5	0.59289	2.669	36	-8.1	0.88142	2.862	40	10.1	0.10540	5.011	38	-1.0	0.49144	3.920	39
ILMN_2270015	AADAT	NM_016228.3	3.6	0.23715	3.910	33	1.0	0.38867	4.018	31	16.6	0.04084	6.008	40	10.0	0.08300	4.129	42
ILMN_1809959	AADAT	NM_182662.1	-11.1	0.96706	1.984	39	-11.0	0.94203	2.993	42	-11.3	0.94730	3.095	39	-8.1	0.86825	2.963	30
ILMN_2357031	AADAT	NM_182662.1	-13.8	0.99473	2.394	29	-5.6	0.73650	3.466	33	-8.8	0.87352	2.869	34	-9.5	0.91041	2.636	35
ILMN_1726986	AADAT	NM_182662.1	-1.0	0.47299	3.258	36	-2.8	0.57444	3.137	44	-3.1	0.58103	2.942	29	4.5	0.22266	4.401	32
ILMN_1689211	AAK1	NM_014911.2	-3.0	0.63109	2.858	51	4.5	0.22530	4.275	49	-1.3	0.47431	4.171	33	-6.5	0.81818	3.095	38
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ILMN_1729835	AANAT	NM_001088.1	-8.8	0.92754	3.966	24	-1.8	0.52569	5.020	31	-9.1	0.88142	3.869	29	-9.9	0.92095	3.435	35
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ILMN_1700461	AARSD1	NM_025267.2	77.8	0.00000	6.798	42	140.1	0.00000	12.790	41	132.4	0.00000	11.890	50	174.5	0.00000	16.124	44
ILMN_1674698	AARSL	NM_020745.1	38.6	0.00395	6.678	41	123.7	0.00000	21.128	27	97.2	0.00000	12.293	34	64.0	0.00264	8.100	44
ILMN_1784269	AASDH	NM_181806.2	50.9	0.00264	5.864	55	134.6	0.00000	10.055	46	35.3	0.01186	4.597	40	191.9	0.00000	12.851	38
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ILMN_1678323	AASS	NM_005763.2	-1.4	0.50461	3.231	43	-3.8	0.63950	3.586	33	-2.8	0.56653	3.188	40	0.6	0.40711	2.903	42
ILMN_2157219	AASS	NM_005763.2	5.7	0.16469	4.489	33	6.5	0.15152	3.607	35	2.8	0.28195	4.186	37	7.1	0.13307	4.197	38
ILMN_1703743	AATF	NM_012138.3	382.8	0.00000	29.404	44	626.3	0.00000	40.235	36	650.1	0.00000	31.912	34	896.0	0.00000	42.914	44
ILMN_1687609	AATK	XM_927215.1	2.1	0.31225	3.711	40	9.7	0.10145	4.703	39	19.3	0.03162	7.000	45	26.1	0.01581	6.247	39
ILMN_2068874	AATK	NM_001080395.1	-11.5	0.97628	2.925	38	0.1	0.42424	3.358	50	-6.8	0.80237	3.123	36	4.1	0.24638	4.806	42
ILMN_1670117	ABAT	NM_020686.3	1.6	0.32543	4.445	26	-9.3	0.90777	2.758	41	0.7	0.37945	3.138	47	1.2	0.37945	3.839	36
ILMN_1805104	ABAT	NM_020686.3	-4.3	0.73123	2.651	31	-9.2	0.90777	3.534	27	2.4	0.30040	4.325	28	16.0	0.03821	4.983	40
ILMN_2404407	ABAT	NM_000663.3	5.6	0.16733	2.798	41	-1.2	0.49144	3.905	43	-5.4	0.72727	2.871	39	7.2	0.13175	4.793	43
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ILMN_1766054	ABCA1	NM_005502.2	1047.2	0.00000	56.915	27	260.9	0.00000	12.101	37	2543.3	0.00000	90.060	49	496.2	0.00000	22.799	49
ILMN_1753388	ABCA10	NM_080282.2	-1.8	0.54414	2.772	32	-5.7	0.74308	2.880	31	2.3	0.30567	3.079	38	-1.5	0.51910	4.234	32
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ILMN_1750250	ABCA12	NM_173076.2	3.7	0.22398	4.297	42	7.5	0.13570	4.818	23	2.6	0.28590	4.186	27	7.3	0.12780	4.773	37
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ILMN_1687636	ABCA2	NM_212533.1	1.0	0.36100	3.851	49	3.7	0.25296	3.872	44	6.1	0.17918	5.047	32	0.8	0.39394	2.832	45
ILMN_1747627	ABCA2	NM_001606.3	7.6	0.11594	4.877	31	10.4	0.09354	6.617	31	40.7	0.00922	7.997	39	63.2	0.00264	12.518	34
ILMN_1758523	ABCA3	NM_001089.1	10.5	0.06851	4.093	52	23.3	0.01845	5.543	46	17.5	0.03821	5.348	39	26.7	0.01318	5.878	39
ILMN_1794965	ABCA4	NM_000350.1	15.1	0.03953	3.494	32	33.6	0.00922	6.532	32	18.6	0.03557	3.227	41	20.7	0.02240	4.791	35
ILMN_1722286	ABCA5	NM_172232.1	-3.6	0.67721	3.821	45	11.4	0.08037	5.226	40	-4.5	0.68511	2.566	36	11.1	0.05929	4.797	40
ILMN_1784176	ABCA5	NM_018672.2	0.4	0.38999	5.653	29	17.6	0.03162	7.680	29	-4.2	0.64559	3.635	41	16.3	0.03689	5.359	34
ILMN_1749001	ABCA5	NM_018672.2	6.8	0.13834	4.558	37	10.8	0.08564	4.798	38	5.2	0.20422	4.588	26	8.7	0.10013	5.040	35
ILMN_1701551	ABCA6	NM_172346.1	2.1	0.31489	3.944	30	4.2	0.23057	4.047	36	-2.7	0.56390	2.437	38	13.1	0.04875	3.446	38

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Data Analysis

Dragons Word Search - Find Hidden Wo

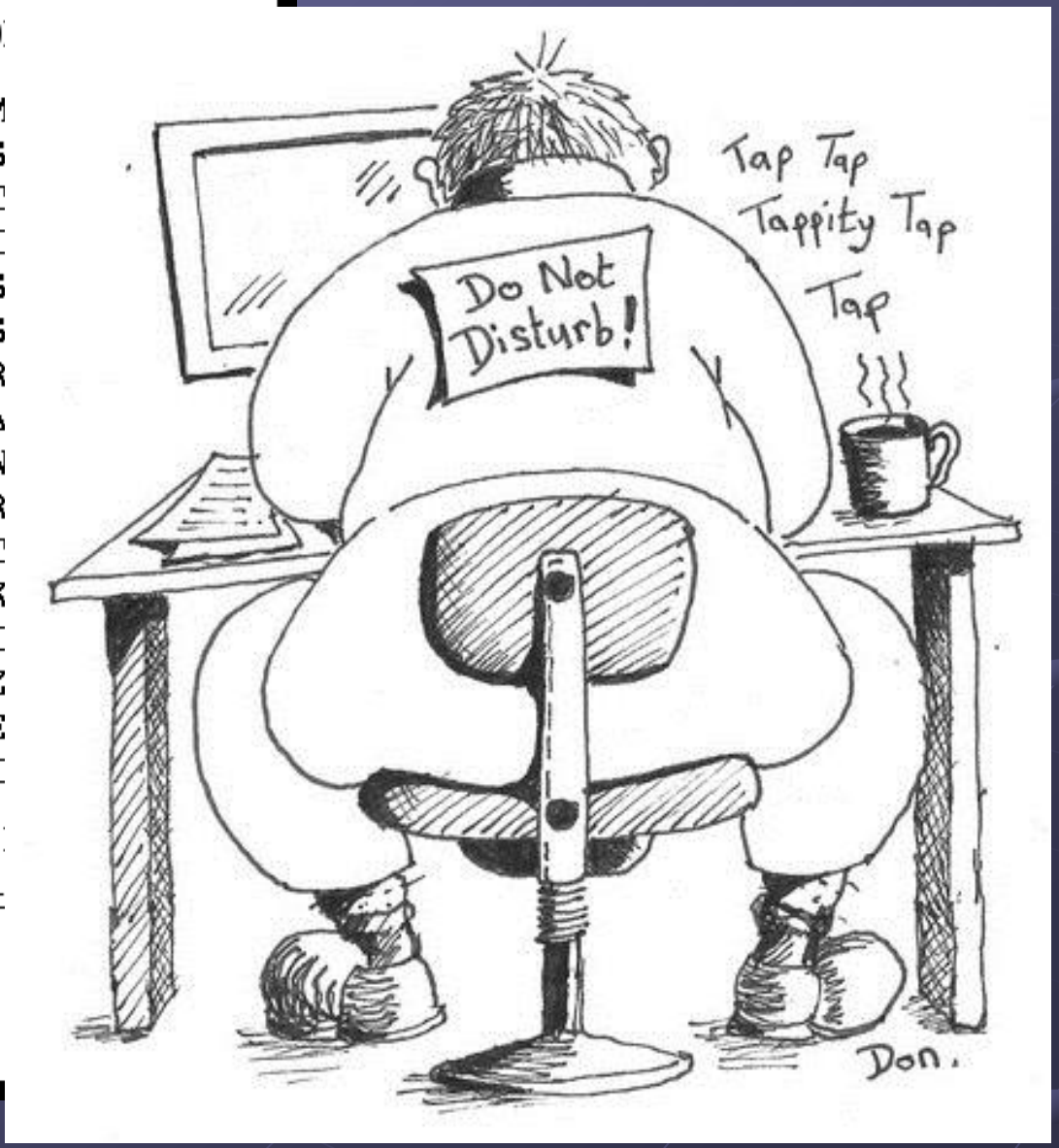
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dragons, breathing, fire, treasure, claws, slaying,

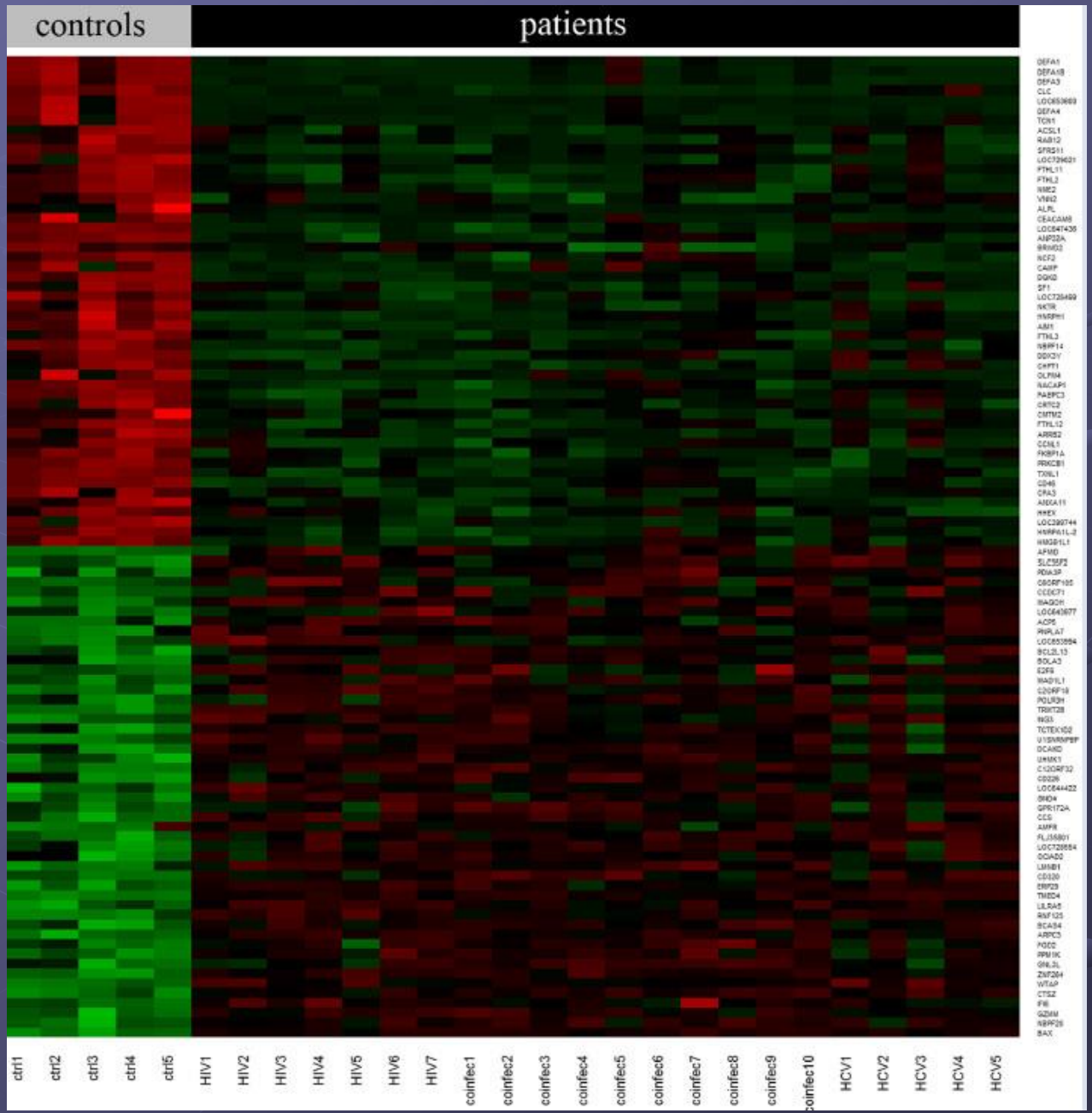
hard word search includes words spelled back

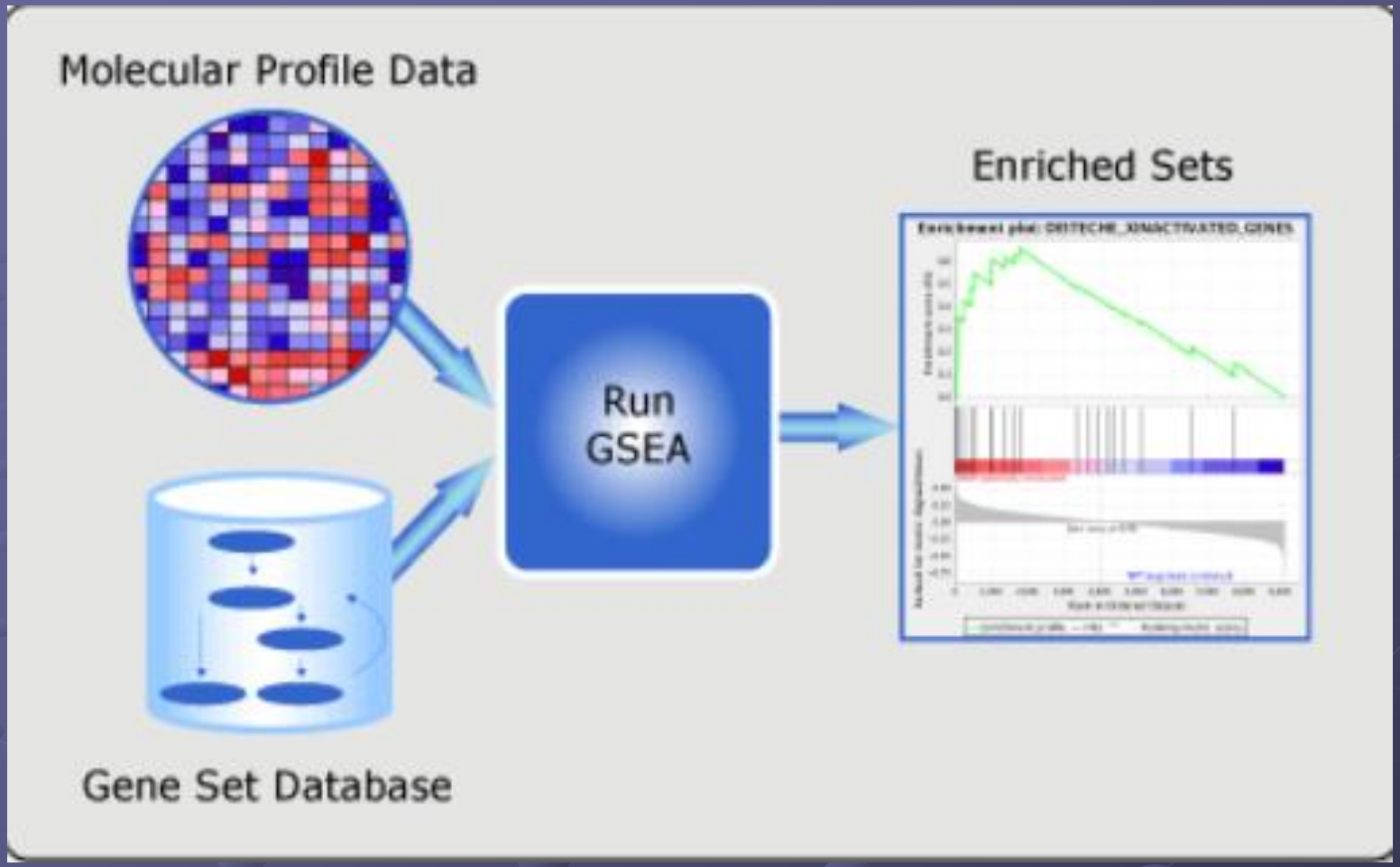
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(c) www.lucylearns.com



Between cases and controls

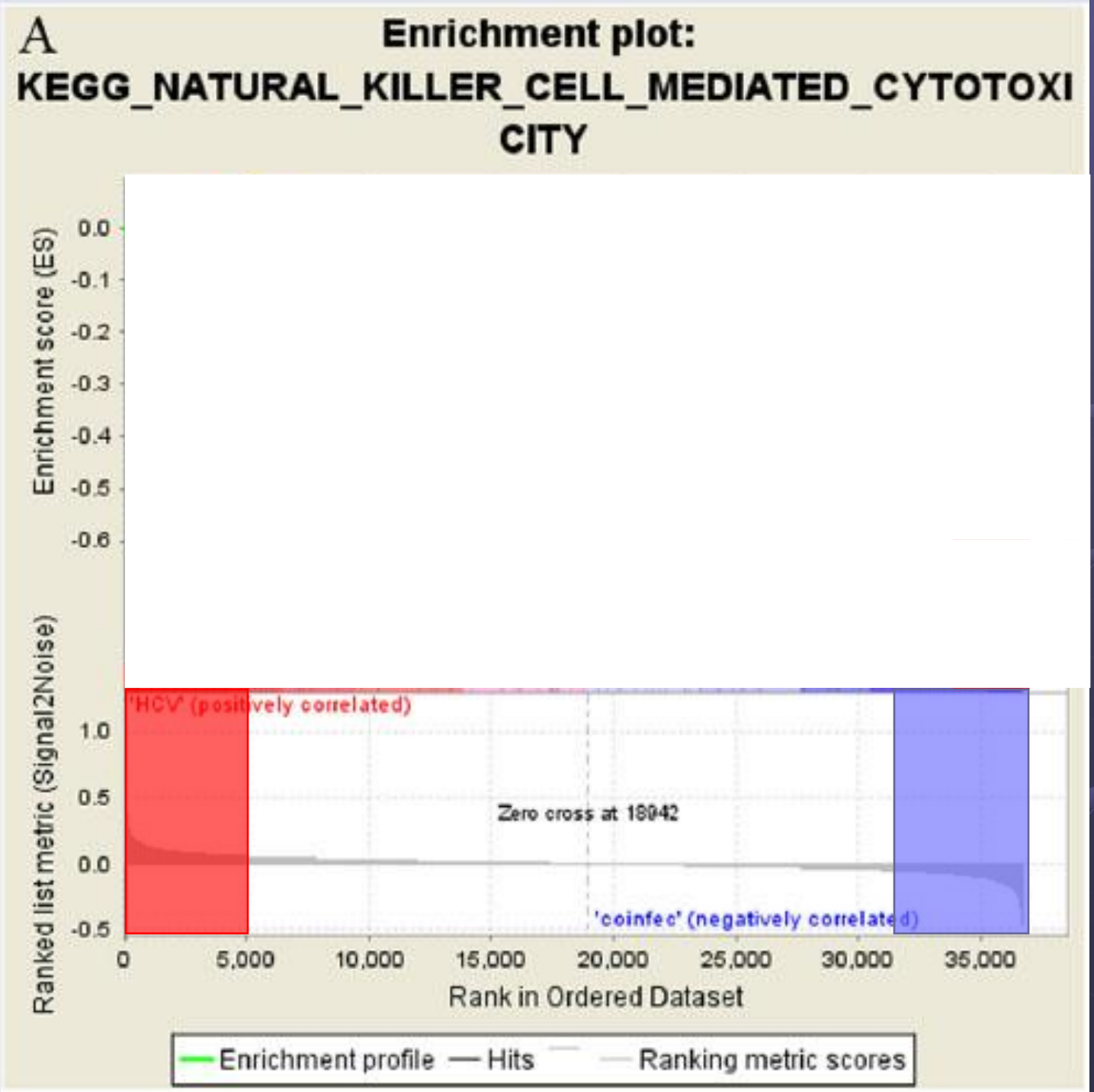




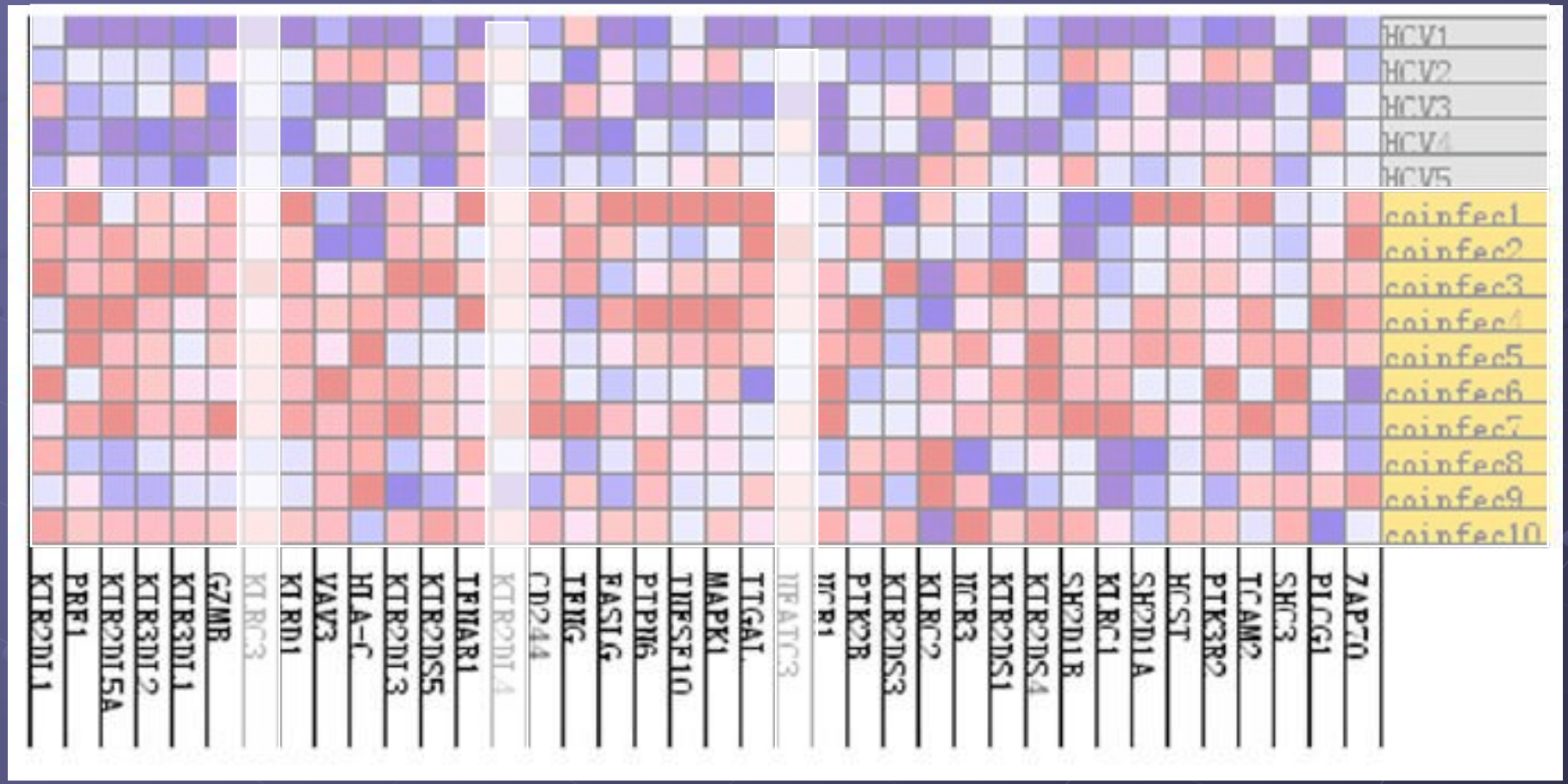
- Gene sets predefined by biological meanings
- To determine the association between members of a gene set and the phenotype group

Subramanian et al., 2005. PNAS. 102(43), 15545-15550.

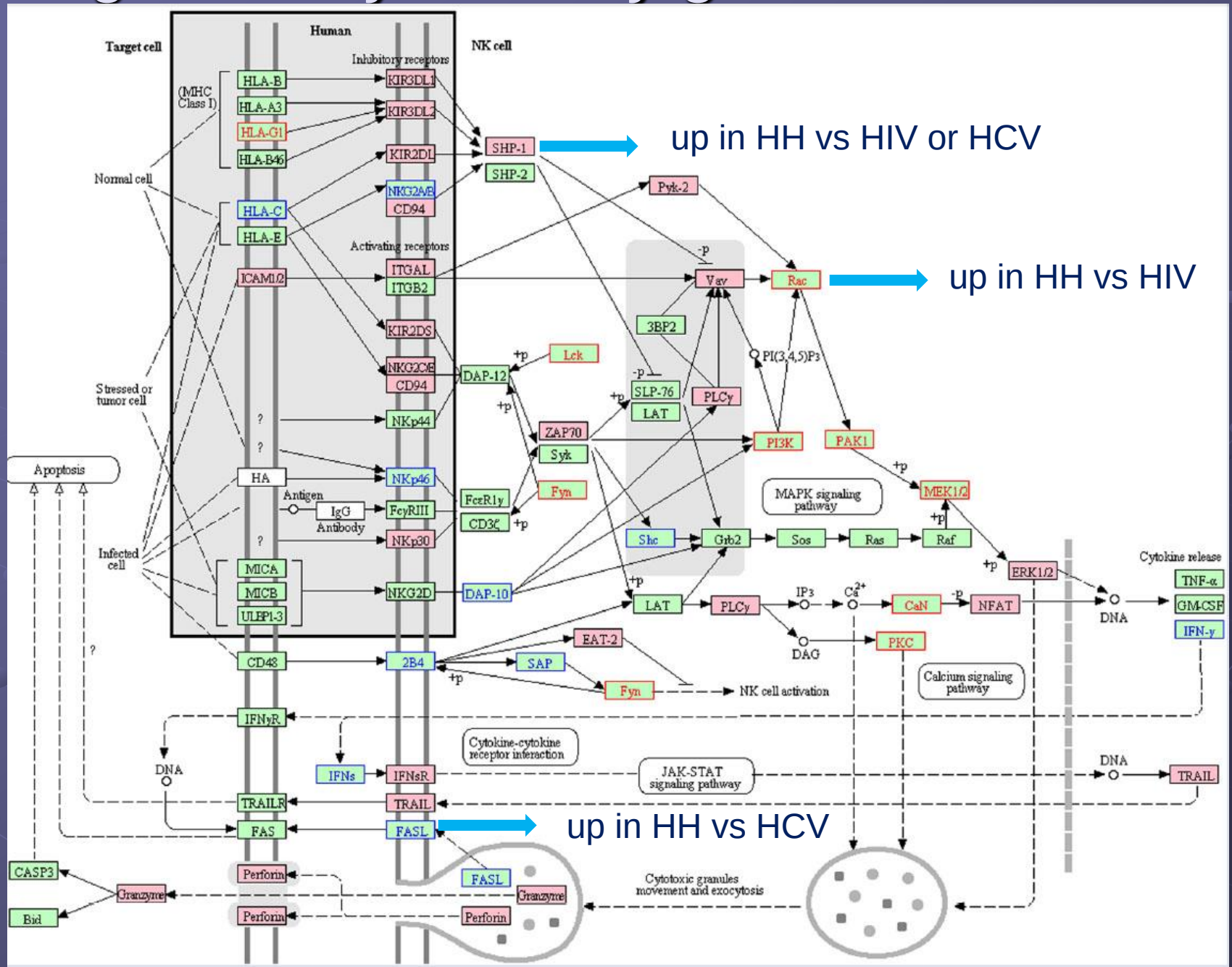
Results: Enrichment Score Plot



Core genes from cytotoxicity pathway



Up-regulated cytotoxicity genes in co-infection



Compensatory effect of cytotoxicity

- Differentiating between HH, HIV and HCV
- 4 KIR genes detected as core genes across 3 pairwise comparisons (KIR2DL1, KIR2DL3, KIR2DL5A, and KIR3DL2)
- KIR2DL3:HLA-C1 homozygosity had the protective effects in HCV+ individuals exposed to low inocula
  Khakoo SI, et al., 2004. Science. 305, 872-4.

Critical pathways dysregulated

Gene Set Name	Comp	Direc	FDR
Cytokine-cytokine receptor interaction	HIVvs.HH	HIV up	0.028
	HCVvs.HH	HCV up	0.003
Cell cycle	HIVvs.HCV	HIV up	0.016
WNT signaling	HIVvs.HCV	HCV up	0.006
P53 signaling	HIVvs.HH	HIV up	0.029
Renal cell carcinoma	HCVvs.HH	HCV up	0.036

FDR: false discovery rate

Conclusion

- Cytotoxicity pathway discriminates between HIV, HCV and HH
- KIR genes may be associated with specific virus-host interactions
- HIV and HCV have different impact on cell proliferation and differentiation

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Genome-wide mRNA and miRNA analysis of peripheral blood mononuclear cells (PBMC) reveals different miRNAs regulating HIV/HCV co-infection

P. Gupta [a], B. Liu [b,1], J.Q. Wu [b,d], V. Soriano [c], E. Vispo [c], A.P. Carroll [b], B.J. Goldie [b,d], M.J. Cairns [b,d,**], N.K. Saksena [a,*]

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ARTICLE INFO

Article history:
Received 11 September 2013
Returned to author for revisions
24 October 2013
Accepted 19 December 2013
Available online 14 January 2014

Keywords:
HIV
HCV
Co-infection
miRNA
Microarray

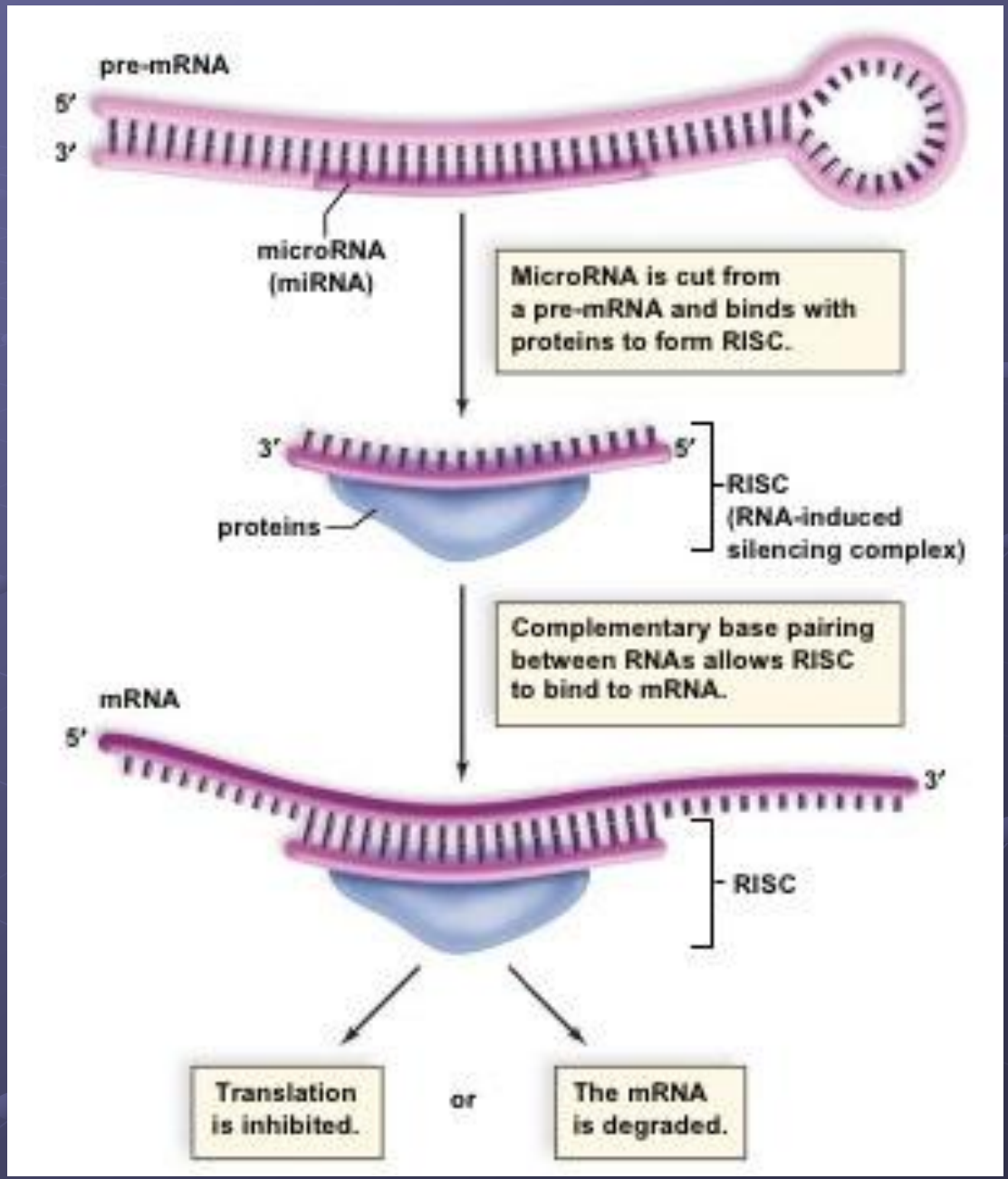
ABSTRACT

Co-infection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is common due to shared transmission routes. The genomic basis of HIV/HCV co-infection and its regulation by microRNA (miRNA) is unknown. Therefore, our objective was to investigate genome-wide mRNA expression and its regulation by miRNA in primary PBMCs derived from 27 patients (5 HCV – mono-infected, 5 HIV-mono-infected, 12 HCV/HIV co-infected, and 5 healthy controls). This revealed 27 miRNAs and 476 mRNAs as differentially expressed (DE) in HCV/HIV co-infection when compared to controls (adj $p < 0.05$). Our study shows the first evidence of miRNAs specific for co-infection, several of which are correlated with key gene targets demonstrating functional relationships to pathways in cancer, immune-function, and metabolism. Notable was the up regulation of HCV-specific miR-122 in co-infection (FC > 50, $p = 4.02E-06$), which may have clinical/biological implications.

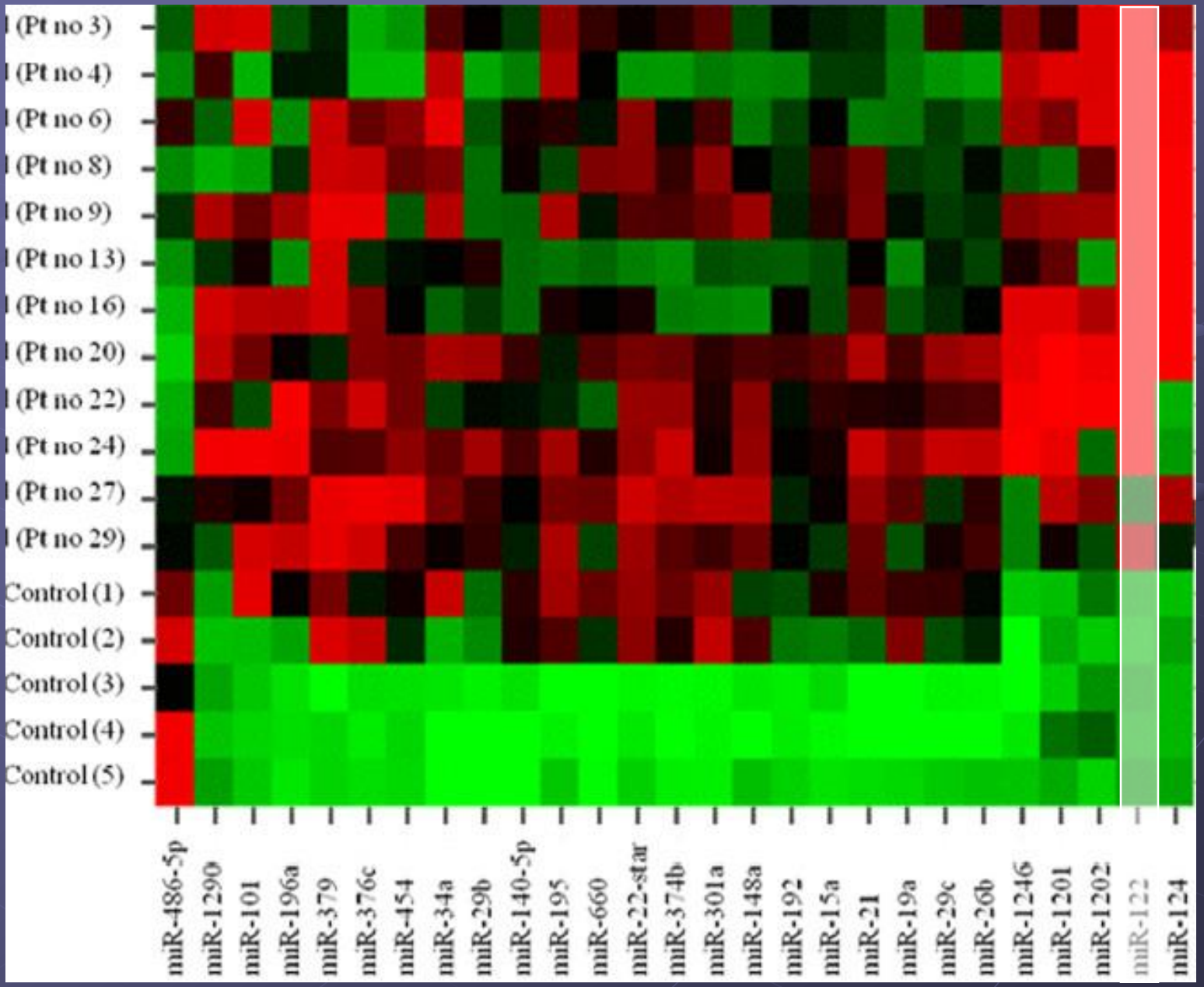
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microRNA regulating gene expression

- Short noncoding RNAs
- Bind to mRNAs
- Regulate protein Expression



Between co-infection and controls



miR-122

- Highest fold change, most significant p
- Liver specific cellular miRNA required for HCV replication
- miR-122 antagonist (Miravirsen) is the first miRNA-targeted drug

Virology 380 (2008) 124–135

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Transcriptional profiles in CD8+ T cells from HIV+ progressors on HAART are characterized by coordinated up-regulation of oxidative phosphorylation enzymes and interferon responses

Jing Qin Wu [a,1], Dominic E. Dwyer [b,2], Wayne B. Dyer [c,2], Yee Hwa Yang [d,3], Bin Wang [a,4], Nitin K. Saksena [a,*,5]

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ARTICLE INFO

Article history:
Received 21 May 2008
Returned to author for revision 9 June 2008
Accepted 25 June 2008
Available online 9 August 2008

Keywords:
Microarray
HIV
CD8+

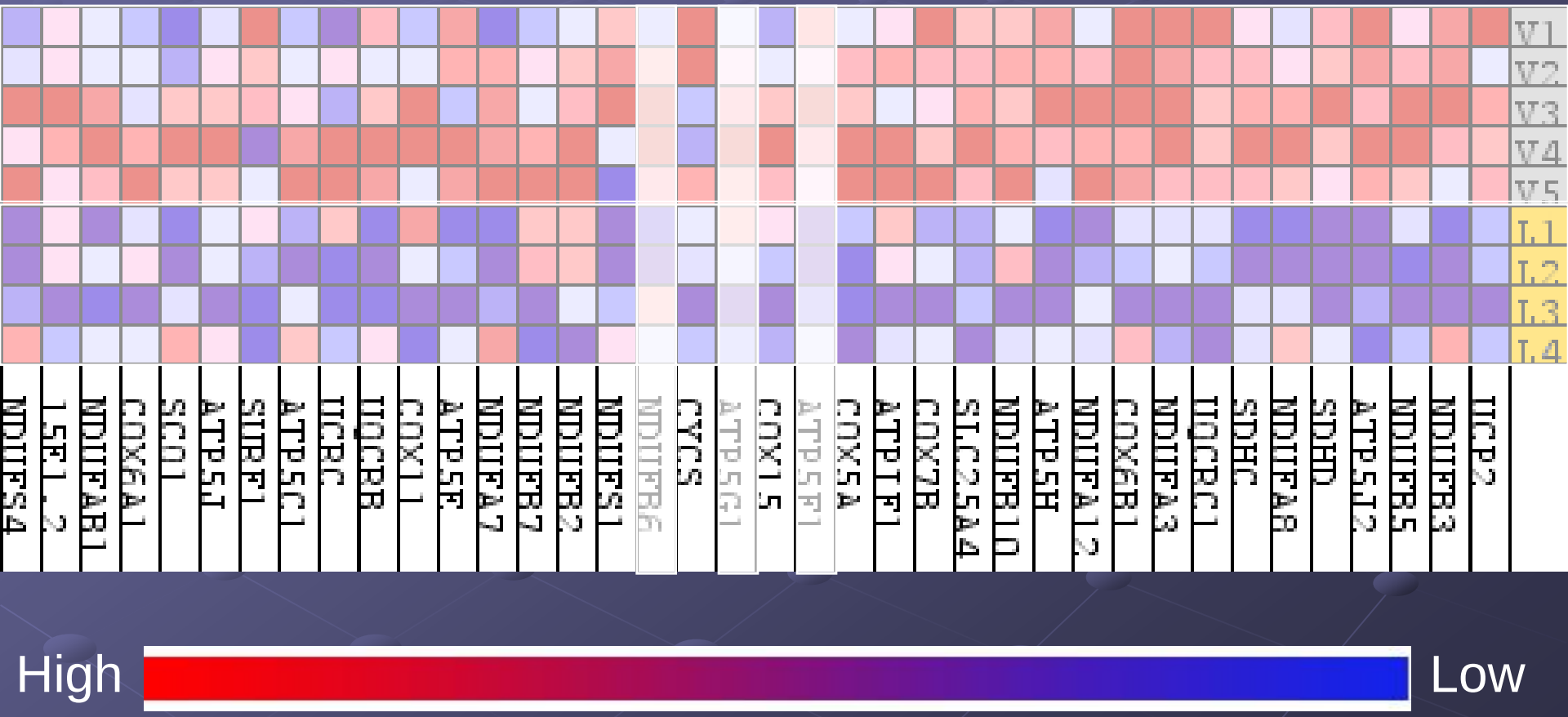
ABSTRACT

The functional impairment and numerical decline of CD8+ T cells during HIV infection has a profound effect on disease progression, but only limited microarray studies have used CD8+ T cells. To understand the interactions of HIV and host CD8+ T cells at different disease status, we used the Illumina Human-6 BeadChips to evaluate the transcriptional profile (>48,000 transcripts) in primary CD8+ T cells from HIV+ therapy-naive non-progressors and therapy-experienced progressors. 68 differentially expressed genes were identified, of which 6 have been reported in HIV context, while others are associated with biological functions relevant to HIV pathogenesis. By GSEA, the coordinated up-regulation of oxidative phosphorylation enzymes and interferon responses were detected as fingerprints in HIV progressors on HAART, whereas LTNP displayed a transcriptional signature of coordinated up-regulation of components of MAPK and cytotoxicty pathways.

Patient groups

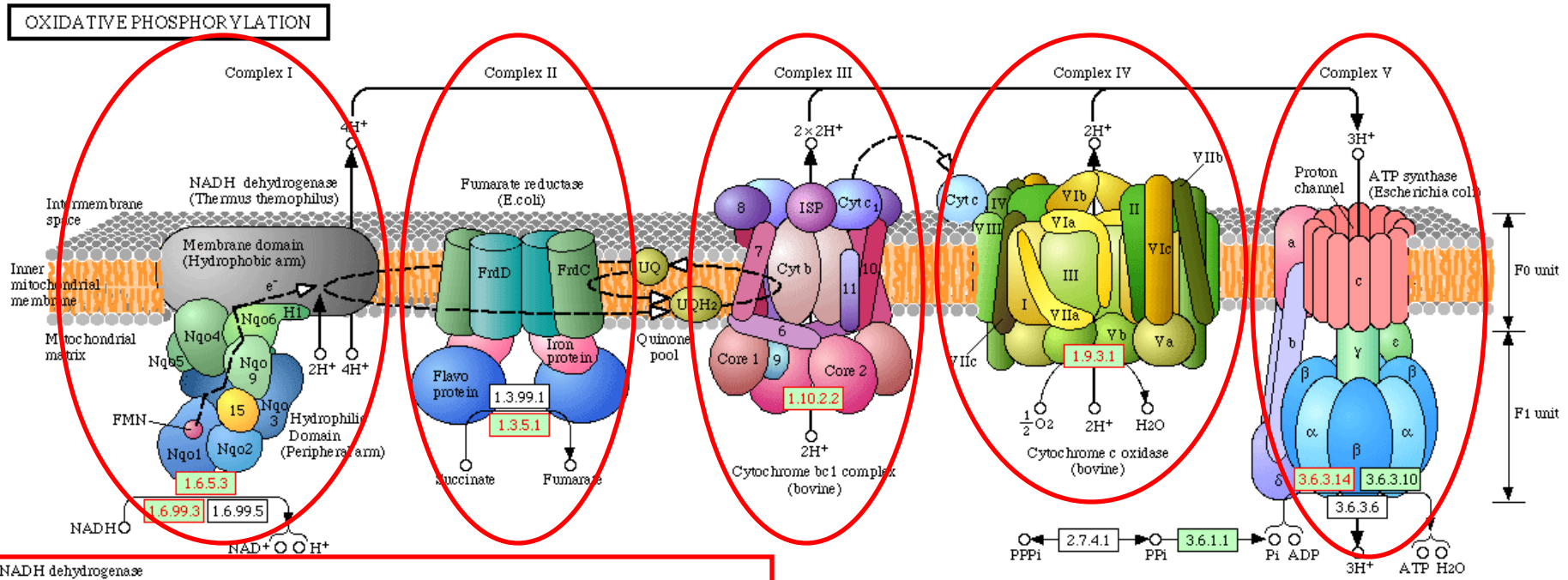
- Long-term non-progressors (LTNP; n=4)
  - Treatment naïve, > 20 years infection
  - CD4 > 500 cells/ul, HIV RNA < 50 copies/ml
- Progressors under therapy (VIR; n=5)
  - Under HAART, CD4 < 500, HIV RNA > 50
- HIV negative controls (NEG; n=5)

OXPHOS genes from electron transport chain



High — Low

Coordinately up-regulated OXPHOS genes



NADH dehydrogenase

E	ND1	ND2	ND3	ND4	ND4L	ND5	ND6													
E	Ndufs1	Ndufs2	Ndufs3	Ndufs4	Ndufs5	Ndufs6	Ndufs7	Ndufs8	Ndufv1	Ndufv2	Ndufv3									
B/A	NuoA	NuoB	NuoC	NuoD	NuoE	NuoF	NuoG	NuoH	NuoI	NuoJ	NuoK	NuoL	NuoM	NuoN						
B/E	NdhC	NdhK	NdhJ	NdhH	NdhA	NdhI	NdhG	NdhE	NdhF	NdhD	NdhB	NdhL	NdhM	NdhN	HoxE	HoxF	HoxU			
E	Ndufa1	Ndufa2	Ndufa3	Ndufa4	Ndufa5	Ndufa6	Ndufa7	Ndufa8	Ndufa9	Ndufa10	Ndufab1	Ndufa11								
E	Ndufb1	Ndufb2	Ndufb3	Ndufb4	Ndufb5	Ndufb6	Ndufb7	Ndufb8	Ndufb9	Ndufb10	Ndufc1	Ndufc2								

Succinate dehydrogenase / Fumarate reductase

E	SDHC	SDHD	SDHA	SDHB
B/A	SdhC	SdhD	SdhA	SdhB
			FrdA	FrdB	FrdC	FrdD

Cytochrome c reductase

E/B/A	ISP	Cyt b	Cyt c1					
E	COR1	QCR2	QCR6	QCR7	QCR8	QCR9	QCR10	

Cytochrome c oxidase

E	COX10		COX3	COX1	COX2	COX4	COX5A	COX5B	COX6A	COX6B	COX6C	COX7A	COX7B	COX7C	COX8	E/B/A	
																COX11	COX15	COX17
B/A	CyoE	CyoD	CyoC	CyoB	CyoA												
		CoxD	CoxC	CoxA	CoxB												
		QoxD	QoxC	QoxB	QoxA												

Cytochrome c oxidase, cbb3-type

B	I	II	IV	III

Cytochrome bd complex

B/A	CydA	CydB

F-type ATPase (Bacteria)

beta	alpha	gamma	delta	epsilon	c	a	b

F-type ATPase (Eukaryotes)

beta	alpha	gamma	OSCP	delta	epsilon	c	a
b	e	f6	f	8			
d	f	h	j	k	g		

V-type ATPase (Prokaryotes)

A	B	C	D	E	F	I	K

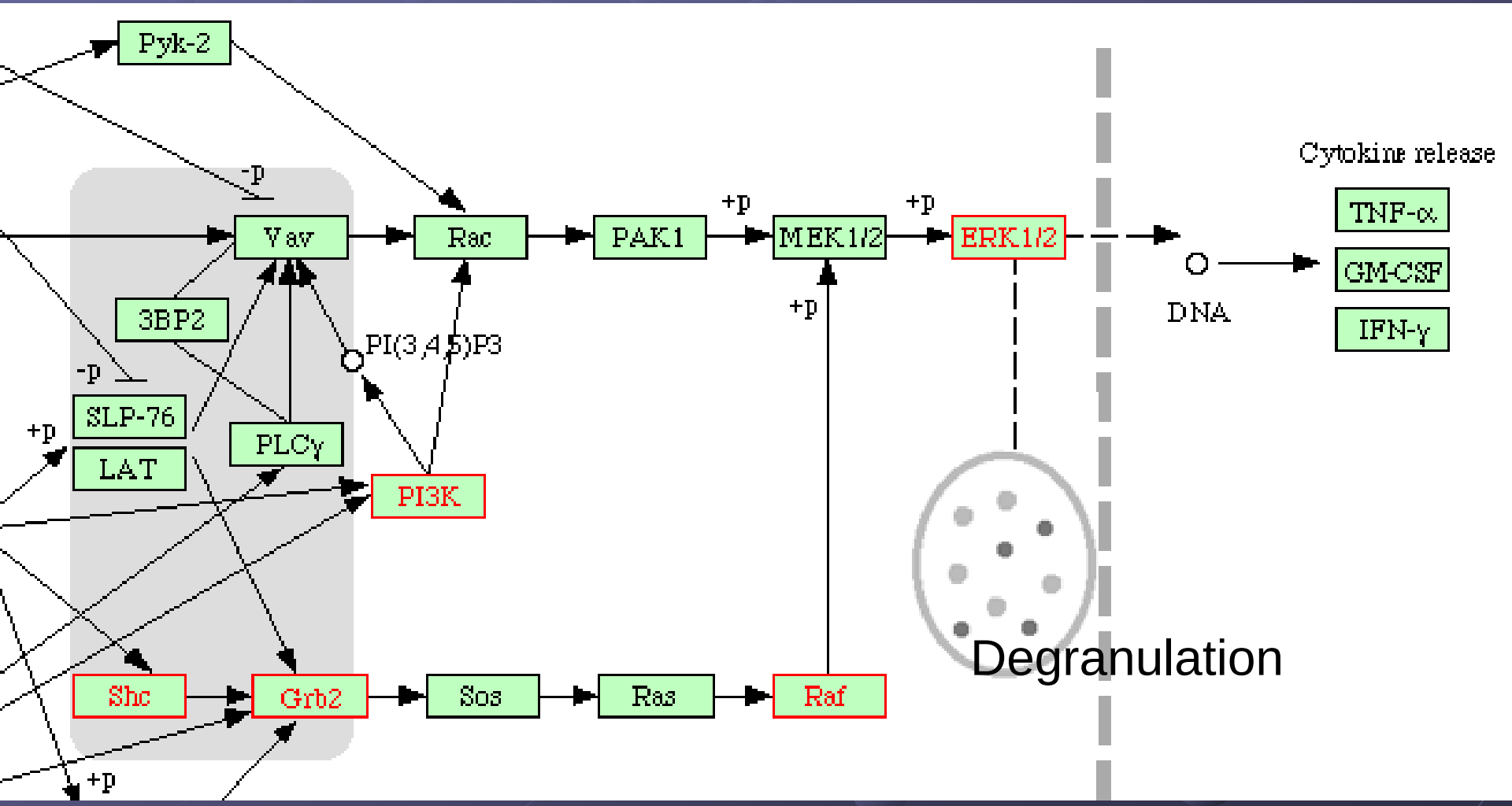
V-type ATPase (Eukaryotes)

A	B	C	D	E	F	G	H
I	AC39	54kD	S1	lipid			

Compensatory effect of mt toxicity

- Backbone of HAART: NRTIs
  - Block DNA polymerase gamma required for mtDNA synthesis
  - Impair adenylate kinase and ADP/ATP translocator
- Lead to mt dysfunction and depletion
- Coordinate up-regulation of OXPHOS genes observed in NRTI treated cell lines
  Divi et al., 2007. Environ. Mol. Mutagen. 48(3-4), 179-189.

Coordinately up-regulated genes of cytotoxicity pathway in LTNP



Conclusion

- VIR under HAART characterized by
  - Coordinately up-regulated OXPHOS genes
  - Interferon responses
- LTNP: coordinately up-regulated components of the cytotoxicity pathway

JOURNAL OF VIROLOGY, Oct. 2008, p. 10326–10327 Vol. 82, No. 20
0022-538X/08/$08.00+0 doi:10.1128/JVI.01386-08
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Letter to the Editor

Transitory Viremic Surges in a Human Immunodeficiency Virus-Positive Elite Controller Can Shift the Cellular Transcriptome Profile: a Word of Caution for Microarray Studies▽

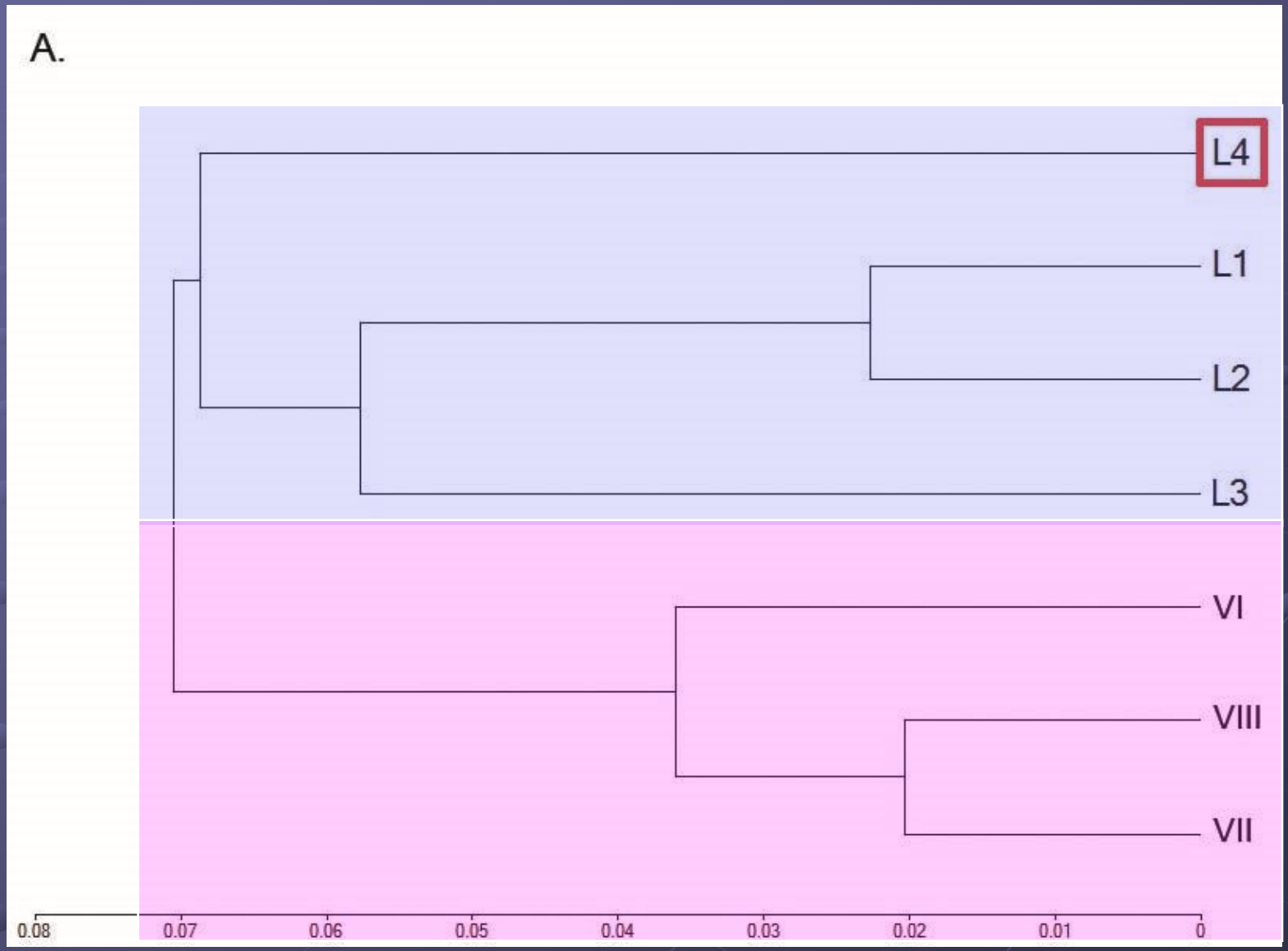
Human immunodeficiency virus (HIV) is capable of infecting diverse blood leukocytes, through which interaction between HIV and cellular transcriptome can be unveiled. Recently, the transcriptional profiles of primary $CD4^+$ and $CD8^+$ T cells from HIV-positive individuals have been shown to distinguish progression from nonprogression (3). This study, together with our study on cell surface antigens using a protein microarray (8), has demonstrated that the $CD8^+$ T-cell transcriptome and its surface are more informative in differentiating HIV disease groups than the $CD4^+$ T cells. This is consistent with our recent findings, which showed that the whole-genome transcriptome in primary $CD8^+$ T cells was able to segregate HIV-positive viremic (VIR) patients on highly active antiretroviral therapy (HAART) from therapy-naïve long-term nonprogressors (LTNPs) (7). Here, we wish to highlight how a subtle surge in plasma viremia in HIV-positive LTNPs can affect the clustering pattern of the cel

TABLE 1. Clinical details of study patients

Time point and participant[a]	Viral load (copies/ml)	CD4 count (cells/μl)	CD8 count (cells/μl)	Collection date (mo/day/yr)
Time point 1				
VIR				
VI	98	617	688	6/6/06
VII	11,500	230	560	6/14/06
VIII	53,900	350	900	6/14/06
LTNP				
L1	<50	690	650	6/28/06
L2	<50	817	513	6/28/06
L3	<50	880	860	7/18/06
L4	<50	760	1,800	7/21/06

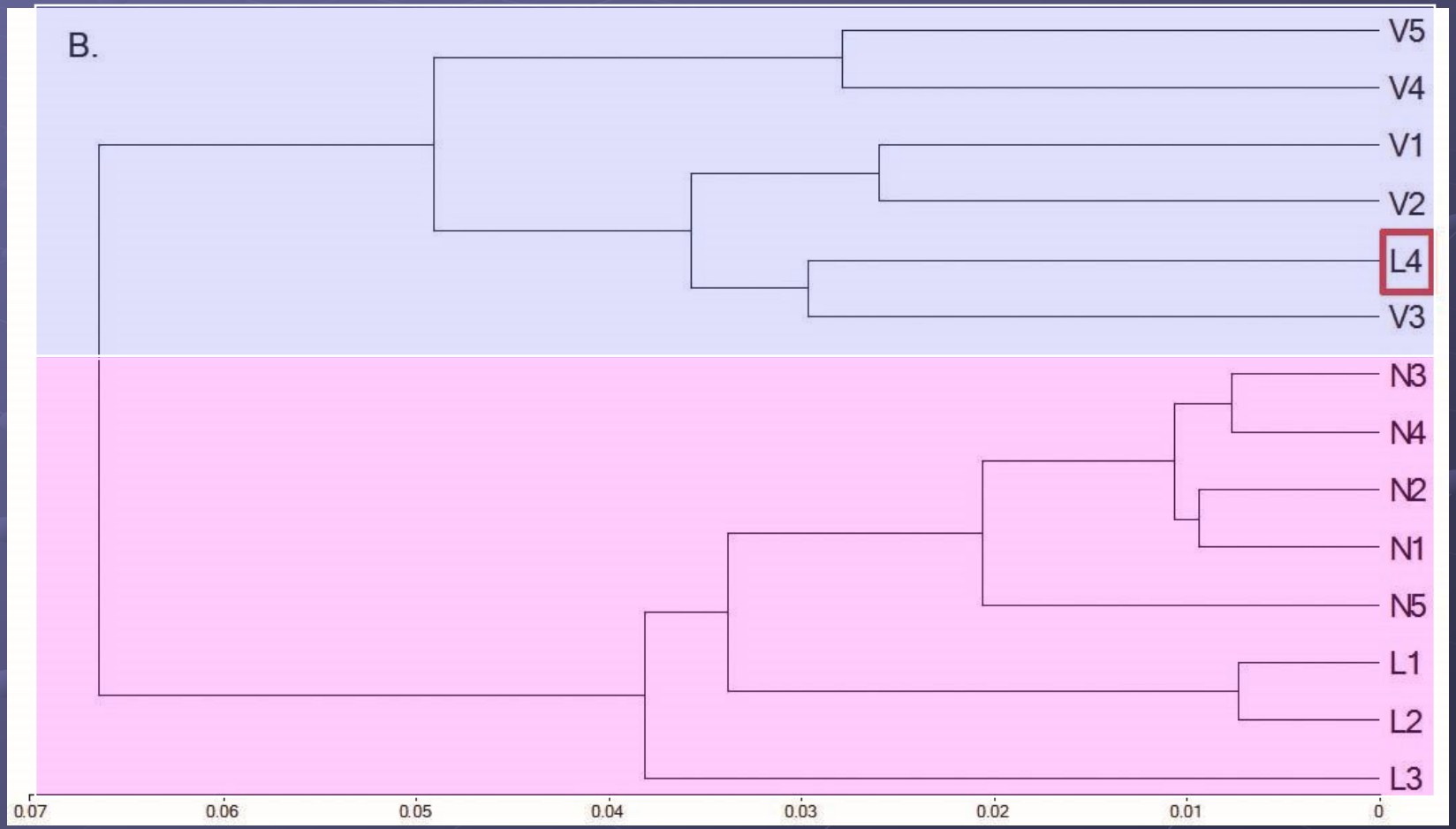
Results: Cluster analysis

Hierarchical clustering of global gene expression using Pearson correlation metric and correlation agglomeration method



Results: Cluster analysis

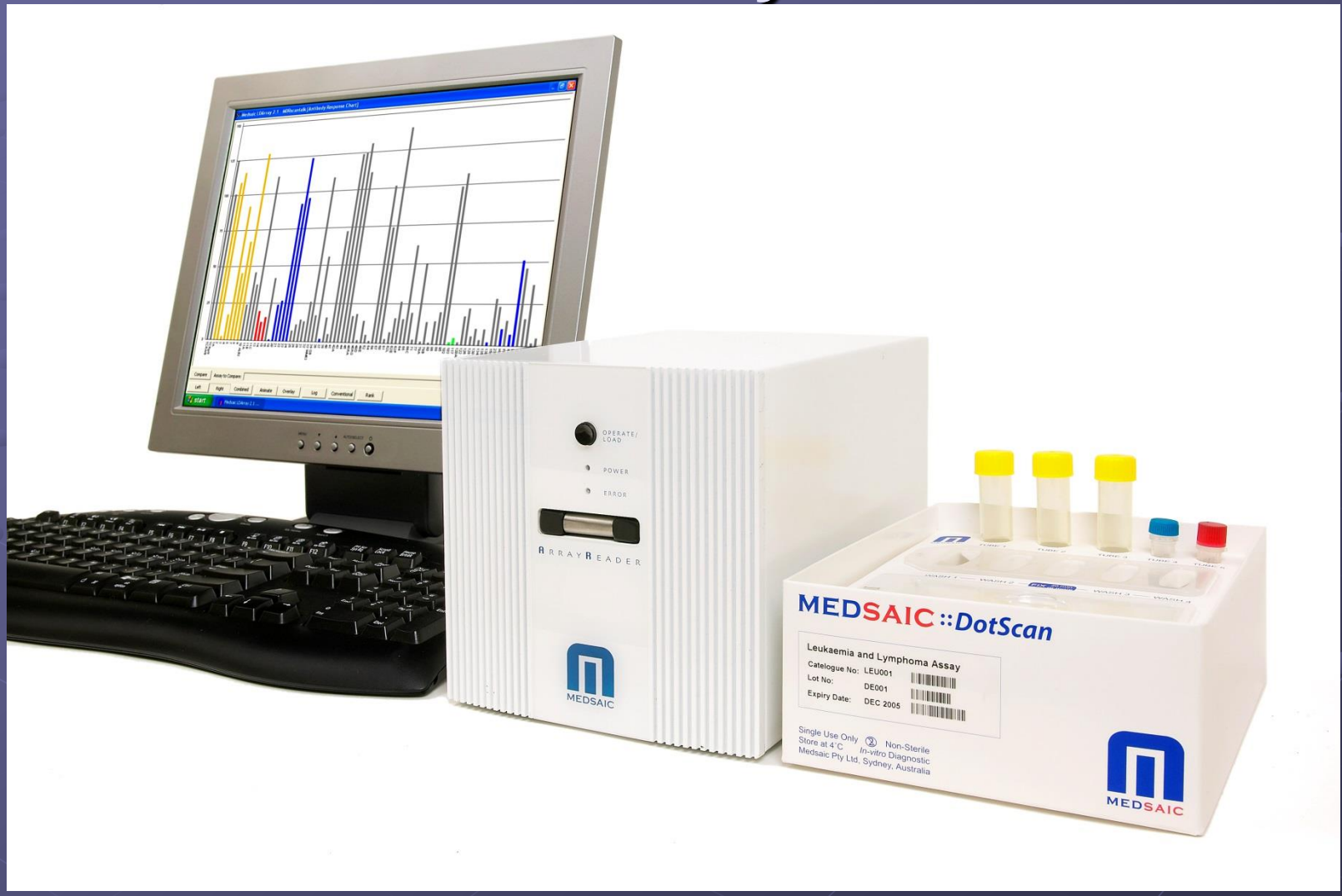
Hierarchical clustering of global gene expression using Pearson correlation metric and correlation agglomeration method



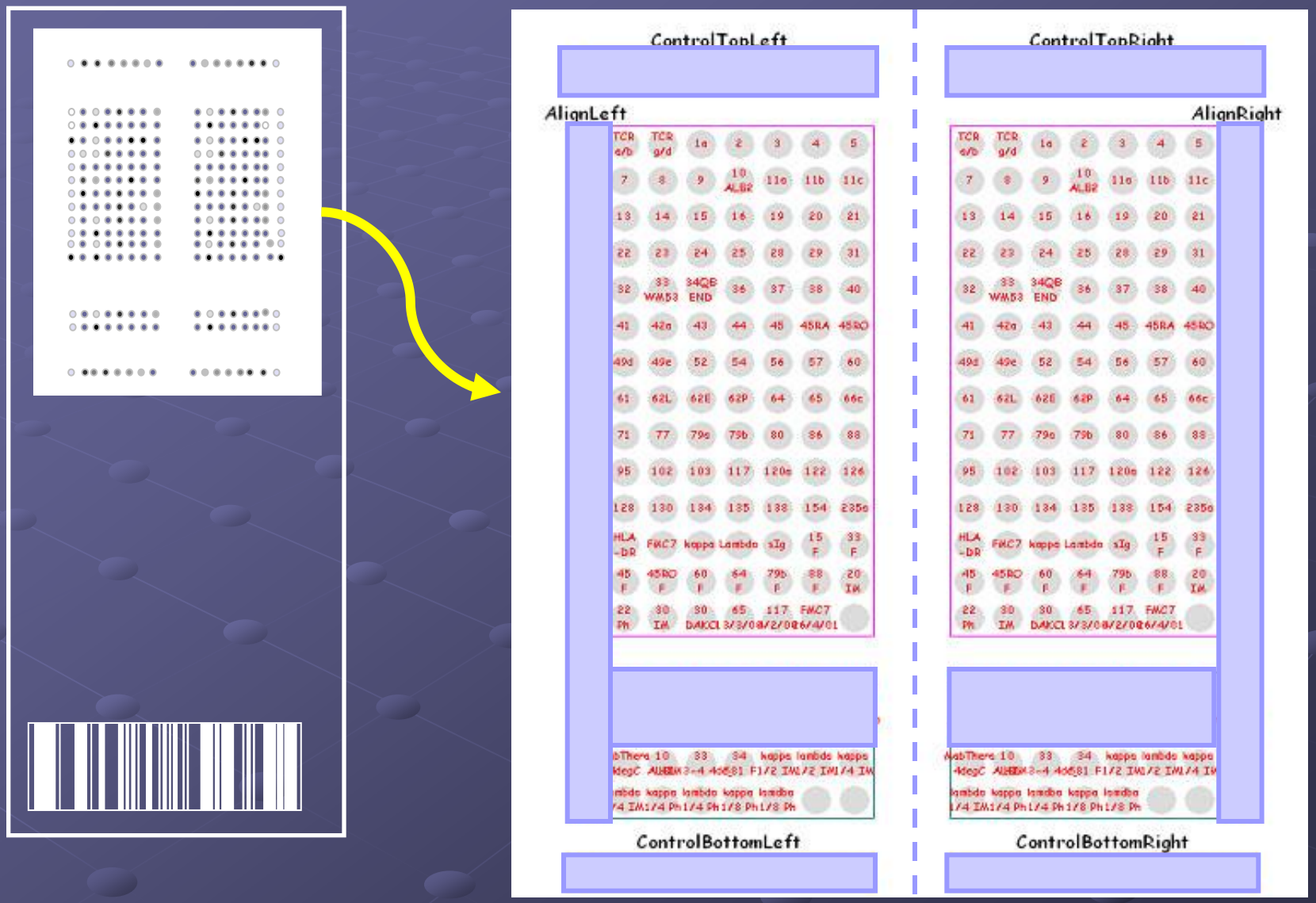
Conclusion

- Plasma viremia is a key determinant of the clustering pattern between VIR and LTNPs
- For microarray studies, current viral load should always be determined for patient samples
- A viral load threshold may exist, above which the transcriptome profile can shift

Antibody Microarray DotScan System



DotScan Chip



Retrovirology

BioMed Central

Research

Highly accessed Open Access

Antibody microarray analysis of cell surface antigens on CD4+ and CD8+ T cells from HIV+ individuals correlates with disease stages

Jing Qin Wu[1], Bin Wang[1], Larissa Belov[2], Jeremy Chrisp[2], Jenny Learmont[3], Wayne B Dyer[3], John Zaunders[4], Anthony L Cunningham[1], Dominic E Dwyer[5] and Nitin K Saksena*[1]

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Published: 26 November 2007

Retrovirology 2007, **4**:83 doi:10.1186/1742-4690-4-83

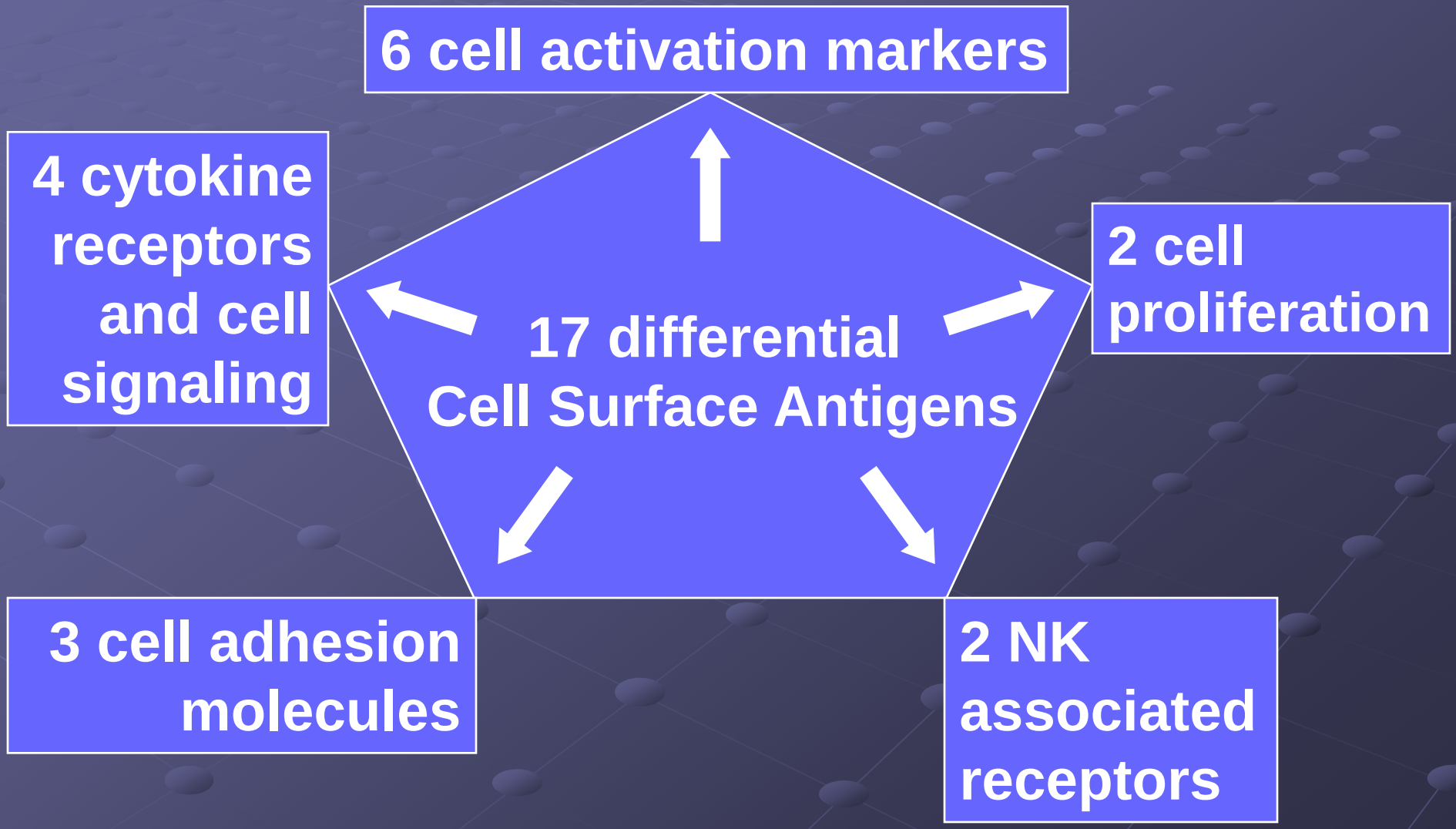
This article is available from: http://www.retrovirology.com/content/4/1/83

Received: 24 August 2007
Accepted: 26 November 2007

Patient Groups

- LTNP: HIV+, remain healthy for > 18 years, treatment naïve (natural controller)
- BDL: plasma viremia below detectable levels (<50 copies/ml plasma), patients on HAART (control of viremia with therapy)
- Viremia: various degrees of plasma viremia, patients on HAART (possible treatment failure)
- HIV negative control: healthy donors

Summary


6 cell activation markers
4 cytokine receptors and cell signaling
2 cell proliferation
17 differential Cell Surface Antigens
3 cell adhesion molecules
2 NK associated receptors

Summary

- Out of 17 statistically significant cell surface antigens, 5 are novel: CD212b1, CD218a, CD183, CD3epsilon and CD9.
- Changes in activation marker expression were more pronounced in CD8+ T cells, changes in cell membrane receptors were more pronounced in CD4+ T cells.

Retrovirology

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Research

Highly accessed Open Access

Longitudinal microarray analysis of cell surface antigens on peripheral blood mononuclear cells from HIV+ individuals on highly active antiretroviral therapy

Jing Qin Wu[1], Wayne B Dyer[2], Jeremy Chrisp[3], Larissa Belov[3], Bin Wang[1] and Nitin K Saksena*[1]

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Published: 4 March 2008

Retrovirology 2008, **5**:24 doi:10.1186/1742-4690-5-24

Received: 29 November 2007
Accepted: 4 March 2008

This article is available from: http://www.retrovirology.com/content/5/1/24

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Patient Samples

- 12 patients, 2 groups:
  - Sustained Responder: steady control of viral load (n=6)
  - Transient Responder: frequent rebounding of viral load under HAART (n=6)
- 33 time points for 3 years, 5-8 points for microarray
- 4 time points for analyzing time-related changes

Time Point 1: Initiation date of therapy

Time Point 2: ~1 year of therapy

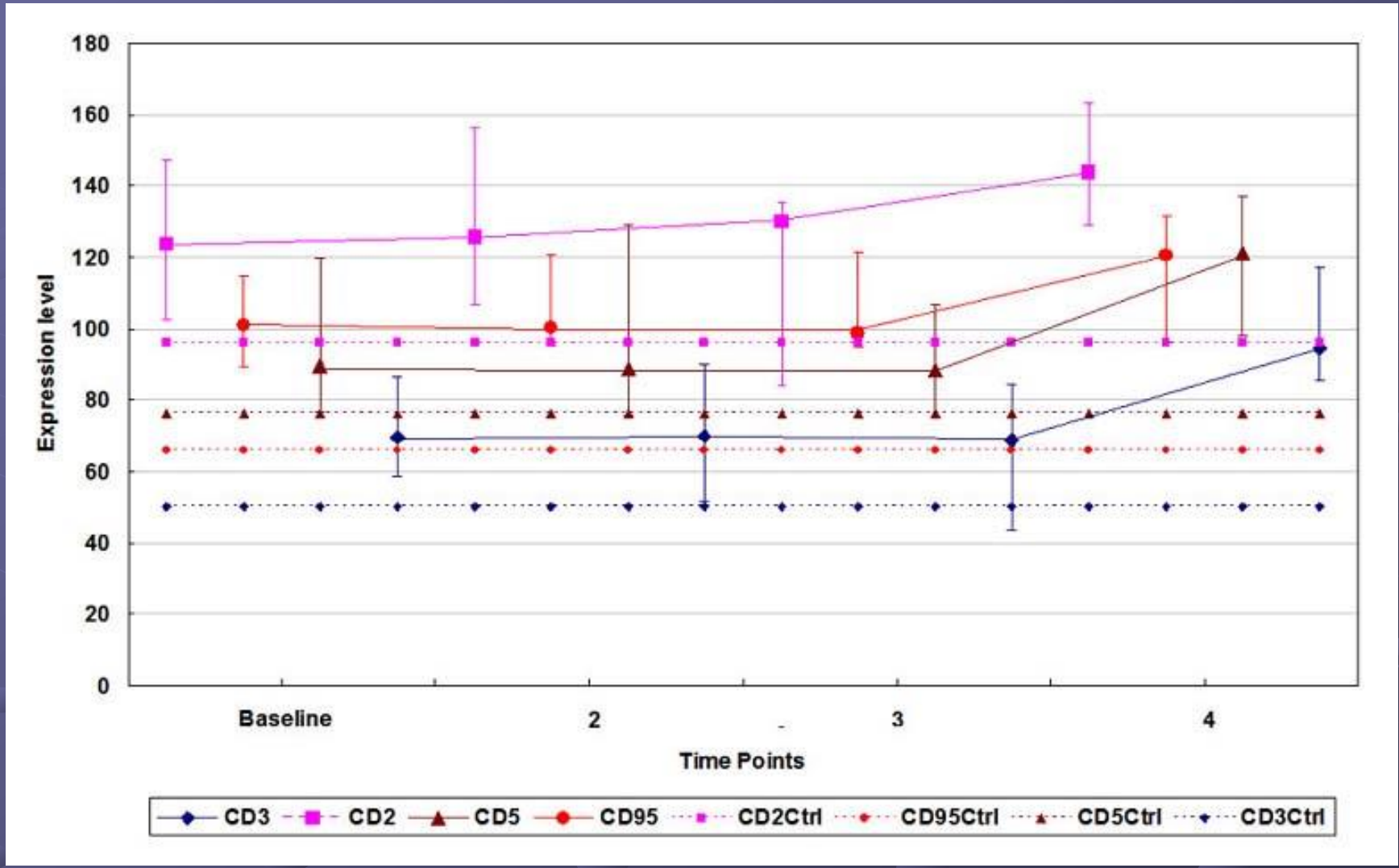
Time Point 3: ~1-1.5 year of therapy

Time Point 4: >= 2 years of therapy

Summary

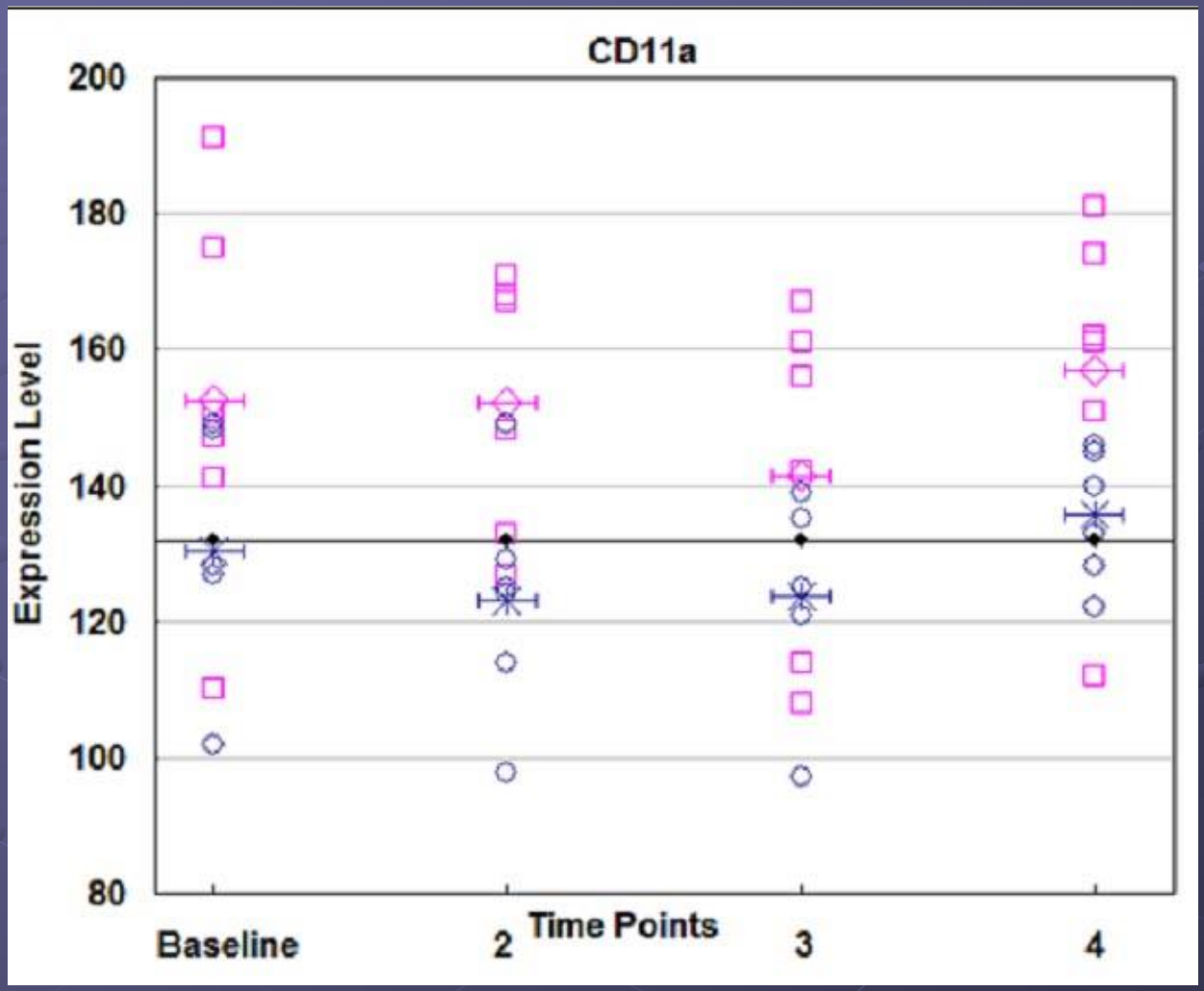
- 7 antigens showed a trend over time
  CD2, CD3, CD5, CD95, CD27, CD28 and CD36
- 10 antigens differentiate between groups
  CD11a, CD29, CD38, CD45RO, CD52
  CD56, CD57, CD62E, CD64 and CD33
- 4 antigens positively correlate with CD4 counts:
  CD9 CD11a CD27 CD28
  5 cell surface antigens negatively correlate with CD8 counts: CD52 CD44 CD49d CD49e CD11c

Results: Antigen expression that shows a trend ($p<0.05$)



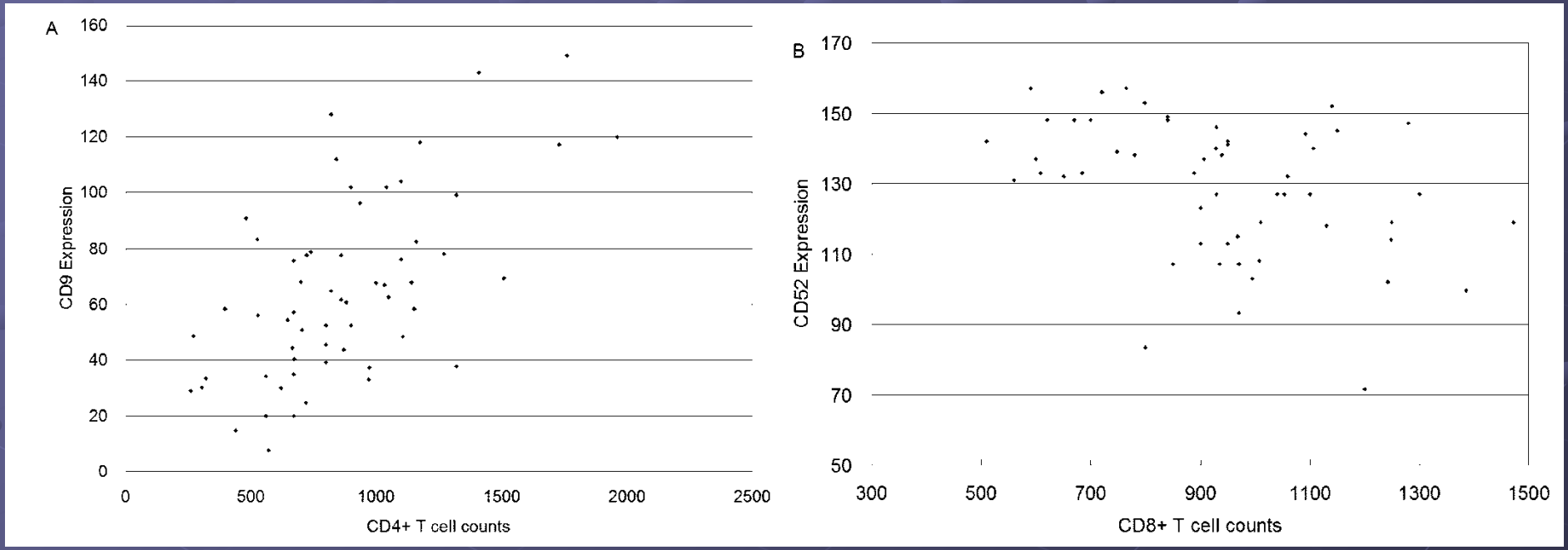
Median values linked by solid lines; bars: 25th and 75th quartile values.
Dashed line: mean values of healthy controls.

Results: Antigens that differentiate SR and TR (p<=0.003)



Pink squares: SR; Blue circles: TR; Bars: group mean value; solid line: NEG.

Results: correlation (p<=0.001)



Acknowledgements

- Dr. Nitin Saksena and Dr. Bin Wang from Uni of Sydney
- Dr. Vincent Soriano and Dr. Eugenia Vispo from Hospital Carlos III, Madrid, Spain
- Dr. Dominic Dwyer and Dr. Choo Beng Chew from Westmead hospital, Sydney
- Dr. Wayne B. Dyer , Blood Bank
- Dr. Jenny Learmont, Blood Bank
- Dr. Yee Hwa Yang, Sydney Bioinformatics
- Dr. Jeremy Chrisp, MedSaic
- Dr. Mervyn Thomas, MedSaic
- Dr. Larissa Belov, MedSaic