

***Molecular profiling of diffuse large B-cell lymphoma
identifies robust subtypes
including one characterized by host inflammatory response***

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**Diffuse Large B-cell Lymphoma
(DLBCL)**

- Most common lymphoid malignancy (~40%)
- Significant clinical & genetic heterogeneity
- Hypothesis:
 - gene expression profiling will reveal disease heterogeneity

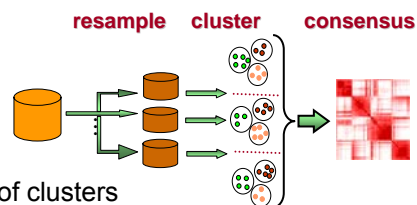
DLBCL expression profiling

- 176 DLBCL samples.
- Nodal biopsies from untreated patients.
- Affymetrix U133A/B chips (~42K probes)
- **Goal:** unsupervised analysis to discover novel substructure

Analysis of DLBCL Substructure by unsupervised analysis

➤ Consensus clustering

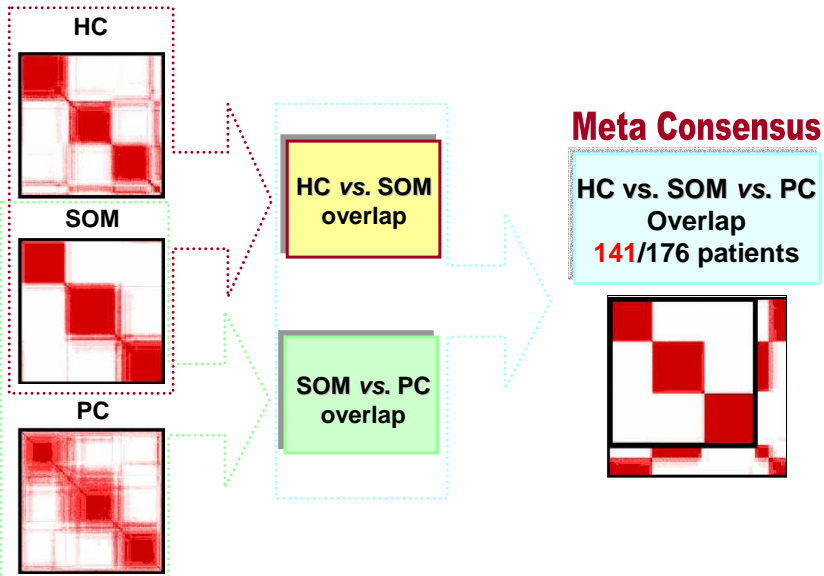
- identifies **robust** clusters
- **resampling-based** method
- automatically selects the **number** of clusters



➤ Used with 3 different clustering algorithms

- Hierarchical clustering (HC)
- Self-organizing Map (SOM)
- Probabilistic Clustering (PC)

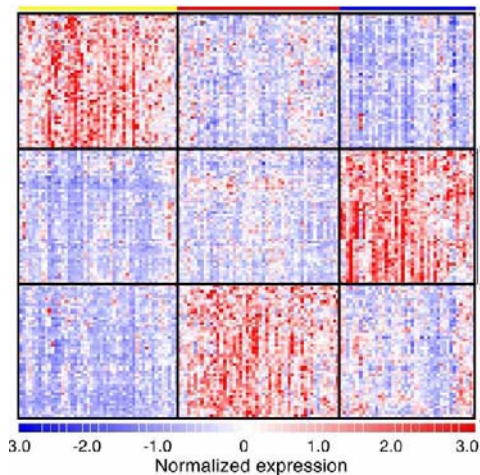
Consensus Clustering of DLBCL



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DLBCL Consensus Clusters

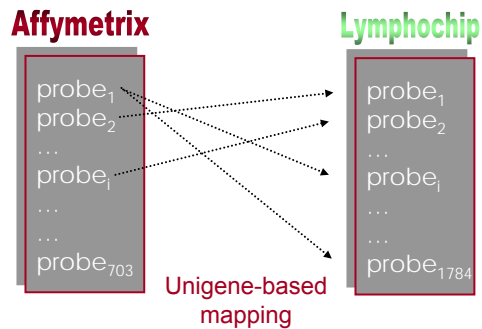
OxPhos BCR/Proliferation Host Response



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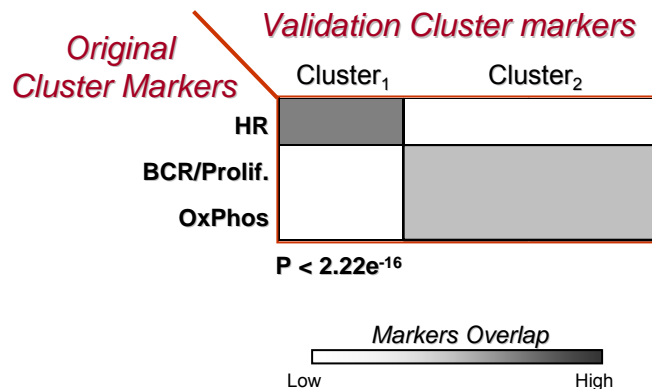
Validation of Consensus Clusters on Independent Database

- 221 DLBCL samples on Lymphochip [Rosenwald, et al., NEJM 2003]
- Cross-platform mapping



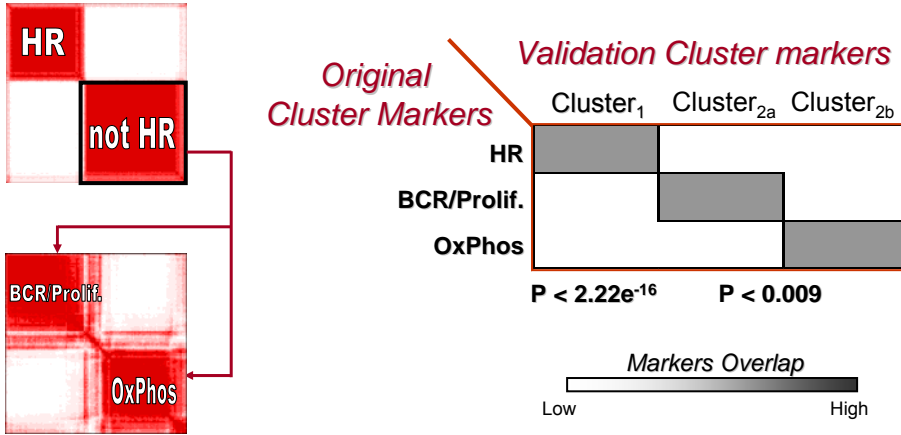
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Validation of Consensus Clusters on Independent Database

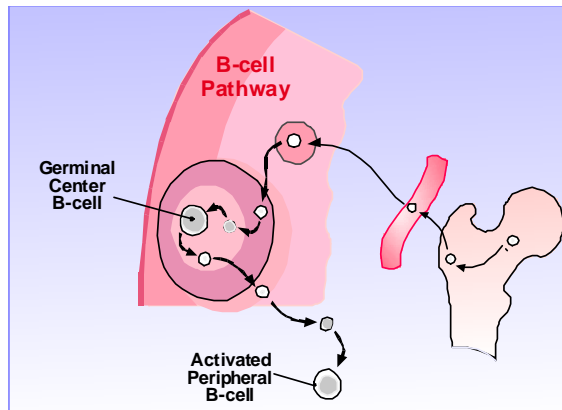


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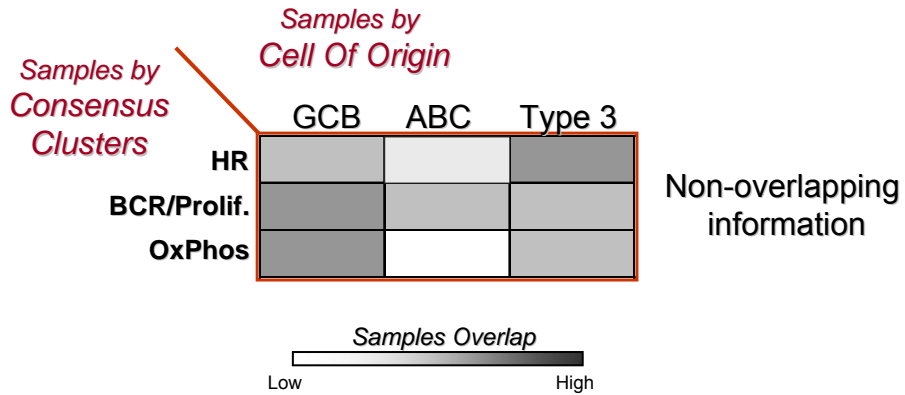
Validation of Consensus Clusters on Independent Database



Consensus Clusters and Cell Of Origin



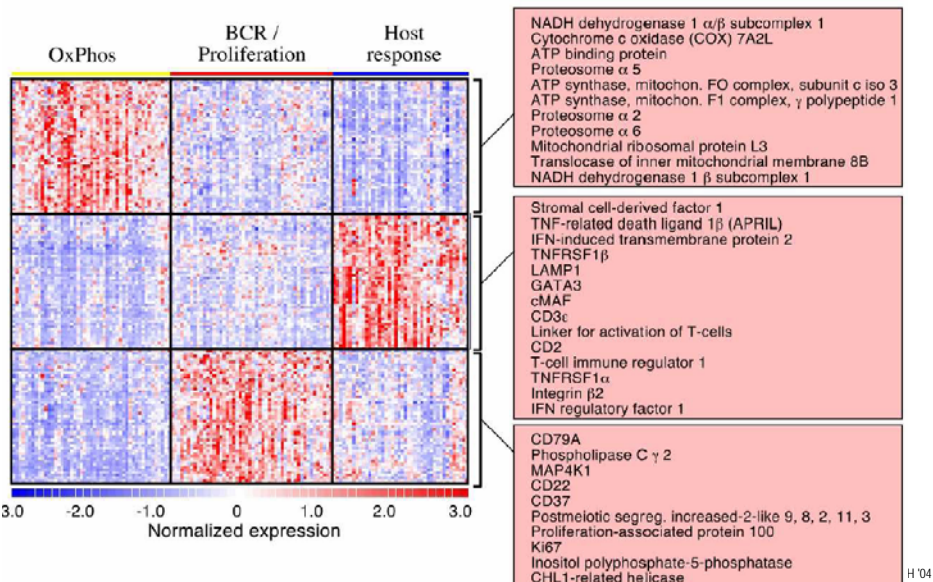
Consensus Clusters and Cell Of Origin



COO assignment based on methodology described in [Wright, et al., PNAS 2003].

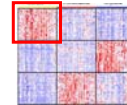
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DLBCL Consensus Clusters



OxPhos cluster

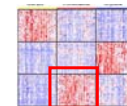
- Genes involved in **oxidative phosphorylation** ($p \leq .002$) and **mitochondrial function** ($p \leq .003$)
 - **BFL-1/A1** (anti-apoptotic BCL-2 family member)
 - members of **NADH dehydrogenase** complex
 - members of the **COX** complex
 - **ATP synthase** components



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BCR/Proliferation cluster

- BCR signaling components
 - CD19, IG, CD79a, BLK, SYK, PLCgamma2, MAP4K
- B-cell transcription factors
 - PAX5, OBF-1, E2A, BCL6, STAT6, MYC
- Cell cycle regulatory genes
 - CDK2, MCM
- DNA repair genes
 - PMS2, H2AX, PTIP, p53



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Host Response (HR) cluster

signature largely defined by the associated host response rather than the tumor itself

- Components of TCR (TCR α/β , CD3), CD2, T/NK cell activation, and complement cascade.
- Co-regulated inflammatory mediators
- More abundant monocyte/macrophage & dendritic cell transcripts
- Interferon-induced genes, TNF ligands/receptors, cytokine receptors.



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Tumor Infiltrating Lymphocytes (TILs)* (morphology)

Consensus clusters

> 20 TILs/HPF

HR

65%

BCR/proliferation

14%

OxPhos

11%

p < .0001

* Morphologically normal (CD2+) lymphocytes with round/oval nuclei and delicately dispersed chromatin.

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TILs and Dendritic Cells in HR tumors (immunostaining)

- Increased # of CD2⁺/CD3⁺ T-cells (p≤.005)
- Increased # of GILT⁺ dendritic cells (DC) (p=.06)
 - Interdigitating DCs (S100⁺, CD1a⁻ CDC123⁻), (p<.009)
 - correlated with TILs (p<.0001)

HR tumors and T-cell/histiocyte-rich LBCLs

- HR tumors appear
 - in younger patients (p=0.04);
 - with higher incidence of splenic (p=0.02) and BM involvement (p=0.03).
- 8/10 patients with T-cell/Histiocyte-rich LBCLs fall in the HR cluster

Genetic abnormalities in DLBCL consensus clusters

Nearly absent in the HR cluster

Genetic abnormality	OxPhos (n=27)	BCR/ Prolif. (n=50)	HR (n=29)	Total (n=116)
t(14;18)	8 (22%)	5 (10%)	1 (3%)	14 (12%)
t(3; ...)	2 (5%)	8 (16%)	1 (3%)	11 (9%)
None	27 (73%)	37 (74%)	27 (93%)	91 (78%)

p = .059

* Columns may not add to 100% because of rounding.

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Summary

- Identified three robust clusters by transcriptional profiling and confirmed in independent series.
- HR cluster characterized by:
 - inflammatory/immune cell infiltrate
 - fewer known genetic lesions
 - distinct clinical features
- Different mechanisms of transformation?

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Participants

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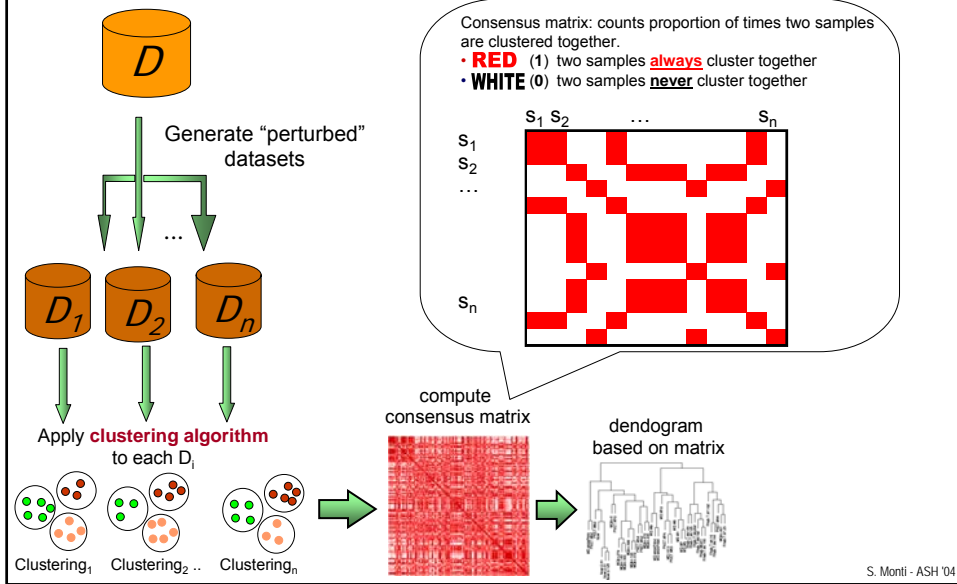
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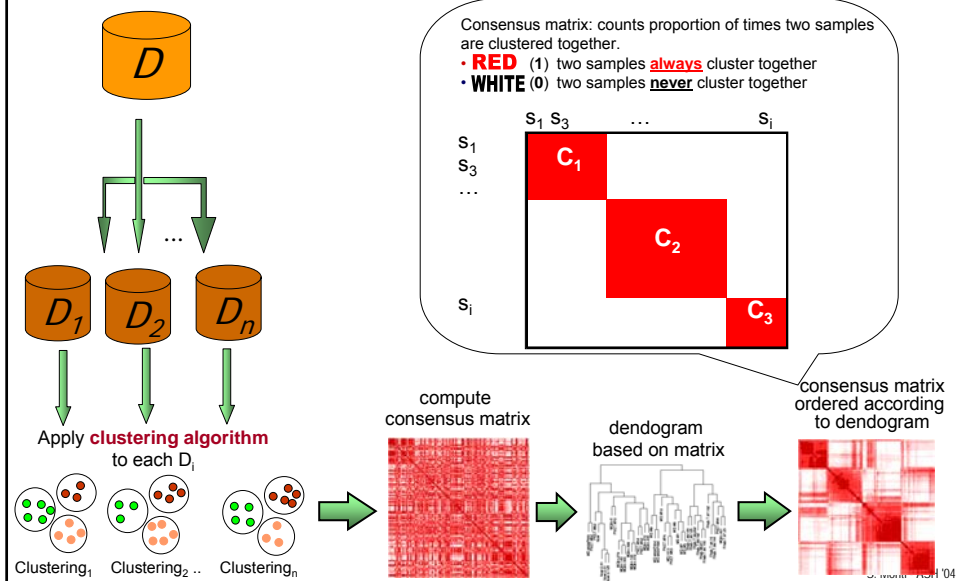
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Appendix

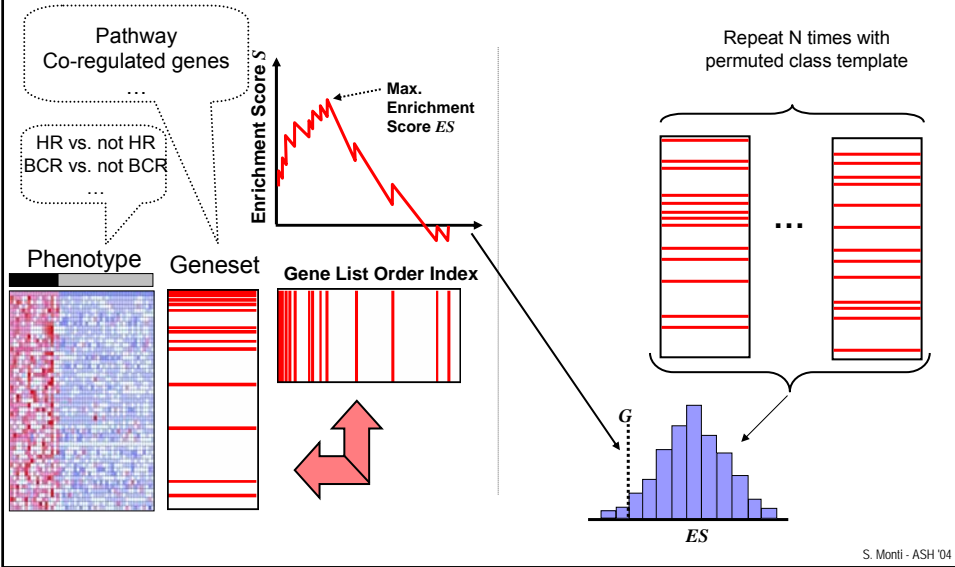
Consensus Clustering



Consensus Clustering



Gene Set Enrichment Analysis



Clusters' annotation by GSEA

