Behavior Approximation for Leukemia ceLL LineS (BALLLLS)

UCSD BISB Bootcamp 2013

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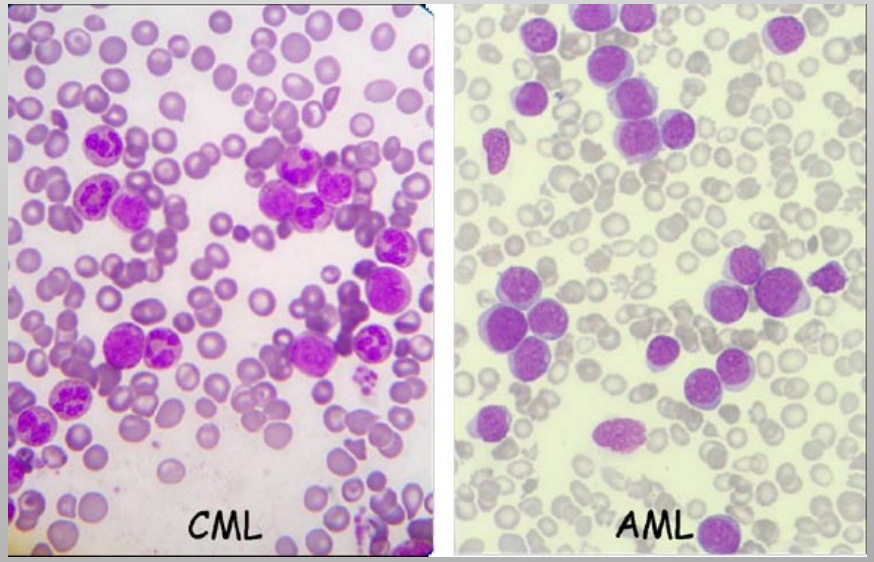
Project Aims

1. Determine gene expression profiles for K562 (CML) and H1-ESC (embryonic stem cell) cell lines from RNA-seq data
2. Cluster gene expression profiles of patients generated from RNA-seq data by The Cancer Genome Atlas
3. Determine if K562 or H1-ESC cell lines can adequately represent AML in vitro

Background: Leukemia

- Chronic Myeloid Leukemia (CML)
  - Slow moving cancer
  - Cancer of differentiated blood cells
- Acute Myeloid Leukemia (AML)
  - Fast moving cancer
  - Abnormal cells accumulate inside bone marrow



Background: Cell Lines

- CML (K562)
  - From Source
  - RNA-seq data:
    - H1-ESC(GSM958737): Embryonic stem Cells
    - K562(GSM958731): 53 year old female patient
    - 80 AML female patients from TCGA (Expression calls already given)
- Processing

RNA-seq Alignment

- Alignment using Tophat

```
tophat --library-type fr-secondstrand -o /oasis/projects/nsf/csd399/serein/H1Rep1_test -p 16 /oas
is/projects/nsf/csd399/serein/bt2/hg19 /oasis/projects/nsf/csd399/serein/data/raw/wgEncodeCaltech
RnaSeqH1hescR1x75dFastqRep1.fastq
```

- Result

```
                Input:  25157723
               Mapped:  15247005 (60.6% of input)
          of these:    3717958 (24.4%) have multiple alignments (724 have >20)
60.6% overall read alignment rate.
```

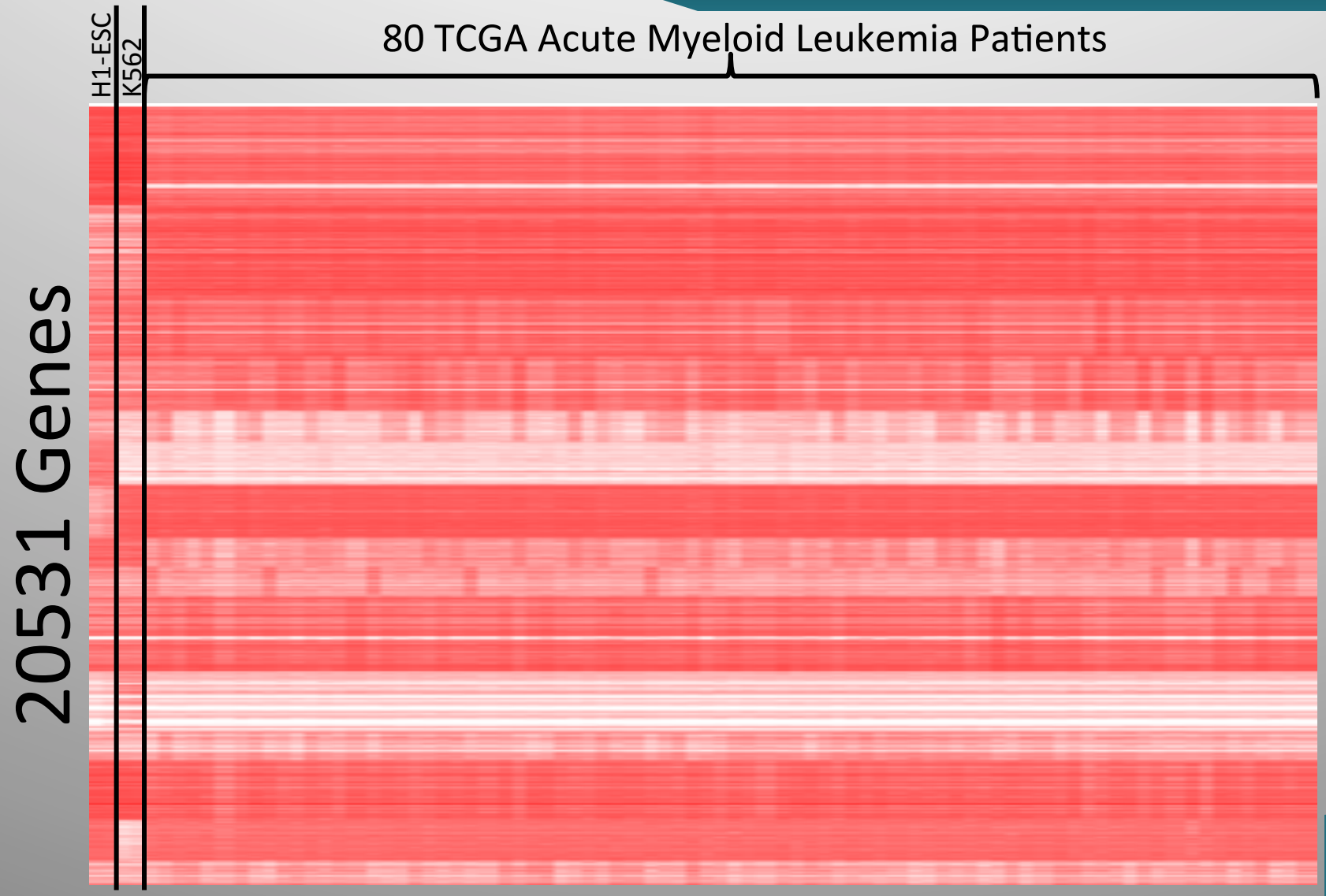
- RPKM
  - Reads per kilo base per million
  - (Read count * 1,000,000) / (total number of reads * kilo base of gene)
- Using DEGseq in R

```
Rscript DEGseq.R H1Rep2.bed hg19.refflat H1Rep2.exp
```

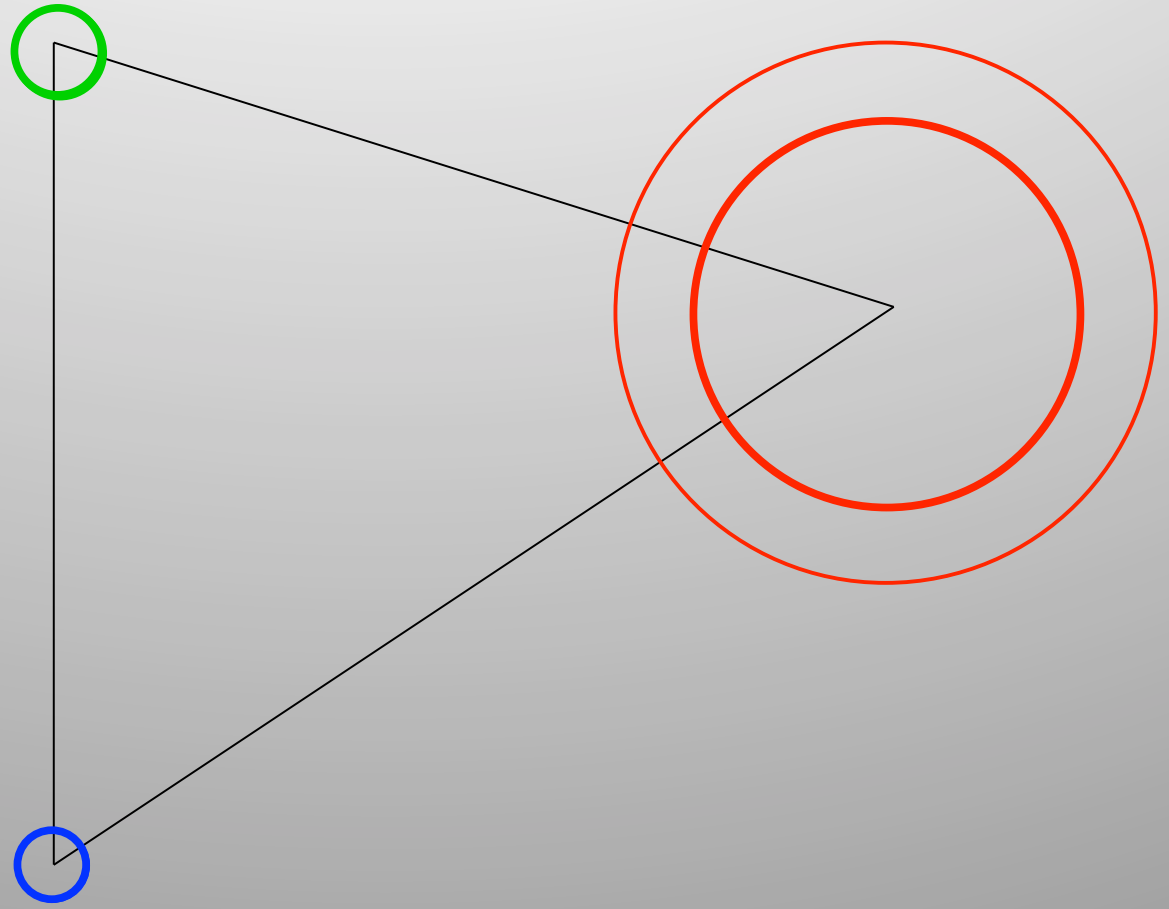
TCGA Patient Clustering

1. Data were normalized to a zero-one scale for each patient and cell line
2. A k-means clustering algorithm was used with k = 3. One profile from each cell line and one patient profile were chosen as the initial guesses for the means.
3. The following distance was used: d(x,y) = 1 – corr(x,y), where corr(x,y) is the Pearson correlation coefficient of profiles x and y.
4. Before clustering all data at once, we applied the algorithm to the patient profiles only. In that case, running the algorithm with k = 2 or k = 3 offered little qualitative improvement over running it with k = 1. We concluded that the patient profiles could safely be treated as a single cluster without subtypes.

Patient Expression Profiles



Cell Line Similarity (Distances shown to scale)



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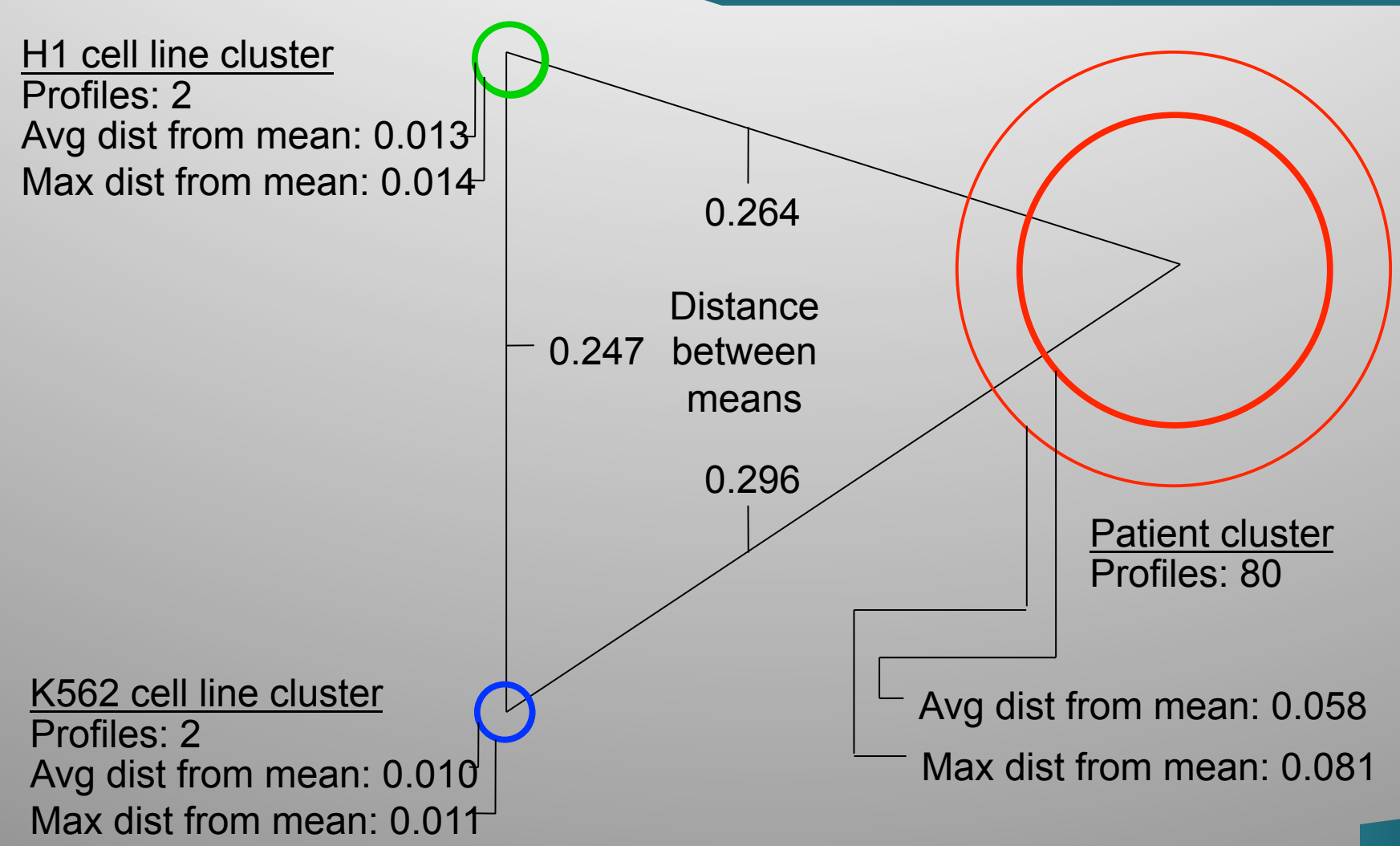
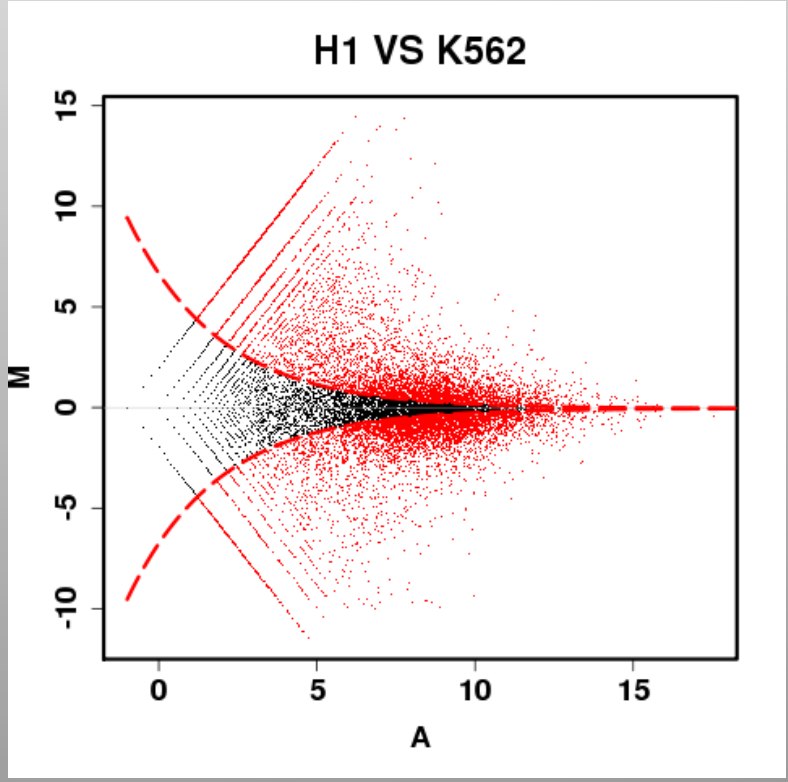


Differential expression analysis

- Search for genes that are differentially expressed between cell lines and patients
  - H1 vs K562
  - K562 vs Patients
  - H1 vs Patients
- Using DEGseq



Differentially Expressed Genes

	Patients vs K562	Patients vs H1-ESC	K562 vs H1-ESC
Number of Genes	9738 genes	10306 genes	7197 genes
Most Enriched Annotations	-Phosphoprotein -Alternative Splicing -Splice Variant -Zinc-Finger -Metal-Binding -Pleckstrin homology	-Alternative Splicing -Splice Variant -Phosphoprotein -Plasma Membrane Part -Golgi Apparatus -Mutagenesis Site	-Alternative Splicing -Splice Variant -Plasma Membrane Part -Biological Adhesion -Cell Adhesion -Phosphoprotein
Most Enriched KEGG Pathways	-Chemokine Signaling Pathway -Hematopoietic cell lineage -Lysosome -MAPK Signaling pathway	-Lysosome -Chemokine Signaling Pathway -MAPK Signaling pathway -B Cell Receptor Signaling Pathway	-Focal Adhesion -Cell Adhesion Molecules (CAMs) -Axon Guidance -Pathways in Cancer -ECM-Receptor Interaction

Conclusions

- The female patients in TCGA for LAML have very similar gene expression profiles
- The cell lines are significantly different from the patients and each other
  - One cell line was not substantially more similar to the patient profiles than the other.
- The cell lines may not be a good in vitro model for LAML (based on TCGA patients)

Discussion

- Many details may have effect on result
  - Pair-end vs single-end
  - Strand information
  - Mapping algorithm and RPKM calculation
- Following to do
  - Get more data for cell lines
  - Get data for CML patient
  - Find TF or miRNA regulating differential expressed genes