Acute promyelocytic leukemia: molecular characterization by cancer immune profiling may identify patients at risk of early death

Ravindra Kolhe, MD, PhD
Associate Professor of Pathology
Associate Director Residency program.
Medical Director, AUMC Cytogenetics laboratory
Director, Georgia Esoteric & Molecular Laboratory
Augusta University, Augusta, GA
rkolhe@augusta.edu

AUGUSTA UNIVERSITY
MEDICAL COLLEGE OF GEORGIA

Georgia Esoteric & Molecular Labs, LLC
At Augusta University

Disclosures

- **Employment or Leadership Position:**
  - Medical College of Georgia at Augusta University, USA.

- **Honoraria, Travel Funding & Research Support :**
  - Illumina, Affymetrix, Thermofisher, Asuragen, Qiagen, Nanostring, Newlink genetics and BMS.

AUGUSTA UNIVERSITY
MEDICAL COLLEGE OF GEORGIA

Georgia Esoteric & Molecular Labs, LLC At Augusta University

Goal

- Molecular dissection for APL patients with early death.

Goal

- Molecular dissection for APL patients with early death.

- Investigating:
  - Genetics.
  - Epigenetics.
  - Posttranslational modifications.

Goal

- Molecular dissection for APL patients with early death.

- Investigating:
  - Genetics:
    - miRNA expression profile.
    - **Cancer immune profiling.**
    - Sequencing (Targeted NGS and WES)

  - Epigenetics.
  - Posttranslational modifications.

Outline

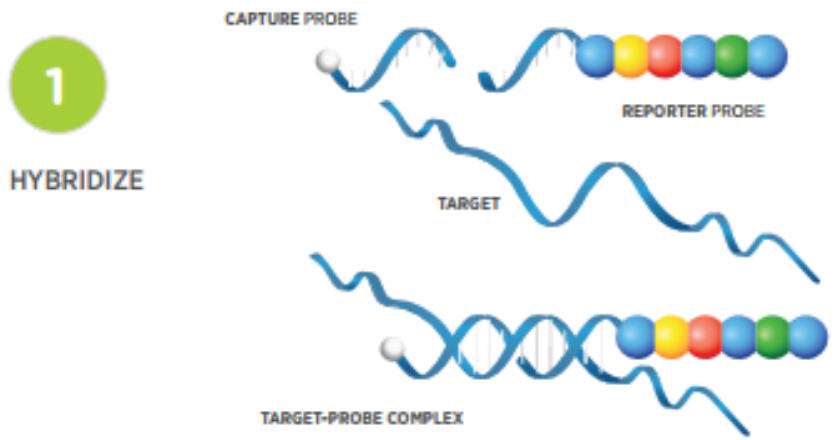
- Background
- Content of the panel.
- Data
- Future plans.

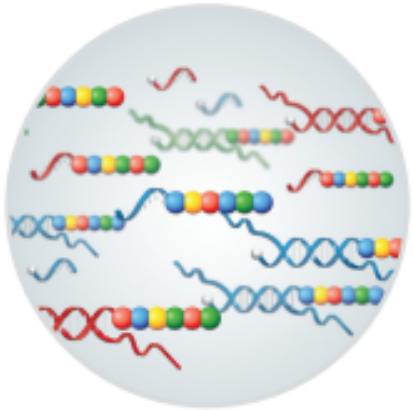
- **Early death in APL** continues to be problem both in clinical trials or real world patients.

- **Understanding the biology of the disease** may help us identify the causes for these poor outcomes and allow for improvement in outcomes.

- Our study investigates the **differences in expression genes involved in cancer immunology** in APL patients with early mortality versus surviving patients.

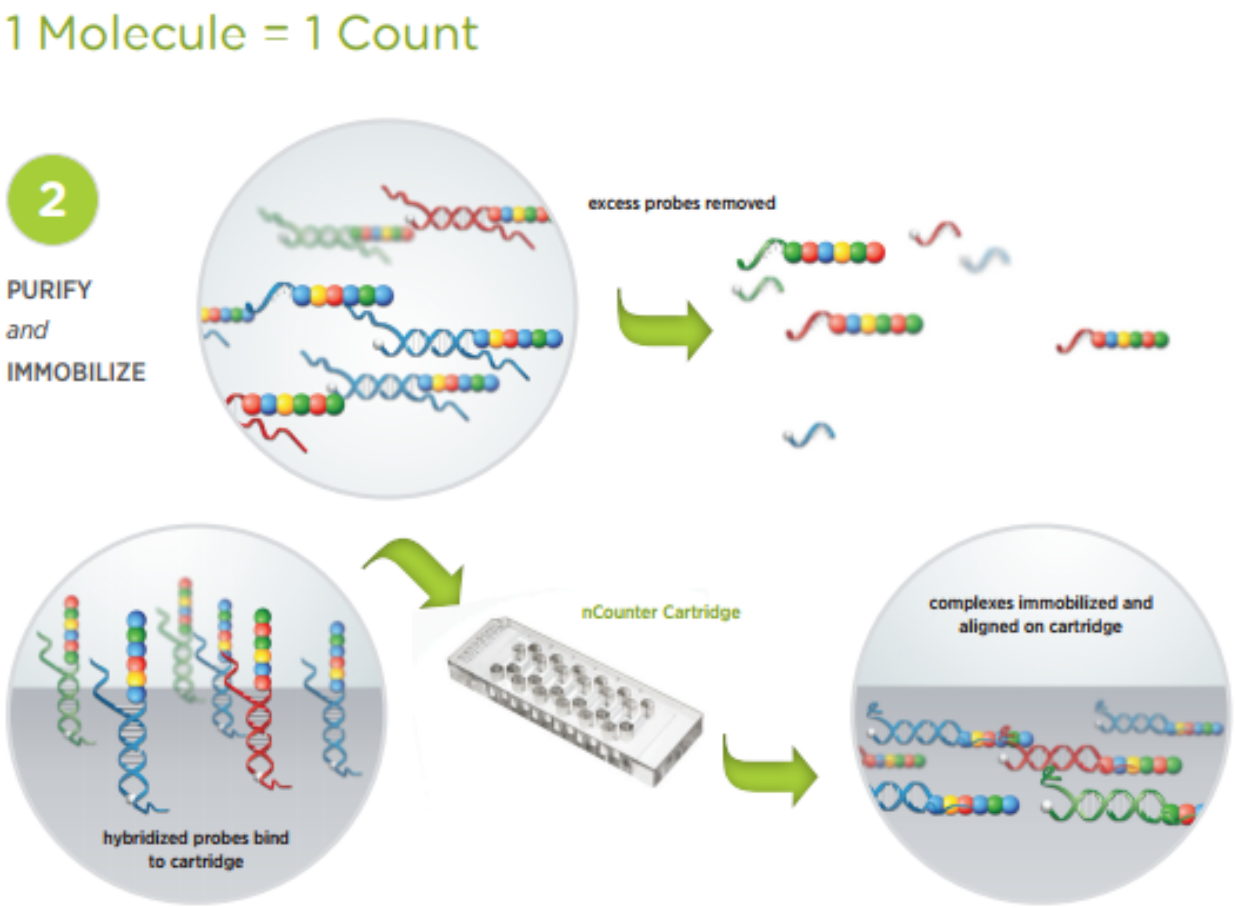
Platform and panel selection



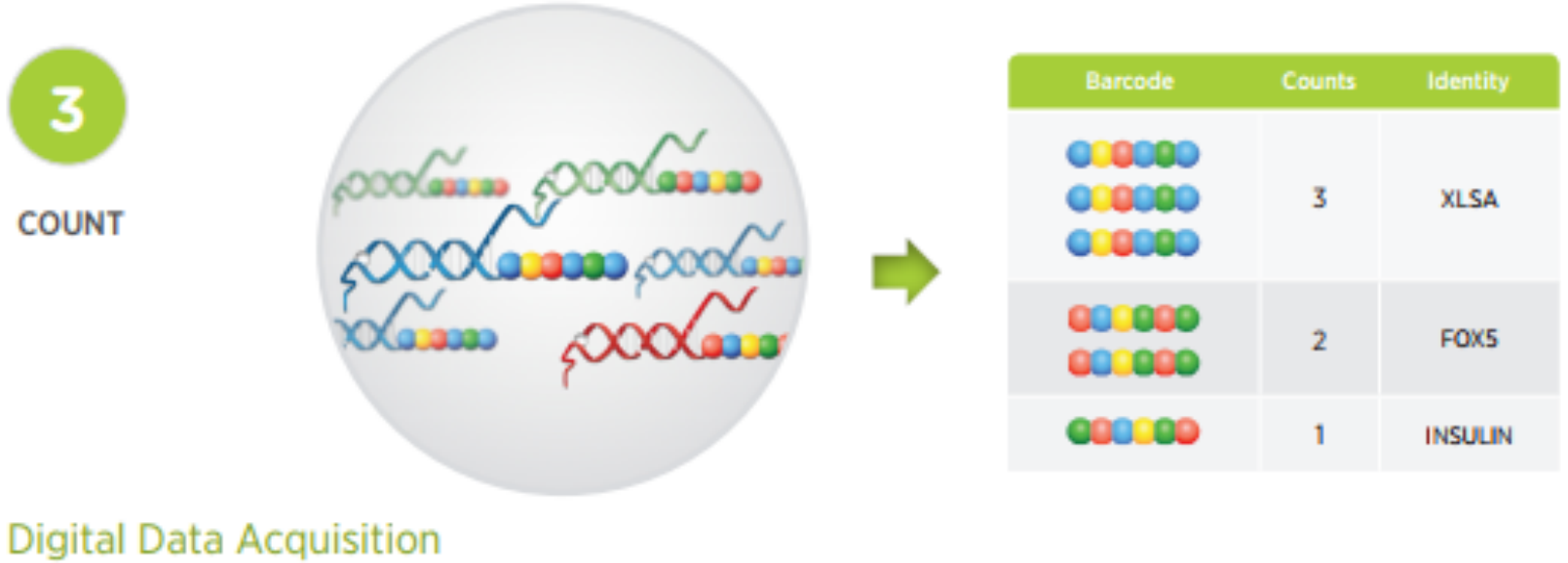


solution phase hybridization

Platform and panel selection



Platform and panel selection



AUGUSTA UNIVERSITY
MEDICAL COLLEGE
OF GEORGIA

Georgia Esoteric & Molecular Labs, LLC
At Augusta University

Platform and panel selection

- Multiplex gene expression analysis with 770 genes from
  - 24 different immune cell types,
  - Common checkpoint inhibitors,
  - CT antigens, and
  - Genes covering both the adaptive and innate immune response.

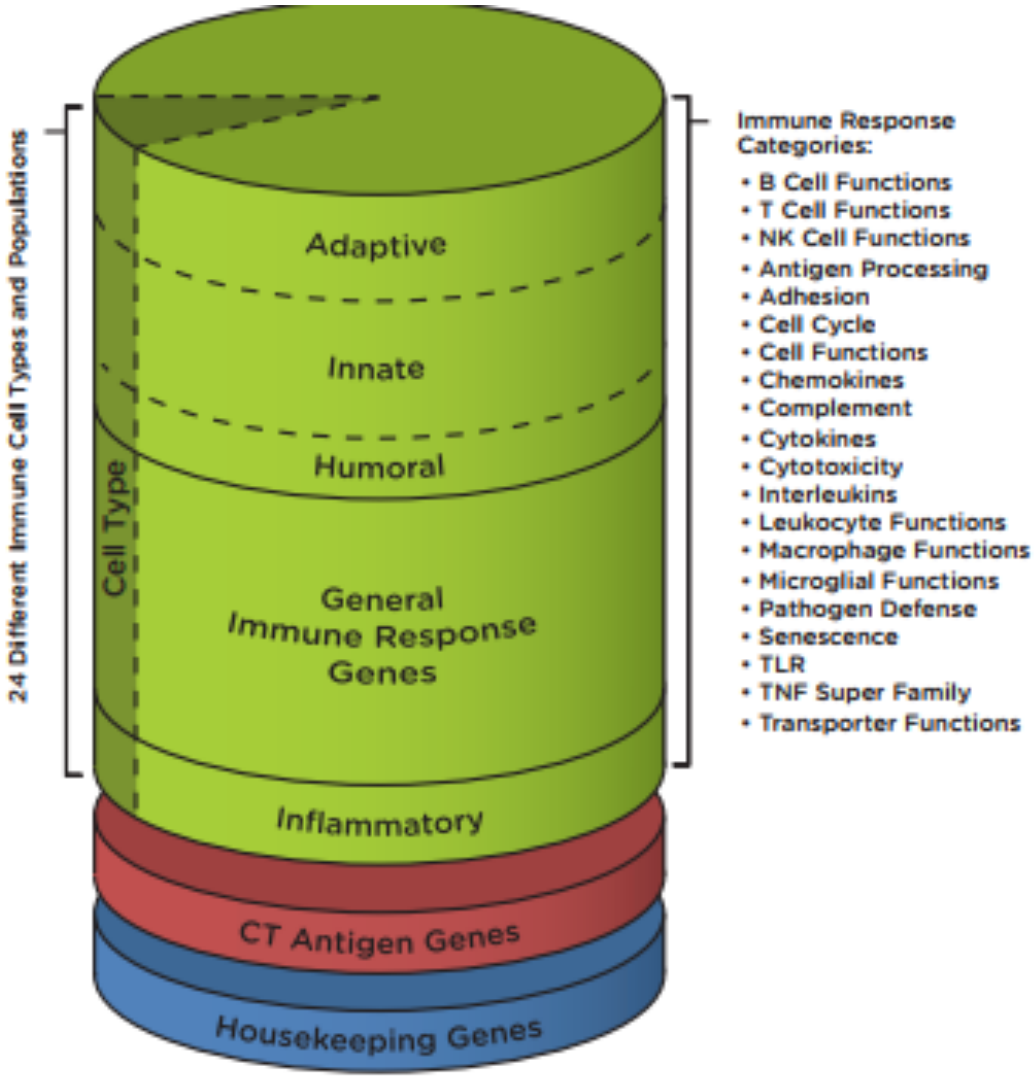


FIGURE 1 Distribution of genes included in the PanCancer Immune Profiling Panel, including genes for identifying Immune Cells (dark green), Immune Response genes (green), CT Antigens (red) and Housekeeping genes (blue). Biological process categories comprising the Immune Response genes are indicated on the right.

AUGUSTA UNIVERSITY
MEDICAL COLLEGE OF GEORGIA

Georgia Esoteric & Molecular Labs, LLC
At Augusta University

Platform and panel selection

- We studied 48 AML samples. Formalin-fixed, paraffin-embedded (FFPE) specimens were obtained from the Pathology archive.

	Survival group	Number of patients
1	1-6 months	12
2	6 months to 1	12
3	1-5 years	12
4	>5 years	12

Platform and panel selection

- We studied 48 AML samples. Formalin-fixed, paraffin-embedded (FFPE) specimens were obtained from the Pathology archive.

	Survival group	Number of patients
1	1-6 months	12
2	6 months to 1	12
3	1-5 years	12
4	>5 years	12

A novel immunohistochemical score to predict early mortality in acute myeloid leukemia patients based on indoleamine 2,3 dioxygenase expression

Platform and panel selection

APL Patient samples

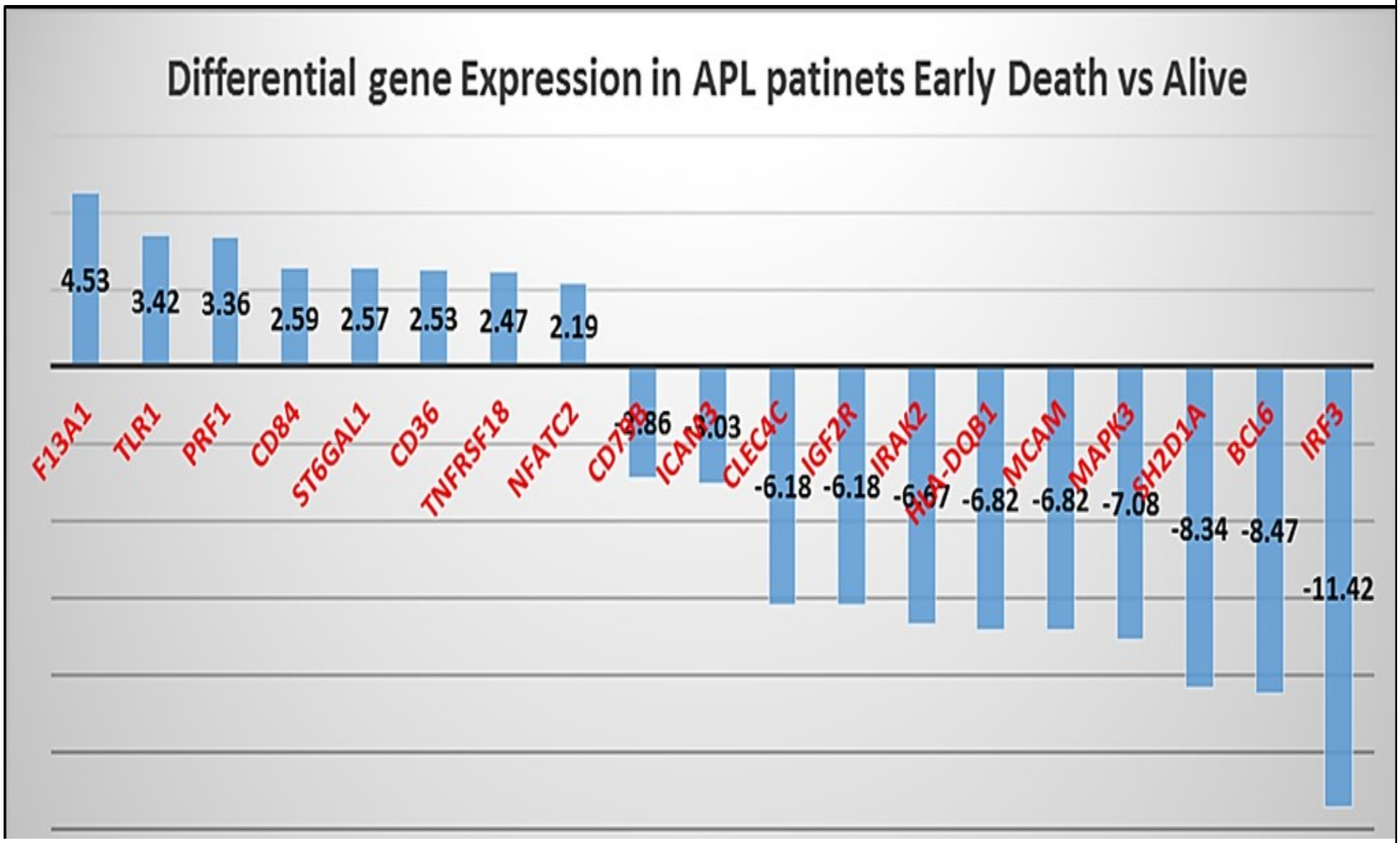
- APL patients who died – **treatment or disease related cause** (n=5) and control match (n=5) were selected.
- Reviewed H&E **slides on diagnostic marrow along** with patient chart. Archival blocks (n= 5 alive and n=5 early death) with slides were retrieved, reviewed and clinical information obtained from patient charts under approved IRB protocol.
- Several patient/disease characteristics were identified including age, sex, race, body mass index (BMI), and cytogenetics were noted.
- Therapy indicators such as treatment received, remission and relapse status was also noted. Seven 10µm sections of each case were used with >90% lesion.

- Total RNA was extracted using the Qiagen kit and analyzed with nanoString nCounter® using PanCancer Immune Profiling Panel designed to Perform multiplex gene expression analysis with 770 genes.

- Following hybridization and data acquisition, we used
  - Partek Genomics Suite ® software for Robust Multi-array Average (RMA) normalization and to determine statistically significant differences in gene expression between experimental groups by ANOVA and pairwise comparisons (two-sided α=0.05).

- The gene expression profiles of dead versus alive patients shows
  - **statistically significant (1.5 to 12X, p<0.05) downregulation of a set of 11 genes, and upregulation of 8 genes (p<0.05).**

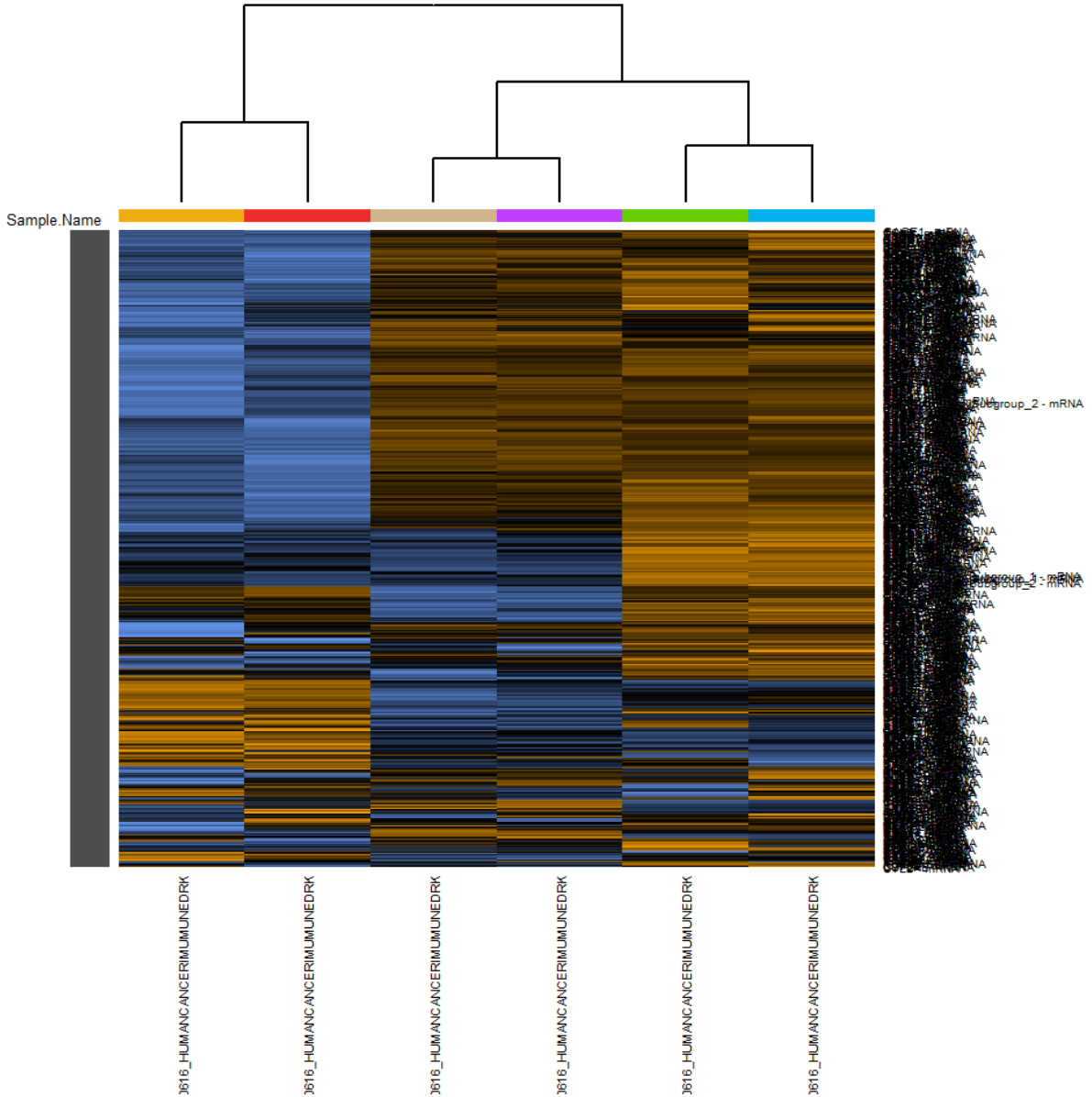
- Although the role of these genes has not been studied in APL, they have been described in other tumors with the dysregulation of cancer immune escape showing a role in
  - **tumor suppression,**
  - **differentiation,**
  - **cell proliferation,**
  - **chemo resistance and inflammation.**



AUGUSTA UNIVERSITY
MEDICAL COLLEGE OF GEORGIA

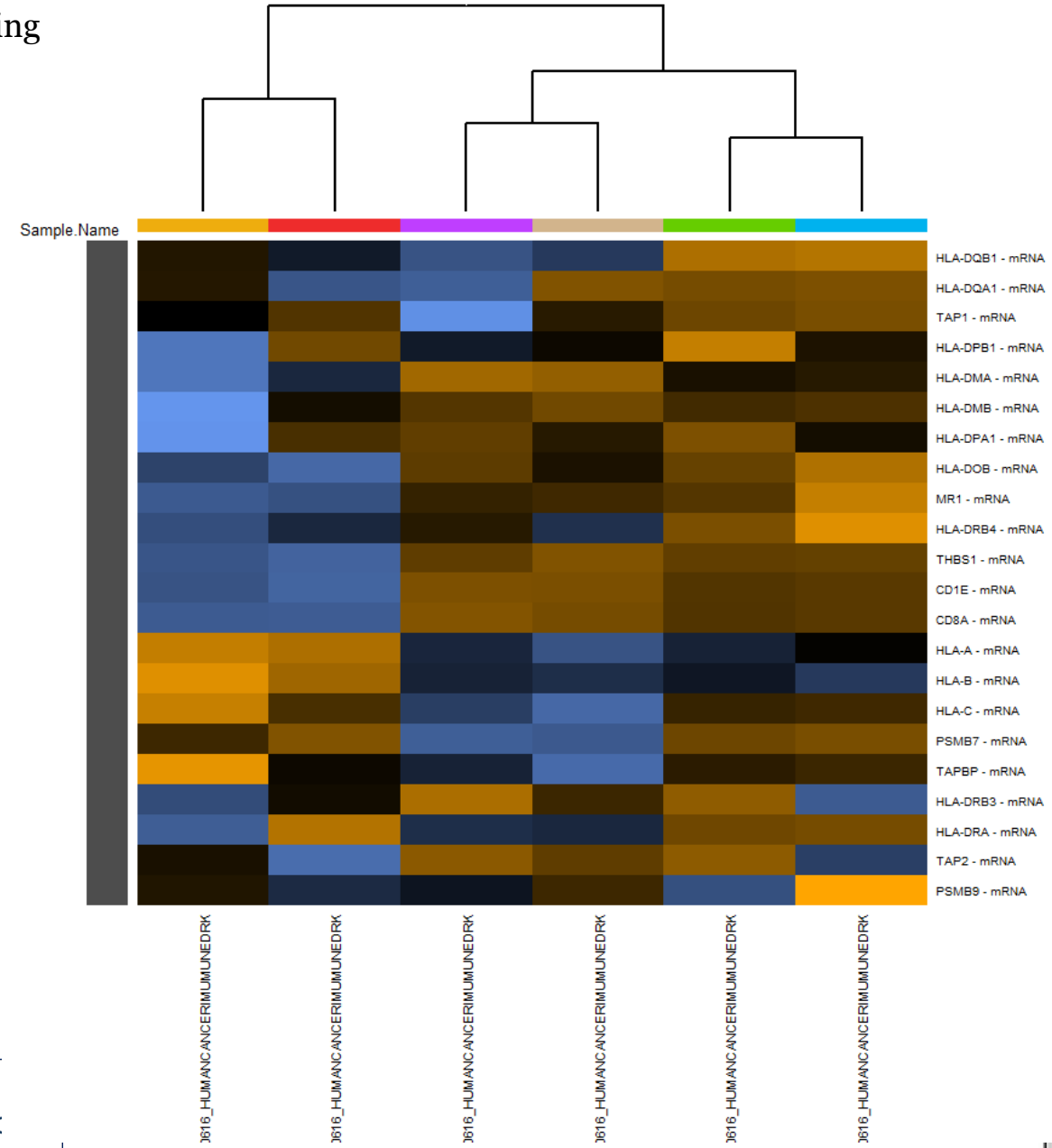
Georgia Esoteric & Molecular Labs, LLC At Augusta University

Heat map for All genes

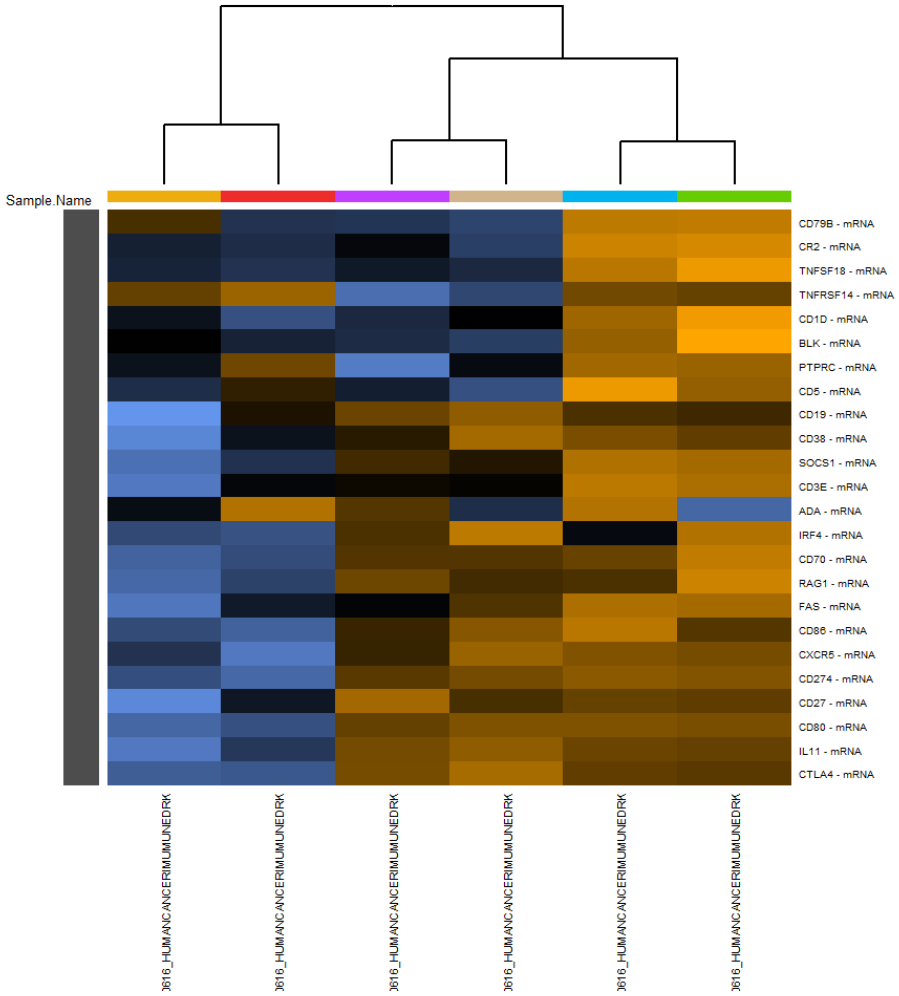


1. Antigen Processing
2. B cell functions
3. Cell cycle.
4. Chemokines.
5. Complement.
6. CT antigens.
7. Cytokines.
8. Interleukins.
9. Leukocyte functions.
10. Macrophage Function.
11. T cells
12. TLR

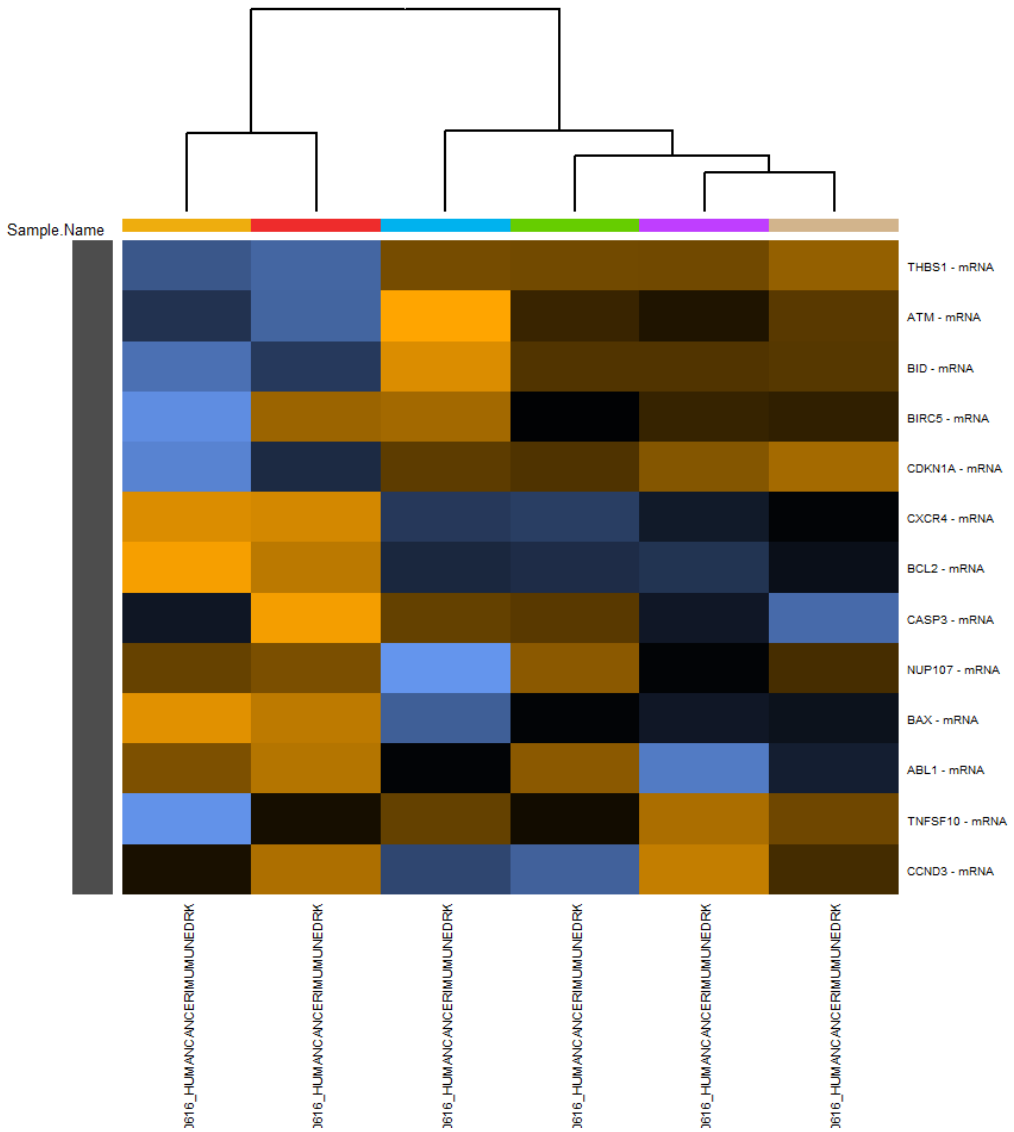
Antigen Processing



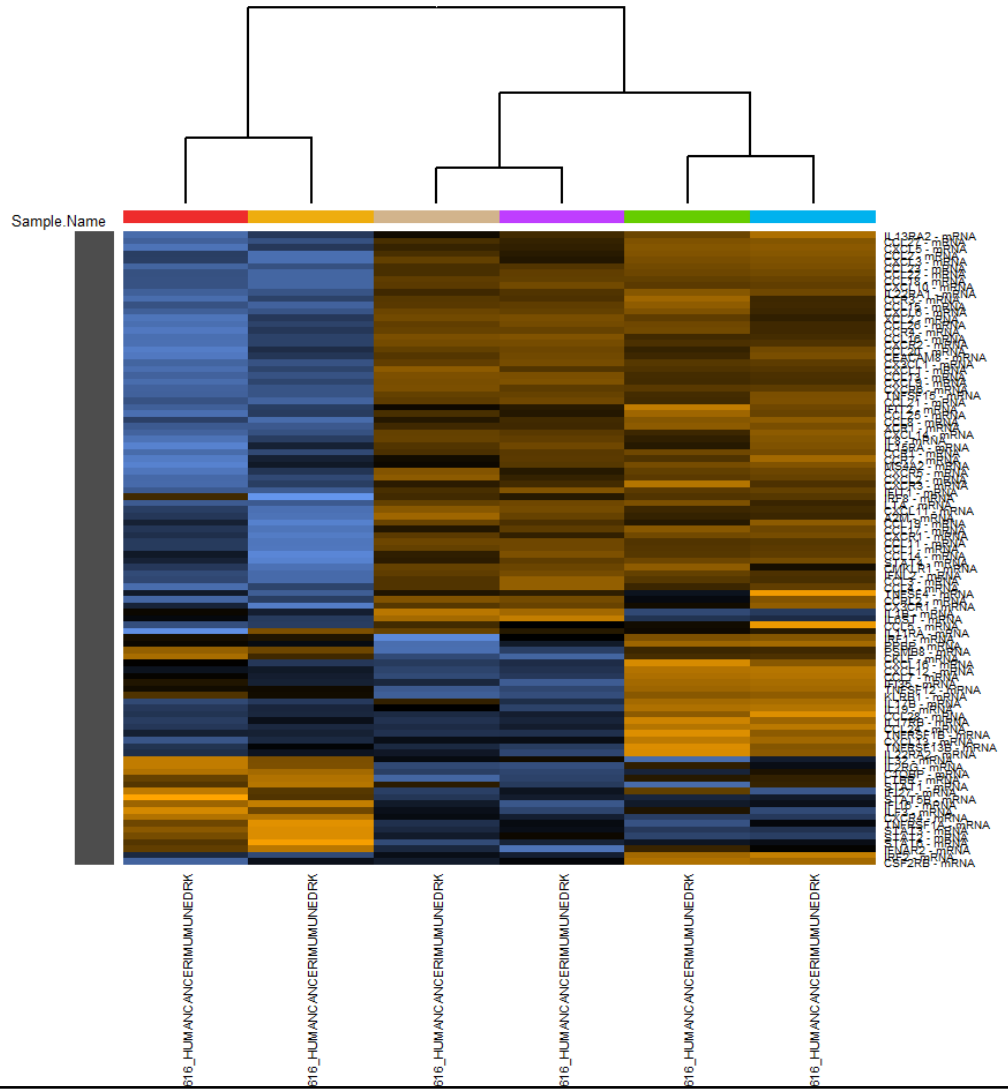
B cell functions



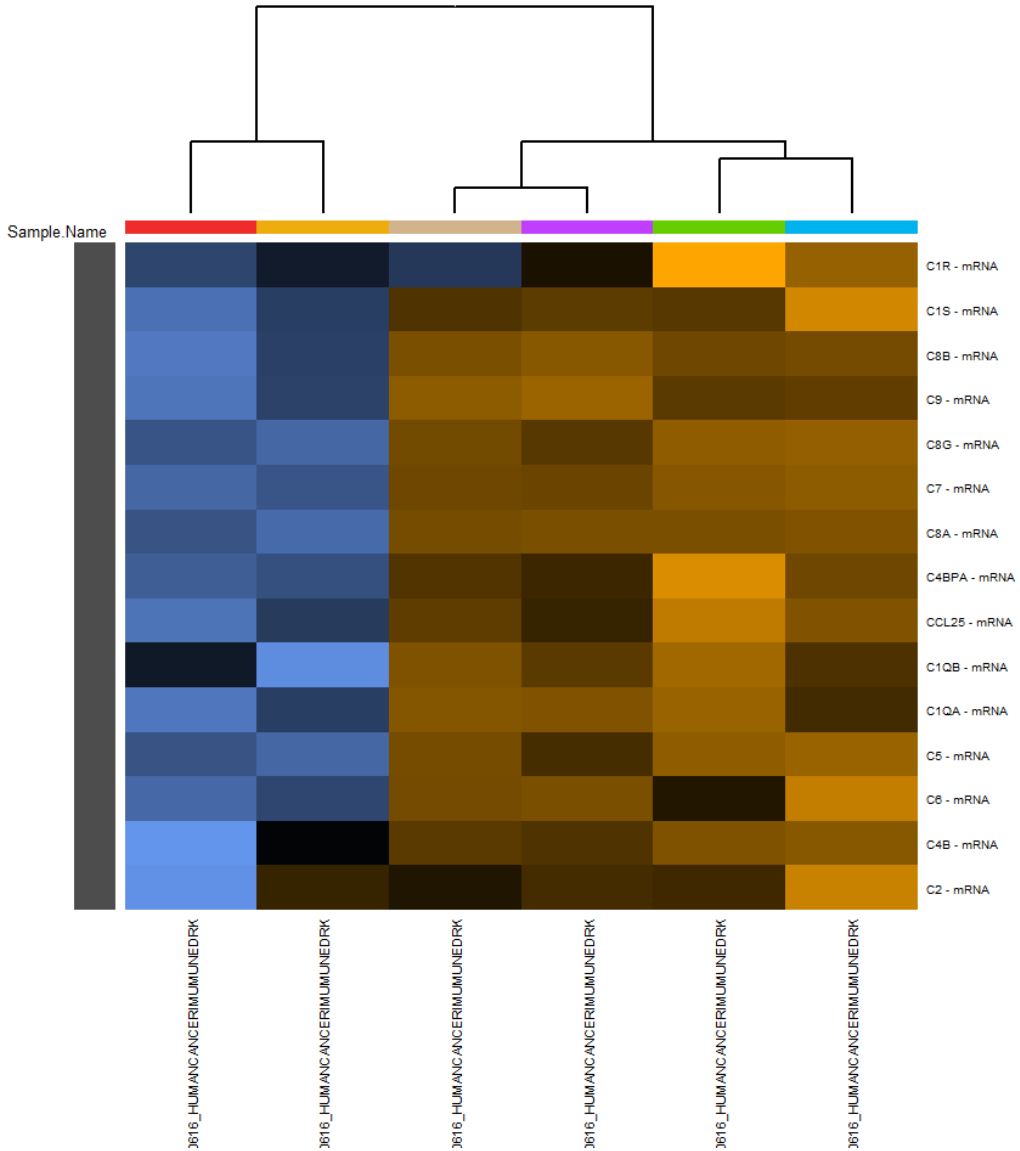
Cell cycle


Sample.Name
THBS1 - mRNA
ATM - mRNA
BID - mRNA
BIRC5 - mRNA
CDKN1A - mRNA
CXCR4 - mRNA
BCL2 - mRNA
CASP3 - mRNA
NUP107 - mRNA
BAX - mRNA
ABL1 - mRNA
TNFSF10 - mRNA
CCND3 - mRNA

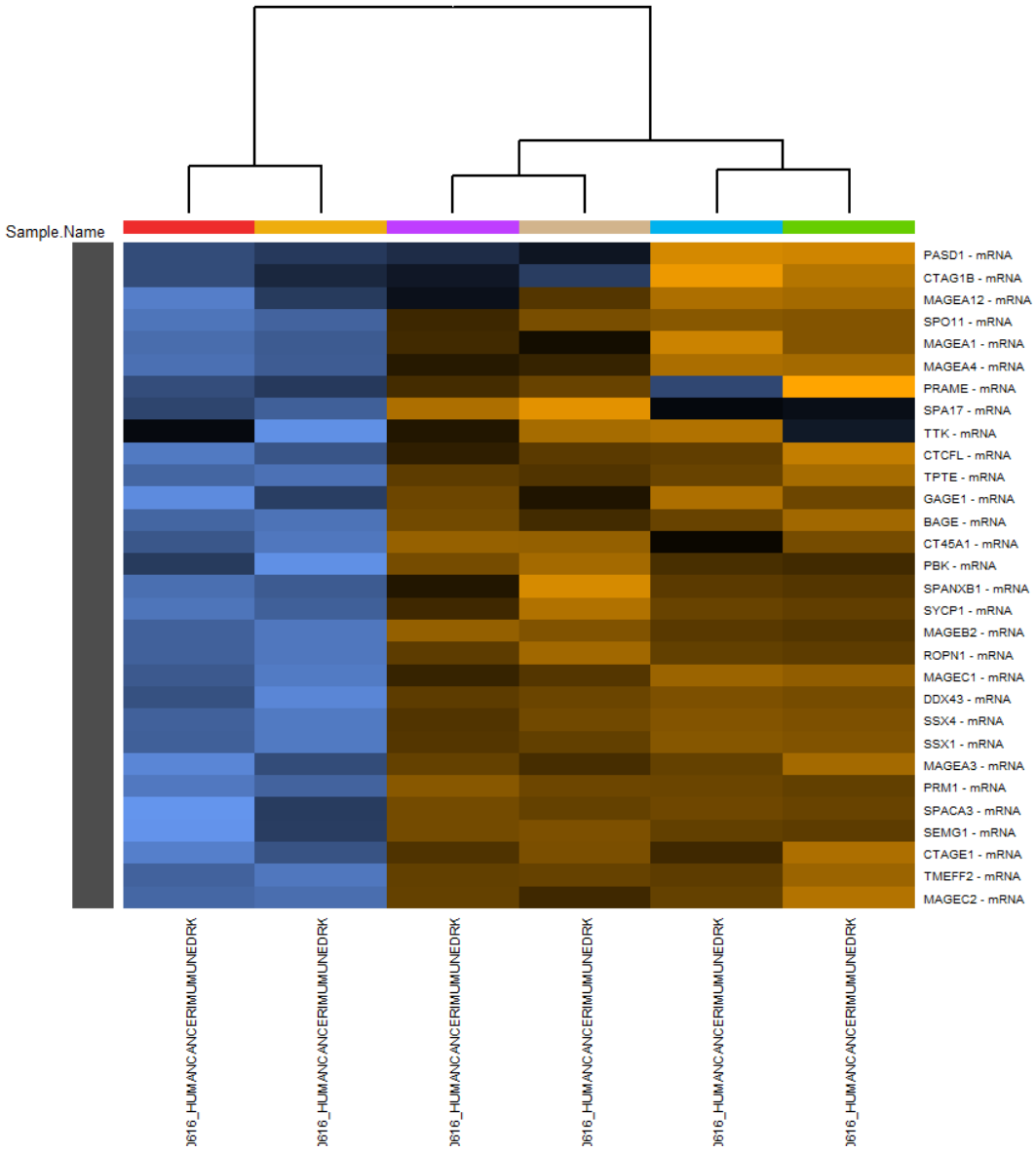
Chemokines



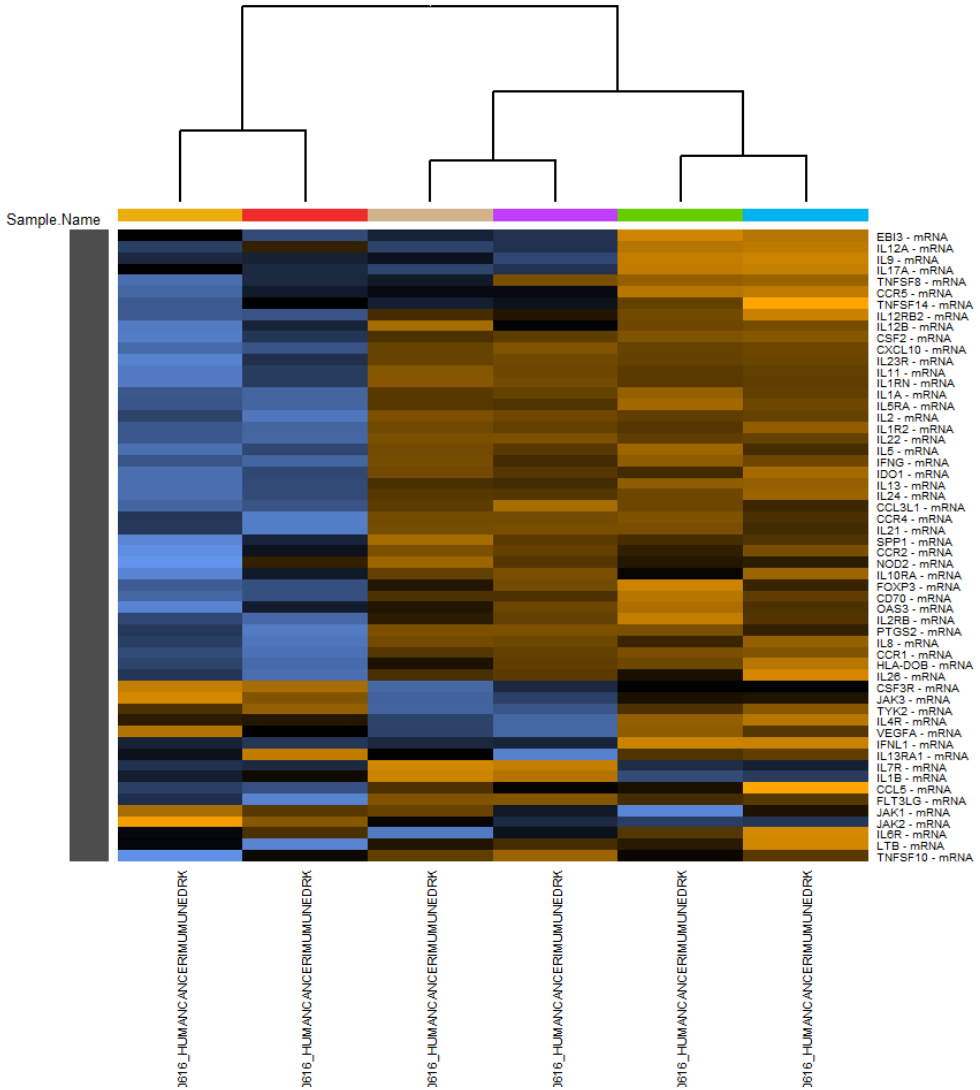
Complement


Sample.Name
C1R - mRNA
C1S - mRNA
C8B - mRNA
C9 - mRNA
C8G - mRNA
C7 - mRNA
C8A - mRNA
C4BPA - mRNA
CCL25 - mRNA
C1QB - mRNA
C1QA - mRNA
C5 - mRNA
C6 - mRNA
C4B - mRNA
C2 - mRNA

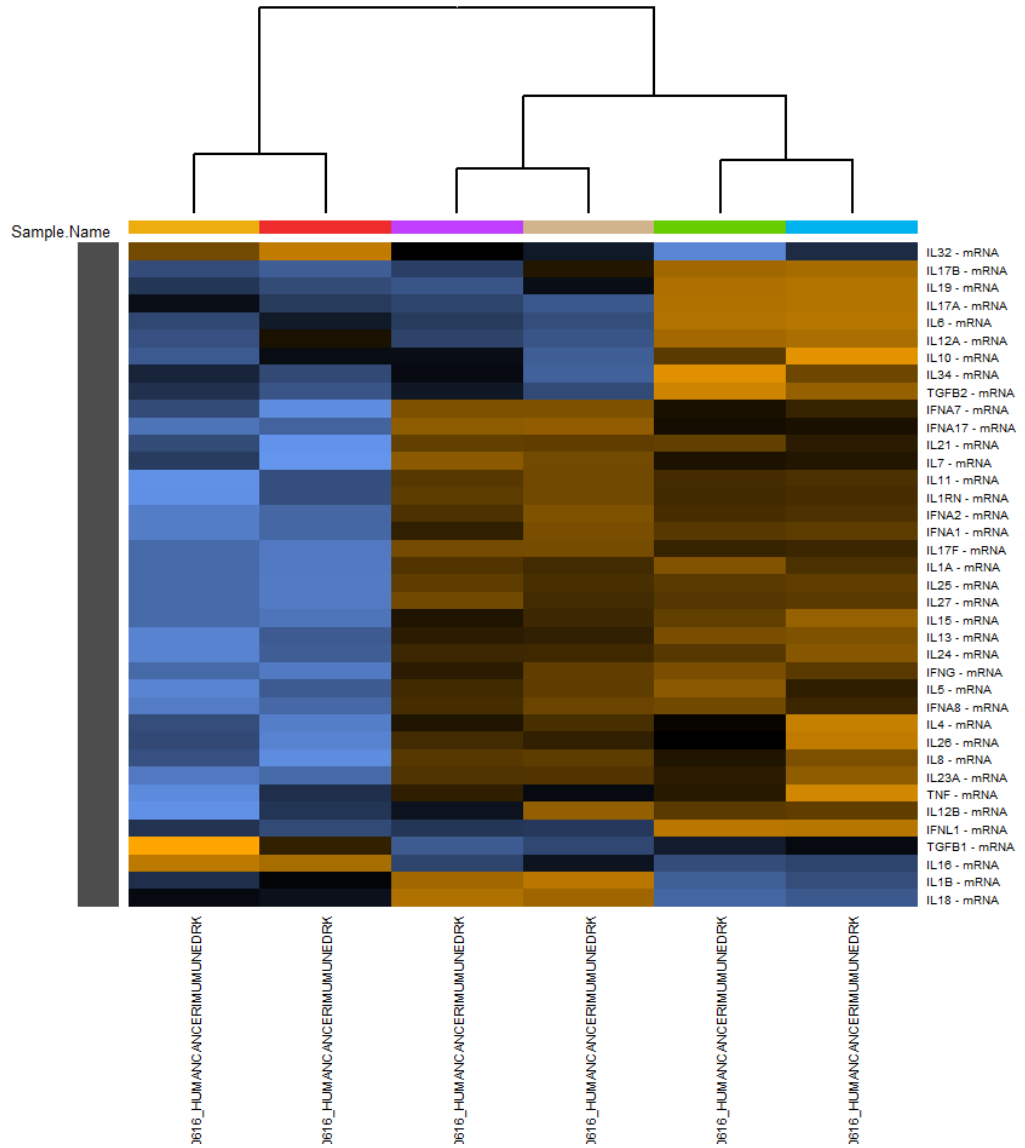
CT antigen


Sample.Name
PASD1 - mRNA
CTAG1B - mRNA
MAGEA12 - mRNA
SPO11 - mRNA
MAGEA1 - mRNA
MAGEA4 - mRNA
PRAME - mRNA
SPA17 - mRNA
TTK - mRNA
CTCFL - mRNA
TPTE - mRNA
GAGE1 - mRNA
BAGE - mRNA
CT45A1 - mRNA
PBK - mRNA
SPANXB1 - mRNA
SYCP1 - mRNA
MAGEB2 - mRNA
ROPN1 - mRNA
MAGEC1 - mRNA
DDX43 - mRNA
SSX4 - mRNA
SSX1 - mRNA
MAGEA3 - mRNA
PRM1 - mRNA
SPACA3 - mRNA
SEMG1 - mRNA
CTAGE1 - mRNA
TMEFF2 - mRNA
MAGEC2 - mRNA

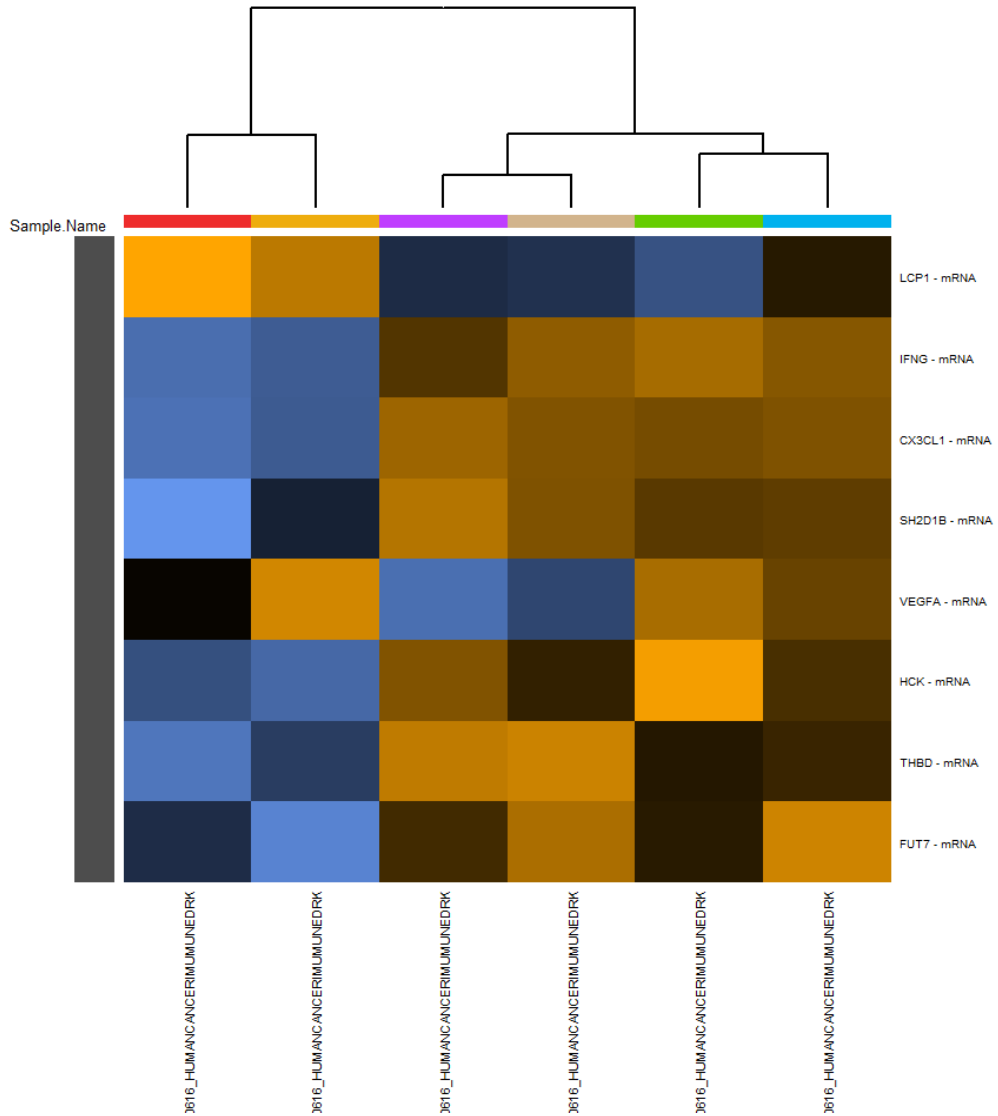
Cytokines


Sample.Name
EBI3 - mRNA
IL12A - mRNA
IL9 - mRNA
IL17A - mRNA
TNFSF8 - mRNA
CCR5 - mRNA
TNFSF14 - mRNA
IL12RB2 - mRNA
IL12B - mRNA
CSF2 - mRNA
CXCL10 - mRNA
IL23R - mRNA
IL11 - mRNA
IL1RN - mRNA
IL1A - mRNA
IL5RA - mRNA
IL2 - mRNA
IL1R2 - mRNA
IL22 - mRNA
IL5 - mRNA
IFNG - mRNA
IDO1 - mRNA
IL13 - mRNA
IL24 - mRNA
CCL3L1 - mRNA
CCR4 - mRNA
IL21 - mRNA
SPP1 - mRNA
CCR2 - mRNA
NOD2 - mRNA
IL10RA - mRNA
FOXP3 - mRNA
CD70 - mRNA
OAS3 - mRNA
IL2RB - mRNA
PTGS2 - mRNA
IL8 - mRNA
CCR1 - mRNA
HLA-DOB - mRNA
IL26 - mRNA
CSF3R - mRNA
JAK3 - mRNA
TYK2 - mRNA
IL4R - mRNA
VEGFA - mRNA
IFNL1 - mRNA
IL13RA1 - mRNA
IL7R - mRNA
IL1B - mRNA
CCL5 - mRNA
FLT3LG - mRNA
JAK1 - mRNA
JAK2 - mRNA
IL6R - mRNA
LTB - mRNA
TNFSF10 - mRNA
3616_HUMANCANCERIMUMUNEDRK
3616_HUMANCANCERIMUMUNEDRK
3616_HUMANCANCERIMUMUNEDRK
3616_HUMANCANCERIMUMUNEDRK
3616_HUMANCANCERIMUMUNEDRK
3616_HUMANCANCERIMUMUNEDRK

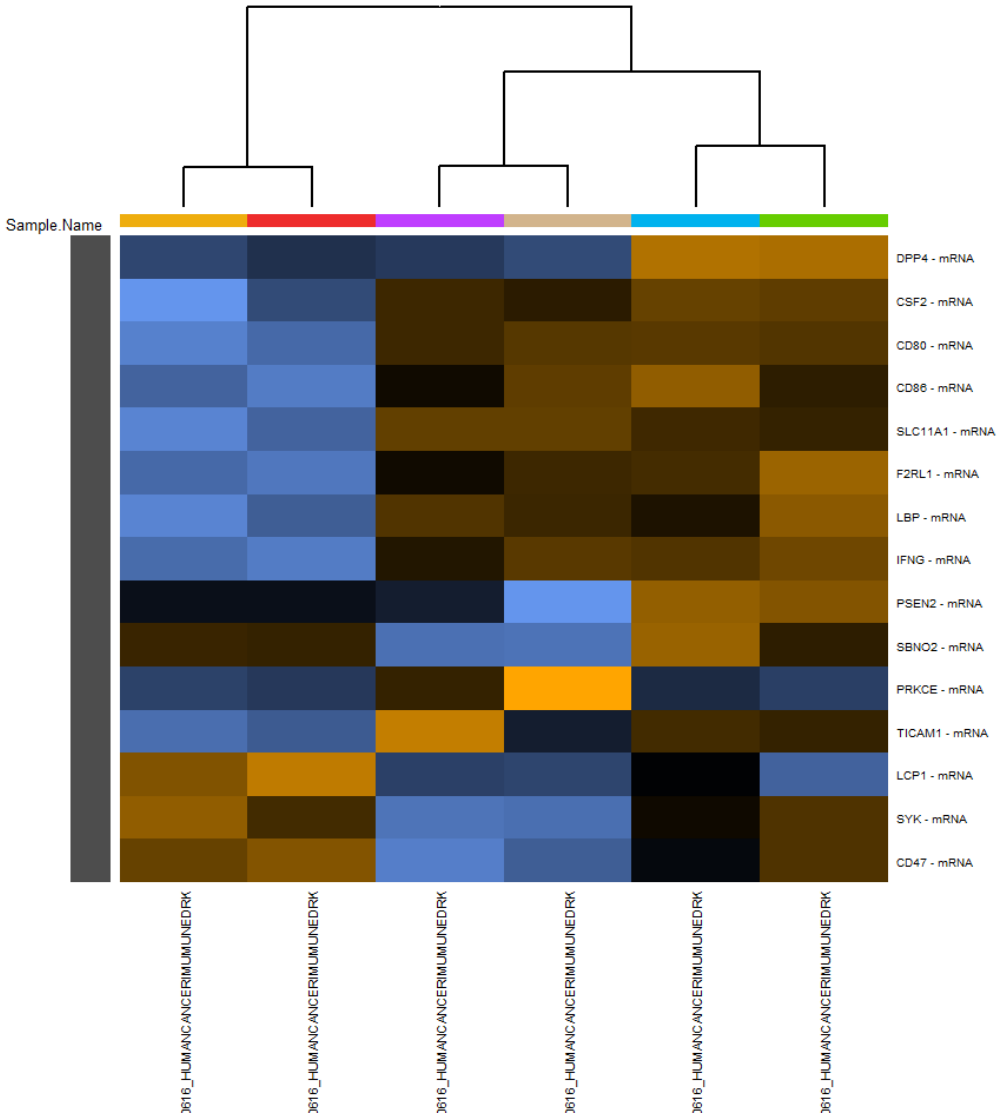
Interleukins



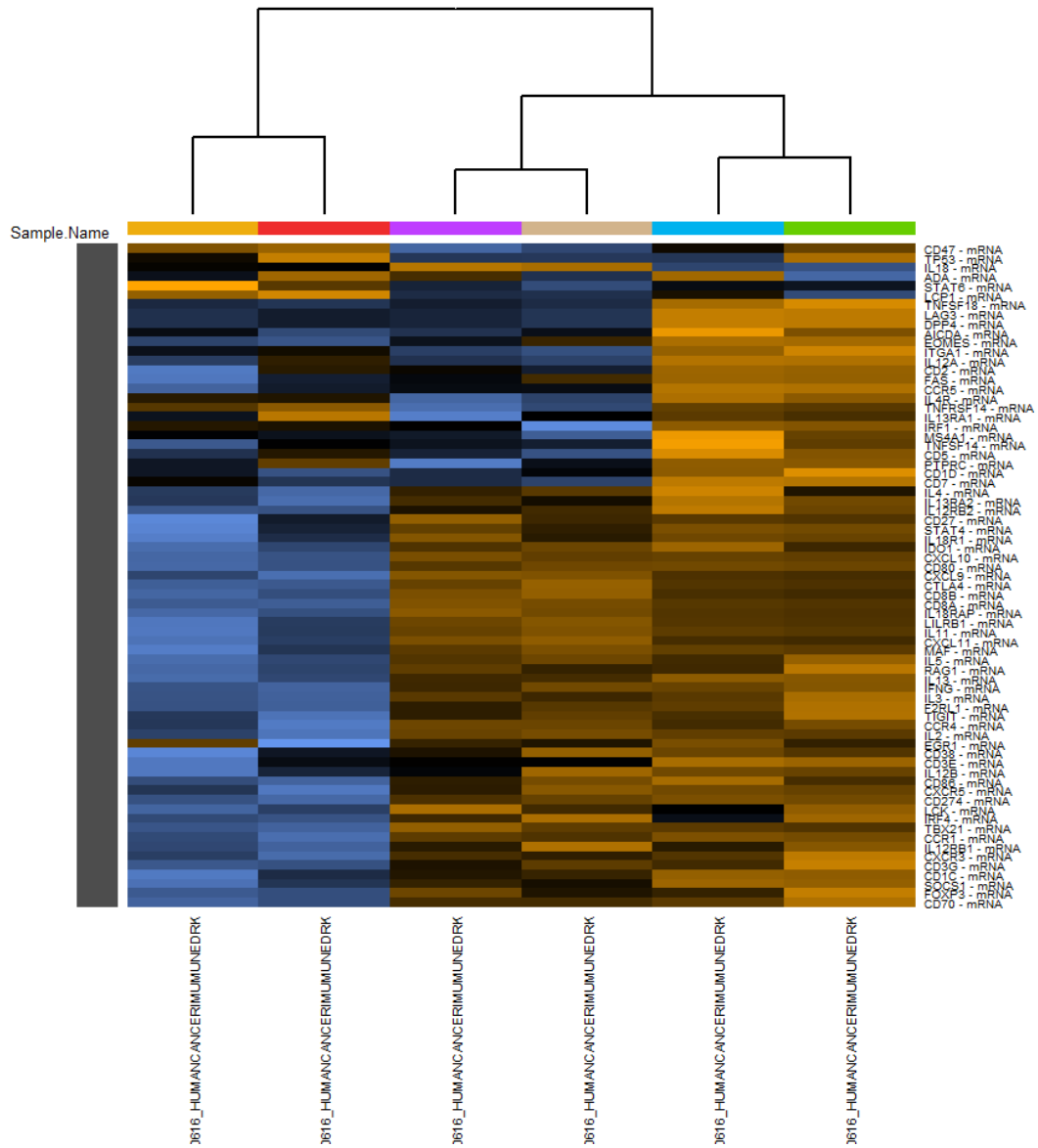
Leukocytes functions



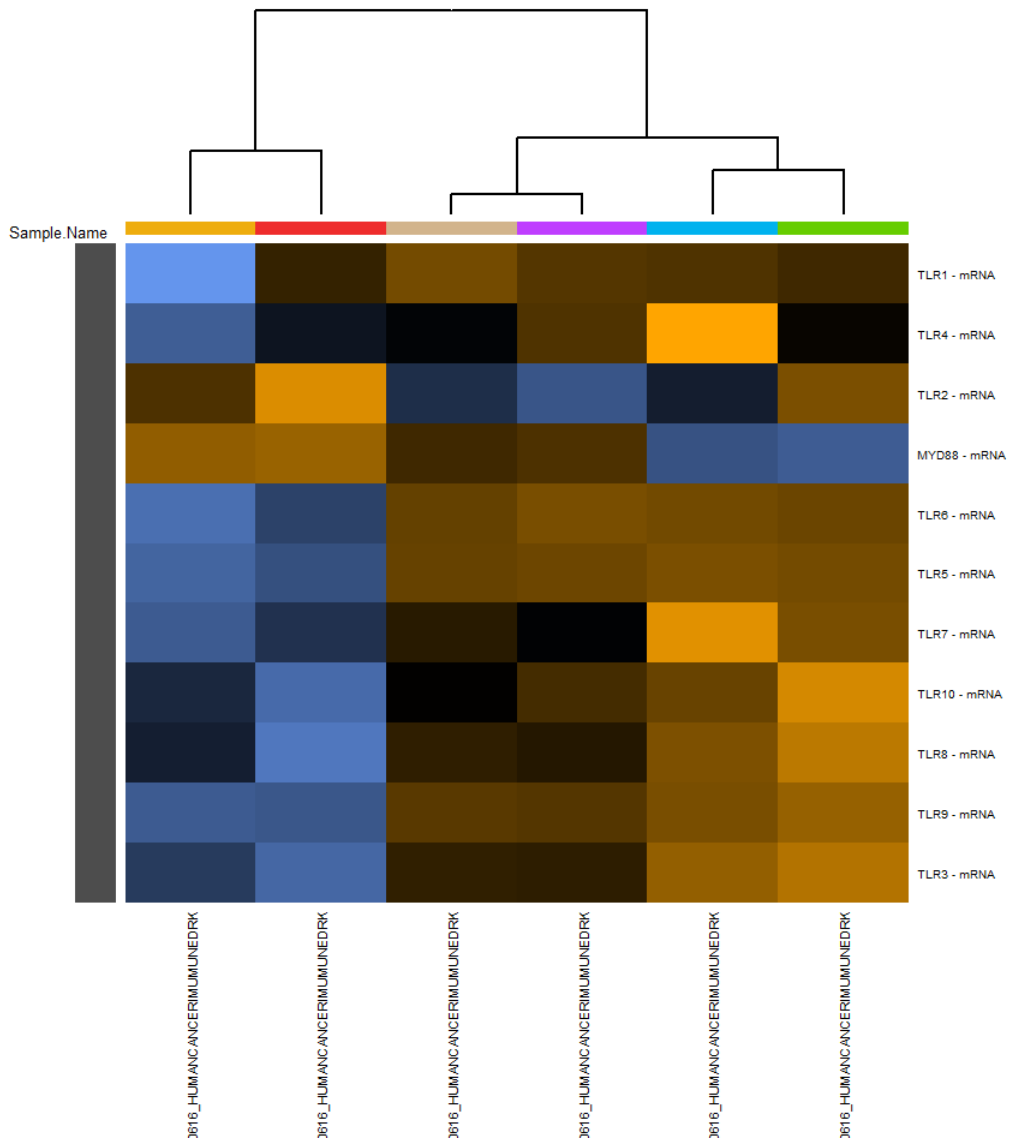
Macrophage functions



T cell functions



TLR


Sample.Name
TLR1 - mRNA
TLR4 - mRNA
TLR2 - mRNA
MYD88 - mRNA
TLR6 - mRNA
TLR5 - mRNA
TLR7 - mRNA
TLR10 - mRNA
TLR8 - mRNA
TLR9 - mRNA
TLR3 - mRNA

- To the best of our knowledge, the current study represents the first cancer immune profiling exploration in understanding the early death in APL patients.

- **Toll-like receptors (TLRs) play a vital role in activating immune responses**. Activation of tumor cell TLRs not only promotes tumor cell proliferation and resistance to apoptosis.

- Previous studies have demonstrated that that prevention of interferon regulatory transcription **factor 3 (IRF3) phosphorylation** (along with interferon (IFN)-β expression, STAT1 phosphorylation) is a critical downstream event in inhibitory effect of Arsenic trioxide (ATO) on Inducible nitric oxide synthase (iNOS) expression.

- We hypothesize that this **dysregulation of cancer immune genes leads to a sustained proinflammatory state and also impart some resistance to an anti-inflammatory** property of ATO that eventually leads to the poor outcomes.

- Cell lines to identify the de-novo status and mechanistic studies.

- Expand the validation in bigger patient sample database.

Acknowlegments

AUGUSTA UNIVERSITY
MEDICAL COLLEGE
OF GEORGIA

Georgia Esoteric &
Molecular Labs, LLC
At Augusta
University

AUGUSTA UNIVERSITY
MEDICAL COLLEGE OF GEORGIA

Georgia Esoteric & Molecular Labs, LLC
At Augusta University

AUGUSTA UNIVERSITY
MEDICAL COLLEGE
OF GEORGIA

Georgia Esoteric &
Molecular Labs, LLC
At Augusta
University

Georgia Esoteric Molecular Laboratory at Augusta University

Dr. Barb DuPont, PhD. FACMG

Dr. Alka Chaubey, PhD. FACMG

Dr. Ashis K Mondal, PhD. MB(ASCP)

Kimya Jones, MS, HT(ASCP)

Olabisi Ojo, MS

Dr. Chetan Pundkar, DVM, MS

AUGUSTA UNIVERSITY
MEDICAL COLLEGE
OF GEORGIA

Georgia Esoteric &
Molecular Labs, LLC
At Augusta
University

Questions.