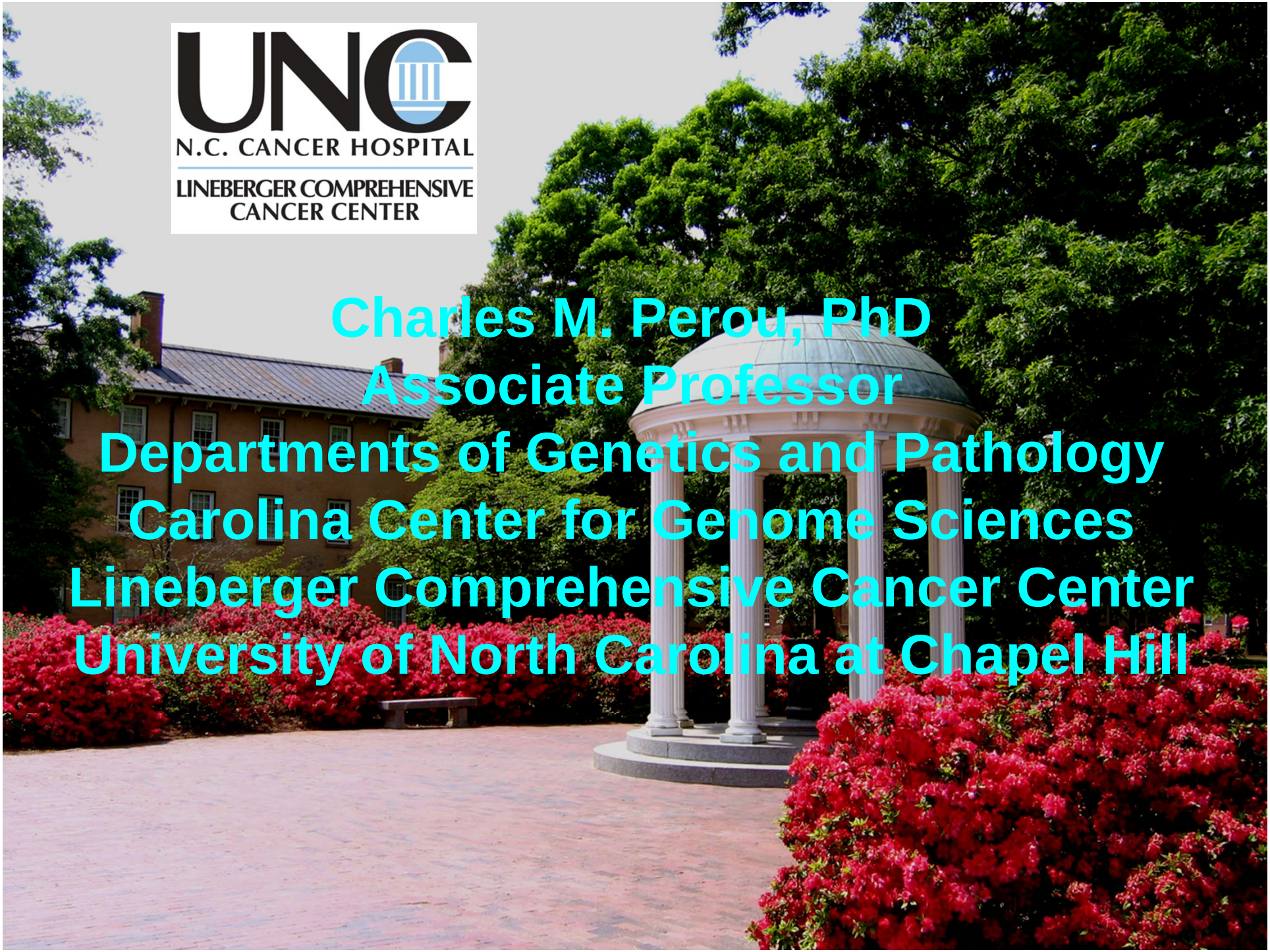

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Identification of GBM Subtypes using Gene Expression Profiling

Agilent Custom 244k Microarray

Affymetrix Human Exon 1.0 Array

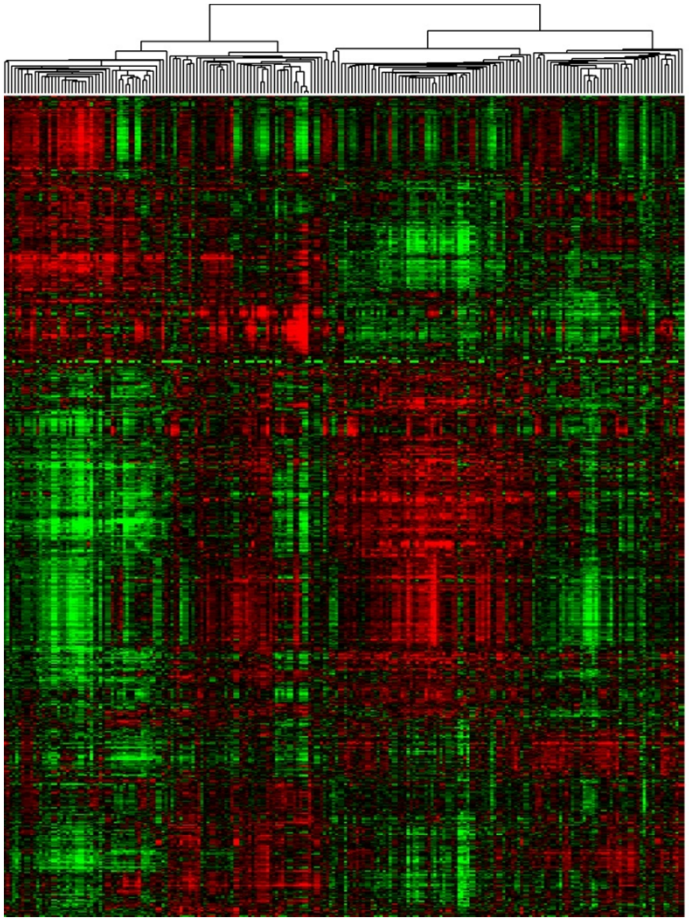
Affymetrix HT-HG-U133A Array

Identify Samples and Genes represented on all 3 platforms

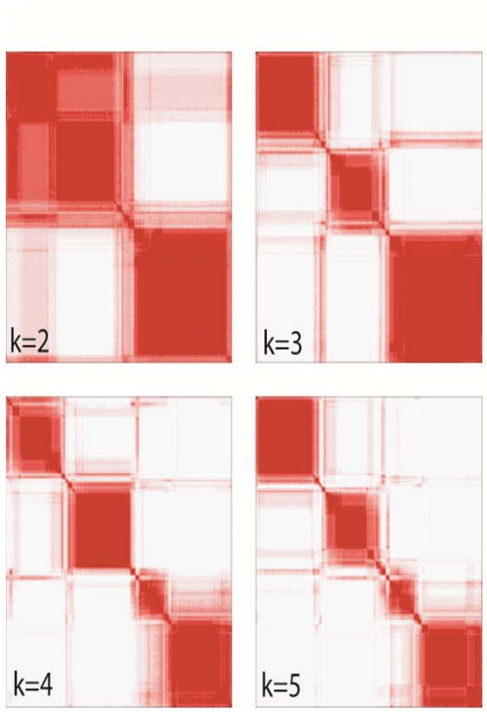
Factor Analysis

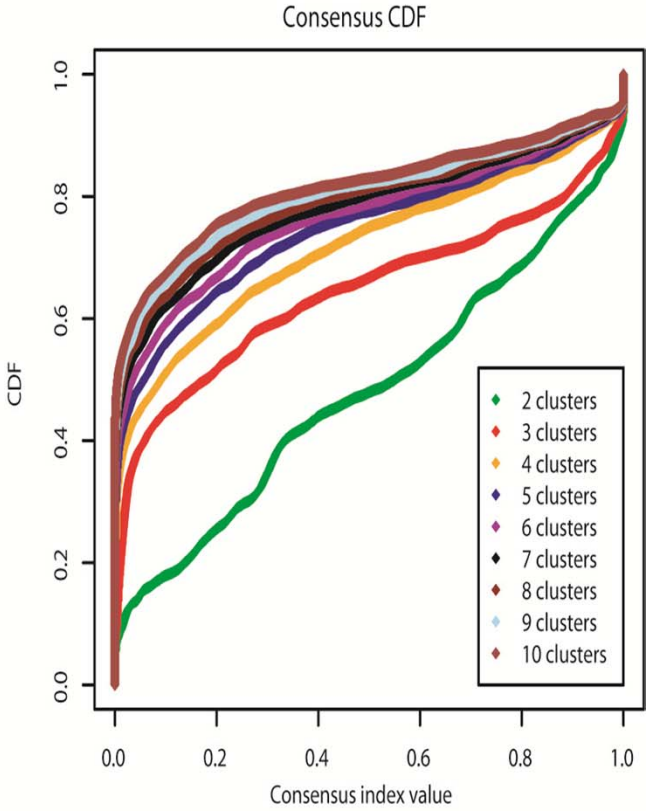
Single unified gene expression measurement for each gene
202 patients
11,681 genes

Identification of GBM Subtypes



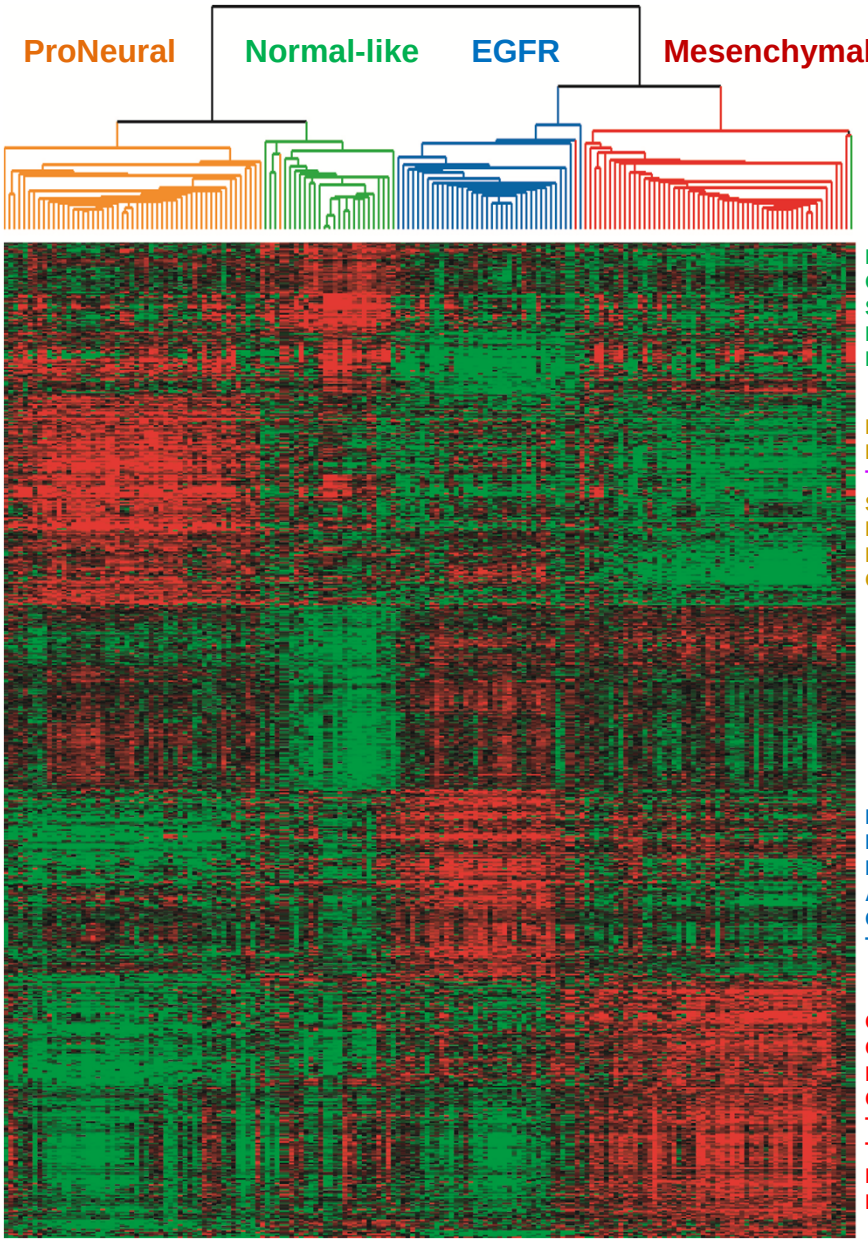
Unsupervised clustering of 1740 variably expressed genes selected using a unified gene expression measure across 3 expression platforms





Census Clustering of the 202 samples X 1740 genes suggests that 4 subtypes of GBM exist

Core TCGA Samples (173) with Subtype-defining genes



Gene Ontology/Pathway:

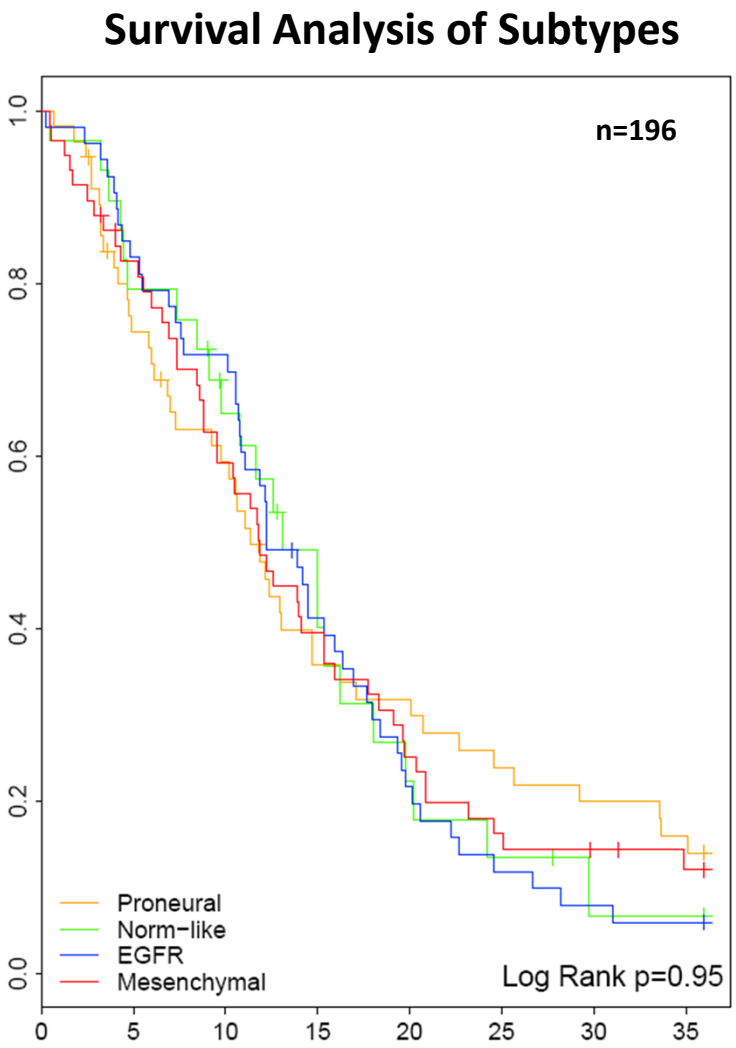
- ProNeural:
  1. nervous system development
  2. neuron differentiation (SOXs)
  3. cell cycle = proliferation
  4. cell adhesion molecules
  5. ErbB signaling pathway

- Normal-like:
  1. nucleotide metabolic process
  2. neurological system process
  3. axon
  4. neuron projection
  5. synaptic transmission

- EGFR:
  1. regulation of transcription
  2. cell migration
  3. nervous system development
  4. cell proliferation
  5. metal ion binding

- Mesenchymal:
  1. immune response
  2. receptor activity
  3. wound healing
  4. cytokine and chemokine mediated signaling pathway
  5. NF-κB Signaling Pathway

Correlations between gene expression subtypes and clinical parameters



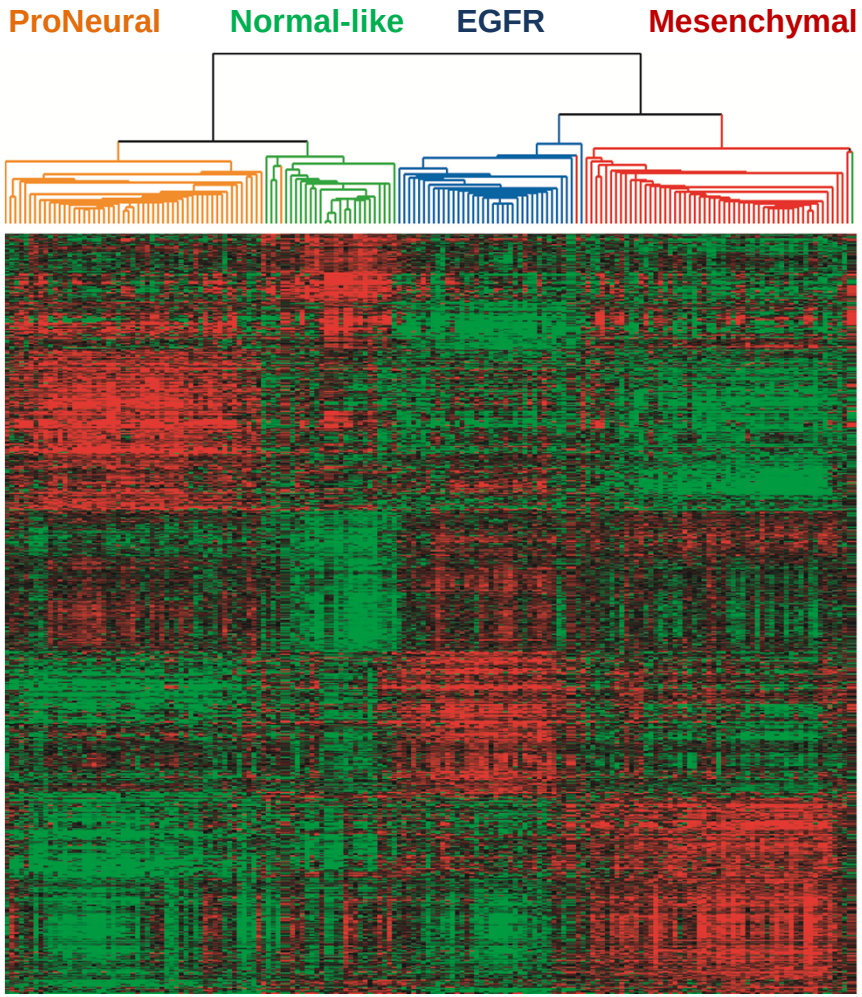
Subtypes are correlated with:

1. **Age (ProNeural – early age)**
2. **Nuclear Atypia (Normal-like=lowest)**
3. **Cellularity (Normal-like=lowest)**
4. **Average % Necrosis (Normal-like=lowest, Mesenchymal=highest)**

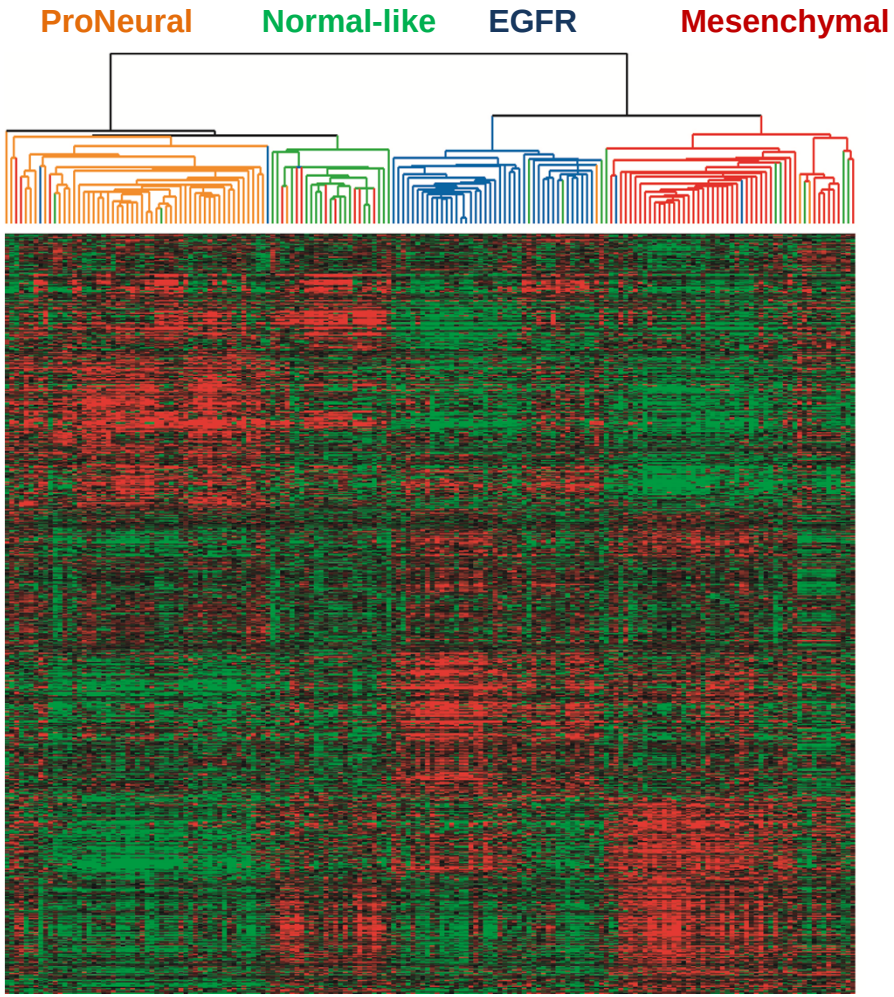
Subtypes are NOT correlated with:

1. **Average % tumor nuclei (>90%)**

Core TCGA Samples (173)



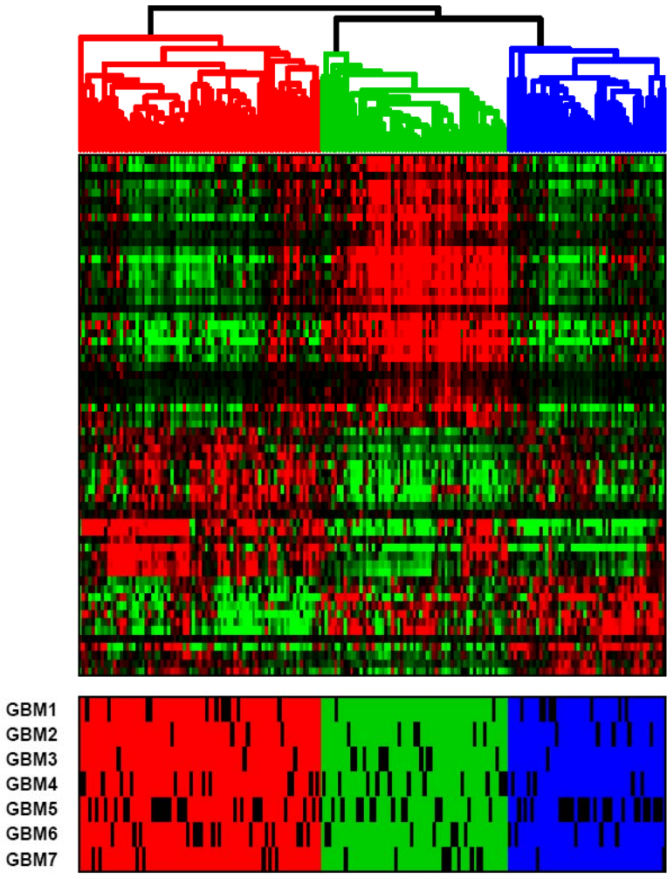
Combined Validation Set (174)



The validation set contains samples from three publically available data sets.

microRNA-based Subtype Discovery

~534 miRNAs assayed using Agilent microarrays with unsupervised clustering analysis

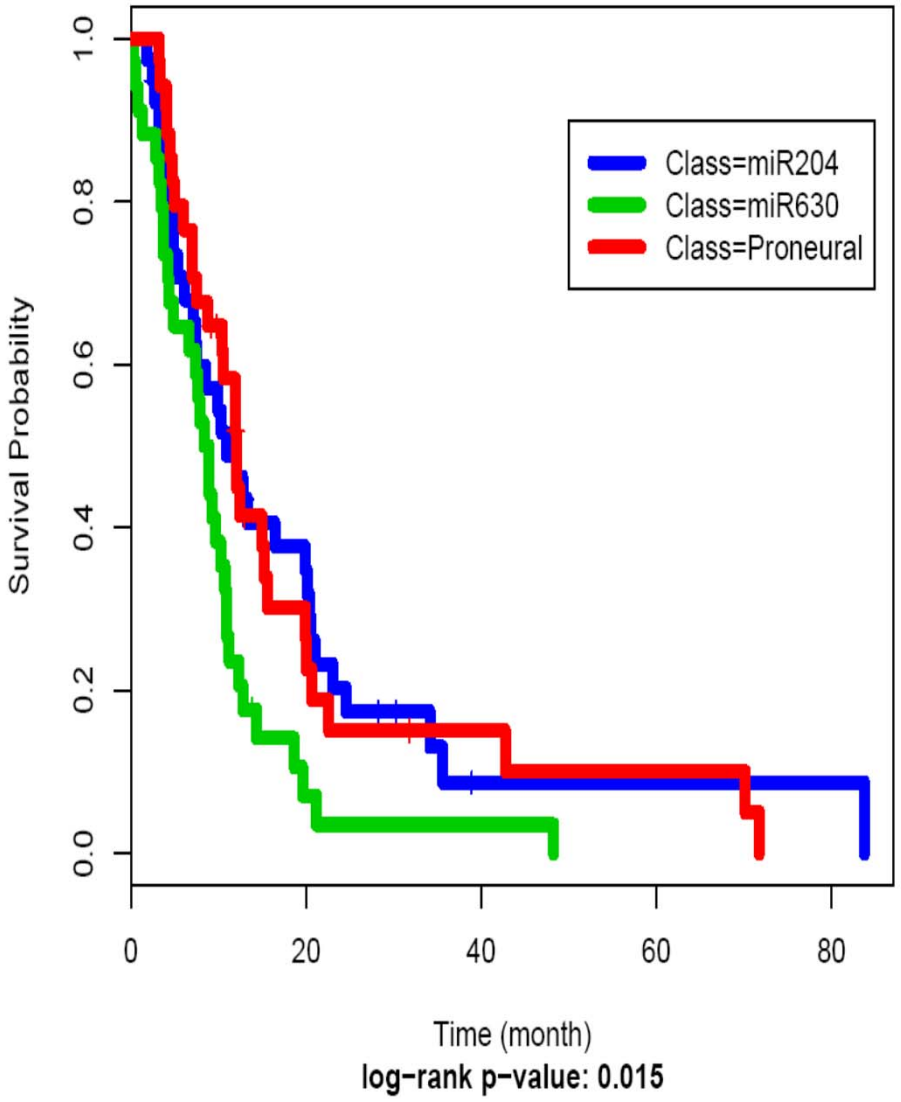


	microRNA		
GeneExpress	ProNeural	miR630	miR204
Norm-like	1	1	5
ProNeural	29	3	3
EGFR	2	7	19
Mes	3	16	14

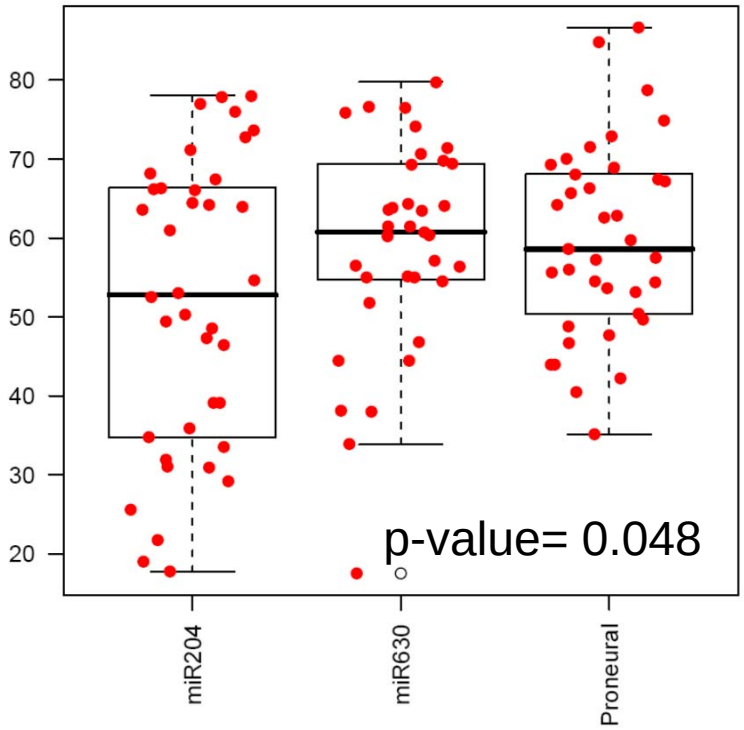
Fisher's exact test p-value = 9.716e-13

miRNA Subtypes Association to Clinical data

Survival Analysis



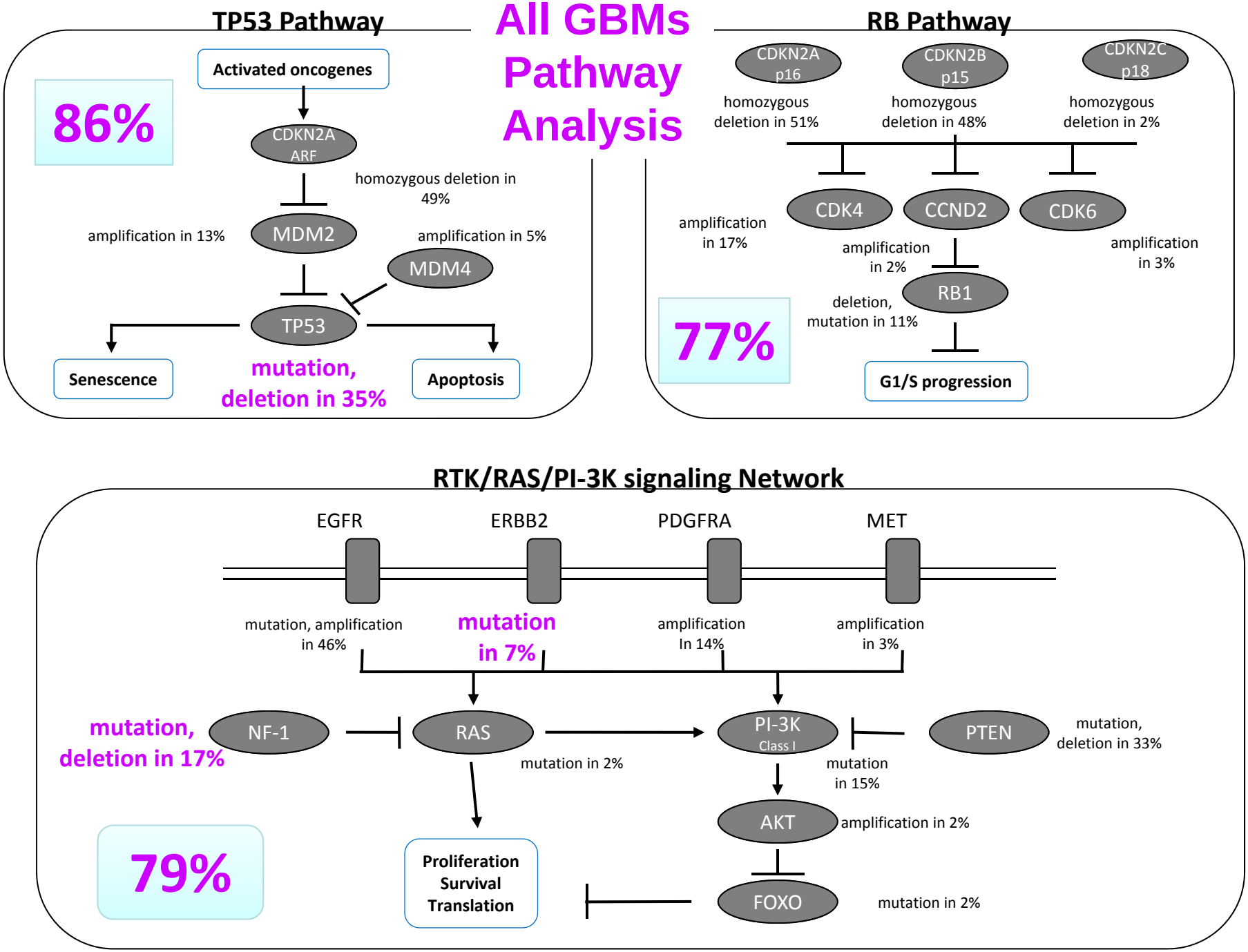
Age Distribution by Subtype

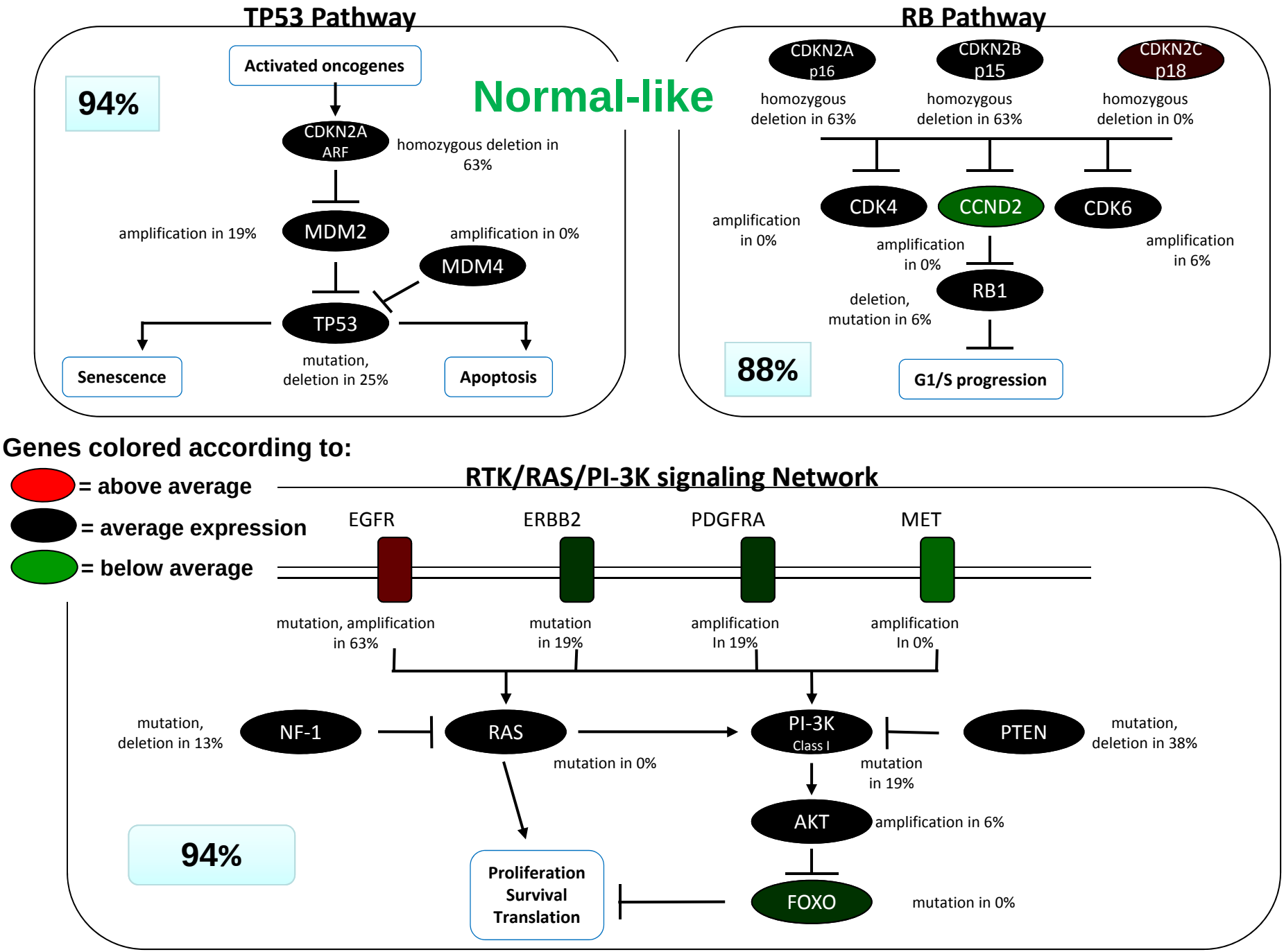


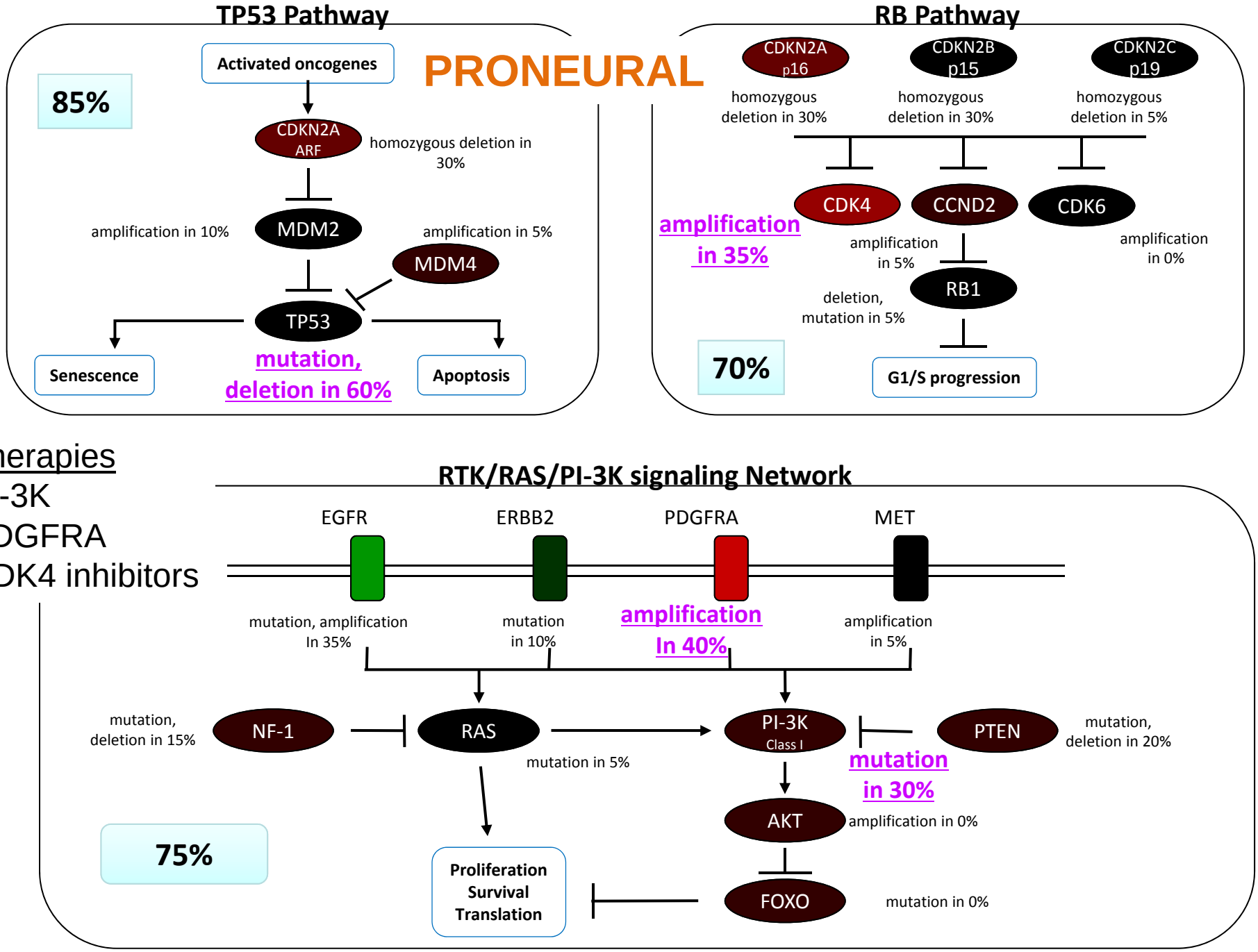
Gender Distribution by Subtype

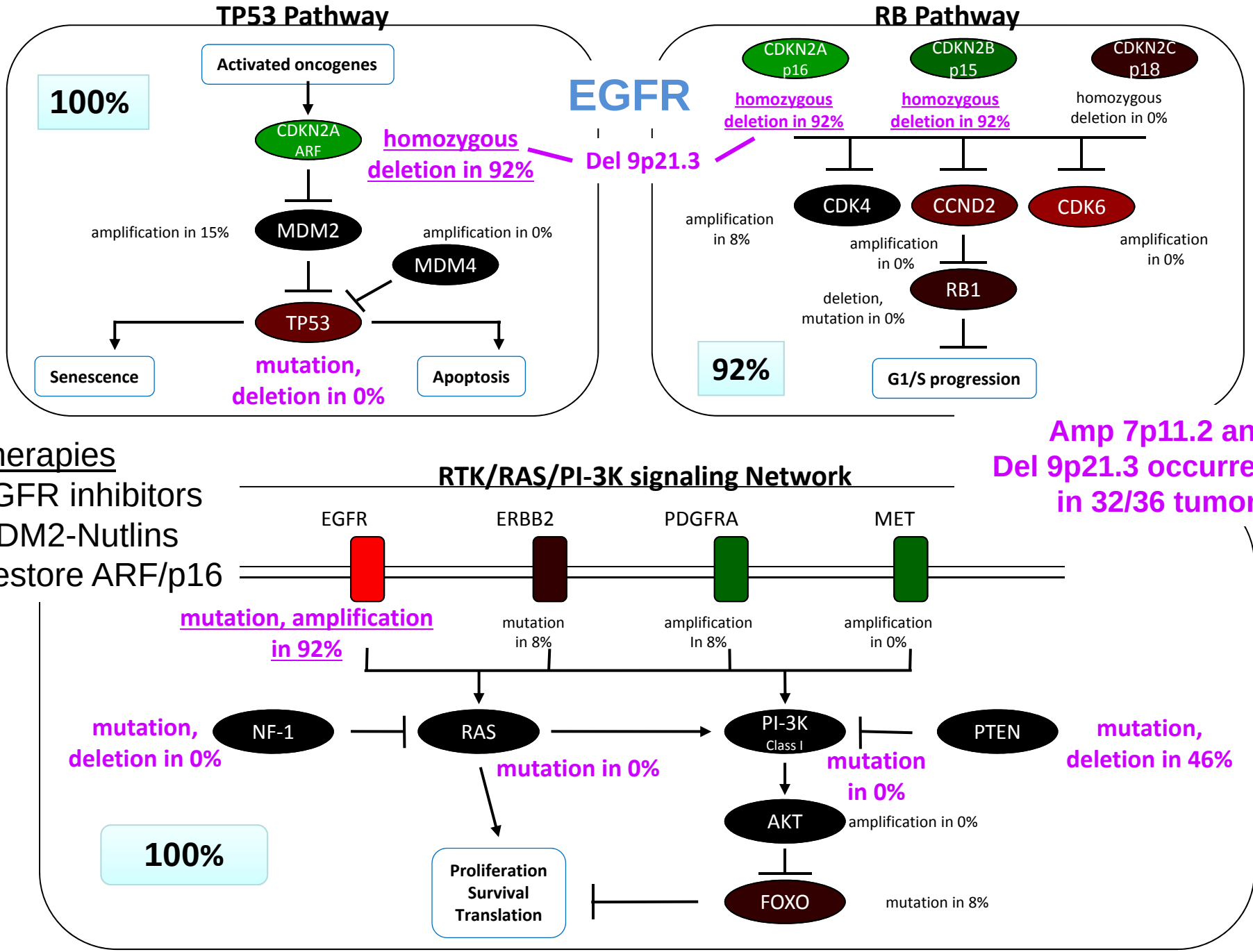
	miRNA cluster		
Gender	miR204	miR630	Proneural
FEMALE	17	17	6
MALE	23	18	29

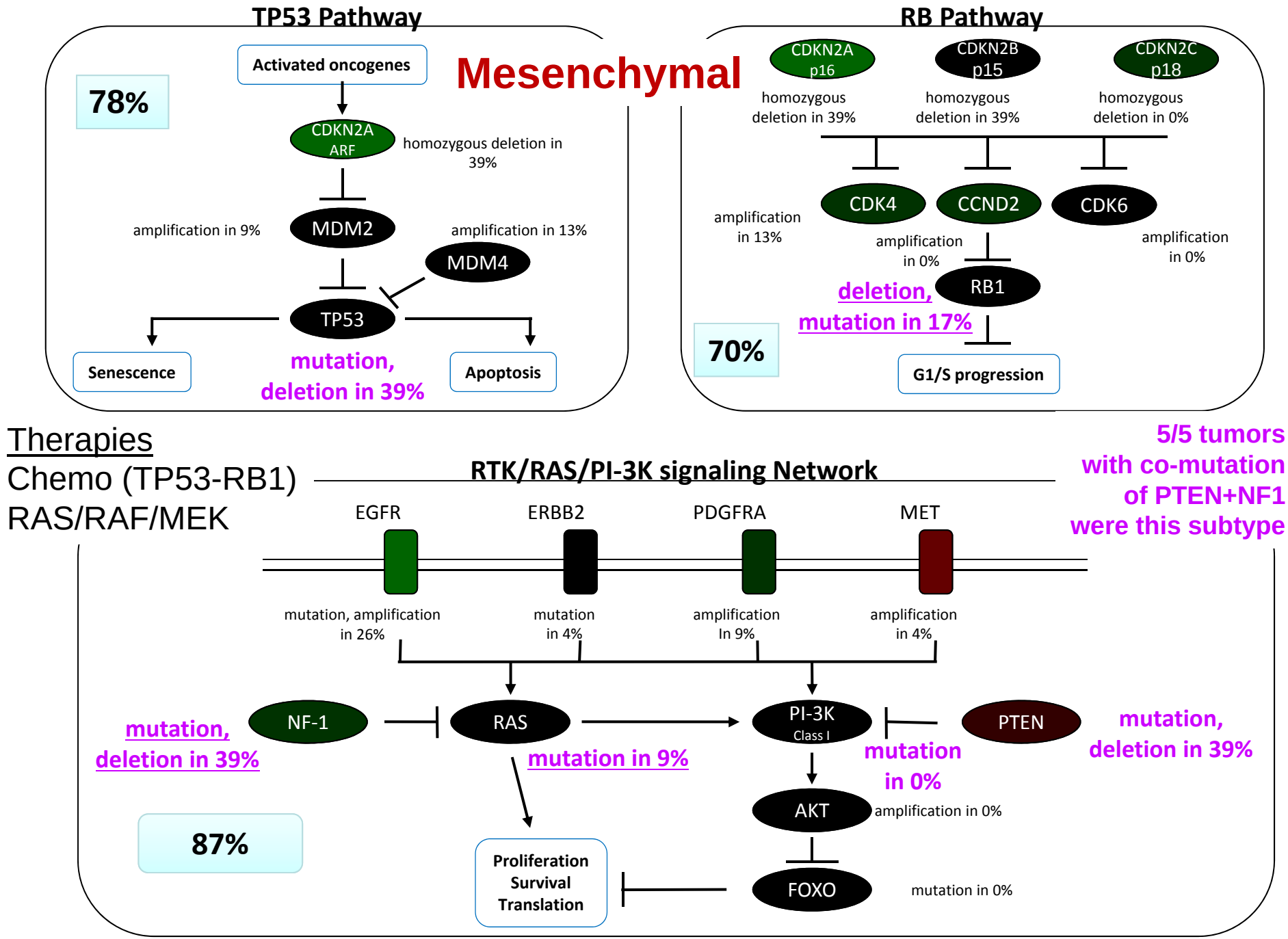
p-value = 0.01432


All GBMs Pathway Analysis
TP53 Pathway
86%
Activated oncogenes
CDKN2A ARF
homozygous deletion in 49%
amplification in 13%
MDM2
amplification in 5%
MDM4
TP53
Senescence
mutation, deletion in 35%
Apoptosis
RB Pathway
CDKN2A p16
CDKN2B p15
CDKN2C p18
homozygous deletion in 51%
homozygous deletion in 48%
homozygous deletion in 2%
CDK4
CCND2
CDK6
amplification in 17%
amplification in 2%
amplification in 3%
RB1
deletion, mutation in 11%
77%
G1/S progression
RTK/RAS/PI-3K signaling Network
EGFR
ERBB2
PDGFRA
MET
mutation, amplification in 46%
mutation in 7%
amplification In 14%
amplification in 3%
mutation, deletion in 17%
NF-1
RAS
mutation in 2%
PI-3K Class I
mutation in 15%
PTEN
mutation, deletion in 33%
AKT
amplification in 2%
79%
Proliferation Survival Translation
FOXO
mutation in 2%


TP53 Pathway
Activated oncogenes
94%
CDKN2A ARF
homozygous deletion in 63%
amplification in 19%
MDM2
amplification in 0%
MDM4
TP53
mutation, deletion in 25%
Senescence
Apoptosis
Normal-like
RB Pathway
CDKN2A p16
CDKN2B p15
CDKN2C p18
homozygous deletion in 63%
homozygous deletion in 63%
homozygous deletion in 0%
CDK4
CCND2
CDK6
amplification in 0%
amplification in 0%
amplification in 6%
deletion, mutation in 6%
RB1
88%
G1/S progression
Genes colored according to:
= above average
= average expression
= below average
RTK/RAS/PI-3K signaling Network
EGFR
ERBB2
PDGFRA
MET
mutation, amplification in 63%
mutation in 19%
amplification In 19%
amplification In 0%
mutation, deletion in 13%
NF-1
RAS
mutation in 0%
PI-3K Class I
mutation in 19%
PTEN
mutation, deletion in 38%
AKT
amplification in 6%
94%
Proliferation Survival Translation
FOXO
mutation in 0%


TP53 Pathway
PRONEURAL
RB Pathway
Activated oncogenes
85%
CDKN2A ARF
homozygous deletion in 30%
amplification in 10%
MDM2
amplification in 5%
MDM4
TP53
Senescence
mutation, deletion in 60%
Apoptosis
CDKN2A p16
CDKN2B p15
CDKN2C p19
homozygous deletion in 30%
homozygous deletion in 30%
homozygous deletion in 5%
CDK4
CCND2
CDK6
amplification in 35%
amplification in 5%
amplification in 0%
RB1
deletion, mutation in 5%
70%
G1/S progression
Therapies
PI-3K
PDGFRA
CDK4 inhibitors
RTK/RAS/PI-3K signaling Network
EGFR
ERBB2
PDGFRA
MET
mutation, amplification In 35%
mutation in 10%
amplification In 40%
amplification in 5%
mutation, deletion in 15%
NF-1
RAS
mutation in 5%
PI-3K Class I
PTEN
mutation, deletion in 20%
mutation in 30%
AKT
amplification in 0%
75%
Proliferation Survival Translation
FOXO
mutation in 0%


TP53 Pathway
Activated oncogenes
100%
CDKN2A ARF
homozygous deletion in 92%
EGFR
Del 9p21.3
amplification in 15%
MDM2
amplification in 0%
MDM4
TP53
Senescence
mutation, deletion in 0%
Apoptosis
RB Pathway
CDKN2A p16
CDKN2B p15
CDKN2C p18
homozygous deletion in 92%
homozygous deletion in 92%
homozygous deletion in 0%
CDK4
CCND2
CDK6
amplification in 8%
amplification in 0%
amplification in 0%
RB1
deletion, mutation in 0%
92%
G1/S progression
Amp 7p11.2 and Del 9p21.3 occurred in 32/36 tumors
Therapies
EGFR inhibitors
MDM2-Nutlins
Restore ARF/p16
RTK/RAS/PI-3K signaling Network
EGFR
ERBB2
PDGFRA
MET
mutation, amplification in 92%
mutation in 8%
amplification In 8%
amplification in 0%
mutation, deletion in 0%
NF-1
RAS
PI-3K Class I
PTEN
mutation, deletion in 46%
mutation in 0%
mutation in 0%
AKT
amplification in 0%
100%
Proliferation Survival Translation
FOXO
mutation in 8%


TP53 Pathway
Activated oncogenes
78%
CDKN2A ARF
homozygous deletion in 39%
amplification in 9%
MDM2
amplification in 13%
MDM4
TP53
Senescence
mutation, deletion in 39%
Apoptosis
Mesenchymal
RB Pathway
CDKN2A p16
CDKN2B p15
CDKN2C p18
homozygous deletion in 39%
homozygous deletion in 39%
homozygous deletion in 0%
amplification in 13%
CDK4
CCND2
CDK6
amplification in 0%
amplification in 0%
deletion, mutation in 17%
RB1
70%
G1/S progression
Therapies
Chemo (TP53-RB1)
RAS/RAF/MEK
RTK/RAS/PI-3K signaling Network
5/5 tumors with co-mutation of PTEN+NF1 were this subtype
EGFR
ERBB2
PDGFRA
MET
mutation, amplification in 26%
mutation in 4%
amplification In 9%
amplification in 4%
mutation, deletion in 39%
NF-1
RAS
mutation in 9%
PI-3K Class I
mutation in 0%
PTEN
mutation, deletion in 39%
AKT
amplification in 0%
87%
Proliferation Survival Translation
FOXO
mutation in 0%

Human Cancer Biospecimen Core Resource

•The International Genomics Consortium and Translational Genomics Research Institute, Phoenix, Ariz.,

Biospecimen Source Sites

Glioblastoma multiforme biospecimens:
•MD Anderson Cancer Center
•Henry Ford Hospital System, Department of Neurosurgery
•University of California at San Francisco Medical Center, Department of Neurological Surgery

Patients who agreed to participate in the study

Cancer Genome Characterization Centers

•Broad Institute of MIT and Harvard
•Harvard Medical School and Brigham and Women's Hospital
•Lawrence Berkeley National Laboratory
•Memorial Sloan-Kettering Cancer Center
•The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University
•The University of Southern California Norris Comprehensive Cancer Center
•Stanford University School of Medicine
•University of North Carolina Lineberger Comprehensive Cancer Center

Genome Sequencing Centers

•Broad Institute Sequencing Platform, The Broad Institute of the Massachusetts Institute of Technology and Harvard University
•Washington University Genome Sequencing Center, Washington University School of Medicine
•Human Genome Sequencing Center, Baylor College of Medicine

University of North Carolina at Chapel Hill

Neil Hayes
Michael Topal
Katherine Hoadley
Matt Wilkerson
Sai Balu
Yuan Qi
Joel Parker
Victor Weigman
Yufeng Liu
Yan Shi

National Cancer Institute

Ana Barker
Daniela Gerhard
Joe Vockley

National Human Genome Research Institute

Francis Collins