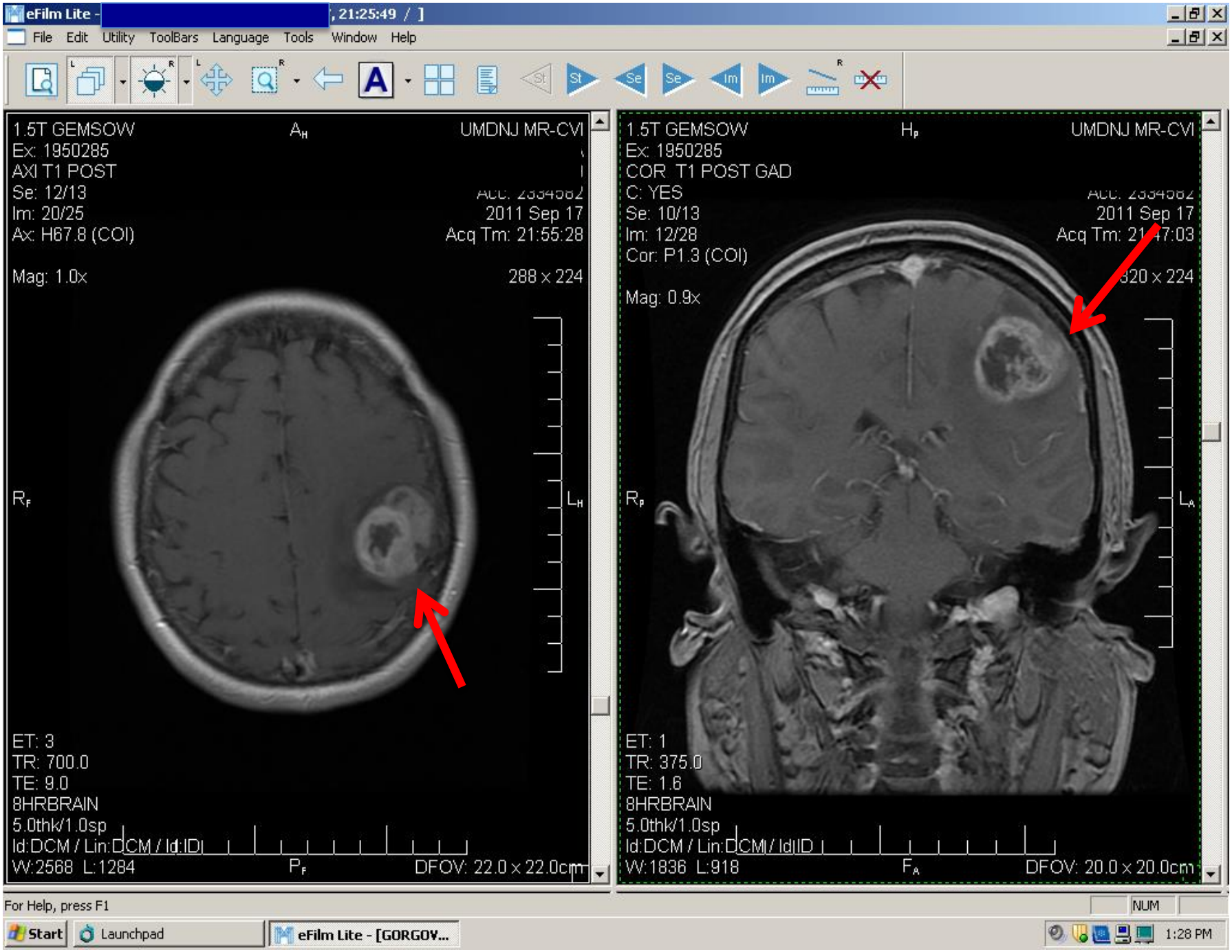
# Gliomas: Of Mice and Men

Eric C Holland

Fred Hutchinson Cancer Research Center

SOLID TUMOR
TRANSLATIONAL RESEARCH

FRED HUTCH
CURES START HERE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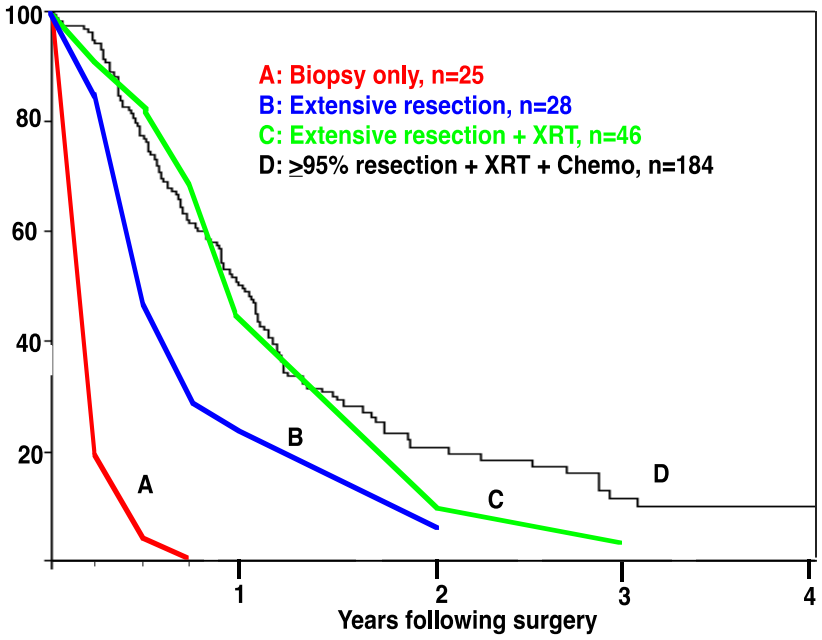
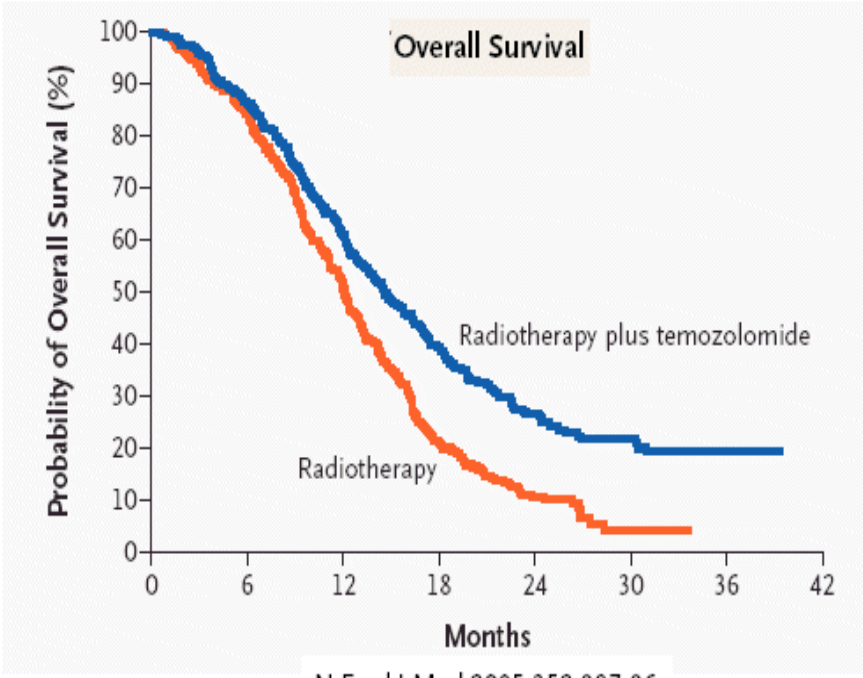
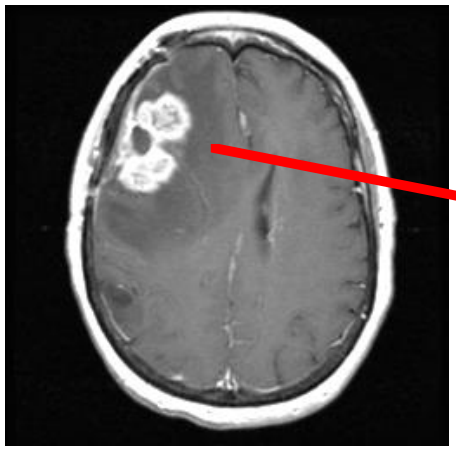
# The standard of care for GBM treatment was established in 2005 in a paper that concluded the addition of chemotherapy to radiation was better than radiation alone




N Engl J Med 2005;352:987-96.

# We can take genes that are abnormal in human brain tumors and use viruses to transfer them into mice.

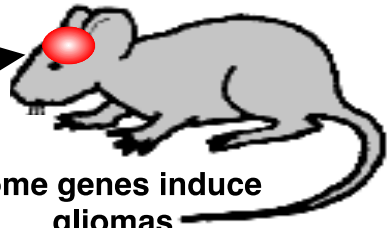


Isolate abnormal genes in gliomas

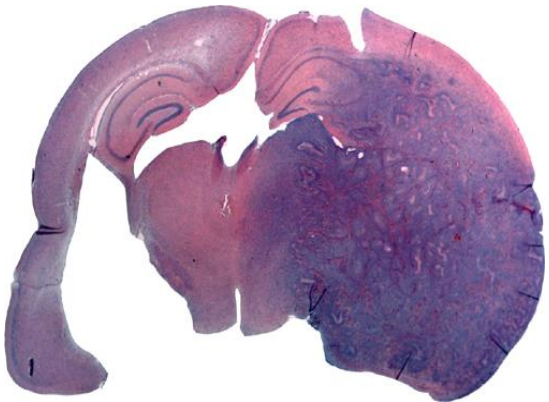
convert genes into viruses

use viruses to transfer genes specifically to the brains of genetically engineered mice

Most genes do not cause brain tumors

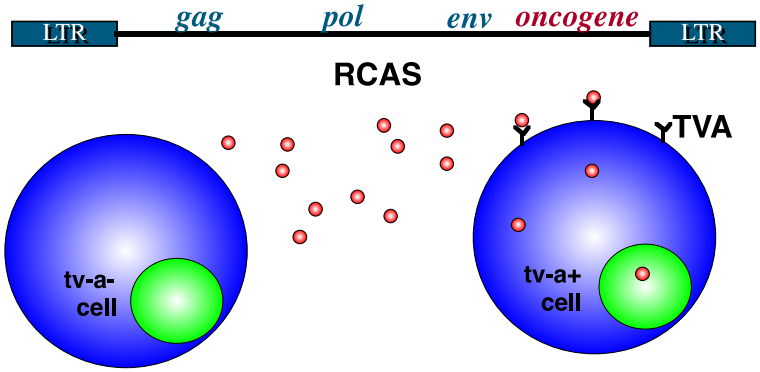


some genes induce gliomas



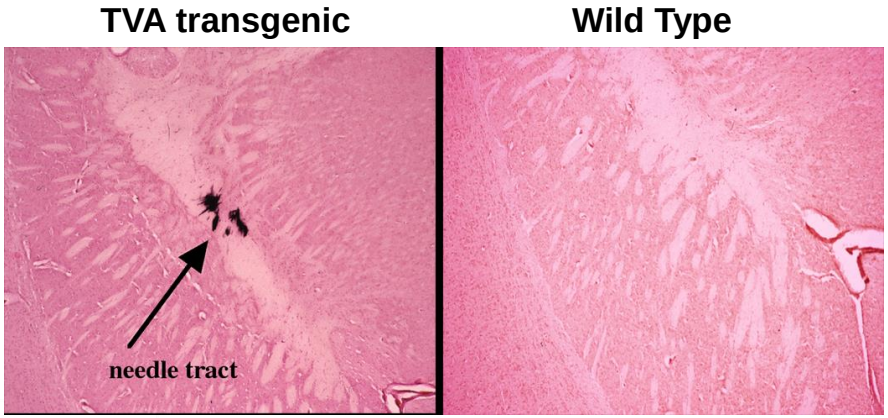
# Modeling cancer formation with post natal, cell type-specific retroviral gene transfer – fulfilling Koch's Postulate

## Somatic cell type-specific gene transfer in mice via RCAS/tv-a

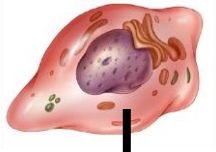



RCAS vectors transfer genes to mammalian cells only if they express tv-a

## Gene transfer to tv-a transgenic mice by RCAS infection in vivo

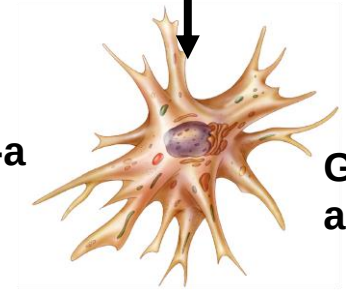



RCAS-PDGFB → Ntv-a



nestin promoter, stem cells

RCAS-PDGFB → Gtv-a

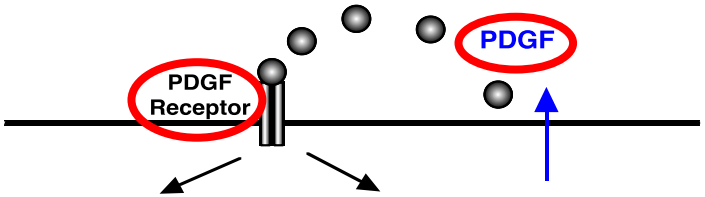


GFAP promoter, differentiated astrocytes

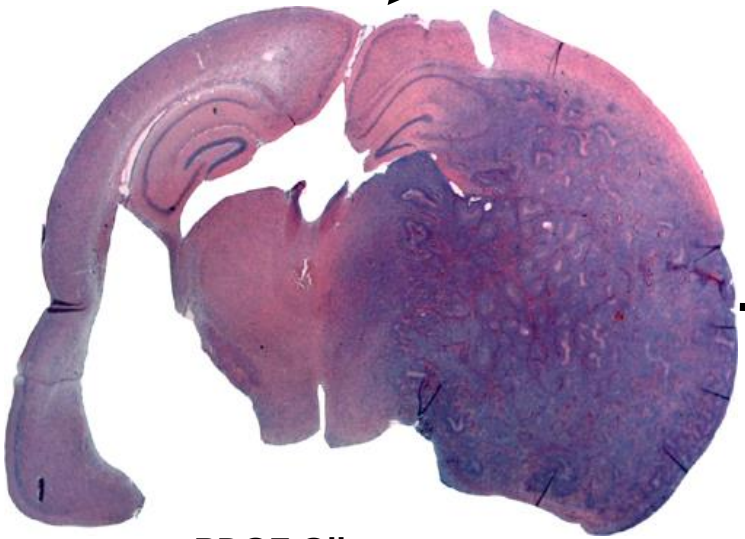
Holland et al, 1998
Dai et al, 2001

# Gain-of-function RCAS/tv-a modeling
# PDGFB is causally related to formation of PN-like GBM

RCAS-PDGFB



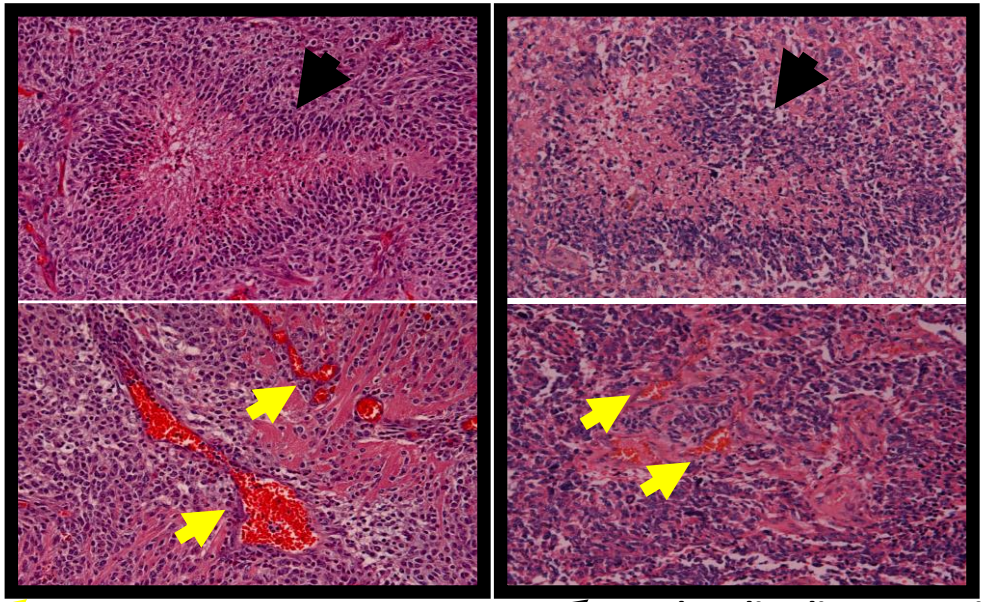

Nestin Tv-a mouse or
GFAP Tv-a mouse,
newborn or adult,
anywhere in the brain



PDGF Glioma

mouse GBM

human GBM



--microvascular proliferation

pseudopalisading necrosis

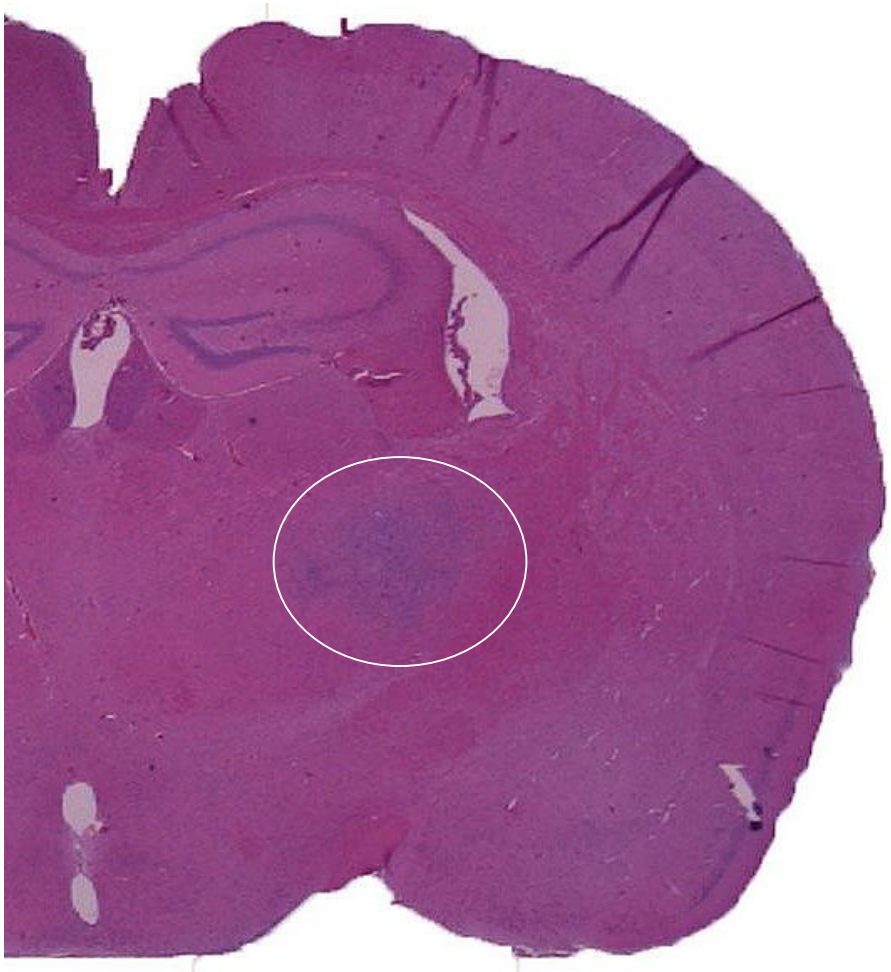
Dai et al, 2001
Shih et al, 2004

P53-/-, ink4a-arf-/- or PTEN-/-

# One reason that surgery cannot cure GBM

## A small localized tumor can be seen in the mouse brain.

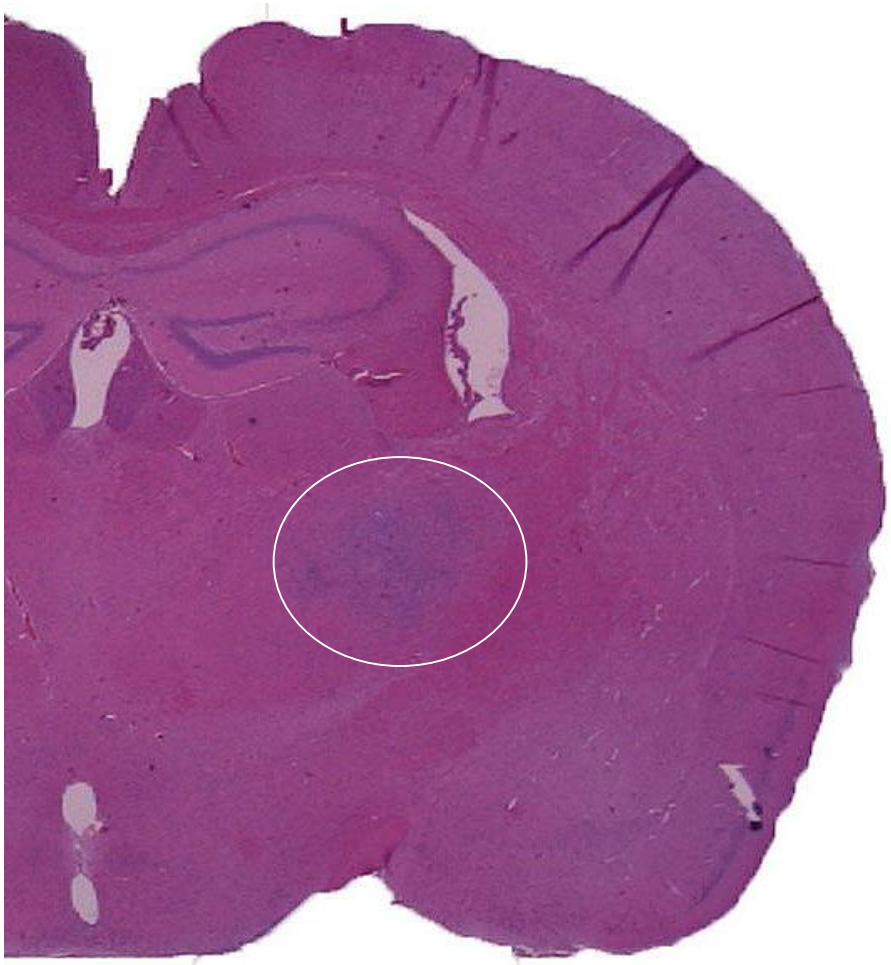
H&E



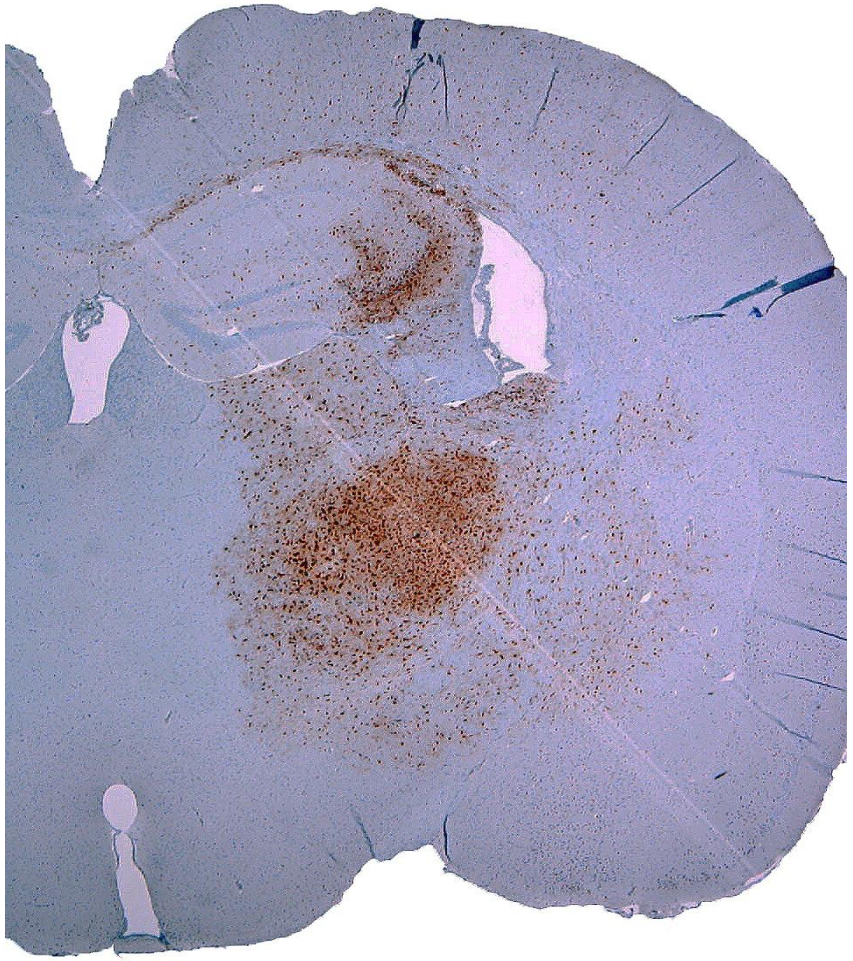
# One reason that surgery can not cure GBM

**But the tumor cells are all over the brain, this is why the tumors come back.**

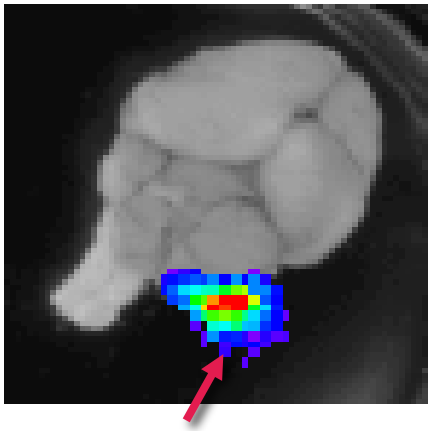
H&E

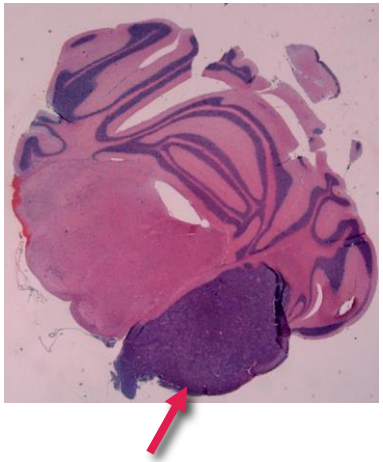


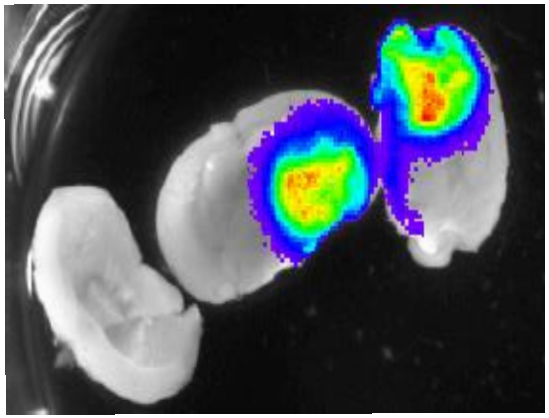
Stain for tumor cells

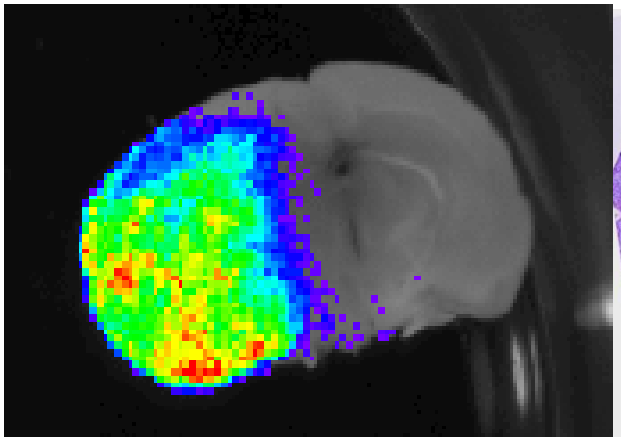


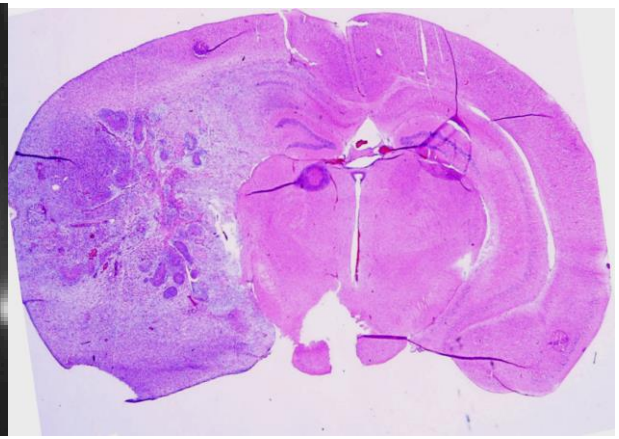
**We can genetically engineer the tumors with a firefly gene that makes brain tumors give off light in response to biological signaling.**

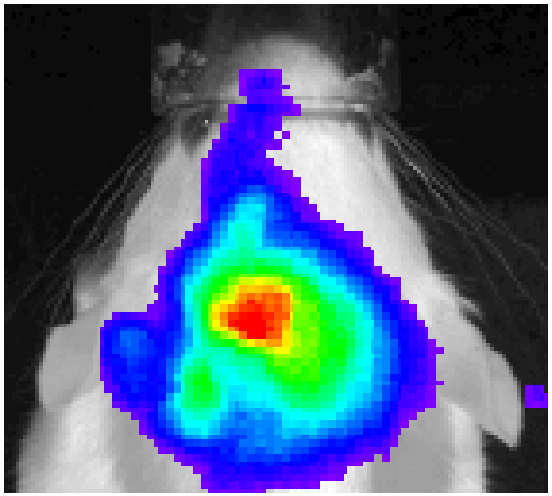






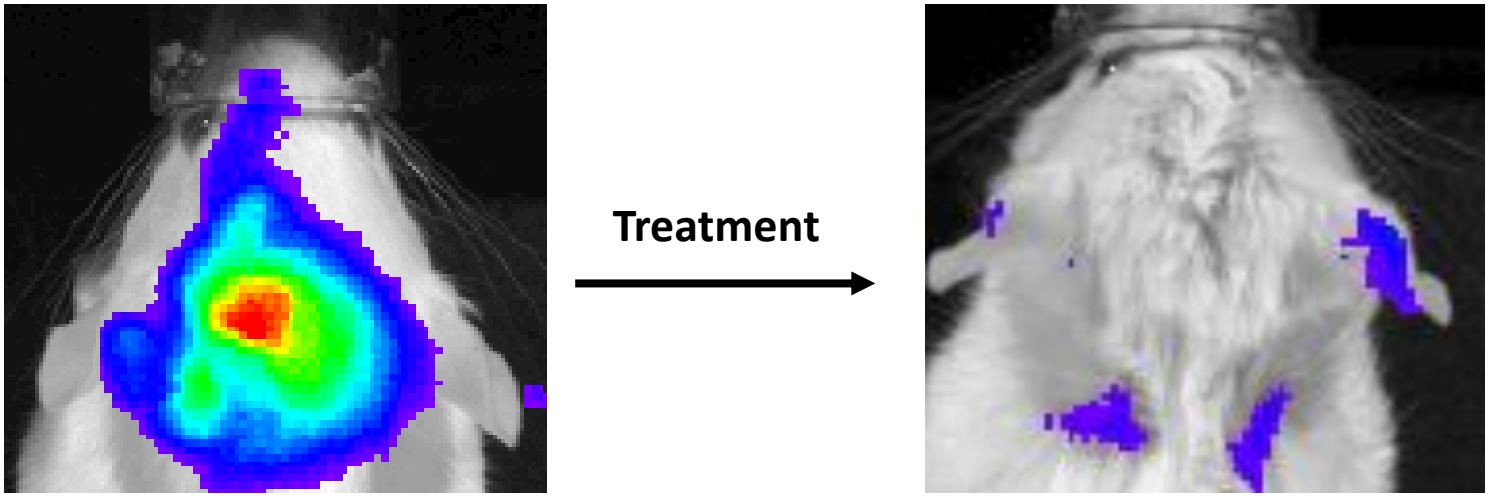






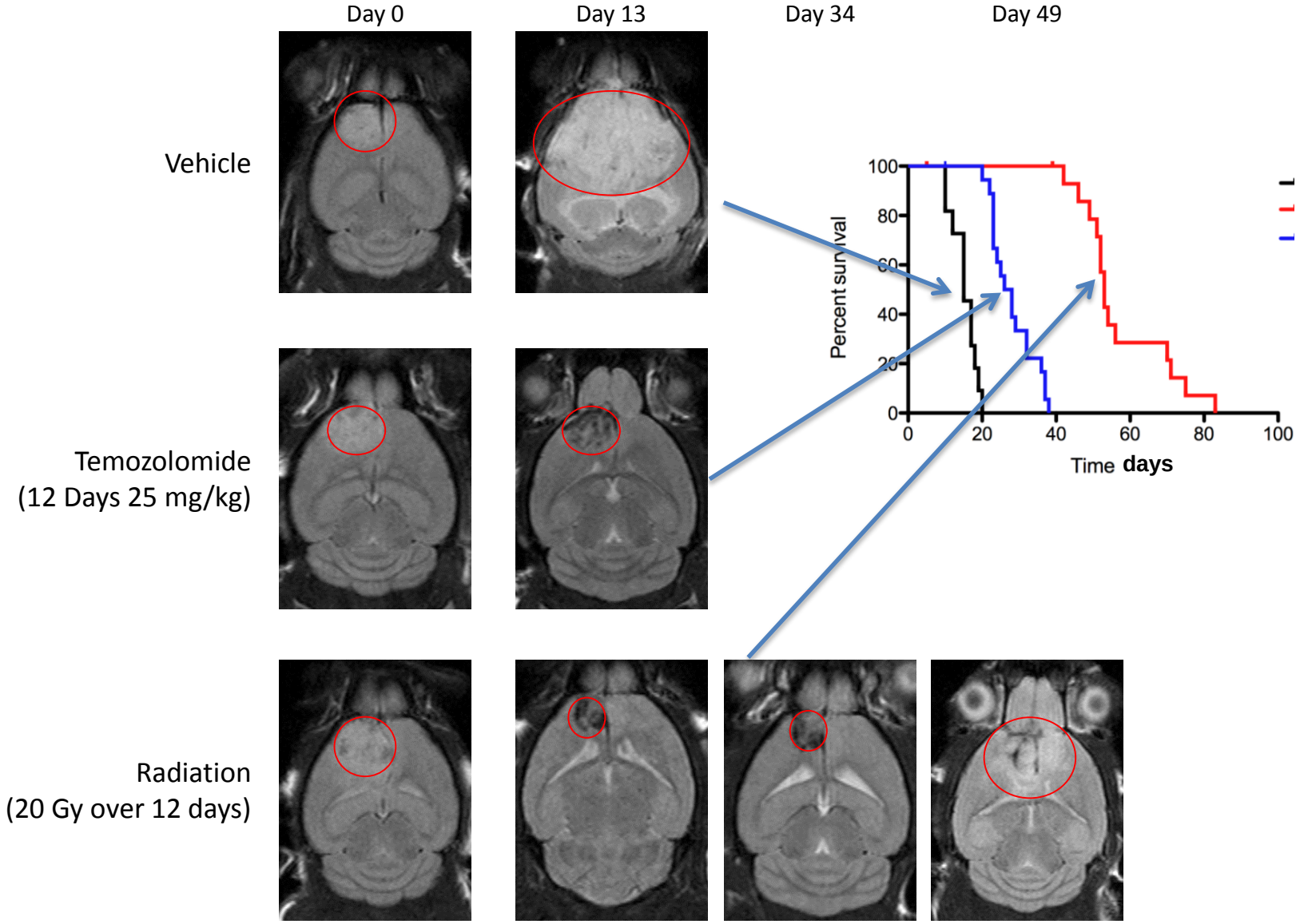
**We can detect that light even in a living mouse with a tumor**

**We can treat the mice the same way we treat people**

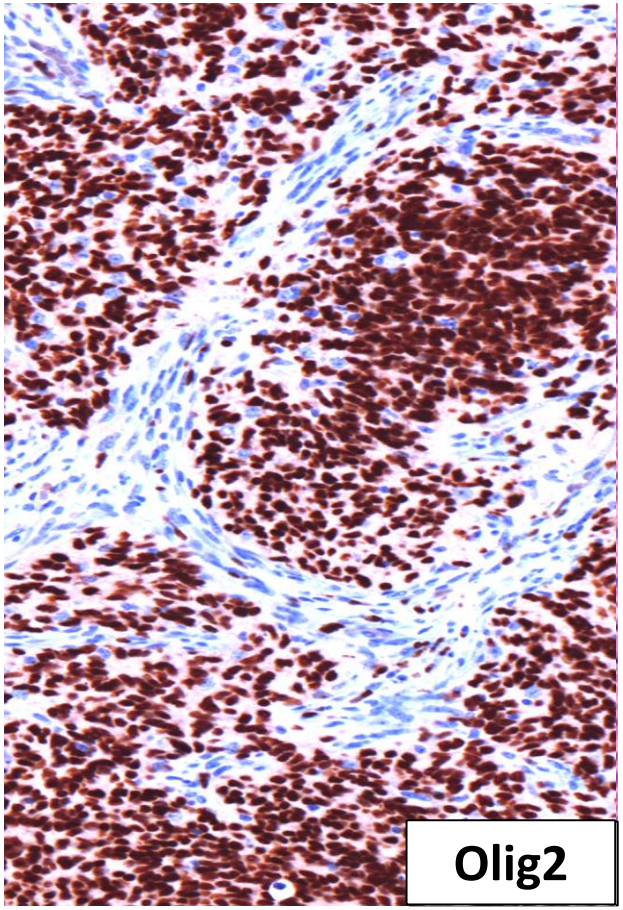


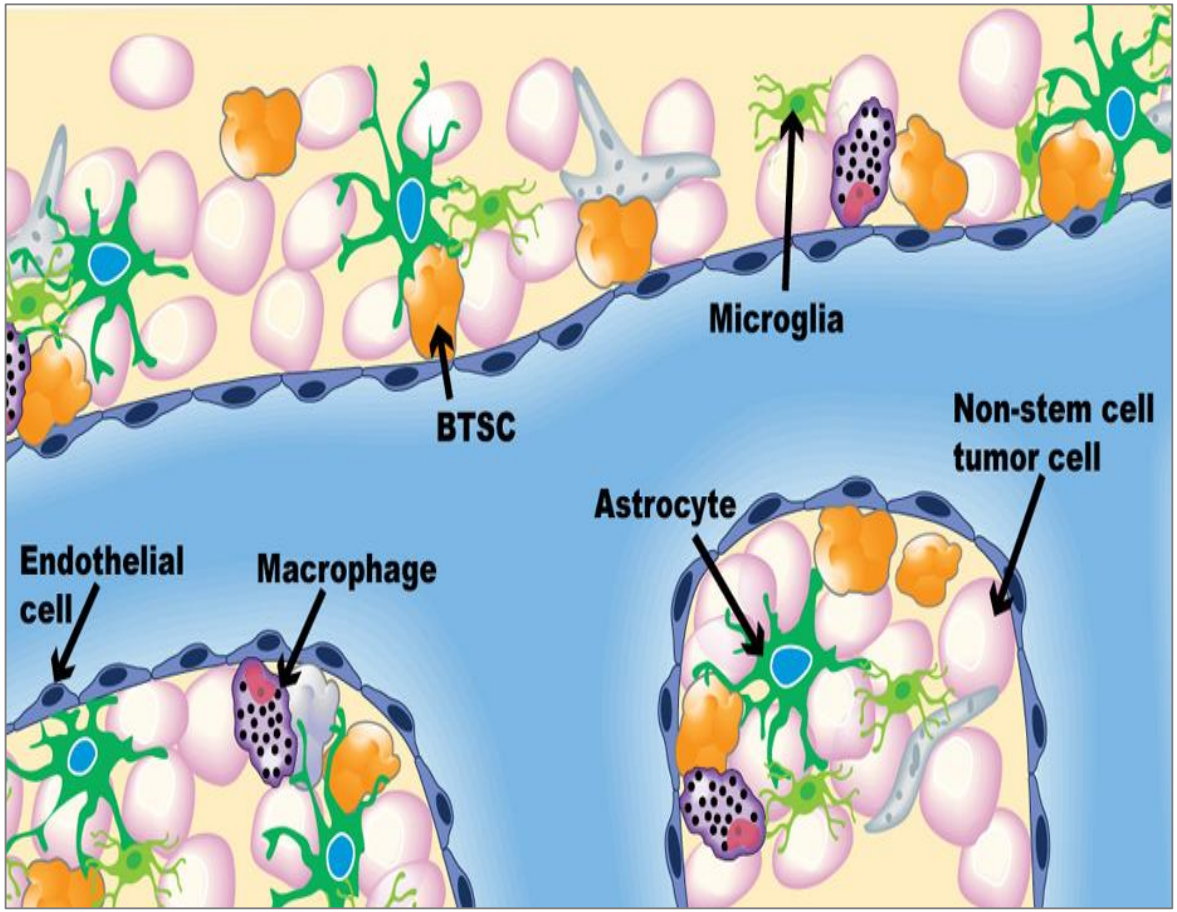

**We can use the light production to follow the tumor's response to therapy**

# MRI responses to temozolomide and XRT



# The GBM itself is heterogeneous, tumor cells intermingled with stromal cells in a complex microenvironment

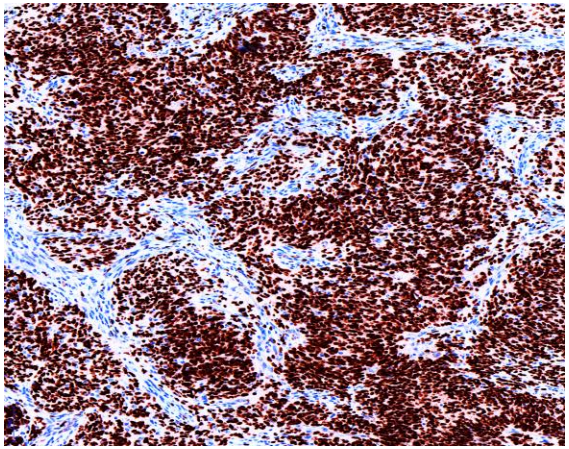


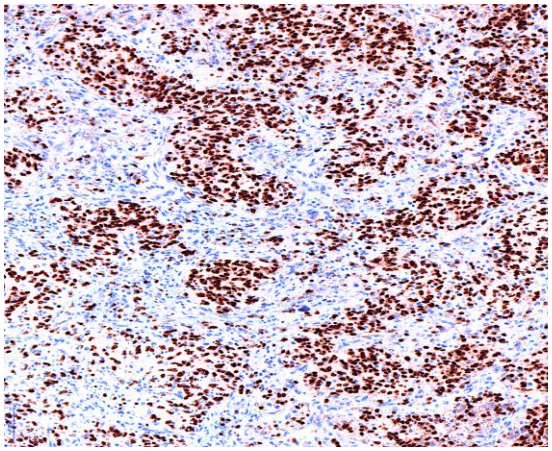

**Cells with stem cell characteristics (radiation resistance) occupy the perivascular space**

# Differentiated, sensitive cells are depleted by irradiation; perivascular cell's stem-like cells are spared (enriched)

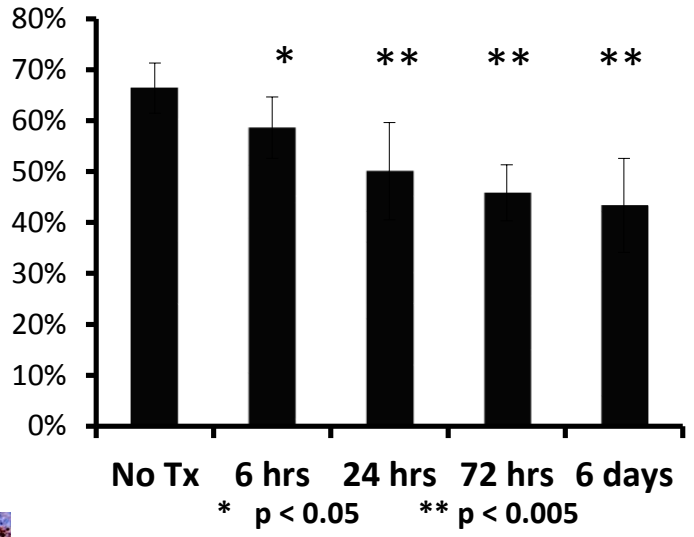
**Untreated**



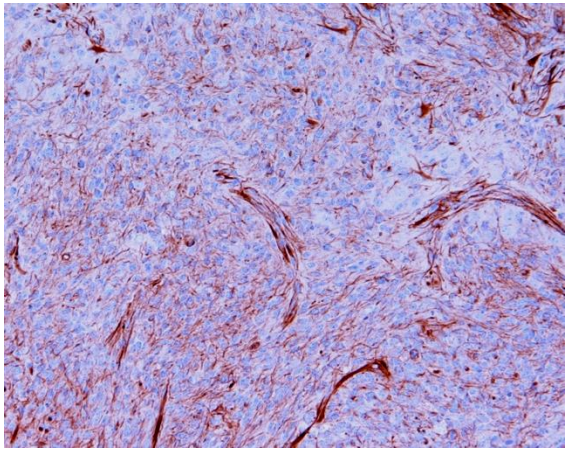
**72 hours**

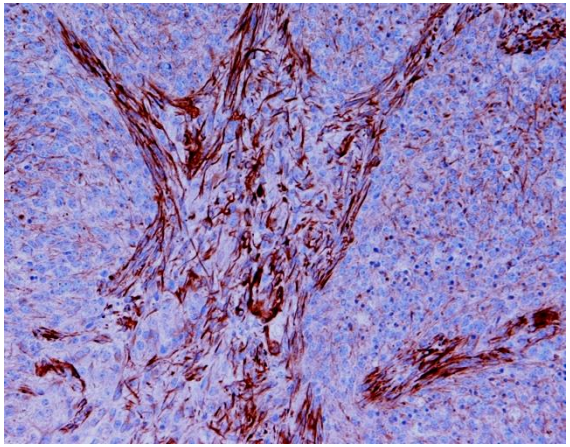


**Percent olig2+**




**Olig2**

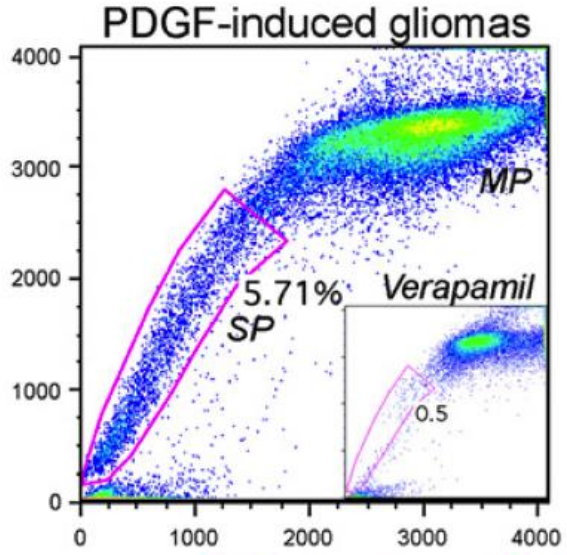


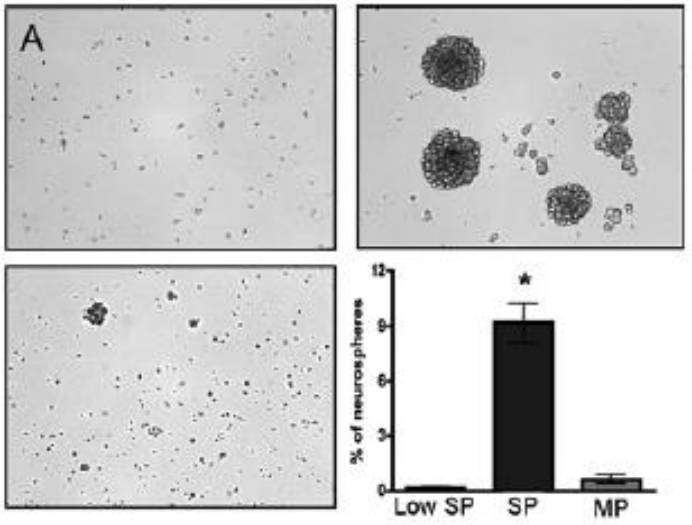


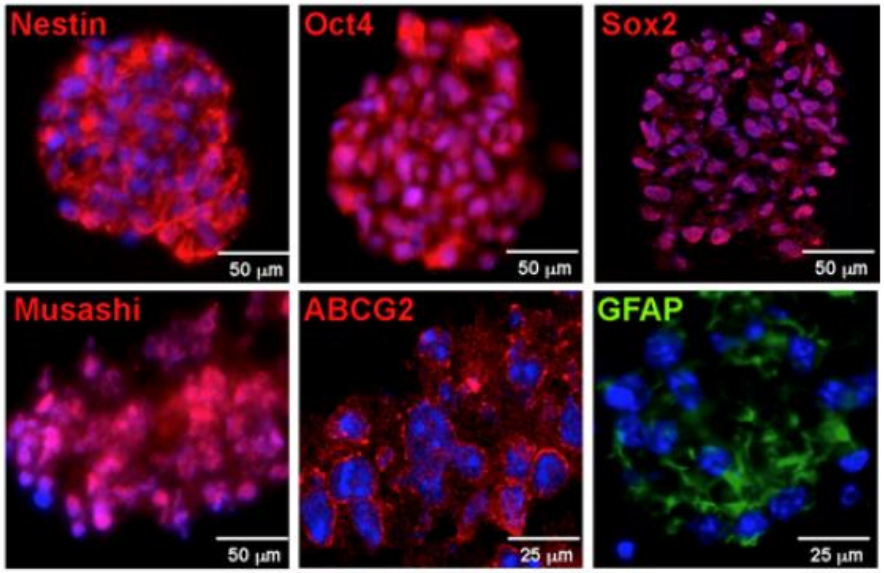
**Nestin**

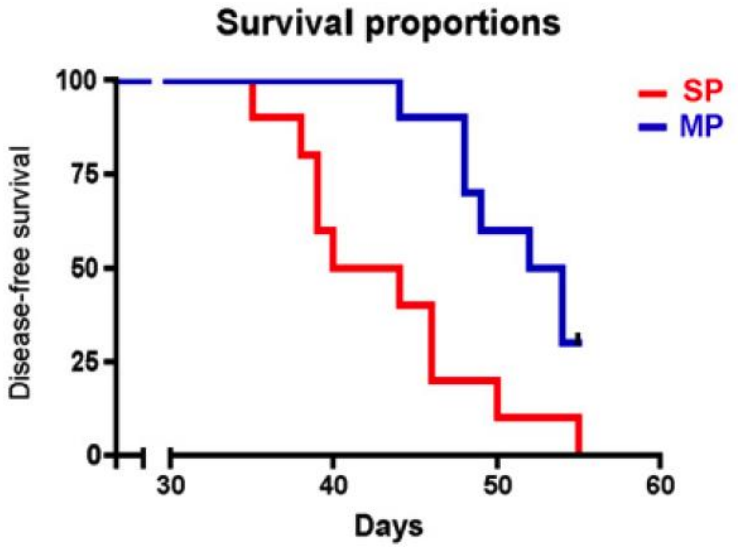
Karim Helmy

# Side-population analysis allows for functional isolation of stem-like cells



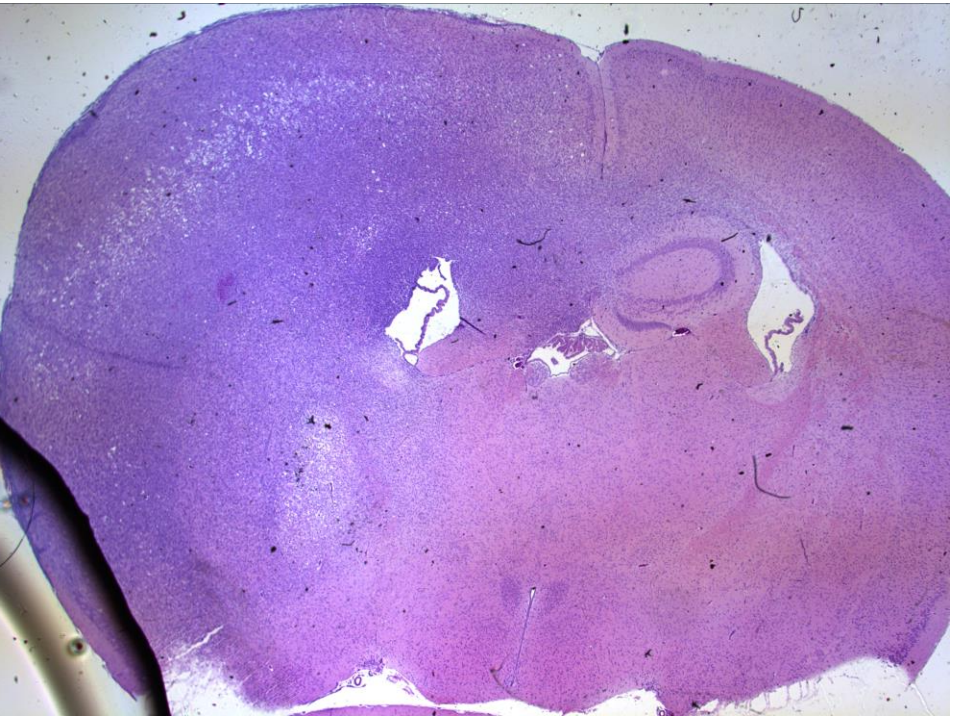



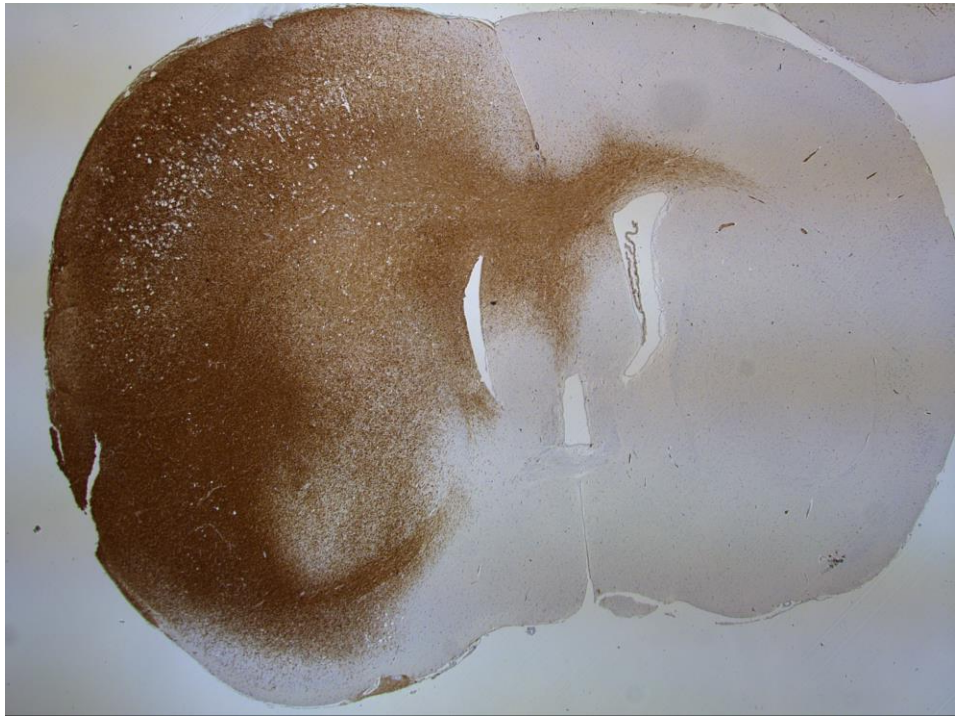




Bleau AM *et al.* Cell Stem Cell 2009

# We are able to create IDH1 mutant gliomas with high 2HG levels



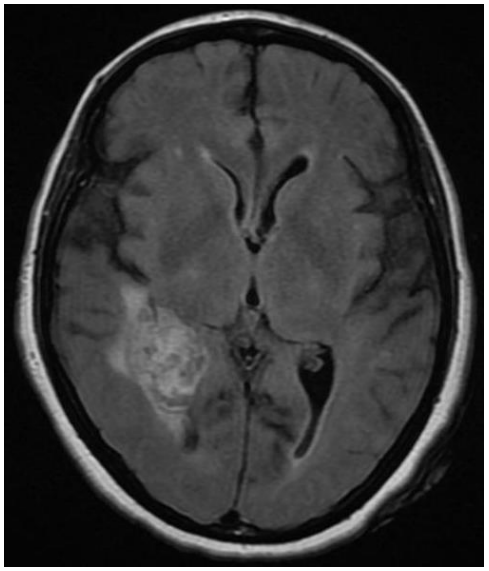
H&E



Anti-IDH1$^{mu}$

# We have a model of supratentorial ependymoma driven by a fusion gene commonly found in the human tumors

Human ST-EP



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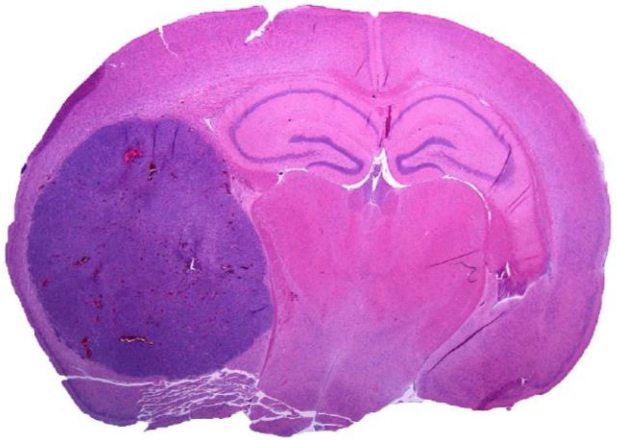

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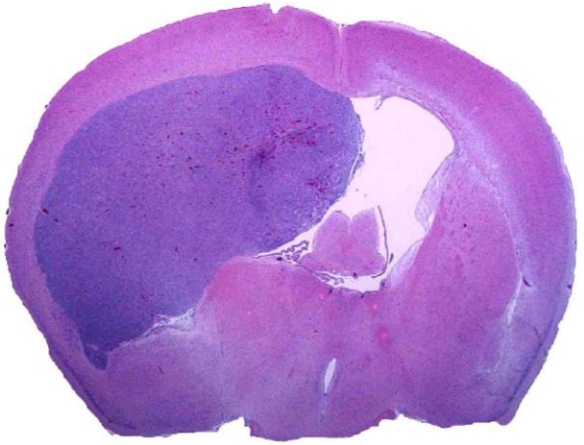

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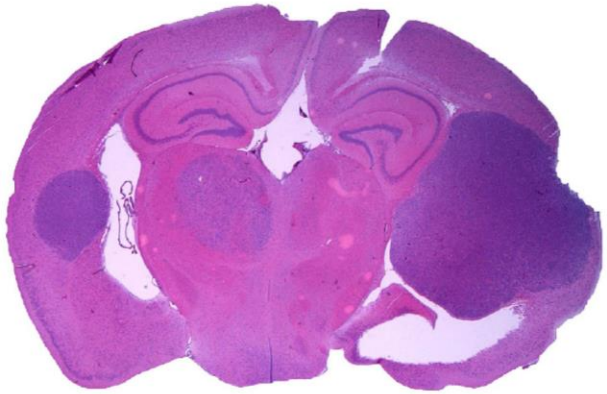

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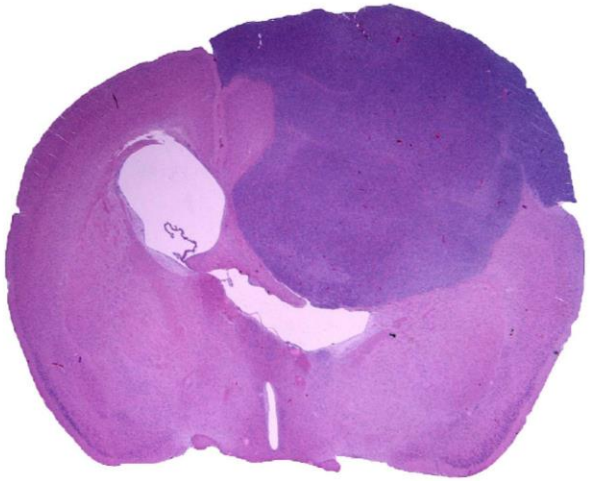

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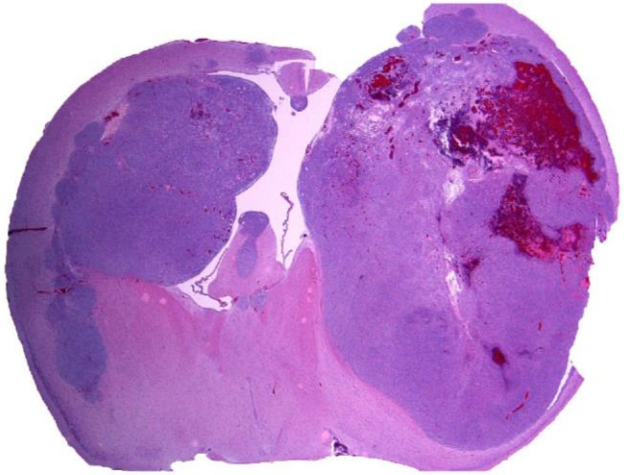


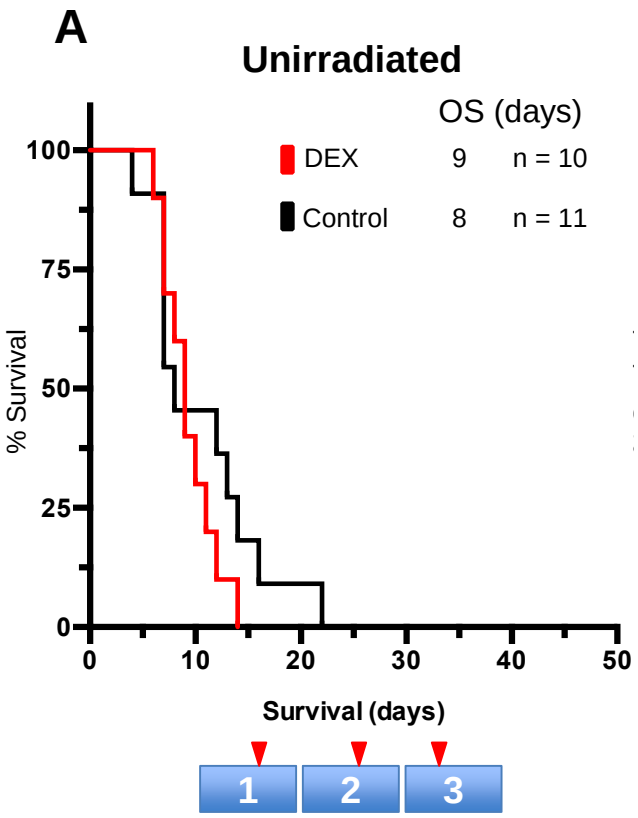
Courtesy by Gordana Juric-Sekhar

**We are using these models to develop new drugs and optimize therapies.**

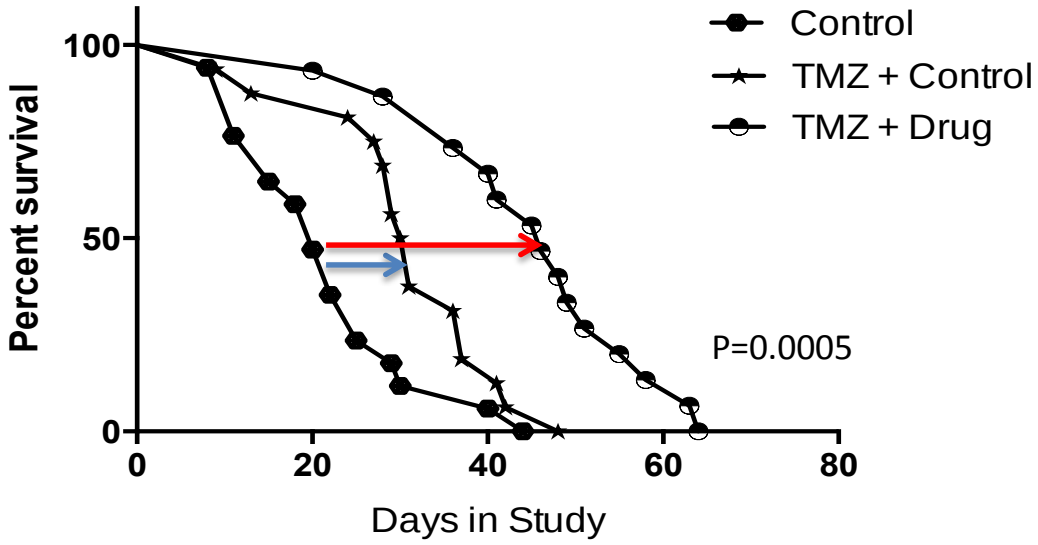
**We are using these models to develop new drugs and optimize therapies.**

**As an example, dexamethasone (DEX) is usually given during radiation therapy for gliomas…**

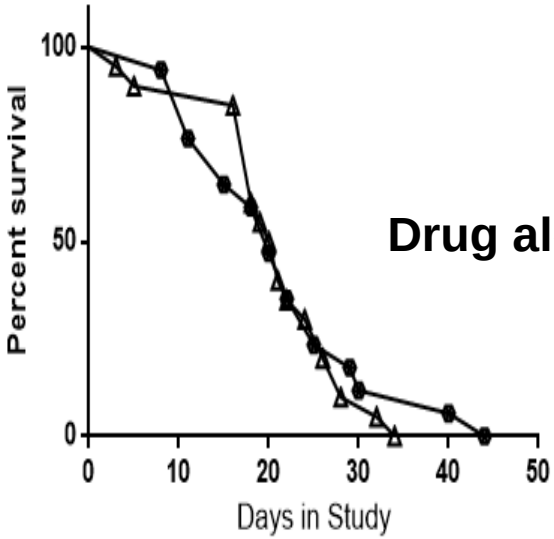
# DEX pre-treatment significantly decreases the survival benefit from IR in PDGF-driven glioma



# We are developing drugs that enhance efficacy of standard therapy.




**Daily dosing of both drugs 5 days per week up to 7 weeks**




**Drug alone vs control**

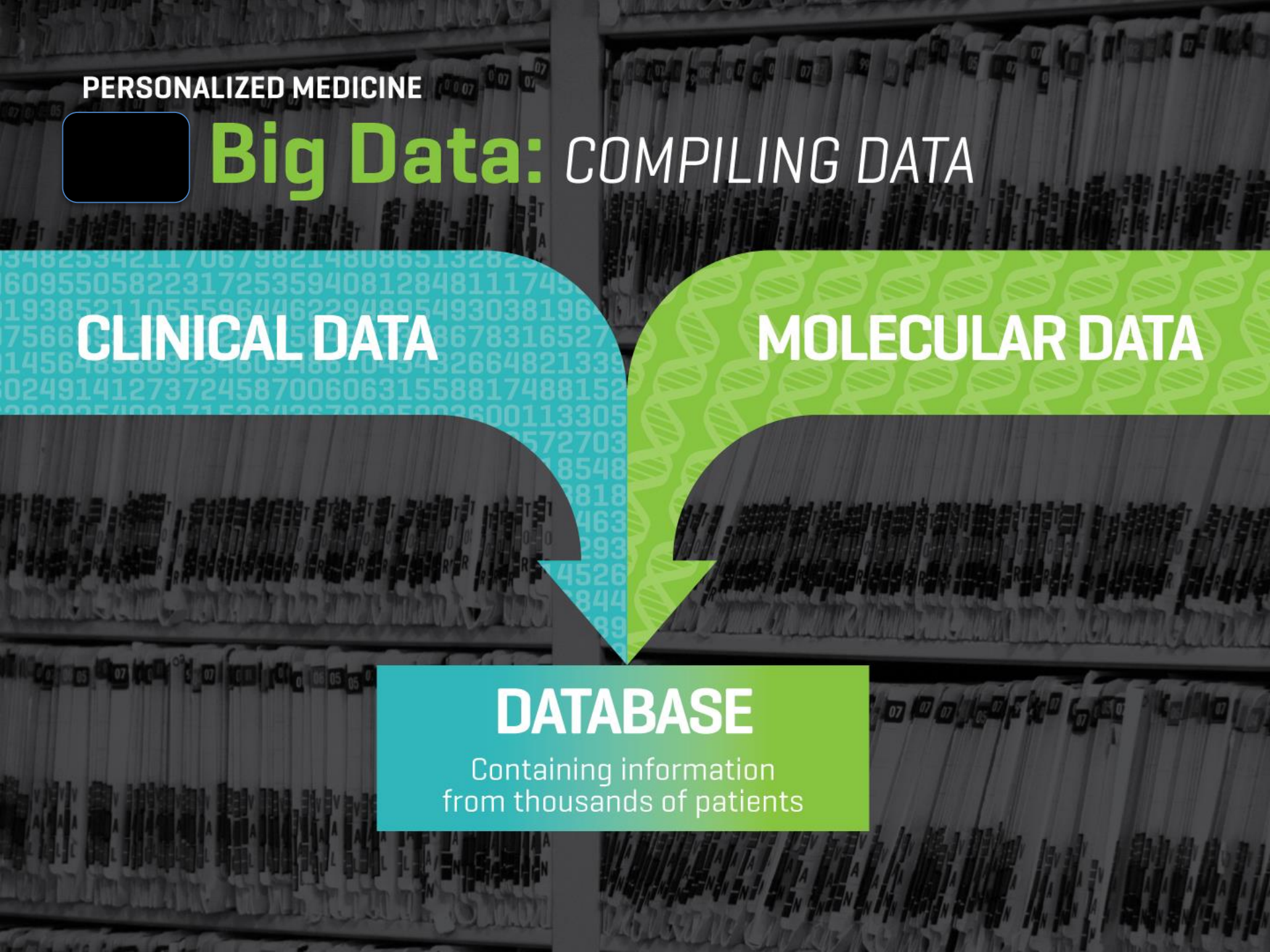
**The mouse work:**

**Genetically engineered mouse models of brain tumors recapitulate genetics, histology and gene expression patterns found in their human counterparts.**

**These models are immune competent and mimic responses to standard therapies.**

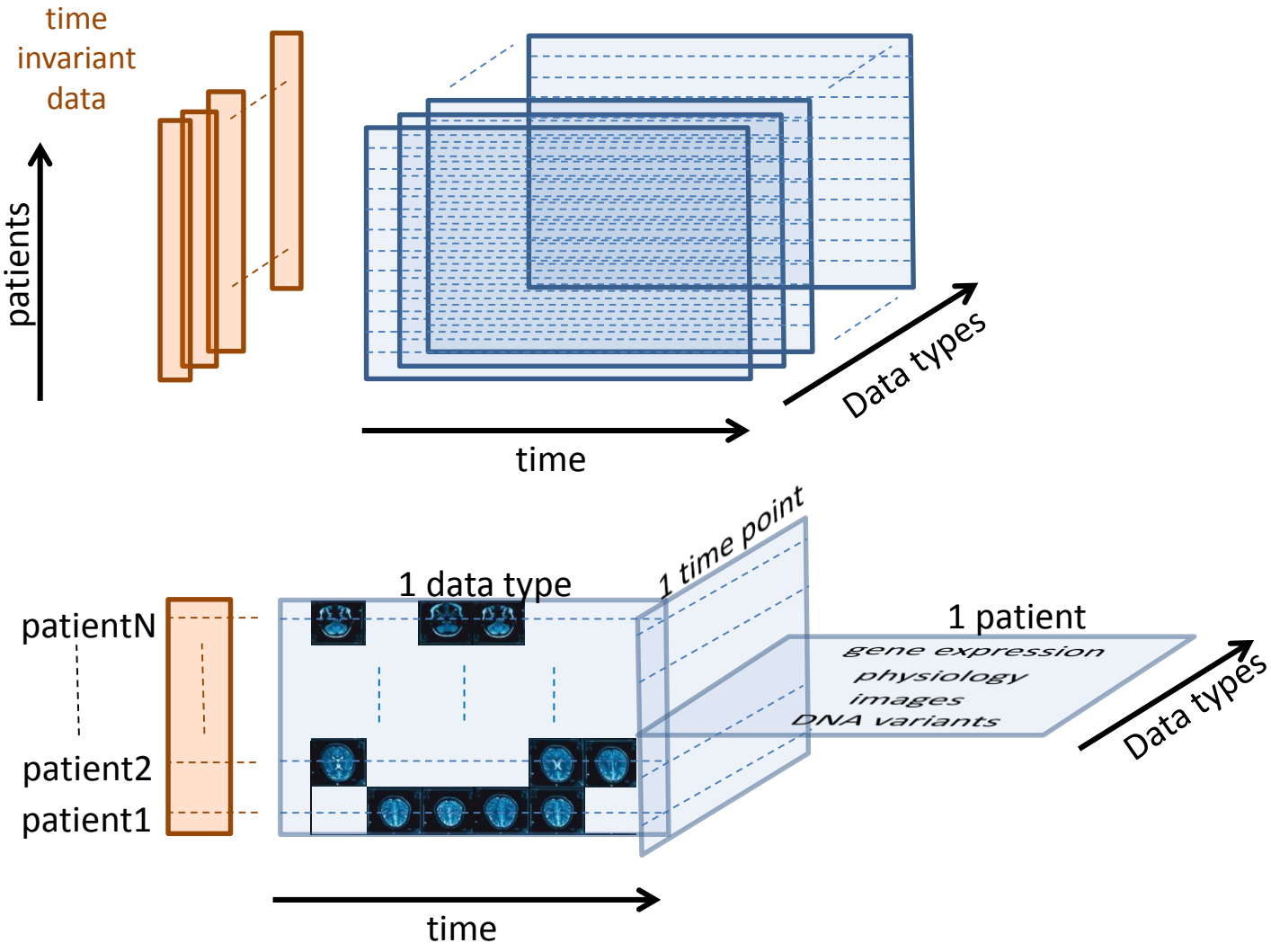
**They are currently being used to contribute to the development of drugs and optimization of therapy.**

# Acknowledgements for the mouse work:


PERSONALIZED MEDICINE
Big Data: COMPILING DATA
CLINICAL DATA
MOLECULAR DATA
DATABASE
Containing information
from thousands of patients

# Oncoscape – the experimental sandbox

Visualizing and Exploring Hypotheses in Translational Research



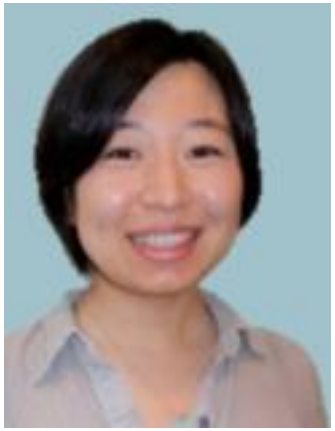

Oncoscape – Big data visualization tools

# The Oncoscape team at the Fred Hutch

Lisa McFerrin

Paul Shannon

Hamid Bolouri



Jenny Zhang

Desert Horse-Grant

# Collapsing very large data into meaningful patterns

**Hamid Bolouri**

# Molecular profiling platforms

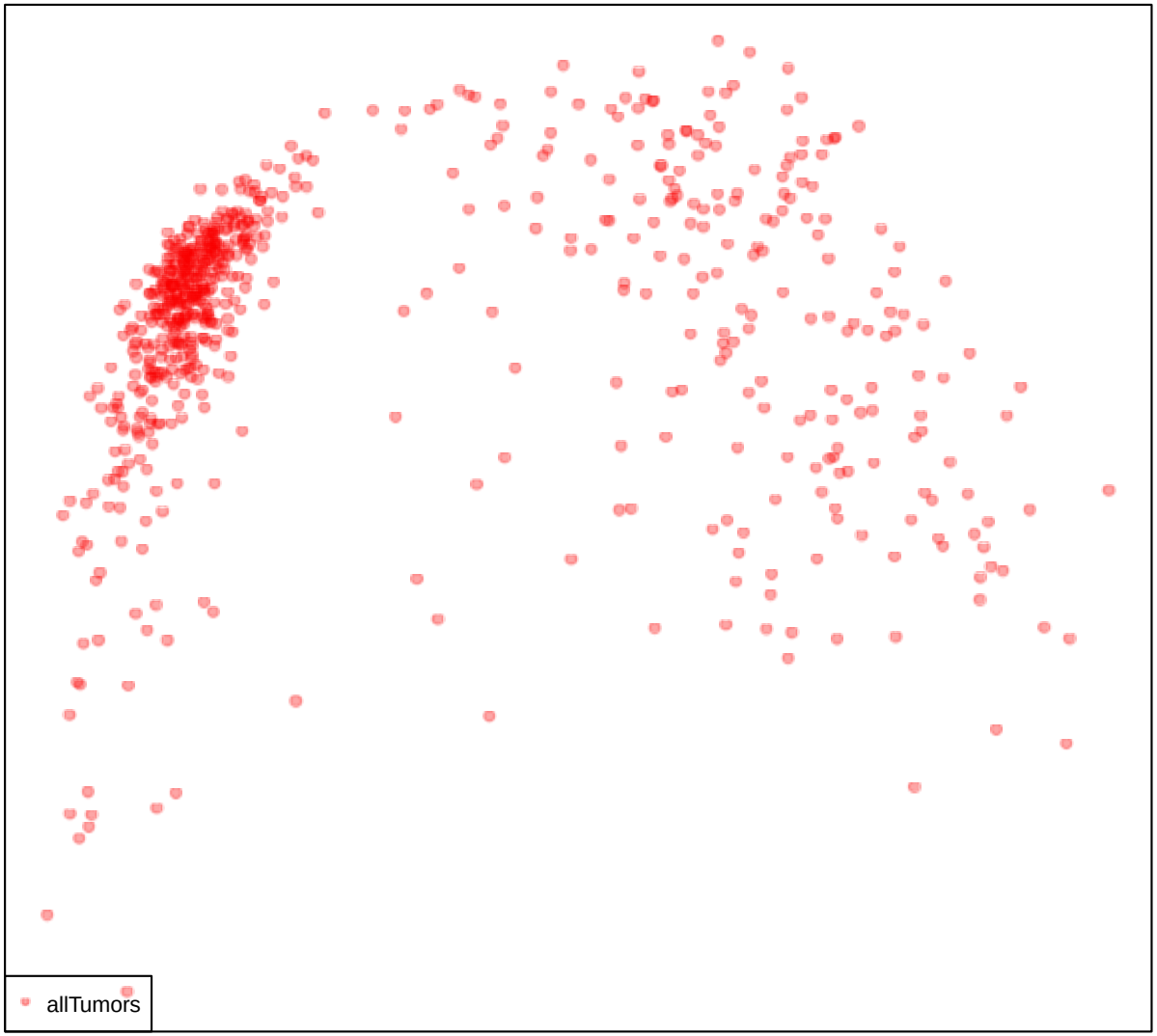
- **Global DNA methylation patterns (450K array)**
- **DNA sequencing**
  - **Whole genome copy number (CNA)**
  - **Whole exome sequencing (SNA)**
- **Gene expression (RNAseq)**

# Multidimensional scaling

- **Similar to Principal Component Analysis**
- **Distance between samples is defined ad-hoc, then samples are projected into low-dimensional space while preserving distance relationships.**
- **In this case it calculates similarity between tumors and plots them in 2 dimensions at a distance based on that similarity**
- **One can use whole genome sequence, copy number alterations across the genome, methylation patterns across the genome, or RNAseq patterns.**
- **We used the combined GBM and LGG database from the TCGA that contained over 1100 patients tumors**

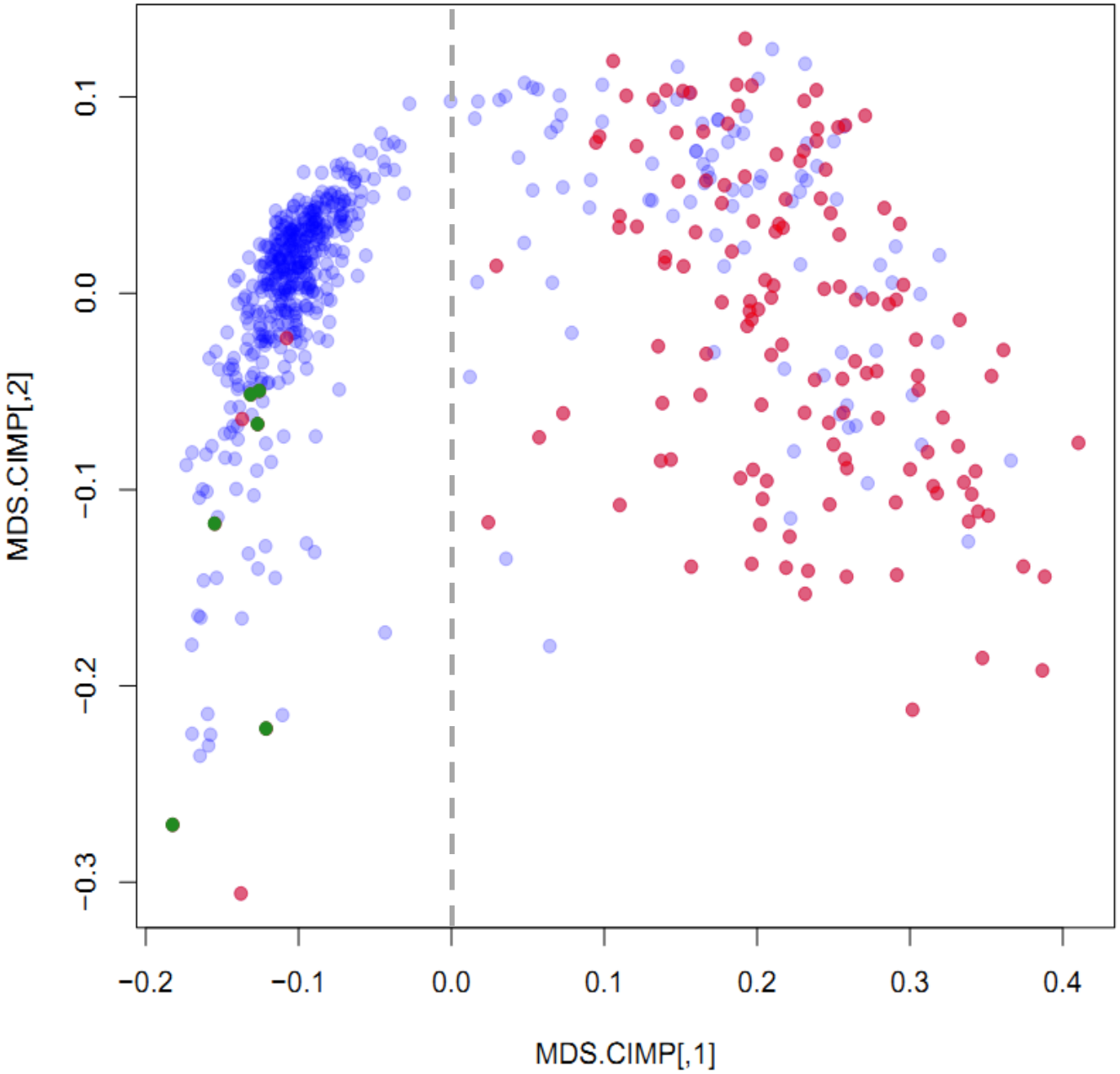
# Epigenetic analysis

- **DNA methylation - subsets of 450K array**



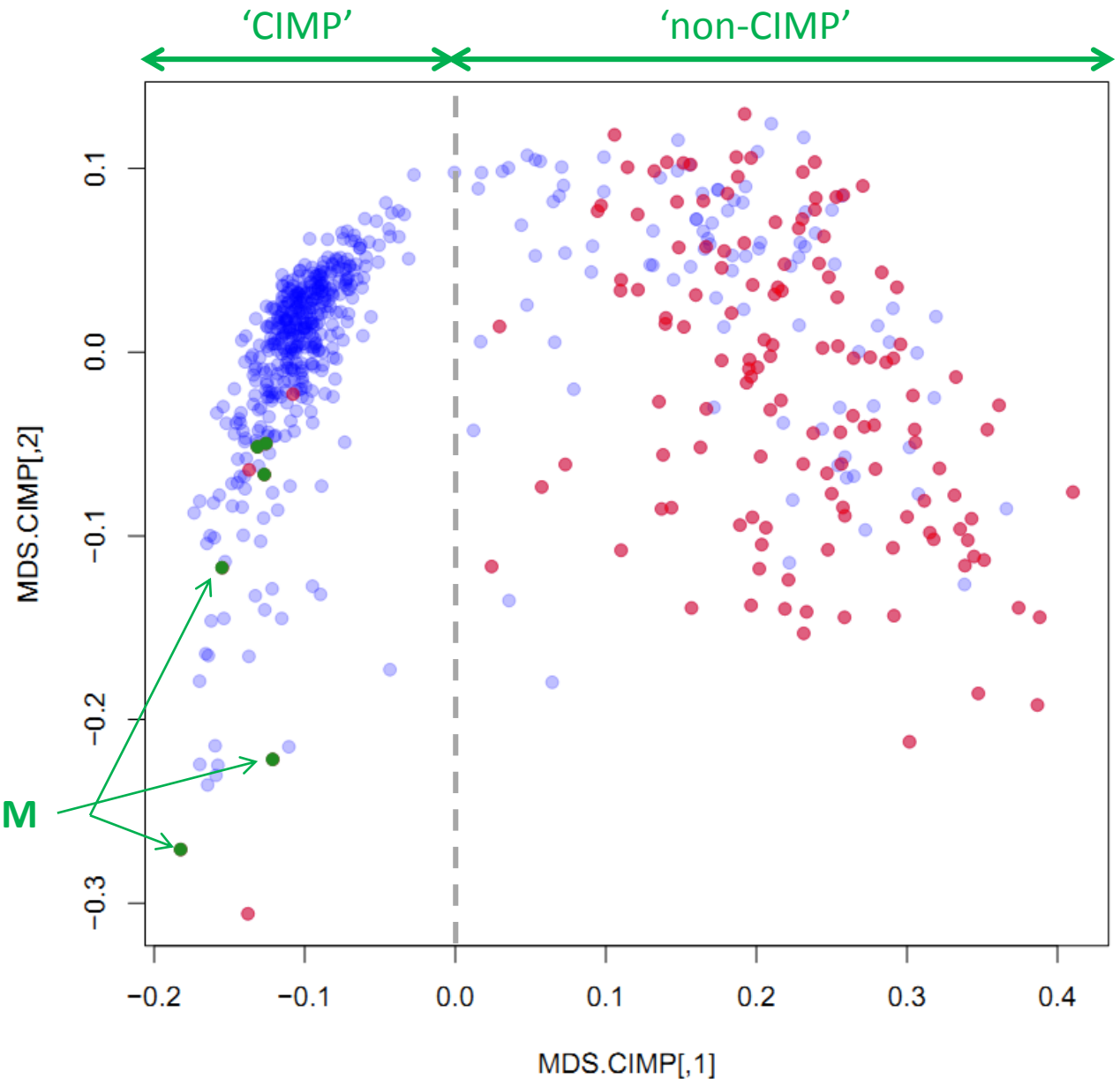

**For starters, we use the 450k methylation array and selected the 1503 probes used by Peter Laird to define the "G-CIMP" status in GBM**

**That data displays the tumors in this pattern**



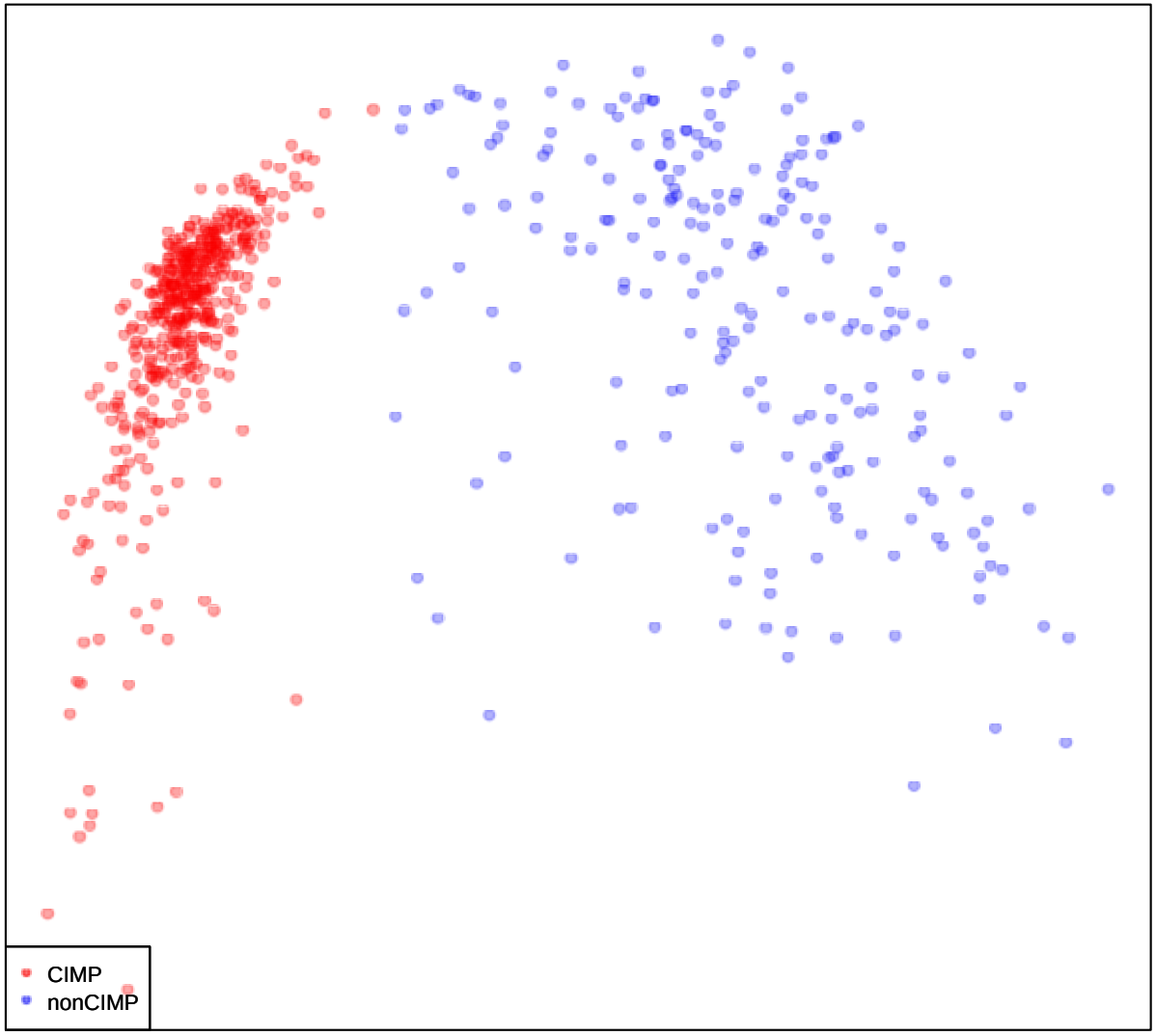

**We painted in the GBM (red) vs LGG (blue)**

**The GBMs identified previously as "CIMP" are located here, but some CIMP-GBM have been missed**




**LGGs are CIMP and non-CIMP**

**Most GBM are non-CIMP**

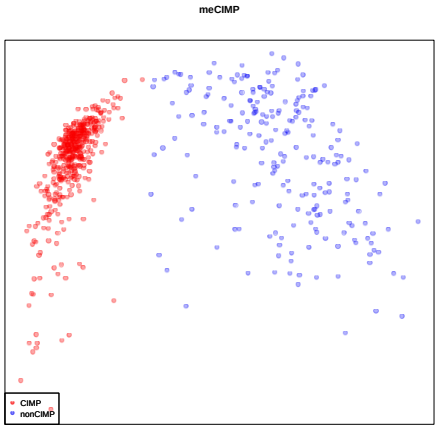



**For this talk, we now define two groups of gliomas as CIMP and non-CIMP by this plot analysis**

**This particular data uses the Laird set of methylation probes that initially defined the CIMP phenotype**

**What about other subsets of methylation probes on the 450K array?**

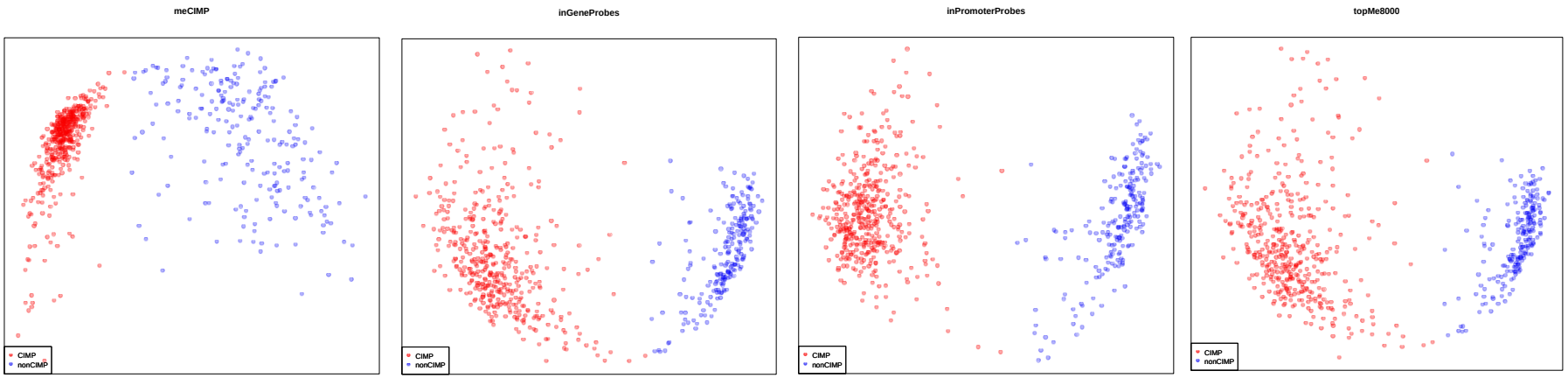
**Does the CIMP status across the genome of the population of gliomas hold true?**




**Laird set**

**What about other subsets of methylation probes on the 450K array?**

**Does the CIMP status across the genome of the population of gliomas hold true?**

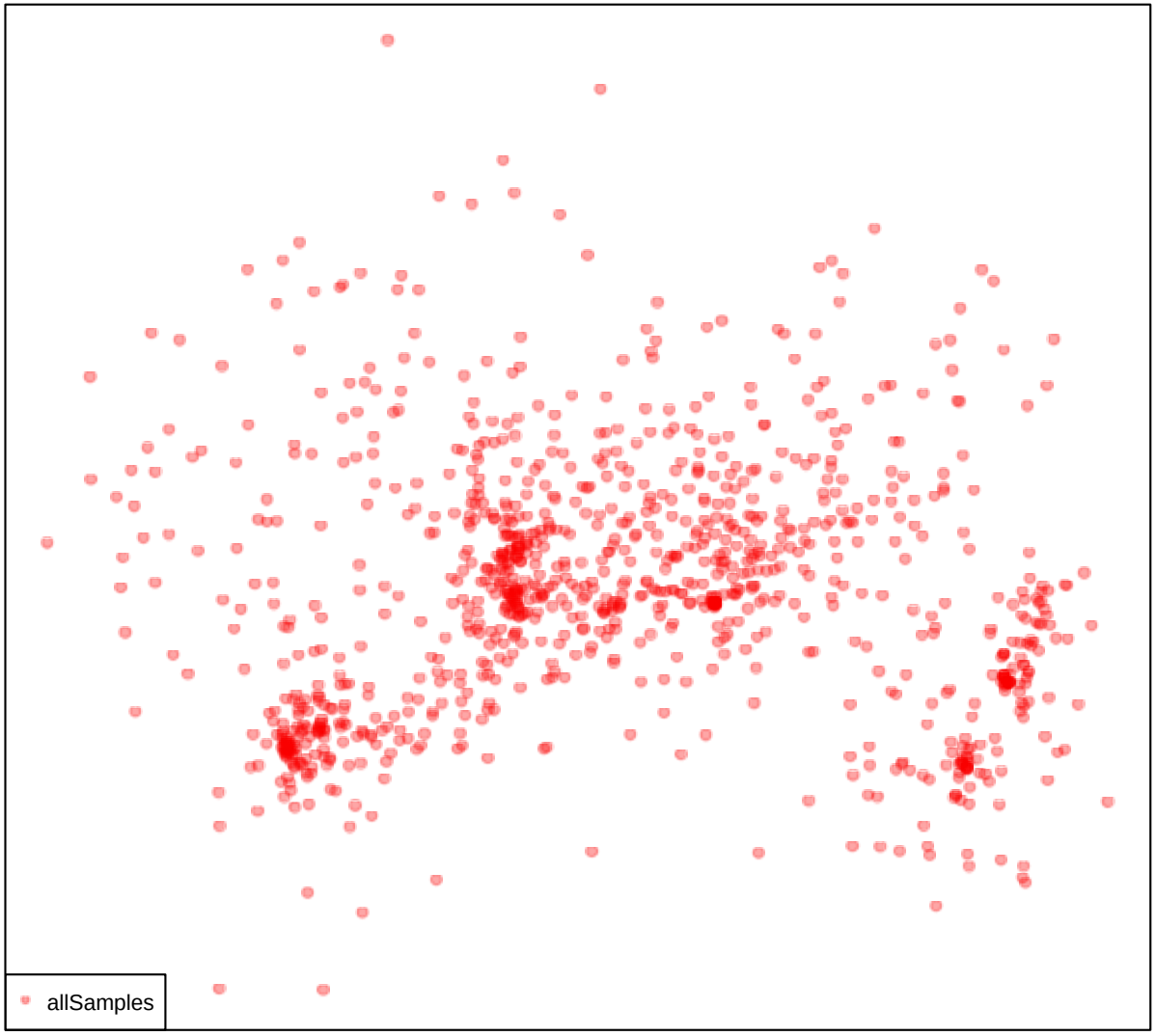



**Laird set** **In gene** **In promoter** **8000 most altered**

**Yes, the definition of CIMP reflects methylation changes across all regions of GBM patients**

# DNA copy number analysis - CNA

- **Affymetrix SNP6.0 arrays**

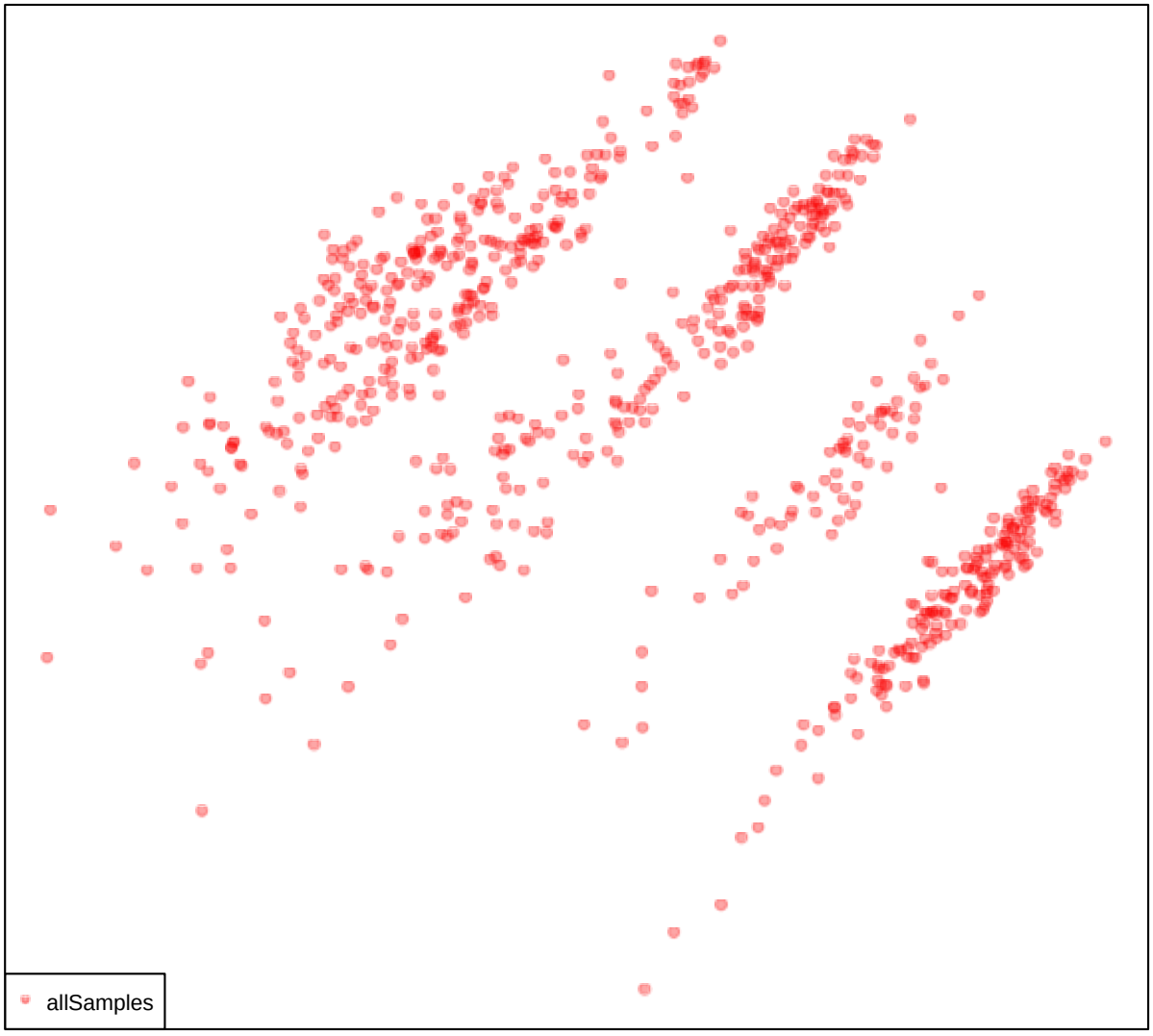



As an example, this is the pattern of GBM/LGG when displayed based on copy number alterations across the genome

**Distribution of gliomas based on DNA copy number alterations**

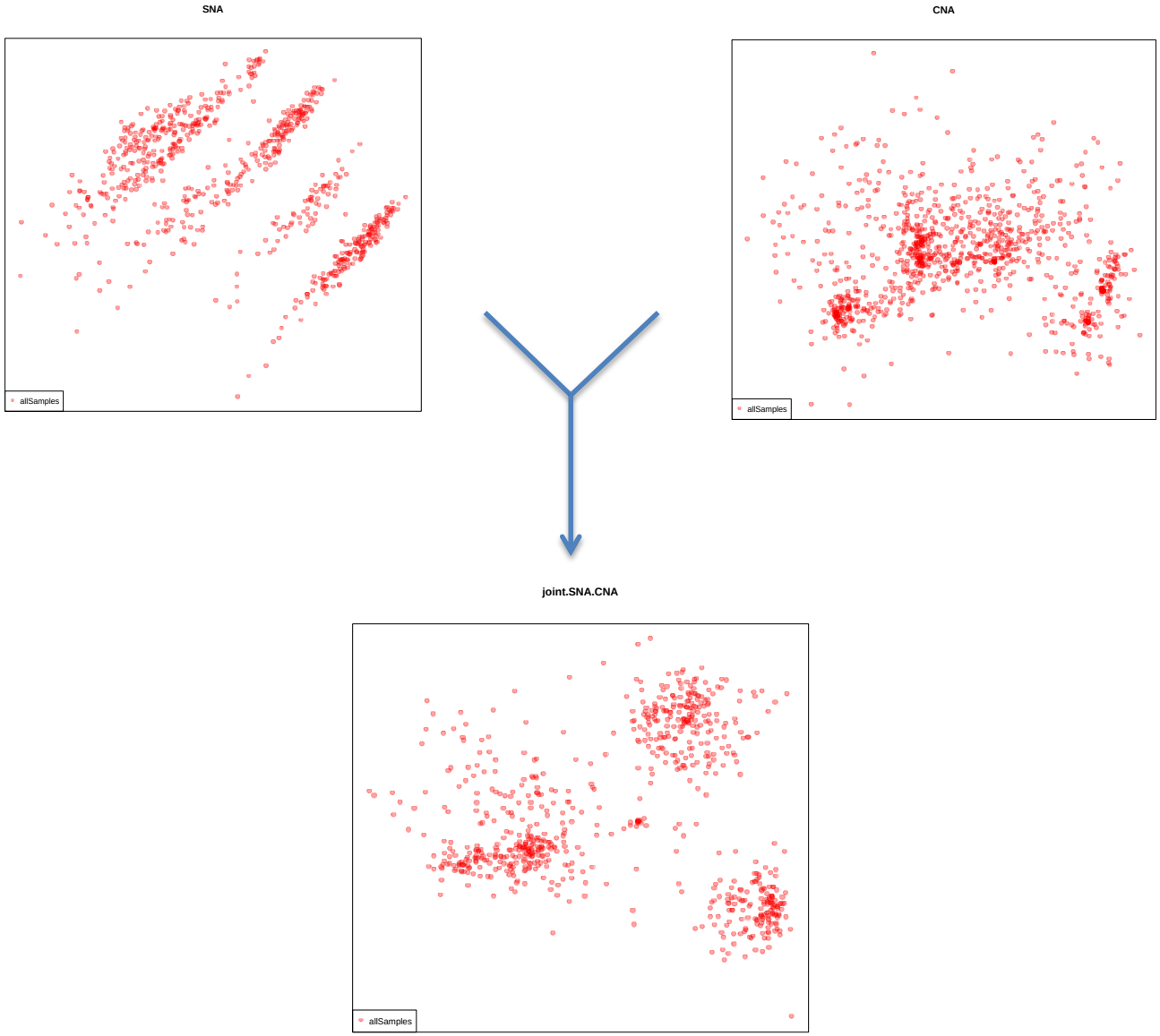
# Mutational analysis - SNA

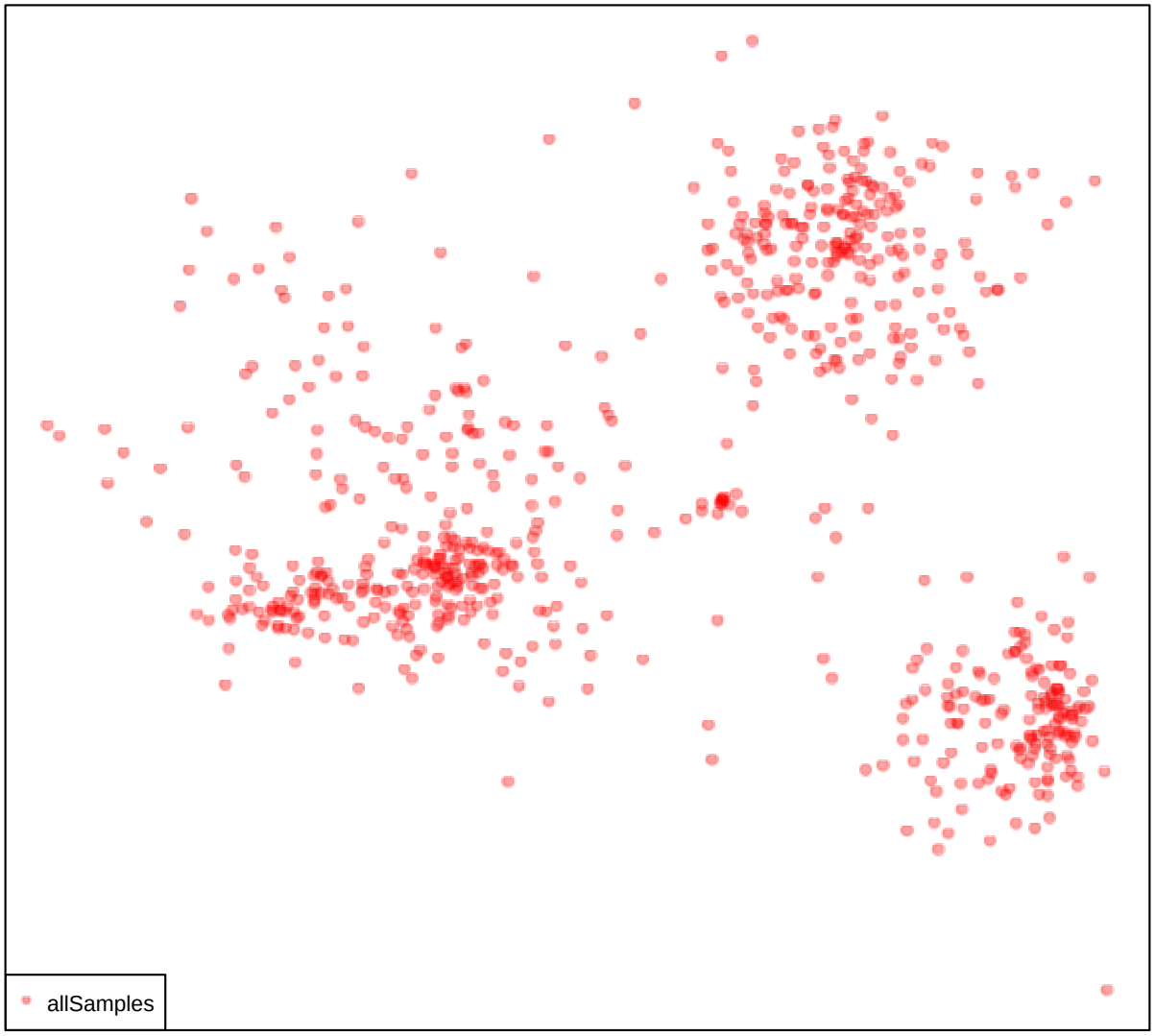
- **Whole exome DNA sequencing**




**Alternatively, we can display this same group of gliomas based on sequence variation (point mutations) across the genome (WGS).**

**We are also able to combine CNA and SNA datasets into one dataset that represents all the DNA data simultaneously.**

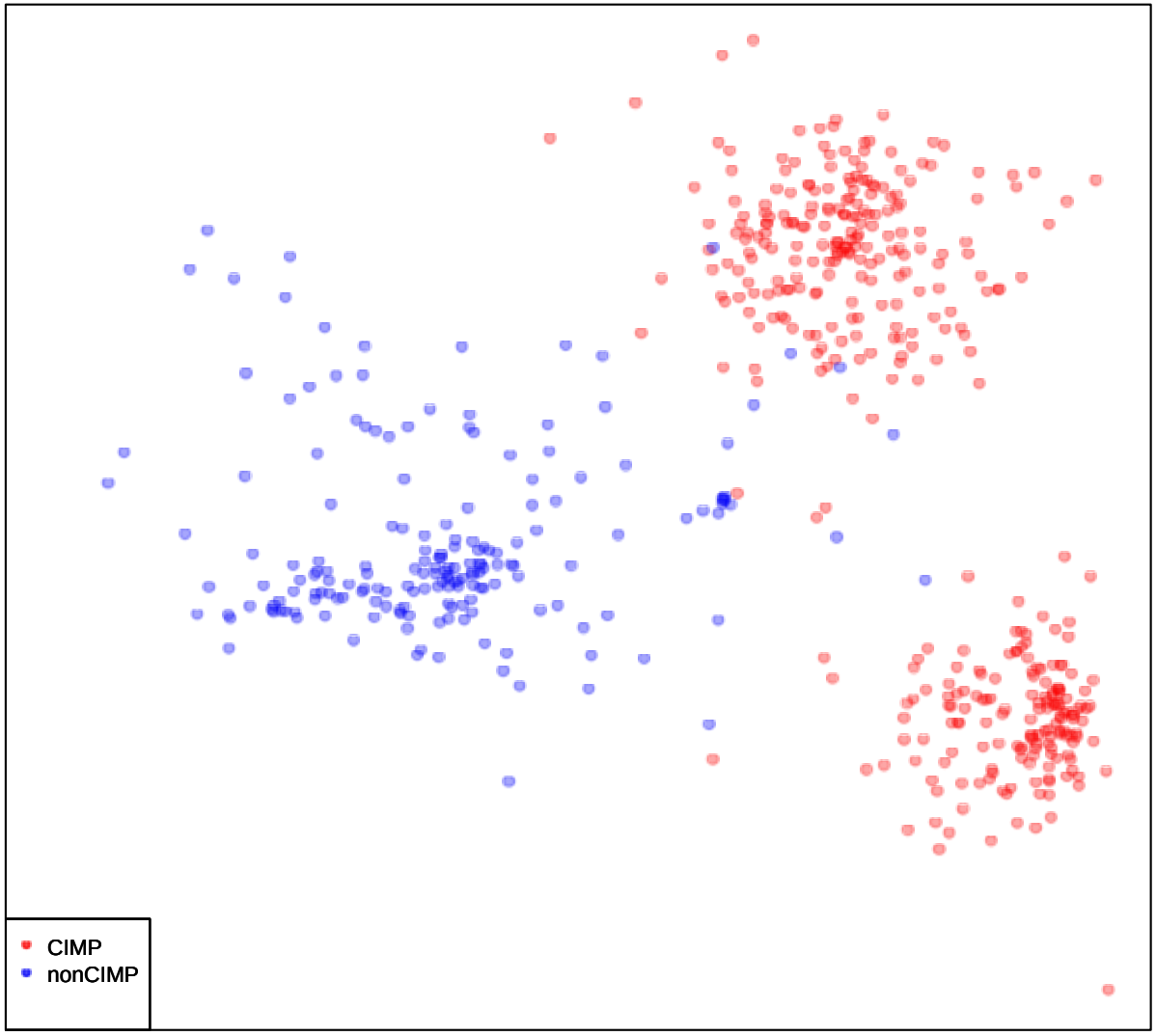





**Combining CNA and SNA data sets**

**This represents the complete DNA structural alterations across the genome for gliomas.**

**From this combined DNA data one sees 3 distinct clouds**

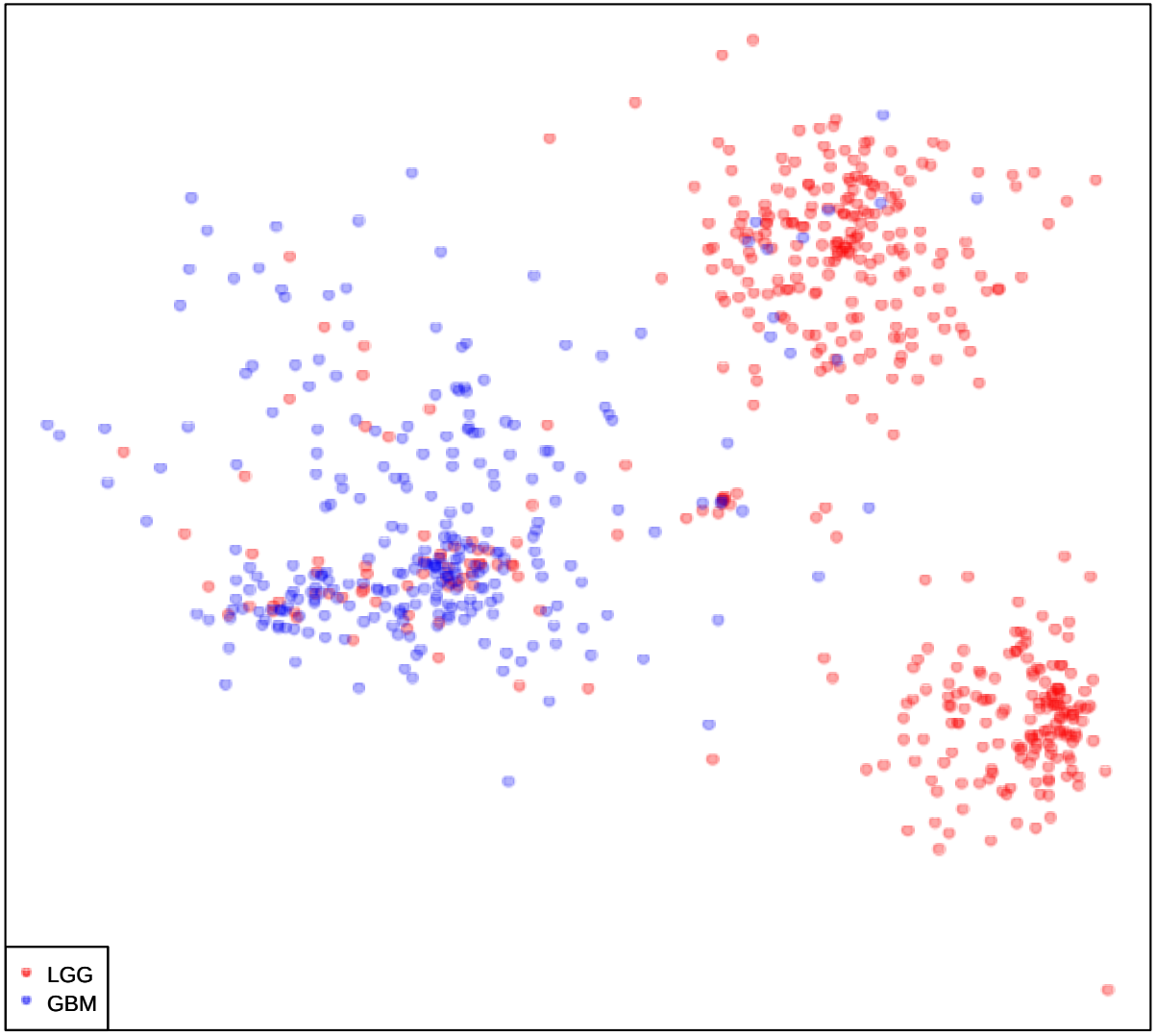



In this plot the majority of CIMP tumors (red) are located in the right two clusters

While the non-CIMP tumors (blue) are found in the third cluster

**Genome structure predicts methylation status**

joint.SNA.CNA

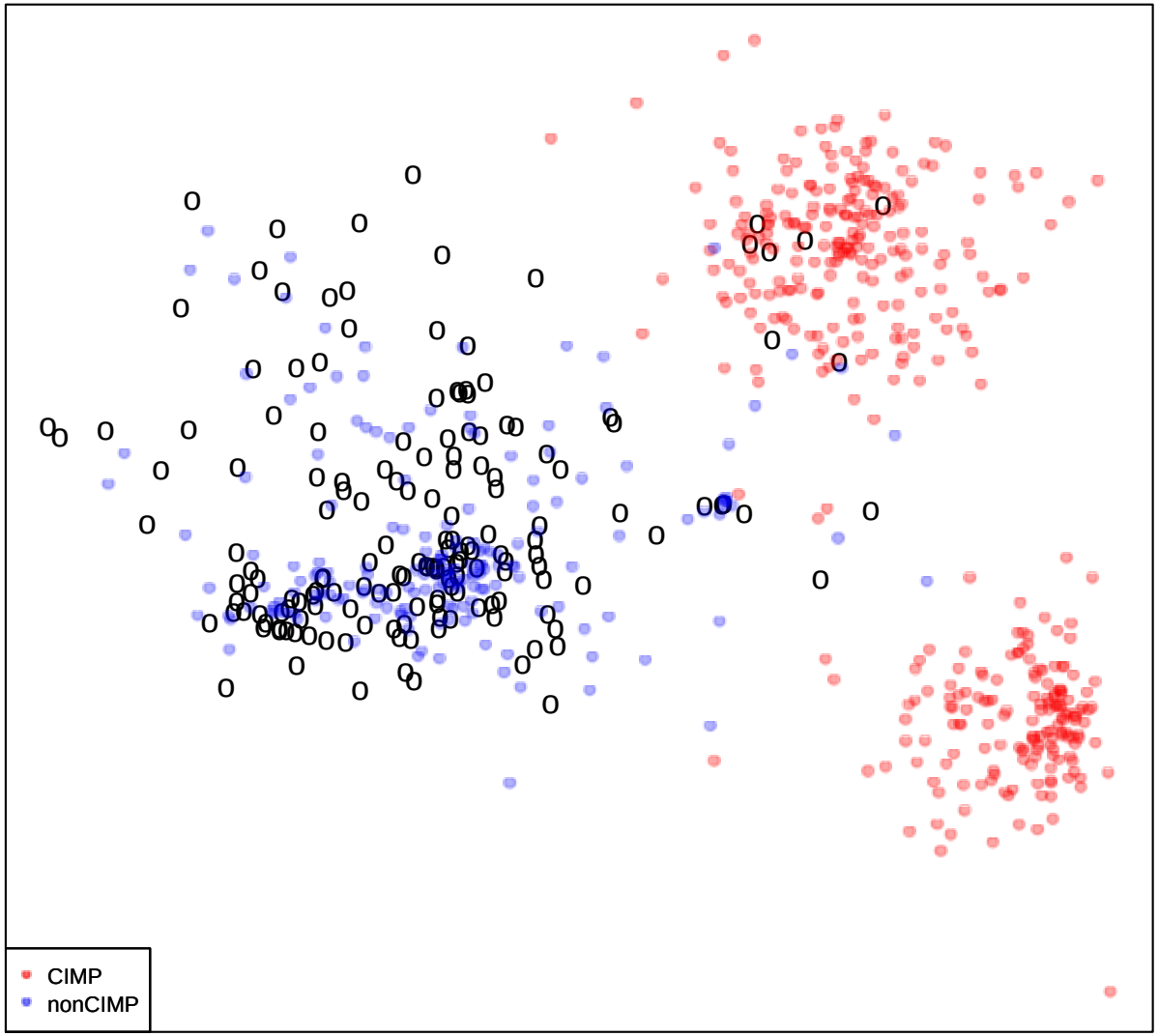



LGG vs GBM

These 3 clouds of tumors represent 2 LGG clusters and one more diffuse GBM cluster

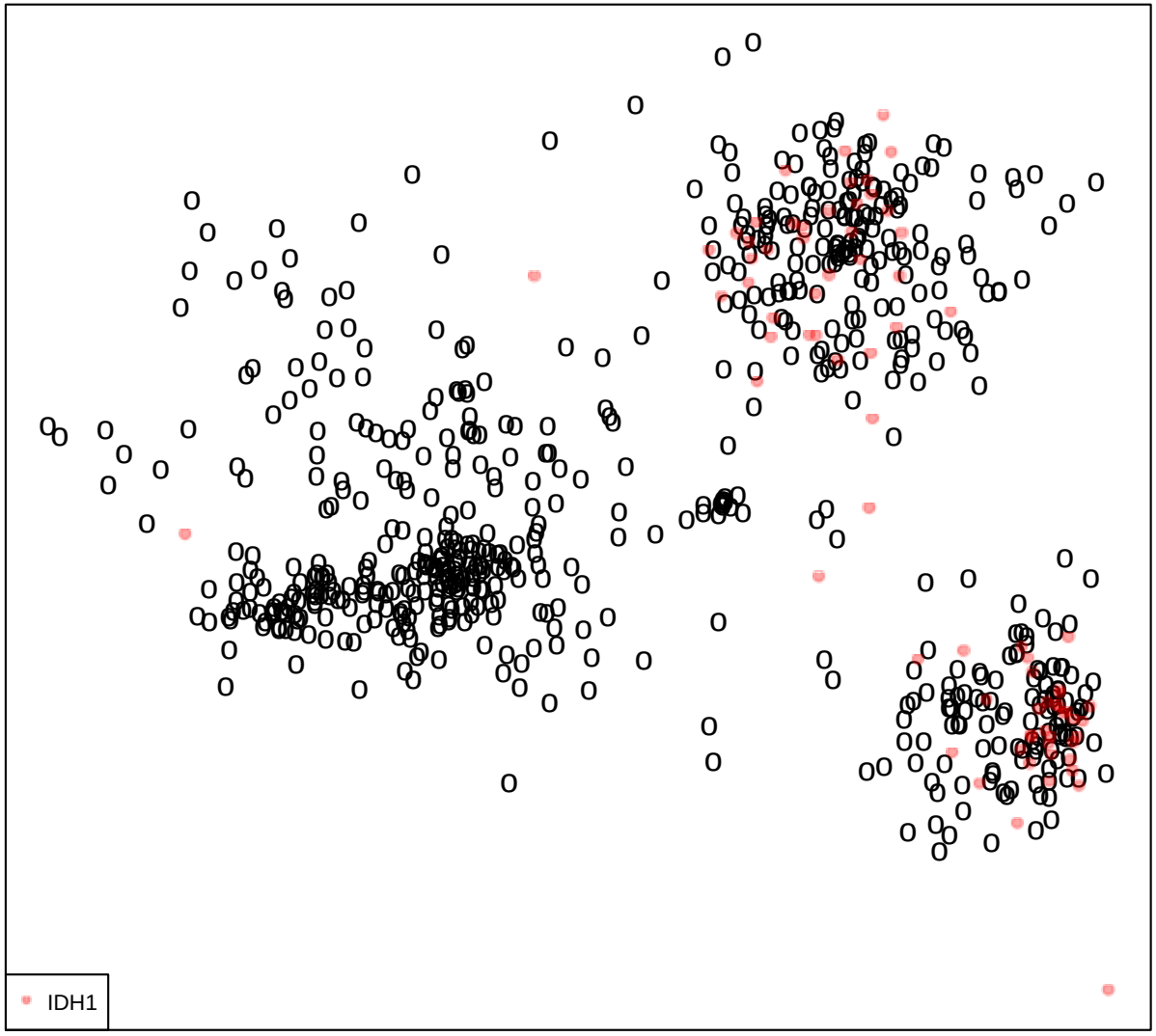
There are a few LGGs in the GBM cloud

and some GBM in the upper LGG cloud



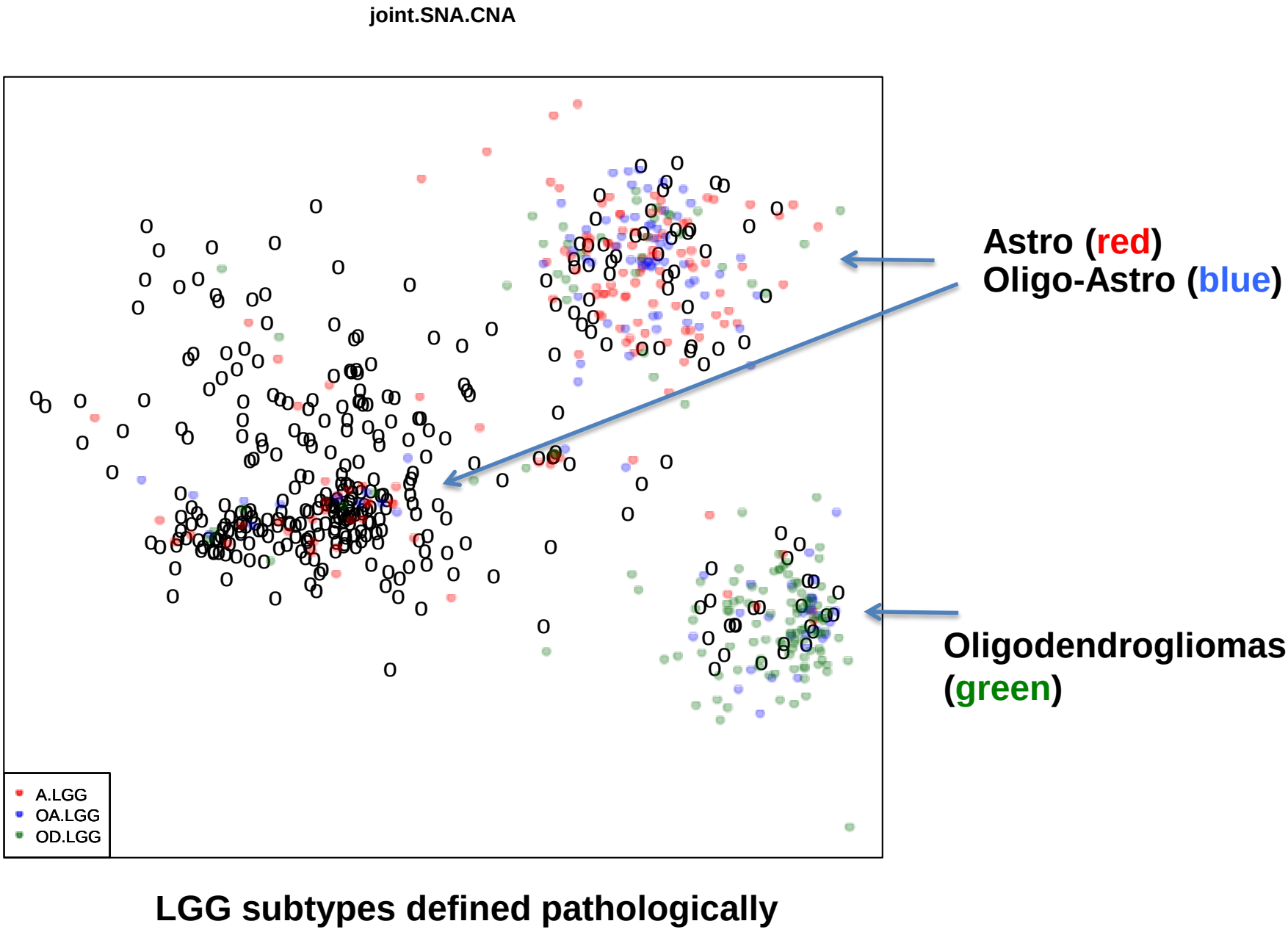

**LGG: CIMP vs non-CIMP**

**Now one gets much better separation of CIMP and non-CIMP LGG tumors**

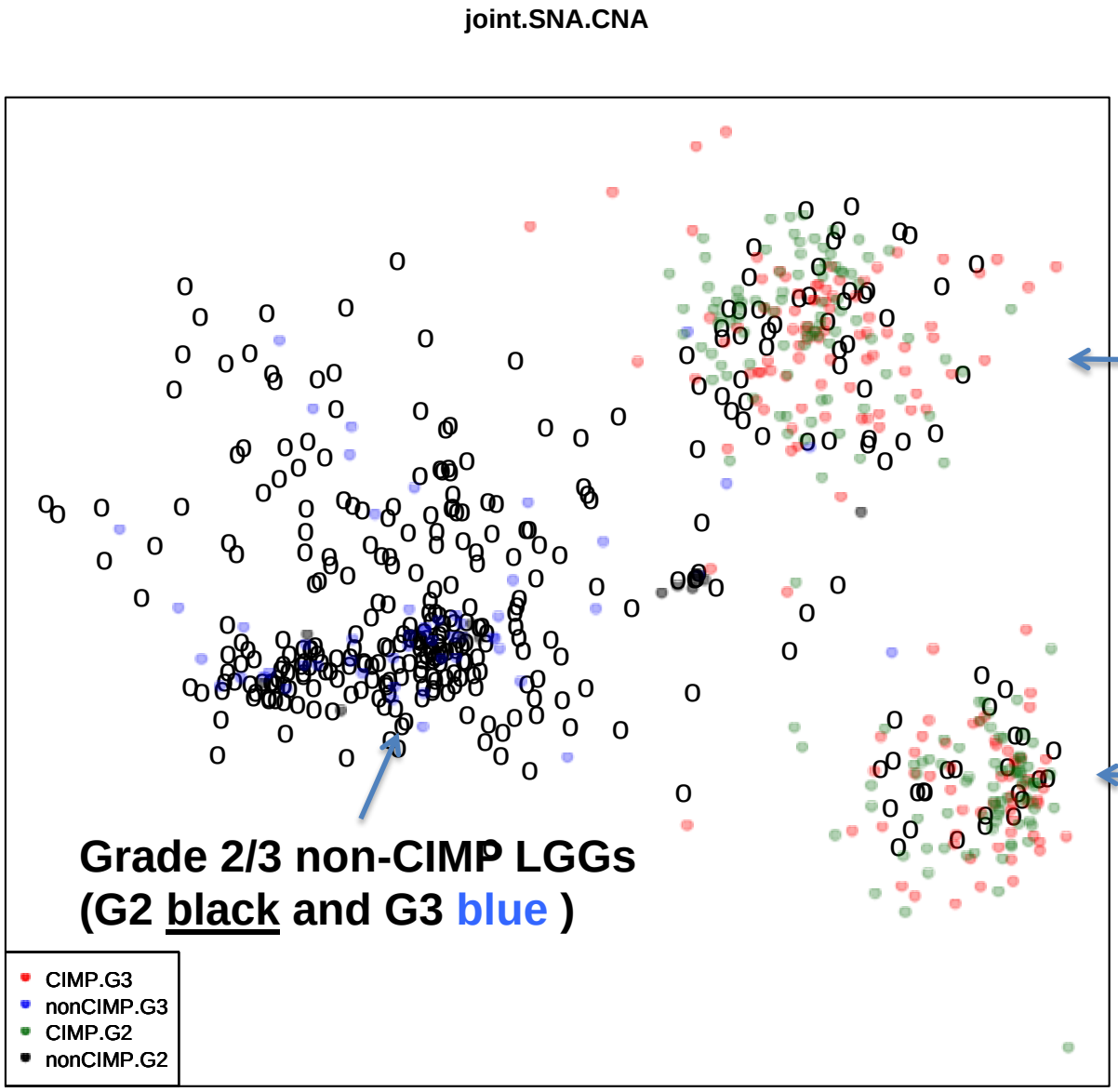



**IDH1 mutant tumors are found in both CIMP clouds**

**But in this dataset IDH1 mutations are seen in only a minority of each**




LGG subtypes defined pathologically




LGGs by grade

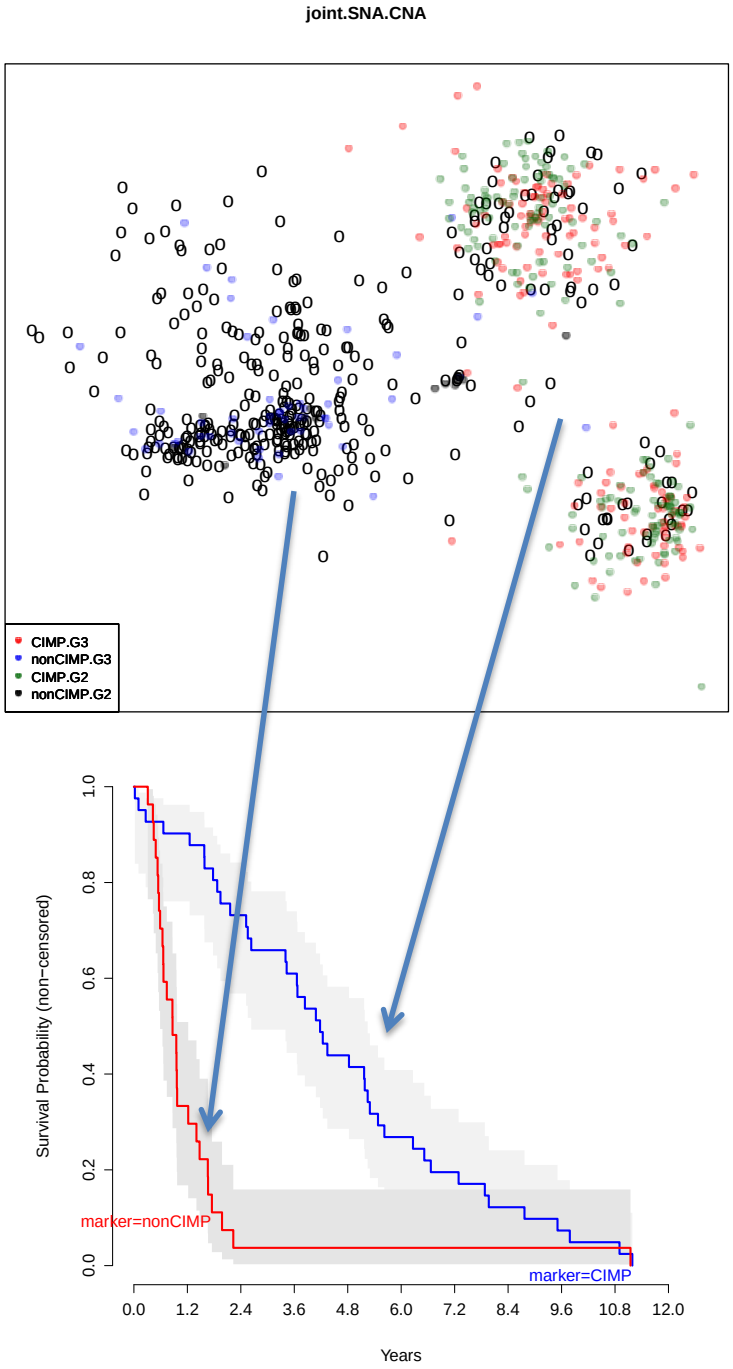
Grade 2/3 astro

Grade 2/3 CIMP LGGs
(G2 green and G3 red )

Grade 2/3 oligo

Grade 2/3 non-CIMP LGGs
(G2 black and G3 blue )

LGG grades are mixed

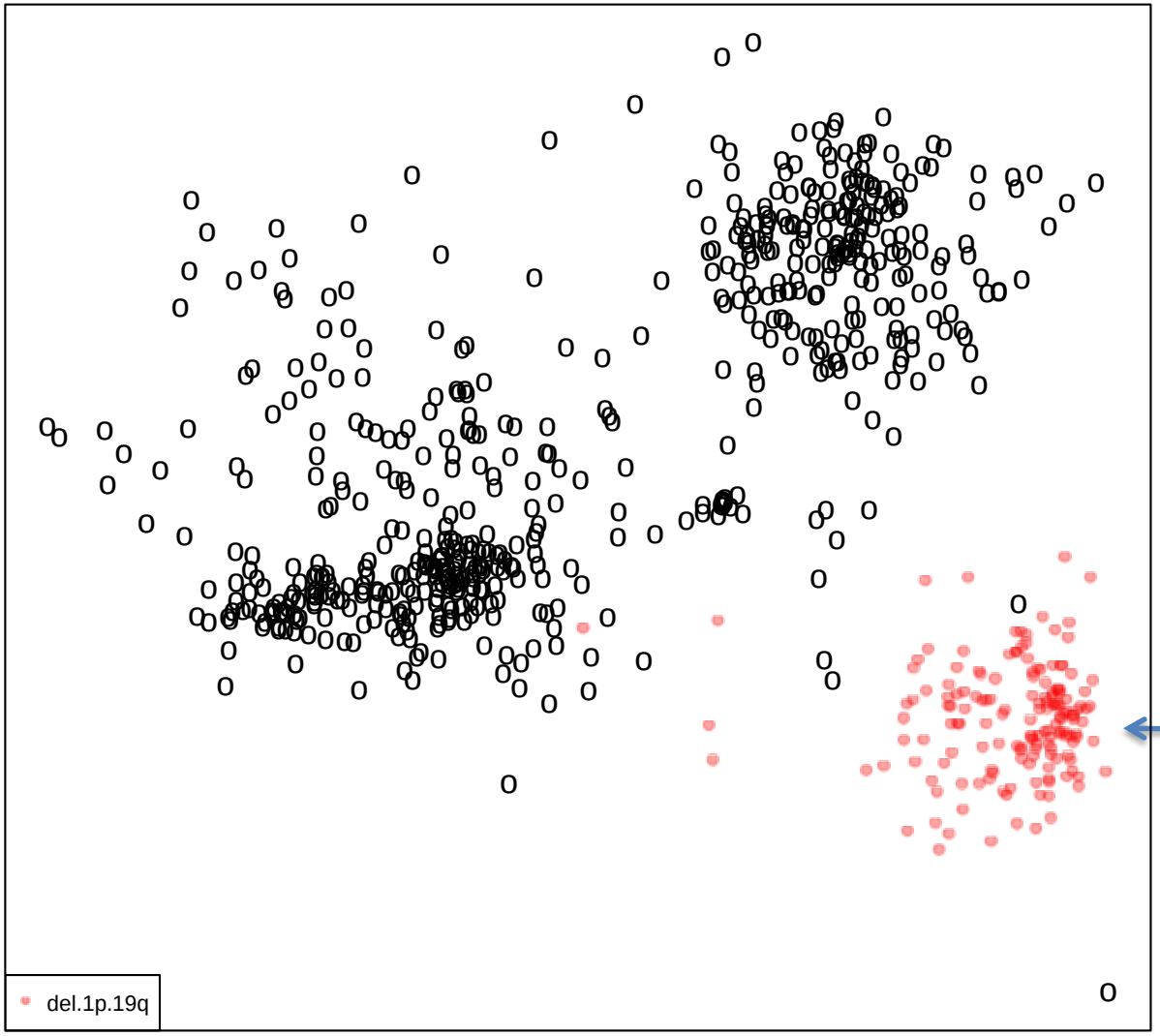



**The non-CIMP LGGs that occupy the GBM region appear to behave like GBM**

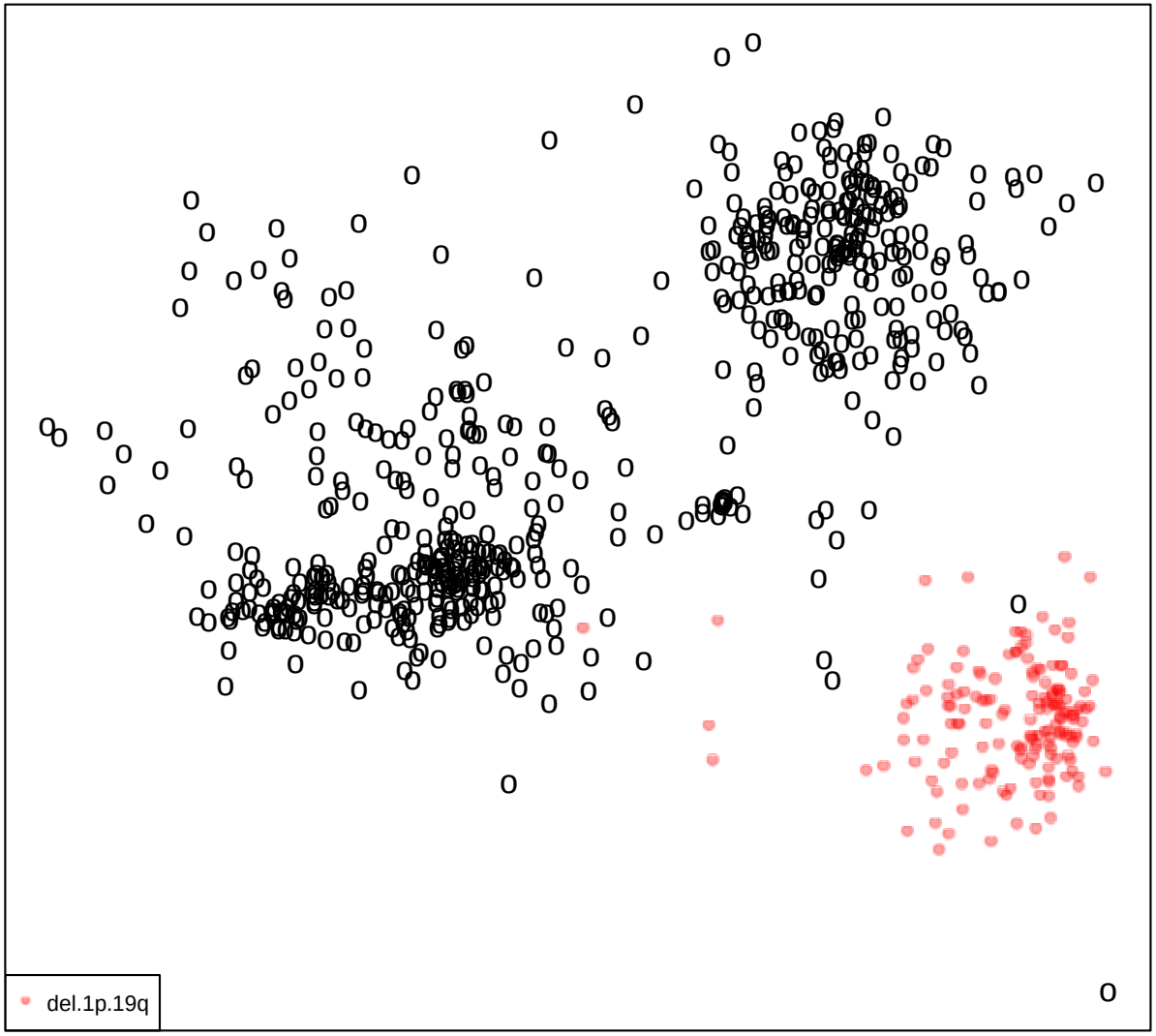
**Based on the TCGA LGGs that we have survival data on (the majority of LGGs in the TCGA do not have survival data)**

joint.SNA.CNA

# 1p19q deleted gliomas

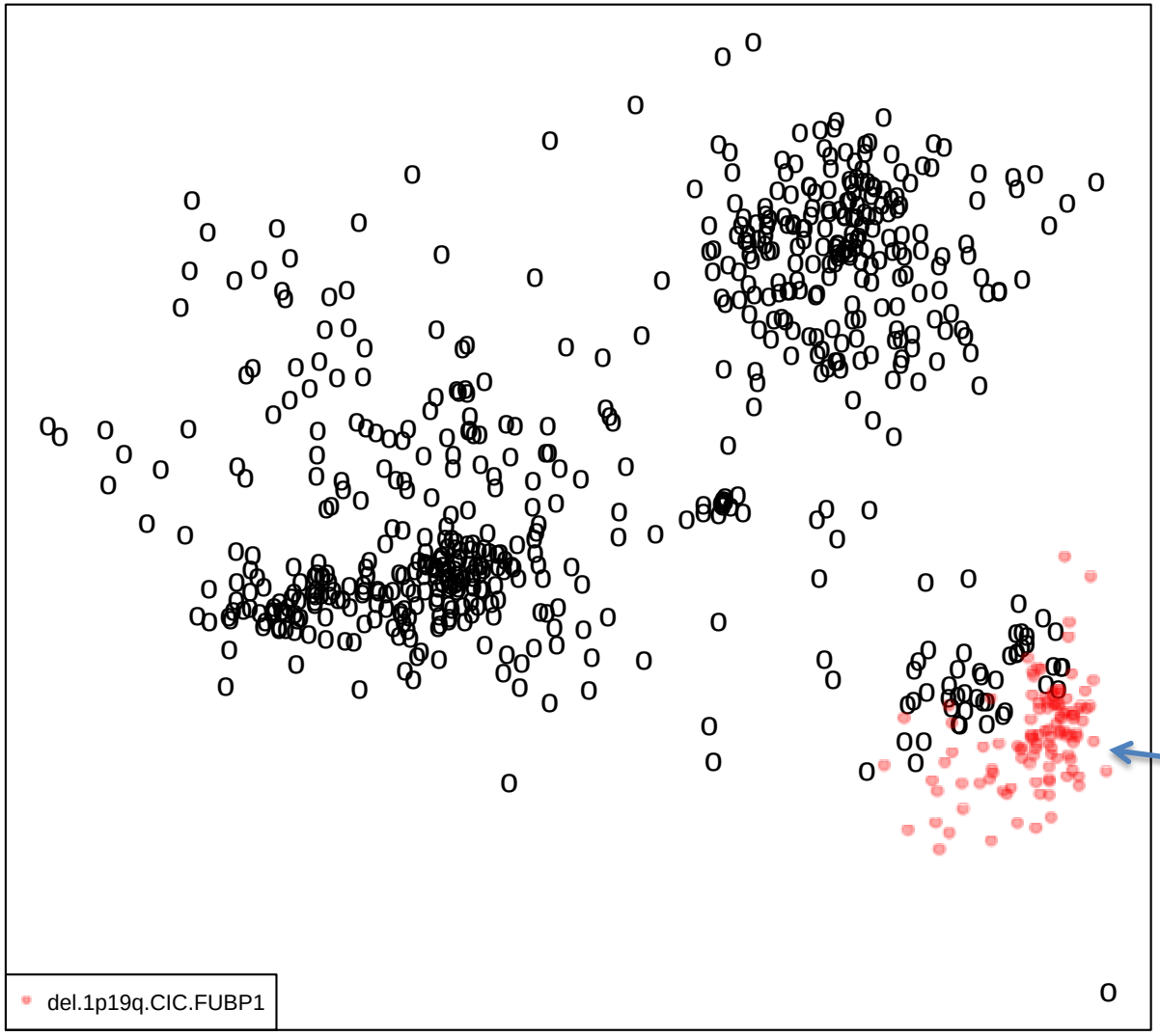



**Not surprisingly, the oligo cloud is essentially 100% deleted**




**Previous publications have shown that the genes FUBP1 (1p) and CIC (19q) are frequently mutated in 1p19q deleted oligodendroglioms.**

**It has been suggested that they are the drivers of these chromosomal losses.**

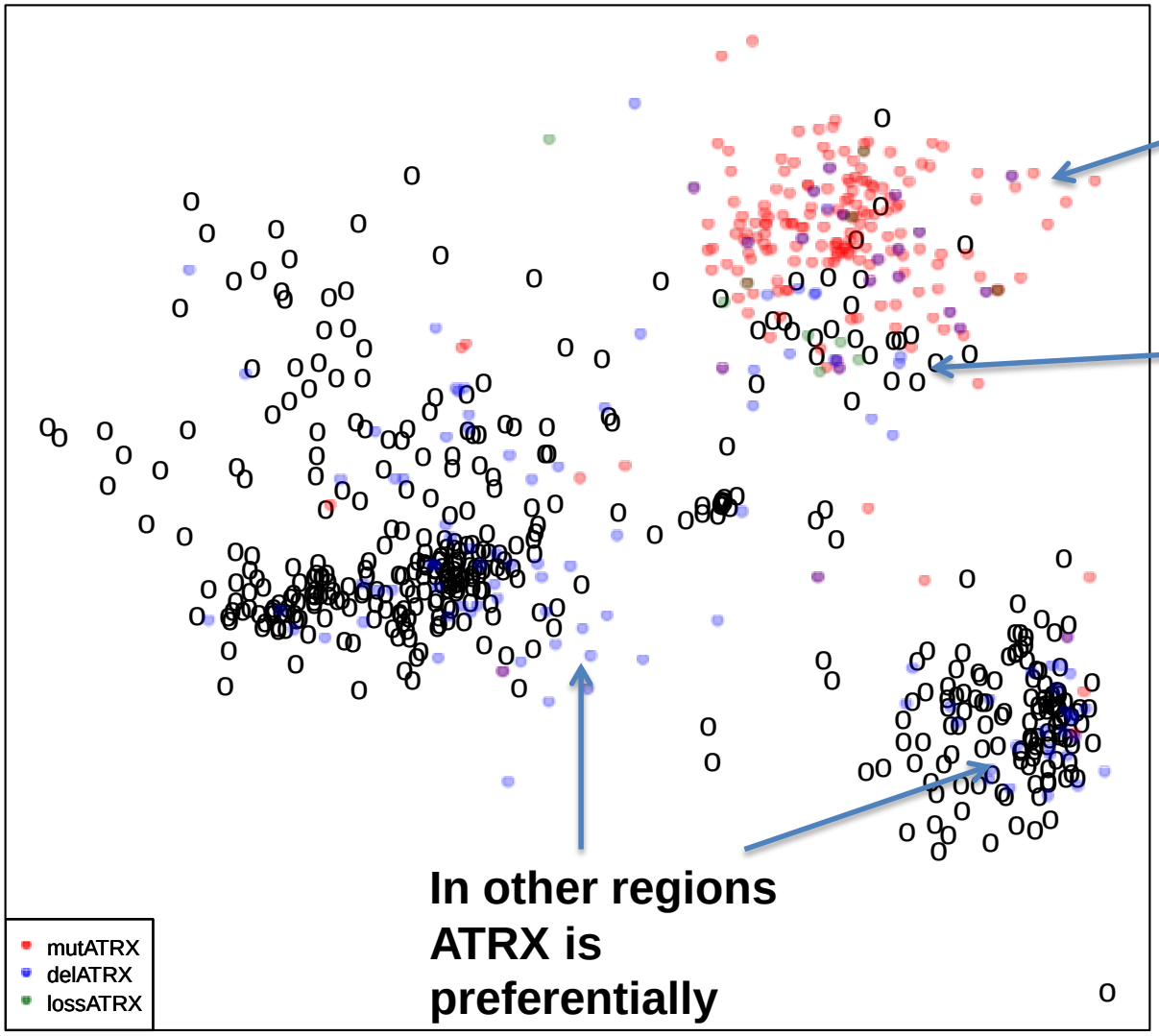



**CIC and FUBP1 mutations**

**Point mutations in CIC and FUBP1 are found specifically in this region of the oligo cloud**

joint.SNA.CNA

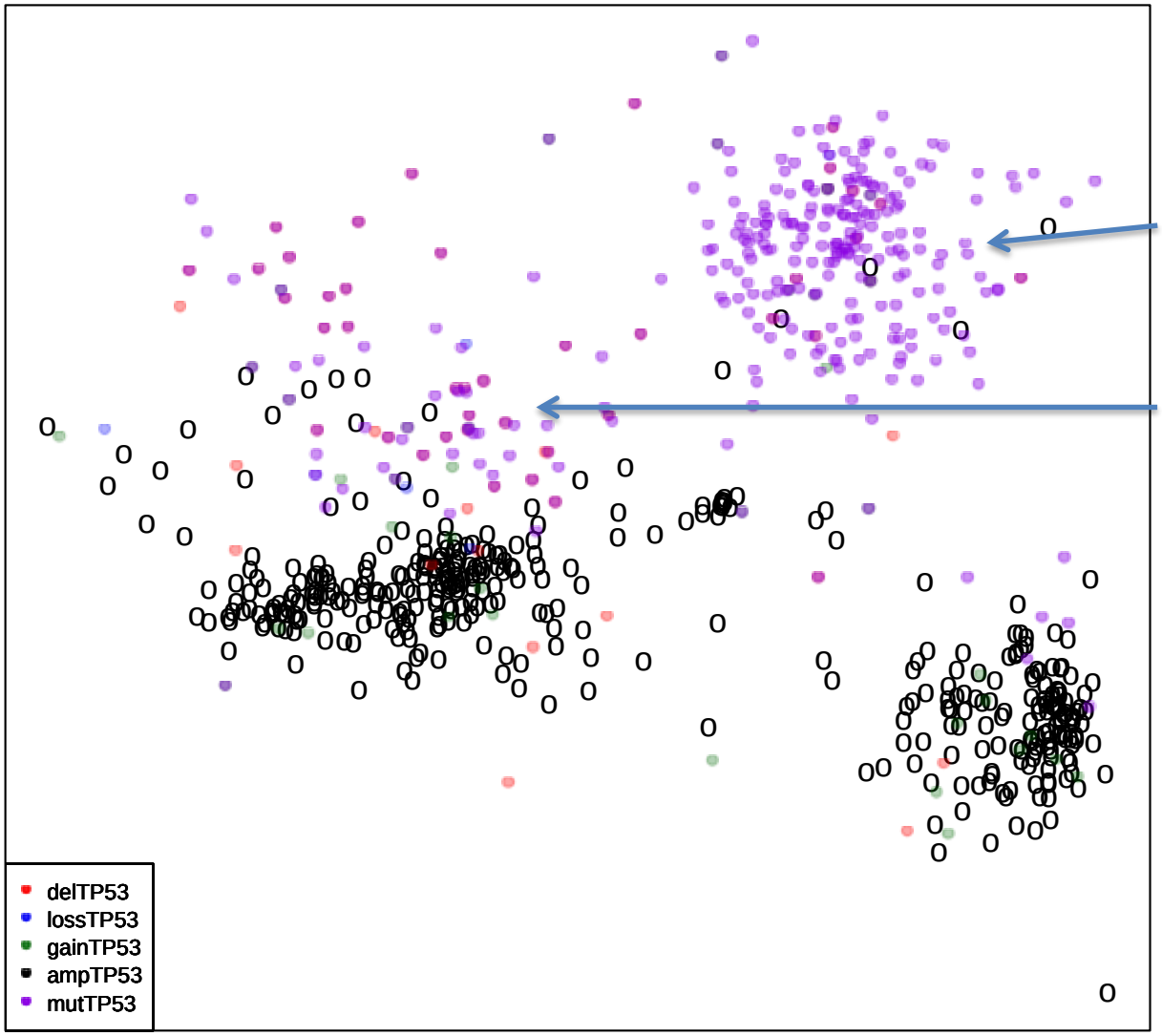
# ATRX




Not surprisingly, the astro cloud is ATRX mutated. (but only the upper portion for some reason).

In other regions ATRX is preferentially deleted, not mutated

joint.SNA.CNA

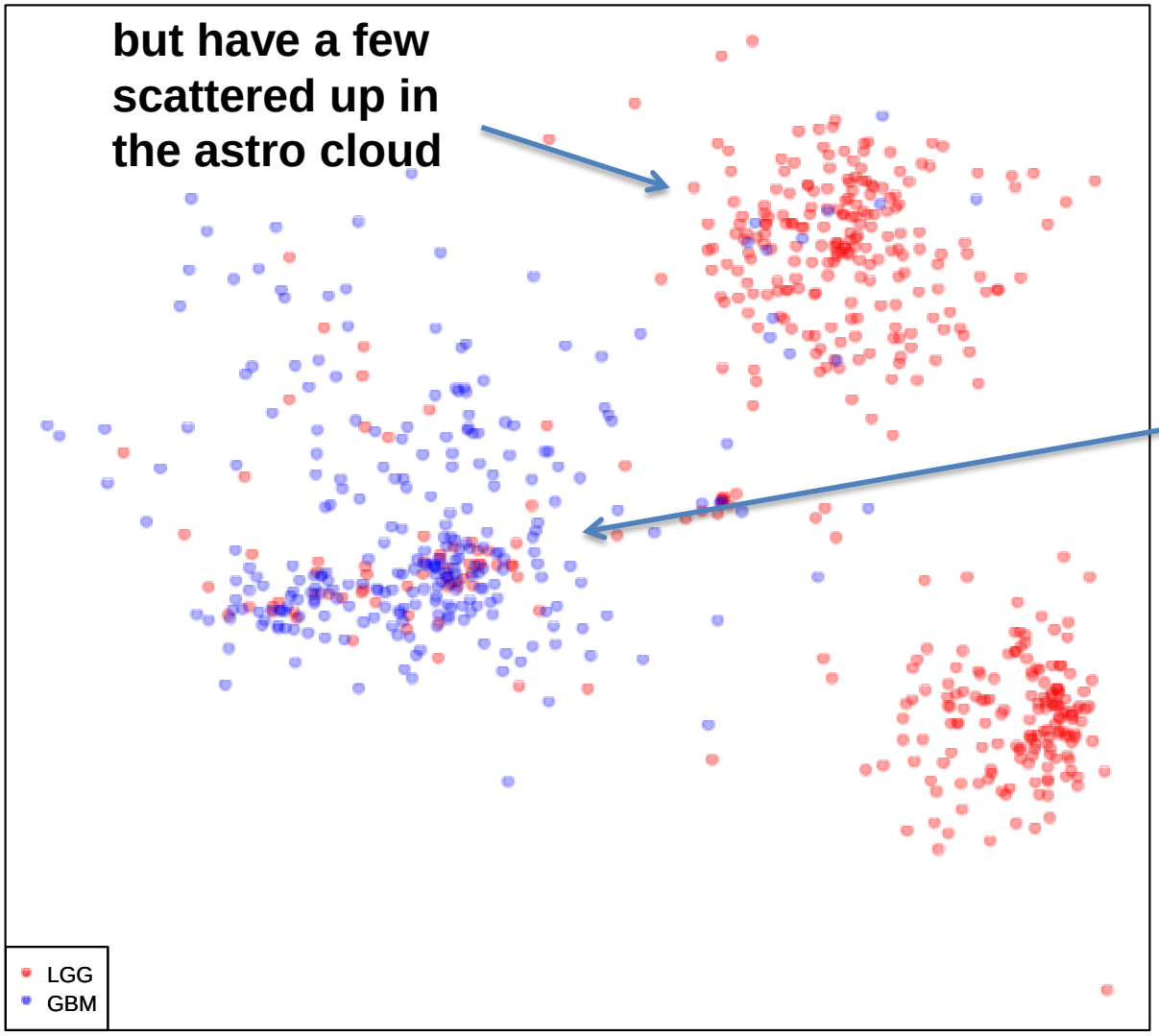



p53

P53 is very specifically mutated in the astro cloud

And in this part of the diffuse GBM region

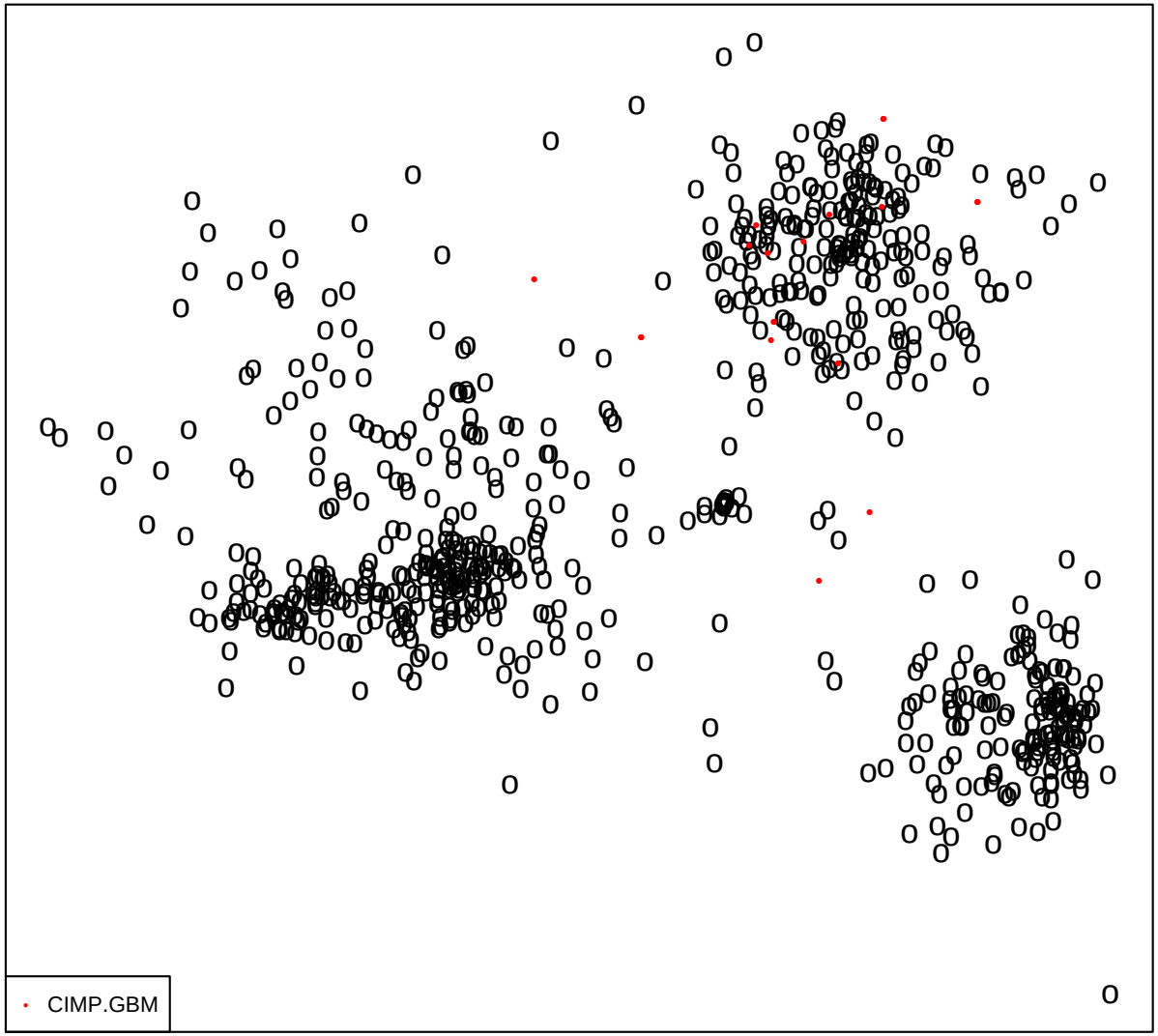
joint.SNA.CNA




Now for the GBMs,

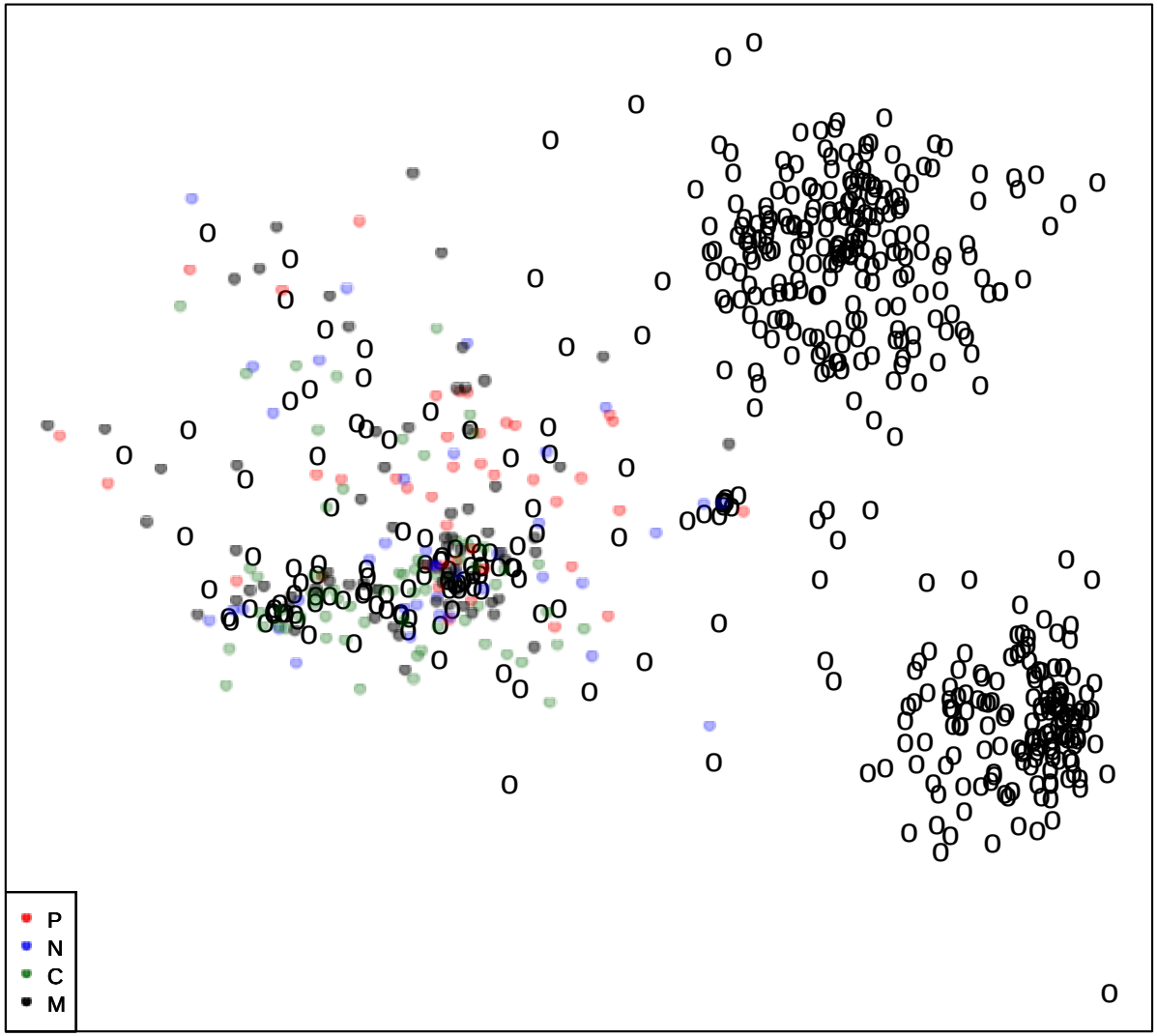
which are located primarily here…

GBM vs LGG



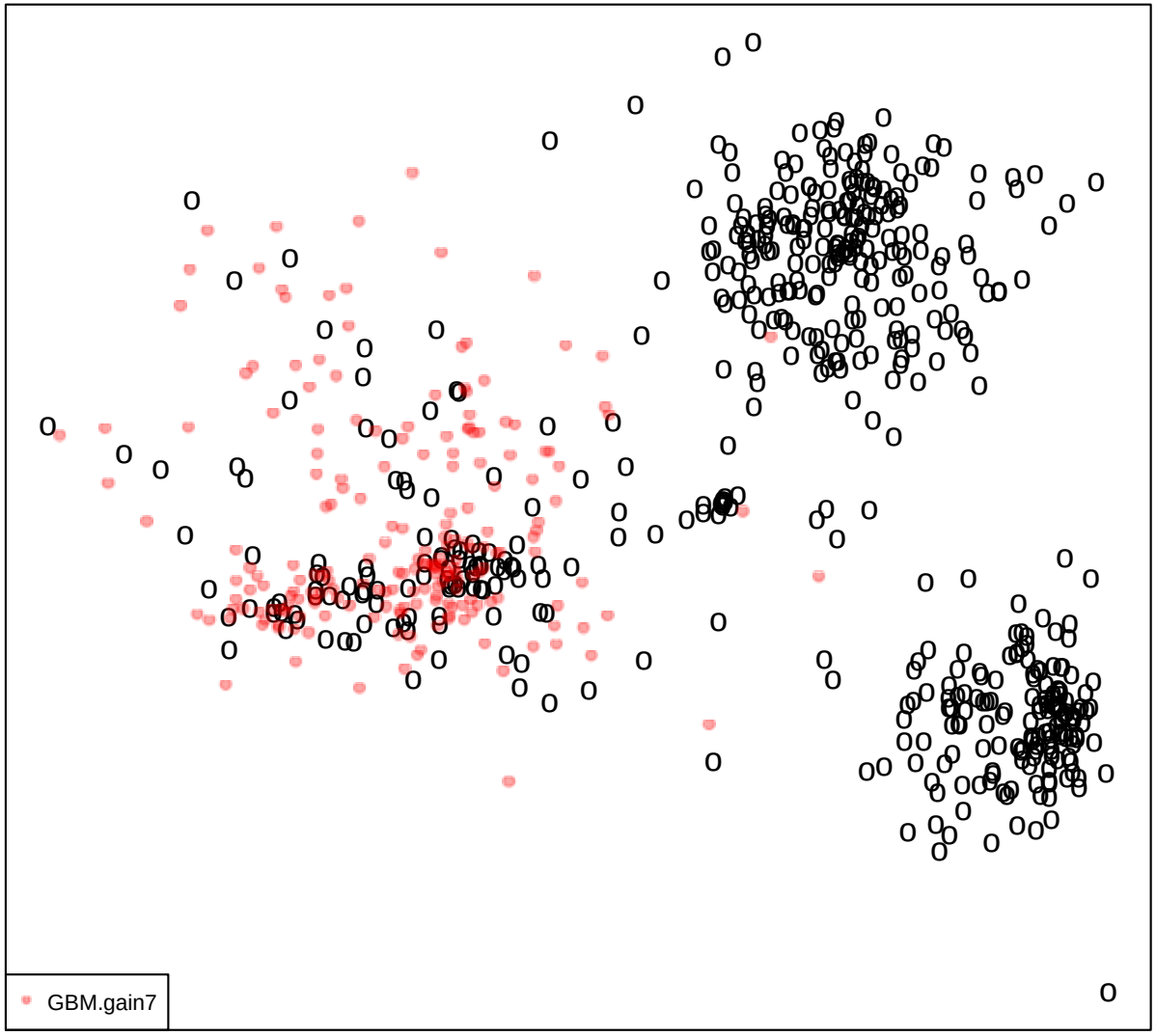

**CIMP GBM**

**The CIMP-GBM are the GBMs that are located in and around the astro cloud**




**Non-CIMP GBM**

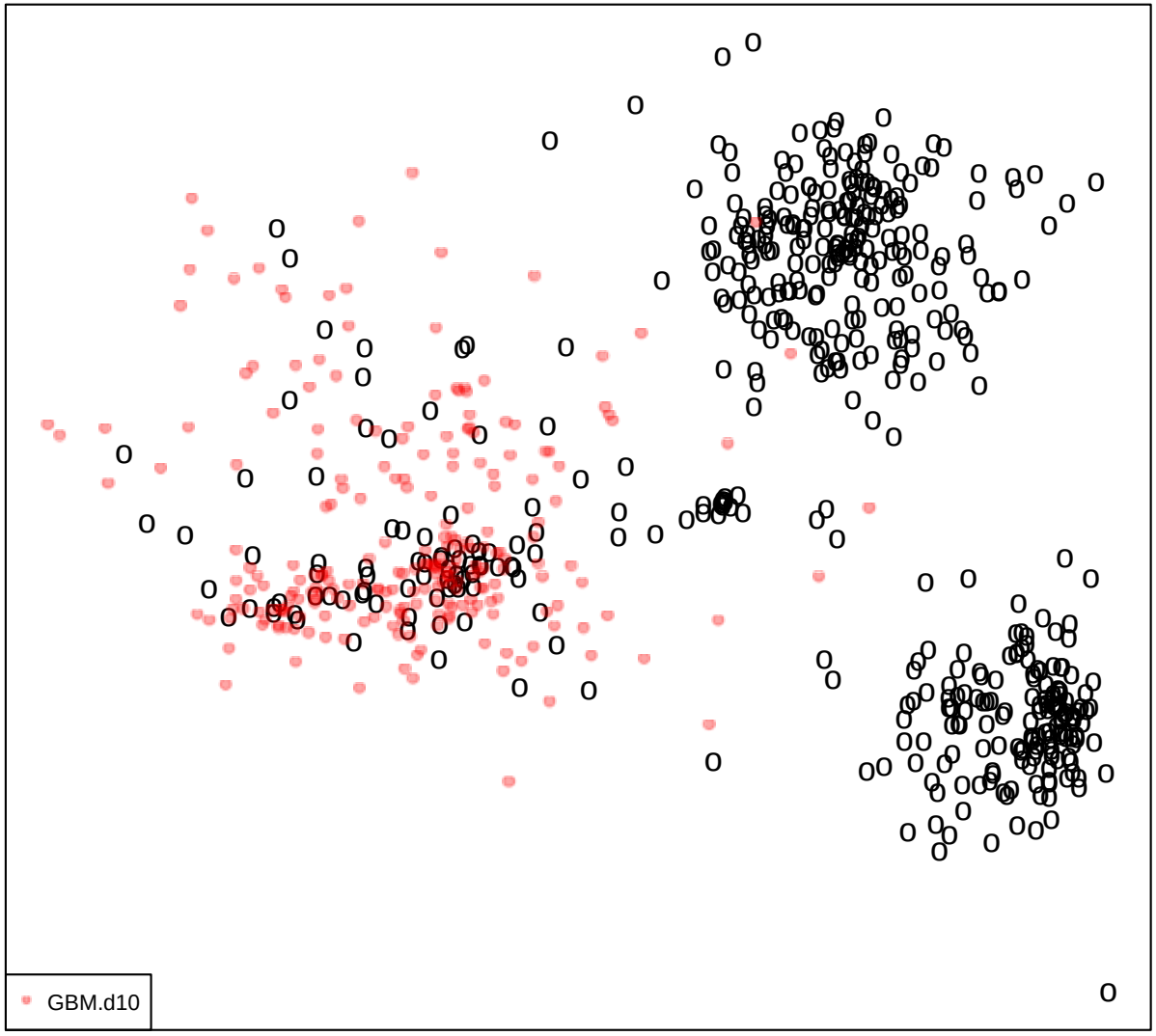
**The non-CIMP GBM expression subgroups are not regionally located**




**GBM: gain of ch7**

**The vast majority of non-CIMP GBM gain several copies of ch7**

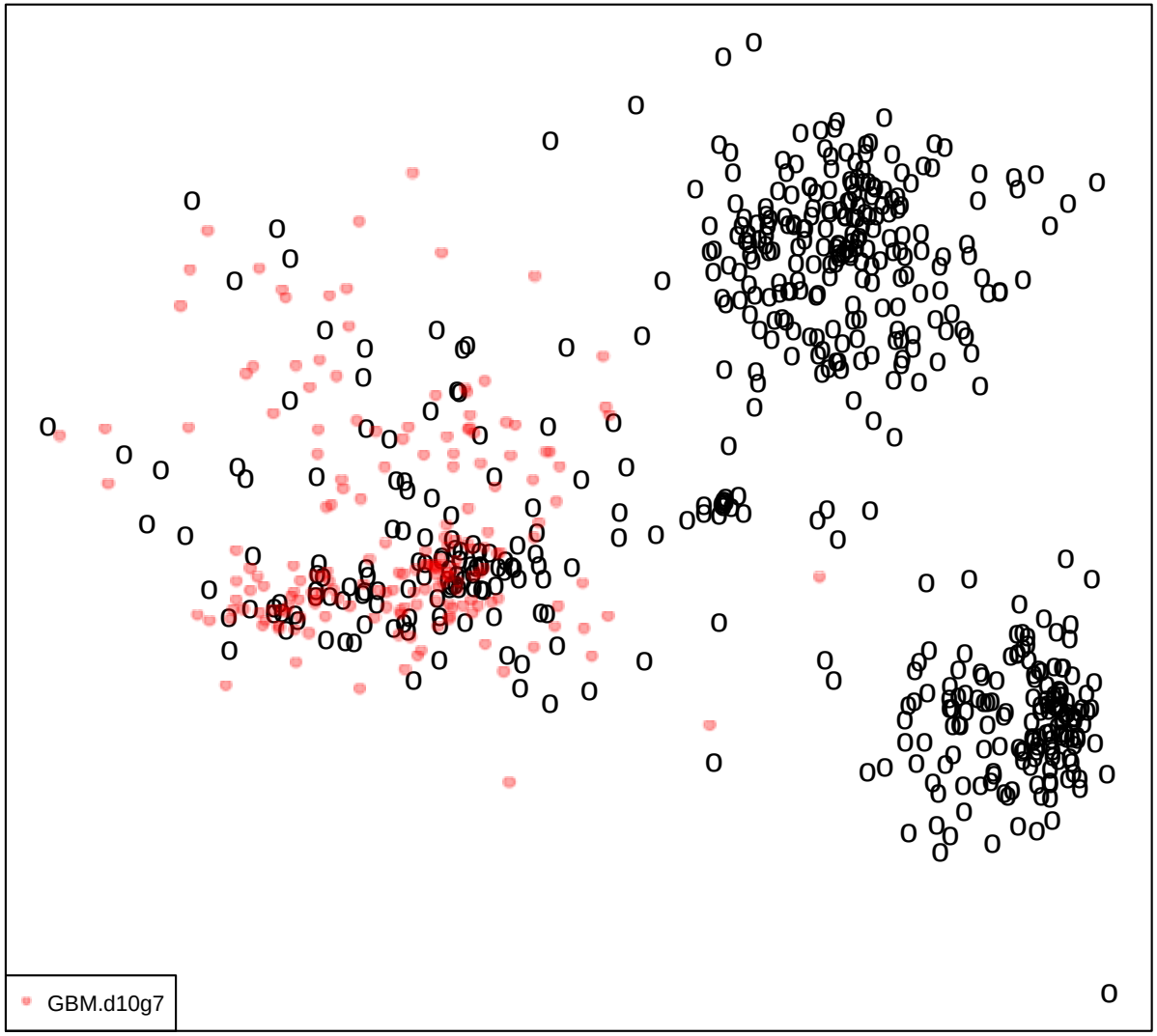
**Most prominent driver of that event is PDGFA**




**GBM: loss of ch10**

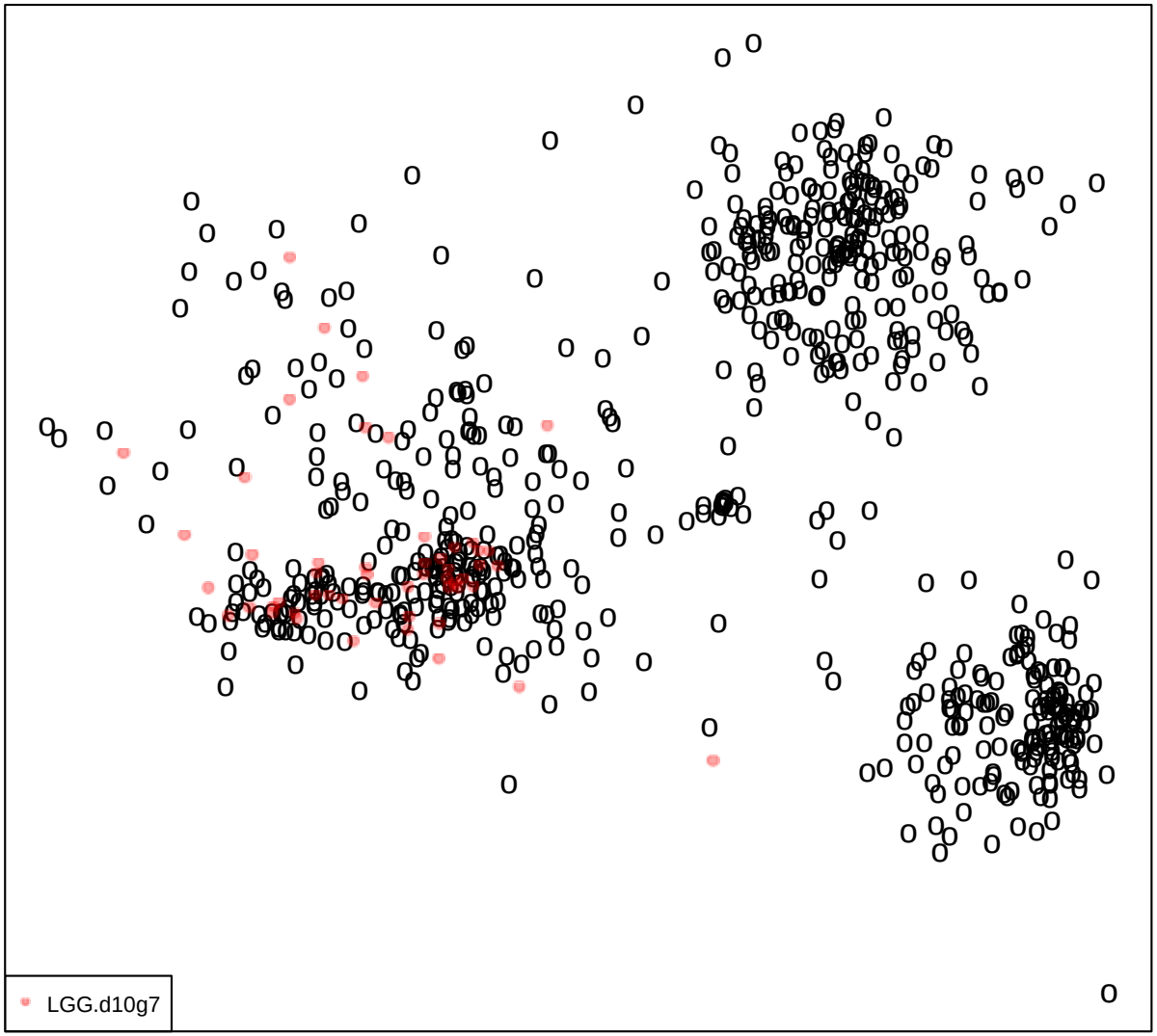
**The vast majority of non-CIMP GBM also lose one copy of ch10**

**The most prominent driver of this event is PTEN**




**GBM: loss of ch10 and gain of ch7**

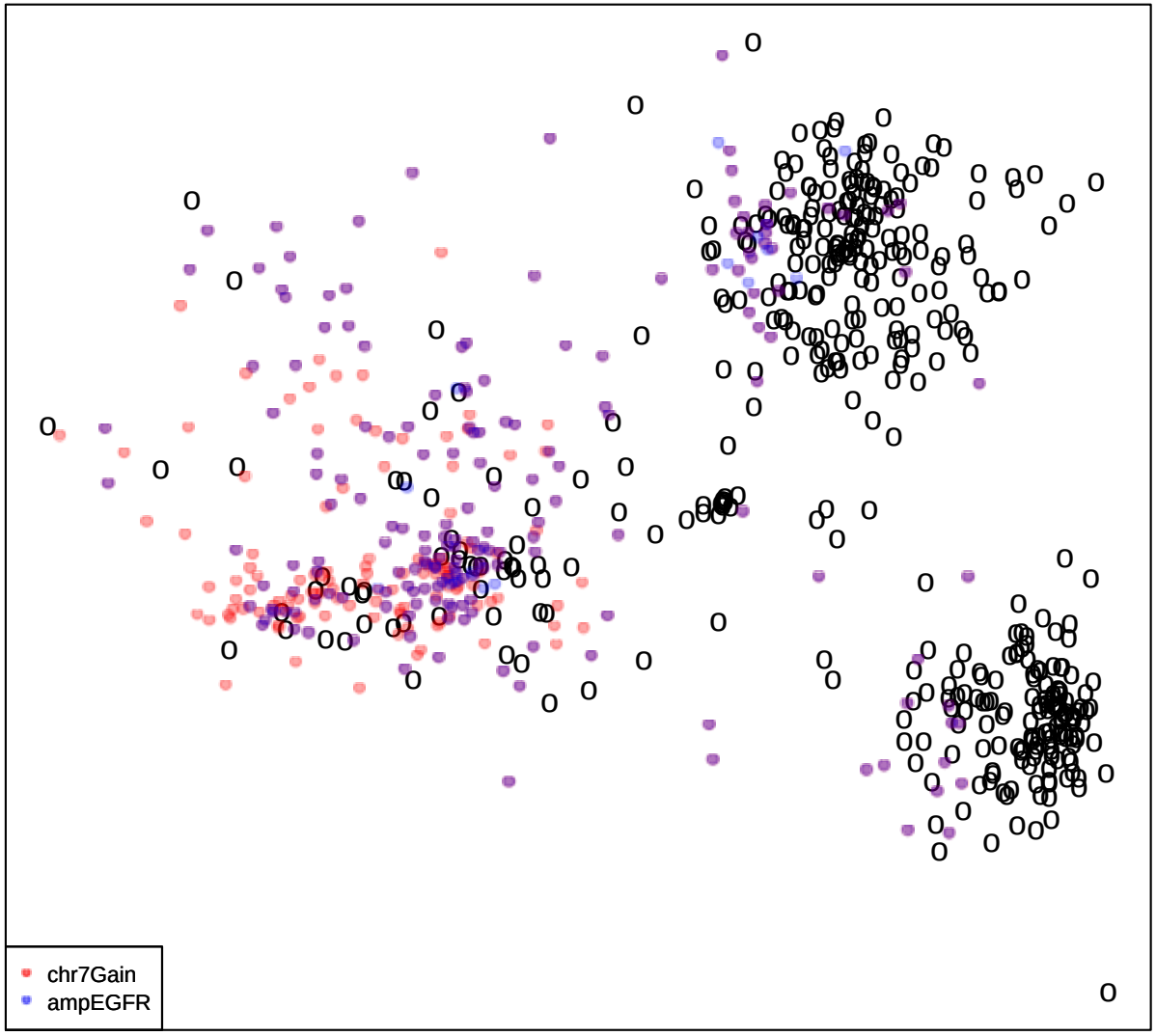
**The combined non-disjunction events of ch7 gain and ch10 loss are the earliest events in GBM evolution**




**LGG: gain of ch7 and loss of ch10**

**The only LGGs that have both gain of ch7 and loss of ch10 are the ones living with GBM and those have a survival similar to GBM.**

**Are these tumors evolving to GBM?**




**EGFR amp (GBM and LGG)**

**EGFR is amplified in some but not all GBM with gain of ch7**

**But in CIMP LGG, all gain of ch7 tumors have EGFR amplification**

**EGFR may be the driver of gain of ch7 in CIMP LGGs**

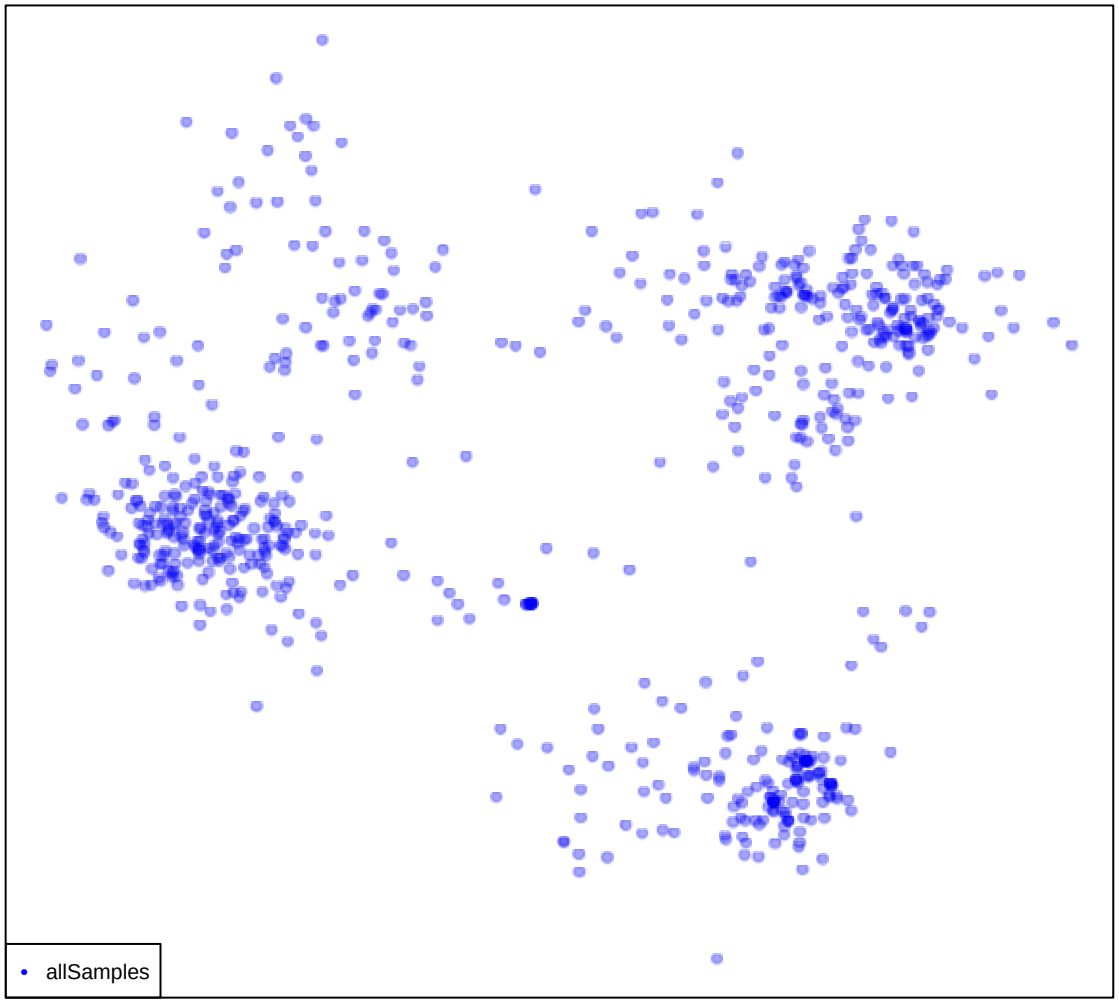
# The data presented uses whole genome copy number and exome sequence


# How much of the genome drives this variance?

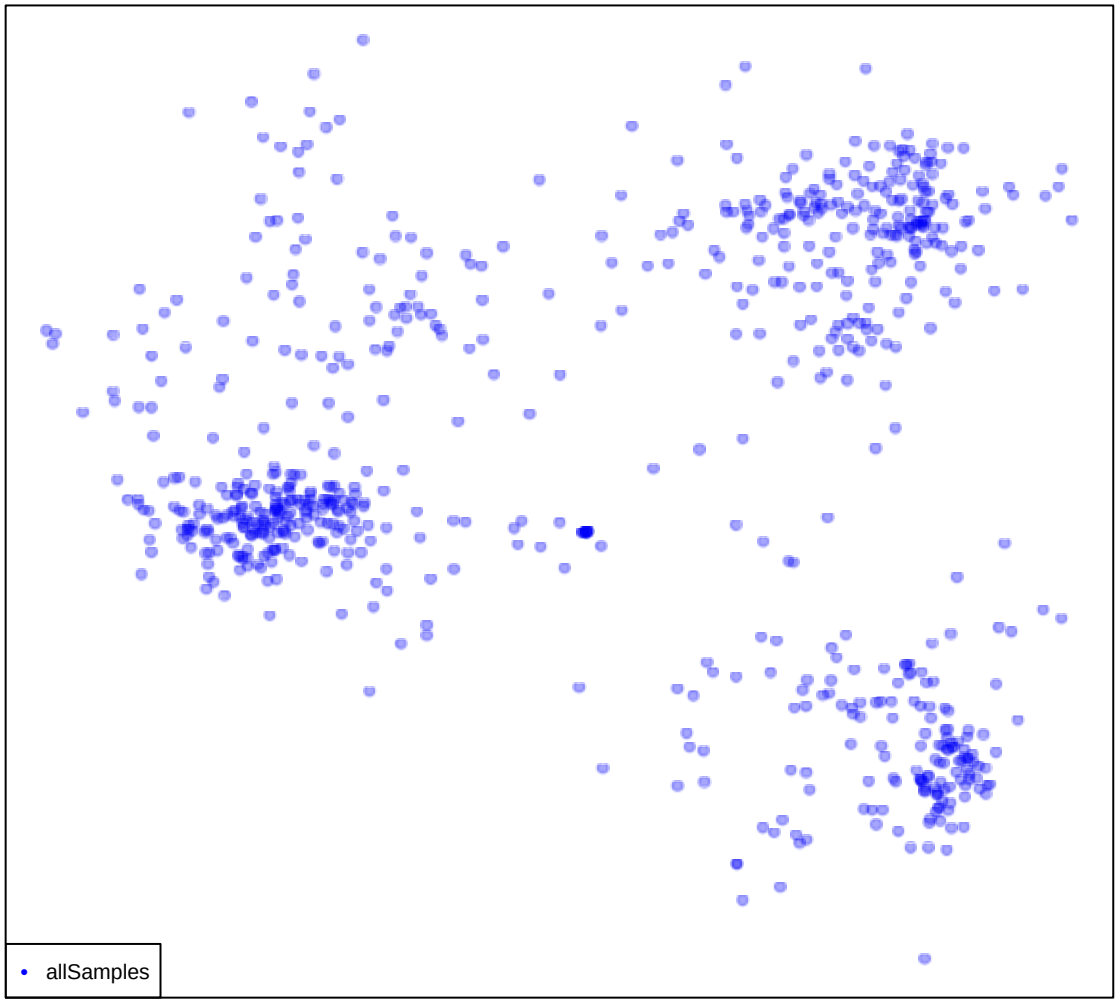
# Can we cluster gliomas based on DNA structural variation with less than the whole genome/exome?

# We went searching for a gene set that could give us tight, genetically homogenous clusters

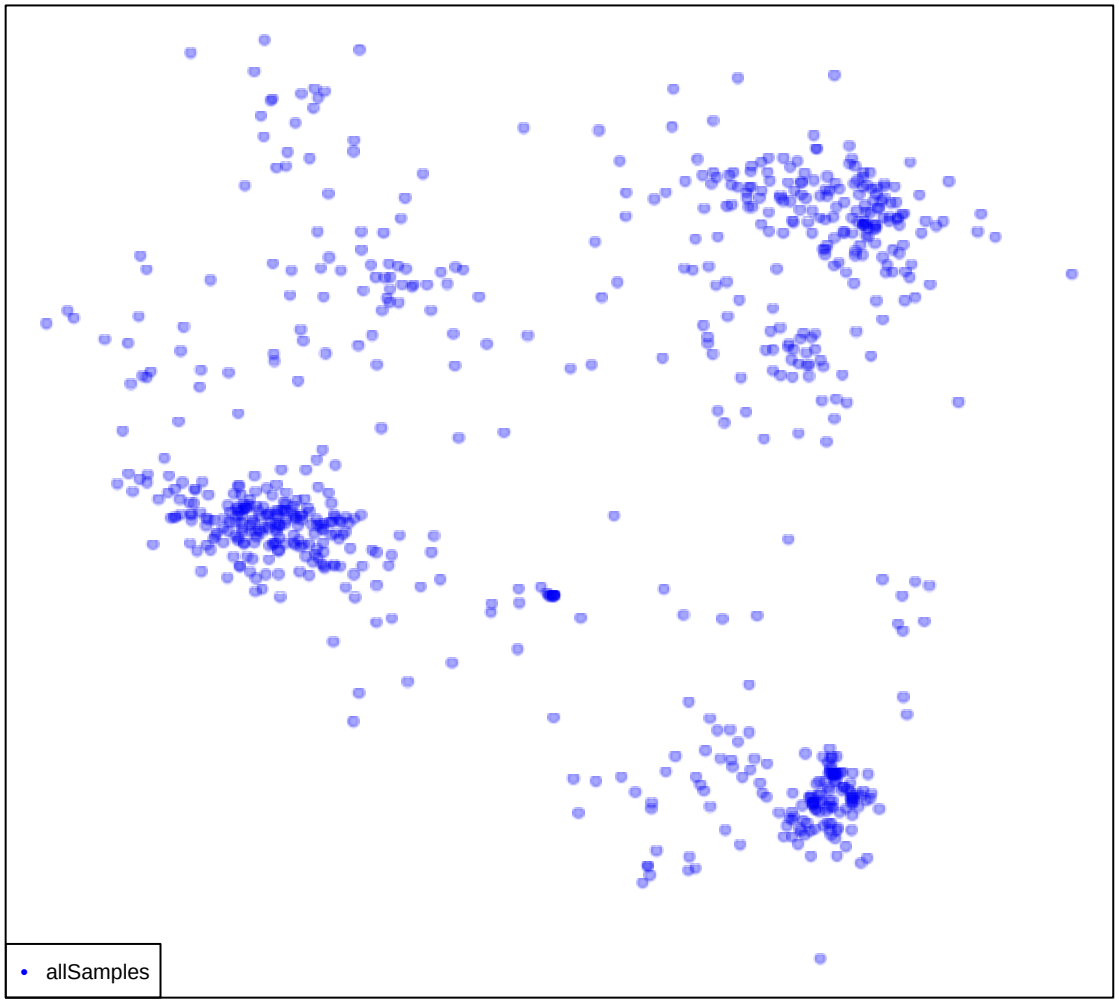
# 97 genes, sequence and copy number



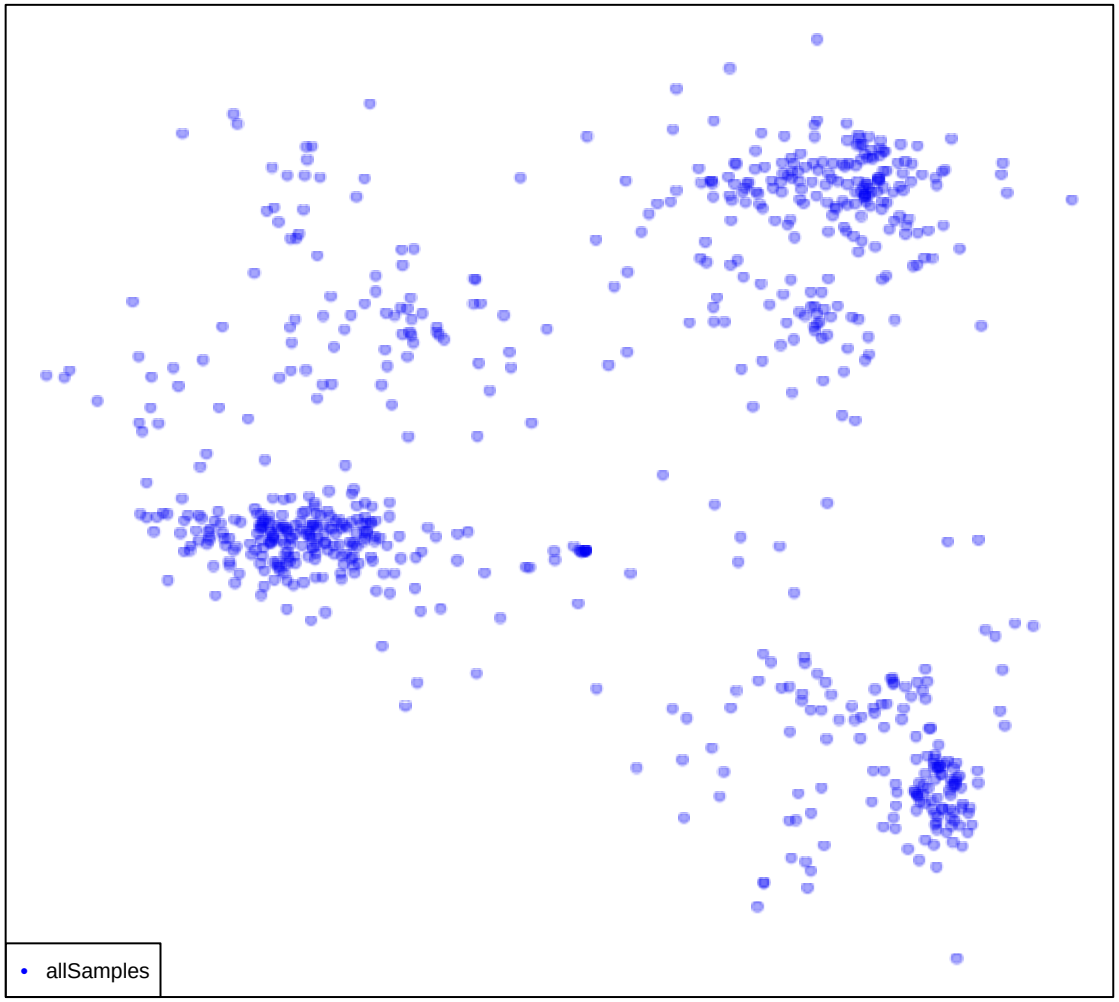
# 134 genes, sequence and copy number



# 236 genes, sequence and copy number



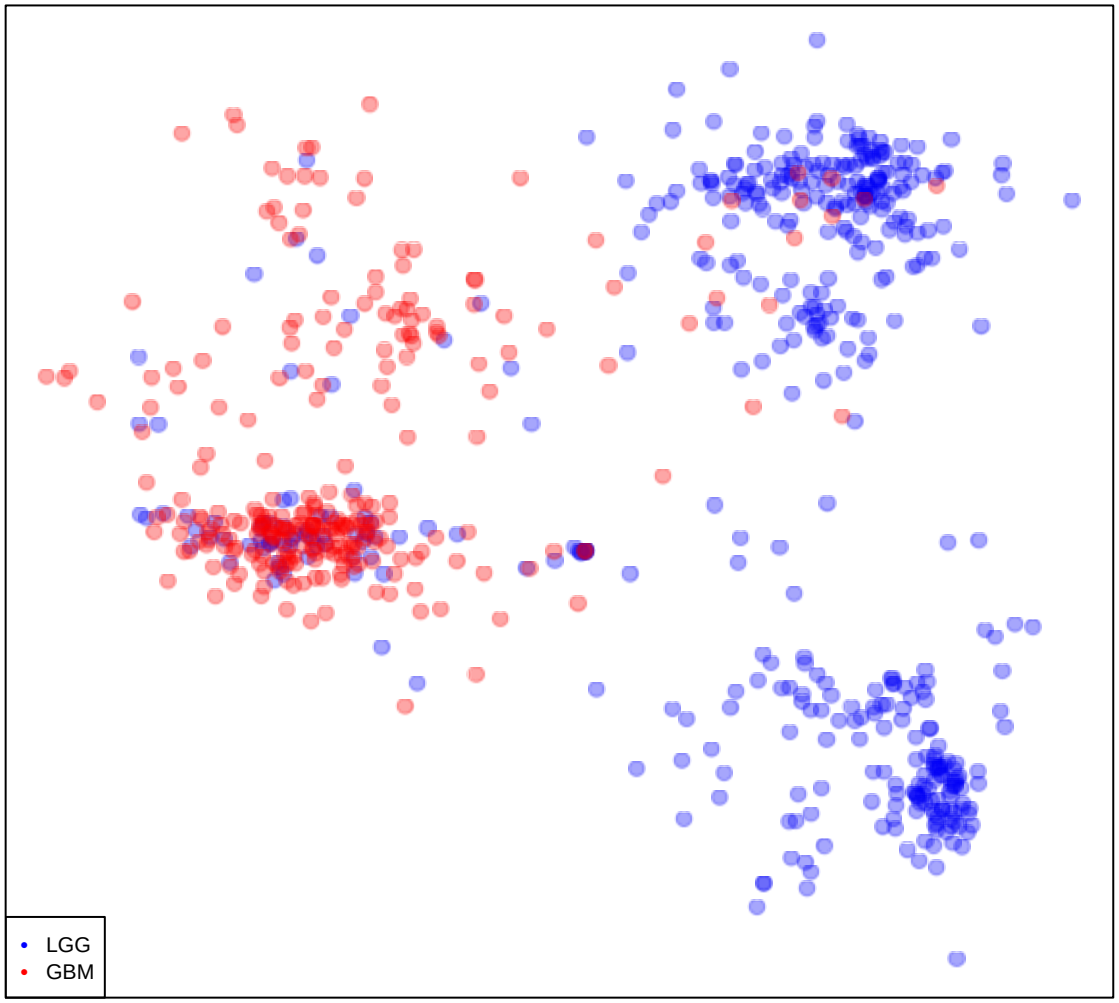
# 274 genes, sequence and copy number




**For the purpose of this talk, will use a 274 gene set because of the molecular data**

**(on the following slides)**

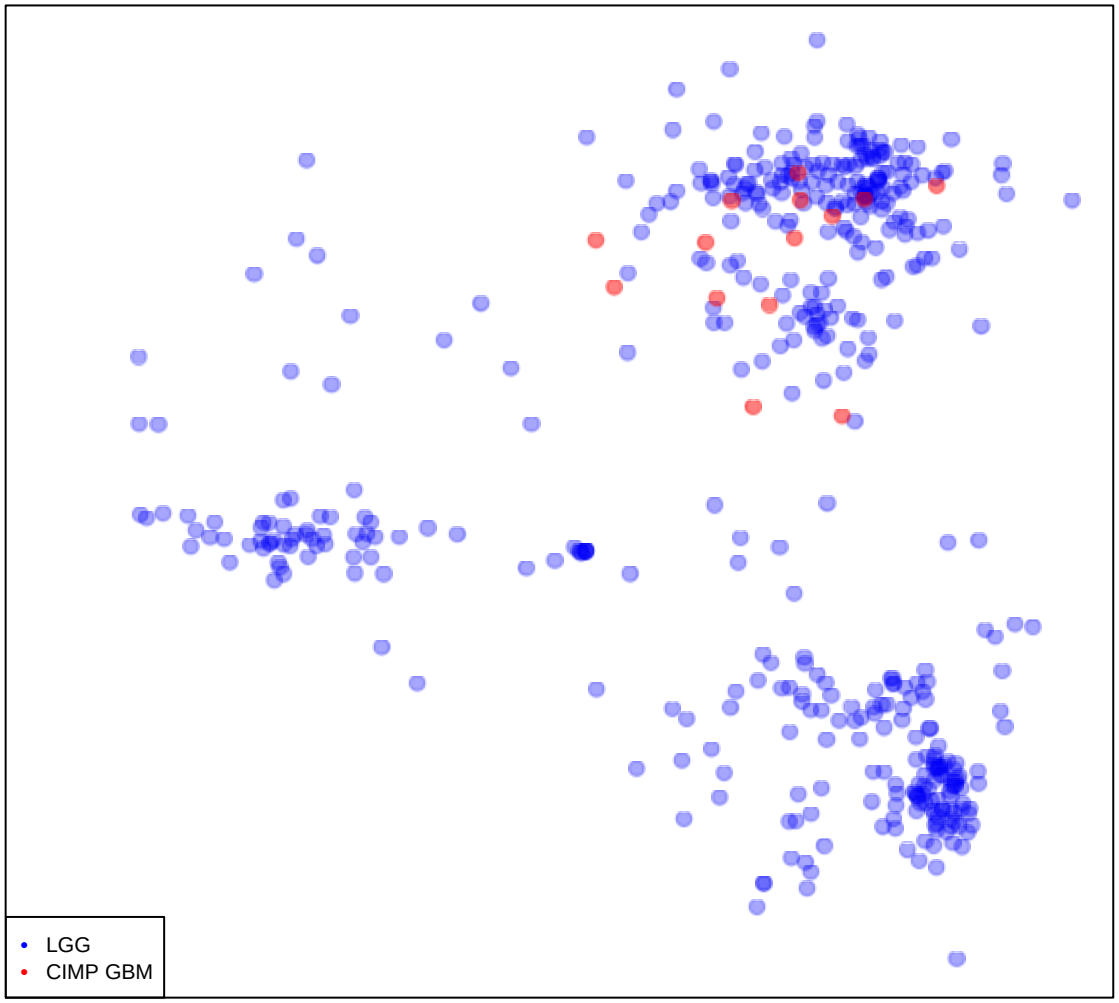
# 274 genes, sequence and copy number




**LGG – blue**

**GBM - red**

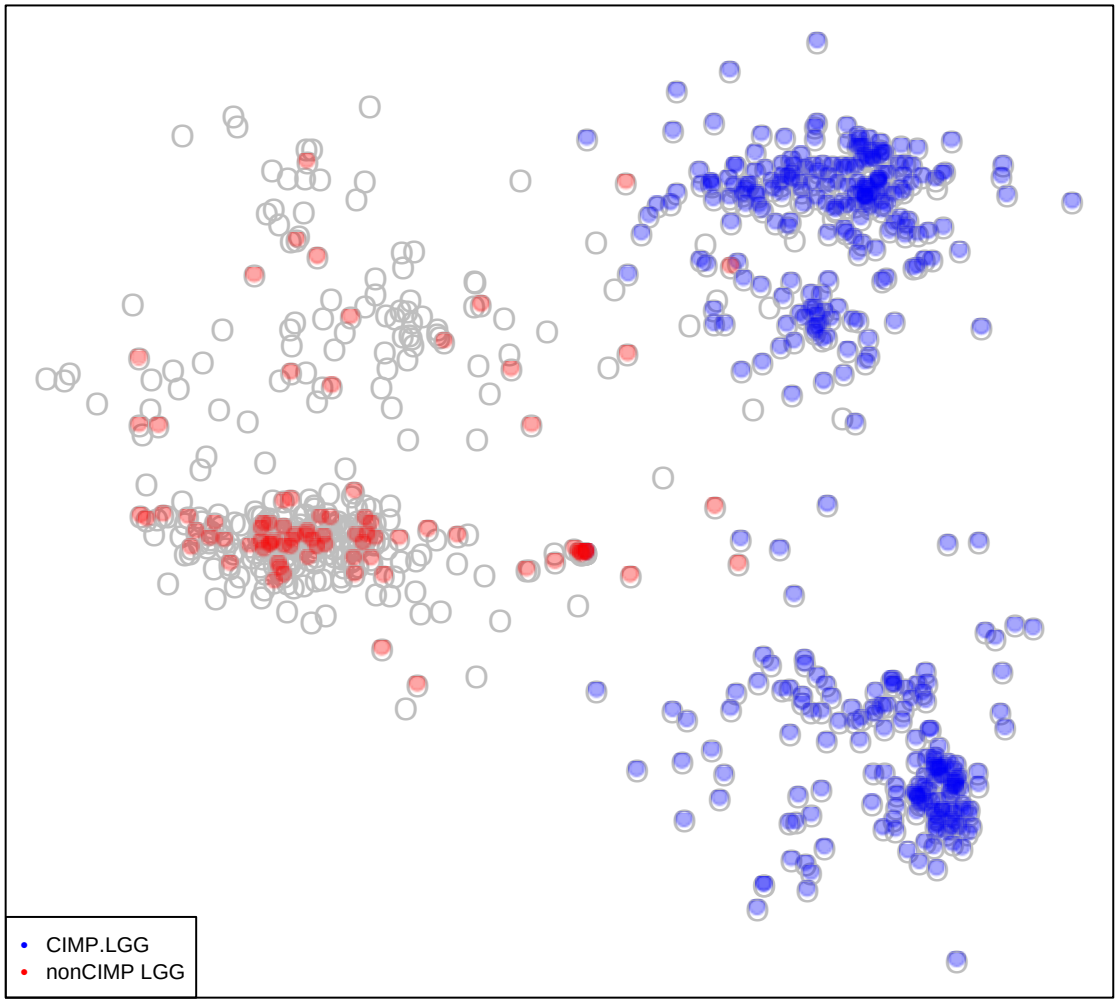
# 274 genes, sequence and copy number




**CIMP GBM – red**

**LGG - blue**

# 274 genes, sequence and copy number

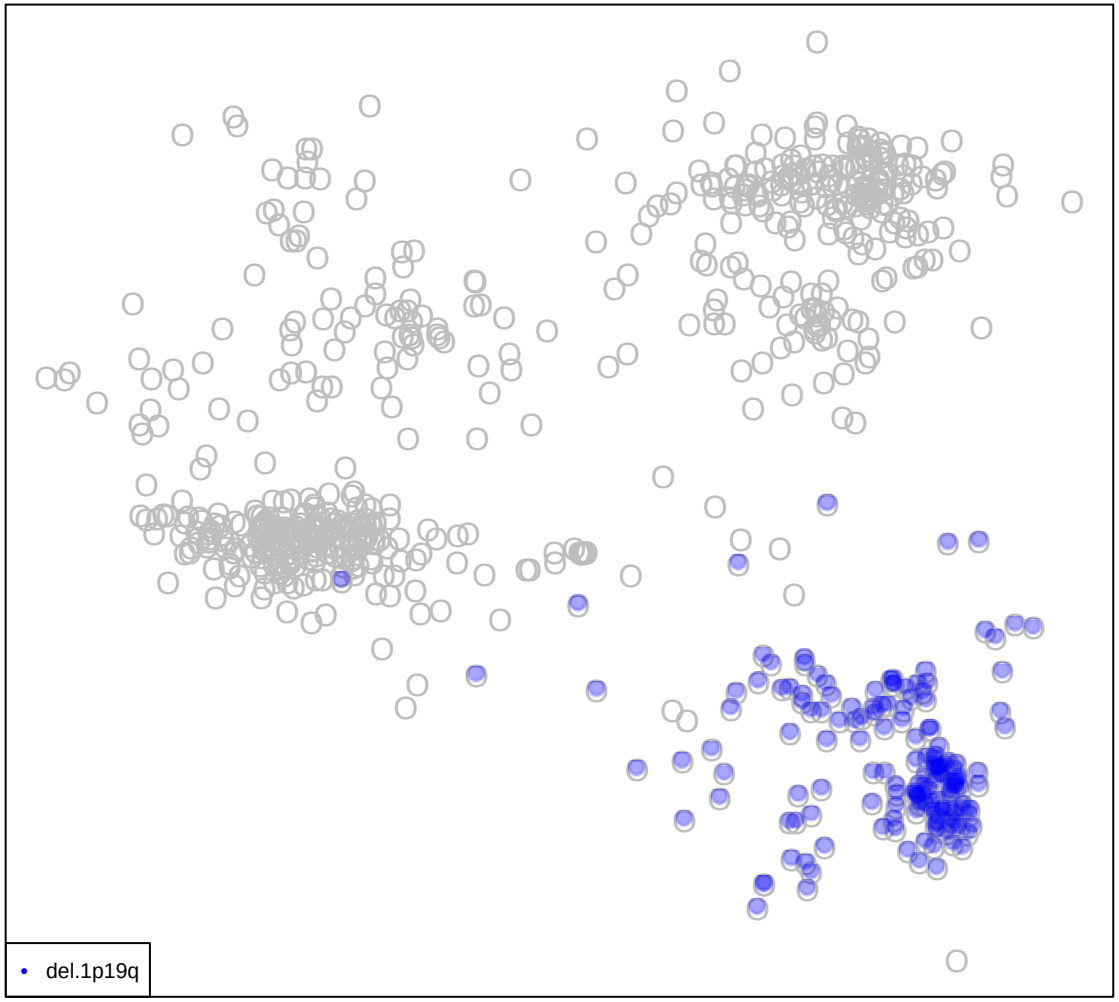



**Low Grade Gliomas**

**CIMP – blue**

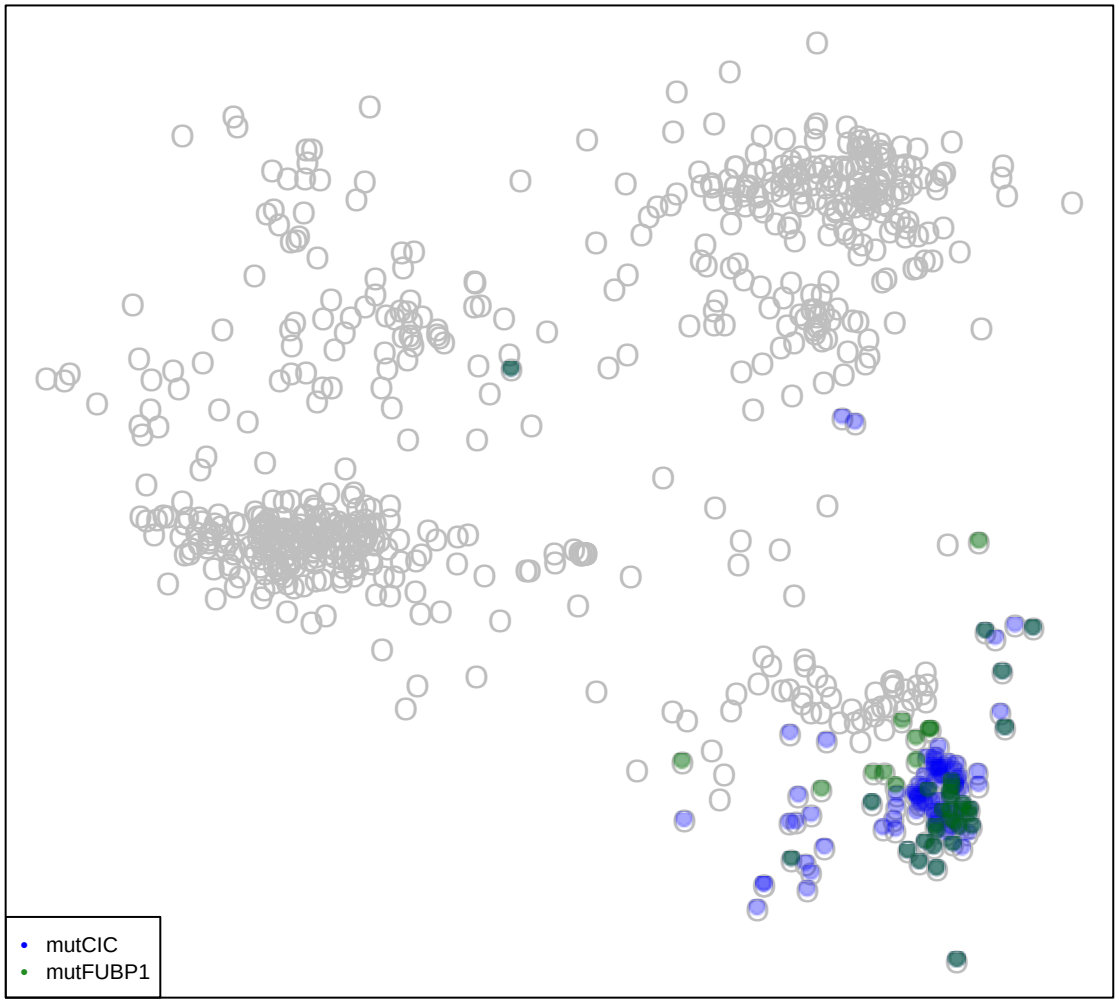
**Non-CIMP - red**

# 274 genes, sequence and copy number



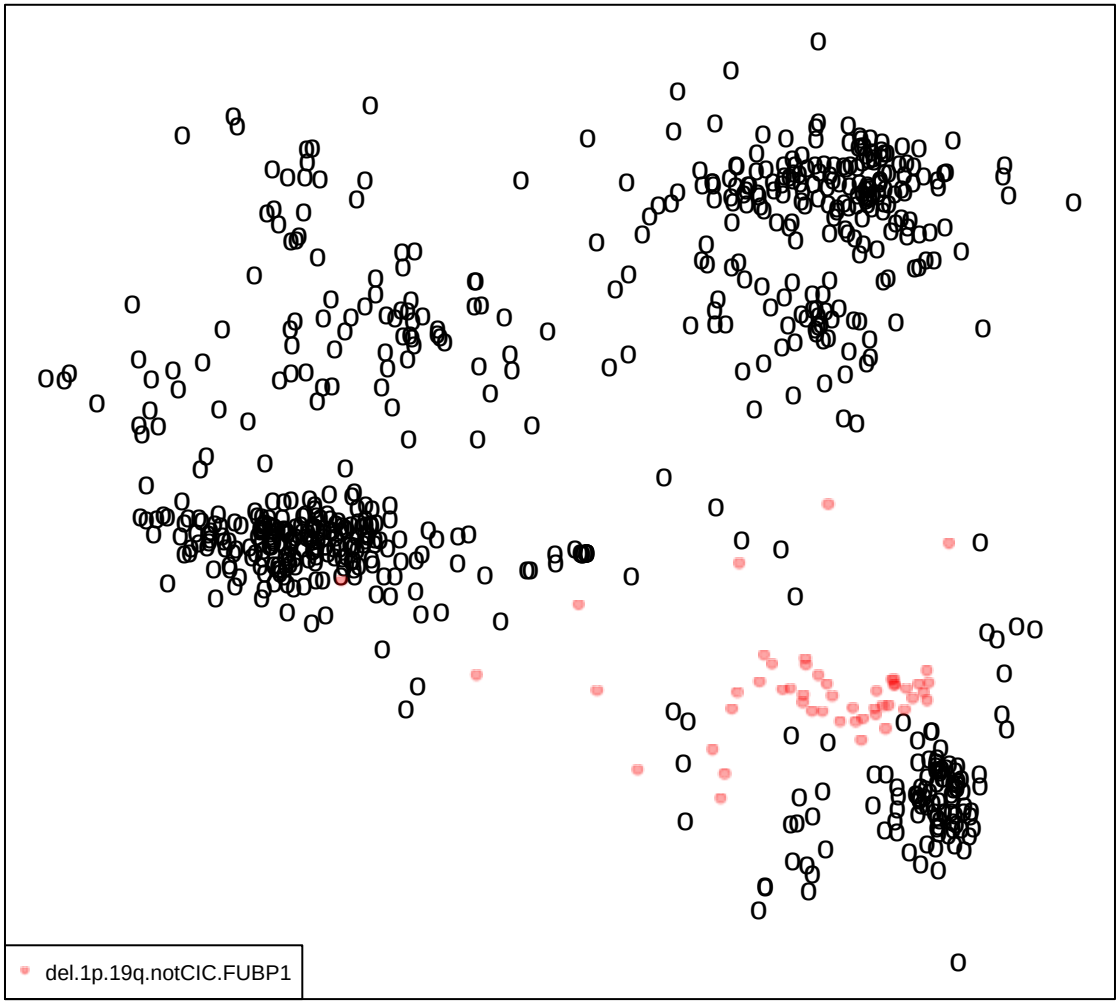

**All gliomas with deletions in 1p19q**

# 274 genes, sequence and copy number



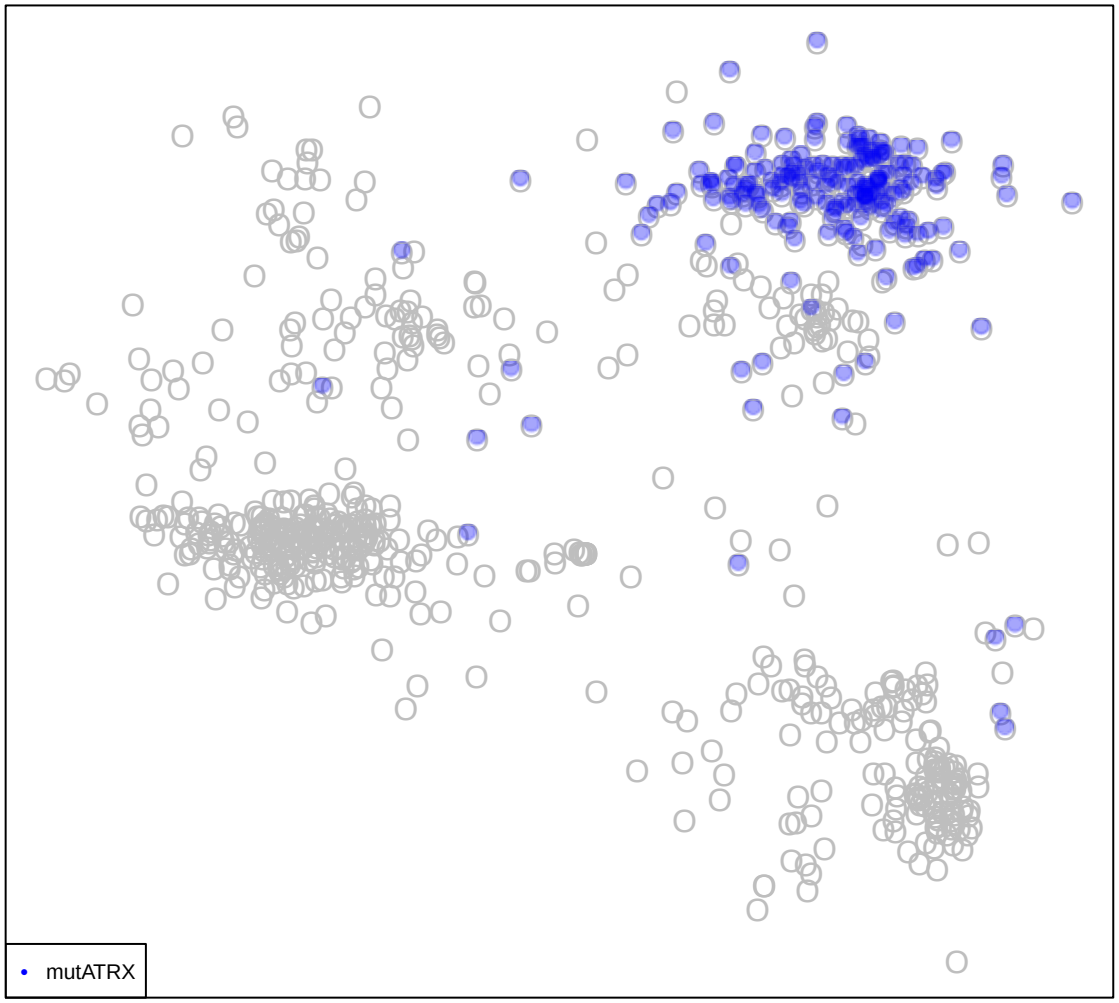

**All gliomas with mutations in CIC or FUBP1**

# 274 genes, sequence and copy number



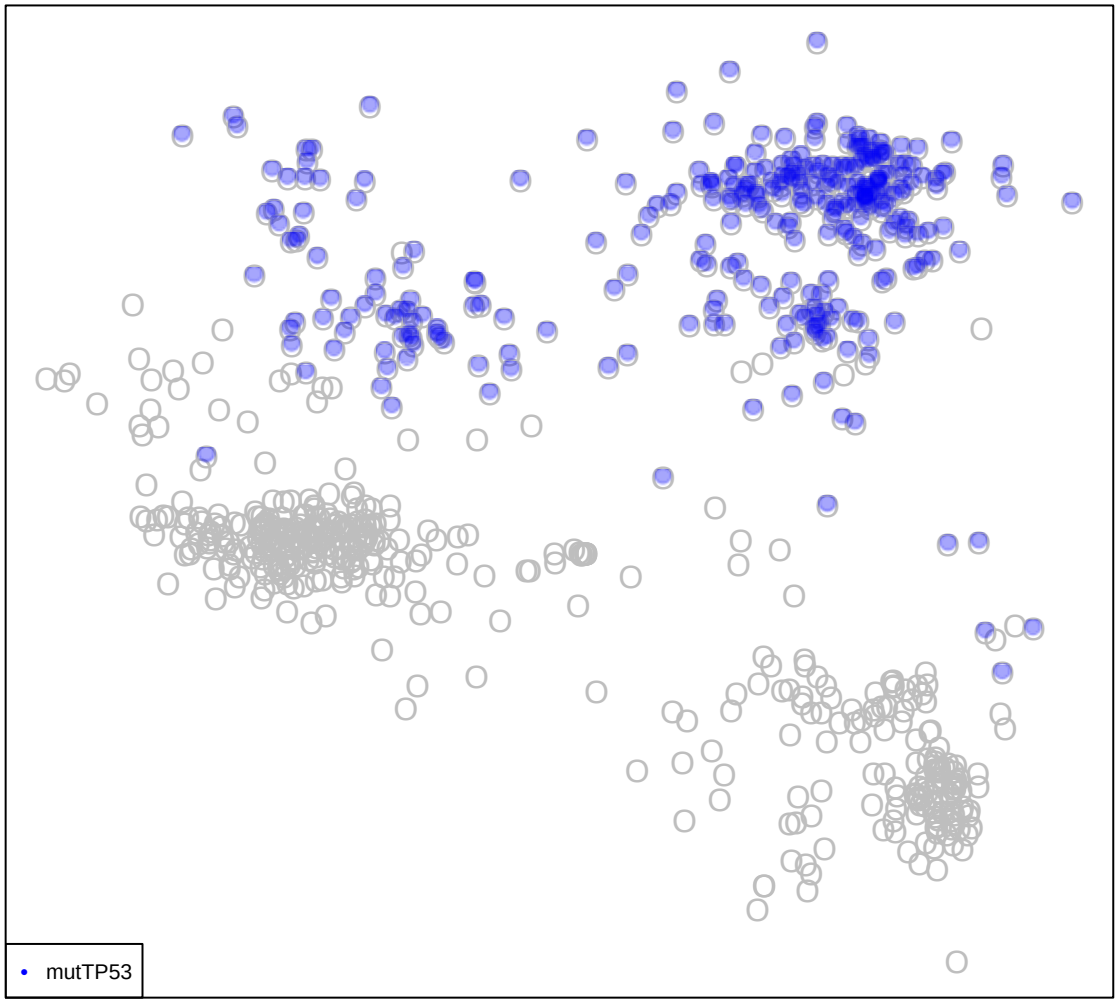

**1p19q deleted gliomas that do not have mutations in CIC and FUBP1**

# 274 genes, sequence and copy number



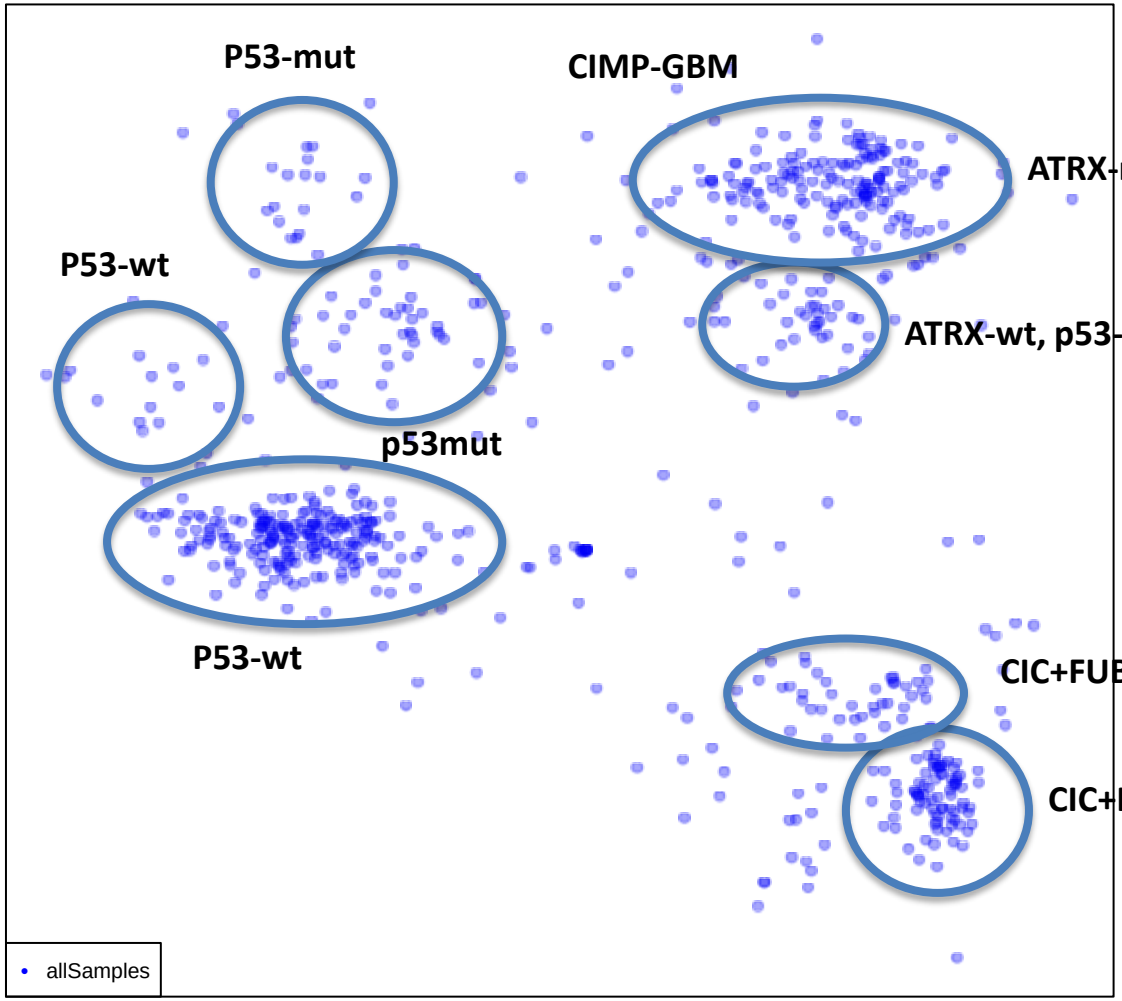

## ATRX

# 274 genes, sequence and copy number




**p53**

# A relatively small number of genes (sequence and copy number) drive this variance



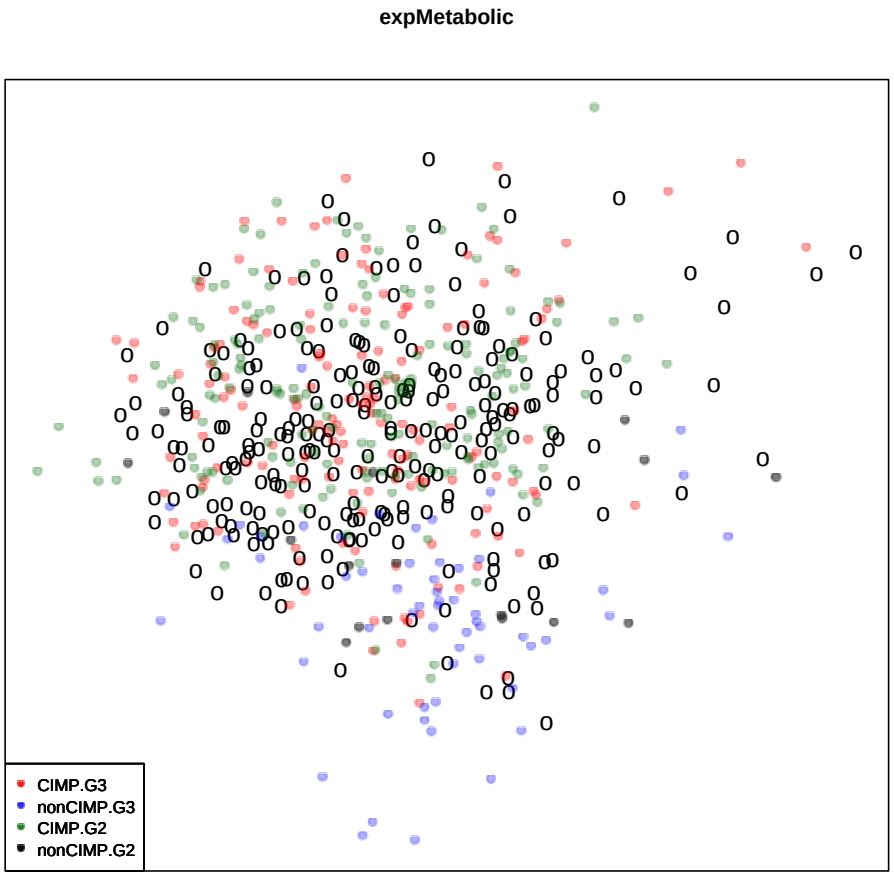
# Some genomic alterations that show striking regional occurrence in the preceding plot

- PIK3CA
- PIK3R1
- PDGFRA
- Met
- VEGFR1
- NRas
- Ch11 deletion
- Ch14 deletion
- Ch1q gain
- Notch1
- etc

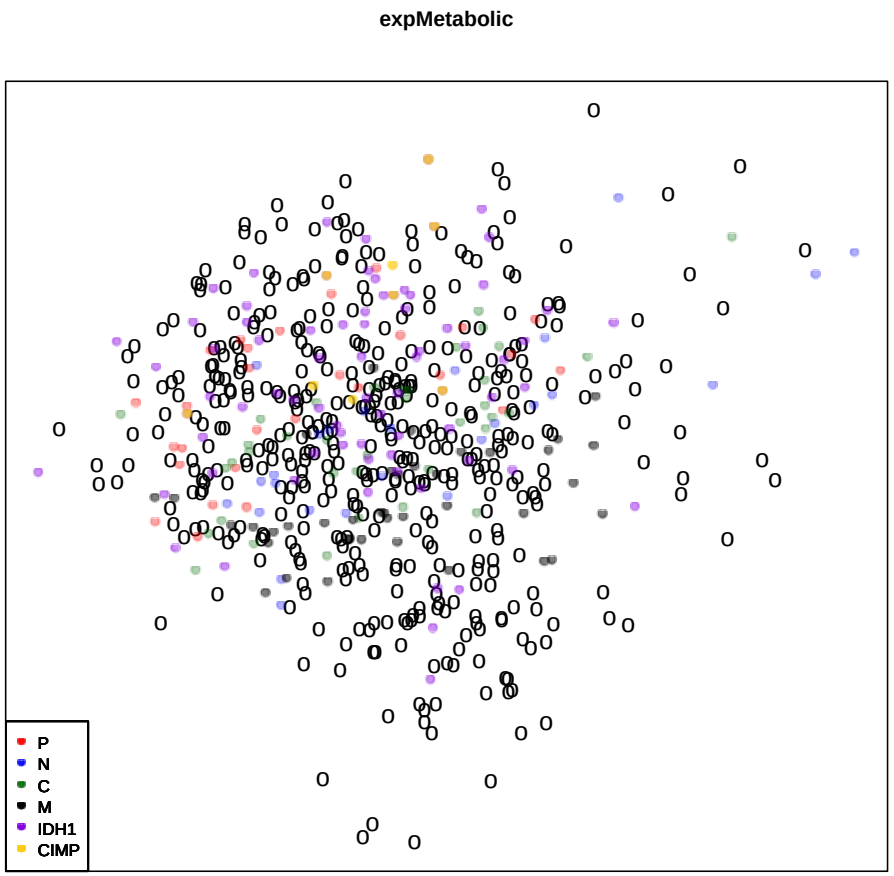
# Displaying tumors using gene expression data

- **Start with RNAseq**
- **Project tumors onto 2 dimensions based on expression of subsets of genes chosen by biologic process**
- **Use genes sets involved in metabolism, stemness, etc.**

# Expression of genes relative to metabolism illustrate differences between non-CIMP LGGs and other gliomas

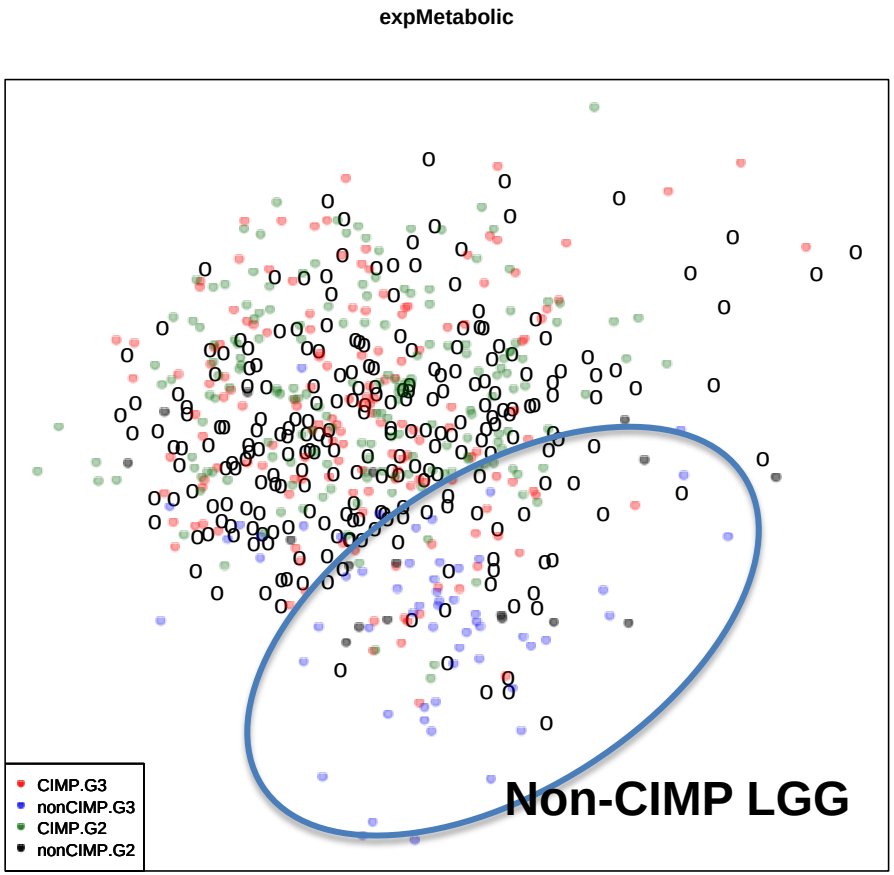



LGG

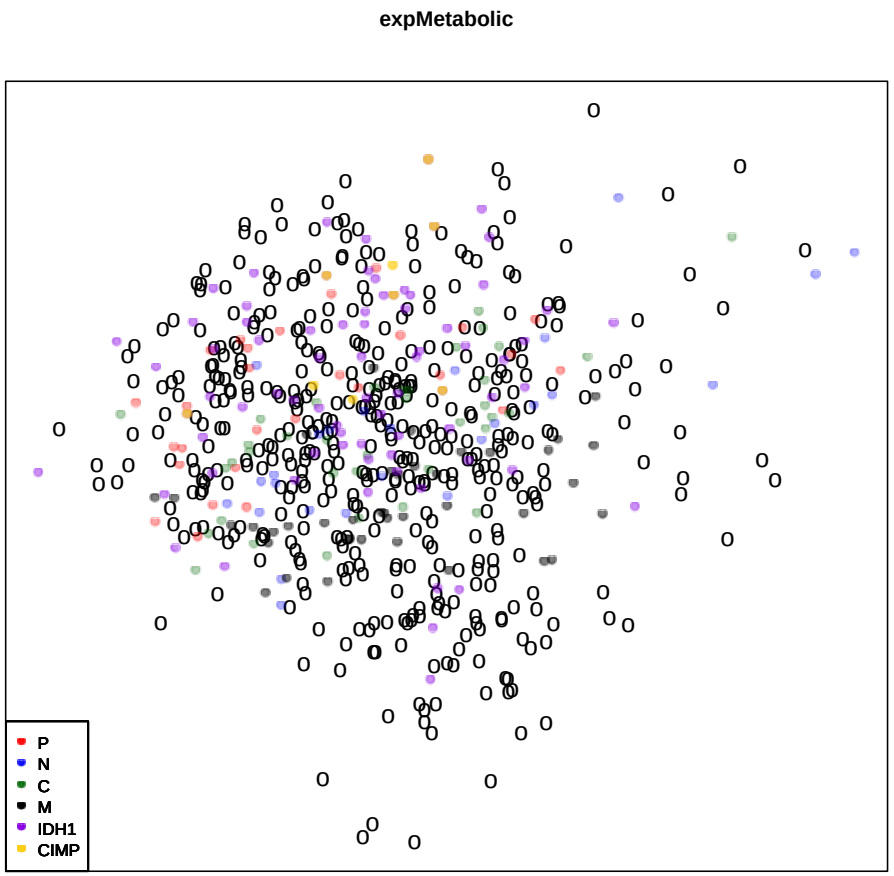



GBM

# Expression of genes relative to metabolism illustrate differences between non-CIMP LGGs and other gliomas

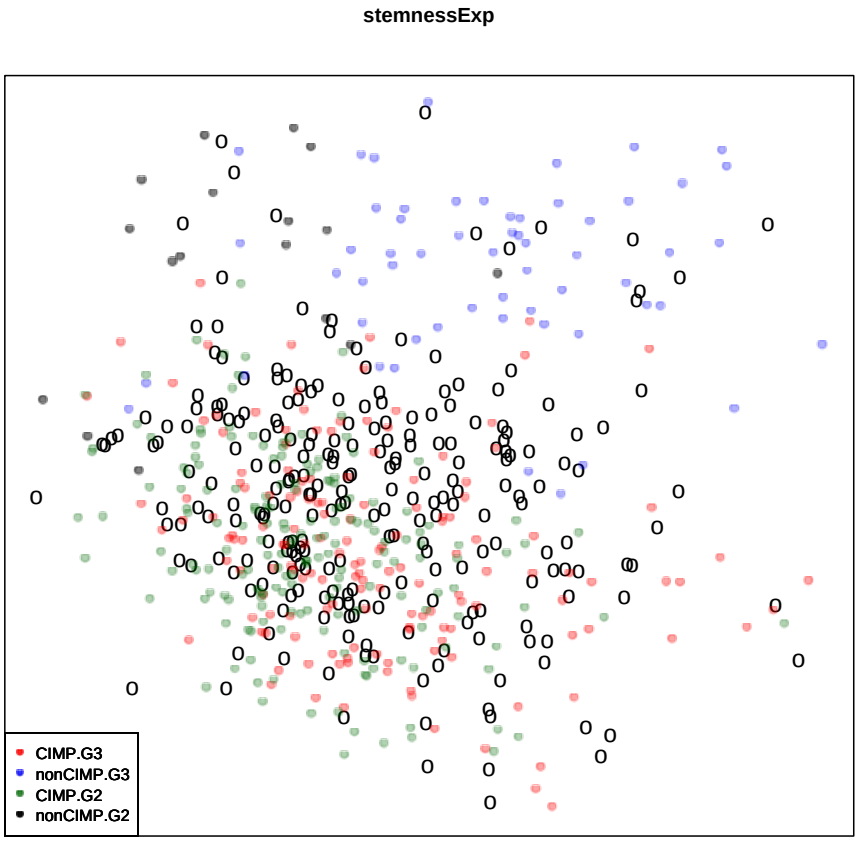



LGG

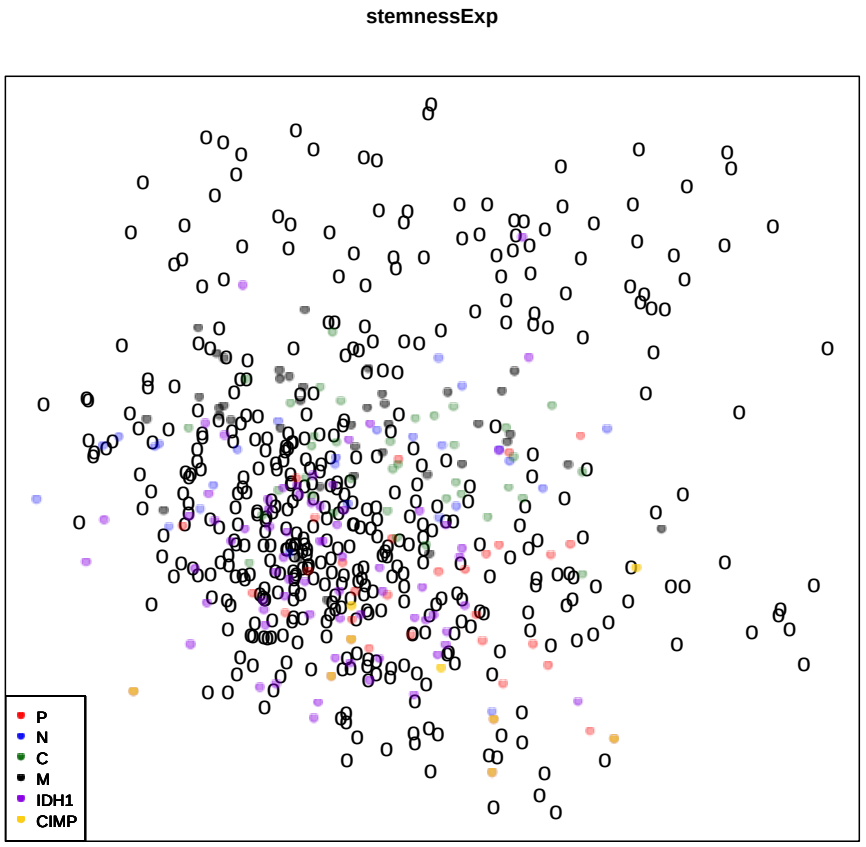



GBM

# Expression of genes relative to stemness also illustrate differences between non-CIMP LGGs and other gliomas



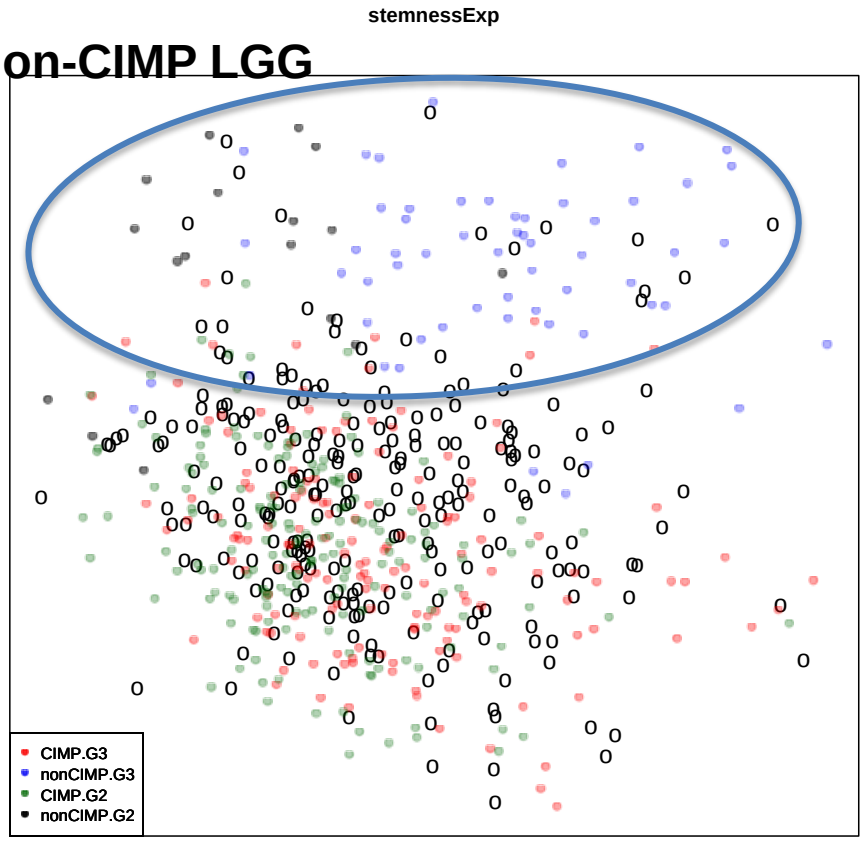

LGG

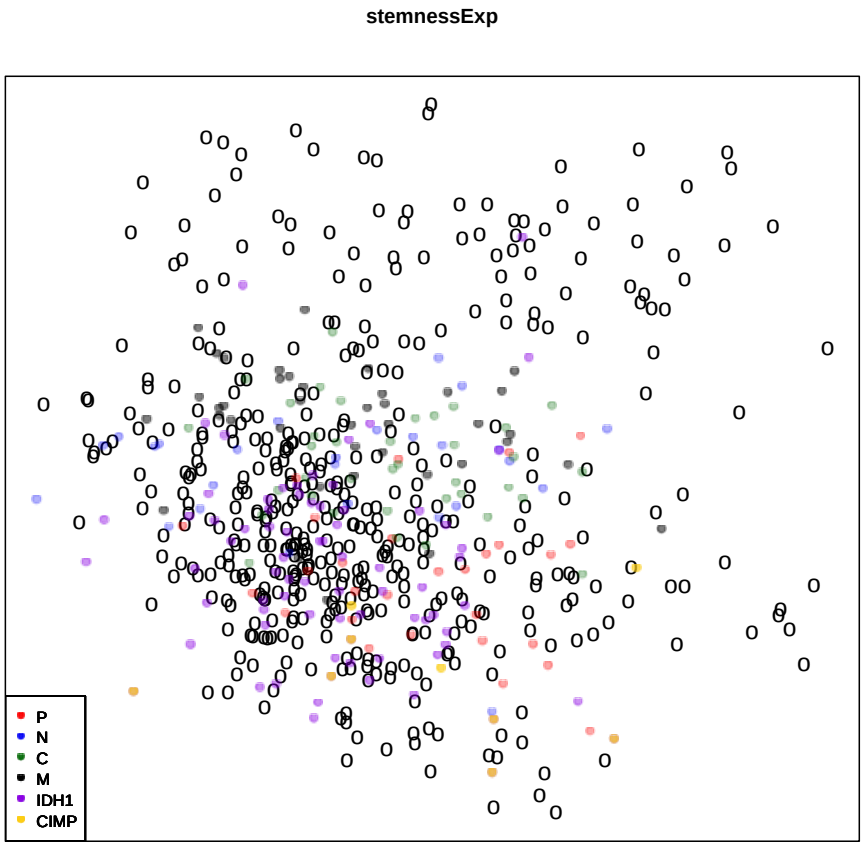



GBM

# Expression of genes relative to stemness also illustrate differences between non-CIMP LGGs and other gliomas

Non-CIMP LGG



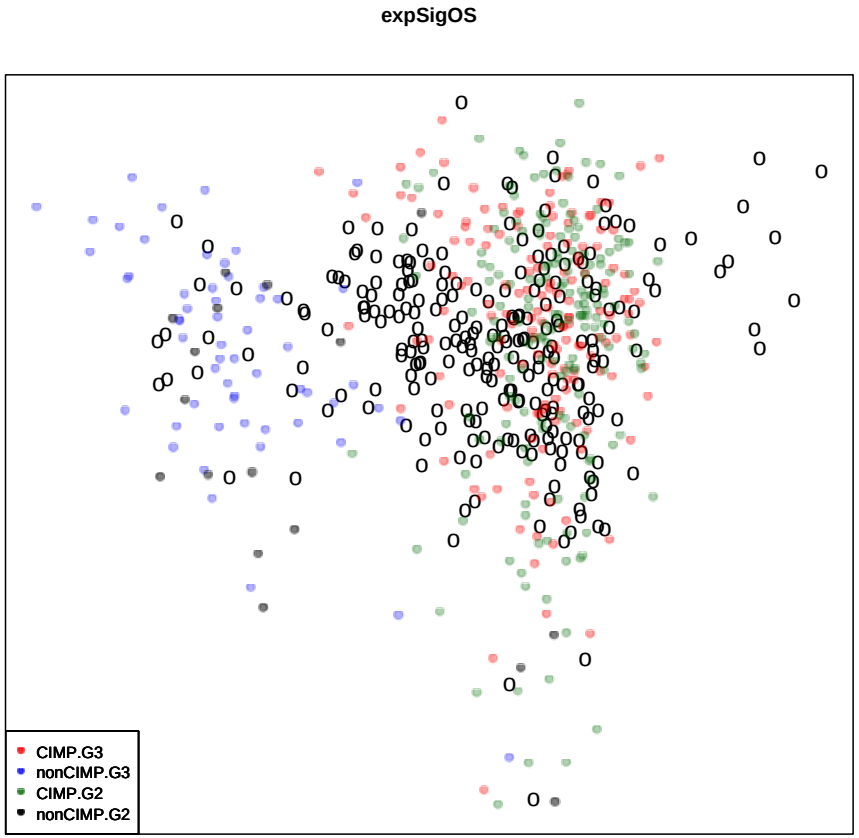




LGG

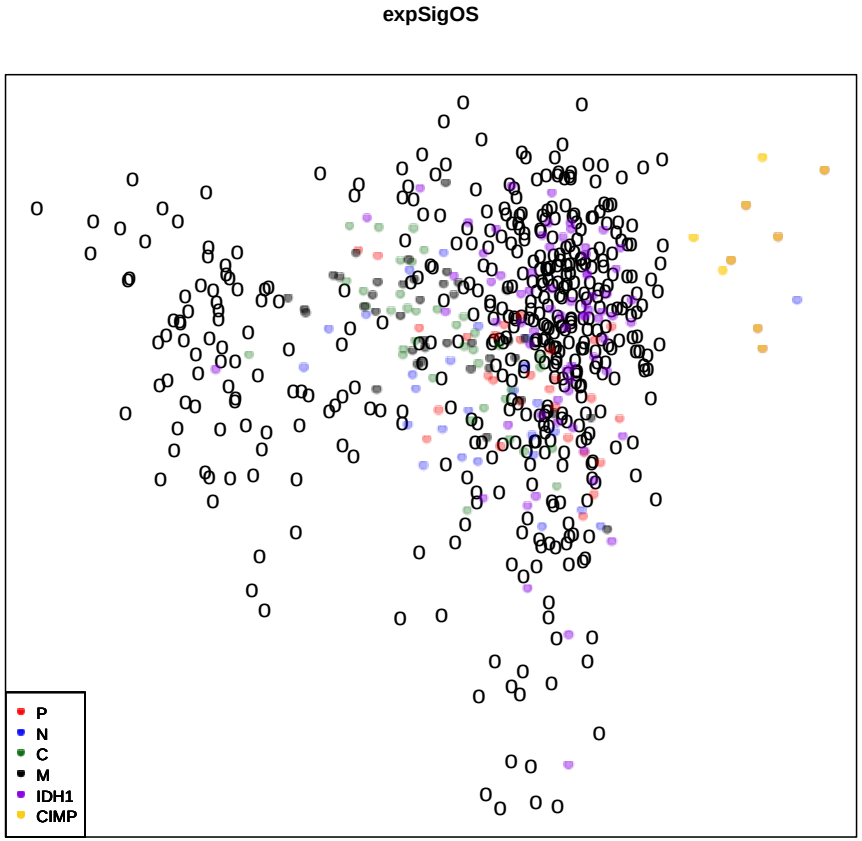
GBM

**We are also able to plot using correlation of overall expression patterns with overall survival**

**This plot shows the non-CIMP LGGs and CIMP-GBM clearly separate from the others.**



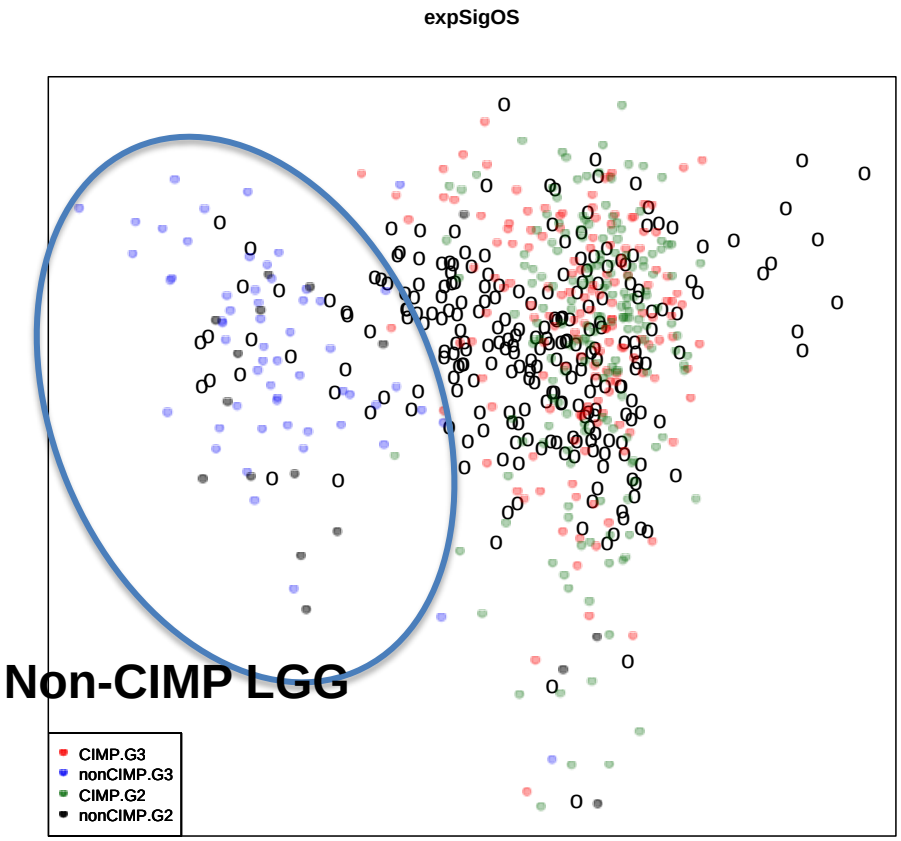

**LGG**

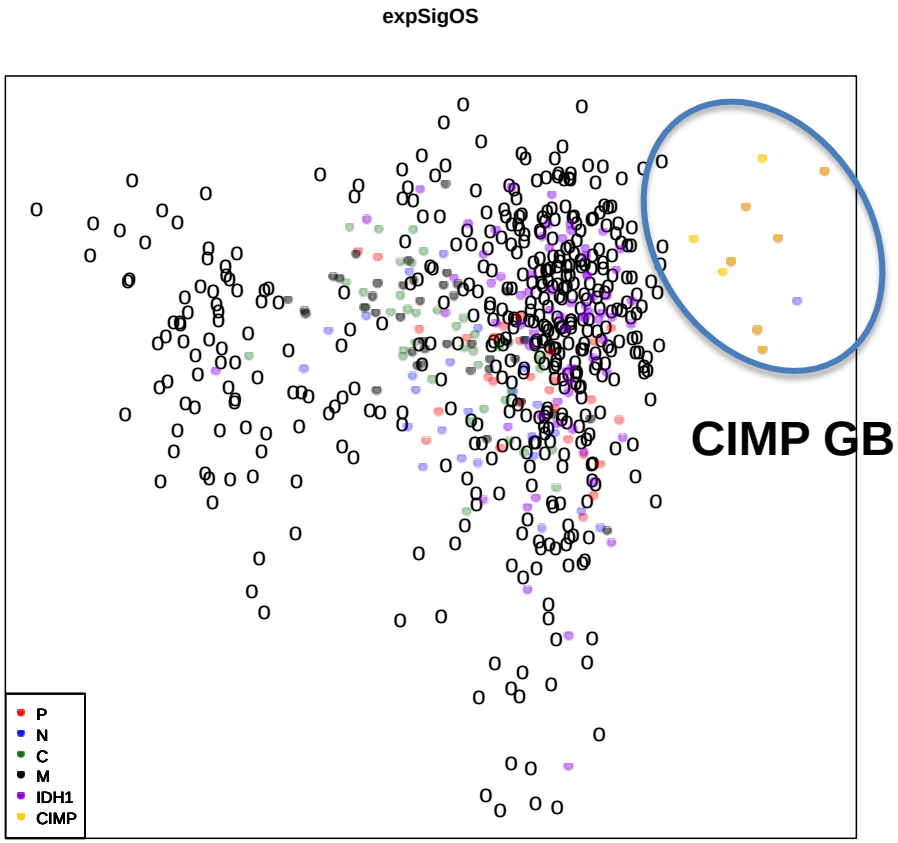



**GBM**

**We are also able to combine overall expression patterns with overall survival**

**This plot shows the non-CIMP LGGs and CIMP-GBM clearly separate from the others.**



# Some conclusions of patterning with gene expression

- **If we limit the gene sets to specific biology we can learn something from the data.**
- **Gene sets relating to metabolism, stem-ness, immunity, glioma-associated genes, and signaling pathways provide insight to the behavior of glioma clusters**
- **Gene expression patterning suggest that non-CIMP LGG are not simply mis-diagnosed GBM**


Thank you ☺
SOLID TUMOR
TRANSLATIONAL RESEARCH
FRED HUTCH
CURES START HERE