Glioblastoma multiforme

- Glioblastoma multiforme (GBM), or Glioblastoma, or Grade IV Astrocytoma is the most common and most aggressive malignant primary brain tumor.

- GBM is usually involving glial cells, and the incidence is about 2~3 cases per 100,000 person life-years.
Genomic Landscape of Primary GBM

- Cross-sectional studies (~200 patients) have identified many new driver genes:

*Science (2012)*,  *Nature Genetics (2013)*.
Treatment

- Stupp Therapy (Roger Stupp):
  - Surgery
  - radiation with daily temozolomide (TMZ)
  - six cycles of five-days-a-week TMZ.

- TMZ alkylates/methylates DNA, and triggers the death of tumor cells
- MGMT repairs this damage, diminishes the therapeutic efficacy

- For now there is no standard treatment for recurrent tumor
Dynamic Landscape of GBM
Questions

How does GBM evolve under therapy?

Can we reconstruct the main routes of GBM evolution?

How spatial information (intratumor heterogeneity) informs evolution?

Are there specific genes associated to GBM relapse?
The cohort

Nature Genetics (2016).
How can we compare evolutionary histories in different patients?
Comparing evolutionary histories

• If every tumor follows a different evolutionary path, how do we extend computational and statistical frameworks to study longitudinal data?

• Aims:
  1) to develop a framework to compare the evolutionary history of different patients -> metric space that allow comparison of evolutionary histories.
  2) To develop a statistical framework and machine learning approaches to longitudinal cohorts.
  3) To develop a statistical/evolutionary framework to test different evolutionary models (e.g. multistage due to hard selective sweeps).
Cancer follows clonal Darwinian evolution
Patients represent forests of phylogenetic trees
Moduli or classifying space of clonal evolution

- To compare two or more histories we would like to find a space that describe all possible evolutionary histories.
  - Moduli space: space whose points correspond to objects of some kind.

- Every tumor has a different history, so every tumor evolution corresponds to a particular point in that space.
Space of trees

Theorem 1: The space of trees is a Polish space (one can define statistics).

Theorem 2: The space of trees is a CAT(0) space (one can define means and variances in a meaningful way).

Zairis, Khiabani, Blumberg, Rabadan (2014)
GBM evolution shows distinct evolutionary trajectories associated to specific molecular mechanisms.
GBM evolution shows distinct evolutionary trajectories associated to specific molecular mechanisms.
Figure 2

- Silent/Missense Ratio

- Mean expression

- HM Score

- Number of mutations

- Initial Recurrence

- Untreated Recurrent Non-HM Recurrent HM

- m=160

- n=87

- P<0.01

- n=93

- HM gene (9,838)

- M gene (3,278)

- NM gene (10,375)
# Considering subclonal mutations

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Recurrent</th>
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<tbody>
<tr>
<td>C</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>S</td>
<td>✔️</td>
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<tr>
<td>X</td>
<td>✔️  ✔️</td>
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- MRCA of initial sample
- MRCA of recurrent sample

**Type I**

- Initial sample
- Recurrent sample

Time
Can we reconstruct the main routes of GBM evolution under therapy?
Type I model of tumor evolution

Birth

Tumor common ancestor

Diagnosis & Treatment

Relapse

Primary sample

Relapse sample

Time
A simple model of tumor evolution

By observing the number and clonality patterns of mutations...

...we can estimate branch lengths and mutation rates
Untreated

Tumor substitution rates:

\[ u_1 \]

\[ u_2 \]

Recurrent

\[ t_{\text{grow}} \]

\[ t_{\text{div}} \]

\[ t_{\text{d}} \]

\[ t_{\text{g}} \]

Age at tumor lineage divergence

Age at diagnosis

Age at recurrence

\[ t_{\text{grow}} \]

\[ t_{\text{div}} \]

\[ t_{\text{d}} \]

\[ t_{\text{g}} \]

Post-treatment substitution rate (Mb-Year)

Pre-treatment mutation rate (Mb-Year)

Time between tumor divergence and diagnosis (years)

Percentile
Ordering genes

Common

TP53
PTEN
NF1
IDH1
ATRX
EGFR
PIK3CA
PREX1
PIK3CG
LTBP4
GLI3
PIK3R1
MT-ND4
PDGFRA
EGFRvIII
MSH6
PTPN11
RB1
PDGFRA
GLI3
NSD1
NF1
MSH6
PTPN11
RB1
PDGFRA
GLI3
NSD1
NF1

Untreated Only

Recurrent Only

Nature Genetics (2016).
GBM evolution shows distinct evolutionary trajectories associated with specific molecular mechanisms.
How spatial information (intratumor heterogeneity) informs evolution?
Expression-based subtyping

![Expression-based subtyping diagram]
Subtype switching in primary GBM

Expression subtype change

Multi-Section
- Switched: 33%
- Not Switched: 67%

Longitudinal
- Switched: 33%
- Not Switched: 67%
Are there specific genes associated to relapse?
Novel MGMT fusions in Recurrent GBM

Fusion gene:
- **NFYC-MGMT**
- **BTRC-MGMT**

**BTRC-MGMT**
- Primers:
  - Primer 1
  - Primer 2
  - Primer 3
  - Primer 4
- Exons:
  - Exon 1
  - Exon 2
  - Exon 3
  - Exon 4
  - Exon 5
- Fusion gene (bp):
  - 300
  - 200
  - 100
- Samples:
  - I: Initial tumor
  - R: Recurrent tumor

**NFYC-MGMT**
- Primers:
  - Primer 1
  - Primer 2
  - Primer 3
  - Primer 4
- Exons:
  - Exon 1
  - Exon 2
  - Exon 3
  - Exon 4
  - Exon 5
- Fusion gene (bp):
  - 300
  - 200
  - 100
- Samples:
  - P: Primary tumor
  - M: Malignant tissue
  - R: Recurrent tumor

**Figures**
- Initial tumor (I)
- Recurrent tumor (R)
### METHYLATION

- **Initial**
  - p-value: 1e-4
- **Recurrence**
  - p-value: 0.4

### EXPRESSION

- **Initial**
  - p-value: 0.2
  - n = 29, n = 32
- **Recurrence**
  - p-value: 4e-4
  - n = 25, n = 46

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- **MGMT UNMET**
- **MGMT MET**
- **MGMT EXP**

- p-value: 5.9e-5
- p-value: 1.1e-5

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- **OS** (Overall Survival)
- **Time (month)**
- **Censored**
Conclusions

- How does GBM evolve under therapy?
  - Type I: fits 70% clonal histories. The clone at diagnosis is not direct ancestor of clone at relapse.
    - Type II cases (22%) have shorter time between diagnosis and relapse (0.7 vs 1.5 years).
    - Type III: enriched in RB1 deletions.
  - Most patients the evolutionary rate ~ 0.03 /Mb.year,
    - Subsets of patients (17%) undergo hypermutation. Hypermutations associated to dinucleotide signatures (CpC/GpG) in genes with higher expression.
    - Common ancestor present years before diagnosis.
  - Expression typing is not stable in primary GBM (63% change).
    - Subtype does not reflect clonal history but changing mixture of cell populations.
    - Unsupervised dimensionality reduction does.

- Can we reconstruct main evolutionary routes?
  - Type I model allows (topological) ordering events.
    - IDH1 -> TP53, PTEN -> EGFR, MSH6, LTBP4

- Are there specific alterations associated to progression?
  - Preserved in evolution: IDH1, FGFR3-TACC3,…
  - Lost in evolution: EGFRviii lost at relapse.
  - Gained in evolution:
    - New relapse specific alterations (> 10%): LTBP4 (TGF-β), MSH6, PREX1, etc.
    - Novel relapse specific MGMT gene fusions, MGMT expression at relapse associated to prognosis.
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