UNDERSTANDING MOLECULAR TESTING IN BRAIN TUMORS: *HOW CLINICALLY USEFUL IS IT?*

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Goals:

1. Describe the most commonly used tests in glioma, including MGMT, 1p19q and IDH1/2, and how these affect clinical decision making.
2. Discuss the utility of broad genetic panels for glioma patients.
3. Describe the most commonly used tests in brain metastases, and how these affect clinical decision making.

Context:

In 2016, the WHO released new diagnostic criteria for CNS tumors that uses both histologic and molecular features to define tumors(1). Major changes include:

1. Use of Isocitrate Dehydrogenase (IDH) to sub-type low grade gliomas and glioblastoma
2. Use of 1p19q co-deletions to define oligodendroglioma
3. Subtyping medulloblastoma based on signaling pathway activity (not covered here)

Glioblastoma: (not glioblastoma multiforme)

O^6-methylguanine-methyl transferase (MGMT)

- Enzyme that repairs the damage done by alkylating chemotherapy
  - Temozolomide(TMZ), lomustine (CCNU), carmustine (BCNU)
  - more MGMT activity = more DNA repair = less tumor killed
  - less MGMT activity = less DNA repair = more tumor killed
- Epigenetic regulation by methylation
  - More methylated = less MGMT activity = more tumor killed
  - Less methylated = more MGMT activity = less tumor killed
- MGMT "positive"-associated with better response to TMZ(2)
- *Does "better" response matter when all you really have is TMZ?*
- Guides treatment in "non-perfect therapy candidates" (older, sicker, etc)
  - MGMT-meth patients benefited in TMZ arms, not in RT alone arms(3-5)
  - MGMT-unmeth patients do not get as significant benefit when using TMZ(2, 6)
- Pseudo-progression
  - when the MRI appears to demonstrate progression, but patient has treatment effect or radiation necrosis
  - approx. 90% of MGMT-meth patients in Brandes JCO 2008 study had pseudoprogression, while only 40% MGMT-unmeth did(7)
  - treatment for pseudo-progression includes time, steroids, BEV
  - treatment for true progression usually an anti-tumor agent

MGMT, Practically speaking

- ***MGMT methylation = good***
- Patients who may not tolerate RT+TMZ can be considered for RT or TMZ alone depending on their MGMT status
- Increasing in-treatment-field enhancement in MGMT positive patient may be pseudo-progression, which should be approached differently than true progression
- MGMT now being used to stratify patients in clinical trials
- Ordering:
  - Who: All GB patients with sufficient tissue
  - Technique: usually, PCR-based test
  - Wait time: 7-10 days

- Tissue Required: 5 unstained slides
- Cost: From $500-2000 USD depending on in-house v. send-out
- Insurance: not universally covered

Low-grade gliomas (LGG)

1. ***Oligodendroglioma and low grade astrocytoma no longer lumped together***
2. 1p19q co-deletion (co-del) and IDH gene mutations used to classify these tumors
3. Prognosis varies depending on the particular molecular characteristics of the tumor

Oligodendroglioma:

- The "new" oligodendroglioma is defined by 1q19q co-deletions
- The "new" astrocytoma *does not* have 1p19q co-del, but may have 1p ***or*** 19q deletion
- 1p19q is a chromosomal co-del of both the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q)
    - Role in pathogenesis not clear
    - Mechanism driving the translocation also not clear
    - 1p19q co-del is a strong marker of improved survival (8, 9)
    - 1p19q co-del may predict response to chemotherapy regimens, like PCV (from EORTC 26951, RTOG 9402)(10, 11)
    - CODEL study will compare RT+PCV to RT+TMZ since TMZ is a more tolerable regimen

1p19q, Practically speaking

- ***1p19q co-deletion = good***
- Patients who have this marker may be better candidates for observation or possibly, chemotherapy alone (no studies have asked this in a robust manner)
- Ordering:
    - Who: All LGG or oligo patients with sufficient tissue
    - Technique: Fluorescence in-situ hybridization (FISH)
    - Wait time: 3-5 days
    - Cost: About $ 850 USD (based on 2016 Medicare rate)
    - Tissue requirement: 3-5 slides
    - Insurance: more often covered

IDH1/IDH2: Isocitrate dehydrogenase 1 or 2

- Mutations are associated with longer progression free and overall survivals
- Not clear that this = more response to chemotherapy, or better behaved tumor(12)
- Wildtype associated with shorter progression free and overall survivals
- May act via HIF-1alpha or by encouraging the formation of an "oncogenic metabolite", 2-hydroxyglurate, which may encourage cells to grow independently of growth factors(13, 14)
- IDH1/2 mutations present in 50-80% of grade 2 and 3 astrocytomas and oligodendrogliomas(15)
- May be found in 5% of primary glioblastoma (80% of transformed)
- Very high proportion of patients with 1p19q deletions will also have IDH1 mutations (in one study, 100%)

IDH1/2, Practically speaking

- ***IDH1 or 2 mutation = good***
- Patients who have this marker may be better candidates for observation or possibly, chemotherapy alone (no studies have asked this in a robust manner)
- Being used as a stratification of overall risk and starting to be used in trials
- Ordering:
    - Who: All patients with sufficient tissue, a histologic diagnosis of LGG, oligodendroglioma, anaplastic oligodendroglioma, or glioblastoma
    - Technique: Immunohistochemistry or PCR with 5-14 day turn around
    - Cost: About $250-500 USD (IHC + professional fee)
    - Tissue requirement: 3-5 unstained slides
    - Insurance: may be covered more often than MGMT

ATRX: alpha-thalassemia/mental retardation syndrome X-linked

- Mutation or loss of expression associated with better prognosis(16)
- ATRX loss of function strongly associates with IDH mutations
- ATRX loss almost exclusively NOT associated with 1p19q deletion
- Did not make WHO classifications this time around

ATRX, Practically speaking

- ***ATRX loss = good***
- Used in combination with IDH and 1p19q, identify worst and best risk patients
- Ordering:
    - Who: Patients with sufficient tissue & histologic diagnosis of low grade glioma
    - Technique: Immunohistochemistry with 5-14 day turn around
    - Cost: About $250-500 USD (based on IHC + professional fee)
    - Tissue requirement: 3-5 unstained slides
    - Insurance: not clear

Seema, now I'm really confused! How do I use these?

For patients with glioblastoma:

- Patients with IDH1/2 mutated tumors may have better prognosis
- MGMT will help predict response to treatment and possibly, help identify patients with pseudo-progression

For patients with lower grade gliomas:

- 1p/19q deletions separate oligodendroglial from astrocytic tumors
- 1p/19q deletions indicate better prognosis patients
- IDH1/2 mutations will identify better prognosis patients
- ATRX loss will identify better prognosis patients
- Tumors without 1p/19q, an IDH mutation or ATRX loss may behave like a glioblastoma!! ("triple negative")

Great, but this looks nothing like the commercial tests my patients are asking for. What are those, and if it tests everything, why shouldn't I use those?

Commercial Tumor Genetic Panels

- Each has proprietary techniques and panels that may detect tumor genetic abnormalities, RNA and protein transcription levels.
- Examples:
    - Oncoplex
    - Foundation One
    - GPS Cancer
    - Your institution's very own internal customized panel (i.e. Stanford's is called "STAMP")
- The good:
    - pretty comprehensive
    - generally, reports are physician friendly with explanations about the abnormality found
    - may link the abnormality to an available drug (for example, EGFR mutation with a list of EGFR TKIs)
    - in house testing makes ordering easy
- The bad:
    - uses tissue that you may need later, and more than if you pick a targeted test
    - 21 day or longer wait period
- ***The ugly****:*
    - may cost ***thousands*** out of pocket (closer to 10K for some!)
    - though you will likely get an "actionable" target, most drugs aren't approved for use in glioma
    - ***the Signature Trial, which used a gene panel to assign treatment to 469 patients with targetable mutations, had a partial + complete remission rate of just 2.4%!!(17)***
    - a targetable mutation does not = a driver mutation

I have backed away from using "comprehensive panels" in glioma patients unless the patient understands both the cost and the low likelihood of a drug that will alter tumor course.

CNS Metastases

- 160,000 patients/year in the US versus 25,000 with glial tumors
- breast CA, lung CA and melanoma have targetable mutations with drugs that confer survival benefit
- many targeted tyrosine kinase inhibitors have CNS penetration and can be used to treat brain metastases with medical management
- use of specific molecular panels is standard of care in these diseases
  - NSCLC:
    - EGFR mutations: erlotinib, gefitinib, afatinib, osimeritinib (for T790M)
    - ALK/ROS mutations: crizotinib, ceritinib, alectinib, brigantinib, lorlatinib
    - PD-1/PDL-1: nivolumab, pembrolizumab
  - Her2+ Breast CA:
    - Lapatinib
  - Melanoma:
    - BRAF v600e: dabrafenib with trametinib, vemurafenib and cobimetinib
    - PD-1/PDL-1: nivolumab, pembrolizumab
- Testing for EGFR or BRAF costs around $600-900 US dollars
- My approach:
  - if they have not seen a sub-specialist, I confer with colleague and order specific tests pre-visit
  - if they will not be seeing a specialist, I will either recommend the specific tests or ask the primary oncologist to consider panel testing.

Take Home

These tests are just like a CBC
You should know why you are ordering it before you order it
You should know what you will do with the result

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