Diagnostic application of SNP-arrays to brain cancers

Adriana Olar

4/17/2018
No disclosures
55 yo M, focal motor seizure
DIAGNOSIS

BRAIN, LEFT FRONTAL LOBE, BIOPSY:
- DIFFUSE GLIOMA, OLIGODENDROGLIAL MORPHOLOGY, WHO GRADE II
- IDH1-R132H MUTANT PROTEIN IMMUNOEXPRESSED (IHC)
- PENDING 1P/19Q CO-DELETION STATUS (SNP-ARRAY)
An Integrated Genomic Analysis of Human Glioblastoma Multiforme


IDH1 (Isocitrate Dehydrogenase 1)

2q33.3

- **IDH1 mutation:**
  G395A → Arg132His (R132H)

Glioma-CpG Island Methyelator Phenotype (G-CIMP)

G-CIMP prevalence and outcome in diffuse glioma: (left) methylation profiling shows an association of CIMP status with tumour grade (red, methylated; green, unmethylated; black line, G-CIMP/IDH1-positive; grey line, G-CIMP/IDH1-negative; white lines, unknown IDH status); (right) Kaplan–Meier survival curves plotting patient outcome by G-CIMP status (red lines, G-CIMP-positive; black lines, G-CIMP-negative)

Noushmehr et al (2010), Cancer Cell
THE DIAGNOSTIC ROLE OF IDH
THE DIAGNOSTIC ROLE OF IDH
The Prognostic Role of IDH

A Glioblastoma

B Anaplastic Astrocytoma

The Prognostic Role of IDH

IDH mutation status is prognostic beyond the histological grade

Hartmann C et al. Acta Neuropathol. 2010
Molecular Genetic Analysis of Oligodendroglial Tumors Shows Preferential Allelic Deletions on 19q and 1p

Julia Reifenberger,∗‡ Guido Reifenberger,∗‡ Lu Liu,∗§ C. David James,† Wolfgang Wechsler,‡ and V. Peter Collins∗§

Table 2. Univariate genetic predictors of response and risk of death in patients with anaplastic oligodendroglialomas*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chemotherapeutic response</th>
<th>Risk of death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response rate, No./total No. (%)</td>
<td>P</td>
</tr>
<tr>
<td>Chromosome 1p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allelic loss</td>
<td>24/24 (100)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intact</td>
<td>3/12 (25)</td>
<td></td>
</tr>
<tr>
<td>Chromosome 19q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allelic loss</td>
<td>23/28 (82)</td>
<td>.126</td>
</tr>
<tr>
<td>Intact</td>
<td>3/6 (50)</td>
<td></td>
</tr>
<tr>
<td>Chromosomes 1p and 19q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allelic loss of both</td>
<td>22/22 (100)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No allelic loss of both</td>
<td>4/13 (31)</td>
<td></td>
</tr>
<tr>
<td>Chromosome 10q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allelic loss</td>
<td>5/8 (63)</td>
<td>.126</td>
</tr>
<tr>
<td>Intact</td>
<td>23/26 (88)</td>
<td></td>
</tr>
<tr>
<td>CDKN2A gene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deleted</td>
<td>5/8 (63)</td>
<td>.363</td>
</tr>
<tr>
<td>Intact</td>
<td>24/30 (80)</td>
<td></td>
</tr>
<tr>
<td>TP53 gene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>3/6 (50)</td>
<td>.123</td>
</tr>
<tr>
<td>Wild-type</td>
<td>28/34 (82)</td>
<td></td>
</tr>
</tbody>
</table>

*Risk of death was calculated as RR (relative risk) and was determined for all Cox models. Corresponding P values are shown with significant values in boldface type. All P values are two-sided. CI = confidence interval.
558 diffuse gliomas (A, O, OA)
B Gliomas Classified According to Molecular Subtype

- LGG with IDH mutation and 1p/19q codeletion (N=84)
- LGG with IDH mutation and no 1p/19q codeletion (N=139)
- LGG with wild-type IDH (N=55)
- GBM with IDH mutation (N=24)
- GBM with wild-type IDH (N=373)

P<0.001 by log-rank test

Overall Survival (%)

Years since Diagnosis
**Diffuse glioma**

<table>
<thead>
<tr>
<th></th>
<th>IDH-mutant</th>
<th>IDH-wild type</th>
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</thead>
<tbody>
<tr>
<td><strong>ATRX-mutant</strong></td>
<td>ATRX-wild type</td>
<td>ATRX-wild type</td>
</tr>
<tr>
<td><strong>TP53-mutant</strong></td>
<td>1p/19q co-deleted</td>
<td>TP53-wild type</td>
</tr>
<tr>
<td><strong>TERTp-mutant</strong></td>
<td>TERTp-mutant</td>
<td>TERTp-mutant</td>
</tr>
<tr>
<td><strong>FUBP1/CIC-mutant</strong></td>
<td>EGFR alterations*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTEN loss</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+7/-10</td>
<td></td>
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<tr>
<td></td>
<td>CDKN2A/B homozygous deletion</td>
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</tbody>
</table>

- **Diffuse astrocytoma**
- **Anaplastic astrocytoma**

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<tbody>
<tr>
<td><strong>Secondary glioblastoma</strong></td>
<td>Oligodendroglioma</td>
<td>Primary glioblastoma</td>
</tr>
</tbody>
</table>
Oligodendroglioma

Molecularly defined entity

• IDH mutations
• 1p/19q co-deletion
FINAL DIAGNOSIS

BRAIN, LEFT FRONTAL LOBE, BIOPSY:
- OLIGODENDROGLIOMA, WHO GRADE II
- IDH1-R132H MUTANT (IHC, NGS)
- 1P/19Q CO-DELETED (SNP-ARRAY)
TREATMENT

• Sequential RT + PCV (old age + subtotal resection/biopsy) (RTOG 9802)
31 yo M, difficulty speaking
INITIAL DIAGNOSIS

1. BRAIN, RIGHT TEMPORAL LOBE, **BIOPSY**:  
   - MIXED OLIGOASTROCYTOMA, WHO GRADE III  
   - *IDH1-R132H* MUTANT (Sanger sequencing, per report)  
   - 1P/19Q CO-DELETED (FISH, per outside report)
RESECTION

Chromosome 1 is intact
BIOPSY

RESECTION (+ 1 mo)
FINAL DIAGNOSIS

1. BRAIN, RIGHT TEMPORAL LOBE, **BIOPSY:**
   - ANAPLASTIC ASTROCYTOMA, WHO GRADE III
   - *IDH1-R132H* MUTANT (Sanger sequencing, per report)
   - 1P/19Q NOT CO-DELETED (SNP-ARRAY)

2. BRAIN, RIGHT TEMPORAL LOBE, **RESECTION:**
   - ANAPLASTIC ASTROCYTOMA, WHO GRADE III
   - *IDH1-R132H* MUTANT (Sanger sequencing, initial biopsy)
   - 1P/19Q NOT CO-DELETED (SNP-ARRAY)
   - *TERT* PROMOTER MUTATION STATUS: WILD-TYPE (Sanger sequencing, outside test)
   - *MGMT* PROMOTER METHYLATION STATUS: UNMETHYLATED (RT-PCR and Methylation specific PCR, outside test)
The F.I.S.H. issue
TREATMENT & FOLLOW-UP

• Intensity modulated radiation therapy
• Adjuvant TMZ
• Stable MRI
62 yo W, aphasia and seizures

T2-Flair

T1-post C
DIAGNOSIS

BRAIN, LEFT TEMPORAL LOBE, BIOPSY:
- DIFFUSE GLIOMA, ASTROCYTIC MORPHOLOGY, MINIMUM WHO GRADE III
- IDH1-R132H MUTANT PROTEIN NEGATIVE (IHC)
- PENDING 1P/19Q CO-DELETION STATUS (SNP-ARRAY)
+MDM2

-9p (CDKN2A/B)
7: EGFR

Exon 1

EGFR amplification

EGFRvIII (exon 2-7 deletion)

Exons 8 - 27
Truncated
Constitutively phosphorylated
Increased intracellular signaling and cell proliferation
Due to intellectual property (unpublished work) this slide was removed.
I apologize for the inconvenience.

For information on EGFRvIII and glioblastoma gene expression subgroups please refer to:

Journal of Pathology
J Pathol 2014; 232: 165–177
Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.4282

Using the molecular classification of glioblastoma to inform personalized treatment

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FINAL DIAGNOSIS

BRAIN, LEFT TEMPORAL LOBE, BIOPSY:
- HIGH-GRADE ASTROCYTOMA, MINIMUM WHO GRADE III
- MOLECULAR GLIOBLASTOMA
  - IDH1/2-WILD TYPE (IHC, NGS)
  - 1P/19Q NOT CO-DELETED (SNP-ARRAY)
  - LOSS OF CHROMOSOME 7/GAIN OF CHROMOSOME 10 PRESENT
- EGFR AMPLIFICATION/EGFRvIII PRESENT
- CDKN2A/B HOMOZYGOUS DELETION PRESENT
TREATMENT & FOLLOW-UP

• Concurrent chemoradiation (TMZ+ RT)
• Early progression (+ 2 months)
• Avastin
• Adjuvant TMZ
53 yo woman

- History of a childhood brainstem/posterior fossa tumor status post resection and radiation (two prior craniotomies at ages 9 and 18).
- The histological diagnosis unknown
- Early imaging not available
DIAGNOSIS

CEREBELLUM, LEFT, CRANIOTOMY AND RESECTION OF MASS:
- NECROSIS, REACTIVE CHANGES, AND ACUTE HEMORRHAGE
- CEREBELLAR ATROPHY
Chromosome 7 – *KIAA1549-BRAF* fusion-duplication
BRAF alterations in Pilocytic astrocytoma

Collins VP, 2015, Acta NP
FINAL DIAGNOSIS

CEREBELLUM, LEFT, CRANIOTOMY AND RESECTION OF MASS:
- NECROSIS, REACTIVE CHANGES, AND ACUTE HEMORRHAGE
- CEREBELLAR ATROPHY
- MOLECULAR EVIDENCE (KIAA1549:BRAF FUSION DUPLICATION DETECTED) OF RESIDUAL NEOPLASM
TREATMENT & FOLLOW-UP

• Management of symptoms (severe left corneal ulcer, whole-body tremor)
• Serial imaging
Endolymphatic sac tumor

Due to intellectual property (unpublished work) this slide was removed. I apologize for the inconvenience.

Please refer to abstract work (#1827) published In Modern Pathology (USCAP 2018).

https://www.nature.com/articles/labinvest201818.pdf
5 yo girl, broad gait, vomiting, neck pain, head tilt

Post-op

T2-Flair

T1-post C
DIAGNOSIS

CEREBELLUM, CLINICALLY RIGHT, CRANIOTOMY AND RESECTION:
- MEDULLOBLASTOMA, LARGE CELL/ANAPLASTIC, WHO GRADE IV
Kool, 2012, Acta NP.
<table>
<thead>
<tr>
<th>Genetic profile</th>
<th>Histology</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulloblastoma, WNT-activated</td>
<td>Classic</td>
<td>Low-risk tumour; classic morphology found in almost all WNT-activated tumours</td>
</tr>
<tr>
<td></td>
<td>Large cell / anaplastic</td>
<td>Tumour of uncertain clinicopathological significance</td>
</tr>
<tr>
<td>Medulloblastoma, SHH-activated, <strong>TP53-mutant</strong></td>
<td>Classic</td>
<td>Uncommon high-risk tumour</td>
</tr>
<tr>
<td></td>
<td>Large cell / anaplastic</td>
<td>High-risk tumour; prevalent in children aged 7–17 years</td>
</tr>
<tr>
<td></td>
<td>Desmoplastic / nodular (very rare)</td>
<td>Tumour of uncertain clinicopathological significance</td>
</tr>
<tr>
<td>Medulloblastoma, SHH-activated, <strong>TP53-wildtype</strong></td>
<td>Classic</td>
<td>Standard-risk tumour</td>
</tr>
<tr>
<td></td>
<td>Large cell / anaplastic</td>
<td>Tumour of uncertain clinicopathological significance</td>
</tr>
<tr>
<td></td>
<td>Desmoplastic / nodular</td>
<td>Low-risk tumour in infants; prevalent in infants and adults</td>
</tr>
<tr>
<td></td>
<td>Extensive nodularity</td>
<td>Low-risk tumour of infancy</td>
</tr>
<tr>
<td>Medulloblastoma, non-WNT/non-SHH, group 3</td>
<td>Classic</td>
<td>Standard-risk tumour</td>
</tr>
<tr>
<td></td>
<td>Large cell / anaplastic</td>
<td>High-risk tumour</td>
</tr>
<tr>
<td>Medulloblastoma, non-WNT/non-SHH, group 4</td>
<td>Classic</td>
<td>Standard-risk tumour; classic morphology found in almost all group 4 tumours</td>
</tr>
<tr>
<td></td>
<td>Large cell / anaplastic (rare)</td>
<td>Tumour of uncertain clinicopathological significance</td>
</tr>
</tbody>
</table>
Copy number changes

Northcott, 2017, Nature
Copy number changes

Kool et al., 2012, Acta NP.
No mutations identified on NGS

<table>
<thead>
<tr>
<th>ABL1</th>
<th>AKT1</th>
<th>ALK</th>
<th>APC</th>
<th>ATM</th>
<th>BRAF</th>
<th>CDH1</th>
<th>CDKN2A</th>
<th>CSF1R</th>
<th>CTNNB1</th>
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</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>ERBB2 (HER2)</td>
<td>ERBB4</td>
<td>EZH2</td>
<td>FBXW7</td>
<td>FGFR1</td>
<td>FGFR2</td>
<td>FGFR3</td>
<td>FLT3</td>
<td>GNA11</td>
</tr>
<tr>
<td>GNAQ</td>
<td>GNAS</td>
<td>HNF1A</td>
<td>HRAS</td>
<td>IDH1</td>
<td>IDH2</td>
<td>JAK2</td>
<td>JAK3</td>
<td>KDR</td>
<td>KIT</td>
</tr>
<tr>
<td>KRAS</td>
<td>MET</td>
<td>MLH1</td>
<td>MPL</td>
<td>NOTCH1</td>
<td>NPM1</td>
<td>NRAS</td>
<td>PDGFRA</td>
<td>PIK3CA</td>
<td>PTEN</td>
</tr>
<tr>
<td>PTPN11</td>
<td>RB1</td>
<td>RET</td>
<td>SMAD4</td>
<td>SMARCB1</td>
<td>SMO</td>
<td>SRC</td>
<td>STK11</td>
<td>TP53</td>
<td>VHL</td>
</tr>
</tbody>
</table>
Figure 2. Amplifications of MYC genes in human medulloblastoma. (A) MYC and MYCN amplifications, shown in red, and the most common recurrent subgroup mutations (missense, indels, frameshift mutations), shown in black, relative to the four medulloblastoma subgroups. Results are reported across three genomic studies (Jones et al. 2012; Pugh et al. 2012; Robinson et al. 2012). (B,C) MYCN and MYC amplifications relative to four medulloblastoma subgroups across three whole genome sequencing (WGS) studies (Jones et al. 2012; Pugh et al. 2012; Robinson et al. 2012). (D,E) MYCN and MYC amplifications relative to four medulloblastoma subgroups in 827 medulloblastomas (Northcott et al. 2012b).
Loss - Chromosome 17: 1- 16557425 (hg19)

Homozygous loss - Chromosome 17: 7571034 - 7579074

TP53 Exons 5-11

NGS Coverage
FINAL DIAGNOSIS

BRAIN, POSTERIOR FOSSA, CRANIOTOMY AND RESECTION:
- MEDULLOBLASTOMA, LARGE CELL/ANAPLASTIC, WHO GRADE IV, SHH-ACTIVATED, **TP53-MUTANT**
- **MYCN** STATUS: AMPLIFICATION PRESENT
- **MYC** STATUS: NEGATIVE FOR AMPLIFICATION
TREATMENT & FOLLOW-UP

• Negative for drop metastases
• Radiation
• ACNS0332 clinical trial, arm A (Carboplatin + RT)
25 yo man

• Balance difficulties
• Nausea
• Headaches
DIAGNOSIS

CEREBELLUM, LEFT, CRANIOTOMY AND RESECTION:
- NODULAR/DESMOPLASTIC MEDULLOBLASTOMA, WHO GRADE IV
Sequencing results

- **IDH1 p.R132C**
- **PTEN p.R173C**
- **ASXL1 p.V1357I** (Foundation One)
- **TERT PROMOTER -124C>T MUTATION PRESENT** (Foundation One)
IDH1 p.R132C

Beta catenin nuclear immunoexpression
YAP1 positive
GAB1 positive

Northcott, 2017, Nature
FINAL DIAGNOSIS

CEREBELLUM, LEFT, CRANIOTOMY AND RESECTION:
  - NODULAR/DESMOPLASTIC MEDULLOBLASTOMA, WHO GRADE IV (SEE COMMENT)
  - SHH-ACTIVATED, TP53 WILD-TYPE
    - IDH1-R132C MUTATION PRESENT (NGS)
    - PTEN-R173C MUTATION PRESENT (NGS)
    - ASXL1-V1357I MUTATION PRESENT (Foundation One)
    - TERT PROMOTER -124C>V MUTATION PRESENT (Foundation One)
  - MONOSOMY 6 and ISOCHROMOSOME 17q: NEGATIVE (See separate SNP-array report)
TREATMENT & FOLLOW-UP

• 4 local recurrences – last one ~ spinal drop metastases - craniospinal axis radiation
• Vismodegib (Hedgehog Pathway Inhibitor)
12 yo M, headache and vomiting
DIAGNOSIS

CEREBELLUM, CRANIOTOMY AND RESECTION:
- LARGE CELL/ANAPLASTIC MEDULLOBLASTOMA, WHO GRADE IV

IMMUNOHISTOCHEMISTRY:
- BETA-CATENIN: NOT TRANSLOCATED TO NUCLEUS
- YAP1: NEGATIVE
- GAB1: NEGATIVE
Medulloblastoma

Ramkissoon, 2017, Neuro-Oncology

Northcott, 2017, Nature
FINAL DIAGNOSIS

BRAIN, LEFT CEREBELLUM, CRANIOTOMY AND RESECTION:
- LARGE CELL/ANAPLASTIC MEDULLOBLASTOMA, WHO GRADE IV
  - NON-WNT/NON-SHH MOLECULAR SUBTYPE
  - GROUP 4 Favored
TREATMENT & FOLLOW-UP

• Spine drop metastases (imaging, LP+), M3 staging
• Concurrent chemotherapy (Vincristine) and craniospinal axis radiation
12 yo M with headaches and vomiting
DIAGNOSIS

CEREBELLUM, CRANIOTOMY AND RESECTION:
- LARGE CELL/ANAPLASTIC MEDULLOBLASTOMA, WHO GRADE IV

IMMUNOHISTOCHEMISTRY:
- BETA-CATENIN: NOT TRANSLOCATED TO NUCLEUS
- YAP1: NEGATIVE
- GAB1: NEGATIVE
Tetraploid

Legend: Gain Loss

-17p

+17q
FINAL DIAGNOSIS

BRAIN, LEFT CEREBELLUM, CRANIOTOMY AND RESECTION:
- LARGE CELL/ANAPLASTIC MEDULLOBLASTOMA, WHO GRADE IV
- NON-WNT/NON-SHH MOLECULAR SUBTYPE
TREATMENT & FOLLOW-UP

• Negative for spine metastases (imaging, LP)
• Planning to start radiation
Due to intellectual property (unpublished work) the next 4 slides were removed. I apologize for the inconvenience.

The slides were presenting a case of a young woman with a spinal cord mass that showed \textit{NMYC} amplification and isodicentric 17q on SNP-array testing (medulloblastoma abnormalities). On microscopy the tumor was AE1/AE3, GFAP and S100 immunopositive and negative for Synaptophysin (technically excluding medulloblastoma). Electron microscopy confirmed ependymoma (cilia and microvilli demonstrated) (Courtesy of Dr. Caterina Giannini, Mayo Clinic). The case illustrated the importance of integrative diagnostics (optic microscopy, electron microscopy, molecular diagnostics, radiology)

\\textbf{!!!! AE1/AE3 immunohistochemistry in the CNS cross-reacts with GFAP (glial tumors will be AE1/AE3 positive!!!!). For DDx with metastatic carcinoma is best to use Cam 5.2, CK7, CK20.}
DNA methylation-based classification of central nervous system tumours

76 histopathological entities
Conclusions

• The high sensitivity and superior coverage of the SNP-array assays offer superior diagnostic accuracy compared to commonly used molecular assays (e.g. F.I.S.H.) in brain tumor diagnostics.

• Offers complementary copy number information that should be interpreted in the clinical, pathological, and radiological context.

• Limitations
• Complexity of cancer
Thank you!

Protesting Against New Technology - The Early Days

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