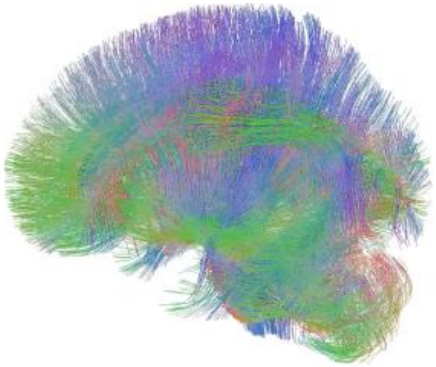


Welcome to the Neuro-Oncology Clinic

Resource Sheet 2

Molecular pathology of Gliomas

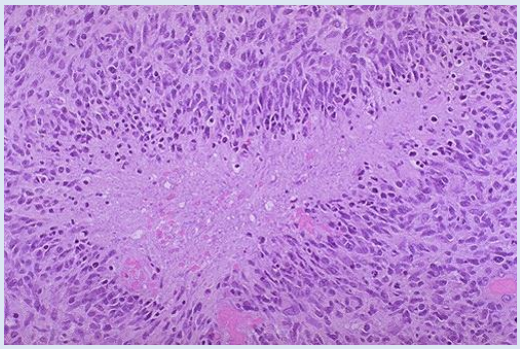
WHO 2016 introduces an integrated diagnosis for gliomas, which comes in two parts. The first is the conventional histopathology report, featuring light microscopy and immunohistochemistry, which is generally available within 7-10 days of the patient's surgery. The second is the molecular pathology testing, which takes an additional 7-14 days.

Quite often, when we first see the patient, we do not have the full integrated diagnostic data!

The old school classification system

Classically, gliomas were defined by the morphological appearance of the predominant tumour cell type and graded according to ultrastructural features from light microscopy.

- **Astrocytic or oligodendroglial morphology.** These are the two precursor cell types from which glial cells were thought to originate. Often tumours contain a mixture of malignant astrocytes & oligodendrocytes and used to be described as mixed astrocytomas.
- **Increased mitotic rate.** A hallmark of cancer. Increased mitosis, as quantified by Mib1 staining of the Ki67 protein, collates very closely with outcome in a whole range of brain tumours, including gliomas.
- **Microvascular proliferation.** Indirect evidence of rapid tumour formation (in the true sense of the word – a ball of cells). Once the ball of cells becomes about 1mm in size, oxygen availability to the centre of the ball of cells is diffusion bound. A tumour needs to induce a blood supply from the surrounding stroma to keep growing.
- **Necrosis.** Even more indirect evidence of rapid tumour growth. The tumour is growing so fast that it outstrips its oxygen supply, and cells in the centre of the tumour die off. On H&E stain, the area looks pink because of the lack of intact nuclei which are dark purple.



Notice the central necrotic area in this H&E section of a glioblastoma

Gliomas are classically divided into low grade (Grades 1 and 2) and high grade (Grades 3 & 4) tumours. Low grade gliomas exhibit increased mitotic rate but typically grow by infiltrating into surrounding brain tissue rather than destroying it through 'tumour' formation. High grade gliomas will typically demonstrate microvascular proliferation and/or necrosis. An astrocytic tumour with increased mitosis, microvascular proliferation and necrosis is a Glioblastoma (grade 4 tumour). A tumour with microvascular proliferation but no necrosis (or vice versa) is a grade 3 tumour.

This classification works reasonably well but reflects an underlying continuous spectrum of phenotypic appearances of glioma. At the same time, int became clear that there were specific genetic mutations that are more closely associated with different grades of tumours…

The new integrated classification

In 2016 a group of the world's leading neuropathologists met to establish a new classification for gliomas on behalf of the WHO. The resulting integrated classification is more complex, but also more powerful in stratifying patients by outcome and optimum therapy type. To keep things simple we will consider the following entities:

Type	Grade				
Diffuse astrocytoma	2				
Oligodendroglioma	2				
Anaplastic Oligo	3				
Glioblastoma	4				

So how do we distinguish these types in the new classification?

Step 1 – light microscopy

The starting point remains the light microscopy appearance of the tumour, low grade or high grade, astrocytoma or oligodendroglioma.

Step 2 - Evaluate IDH mutation status and 1p19q co-deletion status

IDH or isocitrate dehydrogenase is an enzyme in the cell metabolic pathway. Mutations in the IDH enzyme lead to accumulation of alpha-ketoglutarate, which is an oncometabolite (to cut a long and boring story short it promotes oncogenesis in tumour cells). Tumours with a mutation in the IDH1 or IDH2 enzymes tend to be associated with a better prognosis. High grade tumours with an IDH mutation are often those that have grown through transformation from a lower grade tumour, tend to do slightly better than IDH wild-type tumours, and may benefit from adjuvant chemotherapy after radiotherapy. So let's add that onto our simple classification the common IDH status for each type.

Type	Grade	IDH			
Diffuse astrocytoma	2	Mutant			
Oligodendroglioma	2	Mutant			
Anaplastic Oligo	3	Mutant			
Glioblastoma	4	Wild Type			

Co-deletions of chromosomes 1p and 19q are associated with **oligodendrogliomas**. This is helpful because the new classification does not support mixed tumour (oligoastrocytomas) any more. It's also incredibly helpful because it defines a patient population who defintely benefit from adjuvant PCV chemotherapy treatment – be sure to ask about this in clinic. Let's revisit our classification and add to it.

Type	Grade	IDH	1p19q		
Diffuse astrocytoma	2	Mutant	Non-codeleted		
Oligodendroglioma	2	Mutant	Co-deleted		
Anaplastic Oligo	3	Mutant	Co-deleted		
Glioblastoma	4	Wild Type	Non-codeleted		

Step 3 – Add in ATRX and TERT mutations

Okay let's go to the next step. ATRX (alpha thalassemia/mental retardation syndrome X-linked) is an important regulator of DNA transcription. We use an antibody to the wild type version of this protein, which therefore stains positively in normal cells. Mutations in the ATRX gene results in loss of nuclear staining in tumour cells. ATRX is helpful for telling apart astrocytic tumours, in that it is lost in low grade diffuse astrocytomas, and retained in high grade astrocytomas (i.e. Glioblastoma). We use ATRX immunohistochemistry routinely for astrocytomas for this reason. Let's add this to our classification and see what we have.

Type	Grade	IDH	1p19q	ATRX	
Diffuse astrocytoma	2	Mutant	Non-codeleted	Lost	
Oligodendroglioma	2	Mutant	Co-deleted	Retained	
Anaplastic Oligo	3	Mutant	Co-deleted	Retained	
Glioblastoma	4	Wild Type	Non-codeleted	Retained	

If you remember your notes from the hallmarks of cancer papers, you will recall that replicative immortality is a hallmark of cancer, and thus it is not surprising to find mutations in telomerase pathways in a range of tumours. These are the enzymes that restore padding to the ends of chromosomes each time they replicate. TERT (telomerase reverse transcriptase) gene promoter methylations are found in a range of tumours, but are particularly helpful in gliomas. TERT mutations are observed in Glioblastoma, but not in diffuse astrocytoma. You can think of TERT as ATRX in reverse. Let's add this to the mix:

Type	Grade	IDH	1p19q	ATRX	TERT
Diffuse astrocytoma	2	Mutant	Non-codeleted	Lost	Wild type
Oligodendroglioma	2	Mutant	Co-deleted	Retained	Mutant
Anaplastic Oligo	3	Mutant	Co-deleted	Retained	Mutant
Glioblastoma	4	Wild Type	Non-codeleted	Retained	Mutant

There are a couple of other categories in the WHO Glioma classification, but this covers the bulk of the tumours we see. If you are still following and want to see the full picture including diffuse midline gliomas, see the classification table by Prof Brandner on page 4 (warning – not for those of a nervous disposition).

Why does this matter in clinic?

We can pick apart tumour entities and give better prognosis estimates. One such entity is the astrocytic tumour that doesn't look very high grade under the microscope, but the MR imaging and history is worrying. If the tumour is IDHWY, ATRX retained and TERT mutated, we know that this is a 'baby' GBM waiting to rear its ugly head!

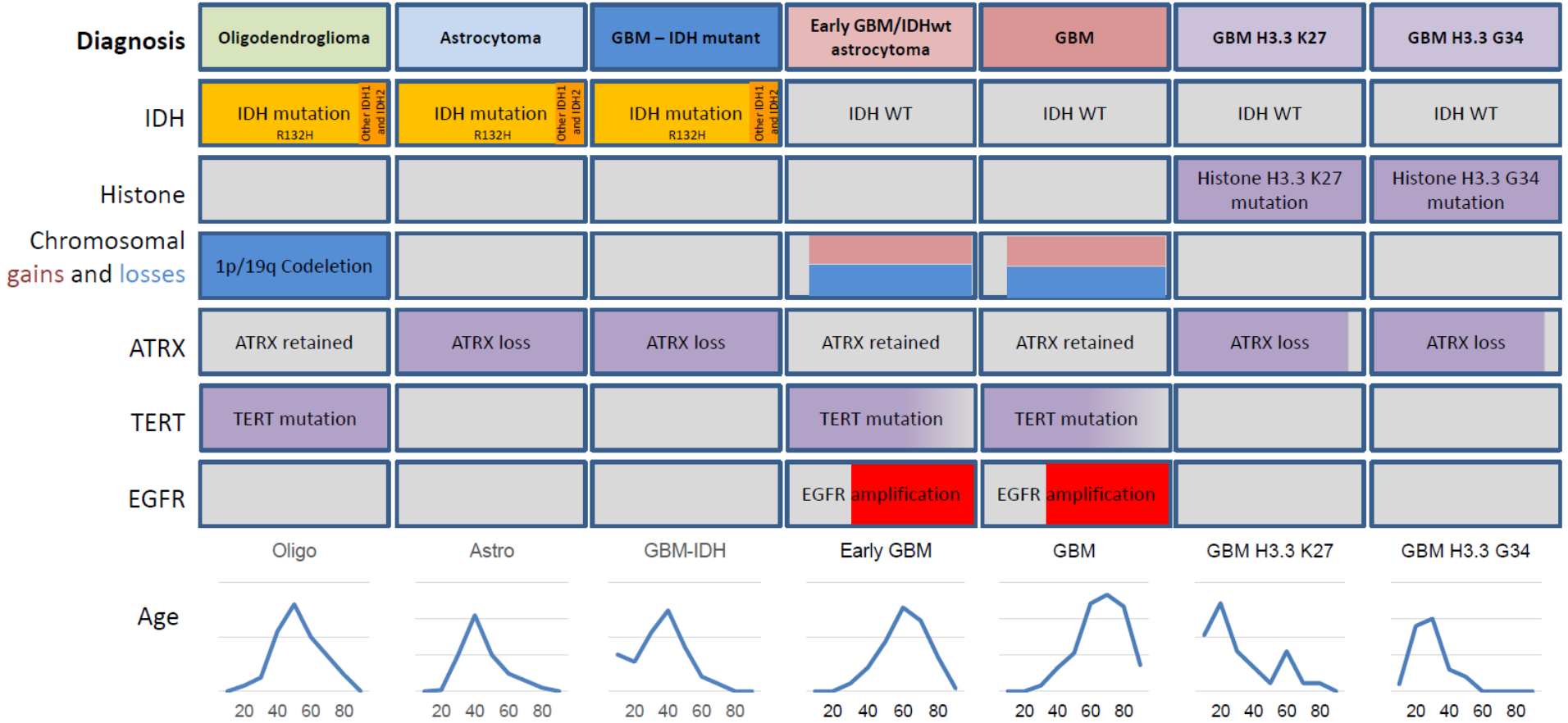
The integrated diagnosis categories also map to different treatment arms as evidence by RCT evidence:

- GBMs do best with combined chemo-radiation therapy and 6 months of adjuvant temozolomide chemotherapy
- All Oligodendrogliomas get a 4 year survival advantage if they have 6 cycles of PCV chemotherapy after radiotherapy
- IDH mutated astrocytomas get a survival advantage if they receive 12 cycles of temozolomide after radiotherapy

Find more teaching resources

To find an electronic copy of this resource sheet, and other materials, go to www.camradiotherapy.org.uk/neuroclinic or just scan this QR code with your phone:

Simplified, schematic guidance for the use of molecular markers for the integrated diagnosis of gliomas



Prof Brandner's schematic chart